

COMPOSITION STRUCTURE PROPERTY RELATIONSHIPS IN BIOACTIVE GLASSES

Sally Watts

Doctor of Philosophy

*Department of Materials
Imperial College London
London SW7 2AZ
United Kingdom*

COMPOSITION STRUCTURE PROPERTY RELATIONSHIPS IN BIOACTIVE GLASSES

Sally Watts

A thesis submitted in partial fulfilment of the requirements for the
degree of

Doctor of Philosophy

And the Diploma of Imperial College

Supervised by: Professor Robert Hill and Dr. Rob Law

*Department of Materials
Imperial College London
London SW7 2AZ
United Kingdom*

February 2010

Hench developed the first bioactive material, Bioglass[®], based on a soda-lime phospho-silicate glass. Most materials, elicit a neutral response when implanted into the human body. Bioglass[®], however, was seen to create a positive response by depositing the body's natural bone substance, Hydroxyapatite on its surface. Although it is recognised that compositional modifications effect bioactivity, there is very little comprehension of the composition-structure-property relationships that result in such bioactivity. The objective of this investigation, therefore, was to study such fundamental relationships with respect to two components often found in bioactive glass compositions – P_2O_5 and MgO .

The first component studied was P_2O_5 . The design of two series was undertaken – the first, a straight substitution of silicon for phosphorus, varying the network connectivity, NC, and the second, a charge compensating series, keeping the NC constant. ^{31}P and ^{29}Si MAS NMR of the two series provided evidence that the glasses were phase separated, with a predominantly Q^2 silicate structure co-existing with phosphorus in a predominantly Q^0 orthophosphate environment. Raman, FTIR, density measurements, differential thermal analysis and dilatometric analysis all further supported the existence of this structure. Dissolution studies in SBF highlighted the importance of phosphorus on bioactivity, with the glass dissolution rates of both series increasing with the addition of phosphorus. Instead of the dissolution of a glass depending solely on ion exchange reactions, as previously thought, it is proposed that dissolution depends upon the balance existing between the NC of the silicate phase and the existence of isolated orthophosphate rich domains. It is hypothesised that phosphorus in a phase separated structure is far more important than previously suspected, with its ability to preferentially dissolve into solution, dominating over the effect of NC on the resultant bioactivity of the glass and apatite formation.

The second component investigated was magnesium oxide and its influence on the glass structure when substituted for calcium oxide. Two series of glasses were designed, the first series with a high sodium content and the second series with a low sodium content. In order to eliminate any influence due to silicate network disruption, all glasses were designed to have a constant NC of 2.04. All physical parameters were seen to be related strongly to the substitution of magnesium oxide, in both series. ^{31}P , ^{29}Si and ^{25}Mg MAS-NMR of the high sodium magnesium glasses highlighted that magnesium, rather than acting to depolymerise the silicate network by acting as a network modifier, was acting partially as an intermediate oxide with a proportion entering the network as MgO_4 tetrahedra. The decreasing T_g and T_s values and increasing thermal expansion coefficients, with increasing MgO substitution, supported this theory; with the significantly weaker bond strength of $Mg-O$, compared to $Si-O$, explaining the experimentally observed weakening of the network. The corresponding results for the low sodium magnesium glasses also pointed to magnesium acting as an intermediate oxide, however, with a smaller proportion entering the silicate network as MgO_4 tetrahedra. It is suggested that magnesium acts as an intermediate oxide in highly disrupted glasses, with a more disrupted glass giving a higher proportion of MgO_4 . Dissolution studies in SBF settled the previously controversial subject of magnesium and bioactivity, with the addition of magnesium resulting in decreased glass dissolution rates and apatite formation in both series.

This work has highlighted the importance of having a detailed understanding of the composition-structure-property relationships which exist in a bioactive glass. It is suggested that, from the contribution this work makes to this understanding, coupled with the knowledge gained from parallel studies, we are now at the point where a specific bioactive glass composition could be engineered, and tailored for a particular biomedical application.

Dedication

This thesis is dedicated to my mum, dad and brother.

Declaration

This research was carried out at Imperial College London during the period of August 2005 to September 2008. This research is my own work and has not been previously submitted to any university for any award.

Sally Watts

February, 2010

Acknowledgements

I wish to extend my sincere gratitude to the following people:

- My supervisors, Prof. Robert Hill and Dr. Rob Law who have been a great inspiration and an absolute pleasure to work for. Their patience and guidance over the years was greatly appreciated.
- The EPSRC for funding this research.
- Richard Sweeney. Thank you for the advice and encouragement over the years – you are a real friend. I will miss our chats!
- Members of the Materials Department – especially Dr. David McPhail and Prof. Trevor Lindley. Thank you for your support and having faith in me.
- Biomaterials research group - Matt, Natalia, Delia, Nasrin (thank you for being my computer saviour!), Adam, Yann – thank you for your help and friendship.
- Two of the kindest and most lovely friends I have in the world and who I will miss greatly – Lin and Hiroko.
- The greatest thanks go to my mum, dad and brother for their love, unfailing encouragement and support. I love you very much and this thesis is for you.

I got there in the end!!

Contents

CONTENTS	6
LIST OF FIGURES.....	10
LIST OF TABLES.....	17
SYMBOLS AND ABBREVIATIONS.....	17
1 INTRODUCTION.....	21
1.1 OBJECTIVES OF STUDY	23
1.2 OUTLINE OF THESIS	23
2 MATERIALS IN MEDICINE	24
2.1 TRANSPLANTATION	24
2.2 IMPLANTATION	25
2.3 IMPLANT MATERIALS	27
2.3.1 <i>Stress Shielding</i>	29
2.4 FUTURE OF IMPLANT MATERIALS.....	29
2.5 BIOLOGICAL RESPONSE TO MATERIALS	30
2.5.1 <i>Toxic Response</i>	30
2.5.2 <i>Biologically Nearly Inert: Fibrous Capsule Formation</i>	30
2.5.3 <i>Bioresorption</i>	31
2.5.4 <i>Bioactive</i>	31
3 GLASS.....	37
3.1 GLASS STRUCTURE.....	40
3.1.1 <i>Zachariasen's Random Network Theory</i>	40
3.1.2 <i>Dietzel's Structural Model</i>	46
3.1.3 <i>Inorganic Polymer Model</i>	48
3.2 PHASE SEPARATION.....	50
4 BIOACTIVE GLASSES.....	55
4.1 MECHANISMS OF GLASS DEGRADATION	56
4.2 APATITE FORMATION	61

4.2.1	SBF	62
4.3	COMPOSITIONAL DEPENDENCE	64
4.3.1	<i>Inorganic Polymer Model and Bioactivity</i>	66
5	EXPERIMENTAL – MATERIALS AND METHODS	69
5.1	RATIONALE	69
5.1.1	<i>Glass Network Connectivity</i>	71
5.2	SERIES 1 – MAGNESIUM SUBSTITUTED GLASSES	73
5.3	SERIES 2 – PHOSPHORUS GLASSES	75
5.4	GLASS PRODUCTION	78
5.4.1	<i>Glass Casting</i>	79
5.5	X-RAY DIFFRACTION	80
5.6	DIFFERENTIAL THERMAL ANALYSIS	81
5.7	DILATOMETRY	81
5.8	DENSITY MEASUREMENTS	81
5.9	MAGIC ANGLE SPINNING NUCLEAR MAGNETIC RESONANCE	82
5.10	INFRARED AND RAMAN SPECTROSCOPY	83
5.10.1	<i>FTIR</i>	83
5.10.2	<i>Raman</i>	83
5.11	DISSOLUTION STUDIES	83
6	RESULTS AND DISCUSSION – PHOSPHORUS GLASSES	85
6.1	³¹ P MAS NMR	85
6.1.1	<i>³¹P MAS NMR of Series I Phosphorus Glasses</i>	85
6.1.2	<i>³¹P MAS NMR of Series II Phosphorus Glasses</i>	91
6.2	²⁹ Si MAS NMR	93
6.2.1	<i>²⁹Si MAS NMR of Series I Phosphorus Glasses</i>	93
6.2.2	<i>²⁹Si MAS NMR of Series II Phosphorus Glasses</i>	98
6.3	SUMMARY OF MAS NMR	99
6.4	FTIR SPECTROSCOPY	101
6.4.1	<i>Summary of FTIR for phosphorus series of glasses</i>	104
6.5	RAMAN SPECTROSCOPY	104
6.6	X-RAY DIFFRACTION	106
6.6.1	<i>Summary of XRD for phosphorus series of glasses</i>	108
6.7	DENSITY	109
6.7.1	<i>Doweidar’s analysis</i>	115
6.7.2	<i>Summary of density results for phosphorus series of glasses</i>	118
6.8	DIFFERENTIAL THERMAL ANALYSIS	118
6.8.1	<i>Glass Transition Temperature</i>	118

6.8.2	<i>Crystallisation Temperatures.....</i>	121
6.8.3	<i>Summary of DTA results for the phosphorus series of glasses</i>	124
6.9	DILATOMETRY.....	125
6.9.1	<i>Summary of dilatometry results for the phosphorus series of glasses.....</i>	129
6.10	DISSOLUTION STUDIES IN SIMULATED BODY FLUIDS.....	129
6.10.1	<i>Variation in Simulated Body Fluid pH.....</i>	130
6.10.2	<i>Summary of SBF pH data for the phosphorus series of glasses.....</i>	133
6.11	X-RAY DIFFRACTION	133
6.11.1	<i>Summary of SBF XRD results for phosphorus series of glasses</i>	142
6.12	FTIR SPECTROSCOPY	143
6.12.1	<i>Summary of SBF FTIR results for the phosphorus series of glasses.....</i>	154
6.13	SUMMARY	154
7	RESULTS AND DISCUSSION – MAGNESIUM SUBSTITUTED GLASSES	163
7.1	³¹ P MAS NMR	163
7.1.1	<i>³¹P MAS NMR of ICIE1 High Sodium Magnesium Glasses.....</i>	163
7.1.2	<i>³¹P MAS NMR of ICIE4 Low sodium Magnesium Glasses</i>	166
7.2	²⁹ Si MAS NMR.....	170
7.2.1	<i>²⁹Si MAS NMR of ICIE1 High Sodium Magnesium Glasses</i>	170
7.2.2	<i>²⁹Si MAS NMR of ICIE4 Low Sodium Magnesium Glasses</i>	176
7.3	²⁵ Mg MAS NMR	180
7.4	SUMMARY OF MAS NMR RESULTS FOR THE MAGNESIUM SERIES OF GLASSES	183
7.5	FTIR SPECTROSCOPY	184
7.6	RAMAN SPECTROSCOPY	186
7.7	X-RAY DIFFRACTION	188
7.8	DENSITY	189
7.9	DIFFERENTIAL THERMAL ANALYSIS	191
7.9.1	<i>Glass Transition Temperature</i>	193
7.9.2	<i>Crystallisation Temperature</i>	195
7.9.3	<i>Summary of DTA results for the magnesium series of glasses.....</i>	197
7.10	DILATOMETRY.....	198
7.10.1	<i>Dilatometric Softening Temperature.....</i>	199
7.10.2	<i>Thermal Expansion Coefficient.....</i>	200
7.10.3	<i>Summary of dilatometry results for the magnesium series of glasses</i>	204
7.11	DISSOLUTION STUDIES IN SIMULATED BODY FLUID.....	205
7.11.1	<i>Variation in Simulated Body Fluid pH.....</i>	205
7.11.2	<i>X-Ray Diffraction.....</i>	207
7.11.2.1	<i>Summary of SBF XRD results for the magnesium series of glasses.....</i>	215
7.11.3	<i>FTIR Spectroscopy.....</i>	216

7.11.3.1	Summary of SBF FTIR results for the phosphorus series of glasses.....	228
7.12	SUMMARY	229
8	CONCLUSION.....	240
8.1	SUGGESTIONS FOR FUTURE WORK	241
9	REFERENCES	245
10	APPENDIX	260

List of Figures

FIGURE 1: COMPARISON OF HUMAN SURVIVABILITY IN YEAR 1900 VERSUS 2000 WITH EFFECT OF AGE ON THE QUALITY OF CONNECTIVE TISSUE [19].....	24
FIGURE 2: RIGHT; HYBRID HIP REPLACEMENT WITH EVIDENCE OF LOOSENING, LEFT; CHARNLEY ELITE HIP REPLACEMENT [26].	27
FIGURE 3: BIOACTIVITY SPECTRA FOR VARIOUS BIOCERAMIC IMPLANTS: (A) RELATIVE RATE OF BIOACTIVITY AND (B) TIME DEPENDENCE OF FORMATION OF BONE BONDING AT AN IMPLANT INTERFACE ((A) 45S5 BIOGLASS [®] , (B) KGS CERAVITAL [®] , (C) 55S4.3 BIOGLASS [®] , (D) A-W GLASS-CERAMIC, (E) HA, (F) KGX CERAVITAL [®] , AND (G) AL ₂ O ₃ -Si ₃ N ₄) [33].	33
FIGURE 4: VOLUME-TEMPERATURE GRAPH: RELATIONSHIP BETWEEN THE GLASSY, LIQUID AND SOLID STATES.	39
FIGURE 5: AN EXAMPLE OF THE BASIC STRUCTURE OF TETRAHEDRAL UNITS IN SILICATE GLASSES [81]. ...	41
FIGURE 6: SCHEMATIC 2D REPRESENTATION OF AN OXIDE IN (A) THE CRYSTALLINE FORM AND (B) THE GLASSY FORM [82].	42
FIGURE 7: SiO ₂ SCHEMATIC – EFFECT OF A NETWORK MODIFYING ATOM [81].	43
FIGURE 8: THE EFFECT OF AN ALKALI OXIDE ON Q TERMINOLOGY [81].	45
FIGURE 9: EFFECT OF OXYGEN/SILICON RATIO ON SILICATE NETWORK STRUCTURES [81].	45
FIGURE 10: FREE ENERGY VERSUS COMPOSITION RELATIONSHIP AND THE RESULTANT PHASE DIAGRAM SHOWING THE REGIONS OF PHASE SEPARATION BY SPINODAL DECOMPOSITION AND BY NUCLEATION AND GROWTH [89].	52
FIGURE 11: EXAMPLE OF PHASE SEPARATION IN A GLASS [85].	52
FIGURE 12: STAGES 1 TO 4 OF HCA FORMATION [66].	58
FIGURE 13: SEM MICROGRAPH OF APATITE FORMATION [123].	63
FIGURE 14: COMPOSITION OF SBF [127].	64
FIGURE 15: COMPOSITIONAL DEPENDENCE (IN WEIGHT PERCENT) OF BONE BONDING AND SOFT-TISSUE BONDING OF BIOACTIVE GLASS AND GLASS-CERAMICS [33].	65
FIGURE 16: ³¹ P MAS NMR SPECTRA (CHEMICAL SHIFT ON X AXIS) FOR SERIES I (FIXED Na ₂ O:CAO) GLASSES. INCREASING PHOSPHATE CONTENT FROM BOTTOM TO TOP. TOP SPECTRA (ICSW4) SHOW DISTINCT PEAK ASYMMETRY.	85
FIGURE 17: ³¹ P MAS NMR CHEMICAL SHIFT VALUES FOR SERIES I GLASSES WITH PHOSPHATE MOLE RATIO.	88
FIGURE 18: ³¹ P MAS NMR CHEMICAL SHIFT VALUES FOR SERIES I GLASSES WITH NETWORK CONNECTIVITY VALUES ASSUMING PHOSPHORUS IN AN ORTHOPHOSPHATE ENVIRONMENT.	88

FIGURE 19: ^{31}P MAS NMR SPECTRA FOR THE ICSW4 GLASS HEAT TREATED AT AROUND 350°C , DELAY TIME 2 S (BOTTOM) AND 60 S (TOP).	90
FIGURE 20: ^{31}P MAS NMR SPECTRA FOR SERIES II (CHARGE BALANCED) GLASSES. INCREASING PHOSPHATE CONTENT FROM BOTTOM TO TOP.	91
FIGURE 21: ^{29}Si MAS NMR SPECTRA FOR SERIES I (FIXED $\text{Na}_2\text{O}:\text{CaO}$) GLASSES. PHOSPHATE CONTENT INCREASES FOR BOTTOM TO TOP.	93
FIGURE 22: ^{29}Si MAS NMR CHEMICAL SHIFT VALUES VERSUS PHOSPHATE CONTENT IN SERIES I GLASSES.	95
FIGURE 23: DIFFERENCE IN NC AND NC' FOR GLASSES IN SERIES I AND DATA FROM NMR FITTING.	96
FIGURE 24: ^{29}Si MAS NMR SPECTRUM FOR THE HEAT TREATED (650°C) ICSW4 GLASS SAMPLE, DELAY TIME=60 SECONDS.	97
FIGURE 25: ^{29}Si MAS NMR SPECTRA FOR SERIES II GLASSES.	98
FIGURE 26: FTIR SPECTRA FOR SERIES I NON-CHARGE BALANCED PHOSPHORUS SERIES. INCREASING PHOSPHATE CONTENT FROM BOTTOM TO TOP.	103
FIGURE 27: FTIR SPECTRA FOR SERIES II CHARGE BALANCED PHOSPHORUS SERIES. INCREASING PHOSPHATE CONTENT FROM BOTTOM TO TOP.	103
FIGURE 28: RAMAN SPECTRA FOR SERIES I, NON CHARGE BALANCED, PHOSPHORUS GLASSES. INCREASING PHOSPHATE CONTENT FROM BOTTOM TO TOP.	105
FIGURE 29: RAMAN SPECTRA FOR SERIES II, CHARGE BALANCED, PHOSPHORUS GLASSES. INCREASING PHOSPHATE CONTENT FROM BOTTOM TO TOP.	106
FIGURE 30: X-RAY DIFFRACTION PATTERNS FOR SERIES I GLASSES. INCREASING PHOSPHATE CONTENT FROM BOTTOM TO TOP.	107
FIGURE 31: X-RAY DIFFRACTION PATTERNS FOR SERIES II GLASSES. INCREASING PHOSPHATE CONTENT FROM BOTTOM TO TOP.	107
FIGURE 32: X-RAY DIFFRACTION PATTERN FOR CRYSTALLINE SAMPLE ICSW4 BEFORE AND AFTER HEAT TREATMENT WITH PHASES IDENTIFIED AND RIETVELD REFINEMENT, WITH MOLAR PROPORTIONS OF PHASES SHOWN (NOTE – ADDITIONAL SODIUM PYROPHOSPHATE PEAK ARE MASKED IN THE REGION AROUND 32° WHICH CONTAINS THE STRONGEST LINES FROM THE OTHER TWO PHASES).	108
FIGURE 33: VARIATION IN DENSITY WITH PHOSPHATE CONTENT FOR SERIES I WITH DOWEIDAR'S THEORETICAL VALUES.	113
FIGURE 34: VARIATION WITH DENSITY WITH PHOSPHATE CONTENT FOR SERIES II WITH DOWEIDAR'S THEORETICAL VALUES.	114
FIGURE 35: DOWEIDAR'S GRAPH TAKEN FROM HIS 2009 PAPER SHOWING DENSITY AS A FUNCTION OF PHOSPHORUS CONTENT. THE SQUARE AND DIAMOND SYMBOLS REPRESENT THIS STUDY'S SERIES I AND SERIES II EXPERIMENTAL VALUES WHILE THE TRIANGULAR SYMBOL REPRESENTS LUSVARDI'S <i>ET AL.</i> 'S [183] EXPERIMENTAL DENSITY VALUES. THE CLOSED TRIANGULAR SYMBOLS REPRESENT DOWEIDAR'S THEORETICAL VALUES. ERROR LIMIT IN THE EXPERIMENTAL DENSITIES IS $\pm 0.02\text{G}/\text{CM}^3$ [182].	117

FIGURE 36: DOWEIDAR'S GRAPH TAKEN FROM HIS 2009 PAPER SHOWING V_M AS A FUNCTION OF PHOSPHATE CONTENT USING THIS STUDY'S AND LUSVARDI'S <i>ET AL.</i> 'S [183] RESULTS. SYMBOLS AS WITH FIGURE 35 – CLOSED TRIANGULAR SYMBOLS REPRESENT CALCULATED MOLAR VOLUMES [182].	117
FIGURE 37: DTA TRACES FOR SERIES I (ICSW1 MAGNETA, ICIE1 RED, ICSW2 YELLOW, ICSW5 CYAN AND ICSW4 BLACK).	120
FIGURE 38: DTA TRACES FOR SERIES II (ICSW1 MAGENTA, ICSW6 BLUE, ICSW7 YELLOW, ICSW8 SIAN, ICSW10 BROWN, ICSW9 PURPLE).	120
FIGURE 39: GLASS TRANSITION TEMPERATURES FOR SERIES I.	121
FIGURE 40: GLASS TRANSITION TEMPERATURES FOR SERIES II.	121
FIGURE 41: CRYSTALLISATION TEMPERATURES FOR SERIES I (T_{c1} BLUE, T_{c2} PINK).	123
FIGURE 42: CRYSTALLISATION TEMPERATURES FOR SERIES II (T_{c1} BLUE, T_{c2} PINK).	123
FIGURE 43: $T_x - T_g$ FOR SERIES I AND II.	124
FIGURE 44: TEC'S (α) FOR SERIES I AND II WITH CALCULATED (APPEN) VALUES.	126
FIGURE 45: SOFTENING TEMPERATURES FOR SERIES I GLASSES.	128
FIGURE 46: SOFTENING TEMPERATURES FOR SERIES II GLASSES.	128
FIGURE 47: VARIATION IN SBF pH WITH TIME FOR SERIES I PHOSPHORUS GLASSES.	131
FIGURE 48: VARIATION IN SBF pH WITH TIME FOR SERIES II PHOSPHORUS GLASSES.	132
FIGURE 49: XRD PATTERNS FOR ICSW1 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	133
FIGURE 50: XRD PATTERNS OBTAINED FOR ICIE1 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	134
FIGURE 51: XRD PATTERNS OBTAINED FOR ICSW2 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	135
FIGURE 52: XRD PATTERNS OBTAINED FOR ICSW3 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	135
FIGURE 53: XRD PATTERNS OBTAINED FOR ICSW3 (AS WITH FIGURE 54). EXHIBITS THE BRAGG PEAKS ASSOCIATED WITH CRYSTALLINE HCA FORMATION.	136
FIGURE 54: XRD PATTERNS OBTAINED FOR ICSW5 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	136
FIGURE 55: XRD PATTERNS OBTAINED FOR ICSW4 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	137
FIGURE 56: CRYSTALLITE SIZE ANALYSIS FROM 002 BRAGG PEAK FOR BOTH SERIES OF GLASSES.	138
FIGURE 57: XRD PATTERNS OBTAINED FOR ICSW6 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	139
FIGURE 58: XRD PATTERNS OBTAINED FOR ICSW7 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	139
FIGURE 59: XRD PATTERNS OBTAINED FOR ICSW8 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	140

FIGURE 60: XRD PATTERNS OBTAINED FOR ICSW10 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	140
FIGURE 61: XRD PATTERNS OBTAINED FOR ICSW9 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	141
FIGURE 62: FTIR SPECTRA OF SERIES I GLASSES AFTER 15 MINUTES OF IMMERSION IN SBF.	144
FIGURE 63: FTIR SEPCTRA OF SERIES I GLASSES AFTER 1 HOUR OF IMMERSION IN SBF.	144
FIGURE 64: FTIR SPECTRA OF SERIES I GLASSES AFTER 4 HOURS OF IMMERSION IN SBF.	144
FIGURE 65: FTIR SEPCTRA OF SERIES I GLASSES AFTER 16 HOURS OF IMMERSION IN SBF.	145
FIGURE 66: FTIR SPECTRA OF SERIES I GLASSES AFTER 24 HOURS OF IMMERSION IN SBF.	145
FIGURE 67: FTIR SPECTRA OF SERIES I GLASSES AFTER 48 HOURS OF IMMERSION IN SBF.	145
FIGURE 68: FTIR SPECTRA OF SERIES I GLASSES AFTER 1 WEEK OF IMMERSION IN SBF.	146
FIGURE 69: FTIR SPECTRA OF SERIES I GLASSES AFTER 21 DAYS OF IMMERSION IN SBF.	146
FIGURE 70: FTIR SPECTRA OF SERIES II GLASSES AFTER 15 MINUTES OF IMMERSION IN SBF.	147
FIGURE 71: FTIR SPECTRA OF SERIES II GLASSES AFTER 1 HOUR OF IMMERSION IN SBF.	147
FIGURE 72: FTIR SPECTRA OF SERIES II GLASSES AFTER 4 HOURS OF IMMERSION IN SBF.	147
FIGURE 73: FTIR SPECTRA OF SERIES II GLASSES AFTER 16 HOURS OF IMMERSION IN SBF.	148
FIGURE 74: FTIR SPECTRA OF SERIES II GLASSES AFTER 24 HOURS OF IMMERSION IN SBF.	148
FIGURE 75: FTIR SPECTRA OF SERIES II GLASSES AFTER 48 HOURS OF IMMERSION IN SBF.	148
FIGURE 76: FTIR SPECTRA OF SERIES II GLASSES AFTER 1 WEEK OF IMMERSION IN SBF.	149
FIGURE 77: FTIR SPECTRA OF SERIES II GLASSES AFTER 21 DAYS OF IMMERSION IN SBF.	149
FIGURE 78: 3D CONTOUR PLOT OF ICSW10 GLASS (TIME AXIS NOT LINEAR)	150
FIGURE 79: AREA OF ν_4 PHOSPHATE FTIR REGION PLOTTED AGAINST (002) BRAGG PEAK AREA FROM XRD AFTER 21 DAYS IN SBF.	153
FIGURE 80: COMPARISON OF ICSW9 AND 45S5 BIOGLASS® BY FTIR AFTER 24 HOURS IN SBF.	154
FIGURE 81: ^{31}P SPECTRA FOR THE ICIE1 MAGNESIUM SUBSTITUTED SERIES OF GLASSES	163
FIGURE 82: THE EFFECT OF ^{31}P ORTHOPHOSPHATE (Q^0) SHIFT WITH INCREASE IN MAGNESIUM CONTENT (WHILE SODIUM CONTENT REMAINS CONSTANT). ILLUSTRATES THE PROGRESSIVE PREFERENTIAL ASSOCIATION OF SODIUM WITH THE PHOSPHATE THROUGH THE SERIES. LINE INDICATES A LINEAR FIT.	165
FIGURE 83: ^{31}P MAS NMR SPECTRA FOR THE ICIE4 MAGNESIUM SERIES OF GLASSES.	166
FIGURE 84: ^{31}P MAS NMR CHEMICAL SHIFT VALUES FOR ICIE4 LOW SODIUM MAGNESIUM GLASSES.	168
FIGURE 85: ^{31}P MAS NMR LINEWIDTHS FOR ICIE4 LOW SODIUM MAGNESIUM GLASSES.	169
FIGURE 86: ^{29}Si MAS-NMR SPECTRA. INCREASING MAGNESIUM CONTENT FROM BOTTOM (ICIE1) TO TOP (ICIE1100MG). ILLUSTRATES THE PROGRESSIVE FORMATION OF A Q^3 SHOULDER ON A PREDOMINANTLY Q^2 PEAK.	170
FIGURE 87: HYPOTHETICAL SCHEMATIC MODEL OF GLASS STRUCTURE. ILLUSTRATES THE TWO PHASE GLASS WITH (A) MAGNESIUM WITHIN THE SILICATE NETWORK AS MgO_4 UNITS CHARGE BALANCED BY CA AND (B) SODIUM ASSOCIATED WITH THE ORTHOPHOSPHATE SPECIES.	173

FIGURE 88: NETWORK CONNECTIVITY CALCULATED FROM NMR FITTING (TABLE 19) (NC(NMR)) COMPARED TO NETWORK CONNECTIVITY ASSUMING MgO ACTS AS A MODIFIER (NC'). IN THE NC' CALCULATION, IT WAS ASSUMED THAT Mg IS ONLY ACTING AS A NETWORK MODIFIER. THE EQUATION OF THE LINE IS FOR NC(NMR).....	174
FIGURE 89: THE ABSOLUTE AMOUNT OF MgO (MOL %) ENTERING THE GLASS NETWORK WITH Mg SUBSTITUTION. THIS ASSUMES THAT THE MgO IS ONLY IN TETRAHEDRAL CO-ORDINATION. AS THE MgO CONTENT IS INCREASING ACROSS THE SERIES, THIS CORRESPONDS TO A CONSTANT PROPORTION (~ 14%) OF MgO ACTING AS A NETWORK FORMER COMPARED TO NETWORK MODIFYING MgO.....	175
FIGURE 90: ²⁹ Si MAS NMR SPECTRA FOR THE ICIE4 MAGNESIUM SERIES OF GLASSES	177
FIGURE 91: ²⁹ Si CHEMICAL SHIFT VALUES FOR THE ICIE4 LOW SODIUM MAGNESIUM SERIES OF GLASSES	178
FIGURE 92: ²⁵ Mg STATIC NMR FOR ICIE1 MAGNESIUM SERIES	181
FIGURE 93: ²⁵ Mg STATIC NMR FOR ICIE4 MAGNESIUM SERIES	181
FIGURE 94: FTIR SPECTRA FOR HIGH SODIUM ICIE1 MAGNESIUM GLASSES. INCREASING MAGNESIUM CONTENT FROM BOTTOM TO TOP.	184
FIGURE 95: FTIR SPECTRA FOR LOW SODIUM ICIE4 MAGNESIUM GLASSES. INCREASING MAGNESIUM CONTENT FORM BOTTOM TO TOP.	185
FIGURE 96: RAMAN SPECTRA FOR HIGH SODIUM ICIE1 GLASSES. INCREASING MAGNESIUM CONTENT FROM BOTTOM TO TOP.	186
FIGURE 97: RAMAN SPECTRA FOR LOW SODIUM ICIE4 MAGNESIUM GLASSES. INCREASING MAGNESIUM CONTENT FROM BOTTOM TO TOP.	187
FIGURE 98: X-RAY DIFFRACTION PATTERNS FOR HIGH SODIUM ICIE1 MAGNESIUM GLASSES. INCREASING MAGNESIUM CONTENT FROM BOTTOM TO TOP.....	188
FIGURE 99: X-RAY DIFFRACTION PATTERNS FOR LOW SODIUM ICIE4 MAGNESIUM GLASSES. INCREASING MAGNESIUM CONTENT FROM BOTTOM TO TOP.....	188
FIGURE 100: VARIATION IN DENSITY WITH MAGNESIUM CONTENT FOR HIGH SODIUM ICIE1 MAGNESIUM GLASSES WITH DOWEIDAR'S THEORETICAL VALUES.	190
FIGURE 101: VARIATION IN DENSITY WITH MAGNESIUM CONTENT FOR LOW SODIUM ICIE4 MAGNESIUM GLASSES WITH DOWEIDAR'S THEORETICAL VALUES.	190
FIGURE 102: DIFFERENTIAL THERMAL ANALYSIS TRACES FOR THE ICIE1 MAGNESIUM SUBSTITUTED SERIES OF GLASSES.....	192
FIGURE 103: DIFFERENTIAL THERMAL ANALYSIS TRACES FOR THE ICIE4 MAGNESIUM SUBSTITUTED SERIES OF GLASSES.....	192
FIGURE 104: GLASS TRANSITION TEMPERATURES FOR THE ICIE1 MAGNESIUM GLASSES	193
FIGURE 105: GLASS TRANSITION TEMPERATURES FOR THE ICIE4 MAGNESIUM GLASSES	194
FIGURE 106: CRYSTALLISATION TEMPERATURES FOR ICIE1 AND ICIE4 MAGNESIUM GLASSES.....	196
FIGURE 107: DILATOMETRIC TRACE FOR THE ICIE1 MAGNESIUM GLASSES.....	199
FIGURE 108: DILATOMETRIC TRACE FOR THE ICIE4 MAGNESIUM GLASSES.....	199
FIGURE 109: COMPARISON OF SOFTENING TEMPERATURES FOR ICIE1 AND ICIE4 MAGNESIUM SUBSTITUTED GLASSES.....	200

FIGURE 110: GRAPH OF EXPERIMENTAL THERMAL EXPANSION COEFFICIENTS, TEC (TOP) AND APPEN'S PREDICTIONS (BOTTOM) AGAINST PERCENTAGE OF MAGNESIUM SUBSTITUTED FOR ICIE1 SERIES OF GLASSES. ILLUSTRATES APPEN'S PREDICTED DECREASING TREND AND THE EXPERIMENTAL INCREASE IN TEC WITH MAGNESIUM SUBSTITUTION. THE TOP LINE IS A GUIDE FOR THE EYE.....	202
FIGURE 111: GRAPH OF EXPERIMENTAL THERMAL EXPANSION COEFFICIENTS, TEC (TOP) AND APPEN'S PREDICTIONS (BOTTOM) AGAINST PERCENTAGE OF MAGNESIUM SUBSTITUTED FOR ICIE4 SERIES OF GLASSES.	203
FIGURE 112: VARIATION IN SBF pH WITH TIME FOR HIGH SODIUM ICIE1 GLASSES.	206
FIGURE 113: VARIATION IN SBF pH WITH TIME FOR LOW SODIUM ICIE4 GLASSES.	207
FIGURE 114: XRD PATTERNS OBTAINED FOR ICIE1 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	208
FIGURE 115: XRD PATTERNS OBTAINED FOR ICIE125MG GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	209
FIGURE 116: XRD PATTERNS OBTAINED FOR ICIE150MG GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	210
FIGURE 117: XRD PATTERNS OBTAINED FOR ICIE175MG GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	210
FIGURE 118: XRD PATTERNS OBTAINED FOR ICIE1100MG GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	211
FIGURE 119: XRD PATTERNS OBTAINED FOR ICIE4 GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	213
FIGURE 120: XRD PATTERNS OBTAINED FOR ICIE425MG GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	213
FIGURE 121: XRD PATTERNS OBTAINED FOR ICIE450MG GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	214
FIGURE 122: XRD PATTERNS OBTAINED FOR ICIE475MG GLASS FOLLOWING IMMERSION IN SBF FOR DIFFERENT TIME PERIODS.	214
FIGURE 123: FTIR SPECTRA OF ICIE1 SERIES OF GLASSES FOLLOWING 15 MINUTES OF IMMERSION IN SBF.	217
FIGURE 124: FTIR SPECTRA OF ICIE1 SERIES OF GLASSES FOLLOWING 1 HOUR OF IMMERSION IN SBF. ...	217
FIGURE 125: FTIR SPECTRA OF ICIE1 SERIES OF GLASSES FOLLOWING 4 HOURS OF IMMERSION IN SBF.	218
FIGURE 126: FTIR SPECTRA OF ICIE1 SERIES OF GLASSES FOLLOWING 16 HOURS OF IMMERSION IN SBF.	218
FIGURE 127: FTIR SPECTRA OF ICIE1 SERIES OF GLASSES FOLLOWING 24 HOURS OF IMMERSION IN SBF.	219
FIGURE 128: FTIR SPECTRA OF ICIE1 SERIES OF GLASSES FOLLOWING 48 HOURS OF IMMERSION IN SBF.	219
FIGURE 129: FTIR SPECTRA OF ICIE1 SERIES OF GLASSES FOLLOWING 1 WEEK OF IMMERSION IN SBF. ...	220
FIGURE 130: FTIR SPECTRA OF ICIE1 SERIES OF GLASSES FOLLOWING 21 DAYS OF IMMERSION IN SBF.	220

FIGURE 131: FTIR SPECTRA OF ICIE4 SERIES OF GLASSES FOLLOWING 15 MINUTES OF IMMERSION IN SBF.	223
FIGURE 132: FTIR SPECTRA OF ICIE4 SERIES OF GLASSES FOLLOWING 1 HOUR OF IMMERSION IN SBF...	223
FIGURE 133: FTIR SPECTRA OF ICIE4 SERIES OF GLASSES FOLLOWING 4 HOURS OF IMMERSION IN SBF.	224
FIGURE 134: FTIR SPECTRA OF ICIE4 SERIES OF GLASSES FOLLOWING 16 HOURS OF IMMERSION IN SBF.	224
FIGURE 135: FTIR SPECTRA OF ICIE4 SERIES OF GLASSES FOLLOWING 24 HOURS OF IMMERSION IN SBF.	225
FIGURE 136: FTIR SPECTRA OF ICIE4 SERIES OF GLASSES FOLLOWING 48 HOURS OF IMMERSION IN SBF.	225
FIGURE 137: FTIR SPECTRA OF ICIE4 SERIES OF GLASSES FOLLOWING 1 WEEK OF IMMERSION IN SBF...	226
FIGURE 138: FTIR SPECTRA OF ICIE4 SERIES OF GLASSES FOLLOWING 21 DAYS OF IMMERSION IN SBF.	226
FIGURE 139: FTIR SPECTRA OF ICIE125MG GLASS FROM 15 MINUTES TO 21 DAYS OF IMMERSION IN SBF.	227
FIGURE 140: FTIR SPECTRA OF ICIE1100MG GLASS FROM 15 MINUTES TO 21 DAYS OF IMMERSION IN SBF.	227
FIGURE 141: FTIR SPECTRA OF ICIE425MG GLASS FROM 15 MINUTES TO 21 DAYS OF IMMERSION IN SBF.	228
FIGURE 142: FTIR SPECTRA OF ICIE475MG GLASS FROM 15 MINUTES TO 21 DAYS OF IMMERSION IN SBF.	228
FIGURE 143: IMAGE OF A PERCOLATION CHANNEL IN A MAGNESIUM SUBSTITUTED SODA LIME SILICATE GLASS. MG IONS (LIGHT YELLOW) ENTER INTO THE Si-O (Si, LIGHT BLUE AND O, RED) NETWORK ACTING AS A NETWORK FORMER. NA IONS (LIGHT GREEN) ARE FREE TO DIFFUSE HOPPING AMONG AFFINITY SITES [239].	234
FIGURE 144: SNAPSHOT OF THE 3D STRUCTURE OF A MAGNESIUM SUBSTITUTED SODA LIME PHOSPHOSILICATE BIOACTIVE GLASS. VIOLET TETRAHEDRA REPRESENT THE ORTHOPHOSPHATE UNITS, YELLOW SPHERES REPRESENT THE MAGNESIUM IONS, GREEN SPHERES REPRESENT THE CHARGE MODIFYING SODIUM IONS, CYAN SPHERES REPRESENT THE CHARGE MODIFYING CALCIUM IONS WHILE THE GREY STICKS REPRESENT THE Si-O FRAMEWORK [241].	235
FIGURE 145: MG-O-MG BRIDGES FORMED BY MgO_4 TETRAHEDRA IN THE SILICON NETWORK. SILICON, MAGNESIUM, OXYGEN AND SODIUM IONS ARE REPRESENTED AS BLUE, YELLOW, RED AND GREEN SPHERES [239].	235
FIGURE 146: SCHEMATIC OF THE STRUCTURE OF BIOACTIVE GLASSES; BOs COLOURED AS RED. THE FRAGMENT IN (A) IS A THREE-MEMBERED SILICATE CHAIN, WITH NO COVALENT LINKS TO THE REST OF THE STRUCTURE, WHOSE DISSOLUTION WILL BE FASTER THAN THAT OF (B) A FIVE-MEMBERED RING, COVALENTLY CROSS-LINKED THROUGH THE ADDITIONAL Si-O BONDS COLOURED IN BLUE.	238

List of Tables

TABLE 1: MAJOR BIOMATERIALS AND THEIR USES [33].	28
TABLE 2: FACTORS EFFECTING IMPLANT-TISSUE INTERFACIAL RESPONSE.	31
TABLE 3: CLASSIFICATION OF FIELD STRENGTHS FOR DIFFERENT CATIONS.	47
TABLE 4: SELECTED BIOACTIVE GLASSES (IN MOL%) AND THEIR RESULTANT NETWORK CONNECTIVITY VALUES (ASSUMING PHOSPHORUS ENTERS THE NETWORK) [43].	72
TABLE 5: MAGNESIUM SUBSTITUTED ICIE1 MOLAR GLASS COMPOSITIONS (MOL%).	74
TABLE 6: MAGNESIUM SUBSTITUTED ICIE4 MOLAR GLASS COMPOSITIONS (MOL%).	75
TABLE 7: GLASS COMPOSITIONS (IN MOL%) FROM SERIES I (FIXED $\text{Na}_2\text{O}:\text{CaO}=1:0.87$) AND SERIES II (CHARGE BALANCED), SHOWING MOLAR MOL%, NETWORK CONNECTIVITY (NC) ASSUMING P_2O_5 ENTERS THE GLASS NETWORK, MODIFIED NETWORK CONNECTIVITY (NC') ASSUMING ISOLATED ORTHOPHOSPHATE UNITS.	77
TABLE 8: MELT OBSERVATIONS IN THE MAGNESIUM AND PHOSPHORUS SERIES OF GLASSES	79
TABLE 9: ^{31}P MAS NMR PEAK POSITIONS AND FULL WIDTH HALF MAXIMUMS (FWHM) FOR GLASSES IN SERIES I.	86
TABLE 10: ^{31}P MAS NMR PEAK POSITIONS AND FULL WIDTH HALF MAXIMUMS (FWHM) FOR GLASSES IN SERIES II.	92
TABLE 11: ^{29}Si MAS NMR PEAK POSITIONS AND FULL WIDTH HALF MAXIMUMS (FWHM) FOR GLASSES IN SERIES I.	94
TABLE 12: ^{29}Si MAS NMR PEAK POSITIONS AND FULL WIDTH HALF MAXIMUMS (FWHM) FOR GLASSES IN SERIES.	99
TABLE 13: DENSITIES OF SILICATE Q SPECIES AND PHOSPHATE SPECIES.	111
TABLE 14: FIRST TIME POINT AT WHICH APATITE IS OBSERVED BY X-RAY DIFFRACTION.	142
TABLE 15: FTIR VIBRATIONAL MODES USED TO ASSESS APATITE FORMATION [154].	143
TABLE 16: TIME FOR CRYSTALLINE APATITE FORMATION OBSERVED BY SPLITTING OF THE FTIR P-O BENDING MODE AT AROUND 550cm^{-1} (ICSW4 SAMPLE PARTIALLY CRYSTALLINE) [195, 196].	152
TABLE 17: NMR DATA FOR ^{31}P NMR SPECTRA OF THE SERIES OF MAGNESIUM CONTAINING BIOACTIVE GLASSES WHERE W CORRESPONDS TO PEAK WIDTH.	164
TABLE 18: THE SPECTRAL PARAMETERS DETERMINED FORM THE ^{31}P STUDIES OF ICIE4 MAGNESIUM SERIES OF GLASSES.	167
TABLE 19: NMR DATA FOR ^{31}P NMR SPECTRA OF THE SERIES OF MAGNESIUM CONTAINING BIOACTIVE GLASSES WHERE X CORRESPONDS TO PEAK POSITION, W PEAK WIDTH AND A FRACTION AREA.	171

TABLE 20: THE SPECTRAL PARAMETERS DETERMINED FORM THE ^{29}Si STUDIES OF ICIE4 MAGNESIUM SERIES OF GLASSES.	177
TABLE 21: FIRST TIME POINT AT WHICH APATITE IS OBSERVED BY X-RAY DIFFRACTION.	215

Symbols and abbreviations

BO	Bridging oxygen
NBO	Non-bridging oxygen
DTA	Differential thermal analysis
HA	Hydroxyapatite
HCA	Hydroxycarbonate apatite
ICIE1	Imperial College Hill <i>et al.</i> base glass
ICSW	Imperial College phosphorus series glass
MAS NMR	Magic angle spinning nuclear magnetic resonance
mol%	Mole percent
CLD	Cross-link density
NC	Network connectivity
NC'	Network connectivity taking account of phosphorus in orthophosphate environment
NMR	Nuclear magnetic resonance
Q^0	SiO_4 structural unit with four non-bridging oxygens
Q^1	SiO_4 structural unit with three non-bridging oxygens
Q^2	SiO_4 structural unit with two non-bridging oxygens
Q^3	SiO_4 structural unit with one non-bridging oxygen
Q^4	SiO_4 structural unit without non-bridging oxygens

RO	Any alkaline earth metal oxide
R ₂ O	Any alkali metal oxide
SBF	Simulated body fluid
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
T.E.C	Thermal expansion coefficient
α	Thermal expansion coefficient
T _c	Glass crystallisation temperature
T _{c1}	First glass crystallisation temperature
T _{c2}	Second glass crystallisation temperature
T _g	Glass transition temperature
T _s	Dilatometric softening temperature
wt%	Weight percent
XRD	X-ray diffraction

1 Introduction

The concept of a bioactive material, capable of bone bonding, was originally suggested by Larry Hench in the late 1960s to early 1970s in response to the need to develop an innovative material capable of repairing large bone injuries suffered by servicemen during the Vietnam War. Hench used silica glass as a host material, combining it with other components, such as calcium, to pack between bone fragments to fuse the shattered bones of injured soldiers. In essence, the body is tricked into believing that this loaded glass powder is bone, resulting in the stimulation and re-growth of new bone between the fractures [1] .

The bioactivity of glasses was initially observed for silicate glasses based on the system $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$. Bioglass[®], otherwise known as 45S5, is the trade name given to the original glass developed in the system with the composition of 46.1 mol% SiO_2 , 26.9 mol% CaO , 24.4 mol% Na_2O and 2.5 mol% P_2O_5 . Since then, there have been many variations on the original composition, Bioglass[®], with several glass compositions having been studied, tissue bonding mechanisms proposed, and clinical applications developed [2]. As a result, bioactive glasses are approved worldwide as materials for a wide range of clinical and cosmetic applications [3].

The first bioactive glass device cleared for marketing in the United States was a device to treat the conductive hearing loss, by replacing the bones in the middle ear [4]. Since then, and over the last 30 years, there have been great advances in the field of bioactive glasses with bioactive glasses and ceramics particularly prevalent in the area of mineralised tissue regeneration in orthopaedic applications [5]. More recently, these materials are finding their way into ever expanding and diverse applications including acting as remineralising agents in toothpastes, (Sensishield marketed in the EU by Periproducts contains Novamin (45S5 Bioglass[®]) as the active ingredient) [6] and acting

as additives in relaxing and hair straightening products (L'Oreal also have a patent [7] for Bioglass[®] as an additive to products for relaxing / straightening hair). This has opened up the possibility for bioactive glasses to be used in a variety of consumer products.

Although the use of Bioglass[®] within the biomedical and, more recently, cosmetic field is ever expanding, it is suggested that the design of such products has been based on a trial and error approach, rather than one based on a thorough understanding of the glasses and the phenomenon of bioactivity itself. Although many theories have been put forward to explain the mechanism of bioactivity there is not, as yet, a unified theory or understanding of how a glass's composition results in its behaviour within the body. Furthermore, theoretical research scientists studying within the field of bioactive glasses seem to follow a similar trial and error approach to that of the commercial product designers, with only a few research groups understanding and vocalising the importance of a methodical and systematic approach [8] [9] [10] [11] [12] [13] [14] [15] [16].

Hill *et al.* [17] have made great strides in the last few decades to undertake a logical and thorough approach to the area of bioactive glass design by using the network connectivity model, in partnership with materials characterisation techniques such as, primarily, MAS-NMR to investigate the effect of each constituent within a bioactive glass system. The work has focused on a similar composition to that of Bioglass[®], a silicate glass named ICIE1 with a composition of 49.46 mol% SiO₂, 23.08 mol% CaO, 26.38 mol% Na₂O and 1.07 mol% P₂O₅. Hill *et al.* [17] already have a good understanding of the effect of silicon, sodium, calcium and strontium on bioactivity and this study aims to follow up on such work by investigating the effect of phosphorus and magnesium. In a fully characterised glass system, in theory, it should be possible to custom design glass compositions for specific biomedical applications. This study aims to contribute to this ultimate goal.

1.1 Objectives of Study

The aim of this study was to characterise the glass structures of a series of bioactive glasses using ^{29}Si and ^{31}P MAS NMR spectroscopy, density analysis, differential thermal analysis, dilatometry, Fourier transform infra-red spectroscopy and Raman spectroscopy to -.

1. understand the structural behaviour of phosphorus within a bioactive glass system.
2. investigate the effect of phosphorus on bioactivity.
3. understand the structural behaviour of magnesium within a bioactive glass system and, more specifically, understand the influence of replacing Ca^{2+} with Mg^{2+} in a bioactive glass.
4. investigate whether the level of disorder within a bioactive glass effects the structural behaviour of magnesium.
5. investigate the effect of magnesium on bioactivity, in both a disordered and ordered bioactive glass system.
6. incorporate the structural understanding gained on phosphorus and magnesium into the network connectivity model.

1.2 Outline of Thesis

This thesis contains two distinct parts. The first part details the background information in the form of a review and examination of the relevant literature. This begins by discussing the development of the use of man made materials in the medical field (Chapter 2) and then moves on to discuss glasses and glass structure in general (Chapter 3). The literature review is then finalised with an analysis of the theory behind bioactive glass structure (Chapter 4).

Part two of the thesis details the experimental procedures carried out over the three years of analysis and the rationale behind such experiments. The experimental results gained are then displayed, discussed and conclusions theorised. The thesis finalises with an overall discussion on the impact of the thesis and suggestions for future work.

2 Materials in Medicine

The life expectancy of an average adult in the US has increased from 47 to 77 years in the last century alone [18]. This major increase in human survivability is largely attributed to a combination of factors, including nutrition, public health and the discovery of antiseptics and antibiotics. This, in turn, has resulted in many people outliving the quality of their connective tissues and organs. For centuries, when tissues became diseased or damaged, a physician had little recourse but to remove the offending part leading to only a marginally improved quality of life [19]. The last 40 years however, has seen a revolution in medical care with a shift in emphasis from palliative treatment to surgical intervention to repair and re-construct diseased or damaged parts of the body [20] [21].

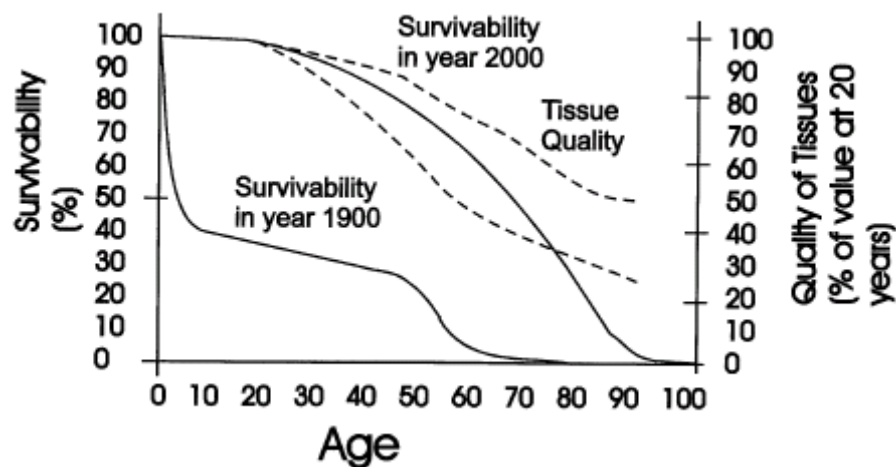


Figure 1: Comparison of human survivability in year 1900 versus 2000 with effect of age on the quality of connective tissue [19].

2.1 Transplantation

Current clinical procedures for the repair of diseased tissues/organs involve either transplantation or implantation [22]. The transplantation of tissue from the patient's own body (autografts) is seen as the "method of choice", primarily due to the minimal

immunological and ethical concerns it raises. However, the transfer of tissue grafts requires the sacrifice of tissue from a donor site, resulting in certain associated site morbidity (scar formation, potential injury and impairment to donor site etc.) [23]. A partial solution to some of these limitations is the use of tissue from living donors (heart, kidney, and lung) or cadavers (freeze dried bone). However, availability, immunological responses due to genetic differences, the need for immunosuppressant drugs, the risk of infection, which has been reported in 8-11% of cases, and the transmission of diseases such as HIV and human T lymphotropic virus all limit the use of allografts [19] [24] [25] [26].

Because of the world wide shortage of organs for clinical implantation, 60% of patients awaiting replacement organs die on the waiting list. The current demands for transplant organs and tissues is far outpacing the supply, and all manner of projections indicate that this gap will continue to widen [27] [28]. Recent advances in understanding the mechanisms of transplant organ rejection have brought science to a stage where it is reasonable to consider that organs from other species (xenografts) may soon be engineered to minimise the risk of rejection and used as an alternative to human tissues, possibly ending organ shortages [29]. Non-living, chemically treated xenografts are routinely used as heart valve replacements (porcine) and bone substitutes (bovine) but at present the clinical results are less than optimal [19]. Disease transmission (xenozoonosis) and permanent alteration to the genetic code of the animals, as well as the many novel medical, legal and ethical issues involved are a definite cause for concern and are the foundation for great controversy.

2.2 Implantation

An alternative to the ethical and legal mine field of transplantation, as a method for the repair and replacement of tissues and organs, is the development and modification of man-made materials to interface with living, host tissues, e.g. implants made from biomaterials. Implant technology has many major bonuses over transplantation including the reliability, availability and reproducibility associated with devices that can

be manufactured under good practice, international standards, regulations and quality assurance [19].

Some of the earliest biomedical applications were as far back as ancient Phoenicia (1550BC – 300BC) where loose teeth were bound together with gold wires. Artificial legs, eyes and teeth were routinely used in the Vidic period in India (1500 to 1800BC) and the Mayan people used to fashion teeth from seashells (roughly 600 AD), achieving, what we now refer to as, bone integration [30] [31]. With the creation of anesthesia more invasive implant procedures were made possible, including the repair of complex fractures by the attachment of metal plates and screws to the fractured ends of bones. By the 1960's total hip replacements were in development, closely simulating the original hip joint and made up of a separate cup component along with a ball and stem for securing into the femur. The prosthesis designed by Charnley was particularly successful, providing enhanced mobility for thousands of patients and initiating advances in artificial joint design.

As expectations of health increased in parallel with rising affluence, researchers began to study how other types of implants might help ameliorate a wider range of health problems [30]. Today, most of the major disciplines of surgery including orthopedics, cardiovascular surgery, dental and maxillofacial surgery, plastic and reconstructive surgery and ophthalmology all utilise implants to some extent (Figure 2).



Figure 2: Right; hybrid hip replacement with evidence of loosening, left; Charnley elite hip replacement [26].

2.3 Implant Materials

A biomedical implant is defined, in simple terms, as an artificial device used for restoring the functionality of a damaged natural organ or tissue of the body. In other words it is expected to perform the functions of the natural organ or tissue without adverse effect to other parts of the body [30]. The functional requirements of an implant material may be of mechanical, physical or even chemical origin. For instance in most cases, especially within the fields of dentistry and orthopedics, an implant acts as a structural member, thus the optimisation of mechanical parameters, such as yield and ultimate strength, hardness, fatigue resistance, and elastic moduli, are essential [32]. Whatever the precise functional characteristic of the implant, however, we usually find that these requirements are relatively easy to satisfy from the materials selection point of view. With exception of the wear properties, and possibly one or two miscellaneous examples, there are sufficient engineering materials available to meet the purely mechanical or physical specifications [32].

Table 1: Major biomaterials and their uses [33].

Material	Major Uses
Metals and Alloys	
316 stainless steel	Orthopaedic fracture plates, joint replacement prothesis
Cobalt-chromium alloys	Orthopaedic fracture plates, denture bases, heart valves
Titanium and its alloys	Dental implants, cranial plates
Platinum metals	Electrodes
Plastics, elastomers and fibres	
Polymethylmethacrylate	Dentures, cements for fixation of orthopaedic prothesis, contact lenses
Polydimethylsiloxane	Soft tissue reconstruction (e.g. breast, facial contours)
Hydrogels	Blood compatible surfaces
Cyanoacrylates	Adhesives
Polylactic and glycolic acids	Absorbable sutures and prothesis
Ceramics and glasses	
Oxide ceramics (e.g. alumina)	Joint replacement prostheses
Ion leachable glasses	Dental cements, coatings for orthopedic prostheses
Carbons	Heart valves, dental implants

Although the physical and mechanical properties of an implant may be easy to satisfy, the main problem faced with all implants is that of its interaction with the physiological environment – both the effect of the material/device on the recipient and that of the host tissues on the device, can lead to device failure. Because the success of implants depends so much on the nature of this interfacial reaction, the history of implant material development has been largely one of seeking the most physiologically inert materials, with little attention paid to how the material would interact with living tissue [32]. In fact, through the mid-1960s, the goal of biomaterials development was the creation of materials that were as chemically inert as possible [34]. It was only by minimizing the materials' interaction with the host that biocompatibility and long term survival of implants was achieved. Thus the most widely used materials of the time were primarily highly un-reactive, such as special medical grades of stainless steel,

cobalt-chrome alloys, titanium alloys as well as various polymeric materials such as ultra-high molecular weight polyethylene and polymethylmethacrylate and ultra stable ceramics such as alumina and zirconia. Such materials were substantially subject to corrosion and eventual failure as a result of the extremely aggressive nature of body fluids. Furthermore, in the case of metallic materials, utilised in the repair of skeletal systems, long term implantation was often followed by catastrophic failure attributed to the progressive deterioration of the bone in contact with the biomaterial [35] [36].

2.3.1 Stress Shielding

All bio-inert materials elicit the formation of a thin, non-adherent fibrous capsule between the material and living bone. Many factors contribute to the gradual breakdown of this interface including micro motion, wear debris and most significantly, stress shielding [37]. Stress shielding occurs when there is elastic moduli mismatch between the bone and implant material (for example 60-80 MPa for cortical bone compared to 350 MPa for titanium). A consequence of this mismatch is that the implant will shield the bone from mechanical loading resulting in resorption of bone minerals and a resultant loss in bone density. The loss of bone stock at the interface of an implant weakens the bone, eventually giving rise to implant/tissue interfacial complications [38].

2.4 Future of Implant Materials

The combined demands of a longer human lifespan and a longer fraction of the life span with an active lifestyle, have lead to a need for high performance biomedical materials that can last for up to 30 plus years for orthopedic implants [30]. The use in the medical field of materials such as stainless steel, alumina and polyethylene that were originally developed for general engineering applications, rather than for tissue replacement in human bodies has obviously been successful and it is certain that most of these proven materials will be continually used in the healthcare industry (Table 1). However, there are also shortcomings of these materials for their intended medical applications.

Implants or prostheses should ideally, in most cases, survive the lifetime of the patient without failure or need for intervention. To satisfy this growing need for very long term tissue repair, a shift of emphasis on the replacement of tissues to the regeneration of tissues is needed and an understanding, use and appreciation of biological approaches is essential [39]. Since the 1970's the quest for better biocompatibility of implant materials resulted in a focus on the use of biomaterials that can actively interact with tissues and induce their intrinsic repair and regenerative potential [40]. The demands of implant technology changed from maintaining an essentially physical function without eliciting a host response, to providing a more integrated interaction with the host [41].

2.5 Biological Response to Materials

All materials intended for application in humans such as biomaterials, medical devices and prosthesis undergo tissue responses when implanted into living tissue [42]. In order to understand the success and failure of implant materials, regardless of whether they are ceramic, metal or polymer, it is essential to understand the types of implant/tissue response that occur in the body [38]. There are four basic types of response.

2.5.1 Toxic Response

Those materials that result in the death of the surrounding tissue or those which release chemicals which can migrate within the tissue fluids to cause systematic toxicity [33]. Such materials are considered to be highly unsuitable as implant materials.

2.5.2 Biologically Nearly Inert: Fibrous Capsule Formation

In response to those materials that are considered biocompatible or biologically nearly inert, a non-adherent fibrous capsule forms in order to isolate the implant from the host. This is the most common response. It is a protective mechanism and in time can lead to complete encapsulation of an implant within the fibrous layer [33]. The thickness of the fibrous capsule is dependant on the period of phagocytic activity and is dependant on many factors. These factors are summarized in Table 2 below [43] [44].

Table 2: Factors effecting implant-tissue interfacial response.

Tissue Side	Implant Side
Type of Tissue	Composition of Implant
Health of Tissue	Phases in Implant
Age of Tissue	Phase Boundaries
Blood circulation in Tissue	Surface Morphology
Blood Circulation at Interface	Surface Porosity
Motion at Interface	Chemical Reactions
Closeness of Fit	Closeness of Fit
Mechanical Load	Mechanical Load

2.5.3 Bioresorption

Bioresorption is the regeneration of tissues rather than their replacement. Resorbable biomaterials are designed to degrade gradually over time and be replaced by the natural host tissue [45] leading to only a very thin or non-existent interfacial thickness. Complications involved in bioresorbable materials include the maintenance of the interfacial strength and stability during degradation and the matching of resorption rates to the repair rates of the body, which themselves vary enormously depending on a number of variables (Table 2). It is also essential that the materials used in bioresorbable implants consist of only metabolically acceptable substances, due to the large quantity of material to be handled by the surrounding cells.

2.5.4 Bioactive

Bioactive fixation is intermediate between resorbable and bioinert behavior. When a bioactive material is implanted in the body, a series of biophysical and biochemical reactions occur at the implant tissue interface eventually resulting in strong chemical interfacial bonding [44]. The bond formed inhibits interfacial movement, resists substantial mechanical forces and imitates the nature of the interface that is produced when natural tissues repair themselves.

Dissolution, precipitation and an ion-exchange reaction between the bioactive implant and surrounding body fluids results in the formation of a biologically active hydroxyapatite layer on the implant that is chemically and crystallographically equivalent to the mineral phase in bone [41]. Simultaneously, parallel reactions occur such as adsorption and incorporation of biological molecules that lead to the attachment of surrounding cells [46].

For the various bioactive materials, the mechanism of bonding, the time dependence of bonding, the strength of the bonding and the thickness of the bonding zone are different. They are chiefly determined by the physiochemical properties of the material surface however mechanical strength, geometry and porosity are also important factors. Bioactive materials very quickly become attached to tissue before the process of fibrous encapsulation can take place, thus resulting in a desirable stability at the interface and minimal motion-induced damage to the traumatized tissue [47]. The rate at which the interface between material and implant is formed can be termed as bioactivity. Figure 3 illustrates the bioactivity and time dependence for a number of biomaterials.

Bioactive materials can be split into 2 classifications [48] –

Class A – Osteoproduktive materials. Osteoproduction is the process whereby a bioactive surface is colonised by osteoprogenitor cells present in the defect environment as a result of surgical intervention [49]. Bioactivity occurs when the material elicits an intracellular as well as an extracellular response at the interface. A Class A biomaterial can bond with both bone and soft tissue.

Class B – Osteoconductive materials. Osteoconduction simply provides a biocompatible surface along which bone tissue can migrate and only occurs when the material elicits an extracellular response [45].

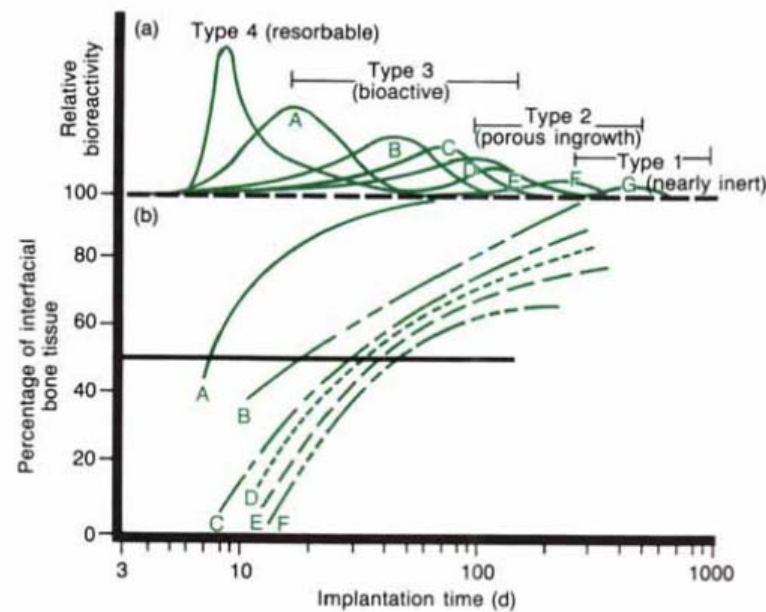


Figure 3: Bioactivity spectra for various bioceramic implants: (a) relative rate of bioactivity and (b) time dependence of formation of bone bonding at an implant interface ((A) 45S5 Bioglass®, (B) KGS Ceravital®, (C) 55S4.3 Bioglass®, (D) A-W glass-ceramic, (E) HA, (F) KGX Ceravital®, and (G) $\text{Al}_2\text{O}_3\text{-Si}_3\text{N}_4$) [33].

The first material with proven bioactive behaviour was Bioglass®, a glass developed by Hench and co-workers at the University of Florida. The concept of bioactivity was defined as -

"A bioactive material is one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material" [5].

The compositional range of bioactive bonding has been expanded considerably to include a large number of bioactive materials with a wide range of rates of bonding and thickness of interfacial bonding layers. They include bioactive glasses such as Bioglass®, bioactive glass-ceramics such as Ceravital®, A/W glass ceramics and machinable glass-ceramics such as Bioverit®, dense calcium phosphate ceramics such as synthetic hydroxyapatite (HA), bioactive composites such as PE-HA mixtures and a series of bioactive coating materials [44]. All of these materials form an interfacial bond with the adjacent tissue. However, the time dependence of bonding, the strength

of bond, the mechanism of bonding and the thickness of the bonding zone differ for the various materials [31].

By the mid-1980s, after many years of clinical testing, bioactive materials had gradually gained their recognition and had reached clinical use in a variety of orthopaedic and dental applications. Bioactive materials have the advantage of being compatible with the human body environment. Their biocompatibility is a direct result of their chemical compositions which contain ions commonly found in the physiological environment (such as Ca^{2+} , K^+ , Mg^{2+} , Na^+ etc) and of other ions showing very limited toxicity to body tissues (such as Al^{3+} and Ti^{2+}) [50].

Due to their close chemical and crystal resemblance to the mineral phase of bone, HA exhibits excellent biocompatibility. Synthetic HA ceramics began to be routinely used as porous implants, powders and coatings on metallic prostheses [33]. Other applications included immediate tooth root replacement [51], augmentation of the alveolar ridge [52] and use within maxillo-facial reconstruction [53].

Bioactive glass-ceramics are created by controlled crystallisation of bioglasses during heat treatment. The overall properties are dependant on the overall chemical composition and morphology, as well as the composition of the crystalline phases or phases [54], with the composition of the residual glass phase being the most important factor in determining the bioactivity of these materials [55]. Glass-ceramics have superior mechanical properties when compared to glasses, with increasing crystallisation resulting in an increase in strength and overall chemical stability. An increase in the content of crystalline phases, however, will result in a decrease in the level of bioactivity [54], as a result of the reduction in the rate of ion exchange reactions [56]. Glass-ceramics are favoured for load bearing applications, however a compromise between the actual mechanical strength and level of bioactivity must be made [54]. Ceravital[®] refers to a range of glass-ceramics based on the original 45S5 Bioglass[®] but with lower alkali content. Even though the original idea was to use these materials in load bearing conditions for the replacement of bone, their mechanical properties were

not found compatible for such applications. These glass-ceramics have, however, had widespread clinical success as coatings on prosthesis [33] and as replacements for the ossicular chain of the middle ear [57]. Apatite-wollastonite (A/W) glass-ceramics are based on the $\text{SiO}_2\text{-CaO-MgO-P}_2\text{O}_5\text{-CaF}_2$ system. Both oxyfluorapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH}_1\text{F}_2)$) and wollastonite (CaOSiO_2) are homogeneously distributed in a glass MgO-CaO-SiO_2 matrix phase, taking the shape of rice grains 50 -100 nm in size [58, 59]. A/W ceramics are used regularly in iliac crest donor site repair, in the replacement of vertebrae, in hip surgery and as a filler material for extensive bone lesions [60] [33]. Commercially it is available as Cerabone[®].

The first clinical use of bioactive glass was for the reconstruction of the bony ossicular chain of the middle ear in the treatment of conductive hearing loss. It was discovered that it not only bonded with the remaining ossicle bone stock, but also to the tympanic membrane via collagen attachment. This prevented the main modes of failure from occurring, by providing implant immobilisation and preventing implant extrusion. Another early use of 45S5 Bioglass[®] was as a natural tooth-form implant following extraction. This clinical success in-turn resulted in the development of a totally submerged, ridge-maintenance implant to preserve the alveolar ridge following tooth extraction, resulting in a 90% retention rate [61]. Further clinical applications for the original bioactive glass composition, 45S5 Bioglass[®] include implants for the orbital floor [62], the repair of periodontal defects and as a filling material in the replacement of lost bone [31]. Thanks to advances in related medical technologies, bioactive glasses are now undergoing a new stage of development. More recent applications of bioactive glasses are as components of bioactive bone cements [63], as drug delivery matrices and as scaffolds for cells [64] or growth factors [65] in the field of tissue engineering [43].

The worldwide global market for bioactive materials increased from \$377.7 million in 2004 to \$431.4 million in 2005 with sales reaching approximately \$473.9 million in 2006. At an average growth rate of 17.2% this market will gross \$1 billion by 2011. Glass and glass-ceramics had the highest market share throughout the forecast period,

worth more than 60% of the total market in 2006. At an average annual growth rate of 16.5% , it will grow to \$600 million by 2011 [66].

Bioactive materials have evolved to become an integral and vital segment of the modern health-care delivery system and a necessary response to the medical needs of a rapidly ageing population. It is fair to comment, however, that most existing biomaterials were developed based on trial and error optimisation. Further studies on composition, microstructure and molecular surface chemistry are needed to obtain long-term reliability, the optimisation of both biochemical and biomechanical compatability and the ability to tailor compositions for specific clinical applications [44].

3 Glass

From our earliest origins, man has been making use of glass. The ease of formation of sharp edges on obsidian, a natural form of glass, allowed the production of knives, arrow heads, spears and other cutting tools [67] [68]. The earliest man-made glass objects, mainly non-transparent glass beads, are thought to date back to around 3500BC, with finds in Egypt and Eastern Mesopotamia [69].

The scientific use of glasses began over seven hundred years ago. Eye glasses were first developed in Italy in the thirteenth century and the first lenses for telescopes were fashioned in the late sixteenth century. During the late nineteenth century, the collaboration of Abbe, Schott and Zeiss at the University of Jena, Germany sparked a new era of scientific excellence in glass research [70]. This early research was of great importance. It represented the first attempt to study systematically the relationships between the composition of a glass and its physical and chemical properties [71].

To this day, the terms and definitions used in glass science remain highly ambiguous. In everyday language glass is described as a hard, brittle, transparent, amorphous material. This definition is far from adequate from a scientific or technical standpoint. Ambiguity, however, also creeps into the internationally recognised definition for a glass -

“a glass is an inorganic product of fusion which has been cooled to a rigid condition without crystallisation” – ASTM Standard [72].

This definition is again far from adequate as it defines all glasses as being inorganic (ignoring organic, metallic or H-bonded materials) and restricts all glasses to a class of supercooled liquids [73]. In reality there are many alternative glass processing routes such as sol-gel, chemical vapor deposition and n-bombardment. This classically

represents the confusing nature of the characterisation of glasses within modern day literature.

A more applicable definition of glass is “an amorphous solid without long-range structural order that undergoes a glass transition” [72]. This classification takes account of the two common characteristics of glasses. Firstly, that no glass has a long-range periodic arrangement of atoms and even more importantly, every glass exhibits time-dependent glass transformation behavior [67].

Glasses are characterised by certain well-defined properties which are common to all of them and different from those of liquids and crystalline solids. As discussed, X-ray diffraction spectra illustrate that glasses lack any kind of regular arrangement of atoms and thus have no long-range order. They more resemble a liquid than a crystalline solid, in terms of their atomic distribution [74]. Additionally, unlike crystals, glasses do not exhibit a sharp or defined melting point and do not cleave in preferred directions. They show elasticity, as do crystalline solids but, as with liquids, they flow under shear stress. Therefore, we can see that the glassy form combines the “short-term” rigidity characteristic of the crystalline state with a little of the “long-term” fluidity of the liquid state [74].

More detailed aspects of the interrelationships between the liquid, crystalline and glassy forms of a material are best explained in terms of the volume-temperature diagram for a glass-forming substance shown in Figure 4 [71]. The classical way to produce a glass is to cool the initial liquid so quickly that crystallisation does not have time to occur. With the decreasing temperature, an increase in viscosity causes the progressive freezing of the liquid to its final solidification. It is this continuous transition from a liquid to a solid that provides another distinction between glasses and crystalline solids [75].

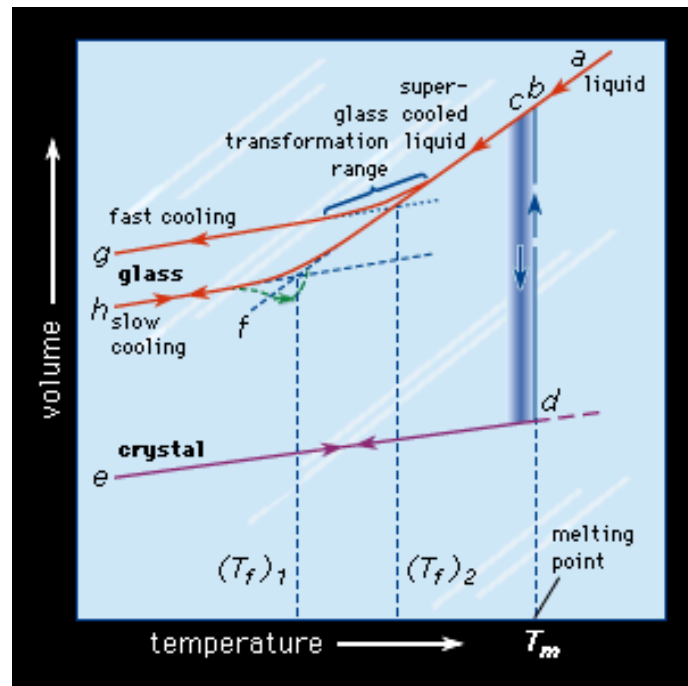


Figure 4: Volume-Temperature Graph: Relationship between the glassy, liquid and solid states.

On cooling a liquid from the initial state a , the volume will decrease steadily along ab . If the rate of cooling is slow enough, and sufficient nuclei are present, crystallisation will occur at the melting point, T_m . The volume will then decrease markedly from b to d , thereafter the solid will contract with falling temperature along d to e .

However if the rate of cooling is sufficiently rapid, crystallisation does not take place at T_m ; the volume of the supercooled liquid decreases along $abcf$, which is a smooth continuation of ac . At a certain temperature T_{f2} , the volume-temperature graph undergoes a significant change in gradient and continues almost parallel to the contraction graph de of the crystalline form.

T_{f2} (T_g) is called the glass transition temperature and only below this temperature is the material a glass [74]. The atomic arrangement within the liquid is frozen and the liquid has an aperiodic structure. T_g is a kinetic parameter and thus parametrically varies with the rate of cooling. It is, therefore, more appropriate to call it the glass transition/transformation range. High rates of cooling will raise the temperature at

which T_g occurs. The glass transition temperature is also taken as the temperature at which discontinuities in temperature-dependence can be detected. The transition is a region of temperatures where the rate of structural relaxation is slow enough to be detected. The atoms in a glass are frozen into fixed positions. Temperature-dependent properties change because the amplitude of these atoms, around their fixed position, is influenced by temperature. As the temperature lowers towards the glass transition temperature the atom mobilities decrease and a certain time is required before these atoms relax back to their equilibrium positions [76]. The structure, therefore, is unable to maintain equilibrium in the region of glass transition [77].

3.1 Glass Structure

There are three main groups of commercial glasses: the soda-lime silica glasses, the borosilicate glasses and the lead silicate glasses. Glass science however comprises a multitude of different glass compositions. Despite this vast compositional range, as with the general definition of a glass, the fundamental basic structure is still to this day the subject of theorising and assumption [78]. Different theories of glass formation have been developed over the last 100 years, which have outlined a wide variety of viewpoints on the molecular structure of glass [79]. It is important to note, however, that although these hypotheses describe glass formation in a number of allied systems, a unified hypothesis, that is able to explain the phenomenon of glass formation in all known systems, has yet to be developed [74].

3.1.1 Zachariasen's Random Network Theory

Zachariasen's theory on the structure of glasses emerged in 1932 in his paper entitled "The Atomic Arrangement in Glass" [80]. The paper represented an enormous leap forward in the knowledge of glass structure-property relationships and still to this day remains one of the most cited papers in glass literature.

Zachariasen postulated that glasses were structurally composed of a random three-dimensional infinite network. This network is constructed from building units of low co-ordination number e.g. SiO_4 tetrahedra (Figure 5) or BO_3 triangles. These building units are connected to each other in an irregular fashion and it is this irregularity of

conformation that differentiates a glass from its crystalline counterparts [70]. Zachariasen contends that it is the ability to form such networks that provides the ultimate condition for glass formation [67].

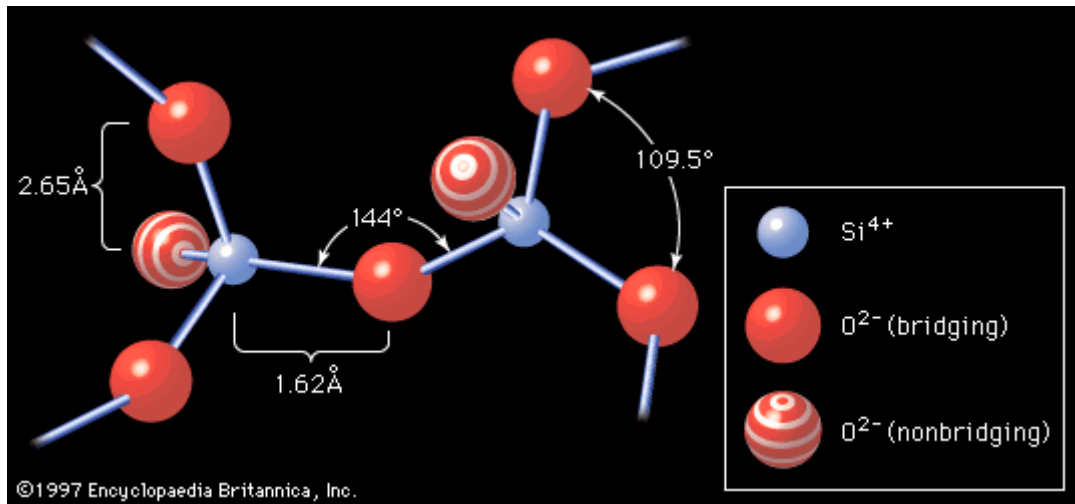


Figure 5: An example of the basic structure of tetrahedral units in silicate glasses [81].

After establishing that the formation of a vitreous network is necessary for glass formation, Zachariasen considered the structural arrangements which could produce such a network [67]. According to his theory, there are a number of rules which an oxide must obey if it is to be a glass former, i.e. the glass formation of simple compounds such as SiO_2 , B_2O_5 , P_2O_5 , GeO_2 , As_2S_3 , and BeF_2 :

1. No oxygen atom can be linked to more than two M atoms (this implies that network formers must have covalently bonded oxygens). Higher co-ordination numbers for the oxygen cations prevent the variation in cation-oxygen-cation bond angles necessary to form a non-periodic network [67].
2. There must be a low number of oxygen atoms surrounding each M. Therefore, M atoms must have a low co-ordination number.
3. The M-O_x polyhedra must share corners with each other, not edges or faces (a requirement of the cations to be as far away from each as possible).

4. For a three-dimensional glass network, at least three of the corners of a polyhedron must be shared with neighboring polyhedra.

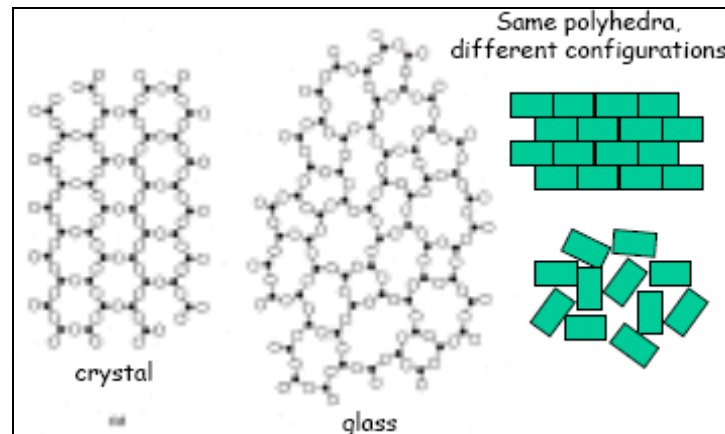


Figure 6: Schematic 2D representation of an oxide in (A) the crystalline form and (B) the glassy form [82].

Zachariasen also considered the more complex glasses which contain a number of oxides in addition to the glass-forming oxides such as SiO_2 and B_2O_3 . For these the rules were modified [83] –

- The oxide glass forms on the basis that oxygen tetrahedra or triangles are surrounded by a high percentage of the cations.
- The triangles or tetrahedra only share corners with each other.
- Some oxygens are only linked to two network cations, therefore there is a distinction in the oxygen's bridging characteristics (bridging and non-bridging).

It should be noted that Zachariasen only states that a glass *may* be formed under such conditions: he further states that the melt must be cooled under proper conditions for glass formation actually to occur - thus anticipating the later theories based on the kinetics of the glass formation process [67].

Practically all of the glass systems in common use, however, will be far more complicated than the simple binary compounds that satisfy Zachariasen's rules. For example a typical bioactive glass may contain two glass formers (SiO_2 and P_2O_5) and also usually contain other elements such as Ca^{2+} , Na^+ and Mg^{2+} . If such large cations, such as Ca^{2+} , are introduced into such simple glasses i.e. in their low-order networks, the bridges formed by the oxygen atoms, between adjacent tetrahedra, will be ruptured [70].

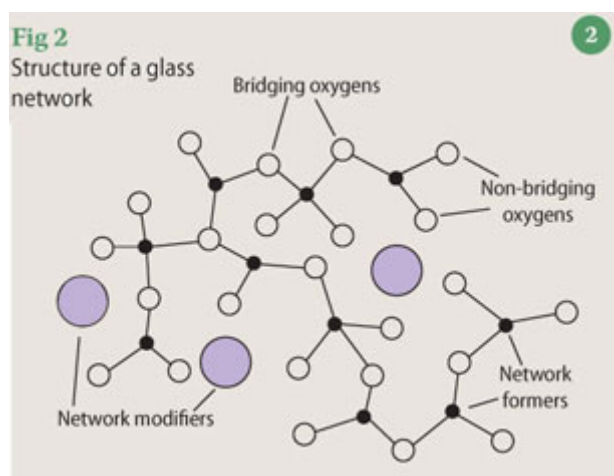


Figure 7: SiO_2 schematic – effect of a network modifying atom [81].

The anions that accompany the cation when introduced (i.e. O^{2-} in the case of Na_2O) will assume sites at the free ends of the separated tetrahedral while the cation situates itself in the interstice created by the separation. Zachariasen suggests that this effectively opens up the glass network [80]. Thus it is evident that different elements must play varying roles within the structure of a glass [70]. Zachariasen was able to divide all cations that participate in glass formation into three groups –

- a) Network Formers: predominantly having co-ordination numbers of three or four (e.g. Si, B, P, Ge, As, Be). These oxides are considered to be the backbone of all oxide glasses and build up three dimensional random networks as shown in Figure 7 [78].
- b) Network Modifiers: normally having co-ordination numbers of six or above (e.g. Na, Ca, K, Ba). Network modifying oxides are incapable of building a continuous network [43]. Instead these cations disrupt or modify the network

and create non-bridging oxygens. For each additional oxygen introduced in the form of a network modifying oxide, one A-O-A bridge is broken resulting in two non-bridging oxygens (Figure 8). The progressive addition of network modifying cations to the silica glass results in a gradual breakdown of the silica network [55].

- c) Intermediate Oxides: predominantly having co-ordination numbers of four or six (e.g. Al, Mg, Zn, Pb, Nb). These are oxides that are not capable of forming glasses on their own, but can take part in the network or take up a modifying role.

In a large proportion of glass literature, silica structures similar to the figure below are defined using the idea of the Q structure. The theory uses the terminology of Q^n where Q^n represents a silicon atom in coordination with n bridging oxygen atoms ($n = 0$ to 4).

Silica glasses are based on the tetrahedral units of (SiO_4^{4-}) . The centred silicon atom with an outer electronic configuration of $3s^2 3p^2$ take up a tetrahedral hybrid state sp^3 and contribute an electron to each bond. Two bonding situations can then occur [84]. Each oxygen atom with an electronic configuration of $1s^2 2s^2 2p_x^2 2p_y^1 2p_z^1$ can either use their two unpaired electrons in a σ covalent bond with two other silicon atoms (bridging oxygen BO) or on the other hand, each oxygen can use one unpaired electron in a σ covalent bond with the neighbouring silicon atom. The other unpaired electron is then left to ionically interact with alkaline or alkaline-earth metals – network modifiers (Na^+ , Ca^{2+} , K^+) forming non-bridging oxygen bonds (NBO). The effect of a network modifier is to disrupt the silicate network leading to an increment of the concentration of NBOs [84].

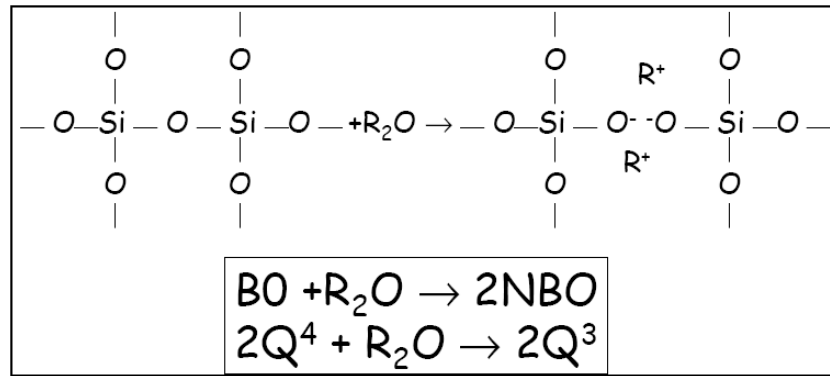


Figure 8: The effect of an alkali oxide on Q terminology [81].

The figure below, Figure 9, summarises silicate network structures and their corresponding Q structure terminology.

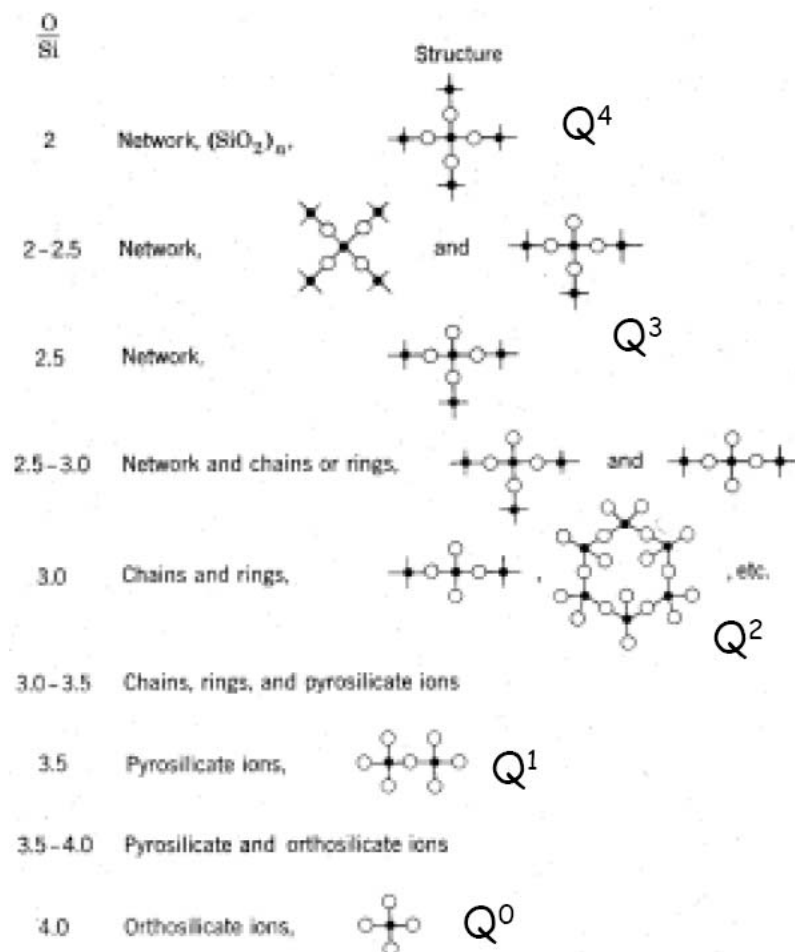


Figure 9: Effect of oxygen/silicon ratio on silicate network structures [81].

A large number of properties can be explained or predicted by the Zachariasen-Warren concept and thus this theory has dominated glass science for many decades. Its importance lies in the fact that it was the first theory to apply crystal chemical principles to the study of glass structure and the first to classify the structural roles for component oxides.

However, the random network theory assumes purely statistical distribution of cations throughout the glass network and is not ideal for predicting all glass systems. For example, Zachariasen can not explain some discontinuities seen in a number of composition-property glass curves thus indicating the influence of changes in short range order in the glass systems [85]. For example, in a random homogenous network the effect of a modifier should be in direct proportion to the concentration according to Zachariasen. However, for many properties, such as activation energy for viscous flow and ionic diffusion, it is found that the effect of the modifier increases sharply and then levels off [78].

3.1.2 Dietzel's Structural Model

Dietzel extended the approach of Zachariasen to consider the behaviour between different cations and anions. He was able to characterise elements in Zachariasen's former-intermediate-modifier scheme in terms of the relationship between size and polarizability of the constituent ions to their respective charges [70]. He described the interacting forces between the valency (Z) and the distance between the oxide ions (A) as *field strength* (Z/a^2). The classification of different cations according to their field strengths is illustrated in Table 3 below.

Table 3: Classification of field strengths for different cations.

Element	Valency	Interval Spacing between oxide ions (Å)	Field Strength of O ²⁻ ions across the interval (Z/a ²) (Å ⁻²)	Ion Group (Z/a ²)
K	1	2.77	0.13	Network Modifier (0.1-0.4)
Na	1	2.30	0.19	
Li	1	2.10	0.23	
Ba	2	2.86	0.24	
Sr	2	2.69	0.28	
Mg	2	2.10	0.45	Intermediate Oxide (0.5-1.0)
Zn	4	2.28	0.77	
Al	3	1.89	0.84	
Si	4	1.60	1.57	Network Former (1.4-2.0)
B	3	1.50	1.34	
P	5	1.55	2.1	

Dietzel further proposed that for a stable binary glass to form, the difference in field strength must exceed 0.3 and for values above this, the tendency for glass formation increases [70]. For example, a ΔF value of greater than 1.33 for binary melts will readily form vitreous solids.

When a binary melt is cooling, cations compete for their oxygen environment to achieve densest packing. If the field strengths are very similar, two separate glass phases will often form. However, if the field strength of one of the cationic species is significantly greater than the other, then the cation of the larger field strength will have a greater affinity for anions thus forming the most closest packing structure of that cation (e.g. SiO_4^{4-}). This will leave the weaker cation in an unstable condition where it will be forced to adopt a higher coordination state and connect itself to the cation-anion network already formed. This situation then promotes compound formation and crystallisation or glass formation [85] [73] [70].

3.1.3 Inorganic Polymer Model

A further useful approach to glass structures is that taken by Holliday [86], where inorganic glasses are viewed as inorganic polymers. It is obvious that there are certain similarities between organic and inorganic polymers, for example both classes of materials exhibit glass transition temperatures and high melt viscosities [70]

Ray defines an inorganic polymer as a substance of giant molecules composed of atoms other than carbon and linked together by mainly covalent bonds. By applying this concept, silicate glasses may be regarded as inorganic polymers of oxygen cross-linked by silicon atoms [87]. Taking concepts from polymer science, the properties of glass can thus be linked to the network connectivity or cross-link density of the structure. Utilising such theories, predictions can be made as to the behaviour of properties such as solubility, thermal expansion coefficient and surface reactivity. For example, the lower the network connectivity the greater its solubility, the lower its glass transition and the greater its reactivity.

The network connectivity of a glass is defined as the average number of bonds that link each repeat unit in the network. The calculation of network connectivity of a glass is based on the relative number of network-forming oxide species (those which contribute BO species) and network modifying species (those which result in the formation of NBOs) present [87]. This idea then may then be developed to define the cross-link density of the glass which is the average number of additional cross-linking bonds above two, for the elements other than oxygen forming the glass network [70]. Thus a glass with a cross-link density of 0 has an equivalent network connectivity of 2 and corresponds to a linear polymeric chain. The composition CaO-SiO₂ would thus have a NC of 2. Slight changes in the composition of glasses with a NC of 2 will have a dramatic effect on the properties of the glass. For example, a slight increase in SiO₂ content will increase the resultant network connectivity and decrease the reactivity of the glass. A pure silica glass thus has a NC of 4 and a cross-link density of 2. This

corresponds to a fully cross-linked inorganic polymer. The addition of metal cations to the silicate network will result in a disruption of the network which will in turn decrease NC. The magnitude of this decrease reflects the degree of disruption introduced into the glass network [43].

The inorganic polymer model is analogous to previous work conducted by Stevel [55], where he expresses the mean number of bridging and non-bridging oxygens in each polyhedron in the glass network. The parameter is also calculated from the molar composition of each glass.

$$NC = \frac{No. BOs - No. NBOs}{No. network former oxides}$$

Equation 1: Equation for the network connectivity of a glass.

The network connectivity is an average value and assumes that the glass is homogeneous in nature. A number of further structural assumptions are also made when calculating the network connectivity of a glass. These assumptions are based on information obtained from Fourier transform infrared (FTIR), Raman and nuclear magnetic resonance (NMR) spectroscopies [88] [17] -

- Silicon exists as a Si^{4+} ion surrounded by four oxygens in a tetrahedron configuration.
- The phosphorus exists as P^{5+} ion in the glass network and is present as PO_4 tetrahedra with a double bond between one of the oxygens and the phosphorus.
- Boron exists as BO_3 or BO_4^- , whilst aluminium exists as AlO_4^- tetrahedra.
- In the case of BO_4^- and AlO_4^- , the charge is balanced by PO_4^+ tetrahedra (without the phosphorus-oxygen double bond). In the absence of phosphorus the charge is balanced by metal cations.

A major weakness to the application of the structural approach to glass formation is that the nature of the bonding is neglected, which in principle should determine the structure

of glasses. Within Zachariasen's theory, it seems irrelevant whether SiO_2 is ionic or covalent, as is evident from the listed rules. Zachariasen used the ionic model as a result of considering radius ratios etc, however the resulting continuous random network model requires that the glass forming compounds have strong and highly covalent directional bonds. Attempts have been made to relate the glass forming tendencies of inorganic materials to the nature of bonding involved. Indeed, Smekel (1951) recognised the importance of mixed covalent ionic bonding in glass forming materials.

It is, therefore, evident that the prediction of the glass forming ability of a random element in a random composition is in no way simple. To this day a grand unified theory on glass formation has not materialised and in many ways, depending on what one is looking for in a glass with regards to the properties concerned, one tends to accept or reject a particular model with respect to others [78].

3.2 Phase Separation

Liquid-liquid immiscibility, or phase separation, is a common phenomenon in liquid systems. It should not be surprising to find that it is also a common phenomenon in melts [67]. In fact, many more binary glass systems exhibit phase separation than exhibit homogeneous behaviour. Depending on the actual composition of a glass and the bonding involved, undercooling results in aggregation processes comprising of all transitions between a homogeneous mixture and a completely separated phase. The possible reasons for phase separation involve the thermodynamics of mixed phases and the application of mechanisms of nucleation and growth [85]. If the mixing of two components result in a lowering of the overall free energy then the mixture will remain homogeneous. However if the separation of the mixture into two components yields a lower free energy, then phase separation will occur, but only if allowed by kinetic constraints. The thermodynamic basis for phase separation can be derived by considering the free energy of mixing ΔG_m

$$\Delta G_m = \Delta H_m - T\Delta S_m$$

Equation 2: Gibbs free energy equation.

Where ΔH_m is the enthalpy of mixing and is defined as:

$$\Delta H_m = \alpha X_1 X_2$$

Where α is a constant related to bond energies among the components. ΔS_m , the entropy of mixing is defined as:

$$\Delta S_m = -R(X_1 \ln X_1 + X_2 \ln X_2)$$

Where X_1 is the concentration expressed as a mole fraction of phase 1, X_2 is the concentration expressed as a mole fraction of phase 2 and R is the gas constant.

For the entropy of mixing, we find that since X_1 and X_2 are below unity, the term in brackets will always be negative. The value of the Gibbs free energy for mixing will be either be positive or negative depending on the value of α . If it is negative, then the Gibbs free energy of mixing will be negative and the system will not phase separate. If however α is positive, there will be competition between the contributions from enthalpy and entropy and the sign on the ΔG_m will depend on temperature.

If the temperature is high enough, at T_c (the critical temperature), the $T\Delta S_m$ term will dominate and the free energy of mixing will always be negative resulting in an homogeneous system. Between absolute zero and T_c the competition between the terms will result in a saddle in the free energy versus composition graph (Figure 10). This curve has two minimum points which approach each other with increasing temperature, until they meet at T_c . Drawing a tangent between the two points shows that the free energy is minimised if the liquid separates into two phases with compositions A (c_a , on Figure 10 below) and B (c_b , on Figure 10 below). The compositions are a function of temperature, with a decreasing difference in composition as the temperature tends to T_c . The resulting phase diagram exhibits a symmetrical dome below which the liquid will spontaneously separate into two components (if

allowed by kinetics) whose compositions are given by their location on the dome (phase boundary/immiscibility limit). The region inside the dome is termed the miscibility gap or immiscibility region [67].

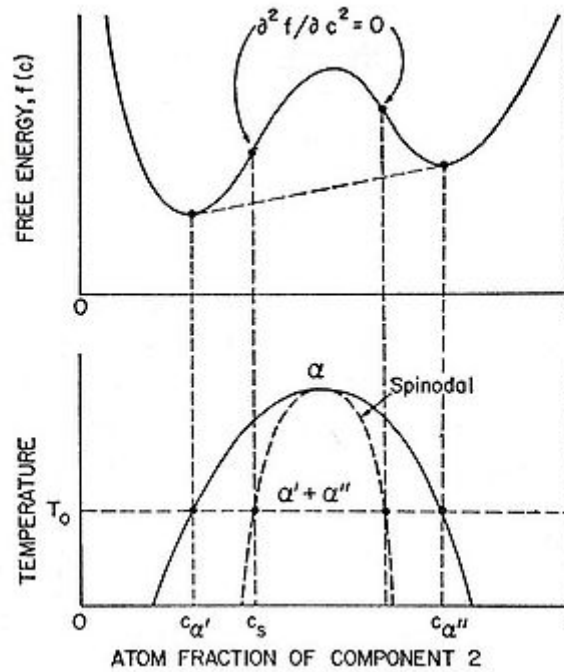


Figure 10: Free energy versus composition relationship and the resultant phase diagram showing the regions of phase separation by spinodal decomposition and by nucleation and growth [89].

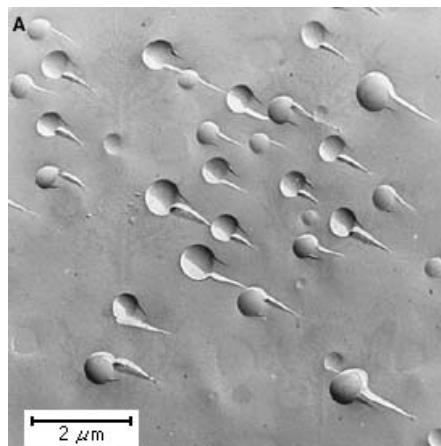


Figure 11: Example of phase separation in a glass [85].

A number of theories can be utilised to derive the free energy curves. The simplest and most well known is often called the regular solution or nearest neighbour model [90] [91]. The model assumes that each component is distributed randomly with a certain fixed number, Z , of neighbours. In the case of metallic alloys or organic liquids, it is simple to see that the elementary mixing units are the metal atoms or small molecules. However, in a network compound such as a silicate glass it is often difficult to identify the mixing units that should be used for theoretical calculations, and this uncertainty leads to weaknesses in theoretical descriptions of miscibility in silicates [72].

Warren and Pincus [92] hypothesised that the greater tendency of a binary silicate melt to phase separate was a result of an increase in the cation field strength. The two tendencies for bonding – the silicon ion tetrahedrally coordinates the four oxygen ions or the other cation surrounds itself with negatively charged non-bridging oxygens of the network. If the cation field strength is low, its coordination is incomplete and a single phase dominated by the silicon-oxygen network results. On the other hand, if the cation field strength is higher, it is better able to coordinate the NBOs and these groups then form a separate phase, the other phase being predominantly silica [72]. Levin and Block [93] introduced a similar argument, suggesting that the larger the coordination number of the cation, the larger the field strength thus the energy of mixing can be considered positive, leading to phase separation. Charles [94] however, rejected this idea, for alkali and alkaline earth silicate glasses, and suggested a theory based on the deformation of the silicate chains in the glass network. He argued that, as the cation concentration increases, the distortion of Si-O-Si bonds will increase, the positive value for energy of mixing will increase and thus phase separation will occur [72].

Again it is evident that for a complex multi component glass it is very difficult to hypothesise, with any degree of certainty, whether the system will phase separate. The prediction is further hindered with the depiction of immiscibility regions in ternary/quaternary systems being far more confusing than those for binary systems. It is often thought that the lack of observation of visible light scattering or opalescence is an indication that phase separation has not occurred. This is rarely true unless extremely

fast quenching methods have been employed. Bulk glasses formed above the metastable immiscibility region are virtually always phase separated on a microscopic level. It is therefore always necessary to do an electron microscopic probe on the structure before a statement of homogeneity is made [67].

4 Bioactive Glasses

Bioactive glasses are a subset of inorganic bioactive materials, which are capable of reacting with physiological fluids to form tenacious bonds to bone through the formation of a bone-like hydroxyapatite layer and the biological interaction of collagen with the material's surface [95] [96]. The base components in most bioactive glasses and glass-ceramics, made by the traditional high temperature melt-quench technique, are SiO_2 , Na_2O , CaO and P_2O_5 . The first, and most well known, composition is 45S5 Bioglass[®], which contains 45% SiO_2 -24.5% Na_2O -24.5% CaO -6% P_2O_5 , in weight percent, and was originally based on a ternary eutectic in the SiO_2 - NaO - CaO phase equilibrium diagram. The compositions of several typical bioactive glasses are given in Table 4.

There are three key compositional features to these glasses that distinguish them from traditional soda-lime silicate glasses. Firstly these glasses have less than 60mol% SiO_2 , secondly they all have a high Na_2O and high CaO content and finally they all have a high $\text{CaO}/\text{P}_2\text{O}_5$ ratio. These specific features give rise to glasses that are highly reactive when exposed to an aqueous medium, resulting in fast bonding with living tissue and promotion of bone growth [97]. The primary disadvantage of bioactive glasses is their mechanical weakness and low fracture toughness, primarily as a result of their amorphous two dimensional glass network. The tensile strength of most of the compositions are in the region of 40-60MPa which make them highly unsuitable for load bearing applications, however this does not limit their use for coating applications, as buried implants, in low-loaded or compressively loaded devices, in the form of powders or as the bioactive phase in composites [33].

The introduction of bioactive glasses to the field of implant technology, however, was initially very limited. The medical community was slow to accept that the well known

properties of glass, such as its brittle and fragile nature, would not result in a detrimental effect to the tissues and blood vessels. Exhaustive tests were carried out to investigate what the effect of a particulate glass might be on tissues. Fortunately the constituent chemicals; Si, Na, Ca and P are all present within the body, and at concentrations that do not disturb the adjacent tissues. Glass particles can be digested by phagocytic cells and without any detrimental effects to the viability or behavior of cells. Many other tests illustrated that bioactive glasses, whether in solid or particulate form, did not elicit any kind of toxic response in the body. They were shown to bond to osseous tissues and to soft tissues through a compliant interface and it was these unique properties that defined the clinical applications for which they have been used in the last 20 or more years [33].

4.1 Mechanisms of Glass Degradation

A number of mechanisms have been proposed to explain the bioactive behavior of glasses and glass-ceramics. The most widely accepted mechanism is that associated with the research of Hench and co-workers [98] [5] – this is discussed further. There are, however, alternative theories as to the mechanisms of glass degradation and bone bonding from Kokubo *et al.* [99] [100], Andersson *et al.* [101] [102] [103], Li *et al.* [104] [105], and Hill *et al.* [17]. Rather than distinct, these different mechanisms give additional explanations for specific details concerning the apatite formation [106].

The basis of the tissue bonding property of bioactive glasses is the chemical reactivity of the glass in body fluids. It seems generally accepted that a layer of biologically active HCA must be deposited on the implant before it bonds to tissue [97]. The bonding mechanism of surface-active glasses is a complex combination of 11 physiochemical and ultrastructural phenomena [107]. The first 5 reactions that occur on the glass side of the interface do not depend on the presence of tissues. They occur in distilled water, tris-buffer solution and simulated body fluid (SBF) and have been well established [108] [109] [110] [99] and studied using FTIR, Auger electron microscopy and electron microprobe analysis [44]. Bonding to tissue however, requires an additional series of reactions (stages 6-11). These are less established and the actual

details of the sequence of cellular events at a molecular biology and genetic level are still being fully established. It has, however, recently been shown that the unique biological behavior of bioactive glasses is due to the activation of seven families of genes that are present in bone forming cells. These genes are activated to produce various proteins that serve as growth factors and enhance the proliferation of new bone cells (osteoblasts) [111]. The cells not only expand in number but also generate collagen and other extracellular matrix (ECM) proteins that mineralize and form new bone. An important aspect of this understanding is that the biological response is due to the controlled release of critical concentrations of soluble silica and calcium ions from the bioactive surface, fundamentally as a result of the leaching and dissolution of the bioactive surface. The surface layer generated during the corrosion reaction serves as a biological active substrate for anchoring and growth of new cells and tissue [111] [112] [113] [96] [40].

There is increasing evidence to suggest that tissue bonding is the result of multiple and parallel reactions at the material-tissue interface, rather than a hierarchical sequence of reactions. For example, cells that have adhered to the ever-reacting surface may interact with the material and produce some of the surface changes. In the inverse direction i.e. from material to environment, there is both a solution-mediated as well as surface controlled effect on cellular activity, matrix deposition and mineralization. All phenomena, collectively, lead to the gradual incorporation of the bioactive implant into developing bone tissue [114]. These reactions are listed below [115] and stages 1-5 are schematically represented in Figure 12.

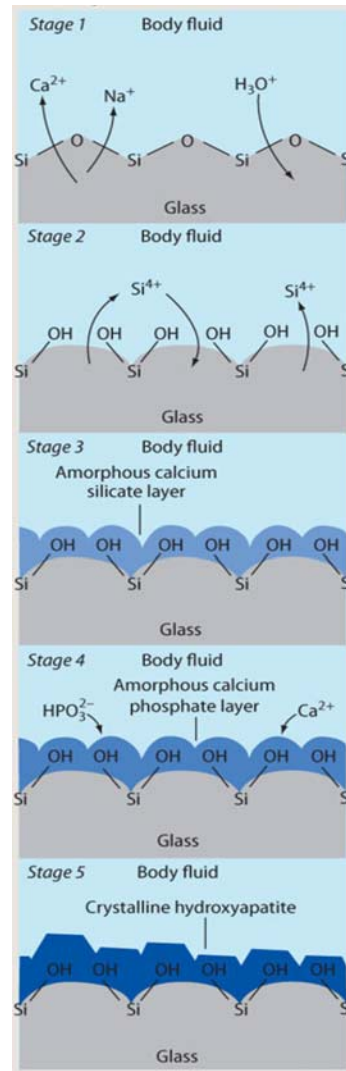
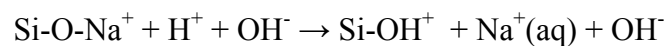


Figure 12: Stages 1 to 4 of HCA formation [66].

1) Rapid exchange of alkali or alkaline earth elements such as Na⁺ and Ca²⁺ with H⁺ or H₃O⁺ ions.

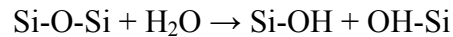
- Diffusion controlled with $t^{1/2}$ dependence, causing hydrolysis of silica groups, which creates silanols;



- Rapid exchange of alkali or alkaline earth ions due to their modifying nature.
- Interfacial pH increases due to exchange of H⁺ with cations.

2) Network dissolution by breaking of Si-O-Si-O-Si- bonds through action of OH⁻ ions.

- Local network breakdown releases silica into solution and forms Si-OH (silanols) at the surface;



- Interface controlled reaction with $t^{1.0}$ dependence.

3) Condensation and re-polymerisation of a SiO₂ rich layer on the surface.

- Porous surface depleted in alkali and alkaline earth cations.

4) Migration of Ca²⁺ and PO₄³⁻ groups to the surface through the SiO₂-rich layer, forms a CaO-P₂O₅-rich film on top of SiO₂-rich layer.

- Followed by growth of amorphous CaO-P₂O₅-rich film by incorporation of soluble calcium and phosphates from solution.
- When formed *in vitro*, the CaP layer is located on top of the silica gel while when formed *in vivo*, it is formed within the gel layer.
- The CaO-P₂O₅ layer protects the glass surface from further attack.

5) Crystallisation of amorphous CaO-P₂O₅ layer film by incorporation of carbonate and hydroxyl anions from solution.

- Forms a hydroxycarbonate apatite structure.
- Glancing angle XRD studies of bioactive glass samples immersed in SBF highlighted the presence of only two lines of the HA pattern suggesting some sort of crystal orientation [43].

6) Adsorption and desorption of biological growth factors, in the HCA layer, to activate differentiation of stem cells .

7) Action of macrophages.

- Remove debris from the site to allow cells to occupy the space.

8) Attachment of stem cells.

9) Differentiation of stem cells to form osteoblasts (bone growing cells).

- 10) Generation of extracellular matrix by the osteoblasts to form bone.
- 11) Mineralisation of matrix to enclose bone cells in a living composite structure.

These reactions take place within the first 12 to 24 hours of implantation. Therefore, by the time osteogenic cells, such as osteoblasts and mesenchymal stem cells, infiltrate the tissue defect (which normally takes 24-72 hours) they encounter a bone-like surface, complete with organic components, and not a foreign material. It is this sequence of events, in which bioactive glass participates in the repair process that allows for the creation of a direct bond of the material to tissue [61]. Within 4 weeks the interface is completely bonded to bone without any intervening fibrous tissue [33].

The overall rate of change of the glass surface can be quantized by equation –

$$R = -k_1t^{0.5} - k_2t^{1.0} + k_3t^{1.0} + k_4t^{ty} + K_5t^{tz}$$

Equation 3: Rate of change of the glass surface.

Where k_1 describes the rate of alkali-proton exchange, k_2 the rate of interfacial network dissolution, k_3 the rate of silica polymerisation, k_4 the formation of multivalent containing multilayers and k_5 the rate of HCA crystallisation. For a bioactive bond to form, stages 4 and 5 must replicate the biomineralisation rate *in vivo*. If these stages are too rapid, the implant will resorb, while if the stages are too slow, bioactivity will not occur.

The bioactive properties of a material are determined by the rate of formation of the HCA layer at its surface (stage 5). The time required for the formation of the HCA (stage 4) layer and the time for onset of crystallization (stage 5) depend on many factors including glass composition, glass structure, glass geometry, solution composition and ratio of surface area to surface volume [116]. For example, the rate of HCA formation is greatly influenced by the dissolution of the glass (stages 1 and 2).

The level of bioactivity of a specific material can be related to the time taken for more than 50% of the interface to bond with bone ($t_{0.5bb}$):

$$\text{Bioactivity index, } I_B = 100 / t_{0.5bb}$$

Equation 4: Bioactivity index.

Materials exhibiting an I_B value greater than 8 (class A), e.g. 45S5 Bioglass[®], will bond to both soft and hard tissue. Materials with an I_B value less than 8 (class B), but greater than 0, e.g. synthetic HA, will bond only to hard tissue. The thickness of the interfacial bond is approximately proportional to the I_B value, while the failure strength of the bioactive bond appears to be inversely proportional to the thickness of the bonding zone. Thus a very high I_B value gives a thick bonding zone but a low shear strength [33].

4.2 Apatite Formation

There are over 85 chemically distinct apatites. The general formula of stoichiometric apatite is $(A(1)_2)(BO_4)_3X$ where $(A(1)A(2))^{2+}$, B^{5+} and X^{-1} are a range of elements. The most common apatites are calcium phosphates ($Ca_3(PO_4)X$) and these include Hydroxyapatite (Hap: $X=OH$), Fluorapatite (Fap: $X=F$) and carbonate apatites (CO_3Aps : $X=CO_3$) [117].

Hydroxyapatite is a naturally occurring mineral and the predominant mineral component of bone and tooth enamel. Naturally occurring bone mineral is made up of nanometer sized, poorly crystalline calcium phosphate with an hydroxyapatite structure. The ideal stoichiometric crystalline hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ with atomic Ca/P ratio of 1.67 is very different from naturally occurring apatite, in that biological apatites are always calcium deficient and carbonate substituted (3.2-5.8wt% carbonate) and also contain other minor elements (as described below) as well as organic materials (enamel ~ 1.0wt% and bone ~ 25wt%) –



Where M represents Mg, Na, K and trace elements Sr,Pb,Ba etc and where Y represents acid phosphate, HPO_4 , sulfates, borates, vanadates etc. These elements will significantly change the lattice parameters and the crystal properties of apatite [118].

4.2.1 SBF

The development of a salt solution which represented similar concentrations to that of ions present in blood plasma began as early as 1943 when Earle and co-workers developed a balanced salt solution for use in cell culture media. Since then, simulated body fluids (SBF) have been produced in order to provide insight into the reactivity of the inorganic component of blood plasma, and predict the bioactivity of implants, bone scaffolds, as well as other novel biomaterials [119].

It is widely accepted that the essential requirement for an artificial material to bond to bone is the formation of a bone like apatite layer on its surface. Formation of a bone like apatite layer on the bioactive material's surface (Figure 13) can be produced in simulated body fluid (SBF), with ion concentrations almost equal to those in human blood plasma (Figure 14). This synthetic fluid is supersaturated, in calcium and phosphate, with respect to apatite and is also metastable [41]. Therefore, if a material has a functional group effective for apatite nucleation on its surface, it can form the apatite spontaneously [41]. Thus, a glass is considered to be bioactive if a layer of apatite is formed on its surface in SBF [120].

Studies into the apatite formation on a CaO-SiO_2 surface by Ebisawa *et al.* indicate that the deposition of apatite is a consequence of the chemical reaction between the dissolved calcium and silicon from the glass and the phosphorus from the SBF solution (Figure 14) [121]. The silicon provides sites for the nucleation of apatite while the calcium increases the extent of supersaturation of the SBF [122].

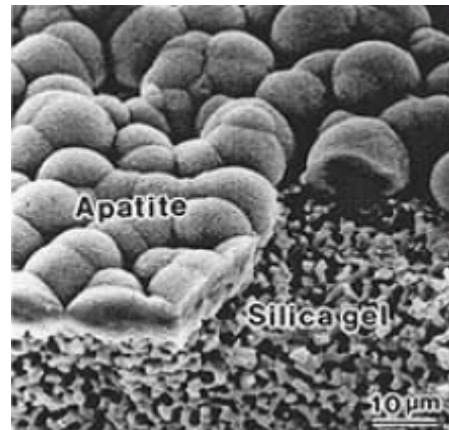


Figure 13: SEM micrograph of apatite formation [123].

Pereira *et al.* found that HCA nucleated faster in SBF than Tris-buffer (a simple organic buffer solution), which they assigned to the higher phosphorus concentrations aiding HCA nucleation [124]. However, Tsuru *et al.* [125] suggested that this would be a result of the dissolution of Ca^{2+} ions increasing the degree of supersaturation (which is already supersaturated with respect to HCA formation) hence making it much easier for HCA nucleation. Zhong and Greenspan [126] have shown the opposite and found that the classic behavior response of the formation of a crystallised HCA layer, occurs faster in Tris-buffer rather than SBF.

As with all *in vitro* test systems, the conditions whereby a HCA layer forms in SBF do not necessarily mimic the *in vivo* condition [114]. In fact, not all bioactive compositions that form a calcium phosphate layer *in vitro* will end up bonding with bone *in vivo*. SBF cannot mimic the complexity of body fluids.

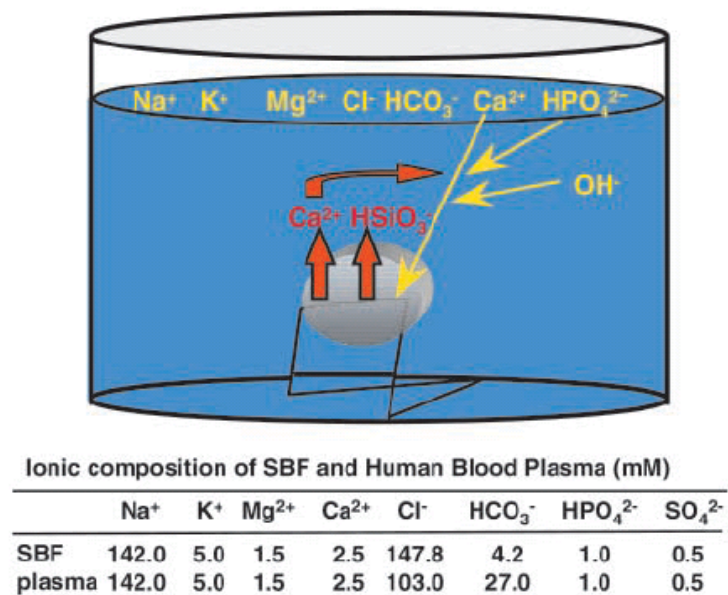


Figure 14: Composition of SBF [127].

4.3 Compositional Dependence

The compositional dependence of both hard and soft tissue bonding for the Na₂O-CaO-P₂O₅-SiO₂ system, with constant 6 wt% P₂O₅ were studied by Hench and co-workers and are illustrated in Figure 15.

Figure 15 establishes the bioactive-bonding-boundary of the compositions studied. Within region A the glass compositions are predicted by Hench to be bioactive, with the central region, labelled S, highlighting soft tissue bonding. Glasses within region B (such as window glass) behave as nearly inert materials and they will elicit a fibrous capsule at the interface of the implant and tissue. Glasses within region C are resorbable, dissolving within 10-30 days while the compositions in region D are non-glass forming due to their very low silica content [33].

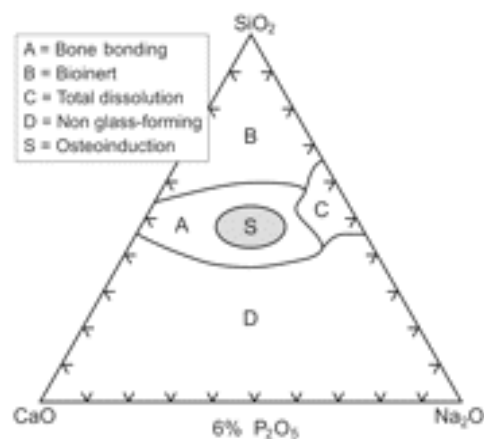


Figure 15: Compositional dependence (in weight percent) of bone bonding and soft-tissue bonding of bioactive glass and glass-ceramics [33].

A slight deviation in composition can radically alter the dissolution kinetics or even the basic mechanisms of bonding [14] [13]. It is widely accepted that increasing the silica content of bioactive glasses greatly inhibits the level of bioactivity by reducing the availability of modifier ions to the solution and inhibiting development of the silica gel layer on the surface [115] [35] [128]. For example, glasses containing up to approximately 53mol% SiO_2 crystallisation occurs extremely rapidly, within 2 hours of implantation, resulting in a rapid bond to bone and also an adherent collagen bond with soft tissue. In comparison, if the silica content is increased to 53-58mol% the glasses will require at least 2-3 days to form a crystalline HCA layer on their surface. Compositions with 60mol% or over do not form a crystalline HCA layer at all, even after 4 weeks in SBF [33].

Na_2O causes a rise in the rate of apatite formation by increasing the local pH of the solution at the implant-tissue interface; which in turn accelerates the dissolution of the glass. A change in the $\text{Na}_2\text{O}/\text{CaO}$ ratio has been documented as altering the level of bioactivity [47], as has the addition of alkali oxides effecting the rate of *in vitro* apatite formation [17]. Contrary to this, the addition of multivalent cations, such as aluminium, stabilises the glass structure by eliminating non-bridging oxygens, thus resulting in a decrease of HCA formation [129]. An addition of as little as 3 wt% Al_2O_3 to 45S5 totally inhibits surface bonding [44]. Brink *et al.* [130] [131] studied the *in-*

vivo bone-bonding ability of 26 melt glasses in the system Na_2O - K_2O - CaO - MgO - B_2O_3 - P_2O_5 - SiO_2 and concluded that the compositional limits for bioactivity include: 14-30mol% of alkali metal oxides (NaO and K_2O), 14-30mol% of alkaline-earth metal oxides (CaO and MgO) and less than 59mol% SiO_2 .

Most bioactive glasses, including those developed by Hench *et al.* are silicate glasses based on the SiO_2 - CaO - Na_2O - P_2O_5 system, but research has showed that the presence of all the constituents is not a prerequisite for bioactivity, and that the other constituents such as CaF_2 , B_2O_3 , MgO and Fe_2O_3 effect the bioactivity to various extents [11]. In a multi-component system it is especially difficult to find a simple relationship between composition and tissue bonding that can be expressed in a two-dimensional diagram such as Figure 15 [33].

The compositional dependence of bioactivity has received much attention. Despite this, and the immense number of papers published on bioactive glass systems, it still remains very difficult to predict, with any degree of confidence, whether or not a purely random glass composition will be bioactive. The bone bonding behaviour of a number of bioactive glasses cannot be explained in terms of the Hench's accepted model for bioactivity. This notably includes Ebisawa's [121] $\text{CaO}.\text{SiO}_2$ composition which, although sodium free still bonds to bone. This questions the requirement of the exchange of sodium ions for protons as a step for bioactivity and raises uncertainties regarding Hench's accepted model.

4.3.1 Inorganic Polymer Model and Bioactivity

Hill [17] suggested an alternative view with respect to the degradation of bioactive glasses. Although not regarded as an alternative explanation to that proposed by Hench [98] [33], Hill simply realised the inadequacy of the model in predicting the reactivity of a bioactive glass as a function of its composition. As with Strnad [55] and Rawlings [11] he recognised the importance of the degree of disruption of a glass in determining its bioactivity. Following the approach of Holliday and Ray [86] [87], who viewed

silicate glasses as inorganic polymers of oxygen cross linked by silicon atoms, Hill introduced the concept of network connectivity to the field of bioactive glasses.

Glasses with a corresponding low network connectivity are considered to have highly disrupted glass networks. The network connectivity (or cross-link density) of a glass can be used to predict a number of structural properties including surface reactivity, solubility and thermal expansion coefficient, thus the lower the value, the lower the glass transition temperature and the greater the reactivity and solubility.

As bioactivity arises from the ability of a glass to undergo surface dissolution in a physiological environment, the degree of solubility of a composition is indicative of its bioactivity level. The application of the inorganic polymer model to glass structure thus allows the prediction of bioactivity. A glass of low network connectivity is predicted to be bioactive provided it does not give rise to toxic products of degradation [43].

All of the very reactive bone bonding bioactive glass compositions have network connectivities below 2 (referred to as invert glasses), with the most widely studied 45S5 glass with a NC value of 1.90. This means that the silicate structural units present are of low molar mass and are capable of dissolving and going into solution without breakage of the silicon-oxygen-silicon bonds, as proposed in the second step of Hench's model [98] [33]. The dissolution of the network in this way is the primary initiation of the series of reactions that result in bioactivity, this breakdown of structural units gives rise to the release of the units into the external solution and thus accounts for the observed increase in alkali ion concentration. Following the breakup of the silicate network, the sequence of stages, as proposed by Hench, can apply to fully describe the bioactive behaviour [43].

The bioactivity of a glass can only be predicted from this connectivity model to a certain degree, working best for glasses in the $\text{SiO}_2\text{-CaO-Na}_2\text{O}$ system. The model however starts to breakdown when used on phosphate containing compositions. This

therefore suggests that the model misunderstands the role of phosphorus in some way. More recently however the model has been corrected to take account of the actual role of phosphorus within bioactive glasses – this is discussed in detail later on in the project

5 Experimental – Materials and Methods

5.1 Rationale

Despite the vast number of papers and investigations into bioactive glasses and glasses in general, the relationships which exist between a glass's composition, its structure and its properties are still not fully characterised and understood. The main objective of this work is to gain a greater understanding of how a glass's composition can influence its physical, chemical and bioactive behaviour. The over-riding objective is to gain a comprehensive understanding of such relationships with the view to developing new bioactive glass compositions with enhanced properties for biomedical applications.

As commented by Tilocca *et al.* [15] the growing technological importance of bioactive glasses has not been supported by a corresponding growth in fundamental understanding of the nature of their bioactivity, and trial-and-error approaches still represent the most common way to systematically optimise new applications of bioactive glasses [15]. Furthermore, most work on bioactive glass systems, reported in literature, is carried out on a weight percent basis. This method, rather than helping to reveal the composition-property relationships actually masks these principles by ignoring the degree of disruption of the silicate network [17]. Therefore, in this study, the inorganic polymer model has been used to design glass compositions on a mole percent basis, thus providing a greater understanding of the resulting properties with respect to structural and compositional parameters.

The base composition used in both series is that of Elgayar *et al.*'s [78] soda lime phosphosilicate ICIE1 glass – its composition is tabulated in Table 4. The effect of CaO substitutio on the series has already been investigated by Elgayar *et al.* [78], as has the effect of strontium substitution by Fredholm *et al.* [132]. Therefore, there already

exists a good understanding as to the effect of such structural species on $\text{SiO}_2\text{-P}_2\text{O}_5\text{-Na}_2\text{O-CaO}$ glass systems. The effect of P_2O_5 on the quaternary glass system still remains an area for theorising and assumptions, as does the effect of MgO substitution on the system. This project therefore investigates the two glass species, P_2O_5 and MgO and tries to gain an understanding as to their structural roles and their influence on the bioactive behaviour of a soda lime phosphosilicate glass.

Two series of glasses have been studied –

- **Magnesium Substituted Series** (further discussed in Chapter 5.2) - The first series was designed to determine the influence of substituting magnesium for calcium in a $\text{SiO}_2\text{-P}_2\text{O}_5\text{-Na}_2\text{O-CaO}$ glass system. Two sub-sets of the magnesium series were designed in order to understand whether the behaviour of magnesium in a glass system is influenced by the network disorder of that glass system. Therefore, magnesium was substituted for calcium on a mole percent basis in both the ICIE1 series of glasses and the ICIE4 series of glasses. Within both series, in order to fully understand the role of magnesium as an alkaline-earth ion, it was necessary to eradicate any interference due to variations in network polymerisation. Therefore all glasses in each series were designed to have the same network connectivity of 2.04.
- **Phosphorus Series** (further discussed in Chapter 5.3) - The effect of the variation in phosphate (P_2O_5) content on the structure of two series of bioactive glasses in the quaternary system $\text{SiO}_2\text{-Na}_2\text{O-CaO-P}_2\text{O}_5$ was studied. The first series (I) was a simple substitution of P_2O_5 for SiO_2 keeping the $\text{Na}_2\text{O:CaO}$ ratio fixed (1:0.87). The second series (II) was designed to ensure charge neutrality in the orthophosphate (PO_4^{3-}) complex, therefore as P_2O_5 was added, the Na_2O and CaO content was varied to provide sufficient Na^+ and Ca^{2+} anions to charge balance the orthophosphate species present.

5.1.1 Glass Network Connectivity

Hill [17] has theorised the use of the inorganic polymer model in order to understand the structure and bioactivity of bioactive glasses. Table 5 illustrates a number of commercially available bioactive glass compositions and their resulting network connectivity values.

The network connectivity of a glass is determined from its molar compositions using the equation below. It is based on the relative numbers of network-forming oxide species (those which contribute BO species) and network-modifying species (those which result in the formation of NBO species) present –

$$NC = 2 + \frac{[(2 \times SiO_2) + (2 \times P_2O_5)] - [(2 \times CaO) + (2 \times Na_2O)]}{SiO_2 + (2 \times P_2O_5)}$$

Equation 5: Network connectivity calculation for a soda lime phosphosilicate glass..

For example, the network connectivity for the base glass used in this investigation, ICIE1, is calculated below –

$$NC = 2 + ((2xSiO_2 + 2xP_2O_5) - (2xCaO + 2xNa_2O))/(SiO_2 + 2xP_2O_5)$$

$$NC = 2 + ((2x49.46 + 2x1.07) - (2x23.08 + 2x26.38))/(49.46 + 2x1.07)$$

$$NC = 2 + 2.14/51.60$$

$$NC = 2 + 0.04$$

$$NC = 2.04$$

It should be noted, however, that this network connectivity calculation assumes phosphorus enters the silicate network. Modified network connectivity calculations (NC') are discussed further on in this study.

Table 4: Selected bioactive glasses (in mol%) and their resultant network connectivity values (assuming phosphorus enters the network) [43].

Glass	SiO ₂	Na ₂ O	CaO	P ₂ O ₅	MgO	ZnO	K ₂ O	NC
45S5	46.16	24.32	26.92	2.60	0.00	0.00	0.00	1.90
ICIE1	49.46	26.38	23.08	1.07	0.00	0.00	0.00	2.04
ICIE4	49.46	6.60	42.87	1.07	0.00	0.00	0.00	2.04
55S4.3	56.52	19.41	21.46	2.61	0.00	0.00	0.00	2.59
B3	35.12	0.00	50.29	7.15	7.15	0.00	0.00	1.39
BG1	49.44	47.89	0.00	2.67	0.00	0.00	0.00	2.15
BG2	48.94	37.93	10.48	2.64	0.00	0.00	0.00	2.12
6P44-a	45.19	23.37	13.80	2.60	10.81	0.00	4.24	1.82
6P50	49.80	15.02	16.71	2.54	13.26	0.00	2.68	2.17
S55-5P4	57.00	29.00	12.00	2.00	0.00	0.00	0.00	2.59
S53P4	54.00	23.00	22.00	2.00	0.00	0.00	0.00	2.38
E1	31.09	0.00	31.72	10.57	26.62	0.00	0.00	1.36
CS-Na	48.38	2.75	48.88	0.00	0.00	0.00	0.00	1.87
CS-P	49.14	0.00	49.64	1.22	0.00	0.00	0.00	2.03
1.25C-S	44.44	0.00	55.56	0.00	0.00	0.00	0.00	1.50
CS	50.00	0.00	50.00	0.00	0.00	0.00	0.00	2.00
H	46.20	24.30	26.90	2.60	0.00	0.00	0.00	1.91
HZ5	44.40	23.40	25.90	2.50	0.00	3.80	0.00	1.75
HZ10	42.50	22.50	24.80	2.40	0.00	7.80	0.00	1.57
HZ20	38.80	20.50	22.60	2.20	0.00	15.90	0.00	1.17
HP5	43.70	24.40	26.90	5.00	0.00	0.00	0.00	1.90
HP5Z5	42.10	23.40	25.90	4.70	0.00	3.90	0.00	1.75
HP8	40.00	24.40	26.90	8.70	0.00	0.00	0.00	1.91

5.2 Series 1 – Magnesium Substituted Glasses

As discussed previously, the role of two group II alkaline earth ions, calcium and strontium, with respect to their network modifying roles within bioactive glasses have already been investigated by Elgayar *et al.* [78] and Fredholm *et al.* [132], respectively. Therefore, it seemed logical to investigate a third member of the alkaline earth group, magnesium, and its role structurally within a bioactive glass.

Magnesium has often been incorporated into bioactive glasses [120, 133-141], however despite this there have been few systematic studies into the structural role of MgO and its resultant bioactive behaviour. Conventionally magnesium is thought to behave as a network modifier within a glass structure. Also if the periodic nature of the alkaline earth metals is accepted and the network modifying property, as discovered by Elgayar *et al.* [78] and Fredholm *et al.* [132], of calcium and strontium replicated through the group, it is suggested that magnesium, when substituted into a bioactive glass, will simply occupy a modifying role. However on examination of a number of elemental properties such as charge to size ratio and Pauling electronegativity, Mg^{2+} falls on the boundary between being a network modifier and intermediate oxide. Circumstantial evidence exists that MgO may act as an intermediate oxide in highly disrupted silicate glasses from the extension of the glass forming region in ternary diagrams towards lower SiO_2 mole fractions and this point has been recognised by McMillan [142].

Stebbins and Fiske [143], Oliveira [137], Kohara [144], Karakassides [145], Dietzel [146], Shimoda [147, 148] and Doweidar [149] acknowledge the possibility of MgO acting as an intermediate oxide and all comment on the importance of clarifying its structural role within a glass. It is suggested that the lack of structural understanding of magnesium within glasses has lead to the many conflicting views on its contribution with respect to bioactivity. MgO has been documented as decreasing apatite formation in SBF [139] but also, on the other hand, improving the early stages of mineralisation [135, 137] and contributing to an intimate contact with living tissue [150].

Magnesium is a very interesting cation with respect to its role within the basic glass structure, its beneficial properties within the engineering of bioactive glasses and its bioactive properties. Magnesium is involved in over 300 reactions in the human body and among other things is known to activate phagocytosis and regulate active calcium transport. As a result, there has been growing interest in its role not only in wound healing [151] but also in bone metabolism, with a 2 year study highlighting the positive effects on fracture prevention and bone density [152]. Such influential roles with respect to bone metabolism and wound healing are extremely attractive when considering the potential end applications of magnesium containing bioactive glasses. It is already widely used within the bioactive field in general and is present in, for example, Ceravital[®]. It is surprising therefore that, although there has been investigation into the role of magnesium with respect to bioactivity, there is little or no understanding of its role in respect of composition-structure-property relationships within bioactive glasses. This investigation aims to fully comprehend and utilise such relationships.

Magnesium oxide was substituted for calcium oxide on a molar basis in the ICIE1 and ICIE4 series of glasses, keeping the calculated network connectivity values constant, at 2.04, in order to try and eliminate any influence due to network disruption – the resulting glass compositions are tabulated in Tables 5 and 6 below.

Table 5: Magnesium substituted ICIE1 molar glass compositions (mol%).

	SiO ₂	Na ₂ O	CaO	P ₂ O ₅	MgO
ICIE1	49.46	26.38	23.08	1.07	0
ICIE125MG	49.46	26.38	17.31	1.07	5.77
ICIE150MG	49.46	26.38	11.54	1.07	11.54
ICIE175MG	49.46	26.38	5.77	1.07	17.31
ICIE1100MG	49.46	26.38	0	1.07	23.08

Table 6: Magnesium substituted ICIE4 molar glass compositions (mol%).

	SiO ₂	Na ₂ O	CaO	P ₂ O ₅	MgO
ICIE4	49.46	6.60	42.87	1.07	0
ICIE425MG	49.46	6.60	32.15	1.07	10.72
ICIE450MG	49.46	6.60	21.44	1.07	21.44
ICIE475MG	49.46	6.60	10.72	1.07	32.15

As Tables 5 and 6 above show, the ICIE1 base glass is a high sodium containing calcium phosphosilicate glass. ICIE4, on the other hand is the equivalent low sodium containing glass. As the network connectivities of the two series are the same, when substituting magnesium into the two glass systems the resultant data should highlight the difference between a highly disruptive glass system, such as ICIE1 series, compared to a less disrupted glass system, such as the ICIE4 series. By comparing the two sets of data it was hypothesised that a greater understanding of magnesium's behaviour within soda lime phosphosilicate glasses, of differing levels of disorder, would result.

*Please note that 100% magnesium substituted glass in the ICIE4 series was unable to be cast as a glass.

5.3 Series 2 – Phosphorus Glasses

Phosphate is a component of many bioactive glasses. Its role within the glass structure is, however, highly ambiguous. This has resulted in a rather unclear and controversial position as to its effect on bioactivity. Phosphorus is, on the one hand, reported to increase bioactivity [107] and on the other have an adverse effect on bone bonding [138]. Phosphorus is a key element in all known forms of life. Inorganic phosphorus, in the form of the phosphate PO_4^{3-} , plays a major role in biological molecules such as DNA and RNA where it forms part of the structural framework of these molecules. Living cells also use phosphate to transport cellular energy in the form of adenosine triphosphate (ATP). An average adult human contains about 0.7 kg of phosphorus, about 85-90% of which is present in bones and teeth in the form of apatite, and the

remainder in soft tissues and extracellular fluids (~1%)[153]. In Hench's model [33, 107] phosphates play an important role in bioactivity, forming part of a silica and CaO-P₂O₅ rich bi-layer after leaching of Na⁺ and Ca²⁺ from the glass. This surface promotes the formation of hydroxycarbonate apatite (HCA), necessary for tissues to bond to the implant. However, this model does not predict the bioactivity of some glass compositions which do not contain phosphate [121] and also neglects the network connectivity of the glass [17] [86] [87]. The observed bioactivity of phosphorus-free compositions shows that apatite growth can also proceed by incorporating phosphate from the physiological solution [121] [139] [102] [154]. However, while not strictly necessary for bioactivity, the incorporation of a small P₂O₅ fraction in the glass does enhance its HA deposition and bone-bonding ability [121] [102] [130].

There are many suggestions as to why phosphorus is, or is not, important with respect to bioactivity. There has not, however, to the knowledge of the author, been a systematic study of phosphorus containing bioactive glasses. This has, therefore, been undertaken in this investigation - the objective, to gain an explicit understanding as to the actual structural role of phosphorus within bioactive glasses. Conventionally phosphorus is thought to enter the glass network, resulting in P-O-Si bonding; however Lockyer *et al.* [10] and Elgayar *et al.* [14] discuss, and have reported, NMR data supporting phosphorus residing in an orthophosphate environment. The assumption that phosphorus does indeed dwell within such an environment proved the basis for the investigation.

In order to finally clarify the role of phosphorus in a soda lime phosphosilicate glass, two series of glasses were designed –

- Firstly a simple straight molar substitution of P₂O₅ for 2SiO₂ was created on the grounds that the charge deficient orthophosphate species would remove charge balancing cations from their modifying roles in the silicate network and a less disrupted glass would be seen.

- The rationale behind the second series of glasses centred on the need for charge neutrality with respect to the orthophosphate complexes. Therefore the series was designed where the silica content was kept constant, however the NaO and Ca₂O content adjusted accordingly with the increasing phosphorus content, thus resulting in no change to the network connectivity.

Table 7 below tabulates the resulting glass compositions for both series of phosphorus glasses – the series I non-charge balanced phosphorus glasses and the series II charge balanced phosphorus glasses.

Table 7: Glass compositions (in mol%) from Series I (fixed Na₂O:CaO=1:0.87) and series II (charge balanced), showing molar mol%, network connectivity (NC) assuming P₂O₅ enters the glass network, modified network connectivity (NC') assuming isolated orthophosphate units.

	SiO ₂	Na ₂ O	CaO	P ₂ O ₅	NC	NC'
<i>Series I – Non-charge balanced</i>						
ICIE1	49.46	26.38	23.08	1.07	2.04	2.13
ICSW2	47.32	26.38	23.08	2.14	2.00	2.18
ICSW3	43.04	26.38	23.08	4.28	1.92	2.30
ICSW5	38.76	26.38	23.08	6.42	1.83	2.44
ICSW4	34.48	26.38	23.08	8.56	1.75	2.62
<i>Series II – Charge balanced</i>						
ICSW1	51.60	26.38	23.08	0.00	2.08	2.08
ICSW6	51.60	28.09	24.58	1.07	2.00	2.08
ICSW7	51.60	29.81	26.07	2.14	1.92	2.08
ICSW8	51.60	33.23	29.07	4.28	1.79	2.08
ICSW10	51.60	36.64	32.08	6.42	1.67	2.08
ICSW9	51.60	40.08	35.06	8.56	1.56	2.08

5.4 Glass Production

Batch calculations were performed to give 200g of final glass. All glasses were then made using Analar grade reagent chemicals (SiO_2 , P_2O_5 , CaCO_3 , Na_2CO_3 , and MgCO_3 from Sigma). To prepare each glass sodium carbonate, calcium carbonate/magnesium carbonate, silica and phosphorus pentoxide were weighed on electronic scales to the nearest 0.01 g and mixed vigorously in a plastic container. For the P_2O_5 containing glasses, phosphorus pentoxide was always added last due to its extremely hydrophilic nature.

The pre-mixed batches were then melted in platinum-rhodium or platinum-gold crucibles at 1390°C for one and a half hours. The molten glass was also agitated over the one and a half hours to assist the melting reaction. The melts were then rapidly quenched in cold water (rapid melt-quench technique) and the glass frit collected in a sieve and dried overnight at 120°C .

100g of the dried frit was then ground in a Gyro Mill (Glen Creston) for seven minutes and sieved for forty five minutes in a mechanical shaker to obtain $<45\mu\text{m}$ particles. The size of the charge and grinding time was kept constant in order to maintain control over the particle size distribution and in turn allow a confident comparison and analysis of results.

It is important to note that, before a systematic study of a glass series can be undertaken with any degree of scientific accuracy, one of the biggest problems to overcome is the consistency in the fabrication of the multicomponent glasses. Deceptive results can easily be produced if problems such as inhomogeneity, phase separation, incomplete melting, crystallisation or loss of volatile species are not appreciated and addressed [155]. Once dry, therefore, the frit was inspected visually for heterogeneities, crystallisation or amorphous phase separation with any glass batches with any visual sign of inconsistencies discarded or the visual appearance noted.

5.4.1 Glass Casting

Rods and discs were cast for each composition by re-melting the glass frit for half an hour at approximately 1390°C, in a bench top Carbolite electric furnace. The glass was then poured into a pre-heated graphite mould and annealed overnight at a temperature corresponding to the glass transition temperature of each glass.

Bars of approximately 25mm in length and 6mm in diameter were cut from the cast samples using a precision saw and diamond blade. In order to ensure the ends were parallel the samples were then ground using silicon carbide paper. The samples were then cleaned in an ultrasonic acetone bath and then air dried. The following observations in Table 8 were taken on the pouring of the glasses, proving helpful in determining the nature of each glass. It should be noted, not all samples could be cast in rod form for dilatometry due to devitrification (ICSW4) and/or phase separation (other samples).

Table 8: Melt observations in the magnesium and phosphorus series of glasses

Glass Sample	Melt Observations
ICIE1	Frit and cast block clear, flowed well
ICIE125MG	Frit and cast block clear, very fluid – had to wait before pouring
ICIE150MG	Frit and cast block clear, flowed well
ICIE175MG	Frit and cast block clear, very fluid
ICIE1100MG	Frit and cast block clear, flowed well
ICIE4	Frit and cast block clear, flowed well
ICIE425MG	Frit and cast block clear, flowed well
ICIE450MG	Frit and cast block clear, viscous
ICIE475MG	Frit and cast block milky and very viscous

ICSW1	Frit clear, UNABLE TO CAST
ICSW2	Frit and cast block clear, flowed well
ICSW3	Frit and cast block clear, flowed well
ICSW5	Frit and cast block milky in colour, however clear at the bottom of the crucible
ICSW4	Frit and cast block milky in colour
ICSW6	Frit clear, didn't flow too well, UNABLE TO CAST
ICSW7	Frit clear, UNABLE TO CAST
ICSW8	Frit and cast block clear, flowed well
ICSW10	Frit clear but opalescent at sides and bottom of crucible, Clear cast sample
ICSW9	Frit clear, UNABLE TO CAST

5.5 X-Ray Diffraction

A Phillips Powder Diffractometer with a copper (Cu K_α) x-ray source (Philips PW 1700 series diffractometer, Philips, Endhoven, NL) was used to characterise the non heat treated glass samples. The powder samples ($<45\mu\text{m}$ particle size) were scanned between $2\theta = 5-80^\circ$ with a step size of $2\theta = 0.04$, in order to try and determine the amorphous nature of each glass.

In order to try and verify the existence of any crystalline phases the frit was heat treated using a Centurion Quartz porcelain furnace, then ground to $<45\mu\text{m}$ particle size powder. DTA analysis was undertaken prior to heat treatment in order to determine the actual temperature at which the samples were to be heat treated - corresponding to the peak crystallisation temperature. The powder samples were heat treated to such a

temperature at a rate of 10°C/min, held for one hour, and then allowed to cool to room temperature. XRD was then carried out and the results analysed using software containing a database of standard diffraction files. Rietveld refinement was performed with GSAS and EXPGUI software.

5.6 Differential Thermal Analysis

The glasses were characterised by simultaneous DTA/TGA using a Stanton Redcroft DTA 1600. Alumina was used as the reference material and both reference and sample were contained within platinum-rhodium alloy crucibles. The <45µm particle size powders were heated from room temperature to 1300°C at a heating rate of 10°C/min in an inert Argon atmosphere. The glass transition temperature (T_g) and crystallisation temperatures (T_c) for each glass were then calculated from the corresponding traces, with T_g represented by a small negative change in slope of the baseline and the crystallisation temperature represented by the presence of an exotherm.

5.7 Dilatometry

A Netzsch Dil 402C dilatometer was used in order to determine the glass transition temperature (T_g), the dilatometric softening temperature (T_s) and the thermal expansion coefficient (α) for each glass. The 25mm x 6mm cut samples were analysed from room temperature up until the point just before melting, at a heating rate of 5°C/min. The α , T_s and T_g were determined by using system software and the α taken between 25°C and 400°C.

5.8 Density Measurements

Using the Archimedes principle, the density of each cast sample, at room temperature, was determined. The weight of each glass was measured in air and in distilled water, using a balance measuring to ± 0.1 mg. The weight was measured three times and an average taken in order to try and minimise the sources of error.

The sample density, ρ , was determined by the following equation –

$$\rho = \frac{m_d \times \rho_w}{m_d - m_s}$$

Equation 6: Density calculation

where m_d = mass of sample dry, ρ_w = density of water at the measurement temperature and m_s = mass of sample submerged in water. Care was taken to make sure that all of the cast samples were bubble free and that the glass samples were fully wetted when immersed in the distilled water.

5.9 Magic Angle Spinning Nuclear Magnetic Resonance

^{29}Si MAS NMR and ^{31}P MAS NMR were used to analyse the different glass samples using a Bruker DSX-200 NMR spectrometer operating at Larmor frequencies of 39.77MHz and 81.9MHz, respectively.

Samples were packed into a 5mm zirconia rotor and spun at the magic angle (54.7°) to remove anisotropy effects. The ^{29}Si and ^{31}P MAS NMR spectra of the glasses were recorded at spinning frequencies from 3-12 kHz using pulse acquisition of $\pi/2$ for silicon and $\pi/4$ for phosphorus.

The ^{29}Si and ^{31}P MAS NMR samples were all spun with a recycle/delay time set to generally 2 s (this however was increased to 60 s if the glass species were not thought to be completely relaxed).

All spectra were recorded at an ambient probe temperature with ^{29}Si referenced relative to polydimethylsilane (PDMS) and 85% H_3PO_4 for ^{31}P .

Natalia Karpukhina ran ^{25}Mg static NMR on the ICIE1 and ICIE4 magnesium series of glasses. Measurements were done at high field (18.8 T) on the 800MHz Bruker spectrometer at the resonance frequency of 49.0 MHz, using a static probe and 4 mm zirconia rotors. Hahn echo pulse program was adopted for the quadrupolar nuclei phase cycle [156]. Around 24k scans were acquired for each sample with 2 s recycle delay. A saturated solution of magnesium acetate was used for referencing the chemical shift scale.

5.10 Infrared and Raman Spectroscopy

5.10.1 FTIR

Information on the IR active Si-O groups present in the glasses was obtained using a Bruker IFS 28 Fourier transform infrared spectrometer in the mid-IR range of 550 to 2000cm^{-1} with a resolution of 0.2cm^{-1} . Peak analysis was performed using SciDAVis 0.1.3 software.

5.10.2 Raman

Raman spectroscopy is an adequate technique for the analysis of silica-based glasses because it can detect local changes in the environment of the Si-O-Si bonds [157]. The glasses were therefore analysed using a Fourier transform Raman spectrophotometer equipped with a 2W Nd: YAG laser ($1.06\mu\text{m}$). The spectra were collected using 380mW laser power, 520 scans and 4cm^{-1} resolution.

5.11 Dissolution Studies

A glass's bioactivity is generally measured by its ability to form a bone-like hydroxyl carbonate apatite layer on its surface, when implanted into the human body. Due to many practical and ethical issues *in vivo* studies are not usually utilised at such an early stage of bioactive analysis. Instead the dissolution of glasses in simulated body fluid is used as an alternative to the more invasive *in vivo* studies and is a relatively good indicator as to bioactivity. Although *in vitro* studies only simulate the ionic composition of physiological fluids and therefore the inorganic reactions taking place *in vivo*, it is

commonly accepted that the rate of apatite layer formation is related to *in vivo* bioactivity. Glass powdered samples were therefore soaked in simulated body fluid (SBF) in order to estimate the rate of glass dissolution. Simulated body fluid was prepared according to Kokubo *et al.* [158]. The reagents are tabulated in Figure 14 in Chapter 4 and were added in order to make up a litre of SBF. 0.15g of each glass (of diameter <45µm) were immersed in 150ml of SBF solution, in sealed containers, for up to 21 days and were mechanically agitated at 60rpm using a vibrating plate incubator set to 37°C. At various time points (15 minutes, 1 hour, 4 hours, 16 hours, 24 hours, 48 hours, 1 week and 21 days) the samples were filtered and dried to constant weight for analysis by FTIR spectroscopy, Raman spectroscopy and XRD. The pH of the remaining solution was also measured.

6 Results and Discussion – Phosphorus Glasses

6.1 ^{31}P MAS NMR

6.1.1 ^{31}P MAS NMR of Series I Phosphorus Glasses

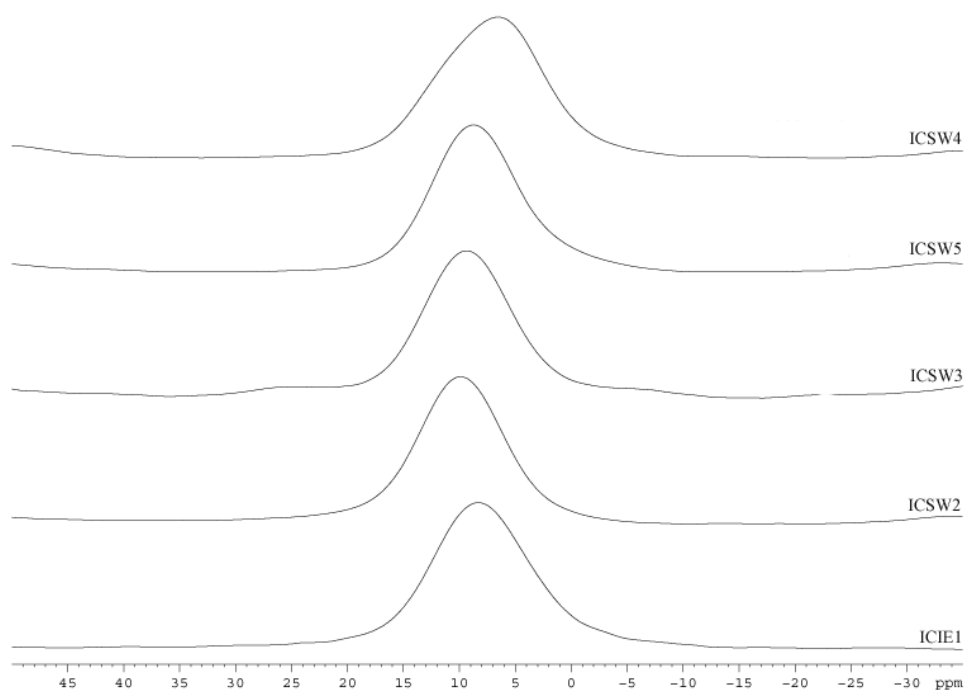


Figure 16: ^{31}P MAS NMR spectra (Chemical shift on x axis) for series I (fixed $\text{Na}_2\text{O}:\text{CaO}$) glasses. Increasing phosphate content from bottom to top. Top spectra (ICSW4) show distinct peak asymmetry.

Figure 16 illustrates the ^{31}P MAS-NMR spectra for the series I phosphorus substituted (fixed $\text{Na}_2\text{O}:\text{CaO}$) glasses. Table 9 also summarises the peak positions and full width half maximums (FWHM) of ^{31}P spectra for these glasses.

All spectra illustrate a symmetric peak with a chemical shift range of *ca.* 7 to 10ppm, with an approximate constant linewidth *ca.* 9ppm.

Table 9: ^{31}P MAS NMR peak positions and full width half maximums (FWHM) for glasses in series I.

Glass	^{31}P peak (ppm)	^{31}P FWHM (ppm)
ICIE1	8.4	10.9
ICSW2	10.0	9.0
ICSW3	9.5	9.0
ICSW5	9.0	9.2
ICSW4	7.0	9.9

These linewidths and chemical shift values correspond to phosphorus in an orthophosphate environment (PO_4^{3-}).

Conventionally phosphorus is thought to enter the glass network. The ^{31}P MAS NMR data, however, corresponds to an orthophosphate structure, therefore indicating that phosphorus is associated with removing sodium and calcium ions. These cations must come from their modifying roles in the silicate network therefore changing the Q^n structure of the silicate. These results agree with those of Lockyer *et al.* [10], Elgayar *et al.* [14] and Turner *et al.* [159], where a single resonance with a line width consistent with an orthophosphate like environment was reported.

Figure 16 and Table 9 show that the ^{31}P MAS NMR chemical shifts for the glasses all lie between those for a sodium orthophosphate at *ca.* 16ppm and a calcium orthophosphate at *ca.* 3.0-0.0ppm. This suggests, therefore, that the phosphorus is present as a mixed sodium calcium orthophosphate. Figure 17 highlights the relationship between the chemical shifts of these mixed sodium calcium

orthophosphates and the percentage of phosphorus substituted, and Figure 18 also illustrates the relationship with modified network connectivity (NC'). The original network connectivity is calculated using the equation 7 below, assuming P₂O₅ enters the glass network.

$$NC = 2 + \frac{[(2 \times SiO_2) + (2 \times P_2O_5)] - [(2 \times CaO) + (2 \times Na_2O)]}{SiO_2 + (2 \times P_2O_5)}$$

Equation 7: Original network connectivity calculation (calculated using the mole percent values for each glass) assuming phosphorus enters the glass network.

Equation 8 below shows the modified network connectivity (NC'), as plotted in Figure 18. The modified network connectivity, NC', was calculated assuming P₂O₅ is present as orthophosphate species, with cations such as Na⁺ and Ca²⁺ required to maintain a charge balance, hence increasing the connectivity of the silicate network.

$$NC' = 2 + \frac{(2 \times SiO_2) - [(2 \times CaO) + (2 \times Na_2O) + (2 \times P_2O_5 \times 3)]}{SiO_2}$$

Equation 8: Modified network connectivity calculation (calculated using the mole percent values for each glass), assuming phosphorus is present as an orthophosphate species.

Figure 23 shows the difference in NC and NC' for series I, and the actual NC calculated from the peak fitting which will be discussed below.

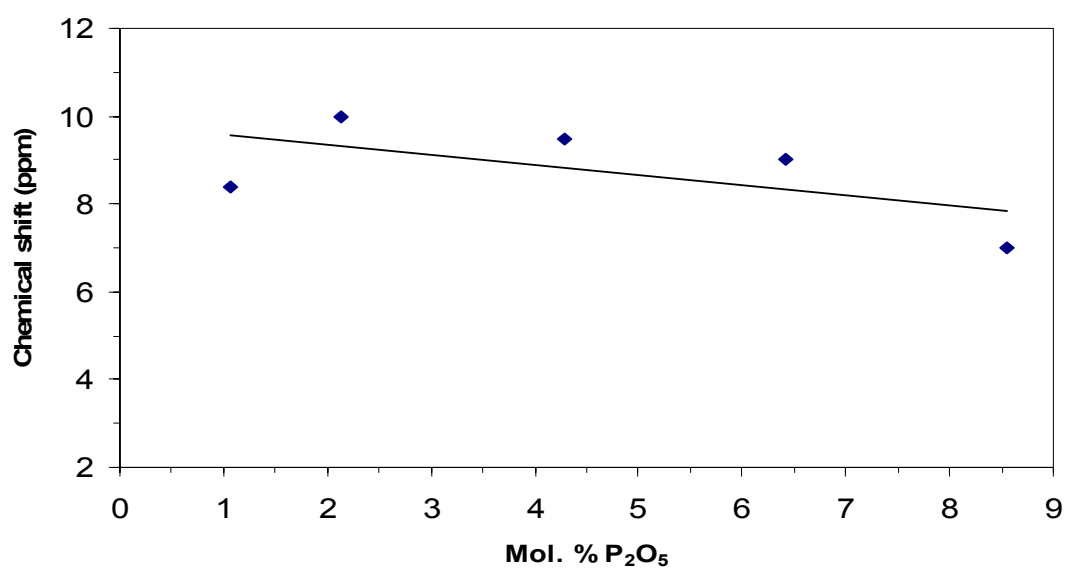


Figure 17: ^{31}P MAS NMR chemical shift values for series I glasses with phosphate mole ratio.

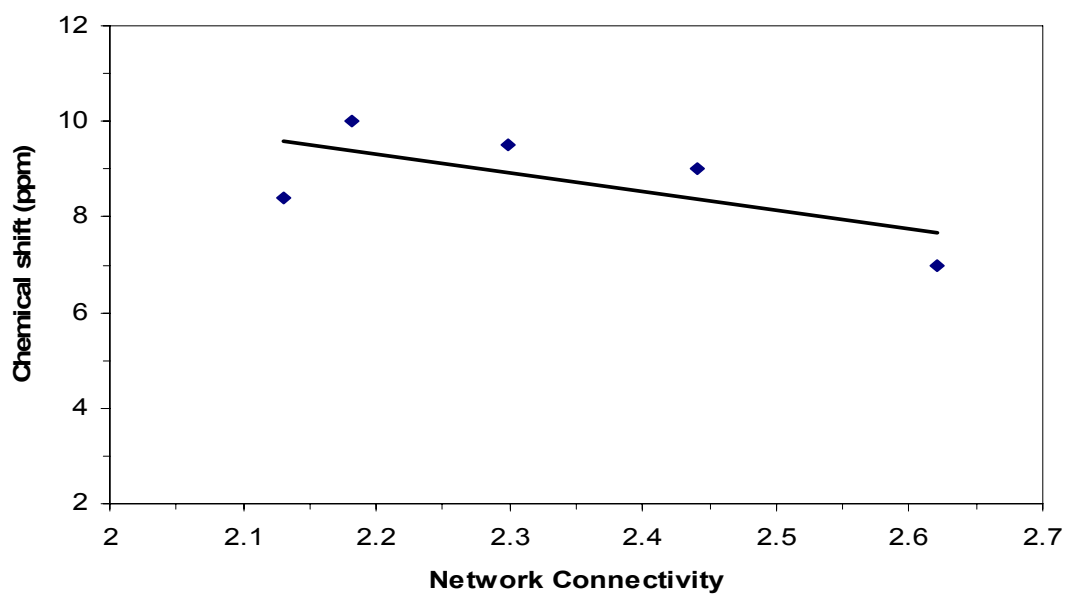


Figure 18: ^{31}P MAS NMR chemical shift values for series I glasses with network connectivity values assuming phosphorus in an orthophosphate environment.

Figure 18 shows that, as P_2O_5 is added (and NC' increases), and the proportion of Q^3 (SiO_4 tetrahedra with three bridging oxygens and one non-bridging oxygen) species

increases, there is a *very slight* shift upfield of the ^{31}P peak to smaller ppm values, corresponding to that of a calcium orthophosphate environment. This could suggest that, as the glass moves towards a Q^3 structure, calcium starts to preferentially associate with the phosphate phase. It should be noted, however, that this trend is in no way distinct or definite and within experimental error it may be more relevant to assume no preferential association of the cations within the phases, as discussed by Lockyer *et al.* [10]. Grussaute *et al.* [160] also observe this phenomenon in similar quaternary $\text{Na}_2\text{O}-\text{CaO}-\text{P}_2\text{O}_5-\text{SiO}_2$ glasses, with increasing P_2O_5 content, stating that each phosphorus nucleus is surrounded by the same number of Na and Ca cations, rather than segregation into stoichiometric compounds.

In this series of glasses the $\text{CaO}:\text{Na}_2\text{O}$ ratio is 23.08:26.38 or 1:1.14. If there is no preferential association within the phosphate phase it would be expected that the ratio of Na:Ca orthophosphate would be consistent with that of the overall glass composition. This would give a chemical shift for a 1:1 glass at *ca.* 9ppm. In this series of glasses with a 1.00/1.14 ratio of $\text{CaO}:\text{Na}_2\text{O}$, chemical shifts of *ca.* 10.3ppm would therefore be expected. It is evident from the experimentally observed chemical shifts (7 to 10ppm) that there is some kind of phenomenon in the structure effecting the values, excluding experimental error.

On closer inspection of the ICSW4 spectra (the highest phosphate containing glass) a slight asymmetry is apparent. The NC' (2.62) of this glass falls into the regime of glasses reported by Grussaute *et al.* [160]. Grussaute's glasses were calculated to have NC' values greater than 2.5 and their corresponding ^{31}P MAS NMR spectra illustrated the presence of pyrophosphate as well as orthophosphate type species. From the XRD data discussed below it is evident that this ICSW4 glass is partially crystalline. There is, however, no corresponding sharpening of the spectrum. This possibly implies that the magnetization of the crystalline phase was not fully relaxed in the NMR experiment. The sample was run with a longer delay time (60 seconds – not shown) but no sharpening of the phosphate resonance was observed. This then necessitated the heat treatment of the glass and a longer delay time to try and grow and determine the

crystalline phase(s) present. The resulting spectrum, at a delay time of 2 seconds, is shown in Figure 19.

To further refine the peaks, the heat treated sample was then run with a 60 second delay time (top spectra of Figure 19). A sharp peak at *ca.* 3ppm is evident – the sharpness suggests a crystalline phase as a result of the highly ordered environment and its chemical shift suggest a calcium orthophosphate. Up field from this peak is a broader peak consisting of three overlapping resonances peaking at *ca.* 8.5ppm, 10.5ppm and 14ppm. These resonances are difficult to assign to specific structures due to the overlapping regions of chemical shifts.

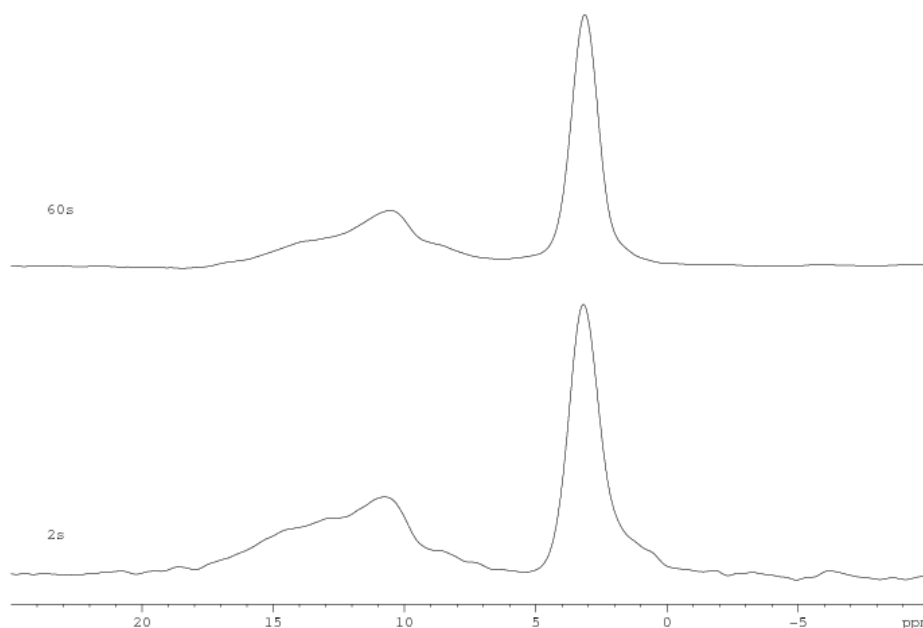


Figure 19: ^{31}P MAS NMR spectra for the ICSW4 glass heat treated at around 350°C , delay time 2 s (bottom) and 60 s (top).

However, if previous data on $\text{SiO}_2\text{-Na}_2\text{O-CaO-P}_2\text{O}_5$ glasses [10, 160] is consulted, the presence of a pyrophosphate species is reported. Lockyer *et al.* [10] assigned two resonances at 13.8 ppm and 2.4 ppm to a sodium orthophosphate like species and a

sodium pyrophosphate like species, respectively. Similarly, Grusssaute *et al.* [160] attributed two well resolved resonances with chemical shifts of 15.5 ppm and 2.9 ppm to Na_3PO_4 and $\text{Na}_4\text{P}_2\text{O}_7$, respectively. Therefore, it is important to consider that the experimentally observed resonance at 3ppm may be as a result of a sodium pyrophosphate type species rather than a calcium orthophosphate type species. Further NMR analysis would first have to be undertaken before an accurate determination of the resonances in the ICSW4 crystalline sample can be assigned. It is important to determine the types of phases present within the crystalline ICSW4 structure as these can usually be closely related to the structure of the parent amorphous glass. This would then help determine whether there are any preferential associations within the phase separated glass structures (i.e. a preference for calcium orthophosphate) or whether the move in chemical shifts, with the addition of phosphorus, implies a preference for the formation of pyrophosphate type species.

6.1.2 ^{31}P MAS NMR of Series II Phosphorus Glasses

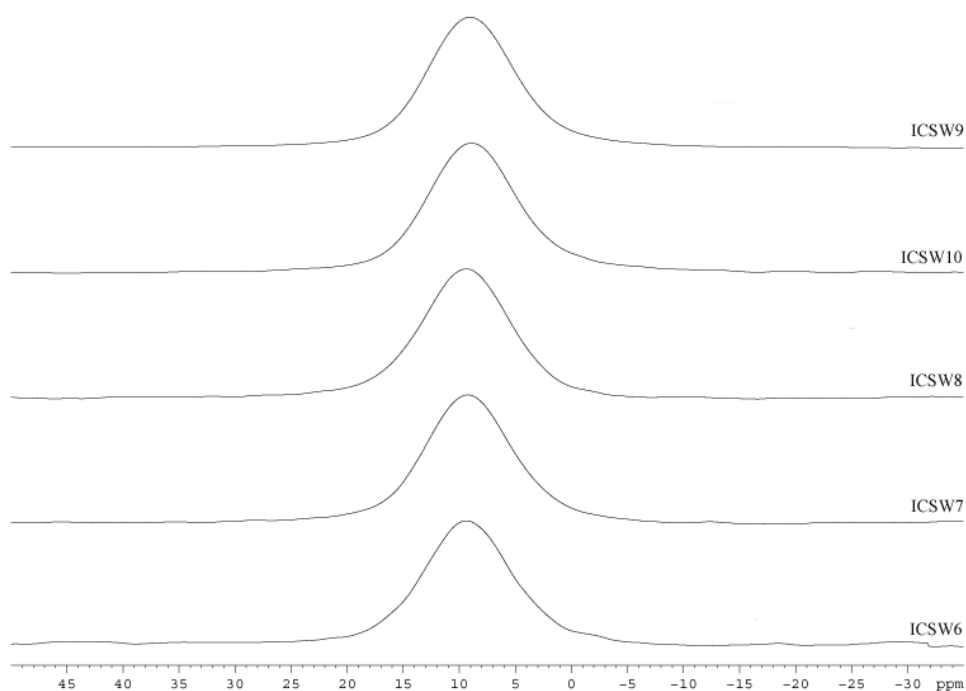


Figure 20: ^{31}P MAS NMR spectra for series II (charge balanced) glasses. Increasing phosphate content from bottom to top.

Figure 20 illustrates the ^{31}P MAS NMR spectra for series II phosphorus glasses, where sodium and calcium have been introduced as charge compensators for the phosphate complex, thus obviating network polymerisation. Table 10 tabulates the the peak positions and full width half maximums (FWHM) of ^{31}P spectra for these glasses.

Table 10: ^{31}P MAS NMR peak positions and full width half maximums (FWHM) for glasses in series II.

Glass	^{31}P peak (ppm)	^{31}P FWHM (ppm)
ICSW6	10.7	9.2
ICSW7	10.9	9.2
ICSW8	10.5	9.5
ICSW10	10.5	9.2
ICSW9	10.9	9.2

All the spectra appear identical, within experimental error, therefore confirming that incorporating phosphate has a minimal effect on the silicate network polymerisation [160]. It can be seen from Table 10 that all the spectra have a single resonance at *ca.* 10.5ppm with a FWHM (9ppm) consistent with that of an orthophosphate environment. As discussed previously, if there is no preferential association of the Na^+ and Ca^{2+} cations with the phosphate phase then the ratio would then be consistent with that of the original glass composition. As previously calculated, this would then result in a resonance of *ca.* 10.3ppm in the ^{31}P spectra of the glasses. This is in very good agreement with the single peak experimentally observed in series II. This implies that the observed peaks can be assigned to a mixed sodium calcium orthophosphate species with no preferential association of the cations with the orthophosphate.

6.2 ^{29}Si MAS NMR

6.2.1 ^{29}Si MAS NMR of Series I Phosphorus Glasses

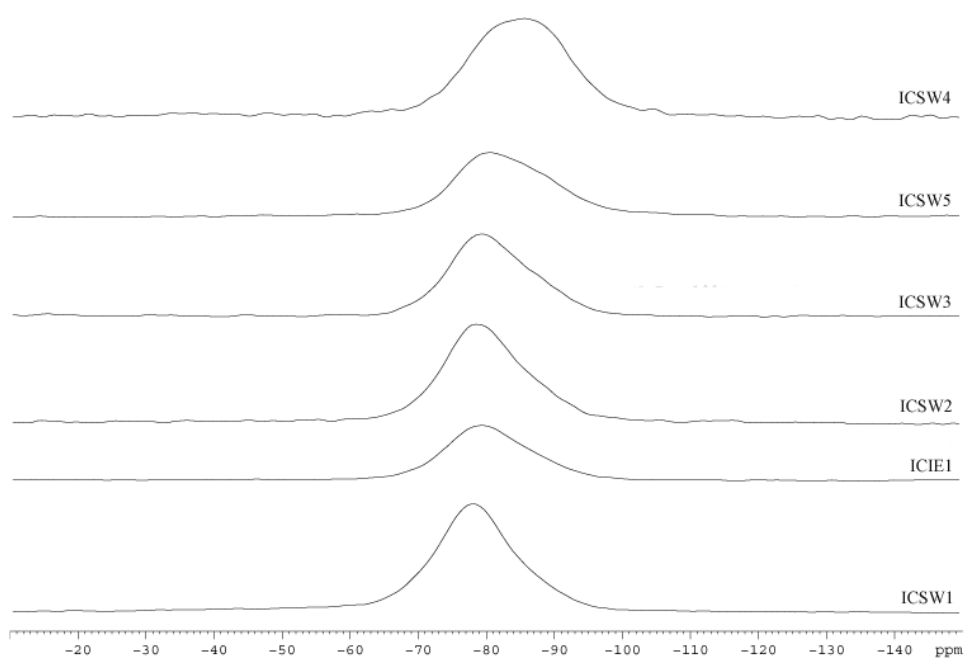


Figure 21: ^{29}Si MAS NMR spectra for series I (fixed $\text{Na}_2\text{O}:\text{CaO}$) glasses. Phosphate content increases for bottom to top.

^{29}Si MAS-NMR spectroscopy was carried out on the first series of ICSW phosphorus glasses as shown in Figure 21 above.

If the spectrum is observed for ICSW1, a $\text{SiO}_2\text{-CaO-Na}_2\text{O}$ glass with no phosphorus present, a highly symmetrical single resonance centred on *ca.* -78 ppm can be seen.

As the phosphorus content is increased in the $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$ glass series, the peak asymmetry becomes evident. For other than the phosphate free glass it can be seen that the spectra are a manifestation of 2 overlapping resonances centred on *ca.* -78ppm and *ca.* -86ppm. Such overlaps are as a result of the amorphous nature of the

samples, i.e. the variations in structural features within the glass, such as variations in O-Si-O bond angles and distances [161-167]. The upfield shift from *ca.* -78 ppm to *ca.* -86 ppm indicates changes in the environment around the silicon atom as phosphorus is introduced into the glass. From these shift values and data on ^{29}Si shifts in relation to Q^n structure and connectivity, the resonance at *ca.* -78 ppm can be assigned to a Q^2 species and the resonance at *ca.* -86 ppm can be assigned to a Q^3 species. The linewidths measured at half height are much larger than the expected chemical shift differences for Q^n and Q^{n+1} species. It should also be noted the absence of any peaks around -200ppm indicating that no six co-ordinated silicon exist. The peaks are well within the chemical shift range of silicon in a four co-ordinate state.

Table 11 tabulates the peak positions and linewidths for the series I glasses.

Table 11: ^{29}Si MAS NMR peak positions and full width half maximums (FWHM) for glasses in series I.

Glass	^{29}Si peak (ppm)	^{29}Si FWHM (ppm)
ICIE1	-78.0	13.0
ICSW2	-79.0	17.2
ICSW3	-78.5	14.9
ICSW5	-80.0	14.7
ICSW4	-81.0	17.5

The peak assigned to the Q^3 species increases intensity with P_2O_5 addition. The experimental findings, that on increasing phosphate content the glass moves from a Q^2 to a Q^3 structure support the hypothesis that phosphorus acts as a network polymerising agent on the silicate network. This is explained by the addition of the PO_4^{3-} complex into the glass requiring cations such as Na and Ca to charge balance, thus removing them from their network modifying role within the silicate network. This therefore decreases the amount of NBOs to give a more cross-linked silicate structure and hence the move towards a Q^3 glass. This is supported by the network connectivity

calculations where it is assumed phosphorus is in an orthophosphate environment (and not part of the silicate network as previously thought).

Figure 22 below highlights the chemical shift values with respect to the percentage of phosphorus content.

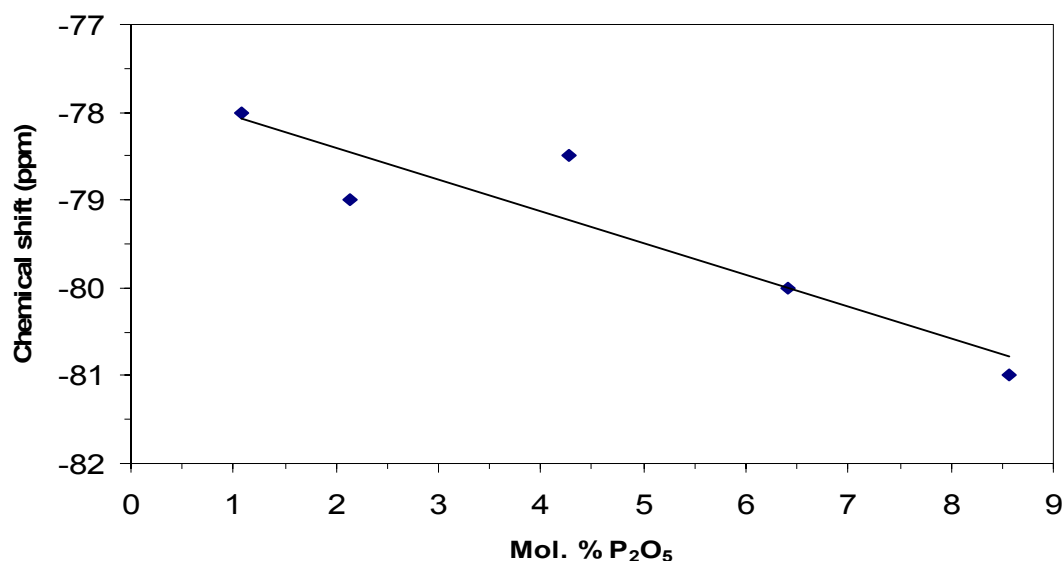


Figure 22: ^{29}Si MAS NMR chemical shift values versus phosphate content in series I glasses.

Figure 23 below illustrates the difference in NC and NC' for series I, and the actual NC calculated from the values of Q^2 and Q^3 proportions from Gaussian peak fitting of the ^{29}Si MAS NMR data for series I. A simple calculation shows that if around 2/3 of the phosphate was present in a separate orthophosphate phase and the remaining 1/3 in the glass network, a good fit can be reached with the ^{29}Si MAS NMR data. However, in reality the glass structure is likely to be much more complex with various Q^n , orthophosphate and pyrophosphate species, preferential segregation of modifiers as well as crystalline components. Further NMR studies (e.g. ^{17}O) are therefore needed to obtain a more complete picture.

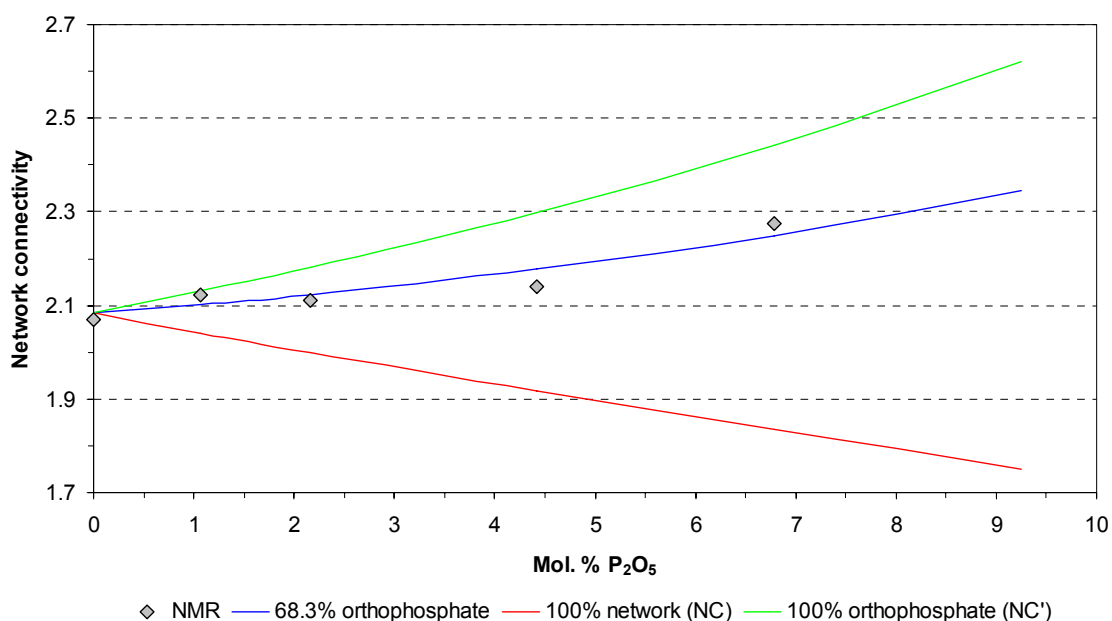


Figure 23: Difference in NC and NC' for glasses in series I and data from NMR fitting.

From the XRD results, it is known that ICSW4 is in fact partially crystalline. It should be noted however that running the as-cast sample for a longer delay time (60s, not shown) did not result in any significant sharpening of the silicon resonance. The powder XRD of the heat treated sample indicates three phases: the six membered ring combeite, sodium-calcium orthophosphate and sodium pyrophosphate. A much sharper peak, or peaks would have been expected, due to the ordering of the structure, as a result of the crystalline nature, but this was not observed. A possible explanation of the lack of observed crystallinity may be due to the much longer spin lattice relaxation time, T_1 , of the crystal phase. At short time delays (2s, not shown) the ^{29}Si within the crystalline phases would be saturated and not detectable. As crystallinity had already been determined from the powder XRD, the heat treated sample was re-run with a longer delay time (60s) to try and pick up the crystalline phase – Figure 24 .

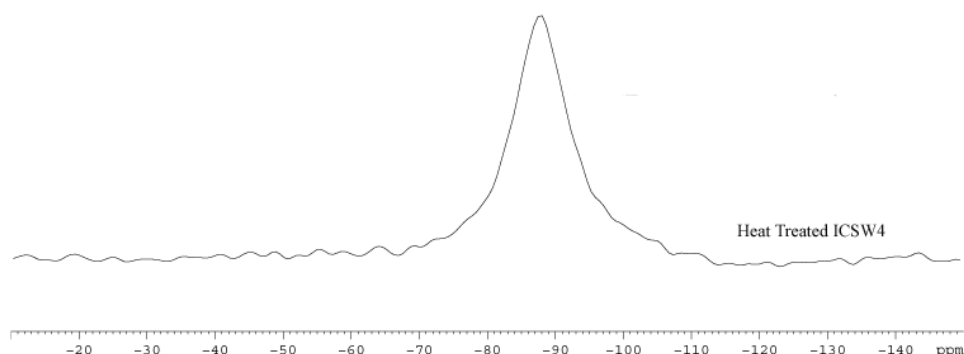


Figure 24: ^{29}Si MAS NMR spectrum for the heat treated (650°C) ICSW4 glass sample, delay time=60 seconds.

From this spectrum it can be seen that the peak has sharpened but still not to a great extent. The peak maximum is at *ca.* -88ppm. This chemical shift value is rather low for a Q^2 silicon crystal phase but this structure would be expected for this series of glasses. Wollastonite (CaSiO_2) and β -wollastonite exhibit chemical shift values in this region [168] [169] and have been reported to crystallise in bioglass based compositions [170]. However, the XRD spectrum showed the presence of combeite ($\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$) [171]. The peak is therefore assigned to combeite. It is suggested that it would be beneficial to run this sample with a longer delay time than 60 seconds to try and resolve further the peak and gather more accurate information – this however would prove very difficult as the run time would extend to a number of days.

6.2.2 ^{29}Si MAS NMR of Series II Phosphorus Glasses

^{29}Si MAS NMR was carried out on the series II glasses and the resulting spectra are shown in Figure 25.

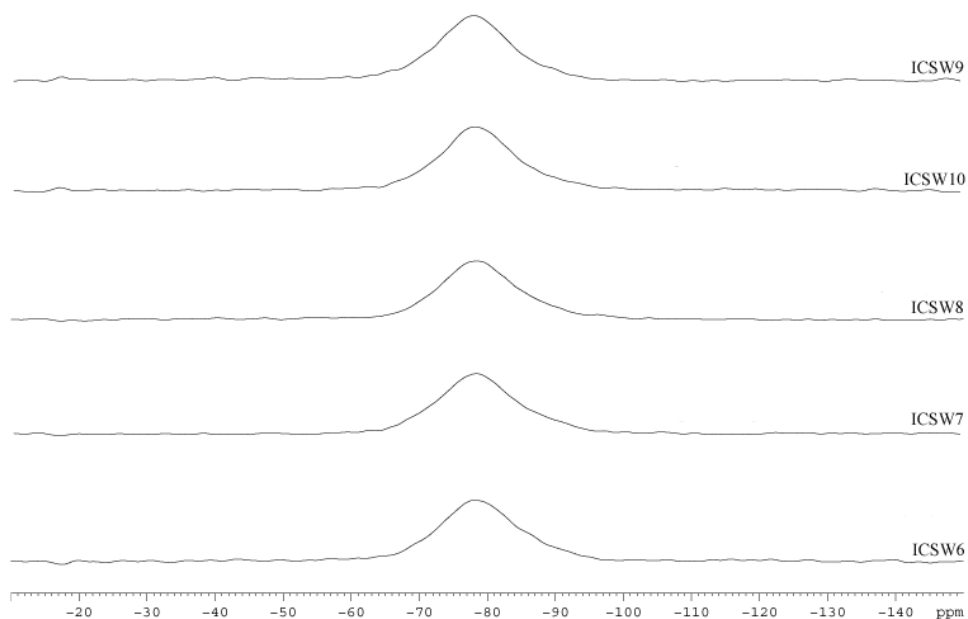


Figure 25: ^{29}Si MAS NMR spectra for series II glasses.

Figure 25 shows that all the spectra are highly symmetrical with a single resonance centred on -78ppm with linewidths between *ca.* 12ppm and *ca.* 13ppm. Even within experimental error, there is no shift of any of the spectra and certainly no presence of any shoulders.

Table 12 tabulates the the peak positions and full width half maximums (FWHM) of ^{29}Si spectra for these glasses.

Table 12: ^{29}Si MAS NMR peak positions and full width half maximums (FWHM) for glasses in series.

Glass	^{29}Si peak (ppm)	^{29}Si FWHM (ppm)
ICSW6	-78.0	13.2
ICSW7	-78.0	13.0
ICSW8	-78.0	12.7
ICSW10	-78.0	11.8
ICSW9	-78.0	12.7

It can be concluded that all the glasses in the series are Q^2 in nature, thus agreeing with the constant NC' value of all the glasses - 2.08. It is also further evidence to support that phosphorus exists within an orthophosphate environment.

In the series II glasses, the silica content remains the same while the sodium and calcium contents increase in order to charge compensate the phosphorus species being added. This therefore removes the need for any modifying ions already associated within the silicate network to charge compensate the PO_4^{3-} species, therefore keeping the number of NBOs attached to silicon constant and the silicate network unaffected.

The single Q^2 resonance at *ca.* -78ppm suggests that the NBOs are preferentially bonded to sodium as a Q^2 sodium silicate exhibits a resonance around -77ppm and Q^2 calcium silicates occur in the -92 to -83ppm region [168] [169].

6.3 Summary of MAS NMR

^{29}Si MAS-NMR spectra of the series I phosphorus glasses consisted of a progression of asymmetry through the series – a result of two overlapping resonances assigned to Q^2 and Q^3 species, with the latter increasing with P_2O_5 addition. The asymmetric peak shift in the ^{29}Si spectra towards lower frequencies, as the phosphate content increased, is attributed to an increase in the average degree of polymerisation of the silicate network.

This, therefore, implies the network polymerising role of phosphorus, and the existence of an orthophosphate species. There is no evidence of any disruption of the glass network from the ^{29}Si MAS-NMR spectra thus eliminating the idea of phosphorus acting as a former and entering the silicate glass network. ^{31}P MAS-NMR spectra clearly illustrates single resonances for all spectra centred on chemical shift values associated with those of an orthophosphate environment. This is direct evidence of the existence of phosphorus within such an environment, rather than the conventional view of phosphorus entering the network. A slight shift to smaller chemical shift values is observed through the series. This shift can either be assigned to the preferential association of calcium with the orthophosphate type species or alternatively the formation of a pyrophosphate type species. Further analysis would have to be undertaken before a definitive conclusion can be made.

^{29}Si MAS-NMR spectra of the charge balanced phosphorus glasses resulted in a series of single resonances all centred on a chemical shift associated with a Q^2 glass. No evidence of any shoulders or peak shift is seen, therefore concluding that, on addition of phosphorus, the silicate network remains unchanged as a result of the addition of charge compensating cations. The ^{31}P spectra also consisted of a series of single resonances all centred on a chemical shift associated with that of an orthophosphate environment.

It can, therefore, be concluded, from both series, that within the test glasses phosphorus exists as an orthophosphate complex, rather than taking part in or entering the silicate glass network, as traditionally thought. The design of the two series, based upon such an assumption results in a definitive and clear cut set of data. The evidence for the existence of phosphorus within an orthophosphate environment and therefore the possibility of phase separation into phosphate rich clusters has significant implications with respect to the bioactive properties of the glass. It is suggested that the orthophosphate rich phase will very likely be extremely soluble and when exposed to water, dissolve much more rapidly than the silicate phase, effectively increasing the level of phosphorus super saturation of the external environment. This would then result in an increase of the rate of apatite deposition on the glass surface [13]. However,

it should not be neglected that the resultant polymerisation of the silicate network points towards a less degradable bioactive glass and would, therefore, serve to reduce bioactivity. The effect of phosphorus on bioactivity therefore is a necessary and essential extension to this investigation and is discussed further on in this study.

6.4 FTIR Spectroscopy

Infrared Spectroscopy is one of the most powerful tools for identifying types of chemical bonding within a structure and has been at the forefront of materials analysis for over 70 years. All molecular bonds vibrate. The frequency of this vibration is dependent on the type of elements present and the type of bonds between these elements. For any given bond, there is a number of specific frequencies at which it can vibrate. For example, complex molecules, such as CH_2 , can vibrate in many different ways, including symmetrical stretch, asymmetrical stretch, scissor, rock, wagg and twist.

An infrared spectrum represents a fingerprint of a sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material has a certain unique combination of atoms and bonding, no two compounds will produce the same IR spectrum. IR spectroscopy is therefore used as a positive identification of a compound [172].

Figures 26 and 27 illustrate the infrared absorption spectra of the individual glasses in the series I, non-charged balanced, phosphorus series and series II, charge balanced, phosphorus series, respectively.

Infrared spectroscopy is extremely accurate in picking up vibrations associated with non-bridging oxygen and silicon interactions (Si-O-NBO bonding) [173] -

1. The Si-O(s) with one non-bridging oxygen (1 NBO) per SiO_4 tetrahedra (i.e. Q^3 bonding) is located in the range of $1020\text{-}1052\text{cm}^{-1}$ (illustrated by dashed line)

2. The Si-O-2NBO species (i.e. Q^2 bonding) is located at *ca.* 920-960 cm^{-1} (illustrated by dotted line)

Figure 26 shows that, for the non-charge balanced series, there is an evolution of the peak associated with Q^3 species (around 1020-1052 cm^{-1} – dashed line) as we move through the series and phosphorus is added. This is further evidence to support the MAS NMR results which illustrate that as phosphorus is added, more Q^3 species form within the glass. This is as a result of the formation of charge deficient orthophosphate species. The charge modifiers are removed from their roles in the silicate structure to charge balance the PO_4^{3-} type species. The effect is a change in polymerisation of the silicate network with a move from a predominantly Q^2 glass to a predominantly Q^3 glass.

If we compare the infrared spectra for the non-charge balanced series (Figure 27) with those of the charge balanced series we can see that this effect, i.e. the polymerisation of the network through the series, is not seen. There is no obvious evolution of the peak associated with Q^3 silicate species. The peak heights remain constant, within experimental error, throughout the series. This is analogous with the previous NMR data and network connectivity data, which also show no change in the silicate network polymerisation as phosphorus is added through the series. As discussed before, this is explained by the fact that as phosphorus is added, charge balancing sodium and calcium ions are also added to accommodate for the formation of the charge deficient orthophosphate type species. As calcium and sodium have been added to account for this phenomenon there is no need for the original charge modifiers to be removed from their role in the silicate network. Hence no change is seen in Q structure of the silicate network.

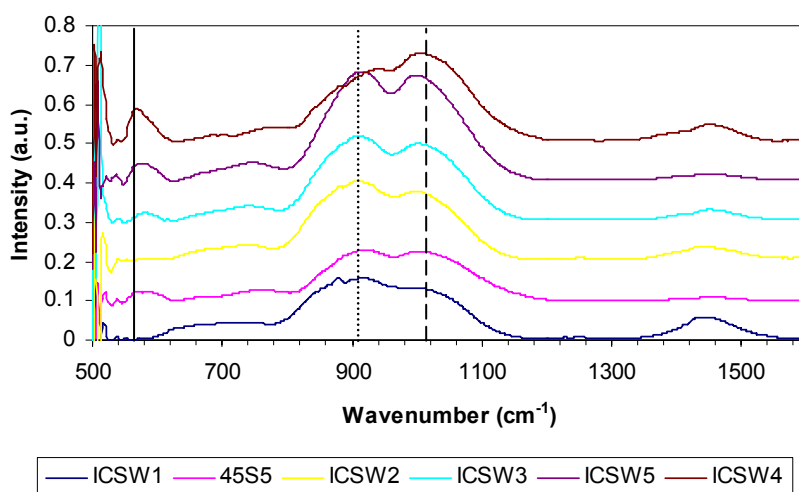


Figure 26: FTIR spectra for series I non-charge balanced phosphorus series. Increasing phosphate content from bottom to top.

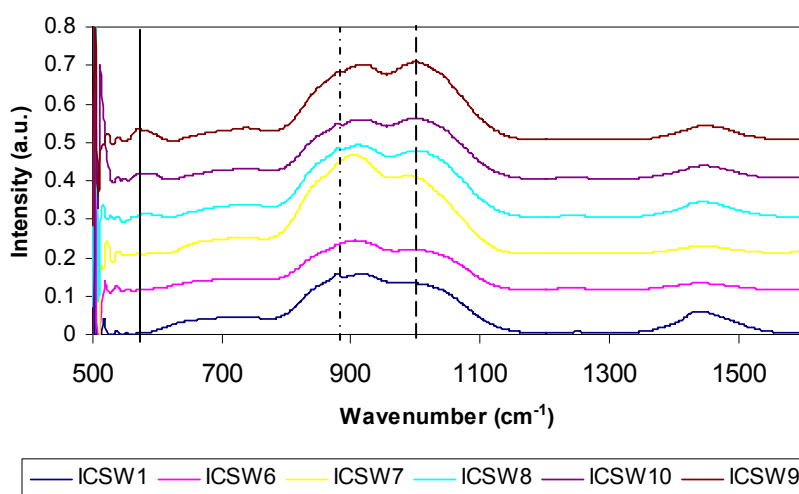


Figure 27: FTIR spectra for series II charge balanced phosphorus series. Increasing phosphate content from bottom to top.

Further examination of Figures 26 and 27 illustrates that both series have a peak in the region of 520 to 600 cm^{-1} , at approximately 580 cm^{-1} (illustrated by block line). As phosphorus is added through the non-charge balanced series, it is evident that the peak height increases. The same phenomenon is seen in the charge balanced series, however to a much lesser extent. There are four vibrational modes theoretically present for phosphate ions – ν_1 , ν_2 , ν_3 and ν_4 . The evolution of the peak in the region of 520 to 600 cm^{-1} can thus be assigned to the formation of PO_4^{3-} orthophosphate type species within the glass

and corresponds to the O-P-O antisymmetric angle bending mode. There is, however, a Si-O-Si bending vibration in the same region [174] [175] [154] [176]. It is interesting to note the difference between the two series of glasses. The evolution of the peak in the 520-600 cm^{-1} regions is extremely prominent in the non-charge balanced series and although evident in the charge balanced series, is not so prominent. No explanation can be thought of as to why the non charge balanced series display a more prominent increase in phosphorus peak height in comparison to the charge balanced series.

6.4.1 Summary of FTIR for phosphorus series of glasses

- FTIR analysis illustrated the evolution of the peak associated with Q^3 species, and accompanying decrease in the peak height for the Q^2 silicate species, through the non charge balanced series of glasses.
- This phenomenon was not observed in the spectra for the charge balanced series of glasses, where the peaks associated with the Q^2 and Q^3 silicate species remained relatively unchanged through the series.
- This suggests that the non charge balanced series is becoming more cross-linked with the addition of phosphorus while the Q structure is unaffected with the addition of phosphorus in the charge balanced series of glasses.
- It can, therefore, be concluded, from both series, that within the test glasses phosphorus exists as an orthophosphate complex, rather than taking part in, or entering, the silicate glass network.

6.5 Raman Spectroscopy

Figure 28 below, shows the Raman spectra for series I, non charge balanced phosphorus glasses. It is evident that the spectra are near enough featureless as with Figure 29 below, for the charge balanced series of glasses. It is unsure why the spectra are so poor.

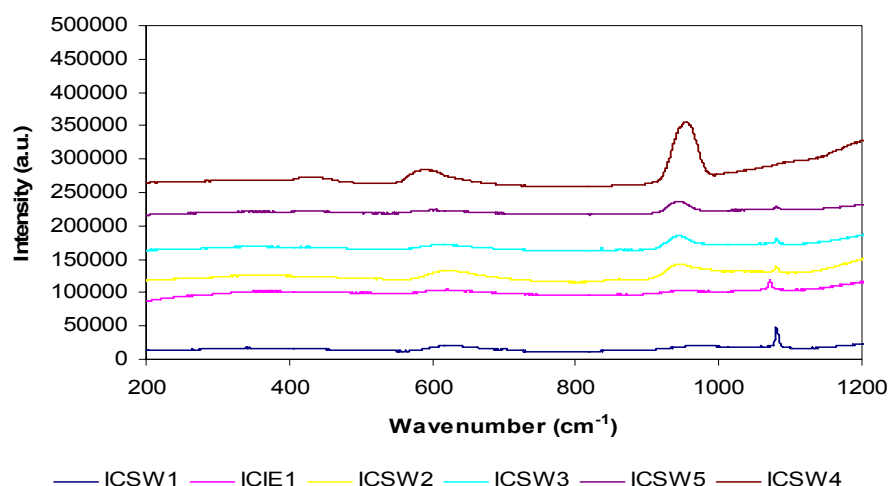


Figure 28: Raman spectra for series I, non charge balanced, phosphorus glasses. Increasing phosphate content from bottom to top.

Due to the quality of the spectra, only a menial analysis of the spectra can be undertaken. On close inspection, however, it can be seen that the bands associated with Si-O-Si bending, Si-O-NBO and Si-O-Si stretching are present at approximately 600cm^{-1} , 970cm^{-1} and 1080cm^{-1} , respectively [84] [157].

Also it is evident that these bands do not remain the same with respect to intensity and broadness as phosphorus is added and the network connectivity increases. Thus it can be concluded that the addition of phosphorus promotes a local distortion in molecular symmetry.

Figure 29 below also has the same characteristic bands associated with Si-O-Si bending, stretching and Si-O-NBO. Interestingly, these bands seem to be, within experimental error, very similar with respect to intensity and broadness as phosphorus is added. This suggests that there is little distortion of the silicate network. This is in contrast to the non-charge balanced series.

Therefore, it can be concluded that, although the spectra are of poor quality, we can gather some basic information on the form of the silicate network in the two series of glasses. The non-charge balanced series of glasses experience a distortion in the silicate network as phosphorus is added. In contrast, the charge balanced series experience very little distortion of the silicate network. These results agree with the NMR and FTIR data (and of course the actual design of the glasses) and are further evidence to support the changing network connectivity in the non-charge balanced series and the constant network connectivity in the charge balanced phosphorus series.

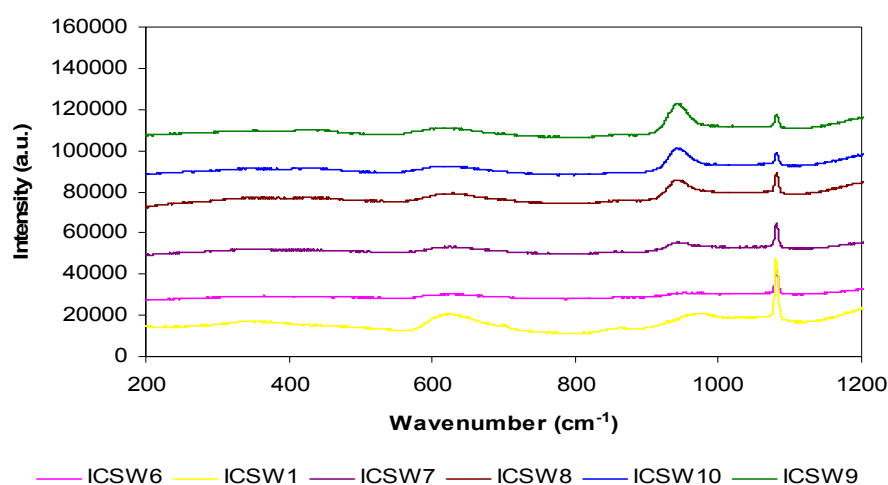


Figure 29: Raman spectra for series II, charge balanced, phosphorus glasses. Increasing phosphate content from bottom to top.

6.6 X-Ray Diffraction

XRD is a limited technique for amorphous materials as only long range periodic order or disorder can be probed. X-ray diffraction was, therefore, used to simply confirm the glassy nature of the ICSW phosphate glasses series I and II. Figure 30 and Figure 31 indeed show the characteristic amorphous halo for glasses in series I and series II, respectively.

There is one exception within both series – ICSW4. The XRD pattern of the heat treated ICSW4 in Figure 30 exhibits characteristic sharp crystalline peaks. Initially a

match using Xpert software was undertaken to try and determine the crystalline phase present as the resulting crystalline phase/s present are often a good indication of the phases present within the corresponding amorphous glasses. It was, however, obvious that more than one crystalline phase was present within the glass making it much more difficult to try and determine the actual phases present.

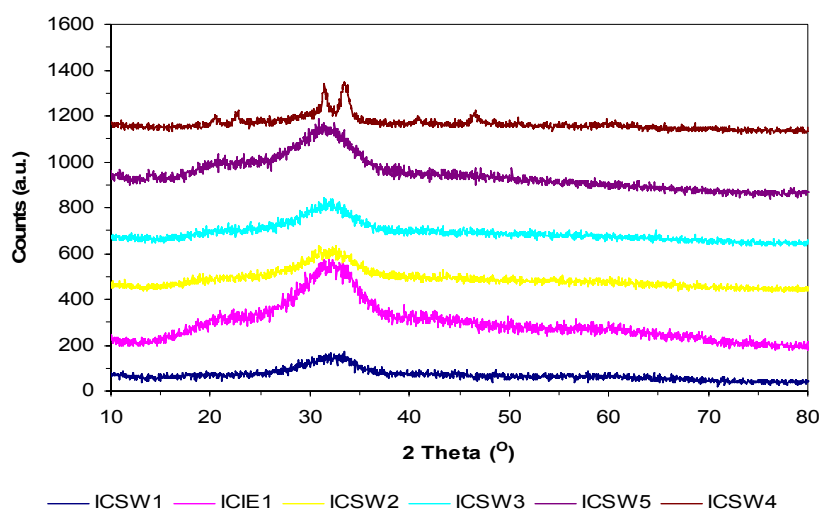


Figure 30: X-Ray diffraction patterns for series I glasses. Increasing phosphate content from bottom to top

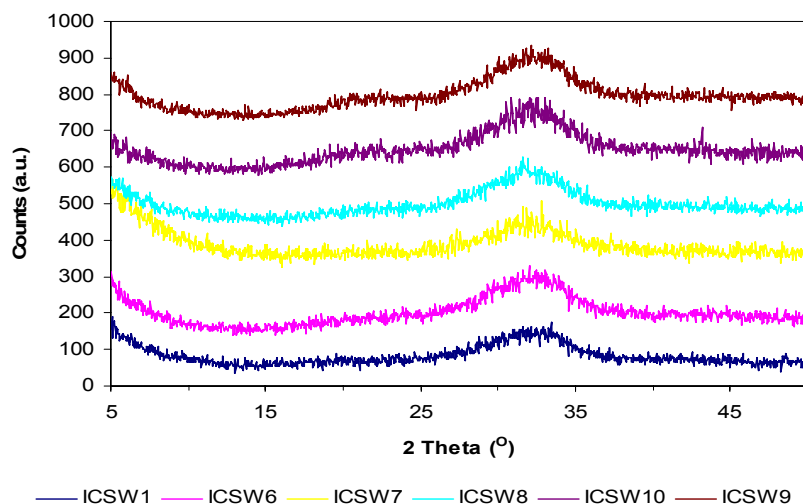


Figure 31: X-Ray diffraction patterns for series II glasses. Increasing phosphate content from bottom to top.

Rietveld refinement was therefore performed on the spectrum. Three phases were identified: NaCaPO_4 (sodium calcium orthophosphate, 68 mol. %), $\text{Na}_2\text{Ca}_2\text{Si}_3\text{O}_9$ (combeite, 28 mol. %) and $\text{Na}_4\text{P}_2\text{O}_7$ (sodium pyrophosphate, 4 mole %). This is consistent with the ^{31}P NMR observation of the presence of predominantly orthophosphate and a smaller amount of pyrophosphate. The ^{29}Si NMR spectra of the heat treated sample showed a broad peak around -88ppm which was thought to be a Q^2 or Q^3 silicate. However, Schneider *et al.* [177] observed three overlapping chemical shift anisotropies (CSAs) for combeite at around -88, -90 and -93 ppm, which fits with the NMR and XRD and Rietveld data of ICSW4. The presence of three CSAs is due to closely spaced, but three distinct crystallographic sites for silicon in the combeite lattice.

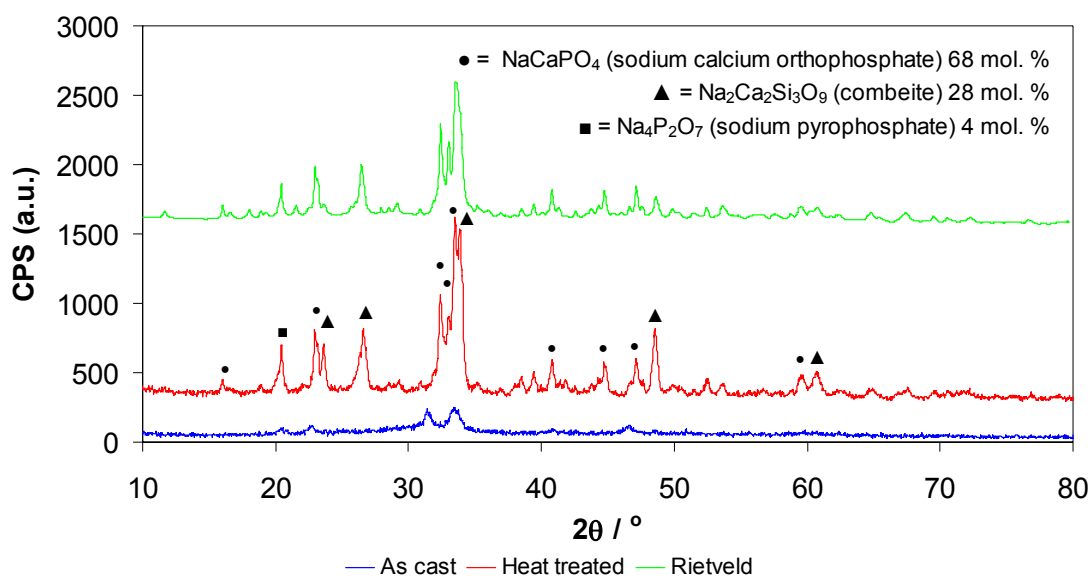


Figure 32: X-Ray diffraction pattern for crystalline sample ICSW4 before and after heat treatment with phases identified and Rietveld refinement, with molar proportions of phases shown (note – additional sodium pyrophosphate peak are masked in the region around 32° which contains the strongest lines from the other two phases).

6.6.1 Summary of XRD for phosphorus series of glasses

- The spectra for the two series of glasses illustrated that all glasses were amorphous.
- The exception to this was the ICSW4, highest phosphorus containing, glass of the non charge balanced series.

- The crystalline phases present in the ICSW4 glass were identified as sodium calcium orthophosphate, sodium pyrophosphate and combeite. This fits with the NMR data for the glass and suggests that a pyrophosphate type environment may exist in the higher phosphorus glasses.

6.7 Density

Density analysis is widely used for assessing the character and degree of structural changes in glass. Doweidar's model correlates the type of structural units within a glass system with its physical density. Doweidar analysed the density and structure of various types of silicate glasses, such as R_2O-SiO_2 , $RO-SiO_2$ and $Na_2O-Al_2O_3-SiO_2$ systems (R_2O being an alkali oxide and RO an alkaline earth oxide), to estimate the volume of each structural unit present. He found the volume of structural units in silicate glasses, modified with more than one type of modifier oxide, the same as in the corresponding R_2O-SiO_2 or $RO-SiO_2$ glass. Similar trends were also found in mixed alkali borate glasses [178] [179] [180].

Depending on the type of glass and the structural make up, Doweidar observed natural correlations between the glass density, the concentration of the structural units present and their volume [181]. The resultant working model is now applicable to many types of glass systems and is capable of estimating the densities of sophisticated glass systems with a good degree of scientific accuracy [149].

Elgayar *et al.* [14] and Wallace *et al.* [43] have both successfully applied Doweidar's model to multicomponent glass systems. However, in both Elgayar *et al.*'s and Wallace *et al.*'s glasses, as the content of P_2O_5 was fixed and negligible, when employing Doweidar's model, the existence of P_2O_5 within the glass structure was disregarded. Doweidar does not consider the species in the development or application of any of his models [43]. As a result, although good agreement is observed between Elgayar *et al.*'s and Wallace *et al.*'s own measured densities and those calculated by his model, inconsistencies are obvious in both sets of results [43] [78]. It is suggested that the variations observed, as well as a result of normal experimental deviation, are a direct

consequence of the exclusion of the P_2O_5 component in Doweidar's theoretical model. For Doweidar's model to be applied to a glass system, with any degree of scientific certainty, it is paramount that the effect of the addition of any species to a glass structure is thoroughly understood. To the author's knowledge, this is the first time that the effect of P_2O_5 content on Doweidar's model has been experimentally investigated.

Figures 33 and 34 below show the experimental density measurements for phosphorus series I and phosphorus series II, respectively. Also on each Figure are plotted the density values as predicted by Doweidar's model (red trend line). When calculating Doweidar's densities, in line with Elgayar *et al.*'s and Wallace *et al.*'s treatment, all glass compositions were normalised to exclude the phosphate content present.

Doweidar assumes a binary distribution of silicate Q species and, on normalisation, all compositions fall within the $33 \leq x_t \leq 50\text{mol}\%$ region (where x_t is the total modifier mol%). This signifies a Q^2 and Q^3 silicate distribution within both glass series; in agreement with their respective ^{29}Si MAS NMR results.

The relative proportions of the Q^n species were calculated using the following formulas (a), (b), (c), (d), where "a" denotes Na^+ containing Q^n species and "b", Ca^{2+} containing Q^n species:

$$Q^{2a} = [3 \times (f_{Na_2O} + f_{CaO}) - 1] \times \left(\frac{f_{Na_2O}}{f_{Na_2O} + f_{CaO}} \right) \quad 6(a)$$

$$Q^{2b} = [3 \times (f_{Na_2O} + f_{CaO}) - 1] \times \left(\frac{f_{CaO}}{f_{Na_2O} + f_{CaO}} \right) \quad 6(b)$$

$$Q^{3a} = [2 - 4 \times (f_{Na_2O} + f_{CaO})] \times \left(\frac{f_{Na_2O}}{f_{Na_2O} + f_{CaO}} \right) \quad 6(c)$$

$$Q^{3b} = [2 - 4 \times (f_{Na_2O} + f_{CaO})] \times \left(\frac{f_{CaO}}{f_{Na_2O} + f_{CaO}} \right) \quad 6(d)$$

Equation 9: Doweidar's equations to calculate Q species.

and where $f_{M_xO_y}$ is the fraction of oxide component M_xO_y . Each value was then normalised to give the fraction of each species (x_n) and the resultant densities calculated using the following equation:

$$\rho = x_{2a}\rho_{2a} + x_{2b}\rho_{2b} + x_{3a}\rho_{3a} + x_{3b}\rho_{3b}$$

Equation 10: Doweidar's equation for density.

Table 13 shows the resultant densities of each Q^n species.

Table 13: Densities of silicate Q species and phosphate species.

Species	$\rho / \text{g.cm}^{-3}$
$Q^{2a} (\text{Na}^+)$	2.57
$Q^{2b} (\text{Ca}^{2+})$	2.90
$Q^{3a} (\text{Na}^+)$	2.50
$Q^{3b} (\text{Ca}^{2+})$	2.68
Na_3PO_4	2.54
$\text{Ca}_3(\text{PO}_4)_2$	3.14

From the experimental ^{29}Si and ^{31}P MAS NMR results, it is hypothesised that P_2O_5 is participating within the glass structure as a separate orthophosphate phase, charge balanced by Na^+ and Ca^{2+} ions, at a ratio consistent with that of the parent glass. The two series were purposively designed to illustrate and corroborate this hypothesis, with series I characterised with a fixed ratio of $(\text{CaO}/\text{Na}_2\text{O}) = 0.87$ and series II designed to maintain the network connectivity value at a constant value (with the content of Na_2O and CaO adjusted to take account of the charge deficient isolated phosphate species).

Examining the experimental density values for both series and their respective theoretical values, the deficiency in Doweidar's model becomes most apparent. At higher proportions of P_2O_5 , it is no longer acceptable to simply ignore the existence of a species within the glass structure. From our understanding of phosphorus' role, therefore, a modified model was formulated (blue trend line), in which the effect of P_2O_5 on density was taken into consideration. Using the density values of sodium and calcium orthophosphate, as tabulated in Table 13, Equation 10 was modified to incorporate the fractions of sodium and calcium orthophosphate within the glass system (blue trend line on Figure 33).

On examination of the series I experimental glass densities in comparison to those predicted by Doweidar, there is little correlation. The series I experimental density values are unaffected by the percentage of phosphorus added, with no real trend obvious to the eye. It should be noted, however, that the end point on the graph, ICIE4, is a partially crystalline glass. Therefore, this explains the inconsistency and significantly higher density. This point, therefore, should be disregarded from the overall density analysis.

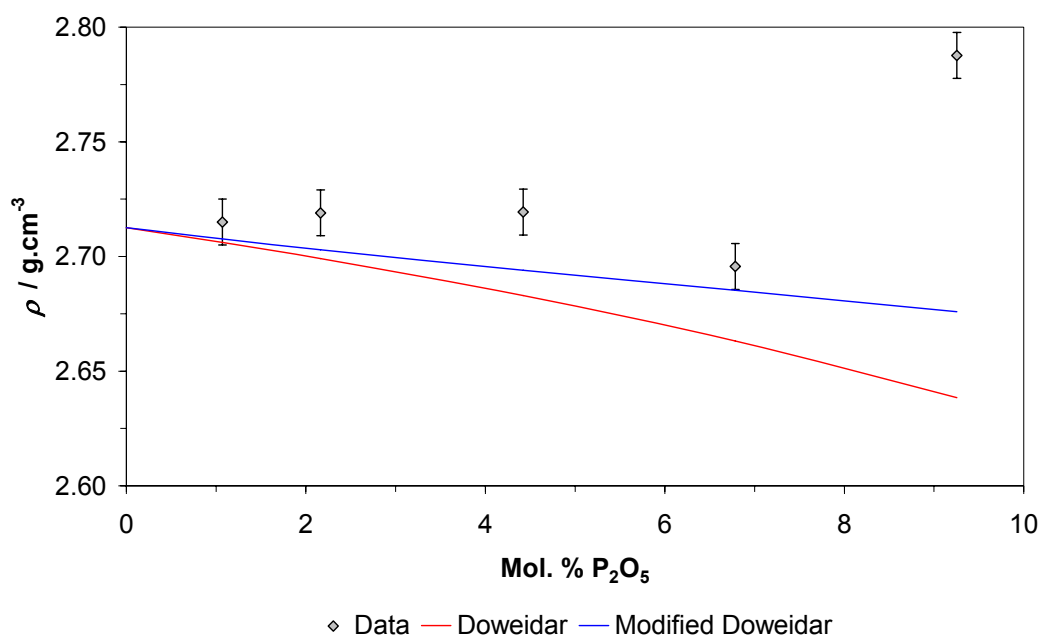


Figure 33: Variation in density with phosphate content for series I with Doweidar's theoretical values.

Within series I, the glasses should display a decreasing trend in density, as phosphorus is substituted, as a result of the removal of sodium and calcium ions from their charge balancing role in the silicate structure in order to counter the charge deficiency resulting from the formation of orthophosphate type species. This lack of charge balancing ions in the silicate network results in the move from predominantly Q^2 to predominantly Q^3 species. Following Doweidar's original assessment (Q^3 silicate units are less dense than their corresponding Q^2 counterparts), a resultant decrease in density will occur, as highlighted by the red trend line. We know from the previous ^{29}Si MAS NMR results that Q^2 and Q^3 silicate species, charge balanced by sodium and calcium are present within the glass structure and indeed, as phosphorus is added at the expense of silica, there is a move to Q^3 charge balanced species. The actual density data, however, obviously does not follow this trend line. This implies that there is some additional event that is suppressing the theoretical densification of the structure.

As recognised earlier, there becomes a point where, when utilising a theoretical model, it is no longer scientifically acceptable to disregard a potential variable, in this case the

addition of phosphorus to the glass structure. By accounting for this proposed phenomenon in Doweidar's model (blue trend line), there is a definite move towards the experimental values. The modified model however still does not predict the actual density values with any significant degree of scientific accuracy and significant deviations are apparent from the actual experimental results, even if lessened.

Figure 33 shows that the actual experimental density values are higher than those predicted, either by the original or modified Doweidar model. In the modified calculations (blue trend line), the existence of a higher density orthophosphate component has already been incorporated. Furthermore, apart from ICSW4 glass, the presence of a crystalline phase is ruled out from XRD analysis. The results imply that a higher density type species may be present. It is suggested that this species may be some kind of sodium/calcium pyrophosphate.

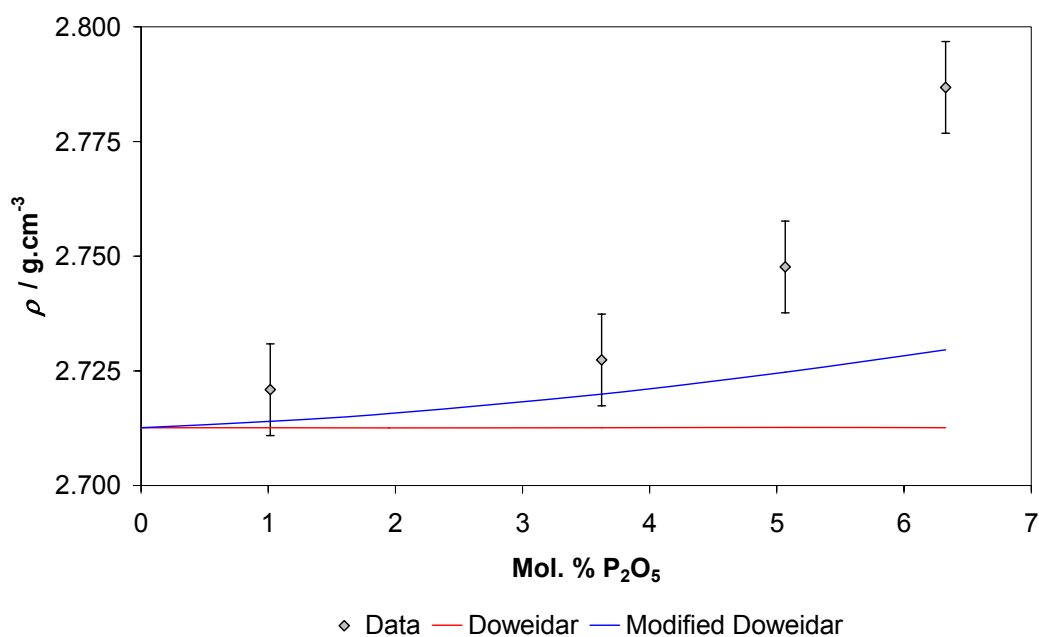


Figure 34: Variation with density with phosphate content for series II with Doweidar's theoretical values.

Examination of series II shows that the addition of phosphorus to the series increases the density of the glasses. Again, as with series I, there is no agreement with Doweidar's theoretical values. I suggest that, as well as the simple effect of the silicate

species present, another phenomenon is influencing the density of the glass series. As charge balancing cations are added to compensate for the formation of orthophosphate species, the silicate Q structure of the glasses remains constant, as illustrated by ^{29}Si MAS NMR results. This would suggest that, in line with Doweidar's thinking, the density would remain exactly the same as the Q species remain constant, as do the type of modifier ions present. As postulated by Doweidar, the concentration of the modifier ions does not effect the volume of the structural species, only the type of species themselves. This is indicated by the horizontal trend line, highlighting no change in density with the addition of phosphorus. Although the proportion of silica is decreasing, phosphorus pentoxide is added at its expense. When this is accounted for in the modified Doweidar model, a resultant trend in increasing density is observed, as a result of the formation of sodium and calcium orthophosphate type species. This still does not match the marked increase in experimental density. As with series I, this suggests that, as phosphorus is added, an even denser species is forming. Again, as a result of the XRD analysis this cannot be assigned to crystalline species, therefore it is suggested that, as with Series I, a pyrophosphate type species is formed.

6.7.1 Doweidar's analysis

Doweidar, himself, comments on the experimental results collected in this study (as published in the Journal of Non Crystalline Solids) and their significance and impact on his own theoretical model in his 2009 paper entitled "Density-Structure Correlations in $\text{Na}_2\text{O-CaO-P}_2\text{O}_5\text{-SiO}_2$ bioactive glasses" [182].

Doweidar concludes that the structural information gathered by the incorporation of P_2O_5 in $\text{Na}_2\text{O-CaO-SiO}_2$ bioactive glasses can be employed to develop relations that can be used for predicting this type of glass. Furthermore, he comments that the agreement between the experimental and calculated densities of the series may be taken as evidence for the validity of his presented model [182].

Although Doweidar recognises this study's approach taken when modifying his model, he employs a slightly different angle with respect to the effect of phosphorus. He

accepts that, from our previous MAS NMR results, P_2O_5 forms a separate phase of Na_3PO_4 and $Ca_3(PO_4)_2$, in the same proportion of sodium and calcium in the parent glass. He believes that the P_2O_5 phase has the priority to be fully saturated with respect to the modifier oxide, and indeed at the expense of the silicate structure.

Therefore, as a starting point, to predict the density of a phosphorus containing glass, he calculates the amount of Na_2O and CaO consumed as a result of the formation of the separate orthophosphate species and subtracts this from the overall content of the Na_2O and CaO in the glass. Doweidar then suggests that the next step is to calculate the amount of sodium and calcium orthophosphate in the glass and then allow the residual sodium and calcium modifiers to act as usual within the silicate network, to generate and yield the overall number of Q^n species present.

Figure 35 below presents the correlation between Doweidar's own modified model and the series I and series II experimental results. Doweidar comments that the experimental and calculated densities are consistent, except, in both cases, the two end compositions of the series.

Figure 36 demonstrates the experimental molar volumes and the calculated values as a function of phosphorus content. Again, with only some scattering observed, and the two end values inconsistent, Doweidar comments on the excellent correlation and its evidence for the validity of the predicted orthophosphate volumes in the sodium and calcium phosphate phases.

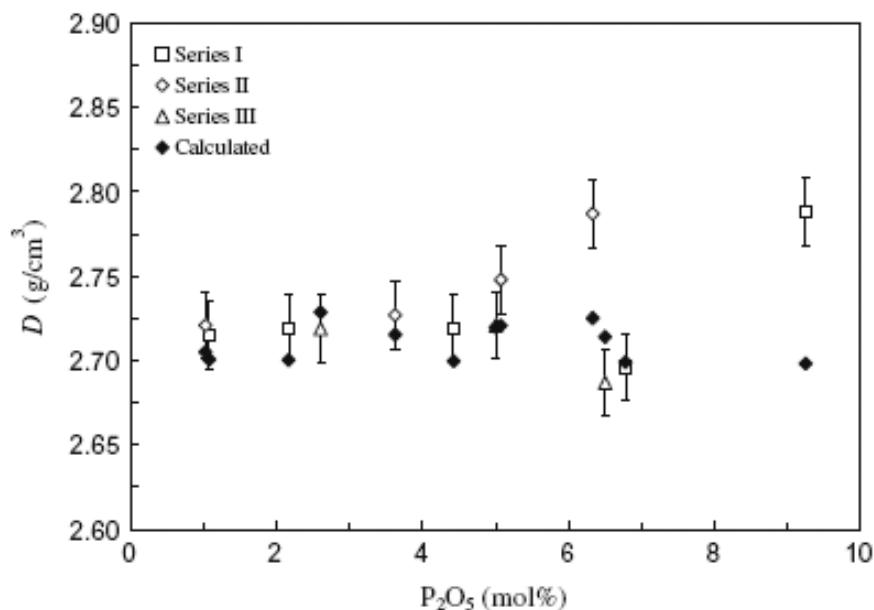


Figure 35: Doweidar's graph taken from his 2009 paper showing density as a function of phosphorus content. The square and diamond symbols represent this study's series I and series II experimental values while the triangular symbol represents Lusvardi's *et al.*'s [183] experimental density values. The closed triangular symbols represent Doweidar's theoretical values. Error limit in the experimental densities is $\pm 0.02 \text{ g/cm}^3$ [182].

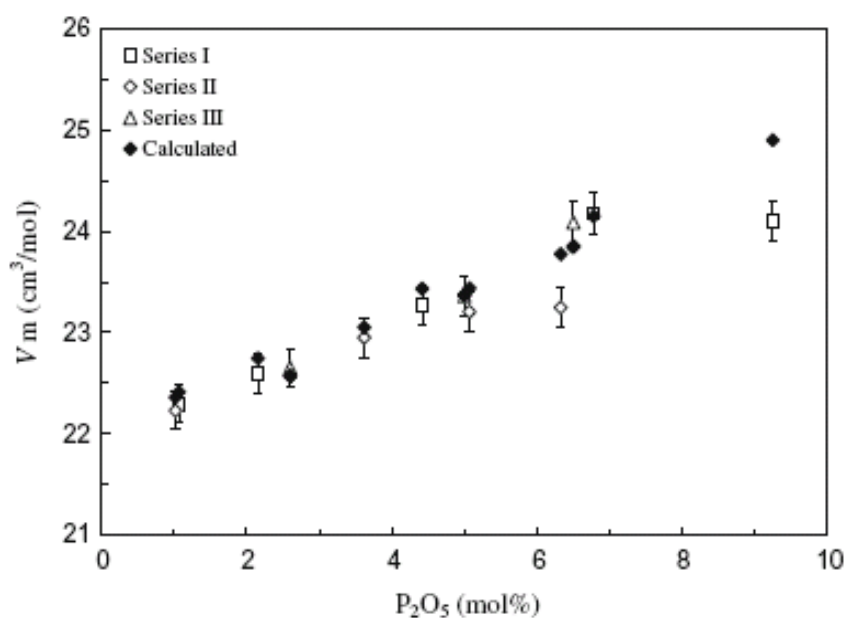


Figure 36: Doweidar's graph taken from his 2009 paper showing V_m as a function of phosphate content using this study's and Lusvardi's *et al.*'s [183] results. Symbols as with Figure 35 – closed triangular symbols represent calculated molar volumes [182].

The above analysis highlights that Doweidar's model, used initially for simple silicate [179] [180] and mixed silicate glasses [149], can be developed to be applicable for far more complex glass systems [182].

6.7.2 Summary of density results for phosphorus series of glasses

- The experimental density values were seen to be unaffected by the addition of phosphorus in the series I glasses and were seen to increase with the addition of phosphorus in the series II glasses.
- There was little correlation between the experimental density values and Doweidar's predicted density values. This clearly highlights the inadequacy of Doweidar's model for phosphorus containing glasses.
- However, using the data from this thesis, published in the Journal of Non Crystalline Solids in 2009, Doweidar subsequently modified his model to take account of phosphorus in soda lime silicate glasses. The experimental density values were then observed to correlate well with Doweidar's modified values; thus providing evidence for the existence of phosphorus in an orthophosphate type environment.

6.8 Differential Thermal Analysis

Figures 37 and 38 illustrate the DTA traces for the phosphorus glasses, series I and series II, respectively. From these traces the glass transition temperature (T_g) and crystallisation temperature (T_x) for each glass were determined. In general the glasses manifested a T_g and two exotherms corresponding to T_{xi} and T_{xii} crystallisation temperatures. Only glass ICSW4 (9.25 mol. % P_2O_5 from series I) showed a third crystallisation exotherm (T_{xiii}).

6.8.1 Glass Transition Temperature

If T_g is plotted against the percentage of phosphorus added for both series (Figures 39 and 40) it is clear that as phosphorus is increased, T_g decreases. This contradicts the theory that phosphate addition results in a polymerisation of the silicate network. This

also contradicts the NMR findings. In series I, phosphorus was substituted for silica on a mole percent basis. This then requires Na^+ and Ca^{2+} ions to charge compensate the phosphorus complexes, removing the cations from their modifying role and thus polymerising the network. The expectation would have been a corresponding increase in T_g . In the case of series II, the need for charge balancing cations is compensated for and thus the silicate network remains unchanged. The expectation would have been to see no real change in T_g , however, experimentally, it was seen, once again, to decrease with increasing phosphorus content.

There is, therefore, an obvious difference between the expected behaviour of the glass and the actual behaviour, with respect to transition temperatures. For both series, the expected T_g correlation theories are based on the degree of disruption of the silicate network. This theory in turn bases itself on the idea that T_g is affected solely by the silicate phase. Elgayar *et al.* [78] have already discussed the effect of Na_2O replacement of CaO and the loss of ionic bridges on T_g . However, in the two phosphorus series studied, there is no change with respect to any modifying cations so there must be a different phenomenon occurring with the addition of phosphorus.

From the ^{31}P MAS NMR data it is already known that the glasses have a phase separated phosphate structure. An expectation might be a second glass transition temperature corresponding to the phosphate phase. However, from the traces, there seems to be no evidence of this. This could mean it is simply being masked by the other features in the DTA trace. Conversely, the amount of phosphorus present, or the actual size of the phosphate phase, may be too small to be picked up by differential thermal analysis.

However, it is suggested that what may be observed is a composite glass transition temperature (i.e. as a result of a number of competing factors) due to the fact that T_g decreases when the NC' is the same (series II) and even when NC' increases (series I). It is suggested that as the phosphorus content increases, the corresponding phosphate

phase size increases, then surpassing some critical size dimension to be of significance to effect the T_g values.

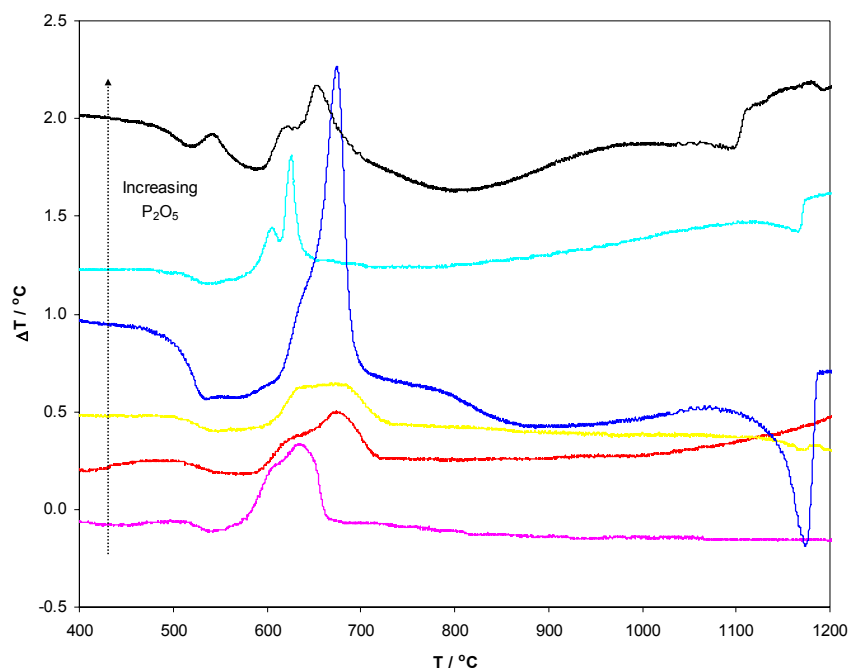


Figure 37: DTA traces for series I (ICSW1 magneta, ICIE1 red, ICSW2 yellow, ICSW5 cyan and ICSW4 black).

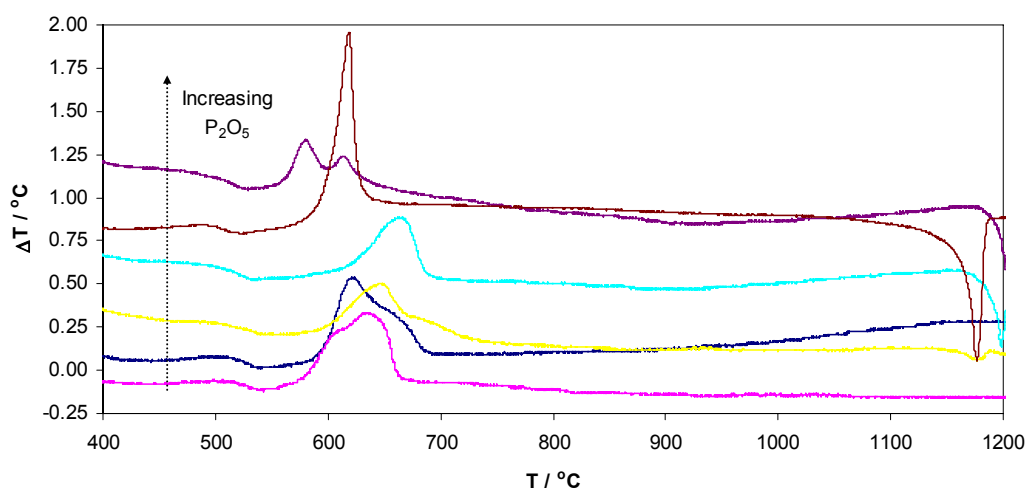


Figure 38: DTA traces for series II (ICSW1 magenta, ICSW6 blue, ICSW7 yellow, ICSW8 cyan, ICSW10 brown, ICSW9 purple).

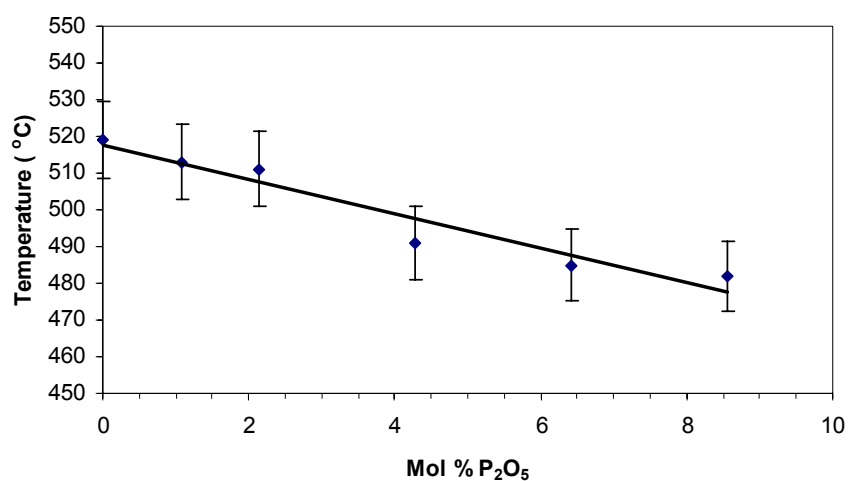


Figure 39: Glass transition temperatures for series I.

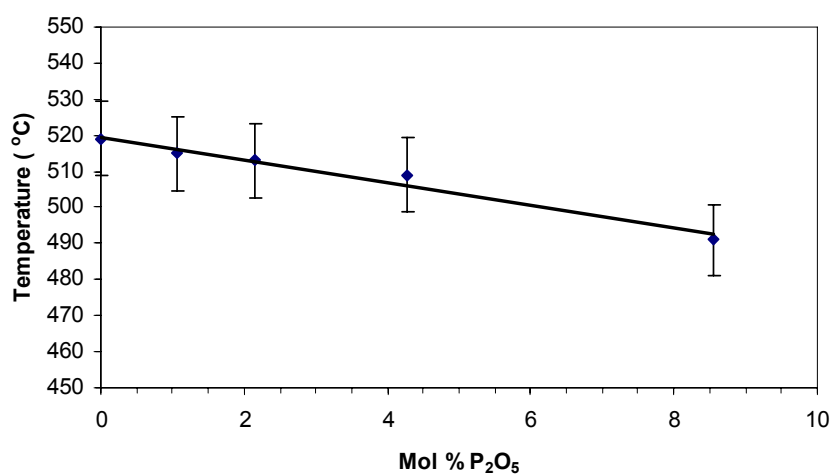


Figure 40: Glass transition temperatures for series II.

6.8.2 Crystallisation Temperatures

Figures 41 and 42 illustrate the crystallisation temperatures with respect to the phosphorus content.

The traces show the presence of two crystallisation exotherms in both series, which often overlap. These two exotherms could correspond to the crystallisation of two

separate crystal phases or conversely, the crystallisation of the same phase in two phase separated regions or by two different mechanisms. It is suggested that it is most probably as a result of the crystallisation of two separate crystal phases i.e. crystal phase A and crystal phase B. It should not be neglected that the order of crystallisation temperatures can switch within the series – for example in one glass, T_{xi} can correspond to crystal phase A and T_{xii} to crystal phase B while in another glass, T_{xi} could correspond to crystal phase B and T_{xii} to crystal phase A, depending on composition and the crystallisation mechanisms involved.

In both series I and II, T_{xi} and T_{xii} seem to be relatively independent of phosphate content, with a small decrease in temperatures observed with increasing P_2O_5 . This suggests that phosphate aids crystallisation at lower temperatures. For series I however, as the network polymerises, it is suggested that more energy would be required to re-arrange the ions into a crystal state thus resulting in a higher crystallisation temperature. This trend however is not seen, suggesting that, once again, the addition of phosphate somehow aids crystallisation. This, however, is in no way conclusive and the trend seen could be simply due to experimental errors.

Within series I, the observed endotherms are all broad and weak and are attributed to the melting of the residual glassy phase. In series II, however, ICSW8 and ICSW9 exhibit very sharp and definite melting endotherms with an onset of melting at approximately 1180°C. This suggests that ICSW8 and 9 crystallise to the same phase. This endotherm is missing from all the other glasses in the series, with exception to ICSW6 – which shows a much weaker endotherm, however, at the same temperature. This suggests that the other members of the series do not crystallise to this unknown phase.

From the DTA information on crystallisation exotherms and melting endotherms, it can be predicted that there are probably three crystalline phases occurring; one of which is common to ICSW8 and ICSW9 with a very small amount of the same phase in ICSW6.

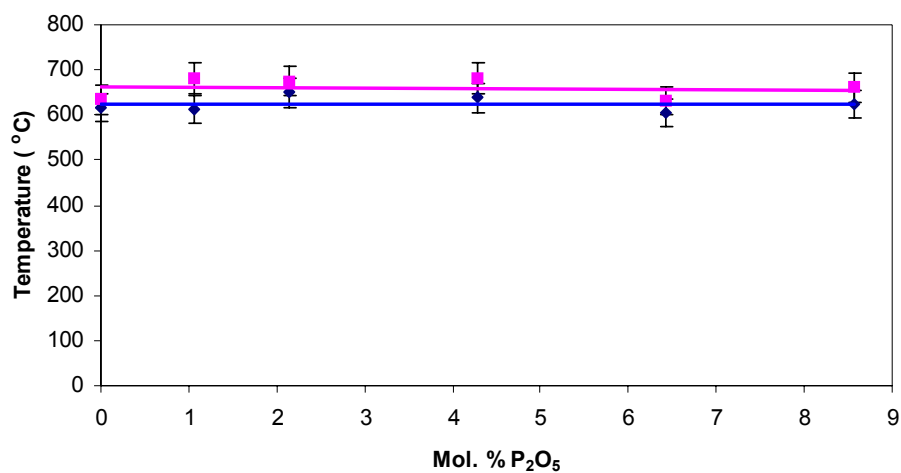


Figure 41: Crystallisation temperatures for series I (T_{c1} blue, T_{c2} pink)

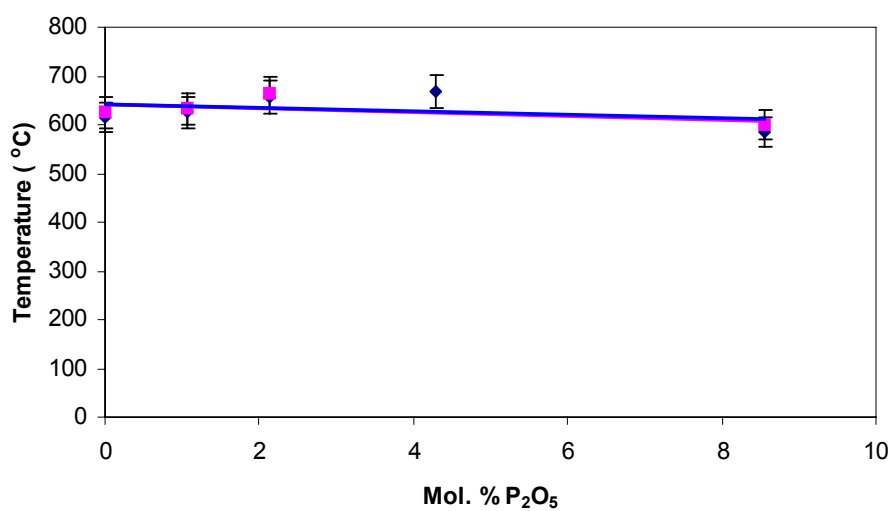


Figure 42: Crystallisation temperatures for series II (T_{c1} blue, T_{c2} pink)

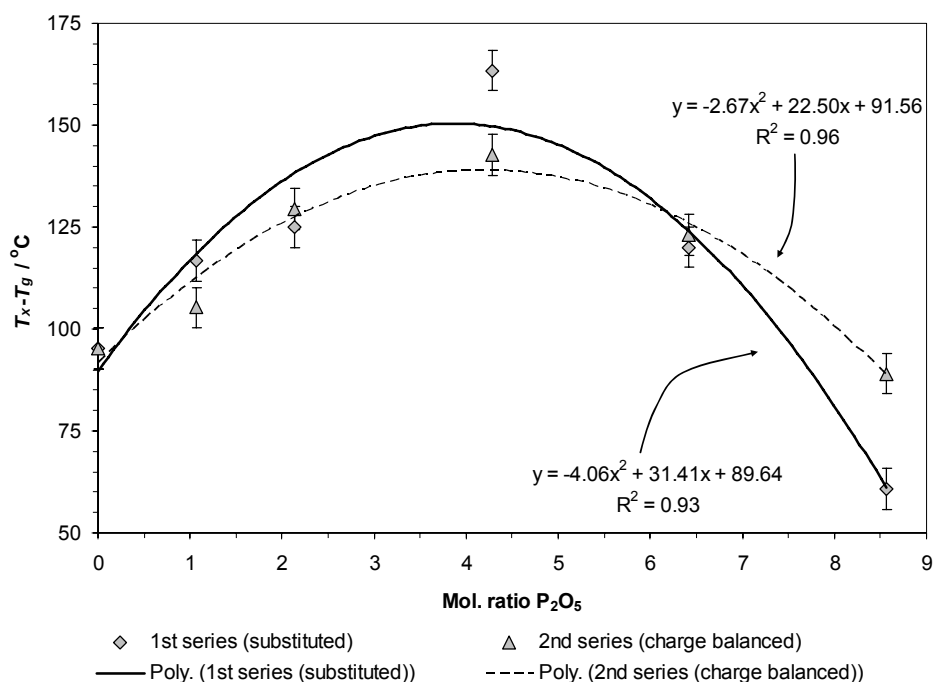


Figure 43: $T_x - T_g$ for series I and II.

Figure 43 illustrates the working range, or glass stability ($T_{xi} - T_g$), with respect to the amount of P_2O_5 added. It can be seen that $T_{xi} - T_g$ increases to a maximum in both series at a molar ratio of around 4 mol% for series I and 3 mol% for series II, then decreases. This working range is important in processes such as sintering and fibre drawing and should ideally be maximised for both to avoid crystallisation at temperatures where the glass viscosity is sufficient to allow viscous flow to occur.

6.8.3 Summary of DTA results for the phosphorus series of glasses

- The glass transition temperatures for both series of glasses were seen to decrease with the addition of phosphorus.
- This suggests that the temperatures recorded by the DTA equipment were composite glass transition temperatures and the glasses are phase separated.
- The crystallisation temperatures were also seen to decrease very slightly with the addition of phosphorus in both series suggesting that crystallisation is in some way aided by the addition of phosphorus.

6.9 Dilatometry

Figure 44 shows the relationship between the thermal expansion coefficient (α) and phosphate content for both series of phosphate glasses. Due to problems with casting, as a result of phase separation and / or crystallisation, only 3 samples of series I and 2 samples of series II could be cast, and thus run in the dilatometer. It is important to note that these glasses have a phase separated structure. It is easy to visualise that each phase will contribute to a given extent to the overall expansion of the glass – a situation analogous to that of composite structures. The composite glass thermal expansion coefficient, α_c , is given by Equation 11.

$$\alpha_c = (\alpha_a \times V_a) + [\alpha_b \times (1 - V_a)]$$

Equation 11: Composite thermal expansion coefficient.

where α_a , is the thermal expansion coefficient of phase a , V_a the volume fraction of phase a and α_b the thermal expansion coefficient of phase b .

Due to the polymerisation of the silicate matrix through series I, it is suggested that the glasses would be less able to expand, thus resulting in smaller α values. Conversely, the thermal expansion coefficient values are seen to increase with phosphate substitution. The observed α values for the series I glasses thus imply that, as phosphate is substituted, a composite thermal expansion coefficient may result. As phosphate is added and the amount of the phosphate phase is increased, the weaker P-O bond strength, compared to the Si-O strength of the silicate phase, would act to weaken the glass structure, thus explaining the increase in measured α values across the series.

The shape and dimensions of the secondary phosphate phase are factors which would effect the thermal expansion of a phase separated structure. Dilatometry is known to be governed dramatically by the dominant matrix phase of a glass. The results suggest that, as the phosphate content increases, the dimensions of the secondary phase are significant enough to contribute to the thermal expansion of the glass. It would be of

interest to examine the phase size and dimensions of the silicate and phosphate phases by scanning electron microscopy (SEM), for example, paying special attention to the possible progressive formation of the secondary phosphate phase through the series. It is suggested that a proper understanding of the thermal expansion of phase separated structures would not be achieved, therefore, without the knowledge of such factors.

Figure 44 also shows the α values calculated from Appen's model [184]. The fraction of each component in the glass compositions (f_{MxOy}) were multiplied by Appen factors [184] (a_{MxOy}) giving α .

$$\alpha = f_{SiO_2} a_{SiO_2} + f_{CaO} a_{CaO} + f_{Na_2O} a_{Na_2O} + f_{P_2O_5} a_{P_2O_5}$$

Equation 12: Theoretical thermal expansion coefficient calculated using Appen factors.

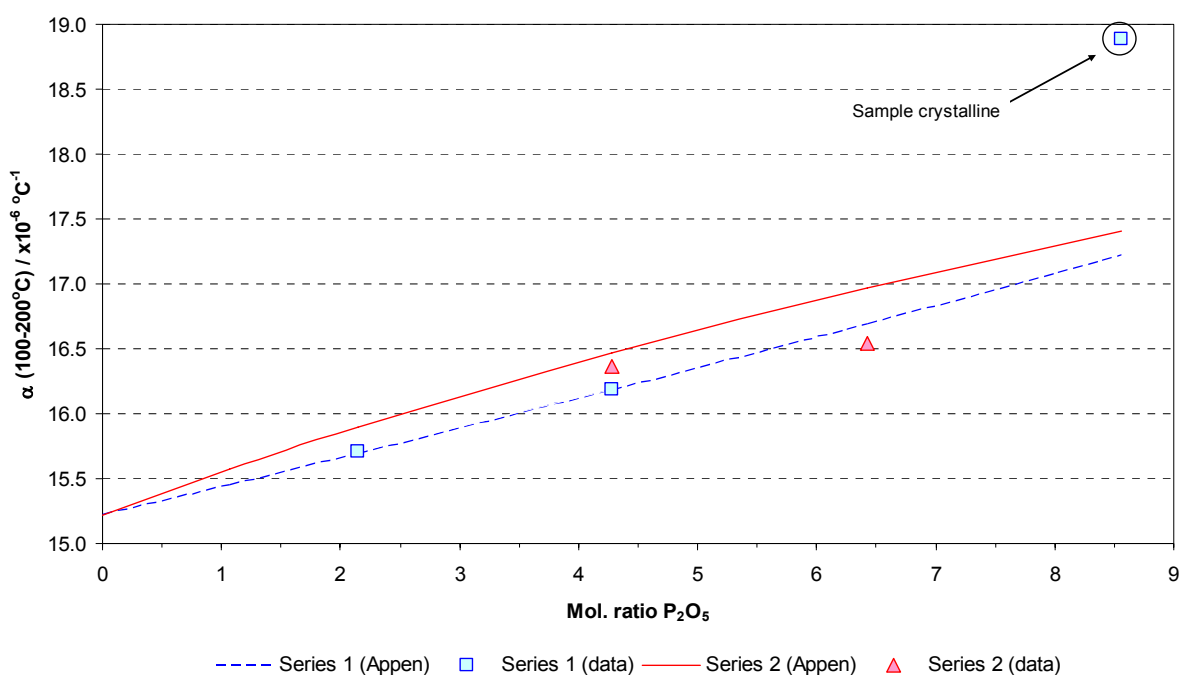


Figure 44: TEC's (α) for series I and II with calculated (Appen) values.

The values over the 100-200°C range show relatively good agreement with the Appen figures. It is difficult, however, to comment on the reliability of experimental data when only 3 data points and 2 data points are available for series I and II respectively.

Figures 45 and 46 illustrate the relationship between the dilatometric softening points, T_s , and percentage of phosphate. It can be seen that T_s increases with P_2O_5 substitution in series I. As P_2O_5 substitution increases, the network connectivity of the dominant silicate phase increases due to formation of a separate phosphate phase. It would, therefore, be expected to observe this increase in the softening point of the glass (i.e. a more cross-linked network through the series resulting in more energy needed for the glass to flow). In series II, however, the silicate phase remains unchanged with respect to Q structure, as a result of the addition of modifying ions to the system. It would, therefore, be expected that the softening points would remain constant. However, they are also seen to increase with phosphate substitution. This suggests, that as with the thermal expansion coefficients, the phosphorus phase is playing some role with respect to the softening point of the overall glass.

If, however, we compare these results to the glass transition results discussed above in the DTA chapter, a discrepancy arises. Both glass transition and softening temperatures are parameters which reflect the structural nature and the degree of disruption of the glass. It is therefore very surprising to observe these conflicting trends, with the glass transition temperatures falling but the softening temperatures increasing with the increase in phosphorus content, in both of the series. The T_g values suggest that the glasses are becoming less cross-linked, while the T_s values are suggesting that the glasses are becoming more cross-linked.

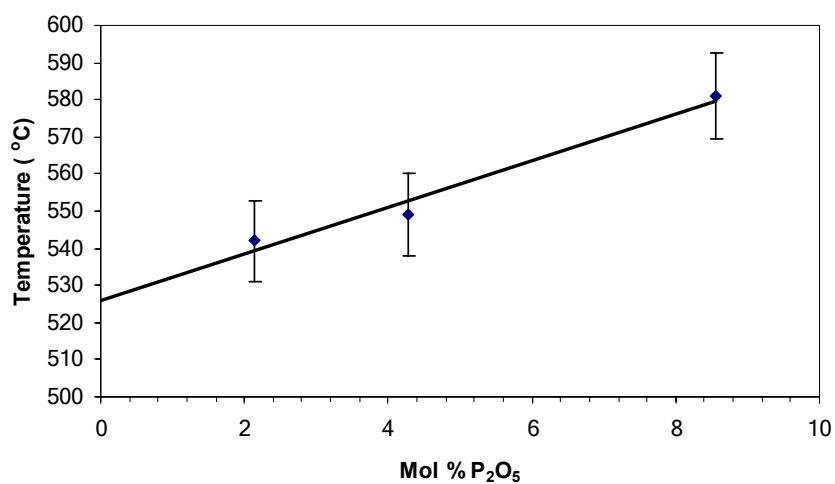


Figure 45: Softening temperatures for series I glasses.

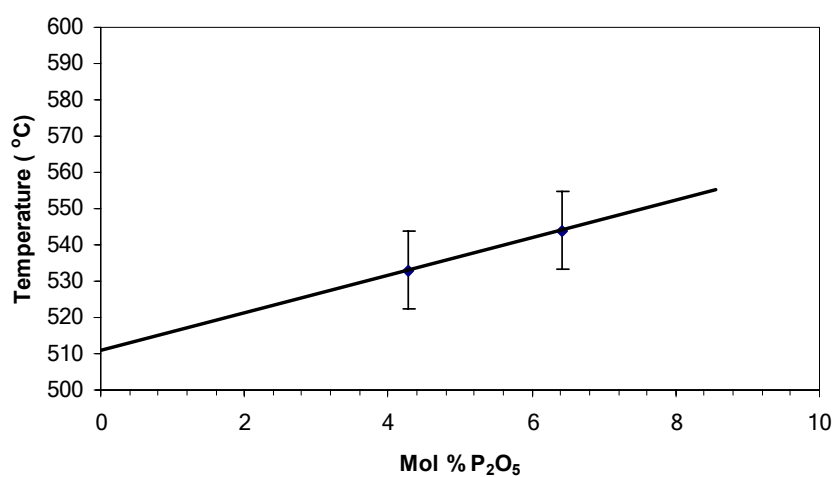


Figure 46: Softening temperatures for series II glasses.

As discussed, earlier, dilatometry is reflective of only essentially the dominant matrix phase in a glass. Therefore, it is suggested that this phenomenon manifests itself in these softening values – reflecting only the degree of disruption of the silicate phase and disregarding the effect of the phosphate phase. The T_g values, on the other hand, are reflecting both of the artifacts. This is further evidence to support that, as phosphorus is added to the glass, the phase size may be reaching some critical size dimension to be of significance to effect the glass transition temperature. It is suggested that dilatometry, as a result of its very nature, is unable to pick up such delicate changes in the glass structure. Why, however, the thermal expansion coefficients, measured by the dilatometer, seem to be able to pick up such changes is difficult to answer. In conclusion, the dilatometric results and the DTA results seem to be rather contradictory, therefore before any real definitive statements can be made on the effect of phase separation on a soda lime phosphosilicate glass, further experimental analysis would be required.

6.9.1 Summary of dilatometry results for the phosphorus series of glasses

- The thermal expansion coefficients were seen to increase with phosphorus content in both series of glasses thus implying a weakening of the glass structure.
- As with the glass transition temperatures, this suggests that a composite thermal expansion coefficient is being picked up as a result of the phase separated structure, with the weaker P-O bonds compared so the Si-O bonds weakening the glass structure.
- The softening temperatures were seen to increase with phosphorus content, in both series. These results are contradictory to the glass transition and thermal expansion coefficient data.

6.10 Dissolution Studies in Simulated Body Fluids

The ultimate aim of this investigation is to understand how a glass's structure influences its bioactive behaviour. Therefore, having analysed the structure of the two phosphorus glass series we then moved on to analysing the glass's bioactive nature. Ultimately, the

objective was to gain an understanding of the underlying mechanisms linking the two – the glass's structure and its resultant bioactivity.

When implanted into the human body, a glass's bioactivity is generally measured by its ability to form a bone-like hydroxyl carbonate apatite layer on its surface. Due to many practical and ethical issues, *in vivo* studies are not usually utilised at such an early stage of bioactive analysis. As an alternative, the dissolution of glasses in simulated body fluid (SBF) is used as an alternative and an indicator as to bioactivity. Simulated body fluid emulates the ionic concentration of human blood plasma. When immersing a bioactive glass into SBF a series of reactions is initiated which results in the formation of a HA layer on the surface. Ultimately, the *in vivo* bone bioactivity of a material can be predicted from the apatite formation on its surface in SBF. It should be noted, however, that SBF studies are only one indication of the bioactivity of a glass and thus should be complemented by cell studies and followed by animal and eventually human trials.

6.10.1 Variation in Simulated Body Fluid pH

Figure 47 and 48 show the variation in pH with time for series I and series II phosphorus glasses, respectively. The pH of SBF before the addition of any samples is 7.25.

As a general trend, for both series of glasses, the SBF becomes more basic with time. This is to be expected as a result of the ion exchange mechanism taking place at the glass surface, as predicted by Hench [95]. The initial stage of the reaction kinetics is the exchange of Na^+ and Ca^{2+} ions from the glass with H^+ and H_3O^+ from the solution. This results in the local pH rise, which further acts to catalyse the degradation of the glass resulting in more alkali ions being released. This pH rise also signifies the breaking of the silicon oxygen bonds at the surface of the glass through the action of the OH^- ions. Elgayar *et al.* [78] observe a gradual decrease in pH with time and assigned it to the formation of silanols ($\text{Si}(\text{OH})_4$) as predicted in stages 2 and 3 of Hench's glass dissolution model. This is also observed with these two series of glasses.

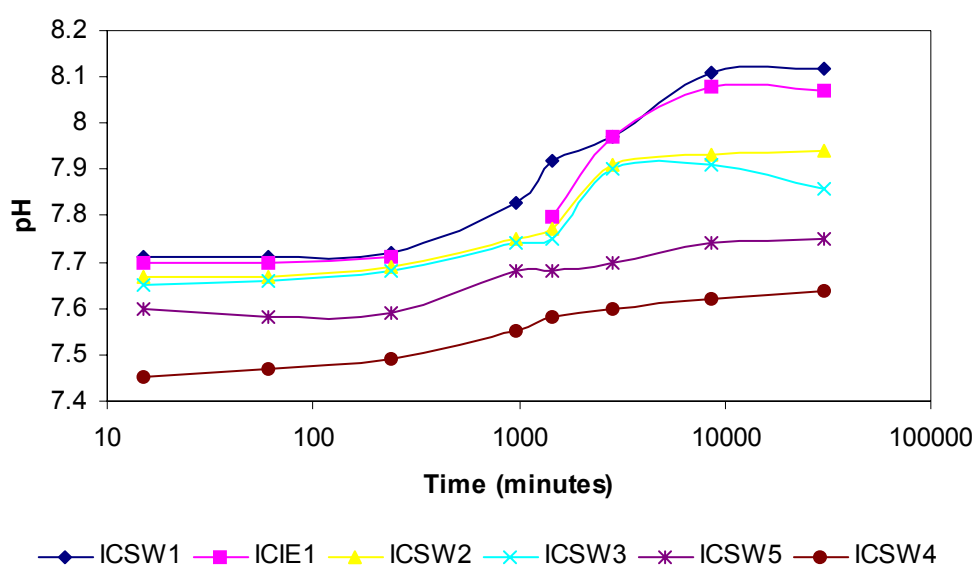


Figure 47: Variation in SBF pH with time for series I phosphorus glasses.

The pH of the solution is also influenced by phosphorus content. Although pH increases with time for all of the glasses in both series, the magnitude of this increase decreases with phosphorus content.

In other words, a shift to a less alkaline environment is observed as phosphorus content increases. This can be assigned to the release of phosphorus into the solution which will buffer the alkalinity caused by the sodium and calcium ions. The observed decrease in pH with phosphorus content will ultimately aid the deposition of apatite as although an alkaline environment is advantageous, the optimum pH in physiological fluid is 7.2 [185]. It has also been noted that extreme alkalinity is unfavourable for cell proliferation and differentiation. A variation in pH effects the activity of cultured human osteoblasts of trabecular bone. Kasinger [186] states that the optimum pH is 7.2 with any deviation resulting in an inflammatory response. The buffering effect of phosphorus therefore could prove extremely valuable.

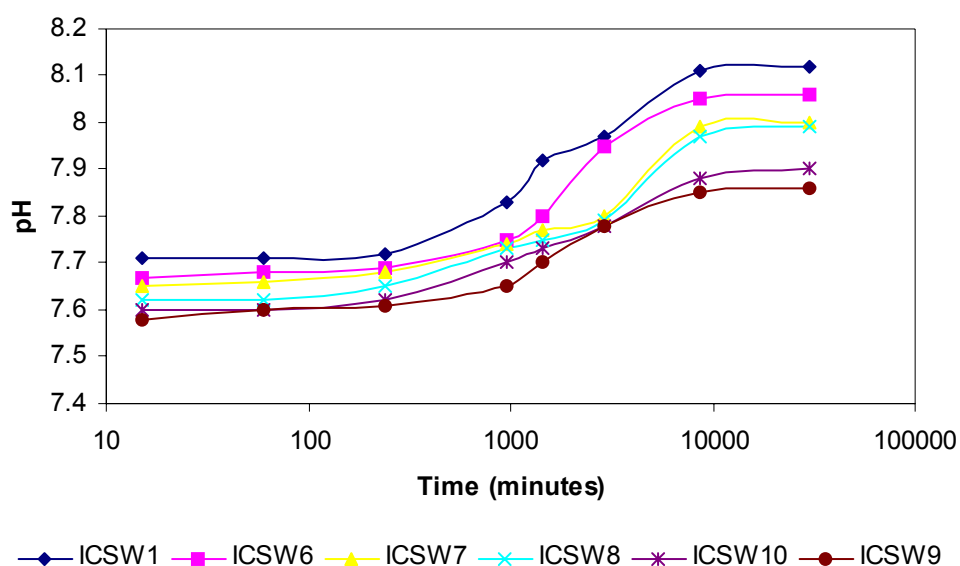


Figure 48: Variation in SBF pH with time for series II phosphorus glasses.

The data shows that the non charge balanced series I glasses have a greater spread of pH values in comparison to the charge balanced, series II glasses. With the series I non-charge balanced glasses the overall pH change decreases with increasing network connectivity i.e. as we move through the series. The charge balanced series II glasses have constant network connectivity through the series, in other words the silicate structure remains constant, in comparison to series I where the silicate network becomes more cross-linked with the addition of phosphorus. It is suggested, therefore, that the more cross-linked glasses in the non-charge balanced series are unable to release sodium and calcium ions into solution as readily therefore resulting in a less alkaline solution and the observed pH decrease through the series.

The series I non-charge balanced series, therefore, has two variables effecting the resultant pH, the phosphorus addition and the cross-linking effect. Series II, in comparison, has a constant silicate structure through the series with only phosphorus addition effecting the resultant pH, hence the smaller spread in pH values.

6.10.2 Summary of SBF pH data for the phosphorus series of glasses

- The pH of the SBF solution was seen to increase with time for both series of glasses.
- Furthermore, this increase was less marked for the higher phosphorus glasses in both series. This can be explained by the release of phosphorus into solution buffering the alkalinity rise.

6.11 X-Ray Diffraction

Figures 49 to 55 show the XRD traces for the non-charge balanced phosphorus series I glasses after immersion in SBF. Each Figure illustrates the original glass's XRD trace, the XRD trace after 4 hours of immersion in SBF, after 16 hours, after 24 hours, after 48 hours, after 1 week and finally after 21 days of immersion in SBF.

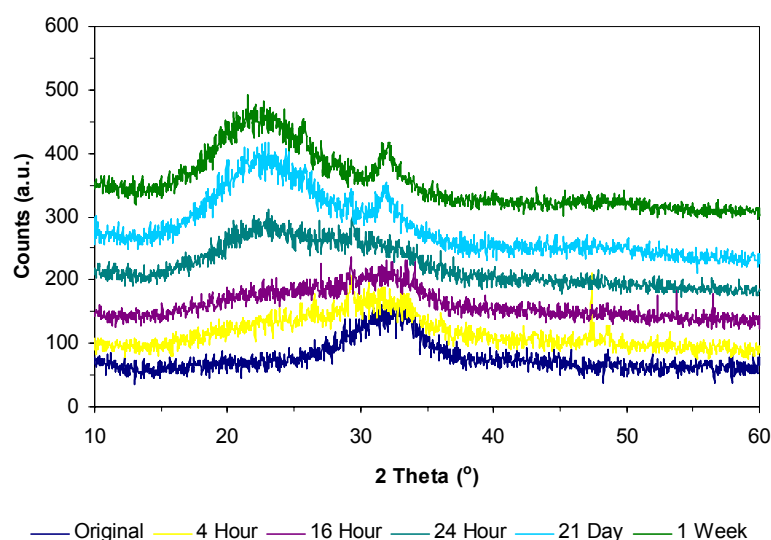


Figure 49: XRD patterns for ICSW1 glass following immersion in SBF for different time periods.

Figure 49 above illustrates that the ICSW1 zero phosphate glass exhibits apatite formation after 21 days. After 16 hours and 24 hours of immersion the spectra only exhibit a flattening of the characteristic amorphous halo. It is therefore suggested that no HCA is formed at these time points.

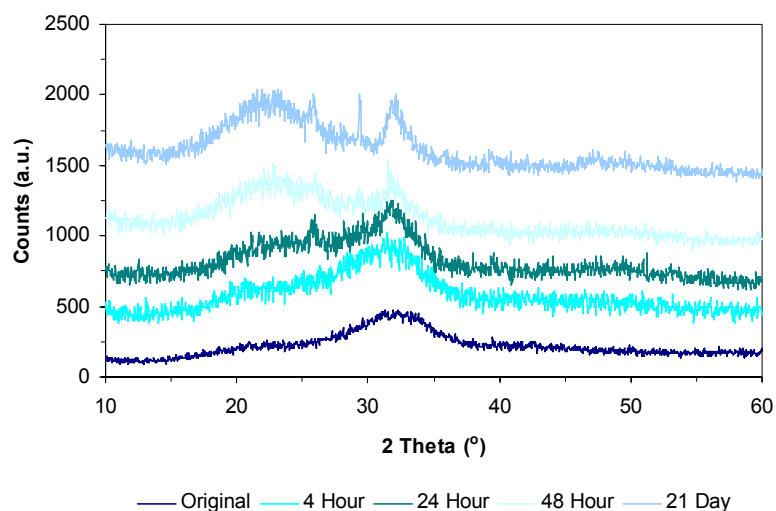


Figure 50: XRD patterns obtained for ICIE1 glass following immersion in SBF for different time periods.

Figure 50 above shows that the ICIE1 glass exhibits apatite formation after 24 hours of immersion in simulated body fluid. The most prominent peaks are at 25.9° and 31.9° and are associated with the (002) and (211) reflections. These peaks become more prominent and sharper in nature with time.

Figure 52 below illustrates the spectra for the ICSW3 glass. This Figure has been replicated below, as Figure 53 to exhibit the Bragg peaks associated with crystalline HCA formation. The Figure clearly shows the formation of apatite with time, with the presence of apatite first seen at 16 hours.

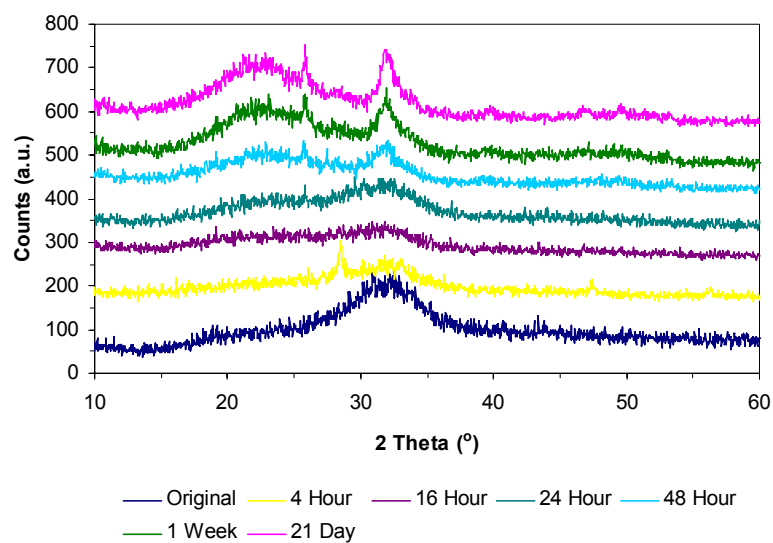


Figure 51: XRD patterns obtained for ICSW2 glass following immersion in SBF for different time periods.

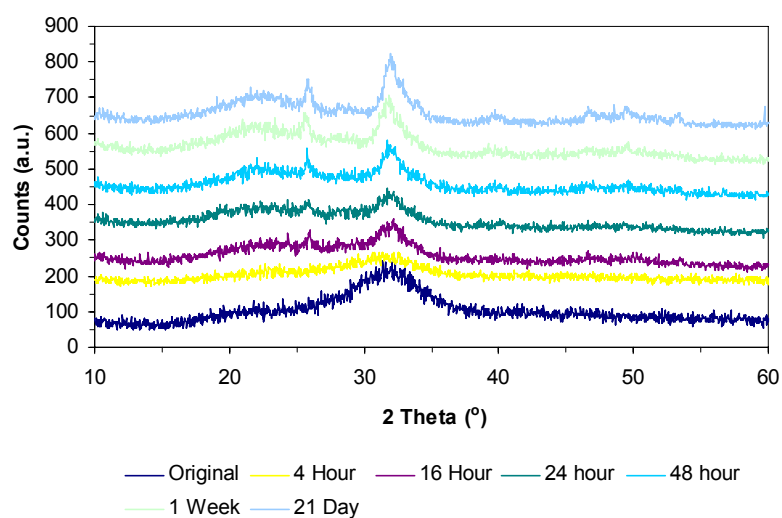


Figure 52: XRD patterns obtained for ICSW3 glass following immersion in SBF for different time periods.

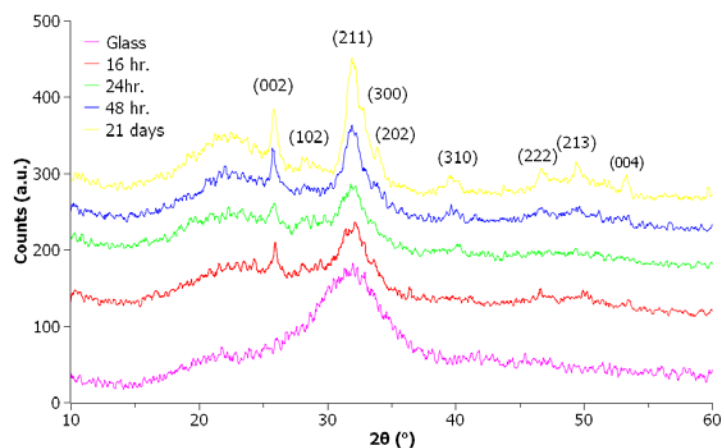


Figure 53: XRD patterns obtained for ICSW3 (as with Figure 54). Exhibits the Bragg peaks associated with crystalline HCA formation.

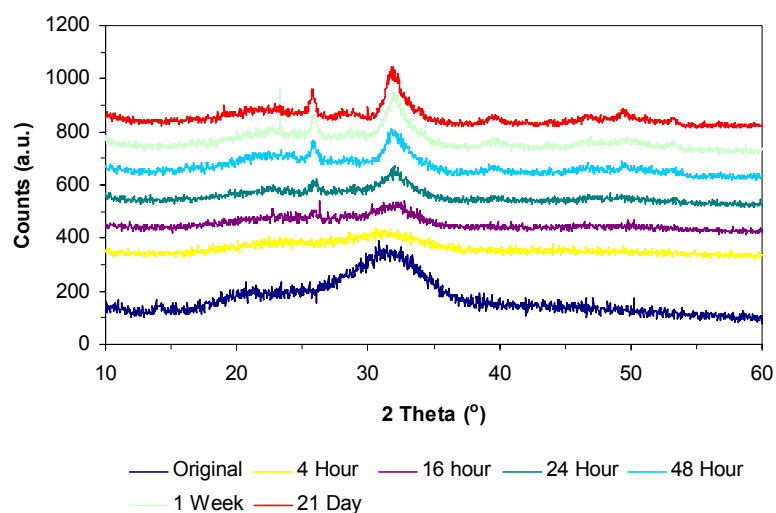


Figure 54: XRD patterns obtained for ICSW5 glass following immersion in SBF for different time periods.

Figure 54 above clearly illustrates that apatite is present in the ICSW5 glass after only 16 hours of immersion.

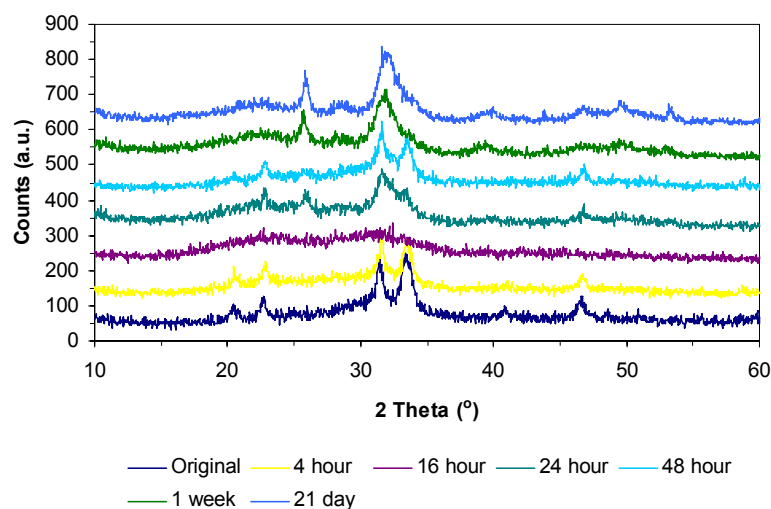


Figure 55: XRD patterns obtained for ICSW4 glass following immersion in SBF for different time periods.

Figure 55 above also illustrates apatite formation in the ICSW4 specimen. It should be noted, however, that from previous X-Ray analysis, ICSW4 is in fact crystalline in nature. Even so, this does not seem to stop apatite formation, but if compared to its nearest counterpart, ICSW5, it seems that the crystalline nature may have stunted the formation of apatite.

Although noisy, the traces clearly show the formation of the Bragg peaks associated with apatite crystallisation with increasing immersion time. The main HCA Bragg peaks are labelled on Figure 53, the spectrum associated with the ICSW3 glass. The most prominent peaks are those at 25.9° and 31.9° , the Bragg peaks corresponding to the (002) and (211) reflections [187] [174]. The region in which the (211) peak is situated, approximately 30 to 35° , also contain a number of additional peaks, in particular those relating to the (112), (300) and (202) reflections [187] [174]. These features, however, are very much masked by the noisy nature of the spectrum and by the significant degree of peak overlap. As a result, crystallite analysis was only undertaken using the (002) peak. Figure 56 shows the resultant crystallite size, calculated using the Scherrer equation from the peak width and position of the (002) peak for each glass in both series, after immersion in SBF for 21 days. There is no real change in crystallite size with increasing phosphate content for the first series of phosphorus glasses, the

non-charge balanced series. Conversely, the crystallite size decreases slightly with increasing phosphate content through the series II phosphorus glasses. The trend, however, is in no way distinct and the actual variation across the series is in fact less than the error in the peak fitting.

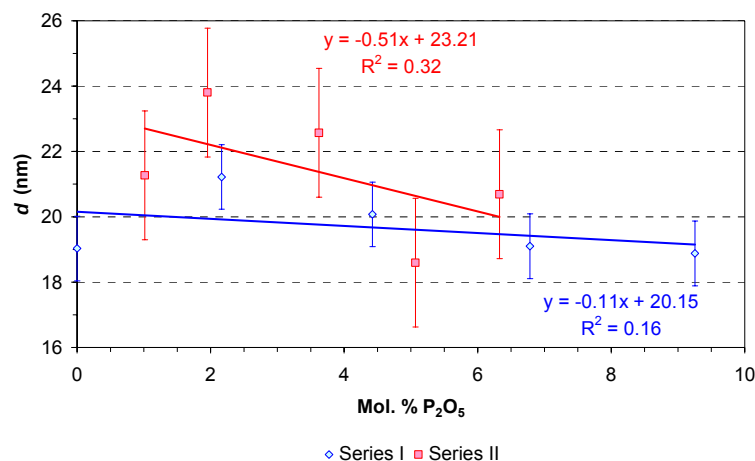


Figure 56: Crystallite size analysis from 002 Bragg peak for both series of glasses.

Comparing the formation of the Bragg peaks associated with apatite crystallisation over time across the series, it is evident that the higher phosphorus containing glasses form apatite quicker than the lower phosphorus containing glasses. This is a surprising result when hypothesising on the basis of the network connectivity model. It would be expected that the cross-linking effect through the series would result in a less degradable glass, which would thus cause less bioactive behaviour. In fact, the opposite is observed. This implies that the addition of phosphorus has a much greater effect on bioactivity than originally thought.

Figures 57 to 61 illustrate the XRD spectra for the charge balanced series of glasses after immersion in SBF.

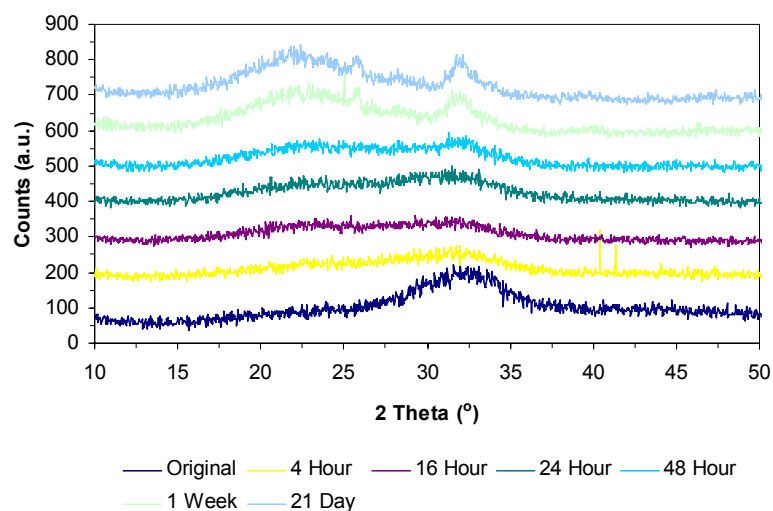


Figure 57: XRD patterns obtained for ICSW6 glass following immersion in SBF for different time periods.

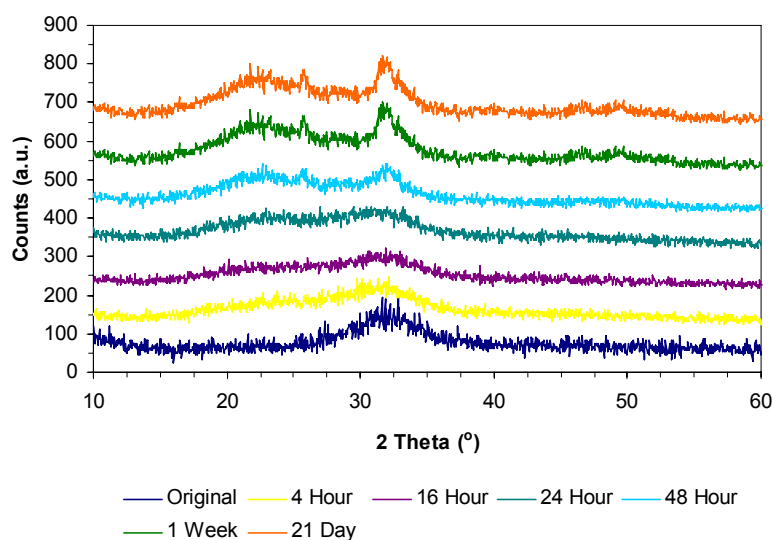


Figure 58: XRD patterns obtained for ICSW7 glass following immersion in SBF for different time periods.

Figure 57 above illustrates that the ICSW6 phosphate glass exhibits apatite formation only after 21 days of immersion. Up until then the spectra only illustrate a flattening of the characteristic amorphous halo.

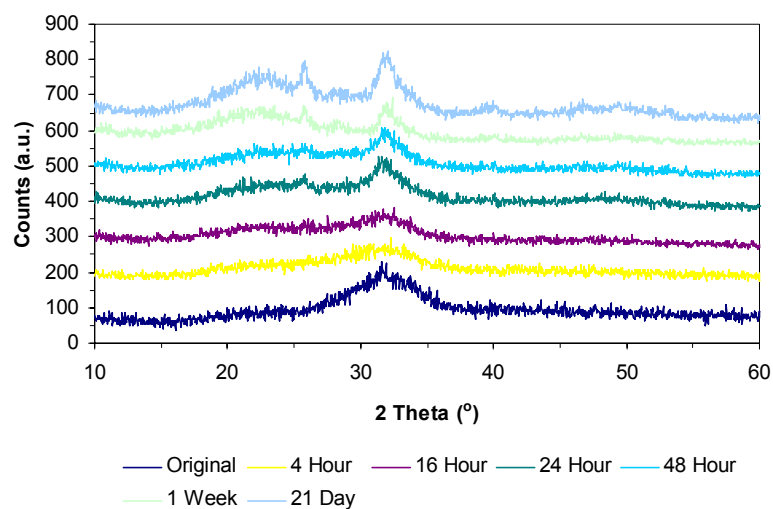


Figure 59: XRD patterns obtained for ICSW8 glass following immersion in SBF for different time periods.

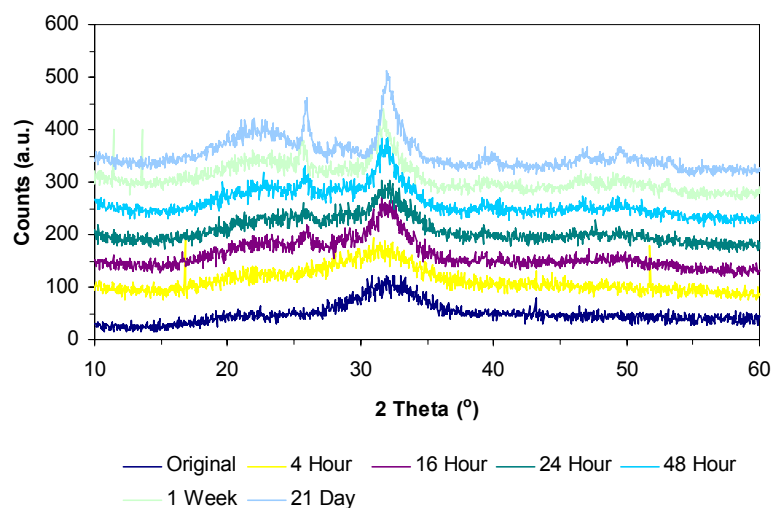


Figure 60: XRD patterns obtained for ICSW10 glass following immersion in SBF for different time periods.

Figure 59 above shows that the ICSW8 glass exhibits apatite formation after 24 hours of immersion in simulated body fluid. The higher phosphorus containing glass, ICSW10, however shows that apatite is formed earlier at 16 hours, as seen in Figure 60 above. Furthermore, this is also observed in Figure 61 below, for the highest phosphorus containing glass, ICSW9. Indeed, it would be interesting to undertake further simulated

body fluid studies and pin point exactly at what time apatite is first formed for this glass.

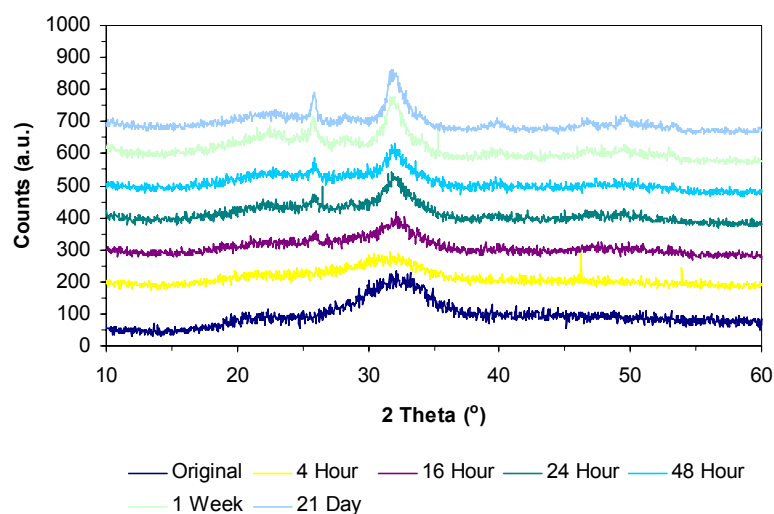


Figure 61: XRD patterns obtained for ICSW9 glass following immersion in SBF for different time periods.

When making a comparison between the XRD spectra for the non charge balanced glasses with those for the charge balanced series, it is immediately evident that the same phenomenon is occurring, that is; the higher phosphorus containing glasses develop the characteristic apatite peaks quicker than their lower phosphate counterparts. Table 14 below tabulates the time at which apatite is first picked up by X-ray diffraction for both series of phosphorus glasses. It can be concluded that phosphorus aids apatite formation in the glasses studied. This trend is unaffected by whether the network connectivity changes with increasing phosphorus content, as in Series I, or whether the silicate network remains constant, as in Series II. It is important to note that the ICSW4 glass in series I does not fit in with this trend. It has already been discussed, however, that this glass is crystalline in nature, and as such will act to inhibit apatite formation thus resulting in the observed decrease in bioactive behaviour in the glass.

Table 14: First time point at which apatite is observed by X-Ray diffraction.

	15 minutes	1 hour	4 hours	16 hours	24 hours	48 hours	1 week	21 days
<i>Series I</i>								
ICSW1							√	√
ICIE1						√	√	√
ICSW2						√	√	√
ICSW3				√	√	√	√	√
ICSW5				√	√	√	√	√
ICSW4							√	√
<i>Series II</i>								
ICSW1							√	√
ICSW6							√	√
ICSW7						√	√	√
ICSW8					√	√	√	√
ICSW10				√	√	√	√	√
ICSW9				√	√	√	√	√

6.11.1 Summary of SBF XRD results for phosphorus series of glasses

- The XRD spectra for both series of glasses illustrated that the higher phosphorus glasses formed apatite in SBF at earlier time points than their lower phosphorus counterparts.
- The cross-linking effect in the series I glasses did not seem to effect the apatite forming ability of the glasses.
- It is therefore considered that the amount of phosphorus in the glasses is more important than first thought.

6.12 FTIR Spectroscopy

As discussed above, one of the indications of whether a glass is bioactive is the formation of a HCA layer on its surface after immersion in SBF. The graphs below illustrate the infrared spectra of each glass after immersion in SBF for 8 time intervals - 15 minutes, 1 hour, 4 hours, 16 hours, 24 hours, 48 hours, 1 week and 21 days.

Table 15 below documents a number of the vibrational modes utilised to determine whether HCA has been formed on a bioactive glass.

Table 15: FTIR vibrational modes used to assess apatite formation [154].

Wavenumber (cm ⁻¹)	Vibrational mode	
1350 – 1080	P = O	Stretch
940 – 860	Si – O – Si	Stretch
890 – 800	C- O	Stretch
1175 – 710	Si – O – Si	Tetrahedral (including Q ² and Q ³ bonding)
610 – 600	P- O	Bend/crystal
560 – 550	P- O	Bend/glass
530 – 515	P- O	Bend/crystal
540 – 415	Si – O – Si	Bend

Chapter 6.4, documented the FTIR spectra for the original phosphorus glasses before immersion in SBF. These are also plotted on Figures 62 to 77 below for comparison with the samples after immersion in SBF. Features associated with the P-O bending mode, Si-O bending vibrations, Si-O (Q²) and Si-O (Q³) of the parent glass are prominent within the spectra.

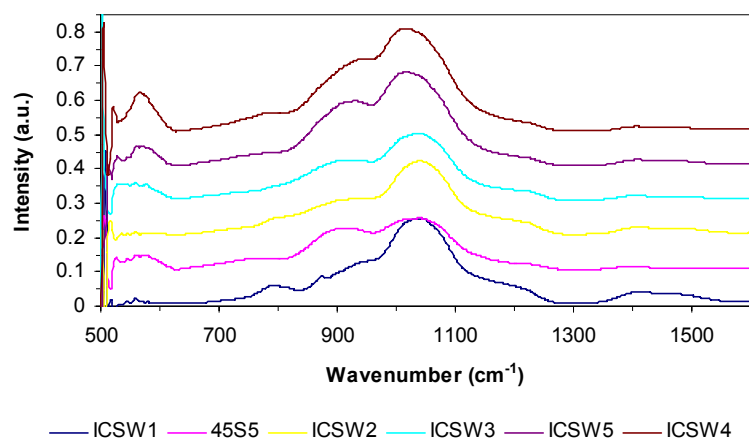


Figure 62: FTIR spectra of series I glasses after 15 minutes of immersion in SBF.

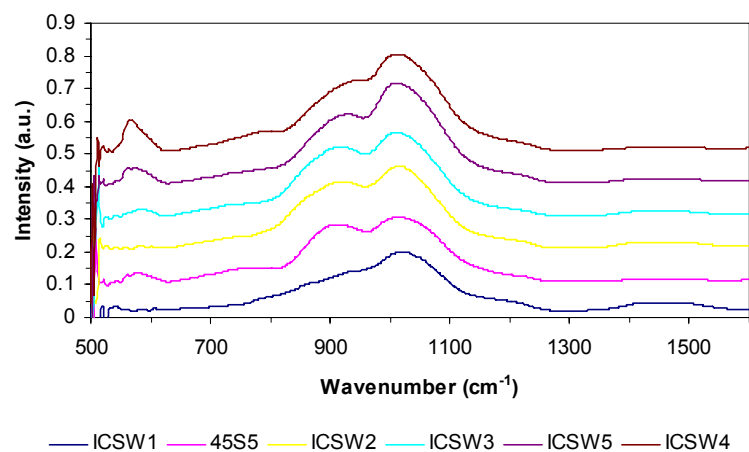


Figure 63: FTIR spectra of series I glasses after 1 hour of immersion in SBF.

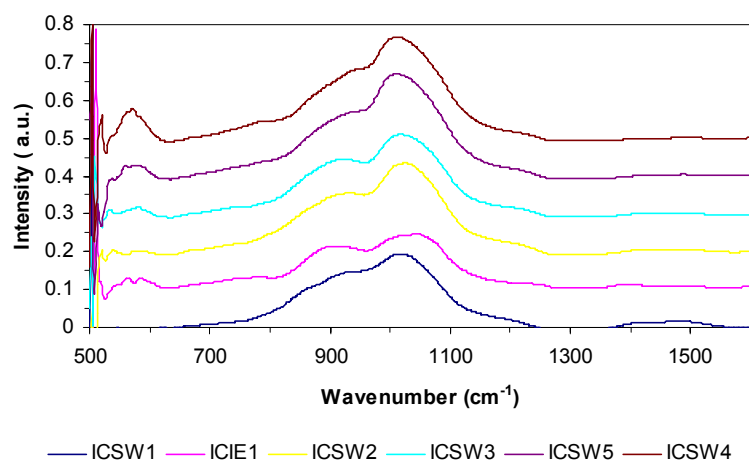


Figure 64: FTIR spectra of series I glasses after 4 hours of immersion in SBF.

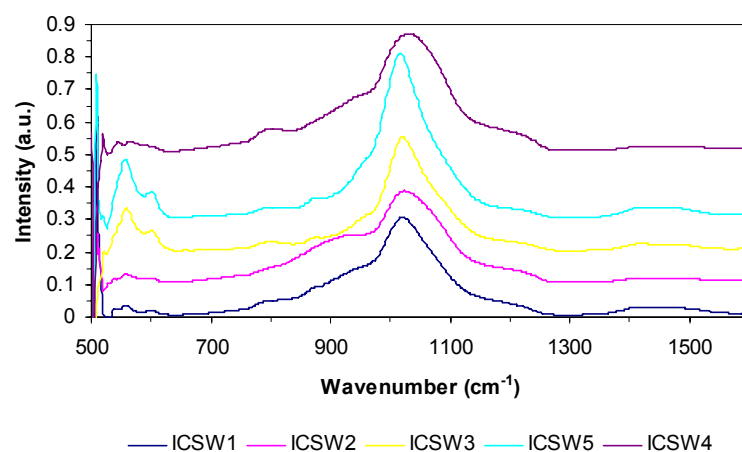


Figure 65: FTIR spectra of series I glasses after 16 hours of immersion in SBF.

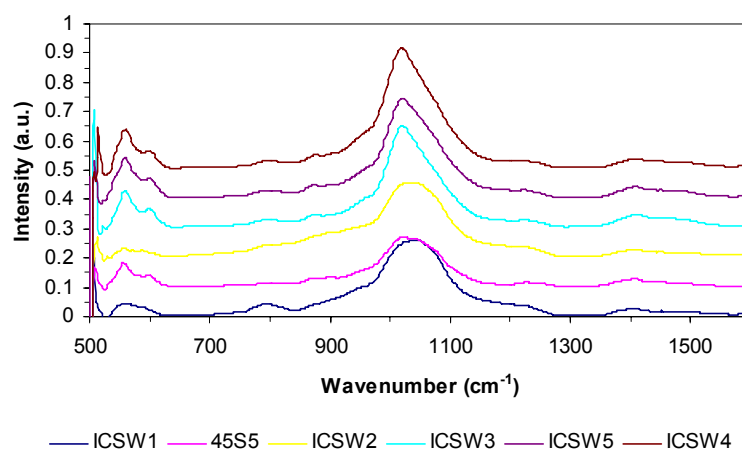


Figure 66: FTIR spectra of series I glasses after 24 hours of immersion in SBF.

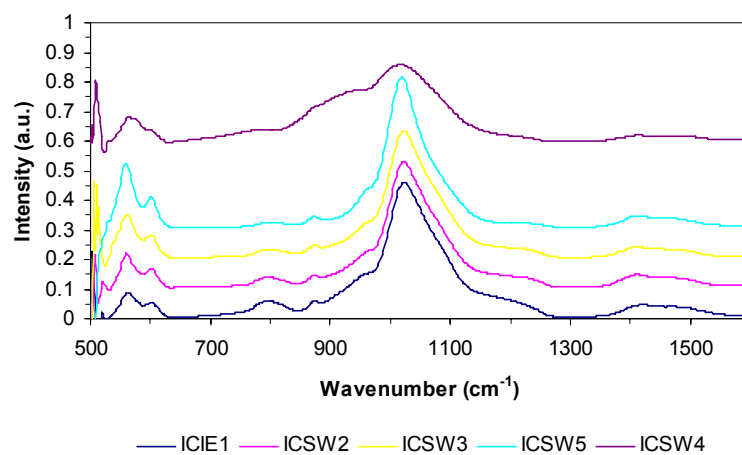


Figure 67: FTIR spectra of series I glasses after 48 hours of immersion in SBF.

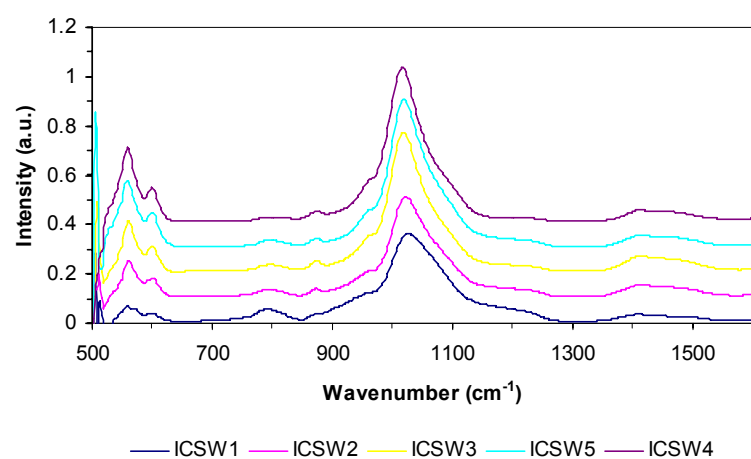


Figure 68: FTIR spectra of series I glasses after 1 week of immersion in SBF.

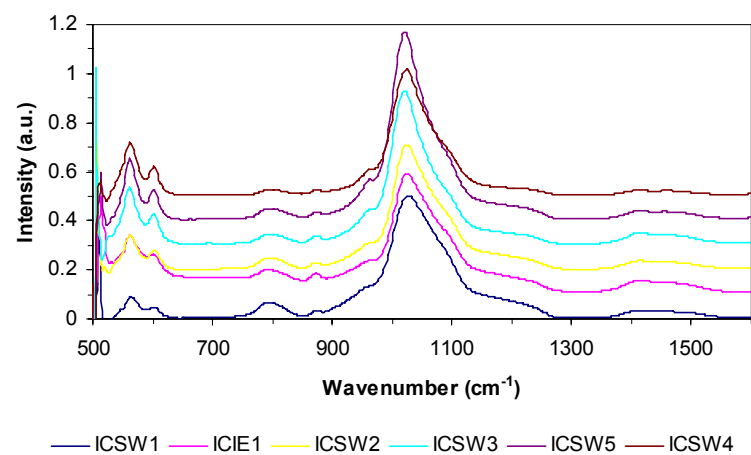


Figure 69: FTIR spectra of series I glasses after 21 days of immersion in SBF.

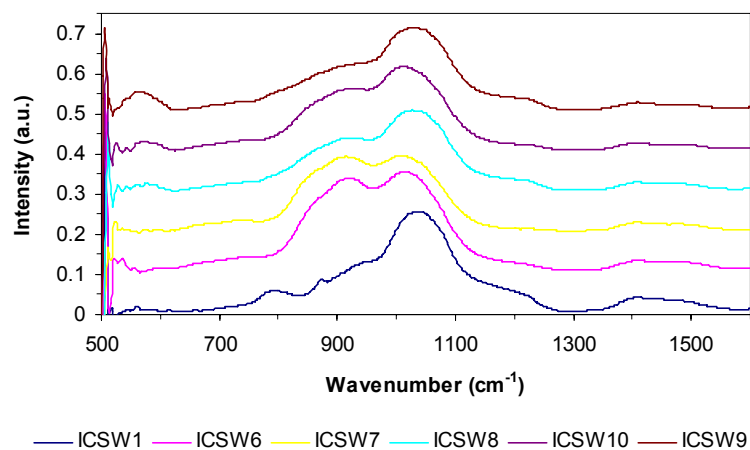


Figure 70: FTIR spectra of series II glasses after 15 minutes of immersion in SBF.

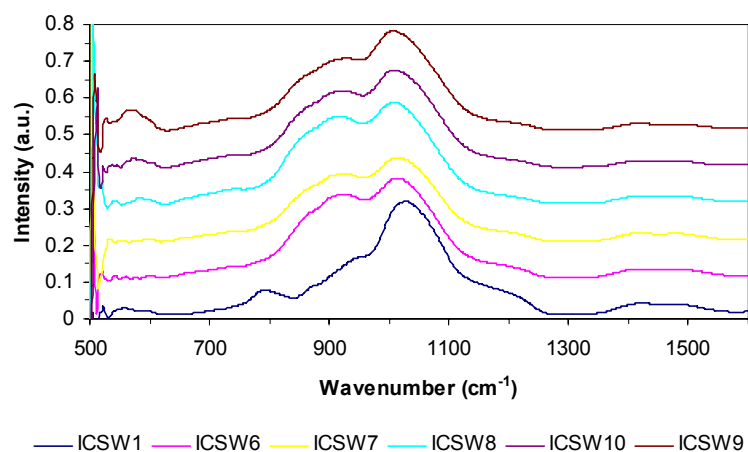


Figure 71: FTIR spectra of series II glasses after 1 hour of immersion in SBF.

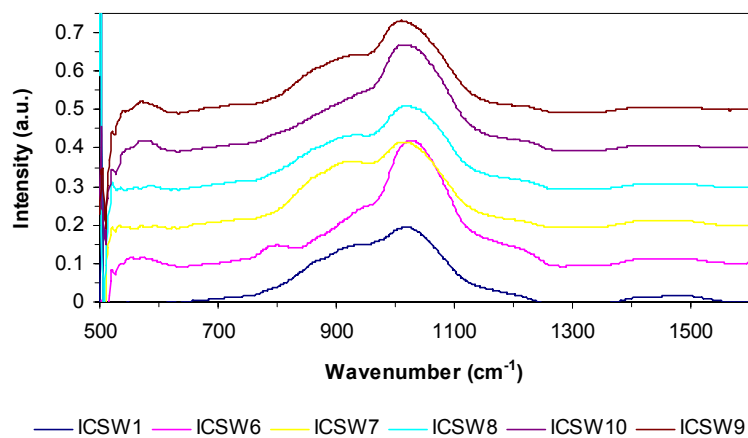


Figure 72: FTIR spectra of series II glasses after 4 hours of immersion in SBF.

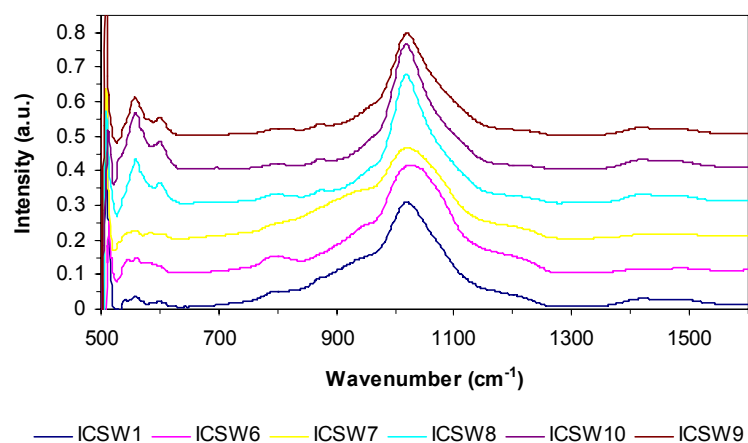


Figure 73: FTIR spectra of series II glasses after 16 hours of immersion in SBF.

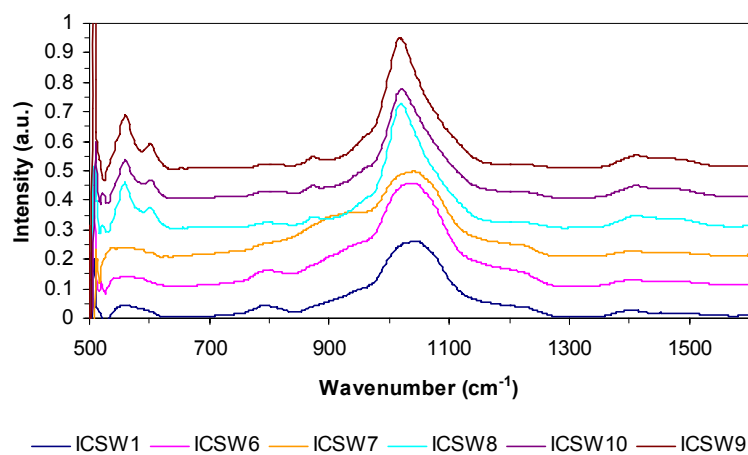


Figure 74: FTIR spectra of series II glasses after 24 hours of immersion in SBF.

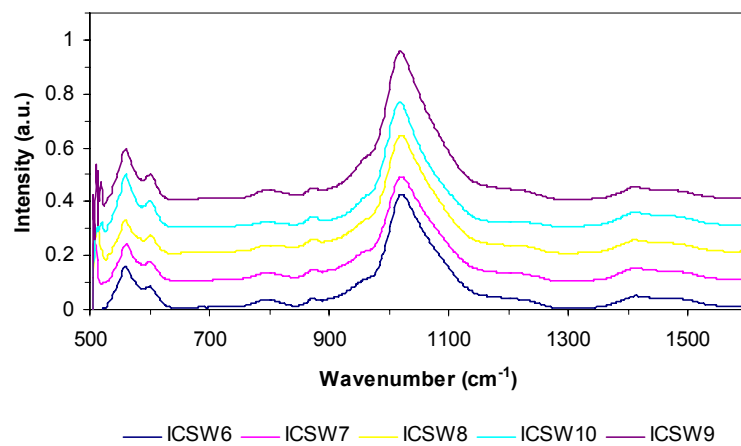


Figure 75: FTIR spectra of series II glasses after 48 hours of immersion in SBF.

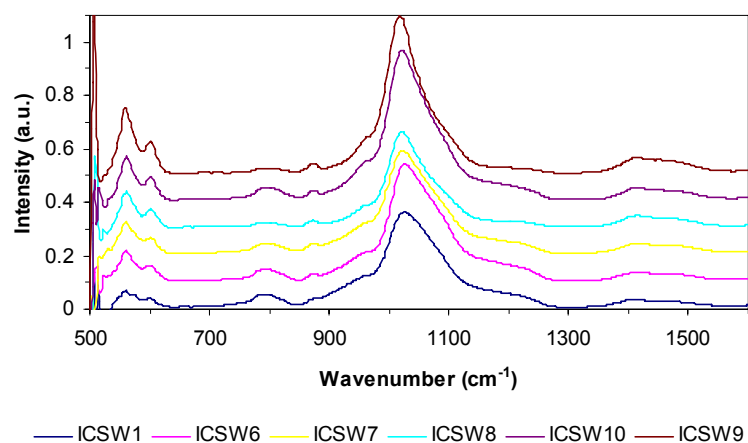


Figure 76: FTIR spectra of series II glasses after 1 week of immersion in SBF.

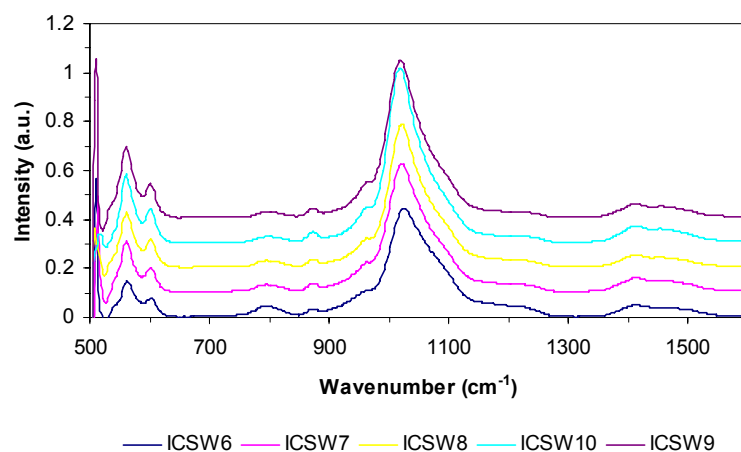


Figure 77: FTIR spectra of series II glasses after 21 days of immersion in SBF.

Figure 78 below illustrates the 3D contour plot for ICSW10. This clearly shows the evolution of the characteristic peaks connected to HCA growth. On close examination, the vibrations associated with the main silicate glass network decrease in intensity with immersion time and the bands associated with the formation of crystalline hydroxyl-carbonate apatite become more prominent.

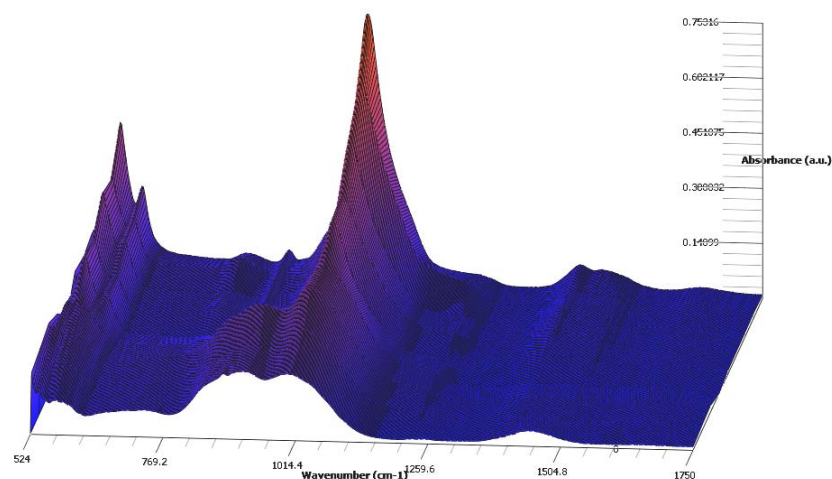


Figure 78: 3D contour plot of ICSW10 glass (time axis not linear)

After only a short period of immersion, up to approximately 4 hours, the formation of non-bridging oxygens as a result of network dissolution is not significant enough to observe any changes to the Si-O (Q^2) band intensity. Therefore, Hench's first two stages associated with 1) ionic exchange and 2) hydrolysis of the vitreous network are not picked up by the FTIR spectra [188]. Furthermore, after only a short period of immersion in SBF the plot (along with all the other glasses) illustrate only a single amorphous P-O peak at approximately 600 cm^{-1} [78]. This peak represents the presence of either octocalcium phosphate (OCP) or amorphous calcium phosphate (ACP), both of which are precursors to hydroxyapatite [189].

After a longer immersion time of approximately 4 hours however, the intensity of the Si-O (Q^3) band increases and the Si-O (Q^2) band decreases until it eventually disappears. This is indicative of the recondensation and repolymerisation of the SiO_2 layer, as predicted in Hench's model and is clear evidence to illustrate the initiation of bioactive behaviour. Furthermore, as the immersion time increases the spectra illustrates, in particular, the gradual sharpening and increase in intensity of the P-O bend and P-O stretch at approximately 580 and 1010 cm^{-1} respectively [154] [175].

The characteristic splitting of the P-O bend (into bands at 560 and 600 cm^{-1}) which is indicative of crystalline apatite formation, is also extremely prominent [190]. Furthermore, after exposure to SBF, the appearance of specific carbonate bands in particular the C-O stretch (ν_2) at approximately 860 cm^{-1} and C-O asymmetric stretching (ν_3) at 1410 and 1450 cm^{-1} respectively [154] [191] [192] is further evidence for crystalline hydroxyl-carbonate-apatite formation. With time, these carbonate bands increase in sharpness and intensity. This is as a result of carbonate being incorporated into the apatite layer to form HCA or could also be a result of calcium-carbonate deposition [193].

On examination of the glasses after 21 days of immersion in SBF it is evident that the carbonate band at approximately 860 cm^{-1} shifts to a higher wavenumber as the content of phosphorus increases through series I. In comparison, as the phosphorus content is increased through the charge balanced series II, although the carbonate band moves to a higher wavenumber initially, it gradually levels off with the higher phosphorus containing glasses (at approximately 4mol%). Furthermore, the Si-O bending vibration at approximately 800 cm^{-1} reappears at higher wavenumber, as also observed by Serra *et al*, after immersion in SBF [194]. Also there is a symmetric stretching vibration mode of the Si-O bond in the SiO_4 tetrahedron near 796 cm^{-1} [188]. Both suggest some degree of repolymerisation and could be an indication of possible condensation of the silica gel formed in SBF, and the third step of Hench's model (condensation and repolymerisation). Furthermore, the silica gel is also characterised by the Si-O-Si band of glass which becomes finer, however this is not evident in these particular spectra, and by the Si-OH stretching vibration mode near 952 cm^{-1} [188].

Table 16: Time for crystalline apatite formation observed by splitting of the FTIR P-O bending mode at around 550cm^{-1} (ICSW4 sample partially crystalline) [195, 196].

ID	Mol. % P_2O_5	Time
<i>Series I</i>		
ICSW1	0.00	1 week
ICSW2	2.16	2 days
ICSW3	4.42	16 hours
ICSW5	6.78	16 hours
ICSW4	9.25	1 day
<i>Series II</i>		
ICSW6	1.02	2 days
ICSW7	1.95	2 days
ICSW8	3.62	16 hours
ICSW10	5.07	16 hours
ICSW9	6.33	16 hours

Examining all the spectra as a whole it is obvious that the higher phosphorus containing glasses seem to show the characteristic bands associated with the formation of HCA at earlier time points thus suggesting that these glasses are more bioactive. Indeed, Table 16 summarises the time interval at which the characteristic splitting of the P-O bending mode at approximately 550cm^{-1} is first seen, with any degree of certainty. This table shows that, as a general trend, apatite formation is faster the more phosphorus there is in the parent glass, irrespective of the series thus irrespective of whether network connectivity increases (as in series I non charge balanced series) or if it stays the same (as in series II charge balanced series). It is important to note, however, that the highest phosphorus containing glass, ICSW4 does not fit in with this trend. It has already been discussed that this glass is in fact crystalline, as is evident from previous XRD and NMR spectra. The crystalline nature of ICSW4 therefore accounts for the slower rate of apatite formation.

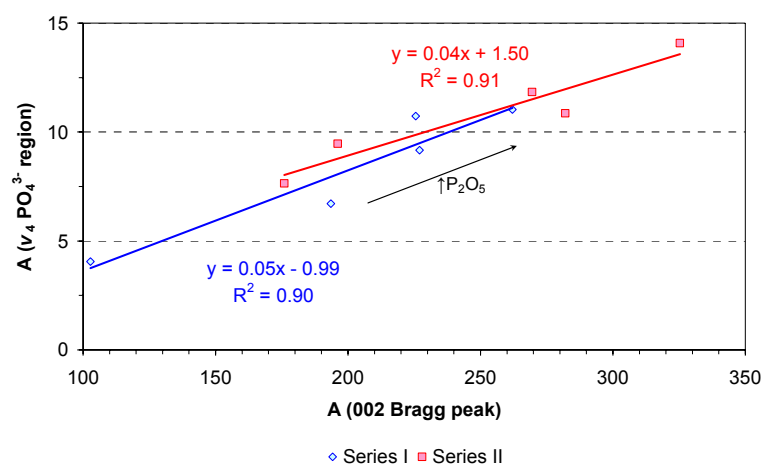


Figure 79: Area of ν_4 phosphate FTIR region plotted against (002) Bragg peak area from XRD after 21 days in SBF.

Figure 79 illustrates the relationship between the area of the P-O bending mode, otherwise known as the ν_4 phosphate region, with the area of the (002) Bragg reflection from the XRD data presented in the previous chapter. There is a linear relationship between the two areas, with both areas increasing with phosphate content. Thus, once again, this general trend is more evidence to suggest that increasing phosphate content results in increasing bioactivity. Figure 79 plots the areas for both series of glasses and the same trend is seen. This suggests that phosphorus is more important for mineralisation than first thought and is less affected by the parent glass's network connectivity. Elgayer *et al.* [78] studied the importance of network connectivity on apatite formation, concluding that the network connectivity of the glass is the determining feature for a glass's bioactivity [193]. Although this is not disputed, these spectra seem to suggest that network connectivity should not be the only feature considered when predicting bioactivity in a soda lime phosphosilicate glass – the amount of phosphorus is extremely influential.

As a definitive display of the effect of phosphorus content on apatite formation, the FTIR spectra of 45S5 parent glass and 45S5 after immersion in SBF for 24 hours is plotted for a comparison with the FTIR spectra for the high phosphorus content parent glass ICSW9 and ICSW9 after immersion in SBF for 24 hours in Figure 80. It can

clearly be seen that the apatite formed on ICSW9 glass is highly crystalline with intense, narrow, split peaks in the 550cm^{-1} region, whereas the phosphate peak can hardly be resolved from background in 45S5. This is strong evidence to illustrate the superior bioactive characteristics of the higher phosphate containing glasses.

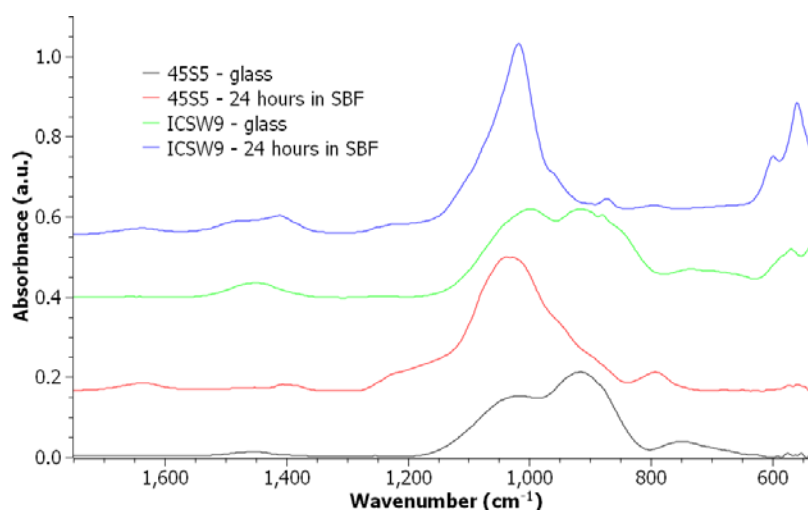


Figure 80: Comparison of ICSW9 and 45S5 Bioglass® by FTIR after 24 hours in SBF.

6.12.1 Summary of SBF FTIR results for the phosphorus series of glasses

- The FTIR spectra for the higher phosphate glasses illustrated the characteristic peaks associated with apatite formation at earlier time points compared to the lower phosphorus containing glasses.
- It is therefore evident that phosphorus aids apatite deposition in bioactive glasses.
- The polymerising effect in the non charge balanced series did not seem to effect the time at which apatite was formed.

6.13 Summary

As discussed previously, the role of phosphorus within a bioactive glass system is highly controversial. Although it forms part of the original bioactive glass composition,

45S5, no real attempt has been made to methodically and systematically analyse its role within a glass structure and, as such, explain its function in, or contribution to, bioactivity.

Following Hench's model, it is thought that phosphorus must be present in the implant material for the implant to be able to bond to living tissue [97] [197]. It is hypothesised by Hench that it plays an important role, in that it forms part of the silica and CaO-P₂O₅ rich bilayer following the leaching of sodium and calcium ions from the glass. In turn, this surface bilayer promotes the HCA layer necessary for the tissue to bond to the implant.

This model, however, is contrary to, and is unable to predict, the experimentally observed bioactive behaviour of phosphorus free glass systems, such as amorphous wollastonite [121]. This experimentally observed bioactivity, however, illustrates that the nucleation and crystallisation of apatite on the implant surface can proceed by simply incorporating the phosphate species from the physiological fluid [15]. Following such an observation the effect of phosphorus on the SiO₂-CaO system was intensively analysed [102] [103] [139] [138] with Ebisawa *et al.* [138] and Ohura *et al.* [139] both observing the increased formation of a bioactive surface layer with the addition of P₂O₅. It was proposed that, although not essential to the mechanism of bioactivity, the presence of phosphorus within a glass system would act to aid HA deposition and the resulting bone bonding ability of the resultant glass. Ebisawa *et al.* [121] equated this phenomenon to the additional phosphorus leaching from the glass into solution and increasing the local supersaturation thus accelerating the HA precipitation. Karlsson *et al.* [198] suggested it would additionally act to buffer the local pH at the glass surface thus preventing excessive acidity, which is known to be detrimental to bone bonding. By contrast, however, some phenomenological models [102] have shown that even the smallest amounts of phosphorus act to decrease bioactive behaviour. Furthermore, it is interesting to note that when P₂O₅ is the main glass former, at percentages in the region of 45 to 50mol%, there is no evidence of any bioactive behaviour [188].

Such a wide range of analysis on differing glass systems, resulting in a diversity of experimental data and resulting conclusions has led to the ambiguous and confusing assumptions of phosphorus's role in bioactive glass systems. One of this study's objectives was to investigate the structural and bioactive role of phosphorus within the quaternary $\text{SiO}_2\text{-CaO-Na}_2\text{O-P}_2\text{O}_5$ system with the ultimate aim of clarifying phosphorus's role in bioactivity and incorporating it within the inorganic polymer and network connectivity model.

As discussed previously, the inorganic polymer and network connectivity model has been used to predict the bioactivity of glasses in the ternary $\text{SiO}_2\text{-CaO-Na}_2\text{O}$ system with great affect. It does not, however, provide an adequate description when taking into account the addition of P_2O_5 . As discussed by Wallace *et al.* [43], phosphorus is assumed to undertake a tetrahedral configuration within the silicate glass network under the inorganic polymer model, (with one of the oxygen atoms double bonded to the central phosphorus atom). As such, using the network connectivity calculation, increasing the amount of phosphorus within a system would result in a decrease in bioactivity. This assumption has been shown to be incorrect in previous studies, with MAS NMR indicating that phosphorus does not co-polymerise with the silicate portions of the glass but forms phosphate rich regions within the glass [199] [200] [78] [10]. This has been further recognised by recent molecular dynamics studies [201]. Although these studies recognise that the assumption that phosphorus takes part within the silicate network is incorrect, there remains differing theorising as to the existence of phase separation within the glass systems. For example Lockyer *et al.* [10] assume that the presence of phosphorus as an orthophosphate type species is due to the effects of annealing and, although they noted no evidence of phase separation during their work, suggest that glass-in-glass immiscibility may exist on a microscopic level [43]. Conversely Elgayer *et al.* [78] theorise that, as a result of dielectric data indicating the presence of two relaxation processes corresponding to alkali ion hopping in two phases bioactive glasses undergo amorphous phase separation into silicate rich and orthophosphate rich phases.

In order to definitively conclude on the structural arrangement of phosphorus, the assumption that phosphorus dwells within an orthophosphate environment provided the basis for the design of the phosphorus containing soda lime silicate glasses studied. To test this hypothesis, two series of phosphorus glasses were designed – firstly a straight substitution of P_2O_5 for $2SiO_2$, with a constant $Na_2O:CaO$ ratio, (with the resulting polymerisation of the network) and secondly a charge compensated series (keeping the network polymerisation constant).

^{29}Si MAS NMR of the series I glasses exhibited an increase in peak asymmetry through the series – a result of two overlapping resonances assigned to Q^2 and Q^3 species, with the later increasing with P_2O_5 addition. The asymmetric peak shift in the ^{29}Si spectra towards lower frequencies, as the phosphorus content increased, was attributed to an increase in the average degree of polymerisation of the silicate network. This, therefore, implied the network polymerising role of phosphorus, and the existence of an orthophosphate species. There was no evidence of any disruption of the glass network from the ^{29}Si spectra thus eliminating the idea of phosphorus acting as a former and entering the silicate glass network. ^{31}P MAS NMR spectra of the series I glasses clearly illustrated single resonances for all spectra centred on chemical shift values associated with those of an orthophosphate environment, providing direct evidence of the existence of phosphorus within such an environment, rather than the conventional view of phosphorus entering the network.

^{29}Si MAS NMR spectra of the series II glasses resulted in a series of single resonances all centred on a chemical shift associated with a Q^2 glass. No evidence of any shoulders or peak shift was observed therefore implying that, on addition of phosphorus, the silicate network remained unchanged as a result of the addition of charge compensating cations. The ^{31}P spectra also consisted of a series of single resonances all centred on a chemical shift associated with that of an orthophosphate environment.

It can therefore be confirmed from both series that, within the test glasses studied, phosphorus exists as an orthophosphate complex rather than entering the silicate glass network as traditionally thought.

XRD spectra confirmed the amorphous nature of all of the glasses studied. The exception to this was the ICSW4 glass with the highest network connectivity of 2.63, which was the only glass to exhibit any asymmetry in the ^{31}P MAS NMR data. This asymmetry indicated the presence of a Q^1 phosphate species, in addition to the Q^0 orthophosphate species exhibited in the other glasses in both series. Previous studies [160] [202] [203] have highlighted the existence of pyrophosphate species as well as orthophosphate type species in glasses of similar compositions to those studied. Three phases crystallised from the heat treated ICSW4 sample: sodium calcium orthophosphate, sodium pyrophosphate and combeite and these results proved consistent with the ^{29}Si and ^{31}P MAS NMR chemical shifts experimentally obtained. This therefore suggests that soda lime phosphosilicate glasses with high network connectivity values may have a preference to form a small fraction of Q^1 (or $\text{Si-O-P}(\text{-O}_3)$) pyrophosphate species (where one of the four tetrahedral oxygens forms a bridge with an adjacent silicon atom) which coexist with the remaining majority of orthophosphate type species [15] [202] [203]. Molecular dynamics analysis performed by Tilocca and Cormack [201] on phosphosilicate glass systems resulted in similar conclusions. Furthermore, Tilocca *et al.* also, as discussed previously, assign the polymerisation of the silicate network, with increasing phosphorus content, to the higher affinity of the sodium and calcium ions to the phosphate groups, which are able to strip the cations from their modifying role within the silicate network resulting in the observed repolymerisation of the network [201]. However, Tilocca and Cormack also highlight a further factor that results in the observed increase in network connectivity of the silicate phase hypothesising that the phosphorus in the glass can replace the sodium and calcium ions in their charge balancing role therefore suggesting that the cross-linking of the silicate network can occur by either the formation of new Si-O-Si or Si-O-P links [201] [15].

Taking into account that phosphorus exists in orthophosphate environment provided the basis for modifications to Doweidar's density model which resulted in an improved correlation with the actual experimental density values obtained. Furthermore, the experimental densities were seen to increase more rapidly than expected, with increasing phosphorus content, thus indicating the presence of a higher density component such as a pyrophosphate type species, for example in the ICSW4 glass. Theoretical thermal expansion coefficients (based on Appen values) also illustrated a good match to experimental values. This can be explained by the increase in the amount of a separate phosphate phase, with P_2O_5 addition, containing weaker P-O bonds compared to the Si-O bonds in the silicate phase, thus resulting in the "composite" glass expanding more with temperature. The glass transition temperature decreased with phosphate addition for both series thus suggesting that the separate phosphate phase has a strong effect on the thermal properties of the resultant glass. The softening temperatures were seen to increase with phosphorus content for both series, however, which contradicts this observed trend in glass transition temperature. This, however, could provide further evidence to support the existence of a composite glass transition temperature as a result of a phase separated structure.

Although the physical analysis of the phosphorus containing glasses was in no way as clear cut compared to that of the MAS NMR analysis, the results still illustrated the existence of a phase separated structure. It is suggested that further analysis would need to be undertaken before a proper understanding of the effect of phase separation on the physical properties of the resultant glasses could be understood, especially to enable a glass to be tailored to a specific application based upon the understanding of phase separation alone.

The evidence for the existence of phosphorus within an orthophosphate environment and the phase separation into phosphate rich clusters has significant implications with respect to the bioactive properties of the glass. The Hench model assumes congruent dissolution, which on reflection would be highly improbable following the discovery that the glasses undergo phase separation into a silicate rich phase and a phosphate rich

phase. It was initially hypothesised that the orthophosphate rich phase would be extremely soluble when exposed to water and thus dissolve much more rapidly than the silicate phase therefore effectively escalating the level of phosphorus super saturation of the external environment. This would result in an overall increase in the rate of apatite deposition on the glass surface [43]. The effect of the corresponding polymerisation of the silicate network on the ability of the glass sample to degrade into solution, however, was not discounted and it was this factor that was thought to be the rate determining step for bioactivity. Therefore, before bioactive studies were undertaken it was initially hypothesised that the first series, with the polymerisation of the silicate network through the series, would result in a decrease in bioactivity. Conversely, it was hypothesised, that the constant network connectivity values through the second series would result in either similar bioactive glasses if the effect of the polymerisation of the network was rate determining, or an increase in bioactivity due to the increase in phosphorus dissolution through the series if the effect of silicate polymerisation was to play a minimal role.

Dissolution studies on the two series of glasses illustrated the formation of apatite within the 21 days of immersion in simulated body fluid. From both FTIR and XRD studies, it was definitively shown that, as the phosphorus content increased, the rate of formation of apatite increased. This phenomenon was observed across both series. This clearly indicates that the phosphate content is more important than the network connectivity value of the silicate phase on the bioactivity of the resultant glass, in the range of glasses studied. This assumption, however, is dependant on the presence of solely orthophosphate species as ICSW4 was clearly observed not to fit in with the over-riding trends. It was known however, as previously discussed, that the sample was partially crystalline and it is suggested that the crystallinity would result in a slower rate of apatite formation.

It is hypothesised that the increased rate of apatite formation with increasing phosphate content, irrespective of the network connectivity of the silicate phase, can be explained as a result of phase morphology. For example, if we consider a phase separated glass

such as Pyrex, a glass which contains sodium borate rich droplets surrounded in a silica rich matrix, the sodium borate rich droplets at the glass surface preferentially degrade when exposed to a corrosive medium, leaving those in the bulk unaffected. A comparable structure could be imagined in the phosphorus containing glasses studied. Furthermore, the presence of orthophosphate groups in isolated nanometre size phases may promote a microstructure with areas where the chemical connection of the glass structure is disrupted. As suggested by Aguiar *et al.* [84] [157] (however with respect to nano crystalline phosphate phases) these weakened areas could provide preferential sites for chemical sites in SBF. Similarly Vycor[®], a further phase separated borosilicate glass also exhibits preferential dissolution. Vycor[®] however exists as a borate phase interconnected with a silicate phase and the glass can be easily etched to form a porous structure. It is, therefore, easy to consider that the phosphorus containing glasses may exist in an analogous structure with an interconnected orthophosphate morphology resulting in continuous leaching on exposure to SBF resulting in the enhanced apatite formation kinetics observed.

As an extension to the molecular dynamic simulations of phosphosilicate glass structures Tilocca *et al.* [12] discussed the resultant effect on bioactivity drawing similar conclusions to those above. The conclusion was also drawn that a balancing effect is present between the high phosphorus containing glasses (with high network connectivity values), forming Si-O-P bonds, thus reducing the bioactivity, versus the low phosphorus containing glasses, with phosphorus in the form of free orthophosphate type species, whose relatively fast initial release act to accelerate the deposition of hydroxyapatite and enhance the bioactive behaviour [15]. As commented, the balance between these opposite effects will ultimately determine the bioactivity of the phosphorus containing glass [15]. It is remarkable that from the bioactive data of the compositions modelled, Tilocca and Cormack concluded that the positive effect on bioactivity occurs only for low mole percentages of phosphorus and the negative effect only for compositions with phosphorus in the region of 10mol% [201]. This sits perfectly with the experimental data produced in this study. It is further suggested that, as a result of the formation of the Si-O-P linkages, there is a preference for the

formation of crystalline phases thus explaining the reason why glasses containing more than 10mol% phosphorus were unable to be formed.

7 Results and Discussion – Magnesium Substituted Glasses

7.1 ^{31}P MAS NMR

7.1.1 ^{31}P MAS NMR of ICIE1 High Sodium Magnesium Glasses

The ^{31}P MAS NMR spectra show a single symmetric resonance, as shown in Figure 80, with a chemical shift range in the region 8.5 to 12.7ppm and with an approximate constant linewidth of *ca.* 29ppm.

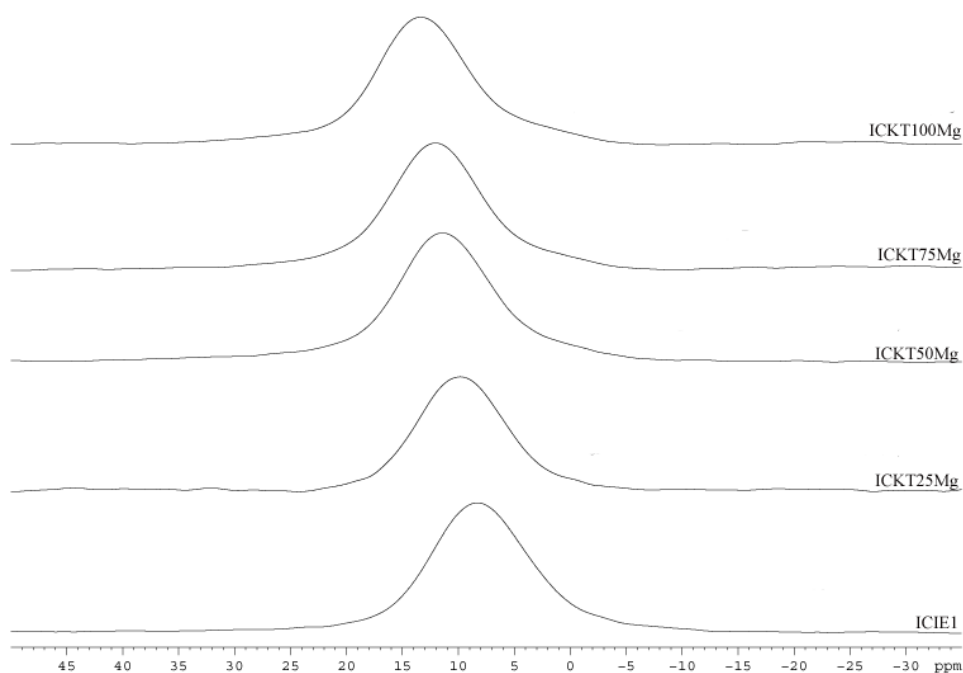


Figure 81: ^{31}P spectra for the ICIE1 magnesium substituted series of glasses

Table 17: NMR data for ^{31}P NMR spectra of the series of magnesium containing bioactive glasses where w corresponds to peak width.

Glass	^{31}P peak (ppm)	^{31}P w (Hz)
ICIE1	8.2	687
25Mg	9.7	526
50Mg	10.5	639
75Mg	11.7	642
100Mg	12.7	642

The ^{31}P NMR chemical shifts for the ICIE1 magnesium substituted series correspond to the existence of an orthophosphate environment with no evidence of the existence of Si-O-P bonds agreeing with the findings of Lockyer *et al.* [10] and Elgayar *et al.* [14]. Previously phosphorus was assumed to enter the glass network forming Si-O-P bonds [204]. However, the absence of peaks ca. -215ppm in the ^{29}Si MAS NMR and ca. -33 to -40ppm in the ^{31}P MAS NMR spectrum rule this out [205]. Furthermore, the experimental observation of the orthophosphate arrangement along with the fact that the glasses are optically clear suggest that the phosphorus in the glass may have undergone amorphous phase separation into a sub-micron (below light scattering dimensions) phosphate phase.

Initially we observe the ICIE1 glass to have a chemical shift of ca. 8.2ppm between that of a $\text{Na}_3(\text{PO}_4)$ species at 14ppm [206] and a $\text{Ca}_3(\text{PO}_4)_2$ species at 0ppm or 3ppm [206] [159], thus implying that both types of modifier ion are associated with the orthophosphate unit. As we move through the series, it would be expected that substituting magnesium for calcium would simply result in the direct replacement in the phosphate environment of Ca for Mg as reported by Oliviera *et al.* [137], or in the case of Lockyer *et al.* [10] and Elgayar *et al.* [14], a mixed, non preferential, two cation orthophosphate at a ratio consistent with that of the overall glass. These resultant chemical shifts however are not evident.

Although Mg^{2+} (1.31) is slightly more electronegative on the Pauling scale, than Ca^{2+} (1.00) and Na^+ (0.93), the observed experimental shift is contrary to what is expected [10], with the final, fully substituted composition shifting to a more positive (away from 0ppm) region of the spectra *ca.* 12.7ppm – close to that associated with a pure sodium orthophosphate species.

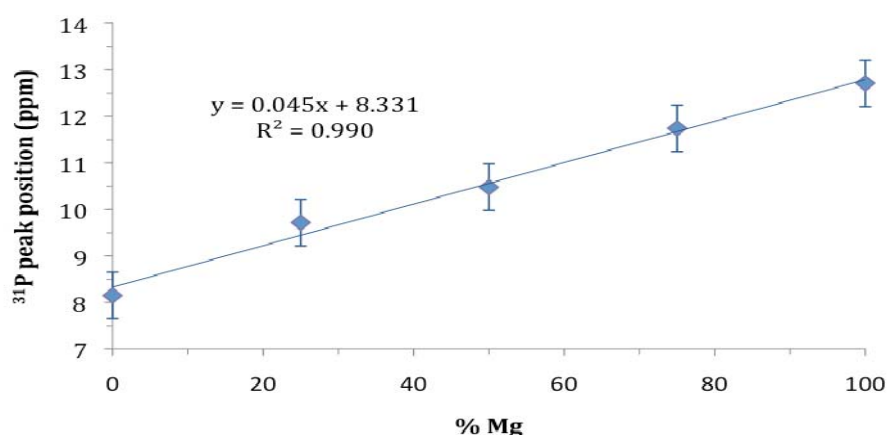


Figure 82: The effect of ^{31}P orthophosphate (Q^0) shift with increase in magnesium content (while sodium content remains constant). Illustrates the progressive preferential association of sodium with the phosphate through the series. Line indicates a linear fit.

This positive shift (away from 0ppm – Figure 82), towards a sodium orthophosphate type species and away from magnesium phosphate (0.5ppm for the amorphous $\text{Mg}_3(\text{PO}_4)_2$ form [207] [208]), implies that as magnesium is added, at the expense of calcium, sodium preferentially associates with the phosphate phase. It appears there is only a minimal association of the magnesium with the phosphate phase and is an indication that an increase in magnesium content may lead to more Mg^{2+} ions participating in the silicate phase (this point is discussed in greater detail later)

It would be extremely beneficial to heat treat the above samples and analyse the crystal phases formed to give some idea as to structures present within the glass. This would

then aid in determining the validity of the existence of preferential associations within the glass structure.

7.1.2 ^{31}P MAS NMR of ICIE4 Low sodium Magnesium Glasses

The ^{31}P MAS NMR spectra all show a symmetric resonance with a chemical shift range in the region of 5.0 to -3.3ppm with a corresponding FWHM linewidth in the range of 10.0 to 11.7ppm.

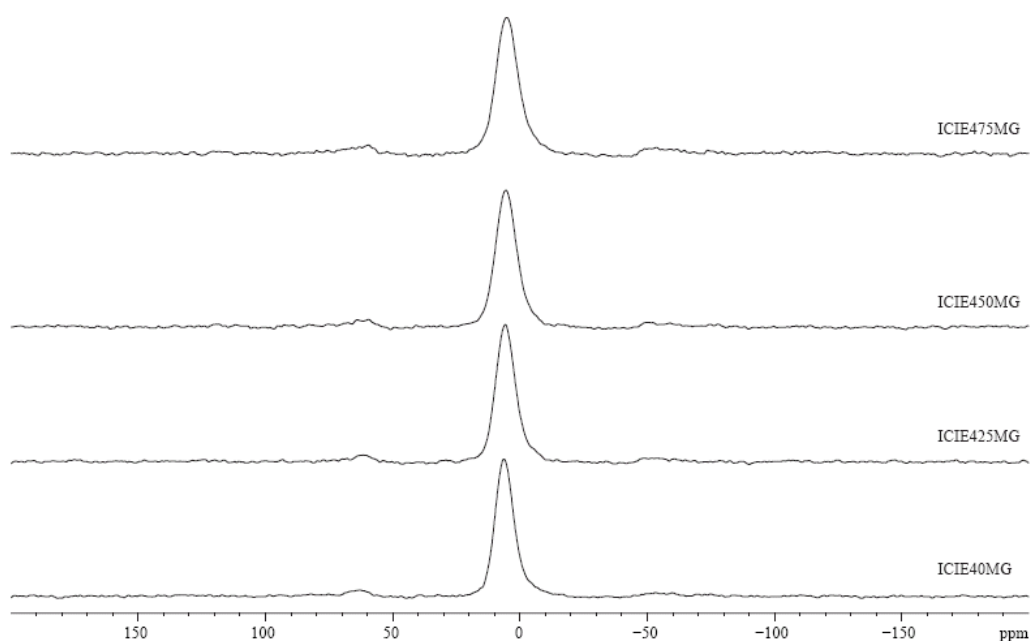


Figure 83: ^{31}P MAS NMR spectra for the ICIE4 magnesium series of glasses.

As discussed previously, with the ICIE1 Magnesium series of glasses, phosphorus was assumed always to take a forming role within a glass structure. However our results, along with those from previous studies [10] [14] [137], illustrate a single resonance with a chemical shift and linewidth consistent with that of an orthophosphate-like environment [209] [159]. Analogous with the ICIE1 series of glasses, all compositions were optically clear implying that the glass structure has undergone amorphous phase separation into a sub-micron second orthophosphate phase.

Table 18: The spectral parameters determined from the ^{31}P studies of ICIE4 magnesium series of glasses.

Glass	^{31}P NMR	
	Peak Position (ppm)	Linewidth (ppm)
ICIE40MG	5.0	10.0
ICIE425MG	1.7	10.0
ICIE450MG	-1.7	10.8
ICIE475MG	-3.3	11.7

The initial composition of the series, ICIE4 with 0% magnesium substitution, has a chemical shift of *ca.* 5ppm (Table 18) between that of $\text{Na}_3(\text{PO}_4)$ unit [209] and a $\text{Ca}_3(\text{PO}_4)_2$ unit [159] suggesting that both types of modifier ion are associated with the orthophosphate species. Furthermore, if the sodium and calcium cations are charge balancing at a ratio consistent with the overall glass composition ($\text{CaO}:\text{Na}_2\text{O}$ of 1:0.15) a chemical shift of 4.6ppm would be expected. This is very close to the experimental value of 5ppm. Therefore, within experimental error, it is concluded that there is no evidence of a preferential association with either the sodium or calcium cations and they are therefore present at a ratio consistent with that of the overall glass composition.

As magnesium is substituted for calcium, in the ICIE4 series of glasses, a direct replacement of magnesium for calcium in the modifying role, as reported by Lockyer *et al.*, Elgayar *et al.* and Oliveira *et al.* would be anticipated [10] [14] [137]. This would then cause the shift in peak maxima to a more negative region of the spectra resulting in a final chemical shift of 0ppm that associated with a pure magnesium orthophosphate type species. We experimentally observe this shift (Table 18 and Figure 84). However, on closer inspection, the final peak maxima, corresponding to a 75% substitution of magnesium for calcium, exceeds this shift to a pure magnesium orthophosphate, to that of -3.3ppm. In fact for this composition we would expect an intermediate shift associated with a mixed sodium calcium magnesium orthophosphate as a result of only

75% substitution and all modifiers present within the glass, able to act in a modifying role. Thus a much more positive chemical shift would be anticipated. The fact that the experimental chemical shift is far from what is anticipated (i.e. a much too negative chemical shift) suggests that another phenomenon is taking place rather than a direct replacement of cations.

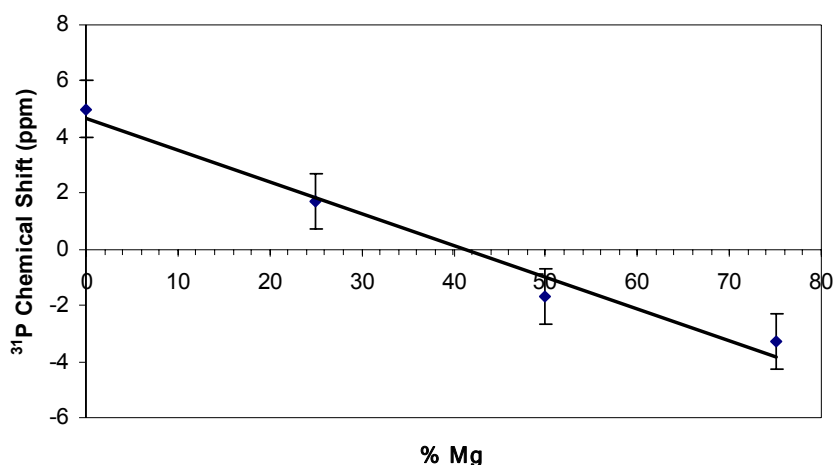


Figure 84: ^{31}P MAS NMR chemical shift values for ICIE4 low sodium magnesium glasses.

Figure 85 illustrates the increase in linewidths with substitution of magnesium for calcium in the ICIE4 magnesium series of glasses. The greater full widths at half maximum imply that there is a greater range of environments than is found in the original ICIE4, 0% substituted glass. This therefore suggests that a further phosphorus environment could be present in the glass. Lockyer [10], Elgayar [14], Leonova [210] and Grussaute [160] all reported the existence of a pyrophosphate type species in similar glass compositions.

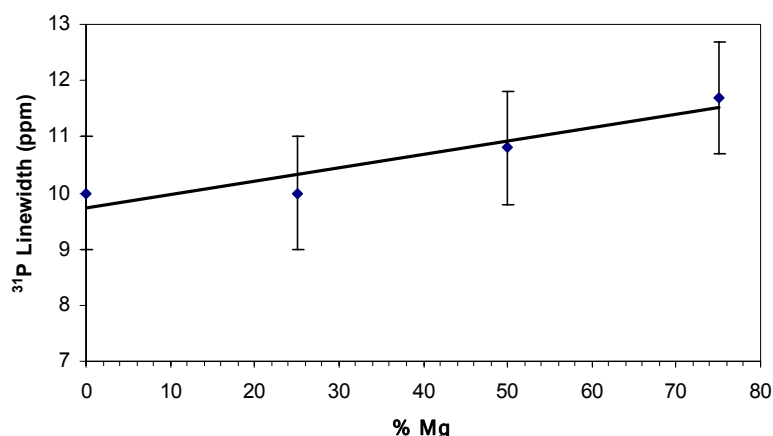


Figure 85: ³¹P MAS NMR linewidths for ICIE4 low sodium magnesium glasses

The chemical shifts of ICIE425MG, ICIE450MG and ICIE475MG all fall within the range typically found for ³¹P in Q¹ tetrahedra and may, as such, conform to a proportion of P-O-P bonding. The chemical shifts of a Na₄P₂O₇ (2.3ppm), Mg₂P₂O₇ (-5.9ppm) and Ca₂P₂O₇ (-8.9ppm) [211] thus imply that the glasses are associated with a mixed sodium magnesium pyrophosphate type species as well as a, previously discussed, mixed sodium calcium orthophosphate environment.

The resonance of *ca.* -3.3ppm is associated with ³¹P nuclei experiencing significantly larger chemical shift anisotropy relative to those of the more symmetrical orthophosphate tetrahedral. This is evident from the ³¹P spectra for ICIE475MG which show that the more shielded ³¹P nuclei generate more intense spinning sideband amplitudes than that of the Q⁰ units and is further evidence to support that a proportion of pyrophosphate is present within the higher magnesium substituted glasses [210].

It is interesting to note the different behaviours between the ICIE1 and ICIE4 magnesium series of glasses with respect to their ³¹P environments. Essentially the two series are exactly the same regarding silicon and phosphorus content and their resultant network connectivity values. The only difference between the two series of glasses is their percentage of calcium and sodium. It is obvious however that this results in a

dramatic difference in behavior, with the ICIE1 high sodium series illustrating a preference for sodium orthophosphate type species on substitution of magnesium, while the ICIE4 low sodium series highlights a preference for the formation of pyrophosphate type environment.

7.2 ^{29}Si MAS NMR

7.2.1 ^{29}Si MAS NMR of ICIE1 High Sodium Magnesium Glasses

Figure 86 illustrates the ^{29}Si MAS NMR spectra for the magnesium substituted series of glasses. The spectrum for 0% magnesium substituted glass consists of a peak with a resonance maximum *ca.* -79ppm. The 25% magnesium substituted glass displays a similar spectrum, however, as we move through the series to the 50% magnesium substituted glass, the presence of a shoulder to the right of the peak *ca.* -90ppm becomes apparent, moving the centre of gravity of the peak to the right. This shoulder becomes more pronounced across the series.

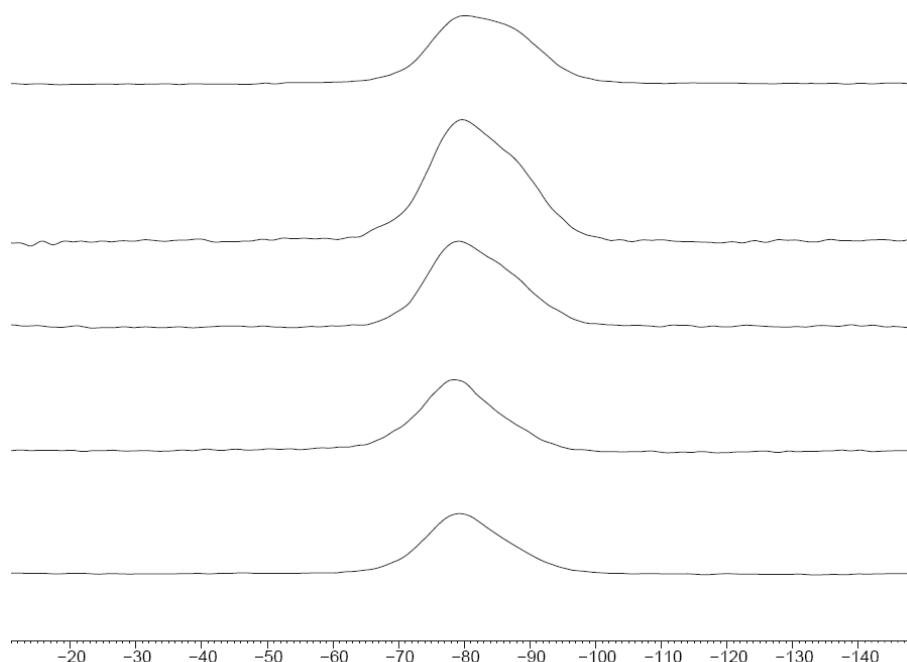


Figure 86: ^{29}Si MAS-NMR spectra. Increasing magnesium content from bottom (ICIE1) to top (ICIE1100Mg). Illustrates the progressive formation of a Q^3 shoulder on a predominantly Q^2 peak.

Table 19: NMR data for ^{31}P NMR spectra of the series of magnesium containing bioactive glasses where x corresponds to peak position, w peak width and A fraction area.

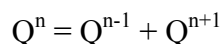
Glass ID	$^{29}\text{Si Q}^2 x$ (ppm)	$^{29}\text{Si Q}^2 w$ (Hz)	$^{29}\text{Si Q}^2 A$ (%)	$^{29}\text{Si Q}^3 x$ (ppm)	$^{29}\text{Si Q}^3 w$ (Hz)	$^{29}\text{Si Q}^3 A$ (%)
ICIE1	-80.6	461	83	-89.8	316	17
25Mg	-79.4	399	80	-87.6	258	20
50Mg	-80.6	451	77	-90.3	376	23
75Mg	-80.1	410	68	-88.8	433	32
100Mg	-80.0	396	59	-88.7	424	41

It can be assumed that the development of the asymmetric line shape and right hand shoulder, across the series, is a progressive superposition of two overlapping signals. The fitting data of these two peaks, from Gaussian deconvolution, can be found in Table 19. With magnesium substitution, the Q^3 shoulder grows from 17% (0% Mg substitution) to 41% (100% Mg substitution) area of the signal. This is clear evidence that, as magnesium is added, a proportion of the magnesium ions form tetrahedral, MgO_4 , which require charge balancing cations [212] [213]. These MgO_4 units remove modifying ions (e.g. Ca^{2+}) from the silicate network resulting in the observed polymerisation going from Q^2 to Q^3 .

Aguiar *et al.* [84] also studied the effect of magnesium substitution in 2 phosphosilicate glasses and obtained similar ^{29}Si MAS NMR spectra, with a peak signal between 75ppm to 80ppm and a shoulder at approximately 85ppm to 90ppm. However, Aguiar *et al.* assigned these peaks to Q^1 and Q^2 species and did not provide an explanation for the change in Q structure in the glass. It is submitted that Aquiar *et al.*'s experimental NMR results are classic Q^2 peaks with a Q^3 shoulder.

In spite of such signal overlap the ^{29}Si NMR spectra of the magnesium substituted series reveal that they are all predominantly populated by Q^2 silicate species with the addition of Q^3 species at higher magnesium contents. There seems to be no evidence of any

further Q species within the series, therefore a binary distribution model for the glasses is suggested agreeing with earlier work by Lockyer *et al.* [10] and Elgayar *et al.* [78]. It is noted, however, from previous work that more than two Q^n species can coexist at all compositions, suggesting a model based on the disproportionation reaction shown by Equation 13 [136, 214].



Equation 13: Disproportionation reaction.

Closer inspection of the magnesium substituted glass spectra illustrate no evidence for peaks at -105ppm or -60 ppm, therefore it can be concluded that there are little or no Q^4 or Q^0 species present. As noted by Elgayar *et al.* [14] difficulty can arise with respect to the identification of Q^1 silicate species with ^{29}Si spectra alone due to the close proximity of Q^2 species (-70ppm $Q^1 \rightarrow$ -80ppm Q^2) [215]. Further hindrance can also occur in mixed metal glasses due to the spread of ^{29}Si chemical shifts for each Q^n species, for example $\delta_{\text{Si}} = -76\text{ppm}$ for $Q^2(\text{Na})$ [216] and $\delta_{\text{Si}} = -81.5\text{ppm}$ for $Q^2(\text{Ca})$ [164]. Therefore, it is questionable as to whether any Q^1 species are being masked due to the overlap of silicon chemical shifts. At present this remains unanswered however for the purpose of this study a binary distribution of Q^2 and Q^3 is assumed.

Using Doweidar's model it is assumed that throughout the series of glasses the binary distribution of Q^2 and Q^3 species will remain constant. Also, using network connectivity calculations to model the glass structure, the connectivity is calculated to remain constant at 2.13 (calculated assuming P is present as orthophosphate as illustrated from the previous ^{31}P MAS NMR data). Both of these models presume that magnesium acts as a network modifier. Therefore, in a direct substitution of magnesium for calcium it is predicted that the connectivity/Q structure will remain constant due to both cations creating the same number of NBOs. This would then result in a series with corresponding constant ^{29}Si chemical shift values (disregarding any small shift due to the shielding effects of Mg^{2+} and Ca^{2+} – Galliano *et al.* [200] did not

find any changes in the ^{29}Si spectra when replacing cations in a glass series containing silicon, phosphorus and alkaline earth elements).

However, progressive formation of a right hand shoulder is observed with a peak resonance associated with that of a Q^3 species. This is contradictory to the network connectivity model, in which it is assumed that magnesium acts as a modifying ion, and implies that the progressive polymerisation of the glass structure is a result of magnesium entering the silicate network.

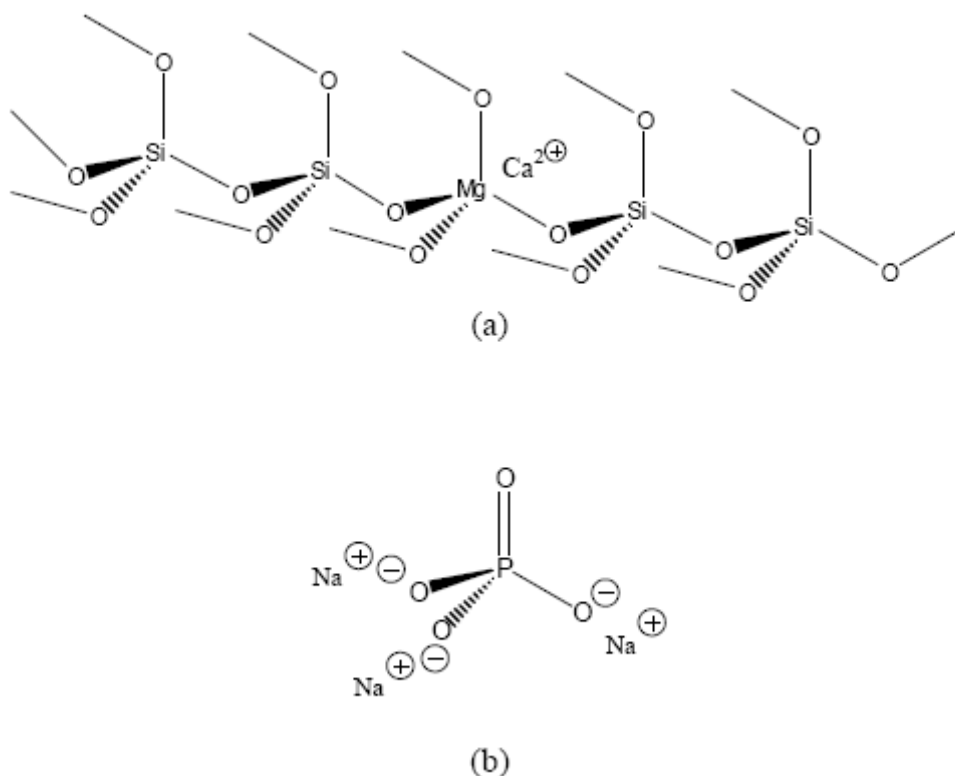


Figure 87: Hypothetical schematic model of glass structure. Illustrates the two phase glass with (a) magnesium within the silicate network as MgO_4 units charge balanced by Ca and (b) sodium associated with the orthophosphate species.

Figure 87 illustrates a schematic of the glass structure. It highlights the preferential association of sodium ions with the phosphate phase, as shown from ^{31}P MAS NMR data, and the tendency of magnesium to enter the silicate network as MgO_4 tetrahedra, charge balanced by calcium/sodium ions. Though shown here as a tetrahedral in Figure 87, other higher co-ordinate states e.g. octahedra are possible for Mg, though they would be outside of the network [213].

Figure 88 shows the calculated network connectivity of the glasses, assuming i) the phosphate enters the network (NC) or ii) phase separation into an orthophosphate phase (NC'). As we have discussed earlier, NC' is a more reasonable assumption for network connectivity as there is little evidence for Si-O-P bonds present. An empirical determination of the network connectivity was derived from peak fitting of the Q^2 and Q^3 ^{29}Si MAS NMR data, the NC(NMR), and is also given in Table 19. The NC(NMR) values are clearly higher, deviating more as magnesium is substituted into the series.

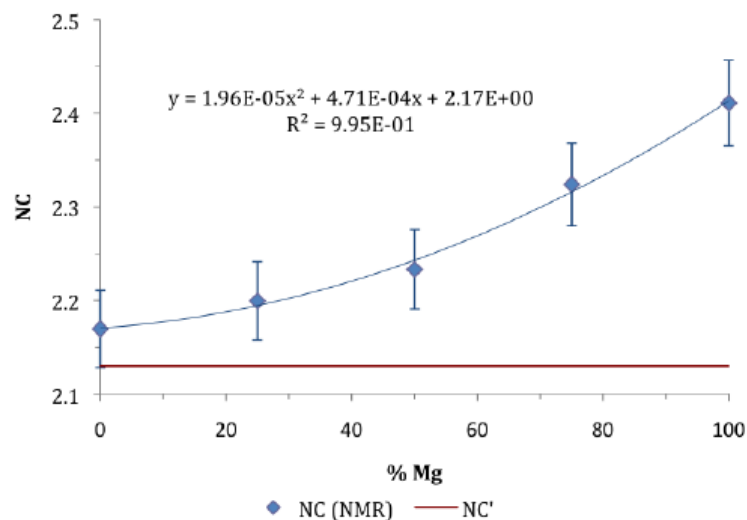


Figure 88: Network connectivity calculated from NMR fitting (Table 19) (NC(NMR)) compared to network connectivity assuming MgO acts as a modifier (NC'). In the NC' calculation, it was assumed that Mg is only acting as a network modifier. The equation of the line is for NC(NMR).

The deviation from the linear NC' must be due to the formation of tetrahedral MgO_4 species which go into the network and will require sodium and/or calcium ions for charge balancing. This removes cations from the silica network and has the net effect

of increasing network connectivity. These NC(NMR) values were used to calculate the proportion of magnesium acting either as modifiers and/or those forming tetrahedral MgO_4 species. From the difference between the calculated NC' and the NC(NMR), it is possible to calculate the amount of tetrahedral MgO_4 . In this calculation, however, we have assumed, for the sake of simplicity, that Mg is only present as tetrahedral MgO_4 . Figure 89 shows the absolute molar amount of magnesium acting as a network former, in a tetrahedral state, for all glass compositions.

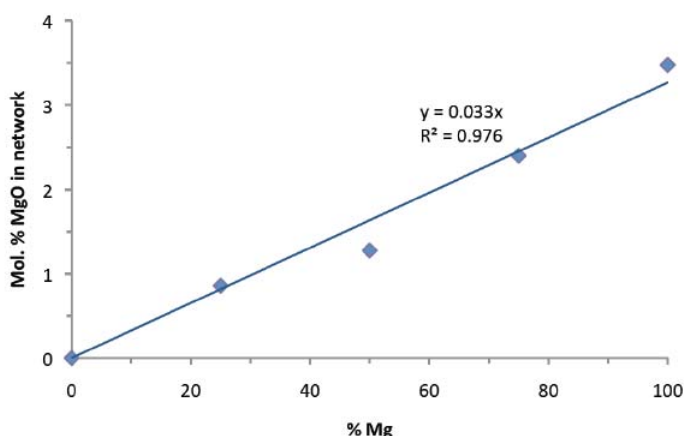


Figure 89: The absolute amount of MgO (mol %) entering the glass network with Mg substitution. This assumes that the MgO is only in tetrahedral co-ordination. As the MgO content is increasing across the series, this corresponds to a constant proportion (~ 14%) of MgO acting as a network former compared to network modifying MgO.

However, as the amount of magnesium increases, the proportion in the network as compared to the proportion acting as modifiers is actually constant across the series, ~14% of the magnesium enters the network as MgO_4 tetrahedra whereas around 86% occupies the traditional modifier role regardless of the composition.

Although Elgayar *et al.* [14] report a 1:1 cation distribution between the silicate and phosphate phase with no preferential association, it should be noted that although working with ICIE1 compositions, magnesium is not present within the system analysed. It is therefore hypothesised that upon introduction of magnesium into the bioactive glass the cationic distribution no longer behaves in an even/equilibrium manner. This then raises the question of why the two alkaline earth cations, Ca and Mg,

behave so differently with respect to the kinetics of the system, and more relevantly mobility, within the bioactive glass studied

F. Natrup *et al.* [217] discuss such roles of the alkaline earth oxides within silicate glasses and believe that the mobility of the alkaline-earth ions strongly depend on the type of alkali ion present in the glass. He further postulates that alkaline-earth ions are most mobile when their ionic radius are similar to that of the alkali metal ion – that is similar ionic radii of the cations in alkali alkaline-earth silicate glasses facilitate the motion of the alkaline-earth ions. From this theory, in the case of the Ca:Na system (studied by Elgayar *et al.* [14]), the similarity of ionic radii (Ca^{2+} 106 Å and Na^{+} 98 Å) would suggest the ease of motion of calcium within the glass – hence no preferential association. However for the Mg:Na system the differing ionic radii (Mg^{2+} 78 Å and Na^{+} 98 Å) would hinder the motion of magnesium within the glass. This is supported by B. Roling *et al.* [218] where his data showed that the lowest alkaline-earth mobilities are found in glasses containing Mg or Ba and on the other hand the Na mobility is highest in the sodium magnesium system.

7.2.2 ^{29}Si MAS NMR of ICIE4 Low Sodium Magnesium Glasses

Figure 90 illustrates the ^{29}Si MAS NMR spectra for the ICIE4 Magnesium series of glasses. The spectra contain a single symmetric resonance with peak maxima in the range of -82.0 to -85.5 ppm and FWHM linewidths from 20 to 24 ppm. The chemical shift values for the glasses can be assigned due to the presence of predominantly Q^2 silicate species, agreeing with the constant network connectivity value of 2.13 through the series. There seems to be no evidence of any further Q species therefore a binary distribution model is assumed.

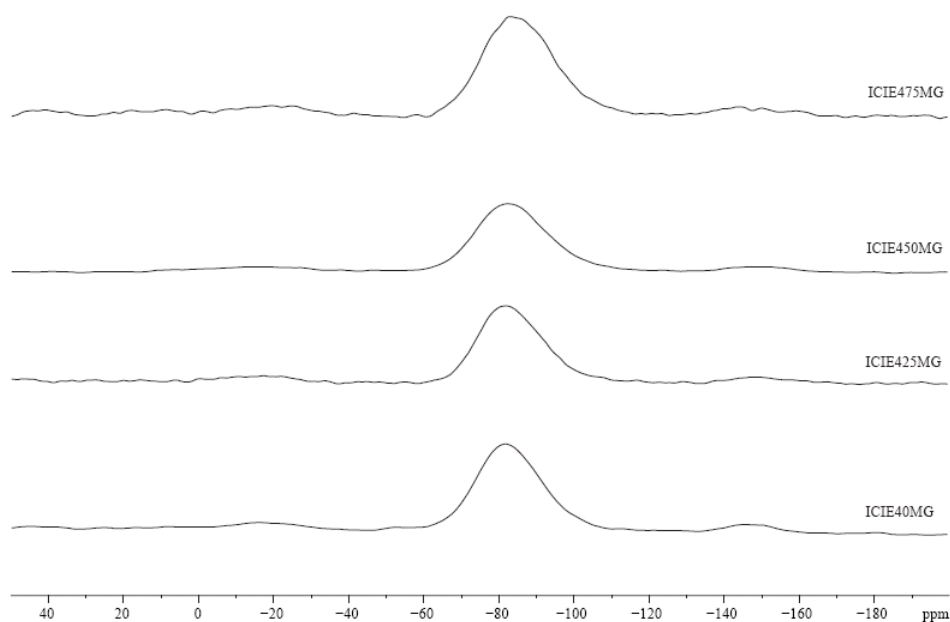


Figure 90: ^{29}Si MAS NMR spectra for the ICIE4 magnesium series of glasses

Taking into account the chemical shift values reported by Murdoch *et al.* [219] for Q^2 species in Na_2SiO_3 ($\delta_{\text{Si}} = -76\text{ppm}$), CaSiO_3 ($\delta_{\text{Si}} = -81.5\text{ppm}$), $\text{CaMgSi}_2\text{O}_6$ ($\delta_{\text{Si}} = -82.0\text{ppm}$) and MgSiO_3 ($\delta_{\text{Si}} = -83.5\text{ppm}$), it is suggested that the chemical shift of -82ppm for the initial ICIE40MG glass can be assigned to a Q^2 calcium silicate species. This is in good agreement with Elgayar *et al.* [78] where a chemical shift of -81.4ppm was reported for the same glass.

Table 20: The spectral parameters determined from the ^{29}Si studies of ICIE4 magnesium series of glasses.

Glass	^{29}Si NMR	
	Peak Position (ppm)	Linewidth (ppm)
ICIE40MG	-82.0	20.0
ICIE425MG	-82.0	20.0
ICIE450MG	-84.0	22.5
ICIE475MG	-85.5	24.0

Figure 91 illustrates that as MgO is substituted for CaO the peak maxima are shifted proportionally to a more negative chemical shift value. As the network connectivities of the glasses are constant through the series, that is the substitution makes no change to the number of non-bridging oxygens, the experimental shift must be assigned to the modifying cations. However, this observed shift of the peak maxima is difficult to explain in such terms. Even with a full substitution of magnesium for calcium the maximum chemical shift possible, that associated with MgSiO_3 , is -83.5ppm. This is additionally assuming that all the magnesium ions preferentially associate with the NBOs of the silicate network and the remaining calcium and sodium ions are in some way blocked from a modifying role in the silicate network. This could be explained if all the sodium and calcium ions preferentially associated with the phosphate phase, however the chemical shifts do not represent such a phenomenon.

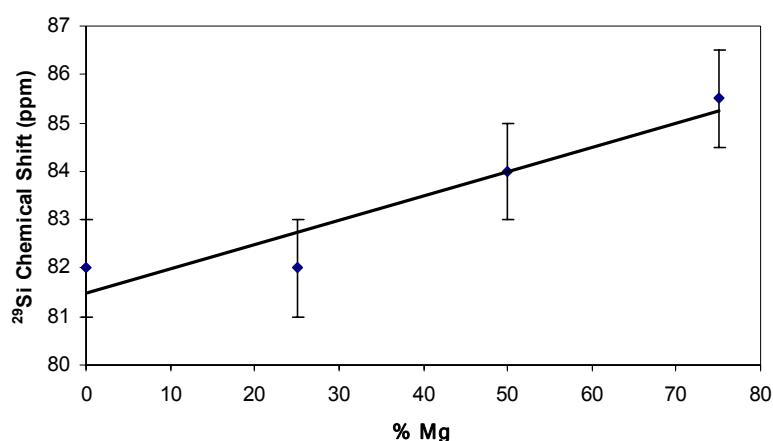


Figure 91: ^{29}Si chemical shift values for the ICIE4 low sodium magnesium series of glasses

It is therefore acceptable to assign the shift in peak maxima to the same trend observed in the ICIE1 high sodium magnesium series of glasses. In the ICIE1 series the development of an asymmetric line shape and right hand Q^3 shoulder, across the series, was attributed to the progressive polymerisation of the silicate network as a result of magnesium entering the network as MgO_4 tetrahedra. It is therefore assumed that in the ICIE4 low sodium magnesium series, the shift to lower frequencies, those associated with Q^3 silicate species, is a result of magnesium acting as a network former and

polymerising the silicate structure. As there is no indication of a separate shoulder, as in the ICIE1 series, the proportions of Q^3 , hence the amount of magnesium going into the network cannot be predicted by fitting procedures. However the lack of a prominent Q^3 shoulder suggests that the proportion of magnesium entering the network is much less than that in the ICIE1 magnesium series of glasses.

Correlating this behavior with the results of the ^{31}P MAS NMR suggests that this may not be the case. As is observed from the ^{31}P spectra, on substitution of magnesium for calcium, there is a preference for the formation of pyrophosphate type species. As a species these require less network modifying ions to charge balance therefore it implies that more cations are available to modify the silicate network. In turn this will reduce the number of Q^3 species and from a “ ^{29}Si point of view” will over-ride the effect of magnesium entering the network as MgO_4 tetrahedra. Due to the lack of a prominent Q^3 shoulder the relative proportions of Q^2 and Q^3 cannot be predicted by fitting procedures, with any degree of accuracy. An estimate of the percentage of magnesium entering the network therefore cannot be carried out. It is suggested however that the relative amounts of modifying cations will have a direct effect on such a phenomenon. It can be imagined that a glass with a smaller degree of disruption, as with the ICIE4 low sodium magnesium series of glasses, will result in a lesser need for magnesium to act as an intermediate oxide.

As discussed for the ICIE1 high sodium magnesium series of glasses the interesting question is what causes the intermediate behavior of magnesium. The ICIE4 series of results also add a further dimension to this question – why does a composition with less sodium cause less magnesium to enter the network?

7.3 ^{25}Mg MAS NMR

The structural role of Mg is poorly understood due to the lack of information on its local environment stemming from the difficulty in extracting information from conventional X-ray diffraction, Raman and Infrared spectroscopy. NMR spectroscopy is an effective tool for collecting structural information focused on a specific element in multicomponent systems [148]. NMR has been used to investigate Mg in silicates only with great difficulty. The sensitivity is low due to a low natural abundance of the NMR active-isotope (^{25}Mg) and the low resonance frequency. Also ^{25}Mg is a quadrupolar nucleus, having spin 5/2, leading to additional quadrupolar interactions and broadening. Mg sites in silicates are often disordered, leading to broad lines and complicated lineshapes which are often difficult to observe [212].

Figures 92 and 93 illustrate the ^{25}Mg static NMR spectra for the ICIE1 and ICIE4 magnesium series of glasses, respectively.

The ^{25}Mg of the glasses suffer from a second-order quadrupolar broadening because of low resonance frequency and a relatively large quadrupolar interaction, which smears out the detailed information in the spectra. It is therefore very difficult for the above spectra to estimate an isotropic chemical shift.

The number of NMR studies of ^{25}Mg in crystalline states is limited (Dupree and Smith 1988 [220]; MacKenzie and Meinhold 1994 [221]; Fiske and Stebbins 1994 [143]; MacKenzie and Meinhold 1997 [222]). These studies highlighted that the ^{25}Mg signal is dependant on the local structure around the Mg atom. Particularly apparent are the effects of the coordination states.

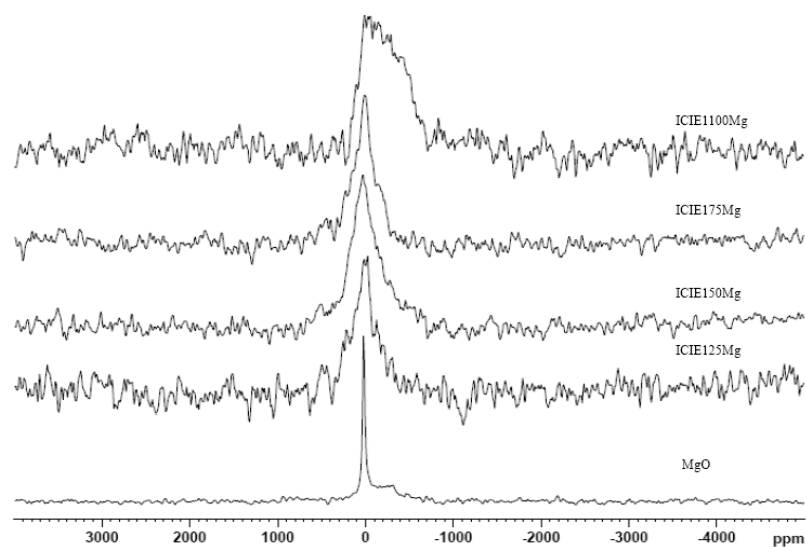


Figure 92: ^{25}Mg Static NMR for ICIE1 magnesium series

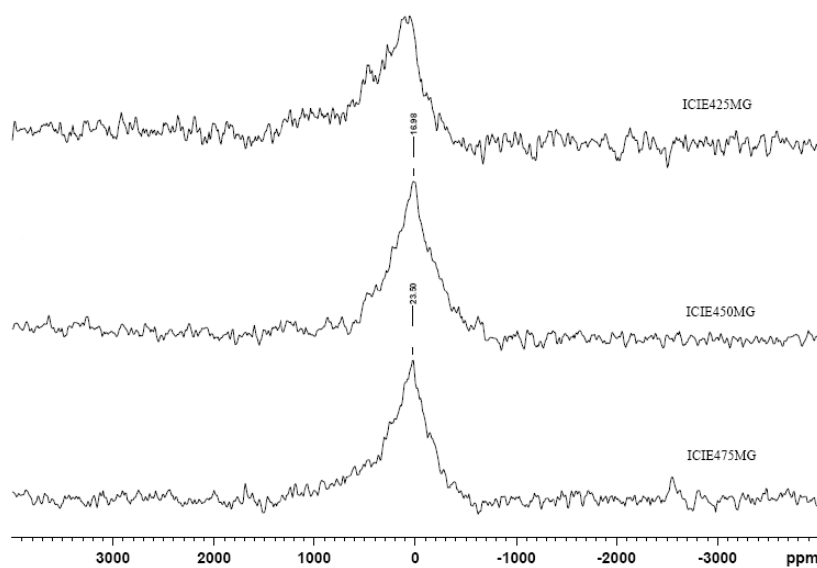


Figure 93: ^{25}Mg Static NMR for ICIE4 magnesium series

As discussed, it is very difficult to predict with any degree of accuracy the actual chemical shifts involved in the above spectra. It is probably therefore more important to observe any changes in the spectral artifacts. By doing so, even though specific species cannot be specified, general information of the environment around the ^{25}Mg cation can be clarified.

On closer inspection, importantly a change in lineshape can be observed as magnesium is substituted for calcium. There is a slight trend through the series suggesting that changes in the environment around ^{25}Mg may be occurring. It is very difficult, however, to draw any definitive conclusions from the data.

In the case of the ICIE4 low sodium magnesium series of glasses there does not seem to be any distinct change in the spectral lineshape. This implies that the Mg environment remains unchanged through the series. However from the ^{29}Si MAS NMR data we would expect to see some kind of variation in the peak shapes or chemical shift values. It is suggested, however, that the discussed problems with ^{25}Mg NMR have resulted in spectra that are very difficult to analyse.

Shimoda [148] showed that the chemical shifts in magnesium silicate melts systematically varied with the addition of a second modifier cation. In general it was seen that the addition of a lower field strength cation moved the peak position to a higher frequency hence suggesting magnesium's move to a lower coordination state, i.e. entering the silicate network as MgO_4 tetrahedra.

The idea that the coordination number of a cation is related to the other cations present within the material is well known and not unique to magnesium. If a cation with a higher field strength is substituted, the replacement cation takes more of the electron density from the O atoms, leaving less for M cation, and consequently a larger coordination number is required to balance the M cation charge. The cation field strength (Z/a^2) increases in the order of K (0.12), Na (0.18), Li (0.26), Ca (0.36), and

Mg (0.46-0.53) [223]. This indicates an increase in bond strength of K-O, Na-O, Li-O, Ca-O and Mg-O. For example a composition with more calcium than magnesium compared to a second composition would be expected to have a higher frequency chemical shift as a result of the lower field strength of calcium [148]. It is, therefore, suggested that in the ICIE1 and ICIE4 series of glasses, when magnesium is substituted for calcium, the higher field strength of magnesium compared to calcium and sodium causes the magnesium ions to adopt a lower co-ordination state and enter the network as MgO_4 tetrahedra. The difference in behavior between the two series however is more difficult to understand.

It is suggested, therefore, that the ambiguity that exists as to the actual role of magnesium in silicate glasses could be a result of the compositional dependence of the size of the average coordination number [212].

7.4 Summary of MAS NMR results for the magnesium series of glasses

^{29}Si MAS NMR illustrated a move from a Q^2 silicate to a Q^3 silicate with the substitution of magnesium for calcium in the ICIE1 series of glasses. This was coupled with a shift to a more positive region of the ^{31}P MAS NMR spectra implying the move from a mixed calcium sodium orthophosphate to a sodium orthophosphate type species with the addition of magnesium. The accompanying ^{25}Mg static NMR spectra were also seen to change in lineshape through the series. This data suggests that, rather than acting as a network modifying cation magnesium is behaving as an intermediate oxide within the glass with a proportion entering the silicate network as MgO_4 tetrahedra thus causing the polymerisation of the silicate network and the preferential association of sodium with the phosphate phase. The ICIE4 series of glasses are hypothesised as behaving in a similar manner, however to a lesser extent, thus resulting in less pronounced spectral features.

7.5 FTIR Spectroscopy

Figures 94 and 95 illustrate the infrared absorption spectra of the individual glasses in the ICIE1 high sodium series and the ICIE4 low sodium series, respectively.

As discussed previously, infrared spectroscopy is extremely accurate in picking up vibrations associated with non-bridging oxygen and silicon interactions (Si-O-NBO bonding) [173] –

3. The Si-O(asymmetric stretching) with one non-bridging oxygen (1 NBO) per SiO_4 tetrahedra (i.e. Q^3 bonding) is located in the range of $1020\text{-}1052\text{cm}^{-1}$
4. The Si-O-2NBO(stretching) species (i.e. Q^2 bonding) is located at approximately $920\text{-}960\text{cm}^{-1}$

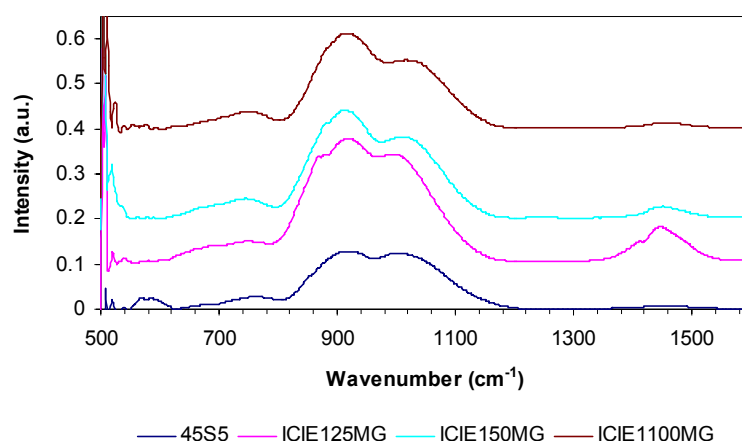


Figure 94: FTIR spectra for high sodium ICIE1 magnesium glasses. Increasing magnesium content from bottom to top.

Figure 94 above exhibit the vibrations associated with Q^2 and Q^3 silicate species. Apart from these two dominant peaks, further features are very difficult to assign. Furthermore, it is very difficult to assess whether the peak heights are changing through the series. Figure 95 below also exhibit vibrations associated with Q^2 and Q^3 species and do not seem to exhibit any change through the series.

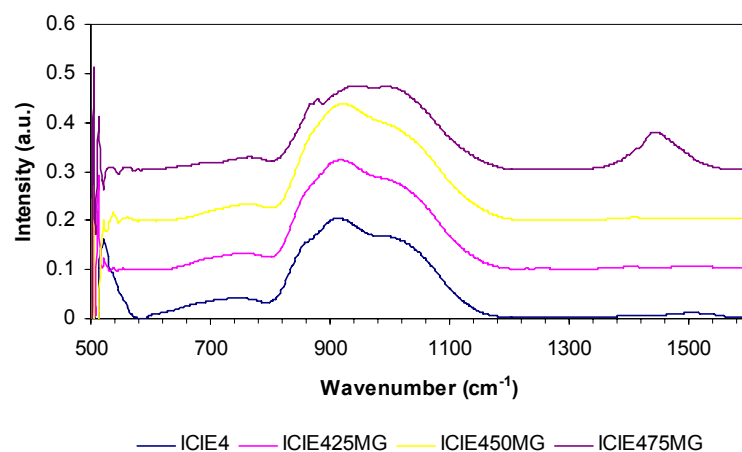


Figure 95: FTIR spectra for low sodium ICIE4 magnesium glasses. Increasing magnesium content form bottom to top.

The ICIE1 series of glasses seem to exhibit an increase in the intensity of the vibration associated with the Q^2 silicate species in comparison to the vibration associated with the Q^3 species. The ICIE4 series of glasses, on the other hand, do not exhibit any real change in the vibrations associated with the silicate species. This therefore suggests that the ICIE1 series of glasses are becoming less cross-linked through the series and the ICIE4 series of glasses are remaining unchanged with respect to the silicate network with the substitution of magnesium. These phenomena are difficult to explain as they are in direct conflict with the NMR data obtained.

7.6 Raman Spectroscopy

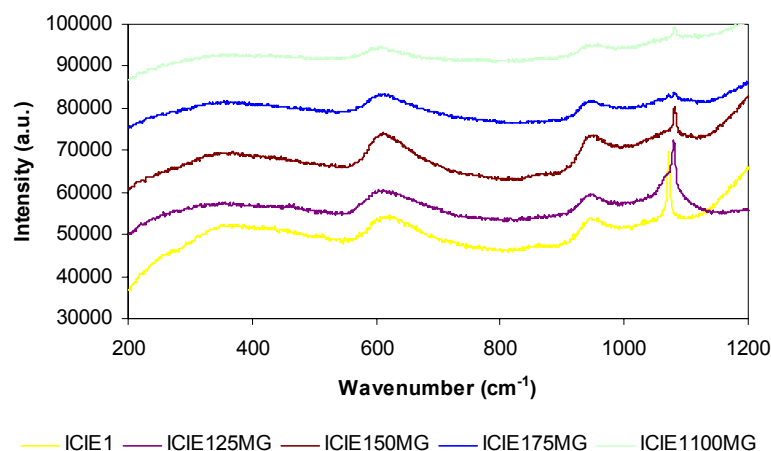


Figure 96: Raman spectra for high sodium ICIE1 glasses. Increasing magnesium content from bottom to top.

Figure 96 above shows the Raman spectra of the ICIE1 high sodium magnesium glasses. The figure reveals the presence of the main optical modes associated with the Si-O-Si groups. Firstly the asymmetric stretching mode at 1000-1200 cm^{-1} , the rocking mode at 560-660 cm^{-1} and lastly the non-bridging silicon-oxygen bond (Si-O-NBO) stretching at 900-970 cm^{-1} [224] [225] [226] [84].

It is well known that the use of vibrational spectroscopy aids in the understanding of the influence of modifier elements on a glass network [84] [194]. Moreover, it has been definitively shown that the introduction of modifying ions into a silicate containing glass promotes structural changes within the silicate network [194]. Figure 96 above highlights the characteristic changes in relative intensities and line changes associated with the incorporation of a modifier element into the SiO_2 matrix. The most prominent change in the Raman features is the change in the ratio between the intensity of the Si-O-NBO and Si-O-Si (s) bands. The ratio decreases as magnesium is introduced into the glass structure which is evidence for the polymerisation of the glass network. Thus it can be concluded that the addition of magnesium promotes a local distortion in molecular symmetry which gives rise to the observed decrease in the ratio of Si-O-NBO/Si-O-Si(s) and thus an increase in network connectivity [157]. Furthermore, the

spectra show that, as magnesium is added, the intensities of the peaks decrease and the bands generally become broader. The accommodation of the cation in the glass network results in a distortion of the Si-O-Si intertetrahedral bonds which in turn causes a spread in angle values and oscillator strength variations [14]. These changes can therefore explain the observed broadening of the bands in the Raman spectra.

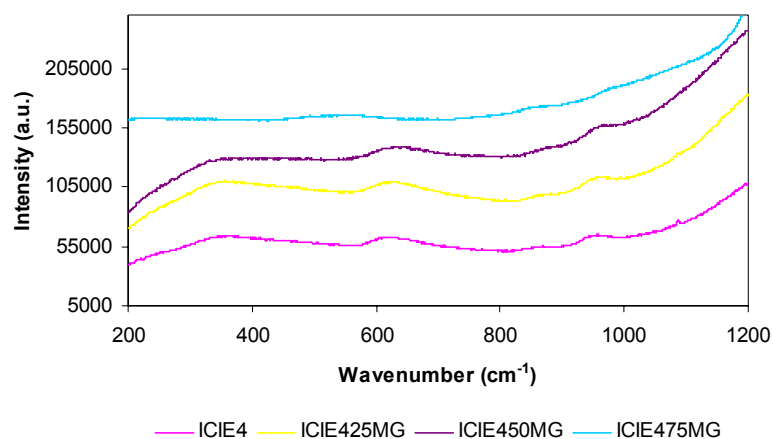


Figure 97: Raman spectra for low sodium ICIE4 magnesium glasses. Increasing magnesium content from bottom to top.

Figure 97 above shows the Raman spectra for the ICIE4 low sodium containing magnesium series. It is evident that, although the Raman spectra for the ICIE1 glasses are not much better, the spectra for these glasses are near featureless. Therefore, as a result, a proper and scientific analysis of the band positions and intensities is extremely difficult. No real conclusion on the structure of the glasses can be made. The only observation that can be made, with any kind of scientific accuracy, is the slight existence of bands at approximately 660cm^{-1} and 980cm^{-1} . These bands could be assigned to the Si-O-Si rocking mode and the Si-O-NBO mode respectively. Due to the poor quality of these spectra no comparison can be made with the ICIE1 series of glasses. It is not known what influenced such a poor series of Raman results.

7.7 X-Ray Diffraction

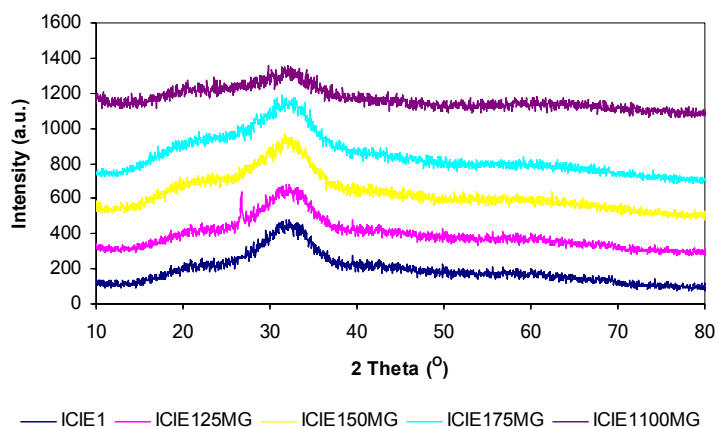


Figure 98: X-Ray diffraction patterns for high sodium ICIE1 magnesium glasses. Increasing magnesium content from bottom to top.

X-ray diffraction was used to confirm the glassy nature of the ICIE1 and ICIE4 magnesium substituted series.

Figures 98 and 99 above and below, respectively, show the characteristic amorphous halo.

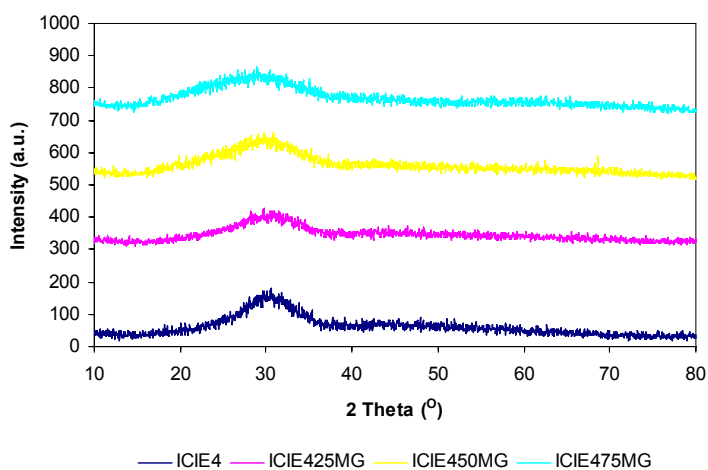


Figure 99: X-Ray diffraction patterns for low sodium ICIE4 magnesium glasses. Increasing magnesium content from bottom to top.

It therefore can be concluded that both series of magnesium glasses were all amorphous in nature. Furthermore, there is no degree of crystallinity within the glasses.

7.8 Density

Density is the most frequently measured property of a glass. It can be measured quickly and accurately and is often used in the quality control of commercial glass manufacture [74]. Density is defined as the mass of a substance per unit volume and thus ultimately has a strong dependence on its composition. It is dependant primarily on the nature of the atoms in the material, on the number of neighboring atoms and on the interaction between them [43]. Density is a strong indicator of the compactness of its own network [227].

Although widely used as an indication of the effect of changing composition on glass structure it is widely argued that the determination of molar volume, mean atomic volume and oxygen molar volume, from experimental density data, are far more useful parameters. These parameters provide important information on particular aspects of a glass's structure such as the coordination numbers of the various elements, degree of anionic packing and deformation [228] [229].

In the 1990's, Doweidar postulated that the density of a glass can be correlated with the volume of its structural units [179] [149] [230]. The volumes of the specific structural units are only dependant on the type of modifier ion associated with them and totally independent on the actual concentration in which they are present [179]. Furthermore, through the study of alkali and alkaline earth silicate glasses as well as alkali aluminosilicate glasses, the volume of the structural units were shown to be constant over the entire region where it exists [178]. The utilisation of such a model has subsequently proved accurate in the determination of the physical densities of alkali and mixed alkali silicate glasses and is often used as mathematical approximation as to the type of structural units present in the glass. The ideas of Doweidar were thus applied and a comparison made between Doweidar's theoretical values and the experimental density values obtained.

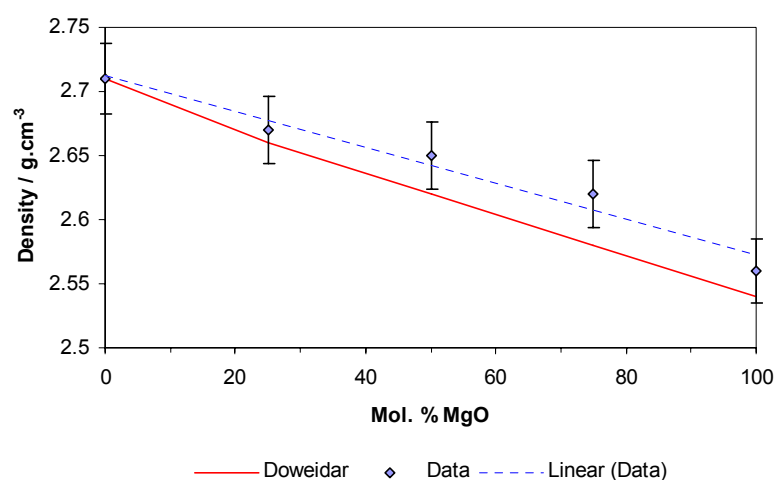


Figure 100: Variation in density with magnesium content for high sodium ICIE1 magnesium glasses with Doweidar's theoretical values.

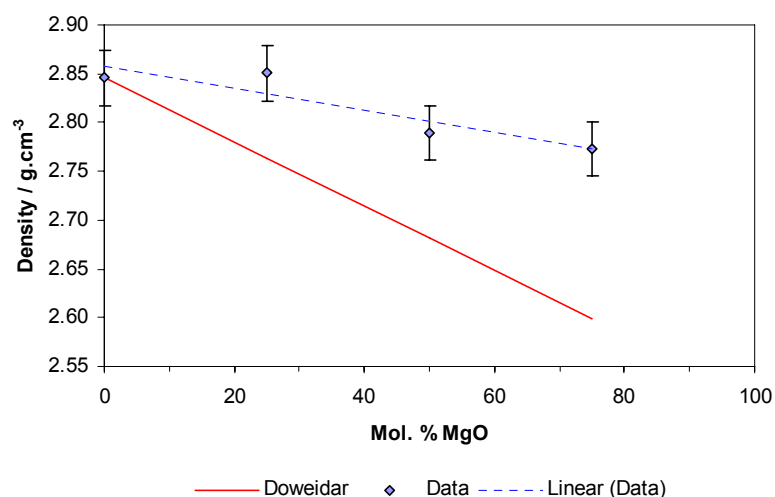


Figure 101: Variation in density with magnesium content for low sodium ICIE4 magnesium glasses with Doweidar's theoretical values.

Figures 100 and 101 illustrate the calculated density values with the data determined experimentally for the ICIE1 magnesium substituted series and the ICIE4 magnesium substituted series, respectively. In both cases, on examination of the experimental data, it can be seen that as MgO replaces CaO the general trend is a decrease in density. According to Galliano *et al.* bulk glass density increases with increasing mean modifier radius [88]. The experimental decrease in density therefore can be attributed to the smaller radius of the magnesium ion (65pm) substituting for the larger calcium ion (99pm). As density is defined as mass per unit volume it should not, however, be

neglected that the mass of the constituent ions will play an important role in the overall density of the glass. Therefore, it could be concluded that not only the smaller ionic radius but the lighter molar mass of magnesium, compared with calcium, contributes to the decrease in bulk glass density across both substituted series.

7.9 Differential Thermal Analysis

Differential Thermal Analysis of the high sodium ICIE1 and low sodium ICIE4 magnesium substituted series was undertaken to determine the glass transition temperature and crystallisation temperature for each glass.

Figures 102 and 103 below illustrate the DTA traces for the high sodium ICIE1 magnesium and low sodium ICIE4 glasses, respectively.

In general the DTA curves for samples ICIE1 to ICIE1-75Mg in the ICIE1 series of glasses have two deflections – one due to a glass transition (T_g), with a negative deviation from the baseline, and one due to an exothermic crystallisation event (T_c). There is a possible second exotherm in the ICIE1 and ICIE1-25Mg glasses, corresponding to a second crystallisation temperature. Also the ICIE1-100Mg glass shows only one transition – due to a glass transition, no crystallisation deflection is evident.

As with the ICIE1 series of glasses we observe two deflections in the ICIE4 series of glasses, one due to a glass transition (T_g), with a negative deviation from the baseline, and one due to an exothermic crystallisation event (T_c). In comparison to the ICIE1 series of glasses there is no evidence of a second exotherm in the traces, therefore only one crystallisation process is involved with the ICIE4 series of glasses.

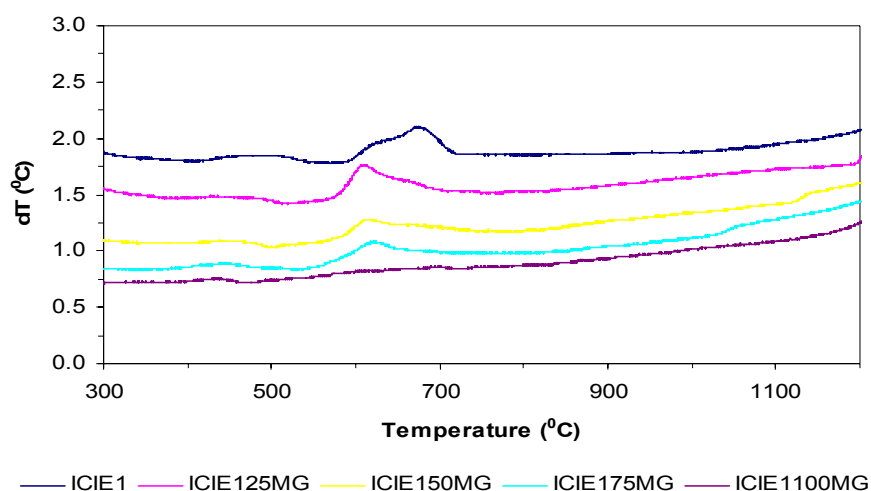


Figure 102: Differential Thermal Analysis traces for the ICIE1 magnesium substituted series of glasses

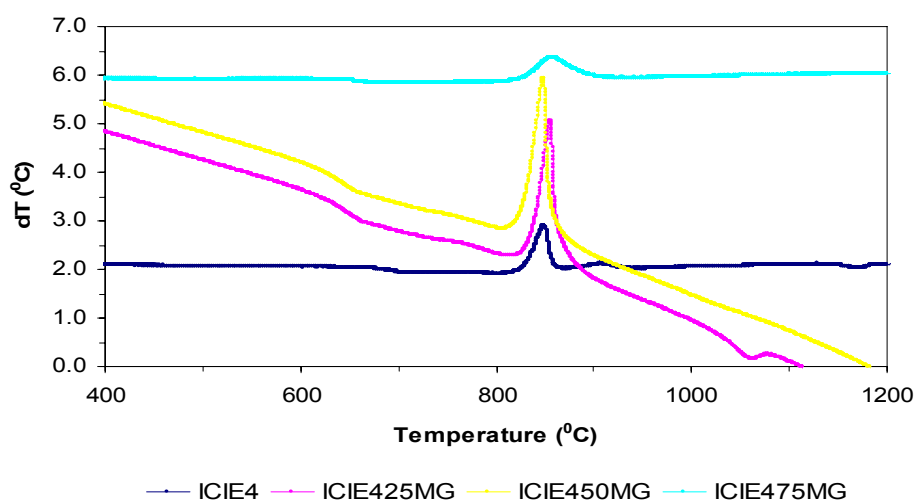


Figure 103: Differential Thermal Analysis traces for the ICIE4 magnesium substituted series of glasses

7.9.1 Glass Transition Temperature

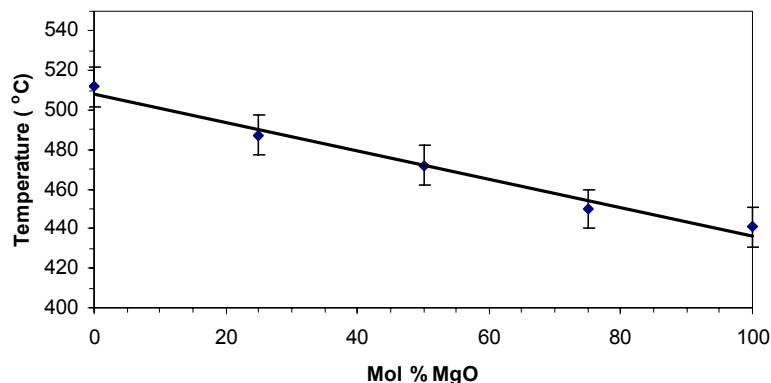


Figure 104: Glass transition temperatures for the ICIE1 magnesium glasses

Figure 104 shows the relationship between T_g and magnesium substitution in the ICIE1 magnesium substituted series. It can be seen that, as magnesium is substituted for calcium, there is a corresponding decrease in the glass transition temperature.

A reduction in T_g is generally achieved by increasing the amount of network modifying oxides relative to the network forming oxides, which in turn depolymerises the network, increasing the amount of NBO to BO and reducing the network connectivity. In the magnesium substituted glasses, however, the theoretical network connectivity should remain constant as, instead of adding more magnesium it is being substituted for another network modifier, calcium.

The behaviour of T_g does suggest, however, that the addition of MgO results in a weakening of the glass network. As we know from the MAS NMR data that the degree of polymerisation increases as a function of Mg addition (a move from a predominantly Q^2 to a predominantly Q^3 glass), the decrease in T_g must be attributed to magnesium entering the network as MgO_4 tetrahedra. As discussed previously, it is hypothesised that the newly formed Si-O-Mg and Mg-O-Mg bonds are, on average, significantly

weaker with respect to the Si-O-Si bonds in the silicate chain (Dowty studied the vibrational spectrum of akermanite, a calcium magnesium silicate ($\text{Ca}_2\text{MgSi}_2\text{O}_7$) which contains MgO_4 units [159]. The bond force constant of the Mg-O bond in akermanite was found to be lower than in MgO and significantly lower than a Si-O bond: 2.00, 3.48 and 9.24 N.cm^{-1} respectively [207]. Therefore, as a result, the strength of the overall network will decrease resulting in the observed reduction in T_g . This phenomenon is also noted by Linati *et al.* [231], however regarding the role of zinc rather than magnesium in a similar glass series. It is interesting to note that magnesium and zinc are indeed very similar ions with respect to charge and ionic radius, $0.72 \text{ \AA}$ and $0.74 \text{ \AA}$ respectively, and it is suggested that the two behave similarly within glass systems [215].

Figure 105 below shows the relationship between the percentage of magnesium substituted into the ICIE4 base glass and the resultant temperature of the glass transition.

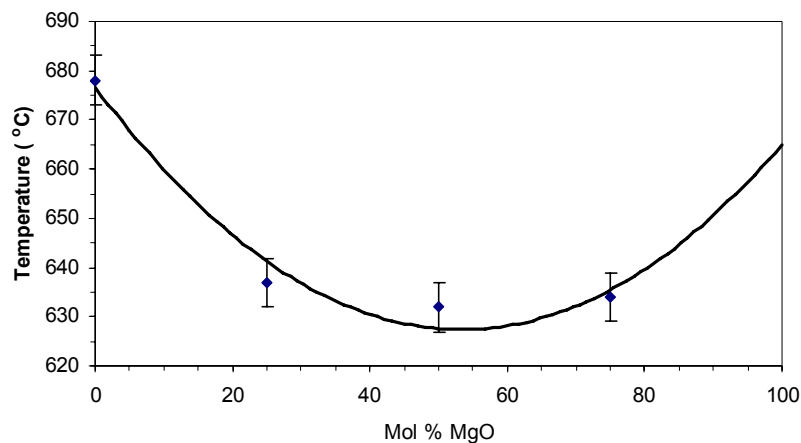


Figure 105: Glass transition temperatures for the ICIE4 magnesium glasses

It is evident that up until approximately 50mol% magnesium substitution the glass transition temperature decreases with increasing magnesium substitution. After approximately 50mol% substitution we then observe a stabilisation of the trend and then an up turn, with the glass transition increasing with increasing magnesium substitution.

Comparing the glass transition temperatures of the two series of magnesium substituted glasses, it is evident that the ICIE4 glasses have significantly higher glass transition temperatures. This can be explained by the higher proportion of the highly disrupting sodium ions in the ICIE1 series of glasses. As a result of the more disrupted network, less energy is required to break up the glass structure, thus resulting in the lower glass transition temperatures of the ICIE1 glass series. Furthermore, the linear relationship of the glass transition temperature with magnesium substitution is not observed with the ICIE4 series of glasses.

As discussed above, the initial decrease in glass transition temperature with increasing magnesium substitution is attributed to the weaker bond strength of Mg-O-Si and Mg-O-Mg compared to Si-O-Si as magnesium enters the silicate network. The up turn observed in the ICIE4 series of glasses is harder to explain. As the glass transition temperature is indicative of a glass's structure any kind of significant change in behaviour, such as the up turn observed, is symptomatic of some kind of structural change.

It is hypothesised that there may be two competing structural factors effecting the glass transition temperature, on substitution of magnesium. As discussed earlier, one is assigned to the weaker bond strength as magnesium enters the network. At approximately 50mol% this factor seems to be overtaken by a second structural effect, which then causes the network to become more compact and/or stronger. To fully understand what this second structural factor is, further experimental work is required.

7.9.2 Crystallisation Temperature

According to Figure 106, T_{c1} linearly increases with increasing MgO content for the ICIE1 series of glasses. Noticeably at low MgO content there is a sharp pronounced crystallisation peak, which reduces in magnitude and broadens as the MgO content increases. In fact, at 100% magnesium there seems to be no evidence of crystallisation

at all. It is suggested that magnesium, therefore, suppresses crystallisation, as discussed below.

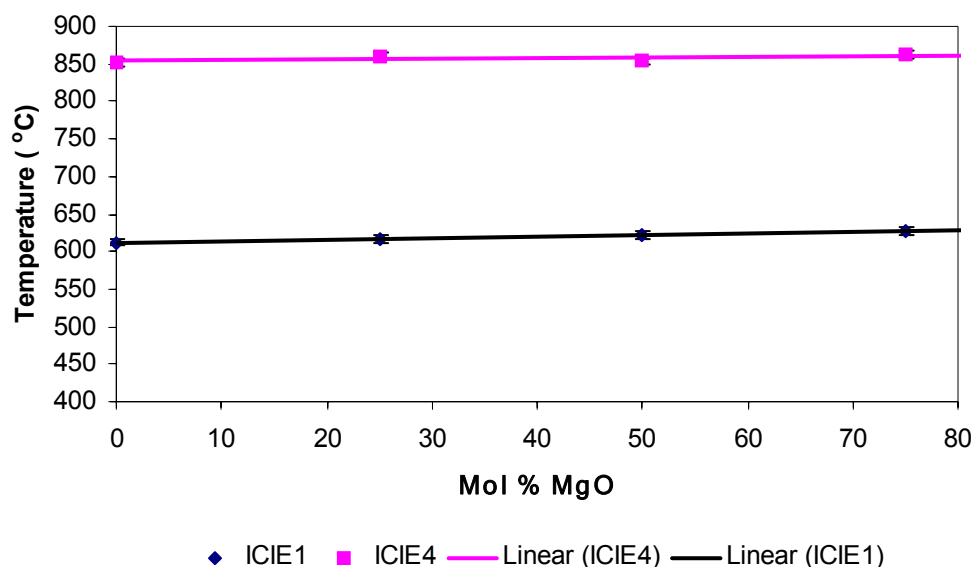


Figure 106: Crystallisation temperatures for ICIE1 and ICIE4 magnesium glasses.

Figure 106 above illustrates the crystallisation temperatures for the ICIE1 and ICIE4 magnesium series of glasses.

As with the ICIE1 series of glasses the crystallisation temperature increases with increasing magnesium substitution. It is therefore hypothesised that magnesium suppresses crystallisation.

It is hypothesised, however, that the increase in crystallisation temperature as magnesium is substituted is a result of the stabilising effect of magnesium entering the silicate network and increasing the network connectivity of the glass. It is hypothesised that when magnesium acts as an intermediate oxide and a proportion enters the silicate network the resultant network connectivity will increase. This will then result in an energy barrier to nucleation. Furthermore, when acting as a modifier, the higher charge to size ratio of magnesium will hold the non-bridging oxygens more tightly therefore also adding to the energy barrier to crystallisation.

When comparing the crystallisation temperatures of the two series, Figure 106 shows that the ICIE1 series have much lower crystallisation temperatures than their corresponding ICIE4 glasses. This, therefore, suggests that less energy is required to crystallise the high sodium ICIE1 glasses and more energy is required to crystallise the low sodium ICIE4 glasses.

Nucleation in a glass can occur by two distinct processes, either by homogeneous nucleation in defect-free regions of the glass or by heterogeneous nucleation where a defect in the glass acts as a nucleus. The existence of a sharp single crystallisation temperature for the two series suggests that the ICIE1 and ICIE4 magnesium substituted glasses undergo homogeneous nucleation or bulk nucleation. As discussed earlier, the presence of sodium within a glass system results in a disruption of the continuity in the glass network. The lower the content of sodium, therefore, the more stable the glass network, with the higher proportion of ionic bridging between the non-bridging oxygens and calcium. This will then form an energy barrier to bulk nucleation and the crystallisation temperature will therefore increase. This therefore explains the higher crystallisation temperatures of the ICIE4 low sodium series of glasses, shown in Figure 106.

7.9.3 Summary of DTA results for the magnesium series of glasses

- For both series of magnesium substituted glasses the glass transition temperature was seen to decrease on substitution of magnesium.
- This was attributed to magnesium entering the silicate network as MgO_4 tetrahedra with the smaller Mg-O bond strength compared to Si-O bonds weakening the glass structure.
- The glass transition temperatures were observed to then increase in the ICIE4 series of glasses at approximately 50 mole percent substitution. This phenomenon was not mirrored in the ICIE1 series of glasses. The up turn in T_g values in the ICIE4 series of glasses is difficult to explain. It is interesting to note, however, that this phenomenon is not isolated. The decrease in glass

transition temperatures and subsequent increase has also been observed in similar magnesium containing glass systems [232].

- The magnesium substituted glasses were also seen to suppress crystallisation with the crystallisation temperatures increasing in both series of glasses with the substitution of magnesium.

7.10 Dilatometry

Dilatometry is the determination of transition points by the observation of volume changes. In this investigation, dilatometry has been used to determine the dilatometric softening point and thermal expansion coefficients of the two magnesium series of glasses.

Magnesium oxide is often added to glasses in order to adjust their thermal expansion coefficients and softening temperatures such that enamelling can be undertaken at temperatures below the $\alpha \rightarrow \beta$ transformation of titanium, a common orthopaedic implant material [134]. The forming properties of glasses for biomedical use have recently been improved by adding magnesium, as well as potassium and boron to soda-lime silica glasses [130].

Figures 107 and 108 illustrate the dilatometric traces for the ICIE1 and ICEIE4 magnesium glasses, respectively.

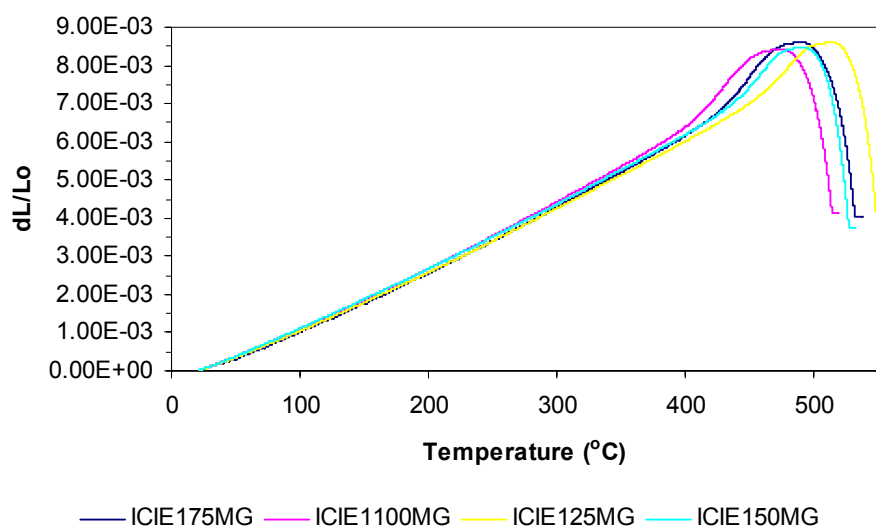


Figure 107: Dilatometric trace for the ICIE1 magnesium glasses

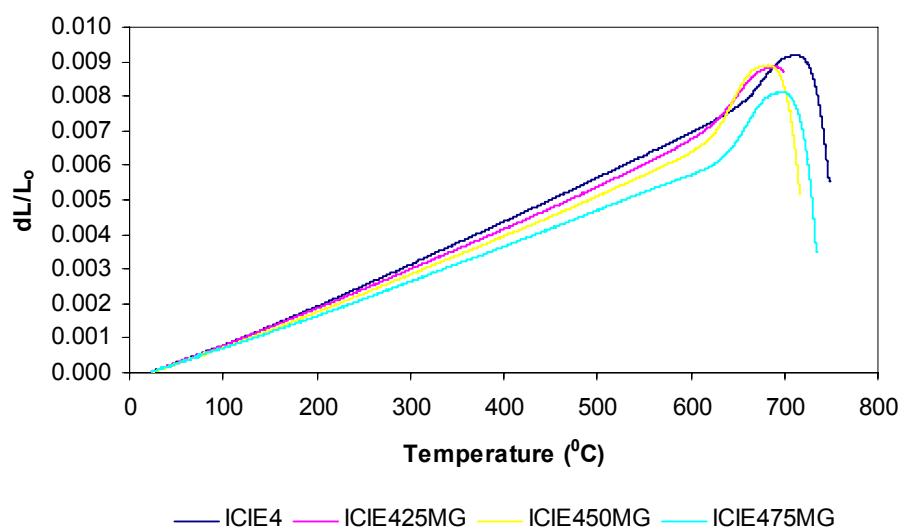


Figure 108: Dilatometric trace for the ICIE4 magnesium glasses.

7.10.1 Dilatometric Softening Temperature

The softening point is related to the glass transition temperature and is the temperature at which the glass network loses cohesion and begins to flow under the force applied during dilatometry [43].

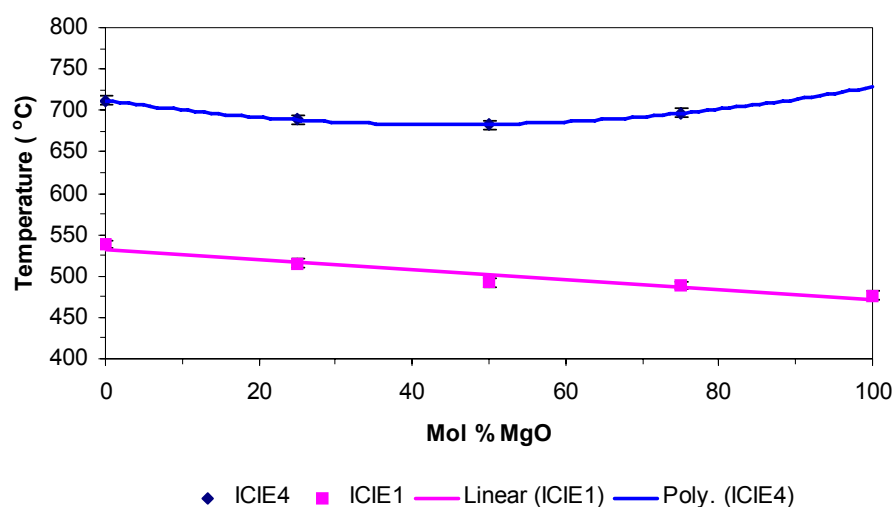


Figure 109: Comparison of softening temperatures for ICIE1 and ICIE4 magnesium substituted glasses

Figure 109 plots the softening points for both series of magnesium glasses. It can be seen that substituting magnesium for calcium, in the high sodium ICIE1 glasses, results in a reduction in the softening temperature, T_s as previously documented by Wallace *et al.* [43]. The same relationship was previously noted for the glass transition temperature, T_g and can be explained by magnesium acting as an intermediate oxide. If magnesium enters the network, as MgO_4 tetrahedra, the weakening of bond strengths will cause the glass structure to weaken therefore giving rise to the observed reduction in T_s .

Figure 109 also illustrates the relationship between the softening temperatures of the ICIE4 series of the glasses and the percentage of magnesium substitution. As can be seen from the graph, the behaviour mirrors that of the corresponding glass transition temperatures. First a decreasing trend is observed and then an up turn is observed at approximately 50mol%.

7.10.2 Thermal Expansion Coefficient

Thermal expansion originates from anharmonic vibrations of atoms within a solid [74]. Increasing temperature causes the atoms in a substance to vibrate about their

equilibrium position more vigorously. These asymmetrical vibrations cause the distance between neighbouring atoms to increase and therefore the solid expands.

Thermal expansion is particularly important with respect to bioactive glasses if used as a coating on metal prosthesis. In order to create proper adhesion between the coating and the substrate there needs to be a correct match between the two TECs. Thermal expansion coefficients for varying metallic implants include –

- Ti $8 \times 10^{-6} \text{ }^{\circ}\text{C}^{-1}$
- Ti6Al4V $8 \times 10^{-6} \text{ }^{\circ}\text{C}^{-1}$
- Vitallium™ $12 \times 10^{-6} \text{ }^{\circ}\text{C}^{-1}$ (an alloy of 60% cobalt, 20% chromium and 5% molybdenum)

The figure below, Figure 110, shows the relationship between the TEC values for the ICIE1 series of glasses and the percentage of magnesium substituted. Also on the graph is incorporated the theoretical TEC values as predicted by Appen.

Appen predicts that TEC values will fall with increasing MgO content. On the examination of Appen's model, it is evident that he has assigned magnesium the role of a modifier. Therefore the greater polarising power, compared to calcium, will act to pull the non-bridging oxygens together more tightly, resulting in less expansion in the glass. Appen's model does not recognise the intermediate nature of magnesium.

Figure 110 illustrates that from 0 to 20mol% there is a decreasing trend in TEC values, agreeing with Appen's predictions. However at just over 20mol% there seems to be a reverse trend away from Appen's theory to slightly increasing TEC.

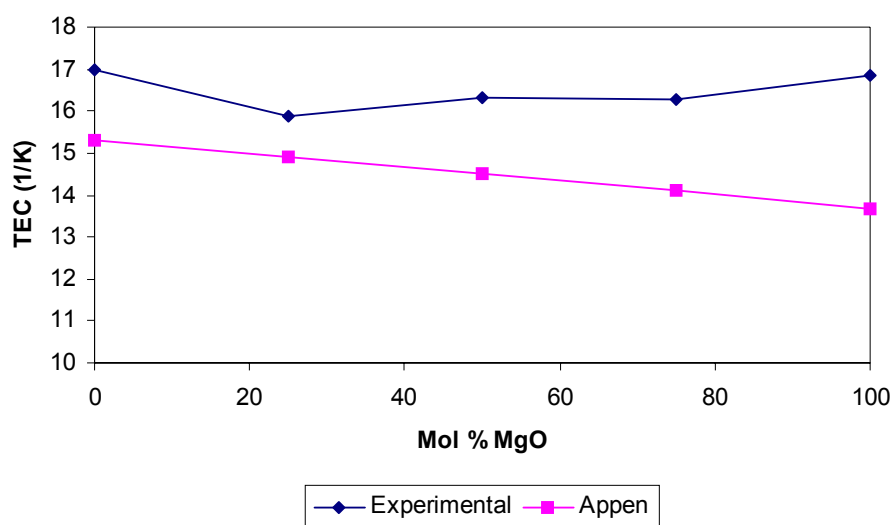


Figure 110: Graph of experimental thermal expansion coefficients, TEC (top) and Appen's predictions (bottom) against percentage of magnesium substituted for ICIE1 series of glasses. Illustrates Appen's predicted decreasing trend and the experimental increase in TEC with magnesium substitution. The top line is a guide for the eye.

This deviation at 20mol% corresponds with other deviations seen in the experimental density values/NMR etc. Once again this could suggest that magnesium is switching its role, at this point, from its traditional role as a modifier to an intermediate oxide within the glass structure. If it were indeed to switch its role to an intermediate oxide, with a proportion of the magnesium ions entering the glass network, an increase in TEC values would occur due to the weakening of the bonds within the glass structure. This increase in TEC values is seen experimentally. However, it should not be neglected that on entering the glass network, the number of NBOs would decrease thus causing the glass to compact and result in a decrease in corresponding TEC values. The two effects of magnesium entering the network, namely the weakening of the bond strengths and the loss of non-bridging oxygens, would then act against each other with respect to the overall effect on the glass's properties. Depending on the magnitude of each effect they could either cancel each other out, or one or the other would be dominant. Clearly this would need to be investigated further in order to fully understand the effect of magnesium acting as an intermediate on thermal expansion. Furthermore, rather than switching roles through the series magnesium may be having as an intermediate oxide in all compositions, and the up turn in TEC values could also be assigned to the weaker

bond strengths simply dominating over the loss of NBOs at 20 mole percent substitution. Again, to fully understand the structural influences at work, further analysis is required.

Figure 111 below illustrates the experimental and theoretical thermal expansion coefficients for the ICIE4 low sodium series of glasses. The thermal expansion coefficients are directly related to the magnesium oxide content of the glass, decreasing linearly with increasing Mg^{2+} content. This relationship has been noted previously by Wallace *et al.* [50]. The thermal expansion coefficient has also been theoretically calculated using Appen factors,

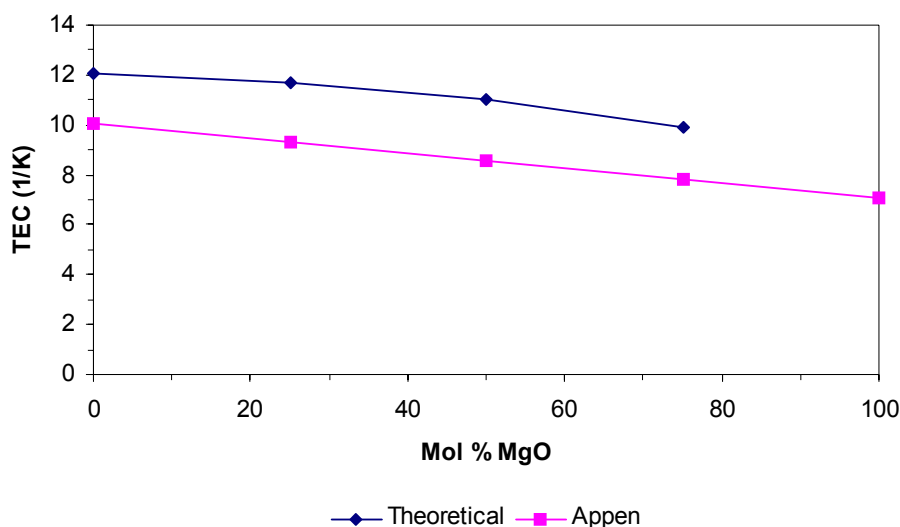


Figure 111: Graph of experimental thermal expansion coefficients, TEC (top) and Appen's predictions (bottom) against percentage of magnesium substituted for ICIE4 series of glasses.

Figure 111 compares the predicted thermal expansion coefficients utilising the above Appen factors with those that have been experimentally measured for the ICIE4 series of glasses. The predicted values result in a trend line parallel with the experimental values. There is however a small difference between the two.

The Appen factors were developed through the measurement of thermal expansion coefficient of many different glass compositions, which were then fitted into an empirical equation. The glass compositions that the Appen factors were based on were mainly, however, soda-lime silicate glasses and with much lower sodium oxide and calcium oxide contents to those of bioactive glasses. The difference is therefore attributed to both experimental error and also, most probably, the presence of P_2O_5 in the ICIE4 glass series, which is not accounted for in the Appen calculations.

It is important to note that although the ICIE1 high sodium series of glasses illustrated a marked deviation from Appen's predicted values, the ICIE4 glass's thermal expansion coefficients follow Appen's decreasing trend through the series. This therefore implies that the magnesium ions are acting in their traditional role in the glass network, as charge modifiers. This however does match with previous results, which suggest that magnesium is also switching its role in the series.

An alternative explanation could be that magnesium is indeed behaving as an intermediate oxide, with a proportion entering the silicate network, but the two competing factors discussed earlier, namely the weaker bond strengths and the loss of non-bridging oxygens is being won out by the loss in NBOs. This would then result in a more rigid network and the observed decreasing thermal expansion values.

7.10.3 Summary of dilatometry results for the magnesium series of glasses

- The thermal expansion coefficients for the ICIE1 and ICIE4 series of glasses were observed to decrease with the increase in magnesium substitution.
- This can be assigned to the higher polarising power of magnesium holding the NBOs more tightly if magnesium is acting as a modifier or the cross-linking effect of magnesium entering the network if magnesium is behaving as an intermediate oxide. Both would result in a more compact network.
- After approximately 20 mole percent magnesium substitution in the ICIE1 series the thermal expansion coefficients were seen to then increase. This was

attributed to either magnesium switching roles and a proportion entering the network as MgO_4 tetrahedra with the weaker Mg-O bonds allowing expansion of the network or if magnesium is acting as an intermediate oxide for all compositions, the weaker bond strength dominating over the loss in NBOs at 20 mole percent. This phenomenon was not observed in the ICIE4 series of glasses.

- The dilatometric softening temperatures for both series of glasses mirrored the behaviour of the glass transition temperatures and decreased with magnesium substitution. The T_s values for the ICIE4 series were then observed to increase at approximately 50 mole percent substitution.

7.11 Dissolution Studies in Simulated Body Fluid

7.11.1 Variation in Simulated Body Fluid pH

As discussed previously, a glass's bioactive nature is fundamentally related to its solubility. It is therefore essential to understand the relationship between the structure of the glass and its dissolution properties. In short, the dissolution characteristics of a glass can be utilised to aid in the understanding of the effect of its composition on the resultant bioactivity.

One way of measuring glass dissolution characteristics is through the analysis of the change in pH of SBF solution with time. It is widely accepted that the first step in the bioactive mechanism is an ion exchange reaction between the sodium ions in the glass structure and the hydrogen ions in the aqueous environment (whether that be *in vivo*, in SBF, in water etc) thus resulting in an increase in the pH levels in the localised region. It is therefore acceptable to use the pH of SBF within a closed environment as an indication of the progress of the ion exchange reaction [13].

Figures 112 and 113 illustrate the pH measurements for the ICIE1 high sodium series of glasses and the ICIE4 low sodium series of glasses, respectively.

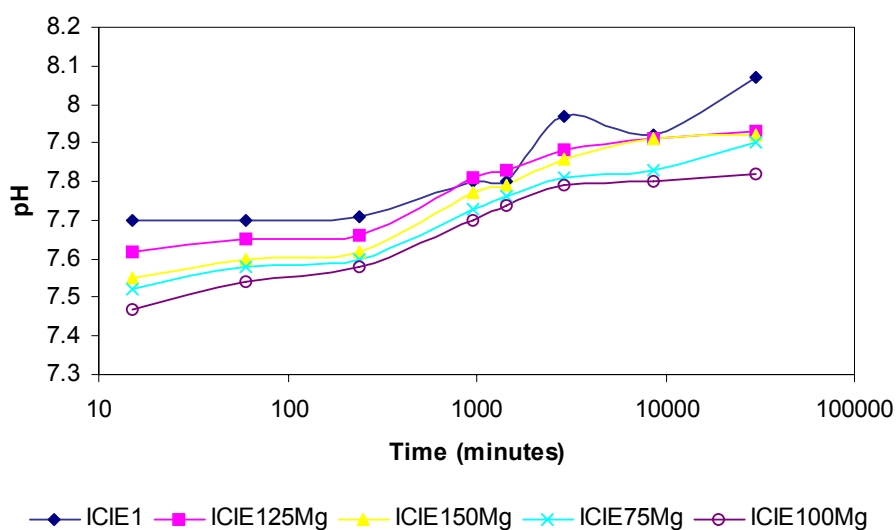


Figure 112: Variation in SBF pH with time for high sodium ICIE1 glasses.

Figure 112 above and Figure 113 below both highlight that the pH of the SBF increases with time for the ICIE1 and ICIE4 series of glasses and similar pH results have been documented by Pereira *et al.* [106]. This increase in pH is evidence of the ion exchange reaction occurring, as discussed above, with Na^+ from the glass exchanging with H^+ and H_3O^+ ions in solution. Again, for both series, the increase observed is clearly related to the MgO content of the glass, with the glasses with higher MgO content giving rise to pH rises of lesser magnitude. This implies that the dissolution rates of the higher MgO content are lower than those with less MgO content. This conflicts with the findings of Wallace *et al* [43] who observed little or no effect of MgO content on the magnitude of the pH increase.

Figure 112 and Figure 113 both show that the addition of magnesium oxide causes a reduction in pH. It is suggested that this illustrates that glass dissolution is suppressed by MgO addition, as a result of the cross-linking effect of magnesium's intermediate role. In other words, the glasses with higher magnesium content are less degradable than those with lower magnesium content. However, the decrease in pH could also be explained by the leaching of Mg^{2+} ions into solution causing a local acidifying effect.

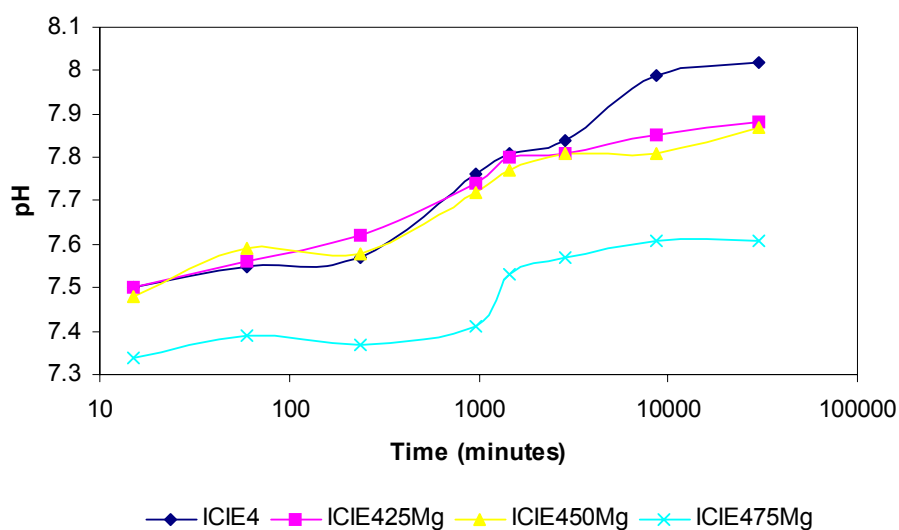


Figure 113: Variation in SBF pH with time for low sodium ICIE4 glasses.

7.11.2 X-Ray Diffraction

The figures below show the XRD traces for ICIE1 magnesium series of glasses after immersion in SBF. Each figure illustrates the original glass's XRD trace, the XRD trace after 4 hours of immersion in SBF, after 16 hours, after 24 hours, after 48 hours, after 1 week and finally after 21 days of immersion in SBF.

The traces clearly show the formation of the Bragg peaks associated with apatite crystallisation with increasing immersion time. It should be noted that the data is very noisy, even after data smoothing.

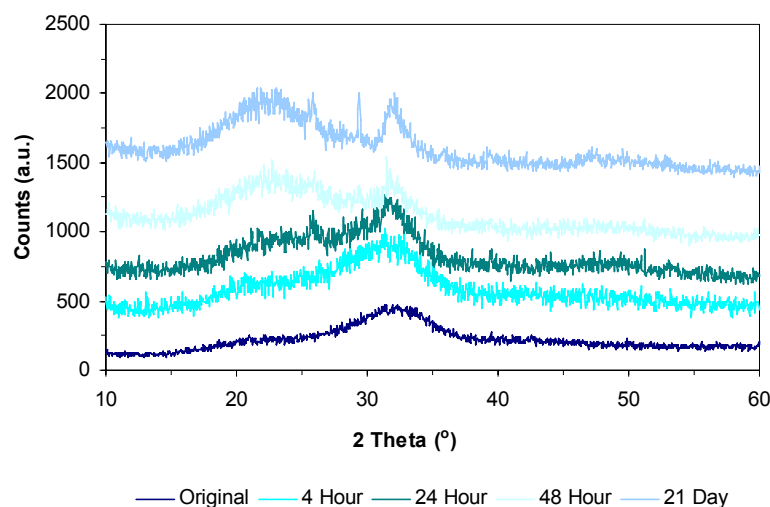


Figure 114: XRD patterns obtained for ICIE1 glass following immersion in SBF for different time periods.

Figure 114 above illustrates the XRD spectra for the ICIE1 glass after immersion in SBF for up to 21 days. It is evident from the spectra that there is a degree of crystallinity after 24 hours of immersion. The level of crystallinity increases with time, with the peaks associated with apatite formation becoming more prominent. The two most prominent features are the Bragg peaks at 25.9° and 31.9° corresponding to the (002) and (211) reflections [174] [187].

Figure 115 below shows the XRD spectra for the ICIE125MG glass after immersion in SBF. After 16 hours of immersion, there are no crystalline peaks evident. There is however a flattening of the characteristic amorphous halo. As discussed by Wallace *et al.* [13] it is likely that, when compared to the quantity of bulk amorphous glass, the quantity of any surface crystallinity was too low to be detected. The same flattening of the amorphous halo is also evident in the 24 hour spectrum. It is only in the 48 hour and subsequent 21 day spectra that exhibit any kind of degree of crystallinity. Again, the two most prominent features are the Bragg peaks at 25.9° and 31.9° corresponding to the (002) and (211) reflections [174] [187]. It can therefore be concluded that HCA is present in the ICIE125MG glass after 48 hours of immersion.

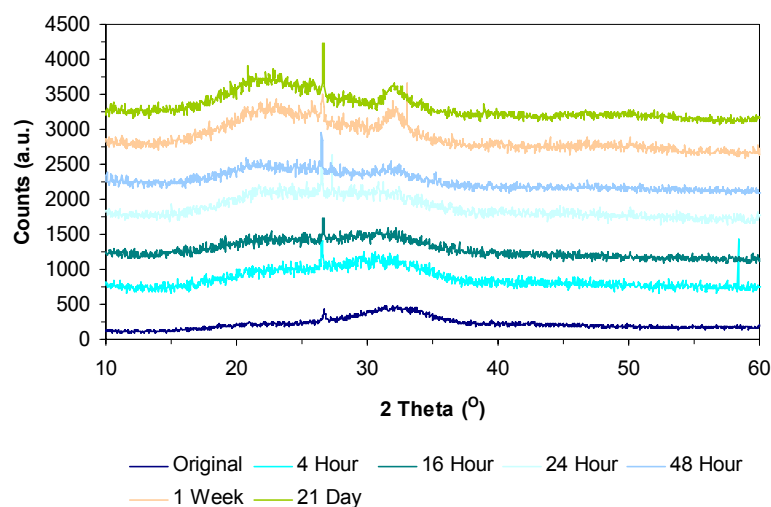


Figure 115: XRD patterns obtained for ICIE125MG glass following immersion in SBF for different time periods.

Figure 116 below illustrates the XRD spectra for the ICIE150MG glass after 21 days of immersion in SBF. As with the ICIE125MG glass, a flattening of the characteristic amorphous halo is seen for the 16 hour, 24 hour and 48 hour spectra. It is only the 21 day spectrum that illustrates any degree of crystallinity with Bragg peaks at 25.9° and 31.9° . It can therefore be concluded that apatite formed only after 48 hours of immersion – at any time between 48 hours and 21 days. The same conclusion can be gathered from the ICIE175MG spectra in Figure 117.

Figure 118 below illustrates the ICIE1100MG spectra. It is obvious straight away that there is no evidence of any crystallinity. Even after the full 21 days of immersion in SBF, no Bragg peaks associated with crystalline HCA are present. There is a flattening of the amorphous halo in all of spectra. It is concluded, therefore, that the ICIE1100MG glass does not exhibit any bioactive behaviour even after a sustained period of time in simulated body fluid.

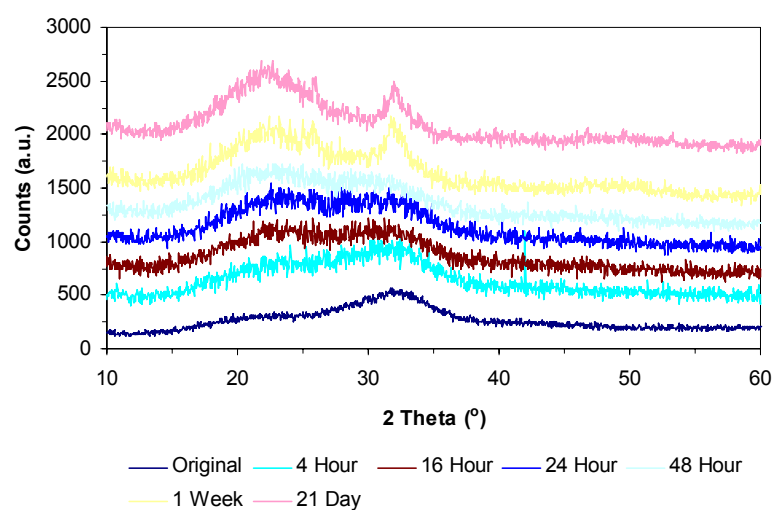


Figure 116: XRD patterns obtained for ICIE150MG glass following immersion in SBF for different time periods.

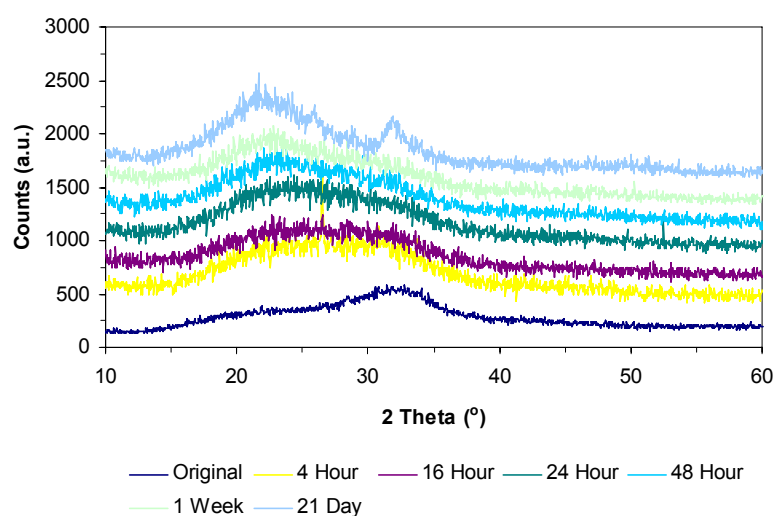


Figure 117: XRD patterns obtained for ICIE175MG glass following immersion in SBF for different time periods.

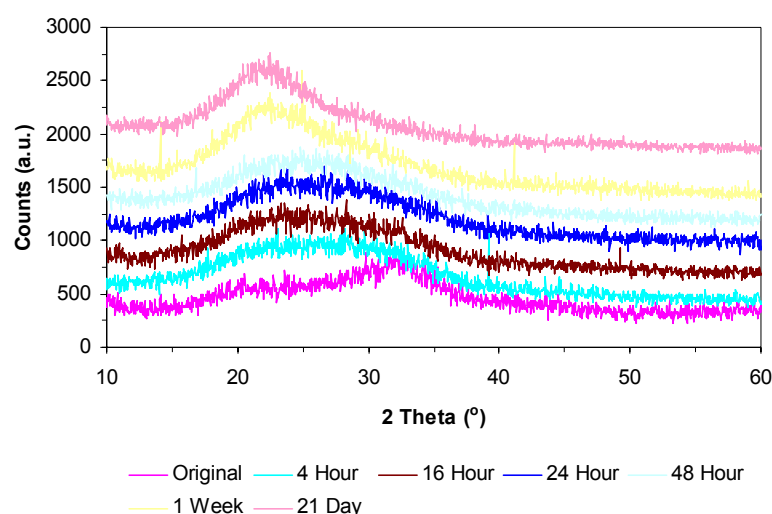


Figure 118: XRD patterns obtained for ICIE1100MG glass following immersion in SBF for different time periods.

If all of the XRD spectra are examined collectively, it is very evident that the presence of crystalline peaks becomes less with the higher magnesium containing glasses. Indeed, the 0% magnesium glass shows HCA formation after 24 hours, the 25% magnesium glass only after 48 hours, the 50% and 75% magnesium containing glass only after 21 days and the 100% magnesium containing glass does not show HCA formation at all. It is therefore concluded that magnesium suppresses apatite formation in the bioactive glass compositions studied.

Figures 119 to 122 show the ICIE4 series of glasses spectra after immersion in simulated body fluid for a period of up to 21 days.

All four figures for the four glasses in the series illustrate exactly the same features. The spectrum for 16 hours, 24 hours and 48 hours for all of the glasses only show a flattening of the characteristic amorphous halo. There is no evidence of any crystalline peaks associated with HCA formation. It can therefore be concluded that for all glasses in the series that have been immersed in SBF for up to 48 hours no apatite formation was picked up by the X-Ray diffractometer.

All four glasses in the series exhibit apatite formation, however, after a period of 21 days in SBF. The data is very noisy, but the Bragg peaks at 25.9° and 31.9° corresponding to the (002) and (211) reflections [174] [187] are evident. On closer inspection, as we move through the series, the sharpness and prominence of the crystalline peaks associated with HCA formation becomes less. Therefore, it is suggested that the same phenomenon discussed above is occurring in the ICIE4 series of glasses, that is that magnesium is suppressing the formation of apatite. It is important to note that there is a large time gap between the spectrum of 48 hours, which illustrates no crystalline peaks, and the spectrum of 1 week which illustrates crystalline peaks associated with HCA. Hydroxyapatite could therefore have formed at any time between 2 days and 1 week. The sharper peaks in the lower magnesium containing glasses suggest that the hydroxyapatite has formed earlier compared with the duller peaks of the higher magnesium containing glasses. It would be interesting, therefore, to undertake further simulated body fluid studies at intermediate time points between 2 days and 1 week. The results would therefore give a greater idea of time at which apatite is first formed on the glasses in the series.

It should be noted that crystallite size analysis was not able to be undertaken for the above two series of glasses due to the noise and degree of peak overlap in the respective spectra.

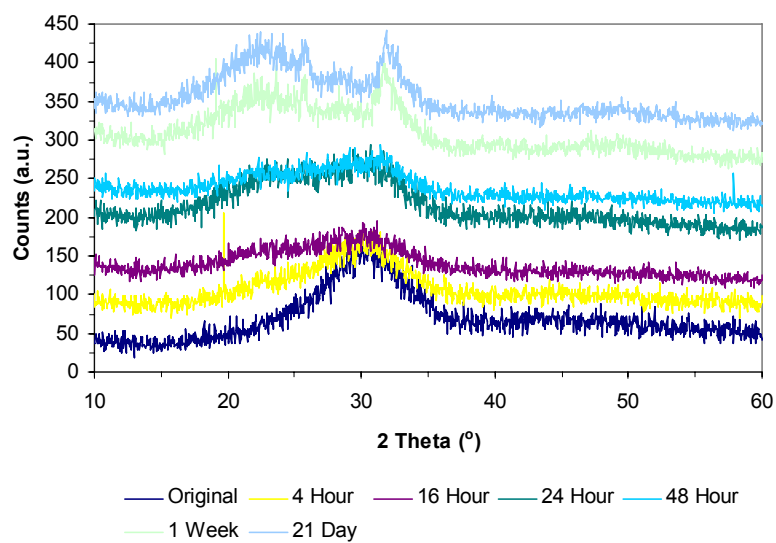


Figure 119: XRD patterns obtained for ICIE4 glass following immersion in SBF for different time periods.

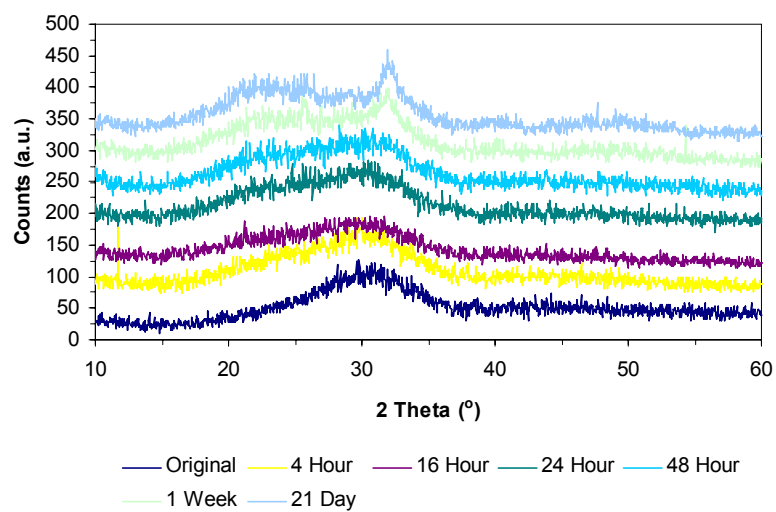


Figure 120: XRD patterns obtained for ICIE425MG glass following immersion in SBF for different time periods.

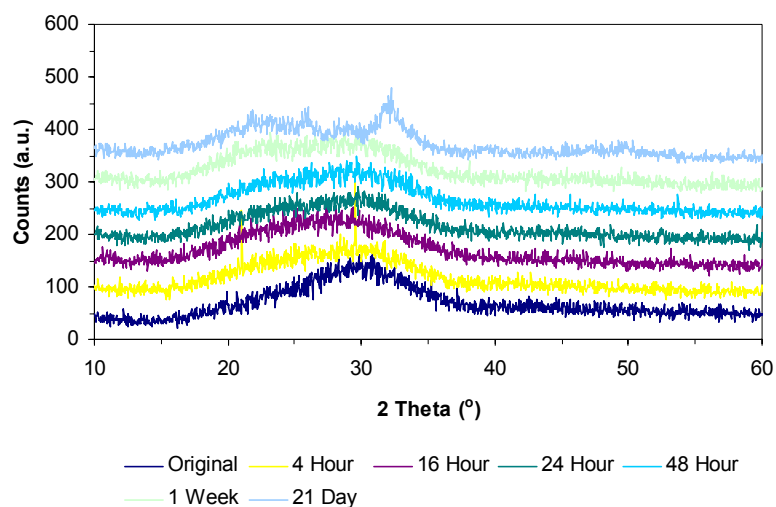


Figure 121: XRD patterns obtained for ICIE450MG glass following immersion in SBF for different time periods.

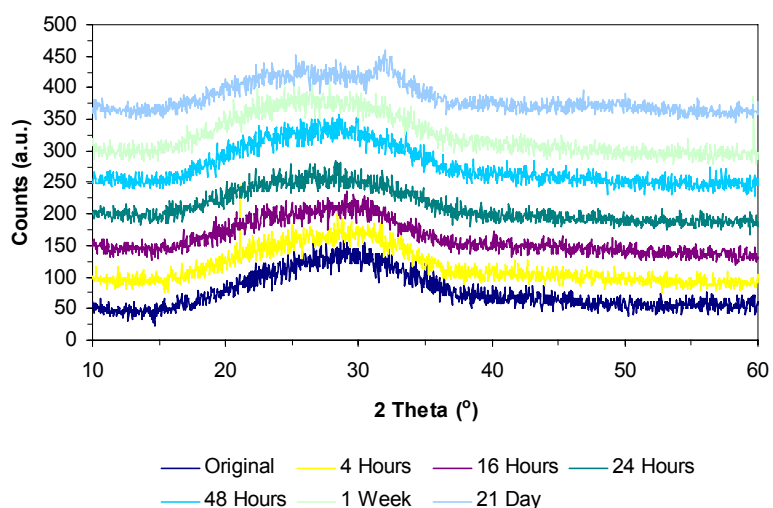


Figure 122: XRD patterns obtained for ICIE475MG glass following immersion in SBF for different time periods.

Table 21 below tabulates the time at which apatite is first picked up by X-ray diffraction for both series of magnesium substituted glasses. It is evident that the lower magnesium containing glasses form apatite quicker than the higher magnesium glasses. Furthermore, the ICIE4 series of glasses seem to form apatite later than their

corresponding ICIE1 glasses. This seems to suggest that the greater the amount of magnesium the more apatite formation is suppressed.

Table 21: First time point at which apatite is observed by X-Ray diffraction.

	15 minutes	1 hour	4 hours	16 hours	24 hours	48 hours	1 week	21 days
<i>ICIE1 series</i>								
ICIE1					√	√	√	√
ICIE125MG						√	√	√
ICIE150MG							√	√
ICIE175MG								√
ICIE1100MG								
<i>ICIE4 series</i>								
ICIE4						√	√	√
ICIE425MG							√	√
ICIE450MG								√
ICIE475MG								

7.11.2.1 Summary of SBF XRD results for the magnesium series of glasses

- The XRD spectra for the higher magnesium substituted glasses illustrate that apatite forms slower than their lower magnesium substituted counterparts.
- Therefore, it can be concluded that magnesium acts to suppress apatite formation in the glasses studied.
- Furthermore the suppressing effect seems more pronounced in the ICIE4 series of glasses. It is suggested that this is a result of the greater amount of magnesium substituted into the parent glasses.

7.11.3 FTIR Spectroscopy

A glass specimen is considered to exhibit bioactive behaviour if a calcium and phosphorus rich layer forms on its surface. Fourier Transform Infra-red Spectroscopy was therefore used to monitor the formation of the HCA layer on glasses immersed in simulated body fluid over a period of 21 days.

Figure 123, 124 and 125 illustrate the FTIR spectra of ICIE1 series of glasses after soaking in SBF for 15 minutes, 1 hour and 4 hours, respectively. On examination of the spectra of the series at each of these time points, the main peaks are those associated with the main glass structure. More specifically absorption bands were located at approximately 1080cm^{-1} and 930cm^{-1} as a result of Si-O group vibrational modes: Si-O stretch and Si-O alkali stretch [233]. There are no characteristic features associated with the formation of HCA. This is clear evidence to show that after 4 hours of immersion in SBF there is no visible bioactive behaviour.

After 16 hours, however, it is evident from Figure 126 that there is a sharpening of the features in the FTIR spectra. This sharpening is particularly noticeable for the band at approximately 1080 cm^{-1} . Furthermore, at 16 hours it can be seen that the second most prominent peak at approximately 930cm^{-1} , that assigned to the Si-O alkali vibration, has virtually disappeared. This loss of a band is even more prominent with the higher magnesium containing glasses. Also after 16 hours of immersion, a new feature is observed at approximately $780\text{-}800\text{cm}^{-1}$. This is assigned to Si-O-Si bond vibration between two adjacent SiO_4 tetrahedra [233]. These changes indicate the existence of a hydrolysis reaction, leaching of sodium and calcium ions and the subsequent formation of a polymerised SiO_2 -gel layer on the glass. The characteristic splitting of the P-O bending vibrations at 570cm^{-1} and 602 cm^{-1} are absent. The existence of this feature is usually evidence of the formation of a crystalline HCA layer, therefore, it is concluded that after 16 hours of immersion in SBF, there is evidence of the initial mechanisms involved in bioactivity but there is no final HCA layer on any of the glasses in the series.

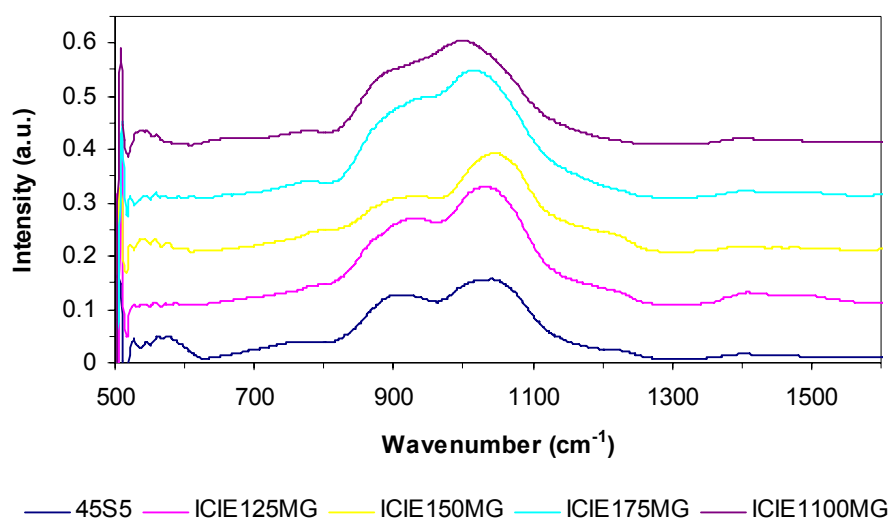


Figure 123: FTIR spectra of ICIE1 series of glasses following 15 minutes of immersion in SBF.

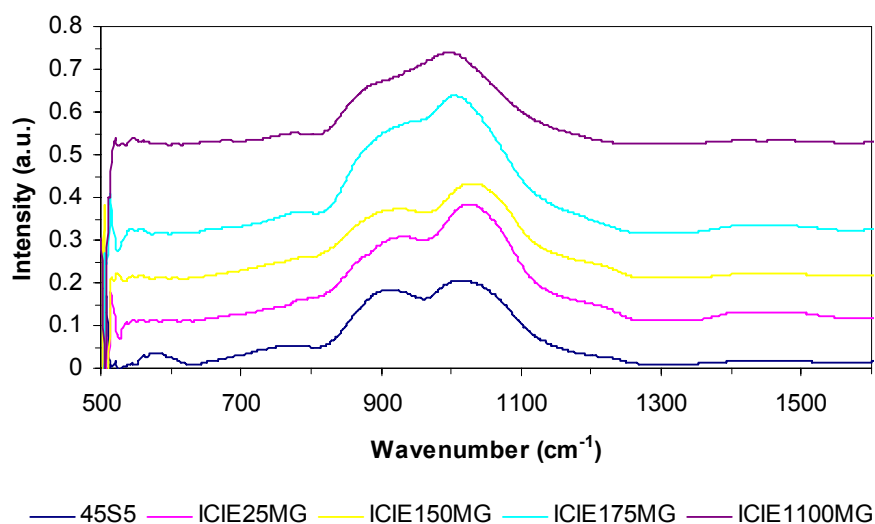


Figure 124: FTIR spectra of ICIE1 series of glasses following 1 hour of immersion in SBF.

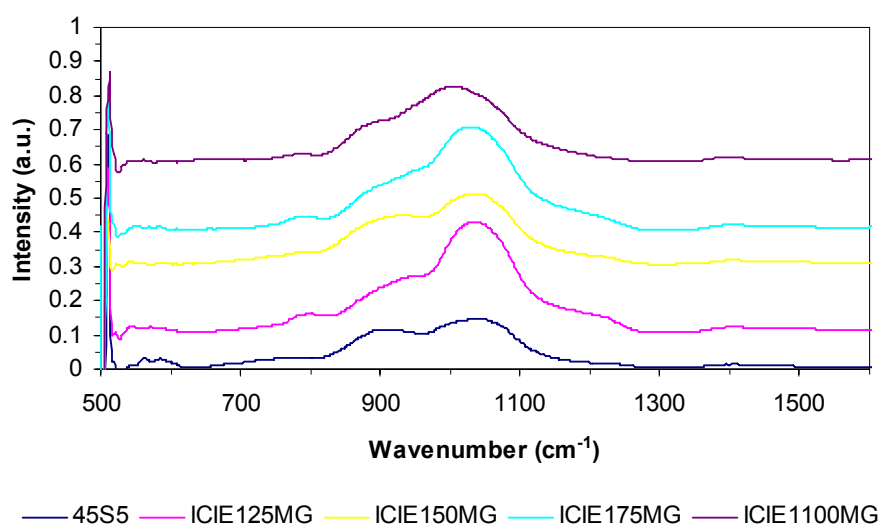


Figure 125: FTIR spectra of ICIE1 series of glasses following 4 hours of immersion in SBF.

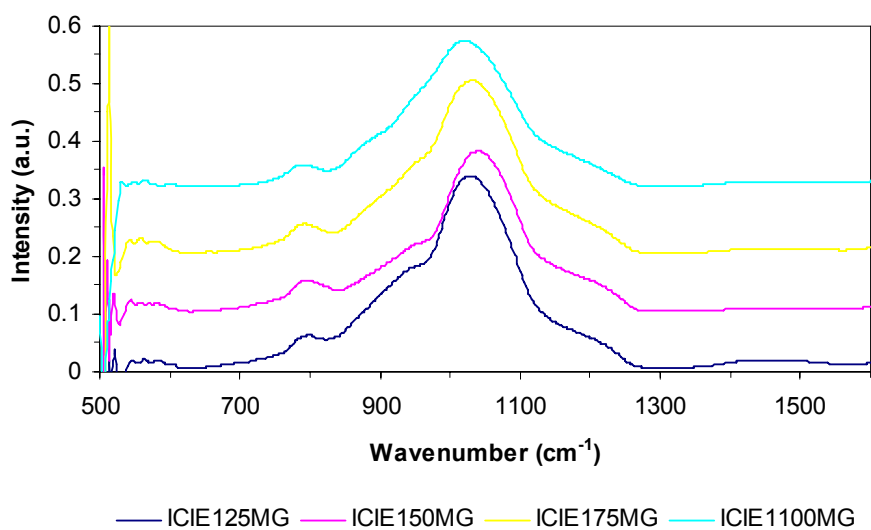


Figure 126: FTIR spectra of ICIE1 series of glasses following 16 hours of immersion in SBF.

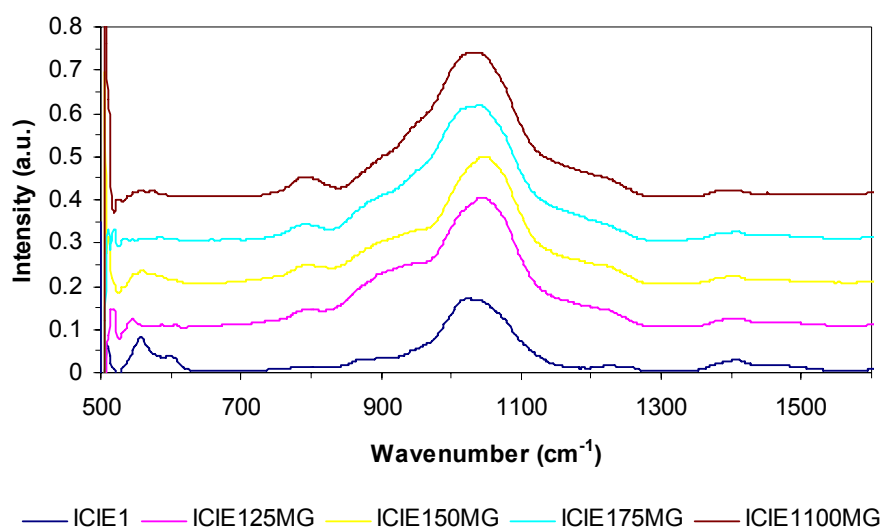


Figure 127: FTIR spectra of ICIE1 series of glasses following 24 hours of immersion in SBF.

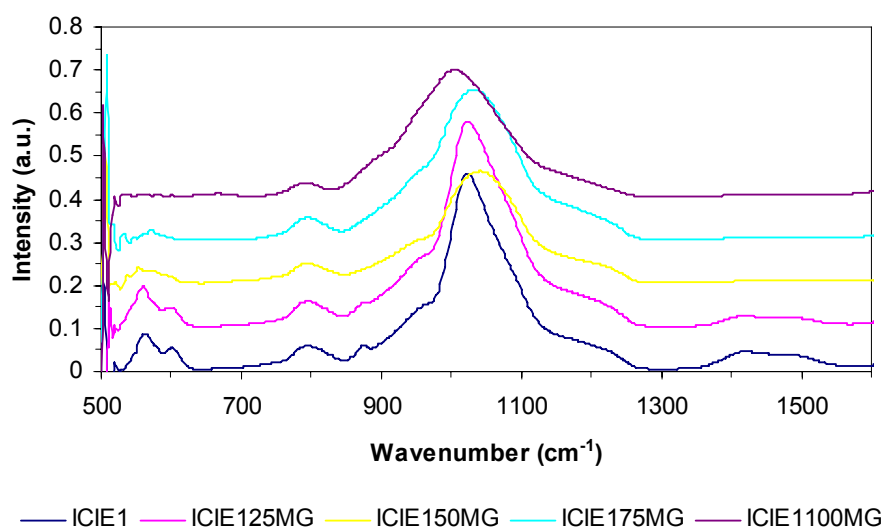


Figure 128: FTIR spectra of ICIE1 series of glasses following 48 hours of immersion in SBF.

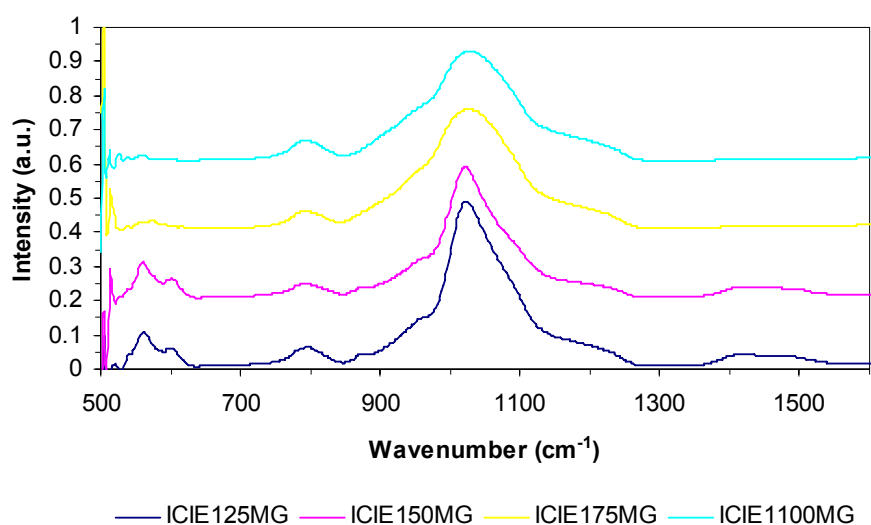


Figure 129: FTIR spectra of ICIE1 series of glasses following 1 week of immersion in SBF.

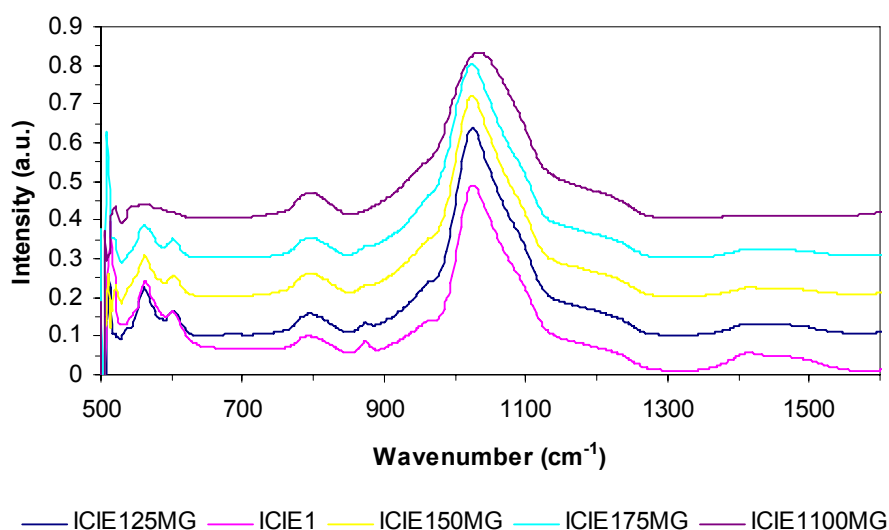


Figure 130: FTIR spectra of ICIE1 series of glasses following 21 days of immersion in SBF.

After 24 hours of immersion, Figure 127 illustrates that the same features as those in the 16 hour spectra are present. Furthermore, the ICIE1 glass also displays the characteristic splitting at approximately 570cm^{-1} and 602cm^{-1} , which correspond to the P-O bending vibrations of PO_4^{3-} tetrahedral in crystalline HCA. The existence of the bands indicate the formation of a crystalline calcium phosphate layer [234]. It can therefore be concluded that the ICIE1 glass only, after 24 hours of immersion in SBF,

forms a bioactive layer. The other magnesium containing glasses in the series, however, do not show the characteristic splitting of the bands.

Further examination of the spectra after 24 hours of immersion show that the band at approximately 780cm^{-1} , first seen after 16 hours of immersion, becomes more prominent and sharper in nature through the series. Furthermore the band seems to shift to lower wavenumbers through the series and as magnesium is added.

After 48 hours of immersion in SBF, Figure 128 illustrates that not only the ICIE1 glass but also the ICIE125MG glass shows the characteristic splitting at approximately 570cm^{-1} and 602cm^{-1} , corresponding to the P-O bending vibrations of PO_4^{3-} tetrahedral in crystalline HCA. The other glasses in the series, ICIE150MG, ICIE175MG and ICIE1100Mg, do not show this feature. Therefore, even after 2 days in SBF, the higher magnesium containing glasses do not exhibit bioactive behaviour. The 48 hour spectra also highlight that the most prominent peak at approximately 1080cm^{-1} becomes less pronounced with the higher magnesium containing glasses. Furthermore, there is a definite shift of this band to higher wavenumbers through the series.

After 1 week of immersion in simulated body fluid, the ICIE125MG and ICIE150MG glasses exhibit the characteristic splitting of the P-O bending vibration at approximately 570cm^{-1} and 602cm^{-1} . Therefore, it is concluded that both these glasses exhibit bioactive behaviour. The higher magnesium containing glasses, however, do not. It is only until 21 days of immersion that the ICIE175MG glass exhibits bioactive behaviour. Even after nearly a month of immersion the fully substituted magnesium glass does not show the characteristic splitting of the P-O bending vibrations.

When analysing all the spectra together, it is evident that the higher magnesium glasses take longer to show any evidence of HCA formation. Indeed, the ICIE1100MG glass does not exhibit any of the features associated with HCA formation at all. It is therefore

concluded that magnesium acts to inhibit crystalline apatite formation in this series of glasses.

The figures below illustrate the FTIR spectra for the ICIE4 low sodium glasses after immersion in SBF for up to a period of 21 days.

After 15 minutes, 1 hour and 4 hours no changes can be observed to the spectra. All spectra up to 4 hours of immersion in SBF simply mirror the features of the unreacted glasses with absorptions bands located at approximately 1100cm^{-1} and 930cm^{-1} . These are, as discussed earlier, associated with the Si-O group vibrational modes: Si-O stretch and Si-O alkali stretch.

After 16 hours of immersion in SBF, the glasses exhibit similar changes in the features of the spectra to those observed for the ICIE1 high sodium glasses. These include the sharpening of the bands and the disappearance of the Si-O alkali band at approximately 930cm^{-1} . Furthermore, new features at around 800cm^{-1} are observed, those relating to Si-O-Si bond vibration between two adjacent SiO_4 tetrahedra. As discussed above these changes are indicative of the initial stages of the bioactive mechanism – hydrolysis, leaching of Na^+ and Ca^{2+} and the formation of a SiO_2 -gel layer.

It is not until after 48 hours of immersion in SBF that any features change in the spectra for the ICIE4 series of glasses. Figure 136 shows that the ICIE4 glass exhibits the characteristic splitting of the P-O bending band at approximately 570cm^{-1} and 600cm^{-1} . These bands correspond to the existence of crystalline HCA. This splitting is only seen for the 0% magnesium glass however and not for the magnesium substituted glasses. Therefore, it can be concluded that, although the glasses exhibit changes that are indicative of the early stages of bioactivity, it is only the ICIE4 glass that exhibits the characteristic features associated with HCA formation.

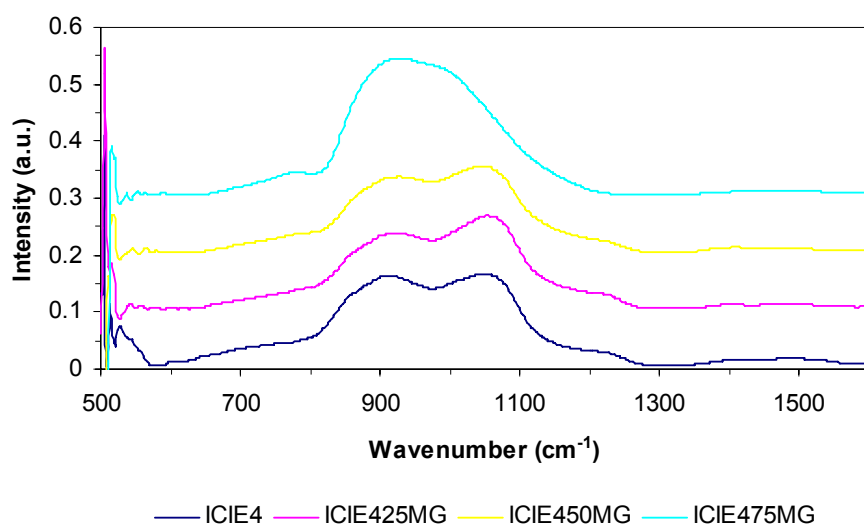


Figure 131: FTIR spectra of ICIE4 series of glasses following 15 minutes of immersion in SBF.

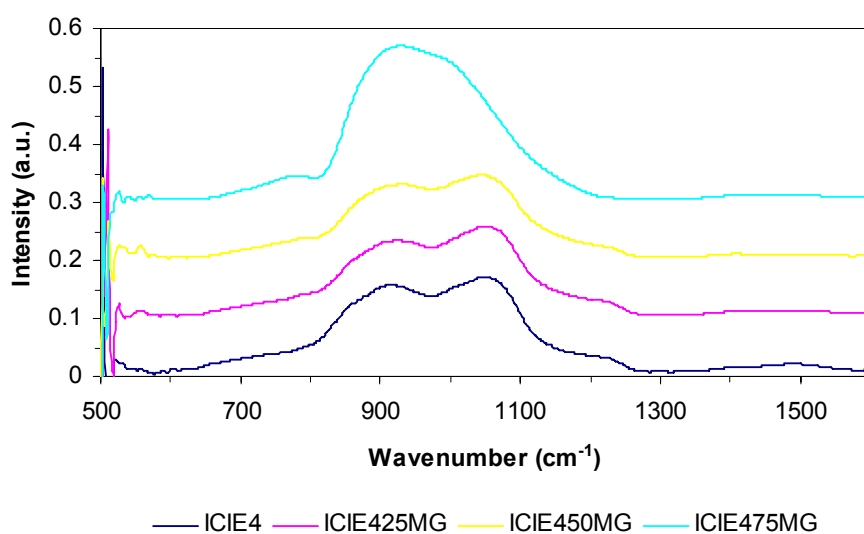


Figure 132: FTIR spectra of ICIE4 series of glasses following 1 hour of immersion in SBF.

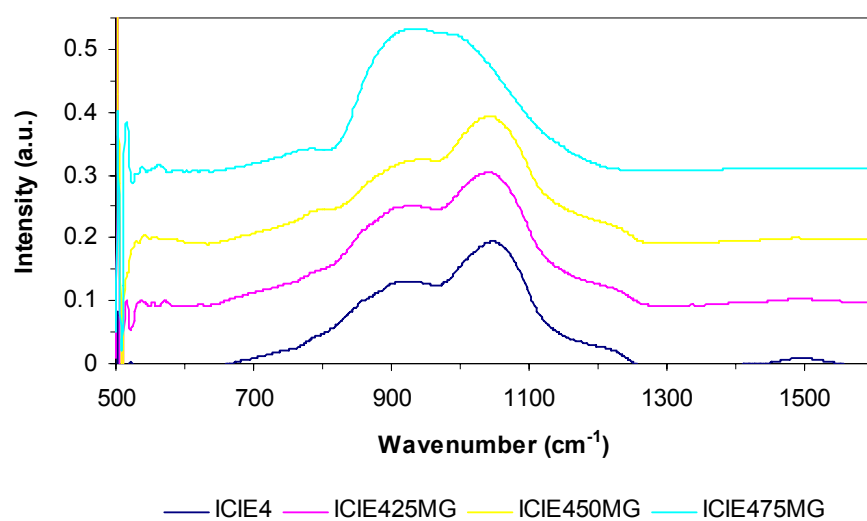


Figure 133: FTIR spectra of ICIE4 series of glasses following 4 hours of immersion in SBF.

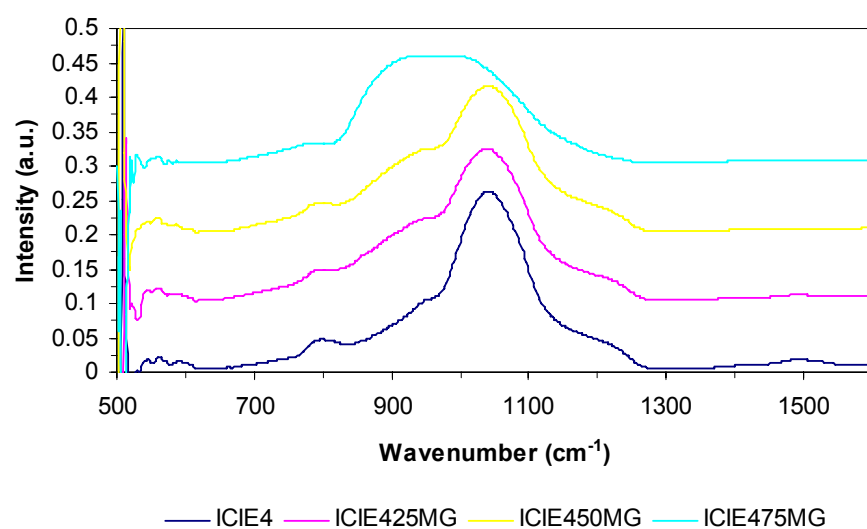


Figure 134: FTIR spectra of ICIE4 series of glasses following 16 hours of immersion in SBF.

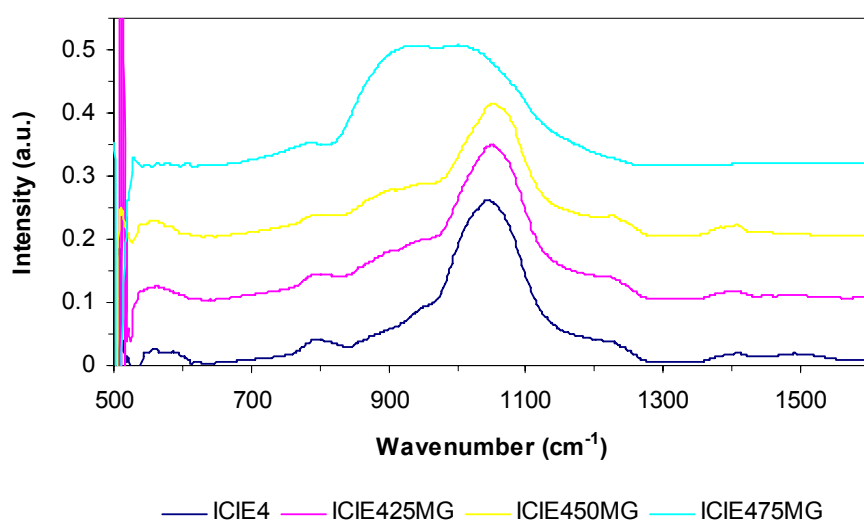


Figure 135: FTIR spectra of ICIE4 series of glasses following 24 hours of immersion in SBF.

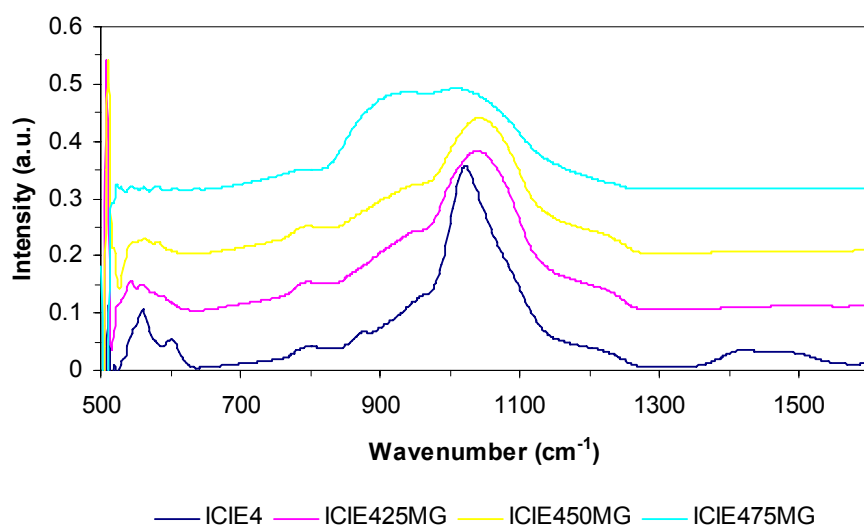


Figure 136: FTIR spectra of ICIE4 series of glasses following 48 hours of immersion in SBF.

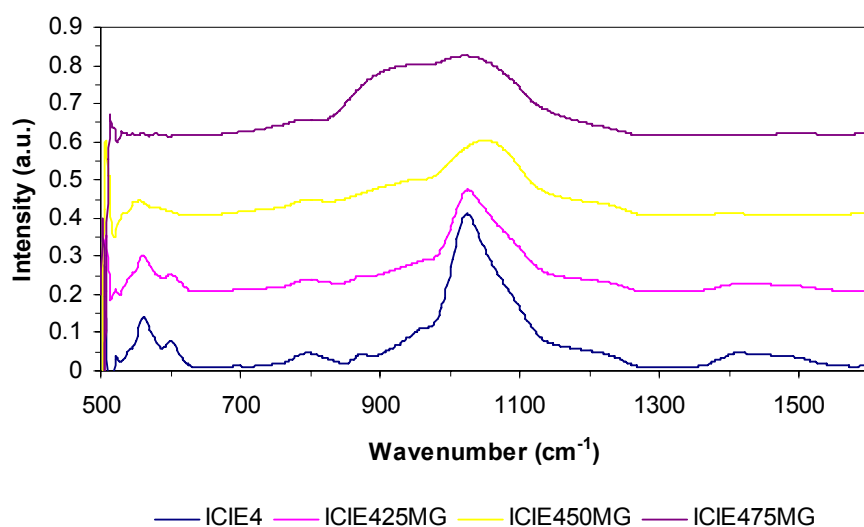


Figure 137: FTIR spectra of ICIE4 series of glasses following 1 week of immersion in SBF.

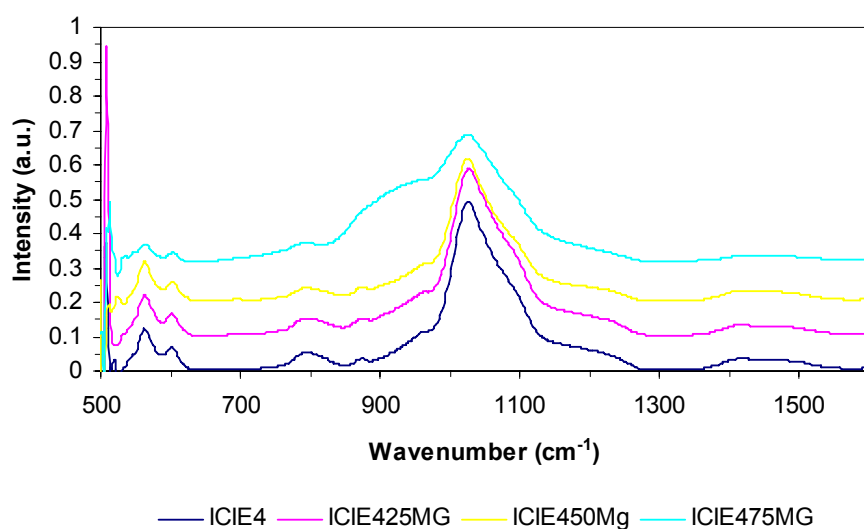


Figure 138: FTIR spectra of ICIE4 series of glasses following 21 days of immersion in SBF.

After 1 week of immersion in SBF, however, the ICIE425MG glass also exhibits this behaviour. After 21 days of immersion in simulated body fluid all of the glasses exhibit the characteristic splitting of the P-O bend (into bands at 560 and 600 cm⁻¹). It can therefore be concluded that all of the glasses form a crystalline hydroxyapatite layer after 21 days of immersion. When examining Figure 138, however, it can be seen that the features associated with HCA formation are more prominent and sharp for the ICIE4 glass thus indicating that the HCA layer is more established.

Figures 139 and 140 below illustrate the spectra for the ICIE125MG and ICIE1100MG glasses, respectively and the evolution of the characteristic FTIR peaks associated with apatite growth. It is clear when comparing the two figures that the characteristic peaks associated with HA formation evolve much quicker and to a larger extent for the ICIE125MG glass.

This phenomenon is also observed in Figures 141 and 142 where the evolution of the HA peaks with time is illustrated for the ICIE425MG glass and the ICIE475MG glass, respectively.

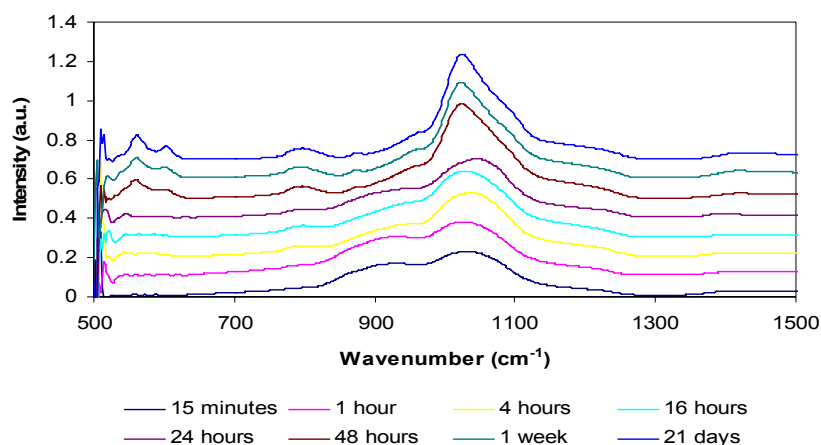


Figure 139: FTIR spectra of ICIE125MG glass from 15 minutes to 21 days of immersion in SBF.

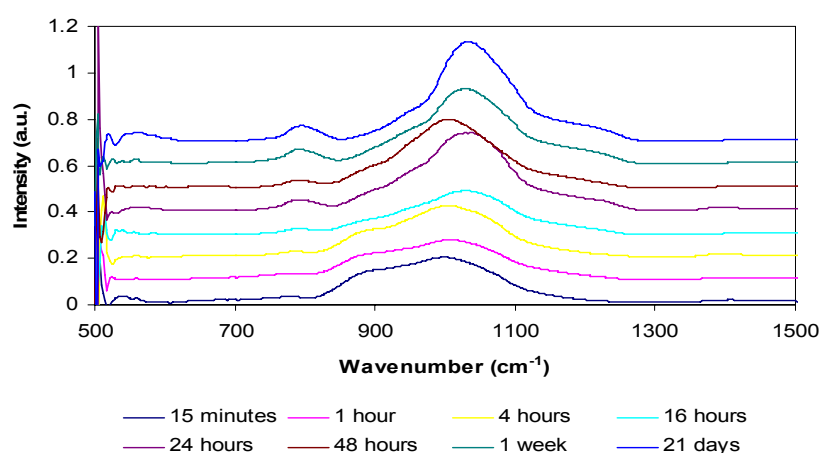


Figure 140: FTIR spectra of ICIE1100MG glass from 15 minutes to 21 days of immersion in SBF.

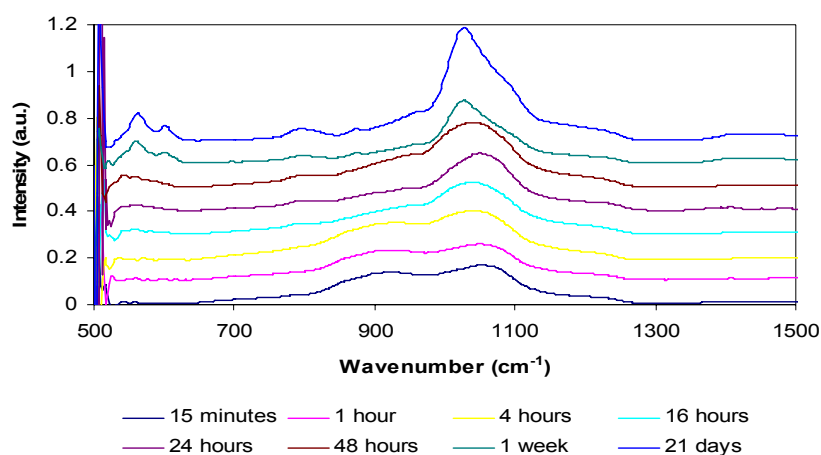


Figure 141: FTIR spectra of ICIE425MG glass from 15 minutes to 21 days of immersion in SBF.

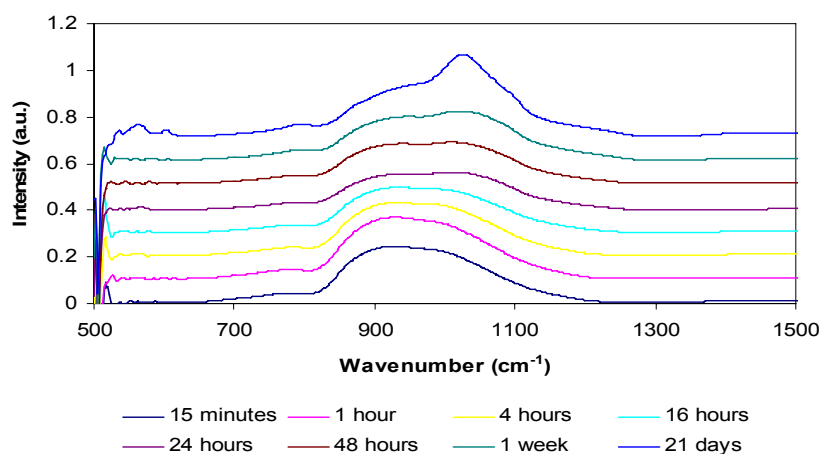


Figure 142: FTIR spectra of ICIE475MG glass from 15 minutes to 21 days of immersion in SBF.

These figures clearly show that magnesium suppresses apatite formation.

7.11.3.1 Summary of SBF FTIR results for the phosphorus series of glasses

- The FTIR spectra for the higher magnesium glasses illustrated the characteristic peaks associated with apatite formation at later time points compared to the lower magnesium containing glasses.
- It is therefore evident that magnesium acts to suppress apatite deposition in bioactive glasses.
- The suppressing effect was more prominent in the ICIE4 low sodium magnesium glasses thus suggesting that the more magnesium in a glass, the less apatite will form (or the slower the rate of apatite formation).

7.12 Summary

Although magnesium is an important ion in all types of glasses, as well as bioactive glasses, the actual role that it undertakes in the glass structure is far from understood. As a result, the effect that magnesium has on a glass's properties and on its bioactive behaviour is very poorly documented and is a source for great controversy.

Following X-Ray absorption spectroscopic investigations, the divalent transition metal cations Fe^{2+} and Ni^{2+} were documented to occupy tetrahedral sites within silicate glasses. It was suggested that magnesium could behave in a similar manner [235] [213]. Over the years there have been several attempts to investigate magnesium's role in a glass structure by using various materials characterisation techniques such as nuclear magnetic resonance [143] [212], X-ray scattering [236] and absorption spectroscopy [237]. As a whole, these works all point to magnesium residing in a highly disordered environment. A truly detailed and comprehensive study of the structural role of magnesium, however, is still to be undertaken.

It is suggested that the lack of structural understanding of magnesium's role within a glass, coupled with the rather haphazard approach to bioactive glass design, has resulted in the conflicting nature of literature regarding magnesium and its role with respect to bioactivity. Indeed, it is one of the controversial topics within the field of bioactive glasses. For example, it is suggested that MgO inhibits mineralisation *in vivo* [120] [138] while conversely it is documented as having no effect on apatite formation [140]. Bioverit[®], however, is a classic example of magnesium containing glass ceramic which has been in clinical use successfully for years.

One of the objectives of this study, therefore, was to undertake a systematic and methodical study on the effect of magnesium substitution within a soda lime phosphosilicate glass. The emphasis of the study was to gain an understanding of magnesium's structural role within a glass and how this role effects a glass's resultant bioactive behaviour. In order to discover whether magnesium behaved in a similar

manner within glass systems of differing levels of disorder, two glass series were designed – the first series; a high sodium content soda lime phosphosilicate glass, the second; a low sodium content soda lime phosphosilicate glass.

The high sodium ICIE1 series of glasses were designed with a systematic substitution of CaO for MgO on a molar basis, with a constant network connectivity value of 2.04 through out the series.

The ^{29}Si MAS NMR data illustrated the presence of a predominantly Q^2 structure (-79ppm) with a progressive shift to a Q^3 structure with increasing MgO substitution (-90ppm). This contradicted the theory that the Q structure would remain constant, on magnesium substitution, as a result of magnesium simply taking up calcium's role as a network modifying cation. The observation of Q^3 species within the glass provided evidence for the progressive polymerisation of the glass structure. This therefore questioned the validity of the assumption of magnesium acting as a network modifier and pointed towards MgO acting as an intermediate oxide with a proportion entering the silicate network as MgO_4 tetrahedra.

The ^{31}P MAS NMR spectra illustrated a single symmetric resonance with a chemical shift range in the region of 8.2-12.7ppm corresponding to the existence of an orthophosphate environment. No evidence was apparent for the existence of Si-O-P bonding, contrary to traditional ideas regarding the behaviour of phosphorus within a bioactive glass, but supporting the conclusions drawn on the structural role of phosphorus within this study. With the substitution of magnesium, a downfield chemical shift towards values associated with a sodium orthophosphate type species was observed. This implied the preferential association of sodium ions with the phosphate phase and it was concluded that magnesium unusually did not enter the phosphate phase (or minimally at that) but was participating within the silicate phase.

Combining the ^{31}P and ^{29}Si MAS NMR data supported the hypothesis that magnesium was behaving as an intermediate oxide in the glasses studied. It is suggested that magnesium was, in effect, unable to participate within the phosphate phase as a result of its involvement in the silicate network. As such, it is suggested that the phosphate phase draws in a more mobile cation such as Na^+ for charge compensation. As the magnesium content increases and more magnesium ions enter the silicate network as MgO_4 tetrahedra, a -2 charge deficiency occurs. MAS NMR data revealed a lack of calcium phosphate type species thus implying that the Ca^{2+} ion may occupy this charge balancing role (along with the proportion of Mg^{2+} ions still acting as network modifiers).

Analysis of the physical properties further supported the hypothesis that a proportion of magnesium was entering the silicate network, with a decrease in T_g and T_s and increase in TEC all highlighting the move to a much weaker glass structure with the substitution of magnesium. Both density and TEC values were observed to stray from their relative models (Doweidar and Appen respectively) at >20mole% substitution implying that magnesium oxide was switching from a modifier to an intermediate oxide through the series.

The low sodium ICIE4 series of glasses were designed similarly to the high sodium ICIE1 series of glasses with a systematic substitution of CaO for MgO on a molar basis, with a constant network connectivity value of 2.04 through out the series.

The ^{31}P MAS NMR spectra all exhibited a symmetric resonance with a chemical shift range in the region of 5.0 - -3.3ppm thus implying that the phosphorus was residing within an orthophosphate environment. With the addition of magnesium, however, a move to more negative chemical shifts was observed and this was attributed to the formation of a pyrophosphate type species.

The ^{29}Si MAS NMR spectra exhibited a single symmetric resonance with peak maxima in the range of -82.0 to -85.5ppm. This implied the presence of a predominantly Q^2 silicate structure, agreeing with the constant network connectivity value of 2.04 through the series. Although no Q^3 shoulders were observed, a slight chemical shift to values associated with Q^3 silicate species was observed. It was therefore concluded that there was evidence, however slight, of magnesium acting as an intermediate oxide (with a proportion entering the silicate network) within the glasses studied. Due to the rather featureless nature of the spectra, quantification of the proportion of magnesium ions entering the network was unachievable. Qualitatively, however, it can be concluded that the proportion was less than that observed for the high sodium ICIE1 series of glasses studied.

Physical analysis of the low sodium ICIE4 series of glasses resulted in data which reflected similar behavior to that recorded for the high sodium ICIE1 series of glasses. The trends, however, were less pronounced and indeed some behavior, such as the observed up turn in glass transition and softening temperatures remains difficult to explain. One explanation was that as magnesium enters the network, the weaker bond strength of Mg-O compared to Si-O results in the initial decrease in glass transition temperature. The formation of MgO_4 tetrahedra, however, would require charge balancing cations and as magnesium is substituted for calcium, more magnesium ions would have to take up this modifying role. Magnesium, with its higher charge to size ratio, would act to pull the NBOs more tightly thus resulting in the up turn observed. The glass transition temperature therefore was suggested as reflecting two competing effects – magnesium entering the network and weakening the network versus the dominance of magnesium also in a modifying role, with the loss of calcium, strengthening the network. Furthermore, it was suggested that this up turn in glass transition temperatures was not observed in the high sodium ICIE1 series of glasses purely as a result that the two competing effects were dominated by the weakening of the structure. In order to fully understand this kind of phenomenon it is suggested that further glass series would need to be analysed. It is important to note, however, that this phenomenon is not isolated, with the same up turn in glass transition temperatures on substitution of magnesium for calcium, observed by Bovo *et al.* [232].

When comparing the two series of magnesium substituted glasses, it was concluded that there was evidence that part of the magnesium oxide in both series was acting as an intermediate oxide and entering the silicate network (Gaussian peak fitting approximated 14% in the ICIE1 glasses studied). It is suggested that MgO prefers to switch its role to an intermediate oxide in highly disrupted silicate glasses, of which bioactive glasses are an example. The high sodium ICIE1 samples represent a more disordered series of glasses compared to the low sodium ICIE4 samples, as a result of the disruptive nature of sodium. It is therefore suggested that it is this disorder that “persuaded” a higher proportion of the magnesium ions to enter into the silicate network and take up a tetrahedral configuration. Bovo *et al.* [232] observed a similar effect with 17% and 30% of the magnesium ions entering the network for 50% SiO₂ containing soda lime silicate glasses and the 45% SiO₂ containing soda lime silicate glasses respectively. Bovo’s results also reflect that the more disrupted glasses (45% SiO₂ series) cause magnesium to enter the silicate glass network more readily.

The second cation field strength (defined as ratio of net charge and the square of the average bond distance to oxygen) has also been suggested to effect magnesium’s role within a glass. It has been hypothesised that the magnesium ion can compete effectively for anionic oxygen contacts with a cation with lower field strength thus reducing its overall coordination number [238]. Therefore it can be visualised that magnesium (0.45) is able to compete effectively with both the lower field strength cations of sodium (0.19) and calcium (0.33) within the soda lime phosphosilicate glasses studied thus causing it to take up a tetrahedral configuration within the silicate glass network.

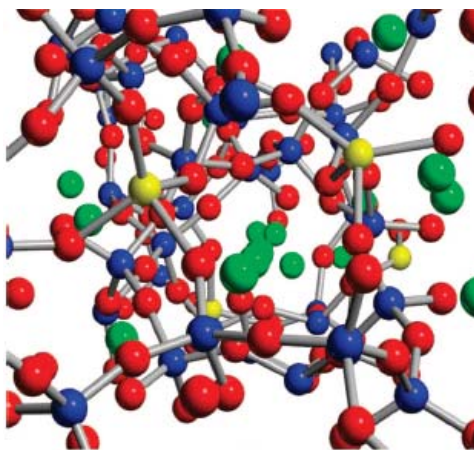


Figure 143: Image of a percolation channel in a magnesium substituted soda lime silicate glass. Mg ions (light yellow) enter into the Si-O (Si, light blue and O, red) network acting as a network former. Na ions (light green) are free to diffuse hopping among affinity sites [239].

The understanding of glass structures and the role of specific elements within these structures can be greatly assisted by the use of atomistic modern computational techniques [239]. Cormack and Du [240] undertook a molecular dynamics simulation of a $\text{SiO}_2\text{-MgO-CaO-Na}_2\text{O}$ glass and interestingly found similar results in that 90% of the Mg ions were observed to be in a four coordinate state participating in the silicate network, forming Si-O-Mg and Mg-O-Mg bonds. The remaining 10% of magnesium ions were in a five co-ordinate state. Furthermore, it was commented that with increasing MgO/CaO ratio, the Na^+ ion diffusivity increased as a direct result of magnesium behaving as a former. This was explained by the fact that magnesium, acting as a former does not act to obstruct the percolation channels and hence the sodium ions are free to diffuse in the structure via a preferential site hopping mechanism as illustrated in Figure 145 above [239] [16]. It could be imagined that the increased diffusivity of sodium ions, as a result of magnesium entering the silicate network, could allow the ions to enter the phosphate phase thus resulting in the observed preferential association of sodium ions with the orthophosphate species.

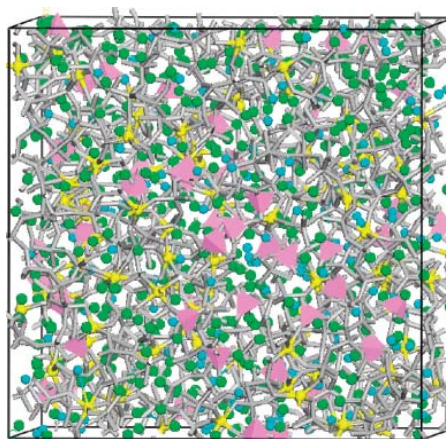


Figure 144: Snapshot of the 3D structure of a magnesium substituted soda lime phosphosilicate bioactive glass. Violet tetrahedra represent the orthophosphate units, yellow spheres represent the magnesium ions, green spheres represent the charge modifying sodium ions, cyan spheres represent the charge modifying calcium ions while the grey sticks represent the Si-O framework [241].

Pedone *et al.* [241] have also documented magnesium acting as a pseudo network former within soda lime phosphosilicate bioactive glasses. Figure 146 above simulates the structure of such a glass. Further molecular simulations on a magnesium substituted soda lime silicate glass system also found that the calcium and magnesium ions exhibited different roles within the glass structure. Calcium was observed in six fold coordination, showing a similar modifying role to that of sodium. Conversely, magnesium ions were in a four fold coordination and exhibited network forming tendencies with MgO_4 tetrahedra interconnecting with the SiO_4 network [239].

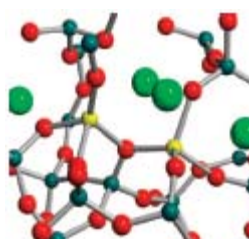


Figure 145: Mg-O-Mg bridges formed by MgO_4 tetrahedra in the silicon network. Silicon, magnesium, oxygen and sodium ions are represented as blue, yellow, red and green spheres [239].

The role of MgO on the bioactive behaviour of glasses has led to contradictory results. This can be easily appreciated as a result of the limited knowledge of the structural role of magnesium within a glass and more specifically as, as discussed above, its ability to behave either as a modifier or as an intermediate oxide.

Dissolution studies were undertaken on both of the series of magnesium containing glasses. All glasses were submerged in simulated body fluid for a number of time periods ranging from 15 minutes to 21 days. The resulting glasses were analysed by XRD and FTIR.

It was proved from the XRD spectra for both series of glasses that although apatite formation was present, it was only really on the unsubstituted glasses or the low magnesium substituted glasses. It was seen that the higher the magnesium substitution the less apatite formation. Indeed, for the 100% magnesium substitution in the high sodium ICIE1 glass, apatite formation was not evident. Similarly, the 75% substituted glass in the low sodium series did not exhibit any bioactive behaviour. FTIR analysis further supported the XRD results. It was concluded that the high magnesium containing glasses were not bioactive in nature and that magnesium acts to suppress apatite formation.

Vallet-Regi *et al.* [242] found that the inclusion of magnesium within a bioactive glass acts to slow down the rate of formation of the apatite layer. Furthermore Ni *et al.* [243] reported that, even following 4 weeks of being immersed in SBF, magnesium containing ceramics were not able to form an apatite layer and commented that the Mg/Ca ratio had an important effect on the apatite-formation ability in CaO-SiO₂-MgO system ceramics. Vallet-Regi *et al.* attributed the slowing of apatite formation on magnesium containing glasses to the magnesium ion, even when only in trace amounts, reducing the overall rate of seeded calcium phosphate crystallisation and significantly slowing the change from the amorphous calcium phosphates to apatite compositions [242]. Conversely it was discussed that the higher Mg-O bond energy would act to decrease the dissolution of the glass into solution as compared with the Ca-O bond.

Therefore if following the nucleation and growth mechanisms of an apatite-like layer proposed by Hench and Kokubo, the higher Mg-O bond energy would hinder the exchange of Ca^{2+} ions with H_3O^+ ions in SBF and thus the subsequent formation of silanol groups [243]. The observed decrease in apatite formation in the magnesium glasses studied could, therefore, be attributed to this effect, when considering the proportion of magnesium acting traditionally as a network modifier. Even more significant, however, is the effect of magnesium entering the silicate network. The move to a more cross-linked glass Q^3 structure would inhibit the dissolution of ions into solution and thus decrease the rate of apatite formation. It is well known and well documented that glasses with high network connectivity values do not exhibit bioactive behaviour, with an empirical upper limit of $\text{NC}=3$ proposed by Kim *et al.* [244] and Stranad *et al.* [55]. This upper limit is based upon the interplay between network connectivity and solubility with low NC denoting an open and fragmented structure which will rapidly dissolve into solution resulting in HA formation in a shorter time period compared to glasses with a highly cross-linked structure. As discussed by Tilocca *et al.* [15], the empirical observation that glasses with $\text{NC}>3$ are bio-inactive means that the hydrolysis of silicate species with an average of three plus BOs endure an excessively high energetic cost, which effects the overall bioactive mechanism to the point where bone bonding can no longer be achieved (Figure 148 below) [15].

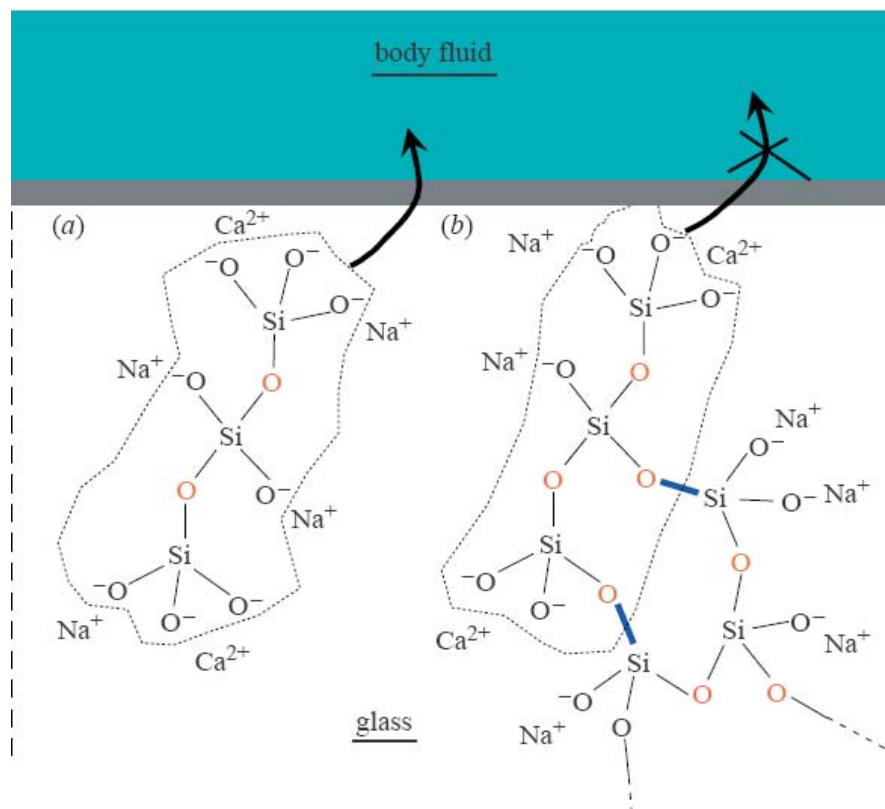


Figure 146: Schematic of the structure of bioactive glasses; BOs coloured as red. The fragment in (a) is a three-membered silicate chain, with no covalent links to the rest of the structure, whose dissolution will be faster than that of (b) a five-membered ring, covalently cross-linked through the additional Si-O bonds coloured in blue.

The structure above graphically represents the difference between a Q^2 silicate structure with magnesium behaving as a modifying ion, which will dissolve easily into solution compared to the Q^3 structure with magnesium entering the network, restricting the dissolution of ions into solution.

Okasaki *et al.* [245] studied the influence of magnesium on apatite precipitation and concluded that magnesium has a tendency, during the precipitation of apatite films, to induce the formation of amorphous structures rather than crystalline precipitates. Posner *et al.* [246] attribute this phenomenon to magnesium entering the hydroxyapatite pre-nuclei in such a way as to distort the structure and prevent any further growth by creating a structural mismatch.

As discussed by Pereira *et al.* [106] a great discussion on the influence of magnesium on apatite crystallisation and bioactive behaviour is still running. The most recent literature on bioactive glasses suggest that it is not the composition of the glass by itself, but the structural arrangement of the ions within the glass network, that governs the type of interaction with the surroundings, either *in vitro* or *in vivo* [106] [17] [198] [137]. Before a detailed understanding of such structural arrangements is achieved a thorough understanding of bioactivity is unattainable. It is submitted that this study on magnesium substituted soda lime phosphosilicate glasses has gone some way to achieving this goal.

8 Conclusion

This work has proved conclusively that the inorganic polymer and network connectivity model can be utilised to design and tailor bioactive glass compositions for specific biomedical applications. More specifically, the work has led to a greater understanding of the roles of phosphorus and magnesium in a glass system, their contribution within the network connectivity model and ultimately their resultant effect on bioactivity.

By designing two series of phosphorus containing glasses, it is concluded that within the soda lime phosphosilicate glass systems studied, phosphorus definitively resides within an orthophosphate type environment resulting in a phase separated structure consisting of silicate rich and phosphate rich domains. The results obtained suggest that glass bioactivity is not determined solely by the dissolution of the glass network, as previously theorised, but is dependant upon the balance between the dissolution of the glass network and the release of phosphorus into solution from the orthophosphate domains. It is concluded that within the glass systems studied, this balance is biased towards the dissolution of phosphorus into solution. The effect of a phase separated structure on the resultant glass properties is intricate and complex and within the context of this study, remains open for further theorising and experimentation.

The substitution of magnesium oxide for calcium oxide has shown that magnesium, rather than behaving as a modifying cation within the glass system will, depending on the disorder of the glass, at times switch to behave as an intermediate oxide with a proportion entering the silicate network as MgO_4 tetrahedra. This has many implications with respect to the understanding of the bioactive behaviour of magnesium containing glasses. It can no longer be assumed that, on substitution of magnesium for another cation, magnesium will simply take up a modifying role, leaving the silicate

network, and hence dissolution, unaffected. It is theorised that, in a highly disordered glass system, magnesium will preferentially enter the silicate network with the greater the proportion of magnesium entering the network the less bioactive the resultant glass. Furthermore, it has been shown that substituting magnesium oxide for calcium oxide results in a number of physical properties changing in the resultant glass. This study has shown that properties such as the glass transition temperature, softening temperature, thermal expansion coefficient and density are all strongly related to the magnesium content of the glass and furthermore that these properties are further related to which role magnesium takes in the glass. A glass composition can therefore be designed with specific properties for a specific biomedical application.

Despite the growing technological importance of bioactive glasses within the field of biomedicine, the composition-structure-property relationships that exist in such glasses is still relatively unknown. As eloquently expressed by Tilocca [15], this fundamental gap and the fact that a “trial and error” approach still represents the most common way of designing new bioactive glass compositions, negatively effects further progress in the field of bioactive glass design. It is hoped that this study has aided in unveiling some of the key structural features with respect to phosphorus and magnesium within a bioactive glass system and has thus, contributed to improve our fundamental understanding of how bioactive materials work.

8.1 Suggestions for Future Work

This study has raised many interesting questions with respect to understanding glasses and their bioactive behaviour. Further work on the following points detailed below, however, would not only further such understanding but may help to strengthen a number of assumptions made in this study.

Phosphorus Studies

- The study of phosphorus within a bioactive glass system clearly illustrated the existence of orthophosphate complexes and a phase separated structure. The materials characterisation results also raised interesting questions as to the effect of such phase separation, and more specifically phase size and dimensions, on physical parameters such as the glass transition temperatures and thermal expansion coefficients. There are a number of potential techniques available for the investigation of amorphous phase separation. In particular, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) would be particularly useful with respect to gaining an idea of the actual phase distributions and dimensions.
- Small Angle Neutron Diffraction (SANS) was also carried out on the samples with a view to investigating the presence, and dimensions, of phase separation. The results proved inconclusive, however, a SANS study on phase separated structures of bioactive glasses would be an extremely beneficial extension to this study.
- Activation energies for ion hopping have previously been investigated by Hill *et al.* [247] in bioactive glasses, proving very informative in the area of phase separation. By using dielectric spectroscopy the activation energies for hopping of the cations within the phosphate and silica phases could be determined. This in turn could provide further evidence for the preferential association of sodium within the phosphate phase of the magnesium glasses (as discussed previously in Chapter 6.12).
- Although this study has been particularly structure-property related, it is important not to forget that the test glasses have been shown to be bioactive in nature. The finding that phosphorus aids bioactivity potentially could have massive implications commercially. An interesting extension to this study would be to apply this knowledge and make up various high phosphate glasses for particular biomedical applications and perform full cell culture studies.

- Furthermore, a deeper understanding of why phosphorus aids apatite deposition is necessary. Linking the above suggestions for studies on phase separation and phase dimensions with SBF type studies may prove very valuable for the understanding of the bioactive behaviour of high phosphate containing glasses.

Magnesium Studies

- Although it is considered that the data collected points towards magnesium acting as an intermediate oxide within the soda lime phosphosilicate glasses studied, other explanations for the observed behaviour should not be neglected. For example, the move from a Q^2 to a Q^3 glass coupled with the decreasing glass transition temperatures and observed suppression of apatite formation on substitution of magnesium could also be attributed to the formation of a polyphosphate type structure. This possibility has not been discussed further in this report, however, because of the knowledge of parallel results in non phosphorus containing glasses. For example, Bovo *et al.* [232] observed the same move from a Q^2 to Q^3 glass and decrease in glass transition temperature with the substitution of magnesium in a zero phosphate containing soda lime silicate glass. Despite this, it is suggested that further structural analysis is required before a definitive statement can be made as to the role of magnesium in the soda lime phosphosilicate glasses studied.
- ^{17}O and ^{25}Mg MAS NMR would prove extremely useful with respect to the clarification of magnesium and its role as an intermediate oxide. Although ^{25}Mg MAS NMR analysis was undertaken, the fact that it is a quadrupolar nuclei made for poor line resolution and difficult analysis. ^{17}O and ^{25}Mg NMR spectra could, however, prove extremely valuable with respect to probing the short range order of the glass structure and providing conclusive evidence for magnesium acting as an intermediate oxide.
- One of the most important questions prompted by the magnesium glass series analysis was - at what point magnesium switches roles from a modifier to an

intermediate? Along with the fact that the results indicated a trend in data deviation at approximately 20 mol% it is suggested that synthesising more glasses between 0 and 30 mol% would be extremely beneficial - the objective being to accurately determine the point at which MgO switches its role for a modifier to an intermediate oxide.

- Another important aspect to the investigation is the reason why magnesium acts as an intermediate oxide in these particular glasses and why in other compositions it acts as a modifier. It was suggested that the highly disruptive nature of the glasses (and the high concentration of non-bridging oxygens) may be a trigger for the switching of roles therefore posing the question as to whether decreasing the modifier content, and hence disruption, would influence the role undertaken by magnesium. The ICIE4 series seemed to support this assumption however, to be able to definitively state the reason why magnesium behaves so differently, it would be necessary to run further analysis on glass systems of varying disorder and analyse the effect on magnesium's role within the glasses.
- As discussed previously, there are commercial implications with respect to magnesium entering the silicate network as concerns cement design. A suggestion for an extension to this study would be to design a suitable composition for a glass ionomer cement and investigate the implications of magnesium replacing aluminium within such a cement.

As discussed at the beginning of this thesis, the ultimate aim of this study was to aid in the understanding of the composition-structure-property relationships within bioactive glasses. I believe that this study has gone along way to help with respect to understanding magnesium and phosphorus's role within a bioactive glass. Therefore, the ultimate suggestion for future work would be to use this new found information, along with the known behaviour of other glass constituents and apply such knowledge to attempt to design a specific glass composition for a specific biomedical application. This could be further expanded to attempting some kind of system for bioactive glass design.

9 References

- [1] P.Hartley,
http://www.projectfacade.com/index.php?/about/glossary_comments/bioactive_glass_facial_implants, (2005).
- [2] M. M. Pereira and L. L. Hench, Encyclopedia of Biomaterials and Biomedical Engineering, 2004.
- [3] A.G.Schott,
<http://www.schott.com/epackaging/english/bioactive/products/cosmetics.html>, (2010).
- [4] L. L. Hench, J. Mater. Sci. - Mater. Med., 17, (2006) 967.
- [5] L. L. Hench, T. K. Greenlee, R. J. Splinter, W.C. Allen, J Biomed Mater Res Symp 2., 2, (1971) 117.
- [6] L. J. Litkowski, G. D. Hack and D. C. Greenspan, Compositions containing bioactive glass and their use in treating tooth hypersensitivity, (2002).
- [7] D. W. Cannell, S. Hashimoto, K. N. Barger and N. V. Nguyen, Hair relaxer compositions comprising a bioactive glass, (2006).
- [8] O. H. Andersson, J. Mater. Sci. - Mater. Med., 1, (1990) 219.
- [9] K. H. Karlsson, Glass Phys. Chem., 24, (1998) 280.
- [10] M. W. G. Lockyer, D. Holland and R. Dupree, J. Non-Cryst. Solids, 188, (1995) 207.
- [11] R. D. Rawlings, J. Mater. Sci. Lett., 11, (1992) 1340.
- [12] A. Tilocca and N. H. deLeeuw, J. Phys. Chem. B, 110, (2006) 25810.

- [13] K. E. Wallace, R. G. Hill, J. T. Pembroke, C. J. Brown and P. V. Hatton, *J. Mater. Sci. - Mater. Med.*, 10, (1999) 697.
- [14] I. Elgayar, A. E. Aliev, A. R. Boccaccini and R. G. Hill, *J. Non-Cryst. Solids*, 351, (2005) 173.
- [15] A. Tilocca, *Proceedings of the Royal Society A*, 465, (2009) 1003.
- [16] A. Cormack, J. Du and T. R. Zeitler, *Phys. Chem. Chem. Phys.*, 4, (2002) 3193.
- [17] R. G. Hill, *J. Mater. Sci. Lett.*, 15, (1996) 1122.
- [18] Wikipedia, Life Expectancy, (2008).
- [19] L. L. Hench, *Biomaterials*, 19, (1998) 1419.
- [20] J. LeFanu, Little Brown, (1999) 104.
- [21] S. R. Simon, *American Academy of Orthopaedics Surgeons*, (1994)
- [22] J. R. Jones, Hench, L. L., *Biomaterials*, 27, 7, (2006) 964-973.
- [23] C. W. Patrick, A. G. Mikos and L. V. McIntire, *Frontiers in tissue science*, Elsevier Science Ltd, (1998).
- [24] J. P. McIntyre, J. F. Shackelford, M. W. Chapman and R. R. Pool, 70, (1991) 1499.
- [25] B. Buck, T. Malinin and M. Brown, *Clin Orthop*, 240, (1989) 129.
- [26] C. B. Hing, R. Y. Ball and J. K. Tucker, *Autobanking of femoral heads for revision total hip replacement, a preliminary report of a new surgical technique*, Endocrine Surgery Masterclass (2000).
- [27] J. Tanigawa, N. Miyoshi and K. Sakurai, *Journal of Applied Polymer Science*, 110, (2008) 608.
- [28] L. Budyanto, Y. Q. Goh and C. P. Ooi, *J. Mater. Sci. - Mater. Med.*, (2008)
- [29] <http://en.wikipedia.org/wiki/Xenotransplantation>, Xenotransplantation, (2008).
- [30] A. W. Batchelor and C. M., *Service Characteristics of Biomedical Materials and Implants*, Imperial College Press, London, (2004).

- [31] B. D. Ratner, A. S. Hoffman, F. J. Schoen and J. F. Lemons, *Biomaterials Science: An Introduction to Materials in Medicine*, Academic Press, (2004).
- [32] D. F. Williams, *Annu. Rev. Mater. Sci.*, (1976) 237.
- [33] L. L. W. Hench, J., *An Introduction to Bioceramics*, World Scientific, Singapore, (1993).
- [34] L. L. Hench and E. C. Ethridge, *Biomaterials, an Interfacial Approach*, New York Academic Press, (1982).
- [35] L. L. W. Hench, J., *Biological Applications of Bioactive Glasses*, Life Chemistry Reports (1996).
- [36] A. Putman and D. Mooney, *Nat Med*, (1996) 824.
- [37] J. Black, *Biomaterials*, 5, (1984) 11.
- [38] L. L. Hench, *Brazilian Journal of Physics*, 22, (1992) 70.
- [39] J. R. Jones and L. L. Hench, *Biomaterials: Bioceramics*, John Wiley, (2006).
- [40] L. L. Hench, J. M. Polak, I. D. Xynos and L. D. K. Buttery, *Mater. Res. Innovations*, 3, (2000) 313.
- [41] G. Heness and B. Ben-Nissan, *Materials Forum*, 27, (2004) 104.
- [42] J. Anderson, *Annu. Rev. Mater. Res.*, 31, (2001) 81.
- [43] K. Wallace, *Design of Bioactive Glass Compositions*, University of Limerick (2000).
- [44] W. P. Cao and L. L. Hench, *Ceram. Int.*, 22, (1996) 493.
- [45] K. De Groot, *Bioceramics of Calcium Phosphate*, CRC Press, (1983).
- [46] P. Ducheyne and Q. Qiu, *Biomaterials*, 20, (1999) 2287.
- [47] K. C. Dee, D. C. Rueger, T. T. Andersen and R. Bizios, *Biomaterials*, 17, (1996) 209.
- [48] L. L. Hench, *Bioactive ceramics: Theory and clinical applications*, Butterworth-Heinemann, Oxford, (1994).

- [49] J. Wilson, A. F. Clark, E. Douek, J. Krieger, W. K. Smith and J. S. Zimet, Clinical applications of bioglass implants, Butterworth-Heinemann, Oxford, (1994).
- [50] M. Wang, Developing novel biomaterials for new challenges, Materials Science and Technology in Engineering Conference - Now, New and Next, Hong Kong (2003).
- [51] H. Denissen, Dental root implants of apatite ceramics. Experimental investigations and clinical use of dental root implants made of apatite ceramics, Vrije Universiteit te Amsterdam (1979).
- [52] A. N. Cranin, G. P. Tobin and J. Gelban, Compend. Contin. Educ. Dent., 8, (1987) 334.
- [53] M. Jarcho, J. Mater. Sci., 11, (1976) 2027.
- [54] H. L. Ren, Y. Yue, C. H. Ye, L. P. Guo and J. H. Lei, Chem. Phys. Lett., 292, (1998) 317.
- [55] Z. Strnad, Biomaterials, 13, (1992) 317.
- [56] A. El-Ghannam, P. Ducheyne and I. M. Shapiro, J. Biomed. Mater. Res., 29, (1995) 359.
- [57] U. M. Gross, C. Muller-Mai and C. Voigt, Ceravital bioactive glass ceramics (chapter 7), World Scientific Publishing Co., Singapore, (1993).
- [58] T. Kokubo, H. Kushitani, C. Ohtsuki, S. Sakka and T. Yamamuro, J. Mater. Sci. - Mater. Med., 3, (1992) 79.
- [59] T. Kokubo, A/W glass-ceramic: processing and properties (Chapter 5), World Scientific Publishing Co, Singapore, (1993).
- [60] T. Yamamuro, A/W glass ceramic: clinical applications (Chapter 6), World Scientific Publishing Co, Singapore, (1993).
- [61] D. C. Greenspan, Developments in biocompatible glass compositions, (2000)
- [62] W. Holand, J. Non-Cryst. Solids, 219, (1997) 192.
- [63] T. Kokubo, S. Yoshihara, N. Nishimura, T. Yamamuro and T. Nakamura, J. Am. Ceram. Soc., 74, (1991) 1739.

- [64] D. C. Greenspan, *Current Opinion in Solid State and Materials Science*, 4, (1999) 389.
- [65] E. M. Santos, S. Radin and P. Ducheyne, *Biomaterials*, 20, (1999) 1695.
- [66] M. Research.com, Bioactive glasses, ceramics, composites, other advanced materials, BCC Research (2006).
- [67] J. E. Shelby, *Introduction to glass science and technology*, Royal Society of Chemistry, (2005).
- [68] <http://www.britglass.org.uk/AboutGlass/HistoryofGlass.html>, History of glass, In: T. B. G. Website, Editor.
- [69] <http://www.glassonline.com/infoserv/history.html>, The discovery of glass, In: G. Online, Editor (1996-2008).
- [70] K. Stanton, The effect of fluorine on microstructural development in apatite-mullite glass-ceramics, University of Limerick (2000).
- [71] H. Rawson, *Inorganic glass-forming systems*, Academic Press, London and New York, Sheffield, (1967).
- [72] R. H. Doremus, *Glass Science*, John Wiley & Sons, New York, Chichester, (1973).
- [73] J. P. Henry, An investigation into the composition-structure-property relationships of novel machinable fluorine based glass-ceramics, University of Limerick (2001).
- [74] A. Paul, *Chemistry of Glasses*, Chapman and Hall, London, (1982).
- [75] D. G. Holloway, *The physical properties of glass*, Wykeham publications, (1973).
- [76] P. H. Gaskell, *Models for the structure of amorphous solids*, VCH Publishers Inc, Weinheim, (1991).
- [77] G. W. Scherer, *Glass formation and relaxation*, VCH Publishers Inc, Weinheim, (1991).

- [78] I. Elgayar, The Influence of Alkali Metal Content and Network Connectivity on Bioactive Glasses, Imperial College of Science, Technology and Medicine, London (2004).
- [79] S. D. Stookey, Annual Report of the Smithsonian Institution, (1962) 323.
- [80] W. H. Zachariasen, Contribution from the Ryerson Physical Laboratory (1932)
- [81] I. Encyclopaedia Britannica, (1997).
- [82] W. D. Kingery, Introduction to Ceramics, Wiley Interscience, New York Chichester, (1976).
- [83] J. K. Rao, Structural chemistry of glasses, Elsevier, (2002).
- [84] H. Aguiar, E. L. Solla, J. Serra, P. Gonzalez, B. Leon, N. Almeida, S. Cachinho, E. J. C. Davim, R. N. Correia, J. M. Oliveira and M. H. Fernandes, J. Non-Cryst. Solids, 354, (2008) 4075.
- [85] W. Vogel, Chemistry of Glass, The American Ceramic Society, Columbus, Ohio, (1985).
- [86] L. Holliday, Inorganic Macromol. Rev. , 1, (1970) 3.
- [87] N. H. Ray, Inorganic Polymers, Academic Press, London, (1978).
- [88] P. G. Galliano, A.L. Cavalier, J. Non-Cryst. Solids, (1995) 311.
- [89] en.wikipedia.org/wiki/Spinodal_decomposition.
- [90] A. H. Cottrell, Theoretical structural metallurgy, Edward Arnold, London, (1948).
- [91] J. W. Christian, The theory of transformations in metals and alloys, Pergamon, London, (1965).
- [92] B. E. Warren and A. G. Pincus, J. Am. Ceram. Soc., 23, (1940) 301.
- [93] E. M. Levin and S. Block, J. Am. Ceram. Soc., 40, (1958) 113.
- [94] R. J. Charles, Phys. Chem. Glasses, 10, (1969) 169.
- [95] L. L. Hench, J. Am. Ceram. Soc., 81, (1998) 1705.

- [96] Q. Chen, J. A. Roether and A. R. Boccaccini, Topics in tissue engineering, 4, (2008)
- [97] L. L. Hench, J. Am. Ceram. Soc., 74, (1991) 1487.
- [98] L. L. Hench and H. A. Paschall, J. Biomed. Mater. Res., 7, (1973) 25.
- [99] T. Kokubo, J. Non-Cryst. Solids, 120, (1990) 138.
- [100] T. Kokubo, Biomaterials, 12, (1991) 155.
- [101] O. H. Andersson, K. H. Karlsson and K. Kangasniemi, J. Non-Cryst. Solids, 119, (1990) 290.
- [102] O. H. Andersson, G. Z. Liu, K. H. Karlsson, L. Niemi, J. Miettinen and J. Juhanoja, J. Mater. Sci. - Mater. Med., 1, (1990) 219.
- [103] O. H. Andersson and K. H. Karlsson, J. Non-Cryst. Solids, 129, (1991) 145.
- [104] P. Li and F. Zhang, J. Non-Cryst. Solids, 119, (1990) 112.
- [105] P. Li, In vitro and in vivo calcium phosphate induction on gel oxides, Leiden (1993).
- [106] D. Pereira, S. Cachinho, M. C. Ferro and M. H. V. Fernandes, J. Eur. Ceram. Soc., 24, (2004) 3693.
- [107] L. L. Hench and J. Wilson, Science, 226, (1984) 630.
- [108] D. E. Clark, C. G. Pantano and L. L. Hench, Corrosion of Glass, New York, (1979).
- [109] L. L. Hench and D. E. Clark, J. Non-Cryst. Solids, 28, (1978) 83.
- [110] C. M. Jantzen, Mater Res Soc, 125, (1988) 143.
- [111] C. G. Pantano, Glass surfaces: Old, new and engineered, Glass Art Society Journal (2004).
- [112] I. D. Xynos, A. J. Edgar, L. L. Hench and J. M. Polak, J Biomed Mater Res, 55, (2001) 151.
- [113] J.-Y. Sun, Y.-S. Yang, J. Zhong and D. C. Greenspan, Journal of Tissue Engineering and Regenerative Medicine, 1, (2007) 281.

- [114] P. Ducheyne and Q. Qiu, *Biomaterials*, 20, (1999) 2287.
- [115] L. L. J. Hench, Jones, J. R. Sepulveda, P., *Bioactive Materials for Tissue Engineering Scaffolds*, (2002).
- [116] I. Notingher, A. R. Boccaccini, J. Jones, V. Maquet and L. L. Hench, *Materials Characterisation*, 49, (2003) 255.
- [117] A. Calver, A 19F MASNMR study: Strontium substitution in Apatite-Mullite-Anorthite glass ceramics, Imperial College (2005).
- [118] R. Z. LeGeros, *Calcium phosphate in oral biology*, Basel, (1991).
- [119] K. Lewis, A. Choi and J. B.-N. Chou, B., *Materials Australia Magazine*, 40, (2007) 32.
- [120] T. Kasuga, K. Nakagawa, M. Yoshida and E. Miyade, *J. Mater. Sci.*, 22, (1987) 3721.
- [121] Y. Ebisawa, T. Kokubo, K. Ohura and T. Yamamuro, *J. Mater. Sci. - Mater. Med.*, 1, (1990) 239.
- [122] F. Branda, R. Fresa, A. Costantini and A. Buri, *Biomaterials*, 17, (1996) 2247.
- [123] T. Kokubo, *J. Aust. Chem. Soc.*, 36, (2000) 37.
- [124] M. M. Pereira, A. E. Clark and L. L. Hench, *J. Biomed. Mater. Res.*, 28, (1994) 693.
- [125] K. Tsuru, C. Ohtsuki, A. Osaka, T. Iwamoto and J. D. Mackenzie, *J. Mater. Sci. - Mater. Med.*, 8, (1997) 157.
- [126] J. P. Zhong and D. C. Greenspan, *Bioglass surface reactivity: From in vitro to in vivo* (1997).
- [127] T. Kokubo, S. Ito, M. Shigematsu, S. Sakka and T. Yamamuro, *J. Mater. Sci.*, 22, (1987) 4067.
- [128] O. H. Andersen, G. P. LaTorre and L. L. Hench, *The kinetics of bioactive ceramics: Part 2 surface reactions of three bioactive glasses*, (1990).
- [129] O. H. Andersen and A. Sodergard, *J. Non-Cryst. Solids*, 246, (1999) 9.
- [130] M. Brink, *J. Biomed. Mater. Res.*, 36, (1997) 109.

- [131] M. Brink, T. Turunen, R. P. Happonen and A. YliUrpo, *J. Biomed. Mater. Res.*, 37, (1997) 114.
- [132] Y. Fredholm and D. R. Hill, *Strontium substitution on bioactive glasses*, Imperial College (2007).
- [133] J. M. Gomez-Vega, E. Saiz and A. P. Tomsia, *J. Biomed. Mater. Res.*, 46, (1999) 549.
- [134] E. Saiz, M. Goldman, J. M. Gomez-Vega, A. P. Tomsia, G. W. Marshall and S. J. Marshall, *Biomaterials*, 23, (2002) 3749.
- [135] J. M. Oliveira, R. N. Correia and M. H. Fernandes, *J. Non-Cryst. Solids*, 273, (2000) 59.
- [136] J. M. Oliveira, R. N. Correia and M. H. Fernandes, *Biomaterials*, 23, (2002) 371.
- [137] J. M. Oliveira, R. N. Correia, M. H. Fernandez and J. Rocha, *J. Non-Cryst. Solids*, 265, (2000) 221.
- [138] Y. K. Ebisawa, T., *J. Mater. Sci. - Mater. Med.*, 1, (1980) 239.
- [139] K. Ohura, T. Nakamura, T. Yamamuro, Y. Ebisawa, T. Kokubo, Y. Kotoura and M. Oka, *J. Mater. Sci. - Mater. Med.*, 3, (1992) 95.
- [140] J. S. Moya, A. P. Tomsia, A. Pazo, C. Santos and F. Guitian, *J. Mater. Sci. - Mater. Med.*, 5, (1994) 529.
- [141] A. J. Salinas, J. Roman, M. Vallet-Regi, J. M. Oliveira, R. N. Correia and M. H. Fernandes, *Biomaterials*, 21, (2000) 251.
- [142] P. W. McMillan, *Glass Ceramics*, Academic Press, London, (1979).
- [143] P. S. Fiske and J. F. Stebbins, *Am. Mineral.*, 79, (1994) 848.
- [144] S. Kohara, K. Suzuya, K. Takeuchi, C. K. Loong, M. Grimsditch, J. K. R. Weber, J. A. Tangeman and T. S. Key, *Science*, 303, (2004) 1649.
- [145] M. A. Karakassides, A. Saranti and I. Koutselas, *J. Non-Cryst. Solids*, 347, (2004) 69.
- [146] A. Dietzel, *Elektrochem.*, 48, (1942) 9.

- [147] K. Shimoda, Y. Tobu, M. Hatakeyama, T. Nemoto and K. Saito, *Am. Mineral.*, 92, (2007) 695.
- [148] K. Shimoda, T. Nemoto and K. Saito, *J. Phys. Chem. B*, 112, (2008) 6747.
- [149] H. Doweidar, *Phys. Chem. Glasses*, 40, (1999) 85.
- [150] E. Leitz Wetzlar GmBh, Germany (1981).
- [151] A. A. Spasov, *Bulletin of experimental biology and medicine*, 131, (2001) 132.
- [152] J. E. Sojka, Weaver, C. M., *Nutr Rev.* 53, (1995) 71.
- [153] <http://en.wikipedia.org/wiki/Phosphorus> (2008).
- [154] O. Peitl and Zanutto, *J. Non-Cryst. Solids*, 292, (2001) 115.
- [155] A. G. Clifford, *Apatite mullite glass ceramics*, University of Limerick (1997).
- [156] F. H. Larsen, H. J. Jakobsen, P. D. Ellis and N. C. Nielsen, *Journal of Physical Chemistry A*, 101, (1997) 8597.
- [157] H. Aguiar, E. L. Solla, J. Serra, P. Gonzalez, B. Leon, F. Malz and C. Jager, *J. Non-Cryst. Solids*, 354, (2008) 5004.
- [158] T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi and T. Yamamuro, *J. Biomed. Mater. Res.*, 24, (1990) 721.
- [159] G. L. Turner, K. A. Smith, R. J. Kirkpatrick and E. Oldfield, *J. Magn. Reson.*, 70, (1986) 408.
- [160] H. Grussaute, L. Montagne, G. Palavit and J. L. Bernard, *Journal of Non-Crystalline Solids*, 263, (2000) 312.
- [161] H. Maekawa, T. Maekawa, K. Kawamura and T. Yokokawa, *J. Non-Cryst. Solids*, 127, (1991) 53.
- [162] J. F. Emerson, P. E. Stallworth and P. J. Bray, *J. Non-Cryst. Solids*, 113, (1989) 253.
- [163] R. Dupree, D. Holland and D. F. Williams, *J. Non-Cryst. Solids*, 81, (1986) 185.
- [164] A. R. Grimmer, M. Magi, M. Hahnert, H. Stade, A. Samoson, W. Wieker and E. Lippmaa, *Phys. Chem. Glasses*, 25, (1984) 105.

- [165] M. Magi, Lippmaa E., Samoson A., Engelhardt G., Grimmer A. R., J. Phys. Chem., 88, (1984) 1518.
- [166] J. F. Stebbins, Nature, 330, (1987) 465.
- [167] J. D. Mackenzie and M. E. Smith, Multinuclear Solid State NMR of Inorganic Materials, Pergamon, (2002).
- [168] M. Magi, E. Lippmaa, A. Samoson, G. Engelhardt and A. R. Grimmer, J. Phys. Chem., 88, (1984) 1518.
- [169] B. L. Sherriff, H. D. Grundy and J. S. Hartman, European Journal of Minerals, 3, (1991) 751.
- [170] T. Nakamura, T. Yamamuro, S. Higashi, S. Kokubo and J. Itoo, Journal of Biomedical Materials Research Part A, 19, (1985) 685.
- [171] M. Schneider, J. Non-Cryst. Solids, 273, (2000) 8.
- [172] ThermoNicolet, Introduction to Fourier Transform Infrared Spectrometry, (2008).
- [173] D. M. Sanders, W. B. Person and L. L. Hench, 28, (1974) 247.
- [174] M. Vallet-Regi, A. M. Romero, C. V. Ragel and R. Z. LeGeros, Journal of Biomedical Materials Research Part A, 44, (1999) 416.
- [175] M. Regina, T. Filgueiras, G. La Torre and L. L. Hench, J. Biomed. Mater. Res., 27, (1999) 1485.
- [176] O. P. Filho, G. La Torre and L. L. Hench, Journal of Biomedical Materials Research Part A, 30, (1996) 509.
- [177] J. Schneider, V. R. Mastelaro, H. Panepucci and E. D. Zanotto, J. Non-Cryst. Solids, 273, (2000) 8.
- [178] H. Doweidar, G. M. El-Damrawi, Y. M. Moustafa and R. M. Ramadan, Physica B., 362, (2005) 123.
- [179] H. Doweidar, J. Non-Cryst. Solids, 249, (1999) 194.
- [180] H. Doweidar, J. Non-Cryst. Solids, 194, (1996) 155.
- [181] H. Doweidar, J. Non-Cryst. Solids, 335, (2009) 577.

- [182] H. Doweidar, *J. Non-Cryst. Solids*, 355, (2009) 577.
- [183] G. Lusvardi, G. Malavasi, L. Menabue, M. C. Menziani, A. Pedone and U. Segre, *J. Eur. Ceram. Soc.*, 27, (2007) 499.
- [184] A. A. Appen, *Chemistry of Glass*, Khimiya, Leningrad (In Russian), (1970).
- [185] A. Krajewski and A. Ravaglioli, *Bioceramics and biological glasses*, Kluwer Academic/Plenum, New York, (2002).
- [186] K. K. Kasinger and W. K. Ramp, *J Cell Biochem*, 68, (1998) 83.
- [187] M. E. Fleet, X. Liu and P. L. King, *Am. Mineral.*, 89, (2004) 1422.
- [188] I. Lebecq, F. Desanglois, A. Leriche and C. Follet-Houttemane, *Journal of Biomedical Materials Research Part A*, (2007) 156.
- [189] J. J. Vidueau and V. Dupuis, *Eur. J. Inorg. Chem.*, 28, (1991) 303.
- [190] P. G., G. Leroy, C. Rey, B. Sombret, P. Huvenne and E. Bres, *J. Mater. Sci. - Mater. Med.*, 8, (1997) 271.
- [191] R. V. Santos and R. N. Clayton, *Am. Mineral.*, 80, (1995) 336.
- [192] C. Rey, B. Collins, T. Goehl, I. Dickson and M. Glimcher, *Calcified Tissue International*, 45, (1989) 157.
- [193] J. Jones, P. Sepulveda and L. L. Hench, *Journal of Biomedical Materials Research Part A*, 58, (2001) 720.
- [194] J. Serra, P. Gonzalez, S. Liste, C. Serra, S. Chiussi, B. Leon, M. Perez-Amor, O. Ylanen and M. Hupa, *J. Non-Cryst. Solids*, 332, (2003) 20.
- [195] M. D. O'Donnell, S. J. Watts, R. V. Law and R. G. Hill, *Journal of Non-Crystalline Solids*, 354, (2008) 3554.
- [196] M. D. O'Donnell, S. J. Watts, R. V. Law and R. G. Hill, *Journal of Non-Crystalline Solids*, 354, (2008) 3561.
- [197] L. L. Hench, *Biomaterials science: An introduction to materials in medicine*, Academic London, (2004).
- [198] K. H. Karlsson, K. Froerg and T. Ringbom, *J. Non-Cryst. Solids*, 112, (1989) 69.

- [199] R. J. Kirkpatrick and R. K. Brow, *Solid State Nuclear Magnetic Resonance*, 5, (1995) 9.
- [200] P. G. Galliano, J. M. P. Lopez, E. L. Varetti, I. Sobrados and J. Sanz, *Mater. Res. Bull.*, 29, (1994) 1297.
- [201] A. Tilocca and A. Cormack, *J. Phys. Chem. B*, 111, (2007) 256.
- [202] V. Fitzgerald, D. M. Pickup, D. C. Greenspan and G. Sarkar, *Adv. Funct. Mater.*, 17, (2007) 3746.
- [203] L. Linati, G. Lusvardi, G. Malavasi, L. Menabue, M. C. Menziani, P. Mustarelli, A. Pedone and U. Segre, *J. Non-Cryst. Solids*, 354, (2008) 84.
- [204] R. Ahsan and M. G. Mortuza, *J. Non-Cryst. Solids*, 351, (2005) 2333.
- [205] R. Dupree, D. Holland and M. G. Mortuza, *Nature*, 328, (1987) 416.
- [206] I. L. Mudrakovskii, V. P. Shmachkova, N. S. Kotsarenko and V. M. Mastikhin, *J. Phys. Chem. Solids*, 47, (1986) 335.
- [207] M. A. Aramendia, V. Borau, C. Jimenez, J. M. Marinas, F. J. Romero, J. R. Ruiz and J. Sol, *State Chem.*, 135, (1998) 96.
- [208] S. N. Scrimgeour, J. A. Chudek, G. A. Cowper and C. H. Lloyd, *Dent. Mater.*, 23, (2007) 934.
- [209] S. Prabhakar, K. J. Rao and C. N. R. Rao, *Chem. Phys. Lett.*, 139, (1987) 96.
- [210] E. Leonova, I. Izquierdo-Barba, D. Arcos, A. Lopez-Noriega, N. Hedin, M. Vallet-Regi and M. Eden, *Journal of Physical Chemistry C*, 112, (2008) 5552.
- [211] B. Moreno, C. O. Rodrigues, B. N. Bailey, J. A. Urbina and S. Moreno, *FEBS Letters*, 523, (2002) 207.
- [212] A. M. George and J. F. Stebbins, *Am. Mineral.*, 83, (1998) 1022.
- [213] S. Kroeker and J. F. Stebbins, *Am. Mineral.*, 82, (2000) 1459.
- [214] P. C. D. Zhang, P. Florian, P. J. Grandinetti and J. F. Stebbins, *J. Non-Cryst. Solids*, 204, (1996) 294.
- [215] P. Zhang, Grandinetti, P.J., and Stebbins, J.F, *J. Phys. Chem. B*, 101, 20, (1997) 4004.

- [216] R. Dupree, Holland, D., Mortuza, M.G. , Phys. Chem. Glasses, 29(1), (1988) 18.
- [217] F. Natrup, H. Bracht, C. Martiny, S. Murugavel and B. Roling, Phys. Chem. Chem. Phys., 4, (2002) 3225.
- [218] B. Roling and M. D. Ingram, Solid State Ionics, 105, (1998) 47.
- [219] J. B. Murdoch, J. F. Stebbins and I. S. E. Carmichael, Am. Mineral., 70, (1985) 332.
- [220] R. Dupree and M. E. Smith, Journal of the Chemical Society, Chemical Communications, (1988) 1483-1485.
- [221] J. D. MacKenzie and M. H. Meinhold, Am. Mineral., 79, (1994) 250.
- [222] J. D. MacKenzie and M. H. Meinhold, Am. Mineral., 82, (1997) 479.
- [223] G. E. Brown, F. Farges and G. Calas, Structure, dynamics and properties of silicate melts, Mineralogical Society of America, Washington D. C., (1995).
- [224] A. E. Geissberger and F. L. Galeener, Phys. Rev. B, 28, (1983) 3266.
- [225] W. A. Pliskin, J. Vac. Sci. Technol., 14, (1997)
- [226] F. L. Galeener and G. Lucovsky, Phys. Rev. Lett., 37, (1976).
- [227] A. A. Megahed, Phys. Chem. Glasses, 40, (1999) 130.
- [228] Hiroa.K., J. Non-Cryst. Solids, (1991).
- [229] N. Soga, J. Non-Cryst. Solids, 73, (1985) 97.
- [230] H. Doweidar, J. Non-Cryst. Solids, 240, (1998) 55.
- [231] L. Linati, G. Lusvardi, G. Malavasi, L. Menabue, M. C. Menziani, P. Mustarelli and U. Segre, J. Phys. Chem. B, 109, (2005) 4989.
- [232] N. Bovo, Structure - properties relationships in bioactive glasses for PAA - based polyalkenoate cements, Imperial College (2007).
- [233] C. Y. Kim, A. E. Clark and L. L. Hench, J. Non-Cryst. Solids, 113, (1989) 195.
- [234] M. M. Pereira, A. E. Clark and L. L. Hench, J. Am. Ceram. Soc., 78(9), (1995) 2463.
- [235] W. E. Jackson, J. M. Leon and G. E. Brown, Science, 262, (1993) 229-233.

- [236] C. D. Yin, M. Okuno, H. Morikawa and F. Marumo, *J. Non-Cryst. Solids*, 55, (1983) 131.
- [237] P. Ildefonse, G. Calas, A. M. Flank and P. Lagarge, *Nuclear Instruments and Methods in Physics Research B*, 97, (1995) 172.
- [238] G. E. Brown, F. Farges and G. Calas, *Mineralogical Society of American Reviews in Mineralogy*, 32, (1995) 317.
- [239] A. Pedone, G. Malavasi, M. C. Menziani, U. Segre and A. Cormack, *Journal of Physical Chemistry C*, 112, (2008) 11034.
- [240] A. Cormack and J. Du, *J. Non-Cryst. Solids*, 283, (2001) 293.
- [241] A. Pedone, G. Malavasi and M. C. Menziani, *Journal of Physical Chemistry C*, 113, (2009) 15723.
- [242] M. Vallet-Regi, A. J. Salinas, J. Roman and M. Gil, *Journal of Materials Chemistry*, 9, (1999) 515.
- [243] S. Ni, J. Chang and L. Chou, *J. Mater. Sci. - Mater. Med.*, 19, (2008) 359.
- [244] C. Y. Kim, A. E. Clark and L. L. Hench, *Journal of Biomedical Materials Research Part A*, 26, (1992) 1147.
- [245] M. Okasaki, *Biomaterials*, 12, (1991) 831.
- [246] A. S. Posner, N. C. Blumental and F. Betts, *Phosphate minerals*, New York, (1984).
- [247] R. G. Hill, I. Elgayar, N. Bubb and D. J. Wood, *Dielectric spectroscopy and dissolution studies of bioactive glasses*, Imperial College, London (2005).

10 Appendix



Contents lists available at ScienceDirect

Journal of Non-Crystalline Solids

journal homepage: www.elsevier.com/locate/jnoncrysolEffect of P₂O₅ content in two series of soda lime phosphosilicate glasses on structure and properties – Part I: NMRM.D. O'Donnell^{a,b,*}, S.J. Watts^a, R.V. Law^{b,*}, R.G. Hill^a^a Department of Materials, Imperial College London, London SW7 2AZ, UK^b Department of Chemistry, Imperial College London, London SW7 2AZ, UK

ARTICLE INFO

Article history:

Received 16 August 2007

Received in revised form 22 February 2008

Available online 28 April 2008

PACS:

61.43.Fs

61.05.Qr

Keywords:

Bioglass

Oxide glasses

NMR, MAS-NMR

Structure

ABSTRACT

The effect of the variation in phosphate (P₂O₅) content on the structure of two series of bioactive glasses in the quaternary system SiO₂–Na₂O–CaO–P₂O₅ was studied. The first series (I) was a simple substitution of P₂O₅ for SiO₂ keeping the Na₂O:CaO ratio fixed (1.00:0.87). The second series was designed to ensure charge neutrality in the orthophosphate (PO₄^{3−}), therefore as P₂O₅ was added the Na₂O and CaO content was varied to provide sufficient Na⁺ and Ca²⁺ cations to charge balance the orthophosphate present. The glass network connectivity (NC) was calculated for each glass and a modification for the presence of a separate P₂O₅ phase was included (NC'). ³¹P and ²⁹Si magic-angle-spinning nuclear magnetic resonance (MAS-NMR) spectroscopy was performed on glass series I and II to determine the structural units present and their relation to glass properties. ³¹P MAS-NMR spectra of series I resulted in a broad resonance around 9 ppm corresponding to orthophosphate in an amorphous environment. The 9.25 mol% P₂O₅ glass shown to be partially crystalline by X-ray diffraction was heat treated, and the ³¹P MAS-NMR spectrum showed a sharp peak around 3 ppm corresponding to calcium orthophosphate or sodium pyrophosphate and overlapping broader peaks at 8.5, 10.5 and 14 ppm possibly corresponding to two mixed calcium–sodium orthophosphate phases and amorphous sodium orthophosphate respectively. ³¹P MAS-NMR spectra of series II resulted in a broad resonance around 10.5 ppm corresponding to orthophosphate in an amorphous environment. ²⁹Si MAS-NMR spectra of glasses from series I showed a shift in the resonance peak from around −78 to −86 ppm indicating an increase in Q³ species in the glass and a reduction in Q² with phosphate addition confirming the presence of orthophosphate. The heat treated sample showed a sharp ²⁹Si-NMR resonance at −88 ppm, indicating a crystalline Q² six-membered combeite (Na₂O · 2CaO · 3SiO₂) silicate-type phase, which was confirmed by powder X-ray diffraction. ²⁹Si MAS-NMR spectra of glasses from series II showed no shift in the resonance at around −78 ppm across the series, confirming an orthophosphate environment.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

The commercial 45S5 Bioglass® composition (46.1SiO₂–26.9CaO–24.4Na₂O–2.5P₂O₅ mol%) originally developed by Hench et al. [1] in the late 1960s has been extensively studied with regards to biological activity, as the material promotes a positive response when implanted into the human body. The proposed mechanism for 'bioactivity' is based on the chemical components in the glass, i.e. phosphate (P₂O₅), silica (SiO₂), soda (Na₂O) and lime (CaO). Very

few systematic studies have been performed to examine the effect of composition on glass properties of this system, either by varying the four components in the 45S5 system or replacing/adding other components such as fluorine, boron, magnesium and zinc. Recent studies [2–5] have suggested that glass structure has a strong influence on bioactivity, neglected in the Hench model, with a marked increase in degradation when the glass moves from a three-dimensional network (Q⁴ and Q³ silicate species) to a disrupted structure, i.e. linear chains (Q²), end members (Q¹) and isolated groups (Q⁰). The purpose of this study is to examine the effect of phosphate addition on glass properties and hence structure, with a view to making glass compositions with enhanced properties for biomedical applications. The phosphate and silicate environment will be probed with magic-angle-spinning nuclear magnetic resonance (MAS-NMR) spectroscopy. This technique has proved powerful for investigating the short-range structure of the amorphous state [6]

* Corresponding authors. Address: Department of Chemistry, Imperial College London, London SW7 2AZ, UK. Tel.: +44 (0)20 7594 6814; fax: +44 (0)20 7594 6757 (M.D. O'Donnell), tel.: +44 (0)20 7594 5860; fax: +44 (0)20 7594 5804 (R.V. Law).

E-mail addresses: m.odonnell@imperial.ac.uk (M.D. O'Donnell), r.law@imperial.ac.uk (R.V. Law).

and for identifying crystalline sites in solid solution compounds such as mixed-apatites [7], which are difficult by conventional techniques such as X-ray diffraction (XRD).

In the Hench model [8], phosphate plays an important role in bioactivity, forming part of a silica and CaO–P₂O₅ rich bilayer after leaching of Na⁺ and Ca²⁺ from the glass. This surface promotes the formation of hydroxycarbonate apatite (HCA), necessary for tissues to bond to the implant. The leached Na⁺ and Ca²⁺ ions may also affect the physiological balance of the solution at the glass interface and modify the local pH. In fact, the alkalization may promote synthesis and cross-linking of collagen and the formation of hydroxyapatite, beneficial for *in vivo* bone growth and repair. However, this model does not predict the bioactivity of glasses such as amorphous wollastonite, 50SiO₂–50CaO mol% [2], which is completely Q² in structure based on the binary Qⁿ distribution model and contains no phosphate or soda. Following the approach of Holliday [9] and Ray [10], Hill [3] introduced the concept of the inorganic polymer model and network connectivity (NC) to bioactive glasses, where these highly disrupted glasses can be visualized as inorganic polymers chains of oxygen and silicon atoms (Q²), cross-linked in places (Q³ and Q⁴) and charge balanced by modifiers such as Na⁺ and Ca²⁺. Providing the glass does not degrade to form toxic compounds, it would be expected that disruption of the silicate network and hence a reduction in NC would result in increased bioactivity. The most bioactive glasses are in fact what are termed invert glasses, where NC < 2 (i.e. an average Qⁿ distribution less than Q²) [11], with 45S5 exhibiting an NC = 1.9 which corresponds to a binary Qⁿ distribution of 90% Q² and 10% Q¹. An invert glass system contains more network modifiers (e.g. Na₂O and CaO) than network formers (e.g. SiO₂). This model can be used to predict the bioactivity of glasses in the ternary SiO₂–CaO–Na₂O system, however it does not provide an adequate description when taking into account P₂O₅ as a network former. This is possibly due to the incorrect assumption that phosphate enters the glass network but instead phase separates into orthophosphate species recognised in recent molecular dynamics studies [12]. This study investigates the role of phosphate in the glass structure, and attempts to incorporate it into the inorganic polymer and network connectivity model (NC').

2. Experimental

2.1. Glass melting

Table 1 shows the mol% compositions of glasses in series I, where P₂O₅ was added to the glass, keeping the Na₂O:CaO ratio fixed (1.00:0.87).

Table 1

Glass compositions from series I (fixed Na₂O:CaO = 1:0.87) and II (charge balanced), showing mol%, network connectivity (NC) assuming P₂O₅ enters the glass network and modified network connectivity (NC') assuming isolated orthophosphate units

ID	Mol%				NC	NC'
	SiO ₂	Na ₂ O	CaO	P ₂ O ₅		
<i>Series I</i>						
ICIE1	49.46	26.38	23.08	1.07	2.04	2.13
ICSW2	47.84	26.67	23.33	2.16	2.00	2.18
ICSW3	44.47	27.26	23.85	4.42	1.92	2.30
ICSW5	40.96	27.87	24.39	6.78	1.83	2.44
ICSW4	37.28	28.52	24.95	9.25	1.75	2.62
<i>Series II</i>						
ICSW1	51.06	26.10	22.84	0.00	2.08	2.08
ICSW6	48.98	26.67	23.33	1.02	2.00	2.08
ICSW7	47.07	27.19	23.78	1.95	1.92	2.08
ICSW8	43.66	28.12	24.60	3.62	1.79	2.08
ICSW10	40.71	28.91	25.31	5.07	1.67	2.08
ICSW9	38.14	29.62	25.91	6.33	1.56	2.08

Table 1 also shows the mol% compositions of glasses in series II, where P₂O₅ was added to the glass and the Na₂O and CaO level adjusted to charge balance the phosphate species (assumed to be PO₄³⁻) to attempt to keep the network connectivity of the glass constant.

Batch calculations were performed to give 200 g of final glass. Reagent grade powders (SiO₂, CaCO₃, Na₂CO₃ and P₂O₅) were batched to the nearest ±0.01 g. For the P₂O₅ containing glasses, this component was batched last due its hydrophilic nature and the containers were kept sealed with plastic film to avoid contamination with atmospheric moisture. Glasses were melted in an electric furnace in platinum crucibles for 90 min at 1390 °C. Glasses for MAS-NMR spectroscopy, XRD and differential thermal analysis (DTA) were frit cast, by pouring the melt directly into cold water, collected in a sieve, followed by drying to constant weight at 120 °C overnight. The glasses were then ground using a milling machine, and sieved to separate into fine (<45 µm) and coarse (>45 µm) particle size distributions.

2.2. Magic-angle-spinning nuclear magnetic resonance (MAS-NMR) spectroscopy

²⁹Si MAS-NMR and ³¹P MAS-NMR were used to analyze the different glass samples using a Bruker DSX-200 NMR spectrometer operating at a Larmor frequencies of 39.77 and 81.9 MHz, respectively. Samples were packed into a 5 mm zirconia rotor and spun at the magic angle (54.7°) to remove anisotropy effects. The ²⁹Si and ³¹P MAS-NMR spectra of the glasses were recorded at spinning frequencies from 3 to 12 kHz using pulse acquisition of π/2 for silicon and phosphorus. The ²⁹Si and ³¹P MAS-NMR samples were spun with a recycle/delay time set to generally 2 s (this however was increased to 60 s if the glass species were not thought to be completely relaxed). All spectra were recorded at an ambient probe temperature with ²⁹Si referenced relative to polydimethylsilane (PDMS) and 85% H₃PO₄ for ³¹P.

3. Results and discussion

3.1. Magic-angle-spinning nuclear magnetic resonance (MAS-NMR) spectroscopy

3.1.1. ³¹P MAS-NMR spectra

Fig. 1 shows the ³¹P MAS-NMR spectra for series I (fixed Na₂O:CaO) glasses.

Table 2 summarizes the peak positions and full width half maximums (FWHM).

All spectra illustrate a symmetric peak with a chemical shift range of 7 to 10 ppm with an approximate constant linewidth of ≈9 ppm. These linewidths and chemical shift values correspond to phosphorus in an orthophosphate environment (PO₄³⁻). Conventionally phosphorus is thought to enter the glass network as a network former. The ³¹P MAS-NMR chemical shift data, however, corresponds to an orthophosphate structure, therefore indicating that phosphorus is associated with removing sodium and calcium ions. These must come from their network modifying roles in the silicate network therefore changing the Qⁿ structure of the silicate. These results correspond with those previously reported [6,13,14] where a single resonance with a linewidth consistent with an orthophosphate like environment was reported. Hill et al. [13] however assigned the presence of orthophosphate species due to the annealing and slow cooling of the glasses, favouring phase separation. However, in the case of the our glasses and the glasses tested by Elgayar et al. [13], all were rapidly quenched in cold water, reducing the possibility of phase separation – the presence of phosphorus in an orthophosphate environment is still observed. Fig. 2 and Table 2 show that the ³¹P MAS-NMR chemical shifts for

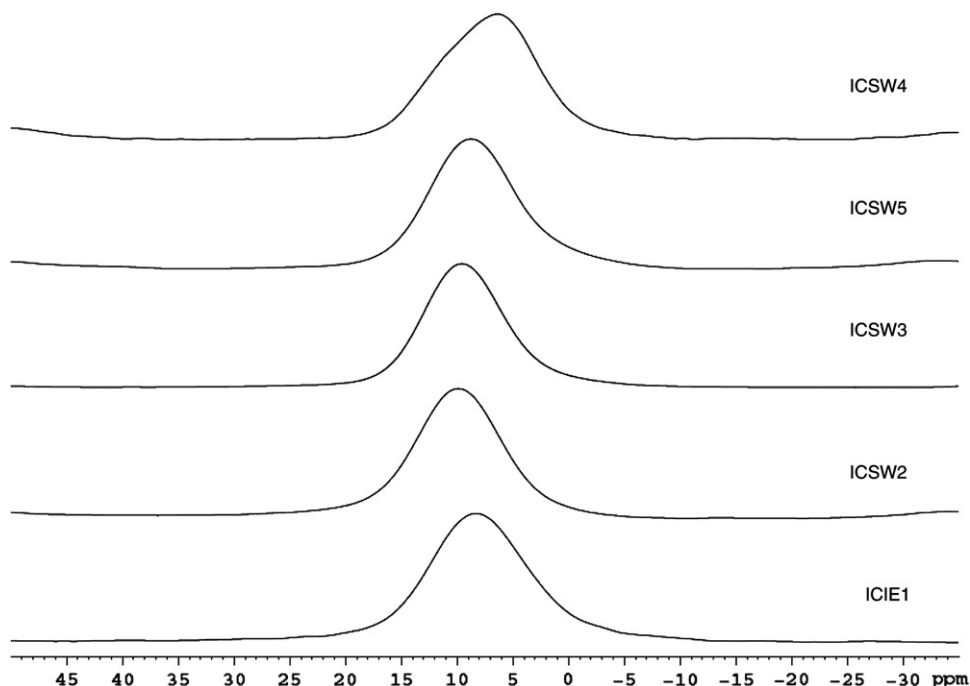


Fig. 1. ^{31}P MAS-NMR spectra for series I (fixed $\text{Na}_2\text{O}:\text{CaO}$) glasses. Increasing phosphate content from bottom to top. Top spectra (ICSW4) show distinct peak asymmetry.

Table 2

^{31}P and ^{29}Si MAS-NMR peak positions and full width half maximums (FWHM) for glasses in series I and II

Glass	^{31}P peak (ppm)	^{31}P FWHM (Hz)	^{29}Si peak (ppm)	^{29}Si FWHM (Hz)
Series I				
ICIE1	8.4	873	−78.0	516
ICSW2	10.0	721	−79.0	683
ICSW3	9.5	721	−78.5	592
ICSW5	9.0	737	−80.0	584
ICSW4	7.0	793	−81.0	695
Series II				
ICSW6	10.7	745	−78.0	524
ICSW7	10.9	745	−78.0	516
ICSW8	10.5	769	−78.0	505
ICSW10	10.5	745	−78.0	469
ICSW9	10.9	745	−78.0	505

the glasses all lie between those for a sodium orthophosphate (16 ppm) and calcium orthophosphate (0–3 ppm).

This therefore suggests that the phosphorus is present as mixed sodium–calcium orthophosphate. The network connectivity (NC) was calculated for each of the glasses and is shown in Table 1. NC was calculated using the following equations, assuming P_2O_5 enters the glass network, substituting the molar fractions of each component in the glass. The modified network connectivity, NC' , was calculated assuming P_2O_5 is present as orthophosphate species, with cations such as Na^+ and Ca^{2+} required to maintain a charge balance, hence increasing the connectivity of the silicate network. The glass network connectivity [3] was calculated using Eqs. (1) and (2).

$$\text{NC} = 2 + \frac{[(2 \times \text{SiO}_2) + (2 \times \text{P}_2\text{O}_5)] - [(2 \times \text{CaO}) + (2 \times \text{Na}_2\text{O})]}{\text{SiO}_2 + (2 \times \text{P}_2\text{O}_5)}, \quad (1)$$

$$\text{NC}' = 2 + \frac{(2 \times \text{SiO}_2) - [(2 \times \text{CaO}) + (2 \times \text{Na}_2\text{O}) + (2 \times \text{P}_2\text{O}_5 \times 3)]}{\text{SiO}_2}. \quad (2)$$

Fig. 1 shows that as P_2O_5 is added (and NC' increases), and the proportion of Q^3 ($[\text{SiO}_4]$ tetrahedra with three bridging oxygens (BO) and one non-bridging oxygen (NBO)) species increases, there

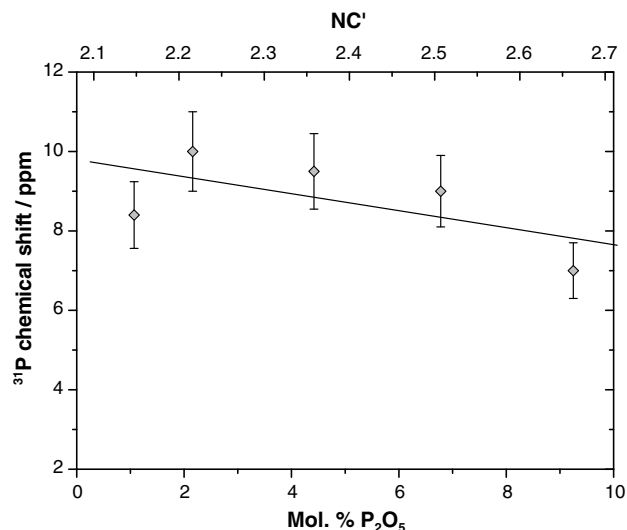


Fig. 2. ^{31}P MAS-NMR chemical shift values for series I glasses with phosphate mole ratio and NC' (error bars ± 1 ppm).

is a slight shift upfield of the ^{31}P peak to smaller ppm values, corresponding to that of a calcium orthophosphate environment. This could suggest that as the glass moved towards a Q^3 structure, calcium starts to associate preferentially with the phosphate phase. It should be noted however that this trend is in no way distinct or definite. In fact within experimental error it may be more relevant to assume no preferential association of the cations within the phases as discussed by Lockyer et al. [6]. Grussaute et al. [15] also observe this phenomenon in similar quaternary $\text{Na}_2\text{O}-\text{CaO}-\text{P}_2\text{O}_5-\text{SiO}_2$ glasses, with increasing P_2O_5 content, stating that each phosphorus nucleus is surrounded by the same number of Na and Ca cations, rather than segregation into stoichiometric compounds. In this series of glasses, the $\text{CaO}:\text{Na}_2\text{O}$ ratio was 1.00/1.14. If there was no preferential association within the phosphate phase, it would be expected that the ratio of Na:Ca orthophosphate to be

consistent with that of the overall glass composition. This would give a chemical shift for a 1:1 glass at ≈ 9 ppm. In this series of glasses with a 1.00/1.14 ratio of $\text{CaO}:\text{Na}_2\text{O}$, chemical shifts of ≈ 10.3 ppm would be expected. On closer inspection of the ICSW4 spectrum (the highest phosphate containing glass) a slight peak asymmetry is apparent. The NC' (2.62) of this glass fell into the regime of glasses reported by Grussaute et al. [15] ($\text{NC}' > 2.5$) who observed the presence of pyrophosphate as well as orthophosphate with ^{31}P NMR for glasses with NC' similar to ICSW4. From the XRD data (part II) it was known that this glass was in fact partially crystalline, there is however no corresponding sharpening of the spectrum suggesting possibly that the magnetization of the crystalline phase is not fully relaxed in the NMR experiment. We ran this sample for longer delay time (60 s, not shown) and no sharpening of the phosphate resonance was seen. This then necessitated the heat treatment of the glass and a longer delay time was used to grow and determine the crystalline phase(s) present. The resulting spectrum at a delay time of 2 s is shown in Fig. 3, bottom.

To further refine the peaks, the heat treated sample was then run with a 60 s delay time (Fig. 3, top). If previous data on $\text{SiO}_2\text{--Na}_2\text{O--CaO--P}_2\text{O}_5$ glasses [6,15] is consulted, the presence of a pyrophosphate species is reported. Lockyer assigned two resonances at 13.8 ppm and 2.4 ppm to a sodium orthophosphate like species and a sodium pyrophosphate like species respectively [6]. Similarly Grussaute et al. [15] attributed two well resolved resonances with chemical shifts of 15.5 ppm and 2.9 ppm to Na_3PO_4 and $\text{Na}_4\text{P}_2\text{O}_7$. The resonance at 3 ppm is possibly a result of a sodium pyrophosphate type species rather than a calcium orthophosphate type species. It is important to try and determine the types of phases present within the crystalline ICSW4 structure as these can usually be closely related to the structure of the parent amorphous glass. This would help determine whether there is in fact any preferential associations, as suggested within the phase separated glass structures. This will be further investigated by XRD in part II of this study.

Fig. 4 shows the ^{31}P MAS-NMR spectra for series II glasses where sodium and calcium have been introduced to charge balance the phosphate thus mitigating silicate network polymerization.

All the spectra appear identical within experimental error, therefore confirming that incorporating phosphate has a minimal affect on the silicate network condensation [15]. It can be seen

from Table 2 that all the spectra have a single resonance at ≈ 10.5 ppm with a FWHM consistent with that of an orthophosphate environment (9 ppm). If there is no preferential association of the Na^+ and Ca^{2+} cations with the phosphate environment, then the ratio would then be consistent with that of the original glass composition. As previously calculated this would then result in a resonance of ≈ 10.3 ppm in the ^{31}P MAS-NMR spectra of the glasses. This is in very good agreement with the single peak experimentally observed in series II, thus suggesting the peak can be assigned to a mixed sodium calcium orthophosphate species with no preferential association of one of the cations with the orthophosphate.

3.1.2. ^{29}Si MAS-NMR spectra

^{29}Si MAS-NMR spectroscopy was carried out on the first series of ICSW phosphorus glasses as shown in Fig. 5.

If the spectrum is observed for ICSW1 a $\text{SiO}_2\text{--CaO--Na}_2\text{O}$ glass with no phosphorus present, a highly symmetrical single resonance centered on -78 ppm can be seen. As the phosphorus content is increased in this $\text{SiO}_2\text{--CaO--Na}_2\text{O}$ glass series, the peak asymmetry, becomes more evident. For all glasses, except the phosphate-free glass, it can be seen that the spectra are a manifestation of two overlapping resonances centered on -78 and -86 ppm. Such overlaps are as a result of the amorphous nature of the samples, i.e. the variations in structural features within the glass such as variations in O–Si–O bond angles and distances [16–24]. The upfield shift from -78 to -86 ppm, indicates changes in the environment around the silicon atom as phosphorus content is increased in the glass. From these isotropic chemical shift values and previous data on ^{29}Si chemical shifts in relation to Q^n structure and connectivity, the resonance at -78 ppm can be assigned to a Q^2 species and the peak at -86 ppm can be assigned to a Q^3 species. The peak assigned to the Q^3 increases intensity with P_2O_5 addition, indicating the phosphate is removing modifier ions (Na^+ and Ca^{2+}), decreasing the number of NBOs, on the silicate chains and forming a more cross-linked network. Table 2 tabulates the peak positions and linewidths for the series I glasses. The experimental findings, that on increasing phosphate content, the glass moves from a Q^2 to a Q^3 structure, supports the idea that phosphorus acts as a network polymerizing agent for the silicate network.

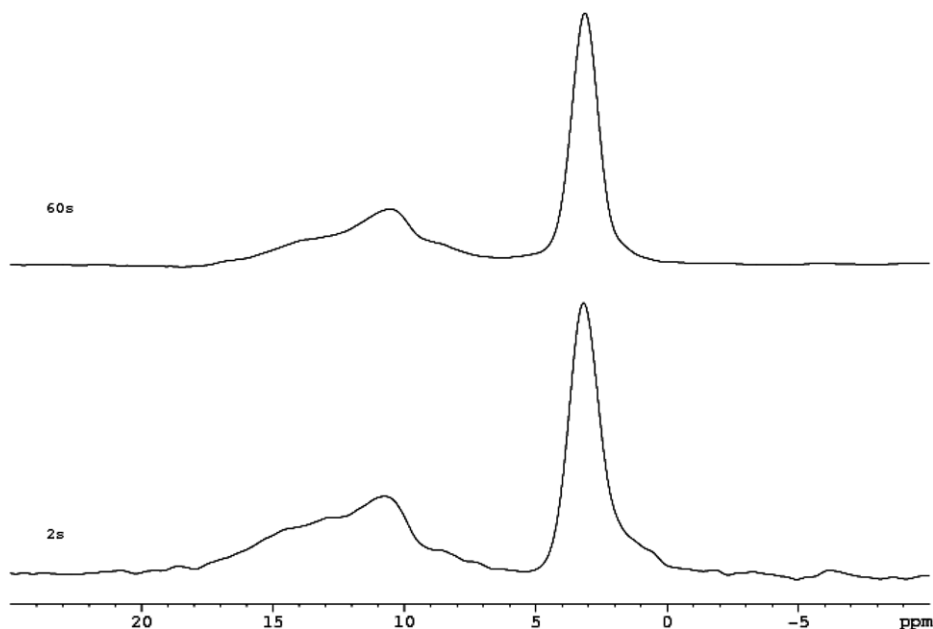


Fig. 3. ^{31}P MAS-NMR spectra for heat treated around 650°C ICSW4 glass, delay time 2 s (bottom) and 60 s (top).

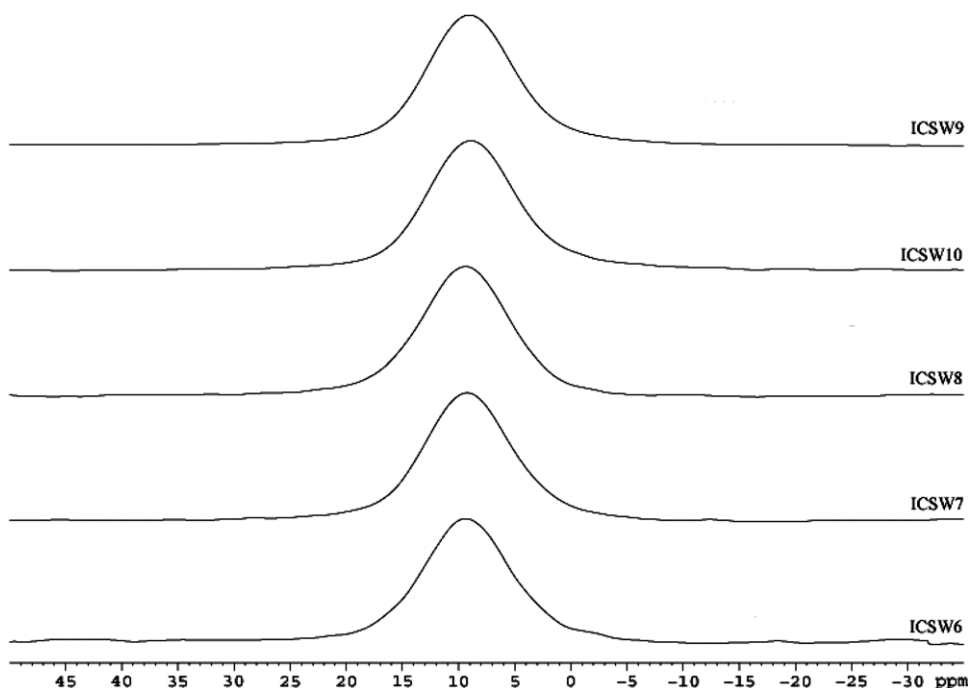


Fig. 4. ^{31}P MAS-NMR spectra for series II (charge balanced) glasses.

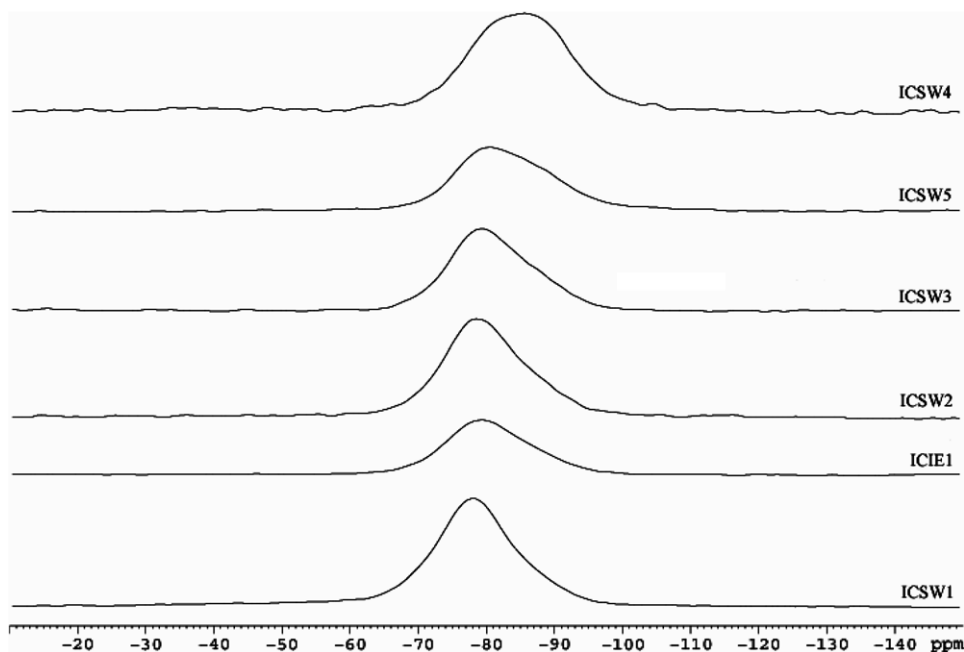


Fig. 5. ^{29}Si MAS-NMR spectra for series I (fixed $\text{Na}_2\text{O}:\text{CaO}$) glasses.

This is explained by the addition of the PO_4^{3-} to the glass, requires cations such as Na^+ and Ca^{2+} to charge balance, therefore removing them from their network modifying role within the silicate network.

This is supported by the network connectivity calculations where it is assumed phosphorus is in an orthophosphate environment (and not part of the silicate network as previously thought) as shown by Eq. (2). Fig. 6 highlights the chemical shift values with respect to the percentage of phosphorus substituted and the resultant NC' values.

From the XRD results (see part II) it is known that ICSW4 was in fact partially crystalline. Running the as-cast sample for a longer

delay time (60 s, not shown) did not result in any significant sharpening of the silicon resonance. The powder XRD of the heat treated sample indicates three phases: the six-membered ring combeite ($\text{Na}_2\text{O} \cdot 2\text{CaO} \cdot 3\text{SiO}_2$), sodium–calcium orthophosphate and sodium pyrophosphate. A much sharper peak or peaks would have been expected due to the ordering of the structure due to crystallinity, however this not observed. A possible explanation of the lack of observed crystallinity maybe due to the much longer spin lattice relaxation time, T_1 , of the crystal phase. At short time delays (2 s, not shown) the ^{29}Si within the crystalline phases would be saturated and not be detectable. As crystallinity had already been determined from the powder XRD, the heat treated sample was

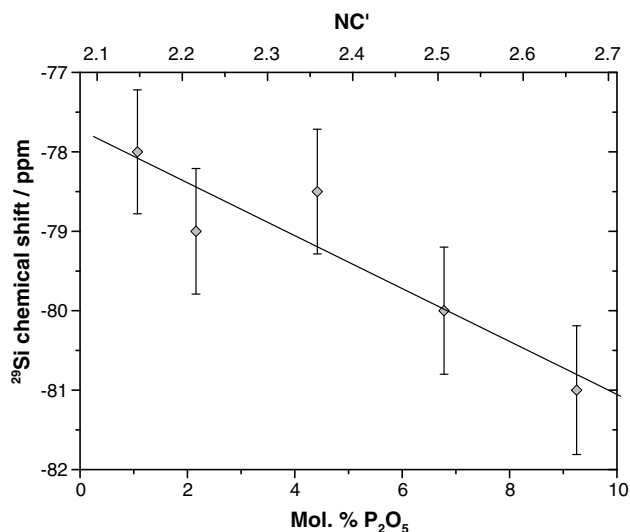


Fig. 6. ²⁹Si MAS-NMR chemical shift values versus P₂O₅ content and NC' in series I glasses (error bars ± 1 ppm).

re-run with a longer delay time (60 s) to try and pick up the crystalline phase – Fig. 7.

From this spectrum it can be seen that the peak has sharpened slightly. The peak maximum is at -88 ppm, within the broad amorphous resonance. This chemical shift is rather low for a Q^2 phase but this structure would be expected for these glasses. Wollastonite (CaSiO₃) and β -wollastonite exhibit chemical shift values in this region [22,25] and have been shown to crystallise in bio-glass based compositions [26]. However, XRD showed the presence of combeite [27]. We therefore assign this peak to combeite.

The ²⁹Si MAS-NMR spectra were carried out on series II glasses and are shown in Fig. 8.

Fig. 8 shows that all the spectra are highly symmetrical with a single resonance centered around -78 ppm with linewidths between 12 and 13 ppm. Even within experimental error there is no shift in any of the spectra and certainly no presence of any shoulders. It can be concluded that all the glasses in the series are Q^2 in nature, agreeing with the constant NC' value of all the glasses: 2.08, shown in Table 1. It also supports the fact that phosphorus exists within an orthophosphate environment. In the series II, the sodium and calcium contents increase to charge compensate the phosphorus species being added. This removes the

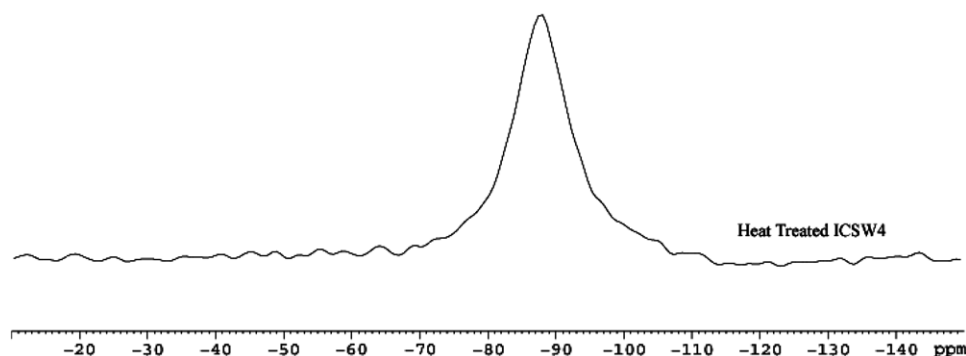


Fig. 7. ²⁹Si MAS-NMR spectrum for the heat treated (650 °C) ICSW4 glass sample, delay time = 60 s.

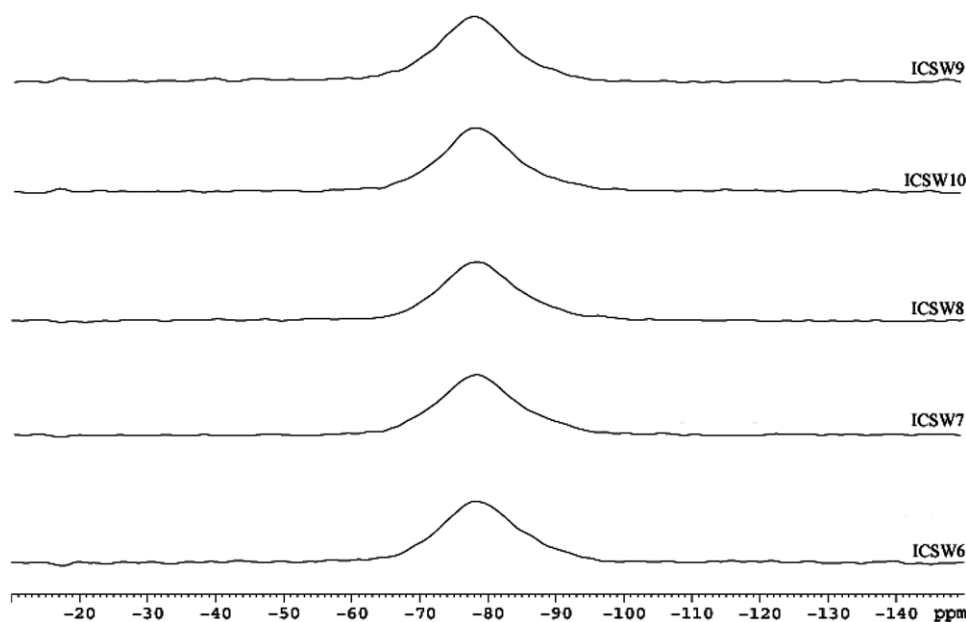


Fig. 8. ²⁹Si MAS-NMR spectra for series II glasses.

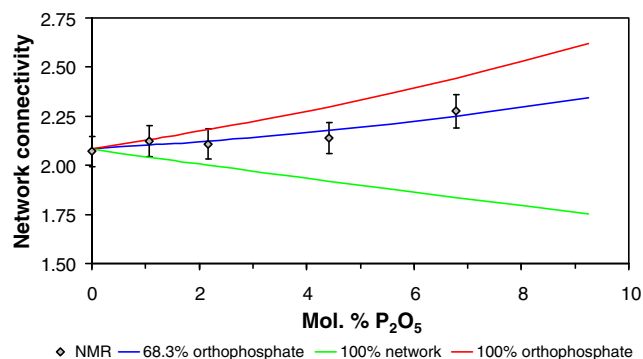


Fig. 9. Difference in NC and NC' for glasses in series I and data from NMR fitting.

need for any modifying ions already associated within the silicate network to charge compensate the PO_4^{3-} species, therefore keeping the number of NBOs attached to silicon constant and the silicate network unaffected. The single Q^2 resonance at -78 ppm suggests that the non-bridging oxygens are preferentially bonded to sodium as a Q^2 sodium silicate exhibits a resonance around -77 ppm and Q^2 calcium silicates occur in the -92 to -83 ppm region [22,25,28].

Fig. 9 shows the difference in NC and NC' for series I, and the actual NC calculated from the values of Q^2 and Q^3 proportions from fitting of the ^{29}Si -NMR data for series I.

A good fit can be obtained to the ^{29}Si MAS-NMR data, if it is assumed that there is a distribution approximately 2/3 of the phosphate in a separate orthophosphate phase and the remaining 1/3 in the glass network. However, it is realized that this is a very simplistic model and the glass structure is likely to be more complex.

4. Conclusions

The assumption that phosphorus resides in an orthophosphate environment provided the basis for the design of the phosphorus containing soda lime silicate glasses studied here. To test this hypothesis, two series of phosphorus glasses were designed – series I, addition of P_2O_5 with a constant $\text{Na}_2\text{O}:\text{CaO}$ ratio, with the resulting polymerization of the network, and series II, a charge compensated series (keeping the network polymerization and hence network connectivity constant).

4.1. Series

^{29}Si MAS-NMR spectra exhibited an increase in peak asymmetry through the series – a result of two overlapping resonances assigned to Q^2 and Q^3 species, with the later increasing with P_2O_5 addition. The asymmetric peak shift in the ^{29}Si spectra towards lower frequencies, as the phosphate content increased, was attributed to an increase in the average degree of polymerization of the silicate network. This therefore suggested the network polymerizing role of phosphorus, and the existence of an orthophosphate species. There was no evidence of any disruption of the glass network from the ^{29}Si MAS-NMR spectra thus rejecting the idea of phosphorus acting as a former and entering the silicate glass network. ^{31}P MAS-NMR spectra clearly illustrated single resonances for all spectra centered on chemical shift values associated with those of an orthophosphate environment, providing direct evidence of the existence of phosphorus within such an environment, rather than the conventional view of phosphorus entering the glass network.

4.2. Series II

^{29}Si MAS-NMR spectra resulted in a series of single resonances all centered on a chemical shift associated with a Q^2 glass (around

-78 ppm). No evidence of any shoulders or peak shift was seen, therefore concluding that on addition of phosphorus the silicate network remained unchanged as a result of the addition of charge compensators. The ^{31}P spectra also consisted of a series of single resonances all centered on a chemical shift associated with that of an orthophosphate environment.

It can therefore be confirmed from both series that phosphorus exists as an orthophosphate complex, rather than entering the silicate glass network as previously thought. There is strong evidence for the existence of phosphorus, in an orthophosphate environment with associated Na^+ and Ca^{2+} cations, and therefore the possibility of phase separation into phosphate rich clusters has significant implications with respect to the bioactive properties of the glass. It is suggested that the sodium orthophosphate rich phase will be extremely soluble and when exposed to water, dissolve more rapidly than the silicate phase effectively increasing the local level of phosphorus super-saturation of the external physiological environment in a biological implant. This may then result in an increase of the rate of apatite deposition on the glass surface [29]. It however should not be neglected that the resultant polymerization of the silicate network points towards a less degradable bioactive glass and would therefore serve to reduce bioactivity. The affect of phosphorus on bioactivity therefore is a necessary and essential extension to this investigation.

Acknowledgements

MDO and SJW would like to thank the EPSRC (Grant No. EP/C549309/1) for funding this work.

References

- [1] L.L. Hench, J. Am. Ceram. Soc. 74 (1991) 1487.
- [2] Y. Ebisawa, T. Kokubo, K. Ohura, T. Yamamuro, J. Mater. Sci. – Mater. Med. 1 (1990) 239.
- [3] R. Hill, J. Mater. Sci. Lett. 15 (1996) 1122.
- [4] R.D. Rawlings, J. Mater. Sci. Lett. 11 (1992) 1340.
- [5] Z. Strnad, Biomaterials 13 (1992) 317.
- [6] M.W.G. Lockyer, D. Holland, R. Dupree, J. Non-Cryst. Solids 188 (1995) 207.
- [7] R. Hill, A. Calver, S. Skinner, A. Stamboulis, R. Law, in: T. Nakamura, K. Yamashita, M. Neo (Eds.), Bioceramics 18, Pts 1 and 2, Trans Tech Publications, Switzerland/Enfield, NH, 2006.
- [8] L.L. Hench, in: B.D. Ratner (Ed.), Biomaterials Science an Introduction to Materials in Medicine, Academic, London, 2004.
- [9] L. Holliday, Ionic Polymers, Wiley, New York, 1975.
- [10] N.H. Ray, Inorganic Polymers, Academic, London, 1978.
- [11] W. Vogel, Glass Chemistry, Springer, Berlin, 1994.
- [12] A. Tilocca et al., J. Phys. Chem. B 111 (2007) 14256.
- [13] I. Elgayar, A.E. Aliev, A.R. Boccaccini, R.G. Hill, J. Non-Cryst. Solids 351 (2005) 173.
- [14] G.L. Turner, K.A. Smith, R.J. Kirkpatrick, E. Oldfield, J. Magn. Reson. 70 (1986) 408.
- [15] H. Grussaute, L. Montagne, G. Palavit, J.L. Bernard, J. Non-Cryst. Solids 263 (2000) 312.
- [16] H. Maekawa, T. Maekawa, K. Kawamura, T. Yokokawa, J. Non-Cryst. Solids 127 (1991) 53.
- [17] J.F. Emerson, P.E. Stallworth, P.J. Bray, J. Non-Cryst. Solids 113 (1989) 253.
- [18] E. Schneider, J.F. Stebbins, A. Pines, J. Non-Cryst. Solids 89 (1987) 371.
- [19] R. Dupree, D. Holland, D.S. Williams, J. Non-Cryst. Solids 81 (1986) 185.
- [20] R. Dupree, D. Holland, D.S. Williams, J. Phys. 46 (1985) 119.
- [21] A.R. Grimmer, M. Magi, M. Hahnert, H. Stade, A. Samoson, W. Wiek, E. Lippmaa, Phys. Chem. Glasses 25 (1984) 105.
- [22] M. Magi, E. Lippmaa, A. Samoson, G. Engelhardt, A.R. Grimmer, J. Phys. Chem. 88 (1984) 1518.
- [23] J.F. Stebbins, Nature 330 (1987) 465.
- [24] K.J.D. Mackenzie, M.E. Smith, Multinuclear Solid-State NMR of Inorganic Materials, Pergamon, 2002.
- [25] B.L. Sherriff, H.D. Grundy, J.S. Hartman, Eur. J. Mineral. 3 (1991) 751.
- [26] T. Nakamura, T. Yamamuro, S. Higashi, T. Kokubo, S. Ito, J. Biomed. Mater. Res. 19 (1985) 685.
- [27] M. Schneider et al., J. Non-Cryst. Solids 273 (1–3) (2000) 8.
- [28] J.F. Stebbins, in: F.C. Hawthorne (Ed.), Spectroscopic Methods in Mineralogy and Geology, Mineralogical Society of America, Washington, DC, 1988.
- [29] K.E. Wallace, R.G. Hill, J.T. Pembroke, C.J. Brown, P.V. Hatton, J. Mater. Sci. – Mater. Med. 10 (1999) 697.



Effect of P_2O_5 content in two series of soda lime phosphosilicate glasses on structure and properties – Part II: Physical properties

M.D. O'Donnell^{a,b,*}, S.J. Watts^a, R.V. Law^b, R.G. Hill^{a,*}

^a Department of Materials, Imperial College London, London SW7 2AZ, UK

^b Department of Chemistry, Imperial College London, London SW7 2AZ, UK

ARTICLE INFO

Article history:

Received 16 August 2007

Received in revised form 22 February 2008

Available online 26 April 2008

PACS:

61.43.Fs

61.05.cp

65.60.+a

Keywords:

Bioglass

X-ray diffraction

Oxide glasses

NMR and MAS-NMR

Structure

Thermal properties

X-rays

ABSTRACT

The effect of the variation in phosphate (P_2O_5) content on the properties of two series of bioactive glasses in the quaternary system SiO_2 – Na_2O – CaO – P_2O_5 was studied. The first series (I) was a simple substitution of P_2O_5 for SiO_2 keeping the Na_2O : CaO ratio fixed (1:0.87). The second series (II) was designed to ensure charge neutrality in the orthophosphate (PO_4^{3-}), therefore as P_2O_5 was added the Na_2O and CaO content was varied to provide sufficient Na^+ and Ca^{2+} cations to charge balance the orthophosphate present. Network connectivity's of the glasses were calculated, and densities and thermal expansion coefficients predicted using the Appen and Doweidar models, respectively. Theoretical densities were measured using the Archimedes principle. Characteristic temperatures, namely the glass transition temperature, T_g , and crystallization temperatures, T_x , were obtained using differential analysis (DTA). Two crystallization exotherms were observed for both glass series (T_{xi} and T_{xii}). Both T_g and T_x decreased with P_2O_5 addition for both series. The working range of the glasses, T_x – T_g was shown to increase to a maximum at around 4 mol% P_2O_5 then decrease at higher P_2O_5 contents for both series. Thermal expansion coefficients were measured using dilatometry increasing with P_2O_5 addition and showed good agreement with the Appen values. Dilatometric softening points, T_s , were also measured, which increased with P_2O_5 addition. X-ray diffraction (XRD) was performed on the glasses to confirm their amorphous nature. The glass containing 9.25 mol% P_2O_5 from series I exhibited well-defined peaks on the XRD trace, indicating the presence of a crystalline phase.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

There is some controversy as to the role of P_2O_5 in soda lime phosphosilicate glass structure, bioactivity and bone mineralization. The Hench model [1,2] predicts that the presence of phosphate is required in the implant material. However, this model does not predict the bioactivity of some glass compositions which do not contain phosphate [3] and neglect the network connectivity of the glass [4–6]. The Hench model also assumes congruent dissolution, which is not possible as these glasses phase separate into a phosphate rich and silicate rich phase as shown in part I. The phosphate phase will degrade in the presence of water at a much higher rate than the silicate phase. This phase separation results in orthophosphate groups (PO_4^{3-}) in the phosphate phase which are charge balanced by alkali and alkaline earth cations. This has the effect of

reducing the number of non-bridging oxygens in the silicate phase resulting in higher connectivity.

Part I of this study examined the structure of two series of bioglasses with varying phosphate contents using solid state MAS-NMR spectroscopy. The purpose of the second part of the study is to use more conventional characterization techniques to validate the NMR findings and assess properties important for processing promising compositions. Glass densities were measured to compare experimental values to calculated values from glass network connectivities based on glass composition (Doweidar's model [7,8]) and using network connectivities obtained from fitting of the ^{29}Si MAS-NMR data. Thermal expansion coefficients were obtained from dilatometry and values compared to calculations using the Appen model [9]. Thermal expansion coefficients are important to characterize, particularly for applications which may involve coatings. If bioactive glass compositions can be identified with thermal expansion coefficients matched to metals typically used in prosthetic implants (or preferably slightly higher than the metal so the glass is in compression), this could provide an important component to the next generation of biomedical devices. Characteristic temperatures were observed

* Corresponding authors. Address: Department of Materials, Imperial College London, London SW7 2AZ, UK. Tel.: +44 (0)20 7594 6814; fax: +44(0)20 7594 6757 (M.D. O'Donnell), tel.: +44(0)20 7594 6783; fax: +44(0)20 7594 6757 (R.G. Hill).

E-mail addresses: m.odonnell@imperial.ac.uk (M.D. O'Donnell), r.hill@imperial.ac.uk (R.G. Hill).

using differential thermal analysis (DTA) to examine the effect of phosphate addition on the glass transition temperature and devitrification behavior. The working range, T_x – T_g , is an important parameter in various applications such as viscous flow sintering (coatings) and fiber drawing. Finally, X-ray diffraction, on amorphous and again on subsequently heat treated samples, was used to investigate if any crystallites were present, and if so how this might relate to the structure of the devitrified material to the parent glass.

2. Experimental

2.1. Glass melting

Details of glass melting can be found in part I of this paper (REF). Glasses for thermal expansion coefficient (TEC) measurements and density were remelted from the frit described above for 30 min and cast into preheated ($\approx T_g - 10$ °C) graphite moulds and annealed in an electric furnace overnight. The rod shaped samples formed were cut into sections of 6 mm in diameter and 25 mm in length using a slow speed diamond saw. The ends of these rods were ground flat using SiC abrasive paper. It should be noted, not all samples could be cast in rod form for dilatometry due to devitrification (ICSW4) and/or phase separation (other samples) in pre-heated or room temperature moulds.

2.2. Density

Using Archimides principles the density of each cast sample at room temperature was determined. The weight of each glass was measured in air and in distilled water using a balance measuring to ± 0.1 mg. The weight was measured three times and an average taken in order to minimize sources of error. The sample density, ρ , was determined by Eq. (1)

$$\rho = \frac{m_d \times \rho_w}{m_d - m_s} \quad (1)$$

where m_d = mass of sample dry, ρ_w = density of water at the measurement temperature and m_s = mass of sample submerged in water. Care was taken to make sure that all of the cast samples were bubble free and that the glass samples were fully wetted when immersed in the distilled water.

2.3. Dilatometry

A Netzsch Dil 402 C dilatometer was used in order to determine the glass transition temperature (T_g), the dilatometric softening temperature (T_s) and the thermal expansion coefficient (α) for each glass. The 25×6 mm cut samples were analyzed from room temperature up until the point just before melting at a heating rate of 5 °C min^{-1} . α , T_s and T_g were determined by using system software and α was measured between 25 and 400 °C.

2.4. Differential thermal analysis (DTA)

The glasses were characterized by simultaneous DTA/TGA using a Stanton Redcroft DTA 1600. Alumina was used as the reference material and both reference and sample were contained within platinum–rhodium alloy crucibles. The <45 μm particle size powders (see part I for details of the milling process) were heated from room temperature to 1300 °C at a heating rate of 10 °C min^{-1} in an inert argon atmosphere. The glass transition temperature (T_g) and crystallization temperatures (T_x) for each glass were then obtained from the corresponding traces, with T_g represented by a small negative change in the slope of the baseline and T_x represented by the presence of an exotherm.

2.5. X-ray diffraction (XRD)

A Phillips powder diffractometer with a copper (CuK_α) X-ray source (Philips PW 1700 series diffractometer, Philips, Endhoven, NL) was used to characterize the glass samples. The powdered samples (<45 μm particle size) were scanned between 2θ values of 5 – 80° with a step size of $2\theta = 0.04^\circ$, in order to try and determine the amorphous nature of each glass. In order to try and verify the existence of any crystalline phases the frit was heat treated using a Centurion Quartz porcelain furnace, then ground to <45 μm particle size powder. DTA analysis was undertaken prior to heat treatment in order to determine the actual temperature at which the samples were to be heat treated at – corresponding to the peak crystallization temperature. The powder samples were heat treated to such a temperature at a rate of 10 °C min^{-1} , held for one hour, and then allowed to cool to room temperature. XRD was then carried out and the results analyzed using software containing a database of standard diffraction files. Rietveld refinement was performed with GSAS and EXPGUI software.

3. Results and discussion

3.1. Density

Figs. 1 and 2 show the density measurements for the glasses from series I and II, respectively.

The plots also show the density values calculated using Doweidar's model [7,8] which assumes a binary distribution of silicate Q^n species. All glass compositions were normalized to exclude the phosphate content, and all fell in the $33 \leq x_t \leq 50$ mol% region (where x_t is the total modifier mol%), therefore the Q^n distribution was Q^3 and Q^2 in the binary distribution model. The relative proportions of the Q^n species were calculated using the following Eqs. (2a),(2b),(2c),(2d), where a denotes Na^+ containing Q^n species and b Ca^{+2} containing Q^n species:

$$Q^{2a} = [3 \times (f_{\text{Na}_2\text{O}} + f_{\text{CaO}}) - 1] \times \left(\frac{f_{\text{Na}_2\text{O}}}{f_{\text{Na}_2\text{O}} + f_{\text{CaO}}} \right), \quad (2a)$$

$$Q^{2b} = [3 \times (f_{\text{Na}_2\text{O}} + f_{\text{CaO}}) - 1] \times \left(\frac{f_{\text{CaO}}}{f_{\text{Na}_2\text{O}} + f_{\text{CaO}}} \right), \quad (2b)$$

$$Q^{3a} = [2 - 4 \times (f_{\text{Na}_2\text{O}} + f_{\text{CaO}})] \times \left(\frac{f_{\text{Na}_2\text{O}}}{f_{\text{Na}_2\text{O}} + f_{\text{CaO}}} \right), \quad (2c)$$

$$Q^{3b} = [2 - 4 \times (f_{\text{Na}_2\text{O}} + f_{\text{CaO}})] \times \left(\frac{f_{\text{CaO}}}{f_{\text{Na}_2\text{O}} + f_{\text{CaO}}} \right), \quad (2d)$$

where $f_{M_xO_y}$ is the fraction of oxide component M_xO_y . These values were then normalized to give the fractions of each species (x_n) and the density calculated using this Eq. (3):

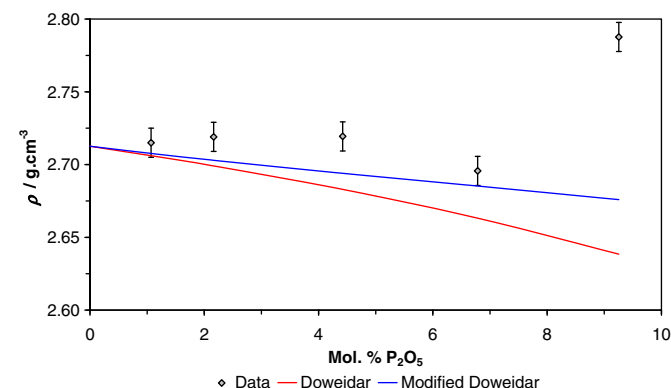


Fig. 1. Variation in density with P_2O_5 content for series I with calculated values.

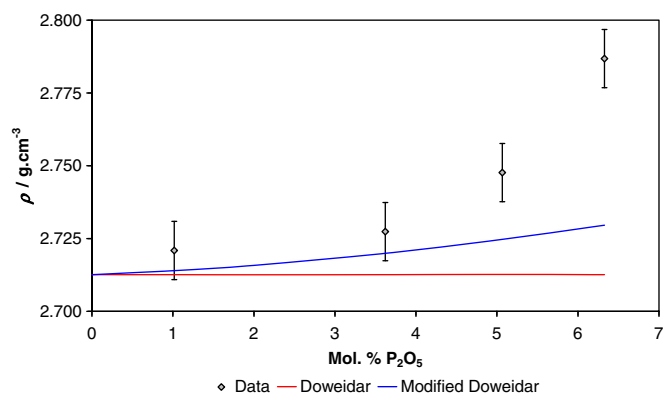


Fig. 2. Variation in density with P₂O₅ content for series II with calculated values.

$$\rho = X_{2a}\rho_{2a} + X_{2b}\rho_{2b} + X_{3a}\rho_{3a} + X_{3b}\rho_{3b}. \quad (3)$$

The densities of the Q^n species can be found in Table 2.

The plots also show modified Doweidar values, calculated assuming the P₂O₅ enters the glasses as a separate orthophosphate phase, based on the MAS-NMR of part I, and is charge balanced by Na⁺ and Ca²⁺ in the same proportion as that present in the overall glass compositions. Using density values of sodium and calcium orthophosphate (Table 2) Eq. (3) was modified to incorporate the fractions of Na₃PO₄ and Ca₃(PO₄)₂ in the separate orthophosphate phase. It can be seen from Figs. 1 and 2 that the experimental density values deviate from the calculated values, however the modified (orthophosphate) values show better correlation. As the densities seem to increase more rapidly than predicted with P₂O₅ content, this would seem to indicate the presence of a higher density component in the sample than the silicate glass such as an amorphous orthophosphate, pyrophosphate and in the case of ICSW4 crystalline phases.

3.2. Dilatometry

Fig. 3 shows the relationship between the thermal expansion coefficient (α) and phosphate content for both series of phosphate glasses.

Due to problems with casting as a result of phase separation and/or crystallization, only three samples of series II and two samples of series I could be run in the dilatometer – their corresponding α values are tabulated in Table 1. For series I in Fig. 3, the expectation would be that due to the polymerization of the silicate network, as phosphate is added, the glass would then find it harder to expand resulting in lower α values down the series. Actually the

Table 2

Densities of species thought to be present in the glasses studied [14]

Species	ρ (g cm ⁻³)
Q^{2a} (Na ⁺)	2.57
Q^{2b} (Ca ²⁺)	2.90
Q^{3a} (Na ⁺)	2.50
Q^{3b} (Ca ²⁺)	2.68
Na ₃ PO ₄	2.54
Ca ₃ (PO ₄) ₂	3.14

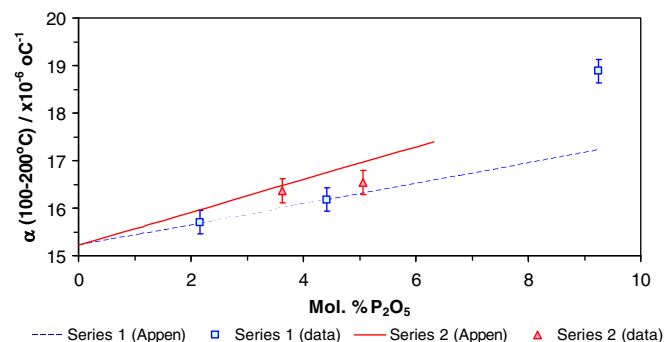


Fig. 3. TECs (α) for series I and II with calculated (Appen) values.

opposite is observed – an increasing α with increasing phosphate content. It must not be forgotten however that these glasses are in fact phase separated. The composite glass thermal expansion coefficient, α_c , given by Eq. (4)

$$\alpha_c = (\alpha_a \times V_a) + [\alpha_b \times (1 - V_a)], \quad (4)$$

where α_a is the thermal expansion coefficient of phase a , V_a the volume fraction of phase a and α_b the thermal expansion coefficient of phase b . The results therefore indicate that as phosphate is added, a composite thermal expansion coefficient may result. As phosphate is added and the amount of the phosphate phase is increased, the weaker P–O bond strength would result in a weaker glass structure thus explaining the increase in α across the series. Consideration however also has to be taken into the actual shape and dimensions of the secondary phosphate phase. Dilatometry is known to be governed dramatically by the dominant matrix phase of a glass. The results therefore suggest that as the phosphate content increases the dimensions of the secondary phase are significant enough to contribute to the thermal expansion of the glass.

Fig. 3 also shows the TEC values calculated from Appen's model [9]. The fraction of each component in the glass compositions

Table 1

Glasses from series I (fixed Na₂O:CaO = 1:0.87) and II (charge balanced), showing characteristic temperatures, glass transition T_g , dilatometric softening point, T_s , and crystallization T_x , thermal expansion coefficient (100–200 °C), α , and densities, ρ

ID	T_g (°C)	T_s (°C)	T_{xi} (°C)	T_{xii} (°C)	T_{xiii} (°C)	$T_{xi}-T_g$ (°C)	α ($\times 10^{-6}/^{\circ}\text{C}^{-1}$)	ρ (g cm ⁻³)
Series I								
ICIE1	513	–	629	676	–	117	–	2.715
ICSW2	511	542	636	679	–	125	15.70	2.719
ICSW3	491	549	655	674	–	163	16.18	2.719
ICSW5	485	–	605	625	–	120	–	2.696
ICSW4	482	581	543	617	656	61	18.88	2.788
Series II								
ICSW1	519	–	614	642	–	95	–	–
ICSW6	515	–	620	653	–	105	–	2.721
ICSW7	513	–	642	692	–	129	–	–
ICSW8	509	533	652	669	–	143	16.37	2.727
ICSW10	496	544	619	–	–	123	16.54	2.748
ICSW9	491	–	580	613	–	89	–	2.787

Table 3
Appen factors (a_{Mo}) for components in the glasses studied [9]

Component	Appen factor
SiO ₂	3.8
Na ₂ O	39.5
CaO	13.0
P ₂ O ₅	14.0

($f_{M_xO_y}$) were multiplied by the Appen factors ($a_{M_xO_y}$) shown in Table 3 giving α as shown by Eq. (5)

$$\alpha = f_{SiO_2} a_{SiO_2} + f_{CaO} a_{CaO} + f_{Na_2O} a_{Na_2O} + f_{P_2O_5} a_{P_2O_5}. \quad (5)$$

The values over the 100–200 °C range show relatively good agreement with the Appen figures. Table 1 summarizes the dilatometric softening points, T_s , of glasses which were cast into rods. It can be seen that T_s increases with P₂O₅ addition. As P₂O₅ addition increases the network connectivity of the dominant silicate phase due to formation of a separate phosphate phase (see part I), it would be expected to see an increase in the softening point of the glass.

3.3. Differential thermal analysis (DTA)

Figs. 4 and 5 illustrate the DTA traces for the phosphate glasses, series I and II. From these traces the glass transition temperature and crystallization temperature for each glass were determined.

In general the glasses manifested a T_g and two exotherms corresponding to T_{xi} and T_{xii} crystallization temperatures. Only glass ICSW4 (9.25 mol% P₂O₅ from series I) showed a third crystallization exotherm (T_{xiii}). If T_g is plotted against the amount of phosphate added for both series (Fig. 6) it is clear that as phosphate is increased, T_g decreases.

This apparently contradicts the theory that phosphate addition is charge balanced resulting in polymerization of the silicate network and also the corresponding NMR results discussed in part I. In series I, phosphate was simply added to the glass requiring Na⁺ and Ca²⁺ ions to charge compensate the phosphate complexes, removing them from their modifying role and thus polymerizing the network. The expectation would have been a corresponding increase in T_g . In the case of series II, the need for charge balancing is compensated for and thus the silicate network remains unchanged. The expectation would have been to see no real change in T_g , however experimentally it was seen once again to decrease with increasing phosphate. There is therefore an obvious difference be-

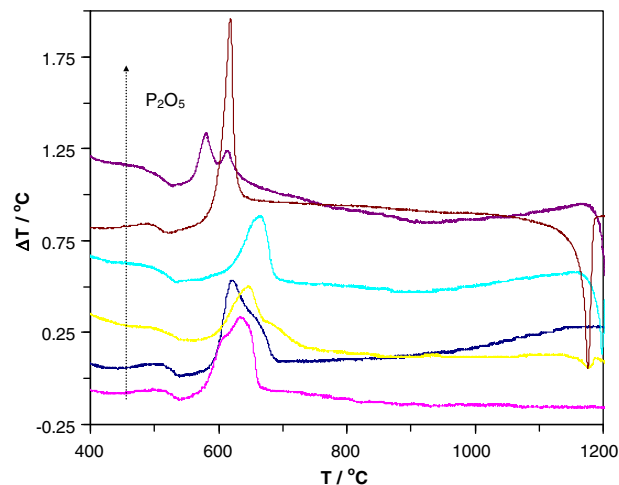


Fig. 5. DTA traces for series II (ICSW1 magenta, ICSW6 blue, ICSW7 yellow, ICSW8 cyan, ICSW10 brown, ICSW9 purple). (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

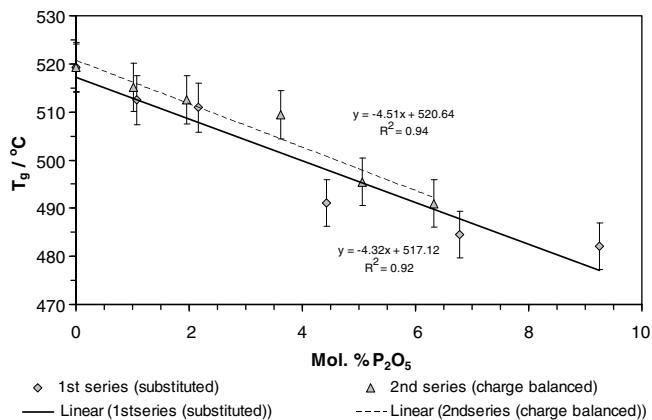


Fig. 6. T_g for series I and II.

tween the expected behavior of the glass and the actual behavior with respect to T_g . For both series the expected T_g correlation theories are based on the degree of disruption of the silicate network. This theory in turn bases itself on the idea that T_g is affected solely by the silicate phase. Elgayar et al. [10] have already discussed the affects of Na₂O replacement of CaO and the loss of ionic bridges affecting T_g . However there is no change with respect to any modifying cations so there must be another phenomena occurring with the addition of phosphate and its corresponding affect on the glass transition temperature. From the ³¹P MAS-NMR data discussed in part I, it is already known that the glasses have a clustered or phase separated phosphate structure. An expectation might be a second glass transition temperature corresponding to the phosphate phase. From the traces however there seems to be no evidence of one. This could mean it is simply being masked by the other features in the DTA trace or even due to the small amount of phosphate in the glass and/or the actual phase size being too small, that it is not detectable. It is however suggested that what is being observed is a composite glass transition temperature (i.e. due to a number of competing factors) due to the fact that T_g decreases when the NC' is the same (series II) and even when NC' increases (series I). It could also be suggested that as the phosphate content increases the corresponding phosphate phase size increases, then surpassing some critical size dimension to be of significance to affect the T_g values.

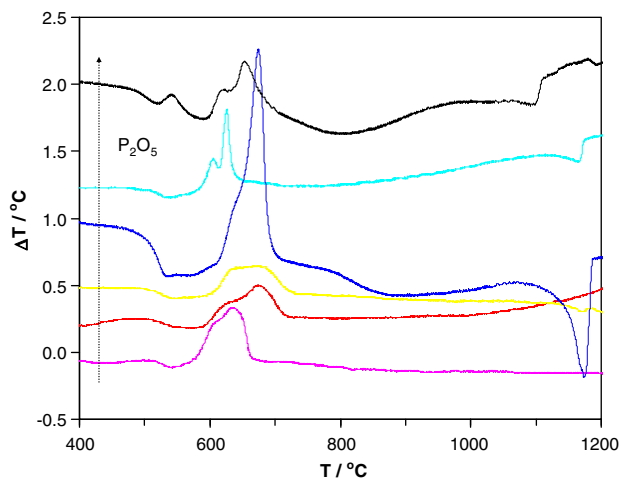


Fig. 4. DTA traces for series I (ICSW1 magenta, ICIE1 red, ICSW2 yellow, ICSW5 cyan, ICSW4 black). (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

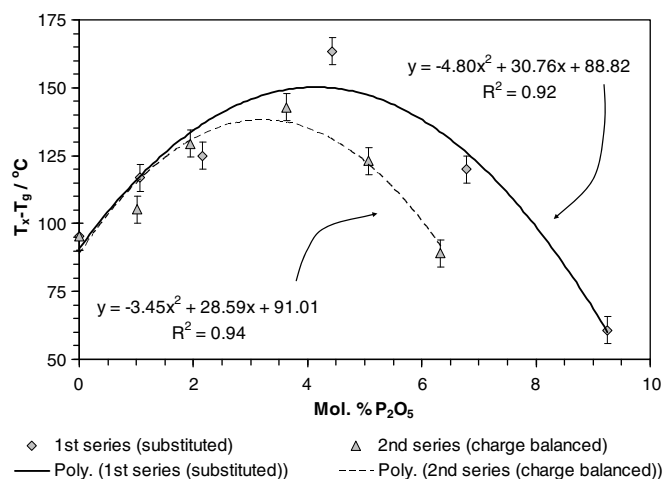


Fig. 7. $T_{xi}-T_g$ for series I and II.

Fig. 7 illustrates the working range or glass stability ($T_{xi}-T_g$) with respect to the amount of P_2O_5 added.

It can be seen that $T_{xi}-T_g$ increases to a maximum in both series at 4 mol% P_2O_5 for series I and 3 mol% P_2O_5 for series II then decreases. This working range is important in processes such as sintering and fiber drawing and should ideally be maximized for both to avoid crystallization at temperatures where the glass viscosity is sufficient to allow viscous flow to occur. The traces show the presence of two crystallization exotherms in both series which often overlap. These two exotherms could correspond to, most probably, the crystallization of two separate crystal phases or, maybe, the crystallization of the same phase in two phase separated regions or by two different mechanisms. It is suggested that it is most likely to be the crystallization of two separate crystal phases, i.e., crystal phase A and crystal phase B. It should be noted that the order of crystallization temperatures observed could switch within the series – for example in one glass T_{xi} could correspond to crystal phase A and T_{xii} to crystal phase B while in another glass T_{xi} could correspond to crystal phase B and T_{xii} to crystal phase A, depending on composition and the crystallization mechanisms involved.

In both series I and II, T_{xi} and T_{xii} seem to be relatively independent of phosphate content (Table 1), with a small decrease with increasing P_2O_5 content. This suggests that phosphate aids crystallization at lower temperatures. For series I however, with the polymerization of the network across the series it would be expected that more energy would be required to re-arrange the ions into a crystal state thus an increase in crystallization temperature would be expected across the series. This trend however is not seen suggesting that once again the addition of phosphate somehow aids crystallization.

With respect to the observed endotherms, within series I, all are broad and weak and seem to be probably as a result of the melting of the residual glassy phase. In series 2 however ICSW8 and ICSW9 exhibit very sharp and definite melting endotherms with an onset of melting approximately 1180 °C. This suggests that ICSW8 and 9 crystallize to the same phase. This endotherm is missing from all the other glasses in the series with exception to ICSW6 – which shows a much weaker endotherm however at the same temperature. This suggests that the other members of the series do not crystallize to this unknown phase. From the DTA information on crystallization exotherms and melting endotherms it can be predicted that there are probably three crystalline phases occurring one of which is common to ICSW8 and ICSW9 with a very small amount of the same phase in ICSW6.

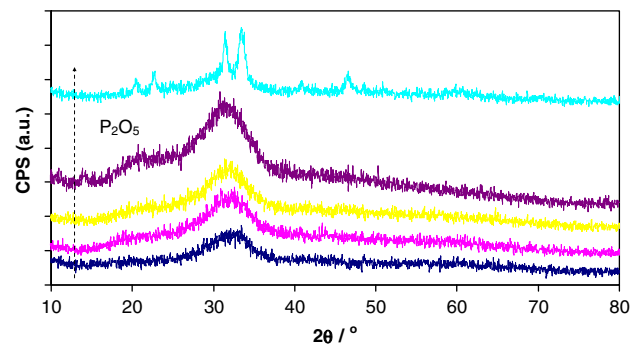


Fig. 8. X-ray diffraction patterns for series I cast glasses with increasing P_2O_5 content.

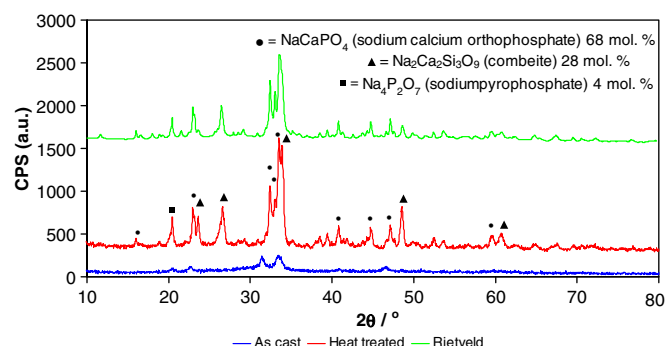


Fig. 9. X-ray diffraction pattern for crystalline sample ICSW4 before and after heat treatment with phases identified and Rietveld refinement, with molar proportions of phases shown (note – additional sodium pyrophosphate peak are masked in the region around 32° which contains the strongest lines from the other two phases).

3.4. X-ray diffraction (XRD)

XRD is a limited technique for amorphous materials as only long range periodic order or disorder can be probed. X-ray diffraction was therefore used just to confirm the glassy nature of the ICSW phosphate glasses series I and II. Fig. 8 indeed shows the characteristic amorphous halo for glasses in series I. There is one exception within both series – ICSW4, which also gave higher density and TEC values shown in (1) and (3) respectively. The XRD pattern of heat treated ICSW4 in Fig. 9 exhibits characteristic sharp crystalline peaks.

Rietveld refinement was performed on this trace and three phases were identified: $NaCaPO_4$ (sodium calcium orthophosphate, 68 mol%), $Na_2O \cdot 2CaO \cdot 3SiO_2$ (combeite, 28 mol%) and $Na_4P_2O_7$ (sodium pyrophosphate, 4 mol%). This is consistent with the ^{31}P NMR observation of the presence of predominantly orthophosphate and a smaller amount of pyrophosphate in part I. The ^{29}Si NMR spectra of the heat treated sample showed a broad peak around -88 ppm which was thought to be a Q^2 or Q^3 silicate. However, Schneider et al. [11] observed three overlapping chemical shift anisotropies (CSAs) for combeite at around -88 , -90 and -93 ppm which fits with the NMR and XRD and Rietveld data of ICSW4. The presence of three CSAs is due to closely spaced, but three distinct crystallographic sites for silicon in the combeite lattice.

4. Conclusions

The modified Doweidar model, taking into account the effect of the P_2O_5 not entering the glass network, seems to provide a closer

fit to experimental density values. The densities of series I increased and series II decreased with P_2O_5 addition. Theoretical thermal expansion coefficients (Appen) also showed a relatively good match to experimental values. This can be explained by the increase in the amount of the separate phosphate phase with P_2O_5 addition which contains weaker P–O bonds compared to the Si–O phase, and hence the ‘composite’ glass expands more with temperature. The only values to deviate significantly in the density and TEC measurements were samples which crystallized, confirmed by NMR and XRD.

The glass transition temperature decreased with phosphate addition for both series approximately linearly indicating the separate phosphate phase has a strong affect on thermal properties. The working range ($T_x - T_g$) increased for both series with phosphate addition to a maximum of around a molar fraction of 4 P_2O_5 , then decreased.

XRD confirmed the amorphous nature of all the samples except ICSW4 (9.25 mol% P_2O_5) from series I, which was the only sample to show any peak asymmetry in the ^{31}P NMR data. This peak asymmetry indicated the presence of both Q^0 and Q^1 phosphate species in the as-cast samples as opposed to the other glasses in series I and II which only contained Q^0 orthophosphate. This phase was then heat treated to increase the degree of crystallinity and three phases were identified: $NaCaPO_4$, $Na_2CaSi_3O_8$ and $Na_4P_2O_6$. The $NaCaPO_4$ phase is in agreement with the conclusions from the NMR data in part I, i.e. presence of a separate orthophosphate phase, however the stoichiometry does not fit with the observed ^{31}P chemical shift of the heat treated sample. This possibly indicates a non-stoichiometric Ca–Na solid solution orthophosphate or the presence of defects such as vacancies. Van Wazer stated the presence of orthophosphate groups in anhydrous sodium-phosphate glasses was highly unlikely due to the high concentration of negative charge on the orthophosphate ion pushing the following equilibrium towards the left (terminal groups) [12]:



However, in the presence of water the formation of orthophosphate becomes more likely, due to the similar electronegativity of hydrogen and phosphorous, enabling the proton to form a covalent bond with an oxygen, or satisfy a charge in the phosphate ion [12]. In the glasses studied here, as the modifier content far exceeds the phos-

phorous content (by at least 4.4:1.0) saturation occurs resulting in a higher probability of orthophosphate formation. Hydrolysis from the melt (during frit casting) could also contribute to orthophosphate presence. However from part I, only ICSW4, the highest phosphate containing glass which devitrified on casting, did contain a shoulder in the ^{31}P NMR possibly corresponding to a nanocrystalline pyrophosphate.

All the glasses were amorphous on casting except ICSW4, which had the highest NC' of the series (2.62). Previous studies [13] have shown the presence of pyrophosphate as well orthophosphate of bioglasses with similar connectivities. Three phases crystallized from the heat treated ICSW4 sample: sodium calcium orthophosphate, sodium pyrophosphate and combeite. These phases are consistent with the observed ^{29}Si and ^{31}P NMR chemical shifts shown in part I of this study: 14 ppm ($NaCaPO_4$), 3 ppm ($Na_4P_2O_7$) and –88 ppm ($Na_2Ca_2Si_3O_9$).

Acknowledgements

MDO and SW would like to thanks EPSRC (Grant No. EP/C549309/1) and Imperial College London for funding this work. MDO would like to thank Dr. Steve Skinner for useful discussions on Rietveld refinement.

References

- [1] L.L. Hench, J. Am. Ceram. Soc. 74 (1991) 1487.
- [2] L.L. Hench, in: B.D. Ratner (Ed.), Biomaterials Science an Introduction to Materials in Medicine, Academic, London, 2004.
- [3] Y. Ebisawa, T. Kokubo, K. Ohura, T. Yamamuro, J. Mater. Sci. – Mater. Med. 1 (1990) 239.
- [4] R. Hill, J. Mater. Sci. Lett. 15 (1996) 1122.
- [5] L. Holliday, Ionic Polymers, Wiley, New York, 1975.
- [6] N.H. Ray, Inorganic Polymers, Academic, London, 1978.
- [7] H. Doweidar, J. Non-Cryst. Solids 240 (1998) 55.
- [8] H. Doweidar, J. Non-Cryst. Solids 249 (1999) 194.
- [9] A.A. Appen, Khimii stekla, Khimii, Leningradskoe otd-nie, Leningrad, 1970.
- [10] I. Elgayar, A.E. Aliev, A.R. Boccaccini, R.G. Hill, J. Non-Cryst. Solids 351 (2005) 173.
- [11] J. Schneider, V.R. Mastelaro, H. Panepucci, E.D. Zanotto, J. Non-Cryst. Solids 273 (2000) 8.
- [12] J.R. Van Wazer, J. Am. Chem. Soc. 72 (1950) 644.
- [13] H. Grussaute, L. Montagne, G. Palavit, J.L. Bernard, J. Non-Cryst. Solids 263 (2000) 312.
- [14] J.A. Kerr, CRC Handbook of Chemistry and Physics, CRC, Florida, 2000.

The effect of phosphate content on the bioactivity of soda-lime-phosphosilicate glasses

M. D. O'Donnell · S. J. Watts · R. G. Hill ·
R. V. Law

Received: 9 November 2008 / Accepted: 5 March 2009 / Published online: 28 March 2009
© Springer Science+Business Media, LLC 2009

Abstract We report on the bioactivity of two series of glasses in the $\text{SiO}_2\text{--Na}_2\text{O--CaO--P}_2\text{O}_5$ system after immersion in simulated body fluid (SBF) after 21 days. The effect of P_2O_5 content was examined for compositions containing 0–9.25 mol.% phosphate. Both series of glasses degraded to basic pH, but the solutions tended towards to neutrality with increasing phosphate content; a result of the acidic phosphate buffering the effect of the alkali metal and alkaline earth ions on degradation. Bioactivity was assessed by the appearance of features in the X-ray diffraction (XRD) traces and Fourier transform infrared (FTIR) spectra consistent with crystalline hydroxyl-carbonate-apatite (HCAp): such as the appearance of the (002) Bragg reflection in XRD and splitting of the P–O stretching vibration around 550 cm^{-1} in the FTIR respectively. All glasses formed HCAp in SBF over the time periods studied and the time for formation of this crystalline phase occurred more rapidly in both series as the phosphate contents were increased. For P_2O_5 content >3 mol.% both series exhibited highly crystalline apatite by 16 h immersion in SBF. This indicates that in the compositions studied, phosphate content is more important for bioactivity than network connectivity (NC) of the silicate phase and compositions showing rapid apatite formation are

presented, superior to 45S5 Bioglass[®] which was tested under identical conditions for comparison.

1 Introduction

Bioactive glasses and ceramics have been important for mineralised tissue regeneration in orthopaedic applications for over 30 years [1]. However, recently these materials are finding usage in products such as toothpaste as remineralising agents [2]. The toothpaste Sensishield marketed in the EU by Periproducts contains Novamin (45S5 Bioglass[®]) as the active ingredient and there is also a toothpaste on the market which contains a zinc substituted hydroxyapatite [3]. L'Oreal also have a patent [4] for Bioglass as an additive to products for relaxing/straightening hair. This opens up the possibility for bioactive glasses to be used in a variety of consumer products.

This study reports the bioactivity of two series of glasses of which structure and properties have been previously reported [5, 6]. In series I phosphate was added to the glass, replacing SiO_2 and the Ca to Na ratio was kept constant. In series II, it was assumed phosphate did not enter the silicate network, but formed a separate orthophosphate phase and sufficient Ca and Na was added to ensure charge neutrality in the PO_4^{3-} complex formed. As the previous study showed, the formation of this type of phosphate structure resulted in removal of network modifiers in series I, polymerising the silicate network and resulting in an increase in Q^3 structural units and a reduction in Q^2 . In series II, as sufficient modifier was added to charge balance the orthophosphate species, no change in the silicate structure was seen by solid state NMR; effectively the silicate network remained Q^2 , $[\text{Si}_n\text{O}_{3n}]^{2n-}$ chains of infinite molar mass.

M. D. O'Donnell · S. J. Watts · R. G. Hill
Department of Materials, Imperial College London,
London, UK

M. D. O'Donnell · R. V. Law
Department of Chemistry, Imperial College London,
London, UK

M. D. O'Donnell (✉)
BioCeramic Therapeutics Ltd., London, UK
e-mail: mdo@bioceramictherapeutics.com

Simulated body fluid (SBF) has been used *in vitro* in a large number of studies to predict *in vivo* bioactivity in particular in glass–ceramic systems to assess the formation of hydroxyl-carbonated-apatite at the surface of the material (HCAp). The formation of HCAp after a reasonable time period in SBF is a good indicator that the material will form a bond and integrate well with bone *in vivo*. Although SBF experiments should be complemented by cell studies, followed by animal and human trials, SBF data can be used to screen out materials for these expensive and possibly unnecessary studies. The glasses studied here are modifications of the original 45S5 Bioglass[®] composition [1] and glasses studied by Elgayar et al. [7], with phosphate added to investigate the effect on bioactivity. The two most obvious negative effects *in vivo* might be degradation of the phosphate component of the glass producing an excessively acidic pH and hence cytotoxicity and pyrophosphate (Q^1 phosphate) species producing wide macrophagic activation [8]. It is known that all the glasses in this study contain a separate orthophosphate (Q^0 phosphate) phase. In the compositions studied the degradation and production of acidic pH caused by phosphate would be buffered by the large amount of basic ions (Na and Ca) in the glass and should not result in an acidic pH as seen in polyphosphate glasses [9]. Acidic pH conditions is not desirable for the formation of crystalline apatite. We would not expect the production of pyrophosphate species either as all but one of the glasses studied have a network connectivity (NC) less than 2.5. Typically soda-lime-phosphosilicate glasses with NC >2.5 contain some phosphate in a Q^1 configuration [7, 10].

Compositions are typically designed in weight percentages in the patent literature and also, surprisingly, in the scientific literature. This study avoids that approach as it is difficult to correlate changes in composition with changes in properties. The glasses presented here were designed on a molecular percentage basis. For example in the studies by Gorustovich et al. [11, 12], the authors substituted calcium oxide with strontium oxide on a weight percentage basis. No difference was seen in the two sets of samples *in vivo*. However, as strontium has a much higher relative atomic mass than calcium, replacing the weight of calcium in the glass with strontium would result in an increase in silica content. This would increase the network connectivity (NC [5, 6]) of the glass, slow dissolution and reduce bioactivity. The beneficial properties of adding the strontium to the glass [13], namely speeding dissolution, promoting osteoblast differentiation and function whilst down-regulating osteoclasts, would be cancelled out by the increase in NC. Therefore it is no surprise no real difference was seen in the calcium bioactive glass compared to the strontium sample in the study by Gorustovich et al.

2 Experimental

The processing of these glasses have been previously reported [5, 6]. Compositions can be found in Table 1 also with network connectivity values assuming phosphate enters the glass network (NC) and that a separate orthophosphate phase is formed (NC').

2.1 SBF

Simulated body fluid was prepared according to Kokubo and Takadama [14]. Glass particles of diameter <38 μm were immersed in this solution in sealed containers for up to 21 days and were mechanically agitated at 60 rpm using an incubator set to 37°C. At various time points the samples were filtered and dried to constant weight for analysis by XRD and FTIR. Powders were used rather than monoliths as a number of commercial products are supplied in powdered or granular form such as Novabone[®], Perioglas[®] and Vitoss[®]. The surface area of glass to volume of SBF given in Kokubo method results in problems. For example, for a 5 by 5 mm glass coating on a Ti6Al4V square results in 2.5 ml of SBF, hardly enough to wet the sample.

2.2 XRD

A Phillips powder diffractometer with a copper (Cu K_α) X-ray source (Philips PW 1700 series diffractometer, Philips, Endhoven, NL) was used to characterise the glass samples. The powdered samples were recorded between 10 and 60° 2θ at a scan speed of 0.04° s^{-1} . For crystallite size analysis high resolution scans of the (002) apatite peak

Table 1 Glass compositions used in this study with network connectivity assuming phosphate enters the silicate network (NC) and assuming phosphate forms a separate orthophosphate phase (NC')

ID	Mol. %				NC	NC'
	SiO ₂	Na ₂ O	CaO	P ₂ O ₅		
Series I						
ICSW1	51.06	26.10	22.84	0.00	2.08	2.08
ICSW2	47.84	26.67	23.33	2.16	2.00	2.18
ICSW3	44.47	27.26	23.85	4.42	1.92	2.30
ICSW5	40.96	27.87	24.39	6.78	1.83	2.44
ICSW4	37.28	28.52	24.95	9.25	1.75	2.62
Series II						
ICSW6	48.98	26.67	23.33	1.02	2.00	2.08
ICSW7	47.07	27.19	23.78	1.95	1.92	2.08
ICSW8	43.66	28.12	24.60	3.62	1.79	2.08
ICSW10	40.71	28.91	25.31	5.07	1.67	2.08
ICSW9	38.14	29.62	25.91	6.33	1.56	2.08

were taken between 2θ of 24 and 28° for a step size of 0.01° , collecting data on each step for 5 s. Peak analysis was performed using SciDAVis 0.1.3 software.

2.3 FTIR

FTIR spectra were obtained using a Bruker IFS 28 Fourier transform infrared spectrometer in the mid-IR range of $550\text{--}2,000\text{ cm}^{-1}$ with a resolution of 0.2 cm^{-1} . Powdered samples were pressed into pellets with KBr. Peak analysis was performed using SciDAVis 0.1.3 software.

3 Results and discussion

3.1 SBF

Figures 1 and 2 show the variation in pH with time for series I and II glasses respectively after immersion in SBF. As a general trend the SBF becomes more basic with time. This is to be expected as more sodium and calcium ions are released. The solution also shifts towards acidity as the phosphate content of the glasses is increased in both series. This can be explained by the release of more phosphate from the glass which will buffer the alkalinity caused by the sodium and calcium ions. This decrease in pH is advantageous for bioactivity as a pH of ≈ 7.3 is optimal in physiological fluid for apatite deposition [8].

3.2 XRD

Figures 3 and 4 show XRD traces of selected glasses from series I and II. With progressing time we can see the formation of Bragg peaks associated with apatite crystallisation. The signal to noise of the data is quite low and

even after 21 days the apatite formed is relatively disordered. The most prominent features are the Bragg peaks at 25.9° and 31.9° corresponding to the (002) and (211) reflections [15, 16]. The region containing the (211) peak ($30\text{--}35^\circ$) also contains a number of other features, namely the (112), (300) and (202) reflections [15, 16]. However due to the noise and degree of peak overlap, these lines could not be used for crystallite size analysis. The (002) peak was used for this purpose. Apatite formation can be clearly seen after 16 h. Figure 5 shows the crystallite size obtained from the peak width and position of the 002 peak using the Scherrer equation [17]. After 21 days there is no real variation in the apatite crystallite size. For series II there is possibly a decrease in crystal size with phosphate content, but the variation over the series is less than the error of the peak fitting.

3.3 FTIR

Figures 6 and 7 show FTIR spectra of selected glasses from both series immersed in SBF for up to 21 days. For the glasses containing phosphate, in the $500\text{--}600\text{ cm}^{-1}$ region there is a broad feature corresponding to the P–O bending mode and also a Si–O–Si bending vibration in this region [16, 18–20]. Around 720 cm^{-1} in the glass spectra is the band due to Si–O bending vibrations [21, 22]. The dominant bands at approximately 910 and 990 cm^{-1} can be attributed to Si–O (Q^2) and Si–O–Si stretching vibrations respectively [18, 19]. On exposure to SBF, vibrations from the silicate glass network decrease in intensity and bands due to the formation of crystalline hydroxyl-carbonate apatite become dominant. In particular the P–O bend and P–O stretch sharpen and increase intensity at around 580 and $1,010\text{ cm}^{-1}$ respectively [18, 19]. The P–O bend also shows the characteristic splitting indicative of crystalline

Fig. 1 pH variation of SBF for series I glasses

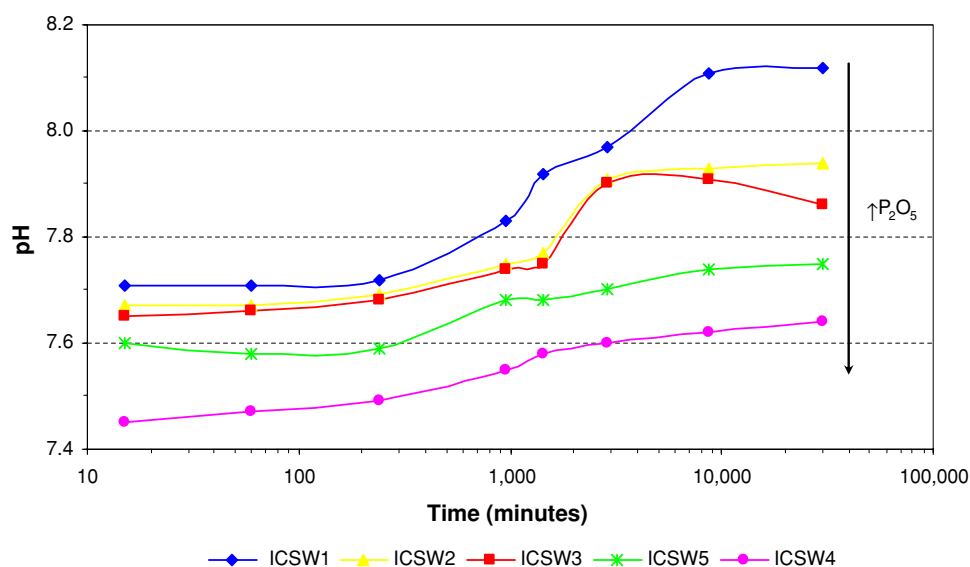


Fig. 2 pH variation of SBF for series II glasses

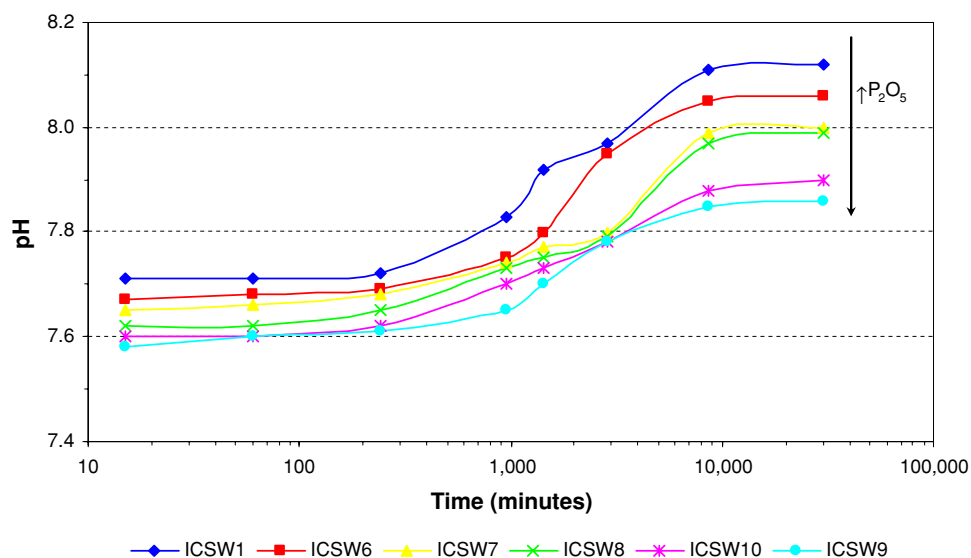


Fig. 3 XRD traces for series I glass ICSW03 (4.42 mol.% P_2O_5) with time of immersion in SBF with the main HCA Bragg peaks labelled

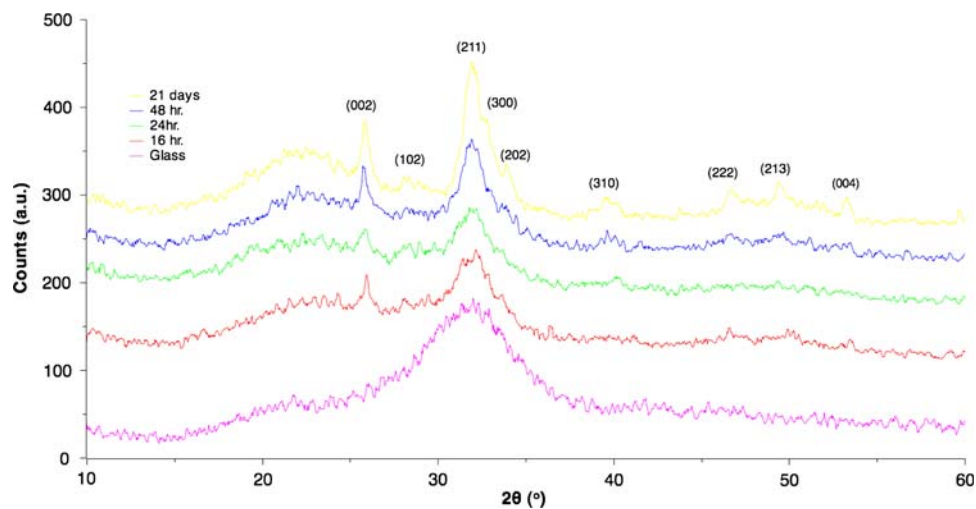
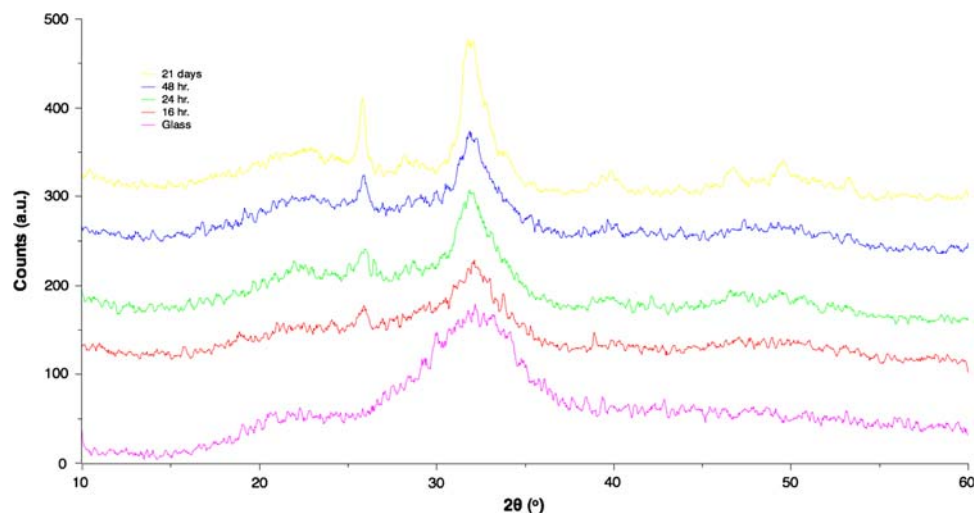


Fig. 4 XRD traces for series II glass ICSW09 (6.33 mol.% P_2O_5) with time of immersion in SBF (see Fig. 3 for Bragg peaks)



apatite formation (into bands at 560 and 600 cm^{-1}) [23]. The appearance of carbonate bands on exposure to SBF is also evidence of crystalline hydroxyl-carbonate-apatite

formation with the C–O stretch (ν_2) around 860 cm^{-1} and C–O asymmetric stretching (ν_3) at 1,410 and 1,450 cm^{-1} [19, 24, 25]. In the samples exposed to SBF the Si–O

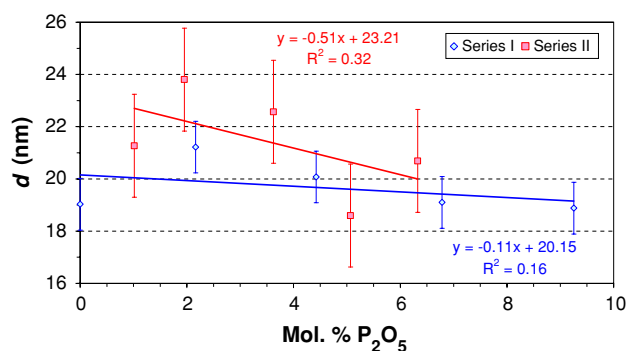


Fig. 5 Crystallite size analysis from 002 Bragg peak for both series of glasses

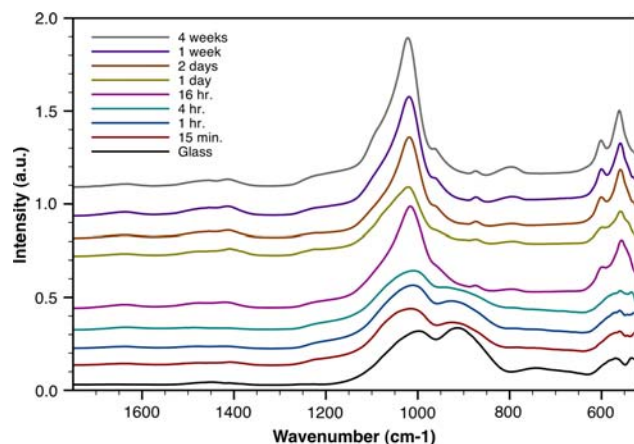


Fig. 6 FTIR spectra of series I glass ICSW05 (6.79 mol.% P_2O_5) with time of immersion in SBF

bending vibration reappears at higher wavenumber ($\approx 800\text{ cm}^{-1}$) compared to the original glasses which indicates some degree of repolymerisation, possibly condensation of the silica gel formed in the SBF. Serra et al. saw this band shift to higher wavenumber with increasing silica, from 44 to 66 wt%, in bioactive glass samples [22]. The band seen around $1,200\text{ cm}^{-1}$ in the 21 day SBF samples is due to a component of the asymmetric stretching mode of Si–O [22, 26, 27].

3.4 General comments

All glasses showed formation of apatite in the 21 day period tested in SBF. Table 2 summarises the time in which the characteristic splitting of the FTIR P–O bending mode at around 550 cm^{-1} was seen indicating crystalline apatite formation. As a general trend, apatite formation is faster as the phosphate content is increased in the parent glass; even in series I which shows an increase in network connectivity with increasing P_2O_5 . This indicates that phosphate content is more important than network

connectivity of the silicate phase for bioactivity in the range of compositions studied as long as the phosphate is present as Q^0 species [5, 6]. The glass ICSW4, containing the highest phosphate content in both series does not fit in with the trend shown in Table 2. It is known from previous studies that this sample was partially crystalline [5, 6] which would account for the slower rate of apatite formation compared to the other glasses.

The area of the P–O bending mode (also known as the ν_4 phosphate region) can be seen to linearly correlate with the area of the (002) Bragg reflection from XRD as presented in Fig. 8. The areas of these features after 21 days in SBF both increase with phosphate in the parent glass. This indicates that as a general trend, as more phosphate is added to the glasses, more apatite forms which suggests the phosphate content is more important in these two series of glasses than NC for mineralisation even though the network connectivity is increasing in series II and fixed in series I. Also as Fig. 5 shows, the size of the apatite crystals are not varying with phosphate content in the parent glass after 21 days in SBF, but the amount of crystals clearly increases due to the increase in the areas of the features in the XRD and FTIR data associated with HCap crystallinity. This result is promising, indicating the formation of biomimetic nanocrystalline Ca-deficient hydroxy-carbonate apatite which will bind to proteins such as fibronectin, vitronectin and collagen which encourage osteoblast attachment and proliferation [28].

Figure 9 compares the bioactivity of glass ICSW9 against 45S5 Bioglass® from FTIR spectra taken on glass powders of identical particle sizes exposed to the same SBF conditions. It can clearly be seen that after 1 day in SBF the apatite forms far more rapidly on the ICSW9 glass with intense, narrow, split peaks in the 550 cm^{-1} region, whereas the phosphate peak can hardly be resolved from the background in 45S5. This unequivocally shows the glasses studied here show superior bioactivity and potential as hard tissue remineralising agents compared to 45S5 Bioglass®.

The increased apatite formation could be a result of phase morphology. Pyrex is comprised of sodium borate rich drops in a silica rich matrix. The drops at the glass surface degrade when exposed to corrosive media, however those in the bulk are not affected. Vycor® is another phase separated borosilicate glass. However the borate phase is interconnected and the glass can be etched to form a porous structure. The high phosphate content glasses presented in this study which show increased apatite deposition may exhibit an interconnected orthophosphate morphology resulting in continuous phosphate leaching on exposure to SBF and enhanced apatite formation kinetics. This may be explained by a rapid increase in the surface area of glass exposed to solution in the initial stages of exposure.

Fig. 7 **a** FTIR spectra of series II glass ICSW10 (5.07 mol.% P_2O_5) with time of immersion in SBF. **b** 3D contour plot (time axis not linear)

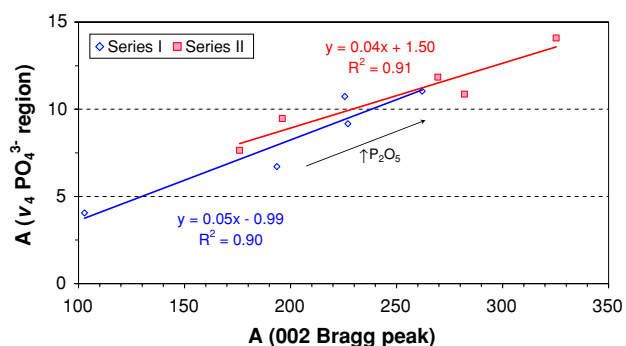
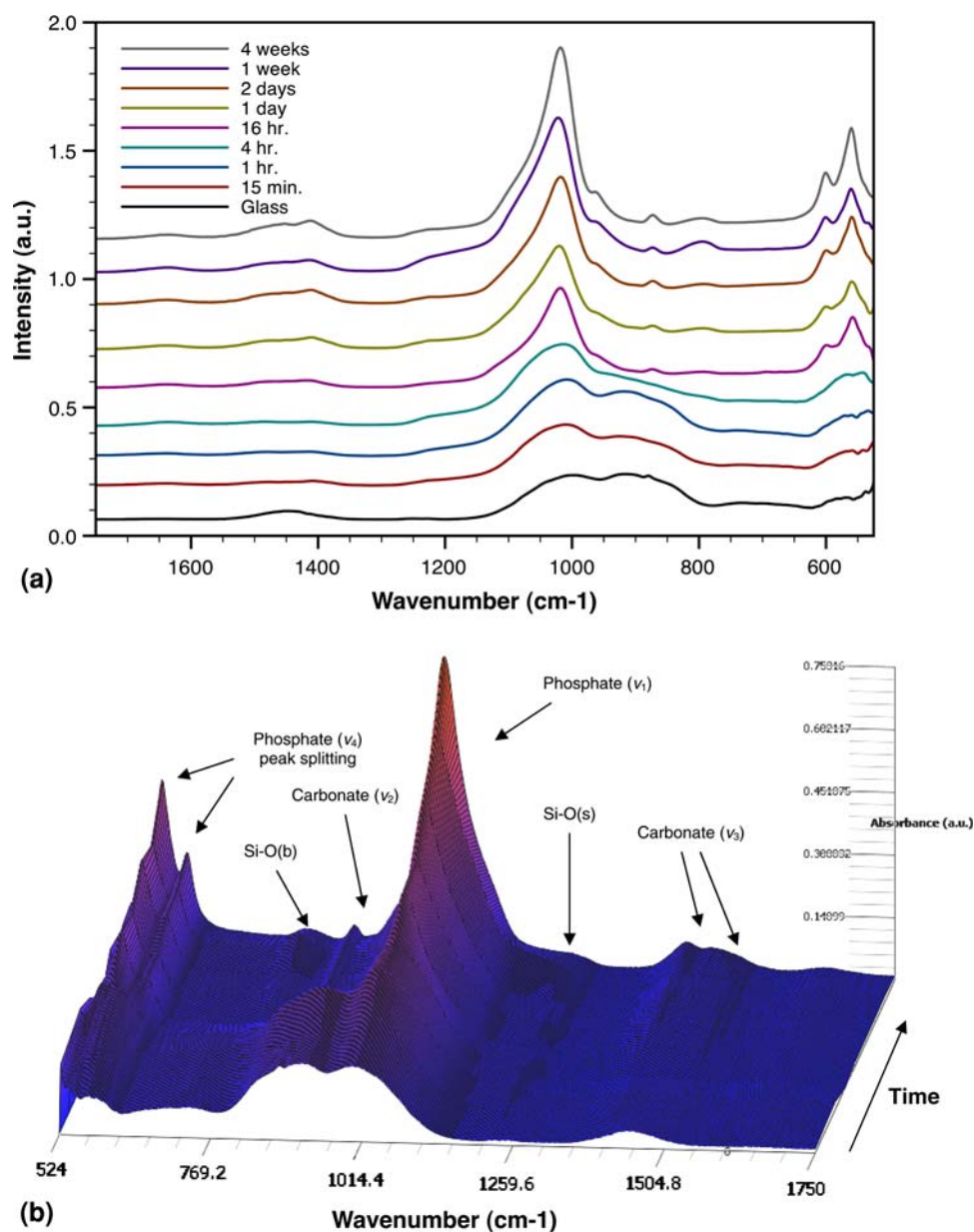


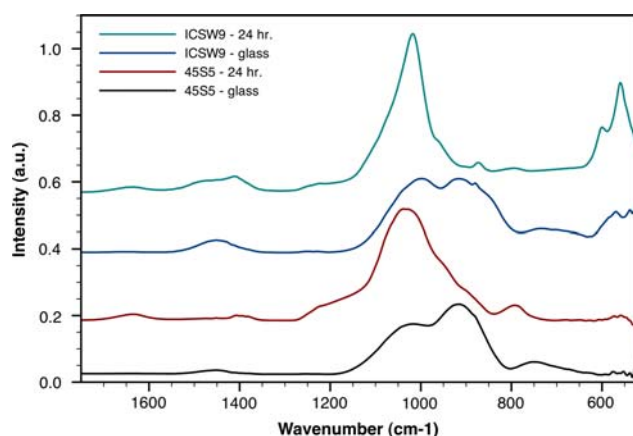
Fig. 8 Area of v_4 phosphate FTIR region plotted against (002) Bragg peak area from XRD after 21 days in SBF

4 Conclusions

In summary, two series of glasses were studied to examine both the effect of network connectivity of the silicate phase and phosphate content on bioactivity. All glasses studied produced a hydroxyl-carbonated-apatite layer on immersion in SBF for up to 21 days. The formation of this layer occurred more rapidly as the phosphate content increased for both series. This is a clear indication that in the compositional ranges studied here, phosphate content is a more important variable than the connectivity of the silicate network or an overriding factor in a process where NC and phosphate dissolution compete. The bioactivity of soda-lime-phosphosilicate glasses can be improved significantly

Table 2 Time for crystalline apatite formation observed by splitting of the FTIR P–O bending mode at around 550 cm^{-1} (sample ICSW4 partially crystalline)

ID	Mol.% P_2O_5	Time
Series I		
ICSW1	0.00	1 week
ICSW2	2.16	2 days
ICSW3	4.42	16 h
ICSW5	6.78	16 h
ICSW4	9.25	1 day ^a
Series II		
ICSW6	1.02	2 days
ICSW7	1.95	2 days
ICSW8	3.62	16 h
ICSW10	5.07	16 h
ICSW9	6.33	16 h

^a Sample partially crystalline**Fig. 9** Comparison of ICSW9 and 45S5 Bioglass[®] by FTIR after 24 h in SBF

by designing compositions with a good understanding of glass structure.

References

- Hench LL, Splinter RJ, Allen WC, Greenlee TK. Bonding mechanisms at the interface of ceramic prosthetic materials. *J Biomed Mater Res.* 1971;5:117–41. doi:10.1002/jbm.820050611.
- Litkowski LJ, Hack GD, Greenspan DC. Compositions containing bioactive glass and their use in treating tooth hypersensitivity. US Patent 6338751; 2002.
- Barry JE, Trogolo JA. Antibiotic toothpaste. US Patent 6123925; 2000.
- Cannell DW, Hashimoto S, Barger KN, Nguyen NV. Hair relaxer compositions comprising a bioactive glass. European Patent EP1709997; 2006.
- O'Donnell MD, Watts SJ, Law RV, Hill RG. Effect of P_2O_5 content in two series of soda lime phosphosilicate glasses on structure and properties—part I: NMR. *J Non-Cryst Solids.* 2008;354:3554–60. doi:10.1016/j.jnoncrysol.2008.03.034.
- O'Donnell MD, Watts SJ, Law RV, Hill RG. Effect of P_2O_5 content in two series of soda lime phosphosilicate glasses on structure and properties—part II: physical properties. *J Non-Cryst Solids.* 2008;354:3561–6. doi:10.1016/j.jnoncrysol.2008.03.035.
- Elgayar I, Aliev AE, Boccaccini AR, Hill RG. Structural analysis of bioactive glasses. *J Non-Cryst Solids.* 2005;351:173–83. doi:10.1016/j.jnoncrysol.2004.07.067.
- Krajewski A, Ravaglioli A. Bioceramics and biological glasses. In: Barbucci R, editor. *Integrated biomaterials science.* New York: Kluwer Academic/Plenum; 2002. p. 189–254.
- Knowles JC. Phosphate based glasses for biomedical applications. *J Mater Chem.* 2003;10:2395–401. doi:10.1039/b307119g.
- Grussaute H, Montagne L, Palavit G, Bernard JL. Phosphate speciation in $\text{Na}_2\text{O}-\text{CaO}-\text{P}_2\text{O}_5-\text{SiO}_2$ and $\text{Na}_2\text{O}-\text{TiO}_2-\text{P}_2\text{O}_5-\text{SiO}_2$ glasses. *J Non-Cryst Solids.* 2000;263:312–7. doi:10.1016/S0022-3093(99)00643-2.
- Gorustovich A, Steimetz T, Cabrini RL, Porto López JM. Osteoconductivity of strontium-doped bioactive glass particles. *Bone.* 2007;41:S1–13.
- Gorustovich A, Steimetz T, Porto López JM. Microchemical characterization of bone around strontium-doped bioactive glass particles. *Bone.* 2007;41:S1–13.
- Fredholm Y, O'Donnell MD, Kapukhina N, Law RV, Hill RG. Strontium containing bioactive glasses: part 1 glass structure and physical properties. *J Non-Cryst Solids* (under review).
- Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials.* 2006;27:2907–15. doi:10.1016/j.biomaterials.2006.01.017.
- Fleet ME, Liu X, King PL. Accommodation of the carbonate ion in apatite: an FTIR and X-ray structure study of crystals synthesized at 2–4 GPa. *Am Mineral.* 2004;89:1422–32.
- Vallet-Regí M, Romero AM, Ragel CV, LeGeros RZ. XRD, SEM-EDS, and FTIR studies of in vitro growth of an apatite-like layer on sol-gel glasses. *J Biomed Mater Res A.* 1999;44:416–21. doi:10.1002/(SICI)1097-4636(19990315)44:4<416::AID-JBM7>3.0.CO;2-S.
- Cullity BD. *Elements of X-ray diffraction.* Reading, MA: Addison-Wesley; 1956.
- Regina M, Filgueiras T, La Torre G, Hench LL. Solution effects on the surface reactions of three bioactive glass compositions. *J Biomed Mater Res.* 1993;27:1485–93. doi:10.1002/jbm.820271204.
- Peitl O, Dutra Zanotto E, Hench LL. Highly bioactive $\text{P}_2\text{O}_5-\text{Na}_2\text{O}-\text{CaO}-\text{SiO}_2$ glass-ceramics. *J Non-Cryst Solids.* 2001;292:115–26. doi:10.1016/S0022-3093(01)00822-5.
- Filho OP, La Torre GP, Hench LL. Effect of crystallization on apatite-layer formation of bioactive glass 45S5. *J Biomed Mater Res A.* 1996;30:509–14. doi:10.1002/(SICI)1097-4636(199604)30:4<509::AID-JBM9>3.0.CO;2-T.
- Sanders DM, Person WB, Hench LL. Quantitative analysis of glass structure with the use of infrared reflection spectra. *Appl Spectrosc.* 1974;28:247–55. doi:10.1366/000370274774332623.
- Serra J, González P, Liste S, Serra C, Chiussi S, León B, et al. FTIR and XPS studies of bioactive silica based glasses. *J Non-Cryst Solids.* 2003;332:20–7. doi:10.1016/j.jnoncrysol.2003.09.013.
- Penel G, Leroy G, Rey C, Sombret B, Huvenne JP, Bres E. Infrared and Raman microspectrometry study of fluor-fluor-hydroxy and hydroxy-apatite powders. *J Mater Sci Mater Med.* 1997;8:271–6. doi:10.1023/A:1018504126866.
- Santos RV, Clayton RN. The carbonate content in high-temperature apatite: an analytical method applied to apatite from the Jacupiranga alkaline complex. *Am Mineral.* 1995;80:336–44.

25. Rey C, Collins B, Goehl T, Dickson I, Glimcher M. The carbonate environment in bone mineral: a resolution-enhanced fourier transform infrared spectroscopy study. *Calcif Tissue Int.* 1989;45:157–64. doi:[10.1007/BF02556059](https://doi.org/10.1007/BF02556059).
26. Galeener FL, Lucovsky G. Longitudinal optical vibrations in glasses: GeO_2 and SiO_2 . *Phys Rev Lett.* 1976;37:1474. doi:[10.1103/PhysRevLett.37.1474](https://doi.org/10.1103/PhysRevLett.37.1474).
27. Lange P. Evidence for disorder-induced vibrational mode coupling in thin amorphous SiO_2 films. *J Appl Phys.* 1989;66:201–4. doi:[10.1063/1.344472](https://doi.org/10.1063/1.344472).
28. Vallet-Regi M, Arcos D. *Biomimetic nanoceramics for clinical use.* Cambridge: RSC Publishing; 2008.



Influence of magnesia on the structure and properties of bioactive glasses

S.J. Watts^{a,b}, R.G. Hill^{a,c,*}, M.D. O'Donnell^{a,b}, R.V. Law^{b,*}

^a Department of Materials, Imperial College, London SW7 2AZ, UK

^b Department of Chemistry, Imperial College, London SW7 2AZ, UK

^c Unit of Dental Physical Sciences, Institute of Dentistry, Barts and the London, Queen Mary College, University of London, Mile End Road London E1 8UQ

ARTICLE INFO

Article history:

Received 2 April 2008

Received in revised form 24 April 2009

Keywords:

Bioglass
Biomaterials
Nuclear magnetic (and quadrupole) resonance
Alkali silicates
Silicates
Soda-lime-silica
NMR, MAS NMR and NQR
Short-range order
Thermal properties

ABSTRACT

Five bioactive glasses based on $49.5\text{SiO}_2\text{--}1.1\text{P}_2\text{O}_5\text{--}(23.0(1-x))\text{CaO--}x\text{MgO--}26.4\text{Na}_2\text{O}$ mol.% were synthesised where CaO was replaced progressively on a molar basis by MgO (where $0 \leq x \leq 1$). The glasses were characterised by ^{31}P and ^{29}Si magic angle spinning nuclear magnetic resonance (MAS NMR) spectroscopy, dilatometry, differential thermal analysis (DTA) and density measurements with the aim of gaining a comprehensive understanding of the structure–property relationships.

The ^{31}P NMR spectra of the glasses exhibited well-defined resonances at ca. 9–14 ppm, corresponding to a Q^0 orthophosphate environment. Full substitution of MgO caused a downfield shift of ca. 5 ppm, implying the preferential association of sodium with the orthophosphate species. The ^{29}Si NMR spectra exhibited a peak at ca. –79 ppm, indicating a Q^2 silicon species, along with a shoulder at ca. –90 ppm, corresponding to a Q^3 species. On addition of MgO, the Q^3 shoulder was seen to progressively increase in magnitude. These results suggest that MgO, rather than depolymerising the silicate network by acting as a network modifier, is behaving in part, as an intermediate oxide. Deconvolution of the ^{29}Si NMR peaks suggest that, although 86% of the magnesium oxide is acting traditionally as a network modifying cation, up to 14% of the magnesium oxide is entering the silicate network as tetrahedral, MgO_4 , removing network modifying ions for charge compensation and resulting in the observed polymerisation of the silicate network. Correspondingly, both the glass transition temperature and dilatometric softening point values were seen to decrease whilst the thermal expansion coefficient values rose, with increasing MgO content. This was due to the significantly lower bond strength, of Mg–O compared to Si–O, weakening the glass network.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

In 1969 Hench et al. [1,2] developed the first amorphous, inorganic bioactive material, Bioglass[®], based on a soda-lime phosphosilicate glass. Most materials, at best, elicit a neutral response when implanted into the human body. Bioglass[®] however was seen to create a positive response by promoting the deposition of the body's natural bone substance, hydroxyapatite. Although it is well recognised that compositional modifications affect the bioactivity of glasses, there is very little understanding of the composition–structure–property relationships that result in such behaviour. The purpose of this investigation, therefore, is to study these fundamental relationships with respect to a component often found in bioactive glass compositions – magnesium oxide.

Network connectivity (NC) is defined as the average number of bonds that link each repeat unit in the silicate network and is based on the relative number of network forming oxides (those which contribute bridging oxygen (BO) species) and number of

network modifying species (those which result in the formation of non-bridging oxygens (NBOs)) [1,2]. To determine the level of bioactive behaviour using such a model, it is critical to have a structural understanding of each species within the glass. Without such knowledge, assessing the bioactive behaviour of a specific glass composition is extremely problematic. Previous studies, in particular using nuclear magnetic resonance (NMR) – a useful technique for probing the short range order in glasses, have clarified the structural role of silicon, aluminium, sodium and calcium in the glass network [3–5]. Thus predicting the bioactive behaviour of glasses containing these particular elements (in any composition), using the network connectivity model, becomes surprisingly straight forward. Therefore, this investigation aims to add to understanding of the network connectivity model with respect to bioactive glasses by clarifying magnesium's structural role within such compositions.

A number of mechanisms have been proposed to explain the bioactive behaviour of glasses and glass–ceramics. The first and most widely accepted mechanism, is that associated with the research of Hench et al. [6,7]. There are, however, alternative theories on the mechanism of glass degradation and bone bonding behav-

* Corresponding authors. Tel.: +44 (0)20 7594 5860; fax: +44 (0)20 7594 6783.

E-mail addresses: r.hill@imperial.ac.uk (R.G. Hill), r.law@imperial.ac (R.V. Law).

our from Kokubo et al. [8], Andersson et al. [9], Li et al. [10] and Hill [11].

Hill suggested an alternative viewpoint with respect to the degradation of bioactive glasses, realising the inadequacy of the mechanism proposed by Hench in predicting the reactivity of a bioactive glass as a function of its composition [11,12]. As with Strnad [13] and Rawlings [14], Hill recognised the importance of the degree of disruption of a glass in determining its bioactivity. Following the approach of Holliday and Ray [15,16], viewing silicate glasses as inorganic polymers of oxygen cross linked by silicon, Hill introduced the concept of network connectivity to the field of bioactive glasses. Properties including thermal expansion, ability to undergo phase separation, surface reactivity and solubility all can be explained on the basis of the degree of polymerisation of the silicate network, network connectivity and the inorganic polymer model [11].

As bioactivity arises from the ability of a glass to undergo surface dissolution in a physiological environment, the degree of solubility of a glass composition must be critical in determining its bioactivity. The application of the inorganic polymer model to a glass structure, therefore, allows its bioactivity to be predicted, with a glass of low network connectivity considered to be highly disrupted and bioactive, provided it does not give rise to toxic degradation products. [17]. All of the very reactive bone bonding bioactive glass compositions have network connectivities below or close to 2.00, with the most widely studied 45S5® glass having a NC value of 1.90.

Magnesia has often been incorporated into bioactive glasses [18–29] however, despite this work, there have been few systematic studies into the structural role of MgO and its resultant bioactive behaviour. Conventionally magnesium oxide is thought to behave like calcium oxide, as a network modifier, within a silicate glass structure. However, on examination of a number of elemental properties such as charge to size ratio and Pauling electronegativity, Mg^{2+} falls on the boundary between being a network modifier and an intermediate oxide. Circumstantial evidence exists that MgO may act as intermediate oxide in highly disrupted silicate glasses from the extension of the glass forming region in ternary diagrams towards lower SiO_2 mole fractions and this point has been recognised by McMillan [30], Fiske and Stebbins [31], Oliveira et al. [24], Kohara et al. [32], Karakassides et al. [33], Dietzel [34], Shimoda et al. [35] and Doweidar [36] acknowledge the possibility of MgO acting as an intermediate oxide and all comment on the importance of clarifying its structural role within a glass.

It is suggested that the lack of structural understanding of magnesium within glasses has led to the many conflicting views on its contribution with respect to bioactivity. MgO has been documented as decreasing apatite formation in SBF [26] but also, on the other hand, improving the early stages of mineralization [24] and contributing to an intimate contact with living tissue. It is proposed that these conflicting reports call for a comprehensive study into magnesium and bioactivity.

Magnesium is involved in over 300 chemical reactions in the body and, among other things, is known to activate phagocytosis

and regulate active calcium transport. As a result, there has been a growing interest in its role, not only in wound healing, but also in bone metabolism with a two year study highlighting the positive effects on fracture prevention and bone density [37,38]. Such influential roles with respect to bone metabolism and wound healing are extremely attractive when considering the potential end applications of magnesium containing bioactive glass compositions. This paper therefore, endeavours to comprehend magnesium's role within a bioactive glass structure and understand the composition–structure–property relationships that exist in such a structure.

2. Experimental

2.1. Glass melting

Magnesium oxide was substituted for calcium oxide, on a molar basis, in a previously studied glass composition [5]. The calculated network connectivity values were kept constant, at 2.13, to eliminate any influence due to network disruption and thus provide a greater understanding of the underlying composition–property relationships in the glass. The resulting glass compositions are given in Table 1.

These are nominal batched values. For the NC(NMR) calculation, we assumed, for the sake of simplicity, that Mg was only in the four co-ordinate state and went into the network as a tetrahedral MgO_4 species. This was based on the assumption that Mg was behaving as it is found in the mineral, akermanite ($Ca_2MgSi_2O_7$) or spinel $MgAl_2O_4$ [31,39–41].

All glasses were prepared using reagent grade chemicals for each case (SiO_2 , P_2O_5 , $CaCO_3$, Na_2CO_3 , and $MgCO_3$). It is important to note that the addition of P_2O_5 was always made last due to its extremely hygroscopic nature. The compositions were melted in platinum–rhodium or platinum–gold crucibles at 1450 °C for one and a half hours. The melts were then rapidly quenched in cold water and the glass frit collected in a sieve and dried overnight at 120 °C. The dried frit was then ground in a Gyro Mill (Glen Creston) for seven minutes and sieved for 45 min in a mechanical shaker to obtain <45 μm particles. Rods and discs were also cast for each composition by re-melting the glass frit for half an hour at approximately 1470 °C. The glass was then poured into a pre-heated graphite mould and annealed overnight at 20 °C below the glass transition temperature (T_g).

2.2. Magic angle spinning nuclear magnetic resonance (MAS NMR)

^{29}Si MAS NMR and ^{31}P MAS NMR were used to analyse the glass samples using a Bruker DSX FT-NMR spectrometer operating at a Larmor frequencies of 39.77 MHz and 81.9 MHz, respectively. Samples were packed into a 4 mm zirconia rotor and spun at the magic angle to remove anisotropy effects. The ^{29}Si and ^{31}P MAS NMR spectra of the glasses were recorded at spinning frequencies from 3–12 kHz using pulse acquisition of $\pi/2$ for both silicon and phosphorus. The ^{29}Si and ^{31}P MAS NMR samples were spun with a re-

Table 1
Glass compositions (nominal batched) of the magnesium substituted glasses in mol.%. Magnesium is substituted for calcium on a molar basis, keeping the network connectivity (NC) constant – where NC is the network connectivity assuming the phosphate enters the silicate network, NC' assuming a separate orthophosphate phase and NC(NMR) calculated from the ^{29}Si NMR peak fitting in Table 2. To simplify the calculations for NC(NMR) it is assumed that Mg is only present as tetrahedral, MgO_4 units.

Glass ID	SiO_2	Na_2O	CaO	P_2O_5	MgO	NC	NC'	NC(NMR)
ICIE1	49.46	26.38	23.08	1.07	0.00	2.04	2.13	2.17
ICIE1-25 Mg	49.46	26.38	17.31	1.07	5.77	2.04	2.13	2.20
ICIE1-50 Mg	49.46	26.38	11.54	1.07	11.54	2.04	2.13	2.23
ICIE1-75 Mg	49.46	26.38	5.77	1.07	17.31	2.04	2.13	2.32
ICIE1-100 Mg	49.46	26.38	0.00	1.07	23.08	2.04	2.13	2.41

cycle delay time set to generally 2 s (this however was increased to 60 s if the glass species were not thought to be completely relaxed). All spectra were recorded at an ambient probe temperature with ^{29}Si referenced relative to polydimethylsiloxane (PDMS) (-22.3 ppm) and 85% H_3PO_4 (0 ppm) for ^{31}P . Peak fitting to determine the peak areas for NC(NMR) was done by WinNMR program (Bruker).

2.3. X-ray diffraction (XRD)

A Phillips Powder Diffractometer with a copper ($\text{Cu K}\alpha$) X-ray source (Philips PW 1700 series diffractometer, Philips, Endhoven, NL) was used to characterise the glass samples. The powder samples ($<45\text{ }\mu\text{m}$ particle size) were scanned between $2\theta = 5\text{--}80^\circ$ with a step size of $2\theta = 0.04^\circ$, in order to investigate the amorphous nature of each glass.

2.4. Differential thermal analysis (DTA)

The glasses were characterised by simultaneous DTA/TGA using a Stanton Redcroft DTA 1600. Alumina was used as the reference material and both reference and sample were contained within platinum–rhodium alloy crucibles. The $<45\text{ }\mu\text{m}$ particle size powders were heated from room temperature to 1300°C , at a heating rate of $10^\circ\text{C}/\text{min}$, in an inert argon atmosphere. The glass transition temperature (T_g) and crystallisation temperatures (T_c) for each glass were then obtained from the corresponding traces.

2.5. Dilatometry

A Netzsch Dil 402C dilatometer was used to determine T_g , the dilatometric softening temperature (T_s) and the thermal expansion coefficient (TEC) for each glass. $25\text{ mm} \times 6\text{ mm}$ cut samples were analysed from room temperature to $T_s + 30^\circ\text{C}$ at a heating rate of $5^\circ\text{C}/\text{min}$. The TEC and T_s were determined by using the system software and the TEC taken between 25°C and 400°C .

2.6. Density measurements

Using Archimedes principle the density of each cast glass sample, at room temperature, was determined. The weight of each glass was measured in air and in distilled water using an accurate balance ($\pm 0.1\text{ mg}$). The weight was measured three times and an average taken in order to minimise the sources of error. Care was taken to make sure that all of the cast samples were bubble free and that the glass samples were fully wetted when immersed in the distilled water. The oxygen density was then calculated from these bulk density values, according to Ray [16].

3. Results and discussion

3.1. Magic angle spinning nuclear magnetic resonance (MAS NMR) spectroscopy

3.1.1. ^{31}P MAS NMR

The ^{31}P MAS NMR spectra show a single symmetric resonance, as shown in Fig. 1, with a chemical shift range in the region $8.2\text{--}12.7\text{ ppm}$ and with an approximately constant linewidth of *ca.* 600 Hz.

Table 2 summarises the peak positions and full width half maximums (FWHM).

The ^{31}P NMR chemical shifts for the magnesium substituted series correspond to the existence of an orthophosphate environment with no evidence of the existence of Si--O--P bonds, agreeing with Lockyer et al. [3] and Elgayar et al. [5]. Previously phosphorus

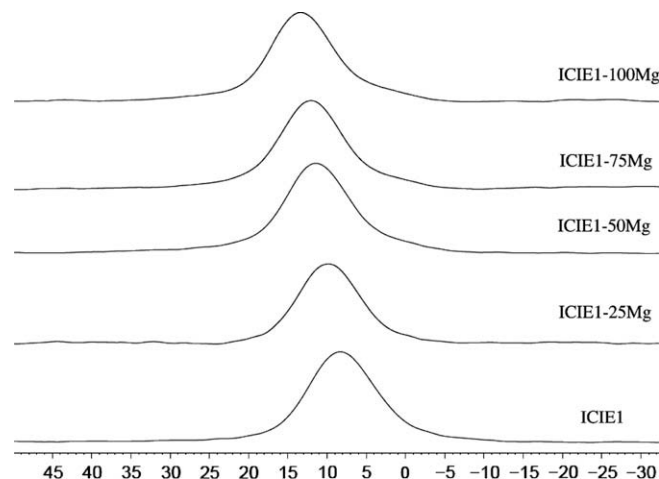


Fig. 1. ^{31}P MAS NMR spectra. Increasing magnesium content from bottom to top, ICIE1 (bottom) to ICIE1-100 Mg (top). This illustrates the existence of orthophosphate species and the progressive preferential association of sodium.

was assumed to enter the glass network forming Si--O--P bonds [42]. However, the absence of peaks at *ca.* -215 ppm in the ^{29}Si MAS NMR and *ca.* -33 to -40 ppm in the ^{31}P MAS NMR spectrum rule this out [43]. However the existence of orthophosphate species, along with the fact that the glasses are optically clear, suggest that the phosphorus in the glass may have undergone amorphous phase separation into a sub-micron (below light scattering dimensions) phosphate phase.

Initially we observe the ICIE1 glass to have a chemical shift of *ca.* 8.2 ppm between that of a $\text{Na}_3(\text{PO}_4)$ species at 14 ppm [44] and a $\text{Ca}_3(\text{PO}_4)_2$ species at 0 ppm or 3 ppm [44,45], thus implying that both types of modifier ion are associated with the orthophosphate unit. As we move through the series, it would be expected that substituting magnesium for calcium would simply result in the direct replacement in the phosphate environment of Ca for Mg as reported by Oliveira et al. [24], or in the case of Lockyer et al. [3] and Elgayar et al. [5], a mixed, non preferential, two cation orthophosphate at a ratio consistent with that of the overall glass. Although Mg^{2+} (1.31) is slightly more electronegative, on the Pauling scale, than Ca^{2+} (1.00) and Na^+ (0.93), the observed experimental shift is contrary to what is expected [3], with the final, fully substituted composition shifting to a more positive (away from 0 ppm) region of the spectra *ca.* 12.7 ppm – close to that associated with a pure sodium orthophosphate species.

This positive shift (away from 0 ppm – Fig. 2), towards a sodium orthophosphate type species and away from magnesium phosphate (0.5 ppm for the amorphous $\text{Mg}_3(\text{PO}_4)_2$ form [46,47]), implies that as magnesium is added, at the expense of calcium, sodium progressively preferentially associates with the phosphate phase. It appears there is only a minimal association of the magnesium with the phosphate phase and is an indication that an increase in magnesium content may lead to more Mg^{2+} ions participating in the silicate phase (this point will be discussed in greater detail later).

3.1.2. ^{29}Si MAS NMR

Fig. 3 illustrates the ^{29}Si MAS NMR spectra for the magnesium substituted series of glasses. The spectrum for 0% magnesium substituted glass consists of a peak with a resonance maximum *ca.* -79 ppm . The 25% magnesium substituted glass displays a similar spectrum, however, as we move through the series to the 50% magnesium substituted glass, the presence of a shoulder to the right of the peak *ca.* -90 ppm becomes apparent, moving the centre of gravity of the peak to the right. This shoulder becomes more

Table 2
The NMR data for ^{29}Si and ^{31}P NMR spectra of the series of magnesium containing bioactive glasses where: x corresponds to peak position, w peak width and A fraction area determined from peak deconvolution.

Glass ID	^{31}P x (ppm)	^{31}P w (Hz)	^{29}Si Q^2 x (ppm)	^{29}Si Q^2 w (Hz)	^{29}Si Q^2 A (%)	^{29}Si Q^3 x (ppm)	^{29}Si Q^3 w (Hz)	^{29}Si Q^3 A (%)
ICIE1	8.2	687	−80.6	461	83	−89.8	316	17
ICIE1-25 Mg	9.7	526	−79.4	399	80	−87.6	258	20
ICIE1-50 Mg	10.5	639	−80.6	451	77	−90.3	376	23
ICIE1-75 Mg	11.7	642	−80.1	410	68	−88.8	433	32
ICIE1-100 Mg	12.7	642	−80.0	396	59	−88.7	424	41

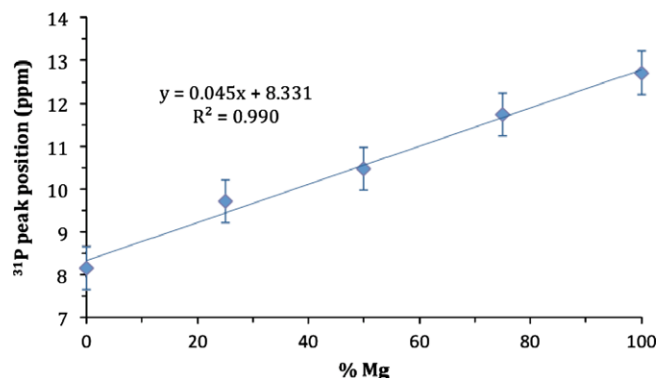


Fig. 2. The effect of ^{31}P orthophosphate shift with increase in magnesium content (while sodium content remains constant). This illustrates the progressive preferential association of sodium with the phosphate through the series.

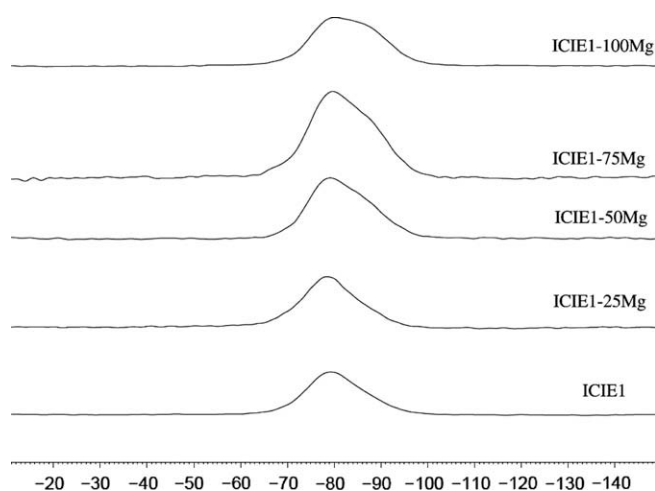


Fig. 3. ^{29}Si MAS NMR spectra. Increasing magnesium content from bottom to top, ICIE1 (bottom) to ICIE1-100 Mg (top). This illustrates the progressive formation of a Q^3 shoulder on a predominantly Q^2 peak.

pronounced across the series. It is assumed that the development of the asymmetric line shape and right hand shoulder, across the series, is a progressive superposition of two overlapping signals. The fitting data of these two peaks, from Gaussian deconvolution, can be found in Table 2. With magnesium substitution, the Q^3 shoulder grows from 17% (0% Mg substitution) to 41% (100% Mg substitution) area of the signal. This is clear evidence that, as magnesium is added, a proportion of the magnesium ions forms tetrahedral, MgO_4 , which requires charge balancing cations. [39,40] These MgO_4 units remove modifying ions (e.g. Ca^{2+}) from the silicate network resulting in the observed polymerisation going from Q^2 to Q^3 .

In spite of such signal overlap, the ^{29}Si NMR spectra of the magnesium substituted series reveal that they are all predominantly

populated by Q^2 silicate species with the addition of Q^3 species at higher magnesium contents. There seems to be no evidence of any further Q species within the series, therefore a binary distribution model for the glasses is suggested, agreeing with earlier work by Lockyer et al. [3] and Elgayar et al. [5]. It is noted, however, from previous work that more than two Q^n species can coexist at all compositions, suggesting a model based on the disproportionation reaction shown by Eq. (1) [23,48].

$$Q^n = Q^{n-1} + Q^{n+1} \quad (1)$$

Closer inspection of the magnesium substituted glass spectra illustrate no evidence of peaks at −105 or −60 ppm, therefore it can be concluded that there are little or no Q^4 or Q^0 species present. As noted by Elgayar et al. [5], difficulty can arise with respect to the identification of Q^1 silicate species with ^{29}Si spectra alone due to the close proximity of Q^2 species (−70 ppm Q^1 → −80 ppm Q^2) [49]. Further hindrance can also occur in mixed metal glasses due to the spread of ^{29}Si chemical shifts for each Q^n species, for example $\delta_{\text{Si}} = -76$ ppm for $Q^2(\text{Na})$ [50] and $\delta_{\text{Si}} = -81.5$ ppm for $Q^2(\text{Ca})$ [51]. Therefore, it is questionable as to whether any Q^1 species are being masked due to the overlap of silicon chemical shifts. At present this remains unanswered however, for the purpose of this study, a binary distribution of Q^2 and Q^3 is assumed.

Using Doweidar's model it is assumed that throughout the series of glasses the binary distribution of Q^2 and Q^3 species will remain constant. Also, using network connectivity calculations to model the glass structure, the connectivity is calculated to remain constant at 2.13 (calculated assuming P is present as orthophosphate as illustrated from the previous ^{31}P MAS NMR data). Both of these models presume that magnesium acts as a network modifier. Therefore, in a direct substitution of magnesium for calcium it is predicted that the connectivity/ Q structure will remain constant, due to both cations creating the same number of NBOs. This would then result in a series with corresponding constant ^{29}Si NMR chemical shift values (disregarding any shift due to the shielding effects of Mg^{2+} and Ca^{2+} – Galliano et al. [52] did not find any changes in the ^{29}Si NMR spectra when replacing cations in a glass series containing silicon, phosphorus and alkaline earth elements).

However, progressive formation of a right hand shoulder is observed with a peak resonance associated with that of a Q^3 species. This is contradictory to the network connectivity model and implies the progressive polymerisation of the glass structure is a result of magnesium entering the silicate network.

Fig. 4 illustrates a cartoon of the glass structure. It highlights the preferential association of sodium ions with the phosphate phase, as shown from ^{31}P NMR data, and the tendency of magnesium to enter the silicate network as MgO_4 tetrahedra, charge balanced by calcium/sodium ions. Though shown here as a tetrahedral in Fig. 4, other higher co-ordinate states e.g. octahedral are possible for Mg, though they would be outside of the network [40].

3.2. Network connectivity

Table 1 shows the calculated network connectivity of the glasses, assuming (i) the phosphate enters the network (NC) or

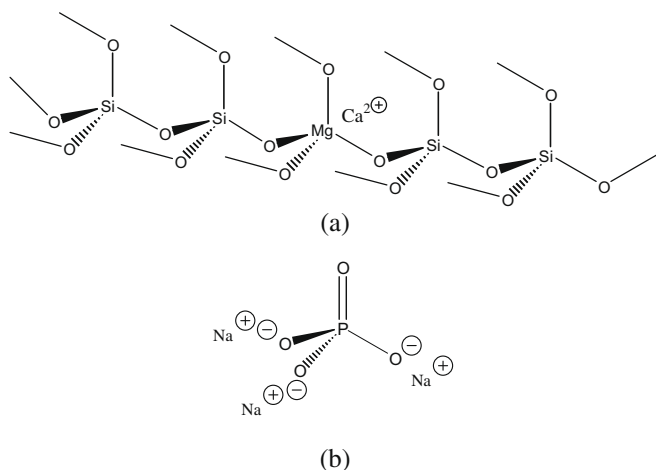


Fig. 4. Cartoon of the glass structure. This illustrates the two regions within the glass with (a) magnesium within the silicate network as tetrahedral MgO₄ units charge balanced by calcium and (b) sodium associated with the orthophosphate species.

(ii) phase separation into an orthophosphate phase (NC'). As we have said earlier NC' is a more reasonable assumption for network connectivity as there is little evidence for Si–O–P bonds present. An empirical determination of the network connectivity was derived from peak fitting of the Q² and Q³ ²⁹Si MAS NMR data (Fig. 3), the NC(NMR), and is also given in Table 1. The NC(NMR) values are clearly higher, deviating more as magnesium is substituted into the series (Fig. 5).

The deviation from the linear NC' must be due to the formation of tetrahedral MgO₄, species which goes into the network, will require sodium and / or calcium ions for charge balancing. This removes cations from the silica network and has the net effect of increasing the network connectivity. These NC(NMR) values were used to calculate the proportion of magnesium acting either as modifiers and/or those forming tetrahedral MgO₄. From the difference between the calculated NC' and the NC(NMR), it is possible to calculate the amount of tetrahedral MgO₄. In this calculation, however, we have assumed, for the sake of simplicity, that Mg is only present as tetrahedral, MgO₄. Fig. 6 shows the absolute molar amount of magnesium acting as a network former, in a tetrahedral state, for all the glass compositions.

However, as the amount of magnesium increases when it is substituted for calcium, the proportion in the network as com-

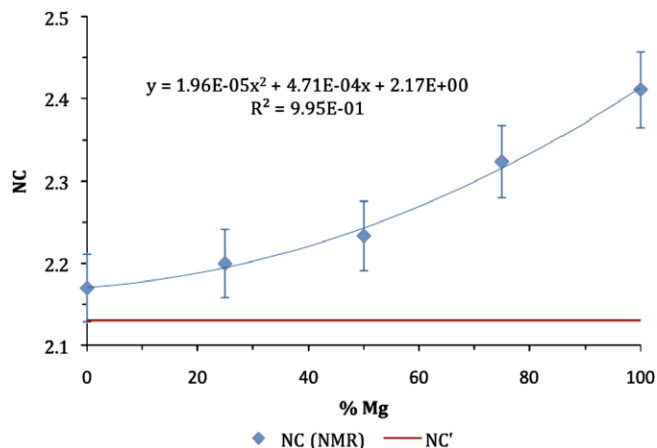


Fig. 5. Network connectivity calculated from NMR peak fitting (Table 2) (NC(NMR)) compared to network connectivity assuming MgO acts as modifier (NC'). In the NC' calculation, it was assumed that Mg is only in a tetrahedral state. The equation of the line is for NC(NMR).

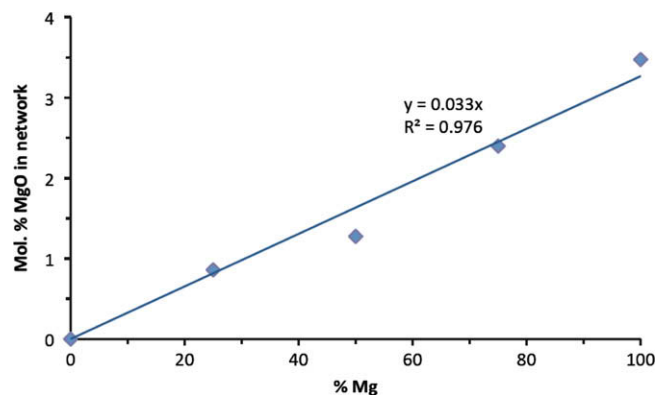


Fig. 6. The absolute amount of MgO (mol.%) entering the glass network with Mg substitution. This assumes that the MgO is only in tetrahedral co-ordination. As the MgO content is increasing across the series, this corresponds to a constant proportion ($\approx 14\%$) of MgO acting as network former compared to network modifying MgO.

pared to the proportion acting as modifiers is actually constant across the series; $\approx 14\%$ of the magnesium enters the network as tetrahedral MgO₄ whereas around 86% occupies the traditional modifier role regardless of the composition.

3.3. Physical properties

XRD of the powdered samples illustrated that they were all amorphous in nature. Fig. 7 displays the resultant DTA traces for the magnesium substituted glasses.

In general the DTA curves for samples ICIE1 to ICIE1–75 Mg have two transitions – one due to a T_g , with a negative deviation from the baseline, and one due to an exothermic crystallisation event (T_c) (Fig. 8). There is a possible second exotherm in the ICIE1 and ICIE1–25 Mg glasses corresponding to a second crystallisation temperature. Also the ICIE1–100Mg glass shows only one transition – due to a glass transition.

The behaviour of T_g suggests that the addition of MgO results in a ‘weakening’ of the glass network. As Mg content increases in the glass, the decrease in T_g is attributed to magnesium entering the network as tetrahedral magnesium species, MgO₄ [35,40]. We hypothesise that the newly formed Si–O–Mg bonds have, on average, a significantly lower bond strength with respect to Si–O–Si bonds in the silicate chain and therefore, as a result, resulting in the observed reduction in T_g . Dowty studied the vibrational spectrum of akermanite, a calcium magnesium silicate (Ca₂MgSi₂O₇) which contains tetrahedral MgO₄ units [53]. The bond force constant of the Mg–O bond in akermanite was found to be lower than in MgO and significantly lower than a Si–O bond: 2.00, 3.48 and 9.24 N cm^{−1}, respectively [53,54]. This phenomenon is also noted by Linati et al. [55], however the study was on the role of zinc rather than magnesium in a similar glass series. However, it is interesting to note that magnesium and zinc are similar ions with respect to charge and ionic radius, 0.72 Å and 0.74 Å accordingly, and it is suggested that the two behave in a similar manner within glass systems [56].

Fig. 9 highlights the behaviour of TEC values with a slight increase after 20 mol.% substituted magnesium, attributed to magnesium entering the network and the weakening of the glass structure allowing greater thermal expansion. Appen's predictions for decreasing thermal expansion with the substitution of the higher polarising magnesium are also plotted in Fig. 9.

The experimental deviation from Appen's trend at >20 mol.% can be ascribed to the change in the structural role of magnesium oxide, from a modifier to an intermediate oxide through the series.

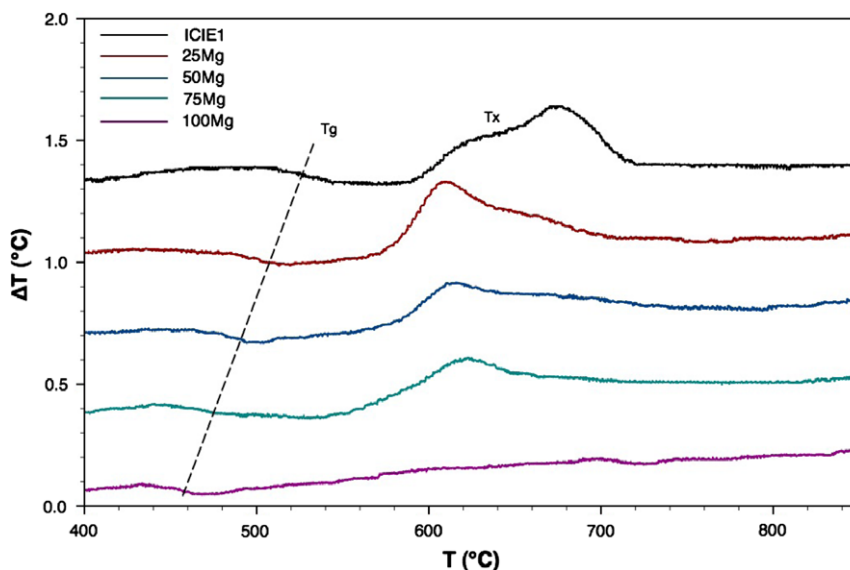


Fig. 7. Differential Thermal Analysis (DTA) traces for the magnesium substituted series of glasses. Increasing magnesium content from ICIE1 (top) to ICIE1-25 Mg to ICIE1-100 Mg to ICIE1-50 Mg to ICIE1-75 Mg (bottom).

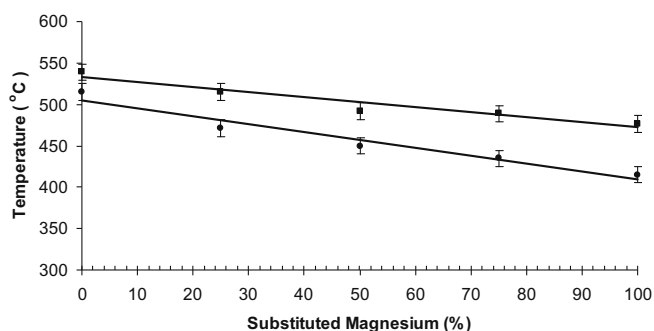


Fig. 8. Graph of glass transition temperature, T_g (bottom line) and softening temperature, T_s (top line) against percentage of magnesium substituted. Illustrates the decreasing trend in T_g and T_s with magnesium substitution.

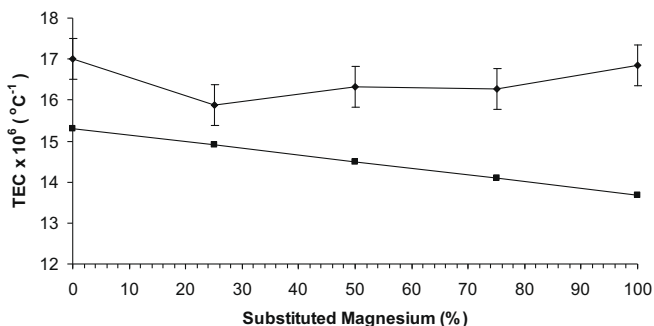


Fig. 9. Graph of experimental thermal expansion coefficients, (TEC) (top) and Appen's predictions (bottom) against percentage of magnesium substituted. This illustrates the difference between Appen's predicted decreasing trend and the experimental increase in TEC with magnesium substitution.

Fig. 10 illustrates the experimental density values along with Doweidar's predicted density values. Doweidar postulated that the density of a glass can be correlated with the volume of its structural units using the conventions of the Q^n units, with the addition of network modifying oxides to calculate the density of a glass [5,36,57].

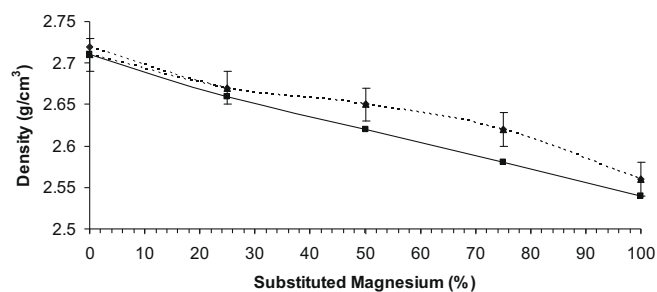


Fig. 10. Graph of experimental density values (top) and Doweidar's predictions (bottom) against percentage of magnesium substituted. This illustrates decreasing trend in experimental and Doweidar's predicted density values with magnesium substitution.

As magnesium is substituted for calcium, the smaller ionic radius [58] and lighter molar mass result in the observed decrease in bulk glass density. This agrees with Doweidar's predictions, confirming that the glasses are predominantly Q^2 in structure. Deviations however are observed from >20 mol.% which could be assigned once again to the change in structural role of magnesium, switching from a modifier to an intermediate through the series.

The substitution of magnesium for calcium is seen to result in a directly proportionate relationship with oxygen density (Fig. 11).

This could be attributed equally to the effect of magnesium as a modifier, the higher polarising power due to the charge-to-size ratio of Mg, binding to the NBOs more closely, or magnesium taking part in the silicate network, with the resultant loss of NBOs. It is important to note that, on entering the network, magnesium would act to weaken the network and to simultaneously decrease the number of NBOs and increase NC. It is argued, however, that the lower bond strengths would contribute to the oxygen density to a much smaller extent than the loss in NBOs.

According to Fig. 12 crystallisation temperature linearly increases with increasing MgO content. Noticeably at low MgO content there is a sharp pronounced crystallisation peak, which reduces in magnitude and broadens as the MgO content increases. Indeed at 100% magnesium substitution there seems to be no evidence of crystallisation.

It is suggested that magnesium suppresses crystallisation. Crystallisation of a bioactive glass is known to inhibit its bioactive

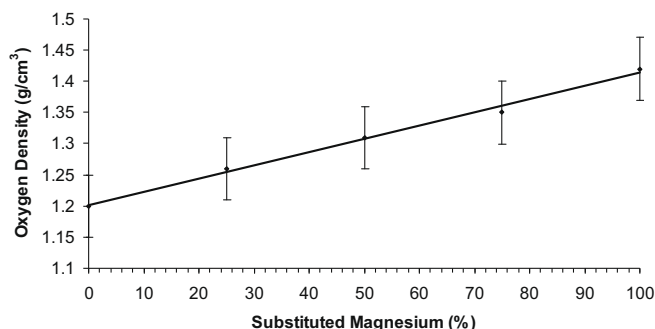


Fig. 11. Graph of oxygen density against percentage of magnesium substituted. Illustrates increasing trend in oxygen density with magnesium substitution.

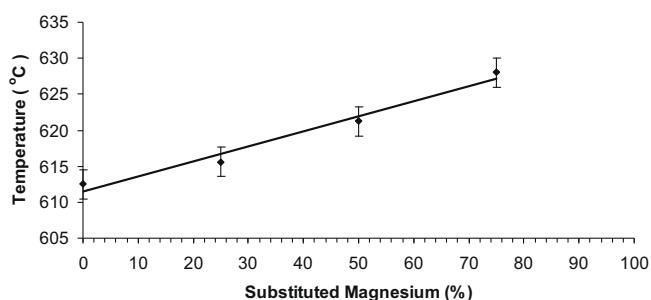


Fig. 12. Graph of crystallisation temperature (T_c) against percentage of magnesium substituted. Illustrates increasing trend in T_c with magnesium substitution.

properties due to the lower reactivity of the crystalline state and increased resistance to the ion exchange reaction [59]. Realising that magnesium acts to inhibit crystallinity is extremely important with respect to the bioactivity of the glass structure. Properties such as T_g and T_c are also critical for the engineering of bioactive glasses with a large window between T_g and T_c essential in ensuring that the glasses sinter well without crystallisation. The experimental decrease in T_g and increase in T_c with magnesium substitution resulting in a large sintering window, also noted by Brink [18] is again extremely beneficial with respect to bioactive glass design for biological implants.

4. Conclusion

Glasses were designed with a systematic substitution of CaO for MgO, on a molar basis, with constant network connectivity throughout the series.

The ^{31}P MAS NMR spectra illustrated a single symmetric resonance with a chemical shift range in the region of 8.2–12.7 ppm corresponding to the existence of an orthophosphate environment. No evidence existed for Si–O–P bonding within the bioactive glass. A downfield shift, towards a sodium orthophosphate type species, was observed on substitution of magnesium, implying preferential association of sodium ions with the phosphate phase.

The ^{29}Si MAS NMR data demonstrated the presence of a predominant Q^2 structure (–79 ppm) with a progressive shift to a Q^3 structure (–90 ppm) with increasing MgO substitution. This provided evidence for magnesia acting more as an intermediate oxide than a modifier, with a proportion entering the silicate network as tetrahedral MgO_4 species.

Analysis of the physical properties further supported this hypothesis with a decrease in T_g and T_c and increase in TEC all highlighting the move to a much weaker glass structure with the substitution of magnesium. Both density and TEC values were

observed to stray from their relative models (Doweidar and Appen, respectively) at >20 mol.% substitution implying that magnesium oxide is switching from a modifier to an intermediate through the series.

It is concluded that there is evidence that part of the magnesium oxide is acting as a network intermediate and entering the silicate network ($\approx 14\%$ in the glasses studied). It is suggested that MgO can only switch to the role of an intermediate oxide in highly disrupted silicate glasses, of which bioactive glasses are an example – this hypothesis, however warrants further investigation. The effect on the bioactive behaviour is a necessary extension to this work and in the case of this series of glasses is currently under investigation. It is suggested that the intermediate nature of MgO, and its polymerising effect, would ultimately act to decrease the resultant bioactivity. However, it was also discovered that magnesium acts to aid the sintering window or ‘working range’ ($T_x - T_g$) and suppress crystallisation, both very beneficial properties with respect to bioactivity.

Although magnesium is an element often found within bioactive glasses there is little understanding of the composition–structure–property relationships within such compositions. This study has enabled us to gain an insight into such relationships, however further investigation and clarification on its structural behaviour and resultant effect on bioactivity is essential.

Acknowledgements

The authors would like to thank the EPSRC (Grant No. EP/C549309/1) for S.J.W. and M.O.O'D for providing the funding for this work.

References

- [1] L.L. Hench, R.J. Splinter, W.C. Allen, T.K. Greenlee Jr., J. Biomed. Mater. Res. 2 (1971) 117.
- [2] L.L. Hench, J. Am. Ceram. Soc. 74 (1991) 1487.
- [3] M.W.G. Lockyer, D. Holland, R. Dupree, J. Non-Cryst. Solids 188 (1995) 207.
- [4] J.B. Murdoch, J.F. Stebbins, I.S.E. Carmichael, Am. Mineral. 70 (1985) 332.
- [5] I. Elgayar, A.E. Aliev, A.R. Boccaccini, R.G. Hill, J. Non-Cryst. Solids 351 (2005) 173.
- [6] L.L. Hench, T.K. Greenlee, R.J. Splinter, W.C. Allen, J. Biomed. Mater. Res. Symp. 2 (2) (1971) 117.
- [7] L.L. Hench, H.A. Paschall, J. Biomed. Mater. Res. 7 (1973) 25.
- [8] T. Kokubo, H. Kushitani, C. Ohtsuki, S. Sakka, T. Yamamuro, J. Mater. Sci. – Mater. Med. 3 (1992) 79.
- [9] O.H. Andersson, K.H. Karlsson, K. Kangasniemi, J. Non-Cryst. Solids 119 (1990) 290.
- [10] P. Li, Q. Yang, F. Zhang, T. Kokubo, J. Mater. Sci. – Mater. Med. 3 (1992) 452.
- [11] R.G. Hill, J. Mater. Sci. Lett. 15 (1996) 1122.
- [12] K. Wallace, PhD Thesis: Design of Bioactive Glass Compositions, University of Limerick, 2000.
- [13] Z. Strnad, Biomaterials 13 (1992) 317.
- [14] R.D. Rawlings, J. Mater. Sci. Lett. 11 (1992) 1340.
- [15] L. Holliday, Inorganic Macromol. Rev. 1 (1970) 3.
- [16] N.H. Ray, Inorganic Polymers, Academic Press, London, 1978.
- [17] K. Wallace, Design of Bioactive Glass Compositions, University of Limerick, 2000.
- [18] M. Brink, J. Biomed. Mater. Res. 36 (1997) 109.
- [19] M. Brink, T. Turunen, R.P. Happonen, A. YliUrho, J. Biomed. Mater. Res. 37 (1997) 114.
- [20] J.M. Gomez-Vega, E. Saiz, A.P. Tomsia, J. Biomed. Mater. Res. 46 (1999) 549.
- [21] E. Saiz, M. Goldman, J.M. Gomez-Vega, A.P. Tomsia, G.W. Marshall, S.J. Marshall, Biomaterials 23 (2002) 3749.
- [22] J.M. Oliveira, R.N. Correia, M.H. Fernandes, J. Non-Cryst. Solids 273 (2000) 59.
- [23] J.M. Oliveira, R.N. Correia, M.H. Fernandes, Biomaterials 23 (2002) 371.
- [24] J.M. Oliveira, R.N. Correia, M.H. Fernandez, J. Rocha, J. Non-Cryst. Solids 265 (2000) 221.
- [25] K. Ohura, T. Nakamura, T. Yamamuro, Y. Ebisawa, T. Kokubo, Y. Kotoura, M. Oka, J. Mater. Sci. – Mater. Med. 3 (1992) 95.
- [26] K. Ohura, T. Nakamura, T. Yamamuro, Y. Ebisawa, T. Kokubo, Y. Kotoura, M. Oka, J. Mater. Sci. – Mater. Med. 3 (1992) 95.
- [27] T. Kasuga, K. Nakagawa, M. Yoshida, E. Miyade, J. Mater. Sci. 22 (1987) 3721.
- [28] J.S. Moya, A.P. Tomsia, A. Pazo, C. Santos, F. Guitian, J. Mater. Sci. – Mater. Med. 5 (1994) 529.
- [29] A.J. Salinas, J. Roman, M. Vallet-Regi, J.M. Oliveira, R.N. Correia, M.H. Fernandes, Biomaterials 21 (2000) 251.

- [30] P.W. McMillan, *Glass Ceramics*, Academic Press, London, 1979.
- [31] P.S. Fiske, J.F. Stebbins, *Am. Mineral.* 79 (1994) 848.
- [32] S. Kohara, K. Suzuya, K. Takeuchi, C.K. Loong, M. Grimsditch, J.K.R. Weber, J.A. Tangeman, T.S. Key, *Science* 303 (2004) 1649.
- [33] M.A. Karakassides, A. Saranti, I. Koutselas, *J. Non-Cryst. Solids* 347 (2004) 69.
- [34] A. Dietzel, *Elektrochem.* 48 (1942) 9.
- [35] K. Shimoda, Y. Tobu, M. Hatakeyama, T. Nemoto, K. Saito, *Am. Mineral.* 92 (2007) 695.
- [36] H. Doweidar, *J. Non-Cryst. Solids* 249 (1999) 194.
- [37] A.A. Spasov, *Bull. Exp. Biol. Med.* 131 (2001) 132.
- [38] J.E. Sojka, C.M. Weaver, *Nutr. Rev.* 53 (1995) 71.
- [39] A.M. George, J.F. Stebbins, *Am. Mineral.* 83 (1998) 1022.
- [40] S. Kroeker, J.F. Stebbins, *Am. Mineral.* 85 (2000) 1459.
- [41] R. Dupree, M.E. Smith, *J. Chem. Soc.-Chem. Commun.* (1988) 1483.
- [42] R. Ahsan, M.G. Mortuza, *J. Non-Cryst. Solids* 351 (2005) 2333.
- [43] R. Dupree, D. Holland, M.G. Mortuza, *Nature* 328 (1987) 416.
- [44] I.L. Mudrakovskii, V.P. Shmachkova, N.S. Kotsarenko, V.M. Mastikhin, *J. Phys. Chem. Solids* 47 (1986) 335.
- [45] G.L. Turner, K.A. Smith, R.J. Kirkpatrick, E. Oldfield, *J. Magn. Reson.* 70 (1986) 408.
- [46] M.A. Aramendia, V. Borau, C. Jimenez, J.M. Marinas, F.J. Romero, J.R. Ruiz, *J. Sol. State Chem.* 135 (1998) 96.
- [47] S.N. Scrimgeour, J.A. Chudek, G.A. Cowper, C.H. Lloyd, *Dent. Mater.* 23 (2007) 934.
- [48] P.C.D. Zhang, P. Floriana, P.J. Grandinetti, J.F. Stebbins, *J. Non-Cryst. Solids* 204 (1996) 294.
- [49] P. Zhang, P.J. Grandinetti, J.F. Stebbins, *J. Phys. Chem. B* 101 (20) (1997) 4004.
- [50] R. Dupree, *Glasses* 29 (1) (1988) 18.
- [51] A.R. Grimmer, M. Magi, M. Hahnert, H. Stade, A. Samoson, W. Wieker, E. Lippmaa, *Phys. Chem. Glasses* 25 (1984) 105.
- [52] P.G. Galliano, J.M.P. Lopez, E.L. Varetto, I. Sobrados, J. Sanz, *Mater. Res. Bull.* 29 (1994) 1297.
- [53] E. Dowty, *Phys. Chem. Miner.* 14 (1987) 122.
- [54] J.A. Kerr, *CRC Handbook of Chemistry and Physics*, CRC Press, Florida, 2000.
- [55] L. Linati, G. Lusvardi, G. Malavasi, L. Menabue, M.C. Menziani, P. Mustarelli, U. Segre, *J. Phys. Chem. B* 109 (2005) 4989.
- [56] R.D. Shannon, *Act. Cryst.* A32 (1976) 751.
- [57] H. Doweidar, *Phys. Chem. Glasses* 40 (1999) 85.
- [58] P.G. Galliano, A.L. Cavalier, *J. Non-Cryst. Solids* (1995) 311.
- [59] K.E. Wallace, R.G. Hill, J.T. Pembroke, C.J. Brown, P.V. Hatton, *J. Mater. Sci. – Mater. Med.* 10 (1999) 697.

The role of Sr^{2+} on the structure and reactivity of SrO-CaO-ZnO-SiO_2 ionomer glasses

Daniel Boyd · Mark R. Towler · Sally Watts ·
Robert G. Hill · Anthony W. Wren · Owen M. Clarkin

Received: 20 June 2006 / Accepted: 29 November 2006
© Springer Science+Business Media, LLC 2007

Abstract The suitability of Glass Polyalkenoate Cements (GPCs) for use in orthopaedics is retarded by the presence in the glass phase of aluminium, a neurotoxin. Unfortunately, the aluminium ion plays an integral role in the setting process of GPCs and its absence is likely to hinder cement formation. However, the authors have previously shown that aluminium free GPCs may be formulated based on calcium zinc silicate glasses and these novel materials exhibit significant potential as hard tissue biomaterials. To further improve their potential, and given that Strontium (Sr) based drugs have had success in the treatment of osteoporosis, the authors have substituted Calcium (Ca) with Sr in the glass phase of a series of aluminium free GPCs. However to date little data exists on the effect SrO has on the structure and reactivity of SrO-CaO-ZnO-SiO_2 glasses. The objective of this work was to characterise the effect of the Ca/Sr substitution on the structure of such glasses, and evaluate the subsequent reactivity of these glasses with an aqueous solution of Polyacrylic acid (PAA). To this end ^{29}Si MAS-NMR, differential scanning calorimetry (DSC), X-ray diffraction, and network connectivity calculations, were used to characterize the

structure of four strontium calcium zinc silicate glasses. Following glass characterization, GPCs were produced from each glass using a 40 wt% solution of PAA (powder:liquid = 2:1.5). The working times and setting times of the GPCs were recorded as per International standard ISO9917. The results acquired as part of this research indicate that the substitution of Ca for Sr in the glasses examined did not appear to significantly affect the structure of the glasses investigated. However it was noted that increasing the amount of Ca substituted for Sr did result in a concomitant increase in setting times, a feature that may be attributable to the higher basicity of SrO over CaO.

Introduction

Glass polyalkenoate cements (GPCs), used in dentistry for restorative purposes [1, 2], are usually formed by the reaction of a fluoro-aluminosilicate glass with an aqueous solution of polyalkenoic acid, usually polyacrylic acid (PAA). The acid attacks and degrades the glass structure, releasing metal cations into the aqueous phase of the setting cement. Once in the aqueous phase, these cations are cross-linked by the carboxylate groups on the PAA chains and form a cement with a microstructure consisting of reacted and unreacted glass particles embedded in a hydrated polysalt matrix [3, 4].

Commercial GPCs are conventionally based on aluminium glass chemistry [4]. Aluminium is present because it can isomorphically replace SiO_4 tetrahedra within the glass structure, causing local charge imbalances within the structure, thus increasing the acid degradability of the glass [5]. However, aluminium has been identified as a potent

D. Boyd (✉) · M. R. Towler · A. W. Wren ·
O. M. Clarkin
Materials & Surface Science Institute, University of Limerick,
National Technological Park, Limerick, Ireland
e-mail: Daniel.Boyd@ul.ie

S. Watts
Department of Chemistry, Imperial College, London, UK

R. G. Hill
Department of Materials, Minerals and Mining, Imperial
College, London, UK

neuro-toxin due to its ability to disrupt both cellular calcium homeostasis [6–8] and promote cellular oxidation [9]. It has been implicated in degenerative brain disorders like Parkinson's and Alzheimer's disease [10–15]. Further to these issues, the use of aluminium containing GPCs for reconstructive otoneurosurgery [16] and total hip replacement [17] has produced negative results; in one case patient death [18]. The removal of aluminium within the glass phase of a GPC is critical if such materials are to be employed for skeletal applications.

The authors have previously shown that bioactive GPCs can be formulated using glasses from the ZnO–CaO–SiO₂ ternary system, because zinc has the ability to work as both a network modifier and an intermediate oxide in a similar way to alumina [19, 20]. Far from acting as a neurotoxin like aluminium, the release of zinc from such cements is likely to have positive effects in vivo as it can increase the DNA of osteoblasts [21], resulting in increased bone mass [22]. Zinc is required for the correct functioning of the immune system, and has also been shown to impart antibacterial properties to GPCs. Zn-GPCs therefore have considerable potential as hard tissue replacement materials.

It is possible to incorporate strontium (Sr²⁺) into the glass structure on a substitutional basis for Ca²⁺, as their respective ionic radii are similar [23]. The beneficial effect of Sr²⁺ on both diseased and healthy osseous tissue is well documented. Nielsen [24] noted that strontium shares some of the same physiological pathways as calcium. For example, Sr sustains secretion of insulin as a response to glucose and is also effective in mediating cell uptake of glucose, roles usually performed by calcium.

Sr has been used in the treatment of osteoporosis for the last 40 years [25], and has numerous beneficial effects on bone [26]. Studies have shown that Sr²⁺ enhances the replication of pre-osteoblastic cells, and stimulates bone formation in cells and calvarial cultures in vitro [27]. Furthermore, clinical trials have shown that the administration of strontium ranelate enhances cell replication and bone formation in vivo [24]. Dahl et al. [26] also note that the administration of Sr to rats (as strontium ranelate or strontium chloride) is associated with increased osteoid and osteoblast surfaces, resulting in increased bone-forming sites. Consequently, strontium administration reduces the incidence of hip fractures and vertebral compression fractures in postmenopausal osteoporosis patients [28]. Further to increased bone formation, evidence has been presented to indicate that Sr also acts as an uncoupling agent in the bone remodelling cycle, apparently diminishing osteoclastic turnover, while maintaining or enhancing osteoblastic activity [26]. It is likely therefore that the release of Sr from hard tissue biomaterials will have a positive effect on bone formation. Indeed, Johal et al. [29] who examined the in vivo

response of Sr²⁺ and Zn²⁺ containing, aluminium-based GPCs showed that the cements containing a high mole fraction of Sr were the most osteoconductive of all cements. However, the effect of substituting calcium with strontium ions in ionomer glasses has not been fully investigated; in terms of both glass structure, and reactivity.

Nicholson [30] has previously shown that SrO added as dry powder to a GPC formulation resulted in decreased setting times at low levels (5 wt%), and increased compressive strengths. However, at higher levels SrO decreased compressive strengths. Unfortunately the study did not investigate SrO as a glass component—which is an imperative point for consideration.

The purpose of this research was to fully characterize a series of ionomeric glasses where SrO incrementally replaces CaO within the glass network, and then to evaluate the reactivity of these glasses when mixed with an aqueous solution of PAA.

Materials and methods

Glass preparation

Appropriate amounts of analytical grade silica, zinc oxide, strontium carbonate and calcium carbonate were weighed out and mixed in a ball mill (1 h), then dried in an oven (100 °C, 1 h). Each batch was then transferred to a mullite crucible for firing (1480 °C, 1 h). The glass melts were shock quenched into water, and the resulting frit was dried, then ground and sieved to retrieve <45 µm glass powder. Glass powders were then annealed at 650 °C for 3 h, and subsequently used for glass characterization and production of cements. Table 1 shows the chemical composition (mole fraction) of the glasses used.

Thermal characterisation of glasses

A combined differential thermal analyser-thermal gravimetric analyser (DTA-TGA, Stanton Redcroft STA 1640, Rheometric Scientific, Epsom, England) was used to measure the glass transition temperature (*T_g*) of each glass. A heating rate of 10 °C min⁻¹ (up to 1,000 °C) was used. Materials were tested in an air atmosphere with alumina as a reference in a matched platinum crucible.

Structural characterisation of glasses

Network connectivity

The network connectivity (NC) of the glasses was calculated with Eq. 1 using the molar compositions of the glass.

Table 1 Glass compositions (mole fraction)

Glass code	SrO	CaO	ZnO	SiO ₂
BT 100	0	0.16	0.36	0.48
BT 101	0.04	0.12	0.36	0.48
BT 102	0.08	0.08	0.36	0.48
BT 103	0.12	0.04	0.36	0.48

$$NC = \frac{\text{No. BOs} - \text{No. NBOs}}{\text{Total No. Bridging species}} \quad (1)$$

Where:

NC = Network Connectivity

BO = Bridging Oxygens

NBO = Non-Bridging Oxygens

X-ray diffraction

Powdered samples of each glass were pressed to form discs (32 mm Ø × 3 mm). Ethyl cellulose was used as a backing material. Samples were analysed using CuK_{α1} radiation emitted from an Xpert MPD Pro 3040/60 X-ray diffraction unit (Philips, Eindhoven, Netherlands).

Magic Angle Spinning Nuclear Magnetic Resonance (MAS-NMR) Spectroscopy

Powdered samples of each glass were examined by ²⁹Si MAS-NMR. The measurements were conducted at a resonance frequency of 39.77 MHz using an FT-NMR spectrometer (DSX-200, Bruker, Germany). The spinning rate of the samples at the magic angle was 5 kHz for ²⁹Si. The recycle time was 2.0 s. The reference material used to measure the chemical shift was tetramethylsilane.

Cement preparation and evaluation

Ciba speciality polymers (Bradford, UK) supplied PAA, coded E9, in aqueous solution (25%w/w). E9, with a number average molar mass *M_n* of 26,100 [31], was freeze-dried, ground and sieved to retrieve a <90 μm powder. Cements were prepared by thoroughly mixing the 2 g of glass powder (<45 μm) with 0.6 g PAA powder and 0.9 ml distilled water on a glass plate. This yielded a cement based on a P:L ratio of 2:1.5, and a PAA concentration of 40 wt%. Complete mixing of cements was undertaken within 30 s. Working times were defined as “the period of time, measured from the start of mixing, during which it is possible to manipulate a dental material without an adverse effect on its properties”. [32]. The net setting time was measured in accordance with ISO9917E [32].

Results & discussion

The glasses synthesized in this work were designed using network connectivity calculations, where, the determination of NC for each glass assumes (based on previous research by the authors [33]) that Zn²⁺ predominantly plays a network-modifying role in CaO–ZnO–SiO₂ glasses. The series was designed such that glasses would be structurally identical to one another (NC = 1.83), and thus facilitate investigating the effect Sr²⁺ on the rheology of resultant cements.

XRD & DTA results

X-ray diffraction patterns obtained from each glass (Fig. 1) indicate that all glasses are amorphous, with the exception of glass BT101 which exhibits a small degree of crystallinity. The diffractogram for BT101 indicates the presence of hardystonite, the only ternary compound on the relevant Ca–Zn–Si phase diagram. The phase separation of this glass is likely due to processing conditions, which vary slightly during glass production. However, irrespective of the partial crystallinity evident in Glass BT101, all glasses exhibit *T_g* values within 5 °C of each other (mean *T_g* = 674 °C; deviation from mean = 2 °C). These results indicate that the glasses in the series are structurally similar.

²⁹Si MAS-NMR results

²⁹Si MAS-NMR was performed to probe the local environment of the ²⁹Si isotope in each glass. The ²⁹Si MAS-NMR results (Fig. 2/Table 2) indicate that the glasses in the series are structurally similar, save for the decreased peak width associated with BT101, a feature attributable to

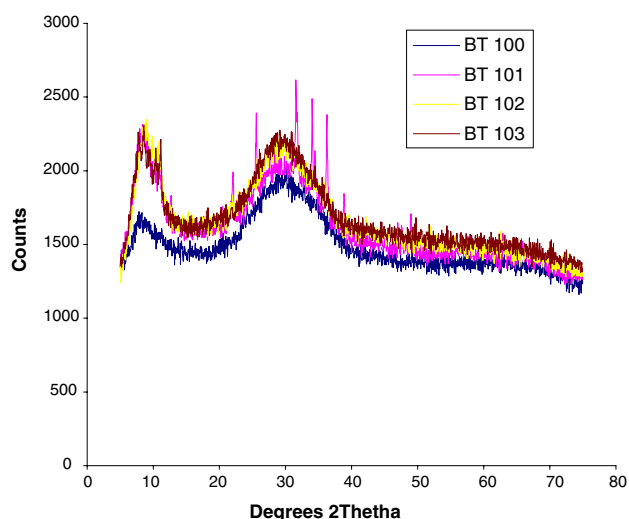


Fig. 1 X-ray diffraction patterns for glasses BT100–BT103

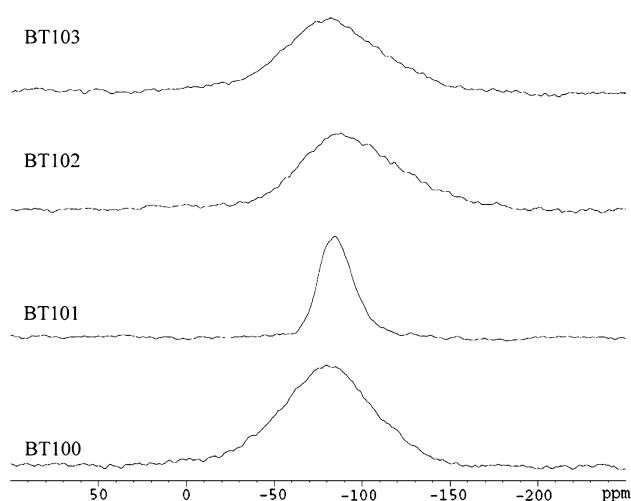


Fig. 2 ^{29}Si MAS-NMR spectra for glasses BT100–BT103

the partial crystallinity of the glass. The ^{29}Si MAS-NMR spectra for each glass (Fig. 2) exhibit a large broad peak at around -70 to -100 ppm for each glass. The chemical shift observed for silicon in a four co-ordinate state is between -60 and -100 ppm [34] indicating that the Si in each glass examined is in a tetrahedral configuration. The chemical shift however depends on the number of NBOs present in the glass. Increasing the number of NBOs moves the peak in a positive direction, whilst increasing the number of next nearest neighbour Si atoms moves the peak in a negative direction. It is accepted that Si resonance's occurring at ~ 86 ppm are associated with Q^3 species, and resonances at ~ 78 ppm are associated with Q^2 structures [35] Oliveira et al. [36] have indicated that ^{29}Si resonances between -60 and -83 ppm have also been associated with the $\text{Q}^{0/1}$ structural unit. In the literature Q^2 structures are known to resonate at -78.9 ppm [35], This shows that the substitution of Ca^{2+} with Sr^{2+} in the glass network does not affect the glass structure, presumably due to their similar ionic radii [23].

The peaks recorded for the glasses in this work are broad, a feature commonly associated with ^{29}Si MAS-NMR spectra. Lockyer et al. [37] have shown that two resonances corresponding to different Q structures can overlap and merge into one peak. Furthermore, Stamboulis et al. [38] have reported peaks widths of -80 to -120 ppm

for commercial ionomeric glasses, while Jones et al. [39] report peak widths between -80 and -110 ppm. Given the width of the peaks for each glass and the precedence for peak merging in the literature, it is assumed that this feature is the result of an assortment of Q^n structures present within each glass network.

Accepting that the ^{29}Si peaks for each glass implicate the presence of a variety of Q^n structures in the network it is fair to conclude, based on the NC calculations and the corroborating chemical shift data that Q^2 structures are pervasive in the glass network. This data is important for three reasons; in the first instance it demonstrates that all the glasses produced in this study are similar, and so a fair analysis of the effect of Sr^{2+} can be made when analysing the rheology of cements produced from each glass. In the second instance, it can be stated that the replacement of CaO with SrO in the glass formulation does not affect the resultant glass network up to additions of 0.12 mole fraction in the glasses examined. This is evinced by the fact that there is no appreciable change in the ^{29}Si chemical shift data or T_g values across the series. Finally, the data herein indicates that the Zn^{2+} ion is predominantly a network-modifying ion in CaO/SrO–ZnO– SiO_2 glasses; and as such substantiates previous reports by the authors [33], which have indicated similarly.

Cement working times and setting times

At this stage it must be emphasized that all glasses are structurally very similar, and that the only variable present is the increasing SrO:CaO ratio across the series. Figure 3 illustrates the working time and setting time of cements produced from each glass, each data point being the average of three tests.

It can be seen that the working times and net setting times of cements produced from respective glasses decreases with increasing substitution of SrO for CaO in the glass, and that this decrease is not due to increased disruption of the glass network. Therefore the decreased working/setting times must be due to some feature independent of the network structure. A possible explanation for the decreased working/setting times might be found in the decreased electronegativity of Sr when compared to Ca. Elements with lower electronegativity tend to form oxides

Table 2 Illustrates decreasing working/setting time for the glass series, irrespective of the structural similarity between the glass networks

Glass designation	Working time (s) (SD)	Net setting time (s)	NC	T_g ($^{\circ}\text{C}$)	Mean chemical shift (ppm)	Predominant Q^n Distribution
BT100	48 (2.5)	103 (2.8)	1.83	672	-80	Q^2
BT101	42 (1.1)	114 (5.29)	1.83	675	-84	Q^2
BT102	32 (0.6)	78 (6.8)	1.83	674	-81	Q^2
BT103	24 (2.6)	60 (9.81)	1.83	677	-81	Q^2

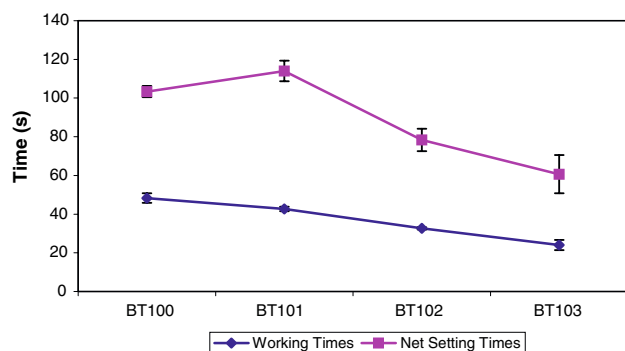


Fig. 3 Effect of SrO/CaO substitution on working times and net setting times

which are more basic than elements with higher electronegativity values. In support of this point the literature indicates that CaO is a moderate base while SrO should be considered a strong base [40]. It is accepted that the reactivity of a glass towards acid depends on its acid base properties [3], and that increased basicity of the glass increases its reactivity with the acid component of GPC formulation. It is the contention of the authors that the mechanism behind the decreased working/setting times associated with Sr substituted Ca–Zn–Si glasses is due to increased basicity of the glass network due to Sr inclusion.

Conclusions

The substitution of Sr for Ca in the CaO–ZnO–SiO₂ glasses examined did not appear to have any effect on the connectivity of the network, as illustrated by the negligible effect the substitution has on T_g 's and Q^n distributions of each glass. Furthermore, the likely reason for concomitant decreases in working/setting times with increased SrO substitution is likely attributable to increased basicity of the Sr containing glasses. Further work is under way to evaluate the effect of the Sr substitution on the mechanical and biological properties of GPCs derived from these glasses.

References

1. A. JOKSTAD and I. A. MJOR, *J. Dent.* **24**(5) (1996) 309
2. J. A. WILLIAMS and R. W. BILLINGTON, *J. Oral Rehabil.* **16**(5) (1989) 475

3. A. D. WILSON and J. W. NICHOLSON, "Acid-base cements, their biomedical and industrial applications", (University Press: Cambridge, 1993)
4. J. W. NICHOLSON, *Biomaterials* **19** (1998) 485
5. S. G. GRIFFIN and R. G. HILL, *Biomaterials* **20** (1999) 1579
6. D. JULKA and K. D. GILL, *Biochim. Biophys. Acta* **1315** (1996) 47
7. G. W. GUO and Y. X. LIANG, *Brain Res.* **888** (2001) 221
8. L. GANDOLFI et al. *Biochim. Biophys. Acta* **1406** (1998) 315
9. P. ZATTA et al. *Coord. Chem.* **228** (2002) 271
10. W. F. FORBES et al. *Exp. Gerontol.* **30**(1) (1995) 23
11. W. F. FORBES and J. F. GENTLEMAN, *Exp. Gerontol.* **33**(2) (1998) 141
12. S. POLIZZI et al. *NeuroToxicology* **23** (2002) 761
13. C. V. SWEGART et al. *Mech. Ageing. Dev.* **112** (1999) 27
14. C. EXLEY, *J. Inorg. Biochem.* **76** (1999) 133
15. P. B. MOORE et al. *Biol. Psychiatr.* **41** (1997) 488
16. G. BABIGHIAN, *J. Laryngol. Otol.* **106** (1992) 954
17. E. ENGLEBRECHT et al. *J. Bone Joint. Surg.* **82-b**(2) (2000) 192
18. E. REUSCHE et al. *Hum. Pathol.* **32**(10) (2001) 1136
19. D. BOYD and M. R. TOWLER, "A preliminary study of zinc based polyalkenoate cements for orthopaedic applications", in World Biomaterials Congress. (Sydney, Australia, 2004)
20. D. BOYD and M. R. TOWLER, *J. Mat. Sci. Mat. Med.* **6** (2004) 843
21. M. YAMAGUCHI and T. MATSUI, *Peptides* **17**(7) (1996) 1207
22. J. OVESEN et al. *Bone* **29**(6) (2001) 565
23. R. D. SHANNON, *Acta Crystallogr.* **A32** (1976) 751
24. S. PORS NIELSEN, *Bone* **35**(3) (2004) 583
25. J. CHRISTOFFERSEN et al. *Bone* **20**(1) (1997) 47
26. S. G. DAHL et al. *Bone* **28**(4) (2001) 446
27. E. CANALIS et al. *Bone* **18**(6) (1996) 517
28. P. J. MEUNIER, R. S. LORENC and I. G. SMITH, *Osteoporos. Int* **13**(supplement 13) (2002) 66
29. K. K. JOHAL et al. *J. Mat. Sci. Mat. Med.* **13**(4) (2002) 375
30. S. DEB and J. W. NICHOLSON, *J. Mat. Sci. Mat. Med.* **10**(8) (1999) 471
31. A. SULLIVAN and R. G. HILL, *J. Mat. Sci.* **35** (2000) 1125
32. ISO9917, Water based dental cements. (1994)
33. D. BOYD et al. *J. Mat. Sci. Mat. Med.* **17** (2006) 397
34. A. STAMBOULIS, R. G. HILL and R. V. LAW, *J. Non-Crystal. Sol.* **333**(1) (2004) 101
35. I. ELGAYAR et al. *J. Non-Crystal. Sol.* **351**(2) (2005) 173
36. J. M. OLIVEIRA et al. *J. Non-Crystal. Sol.* **265**(3) (2000) 221
37. M. W. G. LOCKYER, D. HOLLAND and R. DUPREE, *J. Non-Crystal. Sol.* **188**(3) (1995) 207
38. A. STAMBOULIS, R. V. LAW and R. G. HILL, *Biomaterials* **25**(17) (2004) 3907
39. A. R. JONES et al. *J. Non-Crystal. Sol.* **293–295** (2001) 87
40. J. P. BIRK, "Chemistry", 1st edition, (Houghton Mifflin Company, Boston, and Toronto, 1994)