

Thermoresponsive Gels: How do the structural parameters affect the gelation properties?

Anna Constantinou

Department of Materials
Imperial College London

Submitted in partial fulfilment of the requirements for the
degree of *Doctor of Philosophy*

Declaration of Originality

I declare that the work presented in this PhD thesis is my own, unless stated and appropriately referenced. Any contributions are clearly stated.

Copyright Declaration

The copyright of this thesis rests with the author and is made available under a Creative Commons Attribution Non-Commercial No Derivatives licence. Researchers are free to copy, distribute or transmit the thesis on the condition that they attribute it, that they do not use it for commercial purposes and that they do not alter, transform or build upon it. For any reuse or redistribution, researchers must make clear to others the licence terms of this work.

ABSTRACT

Thermoresponsive polymers are promising biomaterials in Tissue Engineering and in 3D printing. Therefore, this PhD thesis aims to synthesise polymers that form physical gels at body temperature (b.t.). Four different families of thermoresponsive polymers have been successfully synthesised via Group Transfer Polymerisation, and their properties have been investigated, with emphasis on their thermogelling behaviour.

i) In the first study, the ionic, pH- and thermoresponsive 2-(dimethylamino) ethyl methacrylate (DMAEMA) has been combined with the hydrophilic tri(ethylene glycol) methyl ether methacrylate (TEGMA), and the hydrophobic *n*-butyl methacrylate (BuMA). The architecture of diblock terpolymers has been systematically varied for the first time, while statistical and triblock terpolymers have been investigated for comparison.

ii) In the second study, penta(ethylene glycol) methyl ether methacrylate (PEGMA) has been used as the hydrophilic unit, whereas BuMA and DMAEMA have been kept the same as in the first study. Thermoresponsive tetrablock terpolymers of varying architectures and compositions have been systematically investigated for the first time.

iii) The third study is focused on investigating polymers containing a non-ionic thermoresponsive unit, namely di(ethylene glycol) methyl ether methacrylate (DEGMA, C unit). ABC triblock terpolymers of various compositions have been synthesised, with A and B blocks being based on PEGMA and BuMA, respectively.

iv) The fourth study focuses on mimicking Pluronic® F127, which is the commercially-available thermoresponsive polymer. ABA triblock bipolymers with different molar mass (MM) and composition have been produced (A is PEGMA, an B is the *in-house* synthesised hydrophobic monomer di(propylene glycol) methyl ether methacrylate (diPGMA)).

In conclusion, the following structural parameters have been identified as crucial for the thermogelling properties: i) architecture, ii) composition, iii) MM and iv) as expected, the chemistry. The best architecture is the ABC triblock architecture with the hydrophobic monomer as the B block, while the most promising chemistry seems to be the PEGMA-BuMA-DEGMA series.

ACKNOWLEDGMENTS

Firstly, I would like to offer my special thanks to my supervisor, Dr Theoni K. Georgiou. Thank you for the guidance and believing in me throughout this journey; for the mentoring in training and sharing your expertise in GTP and polymer science in general. Also, for the valuable advice you freely gave me, and the opportunities to work and supervise students. It has been a great pleasure to share my knowledge on polymers with them, as I also learned and understood my project better through their questions; supervising students was definitely a part of my PhD I absolutely loved!

I would also like to thank the Department of Materials at Imperial College London for funding my PhD studies.

Special thanks to Prof Costas S. Patrickios for introducing me to the world of polymer science, and Mrs Koulla S. Hellstrom, whose love and passion for chemistry has been decisive for my future.

I would like to thank Theoni's polymer group for all their help and the beautiful moments we shared and will share in the lab. I would also like to especially thank my colleague and friend Dean Carroll, with whom I started this journey. Thank you for supporting me for the last three years, for the long conversations on English language and pronunciation (e.g. stirrer bar), cooking lessons, and for bringing me the so-called "rulers for visual tests", which played an important role for my PhD. And many thanks for all your support in and outside the lab and for all the hours you spent taking care of the GPC machine – you are a GPC expert now! Also, thanks to Xinmo Zhang for this memorable quote "inner peace".

Also, thanks to Natan (Jonathan Ngiam, also known as John Natan, Johnathan etc.), famous for the lizard pose, for sending me thousands of entertaining dog videos, and for offering me free classes of abs-exercise. Thank you for coming to Cyprus and the great company, it was a great week during which I visited Cyprus like I never did before!

I would also like to thank my best friend, whom I met during this PhD journey, who has been with me through thick and thin and through the happy and not so happy moments. Thank you bebişim (Birsen Somuncuoğlu) for being a great sister by heart, for your never-ending support, for the unforgettable moments that we shared in the lab listening to Spanish music while polymerising, and once upon a time for the intense moments we spent in the gym, for the

limitless hours of laughter and craziness, until our bellies hurt (the laughs of the “Cypriot ladies” are famous in the RSM building, and of course loud!). I would also like to thank her for driving me to the monastery of Apostolos Andreas!

Special thanks to my partner, David (Louis Maxime Poussin), for his love, support and patience, especially during the last months. I would also like to thank him for believing in me when I did not and for encouraging me to continue. Thank you for respecting my “crazy hours” of work and my late lunch breaks, for waiting for me to finish my experiments, which were often late, for learning how to make the famous “frappé”, for accepting my “unbalanced” mood of which I am proud, and for doing your best to see me always smiley. Also, a big thank you (I promised) for helping me without hesitation when I sought advice on how to communicate my work (figures, colours, presentations – you know).

At this point I cannot continue without quoting what Mr. Brown said during the movie *Paddington*: “It doesn’t matter that he (Paddington) comes from the other side of the world (Peru), that he is a different species (bear), or that he has a worrying marmalade habit, we love Paddington, and that makes him family. And families stick together”.¹ It doesn’t matter that we come from different places, that we don’t speak the same mother language, you are family, and you are the reason I am glad to have come abroad!

Also, I would like to thank my best friends, Andria Hadjithekli (koumera), and Elena Demetriou (omorfi), whom I’ve known a long time and with whom I can talk for hours every time we meet regardless of how long we’ve been apart. Our friendship will last a lifetime, I am sure. I also wish you that you will finish your PhD soon; I know it is hard, but I also know that you can do it! My friend Nikolas Panayiotou for the long conversations on Skype about PhD life, and my friends Alexios Papadopoulos (Alexei) and Foivia Konstantinou for the unique moments at Androulla’s palace (and not only) with “Gargamel-Δρακουμέλ”. Many thanks for bringing me to hospital when needed, even though it was midnight.

Last but not least, I would like to thank the most important people in my life: my family. The best parents in the world, Christalla and Pericles, for being such great supporters and for doing all they could do to see me and my siblings happy and everything they could to offer us a better life. Thank you for all these years of work and hardship, for prioritising our lives over yours. Thank you for everything! Mum, you are the best example of being a mother, and one day I wish I can be as good mother as you were to me, even though I know it is difficult! Thanks to my big sister Vasoula, who treats me as a second mother, my nieces Christalla, and

Constantina, my nephew Dimitris, and my brother-in-law Pantelis, for all the gifts, and all the pictures and videos they sent me throughout the years, to share with me precious family moments that I could not enjoy. I also thank them for all the times that they helped me organise a surprise-return to my parents. Thanks to my idol and brother Panayiotis (Nios) and my sister-in-law Tanja, for loving me, advising me, and for welcoming me in Berlin and Frankfurt Oder. I wish that you will enjoy your bike-trip and that you will arrive in Cyprus soon. Thanks to my dog Blanca for her unconditional love. Thanks to my grandmother Anna, who tells me every time we speak, “I hope you come back before I die” – I am coming grandma! Wait for me! Thanks to the best aunt Androulla, my cousins Anna, Dimitris, and Maria. Thanks to my cousin Loukia, who supported me every single day, especially the last months. Loukia and Kyriacos, I am sorry that because of my PhD duties I was unable to be present in your wedding and share your happiness, but I will be there for our Eirinaios’ birth! I miss all of you (and the family members that I did not mention separately) and I love you! I promise I am coming soon! And for those who can, you are more than welcome to attend my graduation! I am waiting for you! (Grandma, get ready to visit London again.)

My apologies for the following text in Greek, but as some of my family members cannot read in English, I am translating it for them: “Τέλος, θα ήθελα να ευχαριστήσω τους σημαντικότερους ανθρώπους στη ζωή μου, την οικογένειά μου. Τους καλύτερους γονείς στον κόσμο, Χρυστάλλα και Περικλή, για τη θερμή υποστήριξή τους, και που έκαναν πάντα ό,τι καλύτερο μπορούσαν για να μας κάνουν ευτυχισμένους. Ευχαριστώ για όλα τα χρόνια που δουλέψατε σκληρά και που είχατε τη δική μας ζωή σαν προτεραιότητα. Ευχαριστώ για όλα. Μάμα μου, είσαι πρότυπο μητέρας, κι ελπίζω μια μέρα να μπορέσω να γίνω τόσο καλή μητέρα όσο εσύ (Είναι δύσκολο όμως!); Ευχαριστώ τη μεγάλη μου αδερφή Βασούλα, που μου συμπεριφέρεται σαν δεύτερη μητέρα, τις αδερφότεκνες μου Χρυστάλλα και Κωνσταντίνα, τον αδερφότεκνό μου Δημήτρη και τον γαμπρό μου Παντελή, για όλα τα δώρα και όλες τις φωτογραφίες και βίντεο που μου έστειλαν τα τελευταία χρόνια, θέλοντας να μοιραστούν μαζί μου οικογενειακές στιγμές που δε μπορούσα να ζήσω. Ακόμη, σας ευχαριστώ για όλες τις φορές που με βοηθήσατε να ετοιμάσω έκπληξη στους γονείς μας. Ευχαριστώ το πρότυπό μου και αδερφό μου Παναγιώτη (Νίο) και την νύφη μου Tanja για την αγάπη που μου προσφέρουν και για τη θερμή φιλοξενία στο Βερολίνο και στη Φρανκφούρτη Oder. Σας εύχομαι να περνάτε καλά στο ταξίδι σας με το ποδήλατο και να φτάσετε στην Κύπρο σύντομα. Ευχαριστώ τη σκυλίτσα μου Blanca για την ανιδιοτελή αγάπη της. Ευχαριστώ τη γιαγιάκα μου Άννα, που κάθε φορά που της μιλώ μου λέει «Ελπίζω να ρθεις πίσω να σε δω πριν πεθάνω» - Έρχομαι

γιαγιά μου! Περίμενέ με! Ευχαριστώ τη θεία μου Αντρούλλα (καλύτερη θεία), τα ξαδέρφια μου Άννα, Δημήτρη και Μαρία. Επίσης, ευχαριστώ την ξαδέρφη μου Λουκία, που με υποστήριζε καθημερινώς, ειδικά τους τελευταίους μήνες. Λουκία και Κυριάκο, συγγνώμη που δεν μπορούσα να παρευρεθώ στο γάμο σας και να μοιραστώ τη χαρά σας λόγω του διδακτορικού μου. Θα είμαι κοντά σας για τη γέννηση του Ειρηναίου μας! Σας πεθύμησα όλους (συμπεριλαμβανομένου των μελών της οικογένειάς μου που δεν ανέφερα ξεχωριστά) και σας αγαπώ! Υπόσχομαι ότι θα έρθω σύντομα! Για όσους μπορούν, είστε ευπρόσδεκτοι να έρθετε στην αποφοίτησή μου! Σας περιμένω (Γιαγιά, ετοιμάσου να ρθεις και πάλι στο Λονδίνο.)!”

I feel blessed to have you all in my life!

PUBLICATIONS AND PRESENTATIONS

During this PhD project, the following publications and presentations have been realised.

Publications related to the PhD project:

a. Peer-reviewed Journal Publications:

- The work of Chapter 5.1, excluding Section 5.1.3.5 Gel Points by Rheology, has been already published and can be found in: “<<http://www.mdpi.com/2073-4360/9/1/31>>, Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. A Comprehensive Systematic Study on Thermoresponsive Gels: Beyond the Common Architectures of Linear Terpolymers. *Polymers* **2017**, *9*, 31.”
- Part of Chapter 5.2, (results for PB11-PB25) has been already published and can be found in: “<<https://pubs.acs.org/doi/10.1021/acs.macromol.8b01251>>, Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. Thermoresponsive Tetrablock Terpolymers: Effect of Architecture and Composition on Gelling Behavior. *Macromolecules* **2018**, *51*(18), 7019-7031”.
- Constantinou, A.P.; Georgiou, T. K. Tuning the Gelation of Thermoresponsive Gels *Eur. Polym. J.* **2016**, *78*, 366-375.

b. Bookchapters:

Constantinou, A.P.; Georgiou, T.K. “Thermoresponsive Multi-Block Copolymers” In *Temperature Responsive Polymers: Chemistry Properties and Applications* Khutoryanskiy, V.; Georgiou T.K. John Wiley & Sons, Ltd, 2018.

c. Patents:

- Constantinou, A.P. Georgiou, T.K. Great Britain Patent, P119751GP, *filed 24th of September 2018*

Oral Presentations.

- **Constantinou, A.P.**; Ward, M.A.; Georgiou, T.K. “Thermoresponsive Triblock Copolymers as Injectable Gels” Annual Conference and Expo on Biomaterials, London, UK, 14th to 16th of March 2016.

- **Constantinou, A.P.**; Carroll, D.; Ward, M.A.; Ghasdian, N.G.; Georgiou, T.K. “Triblock and Multiblock Copolymers via Group Transfer Polymerisation” World Congress on Living Polymerizations and Polymers (LPP16), Budapest, Hungary, May-June, 2016.
- Georgiou, T.K.; Lu, B; Tarn, M.D.; Pamme, N.; Ghasdian, N.; Church, E.; Cottam, A.P.; Hornsby, K.; Leung, M.; Ward, M.A.; Taechamahaphant, A.; Carroll, D.; **Constantinou, A.P.** “Amphiphilic Microgels and Macrogels”, IUPAC-sponsored 16th International Conference “Polymers and Organic Chemistry” (POC-16), Heraklion, Crete, Greece, June 2016.
- Georgiou, T.K.; Lu B; Tarn, M.D.; Pamme, N.; Ghasdian, N.; Ward, M.A.; Taechamahaphant, A.; Carroll, D.; **Constantinou, A.P.** “Amphiphilic Microgels and Macrogels”, Warwick Polymer Conference, Warwick, UK, July 2016.
- **Constantinou, A.P.**; Carroll, D. Ward, M.A.; Ghasdian, N.; Lu, B.; Tarn, M. D’ Pamme, N.; Georgiou T.K. “Stimuli Responsive Polymeric Materials” KAUST Research Conference: Polymers-Designing Macromolecules for Applications, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia, 5th - 7th February 2017.
- **Constantinou, A.P.**; Carroll, D.; Georgiou, T.K. “Complex and Multi-Block Copolymers via Group Transfer Polymerisation” European Polymer Congress, EPFlyon2017, Lyon, France 2nd to 7th of July 2017.
- **Constantinou, A.P.**; Zhao, H.; McGilvery C.M.; Porter A.E.; Georgiou, T.K. “Thermoresponsive Gels: How do the structural parameters affect the thermoresponsive properties?” Materials Postgraduate Research Day, Department of Materials, Imperial College London, London, UK, March 2018. *First prize awarded to the postgraduate student in the Department of Materials for the best scientific content of a lecture.*
- **Constantinou, A.P.**; Georgiou, T.K. “Thermoresponsive tetrablock terpolymers: How does the structure affect the properties?”, Young Researchers Meeting – Macrogrouop UK, Royal College of Surgeons Ireland (RCSI), Dublin, Ireland, July 2018.

Poster Presentations

- **Constantinou, A.P.**; Georgiou, T.K. “Thermoresponsive Gels Based on Multiblock Copolymers”, Warwick Polymer Conference, Warwick, UK, July 2016.
- **Constantinou, A.P.**; Zhao, H.; McGilvery C.M.; Porter A.E.; Georgiou, T.K. “Thermoresponsive Gels: Beyond the common architectures of linear terpolymers”,

Materials Postgraduate Research Day, Department of Materials, Imperial College London, London, UK, March 2017. *First prize awarded to the postgraduate student in the Department of Materials for the best poster design and layout.*

- **Constantinou, A.P.**; Zhao, H.; McGilvery C.M.; Porter A.E.; Georgiou, T.K. “Thermoresponsive Gels: Beyond the common architectures of linear terpolymers”, London Polymer Group 3rd Annual Meeting, Imperial College London, London, UK, April 2017.

TABLE OF CONTENTS

	Page
Declaration of Originality	3
Copyright Declaration	4
Abstract	5
Acknowledgments	7
Publications and Presentations	11
Table of Contents	15
List of Figures	19
List of Tables	29
List of Abbreviations	31
Chapter 1: Introduction	37
1.1 Thermoresponsive Polymers	37
1.2 Thermoresponsive Gels	39
1.3 Living-Controlled Polymerisation Methods	40
1.3.1 Group Transfer Polymerisation (GTP)	41
1.4 Polymer Characterisation Techniques	45
1.4.1 Gel Permeation Chromatography (GPC)	45
1.4.2 Nuclear Magnetic Resonance Spectroscopy (NMR)	46
1.4.3 Dynamic Light Scattering (DLS)	47
1.4.4 Rheology	47
1.5 References	50
Chapter 2: Applications of Thermoresponsive Gels	55
2.1 Injectable Gels	56
2.1.1 Pluronic based	62

2.1.2 Other Degradable EG based besides Poloxamer	64
2.1.3 NIPAAm based	68
2.1.4 Poly(organophosphazene)s	71
2.1.5 Summary	72
2.2 3D (bio-)printing	73
2.2.1 3D printing of Thermoresponsive Gels (TRGs) at room temperature (r.t.)	76
2.2.2 3D printing of Thermoresponsive Gels (TRGs) at body temperature (b.t.)	78
2.2.3 Conclusion	79
2.3 References	81
 Chapter 3: Aim of this Thesis	 89
References	91
 Chapter 4: Experimental	 93
4.1 Materials and Methods	93
4.2 Catalyst Synthesis	94
4.3 Monomer Synthesis	95
4.4 Monomer Characterisation	95
4.4.1 Gas Chromatography – Flame Ionisation Detection (GC-FID)	95
4.4.2 Fourier-Transform Infrared (FT-IR) Spectroscopy	96
4.4.3 Nuclear Magnetic Resonance (NMR) Spectroscopy	96
4.4.4 Matrix Assisted Laser Desorption/Ionisation- Time of Flight Mass Spectrometry (MALDI-TOF MS)	96
4.5 Polymers Synthesis and Recovery	96
4.5.1 Block Copolymer Synthesis	97
4.5.2 Statistical Copolymer Synthesis	97
4.5.3 Polymer Recovery	98
4.6 Polymer Characterisation	98
4.6.1 Characterisation in Organic Solvents	98
4.6.1.1 Gel Permeation Chromatography (GPC)	98
4.6.1.2 Proton Nuclear Magnetic Resonance (^1H -NMR) Spectroscopy	99
4.6.2 Characterisation in Aqueous Solutions	99
4.6.2.1 Dynamic Light Scattering (DLS)	100
4.6.2.2 Potentiometric Titration	102

4.6.2.3 Visual Observation of Thermoresponsiveness	102
4.6.2.3.1 Cloud Point (CP)	102
4.6.2.3.2 Visual Gel Point	103
4.6.2.4 Rheological Measurements	104
4.7 References	105
 Chapter 5: Results and Discussion	 107
5.1 Architecture Effect of Novel Thermoresponsive Terpolymers	107
5.1.1 Polymer Synthesis	108
5.1.2 Molecular Mass and Composition	110
5.1.3 Aqueous Solution Properties	114
5.1.3.1 Hydrodynamic Diameters	114
5.1.3.2 Effective pK_{as}	118
5.1.3.3 Cloud Points	119
5.1.3.4 Visual Gel Points	120
5.1.3.5 Gel Points by Rheology	124
5.1.4 Conclusions	125
5.1.5 References	127
5.2 Thermoresponsive Tetrablock Terpolymers	129
5.2.1 Polymer Synthesis	131
5.2.2 Molecular Mass and Composition	132
5.2.3 Aqueous Solution Properties	138
5.2.3.1 Hydrodynamic Diameters	138
5.2.3.2 Effective pK_{as}	144
5.2.3.3 Cloud Points	146
5.2.3.4 Visual Gel Points	148
5.2.3.5 Gel Points by Rheology	153
5.2.4 Conclusions	155
5.2.5 References	158
5.3 DEGMA-Based Thermoresponsive Terpolymers	159
5.3.1 Polymer Synthesis	161
5.3.2 Molecular Mass and Composition	162
5.3.3 Aqueous Solution Properties	166
5.3.3.1 Hydrodynamic Diameters	166

5.3.3.2 Cloud Points	169
5.3.3.3 Visual Gel Points	171
5.3.3.4 Gel Points by Rheology	176
5.3.4 Conclusions	178
5.3.5 References	180
5.4 Mimicking Pluronic® F127	183
5.4.1 Monomer Synthesis	184
5.4.2 Monomer Characterisation	185
5.4.2.1 Determination of Isomers	186
5.4.2.2 Chemical Structure of the Monomer	188
5.4.2.2.1 Chemical Bonds	188
5.4.2.2.2 Chemical Structure	190
5.4.3 Polymer Synthesis	193
5.4.4 Molecular Mass and Composition	194
5.4.5 Aqueous Solution Properties	198
5.4.5.1 Hydrodynamic Diameters	198
5.4.5.2 Cloud Points	201
5.4.5.3 Visual Gel Points	202
5.4.5.4 Gel Points by Rheology	205
5.4.6 Conclusions	206
5.4.7 References	207
 Chapter 6: Conclusion and Future Work	 209
6.1 Conclusion	209
6.2 Future Work	211
 Appendix	 213

LIST OF FIGURES

Figure 1.1: Phase diagrams of thermoresponsive polymer solutions which show: (a) upper critical solution temperature (UCST), and (b) lower critical solution temperature (LCST). The corresponding UCST and LCST values are indicated by red arrow.

Page 37

Figure 1.2: Chemical structures, names, and abbreviations of the most popular thermoresponsive units with LCST.

Page 38

Figure 1.3: Schematic diagram showing the rheological and visual determination of the gel point. The pictures show the transition from a solution phase (left) to the gel phase (right) as the temperature increases. The solid and dashed black lines represent the changes in the shear storage modulus (elastic modulus, G') and shear loss modulus (viscous modulus, G''), respectively. The red arrow corresponds to the gel point determined by rheology, which is determined as the point at which the storage modulus exceeds the loss modulus.

Page 40

Figure 1.4: “Associative” mechanism of GTP. The catalyst (light blue circle) and the initiator (MTS, shown in red square) interact to form a pentacoordinate intermediate group (shown in blue), with which the monomer (shown in green square) interacts. The trimethylsilyl group is transferred to the end of the polymer chain, thus the technique is called “Group Transfer Polymerisation”.

Page 44

Figure 1.5: “Dissociative” mechanism of GTP. The exchange of the silyl group between the catalyst (TBABB, in light blue square) and the initiator (MTS, in red square) take place, and thus an enolate group (in blue square) is formed. The enolate group then interacts with the incoming monomers (in green square) to form the polymer chain.

Page 44

Figure 1.6: Schematic representation of the apparatus for gel permeation chromatography (GPC): (a) solvent reservoir, (b) pump (c) injection point, (d) precolumn, (e) separation column, (f) detector, (g) computer, and (h) waste reservoir. The more recent GPC apparatus are equipped with an autosampler (i).

Page 46

Figure 1.7: (a) a schematic illustration of the rheometer apparatus: a top plate, which rotates, applies a shear stress or shear strain to the sample, while the bottom plate records the response of the sample. The top plate shown is of parallel geometry, however a cone geometry is also available. (b) the expected rheology graph of an LCST thermoresponsive polymer solution

which forms gel. The changes in shear storage modulus (shear elastic modulus, G'), and the shear loss modulus (shear viscous modulus, G'') over a range of temperatures are shown in dark blue solid and red dashed lines, respectively. The gelation point (T_{gel}) is defined as the temperature at which $G' = G''$. Below and above the T_{gel} , a viscous liquid behaviour ($G' < G''$) and an elastic gel behaviour ($G' > G''$) are dominant, respectively.

Page 48

Figure 2.1: The application of the thermoresponsive gels (TRGs) as (a) injectable gels and (b) 3-D printable materials. The sol-phase facilitates the loading of a thermoresponsive polymer solution into a syringe ($T < T_{\text{gel}}$, left) and gelation occurs because of the temperature increase above the gelation point ($T > T_{\text{gel}}$, right).

Page 55

Figure 2.2: The names, the chemical structures, and the abbreviations of the most common thermoresponsive components applied *in vivo* (top), the monomers typically incorporated into the structure to introduce degradability (middle), and the commercially available thermoresponsive polymer (bottom).

Page 58

Figure 2.3: Graph showing the number of publications related to 3D printing of thermoresponsive gels over the years.

Page 73

Figure 4.1: Theoretical self-assembly of (a) an AB diblock bipolymer into core-shell micelles and (b) a BACB tetrablock terpolymer into flower-like micelles. The derivation of the Equations 4.3 and 4.10 is schematically illustrated.

Page 102

Figure 4.2: Pictures which show the different transitions, which might be observed during the visual tests: (a) runny solution phase (transparent, slightly cloudy, cloudy from the left to the right, respectively), (b) viscous solution phase (transparent: left, and cloudy: right), (c) gel phase (transparent: left, and cloudy: right), and (d) two-phased system (syneresis: left, and precipitation: right).

Page 103

Figure 5.1.1: Chemical structures and names, of the monomers used for the GTP of this polymer series. Their abbreviations are also given in brackets: tri(ethylene glycol) methyl ether methacrylate (TEGMA), *n*-butyl methacrylate (BuMA), and 2-(dimethylamino) ethyl methacrylate (DMAEMA).

Page 108

Figure 5.1.2: Chemical reactions of (a) the sequential GTP of an ABC triblock terpolymer (b) the synthesis of a statistical terpolymer by the simultaneous addition of the A, B, C monomers

after the addition of the initiator. In both cases, the A, B, and C correspond to TEGMA, BuMA, and DMAEMA, respectively.

Page 109

Figure 5.1.3: Schematic representation of the triblock (PA1-PA3), diblock (PA4-PA6), and statistical (PA7) terpolymers, and the diblock bipolymers (PA8 and PA9). The TEGMA, BuMA, and DMAEMA units are shown in blue, red, and light green spheres, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

Page 110

Figure 5.1.4: GPC chromatograms of the triblock terpolymer PA1 with theoretical polymer structure TEGMA₉-*b*-BuMA₂₀-*b*-DMAEMA₂₁ (light green dotted line), and its linear precursors: i) TEGMA₉ in blue solid line, and ii) the diblock copolymer TEGMA₉-*b*-BuMA₂₀ in red dotted line, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

Page 110

Figure 5.1.5: ¹H NMR spectra of (a) the TEGMA₉ homopolymer in blue, (b) the TEGMA₉-*b*-BuMA₂₀ diblock copolymer in red, and (c) the TEGMA₉-*b*-BuMA₂₀-*b*-DMAEMA₂₁ triblock terpolymer (Polymer A1) in light green. The corresponding chemical structures are also presented, with the TEGMA, BuMA, and DMAEMA units to be coloured in blue, red, and light green, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

Page 113

Figure 5.1.6: Schematic representation of the theoretical self-assembled structures assumed to be adopted by the copolymers. Spherical micelles are formed by: i) the triblock terpolymers (PA1-PA3), ii) the diblock terpolymers (PA4-PA6), and iii) the diblock bipolymers (PA8 and PA9). The statistical terpolymer (PA7) adopts a random coil configuration. The corresponding polymer structures are also shown. The TEGMA, BuMA, and DMAEMA repeated units are represented by blue, red, and light green spheres, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

Page 117

Figure 5.1.7: Effect of the architecture and the theoretical BuMA content on the pK_a of the DMAEMA units. The pK_a values for polymers with theoretical BuMA weight percentage of 35 w/w% and 45 w/w% are indicated by light green triangles, and red rhombus, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

Figure 5.1.8: Phase diagrams of the soluble polymers in phosphate buffered saline (PBS) and 10% protonation of the DMAEMA units: i) triblock (PA1 & PA3), diblock (PA4-PA6), and statistical (PA7) terpolymers, and ii) diblock bipolymer (PA9). Four phases are observed: i) runny solution in white (square: clear, triangle: slightly cloudy, and circle: cloudy), ii) viscous solution in red (triangle and circle for transparent and cloudy, respectively), iii) the gel phase is shown in blue (triangle and circle for transparent and cloudy, respectively), the two-phase system in light green (rhombus and square for gel syneresis and precipitation, respectively). The corresponding polymer structures are also provided; the TEGMA, BuMA, and DMAEMA units are coloured in blue, red, and light green, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

Figure 5.1.9: Rheological curves of the terpolymer solutions which form a gel visually at 15 w/w% in phosphate buffered saline (PBS), as resulted from temperature ramp measurements. The changes in the shear storage modulus (elastic modulus, G'), shear loss modulus (viscous modulus, G''), and the complex viscosity (η^*) are presented in dark blue, red, and light blue, respectively. The graphs correspond to: (a) the ABC triblock terpolymer (PA1, TEGMA₉-*b*-BuMA₂₀-*b*-DMAEMA₂₁), (b) the BAC triblock terpolymer (PA3, BuMA₂₀-*b*-TEGMA₉-*b*-DMAEMA₂₁), and (c) the B(AC) diblock terpolymer (PA6, BuMA₂₀-*b*-(TEGMA₉-*co*-DMAEMA₂₁)). The corresponding polymer structures are also shown, with blue, red, and light green spheres representing the TEGMA, BuMA, and DMAEMA repeated units, respectively.

Figure 5.2.1: Chemical structures, names, and abbreviations of the monomers used for the polymerisation, which are penta(ethylene glycol) methyl ether methacrylate (PEGMA), *n*-butyl methacrylate (BuMA), and 2-(dimethylamino) ethyl methacrylate (DMAEMA).

Figure 5.2.2: Chemical reaction of the sequential GTP of an ABCA tetrablock terpolymer; where A, B, and C are PEGMA, BuMA, and DMAEMA, respectively.

Figure 5.2.3: Schematic representation of the tetrablock (PB1-PB9, PB11-PB19, and PB21-PB24), triblock (PB20 and PB25), and statistical (PB9) terpolymers of three different PEGMA-BuMA-DMAEMA compositions, which were investigated in this project: i) PB1-PB10 are of 20-40-40 w/w%, ii) PB11-PB20 are of 20-30-50 w/w%, and iii) PB21-PB25 have composition of 20-35-45 w/w%. The PEGMA, BuMA, and DMAEMA units are coloured in light blue, red, and light green, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-

Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

Page 132

Figure 5.2.4: GPC chromatograms of the tetrablock terpolymer PB1 with theoretical polymer structure PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉-*b*-PEGMA_{2.5} (blue solid line), and its linear precursors: i) PEGMA_{2.5} in light blue solid line, ii) the diblock copolymer PEGMA_{2.5}-*b*-BuMA₂₁ in red dotted line, and iii) the triblock terpolymer PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉ in light green dotted line.

Page 133

Figure 5.2.5: ¹H NMR spectra of (a) the PEGMA_{2.5} homopolymer in light blue, (b) the PEGMA_{2.5}-*b*-BuMA₂₁ diblock copolymer in red, (c) the PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉ triblock terpolymer in light green, and (d) the PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉-*b*-PEGMA_{2.5} tetrablock terpolymer (Polymer B1) in blue. The corresponding chemical structures are also shown; the PEGMA, BuMA, and DMAEMA repeated units are coloured in light blue, red, and light green, respectively.

Page 135

Figure 5.2.6: Schematic representation of the micelle structure assumed to be adopted by the tetrablock terpolymers with PEGMA-BuMA-DMAEMA (A-B-C) composition of 20-30-50 w/w%, respectively (PB11-PB19): i) two hydrophilic A blocks (PB11-PB13, top), ii) two hydrophobic B blocks (PB14-PB16, middle), and iii) two thermoresponsive C blocks (PB17-PB19, bottom). The corresponding polymer structures are also given, with PEGMA, BuMA, and DMAEMA units in light blue, red, and light green, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

Page 142

Figure 5.2.7: Effect of the architecture and the composition on the pK_a of the DMAEMA repeated units. The results which correspond to the polymers with theoretical PEGMA-BuMA-DMAEMA weight percentage of 20-30-50 w/w%, 20-35-45 w/w%, and 20-40-40 w/w%, are indicated by light blue squares, light green triangles, and red rhombi, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

Page 145

Figure 5.2.8: Effect of the architecture and composition on the cloud point of the terpolymers which are soluble in water. The results of the polymers with different PEGMA-BuMA-DMAEMA weight percentage are shown with a different symbol: i) light blue squares for 20-30-50 w/w%, ii) light green triangles for 25-35-45 w/w%, and iii) red rhombi for 20-40-40

w/w%. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

Page 146

Figure 5.2.9: Phase diagrams of the terpolymer solutions in phosphate buffered saline (PBS): i) tetrablock terpolymers (PB2, PB4, PB7, PB9, PB12, PB15, PB17, PB19, PB21, PB22, PB23, PB24), and ii) the triblock terpolymers (PB20 and PB25). The effect of the architecture is shown from the left to the right (ABAC, BABC, CABC, ACBC, and ABC, respectively; where A, B, and C blocks are based on PEGMA, BuMA, and DMAEMA, respectively. The effect of the composition is shown from the top to the bottom: (a) 20-30-50 ABC w/w% (top), (b) 20-35-45 ABC w/w% (middle), and 20-40-40 ABC w/w% (bottom). Four phases are identified: i) the runny solution phase is coloured in white (square, triangle, and circle represent the clear, slightly cloudy, and cloudy state, respectively), ii) the viscous solution phase is coloured in red (triangle and circle represent the transparent and cloudy state, respectively), iii) blue colour represents the gel phase (triangles and circles show the transparent or cloudy state of the gel, respectively), the system of two phases is shown in light green (gel syneresis and precipitation are shown in rhombi and squares, respectively). The gelation region is indicated by an approximate black dashed line. The corresponding polymer structures are also given; where PEGMA, BuMA, and DMAEMA are coloured in light blue, red, and light green, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

Pages 149-150

Figure 5.2.10: Possible configurations that are adopted by the BABC architecture. PB15 is given as an example. The corresponding polymer structure is also schematically illustrated with PEGMA, BuMA, and DMAEMA units being represented by light blue, red, and light green spheres, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

Page 152

Figure 5.2.11: Rheological curves of the 15 w/w% terpolymer solutions in phosphate buffered saline (PBS) which form visually a gel, as resulted from temperature ramp measurements. The changes in the shear storage modulus (elastic modulus, G'), shear loss modulus (viscous modulus, G''), and the complex viscosity (η^*) are shown in dark blue, red, and light blue, respectively. The graphs shown correspond to: (a) the polymer B7 with theoretical polymer structure DMAEMA_{9.5}-*b*-PEGMA₅-*b*-BuMA₂₁-*b*-DMAEMA_{9.5} (CACB), (b) the polymer B19

with theoretical polymer structure PEGMA_{5.5}-*b*-DMAEMA₁₃-*b*-BuMA₁₇-*b*-DMAEMA₁₃ (ACBC), (c) the polymer B22 with BABC architecture and structure of BuMA₁₀-*b*-PEGMA_{5.5}-*b*-BuMA₁₀-*b*-DMAEMA_{23.5}, and (d) the ABC triblock terpolymer B25 with theoretical polymer structure PEGMA_{5.5}-*b*-BuMA₂₀-*b*-DMAEMA_{23.5}. The corresponding polymer structures are schematically illustrated with light blue, red, and light green representing the PEGMA, BuMA, and DMAEMA units, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

Page 154

Figure 5.3.1: Chemical structures, names, and abbreviations of the monomers used in this polymer series: penta(ethylene glycol) methyl ether methacrylate (PEGMA), *n*-butyl methacrylate (BuMA), and di(ethylene glycol) methyl ether methacrylate (DEGMA).

Page 160

Figure 5.3.2: Chemical reaction of the GTP synthesis of an ABC triblock terpolymer which is based on PEGMA, BuMA, and DEGMA as A, B, and C units, respectively.

Page 161

Figure 5.3.3: Schematic representation of the ABC triblock terpolymers of various compositions (PC1-PC12), the ABA triblock copolymer (PC3), and CBC triblock bipolymer (PC14), and the random terpolymer (PC15). Light blue, red, and dark blue spheres represent PEGMA (A unit), BuMA (B unit), and DEGMA (C unit).

Page 162

Figure 5.3.4: The GPC chromatogram of the triblock terpolymer PEGMA₁₄-*b*-BuMA₁₄-*b*-DEGMA₁₁ (PC1) is shown in dark blue dotted line. The GPC traces of its linear precursors are also given: i) PEGMA₁₄ in light blue solid line, and ii) PEGMA₁₄-*b*-BuMA₁₄ in red dotted line.

Page 163

Figure 5.3.5: ¹H NMR spectra of (a) the homopolymer PEGMA₁₄, (b) the diblock copolymer PEGMA₁₄-*b*-BuMA₁₄, and (c) the final triblock terpolymer PEGMA₁₄-*b*-BuMA₁₄-*b*-DEGMA₁₁ (PC1) are presented in light blue, red, and dark blue, respectively. Also, the corresponding chemical structures are shown. In these structures, the PEGMA, BuMA, and DEGMA repeated units are coloured in light blue, red, and dark blue, respectively.

Page 166

Figure 5.3.6: Schematic illustration of the spherical micelle adopted by PEGMA₁₄-*b*-BuMA₁₄-*b*-DEGMA₁₁ (PC1). The PEGMA, BuMA, and DEGMA units are shown in light blue, red, and dark blue spheres, respectively. All triblock copolymers in this study, either terpolymers (PC1-PC12) or bipolymers (PC13 and PC14), are assumed to self-assemble in the same way as

shown. The longest hydrophilic block is the one which determines the micelle size.

Page 169

Figure 5.3.7: Effect of experimental DEGMA weight percentage on the cloud points of PEGMA_x-*b*-BuMA_y-*b*-DEGMA_z solutions (1 w/w% in deionised water); x, y, and z is the degree of polymerisation of PEGMA, BuMA, and DEGMA, respectively. The effect of the experimental BuMA weight percentage is also shown; light blue squares, dark blue triangles, and the red rhombi correspond to copolymers of 25, 30, and 35 w/w% of BuMA.

Page 171

Figure 5.3.8: Phase diagrams of the PEGMA-BuMA-DEGMA triblock terpolymers (PC1-PC12) and the triblock bipolymer PEGMA-BuMA-PEGMA (PC3) in phosphate buffered saline (PBS). The effect of DEGMA composition is shown from left to right, whereas the effect of BuMA compositions is shown from the top to bottom: (a) 25 w/w% BuMA content (top), (b) 30 w/w% BuMA content (middle), and (c) 35 w/w% BuMA content (bottom). Four phases are observed: i) runny solution coloured in white (square: clear, triangle: slightly cloudy, and circle: cloudy), ii) viscous solution coloured in red (triangle and circle for transparent and cloudy, respectively), iii) the gel phase is shown in blue (triangle and circle for transparent and cloudy, respectively), the two-phase system is coloured in light green (rhombus and square for gel syneresis and precipitation, respectively). The polymer structures are schematically illustrated, in which the PEGMA, BuMA, and DEGMA units are coloured in light blue, red, and dark blue, respectively.

Pages 174-175

Figure 5.3.9: Rheological properties as a function of temperature for the polymer solutions which form a gel visually at 15 w/w% in phosphate buffered saline (PBS). The (a)-(e) graphs correspond to the terpolymers with 30 w/w% BuMA: (a) PC4 (PEGMA₁₄-*b*-BuMA₁₇-*b*-DEGMA₉), (b) PC5 (PEGMA₁₂-*b*-BuMA₁₇-*b*-DEGMA₁₁), (c) PC6 (PEGMA₁₁-*b*-BuMA₁₇-*b*-DEGMA₁₃), (d) PC7 (PEGMA₁₀-*b*-BuMA₁₇-*b*-DEGMA₁₅), and (e) PC8 (PEGMA₈-*b*-BuMA₁₇-*b*-DEGMA₁₇). The (f) graph corresponds to PC12 (PEGMA₁₁-*b*-BuMA₂₀-*b*-DEGMA₁₁), which has 35 w/w% BuMA. The PEGMA, BuMA, and DEGMA units on the polymer structures are coloured in light blue, red, and dark blue, respectively.

Page 177

Figure 5.4.1: Chemical structures, names, and abbreviations of the monomers used in this polymer series. PEGMA and diPGMA stand for penta(ethylene glycol) methyl ether methacrylate and di(propylene glycol) methyl ether methacrylate, respectively. It should be noted that as the alcohol used for the monomer synthesis is a mixture of isomers, the four possible isomers of the monomer, shown in the Figure, are expected to be formed. The abbreviations of the four isomers are also given.

Figure 5.4.2: Chemical reaction of the synthesis of diPGMA monomer, which is catalysed by triethylamine. diPGMA was synthesised via an esterification reaction between methacryloyl chloride and di(propylene glycol) methyl ether, diPGOH. THF was used as solvent. As the diPGOH used is a mixture of isomers, four possible isomers of the monomer are expected to be formed. The four isomers of the alcohol from top to bottom are the following: i) 2-(2-methoxy-2-methylethoxy)-1-propanol [2-(2-M-2-ME)-1-P], ii) 1-(2-methoxy-1-methylethoxy)-2-propanol [1-(2-M-1-ME)-2-P], iii) 2-(2-methoxy-1-methylethoxy)-1-propanol [2-(2-M-1-ME)-1-P], and iv) 1-(2-methoxy-2-methylethoxy)-2-propanol [1-(2-M-2-ME)-2-P].

Figure 5.4.3: GC-FID chromatograms of the alcohol (diPGOH in black), and the monomer (diPGMA in dark red).

Figure 5.4.4: FT-IR spectra of (a) the alcohol (diPGOH in black), and (b) the monomer (diPGMA in dark red).

Figure 5.4.5: ^1H NMR spectra of (a) the alcohol (diPGOH in black), and (b) the monomer (diPGMA after purification in dark red). The corresponding chemical structures are also presented; the two most abundant isomers are indicated by a square.

Figure 5.4.6: ^{13}C NMR spectra of (a) the alcohol (diPGOH in black), and (b) the monomer (diPGMA after purification in dark red). The corresponding chemical structures are also presented; the two most abundant isomers are indicated by a square.

Figure 5.4.7: Chemical reaction of the GTP synthesis of an ABA triblock copolymer which is based on PEGMA, and diPGMA as A and B units, respectively.

Figure 5.4.8: Schematic representation of the ABA triblock copolymers of various MMs: PD1-PD3 with MM 5100 g mol^{-1} , PD4-PD6 with MM 10100 g mol^{-1} , and PD7-PD9 with MM 13100 g mol^{-1} . Light blue, and dark red spheres represent PEGMA (A unit), and diPGMA (B unit), respectively.

Figure 5.4.9: The GPC chromatogram of the triblock terpolymer PEGMA₇-*b*-diPGMA₅-*b*-PEGMA₇ (PD1) is shown in dark blue dotted line. The GPC traces of its linear precursors are also given: i) PEGMA₇ in light blue solid line, and ii) PEGMA₇-*b*-diPGMA₅ in red dotted line.

Figure 5.4.10: ^1H NMR spectra of (a) the homopolymer PEGMA₇, (b) the diblock copolymer PEGMA₇-*b*-diPGMA₅, and (c) the final triblock copolymer PEGMA₇-*b*-diPGMA₅-*b*-PEGMA₇ (PD1) are presented in light blue, red, and dark blue, respectively. Also, the corresponding chemical structures are shown. In these structures, the PEGMA, and diPGMA repeated units are coloured in light blue, and dark red, respectively.

Figure 5.4.11: Schematic illustration of the spherical micelle adopted by PEGMA₇-*b*-diPGMA₅-*b*-PEGMA₇ (PD1). The PEGMA, and diPGMA repeated units are shown in light blue, and dark red, respectively. All triblock copolymers (PD1-PD9) in this study are assumed to self-assemble in the same way as shown.

Figure 5.4.12: Effect of experimental diPGMA weight percentage on the cloud points of PEGMA_x-*b*-diPGMA_y-*b*-PEGMA_x solutions (1 w/w% in deionised water); x, and y denote the degree of polymerisation of PEGMA and diPGMA, respectively. The effect of the MM is also shown; light blue squares, dark blue triangles, and the red rhombi correspond to copolymers with MM 5100, 10100, and 13100 g mol⁻¹, respectively.

Figure 5.4.13: Phase diagrams of the PEGMA-diPGMA-PEGMA triblock copolymers (PD1-PD9) in phosphate buffered saline (PBS). The effect of diPGMA composition is shown from left to right, whereas the effect of the MM is shown from the top to bottom: (a) MM 5100 g mol⁻¹ (top), (b) MM 10100 g mol⁻¹ (middle), and (c) MM 13100 g mol⁻¹ (bottom). Four phases are observed: i) runny solution coloured in white (square: clear, triangle: slightly cloudy, and circle: cloudy), ii) viscous solution coloured in red (triangle and circle for transparent and cloudy, respectively), iii) the gel phase is shown in blue (triangle and circle for transparent and cloudy, respectively), the two-phase system is coloured in light green (rhombus and square for gel syneresis and precipitation, respectively). The polymer structures are schematically illustrated, in which the PEGMA and diPGMA repeated units are coloured in light blue, and dark red, respectively.

Figure 5.4.14: Rheological properties as a function of temperature for the PD6 polymer solution which form a gel visually at 15 w/w% in phosphate buffered saline (PBS). PD6 has theoretical polymer structure PEGMA₁₀-*b*-diPGMA₁₉-*b*-PEGMA₁₀, MM equal to 10100 g mol⁻¹, and 40 w/w% diPGMA content. The PEGMA and diPGMA units on the polymer structures are coloured in light blue and dark red, respectively.

LIST OF TABLES

Table 2.1: Summary of the *in vivo* studies on injectable gels.

Pages 59-61

Table 2.2: Summary of the studies on thermoresponsive gels (TRGs) used as 3D printable materials.

Page 75

Table 4.1: Equations used for the theoretical calculations of the a) end-to-end distance of the random coil adopted by the statistical copolymer, and b) the diameter of the micelles formed by the block copolymers.

Page 101

Table 5.1.1: Theoretical polymer structure, molar mass ($MM^{\text{theor.}}$), and composition, and experimental molar mass (MM) (number-average MM, M_n), dispersity index ($\bar{D}$), and composition of the copolymers and their linear precursors. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

Page 112

Table 5.1.2: Theoretical polymer structure, theoretical (calculated) and experimental hydrodynamic diameters (d_{hs}), polydispersity indices (PDI), effective pK_a s, and cloud points (CPs) of the aqueous polymer solutions at 1 w/w%. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

Page 116

Table 5.2.1: Theoretical polymer structure, molar mass ($MM^{\text{theor.}}$), and composition, and experimental results of number-average molar mass (M_n), molar mass distribution (MMD, denoted as dispersity index, $\bar{D}$), and composition of the terpolymer and their linear precursors. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, 51(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

Pages 136-138

Table 5.2.2: Theoretical polymer structures, and theoretical diameters of their self-assembled structures, and aqueous polymer solution properties at 1 w/w%: experimental hydrodynamic diameters (d_{hs}), polydispersity indices (PDI), effective pK_a s, and cloud points (CPs). Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, 51(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

Pages 140-141

Table 5.3.1: Theoretical chemical structures, dispersity indices ($\bar{D}$), theoretical and experimental molecular masses ($MM^{\text{theor.}}$ and M_n , respectively) and compositions of the final terpolymers polymers and their precursors (if any).

Pages 164-165

Table 5.3.2: Theoretical polymer structures, architectures, and hydrophobic composition, theoretical and experimental hydrodynamic diameters (d_{hs}), polydispersity indices (PDI), and cloud points (CPs) of 1 w/w% copolymer solutions in deionised water.

Page 167

Table 5.4.1: GC-FID results for the alcohol (diPGOH) and the monomer (diPGMA): the number of peaks identified, the detection time, and the integration of each peak.

Page 186

Table 5.4.2: FT-IR results for the alcohol (diPGOH) and the monomer (diPGMA): the number of peaks present, the characteristic absorptions, the functional groups, the type of vibration, and the intensity are listed.

Page 189

Table 5.4.3: Theoretical chemical structures, dispersity indices ($\bar{D}$), theoretical and experimental molecular masses ($MM^{\text{theor.}}$ and M_n , respectively) and compositions of the final ABA triblock copolymers and their precursors.

Page 196

Table 5.4.4: Theoretical polymer structures, architectures, and hydrophobic composition, theoretical and experimental hydrodynamic diameters (d_{hs}), polydispersity indices (PDI), and cloud points (CPs) of 1 w/w% copolymer solutions in deionised water.

Page 200

LIST OF ABBREVIATIONS

AA	: Acrylic acid
ADMBuLA	: Acryloyloxy dimethyl- γ -butyrolactone
ATRP	: Atom-transfer radical polymerisation
bFGF	: Basic fibroblast growth factor
BMP-2	: Bone morphogenetic protein-2
BSA	: Bovine serum albumin
BuA	: Butyl acrylate
BuLaA	: γ -Butyrolactone acrylate
BuMA, Bu	: <i>n</i> -butyl methacrylate
b.t.	: Body temperature
CA4	: Combrestatin A-4
CGC	: Critical gelation concentration
CGT	: Critical gelation temperature
CL	: ϵ -caprolactone
COX	: Celecoxib
CPH	: 1,6-bis(<i>p</i> -carboxyphenoxy)hexan
CPT	: Camptothecin
CRPC	: Colorectal peritoneal carcinomatosis
DEGA	: Di(ethylene glycol) methyl ether acrylate
DEGMA, DEG	: Di(ethylene glycol) methyl ether methacrylate
di-Gly	: Di(glycine)
diPGOH	: Di(propylene glycol) monomethyl ether
diPGMA, diPG	: Di(propylene glycol) methyl ether methacrylate
DLS	: Dynamic light scattering
DMAAm	: <i>N,N</i> -dimethylacrylamide
DMAEMA, DMA	: 2-(dimethylamino)ethyl methacrylate
DO	: <i>p</i> -Dioxanone
DOX	: Doxorubicin
DTX	: Docetaxel
eDEGA	: Di(ethylene glycol) ethyl ether acrylate
EG	: Ethylene glycol

EGA : Ethylene glycol methyl ether acrylate
EO : Ethylene oxide
ESHU : Serinol hexamethylene urethane
5-Fu : 5-Fluorouracil
FDA : Food and drug administration
GA : Glycolic acid
GC-FID : Gas chromatography flame ionisation detection
GlyLaE : Glycyl lactate ethyl ester
GO : Graphene oxide
GPC : Gel permeation chromatography
GTP : Group transfer polymerisation
HA : Hyaluronic acid
HAMA : Hyaluronan methacrylate
HBC : Hydroxybutyl chitosan
HEMA : 2-Hydroxyethyl methacrylate
HGH : Human growth hormone
HIV : Human immunodeficiency virus
HPMC : Hydroxypropyl methyl cellulose
IleE : Isoleucine ethyl ester
IPGEt : Isopropyl glycidyl ether
LA : Lactic acid
LCST : Lower critical solution temperature
MAA : Methacrylic acid
MALDI-TOF MS : Matrix assisted laser desorption/ionisation-time of flight mass spectrometry
MM : Molar mass
MMD : Molar mass distribution
MOx : 2-Methyl-2-oxazoline
MPEG : Methoxy poly(ethylene glycol)
MSC : Mesenchymal stem cells
MTS : Methyl trimethylsilyl dimethylketene acetal
NEGMA : Nona(ethylene glycol) methyl ether methacrylate
NHC : *N*-Heterocyclic carbene
NHS : *N*-Hydroxysuccinimide

NIPAAm : *N*-Isopropylacrylamide
NMP : Nitroxide-mediated polymerisation
NMR : Nuclear magnetic resonance
OEGMA : Oligo(ethylene glycol) methyl ether methacrylate
P105 : Poloxamer 105 , Pluronic® L35
P124 : Poloxamer 124 , Lutrol® L44
P188 : Poloxamer 188 , Pluronic® F68
P407 : Poloxamer 407 , Pluronic® F127
PBS : Phosphate buffered saline
PCL : Poly(ϵ -caprolactone)
PCLA : Poly(ϵ -caprolactone-*co*-lactide)
PDLA : Poly(*D*-lactide)
PDLLA : Poly(*D,L*-lactide)
pEG : Poly(ethylene glycol)
PEGMA, PEG : Penta(ethylene glycol) methyl ether methacrylate
PEGC : Poly(ethylene glycol) citrate
PG : Propylene glycol
PIC : Poly(isocyanide)
PMMA : Poly(methyl methacrylate)
PNIPAAm : Poly(*N*-isopropylacrylamide)
PLA : Poly(lactic acid)
PLLA : Poly(*L*-lactide)
PLGA : Poly(lactic acid-*co*-glycolic acid)
Pox : 2-*n*-propyl-2-oxazine
PPG : Poly(propylene glycol)
PS : Poly(styrene)
PSu : Propylene sulphide
PTFE : Polytetrafluoroethylene
PTHFC : Poly(tetrahydrofuran carbonate)
PTX : Paclitaxel
PVA : Poly(vinyl alcohol)
QF : Quetiapine fumarate
r.t. : Room temperature
RAFT : Reversible addition-fragmentation chain-transfer

ROP : Ring opening polymerisation
ROS : Reactive oxygen species
SA : Sodium alginate
SEC : Size exclusion chromatography
TBABB : Tetrabutylammonium bibenzoate
TE : Tissue engineering
TEGMA, TEG : Tri(ethylene glycol) methyl ether methacrylate
TMZ : Temozolomide
TRG : Thermoresponsive gel
triMeCa : Trimethylene carbonate
TSUN : 1,4,8-trioxa[4.6]spiro-9-undecanone
UCST : Upper critical solution temperature

*To my Mama and my Papa, Christalla and Pericles,
and my siblings, Vasoula and Panayiotis*

CHAPTER 1: INTRODUCTION

Nature has been, and still is, the ultimate tool of inspiration for scientists. As characterised by Janine M. Benyus in her book “Biomimicry: Innovation Inspired by Nature”, nature is the “*in vivo* genius”.¹ From the chameleon’s skin, capable of changing colour for “camouflage”, to the “Venus flytrap” plant, which closes itself upon physical contact to “trap” its victim, natural “smart materials” exist. Inspired by nature, polymer scientists have synthesised and investigated “smart” polymers, also referred as stimulus-responsive polymers, which respond to an external stimulus, such as temperature, electricity, magnetic field, light, and pH, for decades.^{2,3}

1.1 Thermoresponsive Polymers

This thesis is focused on thermoresponsive polymers, which are polymers that respond to temperature. They are also known as temperature-responsive polymers or thermo-sensitive polymers. In the case of aqueous solvent, the thermoresponse is the change in the hydrophilicity/hydrophobicity of the polymer and thus its solubility in water. This is visually observed as cloudiness, precipitation, or gelation. When the solubility increases as the temperature increases, the polymers possess upper critical solution temperature (UCST) (Figure 1.1 (a)). On the other hand, when the hydrophilicity (and thus solubility) decreases by increasing the temperature, the polymers show lower critical solution temperature (LCST) (Figure 1.1 (b)).

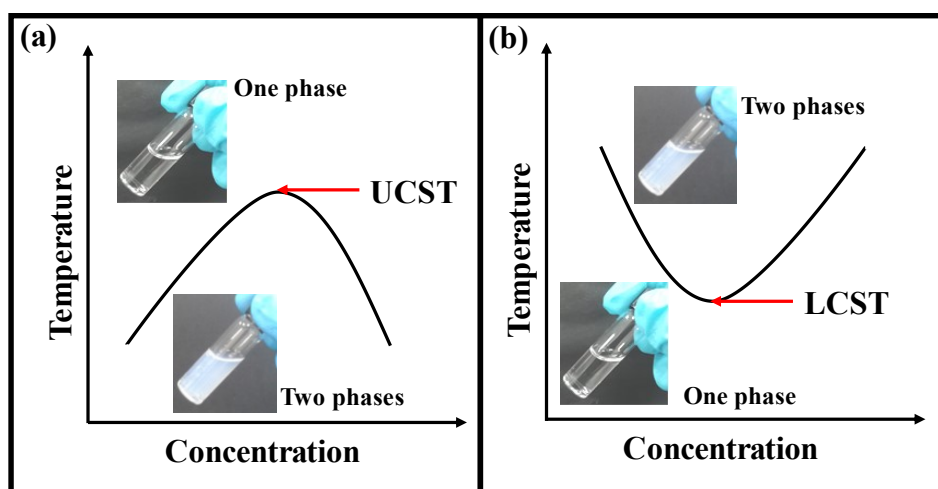


Figure 1.1: Phase diagrams of thermoresponsive polymer solutions which show: (a) upper critical solution temperature (UCST), and (b) lower critical solution temperature (LCST). The corresponding UCST and LCST values are indicated by red arrow.

This PhD thesis focuses on thermoresponsive polymers which present LCST. The most popular thermoresponsive repeated units which show LCST, either in the form of homo- or co-polymer, are *N*-isopropylacrylamide (NIPAAm),⁴ 2-(dimethylamino)ethyl methacrylate (DMAEMA, DMA),⁵ and ethylene glycol (EG, same repeated unit as ethylene oxide, EO).^{6,7} Also, several thermoresponsive units with the EG groups on the side chain have been reported in the literature.⁸ These units are either acrylates or methacrylates with various lengths of EG-based side chains, like ethylene glycol methyl ether acrylate (EGA),⁹ di(ethylene glycol) (m)ethyl ether acrylate (DEGA, or eDEGA, respectively),^{10,11} di(ethylene glycol) methyl ether methacrylate (DEGMA, DEG),¹² and oligo(ethylene glycol) methyl ether methacrylate (OEGMA).¹³ The chemical structures, names, and abbreviations of these thermoresponsive units are shown in Figure 1.2.

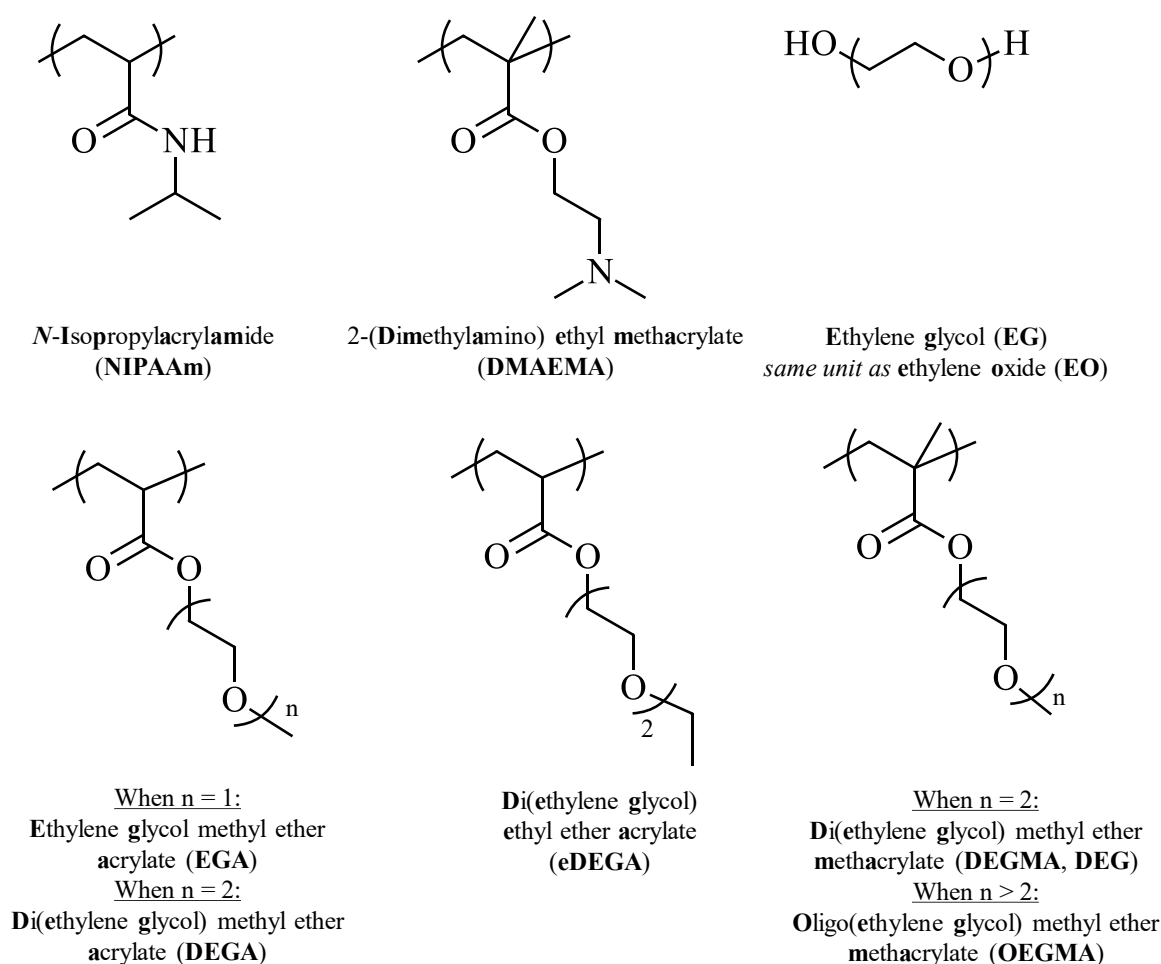


Figure 1.2: Chemical structures, names, and abbreviations of the most popular thermoresponsive units with LCST.

The response of the repeated units with LCST is attributed to the “hydrophobic effect”.^{14,15} This phenomenon can be explained upon consideration of the Gibbs free energy (Equation 1.1), in which T stands for temperature, and ΔG , ΔH and ΔS denote the changes in Gibbs free energy, enthalpy, and entropy, respectively. For polymers that present an LCST behaviour, the most important factor is the entropy (S) and more specifically the S of water. Specifically, S will be higher if there are more free water molecules in solution ($\Delta S > 0$), i.e. phase separation occurs and the polymer is precipitated as the temperature increases ($\Delta T > 0$) (see Figure 1.1 (b)). Hence, the Gibbs free energy decreases ($\Delta G < 0$), and thus, this transition is thermodynamically more favourable.^{14,15}

$$\Delta G = \Delta H - T\Delta S \quad \text{Equation 1.1}$$

1.2 Thermoresponsive Gels (TRGs)

Aqueous thermoresponsive copolymer solutions may respond to the temperature by forming a 3-dimensional (3-D) network, in which water molecules are entrapped. These structures are known as thermoresponsive gels (TRGs) and they are formed via physical entanglements, hydrogen bonds or hydrophobic interactions.¹⁶ The interactions holding the network together are physical, thus, when the stimulus is removed, the solution phase is re-obtained. However, the transition from the solution phase to the gel phase, known as sol-gel transition, is only observed when the polymer possesses the appropriate structural characteristics. The molar mass (MM), the composition/hydrophobicity, the architecture/topology and the stereochemistry of the polymer are known to affect the thermoresponsive behaviour, and their effect on the gelation has been previously summarised.¹⁷ The critical gelation concentration (CGC) and the critical gelation temperature (CGT), i.e. the lowest concentration and the temperature at which the gel formation is observed are interlinked, since it has been proven that the CGT decreases by increasing the concentration of the polymer solution.¹⁸⁻²⁰ The thermoresponse is also affected by the solvent,²¹ the pH,²²⁻²⁴ and the ionic strength.^{22,25}

The sol-gel transition can be observed both visually and rheologically, as it is shown schematically in Figure 1.3. The vial which contains the polymer solution (see Figure 1.3, picture on the left) is visually tested for gelation at different temperatures by the tube-inversion test, i.e. investing the vial/tube upside down. The gel point/gel temperature (T_{gel}) is determined as the temperature at which the polymer solution does not flow, i.e. a stable gel is formed (refer to Figure 1.3, picture on the right). Rheology facilitates the more accurate determination of the

gel point, which is defined as the point at which the shear storage modulus (G') exceeds the shear loss modulus (G''), i.e. the system stores energy as the solid phase is dominant.²⁶

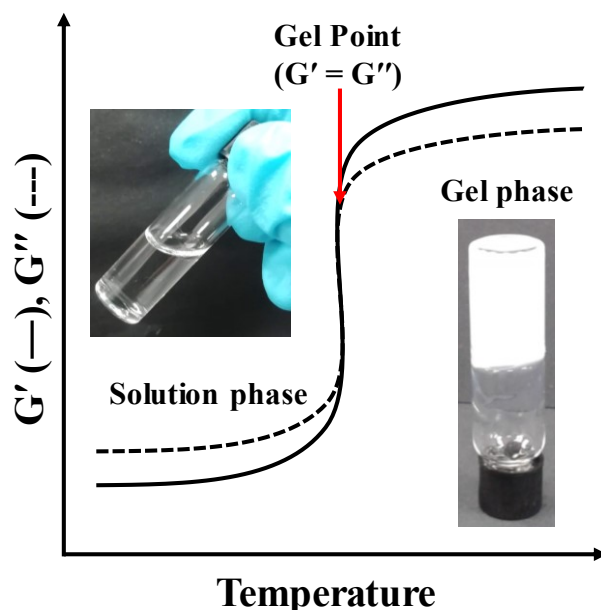


Figure 1.3: Schematic diagram showing the rheological and visual determination of the gel point. The pictures show the transition from a solution phase (left) to the gel phase (right) as the temperature increases. The solid and dashed black lines represent the changes in the shear storage modulus (elastic modulus, G') and shear loss modulus (viscous modulus, G''), respectively. The red arrow corresponds to the gel point determined by rheology, which is determined as the point at which the storage modulus exceeds the loss modulus.

1.3 Living-Controlled Polymerisation Methods

As previously mentioned, the thermoresponsive behaviour, and therefore the gelation depends on the structural characteristics, i.e. MM, composition, and architecture.¹⁷ Therefore, all structural parameters should be controllable and known in order to have a defined and tailor-able sol-gel transition temperature, which is crucial for the final application. Consequently, well-defined polymers (polymers with narrow molar mass distribution, MMD) are required. Well-defined polymers can only be produced via a living or controlled polymerisation method.²⁷ Therefore, this section focuses on defining what are living/controlled polymerisation methods and discussing their advantages.

Currently there is still an on-going scientific debate on what is the definition of a living polymerisation and what are the criteria for this.²⁸⁻³⁴ The IUPAC definition is that living polymerisation is: “A chain polymerisation from which chain transfer and chain termination are absent”, and that “in many cases, the rate of chain initiation is fast compared with the rate of chain propagation, so that the number of kinetic-chain carriers is essentially constant

throughout the polymerisation”.³⁵ Another simpler definition is to define living polymerisations as “those systems where irreversible termination and transfer are sufficiently suppressed so that block copolymers can be synthesised by sequential addition of monomers”.³¹ All definitions agree and suggest well-defined polymers (polymers with narrow MMD) and complex architectures, like block copolymers and star polymers will be able to be synthesised with these methods.²⁸⁻³⁴

Nowadays, there are several living polymerisation methods. The first living polymerisation method to be discovered is the conventional living anionic polymerisation.^{36,37} This technique has been discovered by Michael Szwarc in 1950's, and it has been reported in his paper in *Nature* in 1956.³⁶ With the progress in polymer science over the last 50 years, more living polymerisation techniques have been discovered, including group transfer polymerisation (GTP), living cationic polymerisation, and reversible-deactivation (“living”) radical polymerisation methods, that include atom-transfer radical polymerisation (ATRP), reversible addition-fragmentation chain-transfer (RAFT) polymerisation and nitroxide-mediated polymerisation (NMP).^{27,38-40} GTP has been chosen for the synthesis of all the polymers of this PhD thesis, mostly because it enables the easy and fast synthesis of large amount of polymer ($m > 20\text{g}$), which is essential for the full characterisation of the polymers.

1.3.1 Group Transfer Polymerisation (GTP)

GTP is a living anionic polymerisation which has been invented at Du Pont by Owen W. Webster's group in 1983.⁴¹ From the chemistry point of view, this technique is a Michael addition of silyl ketene acetals (initiator molecules) to α,β -unsaturated carbonyl compounds (monomers). In this reaction, a metal-free nucleophile catalyst is required.^{38,41-44} Monomers that can be polymerised using GTP are α,β -unsaturated esters, ketones, nitriles, and carboxamides.^{41,44}

As a living anionic polymerisation, GTP shares similar advantages as other living polymerisation techniques. Firstly, GTP produces polymers of controlled MM, as the monomers are fully consumed, i.e. the MM is proportional to the number of repeated units added in each initiator molecule.^{41,44} Also, the polymers produced have narrow MMD.^{41,44} Furthermore, GTP can produce complicated architectures, including block copolymers, star and graft copolymers, and polymeric networks (covalently cross-linked gels).⁴²

When GTP is compared to other living or controlled polymerisation techniques, it has several advantages. Firstly, unlike the conventional living anionic polymerisation, which is performed at 0 °C, GTP can be performed at or above room temperature, thus reducing the cost.⁴⁴ Nevertheless, recent studies have reported living anionic polymerisation at room temperature using tubular or microreactor devices.^{45,46} However, the scalability might be restricted using these devices, as opposed to GTP, which can be scaled up to an industrial scale.^{47,48} GTP is also advantageous compared to the conventional living anionic polymerisation, as it is performed at larger concentrations, which again makes GTP more cost-effective.⁴³ GTP also has advantages over the reversible-deactivation (“living”) radical polymerisation methods. GTP is time-effective, as the monomers are fully consumed within 15 minutes.⁴⁹ On account of the complete consumption of the monomers, no intermediate purification steps are required, thus one-pot sequential polymerisation can be performed.^{42,47,50} On the other hand, one-pot synthesis via controlled radical polymerisation techniques can only be achieved after the experimental conditions have been optimised for the specific set of monomers.⁴⁷ Even after the conditions have been optimised, the polymerisation of each block lasts from 30 mins to 8 days.⁴⁷

As the other polymerisation techniques, GTP also possesses limitations. Compared to the conventional living anionic polymerisation and reversible-deactivation (“living”) radical deactivation techniques, production of high MM polymers (i.e. polymers with MM around 60 kg mol⁻¹) is limited with GTP.⁴³ Also, in terms of monomer choice, methacrylate monomers are polymerised best, but acrylates can also be polymerised.^{42,43} However, concerning the GTP of acrylates, the polymerisation occurs faster, and thus the polymer chains do not remain “alive” for long.^{42,43} Therefore, only polymers with MM up to 10 kg mol⁻¹ can be produced with acrylate monomers,⁴³ while our group has synthesised methacrylate based polymers with MM up to 50 kg mol⁻¹. As GTP is an anionic polymerisation, it is not compatible with compounds which contain labile protons.⁴²⁻⁴⁴ Therefore, hydroxyl and carboxyl groups are some examples that are not compatible with an anionic polymerisation.⁴⁴ Nevertheless, protection of these group prior to GTP is possible, and deprotection follows after the polymerisation finishes.^{42,44}

Interestingly, Kakuchi and co-workers investigate new catalyst/initiator systems for GTP.⁵¹⁻⁶⁶ In one of the studies, high MM poly(methyl methacrylate) (PMMA) has been synthesised using a commercially available superbase.⁵⁵ More specifically, the PMMA had experimental MM and *D* equal to 109600 g mol⁻¹ and 1.32, respectively.⁵⁵ The same superbase catalysed the

synthesis of star-shaped methacrylate polymers via the core-first approach.^{62,64,65} It should be noted that these star polymers have been synthesised using *in-house* synthesised initiators.^{62,64,65} Different fluoro-containing catalysts have also been used to synthesise linear^{51,57} and star-shaped methacrylate polymers,⁶³ high MM acrylate polymers (MM > 100000 g mol⁻¹ and narrow MMD),^{61,66} and acrylate-based dodecablock copolymers.⁶⁰ It is worth-noting that the GTP of acrylamides has also been achieved,^{52-54,58} as the GTP of this type of monomers was not feasible before.

Two mechanistic pathways of GTP have been reported in the literature,^{42,43,67-70} and they are shown in Figures 1.4 and 1.5. The GTP mechanism depends on the catalyst used.⁴² The “associative” mechanism had been proposed first (Figure 1.4), however, the “dissociative” one is evidenced to be correct for the conventional system of catalyst/initiator (Figure 1.5).⁴³ Recent studies in which *N*-heterocyclic carbenes (NHCs) are used as catalysts propose the formation of a pentacoordinate intermediate, which agrees with the “associative” mechanism.⁴² These mechanisms are discussed in more detail below.

The first mechanism to be reported is the “associative” one,^{67,68} the mechanistic steps of which are shown in Figure 1.4: the catalyst is shown with light blue circle, and the initiator, methyl trimethylsilyl dimethylketene acetal (MTS), is shown in red square. As can be seen, an intermediate complex is formed (shown in blue square), during which the initiator coordinates towards the nucleophilic catalyst, and it becomes activated.^{43,69} After each monomer is added, the trimethyl silyl group is transferred to the end of the polymer chain.⁴³ The “associative” mechanism is the one related to the name of the technique “Group Transfer Polymerisation”.^{42,43}

However, a “dissociative” mechanism has been proposed,⁷⁰ the mechanistic steps of which are shown in Figure 1.5. In Figure 1.5, the system of initiator, catalyst, and monomer used is MTS, tetrabutylammonium bibenzoate (TBABB), and a methacrylate monomer, indicated by red, light blue, and green squares, respectively. During this mechanism, the catalyst and the initiator interact, thus exchanging the silyl group.^{43,68} An enolate group is formed (shown in blue square), which interacts with the monomer molecules, thus forming the polymer chain.^{43,68}

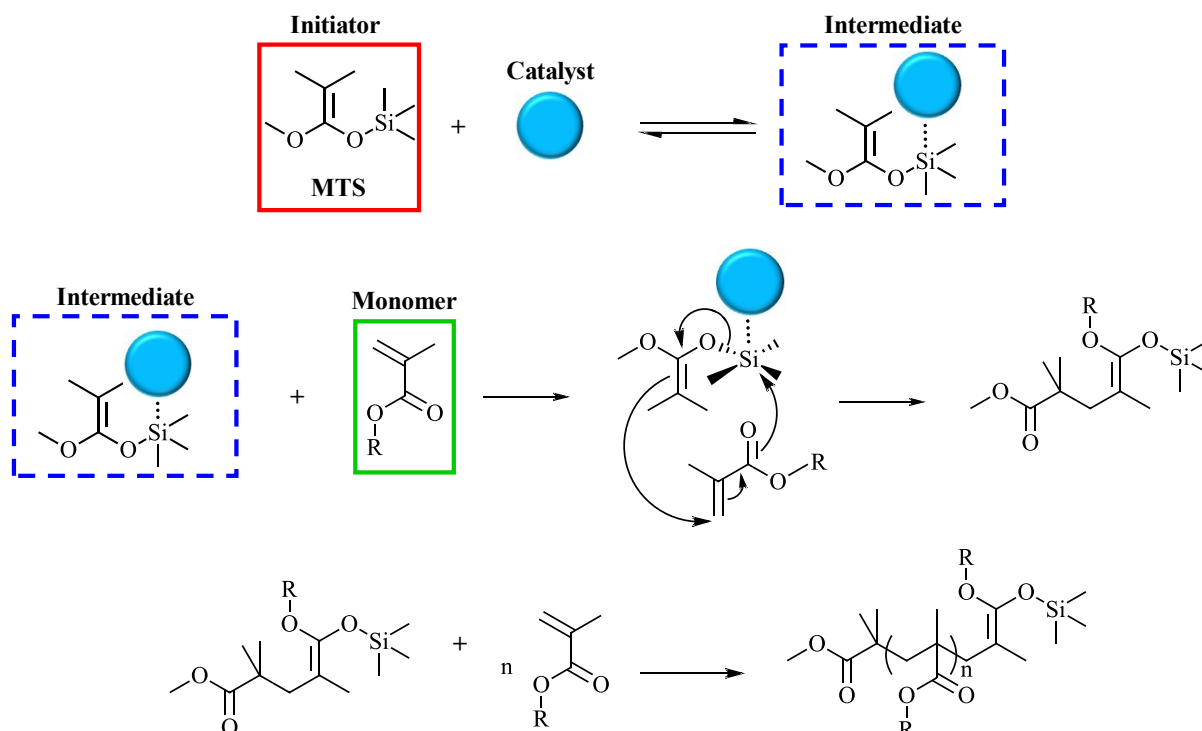


Figure 1.4: “Associative” mechanism of GTP. The catalyst (light blue circle) and the initiator (MTS, shown in red square) interact to form a pentacoordinate intermediate group (shown in blue), with which the monomer (shown in green square) interacts. The trimethylsilyl group is transferred to the end of the polymer chain, thus the technique is called “Group Transfer Polymerisation”.

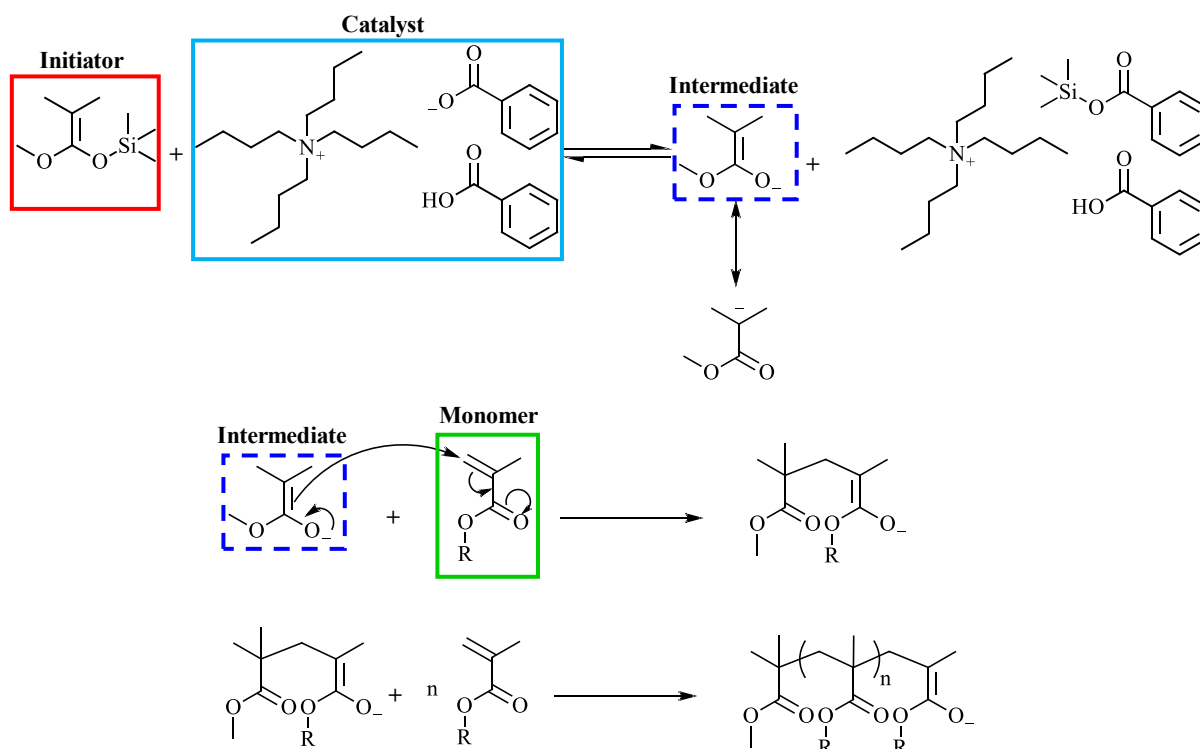


Figure 1.5: “Dissociative” mechanism of GTP. The exchange of the silyl group between the catalyst (TBABB, in light blue square) and the initiator (MTS, in red square) take place, and thus an enolate group (in blue square) is formed. The enolate group then interacts with the incoming monomers (in green square) to form the polymer chain. Note that system of initiator/catalyst shown in this scheme is the system that is used for the synthesis of all polymers throughout this PhD thesis.

1.4 Polymer Characterisation Techniques

Four major characterisation techniques have been used to characterise the polymers in this PhD project, and their theoretical background is discussed below.

1.4.1 Gel Permeation Chromatography (GPC)

Gel Permeation Chromatography (GPC) is an important tool for polymer scientists. It is a size exclusion chromatography (SEC), and as the name indicates, separation of the molecules takes place while they pass through a chromatographic column.^{29,71} In SEC, the molecules are separated because of their different size.^{29,71}

The GPC technique is schematically illustrated in Figure 1.6.^{29,71} As can be seen, the machine is equipped with a chromatographic column, which is formed by packed porous polymer beads (schematically shown in the magnifying lens on the right). The size of the pores determines the separation of the molecules, i.e. column of different pore size separates molecules of different range of MM. The sample to be analysed passes through the chromatographic column. At this point, small molecules (shown in green) pass through the pores of the beads, whereas big molecules (shown in red) pass around the pores. Therefore, the small molecules are trapped in the pores of the beads, and thus they take longer to reach the detector. As a result, the big molecules are detected first. As can be seen in the chromatogram (shown on the left), the peak corresponding to the big molecules appears at lower elution time (or elution volume) than the peak which correspond to the small molecules. The machine is calibrated prior to the measurement by running standard samples, i.e. well-defined polymers of known MM. The calibration curve (log MM *versus* elution time/elution volume) is used to determine the MM of the samples.^{29,71} The GPC provides important quantitative information about the MM characteristics of the molecule/polymer: i) number-average MM (M_n), ii) weight-average MM (M_w), iii) MM which corresponds to the maximum of the peak (M_p), and iv) dispersity index – shows the MMD ($D = M_w / M_n$).²⁹ GPC also provides valuable qualitative information, as it can be used to monitor the steps of a multi-step polymerisation and if for example all the homopolymer has extended to produce a diblock copolymer with the addition of a second monomer or not etc.

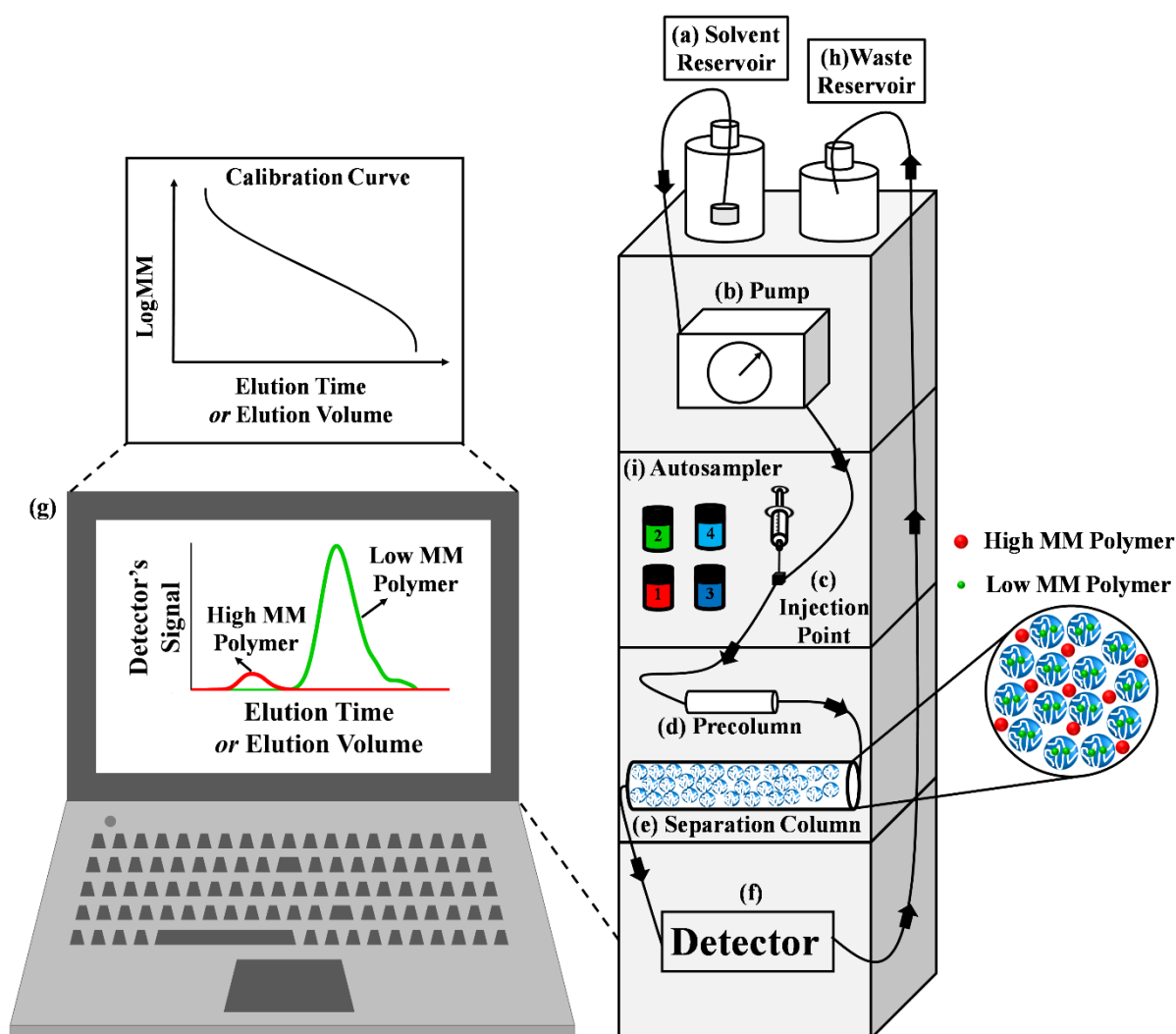


Figure 1.6: Schematic representation of the apparatus for gel permeation chromatography (GPC): (a) solvent reservoir, (b) pump (c) injection point, (d) precolumn, (e) separation column, (f) detector, (g) computer, and (h) waste reservoir. The more recent GPC apparatus are equipped with an autosampler (i).

1.4.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy is a technique which, as the name indicates, focuses on the nuclei of the atoms.²⁹ As NMR is a spectroscopy technique, absorption of radiation takes place from the radiofrequency range (1 – 500 MHz).²⁹

Generally, atoms with odd number of protons and/or neutrons in their nuclei, present nuclear spin quantum numbers (I), because of the charge spinning around the nuclear axis and thus, generating a magnetic moment.²⁹ In the simplest case of ^1H NMR, $I = \frac{1}{2}$ for ^1H . When an external magnetic field (B_0) is applied, this nuclear magnetic moment aligns either (a) parallel to the external magnetic field (low energy alignment) or (b) antiparallel to it (high energy

alignment). The resonant frequency (ν), is the frequency required to change from the (a) state to the (b) state.²⁹

The electronic environment of the atoms provides shielding on the nuclei from B_0 , i.e. the electrons are affected by B_0 and they produce an antiparallel magnetic field.²⁹ Therefore, this antiparallel magnetic field produced by the electrons “protects” the nucleus, and thus the protons feel a slightly different magnetic field compared to B_0 . When electron-donating groups are present, then the protection of the nucleus is enhanced (upfield), whereas in the case of electron-withdrawing groups, the nucleus is deprotected (downfield). Feeling different magnetic field means that the frequency they absorb is also different. The variable shown in an NMR spectrum is the chemical shift (δ), measured in ppm, and it is given by the following equation (Equation 1.2). Therefore, the chemical shift of the peaks gives information about the chemical structure of the compound, while the integration of the peaks (i.e. area under the peak) provides quantitative information.²⁹

$$\delta \text{ (ppm)} = 10^6 \times \frac{(\nu_0^{\text{sample}} - \nu_0^{\text{reference}})}{\nu_0} \quad \text{Equation 1.2}$$

1.4.3 Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS), as indicated by the name, is a technique that is related to the scattering of light by particles.^{29,72} More specifically, a monochromatic beam of light is shone and this light is scattered in all direction upon collision with the particles. A detector records the scattered intensity of the light. In DLS, the Brownian motion (the dynamics) is measured, i.e. the random motion of the particles resulted by the collision with the solvent molecules. The Brownian motion depends on the following parameters: i) viscosity of the solvent, ii) temperature, and iii) size of the particles. The first two are interrelated as the viscosity of the solvent does not only depend on the type (chemical nature) of the solvent, but also on the temperature. The size of the particles affects the Brownian motion as bigger particles move slower, whereas small particles move faster. As this motion is affected by the size of the particles, important information about their size can be obtained using DLS.^{29,72}

1.4.4 Rheology

Rheology is defined as the “science of flow and deformation of matter” (from the Greek $\rho\acute{\epsilon}\omega$ (rheo) = flow + $\lambda\acute{o}\gamma\omicron\varsigma$ (logos) = speech), and it can be studied by using a rheometer.⁷³ This

machine investigates the viscoelastic behaviour of the materials, while they experience an applied shear stress or shear strain. As shown in Figure 1.7 (a), the sample (coloured in light pink) is placed between two plates. The bottom plate is stationary, whereas the top plate rotates, thus applying shear stress or shear strain. The response of the material is recorded i.e. the strain is recorded if a stress is applied and *vice versa*. If a material behaves as an elastic solid, then it can store energy in the system, and therefore the applied stress at the top part of the sample is the same as the one at the bottom part. On the other hand, if the material is a viscous liquid, then the energy is dissipated as heat, and therefore, the applied stress at the top part of the sample is different than at the bottom part. The elastic behaviour of a material is described by the shear storage modulus (shear elastic modulus, G'), while the shear loss modulus (shear viscous modulus, G'') describes the viscous behaviour of a material. When the value of shear storage modulus is higher than the shear loss modulus, the elastic behaviour is dominant, and *vice versa*.⁷³

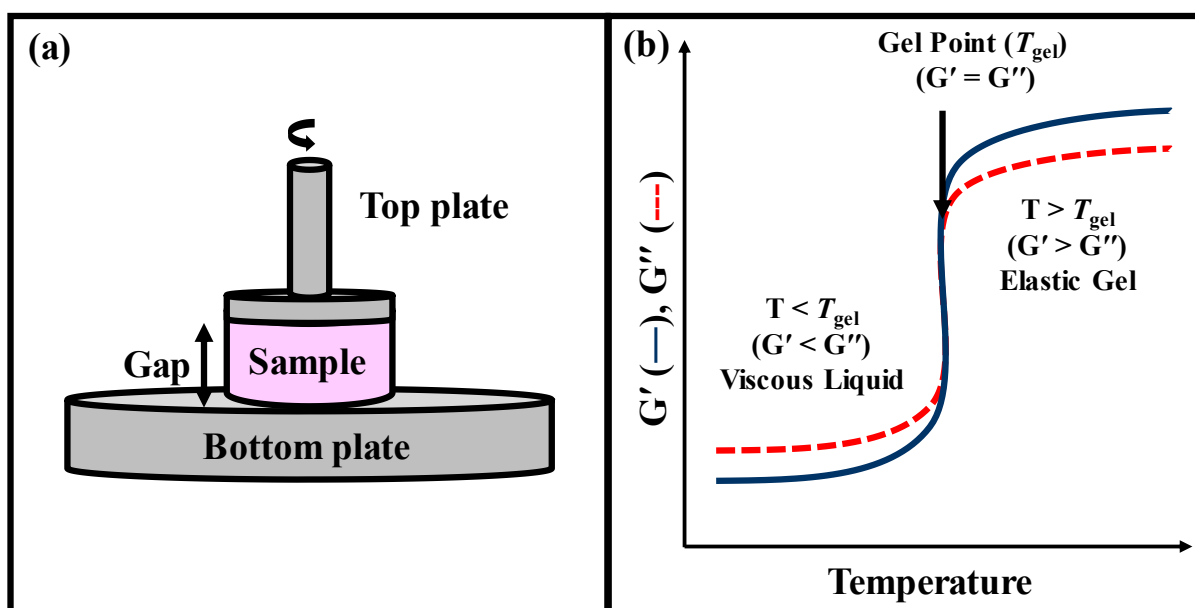


Figure 1.7: (a) a schematic illustration of the rheometer apparatus: a top plate, which rotates, applies a shear stress or shear strain to the sample, while the bottom plate records the response of the sample. The top plate shown is of parallel geometry, however a cone geometry is also available. (b) the expected rheology graph of an LCST thermoresponsive polymer solution which forms gel. The changes in shear storage modulus (shear elastic modulus, G'), and the shear loss modulus (shear viscous modulus, G'') over a range of temperatures are shown in dark blue solid and red dashed lines, respectively. The gelation point (T_{gel}) is defined as the temperature at which $G' = G''$. Below and above the T_{gel} , a viscous liquid behaviour ($G' < G''$) and an elastic gel behaviour ($G' > G''$) are dominant, respectively.

A thermoresponsive polymer solution can be analysed by rheology over a range of temperatures, and thus the T_{gel} can be determined, as discussed before. Figure 1.7 (b) shows the graph expected to be recorded when a thermoresponsive polymer solution with LCST forms gel as the temperature increases. As can be seen, at temperatures below the T_{gel} , the shear loss modulus, G'' , overcomes the shear storage modulus, G' , thus indicating that the liquid phase is prominent. As the temperature increases, both moduli increase rapidly in magnitude, and the instant at which $G'' = G'$ is determined as the T_{gel} . As the temperature increases above the T_{gel} , the shear storage modulus, G' , dominates, proving that an elastic solid/elastic gel is formed.

1.5 References

1. Benyus, J. M. *Biomimicry Innovation inspired by Nature*; Biomimicry Innovation inspired by Nature; HarperCollins Publishers Inc.: USA, 1997; , pp 146-185.
2. Pasparakis, G.; Vamvakaki, M. Multiresponsive polymers: nano-sized assemblies, stimuli-sensitive gels and smart surfaces. *Polym. Chem.* **2011**, *2*, 1234-1248.
3. Roy, I.; Gupta, M. N. Smart Polymeric Materials: Emerging Biochemical Applications. *Chem. Biol.* **2003**, *10*, 1161-1171.
4. Schild, H. G.; Tirrell, D. A. Microcalorimetric detection of lower critical solution temperatures in aqueous polymer solutions. *J. Phys. Chem.* **1990**, *94*, 4352-4356.
5. Bütün, V.; Armes, S. P.; Billingham, N. C. Synthesis and aqueous solution properties of near-monodisperse tertiary amine methacrylate homopolymers and diblock copolymers. *Polymer* **2001**, *42*, 5993-6008.
6. Saeki, S.; Kuwahara, N.; Nakata, M.; Kaneko, M. Upper and lower critical solution temperatures in poly (ethylene glycol) solutions. *Polymer* **1976**, *17*, 685-689.
7. Saeki, S.; Kuwahara, N.; Nakata, M.; Kaneko, M. Phase separation of poly(ethylene glycol)-water-salt systems. *Polymer* **1977**, *18*, 1027-1031.
8. Constantinou, A. P.; Georgiou, T. K. Thermoresponsive Multiblock Copolymers: Chemistry, Properties and Applications. In *Temperature-Responsive Polymers: Chemistry, Properties, and Applications*; Khutoryanskiy, V. V., Georgiou, T. K., Eds.; John Wiley & Son Ltd: UK, 2018; pp 35-65.
9. Steinhauer, W.; Hoogenboom, R.; Keul, H.; Moeller, M. Block and gradient copolymers of 2-hydroxyethyl acrylate and 2-methoxyethyl acrylate via RAFT: Polymerization kinetics, thermoresponsive properties, and micellization. *Macromolecules* **2013**, *46*, 1447-1460.
10. Adelsberger, J.; Bivigou-Koumba, A. M.; Miasnikova, A.; Busch, P.; Laschewsky, A.; Müller-Buschbaum, P.; Papadakis, C. M. Polystyrene-block-poly (methoxy diethylene glycol acrylate)-block-polystyrene triblock copolymers in aqueous solution—a SANS study of the temperature-induced switching behavior. *Colloid Polym. Sci.* **2015**, *293*, 1515-1523.
11. Negru, I.; Teodorescu, M.; Stanescu, P. O.; Draghici, C.; Lungu, A.; Sârbu, A. Thermogelation properties of ABA triblock copolymers of poly(ethylene glycol) (B) and copolyacrylates of oligo(ethylene glycol)s (A) in aqueous solution. *Soft Materials* **2013**, *11*, 149-156.
12. Zhu, W.; Nese, A.; Matyjaszewski, K. Thermoresponsive star triblock copolymers by combination of ROP and ATRP: From micelles to hydrogels. *J. Polym. Sci. Part A* **2011**, *49*, 1942-1952.
13. Luzón, M.; Corrales, T.; Catalina, F.; San Miguel, V.; Ballesteros, C.; Peinado, C. Hierarchically organized micellization of thermoresponsive rod-coil copolymers based on poly[oligo(ethylene glycol) methacrylate] and poly(e-caprolactone). *J. Polym. Sci. Part A* **2010**, *48*, 4909-4921.
14. Southall, N. T.; Dill, K. A.; Haymet, A. D. J. A View of the Hydrophobic Effect. *J Phys Chem B* **2002**, *106*, 521-533.
15. Ward, M. A.; Georgiou, T. K. Thermoresponsive Polymers for Biomedical Applications. *Polymers* **2011**, *3*, 1215-1242.
16. Buwalda, S. J.; Boere, K. W. M.; Dijkstra, P. J.; Feijen, J.; Vermonden, T.; Hennink, W. E. Hydrogels in a historical perspective: From simple networks to smart materials. *J. Controlled Release* **2014**, *190*, 254-273.
17. Constantinou, A. P.; Georgiou, T. K. Tuning the gelation of thermoresponsive gels. *Eur. Polym. J.* **2016**, *78*, 366-375.
18. Thakur, R. R. S.; Fallows, S. J.; McMillan, H. L.; Donnelly, R. F.; Jones, D. S. Microneedle-mediated intrascleral delivery of in situ forming thermoresponsive implants for sustained ocular drug delivery. *J. Pharm. Pharmacol.* **2014**, *66*, 584-595.
19. Hyun, H.; Kim, Y. H.; Song, I. B.; Lee, J. W.; Kim, M. S.; Khang, G.; Park, K.; Lee, H. B. In Vitro and in Vivo Release of Albumin Using a Biodegradable MPEG-PCL Diblock Copolymer as an in Situ Gel-Forming Carrier. *Biomacromolecules* **2007**, *8*, 1093-1100.
20. Hyun, H.; Park, H. S.; Kwon, Y. D.; Khang, G.; Lee, B. H.; Kim, S. M. Thermo-Responsive Injectable MPEG-Polyester Diblock Copolymers for Sustained Drug Release. *Polymers* **2014**, *6*, 2670-2683.

21. Gao, C.; Li, Q.; Cui, Y.; Huo, F.; Li, S.; Su, Y.; Zhang, W. Thermoresponsive diblock copolymer micellar macro-RAFT agent-mediated dispersion RAFT polymerization and synthesis of temperature-sensitive ABC triblock copolymer nanoparticles. *J. Polym. Sci. Part A: Polym. Chem.* **2014**, *52*, 2155-2165.
22. de Souza, J.; Carlos Perbone; Naves, A. F.; Florenzano, F. Specific thermoresponsiveness of PMMA-block-PDMAEMA to selected ions and other factors in aqueous solution. *Colloid Polym. Sci.* **2012**, *290*, 1285-1291.
23. Feil, H.; Bae, Y. H.; Feijen, J.; Kim, S. W. Effect of comonomer hydrophilicity and ionization on the lower critical solution temperature of N-isopropylacrylamide copolymers. *Macromolecules* **1993**, *26*, 2496-2500.
24. Fournier, D.; Hoogenboom, R.; Thijs, H. M. L.; Paulus, R. M.; Schubert, U. S. Tunable pH- and temperature-sensitive copolymer libraries by reversible addition-fragmentation chain transfer copolymerizations of methacrylates. *Macromolecules* **2007**, *40*, 915-920.
25. Florin, E.; Kjellander, R.; Eriksson, J. C. Salt effects on the cloud point of the poly(ethylene oxide)+ water system. *J. Chem. Soc., Faraday Trans. 1* **1984**, *80*, 2889-2910.
26. Cheng, V.; Lee, B. H.; Pauken, C.; Vernon, B. L. Poly(N-isopropylacrylamide-co-poly(ethylene glycol))-acrylate simultaneously physically and chemically gelling polymer systems. *J Appl Polym Sci* **2007**, *106*, 1201-1207.
27. WEBSTER, O. W. Living Polymerization Methods. *Science* **1991**, *251*, 887.
28. Hiemenz, P. C.; Lodge, T. P. *Polymer Chemistry Second Edition*; Taylor & Francis Group, LLC: US, 2007; , pp 608.
29. Young, R. J.; Lovell, P. A. *Introduction to Polymers*; CRC Press Taylor & Francis Group: USA, 2011; , pp 668.
30. Carraher, C. E. J. *Introduction to Polymer Chemistry Third Edition*; Taylor & Francis Group, LLC: US, 2013; , pp 554.
31. Odian, G. *Principles of Polymerization*; John Wiley & Sons, Inc.: US, 2004; , pp 832.
32. Rempp, P.; Merrill, E. W. *Polymer Synthesis*; Heutig and Wepf: US, 1991; , pp 344.
33. Quirk, R. P.; Lee, B. Experimental Criteria for Living Polymerizations. *Polym. Int.* **1992**, *27*, 359-367.
34. Müller, A. H. E.; Matyjaszewski, K. *Controlled and Living Polymerizations: From Mechanisms to Applications*; WILEY-VCH Verlag GmbH & Co: Germany, 2009; , pp 610.
35. McNaught, A. D.; Wilkinson, A. *IUPAC, Compendium of Chemical Terminology, 2nd edition (the "Gold Book")*. Blackwell Scientific Publications: Oxford, 1997; .
36. SZWARC, M. 'Living' Polymers. *Nature* **1956**, *178*, 1168.
37. Szwarc, M. Living polymers. Their discovery, characterization, and properties. *J. Polym. Sci. A Polym. Chem.* **1998**, *36*, IX-XV.
38. Grubbs, R. B.; Grubbs, R. H. 50th Anniversary Perspective: Living Polymerization—Emphasizing the Molecule in Macromolecules. *Macromolecules* **2017**, *50*, 6979-6997.
39. Matyjaszewski, K.; Xia, J. Atom Transfer Radical Polymerization. *Chem. Rev.* **2001**, *101*, 2921-2990.
40. Moad, G.; Rizzardo, E.; Thang, S. Living Radical Polymerization by the RAFT Process – A Second Update. *Australian Journal of Chemistry - AUST J CHEM* **2009**, *62*.
41. Webster, O. W.; Hertler, W. R.; Sogah, D. Y.; Farnham, W. B.; RajanBabu, T. V. Group-transfer polymerization. 1. A new concept for addition polymerization with organosilicon initiators. *J. Am. Chem. Soc.* **1983**, *105*, 5706-5708.
42. Rikkou-Kalourkoti, M.; Webster, O. W.; Patrickios, C. S. Group Transfer Polymerization. *Encyclopedia of Polymer Science and Technology* **2013**.
43. Webster, O. W. Group Transfer Polymerization: A Critical Review of Its Mechanism and Comparison with Other Methods for Controlled Polymerization of Acrylic Monomers. In *New Synthetic Methods* Springer Berlin Heidelberg: Berlin, Heidelberg, 2004; pp 1-34.
44. Hertler, W. R. Group Transfer Polymerisation for Controlled Polymer Architecture. In *Macromolecular Design of Polymeric Materials*; Hatada, K., Kitayama, T. and Vogl, O., Eds.; Marcel Dekker Inc.: USA, 1997; pp 109-122.

45. Nagaki, A.; Takahashi, Y.; Akahori, K.; Yoshida, J. Living Anionic Polymerization of tert-Butyl Acrylate in a Flow Microreactor System and Its Applications to the Synthesis of Block Copolymers. *Macromolecular Reaction Engineering* **2012**, *6*, 467-472.
46. Nagaki, A.; Miyazaki, A.; Yoshida, J. Synthesis of Polystyrenes–Poly(alkyl methacrylates) Block Copolymers via Anionic Polymerization Using an Integrated Flow Microreactor System. *Macromolecules* **2010**, *43*, 8424-8429.
47. Carroll, D. R.; Constantinou, A. P.; Stingelin, N.; Georgiou, T. K. Scalable syntheses of well-defined pentadecablock bipolymer and quintopolymer. *Polym. Chem.* **2018**, *9*, 3450-3454.
48. Haddleton, D. M.; Muir, A. V. G.; Richards, S. N. Industrial Applications of Living Polymerization. In *Macromolecular Design of Polymeric Materials*; Hatada, K., Kitayama, T. and Vogl, O., Eds.; Marcel Dekker, Inc.: New York, 1997; pp 123-137.
49. Ward, M. A.; Georgiou, T. K. Thermoresponsive triblock copolymers based on methacrylate monomers: effect of molecular weight and composition. *Soft Matter* **2012**, *8*, 2737-2745.
50. Ghasdian, N.; Ward, M. A.; Georgiou, T. K. Well-defined clickable copolymers prepared via one-pot synthesis. *Chem. Commun.* **2014**, *50*, 7114-7116.
51. Chen, Y.; Kitano, K.; Tsuchida, S.; Kikuchi, S.; Takada, K.; Satoh, T.; Kakuchi, T. B(C₆F₅)₃-catalyzed group transfer polymerization of alkyl methacrylates with dimethylphenylsilane through in situ formation of silyl ketene acetal by B(C₆F₅)₃-catalyzed 1,4-hydrosilylation of methacrylate monomer. *Polym. Chem.* **2015**, *6*, 3502-3511.
52. Fuchise, K.; Chen, Y.; Takada, K.; Satoh, T.; Kakuchi, T. Effect of counter anions on kinetics and stereoregularity for the strong Brønsted acid-promoted group transfer polymerization of N,N-Dimethylacrylamide. *Macromol. Chem. Phys.* **2012**, *213*, 1604-1611.
53. Fuchise, K.; Sakai, R.; Satoh, T.; Sato, S. I.; Narumi, A.; Kawaguchi, S.; Kakuchi, T. Group transfer polymerization of N,N-dimethylacrylamide using noble efficient system consisting of dialkylamino silyl enol ether as an initiator and strong Brønsted acid as an organocatalyst. *Macromolecules* **2010**, *43*, 5589-5594.
54. Kikuchi, S.; Chen, Y.; Kitano, K.; Takada, K.; Satoh, T.; Kakuchi, T. Organic acids as efficient catalysts for group transfer polymerization of N,N-disubstituted acrylamide with silyl ketene acetal: polymerization mechanism and synthesis of diblock copolymers. *Polym. Chem.* **2015**, *6*, 6845-6856.
55. Kakuchi, T.; Chen, Y.; Kitakado, J.; Mori, K.; Fuchise, K.; Satoh, T. Organic superbase as an efficient catalyst for group transfer polymerization of methyl methacrylate. *Macromolecules* **2011**, *44*, 4641-4647.
56. Fuchise, K.; Chen, Y.; Satoh, T.; Kakuchi, T. Recent progress in organocatalytic group transfer polymerization. *Polym. Chem.* **2013**, *4*, 4278-4291.
57. Kakuchi, R.; Chiba, K.; Fuchise, K.; Sakai, R.; Satoh, T.; Kakuchi, T. Strong Brønsted acid as a highly efficient promoter for group transfer polymerization of methyl methacrylate. *Macromolecules* **2009**, *42*, 8747-8750.
58. Kikuchi, S.; Chen, Y.; Ichinohe, E.; Kitano, K.; Sato, S.; Duan, Q.; Shen, X.; Kakuchi, T. Synthesis and Thermoresponsive Property of Linear, Cyclic, and Star-Shaped Poly(N,N-diethylacrylamide)s Using B(C₆F₅)₃-Catalyzed Group Transfer Polymerization as Facile End-Functionalization Method. *Macromolecules* **2016**, *49*, 4828-4838.
59. Eric, O.; Chen, Y.; Takada, K.; Sato, S.; Satoh, T.; Kakuchi, T. Synthesis of AB block and A₂B₂ and A₃B₃ miktoarm star-shaped copolymers using ω -end-functionalized poly(methyl methacrylate) with a hydroxyl group prepared by organocatalyzed group transfer polymerization. *Polym. Chem.* **2015**, *6*, 7841-7850.
60. Takada, K.; Ito, T.; Kitano, K.; Tsuchida, S.; Takagi, Y.; Chen, Y.; Satoh, T.; Kakuchi, T. Synthesis of homopolymers, diblock copolymers, and multiblock polymers by organocatalyzed group transfer polymerization of various acrylate monomers. *Macromolecules* **2015**, *48*, 511-519.
61. Takada, K.; Fuchise, K.; Kubota, N.; Ito, T.; Chen, Y.; Satoh, T.; Kakuchi, T. Synthesis of α -, ω -, and α,ω -End-Functionalized Poly(n-butyl acrylate)s by Organocatalytic Group Transfer Polymerization Using Functional Initiator and Terminator. *Macromolecules* **2014**, *47*, 5514-5525.
62. Hsu, J.; Chen, Y.; Kakuchi, T.; Chen, W. Synthesis of Linear and Star-Shaped Poly4-(diphenylamino)benzyl methacrylate)s by Group Transfer Polymerization and Their Electrical Memory Device Applications. *Macromolecules* **2011**, *44*, 5168-5177.

63. Chen, Y.; Takada, K.; Fuchise, K.; Satoh, T.; Kakuchi, T. Synthesis of syndiotactic-rich star-shaped poly(methyl methacrylate) by core-first group transfer polymerization using N-(trimethylsilyl)bis(trifluoromethanesulfonyl)imide. *J. Polym. Sci. A Polym. Chem.* **2012**, *50*, 3277-3285.
64. Kikuchi, S.; Chen, Y.; Fuchise, K.; Takada, K.; Kitakado, J.; Sato, S.; Satoh, T.; Kakuchi, T. Thermoresponsive properties of 3-, 4-, 6-, and 12-armed star-shaped poly2-(dimethylamino)ethyl methacrylate]s prepared by core-first group transfer polymerization. *Polym. Chem.* **2014**, *5*, 4701-4709.
65. Chen, Y.; Fuchise, K.; Narumi, A.; Kawaguchi, S.; Satoh, T.; Kakuchi, T. Core-First Synthesis of Three-, Four-, and Six-Armed Star-Shaped Poly(methyl methacrylate)s by Group Transfer Polymerization Using Phosphazene Base. *Macromolecules* **2011**, *44*, 9091-9098.
66. Takada, K.; Fuchise, K.; Chen, Y.; Satoh, T.; Kakuchi, T. Controlled polymerization of methyl acrylate for high-molecular-weight polymers by pentafluorophenylbis(triflyl)methane-promoted group transfer polymerization using triisopropylsilyl ketene acetal. *J. Polym. Sci. A Polym. Chem.* **2012**, *50*, 3560-3566.
67. Webster, O. W. Discovery and commercialization of group transfer polymerization. *J. Polym. Sci. Part A* **2000**, *38*, 2855-2860.
68. Müller, A. H. E. Kinetic Discrimination between Various Mechanisms in Group-Transfer Polymerization. *Macromolecules* **1994**, *27*, 1685-1690.
69. Müller, A. H. E.; Litvinenko, G.; Yan, D. Kinetic analysis of "living" polymerization systems exhibiting slow equilibria. 3. "Associative" mechanism of group transfer polymerization and ion pair generation in cationic polymerization. *Macromolecules* **1996**, *29*, 2339-2345.
70. Müller, A. H. E.; Litvinenko, G.; Yan, D. Kinetic analysis of "living" polymerization systems exhibiting slow equilibria. 4. "Dissociative" mechanism of group transfer polymerization and generation of free ions in cationic polymerization. *Macromolecules* **1996**, *29*, 2346-2353.
71. Bly, D. D. Gel Permeation Chromatography. *Science* **1970**, *168*, 527-533.
72. Stetefeld, J.; McKenna, S. A.; Patel, T. R. Dynamic light scattering: a practical guide and applications in biomedical sciences. *Biophysical Reviews* **2016**, *8*, 409-427.
73. Macosko, C. W., *Rheology: principles, measurements, and applications*; 1994.

CHAPTER 2: APPLICATIONS OF THERMORESPONSIVE GELS

TRGs have received much attention due to their potential application in the tissue engineering (TE) field, and their investigation as injectable gels has been well reported in the literature.^{1,2} For the TE application, an aqueous polymer solution is loaded into a syringe at room temperature (r.t.) and then upon injection into the body, a stable gel is formed, because of the increase in temperature at body temperature (b.t.); this is shown in Figure 2.1 (a). A great deal of research that focuses on the synthesis and characterisation of new thermoresponsive copolymers has been already published.³⁻¹¹ These studies aim to produce TRG which show clear sol-gel transition at b.t..³⁻¹¹

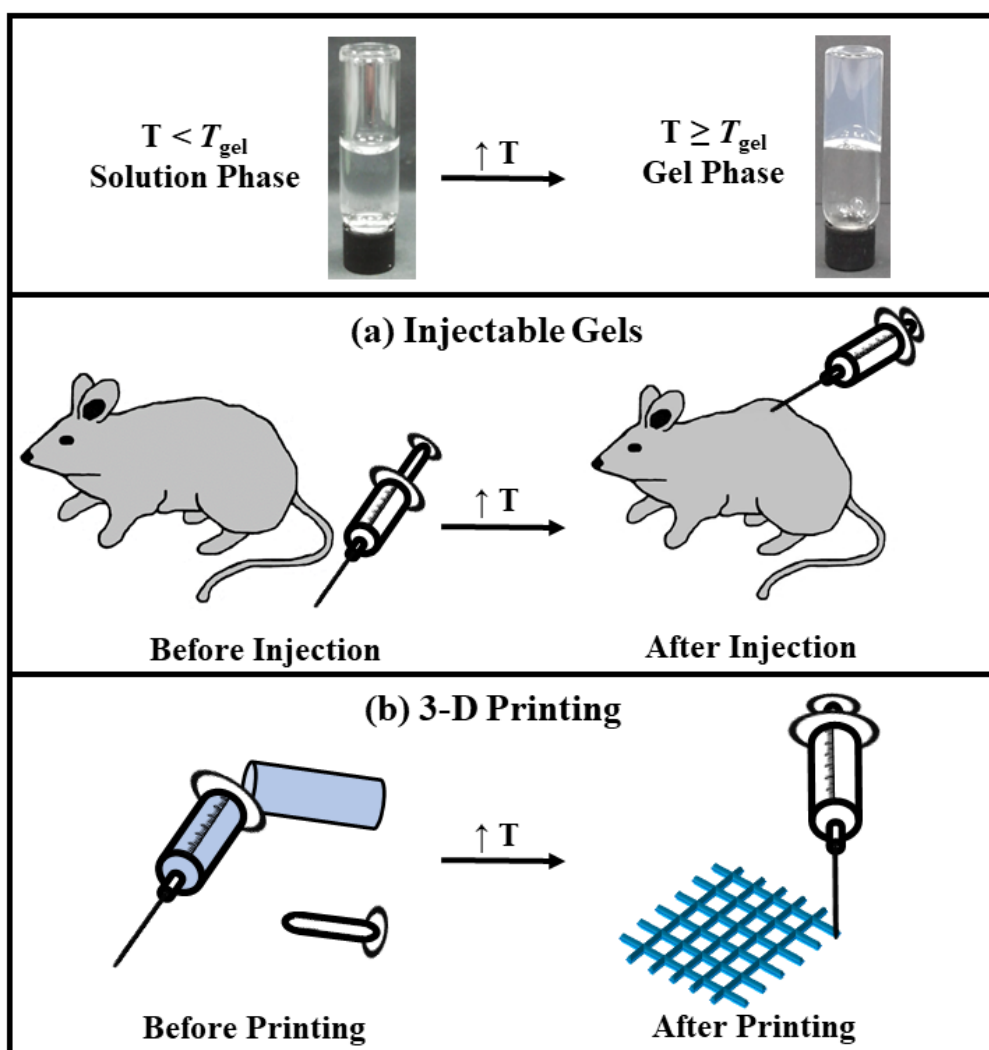


Figure 2.1: The application of the thermoresponsive gels (TRGs) as (a) injectable gels and (b) 3-D printable materials. The sol-phase facilitates the loading of a thermoresponsive polymer solution into a syringe ($T < T_{gel}$, left) and gelation occurs because of the temperature increase above the gelation point ($T > T_{gel}$, right).

In addition to their application in the TE field, TRGs are currently being investigated as 3D printable materials.^{12,13} This application requires a sol-gel transition either at r.t. or at b.t., depending on the temperature at which a stable printed structure is required. As shown in Figure 2.1 (b), the sol phase is useful for loading the ink into a syringe, whereas the gel phase is printed to a stable 3D structure.^{12,13} As defined by Mironov *et al.*, bioprinting is “the use of material transfer processes for patterning and assembling biologically relevant materials - molecules, cells, tissues, and biodegradable biomaterials - with a prescribed organization to accomplish one or more biological functions”.¹⁴ In some cases, the TRG is mixed with cells (referred as bio-ink), thus protecting the cells from the shear stresses while printing.¹² The printed structure can be used either as tissue model for biological studies or as an implant.¹²

Since TRGs can be used as injectable gels and 3D printable materials, the literature review focuses on studies in which the injectability of TRGs has been tested in model animals, and/or stable structures have been successfully printed.

2.1 Injectable Gels

TRGs have been widely studied the last 50 years, with the first systematic study on the TRGs with LCST behaviour dating back to 1984.¹⁵ The progress in polymer science facilitated the synthesis of thermoresponsive polymers and thus many studies have been published on the topic. Natural polymers possessing thermoresponsive properties have also been investigated as TRGs.^{16,17}

Synthetic polymers are generally preferable over natural polymers.^{18,19} Even though natural polymers are bioactive and biodegradable, they require extensive purification prior to use and they also possess the risk of transmitting diseases. Furthermore, synthetic polymers have the advantage that they are tailor-able, i.e. their properties can be tuned to match the requirements of the desired application. Also, the synthesis is reproducible, thus they are characterised by “batch-to-batch uniformity”. In addition, they can be produced in a large scale, they are cheaper, and they have long shelf-time.^{18,19}

As synthetic polymers are advantageous over natural polymers, this review focuses on presenting the studies in which synthetic TRGs with LCST behaviour have been applied *in vivo*. These studies are categorised in four subdivisions depending on the general polymer chemical structure: i) poloxamer based, ii) other degradable EG based besides poloxamer, iii) NIPAAm based, and iv) poly(organophosphazene)s. The majority of the *in vivo* tested

injectable gels shows sol-gel transition between r.t. and b.t., thus the sol phase was loaded into a syringe and upon injection into a model animal, a gel was formed. Nevertheless, injection of the TRGs whilst are in the gel phase was also tested.^{16,20-22} Interestingly, ReGel® polymer, which is based on EG and poly(lactic acid-*co*-glycolic acid) (PLGA), shows a sol-gel transition at temperatures lower than r.t., and has reached to clinical studies.²¹

Prior to the *in vivo* application, gelation at b.t. and *in vitro* biocompatibility are confirmed. In the case of degradable systems, *in vitro* degradability tests are also carried out. When the TRGs are used as drug-delivery matrix, the effect of the drug on the gelation properties is also studied, since it has been previously shown that the incorporation of drugs affects the gelation.^{23,24}

It should be noted that the aim of this review is not to summarise and discuss how the structural characteristics of the polymers affect the gelation properties, since this is well-established and has been previously summarised.²⁵ More specifically, it is well-known that the increase in MM and hydrophobicity of the polymer, as well as the increase in the polymer concentration favour gelation.²⁵ Also, it has been previously observed that when hydrolytically-degradable polymers are concerned, the increase in the hydrophobicity of the polymer and the polymer concentration reduce the degradation rate,²⁶⁻²⁸ as expected. The degradation rate is also well-correlated with the drug-release rate, as they are proportionally related.^{28,29} Therefore, the aforementioned trends are not discussed, and this review is purely focused on giving examples of TRGs that have reached *in vivo* applications in order to identify key steps that are required for reaching clinical studies.

The thermoresponsive (co-)polymers used in these studies are based either on EG, NIPAAm, or poly(organophosphazene)s. These polymers are combined with other species in order to achieve gelation at the desired temperature, to improve the mechanical properties of the gel, and/or to introduce fast-degradability of the structure. The names, the chemical structures, and the abbreviations of the most common thermoresponsive units applied *in vivo*, the monomers which have been used to introduce degradability into the structure, and the commercially available thermoresponsive polymer (Pluronic) are shown in Figure 2.2.

Thermoresponsive component applied <i>in vivo</i>			
Ethylene glycol (EG) <i>same unit as</i> Ethylene oxide (EO)	<i>N</i> -isopropylacrylamide (NIPAAm)	Organophosphazene	
Common monomer to introduce degradability			
ε-Caprolactone (CL)	Glycolide (GL)	Lactide (LA)	Amino Acid
Commercially available thermoresponsive polymer			
Poloxamer <u>Registered Tradenames:</u> Pluronic®, Lutrol®, and Kolliphor® (BASF) Synperonic® (Croda) and Antarox® (Rhodia)			

Figure 2.2: The names, the chemical structures, and the abbreviations of the most common thermoresponsive components applied *in vivo* (top), the monomers typically incorporated into the structure to introduce degradability (middle), and the commercially available thermoresponsive polymer (bottom).

The thermoresponsive systems studied, the model species on which the injectable gels were applied, the model drugs that were used (if used), and the main *in vivo* result are summarised in Table 2.1. Table 2.1 also clarifies the polymer architecture, and the studies are shown in an order of increased complexity of architecture (within the same category of TRG). Additional groups that are chemically bonded on the main thermoresponsive species are also presented. Any polymeric additives that are physically mixed in the gel precursor are also listed, as they affect the gelation temperature and concentration.

Table 2.1: Summary of the *in vivo* studies on injectable gels.

Category	Architecture	Additional covalently-linked species	Polymer Additives	Model Species	Model Drug	Main <i>in vivo</i> Result	Ref.
Poloxamer-based (Poloxamer 407)	Triblock	-	-	Rat	HIV vector	-	30
				Rabbit	QF	↑ Bioavailability	31
				Mouse	PTX & Lapatinib	Control of tumour	32
				Gerbil	Vancomycin	Local release	33
			P188	Rabbit	Piroxicam	Controlled release	34
				Rat	DOX	Controlled release	35
				Mouse	PTX	Control of tumour	36
					Astragalus	Controlled release	37
			P124	Mouse	Metformin	Controlled release	38
			PVA	Rat	Octreotide	Controlled release	39
			HPMC	Rat	PTX, TMZ	Controlled release	40
			HPMC, P188, SA	Rat	Insulin	Controlled release	41
		tetraaniline	-	Rat	-	Successful injection	42
	Graft	chitosan	-	Mouse	Vaccines	Successful release	43
	Pentablock	triMeCa	-	Rabbit	Mitomycin C	Improved health	44
		LA	-	Rat	Antimicrobial peptides	Wound healing	45
				Mouse	DOX	Control of tumour	46
Other Degradable EG-based besides Poloxamer	Diblock	CL, DO	-	Rat	BSA / Bovine insulin	Controlled Release	47,48
		LA, CPH	-	Rat	-	Biocompatibility	49
	Triblock	CL	-	Mouse	-	Degradability	26,28
				Rat	Lidocaine	Controlled release	27
				Mouse	bFGF	Controlled release	29,50
					CPT	Control of tumour	51
		PLGA	-	Mouse	Interleukin-2	Control of tumour	52
				Rat	-	Degradation	24

					5-Fu	Prevented adhesion	53
				Rabbit	-	Degradation	54
					A-phospholipid	Controlled release	55
				Rat, Pig, Dog, Human	PTX (OncoGel®)	Control of tumour	20,21
				Rat	-	Biocompatibility	56
		PCLA	-	Rat		Degradation	22
				Rat	COX	Controlled release	57
				Mouse, Dog	COX	Improved health	58
		CL, GA		Mouse	Liraglutide	Hypoglycaemia	59
		CL, TSUN	HA	Mouse	DOX	Control of tumour	60
		ESHU	-	Rat	-	Biocompatibility	61
				Rabbit	Bevacizumab	Controlled Release	62
		Peptide	-	Rat, Mouse	CA4, DTX	Control of tumour	63
				Rabbits	-	Tissue formation	64
				Rat	-	Degradation	65
	Pentablock	PCL, PLA		Mouse	Vaccines	Control of tumour	66
	Multilock	PG, PTHFC	-	Mouse	DOX	Control of tumour	67
	Star	PLGA	-	Rat	-	Degradation	68
NIPAAm-based	Random	AA, BuLaA	-	Rat	-	Gelation	69
		AA, NHS, ADMBuLA	-	Mouse	-	Degradation	70
		NEGMA, MAA, tetraaniline	-	Rat	-	Biocompatibility	71
		BuA	-	Rabbit	-	Biocompatibility	72
		HAMA	-	Rabbit	-	Tissue formation	73
	PNIPAAm	-	Dextran-CL-HEMA	Rat	BSA	Controller release	74
	Triblock	DMAAm, PS	-	Mouse	Nile red / Rhodamine	Controlled release	75

		DMAAm, PS, CL, PLGA	-	Mouse	Nile red	Controlled release	76
	Pentablock	CL, EG	-	Rat	-	Gelation	77
	Branch	PEGC	-	Rat	Chemokine	Tissue formation	78
			gelatin	Mouse	-	Osteogenic Differentiation	79,80
			-	Mouse	-	Osteoinduction	81
			-	Mouse	-	Bone formation	82
	Graft	HA	-	Mouse	-	Gelation	83
				Rabbit	BSA	Controlled release	84
					-	Cartilage formation	85
				Rat	-	Bone regeneration	86
		Gelatin		Rabbit	-	Chondrogenesis	87
		Chitosan			-		88
Poly (organophosphazene)s	Random	-	-	Mouse	DOX, PTX, DTX	Control of tumour	89-92
		DOX, CPT		Mouse	DOX, CPT	Control of tumour	93,94
		-	-	Rat	HGH	Controlled release	95
		protamine	-	Mouse, monkey	HGH	Controlled release	96
		-	-	Mouse	BMP-2	Controlled release	97
		-	Collagen	Mouse	-	Cell proliferation	98
		-	-	Mouse	DTX / gene	Controlled release	99

Abbreviations: AA – acrylic acid, ADMBuLA – acryloyloxy dimethyl- γ -butyrolactone, bFGF – basic fibroblast growth factor, CPH – 1,6-bis(*p*-carboxyphenoxy)hexan, BMP-2 – bone morphogenetic protein-2, BSA – bovine serum albumin, BuA – butyl acrylate, BuLaA – γ -butyrolactone acrylate, CPT – camptothecin, CL – ϵ -caprolactone, COX – celecoxib, CA4 – Combrestatin, DMAAm – *N,N*-dimethylacrylamide, DO – *p*-dioxanone, DTX – docetaxel, DOX – doxorubicin, EG – ethylene glycol, 5-Fu – 5-fluorouracil, GA – glycolic acid, HGH – human growth hormone, HIV – human immunodeficiency virus, HA – hyaluronic acid, HAMA – hyaluronan methacrylate, HEMA – 2-hydroxyethyl methacrylate, HPMC – hydroxypropyl methyl cellulose, NHS – *N*-hydroxysuccinimide, NIPAAm – *N*-isopropylacrylamide, LA – lactic acid, MAA – methacrylic acid, PTX – paclitaxel, P124 – poloxamer 124, P188 – poloxamer 188, PCL – poly(ϵ -caprolactone), PCLA – poly(ϵ -caprolactone-*co*-lactide), PEGC – poly(ethylene glycol) citrate, NEGMA – nona(ethylene glycol) methyl ether methacrylate, PNIPAAm – poly(*N*-isopropylacrylamide), PLA – poly(lactic acid), PLGA – poly(lactic acid-*co*-glycolic acid), PTHFC – poly(polytetrahydrofuran carbonate), PG – propylene glycol, PS – poly(styrene), PVA – poly(vinyl alcohol), QF – quetiapine fumarate, ESHU – serinol hexamethylene urethane, SA – sodium alginate, TMZ – temozolomide, triMeCa – trimethylene carbonate, TSUN – 1,4,8-trioxa[4.6]spiro-9-undecanone.

2.1.1 Poloxamer Based

The most extensively studied thermoresponsive polymers, which are commercially available, are poloxamers. These are ABA triblock copolymers with A and B blocks based on EO and propylene oxide (PO, same unit as propylene glycol, PG), respectively. Several registered tradenames are available including Pluronic®,¹⁰⁰ Lutrol®, and Kolliphor®, available by BASF, and Synperonic® and AntaroX®, available by Croda and Rhodia, respectively.¹⁰¹ Several poloxamers are commercially available, which differ in the composition and MM.¹⁰¹ The poloxamers which have been incorporated in *in vivo* injectable systems are: i) poloxamer 407 (P407, Pluronic® F127),³⁰⁻⁴⁴ ii) poloxamer 188 (P188, Pluronic® F68),³⁴⁻³⁷ iii) poloxamer 124 (P124, Lutrol® L44),³⁸ and iv) poloxamer 105 (P105, Pluronic® L35).^{45,46} The most commonly used poloxamer in TRG-related studies is poloxamer 407 (Pluronic® F127), with MM equal to 12.5 kg mol⁻¹. In the following text, only the general name poloxamer and its corresponding code are used for simplicity and direct comparison between the studies.

Four studies in which pure poloxamer 407 aqueous solutions, i.e. without any polymer additives, were applied *in vivo* have been found in the literature.³⁰⁻³³ In two of the studies, 15 wt% polymer solution was investigated as a carrier of i) an HIV-1 lentiviral vector in the brain of a rat (HIV stands for human immunodeficiency virus),³⁰ and ii) lipospheres containing quetiapine fumarate (QF) in rabbits.³¹ Interestingly, in the second study, poloxamer 407 protected the lipospheres from *in vivo* degradation by lipases, thus also extending the time of QF being present in the body.³¹ 20 wt% of the polymer solution was also investigated as a carrier of paclitaxel (PTX) and lapatinib particles in tumour-bearing mice.³² Upon injection, fast release of PTX and controlled release of lapatinib were observed, which controlled the tumour growth.³² Lee *et al.* observed cure of chronic otitis in gerbils upon local release of vancomycin from a gel matrix formed by 25 wt% polymer solution.³³

Formulations based on mixture of poloxamers have also been investigated as *in vivo* injectable systems.³⁴⁻³⁸ In all the cases, the concentration of P407, which is the main thermoresponsive component, varied between 15 wt% and 20 wt%.³⁴⁻³⁸ In most of the studies, poloxamer 407 was mixed with poloxamer 188 at several concentrations,³⁴⁻³⁷ while in one of the studies, P407 was mixed with poloxamer 124.³⁸ These mixtures, loaded with drugs (e.g. piroxicam,³⁴ doxorubicin (DOX),³⁵ PTX,³⁶ astragalus polysaccharide,³⁷ and metformin³⁸), were injected in model animals such as rabbits,³⁴ rats,³⁵ and mice.³⁶⁻³⁸ In all the cases, controlled drug release from the gel matrix was observed.³⁴⁻³⁸ Interestingly, in one of

the studies in which PTX-containing liposomes were injected in tumour-bearing mice, tumour growth was controlled by the local sustained release of the drug.³⁶

Other polymers have also been added in poloxamer solutions to enhance the strength of the formed gels, and their mixtures have been investigated upon injection in rats.³⁹⁻⁴¹ Similar to the previous studies, poloxamer 407 was used as the gelling agent,³⁹⁻⁴¹ whereas in one of the studies P188 was also added.⁴¹ The additional polymers that were used are poly(vinyl alcohol) (PVA),³⁹ hydroxypropyl methyl cellulose (HPMC),^{40,41} and sodium alginate (SA).⁴¹ In all the studies, controlled release of drugs, such as octreotide,³⁹ PTX,⁴⁰ temozolomide (TMZ),⁴⁰ and insulin,⁴¹ was observed. Interestingly, the gel matrix delayed the release of the octreotide when compared with the injection of pure octreotide solution, as it was released in one day *versus* six hours, respectively.³⁹

Another approach to modify the gelation properties of the poloxamers is to covalently link the polymer with other molecules,^{42,43} or by further polymerising it to form pentablock terpolymers.⁴⁴⁻⁴⁶ The studies in which these approaches have been applied are discussed below.

In two of the studies, P407 was covalently linked to either chitosan⁴³ or tetraaniline.⁴² In the first study, it was concluded that the chemical bond between the polymers enhanced the stability of the gels when compared to the one formed by the physical mixture of P407 and chitosan.⁴³ The polymer solution based on the grafted copolymer was loaded with PLGA nanoparticles containing vaccines. Upon injection into mice, the vaccines were successfully delivered and caused body response.⁴³ In another study, P407 was covalently linked to tetraaniline, the presence of which enhanced the strength of the gel, which was also injected *in vivo* in rats.⁴²

Ring opening polymerisation (ROP) on poloxamers facilitates the synthesis of pentablock copolymers.⁴⁴⁻⁴⁶ When polymerising trimethylene carbonate (triMeCa) on P407, it was observed that the pentablock terpolymer showed enhanced gelation and improved cytotoxicity compared to P407.⁴⁴ Injection of 5 wt% copolymer solution containing mitomycin C in rabbits, which had previously undergone a glaucoma filtration surgery, improved their health situation.⁴⁴ In two studies by *Li at al.*, L-lactic acid formed the outer blocks of a pentablock terpolymer based on poloxamer 105.^{45,46} In the first study, nanoparticles containing human antimicrobial peptides were incorporated into the poloxamer solution (40 wt%).⁴⁵ This mixture has been applied in rats and it was observed that healing

was promoted, which was attributed to the local and controlled release of the drug as well as to the presence of the humid environment.⁴⁵ In the second study, docetaxel (DTX)-containing micelles and oxaliplatin have been mixed with the polymer solution (35 wt%), and the mixture was injected in mice which suffered from colorectal peritoneal carcinomatosis (CRPC).⁴⁶ It was observed that the controlled release of the anticancer drugs from the gel matrix suppressed the growth of tumour, prevented angiogenesis and tumour cell proliferation, which subsequently prevented metastasis.⁴⁶ However, it should be noted that the concentrations used in the last two studies are relatively high, thus the commercial application of these copolymers might be limited because of high cost.

2.1.2 Other Degradable EG Based besides Poloxamer

Poloxamer's structure is amphiphilic and consists of ether bonds. Ether bonds are not hydrolytically-degradable;¹⁰² however, they can be hydrolysed in a slow rate via oxidation.¹⁰² In a study by Browning *et al.*, it has been observed that when chemically-crosslinked gels formed by poly(ethylene glycol) diacrylamide were implanted in rats, stability over 12 weeks was observed.¹⁰² In order to introduce fast degradability into the polymer structure, polymer scientists have copolymerised poly(ethylene glycol) (pEG) with ester-containing^{20-22,24,26-29,47-60,66-68} or carbamate-containing^{61,62} monomers. These polymers are susceptible to water, and thus their degradation mechanism is through hydrolysis. It has also been reported that enzymatic degradation of ester bonds also takes place.⁷⁵ The most common units that are often combined with pEG-based block to form degradable TRGs are ϵ -caprolactone (CL),^{22,26-29,47-51,57-60,66} lactic acid (LA),^{20-22,24,52-58,66} and glycolic acid (GA).^{20,21,24,52-56,59,68} Nevertheless, peptide-based degradable TRGs have also been applied *in vivo*, the degradation of which is enzymatically catalysed.⁶³⁻⁶⁵

It should be noted that for the purpose of this PhD thesis, poly(ethylene glycol) is abbreviated as pEG, instead of the commonly used PEG. This is to distinguish it from the three-letter abbreviation of penta(ethylene glycol) methyl ether methacrylate (PEGMA, PEG), which will be used in the following chapters.

Diblock copolymers with the A block being based on EG, and the B block being degradable ester-containing were investigated.⁴⁷⁻⁴⁹ In two of the studies, the B block was based on either CL or both CL and *p*-dioxanone (DO), and these copolymers were compared to poloxamer 407.^{47,48} Upon injection of 20 wt% polymer solutions in rats, the gel shape of the

diblock copolymers was preserved for over a month, whereas P407 was destabilised after three days; the lack of stability also limited the drug release from P407 to the same time period. Either bovine serum albumin (BSA) or bovine insulin were sustainably released from the diblock-based gels. The presence of DO increased both the T_{gel} and the release rate.^{47,48} This trend can be attributed to the increased hydrophilicity of DO compared to CL, because of the extra oxygen atom present in its structure. In the third study, the B block was based on LA and 1,6-bis(*p*-carboxyphenoxy)hexane (CPH).⁴⁹ Upon injection in rats, biocompatibility was confirmed.⁴⁹

In several studies, ABA and BAB triblock copolymers with A and B blocks being based on EG and CL, respectively, were investigated.^{26-29,50,51} Concentrated solutions of these copolymers (20-30 wt%) were injected in model animals (e.g. mice^{26,28,29,50,51} and rats²⁷). *In situ* gelation and degradation of the gel were observed.^{26,28} Also, these copolymers were tested *in vivo* as drug delivery systems by loading with lidocaine,²⁷ basic fibroblast growth factor (bFGF)^{29,50} or camptothecine (CPT)-containing particles.⁵¹ In all the cases, sustained drug release was observed.^{27,29,50} Interestingly, in one of the studies, sustained and topical CPT release from the gel matrix controlled both the growth of the CRPC tumour and prevented metastasis.⁵¹

ABA and BAB triblock copolymers with the B block to be based on PLGA instead of poly(ϵ -caprolactone) (PCL) were also evaluated for *in vivo* gel formation and injectability.^{20,21,24,52,53,55,56} These studies are discussed below.

BAB triblock copolymers were investigated *in vivo* as drug delivery systems.^{24,52,53,55} Concentrated copolymer solutions loaded with different drugs were tested in mice,⁵² rats,^{24,53} and rabbits.⁵⁵ *In vivo* degradation^{24,53} and biocompatibility⁵³ were confirmed. Sustained release of interleukin-2 inhibited the growth of tumour in mice,⁵² whereas huperzine A-phospholipid was controllably released for almost two weeks, thus reducing the toxic side effects taking place when burst release occurs instead.⁵⁵ Also, when a TRG loaded with Avastin® was injected in rats' eyes, increased half-life of the drug was confirmed.²⁴ In one of the studies, it was observed that 5-fluorouracil (5-Fu)-loaded hydrogels prevented adhesion in Achilles tendon, which is important in order to avoid pain after the surgery.⁵³

Interestingly, a series of PLGA-pEG-PLGA copolymers known as ReGel® undergoes sol-gel transition at temperatures lower than r.t. and are approved by Food and Drug Administration (FDA) agency.^{20,21} When PTX is incorporated in the ReGel® solution, the system is known

as OncoGel®. The exact chemical structure can be varied in order to control the hydrophobicity, the degradation and the porosity of the formed gel. In 2001, Zentner *et al.* investigated a specific formulation of ReGel® with a total MM equal to 4200 g mol⁻¹ and LA/GA ratio equal to 3/1. 23 wt% copolymer solution of ReGel® fully degraded in 8 weeks after *in vitro* studies, whereas the degradation time was reduced to 4 weeks after injection on rats. In this study, OncoGel® system provided local and sustained release of PTX for 50 days upon injection in tumour-bearing mice, thus controlling the tumour. Non-clinical studies of OncoGel® were carried out in rats, pigs and dogs. Clinical studies were also carried out in patients suffering from cancer.^{20,21} It should be kept in mind that even though ReGel® reached clinical studies, injection while the sample is in gel phase is painful. In an attempt to compare ReGel® with the previously-discussed BAB copolymers, the enhanced properties in ReGel® system can be attributed to the higher MM and hydrophobic content, and to the shorter hydrophilic pEG length. Therefore, it can be concluded that a unique combination of the polymer structural parameters is necessary to achieve the best gelation properties.

pEG-PLGA-pEG copolymers were also applied *in vivo*.^{54,56} Biocompatibility^{54,56} and *in vivo* degradation were confirmed after injection in rats,⁵⁶ and in rabbits' eyes.⁵⁴

In three studies, end-capped PCLA-pEG-PCLA triblock copolymers were investigated; where PCLA stands for poly(ϵ -caprolactone-*co*-lactide).^{22,57,58} In two of the studies, acetyl or 2-(2',3',5'-triiodobenzoyl) (TIB) groups were used as end-capping units,^{22,57} while in the third study, propionyl group was used instead.⁵⁸ TIB groups were introduced into the structure to allow visualisation, but this caused solubility issues because of the increased hydrophobicity.²² Therefore, either mixtures of acetyl- and TIB- or pure acetyl-capped copolymers were studied. 25 wt% copolymer solution with acetyl/TIB ratio of 25/75, which was administered as a gel-phase (T_{gel} at 10-15°C), was injected in rats. *In vivo* degradation of this gel was monitored, and gel disappearance was observed four weeks after injection.²² *In vitro* and *in vivo* sustained release of celecoxib (COX) from acetyl-capped based gels over 90 days and 8 weeks in rats, respectively, was observed.⁵⁷ The propionyl-capped polymers solutions were investigated as effective COX carrier in mice and dogs.⁵⁸ Biocompatibility was confirmed after injection in mice, while intradiscal injection in dogs suffering from intervertebral disc degeneration improved their health. However, this improvement was not correlated to regeneration, at least in the short-term.⁵⁸

BAB triblock copolymers in which A was based on pEG and the B block was based on a random copolymer of CL with either GA⁵⁹ or 1,4,8-trioxa[4.6]spiro-9-undecanone (TSUN)⁶⁰

were also applied *in vivo*. 25 wt% GA-based copolymer solution was mixed with liraglutide, which has antidiabetic function.⁵⁹ The mixture was injected in mice and biocompatibility and hypoglycaemic efficacy were confirmed.⁵⁹ A three-component system consisting of 25 wt% undecanone-based copolymer solution, DOX, and iodine¹³¹-labelled hyaluronic acid (HA) was injected in tumour-bearing mice.⁶⁰ *In vivo* injection showed local administration of the drug and inhibition of tumour growth.⁶⁰

Linear multiblock copolymers based on pEG were also tested as *in vivo* gelling systems.^{66,67} 17 wt% ABCBA polymer solution was loaded with PLGA nanoparticles and vaccines; where A, B and C blocks are pEG, PCL and PLA, respectively.⁶⁶ The mixture was injected in tumour-bearing mice and the release of the vaccines and the tumour growth were controlled.⁶⁶ Concentrated solutions of multiblock copolymers based on pEG, poly(propylene glycol) (PPG), and poly(polytetrahydrofuran carbonate) (PTHFC) were also applied *in vivo*.⁶⁷ Their concentrated solutions loaded with DOX were injected in tumour-bearing mice and inhibition of tumour growth was observed.⁶⁷

An interesting systematic study in which four-star copolymers were investigated as *in vivo* gelators was found in the literature.⁶⁸ The core of the star was based on PLGA of various LA/GA molar ratios, whereas the arms were based on constant methoxy poly(ethylene glycol) (MPEG) block. 30wt% of copolymer solutions were injected in rats and rapid gelation, degradation and good biocompatibility were confirmed.⁶⁸

Incorporation of carbamate bonds also introduces degradability into the system. Two studies, carried out by Wang's group, were based on ABA triblock copolymers with A and B blocks based on EG and serinol hexamethylene urethane (ESHU).^{61,62} Biocompatibility upon injection in rats,⁶¹ and controlled *in vivo* release of bevacizumab upon injection in rabbits' eyes were observed.⁶²

All the studies discussed before are based on hydrolytically degradable systems, the degradation of which can be accelerated in acidic environment. Chen's group investigated polymers which are based on polypeptides and pEG, and thus their degradation is enzyme-catalysed.⁶³⁻⁶⁵ In two of the studies, *L*-alanine and *L*-phenylalanine composed the peptide part.^{63,64} 8 wt% of polypeptide copolymer solution was injected in rats and *in vivo* biocompatibility was confirmed.⁶³ *In vivo* degradation was completed in forty-two days. The same copolymer solution loaded with combrestatin A-4 (CA4) particles and DTX was injected in mice bearing osteosarcoma. Inhibition of tumour growth was observed, which was

attributed to the topical and controlled release of the drugs.⁶³ The polymer solution was mixed with bone marrow mesenchymal stem cells and it was then injected in rabbits with osteochondral defect.⁶⁴ Cartilage regeneration was observed after twelve weeks.⁶⁴ In an interesting study of the group, *L*-methionine was used instead of *L*-alanine and *L*-phenylalanine.⁶⁵ *L*-methionine was incorporated into the structure to protect the cells from reactive oxygen species (ROS). Upon exposure of the gel to hydrogen peroxide (H₂O₂), the thioether group of *L*-methionine is oxidised to sulfoxide and sulfone groups. Thus, the polymer becomes more hydrophilic, and destabilisation and degradation of the gel follow. *In vivo* degradation upon injection in rats was completed after six weeks.⁶⁵

2.1.3 NIPAAm Based

In vivo applications of NIPAAm-based random,⁶⁹⁻⁷² block,⁷⁵⁻⁷⁷ and branch⁷⁸⁻⁸² synthetic copolymers as well as covalently^{73,83-88} or physically mixed^{73,74,79,80} with natural components were reported in the literature.

Four studies in which TRG based on NIPAAm random copolymers were applied in model animals were found in the literature.⁶⁹⁻⁷² These copolymers were synthesised via free radical polymerisation, and their *in vivo* applications are discussed below.

In two of the studies, acrylic acid (AA) was used as a comonomer of NIPAAm.^{69,70} In the first study, γ -butyrolactone acrylate (BuLaA) was used, while in the second study the copolymers were based on *N*-hydroxysuccinimide (NHS) and acryloyloxy dimethyl- γ -butyrolactone (ADMBuLa). Upon injection of the polymer solutions in rats and mice, respectively, *in situ* gel formation, *in vivo* biocompatibility and degradability were confirmed.^{69,70}

In another study, tetraaniline was introduced into the polymer structure to provide electroactive and antioxidant properties needed for myocardial infarction therapy.⁷¹ The random copolymer was based on NIPAAm, nona(ethylene glycol) methyl ether methacrylate (NEGMA), methacrylic acid (MAA), and tetraaniline. 20 wt% of this copolymer solution was injected in rats and absence of extensive inflammation was observed, thus rendering these copolymers as biocompatible. Also, it was observed that the gels were destabilised four weeks after injection.⁷¹

In a very interesting study by Bayat *et al.*, TRG formed by a random copolymer of NIPAAm and butyl acrylate (BuA) was *in vivo* investigated as a possible sealant in ocular trauma.⁷² In

this study, a special syringe was developed, which consisted of a thermal reaction chamber. Prior the *in vivo* injection, an incision was created on the sclera of rabbits. Then, 30 wt% copolymer solution was injected, and hydrogel formation was observed. After inspection of the rabbits, biocompatibility was confirmed. More interestingly, practical workshops in which this copolymer solution was applied *ex vivo* in pig eyes were performed. Military ophthalmologists and clinicians participated in the workshop, and they were briefly instructed on how to apply the injectable gel. On the first attempt, forty-three percent of the participants achieved the application of the TRG, and all of them were successful on the second time.⁷² This proves the ease of the application of TRGs as injectable gels.

In two studies by Gupta *et al.*, ABC triblock copolymers were synthesised via RAFT polymerisation and were tested as *in vivo* drug-release depots.^{75,76} In both studies, B and C blocks were based on *N,N*-dimethylacrylamide (DMAAm), and NIPAAm, respectively.^{75,76} The A block was either based on propylene sulphide (PSu),^{75,76} CL,⁷⁵ and PLGA,⁷⁵ respectively. It should be noted that the A block was synthesised via ROP and these units were incorporated into the structure to provide ROS-degradation,^{75,76} slow hydrolysis/enzymatic degradation,⁷⁵ and fast hydrolysis/enzymatic degradation,⁷⁵ respectively. Interestingly, in one of the studies, the PSu-based copolymers formed gels at the low concentration of 2.5 wt%.⁷⁶ Their selective degradation was confirmed *in vitro* by exposing 5 wt% of the hydrogels under the corresponding conditions.⁷⁵ As mentioned before, the drug release rate is related to the degradation rate, i.e. the slower the degradation is, the slower the release of the drug is. 5 wt% of the copolymer solutions were loaded with Nile red and were injected in mice.^{75,76} *In vivo* degradation was controlled by the degradation mechanism and thus, the PLGA-based polymer released 90% of the stain in two days, whereas the other two copolymers sustained the release for twelve days.⁷⁵ Rhodamine was covalently-linked on the polymer chain and provided *in vivo* imaging, thus confirming the aforementioned results.⁷⁵

ABCBA pentablock terpolymers with A, B, and C being based on NIPAAm, CL, and EG, respectively, were synthesised via ATRP and ROP.⁷⁷ *In situ* gel formation was confirmed upon injection of 20 wt% polymer solution in rats.⁷⁷

Branch copolymers based on poly(PEGC-*co*-NIPAAm) were synthesised via combination of poly(condensation) reaction and free radical polymerisation, resulting in a final *D* of 3.41; PEGC stands for poly(ethylene glycol) citrate.⁷⁸⁻⁸² Incorporation of citrate groups in the structure gives antioxidant properties to the final copolymer.⁷⁸⁻⁸² *In vivo* studies were carried

out on rats and biocompatibility, degradability, controlled release of chemokine and new tissue formation were confirmed.⁷⁸ Interestingly, these copolymers were mixed with gelatin and either multipotent adipose-derived cells,⁸⁰ or immortalised mouse embryonic fibroblasts,⁷⁹ and the mixture was injected in mice.^{79,80} As a result, complete osteogenic differentiation^{79,80} and vascularisation⁷⁹ were observed. In the two latest studies on this material, the polymer was mixed with mesenchymal stem cells (MSC) and either graphene oxide (GO)⁸¹ or strontium ions,⁸² and the mixture was injected in mice. Osteodifferentiation,⁸² mineralisation,^{81,82} osteoinduction,⁸¹ angiogenesis⁸¹ and bone formation⁸² were observed. Nevertheless, one should keep in mind that these copolymers possess broad MMD, and thus polymer chains with different chemical structure possess different properties. These copolymers are also difficult to be reproduced, and therefore, controlled synthetic techniques should be used for the synthesis of well-defined copolymers (MMD closer to 1).

Combination of poly(*N*-isopropylacrylamide) (PNIPAAm) with natural components such as HA,^{73,83-86} gelatin,⁸⁷ chitosan,⁸⁸ and dextran⁷⁴ was also found in the literature. These naturally-based polymers were either mixed with NIPAAm homopolymer^{73,74} or they were covalently-linked.^{73,83-88}

PNIPAAm grafted with HA was investigated as *in vivo* gelator in several studies.⁸³⁻⁸⁶ Their *in vivo* gelation in mice^{83,84} and rabbits^{85,86} was confirmed. In one of the studies, the copolymer solution was investigated as drug-delivery matrix, and it was observed that BSA was controllably released.⁸⁵ When a NIPAAm-g-HA solution was injected in a rabbit with osteochondral defect, rapid gelation was observed, and cartilage replaced the gel matrix twelve weeks post-injection.⁸⁶ When a NIPAAm-g-HA solution mixed with bioactive microvascular fragments was injected in mice bearing osteotomy, improved vascularisation was observed.⁸⁴

In another study, HA methacrylate (HAMA) was copolymerised with NIPAAm and the random copolymer was compared to the physical mixture of HA and PNIPAAm.⁷³ Both the copolymer and the physical mixture were loaded with adipose derived stem cells, and they were intraarticularly injected in rabbits. Three weeks after the injection, no inflammation or alteration in cartilage were observed. Concerning new tissue formation, this surrounded the gel matrix when the physical mixture was used, whereas incorporation of new tissue was observed within the gel depot.⁷³

Grafting of PNIPAAm on either gelatin,⁸⁷ or chitosan⁸⁸ was also reported. 10 wt% of NIPAAm-g-gelatin solution mixed with bone MSC was injected into a defect on rats' cranium.⁸⁷ *In situ* gel formation and enhanced bone regeneration were confirmed.⁸⁷ When a NIPAAm-g-chitosan solution (5 wt%) mixed with MSC was injected in rabbits, chondrogenesis was observed.⁸⁸

Gel matrix formed by a mixture of PNIPAAm homopolymer with conjugated polymer of dextran, CL and 2-hydroxyethyl methacrylate (HEMA) was found to control *in vitro* BSA release and good biocompatibility was confirmed upon injection in rats.⁷⁴

2.1.4 Poly(Organophosphazene)s

A different class of injectable thermoresponsive polymers which were *in vivo* tested is poly(organophosphazene)s, the general structure of which is shown in Figure 1.3, where R represents an organic group.^{89-99,103} These polymers were synthesised by Song's group in Korea. The studies in which poly(organophosphazene)s were applied *in vivo* were published by his group and their collaborators and they are discussed below.^{89-99,103} In these studies, the backbone was functionalised with combinations of different groups, which are as follows: i) the hydrophobic isoleucine ethyl ester (IleE),^{89-99,103} ii) a hydrophilic EG-based group,^{89-99,103} iii) the hydrolysable glycyl lactate ethyl ester (GlyLaE),^{89-92,95,96,98,103} and iv) di(glycine) (di-Gly).^{93,94,96,103}

Poly(organophosphazene)s were used as drug delivery systems in several studies.⁸⁹⁻⁹² Polymer solutions loaded with either DOX,^{90,91} or PTX,⁹² or DTX⁸⁹ were injected in tumour-bearing mice and local administration and control of tumour growth were observed.⁸⁹⁻⁹² When the same gel matrix was used, PTX was found to be released faster than DOX, which indicated the necessity of second injection in order to maintain control of tumour growth.^{90,92} This can be attributed to the different nature of the drug, thus altering the pharmacokinetics of the drug from the gel depot.

In the aforementioned studies, the polymer was physically mixed with the drug.⁸⁹⁻⁹² Another approach that was followed is to covalently link the drug with the polymer.^{93,94} In two of the studies, poly(organophosphazene)s were covalently-linked either to DOX⁹⁴ or camptothecin.⁹³ The polymer solution was injected in tumour-bearing mice and local and slow but prolonged release of the drug to the tumour was confirmed, resulting in inhibition of

the tumour growth.^{93,94} The local release of DOX to the tumour also reduced the side-effects.⁹⁴

Poly(organophosphazene)s were also used as gel matrix for delivering human growth hormone (HGH).^{95,96} In one of the studies, in order to deliver the negatively charged HGH, complexes were formed using a poly(cation) such as poly-*L*-arginine.⁹⁵ These complexes were mixed with the polymer and upon injection in rats, sustained release of the hormone was observed.⁹⁵ In another study, protamine was conjugated on the polymer to electrostatically interact with the negatively charged HGH.⁹⁶ The complexes were injected in both mice and monkeys, whereas mixture of protamine-free polymer with the hormone was also injected in mice for comparison. More controlled release of the hormone was observed when the complex was used.⁹⁶

A similar approach as the one mentioned before was carried out to deliver bone morphogenetic protein-2 (BMP-2).⁹⁷ More specifically, the BMP-2 was interacting with isoleucine through hydrophobic interactions and with an anion-modified unit via electrostatic interactions. The mixture was injected in mice and sustained release was observed, as well as degradation after eight weeks. Interestingly, the controlled release of BMP-2 led to bone formation.⁹⁷

In additional studies of the group, the polymer was mixed with either collagen and pre-osteoblasts,⁹⁸ or a three-component system consisting of polymer, drug, and gene.⁹⁹ In both cases the mixtures were injected in mice.^{98,99} In the first study, cell proliferation was observed, whereas this was not detected in the absence of the collagen additive.⁹⁸ In the second case, controlled delivery of the drug/gene system for up to one month was observed.⁹⁹

2.1.5 Summary

In summary, several TRGs have been studied and these systems possess both advantages and disadvantages. Poloxamers' *in vivo* and successful clinical application as injectable gels is mainly limited by the *in vivo* instability.^{104,105} Attempts to improve the gelation properties of poloxamers have been made by using mixture of poloxamers and/or other additives. A great deal of research has been focused on the *in vivo* application of ester- or carbamate- containing pEG-based polymers, owing to their degradability. Interesting studies based on PNIPAAm have been also carried out, but some of the syntheses were carried out using non-controlled

polymerisation techniques. A group in Korea has also studied poly(organophosphazene)s as injectable gels. Nevertheless, to the best of our knowledge, only the ReGel® system has reached successful clinical applications,²¹ indicating the difficulty to achieve the appropriate combination of structural parameters in order to obtain the desired properties for *in vivo* application.

To conclude, many TRGs have been tested *in vivo*. Several drugs have been incorporated into the gel matrix attempting to treat many different diseases like cancer, osteoporosis and diabetes. In the case of drug-delivery depots, controlled and local drug release were observed when the TRG was used. This indicates the importance of the application of injectable gels in TE. As the ideal TRG is yet to be found, more systematic studies are needed to achieve the best combination of structural parameters which will produce a polymer with improved gelation properties (gelation between r.t. and b.t., enhanced strength, *in vivo* stability, and low CGC) and this is one of the main aims of this PhD thesis.

2.2 3D (bio-)printing

The area of 3D (bio-)printing is a relatively new scientific area that has recently gained interest and therefore there is a limited number of studies where TRGs have been used in 3D printing.^{13,106-121} The first study has been published in 2013¹⁰⁶ and then several other studies followed.

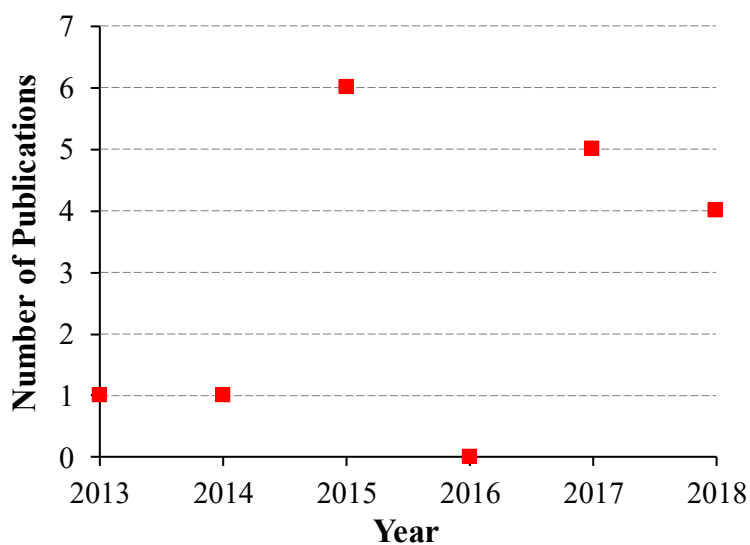


Figure 2.3: Graph showing the number of publications related to 3D printing of thermoresponsive gels over the years.

Prior to 3D printing, the rheological characteristics of the sample should be tested. Firstly, the T_{gel} can be determined by temperature ramp measurements, as explained in Section 1.4.4. Also, the properties of the (bio-)ink at the printing temperature should be investigated. The viscosity of the polymer solution should be measured over a range of shear rates to determine whether the (bio-)ink has shear-thinning properties,^{109,111,112} i.e. the longer the (bio-)ink is being printed, the easier the printing is (the lower the viscosity). Shear recovery experiments should also be performed at the printing temperature to ensure complete and immediate recovery of the (bio-)ink as soon as it exits the needle and is deposited on the substrate. The latter warranties accurate printing.^{109,111,112,115}

As previously mentioned, TRGs can be printed at room or body temperature, depending on the temperature at which a stable structure is desired. It should be mentioned that generally, the material is printed while being in the gel phase, i.e. the heated stage at which the structure is printed, and the syringe where the bio-ink is loaded are at temperatures above the T_{gel} of the polymer solution. This is feasible because of the shear-thinning properties of the gel. Nevertheless, two studies have been found in the literature, at which the syringe was kept at temperatures lower than the T_{gel} , whereas the heated stage was kept at temperatures higher than the T_{gel} .^{114,115} This was preferred in order to ensure shear recovery upon printing.¹¹⁵

This part of the literature review is divided based on the temperature at which a stable structure is printed ($T_{\text{printed scaffold}}$), i.e. the temperature of the heated stage. These studies are summarised in Table 2.2, in which the studies are listed depending on the $T_{\text{printed scaffold}}$. The general polymer structure, the main thermoresponsive component, any polymer additives, and the concentration of the polymer solution printed are also listed. Table 2.2 also clarifies whether the TRG was successfully printed, and if any post-printing processing was carried out.

Table 2.2: Summary of the studies on thermoresponsive gels (TRGs) used as 3D printable materials.

$T_{\text{printed scaffold}}$	Polymer ^a	Thermoresponsive Component	Additives *	Concentration (wt%)	Successful 3D Printing	Post Printing Processing	Ref.
r.t	Pluronic® F127 (EG ₁₀₀ - <i>b</i> -PG ₆₅ - <i>b</i> -EG ₁₀₀)	EG	–	24.5	Yes	Elution	106
				40	Yes	Elution	107
				35	Yes	Elution	108
				20	Yes	Elution	109
					No	No	13
			copper	25 *	Yes	No	110
			graphene	2.5 – 6 *	Yes	No	110
	Pluronic® F127 dimethacrylate	EG	–	25	Yes	Covalent crosslink	109
b.t.	IPGE _{t_x} - <i>b</i> -EG _y - <i>b</i> IPGE _{t_x}	EG	–	15	Yes	No	13
	MO _x - <i>b</i> -PO _{x_y}	PO _x	–	20	Yes	No	111
	Pluronic® F127 (EG ₁₀₀ - <i>b</i> -PG ₆₅ - <i>b</i> -EG ₁₀₀)	EG	acrylated Pluronic® F127	17 *	Yes	Elution	112
			alginate	20 *	Yes	No	113
	HBC	chitosan	–	NA	Yes	No	114
	NIPAAm _x - <i>g</i> -HA _y	NIPAAm	HAMA	15 *	Yes	Elution	115
	PIC	PIC	–	0.5	Yes	Elution	116
	Poly(urethane)	Poly(urethane)	–	25-30	Yes	No	117-121

Abbreviations: EG – ethylene glycol, HA – hyaluronic acid, HBC – hydroxybutylchitosan, NIPAAm – *N*-isopropylacrylamide, IPGEt – isopropyl glycidyl ether, HAMA – methacrylated, MO_x – 2-methyl-2-oxazoline, PIC – poly(isocyanide), PG – propylene glycol, and PO_x – 2-*n*-propyl-2-oxazine.

^a x and y denote the degrees of polymerisation of the different units.

* Other additives are incorporated into the (bio-)ink, apart from cells, which might affect the thermoresponsive properties of the polymer solution. These components are given in the relevant column.

2.2.1 3D printing of Thermoresponsive Gels (TRGs) at room temperature (r.t.)

In most reported studies, poloxamer 407 (known as Pluronic® F127), which is a commercially available TRG, has been used as discussed below.¹⁰⁶⁻¹¹⁰ As a reminder, the chemical structure of Pluronic® F127 is EG₁₀₀-*b*-PG₆₅-*b*-EG₁₀₀.

In the first study, 24.5 wt% Pluronic® F127 was used as a sacrificial material, i.e. the component which was removed/discarded, after the final material was fabricated.¹⁰⁶ This gel was printed in pillar shape and the printed structure was immersed in agarose solution which was gelled by keeping the temperature between 35 °C and 45 °C.¹⁰⁶ This temperature range was chosen to ensure that both gels (Pluronic® F127 and agarose) are in the gel phase. Then, the printed-gel material was cooled down to 4 °C, at which the Pluronic® F127 gel became liquid and was removed, thus a final material with a vascular structure was created.¹⁰⁶

Similarly, in another study, the Pluronic® F127 was again used as a sacrificial scaffold to create channels to mimic vascular structures.¹⁰⁸ In this study, a higher concentration was used, specifically 35 wt% of Pluronic® F127. This was successfully printed in several shapes consisting of 30 printed layers, such as cube, cylinder, and star. Then, the same gel was printed on top of an alginate/gelatin block, and the resulted structure was covered by another alginate/gelatin block. After crosslinking the alginate part, the construct was cooled down to 4 °C, at which the Pluronic® F127 gel was liquified and removed, thus revealing the vascular structure.¹⁰⁸

In an analogous study, different materials were printed in one structure in an attempt to mimic the complicated natural structures.¹⁰⁷ 15wt% gelatin methacrylate solution which underwent UCST-type gelation at 23°C was firstly printed at 20°C. Then, 40wt% Pluronic® F127 was printed at the same temperature and photopolymerisation was followed in order to chemically crosslink the gelatin methacrylate gel. Lowering the temperature at 4°C induced gel-sol transition to the Pluronic®, thus creating open channels within the printed structure.¹⁰⁷

Pluronic® F127 was also used in an interesting study on “gel-in-gel 3D printing”.¹⁰⁹ In this study, 20 wt% of the polymer solution was mixed with an initiator and kept in a bath at the gel state ($G' = 21$ kPa). The ink was based on a functionalised Pluronic® F127 (25 wt% Pluronic® F127 dimethacrylate) and a catalyst. The ink was printed in the gel bath, and the functionalised Pluronic® F127 physical gel was polymerised while printing, because of the generation of free-radicals. Thus, this reaction converted the functionalised physical gel ($G' \approx 20$ kPa) to a chemically crosslinked 3D printed structure ($G' \approx 100$ kPa). The printed structure

could be revealed by cooling the bath below its T_{gel} , thus converting it into the liquid state, which could be easily removed. Graphene oxide and multiwalled carbon nanotubes have been incorporated into the ink, and they have been successfully printed into stable 3D structures.¹⁰⁹

Pluronic® F127 has been utilised as a 3-D printable material in which graphene and copper have been incorporated in order to print electrodes for electrochemical energy storage.¹¹⁰ Two different inks have been fabricated in this study: i) copper (85 wt%) has been dissolved in a 25 wt% Pluronic® F127 solution, and ii) graphene has been mixed with Pluronic® F127 at a weight ratio 1:1 at various concentrations (2.5 wt% to 6 wt%). In this study, a TRG has been incorporated in the inks to provide stabilisation of the copper and graphene by preventing agglomeration. It is noteworthy, that the inks which contain copper or graphene have storage modulus higher than or up to 100 kPa, respectively. Therefore, stable structures have been successfully printed.¹¹⁰

Only two studies report the use of *in-house* synthesised TRG in 3D printing.^{13,111} These studies are discussed in the following paragraphs.

In the first study, two ABA triblock copolymers of different A lengths were fabricated via controlled anionic ROP.¹³ The polymers were based on pEG ($MM = 8000 \text{ g mol}^{-1}$, B block) and isopropyl glycidyl ether (IPGEt, A block), and they were then compared with the Pluronic® F127. Polymer 1 and 2 had A block lengths of 1800 and 2400 g mol^{-1} , respectively. 10 and 15wt% solutions of Polymer 2 and 1, respectively, were gels at r.t., while the Pluronic® was still in the sol phase under the same conditions. 15 wt% of the synthesised copolymers (Polymer 1 *versus* Polymer 2) possessed storage moduli equal to 20.6 and 24.5 kPa at r.t., respectively. On the other hand, 20 wt% of Pluronic® gelled at 17.2°C with elastic modulus only 15.1 kPa. 14 wt% of Polymer 2 and 23 wt% of the Pluronic® were tested in terms of their 3-D printing properties. Eight layers of the 14 wt% copolymer solution were successfully printed and the same structure was not achieved by using 23 wt% of the Pluronic®.¹³

Lorson *et al.* have synthesised and investigated oxazoline/oxazine systems for 3-D printing.¹¹¹ Specifically, the diblock copolymers were based on 2-methyl-2-oxazoline (MOx, A unit) and 2-*n*-propyl-2-oxazine (POx, B unit) and they varied in the block lengths; the B unit is the one showing thermoresponse. 20 wt% of A₉₄-b-B₉₄ copolymer solution was mixed with fibroblast cells, and it was subsequently printed at room temperature (at which the gel phase with shear storage modulus 4 kPa exists).¹¹¹

2.2.2 3D printing of Thermoresponsive Gels (TRGs) at body temperature (b.t.)

3D printing has also been performed at b.t., and the relevant studies are discussed below.¹¹²⁻

121

In an interesting study by Müller *et al.*, Pluronic® F127 was used for 3D printing at b.t.¹¹² Specifically, Pluronic® F127 was mixed with a functionalised (acrylated) Pluronic® F127 at various concentrations, and the thermoresponsive properties were investigated, i.e. gelation temperature and storage modulus at 37 °C. It was observed that the mixture of 17 wt% Pluronic® F127 and 3 wt% functionalised Pluronic® F127 showed sol-gel transition at 23.5 °C, and the storage modulus of the gel at b.t. is 15.8 kPa. UV crosslinking of the printed scaffold led to an increase in the storage modulus to 20.5 kPa. Cells were also incorporated in the mixture thus creating a bio-ink, which was also printed in 3D structures. The elution of the pure (non-functionalised) Pluronic® F127 over time was also monitored.¹¹²

Studies in which printed TRGs promoted cell alignment and orientation were also found in the literature.^{113,114} In one of the studies, 20 wt% Pluronic® F127 solution was mixed with alginate (2 wt%) and cells at r.t. (liquid phase).¹¹³ The mixture was kept at 37 °C, at which physical gelation occurred prior to 3D printing. The bioink was printed into parallel aligned fibers, and crosslinking of the alginate followed by exposure in calcium chloride solution. Interestingly, it was observed that the cells were aligned in the same direction as the fibers.¹¹³ In the second study, 3D printing of hydroxybutyl chitosan (HBC) solution (gel phase at 37 °C) into different shapes was achieved.¹¹⁴ The printed structures were used as a biomaterial on which cells were cultured. Interestingly, the cells could be oriented depending on the shape of the scaffold (square, triangle, rectangle, circle).¹¹⁴

In one of the studies, thermoresponsive poly(NIPAAm) of MM equal to 24 kg mol⁻¹ was grafted with HA.¹¹⁵ 15 wt% of the copolymer solution gels at 29.7 °C ($G' = 3$ kPa) and it remains as a stable gel at b.t.. The copolymer solution was mixed with HAMA (2 wt%) and photoinitiator, and the mixture was printed at b.t.. The printed structure was exposed to UV light for chemically crosslinking the HAMA, and thus increasing the storage modulus of the printed scaffold from 2532 Pa (physical gel) to 8370 Pa (chemically crosslinked gel). A bio-ink was also tested for 3D printing; the bio-ink was prepared by mixing the copolymer/HAMA/photoinitiator solution with chondrocytes. High cell death was observed in the printed structures, which was attributed to the highly crosslinked gel (both physical and chemical crosslinking). The cell death was reduced by removing the copolymer solution at 4 °C (liquid phase), which allowed diffusion of nutrients to the cells.¹¹⁵

Poly(isocyanide) (PIC) with 2-(2-(2-methoxyethoxy)ethoxy)ethyl-(*L*)-alaninyl-(*D*)-isocyanoalanine as a repeated unit was also used as a sacrificial phase for the preparation of scaffolds.¹¹⁶ Specifically, this polymer forms a TRG at temperature above 18 °C. The printed scaffold based on this TRG was immersed in a gelatin methacrylate solution, which was covalently crosslinked upon exposure to UV light. The temperature of this double crosslinked gel (both physical and chemical crosslinks) was lowered to 4 °C, thus promoting gel-sol transition of the TRG, thus revealing a permeable 3D printed structure.¹¹⁶

In five studies by Hsu's group, urethane-based copolymers were synthesised by covalently linking different diols.¹¹⁷⁻¹²¹ In these studies, PCL diols were substituted at low percentage (10-20 mol %) by either PLLA^{119,121} pEG,¹⁵ PDLA¹⁵, and PDLLA^{117,118,121}; where PLLA, PDLA, and PDLLA stand for poly(*L*-lactide), poly(*D*-lactide), and poly(*D,L*-lactide), respectively. In three of the studies, concentrated poly(urethane) solutions were mixed with cells, and the bio-inks were printed:^{117,119,121} i) mesenchymal stem cells (30 wt% copolymer),¹¹⁹ ii) induced pluripotent stem cells (25 wt% copolymer),¹¹⁷ and iii) neural stem cells (25 wt% copolymer)¹²¹. However, in the latter case, the printed structure collapsed when more than eight layers were printed on account of the low storage modulus (0.68 kPa).¹²¹ This shows that a high storage modulus is required for printing stable structures. Implantation of printed biomaterials in injured adult zebrafish found to positively affect their brain function.^{120,121} In an interesting study, a three-component bio-ink (storage modulus equal to 9 kPa) consisting of fibroblasts, a transcription factor, and a poly(urethane) gel was successfully printed.¹¹⁸ Reprogramming of the fibroblasts into neural cells and construction of neural-like tissue was observed.¹¹⁸ Despite the fact the process followed was characterised as green,¹²¹ it may be complicated to be industrially applicable at the moment.

2.2.3 Conclusion

In summary, TRGs have shown promise and can serve as 3D printable materials either at r.t. or at b.t. The most commonly used TRG in 3D printing is Pluronic® F127, which is the commercially available TRG.^{13,106-110,112,113} In some studies, the TRGs are mixed with materials which enhance their stability by providing additional physical interactions between the TRG and the additive^{110,113} or chemical bonds between the additive molecules^{112,115}. Another approach for enhancing their stability is to chemically modify a TRG in a way that can be further polymerised via chemical crosslinking after printing.¹⁰⁹ TRGs are also used as

sacrificial 3D printed structures.^{106-109,112,115,116} This application is useful as it allows the 3D printing of dual hydrogels, which can be converted into permeable 3D structures after eluting the TRG. Permeability is important in TE as cells have access to nutrients, which are critical for their viability.

Since 3D printing of TRGs is a recently emerged scientific field, little work has so far been performed. This demonstrates that many possibilities can be explored and therefore, the synthesis, characterisation and evaluation of new TRGs which are able to serve as 3-D printable materials is one of the aims of this PhD thesis.

2.3 References

1. Buwalda, S. J.; Boere, K. W. M.; Dijkstra, P. J.; Feijen, J.; Vermonden, T.; Hennink, W. E. Hydrogels in a historical perspective: From simple networks to smart materials. *J. Controlled Release* **2014**, *190*, 254-273.
2. Ward, M. A.; Georgiou, T. K. Thermoresponsive Polymers for Biomedical Applications. *Polymers* **2011**, *3*, 1215-1242.
3. Ward, M. A.; Georgiou, T. K. Multicompartment thermoresponsive gels: does the length of the hydrophobic side group matter? *Polym. Chem.* **2013**, *4*, 1893-1902.
4. Ward, M. A.; Georgiou, T. K. Thermoresponsive gels based on ABA triblock copolymers: Does the asymmetry matter? *J. Polym. Sci. Part A: Polym. Chem.* **2013**, *51*, 2850-2859.
5. Ward, M. A.; Georgiou, T. K. Thermoresponsive terpolymers based on methacrylate monomers: Effect of architecture and composition. *J. Polym. Sci. A Polym. Chem.* **2010**, *48*, 775-783.
6. Ward, M. A.; Georgiou, T. K. Thermoresponsive triblock copolymers based on methacrylate monomers: effect of molecular weight and composition. *Soft Matter* **2012**, *8*, 2737-2745.
7. Constantinou, P. A.; Zhao, H.; McGilvery, M. C.; Porter, E. A.; Georgiou, K. T. A Comprehensive Systematic Study on Thermoresponsive Gels: Beyond the Common Architectures of Linear Terpolymers. *Polymers* **2017**, *9*, 31.
8. Constantinou, A. P.; Georgiou, T. K. Thermoresponsive gels based on ABC triblock copolymers: effect of the length of the PEG side group. *Polym. Chem.* **2016**, *7*, 2045-2056.
9. Constantinou, A. P.; Sam-Soon, N.; Carroll, D. R.; Georgiou, T. K. Thermoresponsive Tetrablock Terpolymers: Effect of Architecture and Composition on Gelling Behavior. *Macromolecules* **2018**.
10. Yang, Y. -.; Ali-Adib, Z.; McKeown, N. B.; Ryan, A. J.; Attwood, D.; Booth, C. Effect of block architecture on the gelation of aqueous solutions of oxyethylene/oxybutylene block copolymers. *Langmuir* **1997**, *13*, 1860-1861.
11. Yang, Z.; Pickard, S.; Deng, N.; Barlow, R. J.; Attwood, D.; Booth, C. Effect of block structure on the micellization and gelation of aqueous solutions of copolymers of ethylene oxide and butylene oxide. *Macromolecules* **1994**, *27*, 2371-2379.
12. Skardal, A.; Atala, A. Biomaterials for Integration with 3-D Bioprinting. *Ann. Biomed. Eng.* **2015**, *43*, 730-746.
13. Zhang, M.; Vora, A.; Han, W.; Wojtecki, R. J.; Maune, H.; Le, A. B. A.; Thompson, L. E.; McClelland, G. M.; Ribet, F.; Engler, A. C.; Nelson, A. Dual-Responsive Hydrogels for Direct-Write 3D Printing. *Macromolecules* **2015**, *48*, 6482-6488.
14. Mironov, V.; Reis, N.; Derby, B. Review: Bioprinting: A Beginning. *Tissue Eng.* **2006**, *12*, 631-634.
15. Vadnere, M.; Amidon, G.; Lindenbaum, S.; Haslam, J. L. Thermodynamic studies on the gel-sol transition of some pluronic polyols. *Int. J. Pharm.* **1984**, *22*, 207-218.
16. Gupta, D.; Tator, C. H.; Shoichet, M. S. Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord. *Biomaterials* **2006**, *27*, 2370-2379.
17. Liu, Z.; Yao, P. Injectable thermo-responsive hydrogel composed of xanthan gum and methylcellulose double networks with shear-thinning property. *Carbohydr. Polym.* **2015**, *132*, 490-498.
18. Dhandayuthapani, B.; Yoshida, Y.; Maekawa, T.; Kumar, D. S. Polymeric scaffolds in tissue engineering application: A review. *Int. J. Polym. Sci.* **2011**, 290602.
19. Nair, L. S.; Laurencin, C. T. Biodegradable polymers as biomaterials. *Prog. Polym. Sci.* **2007**, *32*, 762-798.
20. Zentner, G. M.; Rathi, R.; Shih, C.; McRea, J. C.; Seo, M.; Oh, H.; Rhee, B. G.; Mestecky, J.; Moldoveanu, Z.; Morgan, M.; Weitman, S. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. *J. Controlled Release* **2001**, *72*, 203-215.
21. Elstad, N. L.; Fowers, K. D. OncoGel (ReGel/paclitaxel) — Clinical applications for a novel paclitaxel delivery system. *Adv. Drug Deliv. Rev.* **2009**, *61*, 785-794.

22. Sandker, M. J.; Petit, A.; Redout, E. M.; Siebelt, M.; Müller, B.; Bruin, P.; Meyboom, R.; Vermonden, T.; Hennink, W. E.; Weinans, H. In situ forming acyl-capped PCLA-PEG-PCLA triblock copolymer based hydrogels. *Biomaterials* **2013**, *34*, 8002-8011.
23. Bobbala, S.; Tamboli, V.; McDowell, A.; Mitra, A. K.; Hook, S. Novel Injectable Pentablock Copolymer Based Thermoresponsive Hydrogels for Sustained Release Vaccines. *AAPS Journal* **2016**, *18*, 261-269.
24. Xie, B.; Jin, L.; Luo, Z.; Yu, J.; Shi, S.; Zhang, Z.; Shen, M.; Chen, H.; Li, X.; Song, Z. An injectable thermosensitive polymeric hydrogel for sustained release of Avastin1 to treat posterior segment disease. *Int. J. Pharm.* **2015**, *490*, 375-383.
25. Constantinou, A. P.; Georgiou, T. K. Tuning the gelation of thermoresponsive gels. *Eur. Polym. J.* **2016**, *78*, 366-375.
26. Gong, C.; Shi, S.; Dong, P.; Kan, B.; Gou, M.; Wang, X.; Li, X.; Luo, F.; Zhao, X.; Wei, Y.; Qian, Z. Synthesis and characterization of PEG-PCL-PEG thermosensitive hydrogel. *Int. J. Pharm.* **2009**, *365*, 89-99.
27. Gong, C.; Shi, S.; Wu, L.; Gou, M.; Yin, Q.; Guo, Q.; Dong, P.; Zhang, F.; Luo, F.; Zhao, X.; Wei, Y.; Qian, Z. Biodegradable in situ gel-forming controlled drug delivery system based on thermosensitive PCL-PEG-PCL hydrogel. Part 2: Sol-gel-sol transition and drug delivery behavior. *Acta Biomaterialia* **2009**, *5*, 3358-3370.
28. Ma, G.; Miao, B.; Song, C. Thermosensitive PCL-PEG-PCL hydrogels: Synthesis, characterization, and delivery of proteins. *J Appl Polym Sci* **2010**, *116*, 1985-1993.
29. Wu, Q.; Gong, C.; Shi, S.; Wang, Y.; Huang, M.; Yang, L.; Zhao, X.; Wei, Y.; Qian, Z. Mannan Loaded Biodegradable and Injectable Thermosensitive PCL-PEG-PCL Hydrogel for Vaccine Delivery. *Soft Materials* **2012**, *10*, 472-486.
30. Strappe, P. M.; Hampton, D. W.; Cachon-Gonzalez, B.; Fawcett, J. W.; Lever, A. Delivery of a lentiviral vector in a Pluronic F127 gel to cells of the central nervous system. *Eur. J. Pharm. Biopharm.* **2005**, *61*, 126-133.
31. Abo Elela, M. M.; ElKasabgy, N. A.; Basalious, E. B. Bio-shielding In Situ Forming Gels (BSIFG) Loaded With Lipospheres for Depot Injection of Quetiapine Fumarate: In Vitro and In Vivo Evaluation. *AAPS PharmSciTech* **2017**, *18*, 2999-3010.
32. Hu, H.; Lin, Z.; He, B.; Dai, W.; Wang, X.; Wang, J.; Zhang, X.; Zhang, H.; Zhang, Q. A novel localized co-delivery system with lapatinib microparticles and paclitaxel nanoparticles in a peritumorally injectable in situ hydrogel. *J. Control. Release* **2015**, *220*, 189-200.
33. Lee, S. H.; Lee, J. E.; Baek, W. Y.; Lim, J. O. Regional delivery of vancomycin using pluronic F-127 to inhibit methicillin resistant *Staphylococcus aureus* (MRSA) growth in chronic otitis media in vitro and in vivo. *J. Control. Release* **2004**, *96*, 1-7.
34. Xuan, J. J.; Balakrishnan, P.; Oh, D. H.; Yeo, W. H.; Park, S. M.; Yong, C. S.; Choi, H. G. Rheological characterization and in vivo evaluation of thermosensitive poloxamer-based hydrogel for intramuscular injection of piroxicam. *Int. J. Pharm.* **2010**, *395*, 317-323.
35. Xuan, J.; Yan, Y.; Oh, D. H.; Choi, Y. K.; Yong, C. S.; Choi, H. Development of thermo-sensitive injectable hydrogel with sustained release of doxorubicin: Rheological characterization and in vivo evaluation in rats. *Drug Deliv.* **2011**, *18*, 305-311.
36. Mao, Y.; Li, X.; Chen, G.; Wang, S. Thermosensitive Hydrogel System With Paclitaxel Liposomes Used in Localized Drug Delivery System for In Situ Treatment of Tumor: Better Antitumor Efficacy and Lower Toxicity. *J. Pharm. Sci.* **2016**, *105*, 194-204.
37. Yu, Z.; Guo, F.; Guo, Y.; Zhang, Z.; Wu, F.; Luo, X. Optimization and evaluation of astragalus polysaccharide injectable thermoresponsive in-situ gels. *PLOS ONE* **2017**, *12*, e0173949.
38. Baldassari, S.; Solari, A.; Zuccari, G.; Drava, G.; Pastorino, S.; Fucile, C.; Marini, V.; Daga, A.; Pattarozzi, A.; Ratto, A.; Ferrari, A.; Mattioli, F.; Barbieri, F.; Caviglioli, G.; Florio, T. Development of an Injectable Slow-Release Metformin Formulation and Evaluation of Its Potential Antitumor Effects. *Scientific Reports* **2018**, *8*, 3929.
39. Erfani Jabarian, L.; Rouini, M. R.; Atyabi, F.; Foroumadi, A.; Nassiri, S. M.; Dinarvand, R. In vitro and in vivo evaluation of an in situ gel forming system for the delivery of PEGylated octreotide. *Eur. J. Pharm. Sci.* **2013**, *48*, 87-96.

40. Ding, L.; Wang, Q.; Shen, M.; Sun, Y.; Zhang, X.; Huang, C.; Chen, J.; Li, R.; Duan, Y. Thermoresponsive nanocomposite gel for local drug delivery to suppress the growth of glioma by inducing autophagy. *Autophagy* **2017**, *13*, 1176-1190.
41. Yang, G.; Wang, Q.; Gao, Y.; Yang, C.; Hu, L. Combination of coating and injectable hydrogel depot to improve the sustained delivery of insulin. *J. Drug. Deliv. Sci. Technol.* **2018**, *45*, 415-421.
42. Jin, E.; Zhang, Z.; Lian, H.; Chen, X.; Xiao, C.; Zhuang, X.; Chen, X. Injectable electroactive hydrogels based on Pluronic® F127 and tetraaniline copolymer. *Eur. Polym. J.* **2017**, *88*, 67-74.
43. Bobbala, S.; Gibson, B.; Gamble, A. B.; McDowell, A.; Hook, S. Poloxamer 407-chitosan grafted thermoresponsive hydrogels achieve synchronous and sustained release of antigen and adjuvant from single-shot vaccines. *Immunol. Cell Biol.* **2018**, *96*, 656-665.
44. Xi, L.; Wang, T.; Zhao, F.; Zheng, Q.; Li, X.; Luo, J.; Liu, J.; Quan, D.; Ge, J. Evaluation of an Injectable Thermosensitive Hydrogel As Drug Delivery Implant for Ocular Glaucoma Surgery. *PLOS ONE* **2014**, *9*, e100632.
45. Li, X.; Fan, R.; Tong, A.; Yang, M.; Deng, J.; Zhou, L.; Zhang, X.; Guo, G. In situ gel-forming AP-57 peptide delivery system for cutaneous wound healing. *Int. J. Pharm.* **2015**, *495*, 560-571.
46. Li, X.; Fan, R.; Wang, Y.; Wu, M.; Tong, A.; Shi, J.; Xiang, M.; Zhou, L.; Guo, G. In situ gel-forming dual drug delivery system for synergistic combination therapy of colorectal peritoneal carcinomatosis. *RSC Adv.* **2015**, *5*, 101494-101506.
47. Hyun, H.; Kim, Y. H.; Song, I. B.; Lee, J. W.; Kim, M. S.; Khang, G.; Park, K.; Lee, H. B. In Vitro and in Vivo Release of Albumin Using a Biodegradable MPEG-PCL Diblock Copolymer as an in Situ Gel-Forming Carrier. *Biomacromolecules* **2007**, *8*, 1093-1100.
48. Hyun, H.; Park, H. S.; Kwon, Y. D.; Khang, G.; Lee, B. H.; Kim, S. M. Thermo-Responsive Injectable MPEG-Polyester Diblock Copolymers for Sustained Drug Release. *Polymers* **2014**, *6*, 2670-2683.
49. Lai, P.; Hong, D.; Ku, K.; Lai, Z.; Chu, I. Novel thermosensitive hydrogels based on methoxy polyethylene glycol-co-poly(lactic acid-co-aromatic anhydride) for cefazolin delivery. *Nanomedicine* **2014**, *10*, 553-560.
50. Wu, Q. J.; Zhu, X. C.; Xiao, X.; Wang, P.; Xiong, D. k.; Gong, C. Y.; Wang, Y. S.; Yang, L.; Wei, Y. Q. A novel vaccine delivery system: Biodegradable nanoparticles in thermosensitive hydrogel. *Growth Factors* **2011**, *29*, 290-297.
51. Liu, L.; Wu, Q.; Ma, X.; Xiong, D.; Gong, C.; Qian, Z.; Zhao, X.; Wei, Y. Camptothecine encapsulated composite drug delivery system for colorectal peritoneal carcinomatosis therapy: Biodegradable microsphere in thermosensitive hydrogel. *Colloids Surf. B* **2013**, *106*, 93-101.
52. Qiao, M.; Chen, D.; Hao, T.; Zhao, X.; Hu, H.; Ma, X. Injectable thermosensitive PLGA-PEG-PLGA triblock copolymers-based hydrogels as carriers for interleukin-2. *Pharmazie* **2008**, *63*, 27-30.
53. Yuan, B.; He, C.; Dong, X.; Wang, J.; Gao, Z.; Wang, Q.; Tian, H.; Chen, X. 5-Fluorouracil loaded thermosensitive PLGA-PEG-PLGA hydrogels for the prevention of postoperative tendon adhesion. *RSC Adv.* **2015**, *5*, 25295-25303.
54. Hu, C. C.; Chaw, J. R.; Chen, C. F.; Liu, H. W. Controlled release bevacizumab in thermoresponsive hydrogel found to inhibit angiogenesis. *Bio-Med. Mater. Eng.* **2014**, *24*, 1941-1950.
55. Cai, X.; Luan, Y.; Jiang, Y.; Song, A.; Shao, W.; Li, Z.; Zhao, Z. Huperzine A-phospholipid complex-loaded biodegradable thermosensitive polymer gel for controlled drug release. *Int. J. Pharm.* **2012**, *433*, 102-111.
56. Tang, Y.; Singh, J. Biodegradable and biocompatible thermosensitive polymer based injectable implant for controlled release of protein. *Int. J. Pharm.* **2009**, *365*, 34-43.
57. Petit, A.; Sandker, M.; Müller, B.; Meyboom, R.; van Midwoud, P.; Bruin, P.; Redout, E. M.; Versluijs-Helder, M.; van der Lest, C. H. A.; Buwalda, S. J.; de Leede, L. G. J.; Vermonden, T.; Kok, R. J.; Weinans, H.; Hennink, W. E. Release behavior and intra-articular biocompatibility of celecoxib-loaded acetyl-capped PCLA-PEG-PCLA thermogels. *Biomaterials* **2014**, *35*, 7919-7928.
58. Tellegen, A. R.; Willems, N.; Beukers, M.; Grinwis, G. C. M.; Plomp, S. G. M.; Bos, C.; Dijk, M.; Leeuw, M.; Creemers, L. B.; Tryfonidou, M. A.; Meij, B. P. Intradiscal application of a

- PCLA-PEG-PCLA hydrogel loaded with celecoxib for the treatment of back pain in canines: What's in it for humans? *J Tissue Eng Regen Med* **2018**, *12*, 642-652.
59. Chen, Y.; Luan, J.; Shen, W.; Lei, K.; Yu, L.; Ding, J. Injectable and Thermosensitive Hydrogel Containing Liraglutide as a Long-Acting Antidiabetic System. *ACS Appl. Mater. Interfaces* **2016**, *8*, 30703-30713.
 60. Huang, P.; Zhang, Y.; Wang, W.; Zhou, J.; Sun, Y.; Liu, J.; Kong, D.; Liu, J.; Dong, A. Co-delivery of doxorubicin and ¹³¹I by thermosensitive micellar-hydrogel for enhanced in situ synergetic chemoradiotherapy. *J. Controlled Release* **2015**, *220*, 456-464.
 61. Park, D.; Wu, W.; Wang, Y. A functionalizable reverse thermal gel based on a polyurethane/PEG block copolymer. *Biomaterials* **2011**, *32*, 777-786.
 62. Rauck, B. M.; Friberg, T. R.; Medina Mendez, C. A.; Park, D.; Shah, V.; Bilonick, R. A.; Wang, Y. Biocompatible Reverse Thermal Gel Sustains the Release of Intravitreal Bevacizumab In Vivo. *Investigative Ophthalmology and Visual Science* **2014**, *1*, 469-470.
 63. Zheng, Y.; Cheng, Y.; Chen, J.; Ding, J.; Li, M.; Li, C.; Wang, J.; Chen, X. Injectable Hydrogel-Microsphere Construct with Sequential Degradation for Locally Synergistic Chemotherapy. *ACS Appl. Mater. Interfaces* **2017**, *9*, 3487-3496.
 64. Liu, H.; Cheng, Y.; Chen, J.; Chang, F.; Wang, J.; Ding, J.; Chen, X. Component effect of stem cell-loaded thermosensitive polypeptide hydrogels on cartilage repair. *Acta Biomater.* **2018**, *73*, 103-111.
 65. Xu, Q.; He, C.; Ren, K.; Xiao, C.; Chen, X. Thermosensitive Polypeptide Hydrogels as a Platform for ROS-Triggered Cargo Release with Innate Cytoprotective Ability under Oxidative Stress. *Adv. Healthcare Mater.* **2016**, *5*, 1979-1990.
 66. Bobbala, S.; Tamboli, V.; McDowell, A.; Mitra, A. K.; Hook, S. Novel Injectable Pentablock Copolymer Based Thermoresponsive Hydrogels for Sustained Release Vaccines. *The AAPS Journal* **2015**, *18*, 261-269.
 67. Chan, Q. B.; Cheng, H.; Liow, S. S.; Dou, Q.; Wu, Y.; Loh, J. X.; Li, Z. Poly(carbonate urethane)-Based Thermogels with Enhanced Drug Release Efficacy for Chemotherapeutic Applications. *Polymers* **2018**, *10*, 89.
 68. Zou, P.; Suo, J.; Nie, L.; Feng, S. Temperature-responsive biodegradable star-shaped block copolymers for vaginal gels. *J. Mater. Chem.* **2012**, *22*, 6316-6326.
 69. Cui, Z.; Lee, B. H.; Pauken, C.; Vernon, B. L. Degradation, cytotoxicity, and biocompatibility of NIPAAm-based thermosensitive, injectable, and bioresorbable polymer hydrogels. *J. Biomed. Mater. Res. Part A* **2011**, *98 A*, 159-166.
 70. Fitzpatrick, S. D.; Jafar Mazumder, M. A.; Muirhead, B.; Sheardown, H. Development of injectable, resorbable drug-releasing copolymer scaffolds for minimally invasive sustained ophthalmic therapeutics. *Acta Biomater.* **2012**, *8*, 2517-2528.
 71. Cui, H.; Liu, Y.; Cheng, Y.; Zhang, Z.; Zhang, P.; Chen, X.; Wei, Y. In Vitro Study of Electroactive Tetraaniline-Containing Thermosensitive Hydrogels for Cardiac Tissue Engineering. *Biomacromolecules* **2014**, *15*, 1115-1123.
 72. Bayat, N.; Zhang, Y.; Falabella, P.; Menefee, R.; Whalen, J. J.; Humayun, M. S.; Thompson, M. E. A reversible thermoresponsive sealant for temporary closure of ocular trauma. *Science Translational Medicine* **2017**, *9*.
 73. Wang, C.; Eswaramoorthy, R.; Lin, T.; Chen, C.; Fu, Y.; Wang, C.; Wu, S.; Wang, G.; Chang, J.; Ho, M. Enhancement of chondrogenesis of adipose-derived stem cells in HA-PNIPAAm-CL hydrogel for cartilage regeneration in rabbits. *Scientific Reports* **2018**, *8*, 10526.
 74. Wu, D.; Qiu, F.; Wang, T.; Jiang, X.; Zhang, X.; Zhuo, R. Toward the Development of Partially Biodegradable and Injectable Thermoresponsive Hydrogels for Potential Biomedical Applications. *ACS Appl. Mater. Interfaces* **2009**, *1*, 319-327.
 75. Gupta, M. K.; Martin, J. R.; Dollinger, B. R.; Hattaway, M. E.; Duvall, C. L. Thermogelling, ABC Triblock Copolymer Platform for Resorbable Hydrogels with Tunable, Degradation-Mediated Drug Release. *Adv. Funct. Mater.* **2017**, *27*, 1704107.
 76. Gupta, M. K.; Martin, J. R.; Werfel, T. A.; Shen, T.; Page, J. M.; Duvall, C. L. Cell protective, ABC triblock polymer-based thermoresponsive hydrogels with ROS-triggered degradation and drug release. *J. Am. Chem. Soc.* **2014**, *136*, 14896-14902.

77. Abandansari, H. S.; Aghaghafari, E.; Nabid, M. R.; Niknejad, H. Preparation of injectable and thermoresponsive hydrogel based on penta-block copolymer with improved sol stability and mechanical properties. *Polymer (United Kingdom)* **2013**, *54*, 1329-1340.
78. Yang, J.; van Lith, R.; Baler, K.; Hoshi, R. A.; Ameer, G. A. A Thermoresponsive Biodegradable Polymer with Intrinsic Antioxidant Properties. *Biomacromolecules* **2014**, *15*, 3942-3952.
79. Ye, J.; Wang, J.; Zhu, Y.; Wei, Q.; Wang, X.; Yang, J.; Tang, S.; Liu, H.; Fan, J.; Zhang, F.; Farina, E. M.; Mohammed, M. K.; Zou, Y. Z.; Song, D.; Liao, J.; Huang, J.; Guo, D.; Lu, M.; Liu, F.; Liu, J.; Ma, C.; Hu, X.; Haydon, R. C.; Lee, M. J.; Reid, R. R.; Ameer, G. A.; Li, Y.; He, T. A thermoresponsive polydiolcitrate-gelatin scaffold and delivery system mediates effective bone formation from BMP9-transduced mesenchymal stem cells. *Biomedical Materials* **2016**, *11*, 025021.
80. Lu, S.; Wang, J.; Ye, J.; Zou, Y.; Zhu, Y.; Wei, Q.; Wang, X.; Tang, S.; Liu, H.; Fan, J.; Zhang, F.; Farina, E. M.; Mohammed, M. M.; Song, D.; Liao, J.; Huang, J.; Guo, D.; Lu, M.; Liu, F.; Liu, J.; Li, L.; Ma, C.; Hu, X.; Lee, M. J.; Reid, R. R.; Ameer, G. A.; Zhou, D.; He, T. Bone morphogenetic protein 9 (BMP9) induces effective bone formation from reversibly immortalized multipotent adipose-derived (iMAD) mesenchymal stem cells. *American Journal of Translational Research* **2016**, *8*, 3710-3730.
81. Zhao, C.; Zeng, Z.; Qazvini, N. T.; Yu, X.; Zhang, R.; Yan, S.; Shu, Y.; Zhu, Y.; Duan, C.; Bishop, E.; Lei, J.; Zhang, W.; Yang, C.; Wu, K.; Wu, Y.; An, L.; Huang, S.; Ji, X.; Gong, C.; Yuan, C.; Zhang, L.; Liu, W.; Huang, B.; Feng, Y.; Zhang, B.; Dai, Z.; Shen, Y.; Wang, X.; Luo, W.; Oliveira, L.; Athiviraham, A.; Lee, M. J.; Wolf, J. M.; Ameer, G. A.; Reid, R. R.; He, T.; Huang, W. Thermoresponsive Citrate-Based Graphene Oxide Scaffold Enhances Bone Regeneration from BMP9-Stimulated Adipose-Derived Mesenchymal Stem Cells. *ACS Biomater. Sci. Eng.* **2018**, *4*, 2943-2955.
82. Morochnik, S.; Zhu, Y.; Duan, C.; Cai, M.; Reid, R. R.; He, T.; Koh, J.; Szleifer, I.; Ameer, G. A. A thermoresponsive, citrate-based macromolecule for bone regenerative engineering. *J. Biomed. Mater. Res.* **2018**, *106*, 1743-1752.
83. Tan, H.; Ramirez, C. M.; Miljkovic, N.; Li, H.; Rubin, J. P.; Marra, K. G. Thermosensitive injectable hyaluronic acid hydrogel for adipose tissue engineering. *Biomaterials* **2009**, *30*, 6844-6853.
84. Orth, M.; Altmeyer, M. A. B.; Scheuer, C.; Braun, B. J.; Holstein, J. H.; Eglin, D.; D'Este, M.; Histing, T.; Laschke, M. W.; Pohlemann, T.; Menger, M. D. Effects of locally applied adipose tissue-derived microvascular fragments by thermoresponsive hydrogel on bone healing. *Acta Biomater.* **2018**, *77*, 201-211.
85. Ha, D. I.; Lee, S. B.; Chong, M. S.; Lee, Y. M.; Kim, S. Y.; Park, Y. H. Preparation of thermoresponsive and injectable hydrogels based on hyaluronic acid and poly(N-isopropylacrylamide) and their drug release behaviors. *Macromolecular Research* **2006**, *14*, 87-93.
86. D'Este, M.; Sprecher, C. M.; Milz, S.; Nehrbass, D.; Dresing, I.; Zeiter, S.; Alini, M.; Eglin, D. Evaluation of an injectable thermoresponsive hyaluronan hydrogel in a rabbit osteochondral defect model. *J. Biomed. Mater. Res. Part A* **2016**, *104*, 1469-1478.
87. Ren, Z.; Wang, Y.; Ma, S.; Duan, S.; Yang, X.; Gao, P.; Zhang, X.; Cai, Q. Effective Bone Regeneration Using Thermosensitive Poly(N-Isopropylacrylamide) Grafted Gelatin as Injectable Carrier for Bone Mesenchymal Stem Cells. *ACS Appl. Mater. Interfaces* **2015**, *7*, 19006-19015.
88. Cho, J. H.; Kim, S.; Park, K. D.; Jung, M. C.; Yang, W. I.; Han, S. W.; Noh, J. Y.; Lee, J. W. Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly(N-isopropylacrylamide) and water-soluble chitosan copolymer. *Biomaterials* **2004**, *25*, 5743-5751.
89. Cho, J.; Hong, J. M.; Han, T.; Yang, H.; Song, S. Injectable and biodegradable poly(organophosphazene) hydrogel as a delivery system of docetaxel for cancer treatment. *J. Drug Target.* **2013**, *21*, 564-573.
90. Al-Abd, A. M.; Hong, K. -.; Song, S. -.; Kuh, H. -. Pharmacokinetics of doxorubicin after intratumoral injection using a thermosensitive hydrogel in tumor-bearing mice. *J. Control. Release* **2010**, *142*, 101-107.
91. Kwak, M. K.; Hur, K.; Yu, J. E.; Han, T. S.; Yanagihara, K.; Kim, W. H.; Lee, S. M.; Song, S. C.; Yang, H. K. Suppression of in vivo tumor growth by using a biodegradable thermosensitive hydrogel polymer containing chemotherapeutic agent. *Invest. New Drugs* **2010**, *28*, 284-290.

92. Kim, J. H.; Lee, J. H.; Kim, K. S.; Na, K.; Song, S. C.; Lee, J.; Kuh, H. J. Intratumoral delivery of paclitaxel using a thermosensitive hydrogel in human tumor xenografts. *Arch. Pharmacol. Res.* **2013**, *36*, 94-101.
93. Cho, J.; Chun, C.; Kuh, H.; Song, S. Injectable poly(organophosphazene)–camptothecin conjugate hydrogels: Synthesis, characterization, and antitumor activities. *Eur. J. Pharm. Biopharm.* **2012**, *81*, 582-590.
94. Chun, C.; Lee, S. M.; Kim, C. W.; Hong, K.; Kim, S. Y.; Yang, H. K.; Song, S. Doxorubicin–polyphosphazene conjugate hydrogels for locally controlled delivery of cancer therapeutics. *Biomaterials* **2009**, *30*, 4752-4762.
95. Park, M.; Chun, C.; Ahn, S.; Ki, M.; Cho, C.; Song, S. Sustained delivery of human growth hormone using a polyelectrolyte complex-loaded thermosensitive polyphosphazene hydrogel. *J. Controlled Release* **2010**, *147*, 359-367.
96. Park, M.; Chun, C.; Ahn, S.; Ki, M.; Cho, C.; Song, S. Cationic and thermosensitive protamine conjugated gels for enhancing sustained human growth hormone delivery. *Biomaterials* **2010**, *31*, 1349-1359.
97. Seo, B.; Choi, H.; Koh, J.; Song, S. Sustained BMP-2 delivery and injectable bone regeneration using thermosensitive polymeric nanoparticle hydrogel bearing dual interactions with BMP-2. *J. Controlled Release* **2015**, *209*, 67-76.
98. Yoon, J.; Park, K.; Song, S. A thermosensitive poly(organophosphazene) hydrogel for injectable tissue-engineering applications. *Journal of Biomaterials Science, Polymer Edition* **2007**, *18*, 1181-1193.
99. Kim, Y.; Kim, C.; Song, S. Injectable Ternary Nanocomplex Hydrogel for Long-Term Chemical Drug/Gene Dual Delivery. *ACS Macro Lett.* **2016**, *5*, 297-300.
100. Stanley E. Piechota, J. United States Patent US5256396A, 1990.
101. Almeida, M.; Magalhães, M.; Veiga, F.; Figueiras, A. Poloxamers, poloxamines and polymeric micelles: Definition, structure and therapeutic applications in cancer. *Journal of Polymer Research* **2017**, *25*, 31.
102. Browning, M. B.; Cereceres, S. N.; Luong, P. T.; Cosgriff-Hernandez, E. Determination of the in vivo degradation mechanism of PEGDA hydrogels. *Journal of biomedical materials research. Part A* **2014**, *102*, 4244-4251.
103. Park, M.; Cho, C.; Song, S. In vitro and in vivo degradation behaviors of thermosensitive poly(organophosphazene) hydrogels. *Polym. Degrad. Stab.* **2010**, *95*, 935-944.
104. Dumortier, G.; Grossiord, J. L.; Agnely, F.; Chaumeil, J. C. A review of poloxamer 407 pharmaceutical and pharmacological characteristics. *Pharm. Res.* **2006**, *23*, 2709-2728.
105. Edsman, K.; Carlfors, J.; Petersson, R. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. *Eur. J. Pharm. Sci.* **1998**, *6*, 105-112.
106. Müller, M.; Becher, J.; Schnabelrauch, M.; Zenobi-Wong, M. Printing thermoresponsive reverse molds for the creation of patterned two-component hydrogels for 3D cell culture. *J. Vis. Exp.* **2013**, *77*, e50632.
107. Kolesky, D. B.; Truby, R. L.; Gladman, A. S.; Busbee, T. A.; Homan, K. A.; Lewis, J. A. 3D Bioprinting of Vascularized, Heterogeneous Cell-Laden Tissue Constructs. *Adv Mater* **2014**, *26*, 3124-3130.
108. Fitzsimmons, R. E.; Aquilino, M. S.; Quigley, J.; Chebotarev, O.; Tarlan, F.; Simmons, C. A. Generating vascular channels within hydrogel constructs using an economical open-source 3D bioprinter and thermoreversible gels. *Bioprinting* **2018**, *9*, 7-18.
109. Basu, A.; Saha, A.; Goodman, C.; Shafranek, R. T.; Nelson, A. Catalytically Initiated Gel-in-Gel Printing of Composite Hydrogels. *ACS Appl. Mater. Interfaces* **2017**, *9*, 40898-40904.
110. Rocha, V. G.; García-Tuñón, E.; Botas, C.; Markoulidis, F.; Feilden, E.; D'Elia, E.; Ni, N.; Shaffer, M.; Saiz, E. Multimaterial 3D Printing of Graphene-Based Electrodes for Electrochemical Energy Storage Using Thermoresponsive Inks. *ACS Appl. Mater. Interfaces* **2017**, *9*, 37136-37145.
111. Lorson, T.; Jaksch, S.; Lübtow, M. M.; Jüngst, T.; Groll, J.; Lühmann, T.; Luxenhofer, R. A Thermogelling Supramolecular Hydrogel with Sponge-Like Morphology as a Cytocompatible Bioink. *Biomacromolecules* **2017**, *18*, 2161-2171.

112. Michael, M.; Jana, B.; Matthias, S.; Marcy, Z. Nanostructured Pluronic hydrogels as bioinks for 3D bioprinting. *Biofabrication* **2015**, *7*, 035006.
113. Mozetic, P.; Giannitelli, S. M.; Gori, M.; Trombetta, M.; Rainer, A. Engineering muscle cell alignment through 3D bioprinting. *J. Biomed. Mater. Res.* **2017**, *105*, 2582-2588.
114. Tsukamoto, Y.; Akagi, T.; Shima, F.; Akashi, M. Fabrication of Orientation-Controlled 3D Tissues Using a Layer-by-Layer Technique and 3D Printed a Thermoresponsive Gel Frame. *Tissue Engineering Part C: Methods* **2017**, *23*, 357-366.
115. Kesti, M.; Müller, M.; Becher, J.; Schnabelrauch, M.; D'Este, M.; Eglin, D.; Zenobi-Wong, M. A versatile bioink for three-dimensional printing of cellular scaffolds based on thermally and photo-triggered tandem gelation. *Acta Biomater.* **2015**, *11*, 162-172.
116. Celikkin, N.; Simó Padial, J.; Costantini, M.; Hendrikse, H.; Cohn, R.; Wilson, C.; Rowan, A.; Swieszkowski, W. 3D Printing of Thermoresponsive Polyisocyanide (PIC) Hydrogels as Bioink and Fugitive Material for Tissue Engineering. *Polymers* **2018**, *10*, 555.
117. Wong, C.; Chen, Y.; Chien, C.; Yu, T.; Rwei, S.; Hsu, S. A simple and efficient feeder-free culture system to up-scale iPSCs on polymeric material surface for use in 3D bioprinting. *Mater. Sci. Eng. C* **2018**, *82*, 69-79.
118. Ho, L.; Hsu, S. Cell reprogramming by 3D bioprinting of human fibroblasts in polyurethane hydrogel for fabrication of neural-like constructs. *Acta Biomater.* **2018**, *70*, 57-70.
119. Tsai, Y. C.; Li, S.; Hu, S. G.; Chang, W. C.; Jeng, U. S.; Hsu, S. H. Synthesis of Thermoresponsive Amphiphilic Polyurethane Gel as a New Cell Printing Material near Body Temperature. *ACS Appl. Mater. Interfaces* **2015**, *7*, 27613-27623.
120. Hsieh, F.; Hsu, S. 3D bioprinting: A new insight into the therapeutic strategy of neural tissue regeneration. *Organogenesis* **2015**, *11*, 153-158.
121. Hsieh, F. Y.; Lin, H. H.; Hsu, S. H. 3D bioprinting of neural stem cell-laden thermoresponsive biodegradable polyurethane hydrogel and potential in central nervous system repair. *Biomaterials* **2015**, *71*, 48-57.

CHAPTER 3: AIM OF THE THESIS

The aim of this PhD thesis is to identify key design criteria on how the polymer characteristics affect the formation of TRGs and consequently their future applications, and establish important structure-property relationships. This will be achieved by the synthesis and characterisation of novel well-defined thermoresponsive polymers.

Studies on thermoresponsive terpolymers have been previously carried out by our group and their results play a key role in this PhD project.¹⁻⁵ These thermoresponsive polymers are based on a hydrophilic pEG methacrylate, the hydrophobic *n*-butyl methacrylate (BuMA, Bu), and the ionic hydrophilic pH- and thermoresponsive DMAEMA as A, B and C blocks, respectively. It has been proven that the clearest sol-gel transition is achieved when the polymers possess i) ABC architecture,^{1,3} ii) around 7500 g mol⁻¹ target MM,⁴ and iii) 30-40 w/w% hydrophobic content.^{3,4} In additional studies, it has been concluded that the longer the alkyl chain of the B block, and the shorter the EG chain of the A block, the more favoured the gelation is.^{1,5}

When the pEG length has been studied, it has been concluded that a pEG length between two and five EG units is desired for clear sol-gel transition.⁵ Therefore, in this PhD thesis, tri(ethylene glycol) methyl ether methacrylate (TEGMA, TEG)-based terpolymers with varying architectures are synthesised and characterised in terms of their structural characteristics and their thermogelling properties.

Another parameter which has been identified to be of interest is the number and the position of the blocks. This has not been investigated in a significant extent in the literature, and only one non-systematic study on tetrablock copolymers has been reported.⁶ Therefore, the systematic investigation of tetrablock terpolymers of various architectures and compositions is one of the aims of this PhD thesis. These tetrablock terpolymers consist of BuMA and DMAEMA, and in this case, penta(ethylene glycol) methyl ether methacrylate (PEGMA, PEG) has been used as the hydrophilic unit.

As pEG is hydrophilic and biocompatible, it is commonly incorporated into systems that are designed for biological applications.^{7,8} It has been identified in the literature that DEGMA is thermoresponsive below b.t., and more specifically at around 26 °C (MM-dependant).⁹ However, this monomer has only been copolymerised with either PEGMA or NEGMA.^{8,10-14} Random linear and star copolymers have been investigated before, and it has been concluded that only the star copolymers form gels.¹⁰ Therefore, this PhD thesis aims to synthesise linear

DEGMA-based ABC triblock copolymers which present sol-gel transition. The A, B, and C blocks consist of PEGMA, BuMA, and DEGMA, respectively, and the composition is systematically varied.

As Pluronic® F127 is the commercially-available thermoresponsive polymer, it is of great interest to mimic its structure and to produce new thermoresponsive polymers. Therefore, this PhD aims to synthesise and investigate ABA methacrylate-based polymers, the side chains of which consist of EG and PG. An *in-house* synthesised monomer is used to form the hydrophobic B block, namely di(propylene glycol) methyl ether methacrylate (diPGMA, diPG), while the A block is based on PEGMA. The compositions and the MMs of the copolymers are systematically varied to investigate their effect on the thermoresponsive properties of the polymers.

The structural characteristics of the polymers, i.e. the compositions, architectures and MMs, are discussed in detail in the relevant Chapters.

References

1. Ward, M. A.; Georgiou, T. K. Multicompartment thermoresponsive gels: does the length of the hydrophobic side group matter? *Polym. Chem.* **2013**, *4*, 1893-1902.
2. Ward, M. A.; Georgiou, T. K. Thermoresponsive gels based on ABA triblock copolymers: Does the asymmetry matter? *J. Polym. Sci. Part A: Polym. Chem.* **2013**, *51*, 2850-2859.
3. Ward, M. A.; Georgiou, T. K. Thermoresponsive terpolymers based on methacrylate monomers: Effect of architecture and composition. *J. Polym. Sci. A Polym. Chem.* **2010**, *48*, 775-783.
4. Ward, M. A.; Georgiou, T. K. Thermoresponsive triblock copolymers based on methacrylate monomers: effect of molecular weight and composition. *Soft Matter* **2012**, *8*, 2737-2745.
5. Constantinou, A. P.; Georgiou, T. K. Thermoresponsive gels based on ABC triblock copolymers: effect of the length of the PEG side group. *Polym. Chem.* **2016**, *7*, 2045-2056.
6. Chen, J.; Liu, M.; Gong, H.; Cui, G.; Lü, S.; Gao, C.; Huang, F.; Chen, T.; Zhang, X.; Liu, Z. Synthesis of linear amphiphilic tetrablock quaterpolymers with dual stimulus response through the combination of ATRP and RAFT by a click chemistry site transformation approach. *Polym. Chem.* **2013**, *4*, 1815-1825.
7. Lutz, J.; Akdemir, Ö; Hoth, A. Point by Point Comparison of Two Thermosensitive Polymers Exhibiting a Similar LCST : Is the Age of Poly(NIPAM) Over? *J. Am. Chem. Soc.* **2006**, *128*, 13046-13047.
8. Lutz, J.; Hoth, A. Preparation of Ideal PEG Analogues with a Tunable Thermosensitivity by Controlled Radical Copolymerization of 2-(2-Methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate. *Macromolecules* **2006**, *39*, 893-896.
9. Lutz, J. Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials. *J. Polym. Sci. A Polym. Chem.* **2008**, *46*, 3459-3470.
10. Fechler, N.; Badi, N.; Schade, K.; Pfeifer, S.; Lutz, J. Thermogelation of PEG-Based Macromolecules of Controlled Architecture. *Macromolecules* **2009**, *42*, 33-36.
11. Badi, N.; Lutz, J. PEG-based thermogels: Applicability in physiological media. *J. Controlled Release* **2009**, *140*, 224-229.
12. Zarafshani, Z.; Obata, T.; Lutz, J. Smart PEGylation of Trypsin. *Biomacromolecules* **2010**, *11*, 2130-2135.
13. Lutz, J.; Weichenhan, K.; Akdemir, Ö; Hoth, A. About the Phase Transitions in Aqueous Solutions of Thermoresponsive Copolymers and Hydrogels Based on 2-(2-methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate. *Macromolecules* **2007**, *40*, 2503-2508.
14. Lutz, J.; Andrieu, J.; Üzgün, S.; Rudolph, C.; Agarwal, S. Biocompatible, Thermoresponsive, and Biodegradable: Simple Preparation of "All-in-One" Biorelevant Polymers. *Macromolecules* **2007**, *40*, 8540-8543.

CHAPTER 4: EXPERIMENTAL

4.1 Materials and Methods

Materials. The commercially available monomers were purchased from Sigma Aldrich Co Ltd., Irvine, United Kingdom (UK); these monomers are DEGMA (MM = 188.22 g mol⁻¹, 95%), TEGMA (MM = 232.27 g mol⁻¹, 94%), PEGMA (MM = 300 g mol⁻¹, 95%), DMAEMA (98%), and BuMA (99%). Compounds used for the *in-house* synthesised monomer, diPGMA, were also purchased from Sigma Aldrich Co Ltd., Irvine, UK. These reagents are di(propylene glycol) monomethyl ether (diPGOH, mixture of isomers), and methacryloyl chloride (97%), and triethylamine (Et₃N, ≥ 99%). Tetrabutylammonium hydroxide (40% in water) and benzoic acid, used for the catalyst (TBABB) synthesis, were purchased from Acros Organics (the UK distributor is Fisher Scientific UK Ltd., Loughborough, UK). Compounds used for monomer and solvent purification, polymer synthesis and characterisation were also purchased from Sigma Aldrich Co Ltd., Irvine, UK; calcium hydride (CaH₂, ≥ 90%), basic aluminum oxide (Al₂O₃·KOH), magnesium sulphate anhydrous (MgSO₄, ≥ 99.5%), the free-radical inhibitor 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), the NMR solvent deuterated chloroform (CDCl₃, 99.8%), triethylamine (Et₃N, HPLC grade, as additive for mobile phase in chromatography), the initiator, MTS (95%), sodium hydroxide pellets (NaOH, 97%), concentrated hydrochloric acid (HCl, ACS reagent, 37%), hydrochloric acid solution (volumetric, 1M) sodium and potassium metal, and tetrahydrofuran (THF, HPLC grade, polymerisation solvent, ≥ 99.9%). Phosphate buffered saline (PBS, 10x solution), *n*-hexane, chloroform (CHCl₃, analytical reagent grade), polytetrafluoroethylene (PTFE) hydrophilic syringe filters (0.45 µm pore size, 25 mm diameter) and Nylon syringe filters (0.45 µm pore size, 25 mm in diameter) were purchased from Fisher Scientific UK Ltd., Loughborough, UK. Tetrahydrofuran (THF, HPLC grade, not stabilised, mobile phase in chromatography), diethyl ether, and PTFE syringe filters (0.45 µm, 13 mm diameter) were purchased from WR International Ltd, Lutterworth, UK. Poly(methyl methacrylate) (PMMA) standard samples, that were used to calibrate the gel permeation chromatography (GPC) system, were purchased from Fluka, Sigma Aldrich Co Ltd., Irvine, UK.

Methods. Three steps were followed for the purification of the commercially available low MM monomers (DEGMA, TEGMA, DMAEMA, and BuMA): i) they were passed twice through activated basic alumina in order to remove any acidic impurities (traces of methacrylic acid, and the free-radical inhibitor monomethyl ether hydroquinone), ii) DPPH (free-radical

inhibitor) was added to prevent undesirable polymerisation in the flask, and iii) they were then stirred for 3 hours under CaH_2 , which acts as a drying agent. The high MM monomer, PEGMA, was firstly diluted in freshly-purified THF (at a concentration of 50 vol%), followed by steps i) and iii). Step ii) was not applied for the purification of PEGMA, because the monomer cannot be distilled due to its high MM. The preparation (synthesis and purification) of the *in-house* synthesised monomer, diPGMA, is discussed in a separate section. All the monomers were refrigerated prior to polymerisation. The low MM monomers (DEGMA, TEGMA, DMAEMA, BuMA, and diPGMA) were distilled under vacuum prior to use, in order to remove the DPPH, CaH_2 and $\text{Ca}(\text{OH})_2$. The PEGMA/THF solution was filtered directly into the polymerisation by using PTFE 0.45 μm filters to remove the unreacted CaH_2 and the $\text{Ca}(\text{OH})_2$. The initiator, MTS, was distilled under vacuum prior to polymerisation, and the preparation of the catalyst (synthesis and purification) is discussed in a separate section. Two methods were used for purifying the polymerisation solvent, THF: i) the THF was refluxed for 3 days in a mixture of sodium and potassium metals, and it was freshly-distilled prior to use, and ii) the THF was purified using a solvent purification, which is equipped with an activated alumina column (Pure SolvTM Micro 100 Liter solvent purification system, purchased from Sigma Aldrich). All the glassware needed for the distillations and polymerisations were dried in an oven at 140°C overnight, and they were assembled hot under vacuum in order to ensure a moisture-free environment.

4.2 Catalyst Synthesis

The catalyst, TBABB, was synthesised similarly to the procedure reported by Dicker *et al.*¹ 10g of benzoic acid and 80 mL of tetrabutyl ammonium solution (40% in water) were added in a 250 mL round bottom flask (aqueous phase), and the mixture was stirred. As soon as the mixture was homogeneous, it was transferred in a separation funnel, and then 75 mL of CHCl_3 (organic phase) was added. Extraction was carried out three times by keeping the aqueous phase in the funnel and changing the organic phase. The organic phase was kept each time in a beaker, and after the third extraction, 10g of benzoic acid was added in the organic phase. MgSO_4 was used as a desiccant and filtration followed to remove the solid $\text{MgSO}_4/\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. The solvent (CHCl_3) was removed by using a rotary evaporator, after which the product was obtained in a white solid form. The product was dissolved in 100 mL warm THF, and it was recrystallised in 70 mL dried diethyl ether. The product was dried in a vacuum oven.

4.3 Monomer Synthesis

The monomer diPGMA is not commercially available, and therefore was *in-house* synthesised using a standard procedure for methacrylate monomer synthesis.²⁻⁴ The detailed synthetic procedure is reported below.

The monomer diPGMA was synthesised through a reaction of methacryloyl chloride and di(propylene glycol) monomethyl ether at 0°C. All the glassware needed for this reaction was dried overnight in the oven at 140°C prior to use and they were assembled while being hot. Triethylamine, which catalyses the reaction, was stirred over CaH₂ for 3 hours and distilled under vacuum directly prior to the monomer synthesis. A 2-neck 2 L round-bottom flask, with a dropping funnel being connected on one neck, was used for the reaction. In the flask, 75.0 mL of di(propylene glycol) monomethyl ether (0.48 mol, 1 equivalent, mixture of isomers), 135 mL of Et₃N (0.97 mol, 2 equivalents), 250 mL of freshly-purified THF were injected. Then, 56.6 mL of methacryloyl chloride (0.58 mol, 1.2 equivalents) were syringed in the dropping funnel. The methacryloyl chloride was added drop-wise in the flask, while the flask was immersed in an ice-bath. The salt produced (Et₃NH⁺Cl⁻) was removed by filtration, while the solvents were evaporated using a rotary evaporator. The unreacted methacryloyl chloride/methacrylic acid and the humidity were removed by passing the mixture through basic aluminum oxide and stirring over CaH₂ for 3 hours, respectively. The monomer was kept under DPPH during the purification process to prevent unwanted polymerisation. The monomer was distilled twice prior to polymerisation to ensure sufficient purity.

4.4 Monomer Characterisation

4.4.1 Gas Chromatography - Flame Ionisation Detection (GC-FID)

As the alcohol used for the monomer synthesis (diPGOH) is a mixture of isomers, both the *in-house* synthesised monomer, diPGMA, and the alcohol were analysed by GC-FID to identify how many isomers are present and their relative ratios. GC-FID was performed on a Shimadzu GC-2014 instrument equipped with a Shimadzu AO20i autosampler (Shimadzu Scientific instruments, Manchester, UK). Hydrogen was used as the carrier gas, which was supplied by an external hydrogen generator. The GC is fitted with a polar Stabilwax-DA column (30 m length, 0.32 mm ID and 0.25 µm film thickness). The injection volume is 1 µl with a 39 split ratio. The injection and the flame temperatures are 250 °C and 300 °C, respectively. The heating profile is 60 °C to 240 °C at a rate of 5 °C/minute and held for 2.5 minutes.

4.4.2 Fourier-Transform Infrared (FT-IR) Spectroscopy

Both the *in-house* synthesised monomer, diPGMA, and its alcohol precursor, di(propylene glycol) monomethyl ether, were analysed via FT-IR, which provides information on the vibration of the different bonds present in the structure. The experiment was performed using a Nicolet iS10 FT-IR Spectrometer from ThermoScientific (Fisher Scientific UK Ltd, Loughborough, UK).

4.4.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

The *in-house* synthesised monomer, diPGMA, and its alcohol precursor, diPGOH, were analysed via ^1H NMR and ^{13}C NMR, which give information on the chemical composition and confirms the successful monomer synthesis. For this analysis, deuterated chloroform (CDCl_3) was used as the NMR solvent, and the experiment was performed using a 400-MHz Avance Bruker NMR spectrometer (Bruker, UK Ltd., Coventry, UK).

4.4.4 Matrix Assisted Laser Desorption/Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS)

MALDI-TOF MS was performed by using a Bruker Daltonics Ultra flex II MALDI-TOF mass spectrometer, equipped with a nitrogen laser delivering 2 ns laser pulses at 337 nm. Positive ion TOF detection was performed using an accelerating voltage of 25 kV. Solutions were prepared by mixing trans-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (matrix, 20 mg/mL), sodium iodide (cationisation agent, 6 mg/mL), THF (20 μL) and either alcohol or monomer (10 mg/mL). 0.5 μL of the mixture was applied on the target plate. The analysis was performed based on a linear calibration of methyl pEG with various MM (480-1000 g mol^{-1}). However, the results were not conclusive and thus they are not included in the relevant Chapter.

4.5 Polymer Synthesis and Recovery

All the investigated methacrylate polymers in this PhD thesis were synthesised via one-pot GTP. The procedure differs depending on the polymer architecture (block versus statistical), and these synthetic procedures are discussed below. One example of block copolymer synthesis and one example of statistical copolymer synthesis are presented. All the syntheses followed

the same general procedure, and the only differences are the chemical nature of the monomers added, the order of addition, and the amounts; this information can be found in Tables A1 and A2 in the Appendix.

4.5.1 Block Copolymer Synthesis

The block copolymers are synthesised via sequential addition of the monomers, after the addition of the initiator, MTS. The synthesis of PA1 with theoretical polymer structure TEGMA₉-*b*-BuMA₂₀-*b*-DMAEMA₂₁ is presented. In a 250 mL round-bottom flask, TBABB (~10 mg) was added and the flask was then sealed with a rubber septum and purged with inert argon gas, to ensure a moisture-free environment. After this step, freshly-purified THF (60 mL) was syringed in the flask, followed by the addition of 0.37 mL of MTS (0.32 g, 1.8 mmol). Then, the monomers were sequentially added as follows: i) 3.6 mL of TEGMA (3.7 g, 15.9 mmol), ii) 5.8 mL of BuMA (5.2 g, 36.3 mmol), and iii) 6.3 mL of DMAEMA (5.9 g, 37.5 mmol). After each addition, an exotherm was detected: i) from 24.2 to 28.3 °C, ii) 25.2 to 28.9 °C, and 27.6 to 31.8 °C, respectively. After each monomer fully reacted, two samples of 0.1 mL were extracted for GPC and ¹H NMR analysis.

4.5.2 Statistical Copolymer Synthesis

The statistical copolymers are synthesised via simultaneous addition of the monomers in the polymerisation flask. In this project, the monomers were added before the addition of the initiator, and the polymerisation was performed in a water-bath to avoid uncontrolled exotherm reaction. The synthesis of PA7, TEGMA₉-*co*-BuMA₂₀-*co*-DMAEMA₂₁ is discussed. Similar to the block copolymer synthesis, TBABB (~10 mg) was added first to the flask, which was immediately sealed with a rubber septum and purged with argon. 60 mL of THF was syringed in the flask, followed by the addition the three monomers: i) 3.6 mL of TEGMA (3.7 g, 15.9 mmol), ii) 5.8 mL of BuMA (5.2 g, 36.3 mmol), and iii) 6.3 mL of DMAEMA (5.9 g, 37.5 mmol). The MTS (0.37 mL, 0.32 g, 1.8 mmol) was added last to initiate the polymerisation, and an exotherm was detected from 20.9 °C to 25.2 °C. After the polymerisation finished, two samples of 0.1 mL were obtained for GPC and ¹H NMR analysis.

4.5.3 Polymer Recovery

All the solid polymers were recovered via precipitation in cool *n*-hexane. Prior to use, the polymers were placed in a vacuum oven at room temperature for a week, to ensure complete evaporation of *n*-hexane and THF.

4.6 Polymer Characterisation

4.6.1 Characterisation in Organic Solvents

4.6.1.1 Gel Permeation Chromatography (GPC)

The successful syntheses of all the polymers were confirmed via GPC. Specifically, the MM and the MMD of the final copolymers and their linear precursors (if any) were determined using an Agilent SECcurity GPC system, purchased from Agilent technologies UK Ltd., Shropshire, UK. This system is equipped with: i) a Polymer Standard Service (PSS) SDV analytical linear M column (SDA083005LIM, dimensions [mm]: 300 × 8.00, particle size [μm]: 5, separation range [kg mol⁻¹]: 0.1 – 1000), ii) an Agilent guard column (PL1110-1520, PLgel Mixed, dimensions [mm]: 50 × 7.5, particle size [μm]: 5), iii) an Agilent 1250 refractive index (RI) detector, and iv) a “1260 Iso” isocratic pump. The mobile phase (THF with 5 vol% Et₃N) was pumped with a flow rate of 1 mL min⁻¹. The results were based on a PMMA calibration curve; there are six data points on the curve, one resulted from each narrow linear MM PMMA standard sample of 2, 4, 8, 20, 50, and 100 kg mol⁻¹. The calibration curve was plotted by linear fitting of the data points. The samples were filtered through PTFE 0.45 μm filters prior to the measurement. At this point, it should be noted that the column used is designed to analyse polymers up to 1000 kg mol⁻¹, while the polymers studied in this PhD project have *M_n* up to 15 kg mol⁻¹. This might have broadened the dispersities of the polymers, and thus a column with more specific MM range is required for analysing the polymers of this PhD project. However, it should be noted that the column used did not introduce a significant error, and thus the results and comparisons in this PhD thesis are still valid.

The error in the *M_n* and *Đ* values has been calculated by analysing polymer PC12 after precipitation. The same sample was analysed by GPC five times, and each GPC chromatogram was analysed five times. PC12 after precipitation was also analysed using a different column of the same model and the GPC chromatogram was analysed five times. All the resulting *M_n* and *Đ* values have been used to calculate the error of the technique. Therefore, the *M_n* value has an error of 200 g mol⁻¹, whereas the error of the *Đ* value is 0.01.

The experimental MM were compared to the theoretical (targeted ones), which were calculated according to Equation 4.1 below. As shown in Equation 4.1, the theoretical MM ($MM^{theor.}$) is calculated by multiplying the MM of each monomer (MM_i) by the corresponding degree of polymerisation (DP_i). In the case of GTP, 100 g mol⁻¹ is added to the result, to account into the calculation the part of the initiator that stays on the polymer chain after the polymerisation finishes.

$$MM^{theor.}(g\ mol^{-1}) = (\sum_i MM_i \times DP_i) + 100 \quad \text{Equation 4.1}$$

4.6.1.2 Proton Nuclear Magnetic Resonance (¹H NMR) Spectroscopy

Supplementary to the GPC measurements, the successful syntheses of all the final copolymers and their linear precursors (if any) were confirmed via ¹H NMR, which gives information on the chemical composition of each polymer. For this analysis, deuterated chloroform (CDCl₃) was used as the NMR solvent, and the experiment was performed using a 400-MHz Avance Bruker NMR spectrometer (Bruker, UK Ltd., Coventry, UK).

The ¹H NMR spectra were analysed, and the experimental values of the chemical compositions of the polymers were calculated. For these calculations, the following peaks were used: i) DEGMA, TEGMA, and PEGMA are all EG-based methacrylate units, and thus their distinctive peak is the same (the one corresponding to the three methoxy protons) and it appears at 3.35ppm, ii) the distinctive peak of BuMA is the one corresponding to the two methylene protons closest to the ester bond and it appears at 3.9ppm, iii) DMAEMA's distinctive peak appears at 2.25ppm and it corresponds to the protons of the two methyl groups (2 × 3 = 6 protons) attached to the nitrogen atom, and iv) the distinctive peak of diPGMA appears at 4.8ppm and it corresponds to the two methylene protons closest to the ester bond. The analysis will be discussed in more detail in the following chapters.

4.6.2 Characterisation in Aqueous Solutions

All the polymers were tested for solubility in 1 w/w% in deionised (DI) water and in PBS. The self-assembly behaviour, the pH-responsiveness (if applicable), and the thermoresponse of all the soluble polymers was investigated.

4.6.2.1 Dynamic Light Scattering (DLS)

The self-assembly behaviour of 1 w/w% copolymer solutions in DI water was investigated using a Zetasizer Nano ZSP (Malvern Instruments Ltd., Malvern, UK). When the polymers were based on the ionisable unit DMAEMA, the pH of the polymer solutions was adjusted either close to 7 or 6, prior to the DLS measurement; in the results section, the pH at which the measurement was carried out is clearly mentioned. All the polymer solutions were filtered using Nylon 0.45 μm filters to remove any dust or large aggregates. Each polymer solution was analysed three times at 25 $^{\circ}\text{C}$, and the scattered light collected at a backscatter angle of 173° was reported after each measurement. Therefore, the hydrodynamic diameters (d_{hs}) reported are the mean values which correspond to the maximum of the peak by intensity.

The experimental d_{hs} were compared to the theoretical (calculated) diameters. The calculated diameters are based on different theoretical models depending on the polymer architecture. In the case of block architecture, it was assumed that spherical micelles are formed, while when statistical copolymers are concerned, a random coil configuration is expected to be adopted. More specifically, when amphiphilic block copolymers are dissolved in aqueous solvents, they self-assemble in a way that the hydrophobic block(s) form(s) the core of the micelle, whereas the hydrophilic block(s) form(s) the corona of the micelle.

Depending on the polymer architecture, different equations were used, and these are shown in Table 4.1. In the equations, DP stands for the experimental degree of polymerisation, which was calculated by using the experimental values of composition and MM after precipitation, as resulted from ^1H NMR and GPC analysis. Concerning the random coil configuration, the end-to-end distance of the random coil is measured by Equation 4.2. In the theoretical equations of the micelles formed by the block copolymers, the projected length of a methacrylate unit was used, which is equal to 0.254 nm.

Table 4.1: Equations used for the theoretical calculations of the a) end-to-end distance of the random coil adopted by the statistical copolymer, and b) the diameter of the micelles formed by the block copolymers.

a) Statistical Architecture				
Architecture studied	General Structure		End-to-end distance (nm)	No. of Equation
	Full	Abbr.		
Statistical terpolymer	A-co-B-co-C	(ABC)	$\langle d_g^2 \rangle^{1/2} = 2 \times [2 \times 2.20 \times (DP_A + DP_B + DP_C) / 3]^{1/2} \times 0.154$	4.2
b) Block Architecture				
Architecture studied	General Structure		Diameter of the micelle (d) = Equation below $\times 0.254$ (nm)	No. of Equation
	Full	Abbr.		
Diblock bipolymer	A-b-B	AB	$(2 \times DP_A + DP_B)$	4.3
	B-b-C	BC	$(DP_B + 2 \times DP_C)$	4.4
Diblock terpolymer	(A-co-B)-b-C	(AB)C	$(DP_A + DP_B + 2 \times DP_C)$	4.5
	A-b-(B-co-C)	A(BC)	$(2 \times DP_A + DP_B + DP_C)$	4.6
	B-b-(A-co-C)	B(AC)	$[DP_B + 2 \times (DP_A + DP_C)]$	4.7
Triblock bipolymer	A-b-B-b-A	ABA	$(DP_A + DP_B)$	4.8
Triblock terpolymer	A-b-B-b-C	ABC	For $DP_A > DP_C$: $(2 \times DP_A + DP_B)$	4.3
			For $DP_A < DP_C$: $(DP_B + 2 \times DP_C)$	4.4
	A-b-C-b-B or B-b-A-b-C	ACB or BAC	$[DP_B + 2 \times (DP_A + DP_C)]$	4.7
Tetrablock terpolymer ($DP_A < DP_C$)	A-b-B-b-C-b-A or A-b-B-b-A-b-C	ABCA or ABAC	$(DP_A + DP_B + 2 \times DP_C)$	4.9
	A-b-C-b-A-b-B or C-b-A-b-C-b-B	ACAB or CACB	$[DP_B + 2 \times (DP_A + DP_C)]$	4.7
	B-b-A-b-C-b-B	BACB	$(DP_A + DP_B + DP_C)$	4.10
	B-b-A-b-B-b-C	BABC	$(2 \times DP_C)$	4.11
	A-b-B-b-C-b-B	ABCB	$(DP_B + DP_C)$	4.12
	C-b-A-b-B-b-C or A-b-C-b-B-b-C	CABC or ACBC	$(2 \times DP_A + DP_B + DP_C)$	4.6

^a A and B are hydrophilic and hydrophobic repeated units, whereas in case a second hydrophilic unit is present, then it is denoted with C.

^b d_g stands for the diameter of gyration, which is also measured experimentally by DLS.

^c DP stands for degree of polymerisation. In these calculations, the experimental DP was used, which is based on the GPC and ¹H NMR results.

^d 0.254 nm is the projected length of a methacrylate unit.

Figure 4.1 shows schematically the derivation of the equations 4.3 and 4.10, which correspond to an AB diblock and an BACB tetrablock terpolymer respectively; where B is a hydrophobic block, and both A and C are hydrophilic blocks. In these theoretical models, it is assumed that the hydrophobic (B) block fully overlaps, whereas both the hydrophobic (B) and hydrophilic (A and/or C) blocks are fully extended. In the case of spherical micelles, most of the

architectures favour the formation of the conventional core-shell micelles, as shown schematically in Figure 4.1 (a) for an AB diblock copolymer. In only two cases (B-*b*-A-*b*-C-*b*-B, and A-*b*-B-*b*-C-*b*-B), flower-like micelles are assumed to be formed, in which the polymer chains fold in a pedal shape, as shown in Figure 4.1 (b) for a BACB tetrablock terpolymer. The micelle configurations are shown schematically and discussed in more detail in the relevant Chapters. For more information, the reader should refer to Figures 5.1.6, 5.2.6, 5.3.6, and 5.4.11.

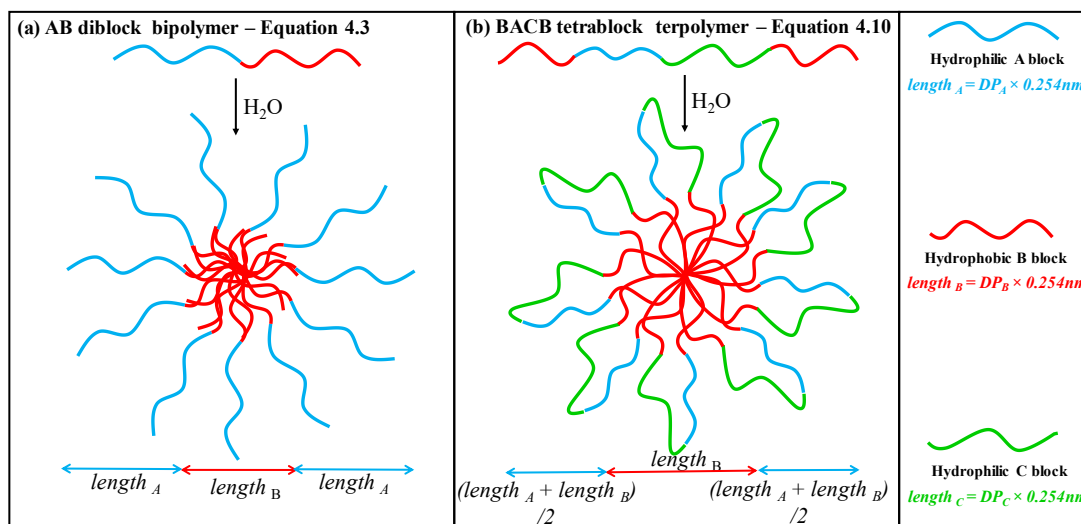


Figure 4.1: Theoretical self-assembly of (a) an AB diblock bipolymer into core-shell micelles and (b) a BACB tetrablock terpolymer into flower-like micelles. The derivation of the Equations 4.3 and 4.10 is schematically illustrated.

4.6.2.2 Potentiometric Titration

The 1 w/w% aqueous solutions of all the DMAEMA-containing polymers were titrated from pH 2 to pH 12 by using a 0.25M NaOH solution. The titrations were performed by using a HI98103 pH-checker (Hanna instruments Ltd., Leighton Buzzard, UK). The effective pK_a values were determined as the half-equivalent point on the titration curve, which is the pH at which 50% of the ionisable DMAEMA units are protonated.

4.6.2.3 Visual Observation of Thermoresponsiveness

4.6.2.3.1 Cloud Point (CP)

The CP values of 1 w/w% polymer solutions in DI water were visually investigated. For this experiment, the vials were immersed in a water-bath, which was heated up to different temperatures by using an IKA RCT stirrer hotplate, and an IKA ETS-D5 temperature controller. The CP was determined as the temperature at which the solution turned to cloudy;

an example of a cloudy solution is presented in Figure 4.1 (a) below (picture on the right). Concerning the first polymer series (Chapter 5.1), the CP points were investigated when the DMAEMA units were neutrally charged and when they were protonated by 10%.

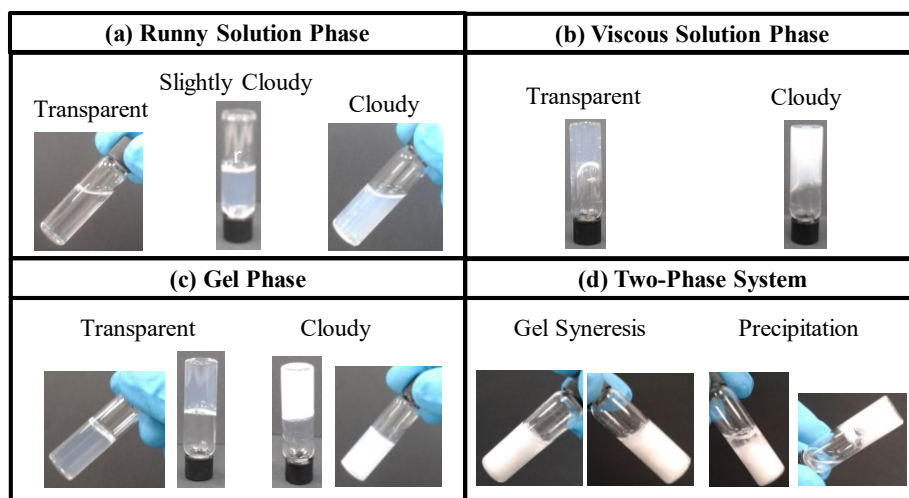


Figure 4.2: Pictures which show the different transitions, which might be observed during the visual tests: (a) runny solution phase (transparent, slightly cloudy, cloudy from the left to the right, respectively), (b) viscous solution phase (transparent: left, and cloudy: right), (c) gel phase (transparent: left, and cloudy: right), and (d) two-phase system (syneresis: left, and precipitation: right). Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

4.6.2.3.2 Visual Gel Point

The polymer solutions in PBS were tested visually for gelation, using the same equipment as in Section 4.6.2.3.1 [Cloud Point (CP)]. The visual gelation point is defined as the temperature at which a stable gel is formed, i.e. the sample does not flow when the vial is inverted. Examples of stable gels are shown in Figure 4.2 (c) (transparent and cloudy on the left and right, respectively). Different concentrations were investigated over a temperature range from 20 to 80 °C, with a heating step of 1 °C, and thus phase diagrams were plotted. On these phase diagrams, detailed transitions were included which are shown in Figure 4.1. These transitions are runny solution [Figure 4.2 (a)], viscous solution [Figure 4.2 (b)], stable gel [Figure 4.2 (c)], and two-phased system [Figure 4.2 (d)]. In the first and second polymer series (Chapters 5.1 and 5.2), the gelation was investigated at concentrations of 1, 2, 5, 10, 15, 20, 25, and 30 w/w%. It should be noted that in the first polymer series, the DMAEMA units were protonated by 10%. Concerning the third and fourth polymer series (Chapters 5.3 and 5.4), solutions of 1, 2, 5, 10, 15, 20, and 25 w/w% were tested.

4.6.2.4 Rheological Measurements

15 w/w% solutions in PBS of the *in-house* synthesised polymers were investigated in terms of their rheological properties by a TA Discovery HR-1 hybrid rheometer (TA Instruments UK, a division of Waters Ltd., Hertfordshire, UK). The rheometer is equipped with a 40 mm parallel Peltier steel plate (996921) and a solvent trap; the latter is used to prevent solvent evaporation. The changes in shear storage modulus (elastic modulus, G') and shear loss modulus (viscous modulus, G'') were investigated over a temperature range from 20 to 80 °C, by performing temperature ramp measurements with a ramp rate of 1 °C min⁻¹. The measurements were performed at a strain of 1%, and angular frequency (denoted as ω) of 1 rad s⁻¹, following previously reported values.⁵ A conditioning step was performed prior to the main measurement (temperature ramp) to ensure homogeneity of the sample. Provided the values of G' and G'' , the complex viscosity (η^*) was calculated using the Equation 4.13: $\eta^* = [(G''/\omega)^2 + (G'/\omega)^2]^{1/2}$.⁶

Contributions:

- Miss Hanyi Zhao assisted with the DLS, titrations and CP experiments for Chapter 5.1 (PA1-PA9).
- Mr Neil Sam-Soon performed the DLS, titrations, and CP experiments for PB11-PB20 in Chapter 5.2.
- Miss Beini Zhan assisted with the polymer precipitation, DLS, CP, and phase diagrams for PC1-PC6, and PC13 in Chapter 5.3.
- Mr Toby Brock assisted with the purification of the *in-house* synthesised monomer, diPGMA, the polymer precipitation, and the DLS experiments in Chapter 5.4 (PD1-PD9).
- Prof David Haddleton and Mr Georgios Patias are acknowledged for performing the GC-FID and MALDI-TOF MS on diPGMA and diPGOH.

4.7 References

1. Dicker, I. B.; Cohen, G. M.; Farnham, W. B.; Hertler, W. R.; Laganis, E. D.; Sogah, D. Y. Oxyanions catalyze group-transfer polymerization to give living polymers. *Macromolecules* **1990**, *23*, 4034-4041.
2. Constantinou, A. P.; Elladiou, M.; Patrickios, C. S. Regular and Inverse Polyampholyte Hydrogels: A Detailed Comparison. *Macromolecules* **2016**, *49*, 3869-3880.
3. Elladiou, M.; Patrickios, C. S. 2-(Pyridin-2-yl)ethanol as a protecting group for carboxylic acids: chemical and thermal cleavage, and conversion of poly[2-(pyridin-2-yl)ethyl methacrylate] to poly(methacrylic acid). *Polym. Chem.* **2012**, *3*, 3228-3231.
4. Elladiou, M.; Patrickios, C. S. ABC Triblock Terpolymers with Orthogonally Deprotectable Blocks: Synthesis, Characterization, and Deprotection. *Macromolecules* **2015**, *48*, 7503-7512.
5. Ge, Z.; Zhou, Y.; Tong, Z.; Liu, S. Thermogelling of double hydrophilic multiblock and triblock copolymers of N,N-dimethylacrylamide and N-isopropylacrylamide: Chain architectural and hofmeister effects. *Langmuir* **2011**, *27*, 1143-1151.
6. Macosko, C. W., *Rheology: principles, measurements, and applications*; 1994.

CHAPTER 5: RESULTS AND DISCUSSION

5.1 Architecture Effect of Novel Thermoresponsive Terpolymers

Note to the readers: This Chapter, excluding Section 5.1.3.5 Gel Points by Rheology, has been already published and can be found in: “<<http://www.mdpi.com/2073-4360/9/1/31>>, Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. A Comprehensive Systematic Study on Thermoresponsive Gels: Beyond the Common Architectures of Linear Terpolymers. *Polymers* **2017**, 9, 31.”

Our group has systematically investigated how the polymer architecture,^{1,2} MM³ and composition^{2,3} affect the thermoresponsive and thermogelling properties of the polymers in aqueous-based solutions. Additional parameter that has been systematically investigated is the length of the side group (either hydrophobic¹ or hydrophilic⁴).

In one of the latest studies of our group, the length of the hydrophilic side group has been varied.⁴ Specifically, the number of EG groups on the side chain of the methacrylate unit has been systematically varied, and a very interesting trend has been observed. In particular, polymers with two, five and nine EG groups were studied by using DEGMA, PEGMA, and NEGMA, respectively. It was revealed that DEGMA-based polymers (two EG units) are too hydrophobic, thus presenting solubility issues. On the other hand, when NEGMA was used (nine EG units), the gelation was interrupted, i.e. gelation was not observed, or observed at high temperatures and concentrations (composition dependant). Therefore, it has been concluded that there might be an optimum value in terms of the thermogelling properties between two and five EG groups.⁴

Therefore, the first Chapter of this PhD thesis discusses the synthesis and characterisation of polymers based on TEGMA, that contains three EG groups in order to investigate if this number of EG produces polymers with better thermogelling properties, i.e. present sol-gel transition at biologically applicable temperatures without presenting any solubility issues. Also, this Chapter aims to investigate novel polymer architectures that have not been trialled before. The terpolymers consist of TEGMA, BuMA, and DMAEMA (A, B and C units, respectively), and their MM and A-B-C composition have been targeted to 8200 g mol⁻¹, and 25-35-40 w/w%, respectively. The architecture of the terpolymers has been systematically varied and the following architectures have been investigated: i) three triblock terpolymers - ABC, ACB, and BAC, ii) three diblock terpolymers - A(BC), (AB)C, and B(AC), (the units in

the brackets have been randomly copolymerised to form a distinct block, which was extended by the addition of the third monomer (or *vice versa*), thus resulting to the final diblock terpolymer), and iii) a statistical terpolymer. Even though triblock and statistical thermoresponsive terpolymers have been investigated before, to the best of our knowledge, this is the first study in which thermoresponsive diblock terpolymers have been investigated and compared to their corresponding triblock ones. For comparison, two BC diblock bipolymers of the same target MM, and B-C content either 46-54 w/w% or 35-65 w/w%, to replicate the BuMA/ DMAEMA or the hydrophobic/hydrophilic [BuMA/ (DMAEMA + TEGMA)] ratios in the terpolymers, have been synthesised and investigated.

The chemical structures, names, and abbreviations of the monomers used for the GTP of this polymer series are shown in Figure 5.1.1 below.

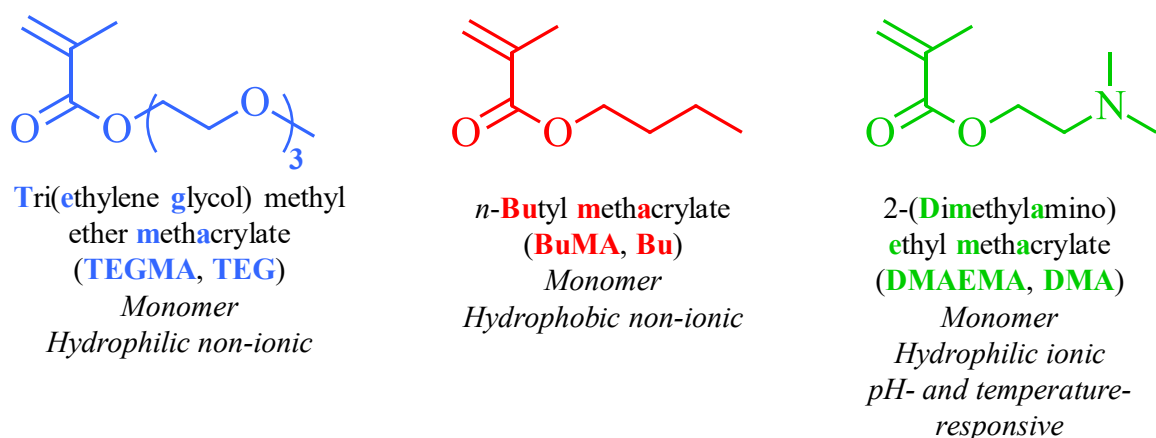


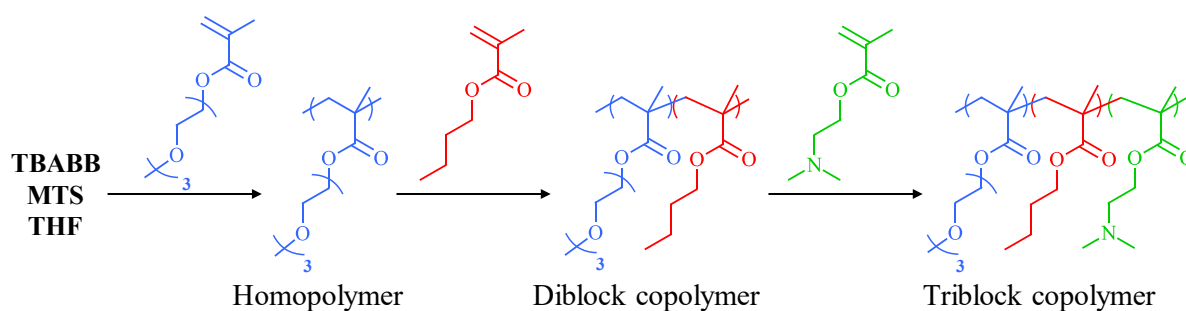
Figure 5.1.1: Chemical structures and names, of the monomers used for the GTP of this polymer series. Their abbreviations are also given in brackets: tri(ethylene glycol) methyl ether methacrylate (TEGMA), *n*-butyl methacrylate (BuMA), and 2-(dimethylamino) ethyl methacrylate (DMAEMA).

5.1.1 Polymer Synthesis

All the copolymers have been synthesised via one-pot GTP. Depending on the polymer architecture (block *versus* statistical), the synthetic route differs, as shown in Figure 5.1.2. Figure 5.1.2 (a) shows the synthesis of an ABC triblock terpolymer with A, B, and C to be based on TEGMA, BuMA, and DMAEMA, respectively. For the synthesis of a block copolymer, the monomers were added sequentially in the polymerisation flask, after the addition of the catalyst (TBABB), solvent (THF), and monofunctional initiator (MTS), as it is schematically illustrated in Figure 5.1.2 (a). All the block copolymers in this polymer series have been synthesised in a similar way; what differs is the amount of the monomers, and the order of addition. When the second block is a random bipolymer, the monomers were syringed in the polymerisation flask at the same time to allow random copolymerisation. For the

synthesis of the statistical copolymer, the monomers were added in the polymerisation flask after the addition of the catalyst and the solvent, and the initiator was added last (Figure 5.1.2 (b)). As soon as the MTS was added, the polymerisation commenced, and all the monomers polymerised statistically along the polymer chain. It should be noted that when the monomers are added at the same time, the term “random” is used, as it is assumed that random copolymerisation takes place. On the other hand, when the MTS is added last, the polymerisation of each monomer is governed by the reactivity ratios of the monomers, and thus the term “statistical” is used. To prevent the temperature rising above the boiling point of the solvent, since the monomers were not added dropwise for this synthesis, a water-bath was used to cool down the reaction.

(a) Triblock terpolymer synthesis:



(b) Statistical terpolymer synthesis:

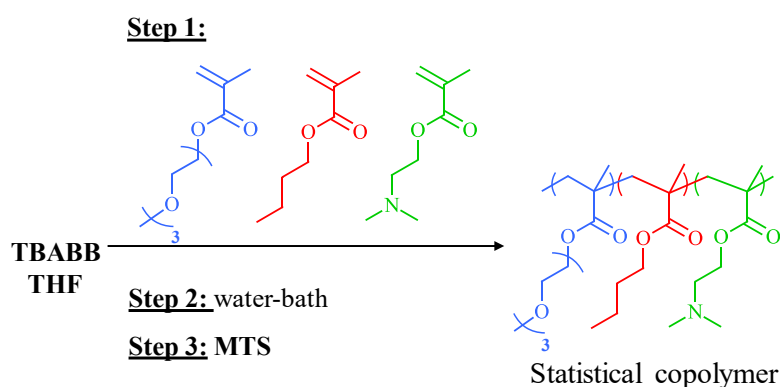


Figure 5.1.2: Chemical reactions of (a) the sequential GTP of an ABC triblock terpolymer (b) the synthesis of a statistical terpolymer by the simultaneous addition of the A, B, C monomers after the addition of the initiator. In both cases, the A, B, and C correspond to TEGMA, BuMA, and DMAEMA, respectively. *Note:* TBABB, MTS, and THF are the catalyst, initiator, and solvent, respectively, used for this PhD thesis.

A schematic representation of the first polymer series is shown in Figure 5.1.3. As can be seen, three triblock terpolymers (PA1-PA3), three diblock terpolymers (PA4-PA6), one statistical terpolymer (PA7) of the same target MM and composition have been investigated. As

mentioned before, two diblock bipolymers (PA8 and PA9) have been synthesised for comparison. In Figure 5.1.3, the TEGMA, BuMA, and DMAEMA units are shown in blue, red, and light green, respectively.

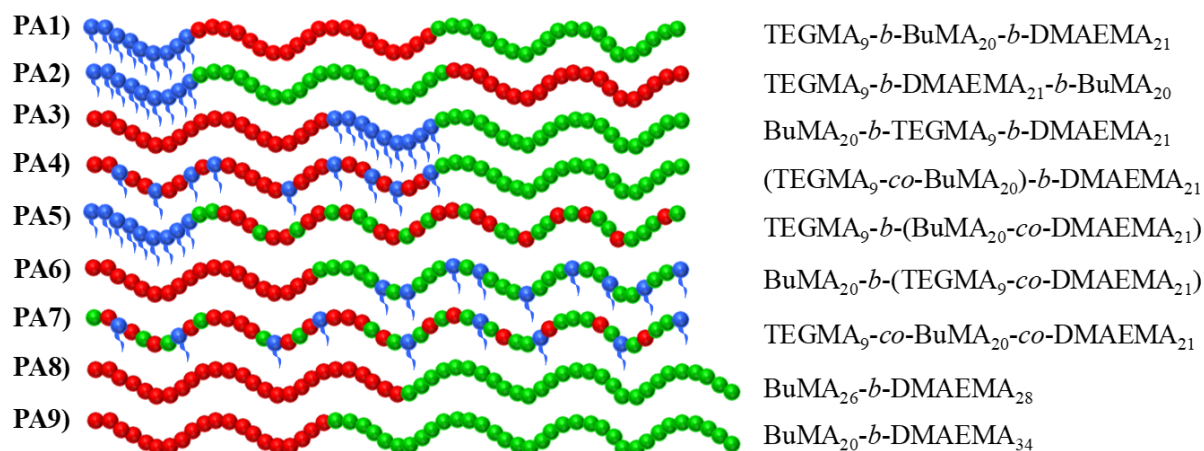


Figure 5.1.3: Schematic representation of the triblock (PA1-PA3), diblock (PA4-PA6), and statistical (PA7) terpolymers, and the diblock bipolymers (PA8 and PA9). The TEGMA, BuMA, and DMAEMA units are shown in blue, red, and light green spheres, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

5.1.2 Molecular Mass and Composition

GPC has been used to confirm the successful syntheses of all the copolymers. Figure 5.1.4 shows the GPC traces of the ABC triblock terpolymer (PA1: TEGMA₉-*b*-BuMA₂₀-*b*-DMAEMA₂₁) in green dotted line. The GPC traces of its precursors are also shown: i) TEGMA₉ in blue solid line, and TEGMA₉-*b*-BuMA₂₀ diblock copolymer in red dotted line.

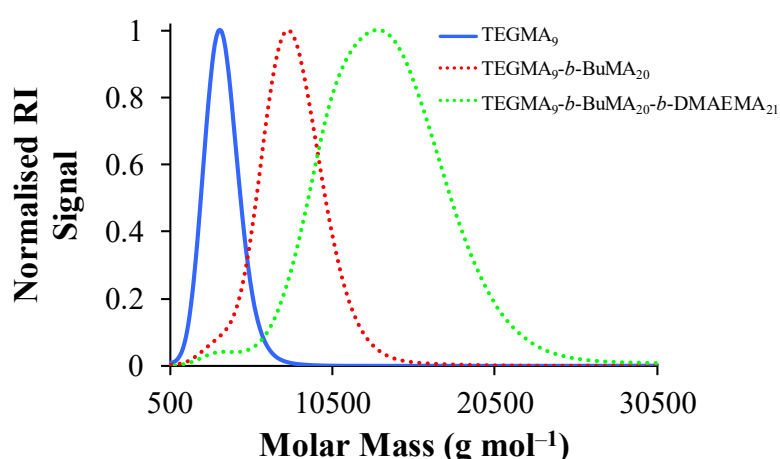


Figure 5.1.4: GPC chromatograms of the triblock terpolymer PA1 with theoretical polymer structure TEGMA₉-*b*-BuMA₂₀-*b*-DMAEMA₂₁ (light green dotted line), and its linear precursors: i) TEGMA₉ in blue solid line, and ii) the diblock copolymer TEGMA₉-*b*-BuMA₂₀ in red dotted line, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

As can be seen in Figure 5.1.4, the peak moves to higher MM values from the homopolymer, to the diblock, and to the final triblock, respectively, thus confirming the successful sequential polymerisation (i.e. chain extension occurs upon addition of the next monomer). Concerning the GPC traces of the final triblock terpolymer, a low intensity shoulder is observed at low MM values. This shoulder corresponds to the TEGMA₉, the polymerisation of which slightly deactivated the polymer chains and prevented chain extension. Also, it should be noted that peaks corresponding to unreacted monomers are not observed, thus confirming the complete consumption of the monomers. Similar observations have been made for all the copolymers and their precursors (if any); their GPC traces can be found in Figure A1 in the Appendix.

The MM of all the copolymers and their linear precursors (if any) have been determined by GPC and they are listed in Table 5.1.1. In Table 5.1.1, in addition to the experimental MM (number-average MM, M_n), the targeted polymer structure and MM are also shown. As can be observed, the M_n values of the final copolymers vary from 8580 to 11000 g mol⁻¹, and they are within the optimum range of MM, which produces polymers with good thermogelling properties, i.e. clear transition from solution to gel phase. When the experimental MM values are compared to the theoretical one ($MM^{\text{theor.}} = 8200 \text{ g mol}^{-1}$), it can be concluded that the M_n values are higher. This is also observed in other GTP studies, and it is attributed to the partial deactivation of the initiator due to the moisture and impurities present in the polymerisation flask.¹⁻⁶

Table 5.1.1 also lists the $\bar{D}$ values, which vary between 1.07 and 1.17. These values are satisfactorily close to the ideal condition of the $\bar{D}$ value being equal to 1, which confirms the living nature of the chosen polymerisation method, GTP.¹⁻⁶ Also, it can be observed that higher values are obtained for the triblock terpolymers (PA1-PA3) and two of the diblock terpolymers (PA5 and PA6). This is expected, as they contain TEGMA, which is a macromonomer, as opposed to the DMAEMA and BuMA which are well-defined low MM monomers. This can be supported by the diblock bipolymers (PA8 and PA9) being better defined ($\bar{D} = 1.11$, and $\bar{D} = 1.10$, respectively), as they are based only on DMAEMA and BuMA. Also, the statistical terpolymer is better defined (lower $\bar{D}$ value) than the TEGMA-based block terpolymers, as the synthesis of the latter requires more additions and sample withdrawals, which introduce deactivation in the polymerisation flask and broadens the $\bar{D}$ values. Contrary to the observations made previously for the block TEGMA-based terpolymers, the $\bar{D}$ value of PA4 ((TEGMA_{9-co}-BuMA₂₀)-*b*-DMAEMA₂₁) is low ($\bar{D} = 1.10$), presumably due to the TEGMA being copolymerised with BuMA, thus leading to a more controlled polymerisation.

Table 5.1.1: Theoretical polymer structure, molar mass ($MM^{theor.}$), and composition, and experimental molar mass (MM) (number-average MM , M_n), dispersity index (D), and composition of the copolymers and their linear precursors. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

No.	Theoretical Polymer Structure ^a	$MM^{theor. b}$ ($g\ mol^{-1}$)	M_n^c ($g\ mol^{-1}$)	D^c	w/w% TEG-Bu-DMA	
					Theoretical	¹ H NMR
PA1	TEG ₉	2125	3000	1.12	100-00-00	100-00-00
	TEG ₉ - <i>b</i> -Bu ₂₀	4960	6600	1.12	42-58-00	42-58-00
	TEG ₉ - <i>b</i> -Bu ₂₀ - <i>b</i> -DMA ₂₁	8200	10700	1.17	25-35-40	25-35-40
PA2	TEG ₉	2125	3000	1.11	100-00-00	100-00-00
	TEG ₉ - <i>b</i> -DMA ₂₁	5365	7600	1.11	38-00-62	38-00-62
	TEG ₉ - <i>b</i> -DMA ₂₁ - <i>b</i> -Bu ₂₀	8200	10800	1.16	25-35-40	26-34-40
PA3	Bu ₂₀	2935	3000	1.12	00-100-00	00-100-00
	Bu ₂₀ - <i>b</i> -TEG ₉	4960	6700	1.08	42-58-00	41-59-00
	Bu ₂₀ - <i>b</i> -TEG ₉ - <i>b</i> -DMA ₂₁	8200	10100	1.13	25-35-40	26-35-39
PA4	TEG ₉ - <i>co</i> -Bu ₂₀	4960	5900	1.09	42-58-00	42-58-00
	(TEG ₉ - <i>co</i> -Bu ₂₀)- <i>b</i> -DMA ₂₁	8200	8800	1.10	25-35-40	26-35-39
PA5	TEG ₉	2125	2800	1.12	100-00-00	100-00-00
	TEG ₉ - <i>b</i> -(Bu ₂₀ - <i>co</i> -DMA ₂₁)	8200	10000	1.14	25-35-40	26-34-40
PA6	Bu ₂₀	2935	4200	1.10	00-100-00	00-100-00
	Bu ₂₀ - <i>b</i> -(TEG ₉ - <i>co</i> -DMA ₂₁)	8200	8600	1.13	25-35-40	26-37-37
PA7	TEG ₉ - <i>co</i> -Bu ₂₀ - <i>co</i> -DMA ₂₁	8200	8800	1.10	25-35-40	26-35-39
PA8	Bu ₂₆	3826	6200	1.07	00-100-00	00-100-00
	Bu ₂₆ - <i>b</i> -DMA ₂₈	8200	11000	1.11	00-46-54	00-47-53
PA9	Bu ₂₀	2935	4000	1.10	00-100-00	00-100-00
	Bu ₂₀ - <i>b</i> -DMA ₃₄	8200	9400	1.10	00-35-65	00-37-63

^a TEG, Bu, and DMA are the abbreviations for tri(ethylene glycol) methyl ether methacrylate (TEGMA), *n*-butyl methacrylate (BuMA), and 2-(dimethylamino) ethyl methacrylate (DMAEMA), respectively.

^b The theoretical MM ($MM^{theor.}$) was calculated according to the Equation 4.1: $MM^{theor.}(g\ mol^{-1}) = (\sum_i MM_i \times DP_i) + 100$.

^c The number-average molar mass (M_n) and the dispersity index (D) of the terpolymers and their linear precursors (if any) were determined by gel permeation chromatography (GPC). The GPC was calibrated with six well-defined linear poly(methyl methacrylate) (PMMA) standard samples of 2, 4, 8, 20, 50, and 100 $kg\ mol^{-1}$.

¹H NMR spectroscopy has been used to confirm the chemical structure of the copolymers and their precursors (if any). The ¹H NMR spectrum of PA1 (TEGMA₉-*b*-BuMA₂₀-*b*-DMAEMA₂₁) is shown in Figure 5.1.5 (c) (in light green). In Figure 5.1.5, the ¹H NMR spectra of its precursors are also presented: (a) TEGMA₉ in blue, and (b) TEGMA₉-*b*-BuMA₂₀ in red. Peaks of low intensity which correspond to the protons of the double bonds of the monomers are still observed at around 5-6 ppm in Figure 5.1.5 (c). This confirms that traces of unreacted monomer are present after the polymerisation finishes. All the expected peaks are observed in the case of the homopolymer, while in the case of the diblock copolymer, additional peaks corresponding to BuMA have appeared. The chemical structure of the triblock terpolymer is revealed by the

appearance of the additional peaks corresponding to DMAEMA. Similar observations can be made for the ^1H NMR spectra of all the copolymers. The TEGMA-BuMA-DMAEMA weight percentages were calculated by using the integration of three distinct peaks per repeated unit, as discussed in the experimental part. These peaks can be found in the ^1H NMR spectra shown in Figure 5.1.5, and they are the ones corresponding to e, f, and l protons (for TEGMA, BuMA, and DMAEMA, respectively). The experimental composition values are listed in Table 5.1.1, and as can be observed, they are equal to the theoretical ones within the experimental error of the NMR spectrometer.

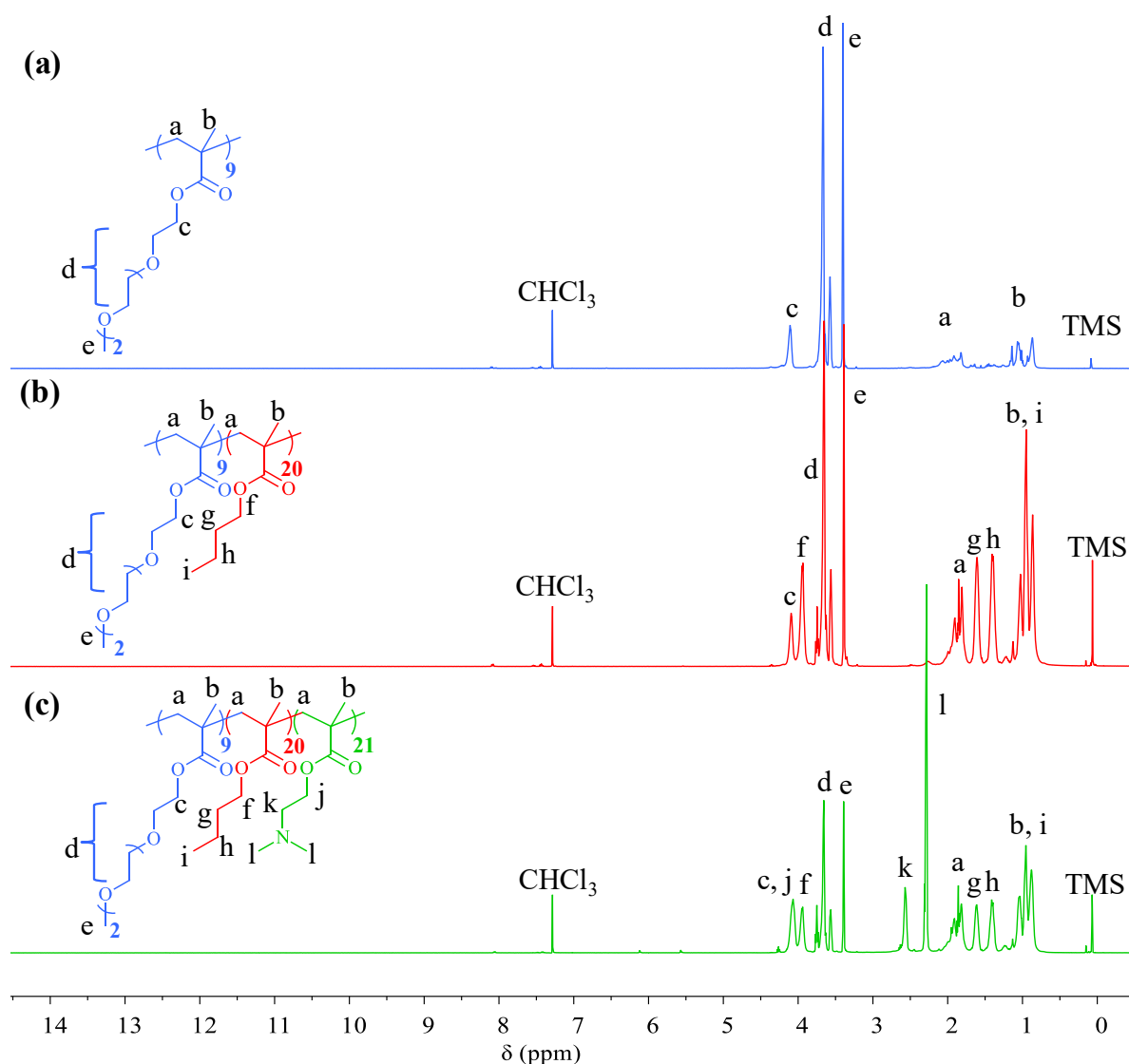


Figure 5.1.5: ^1H NMR spectra of (a) the TEGMA₉ homopolymer in blue, (b) the TEGMA₉-b-BuMA₂₀ diblock copolymer in red, and (c) the TEGMA₉-b-BuMA₂₀-b-DMAEMA₂₁ triblock terpolymer (Polymer A1) in light green. The corresponding chemical structures are also presented, with the TEGMA, BuMA, and DMAEMA units to be coloured in blue, red, and light green, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

5.1.3 Aqueous Solution Properties

5.1.3.1 Hydrodynamic Diameters

The hydrodynamic diameters of the copolymers have been determined by DLS, and the self-assembly behaviour has been investigated at both pH 6 and 7, as the statistical terpolymer was not soluble at pH 7. The results (hydrodynamic diameters, and polydispersity indices, PDI) are reported in Table 5.1.2. It should be noted that in Table 5.1.2, the values reported are the ones corresponding to the maximum of the peak by intensity. Concerning the DLS results, it was observed that in all the cases, a peak which corresponds to the micelles is observed. In some cases, a second peak is present, either at smaller or bigger size than the micelle, which corresponds to unimers (free polymer chains in solution) or bigger aggregates, respectively.

In Table 5.1.2, the theoretical (calculated) diameters are also listed. For the theoretical calculations, it was assumed that the block copolymers self-assemble into core-shell micelles, whereas the statistical terpolymer adopts a random coil configuration. The theoretical structures can be found in Figure 5.1.6: i) triblock terpolymers (top), ii) diblock terpolymers (middle), and iii) statistical terpolymer and diblock bipolymers (bottom – from left to right, respectively). The TEGMA, BuMA, and DMAEMA repeated units are shown in blue, red, and light green, respectively.

It is observed that both the theoretical and experimental hydrodynamic diameters vary depending on the polymer architecture. At this point it should be noted that the theoretical calculations do not take the pH into account. However, it is clearly observed experimentally that it has a strong effect on the size of the micelles. The dependence of the micelle structure and/or size on the pH is expected. At pH 7, which is above the pK_a values of the copolymers, as it will be discussed in the relevant section, the protonation of DMAEMA units varies from 28% to 48% for the more hydrophilic polymers, whereas it drops to 6% to 10% for the more hydrophobic ones. On the other hand, at pH 6, which is below the pK_a values of the copolymers, the DMAEMA units are protonated by 56% to 88%, architecture- and composition-dependant. Several trends concerning the self-assembly behaviour of the copolymers are observed and discussed below.

Firstly, the expected trend is observed for the following block copolymer solutions, regardless the pH, i.e. the polymers form micelles smaller than the theoretical calculation; this trend has been reported before.²⁻⁴ The ABC and BAC architectures (PA1 and PA3, respectively) form micelles smaller than the expected ones, at both pH values. This is also the case for the diblock

bipolymers (PA8 and PA9). This trend is attributed to different assumptions made for the theoretical model: i) the polymer chains are configured in a way that the hydrophobic BuMA block fully overlaps in order to form the core of the micelle, ii) both the hydrophobic BuMA and the hydrophilic (TEGMA and DMAEMA) parts (methacrylate backbone) are fully stretched. However, the BuMA repeated units, as they are hydrophobic, avoid interactions with water not only by fully overlapping, but also by collapsing in the core of the micelle, i.e. they are not extended. Also, a micelle formation is in a dynamic state, so the polymer chains can move and consequently the hydrophobic BuMA part would not always overlap fully.

A second effect is observed for the ACB architecture (PA2) at both pH values, which is the opposite than the one discussed before, i.e. the polymers form micelles that are bigger than the calculated value. As mentioned before, the theoretical model assumes that the BuMA block fully overlaps, however, this might not be the case. Incomplete overlap of the hydrophobic block might allow more polymer chains to participate in one micelle, thus increasing the overall size. This might be the case for the ACB architecture, which has the hydrophobic BuMA block at the end of the polymer chain and forms the bigger micelles theoretically. This argument is supported by the trends observed in Chapter 5.2 on tetrablock terpolymers, as the polymer architectures with the hydrophobic BuMA block at the end of the polymer chain form micelles bigger than the calculated size, regardless the pH.

The size of the micelles formed by the diblock terpolymers depend on both the architecture and the pH. PA4 and PA5, with (AB)C and A(BC) architectures, form smaller micelles than the theoretically predicted ones, the size of which increases by decreasing the pH. On the other hand, concerning the B(AC) architecture (PA6), with the hydrophobic block at the end of the polymer chain, bigger micelles than theoretically are formed at pH 7, similarly to PA2. These micelles become smaller as the pH decreases. The different trends observed in the case of the diblock terpolymers can be attributed to the theoretical model i) considering the copolymer of BuMA with either hydrophilic units as the hydrophobic block which forms the core of the micelle by fully overlapping, and ii) not taking into consideration the change in the pH. Both factors might be important, especially when DMAEMA units are protonated and electrostatic repulsions might play an important role on micelle formation.

Table 5.1.2: Theoretical polymer structure, theoretical (calculated) and experimental hydrodynamic diameters (d_h s), polydispersity indices (PDI), effective pK_a s, and cloud points (CPs) of the aqueous polymer solutions at 1 w/w%. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

No.	Polymer Architecture	Theoretical Polymer Structure ^a	Hydrodynamic diameter (d_h , nm)										Effective $pK_{as} \pm 0.1$	Cloud Points ± 2 °C	
			Theor. ^b	Experimental ± 0.5 ^h										H^+ (%)	
				pH 6				pH 7							
				1 st	2 nd	3 rd	PDI	1 st	2 nd	3 rd	PDI	0			10
PA1	ABC	TEG ₉ - <i>b</i> -Bu ₂₀ - <i>b</i> -DMA ₂₁	22.8 ^c	-	6.5	50.7	0.568	-	8.7	164	0.299	6.7	29	j	
PA2	ACB	TEG ₉ - <i>b</i> -DMA ₂₁ - <i>b</i> -Bu ₂₀	29.8 ^d	-	32.7	-	0.179	-	37.8	-	0.133	6.8	ND ⁱ	j	
PA3	BAC	Bu ₂₀ - <i>b</i> -TEG ₉ - <i>b</i> -DMA ₂₁	27.3 ^d	-	13.5	91.3	0.458	-	18.2	-	0.414	6.9	ND	j	
PA4	(AB)C	(TEG ₉ - <i>co</i> -Bu ₂₀)- <i>b</i> -DMA ₂₁	22.0 ^e	2.0	24.4	-	0.312	1.7	21.0	-	0.446	6.9	j	j	
PA5	A(BC)	TEG ₉ - <i>b</i> -(Bu ₂₀ - <i>co</i> -DMA ₂₁)	19.7 ^f	-	21.0	-	0.166	-	15.7	190	0.396	6.2	32	j	
PA6	B(AC)	Bu ₂₀ - <i>b</i> -(TEG ₉ - <i>co</i> -DMA ₂₁)	25.2 ^d	3.1	18.2	255	0.412	-	28.2	-	0.225	6.7	ND	j	
PA7	(BAC)	TEG ₉ - <i>co</i> -Bu ₂₀ - <i>co</i> -DMA ₂₁	2.8 ^g	-	15.7	-	0.227	ND	ND	ND	ND	6.1	ND	NA	
PA8	BC	Bu ₂₆ - <i>b</i> -DMA ₂₈	34.1 ^c	-	15.7	-	0.378	-	32.7	-	0.091	6.5	ND	j	
PA9	BC	Bu ₂₀ - <i>b</i> -DMA ₃₄	28.3 ^c	2.7	15.7	122	0.431	-	21.0	-	0.207	6.8	ND	j	

^a TEG, Bu, and DMA stand for tri(ethylene glycol) methyl ether methacrylate (TEGMA), *n*-butyl methacrylate (BuMA), and 2-(dimethylamino) ethyl methacrylate (DMAEMA), respectively.

^b The calculations of the theoretical diameters are based on the experimental degrees of polymerisation (DPs); the experimental DPs were calculated by using the experimental MM after precipitation, as determined by GPC, and the experimental composition, as determined by ¹H NMR.

^c The diameter of the spherical micelles formed by the ABC triblock terpolymer (PA1) and the diblock bipolymers (PA8 and PA9) were calculated by using the Equation 4.4: d_h (nm) = (DP_B + 2 × DP_C) × 0.254.

^d The ACB and BAC triblock terpolymers (PA2 and PA3, respectively), and the B(AC) diblock terpolymer (PA6) formed spherical micelles, the diameter of which was calculated by using the Equation 4.7: d_h (nm) = [DP_B + 2 × (DP_A + DP_C)] × 0.254.

^e The Equation 4.5 was used to calculate the diameter of the micelles formed by PA4 with (AB)C architecture: d_h (nm) = (DP_A + DP_B + 2 × DP_C) × 0.254.

^f The Equation 4.6 [d_h (nm) = (2 × DP_A + DP_B + DP_C) × 0.254] was used to calculate the diameter of the micelles formed by PA5 with A(BC) architecture.

^g The statistical terpolymer (PA7) is assumed to form a random coil, the end-to-end distance of which is calculated by the Equation 4.2: $\langle d_g^2 \rangle^{1/2} = 2 \times [2 \times 2.20 \times (DP_A + DP_B + DP_C) / 3]^{1/2} \times 0.154$.

Note: in the equations mentioned above, A, B and C correspond to TEGMA, BuMA and DMAEMA, respectively.

^h The experimental hydrodynamic diameters reported are the mean diameters of the maximum of the peak in DLS histograms.

ⁱ ND stands for not determined, as the polymers were not soluble, and therefore it was unable to test them at the specific pH.

^j The polymers did not show a CP within the temperature range tested.

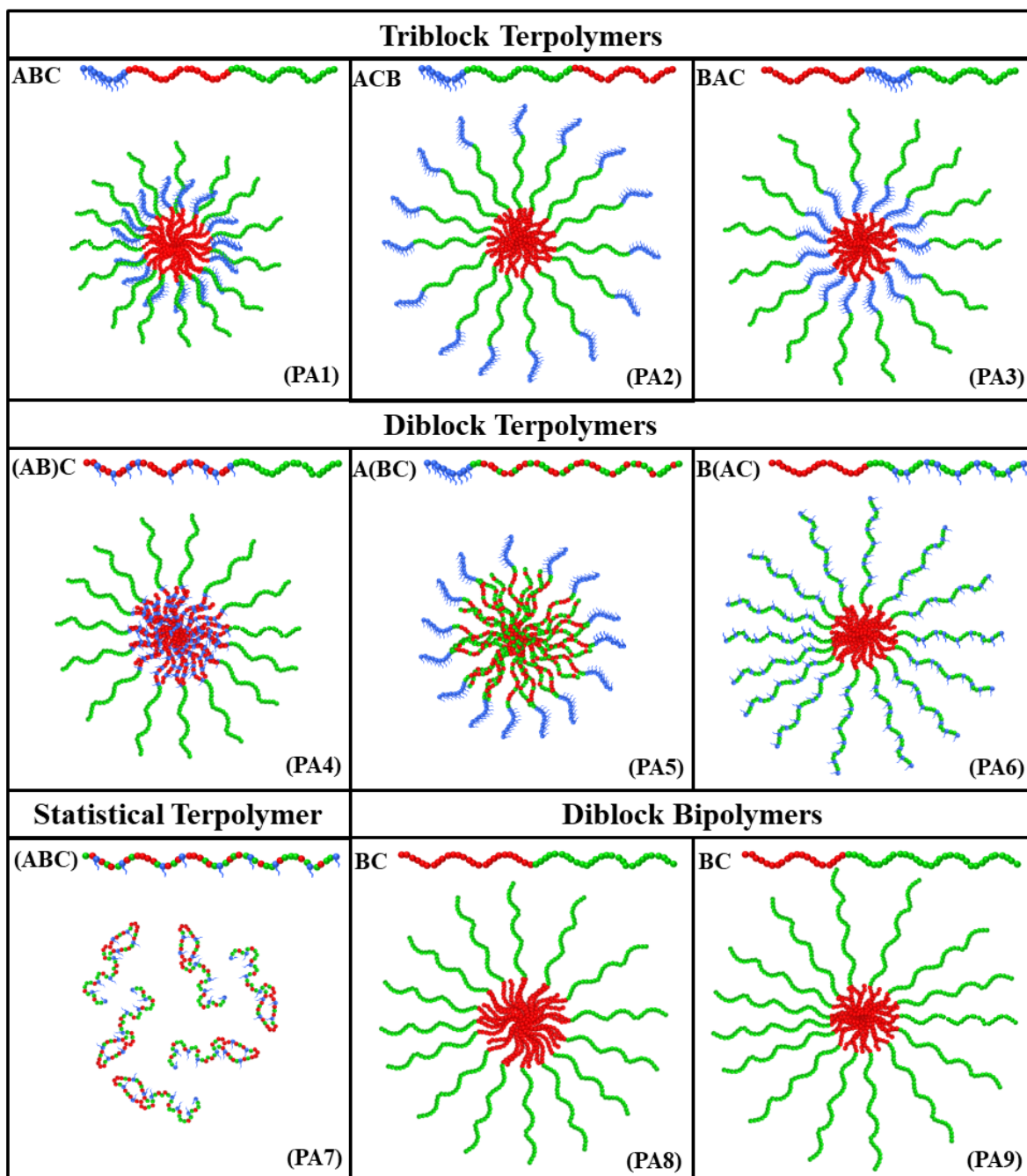


Figure 5.1.6: Schematic representation of the theoretical self-assembled structures assumed to be adopted by the copolymers. Spherical micelles are formed by: i) the triblock terpolymers (PA1-PA3), ii) the diblock terpolymers (PA4-PA6), and iii) the diblock bipolymers (PA8 and PA9). The statistical terpolymer (PA7) adopts a random coil configuration. The corresponding polymer structures are also shown. The TEGMA, BuMA, and DMAEMA repeated units are represented by blue, red, and light green spheres, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

Concerning the statistical terpolymer (PA7), species of size which corresponds to micelles are detected, which is consistent with the latest study of our group;^{1,4} for PA7, the maximum of the peak appears at 15.7 nm, while the theoretical calculation of a random coil is equal to 2.8 nm. The difference can be attributed to the theoretical calculation being based: i) only on the methacrylate backbone, i.e. the presence of side chains has not been taken into account, ii) a random coil configuration. However, the side chains, and especially the long side chain of TEGMA might contribute to the size. Also, the polymer chains might not be coiled, but extended instead;¹ the size of one extended polymer chain of PA7 (x) equals to 14.8 nm according to Equation 5.1.1 below. Also, as has been reported before, aggregation might be favoured by the presence of long side chains.⁷ The absence of micelle structures formed by PA7 has been confirmed by transmission electron microscopy (TEM), and the image can be found in the relevant paper by Constantinou *et al.*⁸

$$x \text{ (nm)} = (DP_{\text{TEGMA}} + DP_{\text{BuMA}} + DP_{\text{DMAEMA}}) \times 0.254 \quad \text{Equation 5.1.1}$$

5.1.3.2 Effective pK_a s

As DMAEMA is an ionisable unit, the polymers have been titrated and their determined pK_a values can be seen in Table 5.1.2 and in Figure 5.1.7. As can be observed, in most of the cases, the pK_a values vary between 6.7 and 6.9 which are in agreement with previously reported pK_a values of DMAEMA units.^{1-4,9-12} The polymer architecture does not seem to have significant effect on the pK_a , supporting the previous studies on linear polymers,^{2,3} star polymers,¹³ and polymeric networks.¹⁴⁻¹⁷ However, the statistical terpolymer (PA7) and the A(BC) diblock terpolymer (PA5) show lower pK_a values (i.e. the DMAEMA is a weaker base). Concerning the statistical architecture, this phenomenon has been reported before,^{1,4,9} and is ascribed to the statistical terpolymer being unable to self-assemble into micelles, as it was confirmed by TEM for PA7, and thus the polymer tends to have solubility issues and it precipitates during titration. Concerning the A(BC) architecture, the random copolymerisation of the DMAEMA with the BuMA weakens the basicity of the DMAEMA, as the DMAEMA units are surrounded by the hydrophobic BuMA ones. A hydrophobicity effect is also observed, as PA8 with 45 w/w% BuMA (its pK_a value is shown in red rhombus in Figure 5.1.7) shows a lower pK_a value. This observation is similar to previous studies, and can be ascribed to the reduced dielectric constant.^{2-4,9} The effect of hydrophobicity is also supported when PA7 is compared to the statistical terpolymer of a previous study by the group, PEGMA_{6-co}-BuMA_{18-co}-

DMAEMA₁₉,⁴ with which they have the same target MM and composition. It can be concluded that the TEGMA (instead of the more hydrophilic PEGMA) has a strong effect, as it reduces the pK_a from 6.7 to 6.1.

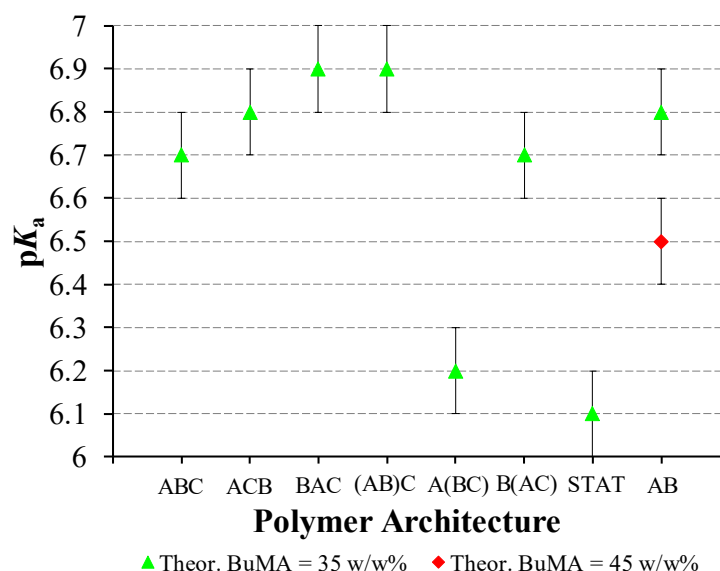


Figure 5.1.7: Effect of the architecture and the theoretical BuMA content on the pK_a of the DMAEMA units. The pK_a values for polymers with theoretical BuMA weight percentage of 35 w/w% and 45 w/w% are indicated by light green triangles, and red rhombus, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

5.1.3.3 Cloud Points

The CPs of the copolymers in water have been investigated by visual tests. The results for both initial pH (no protonation of the DMAEMA units) and at 10% protonation of the DMAEMA units are listed in Table 5.1.2.

As can be seen in Table 5.1.2, only PA1, PA4, and PA5 with ABC, (AB)C, and A(BC) architectures, respectively, have been tested for a CP at initial pH, as the rest of the polymers are insoluble. More specifically, PA1 and PA5 presented a CP at 29 and 32 °C, whereas PA4 did not present a CP within the temperature tested. This observation shows a clear effect of the polymer architecture. Firstly, PA1 forms the smallest micelles in this polymer series, and thus its solubility is enhanced. Concerning PA4 and PA5, in both architectures, the hydrophobic BuMA unit is randomly copolymerised with a hydrophilic unit, thus enhancing the overall hydrophilicity of the final structure, and the solubility of the polymers in water. However, PA4 is more hydrophilic than PA5, as the BuMA is copolymerised with the hydrophilic TEGMA (instead of the hydrophilic and thermoresponsive DMAEMA), and therefore, it does not present a CP. The rest of the polymers are insoluble, and this can be ascribed to the: i) formation

of bigger micelles by PA2 with ACB architecture, ii) absence of the hydrophilic TEGMA (PA9), and both absence of TEGMA and increased hydrophobic BuMA content (PA8), iii) inability of the statistical terpolymer (PA7) to self-assemble into micelles and stabilise itself in water, and iv) hydrophilicity of TEGMA units being masked in the BAC and B(AC) architectures (PA3 and PA6, respectively).

At 10% protonation of the DMAEMA units, none of the polymer solutions tested presents a CP, which shows a strong effect of the protonation of the amino groups on the thermoresponse, especially at low concentrations. This has been previously reported,^{18,19} and it can be attributed to the positive charges being present in the structure which: i) increase the hydrophilicity, and ii) contribute to the presence of electrostatic repulsions.

5.1.3.4 Visual Gel Points

As most of the polymers are not soluble at initial pH (see discussion in the previous Section – Section 5.1.3.3), polymer solutions in PBS have been prepared and the DMAEMA units have been protonated by 10% to promote dissolution. PA2 and PA8 with ACB and BC architecture are not soluble under these conditions, which can be attributed to the architecture and increased hydrophobic content, respectively. Therefore, their phase diagrams have not been constructed. The phase diagrams of the rest of the polymers are shown in Figure 5.1.8, in which four general states can be identified: runny solution phase in white, viscous solution phase in red, gel phase in blue, and two-phase system in green. More details about the transparency/cloudiness of the samples, and the type of two-phase system are given by different shapes.

Concerning the polymer solutions at 1 w/w%, CPs are detected in PBS, unlike in the case of DI water, with the exemption of PA1 with ABC architecture, that forms the smallest micelles. The difference in CPs between DI water and PBS is ascribed to the ionic strength effect, which has been previously observed for DMAEMA units.¹⁸ Also, when the CPs are compared, all the copolymers present a CP above 70 °C, with the exemption of PA5 and PA7 with A(BC) and statistical architecture, respectively, which present a CP at 42 °C and 43 °C, respectively. In both architectures, the thermoresponsive DMAEMA units are randomly copolymerised with the hydrophobic BuMA units, thus the hydrophobicity of the overall structure is increased, and the thermoresponse is enhanced. The increased hydrophobicity of these architectures is also supported by the low pK_a values, as discussed in Section 5.1.3.2.

Concentrated polymer solutions have also been investigated for thermal gelation. The gelation area (i.e. temperature and concentration values at which a stable gel is formed) is approximately shown by black dashed line on the phase diagrams in Figure 5.1.8. The gels can be described as transparent and cloudy, and these states are shown in blue triangles and circles, respectively. As can be seen in Figure 5.1.8, a gelation area is observed for five block copolymer solutions. Specifically, PA1, PA3, PA5, PA6, and PA9 with ABC, BAC, C(AB), B(AC), and BC architectures, respectively, form a gel upon temperature increase. The phase diagrams with a gelation area are divided into two categories, depending on the temperature range that the gelation is observed, and they are discussed in the following paragraphs. On the other hand, PA4 and PA7 with (AB)C and statistical architectures, respectively, do not present a gelation area; their phase diagrams are shown in Figure 5.1.8 (b). At this point it should be noted that the gelation of the statistical terpolymer solution at 30 w/w% is possibly due to the formation of entanglements. The inability of the statistical terpolymer to form a gel at lower concentrations than 30 w/w% is expected, and reported before by our group.⁹ Concerning PA4 with (AB)C architecture, neither a CP was observed in DI water, nor a gelation area in PBS. Both observations can be attributed to its architecture, i.e. copolymerisation of the hydrophilic TEGMA unit with the hydrophobic BuMA unit enhances the hydrophilicity of the polymer structure, and thus the ‘hydrophobic effect’ is interrupted.

Polymers which show sol-gel-sol transition belong to the first category, and their phase diagrams are shown in Figure 5.1.8 (a): PA1 and PA5 with ABC and A(BC) architectures belong to this category. More specifically, PA1 forms a gel at only 10 w/w%, and at a wide temperature range from 35 °C to 59 °C (at 30 w/w%). In all the concentrations that gelation is observed, a transparent gel is formed first, which becomes a cloudy one upon temperature increase. It is not surprising that PA1 with ABC architecture shows a typical phase diagram of a thermoresponsive polymer, as this architecture has been proven before to show the best gelling characteristics, i.e. clear transition from solution to gel phase, without any solubility issues. Nevertheless, the A(BC) architecture (PA5), which belongs to the three novel architectures studied in this project, shows a clear sol-gel transition close to body temperature. As opposed to the transparent gel formed by PA1, the gels of PA5 are cloudy, possibly due to the thermoresponsive units being polymerised in the same block as the hydrophobic BuMA units. It is noteworthy that these two polymers (PA1 and PA5) are the ones which are soluble at 1 w/w% in DI water and present a CP when the solution is not protonated, which confirms that there is a clear effect of the polymer architecture on the thermoresponsive properties.

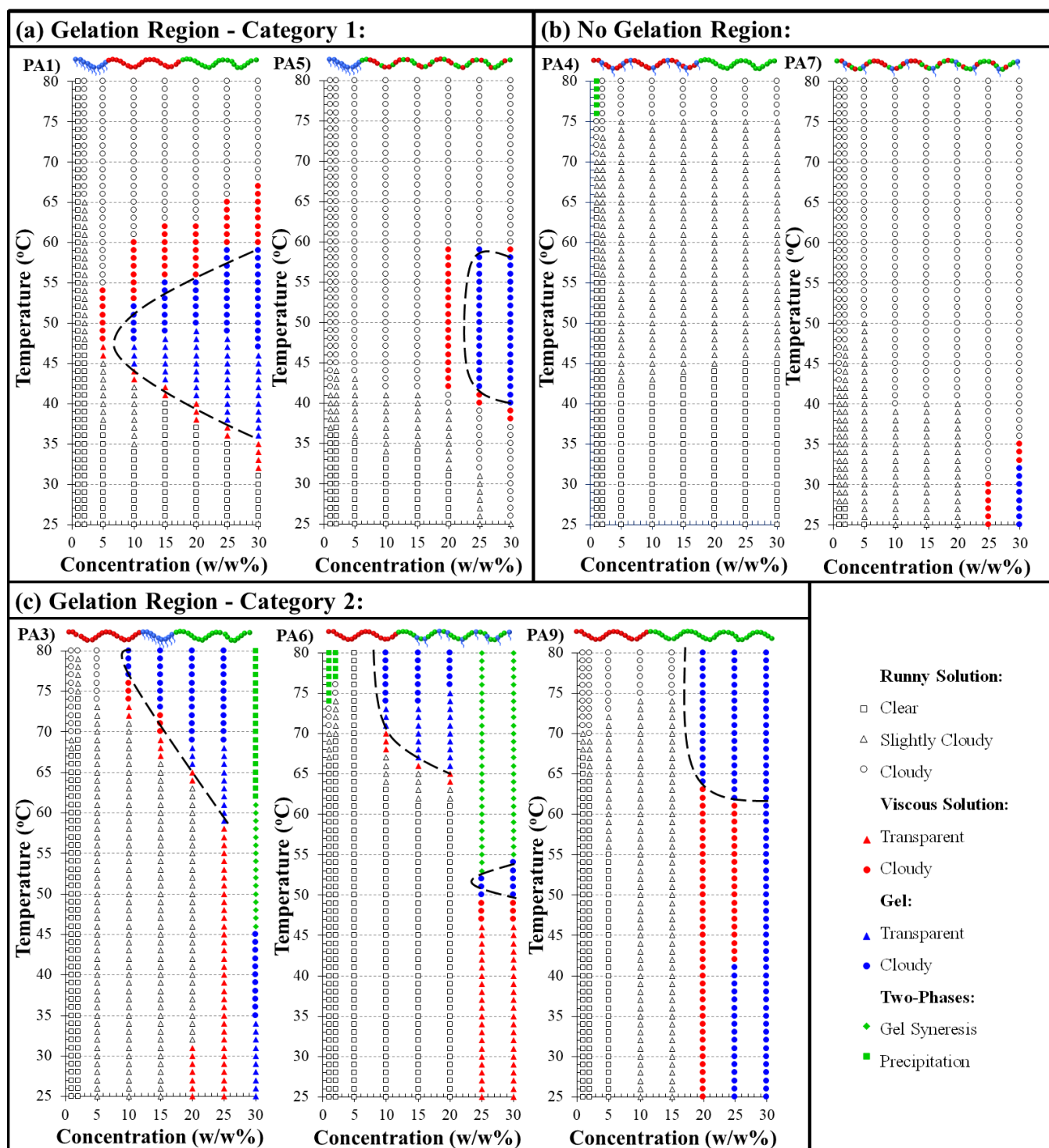


Figure 5.1.8: Phase diagrams of the soluble polymers in phosphate buffered saline (PBS) and 10% protonation of the DMAEMA units: i) triblock (PA1 & PA3), diblock (PA4-PA6), and random (PA7) terpolymers, and ii) diblock bipolymer (PA9). Four phases are observed: i) runny solution in white (square: clear, triangle: slightly cloudy, and circle: cloudy), ii) viscous solution in red (triangle and circle for transparent and cloudy, respectively), iii) the gel phase is shown in blue (triangle and circle for transparent and cloudy, respectively), the two-phase system in light green (rhombus and square for gel syneresis and precipitation, respectively). The corresponding polymer structures are also provided; the TEGMA, BuMA, and DMAEMA units are coloured in blue, red, and light green, respectively. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, *9*, 31.”

In the second category, the polymer solutions form gels at high temperatures ($T \geq 58\text{ }^{\circ}\text{C}$, concentration- and polymer-dependant), which remains stable until the highest temperature tested (i.e. $80\text{ }^{\circ}\text{C}$). In this category, PA3, PA6, and PA9 are included; the corresponding architectures are BAC, B(AC), and BC, respectively. In all the cases, the gelation temperature decreases as the concentration increases, as expected.^{4,20} Also, as the concentration of these polymer solutions increases, viscous solutions and/or gels are formed at room temperature, which is because of increased number of entanglements rather than thermogelation. As the temperature increases, the viscous solutions form gel, and in some cases, gel syneresis is observed at higher temperatures. When gels are observed at room temperature, these gels either get destabilised or remain stable gels as the temperature increases. Also, while PA3 and PA6, which are TEGMA-containing, form transparent gels, PA9, which is based on BuMA and DMAEMA only, does form cloudy ones, because of its increased hydrophobicity; this confirms the observation made before about the cloudy gels formed by PA5 with A(BC) architecture. Concerning the BAC architecture, it forms gels, but at higher temperature than the ABC one. This agrees with the previous study of the group in which the BAC architecture either did not form gels, or the gels were not as stable as the ones formed by the ABC architecture.¹

To summarise, a clear effect of the polymer architecture on the gelation has been demonstrated. When the triblock terpolymers are concerned, the ABC (PA1) shows the best thermoresponsive behaviour (i.e. clear sol-gel transition close to body temperature), whereas the ACB (PA2) is not soluble, and the BAC architecture (PA3) gels at higher temperatures than the ABC, which are not biologically applicable. The trends on the triblock terpolymers confirm the previous studies of our group.^{1,2} Also, new trends are established concerning the novel architectures of diblock terpolymers. Concerning the (AB)C architecture (PA4), no gelation is observed, and the B(AC) (PA6) architecture shows gelation at higher temperatures, which are not biologically applicable. The best architecture of the diblock terpolymers is the A(BC) one (PA5), which shows clear sol-gel transition close to body temperature. Also, the BC architecture is either insoluble (PA8), or forms gels at higher temperatures (PA9), depending on the composition. Therefore, two architectures show promising results: i) the ABC triblock terpolymer, and ii) the A(BC) diblock terpolymer. Both architectures show clear transition from a solution phase to a gel phase, and this transition is observed at temperatures close to body temperature; even though the gelation region is wider for the ABC architecture.

5.1.3.5 Gel Points by Rheology

The copolymer solutions have also been characterised by rheology at a concentration of 15 w/w%; the DMAEMA units have been protonated by 10% to promote solubility. The changes in storage modulus (G'), loss modulus (G''), and complex viscosity (η^*) over a range of temperatures from 30 °C to 80 °C are shown in dark blue, red, and light blue, respectively, in Figure 5.1.9 below. Specifically, three rheological curves are presented, which correspond to the polymer solutions which form a gel visually at the same concentration: (a) PA1 with ABC architecture, (b) PA3 with BAC architecture, and (c) PA6 with B(AC) architecture. The rheological results of the rest of the copolymers can be found in Figure A5 in the Appendix.

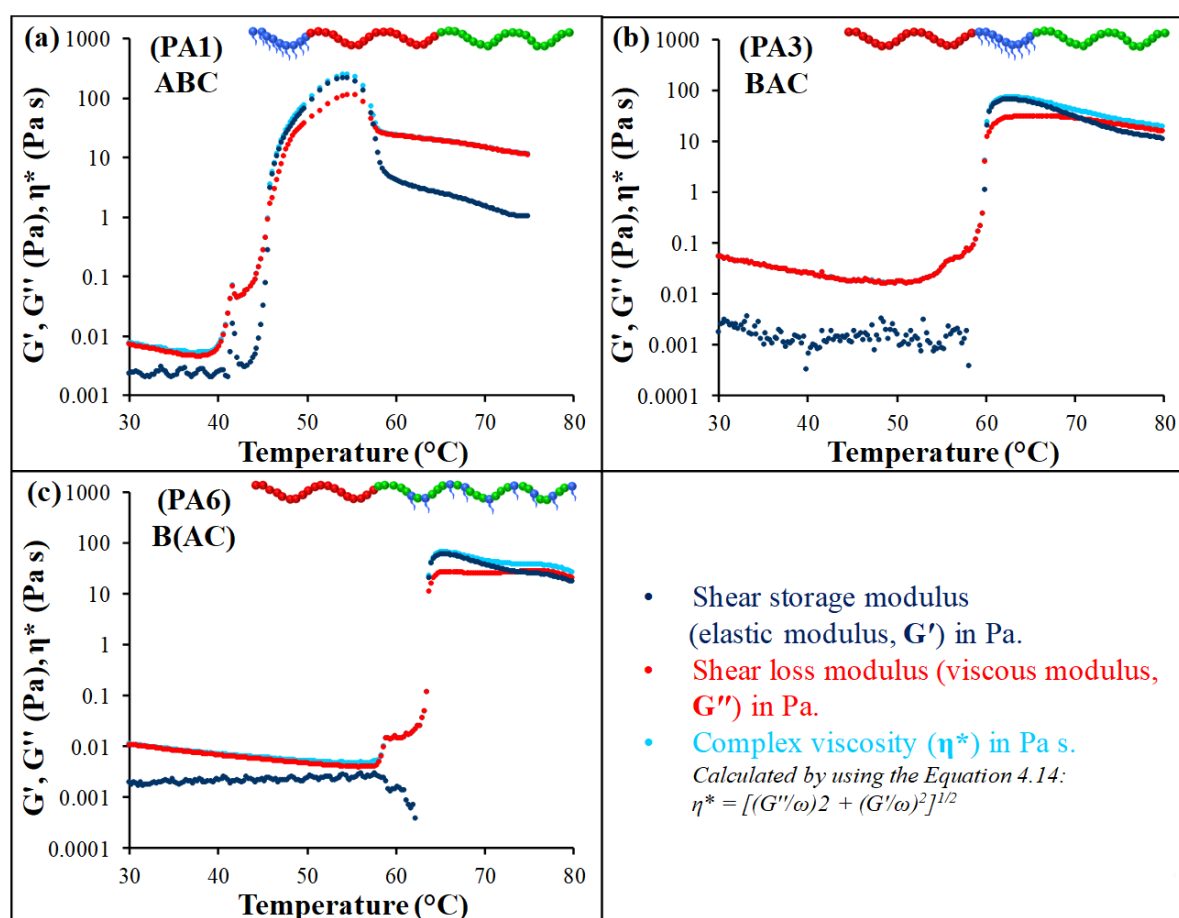


Figure 5.1.9: Rheological curves of the terpolymer solutions which form a gel visually at 15 w/w% in phosphate buffered saline (PBS), as resulted from temperature ramp measurements. The changes in the shear storage modulus (elastic modulus, G'), shear loss modulus (viscous modulus, G''), and the complex viscosity (η^*) are presented in dark blue, red, and light blue, respectively. The graphs correspond to: (a) the ABC triblock terpolymer (PA1, TEGMA₉-*b*-BuMA₂₀-*b*-DMAEMA₂₁), (b) the BAC triblock terpolymer (PA3, BuMA₂₀-*b*-TEGMA₉-*b*-DMAEMA₂₁), and (c) the B(AC) diblock terpolymer (PA6, BuMA₂₀-*b*-(TEGMA₉-*co*-DMAEMA₂₁)). The corresponding polymer structures are also shown, with blue, red, and light green spheres representing the TEGMA, BuMA, and DMAEMA repeated units, respectively.

As can be seen in the graphs in Figure 5.1.9, the viscosity as well as the storage and loss moduli increase as the temperature reaches their thermoresponsive point, and gel formation is observed, which confirms the gelation observed visually. At this point, it should be reminded that a gel is defined by rheology as the point (in this case temperature), at which the storage modulus becomes higher in magnitude than the loss modulus, i.e. $G' > G''$.²¹ On the other hand, a gel is determined visually as the temperature at which a stable gel is formed, i.e. it does not flow upon tube inversion. The gel points by rheology of PA1, PA3, and PA6 are 46 °C, 60 °C, and 64 °C, respectively, while by visual tests, gelation is observed at 43 °C, 73 °C, and 66 °C, respectively. While the gelation point by rheology of PA1 and PA6 agree with the visual observation, the solution of PA3 forms gels at lower temperatures by rheology than visually. This discrepancy can be explained by taking into consideration that even though a solvent trap is used, solvent evaporation might not be completely prevented. Hence, evaporation of water might take place at high temperatures, thus the concentration of the polymer increases, and gelation is observed at lower temperatures.

Also, in an attempt to compare the rheological results of PA3 and PA6, which are the ones that show visually thermoresponse at high temperatures, it can be seen that even though the rheological results of PA6 match the visual tests, the rheological and visual gel points of PA3 differ. This might be explained upon consideration of their polymer architectures. Specifically, PA3 has a BAC architecture, and PA6 has a B(AC) architecture. In the B(AC) architecture, the hydrophilic TEGMA units are closer to the surface of the micelle, whereas in BAC architecture, they are closer to the core of the micelle. Therefore, dipole-dipole interactions between the water molecules and the TEGMA units might be favoured in the case of B(AC) architecture compared to the BAC architecture, and thus evaporation of water affects more the rheological result of PA3 with BAC architecture, compared to the one of PA6 with B(AC) architecture.

5.1.4 Conclusions

In this Chapter, in total nine polymers of varying architectures and compositions have been synthesised using GTP, and they have been investigated in terms of their thermogelling properties. More specifically, these copolymers are based on TEGMA (A unit), BuMA (B unit), and DMAEMA (C unit) and have theoretical MM 8300 g mol⁻¹. The composition of the terpolymers has been kept constant at 23-35-40 w/w% (A-B-C), while the architecture has been varied as follows: i) three triblock terpolymers (ABC, ACB, and BAC), ii) three diblock terpolymers (A(BC), (AB)C, and B(AC)) – the units in the brackets have been randomly

copolymerised), and iii) a statistical terpolymer. For comparison, two BC diblock bipolymers of the same target MM have been synthesised and investigated: i) 46-54 w/w% B-C, which is the hydrophobic/thermoreponsive (B / C) ratio in the terpolymers, and ii) 35-65 w/w% B-C, which is the hydrophobic/hydrophilic $[B / (A + C)]$ ratio in the terpolymers. Several conclusions are drawn and presented below:

1. The copolymers have been successfully synthesised, i.e. they are well-defined (narrow MMD), and their MM values are within the range that has been proven before to induce clear sol-gel transition.
2. The architecture of the terpolymers controls their self-assembly behaviour in aqueous solutions, which is also affected by the degree of protonation of the DMAEMA units.
3. In general, the architecture of the terpolymers does not affect the pK_a values, with the exemption of the statistical terpolymer and the A(BC) diblock bipolymer, in which the pH-responsive DMAEMA units are randomly copolymerised with the BuMA ones.
4. The presence of TEGMA units increases the hydrophobicity of the terpolymers, and thus 10% protonation is required to enhance solubility.
5. The architecture of the terpolymers governs the gelation properties and thus only the ABC triblock terpolymer and the A(BC) diblock bipolymer show gelation at body temperature.
6. The ABC architecture is the most promising one, as it is the one presenting the widest gelation area (wide range of concentrations and temperatures).
7. The rheological results generally agree with the visual results. Some discrepancies can be explained upon consideration of the solvent evaporation taking place at higher temperatures.

5.1.5 References

1. Ward, M. A.; Georgiou, T. K. Multicompartment thermoresponsive gels: does the length of the hydrophobic side group matter? *Polym. Chem.* **2013**, *4*, 1893-1902.
2. Ward, M. A.; Georgiou, T. K. Thermoresponsive terpolymers based on methacrylate monomers: Effect of architecture and composition. *J. Polym. Sci. A Polym. Chem.* **2010**, *48*, 775-783.
3. Ward, M. A.; Georgiou, T. K. Thermoresponsive triblock copolymers based on methacrylate monomers: effect of molecular weight and composition. *Soft Matter* **2012**, *8*, 2737-2745.
4. Constantinou, A. P.; Georgiou, T. K. Thermoresponsive gels based on ABC triblock copolymers: effect of the length of the PEG side group. *Polym. Chem.* **2016**, *7*, 2045-2056.
5. Ghasdian, N.; Ward, M. A.; Georgiou, T. K. Well-defined clickable copolymers prepared via one-pot synthesis. *Chem. Commun.* **2014**, *50*, 7114-7116.
6. Constantinou, A. P.; Sam-Soon, N.; Carroll, D. R.; Georgiou, T. K. Thermoresponsive Tetrablock Terpolymers: Effect of Architecture and Composition on Gelling Behavior. *Macromolecules* **2018**.
7. Hadjiyannakou, S. C.; Vamvakaki, M.; Patrickios, C. S. Synthesis, characterization and evaluation of amphiphilic diblock copolymer emulsifiers based on methoxy hexa(ethylene glycol) methacrylate and benzyl methacrylate. *Polymer* **2004**, *45*, 3681-3692.
8. Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. A Comprehensive Systematic Study on Thermoresponsive Gels: Beyond the Common Architectures of Linear Terpolymers. *Polymers* **2017**, *9*, 31.
9. Ward, M. A.; Georgiou, T. K. Thermoresponsive gels based on ABA triblock copolymers: Does the asymmetry matter? *J. Polym. Sci. Part A: Polym. Chem.* **2013**, *51*, 2850-2859.
10. Simmons, M. R.; Patrickios, C. S. Synthesis and aqueous solution characterization of catalytically active block copolymers containing imidazole. *Macromolecules* **1998**, *31*, 9075-9077.
11. Georgiou, T. K.; Phylactou, L. A.; Patrickios, C. S. Synthesis, characterization, and evaluation as transfection reagents of ampholytic star copolymers: Effect of star architecture. *Biomacromolecules* **2006**, *7*, 3505-3512.
12. Georgiou, T. K.; Vamvakaki, M.; Patrickios, C. S.; Yamasaki, E. N.; Phylactou, L. A. Nanoscopic cationic methacrylate star homopolymers: Synthesis by group transfer polymerization, characterization and evaluation as transfection reagents. *Biomacromolecules* **2004**, *5*, 2221-2229.
13. Georgiou, T. K.; Vamvakaki, M.; Phylactou, L. A.; Patrickios, C. S. Synthesis, Characterization, and Evaluation as Transfection Reagents of Double-Hydrophilic Star Copolymers: Effect of Star Architecture. *Biomacromolecules* **2005**, *6*, 2990-2997.
14. Kali, G.; Georgiou, T. K.; Iván, B.; Patrickios, C. S. Anionic amphiphilic end-linked conetworks by the combination of quasiliving carbocationic and group transfer polymerizations. *J. Polym. Sci. A Polym. Chem.* **2009**, *47*, 4289-4301.
15. Kali, G.; Georgiou, T. K.; Iván, B.; Patrickios, C. S.; Loizou, E.; Thomann, Y.; Tiller, J. C. Synthesis and characterization of anionic amphiphilic model conetworks of 2-butyl-1-octyl-methacrylate and methacrylic acid: Effects of polymer composition and architecture. *Langmuir* **2007**, *23*, 10746-10755.
16. Kali, G.; Georgiou, T. K.; Iván, B.; Patrickios, C. S.; Loizou, E.; Thomann, Y.; Tiller, J. C. Synthesis and Characterization of Anionic Amphiphilic Model Conetworks Based on Methacrylic Acid and Methyl Methacrylate: Effects of Composition and Architecture. *Macromolecules* **2007**, *40*, 2192-2200.
17. Georgiou, T. K.; Patrickios, C. S.; Groh, P. W.; Iván, B. Amphiphilic model conetworks of polyisobutylene methacrylate and 2-(dimethylamino)ethyl methacrylate prepared by the combination of quasiliving carbocationic and group transfer polymerizations. *Macromolecules* **2007**, *40*, 2335-2343.
18. de Souza, J.; Carlos Perbone; Naves, A. F.; Florenzano, F. Specific thermoresponsiveness of PMMA-block-PDMAEMA to selected ions and other factors in aqueous solution. *Colloid Polym. Sci.* **2012**, *290*, 1285-1291.
19. Fournier, D.; Hoogenboom, R.; Thijs, H. M. L.; Paulus, R. M.; Schubert, U. S. Tunable pH- and temperature-sensitive copolymer libraries by reversible addition-fragmentation chain transfer copolymerizations of methacrylates. *Macromolecules* **2007**, *40*, 915-920.

20. Constantinou, A. P.; Georgiou, T. K. Tuning the gelation of thermoresponsive gels. *Eur. Polym. J.* **2016**, *78*, 366-375.
21. Yu, G.; Li, H.; Fairclough, J. P. A.; Ryan, A. J.; McKeown, N.; Ali-Adib, Z.; Price, C.; Booth, C. A Study of Lyotropic Mesophases of Concentrated Solutions of a Triblock Copolymer of Ethylene Oxide and 1,2-Butylene Oxide, E16B10E16, Using Rheometry, Polarized Light Microscopy, and Small-Angle X-ray Scattering. *Langmuir* **1998**, *14*, 5782-5789.

5.2 Thermoresponsive Tetrablock Terpolymers

Note to the readers: Part of this Chapter (results for PB11-PB25) has been already published and can be found in: “<<https://pubs.acs.org/doi/10.1021/acs.macromol.8b01251>>, Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. Thermoresponsive Tetrablock Terpolymers: Effect of Architecture and Composition on Gelling Behavior. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251”. Permission for this reuse has been granted by the ACS, and further permissions related to the material excerpted should be directed to the ACS.

As mentioned in Chapter 3 (Aim of this Thesis), thermoresponsive tetrablock terpolymers have not been investigated in a great extent, as only one study has been found in the literature.¹ In this study, the effects of the pH and the temperature on the micellisation behaviour of ABCD tetrablock terpolymers were investigated.¹ The copolymers were synthesised via RAFT, ATRP and click chemistry. EG, styrene (St), NIPAAm, and DMAEMA were used to form the A, B, C, and D blocks, respectively. It should be noted that both NIPAAm and DMAEMA are thermoresponsive, however, in this study, DMAEMA was incorporated into the structure to provide pH response. It was observed that the hydrophilicity of the DMAEMA was affected by changing the pH, i.e. at low pH, DMAEMA was hydrophilic, thus it takes part in the shell of the micelle, whereas at high pH, DMAEMA was hydrophobic, thus collapsing in the core of the micelle. When the pH of the solution increased from 4 to 9, the LCST of EG₄₃-*b*-St₂₄-*b*-NIPAAm₉₆-*b*-DMAEMA₈₆ decreased, because of the DMAEMA units being more hydrophobic at higher pH, thus enhancing the “hydrophobic effect”. Concerning the effect of temperature, it was observed that when the temperature was increased, the NIPAAm units responded to the temperature by becoming hydrophobic, thus collapsing in the core of the micelle.¹ However, this study has not been carried out in a systematic way, and therefore, the aim of this Chapter is to systematically investigate thermoresponsive tetrablock terpolymers for the first time.

In this study, the architecture of the tetrablock terpolymers, i.e. the position/order of the blocks, has been systematically varied by keeping the same MM and composition within the series. As our group has previously extensively studied thermoresponsive polymers based on PEGMA, BuMA, and DMAEMA,²⁻⁵ consequently the tetrablock terpolymers of this study are based on the same units for comparison (PEGMA, BuMA, and DMAEMA are the A, B, and C units, respectively). In this study, each different monomer is polymerised to form a distinct block,

and one of the blocks is repeated twice, thus tetrablock terpolymers are formed. All possible combinations have been investigated: i) two PEGMA blocks (A blocks) – ABCA, ABAC, and ACAB, ii) two BuMA blocks (B blocks) – BABC, BACB, and ABCB, and iii) two DMAEMA blocks (C blocks) – CABC, CACB, and ACBC. Therefore, in total nine tetrablock architectures have been studied. Three families of tetrablock terpolymers have been synthesised, which differ on the A-B-C composition, i) nine tetrablock terpolymers, and one statistical terpolymer, with target A-B-C composition of 20-40-40 w/w% (PB1-PB10), ii) nine tetrablock terpolymers, and one triblock terpolymer, but of target A-B-C composition of 20-30-50 w/w% (P11-P20), and iii) four selected tetrablock architectures, and one triblock terpolymer with intermediate A-B-C composition of 20-35-45 w/w% (PB21-PB25). The tetrablock architectures studied in the third family are the ABAC, BABC, CABC, and ACBC, and they have been chosen for reasons that will be discussed in Section 5.2.3.4. Concerning the MM of the terpolymers, this has been targeted to 7600 g mol^{-1} for the first family, while for the second and third families, the MM has been targeted to 8300 g mol^{-1} instead, in order to obtain the same experimental MM as in the first family; this difference will be discussed in more detail in Section 5.2.2. Nevertheless, both MM values are within the desirable range which has been proven before to produce polymers which show clear sol-gel transition. Therefore, in total, twenty-two tetrablock terpolymers, one statistical terpolymer, and two triblock terpolymers have been synthesised, and the effects of architecture and composition on the gelation have been systematically investigated and they are presented and discussed in this Chapter.

The chemical structures of the monomers used in this Chapter, and their full and abbreviated names are shown in Figure 5.2.1.

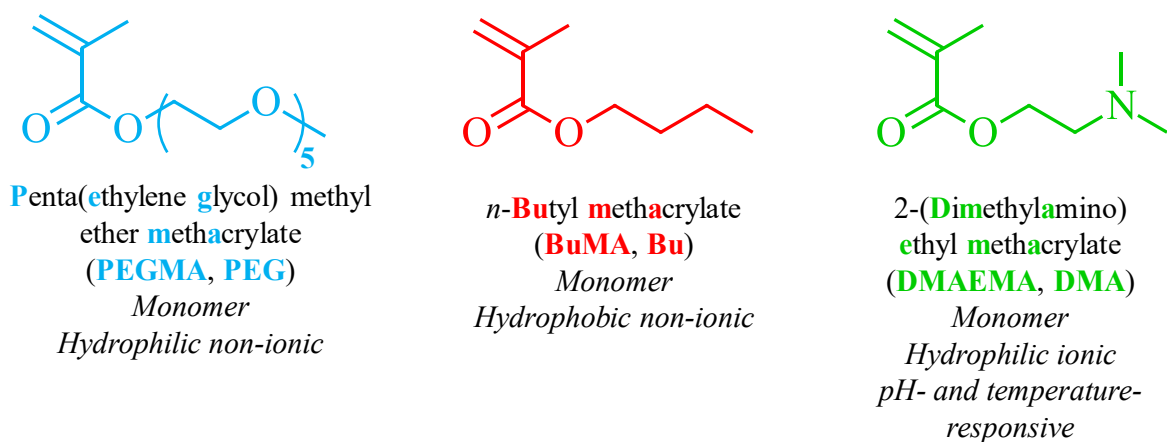


Figure 5.2.1: Chemical structures, names, and abbreviations of the monomers used for the polymerisation, which are penta(ethylene glycol) methyl ether methacrylate (PEGMA), *n*-butyl methacrylate (BuMA), and 2-(dimethylamino) ethyl methacrylate (DMAEMA).

5.2.1 Polymer Synthesis

GTP has been also used to fabricate the polymers of this study. All the copolymers, regardless the architecture, have been prepared via one-pot synthesis. The synthetic route of an ABCA tetrablock terpolymer is schematically illustrated in Figure 5.2.2; where A, B and C correspond to PEGMA, BuMA, and DMAEMA, respectively. As can be seen in Figure 5.2.2, a tetrablock terpolymer is synthesised via sequential addition of the monomers after the addition of the catalyst (TBABB), the solvent (THF), and the monofunctional initiator (MTS), similarly to the synthesis of a triblock terpolymer, which has been previously discussed in Section 5.1.1. The only difference is that an extra addition is required to form the fourth block. All the block copolymers have been synthesised using a similar synthetic route; the amounts and order of additions have been varied to obtain the desirable architectures, compositions, and MMs. The statistical terpolymer has been synthesised by adding the initiator (MTS) last, as discussed in Section 5.1.1.

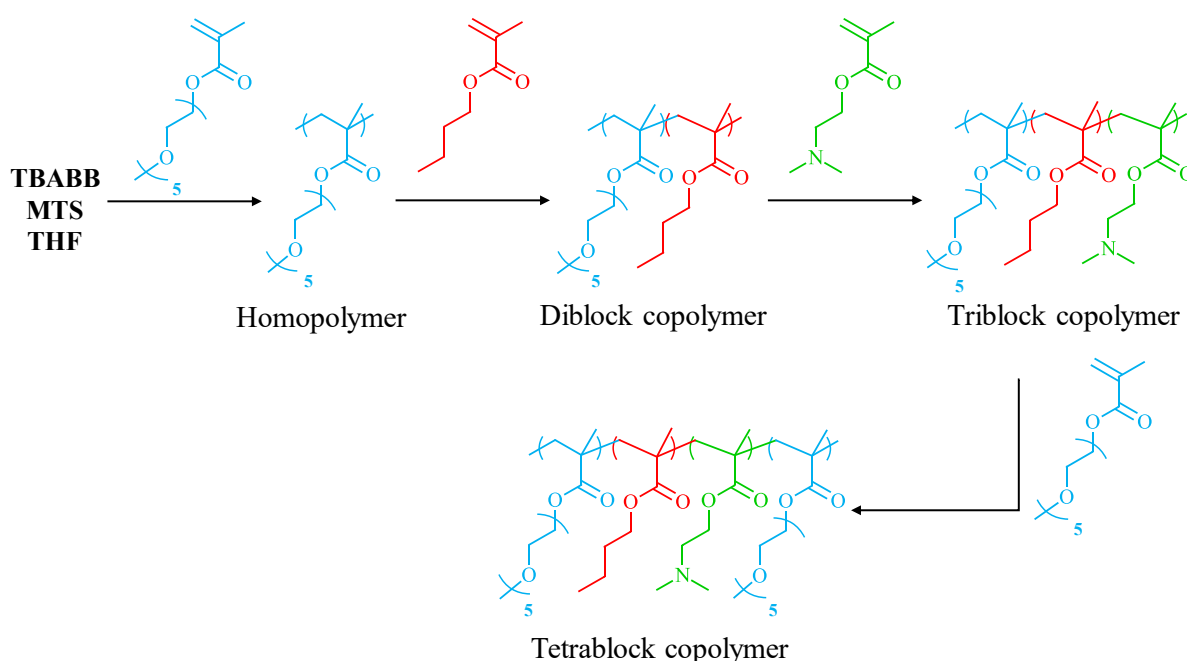


Figure 5.2.2: Chemical reaction of the sequential GTP of an ABCA tetrablock terpolymer; where A, B, and C are PEGMA, BuMA, and DMAEMA, respectively. *Note:* TBABB, MTS, and THF are the catalyst, initiator, and solvent, respectively, used for this PhD project.

The polymer structures are schematically illustrated in Figure 5.2.3; the PEGMA, BuMA, and DMAEMA units are represented by light blue, red, and light green spheres, respectively. As can be observed in Figure 5.2.3, twenty-two tetrablock terpolymers of varying architectures and compositions have been investigated. PB1-PB9 belong to the first family with target MM and A-B-C composition of 7600 g mol^{-1} and 20-40-40 w/w%, respectively. In the second

family, the terpolymers have MM and A-B-C composition of 8300 g mol⁻¹ and 20-30-50 w/w%, respectively (PB11-PB20), whereas the terpolymers of the third family are of 20-35-45 w/w% A-B-C composition (PB21-PB25) and 8300 g mol⁻¹ target MM.

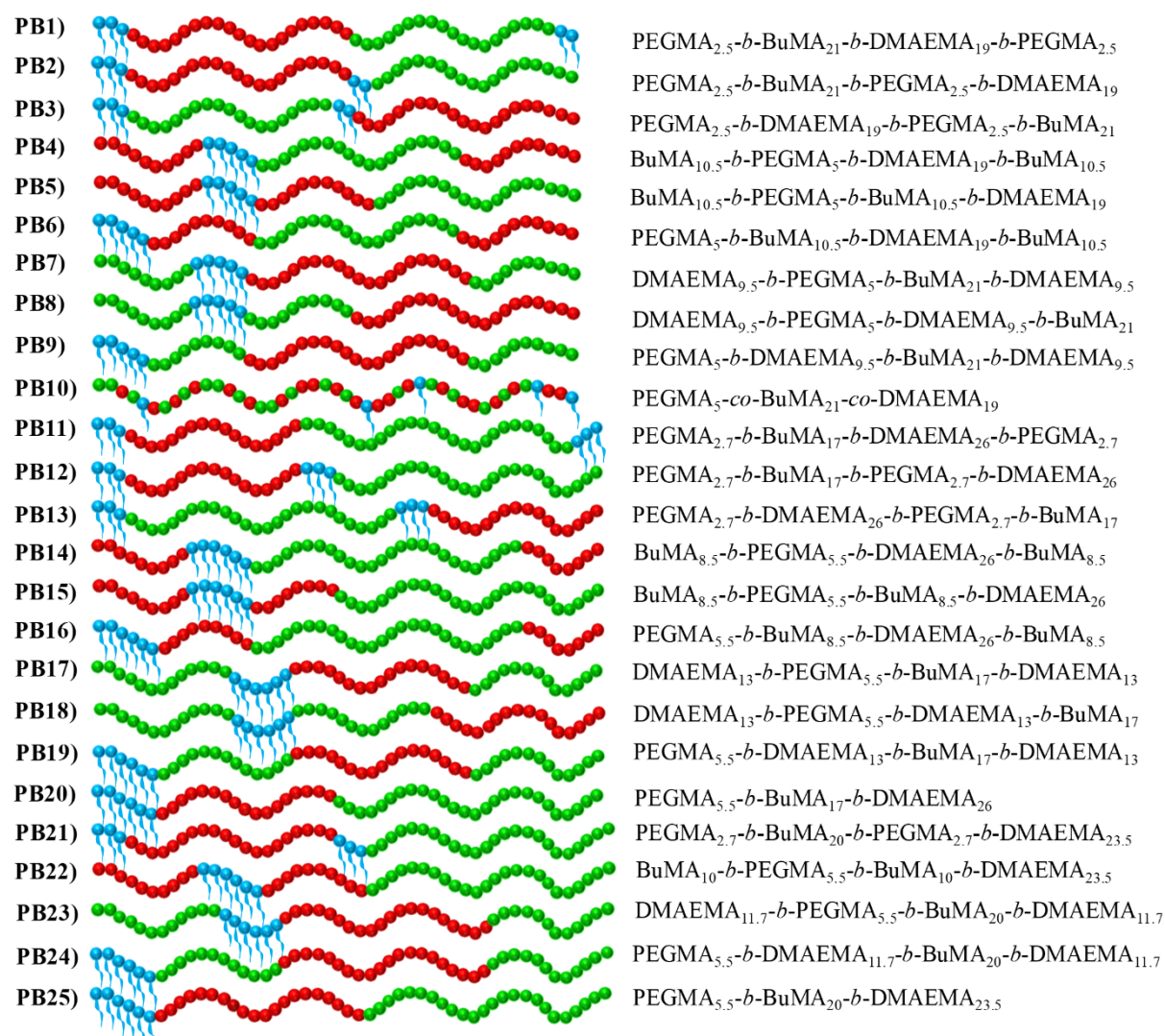


Figure 5.2.3: Schematic representation of the tetrablock (PB1-PB9, PB11-PB19, and PB21-PB24), triblock (PB20 and PB25), and statistical (PB9) terpolymers of three different PEGMA-BuMA-DMAEMA compositions, which were investigated in this project: i) PB1-PB10 are of 20-40-40 w/w%, ii) PB11-PB20 are of 20-30-50 w/w%, and iii) PB21-PB25 have composition of 20-35-45 w/w%. The PEGMA, BuMA, and DMAEMA units are coloured in light blue, red, and light green, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

5.2.2 Molecular Mass and Composition

The final terpolymers and their linear precursors (in the case of tetrablock and triblock terpolymers) have been analysed by GPC in terms of their MM and MMD. The GPC traces of PB1 with theoretical polymer structure PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉-*b*-PEGMA_{2.5}

are shown in Figure 5.2.4 with blue solid line. The GPC traces of its linear precursors are also shown on the same graph: i) light blue solid line for PEGMA_{2.5}, ii) red dotted line for PEGMA_{2.5}-*b*-BuMA₂₁, and iii) light green dotted line for PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉. As can be seen in Figure 5.2.4, the sequential GTP of the tetrablock terpolymer is successful, as after the addition of a new monomer, the MM increases. This indicates that the monomer has been successfully added on the active polymer chains. Also, the “living” nature of GTP is confirmed by the absence of any shoulder, which would indicate significant deactivation of the polymer chains, and prevention of chain extension upon monomer addition. The GPC traces of all the final terpolymers and their linear precursors (if any) are similar, thus confirming the successful polymerisation; these can be found in Figure A2 in the Appendix.

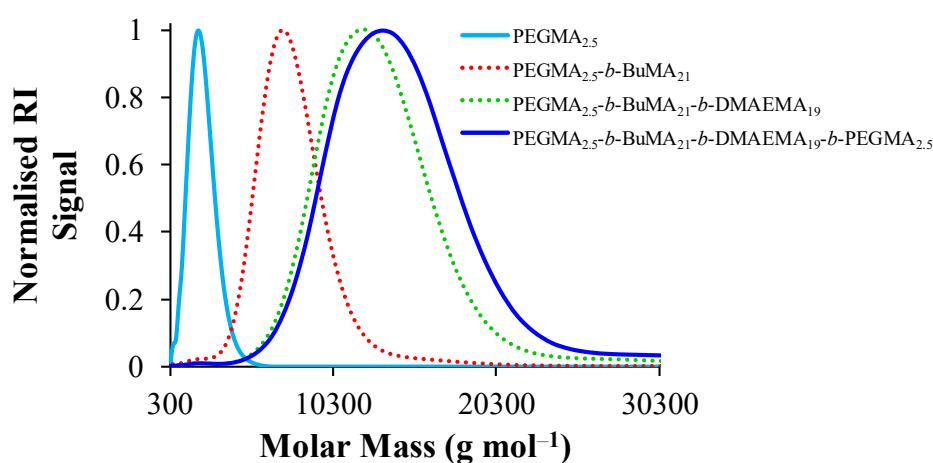


Figure 5.2.4: GPC chromatograms of the tetrablock terpolymer PB1 with theoretical polymer structure PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉-*b*-PEGMA_{2.5} (blue solid line), and its linear precursors: i) PEGMA_{2.5} in light blue solid line, ii) the diblock copolymer PEGMA_{2.5}-*b*-BuMA₂₁ in red dotted line, and iii) the triblock terpolymer PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉ in light green dotted line.

The theoretical MMs, and the experimental MM (M_n) and $\bar{D}$ values, as determined by GPC, are recorded in Table 5.2.1. At this point it should be noted that the difference in theoretical MM (7600 g mol⁻¹ versus 8300 g mol⁻¹) between the first series of tetrablock terpolymers (PB1-PB10) and the other two series (PB11-PB25) is because of the different experimental conditions. Specifically, the first family has been synthesised by using freshly distilled THF from the THF reflux, whereas the THF for the synthesis of the rest of the terpolymers has been purified through a solvent purification system. In our laboratory, the set-up of THF reflux provided THF with higher percentage of humidity compared to the solvent purification system. Therefore, the percentage of deactivation provided by the THF reflux has been taken into consideration, and the target MM has been adjusted in order to produce polymers of final MM being within the desirable range. As can be seen in Table 5.2.1, the M_n values vary between

8500 g mol⁻¹ and 12500 g mol⁻¹, and it has been proven before that polymers within this MM range show clear transition from a solution phase to a gel phase.⁴ These values are higher than either of the targeted theoretical MMs, possibly due to the initiator molecules or active polymer chains being deactivated during the polymerisation; this has been reported before in GTP studies.²⁻⁶ Also, as can be seen in Table 5.2.1, this discrepancy is more pronounced for polymers PB1-PB10, which have been synthesised using THF purified via reflux procedure. This confirms that the solvent purification system produces polymers of experimental MM being closer to the theoretical one, thus the target MM of 8300 g mol⁻¹ (instead of 7600 g mol⁻¹) can be explained.

The $\bar{D}$ values are also listed in Table 5.2.1. and they are sufficiently close to 1, which is attributed to the “living” nature of GTP; this has been reported in other GTP studies.²⁻⁶ More specifically, the most and least well-defined terpolymers have $\bar{D}$ values of 1.10 and 1.23, respectively. These values are slightly higher than the commonly reported $\bar{D}$ values of the triblock terpolymers synthesised by GTP.^{2-5,7} This can be ascribed to the higher number of additions and withdraws when a tetrablock terpolymer is synthesised, compared to the commonly synthesised diblock and triblock copolymers. This introduces humidity in the polymerisation flask, thus leading to deactivation of polymer chains and increases the dispersity values. This explanation can be supported by previous studies on GTP.⁸⁻¹⁰ In Popescu *et al.*'s study, tri-, penta-, hepta-, and nonablock copolymers have been synthesised *via* GTP, and it has been observed that the higher the number of blocks, and the higher the content of the least reactive monomer, the higher the dispersity.⁸

The successful synthesis has been also confirmed by ¹H NMR spectroscopy, and the experimental compositions have been determined by analysing the ¹H NMR spectra. Figure 5.2.5 (d) shows the ¹H NMR spectrum of PB1 with theoretical polymer structure PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉-*b*-PEGMA_{2.5} (shown in blue). Figure 5.2.5 also shows the ¹H NMR of (a) PEGMA_{2.5} homopolymer (in light blue), (b) PEGMA_{2.5}-*b*-BuMA₂₁ diblock copolymer (in red), and (c) PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉ (in light green). No peaks related to unreacted monomers are observed in the ¹H NMR spectra, which confirms the complete consumption of the monomers and their conversion to polymers. As can be seen, additional peaks appear in the ¹H NMR spectra in Figure 5.2.5 (b), and (c), which correspond to BuMA and DMAEMA units, respectively. This confirms the successful sequential synthesis of block copolymers. Concerning the final block, which is PEGMA-based, no additional peaks are observed, as expected, but the integration of the peaks which are related to PEGMA is

increased. Similar observations have been made for all the copolymers. By using the integration of the peaks, the experimental PEGMA-BuMA-DMAEMA weight percentages have been calculated, as explained in the experimental part. The peaks which have been used for the calculations are the ones labelled in Figure 5.2.5 as e, f, and l, for PEGMA, BuMA, and DMAEMA, respectively. The experimental compositions are listed in Table 5.2.1 and they are equal to the theoretical ones, within the error of the technique.

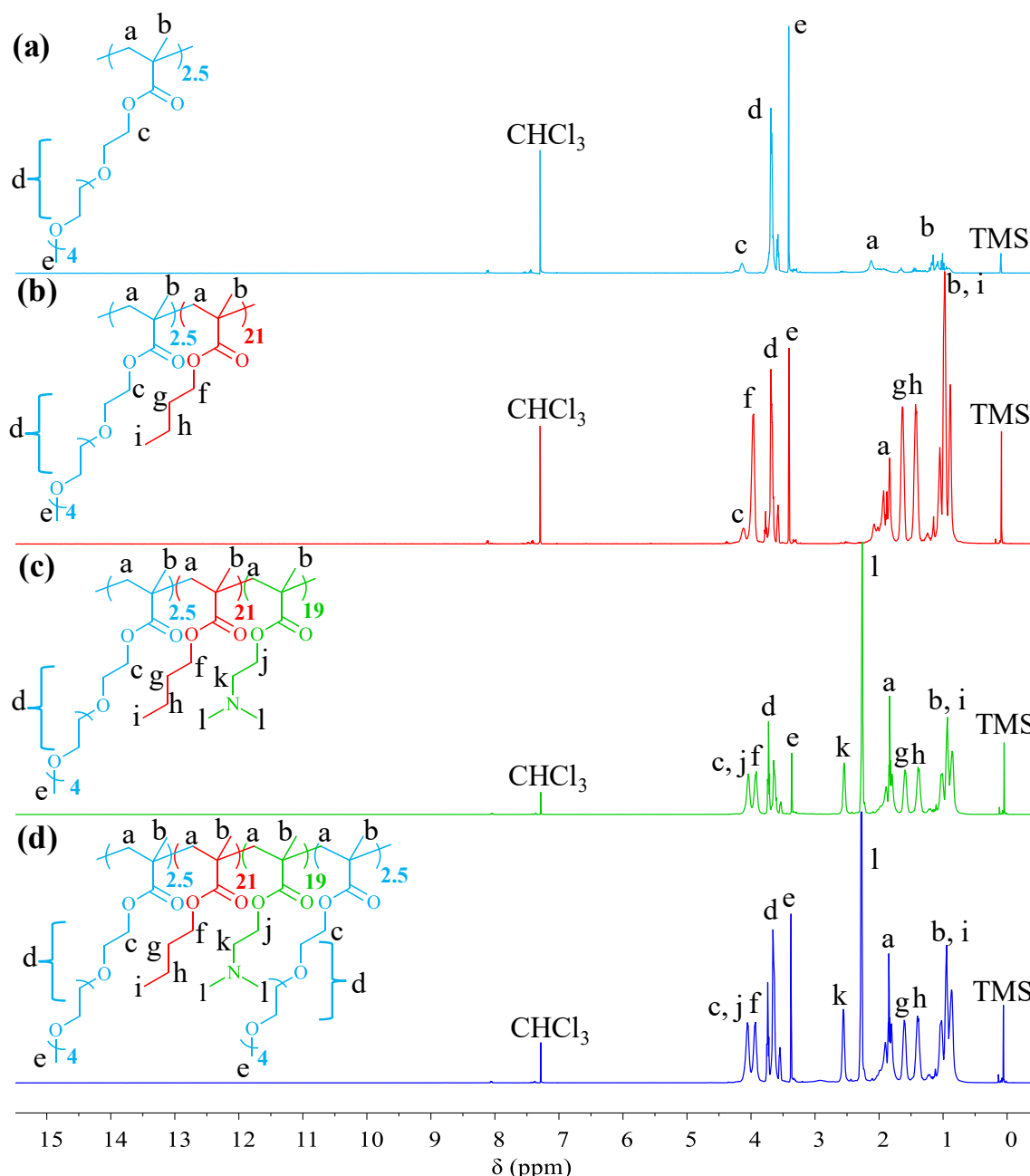


Figure 5.2.5: ^1H NMR spectra of (a) the PEGMA_{2.5} homopolymer in light blue, (b) the PEGMA_{2.5}-*b*-BuMA₂₁ diblock copolymer in red, (c) the PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉ triblock terpolymer in light green, and (d) the PEGMA_{2.5}-*b*-BuMA₂₁-*b*-DMAEMA₁₉-*b*-PEGMA_{2.5} tetrablock terpolymer (Polymer B1) in blue. The corresponding chemical structures are also shown; the PEGMA, BuMA, and DMAEMA repeated units are coloured in light blue, red, and light green, respectively.

Table 5.2.1: Theoretical polymer structure, molar mass ($MM^{\text{theor.}}$), and composition, and experimental results of number-average molar mass (M_n), molar mass distribution (MMD, denoted as dispersity index, $\bar{D}$), and composition of the terpolymer and their linear precursors. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

No.	Theoretical Polymer Structure ^a	$MM^{\text{theor. b}}$ (g mol ⁻¹)	M_n^c (g mol ⁻¹)	$\bar{D}^c$	w/w% PEG-Bu-DMA	
					Theoretical	¹ H NMR
PB1	PEG _{2.5}	850	1600	1.24	100-00-00	100-00-00
	PEG _{2.5} - <i>b</i> -Bu ₂₁	3850	6600	1.11	20-80-00	21-79-00
	PEG _{2.5} - <i>b</i> -Bu ₂₁ - <i>b</i> -DMA ₁₉	6850	11300	1.08	11-44-44	13-44-43
	PEG _{2.5} - <i>b</i> -Bu ₂₁ - <i>b</i> -DMA ₁₉ - <i>b</i> -PEG _{2.5}	7600	12200	1.10	20-40-40	22-39-39
PB2	PEG _{2.5}	850	1500	1.27	100-00-00	100-00-00
	PEG _{2.5} - <i>b</i> -Bu ₂₁	3850	6400	1.13	20-80-00	22-78-00
	PEG _{2.5} - <i>b</i> -Bu ₂₁ - <i>b</i> -PEG _{2.5}	4600	7700	1.11	33-67-00	35-65-00
	PEG _{2.5} - <i>b</i> -Bu ₂₁ - <i>b</i> -PEG _{2.5} - <i>b</i> -DMA ₁₉	7600	12300	1.10	20-40-40	21-38-41
PB3	PEG _{2.5}	850	1700	1.15	100-00-00	100-00-00
	PEG _{2.5} - <i>b</i> -DMA ₁₉	3850	6300	1.12	20-00-80	23-00-77
	PEG _{2.5} - <i>b</i> -DMA ₁₉ - <i>b</i> -PEG _{2.5}	4600	7700	1.12	33-00-67	38-00-62
	PEG _{2.5} - <i>b</i> -DMA ₁₉ - <i>b</i> -PEG _{2.5} - <i>b</i> -Bu ₂₁	7600	11800	1.20	20-40-40	21-39-40
PB4	Bu _{10.5}	1600	2700	1.11	00-100-00	00-100-00
	Bu _{10.5} - <i>b</i> -PEG ₅	3100	4900	1.10	50-50-00	52-48-00
	BuMA _{10.5} - <i>b</i> -PEG ₅ - <i>b</i> -DMA ₁₉	6100	8700	1.12	25-25-50	26-26-48
	Bu _{10.5} - <i>b</i> -PEG ₅ - <i>b</i> -DMA ₁₉ - <i>b</i> -Bu _{10.5}	7600	11100	1.14	20-40-40	21-40-39
PB5	Bu _{10.5}	1600	3000	1.10	00-100-00	00-100-00
	Bu _{10.5} - <i>b</i> -PEG ₅	3100	5600	1.10	50-50-00	50-50-00
	Bu _{10.5} - <i>b</i> -PEG ₅ - <i>b</i> -Bu _{10.5}	4600	8200	1.11	33-67-00	35-65-00
	Bu _{10.5} - <i>b</i> -PEG ₅ - <i>b</i> -Bu _{10.5} - <i>b</i> -DMA ₁₉	7600	12000	1.17	20-40-40	21-40-39
PB6	PEG ₅	1600	2700	1.14	100-00-00	100-00-00
	PEG ₅ - <i>b</i> -Bu _{10.5}	3100	4900	1.14	50-50-00	51-49-00
	PEG ₅ - <i>b</i> -Bu _{10.5} - <i>b</i> -DMA ₁₉	6100	9600	1.19	25-25-50	15-29-56
	PEG ₅ - <i>b</i> -Bu _{10.5} - <i>b</i> -DMA ₁₉ - <i>b</i> -Bu _{10.5}	7600	11600	1.23	20-40-40	22-39-39
PB7	DMA _{9.5}	1600	2300	1.12	00-00-100	00-00-100
	DMA _{9.5} - <i>b</i> -PEG ₅	3100	4300	1.12	50-00-50	53-00-47
	DMA _{9.5} - <i>b</i> -PEG ₅ - <i>b</i> -Bu ₂₁	6100	8100	1.17	25-50-25	27-49-24
	DMA _{9.5} - <i>b</i> -PEG ₅ - <i>b</i> -Bu ₂₁ - <i>b</i> -DMA _{9.5}	7600	9600	1.20	20-40-40	21-39-40
PB8	DMA _{9.5}	1600	2300	1.12	00-00-100	00-00-100
	DMA _{9.5} - <i>b</i> -PEG ₅	3100	4300	1.11	50-00-50	53-00-47
	DMA _{9.5} - <i>b</i> -PEG ₅ - <i>b</i> -DMA _{9.5}	4600	6000	1.12	33-00-67	36-00-64
	DMA _{9.5} - <i>b</i> -PEG ₅ - <i>b</i> -DMA _{9.5} - <i>b</i> -Bu ₂₁	7600	9700	1.20	20-40-40	21-40-39
PB9	PEG ₅	1600	2600	1.14	100-00-00	100-00-00
	PEG ₅ - <i>b</i> -DMA _{9.5}	3100	4400	1.12	50-00-50	53-00-47
	PEG ₅ - <i>b</i> -DMA _{9.5} - <i>b</i> -Bu ₂₁	6100	8600	1.17	25-50-25	26-49-25
	PEG ₅ - <i>b</i> -DMA _{9.5} - <i>b</i> -Bu ₂₁ - <i>b</i> -DMA _{9.5}	7600	11100	1.19	20-40-40	20-40-40
PB10	PEG ₅ - <i>co</i> -Bu ₂₁ - <i>co</i> -DMA ₁₉	7600	9900	1.11	20-40-40	22-38-40

PB11	PEG _{2.7}	920	1800	1.17	100-00-00	100-00-00
	PEG _{2.7} - <i>b</i> -Bu ₁₇	3380	5300	1.11	25-75-00	26-74-00
	PEG _{2.7} - <i>b</i> -Bu ₁₇ - <i>b</i> -DMA ₂₆	7480	10700	1.10	11-33-56	12-33-55
	PEG _{2.7} - <i>b</i> -Bu ₁₇ - <i>b</i> -DMA ₂₆ - <i>b</i> -PEG _{2.7}	8300	11300	1.12	20-30-50	22-28-50
PB12	PEG _{2.7}	920	1600	1.21	100-00-00	100-00-00
	PEG _{2.7} - <i>b</i> -Bu ₁₇	3380	5400	1.10	25-75-00	23-77-00
	PEG _{2.7} - <i>b</i> -Bu ₁₇ - <i>b</i> -PEG _{2.7}	4200	6700	1.10	40-60-00	38-62-00
	PEG _{2.7} - <i>b</i> -Bu ₁₇ - <i>b</i> -PEG ₃ - <i>b</i> -DMA ₂₆	8300	11600	1.12	20-30-50	21-30-49
PB13	PEG _{2.7}	920	1600	1.23	100-00-00	100-00-00
	PEG _{2.7} - <i>b</i> -DMA ₂₆	5020	7400	1.08	17-00-83	20-00-80
	PEG _{2.7} - <i>b</i> -DMA ₂₆ - <i>b</i> -PEG _{2.7}	5840	7900	1.15	29-00-71	32-00-68
	PEG _{2.7} - <i>b</i> -DMA ₂₆ - <i>b</i> -PEG _{2.7} - <i>b</i> -Bu ₁₇	8300	11000	1.16	20-30-50	21-31-48
PB14	Bu _{8.5}	1330	2300	1.11	00-100-00	00-100-00
	Bu _{8.5} - <i>b</i> -PEG _{5.5}	2970	4600	1.10	57-43-00	58-42-00
	Bu _{8.5} - <i>b</i> -PEG _{5.5} - <i>b</i> -DMA ₂₆	7070	8900	1.14	23-18-59	26-19-56
	Bu _{8.5} - <i>b</i> -PEG _{5.5} - <i>b</i> -DMA ₂₆ - <i>b</i> -Bu _{8.5}	8300	11300	1.15	20-30-50	20-30-50
PB15	Bu _{8.5}	1330	2000	1.14	00-100-00	00-100-00
	Bu _{8.5} - <i>b</i> -PEG _{5.5}	2970	4100	1.12	57-43-00	57-43-00
	Bu _{8.5} - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu _{8.5}	4200	5700	1.12	40-60-00	41-59-00
	Bu _{8.5} - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu _{8.5} - <i>b</i> -DMA ₂₆	8300	10600	1.18	20-30-50	21-29-50
PB16	PEG _{5.5}	1740	3000	1.13	100-00-00	100-00-00
	PEG _{5.5} - <i>b</i> -Bu _{8.5}	2970	4900	1.11	57-43-00	57-43-00
	PEG _{5.5} - <i>b</i> -Bu _{8.5} - <i>b</i> -DMA ₂₆	7070	10500	1.17	23-18-59	25-17-58
	PEG _{5.5} - <i>b</i> -Bu _{8.5} - <i>b</i> -DMA ₂₆ - <i>b</i> -Bu _{8.5}	8300	12500	1.18	20-30-50	20-31-49
PB17	DMA ₁₃	2150	2900	1.13	00-00-100	00-00-100
	DMA ₁₃ - <i>b</i> -PEG _{5.5}	3790	5100	1.11	44-00-56	46-00-54
	DMA ₁₃ - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu ₁₇	6250	8300	1.14	27-40-33	27-39-34
	DMA ₁₃ - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu ₁₇ - <i>b</i> -DMA ₁₃	8300	10600	1.17	20-30-50	20-30-50
PB18	DMA ₁₃	2150	3200	1.11	00-00-100	00-00-100
	DMA ₁₃ - <i>b</i> -PEG _{5.5}	3790	5500	1.12	44-00-56	45-00-55
	DMA ₁₃ - <i>b</i> -PEG _{5.5} - <i>b</i> -DMA ₁₃	5840	7900	1.15	29-00-71	29-00-71
	DMA ₁₃ - <i>b</i> -PEG _{5.5} - <i>b</i> -DMA ₁₃ - <i>b</i> -Bu ₁₇	8300	10100	1.19	20-30-50	20-31-49
PB19	PEG _{5.5}	1740	2500	1.18	100-00-00	100-00-00
	PEG _{5.5} - <i>b</i> -DMA ₁₃	3790	4900	1.13	44-00-56	44-00-56
	PEG _{5.5} - <i>b</i> -DMA ₁₃ - <i>b</i> -Bu ₁₇	6250	7800	1.16	27-40-33	27-38-35
	PEG _{5.5} - <i>b</i> -DMA ₁₃ - <i>b</i> -Bu ₁₇ - <i>b</i> -DMA ₁₃	8300	10100	1.19	20-30-50	20-30-50
PB20	PEG _{5.5}	1740	2900	1.14	100-00-00	100-00-00
	PEG _{5.5} - <i>b</i> -Bu ₁₇	4200	6200	1.11	40-60-00	43-57-00
	PEG _{5.5} - <i>b</i> -Bu ₁₇ - <i>b</i> -DMA ₂₆	8300	10700	1.13	20-30-50	22-29-49
PB21	PEG _{2.7}	920	1400	1.25	100-00-00	100-00-00
	PEG _{2.7} - <i>b</i> -Bu ₂₀	3790	4800	1.12	22-78-00	22-78-00
	PEG _{2.7} - <i>b</i> -Bu ₂₀ - <i>b</i> -PEG _{2.7}	4610	6000	1.12	36-64-00	37-63-00
	PEG _{2.7} - <i>b</i> -Bu ₂₀ - <i>b</i> -PEG _{2.7} - <i>b</i> -DMA _{23.5}	8300	9300	1.12	20-35-45	20-35-45

PB22	Bu ₁₀	1535	2000	1.14	00-100-00	00-100-00
	Bu ₁₀ - <i>b</i> -PEG _{5.5}	3175	3900	1.14	53-47-00	55-45-00
	Bu ₁₀ - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu ₁₀	4610	5300	1.13	36-64-00	39-61-00
	Bu ₁₀ - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu ₁₀ - <i>b</i> -DMA _{23.5}	8300	8500	1.15	20-35-45	22-34-44
PB23	DMA _{11.7}	1945	2200	1.16	00-00-100	00-00-100
	DMA _{11.7} - <i>b</i> -PEG _{5.5}	3585	4000	1.16	47-00-53	52-00-48
	DMA _{11.7} - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu ₂₀	6455	7000	1.18	26-45-29	30-42-28
	DMA _{11.7} - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu ₂₀ - <i>b</i> -DMA _{11.7}	8300	8500	1.20	20-35-45	21-34-45
PB24	PEG _{5.5}	1740	2300	1.18	100-00-00	100-00-00
	PEG _{5.5} - <i>b</i> -DMA _{11.7}	3585	4200	1.15	47-00-53	50-00-50
	PEG _{5.5} - <i>b</i> -DMA _{11.7} - <i>b</i> -Bu ₂₀	6455	7500	1.17	26-45-29	36-44-30
	PEG _{5.5} - <i>b</i> -DMA _{11.7} - <i>b</i> -Bu ₂₀ - <i>b</i> -DMA _{11.7}	8300	9600	1.18	20-35-45	21-34-45
PB25	PEG _{5.5}	1740	2400	1.18	100-00-00	100-00-00
	PEG _{5.5} - <i>b</i> -Bu ₂₀	4610	5400	1.14	36-64-00	38-62-00
	PEG _{5.5} - <i>b</i> -Bu ₂₀ - <i>b</i> -DMA _{23.5}	8300	8900	1.12	20-35-45	22-35-43

^a PEG, Bu, and DMA stand for penta(ethylene glycol) methyl ether methacrylate (PEGMA), *n*-butyl methacrylate (BuMA), and 2-(dimethylamino) ethyl methacrylate (DMAEMA), respectively.

^b The theoretical MM ($MM^{theor.}$) was calculated by using the Equation 4.1: $MM^{theor.}(g\ mol^{-1}) = (\sum_i MM_i \times DP_i) + 100$.

^c The number average molar mass (M_n) and the dispersity index (D) were determined by gel permeation chromatography (GPC). The calculation was based on a linear calibration curve, plotted by six well-defined linear poly(methyl methacrylate) (PMMA) standard samples of 2, 4, 8, 20, 50, and 100 kg mol⁻¹.

5.2.3 Aqueous Solution Properties

5.2.3.1 Hydrodynamic Diameters

The self-assembly behaviour of the terpolymers has been investigated by DLS. As some of the polymers in the first family (with 40 w/w% BuMA) are not soluble at pH 7, the terpolymer solutions have been tested at both pH 6 and 7, and the results are listed in Table 5.2.2. It should be noted that the experimental hydrodynamic diameters have been determined as the ones which correspond to the maximum of the peak by intensity. As can be seen, in most of the cases, more than one peaks are observed. These peaks correspond to unimers, micelles, and aggregates (1st, 2nd, and 3rd peak, respectively), and the peak of higher intensity is given in bold.

The theoretical diameters have also been calculated, as discussed in the Experimental Chapter, and they are listed in Table 5.2.2. The theoretical calculations assume that spherical micelles and random coil configurations are adopted by the block terpolymers and statistical terpolymer (PB10), respectively. Two types of micelles are assumed to be formed, depending on the polymer architecture: i) conventional core-shell micelles, in which the polymer chains remain unfolded, are formed by all the polymer architectures with one hydrophobic BuMA block, namely ABCA, ABAC, ACAB, CABC, CACB, ACBC, and ABC, and ii) flower-like micelles,

in which the polymer chains fold in a pedal-shape, resulting to the final flower-shape, are adopted by the terpolymer architectures with two BuMA hydrophobic blocks, namely BACB, BABC, and ABCB. The self-assembly behaviour of the tetrablock terpolymers with 30 w/w% BuMA content (PB11-PB19) is schematically illustrated in Figure 5.2.6: i) 2 PEGMA blocks (PB11-PB13, top), ii) 2 BuMA blocks (PB14-PB16, middle), and iii) 2 DMAEMA blocks (PB17-PB19). The theoretical structures of the statistical terpolymer (PB10) and the triblock terpolymers (PB20 and PB25) are expected to be similar to the ones shown in Figure 5.1.6. In this study, tetrablock terpolymers of the same architecture, but different composition, are expected to form micelles of the same configuration. In Figure 5.2.6, the hydrophilic PEGMA, the hydrophobic BuMA, and the thermoresponsive DMAEMA repeated units are represented by light blue, red, and light green spheres, respectively.

As can be seen in Table 5.2.2, both the theoretical (calculated) and experimental size of the structures varies depending on both the architecture and the composition of the terpolymers. Another experimental parameter which has been investigated is the pH. It can be observed in Table 5.2.2 that the pH affects both the solubility of the polymers as well as the size of structures that they form in water, as expected and discussed in Section 5.1.3. The discussion about the self-assembly behaviour of the terpolymers is organised according to the architecture effect, as this is the novelty of the study.

Generally, two trends are observed; which have been also discussed in the previous Chapter. Firstly, polymers form micelles smaller than expected, and this is attributed to the following assumptions made during the theoretical calculations: i) the methacrylate backbone is fully stretched, ii) the hydrophobic blocks fully interact with each other.^{2-5,11} As discussed before, this is not the case, as the BuMA block is in a collapsed state in the core of the micelle in order to avoid interactions with water, and the hydrophobic blocks do not necessarily fully interact with each other.^{2-5,11} The second trend is the opposite one, according to which, micelles bigger than the calculated values are detected. This might occur because of the micelle structure allowing more polymer chains to participate in the assembly of a micelle, which also leads to incomplete overlap of the hydrophobic blocks.⁷

Table 5.2.2: Theoretical polymer structures, and theoretical diameters of their self-assembled structures, and aqueous polymer solution properties at 1 w/w%: experimental hydrodynamic diameters (d_{hs}), polydispersity indices (PDI), effective pK_a s, and cloud points (CPs). Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

No.	Polymer Architecture	Theoretical Polymer Structure ^a	Hydrodynamic diameter (d_h , nm)								Effective pK_a s ± 0.1	Cloud Points ± 2 °C	
			Theor. ^b	Experimental Peaks ± 0.5 ^k									
				pH 6				pH 7					
				1 st	2 nd	3 rd	PDI	1 st	2 nd	3 rd			PDI
PB1	ABCA	PEG _{2.5} - <i>b</i> -Bu ₂₁ - <i>b</i> -DMA ₁₉ - <i>b</i> -PEG _{2.5}	27.2 ^c	-	8.72	78.8	0.411	-	-	78.8	0.248	6.8	ND
PB2	ABAC	PEG _{2.5} - <i>b</i> -Bu ₂₁ - <i>b</i> -PEG _{2.5} - <i>b</i> -DMA ₁₉	27.5 ^c	-	8.72	78.8	0.440	-	13.5	-	0.165	6.8	ND
PB3	ACAB	PEG _{2.5} - <i>b</i> -DMA ₁₉ - <i>b</i> -PEG _{2.5} - <i>b</i> -Bu ₂₁	31.0 ^d	-	43.8	-	0.127	-	-	142	0.390	6.7	ND
PB4	BACB	Bu _{10.5} - <i>b</i> -PEG ₅ - <i>b</i> -DMA ₁₉ - <i>b</i> -Bu _{10.5}	18.4 ^e	-	-	255	0.257	ND ^l	ND	ND	ND	6.5	ND
PB5	BABC	Bu _{10.5} - <i>b</i> -PEG ₅ - <i>b</i> -Bu _{10.5} - <i>b</i> -DMA ₁₉	17.8 ^f	3.1	50.7	164	0.617	-	11.7	-	0.278	6.9	ND
PB6	ABCB	PEG ₅ - <i>b</i> -Bu _{10.5} - <i>b</i> -DMA ₁₉ - <i>b</i> -Bu _{10.5}	16.5 ^g	-	43.8	295	0.390	ND	ND	ND	ND	6.5	ND
PB7	CABC	DMA _{9.5} - <i>b</i> -PEG ₅ - <i>b</i> -Bu ₂₁ - <i>b</i> -DMA _{9.5}	19.2 ^h	-	15.7	-	0.245	-	15.7	-	0.237	6.6	41
PB8	CACB	DMA _{9.5} - <i>b</i> -PEG ₅ - <i>b</i> -DMA _{9.5} - <i>b</i> -Bu ₂₁	25.2 ^d	-	32.7	-	0.156	-	-	164	0.303	6.6	ND
PB9	ACBC	PEG ₅ - <i>b</i> -DMA _{9.5} - <i>b</i> -Bu ₂₁ - <i>b</i> -DMA _{9.5}	19.4 ^h	-	15.7	78.8	0.262	-	-	78.8	0.279	6.3	ND
PB10	(ABC)	PEG ₅ - <i>co</i> -Bu ₂₁ - <i>co</i> -DMA ₁₉	3.0 ⁱ	-	11.7	295	0.240	ND	ND	ND	ND	6.2	ND
PB11	ABCA	PEG _{2.7} - <i>b</i> -Bu ₁₇ - <i>b</i> -DMA ₂₆ - <i>b</i> -PEG _{2.7}	25.6 ^c	2.7	15.7	122	0.744	-	18.2	342	0.389	6.9	^m
PB12	ABAC	PEG _{2.7} - <i>b</i> -Bu ₁₇ - <i>b</i> -PEG _{2.7} - <i>b</i> -DMA ₂₆	26.0 ^c	2.7	15.7	122	0.850	4.2	21.0	220	0.481	6.9	32
PB13	ACAB	PEG _{2.7} - <i>b</i> -DMA ₂₆ - <i>b</i> -PEG _{2.7} - <i>b</i> -Bu ₁₇	28.0 ^d	-	37.8	-	0.204	-	37.8	-	0.196	6.9	ND
PB14	BACB	Bu _{8.5} - <i>b</i> -PEG _{5.5} - <i>b</i> -DMA ₂₆ - <i>b</i> -Bu _{8.5}	16.3 ^e	-	28.2	164	0.522	3.1	21.0	91.3	0.504	6.7	ND
PB15	BABC	Bu _{8.5} - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu _{8.5} - <i>b</i> -DMA ₂₆	17.1 ^f	2.3	21	342	1.000	3.6	24.4	342	0.474	7.0	36
PB16	ABCB	PEG _{5.5} - <i>b</i> -Bu _{8.5} - <i>b</i> -DMA ₂₆ - <i>b</i> -Bu _{8.5}	16.8 ^g	-	24.4	190	0.538	3.1	21.0	-	0.500	6.8	ND
PB17	CABC	DMA ₁₃ - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu ₁₇ - <i>b</i> -DMA ₁₃	17.5 ^h	2.7	15.7	295	0.441	-	13.5	-	0.300	6.9	40
PB18	CACB	DMA ₁₃ - <i>b</i> -PEG ₆ - <i>b</i> -DMA ₁₃ - <i>b</i> -Bu ₁₇	27.2 ^d	-	37.8	-	0.198	-	32.7	-	0.200	6.9	ND
PB19	ACBC	PEG _{5.5} - <i>b</i> -DMA ₁₃ - <i>b</i> -Bu ₁₇ - <i>b</i> -DMA ₁₃	17.2 ^h	3.6	15.7	220	0.603	-	10.1	-	0.193	6.8	53
PB20	ABC	PEG _{5.5} - <i>b</i> -Bu ₁₇ - <i>b</i> -DMA ₂₆	22.7 ^j	2.3	15.7	531	0.951	3.1	18.2	220	0.500	6.9	39
PB21	ABAC	PEG _{2.7} - <i>b</i> -Bu ₂₀ - <i>b</i> -PEG _{2.7} - <i>b</i> -DMA _{23.5}	18.7 ^c	3.1	15.7	142	0.507	-	11.7	712	0.328	6.9	38
PB22	BABC	Bu ₁₀ - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu ₁₀ - <i>b</i> -DMA _{23.5}	11.5 ^f	2.7	15.7	164	0.469	4.2	24.4		0.378	6.8	38

PB23	CABC	DMA _{11.7} - <i>b</i> -PEG _{5.5} - <i>b</i> -Bu ₂₀ - <i>b</i> -DMA _{11.7}	13.7 ^h	-	13.5	531	0.316	-	11.7	-	0.240	6.6	43
PB24	ACBC	PEG _{5.5} - <i>b</i> -DMA _{11.7} - <i>b</i> -Bu ₂₀ - <i>b</i> -DMA _{11.7}	15.4 ^h	3.12	18.2	-	0.369	-	18.2	-	0.264	6.5	ND
PB25	ABC	PEG _{5.5} - <i>b</i> -Bu ₂₀ - <i>b</i> -DMA _{23.5}	15.9 ^j	2.7	15.7	255	0.520	5.6	-*	-	0.313	6.8	47

^a PEG, Bu, and DMA stand for penta(ethylene glycol) methyl ether methacrylate (PEGMA), *n*-butyl methacrylate (BuMA), and 2-(dimethylamino) ethyl methacrylate (DMAEMA), respectively.

^b The calculated diameters were determined by using the experimental degrees of polymerisation (DPs), which were calculated by using the GPC results of the polymers after precipitation (M_n after precipitation) and the ¹H NMR results (the experimental composition).

^c The diameters of the spherical micelles formed by the ABCA (PB1 and PB11) and ABAC (PB2, PB12, and PB21) tetrablock terpolymers were calculated by using the Equation 4.9: d_h (nm) = (DP_A + DP_B + 2 × DP_C) × 0.254.

^d The ACAB (PB3 and PB13) and CACB (PB8 and PB18) tetrablock terpolymers form spherical micelles, the diameter of which was calculated by using the Equation 4.7: d_h (nm) = [DP_B + 2 × (DP_A + DP_C)] × 0.254.

^e The Equation 4.10 was used to calculate the diameter of the flower-like micelles formed by PB4 and PB14 with BACB architecture: d_h (nm) = (DP_A + DP_B + DP_C) × 0.254.

^f The Equation 4.11 [d_h (nm) = (2 × DP_C) × 0.254] was used to calculate the diameter of the flower-like micelles formed by PB5, PB15, and PB22 with BABC architecture.

^g The ABCB architecture (PB6 and PB16) forms flower-like micelles, and their diameter can be calculated by using the Equation 4.12: d_h (nm) = (DP_B + DP_C) × 0.254.

^h The polymers with CABC and ACBC architecture (CABC: PB7, PB17, and PB23, ACBC: PB9, PB19, and PB24) form spherical micelles with diameter equal to the one given by the Equation 4.6: d_h (nm) = (2 × DP_A + DP_B + DP_C) × 0.254.

ⁱ The statistical terpolymer (PB10) is assumed to adopt random coil configuration, and the end-to-end distance of the random coil is calculated by the Equation 4.2: $\langle d_g^2 \rangle^{1/2} = 2 \times [2 \times 2.20 \times (DP_A + DP_B + DP_C) / 3]^{1/2} \times 0.154$.

^j The calculation of the micelles' diameter formed by the ABC triblock terpolymers (PB20 and PB25) follows the Equation 4.4: d_h (nm) = (DP_B + 2 × DP_C) × 0.254.

Note: in the equations mentioned above, A, B and C correspond to PEGMA, BuMA and DMAEMA, respectively.

^k The experimental hydrodynamic diameters reported are the mean diameters of the maximum of each peak in the DLS histograms by intensity; where more than one peaks is identified, the peak of higher intensity is shown in bold.

^l ND stands for not determined. As the polymers were not soluble, it was unable to test them at the specific pH.

^m The polymers did not show a CP within the temperature range tested.

* In this DLS histogram, bigger micelles and aggregates are present. However, all the peaks merge and therefore only one very broad peak is observed with the maximum appearing in the first peak.

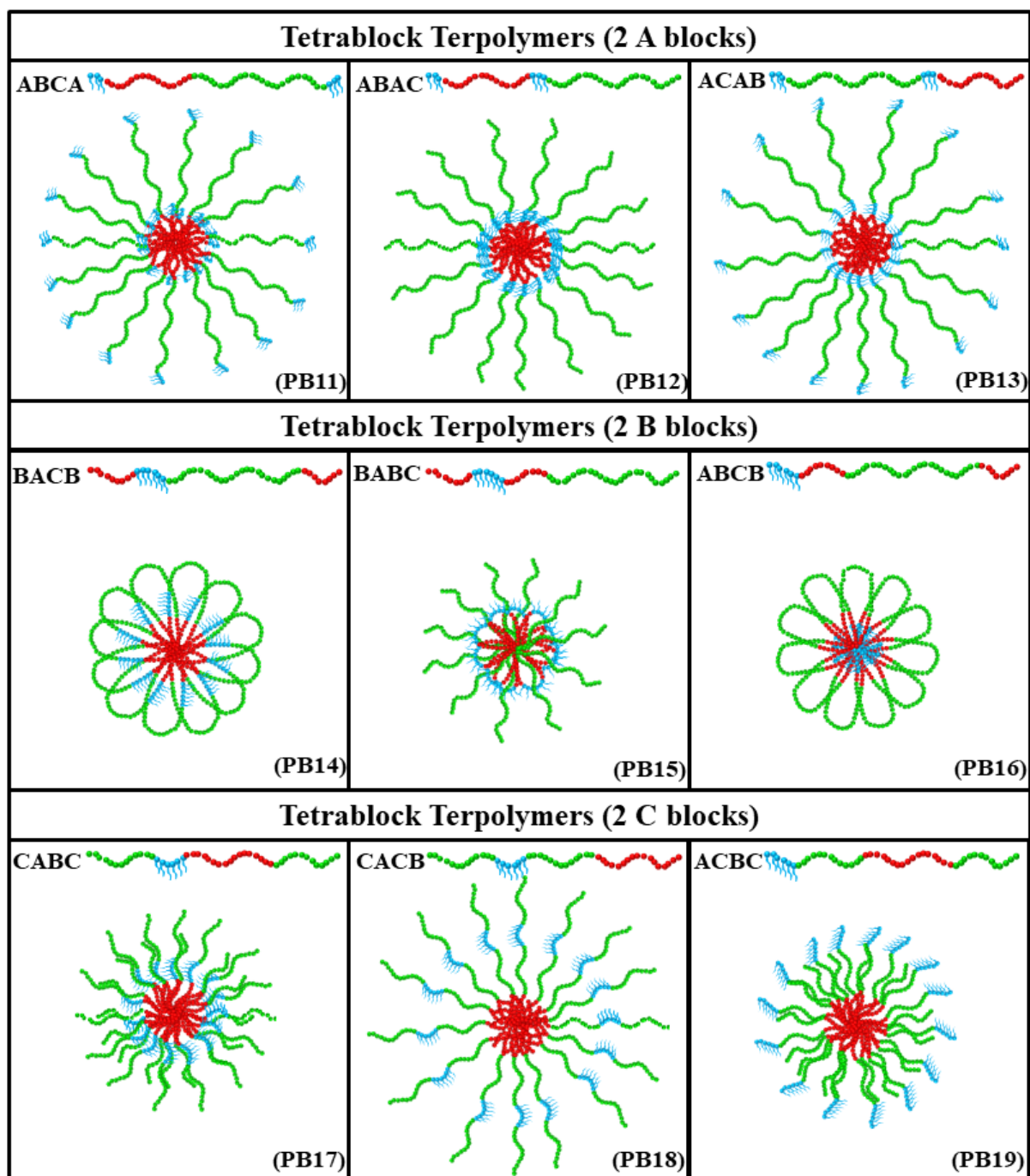


Figure 5.2.6: Schematic representation of the micelle structure assumed to be adopted by the tetrablock terpolymers with PEGMA-BuMA-DMAEMA (A-B-C) composition of 20-30-50 w/w%, respectively (PB11-PB19): i) two hydrophilic A blocks (PB11-PB13, top), ii) two hydrophobic B blocks (PB14-PB16, middle), and iii) two thermoresponsive C blocks (PB17-PB19, bottom). The corresponding polymer structures are also given, with PEGMA, BuMA, and DMAEMA units in light blue, red, and light green, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.” *Note:* It is assumed that the tetrablock terpolymers with A-B-C composition of 20-40-40 w/w%, respectively, (PB1-PB9), form micelles in the same way as PB11-PB19, respectively. The tetrablock terpolymers with A-B-C composition of 20-35-45 w/w% form micelles similarly to the polymers of the same architecture (PB21-PB12, PB22-PB15, PB23-PB17, and PB24-PB19). The statistical terpolymer (PB10) adopts a random coil configuration, similarly to PA7, while the ABC triblock terpolymers (PB20 and PB25) form micelles similarly to PA1; the structures formed by PA7 and PA1 can be found in Figure 5.1.6.

Firstly, as mentioned before, three tetrablock terpolymer architectures with two PEGMA blocks have been investigated in this study: i) ABCA (PB1 and PB11), ii) ABAC (PB2, PB12, and PB21), and iii) ACAB (PB3 and PB13). From Table 5.2.2, it can be observed that polymers with ABAC architecture form micelles smaller than the theoretical values, regardless the composition and the pH. The same trend is observed for PB11 (ABCA architecture) at both pH, which is more hydrophilic compared to PB1 (polymer of the same architecture but higher hydrophobic content). On the other hand, PB1 forms smaller micelles than expected at pH 6, whereas at pH 7, only bigger aggregates are observed. This difference is attributed to the pH-response of DMAEMA units, which changes the hydrophilicity of the structure, i.e. the polymer structure is more hydrophilic (there are more positive charges) at pH 6 compared to pH 7. Concerning the third architecture (ACAB), bigger micelles are formed by PB13 at both pH, and by PB3 at pH 6, whereas only aggregates are detected for PB3 at pH 7, because of the increased hydrophobicity of the structure. The aggregation tendency of PB3 at pH 7, compared to PB13, is ascribed to the composition effect, i.e. the hydrophobic BuMA content in PB3 is higher than in PB13 (40 w/w% *versus* 30 w/w%). The formation of bigger micelles only by the ACAB polymers shows a clear architectural effect. Similar observations have been made for the ACB architecture (discussed in the previous Chapter), in which the hydrophobic block is at the end of the polymer chain, thus bigger micelles are expected to be formed in theory.

When tetrablock terpolymers with two hydrophobic BuMA blocks are concerned, flower-like micelles are expected to be formed. These polymers are: i) BACB (PB4 and PB14), ii) BABC (PB5, PB15, and PB22), and iii) ABCB (PB6 and PB16). In this case, the solubility of the solution is reduced and in most of the cases micelles bigger than theoretical are formed, presumably because of the thermodynamically unfavourable folding of the polymer chains in a pedal-shape. More specifically, PB4 (BACB) is insoluble at pH 7, while only big aggregates are observed at pH 6. On the other hand, PB14, which is of the same architecture as PB4 but more hydrophilic, forms bigger micelles than expected at both pH values. The ABCB architecture shows similar behaviour with BACB, as in both architectures, the two BuMA blocks are separated by either long DMAEMA block, or an AC diblock: i) PB6 is insoluble at pH 7, and ii) bigger micelles are formed by PB6 at pH 6, and PB16 at both pH. Only the BABC architecture, in which the hydrophobic blocks are separated by a short PEGMA block forms smaller micelles in the case of PB5 at pH 7. The same polymer at pH 6, as well as PB15 and PB22 at both pH values, form bigger micelles instead.

When the thermoresponsive DMAEMA block is split in two, different trends are observed. Firstly, the CABC architecture (PB7, PB17, and PB23) always forms smaller micelles, regardless the pH and the composition, whereas the CACB architecture forms either bigger micelles (PB8 at pH 6, and PB18 at both pHs) or tends to aggregate (PB8 at pH 7). The behaviour of the CACB architecture is similar to the one of ACAB, as both tetrablock architectures have the hydrophobic block at the end of the polymer chain. Concerning the ACBC architecture, an intermediate behaviour is observed, which depends on both the composition of the terpolymers, as well as the pH. For example. PB19, which is the most hydrophilic one, forms smaller micelles, whereas PB24 (intermediate hydrophilic content) forms bigger micelles, regardless the pH. PB9 either aggregates or form smaller micelles (at pH 7 and 6, respectively).

Concerning the ABC triblock and statistical architectures, which have been studied before, expected trends are observed. In particular, the ABC terpolymers (PB20 and PB25) form smaller micelles than the theoretically predicted values at both pH values, regardless the composition, as observed and reported before.⁵ On the other hand, the statistical terpolymer (PB10) is insoluble at pH 7, because it does not form micelles; as discussed in previous studies.⁷ Also, at pH 6, it forms bigger structures than the end-to-end distance of its random coil, but as have been proven before by TEM on polymers of similar structure, it does not form micelles.⁷

To summarise, it can be concluded that the architecture of the tetrablock terpolymers controls their self-assembly and micellisation behaviour. Also, the composition is shown to control the micelle formation and aggregation tendency of the terpolymers.

5.2.3.2 Effective pK_a s

The effective pK_a s of the DMAEMA units of the terpolymers are listed in Table 5.2.2 and are shown in Figure 5.2.7. These values vary between 6.2 and 7.0 and they depend on both the polymer architecture and composition. These values are in similar range as previously reported values.^{2-5,12-15} As can be seen in both Table 5.2.2 and Figure 5.2.7, the values follow the hydrophobicity trend, i.e. the higher the hydrophobic content, the lower the pK_a value; the results for terpolymers with 30, 35, and 40 w/w% BuMA are shown in red rhombi, green triangles, and blue squares, respectively. This is attributed to the lower dielectric constant, as the hydrophobicity increases, and it is previously reported in studies on similar polymers.^{3-5,12} The lowest pK_a value is the one presented by the statistical terpolymer (PB10) ($pK_a = 6.2$),

because the statistical architecture is unable to form micelles due to the absence of distinct hydrophobic and hydrophilic blocks, as reported and discussed before.⁷ Also, even though the architecture does not affect the pK_a values when the most hydrophilic series is concerned (PB11-PB20), this is not the case for the polymers of higher hydrophobic content. More specifically, PB4 (BACB) and PB6 (ABCB) present a pK_a value at 6.5, as these copolymers show reduced solubility, because of the formation of flower-like micelles (see Figure 5.2.6 and previous section – 5.2.3.1). This is also the case for PB9 and PB24 (pK_a values at 6.5 and 6.3, respectively), presumably because of their ACBC architecture, with the DMAEMA units surrounding the hydrophobic core, and possibly participating to it, if complete overlap does not take place. A similar behaviour is reported in Section 5.1.3.2, in which the A(BC) architecture (PA5) presents lower pK_a , possibly because of the random copolymerisation of the DMAEMA with the BuMA, which weakens the strength of the DMAEMA base.⁷

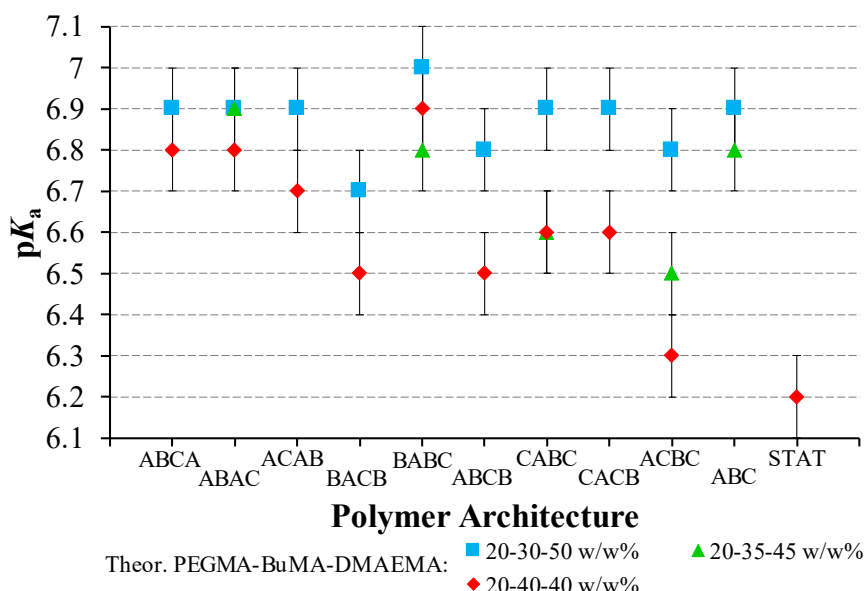


Figure 5.2.7: Effect of the architecture and the composition on the pK_a of the DMAEMA repeated units. The results which correspond to the polymers with theoretical PEGMA-BuMA-DMAEMA weight percentage of 20-30-50 w/w%, 20-35-45 w/w%, and 20-40-40 w/w%, are indicated by light blue squares, light green triangles, and red rhombi, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

5.2.3.3 Cloud Points

Terpolymer solutions have been prepared in DI water at 1 w/w%. However, only the following polymers are soluble and show thermoresponse: i) PB7 with 40 w/w% BuMA and CABC architecture, ii) PB12 (ABAC), PB15 (BABC), PB17 (CABC), PB19 (ACBC), and PB20 (ABC) with 30 w/w% BuMA, and iii) PB21 (ABAC), PB22 (BABC), PB23 (CABC), and PB25 (ABC) with 35 w/w% BuMA. The results can be found in both Table 5.2.2 and Figure 5.2.8. Both architecture and composition effects are observed, and they are discussed below.

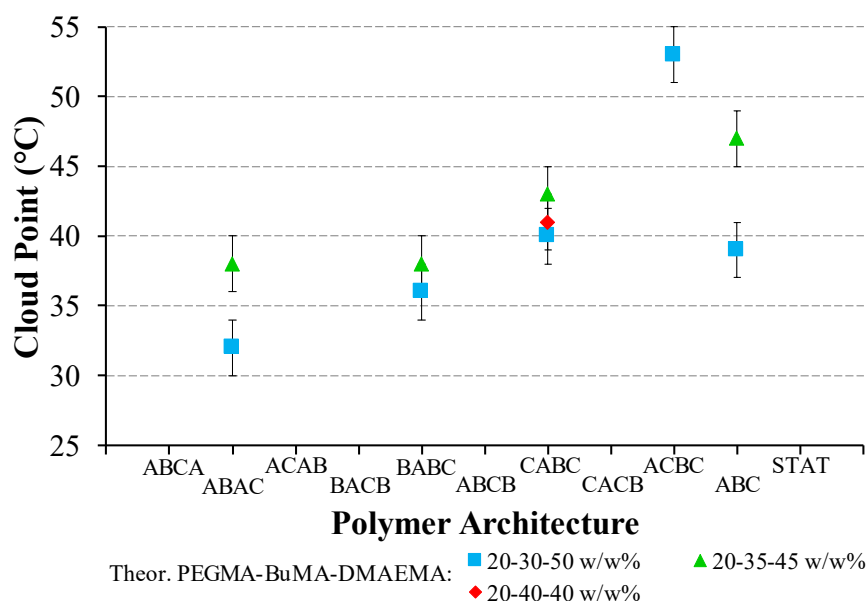


Figure 5.2.8: Effect of the architecture and composition on the cloud point of the terpolymers which are soluble in water. The results of the polymers with different PEGMA-BuMA-DMAEMA weight percentage are shown with a different symbol: i) light blue squares for 20-30-50 w/w%, ii) light green triangles for 25-35-45 w/w%, and iii) red rhombi for 20-40-40 w/w%. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

As can be seen in Figure 5.2.8, an effect of the block architecture on the CP is observed. It is noteworthy that this is the first time that a clear architecture effect on the CP is reported, except from the statistical architecture presenting lower CP than the corresponding triblock architectures.^{2,3} Interestingly, the ABAC architecture presents a lower CP, whereas the ACBC (PB19) one presents the higher CP. Also, the ABC architecture shows either equal or higher CP than the corresponding tetrablock terpolymers. These trends can be explained upon consideration of the polymer architecture and more specifically the position of the A block. In ACBC and ABC terpolymers, the hydrophobic and thermoresponsive block(s) are located next to each other and the hydrophilic block is always at the end of the polymer. As the temperature increases, core-shell micelles are formed with the core being based on both BuMA and

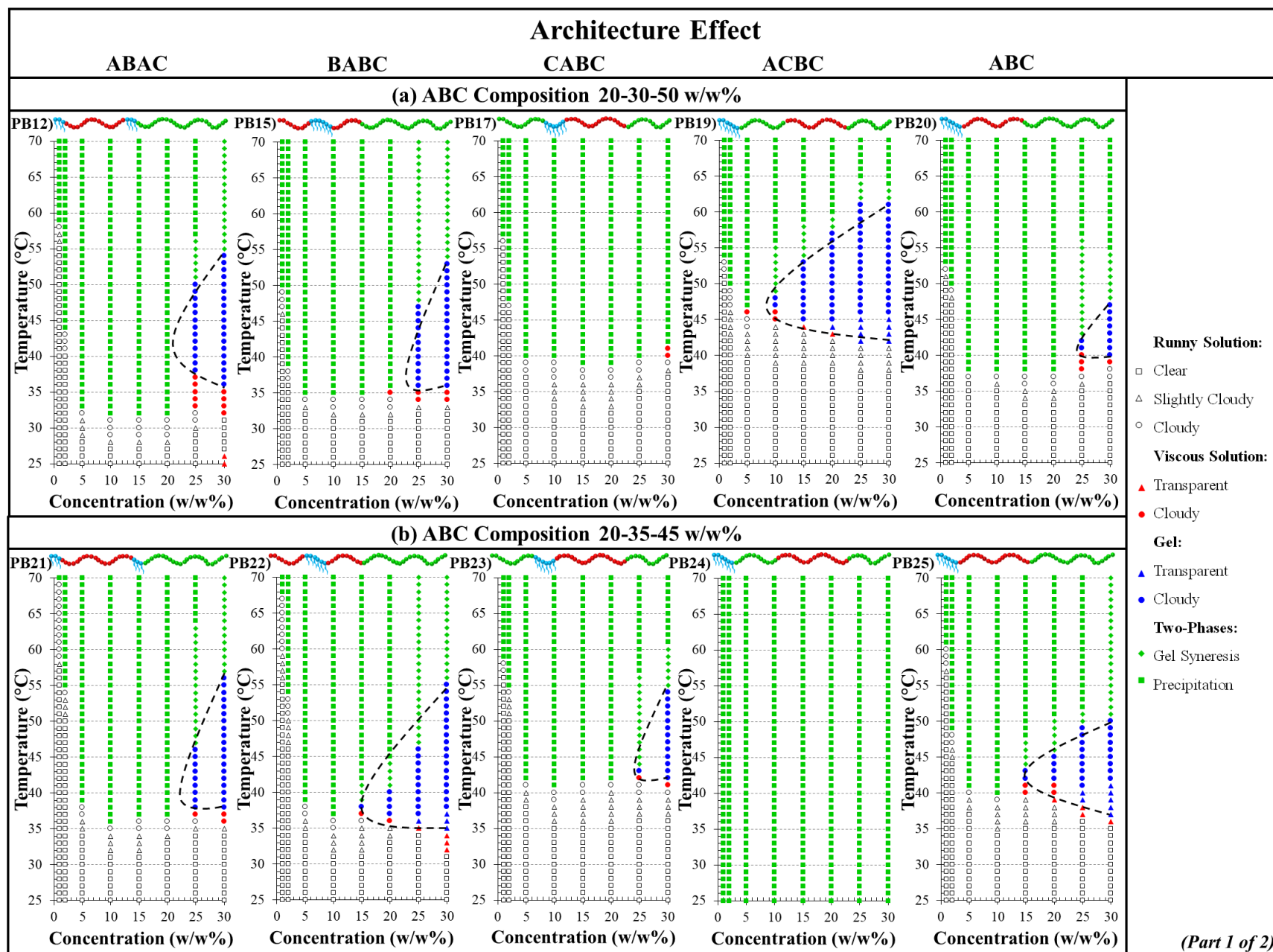
DMAEMA, whereas the shell is based only on PEGMA (A block). These micelles seem to be more stable in solution, thus the CP is observed at higher temperatures. This is not the case in the ABAC architecture, in which the hydrophobic and thermoresponsive block are separated by half of the hydrophilic A block, while the other half A block is in the end of the polymer chain. Thus, in the case of the ABAC architecture, only half the A block can effectively stabilise the micelle at higher temperatures. Concerning the polymers with the BABC and CABC architecture, in which the whole of A block is in the middle of the polymer chain, both show intermediate CPs, as the A block will have to bend in a U shape to form the corona of the micelle at higher temperatures. It should be noted that PB11 (ABCA) is soluble in water at 1 w/w%, but it did not present a CP within the temperature range tested. This can be attributed to the BuMA and DMAEMA blocks being next to each other and surrounded by the hydrophilic PEGMA ones. Thus, as the temperature increases, core-shell micelles with the core based on both BuMA and DMAEMA are formed, which seem to be stable and thus the polymer solution does not respond to temperature.

The composition effect is also shown in Figure 5.2.8; red rhombi, green triangles, and blue squares for terpolymers with 40, 35, and 30 w/w% BuMA. Concerning the two extreme compositions (40 *versus* 30 w/w%), it can be observed that as the thermoresponsive DMAEMA content increases (and the hydrophobic BuMA content decreases), the CP decreases. This opposes to the observations of the previous study, in which it has been observed that the higher the hydrophobic BuMA content, the lower the CP is.⁵ However, in the previous study, the thermoresponsive content has been kept constant, whereas the hydrophobic and hydrophilic contents have been changed.⁵ On the other hand, in the current study, the hydrophilic PEGMA content is kept constant, and the hydrophobic BuMA and thermoresponsive DMAEMA contents change. So, when the hydrophobic content decreases, the thermoresponsive content increases, and its increase has a stronger effect on the CP, thus lowering its value. This can be supported by the CPs of DMAEMA homopolymers, which decrease from 54 °C to 37°C, as the MM increases from 2900 g mol⁻¹ to 9400 g mol⁻¹, respectively. Concerning the CP of PB7, which is the only polymer tested with composition of 40 w/w% BuMA, it can be observed that it presents an intermediate CP compared to the more hydrophilic polymers of the same architecture. This shows that the hydrophobic BuMA content still plays an important role on the CP, thus balancing the DMAEMA effect.

5.2.3.4 Visual Gel Points

As can be seen in Figure 5.2.9, the phase diagrams of the five most promising architectures are presented from the left to the right. These architectures have been determined as the ones which are soluble in PBS when the BuMA content is at 30 w/w% and are: ABAC (PB12), BABC (PB15), CABC (PB17), ACBC (PB19), and ABC (PB20). Therefore, these architectures have been selectively chosen, and polymers of these architectures have been synthesised with intermediate hydrophobic content of 35 w/w% BuMA (PB20-PB25). The effect of composition (PEGMA-BuMA-DMAEMA) is also illustrated from the top to the bottom: (a) 20-30-50 w/w%, (b) 20-35-45 w/w%, and (c) 20-40-40 w/w%. The architectures of which the phase diagrams are not shown in Figure 5.2.9, did not form homogeneous solutions in PBS, regardless the change in the composition. The effects of architecture and/or composition are discussed in the following paragraphs.

For the most hydrophobic series (40 w/w% BuMA, Figure 5.2.9 (c)), only PB7 (CABC) is soluble in PBS, as phase separation is observed in the rest of the phase diagrams shown for this composition. This indicates both a hydrophobic and architecture effects, as i) the high hydrophobic content prevents solubilisation, and ii) despite the high hydrophobic content, the architecture of PB7, namely CABC, enhances solubility. The solubility of PB7 can be attributed to its small micelle size. It should be noted that the corresponding ABC triblock terpolymer has been investigated in a previous study of our group.⁵ As can be seen in the phase diagram of PB7, a gelation area (coloured in blue, and shown with a black dashed line) is observed between 35°C and 45°C, and at a concentration range of 15 w/w% to 30 w/w%. When these results are compared to the corresponding triblock terpolymer, it can be concluded that the CABC architecture disrupts the gelation (the triblock terpolymers gels at only 5 w/w%, and at 20 w/w% a gel is formed at 36 °C and remains stable up to 50 °C).



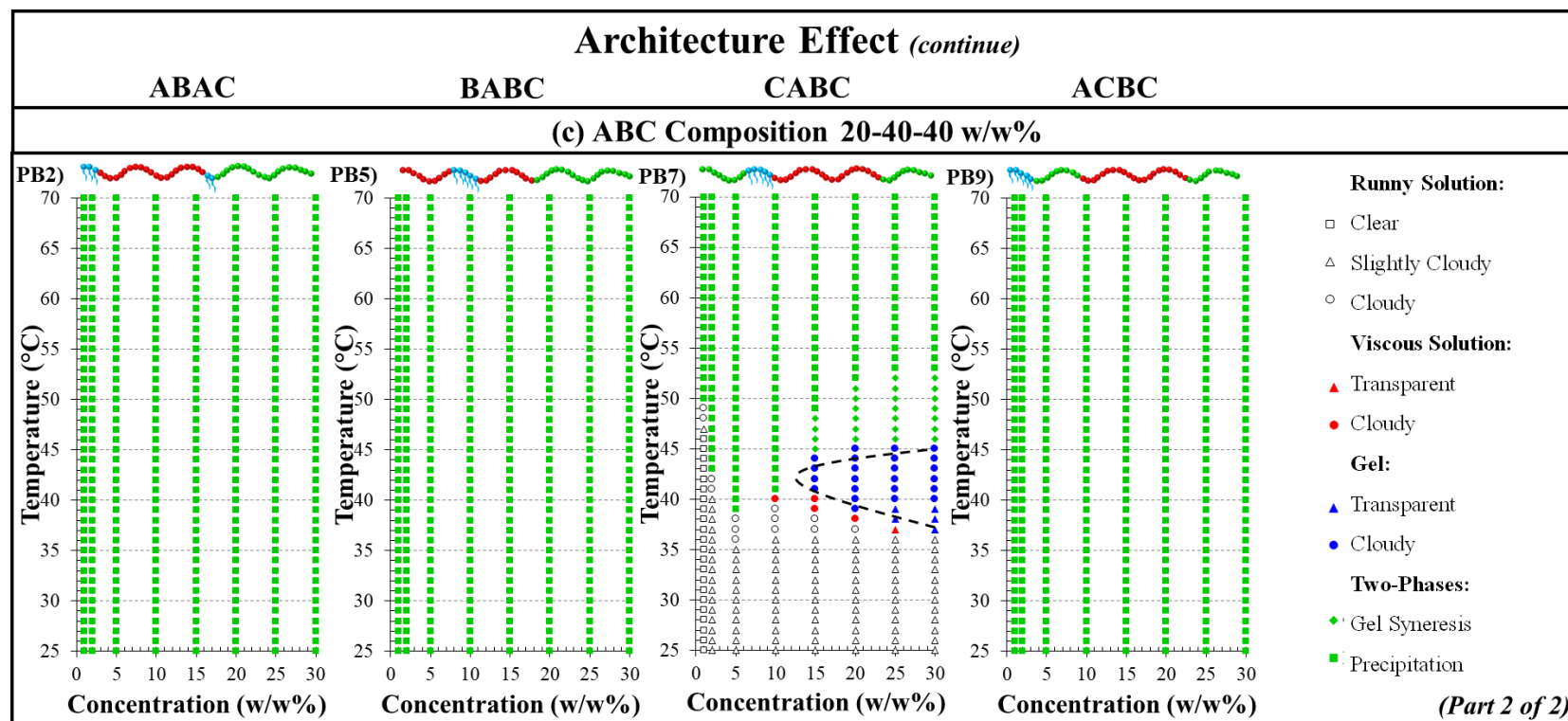


Figure 5.2.9: Phase diagrams of the terpolymer solutions in phosphate buffered saline (PBS): i) tetrablock terpolymers (PB2, PB4, PB7, PB9, PB12, PB15, PB17, PB19, PB21, PB22, PB23, PB24), and ii) the triblock terpolymers (PB20 and PB25). The effect of the architecture is shown from the left to the right (ABAC, BABC, CABC, ACBC, and ABC, respectively; where A, B, and C blocks are based on PEGMA, BuMA, and DMAEMA, respectively). The effect of the composition is shown from the top to the bottom: (a) 20-30-50 ABC w/w% (top), (b) 20-35-45 ABC w/w% (middle), and 20-40-40 ABC w/w% (bottom). Four phases are identified: i) the runny solution phase is coloured in white (square, triangle, and circle represent the clear, slightly cloudy, and cloudy state, respectively), ii) the viscous solution phase is coloured in red (triangle and circle represent the transparent and cloudy state, respectively), iii) blue colour represents the gel phase (triangles and circles show the transparent or cloudy state of the gel, respectively), the system of two phases is shown in light green (gel syneresis and precipitation are shown in rhombi and squares, respectively). The gelation region is indicated by an approximate black dashed line. The corresponding polymer structures are also given; where PEGMA, BuMA, and DMAEMA are coloured in light blue, red, and light green, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

Concerning the copolymers of intermediate hydrophobicity (PB21-PB25), the one with ACBC architecture (PB24) is insoluble in PBS (this is presented as phase separation on the corresponding phase diagram). The ABC triblock terpolymer (PB25) shows a clear gelation area, as expected and observed before for this type of architecture.^{2,3,5,7} The gelation starts at 15 w/w%, and at 30 w/w% a stable gel is formed from 36 °C to 50 °C. When it is compared with its corresponding tetrablock terpolymers, two trends are observed: i) the ABAC (PB21) and the CABC (PB23) terpolymers show narrower gelation region, i.e. a gel is formed at narrower temperature and/or concentration range, and ii) the BABC architecture shows a wide gelation region, similarly to the ABC architecture. It has been proven in our previous studies on block terpolymers that the smaller the size of the core-shell micelles, the more favoured the gelation is.⁷ However, in this case, PB21 and PB23 form micelles of 11.7 nm in size, whereas the micelles formed by PB22 (BABC) are 24.4 nm in size. Therefore, it seems that the flower-like micelles formed by PB22 favour gelation, presumably because some polymer chains cannot fold to U-shape, and thus they act as bridges between different micelles.

Upon increasing the hydrophilicity of the terpolymers, all five architectures are soluble in PBS. In this series, both the BABC and ABC architectures, that showed similar thermoresponsive behaviour at an intermediate hydrophobic content, show narrow gelation area, with gels being formed only at 20 w/w% and 25 w/w%; these terpolymers are PB15 and PB20, respectively. Interestingly, the ABAC architecture at this composition (PB12) behaves similarly to the BABC one (PB15), which is opposite to the previous conclusions. This can be attributed to the two possible theoretical configurations which are assumed to be adopted by PB15; they are shown in Figure 5.2.10. The CABC architecture at this composition (PB17) does not present a gelation region, which is attributed to the increased hydrophilicity of the structure (both the reduced hydrophobic content and the micelle configuration). It is worth reminding that the CABC architecture at higher hydrophobic contents presents a gelation region, but narrower compared to the other architectures. Interestingly, the ACBC architecture (PB19), which is insoluble at the other two compositions, present the wider gelation area than all the terpolymers studied in this Chapter. More specifically, gels are formed at the low concentration of 10 w/w%, and at a temperature range from 42 °C to 61 °C (at 30 w/w%). The enhanced thermoresponsive behaviour of PB19 is because it forms the smallest micelle of all the terpolymers studied in this project (10.1 nm in size), as can be seen in Table 5.2.1 and Figure 5.2.6. This supports our previous conclusions, according to which the smaller the micelle, the more favoured the gelation.⁷ As the temperature at which PB19 forms gel is higher than body temperature, this

polymer is not suitable for biological applications. However, it can be used as an example architecture for future studies, which will aim to lower the sol-gel temperature, by keeping this wide gelation area.

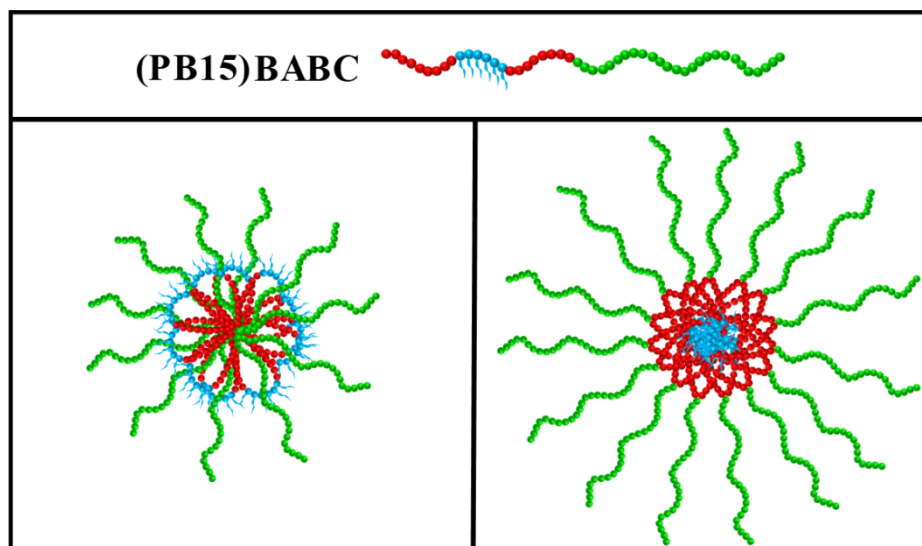


Figure 5.2.10: Possible configurations that are adopted by the BABC architecture. PB15 is given as an example. The corresponding polymer structure is also schematically illustrated with PEGMA, BuMA, and DMAEMA units being represented by light blue, red, and light green spheres, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

To summarise, these findings clearly demonstrate both an architecture and a hydrophobicity effect. Firstly, the number of the blocks (triblock *versus* tetrablock) and the position of the blocks along the polymer chain of the tetrablock terpolymer control the structure of the micelles (core-shell *versus* flower-like), which affects the solubility, and the thermoresponsive behaviour. The BABC architecture favours gelation possibly because of the thermoresponsive block being polymerised next to the hydrophobic one, which might enhance the hydrophobic effect. On the other hand, the CABC architecture clearly disrupts gelation, which can be ascribed to the hydrophilic PEGMA block being polymerised between one of the thermoresponsive DMAEMA blocks and the hydrophobic BuMA block, thus interrupting the hydrophobic effect. The most interesting architecture is the ACBC one, because, even though it is insoluble at the higher hydrophobic BuMA content, it does present a wide gelation region at the more hydrophilic composition. Also, concerning the composition, the lower the hydrophobic content (from bottom to top), the more enhanced the solubility is, and if the polymer is soluble, the gelation region appears at higher concentrations and/or temperatures. This is clearly shown for the CABC architecture: the gelation region is narrower as the hydrophobic BuMA content decreases from 40 w/w% to 35 w/w%, while gelation is not

observed for the most hydrophilic one. The effect of hydrophobicity on the thermoresponsive properties has been established before, and it is related to the hydrophobic effect. Therefore, the architecture and the composition of tetrablock terpolymers need to be carefully chosen to achieve gelation at a desirable temperature and concentration range.

In this study, the thermoresponsive behaviour of tetrablock terpolymers has been systematically investigated for the first time. It is proven that the thermoresponsive and most importantly, the gelation behaviour of the tetrablock terpolymers does strongly depend on the polymer architecture. The hydrophobic content of the tetrablock terpolymers also affects their solubility and thermoresponsive behaviour. Two promising tetrablock architectures are identified, which present a wide gelation area: i) the BABC architecture at 20-35-45 w/w% PEGMA-BuMA-DMAEMA, and ii) the ACBC architecture at 20-30-40 w/w% PEGMA-BuMA-DMAEMA.

5.2.3.5 Gel Points by Rheology

The copolymer solutions in PBS at 15 w/w% have been also investigated by rheology at different temperatures. The rheological curves of the four terpolymers which form a gel visually at this concentration are shown in Figure 5.2.11: (a) PB7 with CABC architecture and 40 w/w% BuMA content, (b) PB19 with ACBC architecture and 30 w/w% BuMA, (c) BABC with 35 w/w% BuMA (PB22), and (d) PB25 with ABC architecture at 35 w/w% BuMA. The storage modulus (G'), loss modulus, (G''), and complex viscosity (η^*) are given in dark blue, red, and light blue, respectively. The results for the rest of the terpolymers can be found in Figure A6 in the Appendix.

As can be seen in Figure 5.2.11, three of the terpolymers form gels both visually and rheologically: i) PB19 with ACBC architecture and 30 w/w% BuMA, ii) PB22 with BABC architecture and 35 w/w% BuMA, and iii) the ABC triblock terpolymer with 35 w/w% BuMA (PB25). The gel point (the temperature at which $G' > G''$) of these terpolymer solutions is identified at 47, 37, and 41 °C, respectively, whereas visually they form a stable gel at 45, 38, and 42 °C, respectively. The rheological gel points are satisfactorily close to the results from visual tests. As the temperature increases further, the loss modulus becomes higher than the loss modulus ($G' < G''$). This is also expected, as gel syneresis and then precipitation occurs. Therefore, the gel is destabilised upon temperature increase, and the liquid phase becomes dominant, i.e. the energy is dissipated in the system as heat. The temperatures at which $G' <$

G'' for PB19, PB22, and PB25 are: 62, 40, and 48 °C. Visually, gel syneresis and precipitation are observed at the following temperatures: 54 and 59 °C for PB19, 39 and 42 °C for PB22, and, 44 and 48 °C for PB25. As can be seen, the rheological results match the temperature at which precipitation is observed, rather than gel syneresis. This can be explained as the transition from stable gel phase to gel syneresis is subtle and the machine might not be sensitive enough to detect it.

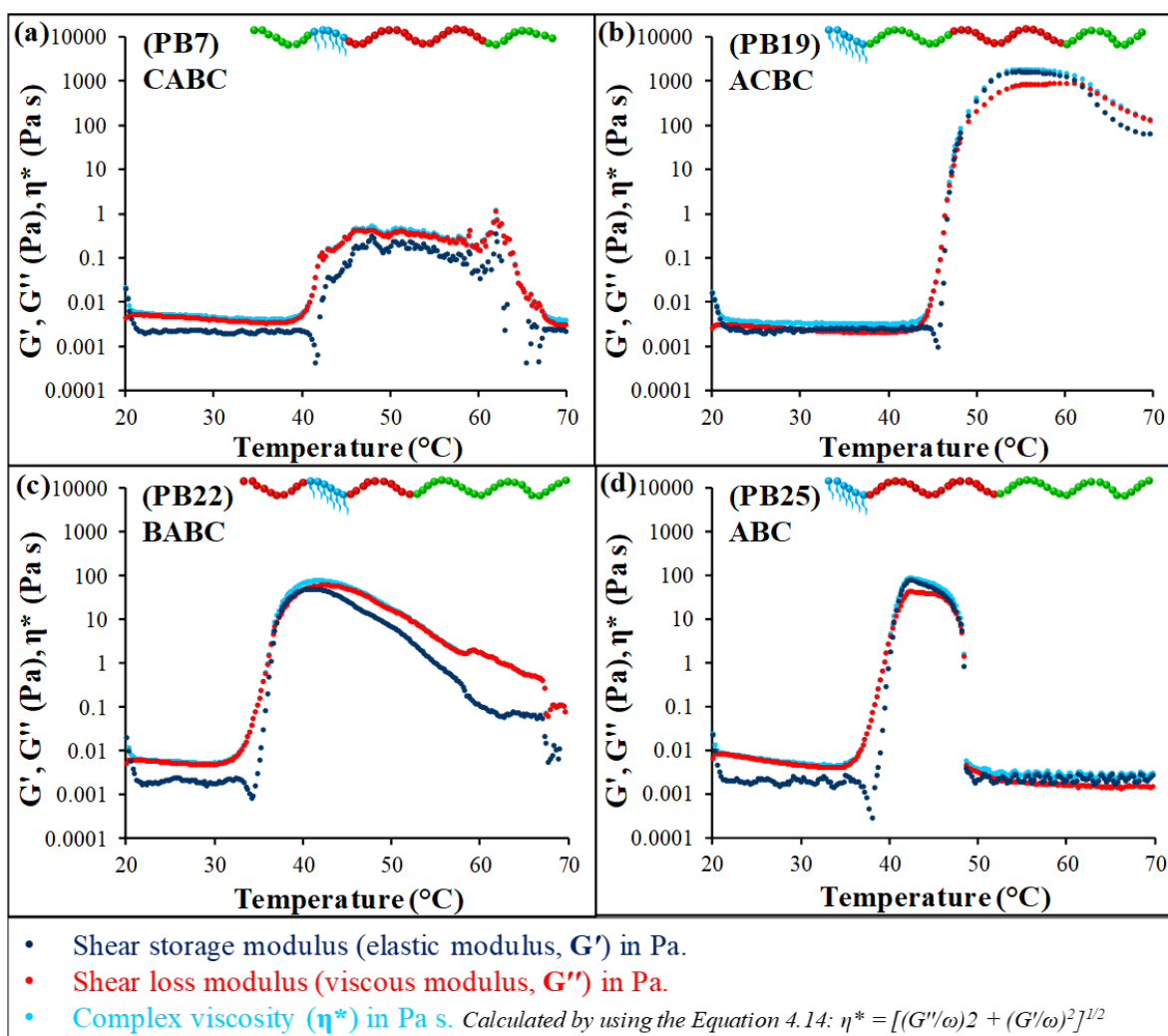


Figure 5.2.11: Rheological curves of the 15 w/w% terpolymer solutions in phosphate buffered saline (PBS) which form visually a gel, as resulted from temperature ramp measurements. The changes in the shear storage modulus (elastic modulus, G'), shear loss modulus (viscous modulus, G''), and the complex viscosity (η^*) are shown in dark blue, red, and light blue, respectively. The graphs shown correspond to: (a) the polymer B7 with theoretical polymer structure DMAEMA_{9.5}-*b*-PEGMA₅-*b*-BuMA₂₁-*b*-DMAEMA_{9.5} (CACB), (b) the polymer B19 with theoretical polymer structure PEGMA_{5.5}-*b*-DMAEMA₁₃-*b*-BuMA₁₇-*b*-DMAEMA₁₃ (ACBC), (c) the polymer B22 with BABC architecture and structure of BuMA₁₀-*b*-PEGMA_{5.5}-*b*-BuMA₁₀-*b*-DMAEMA_{23.5}, and (d) the ABC triblock terpolymer B25 with theoretical polymer structure PEGMA_{5.5}-*b*-BuMA₂₀-*b*-DMAEMA_{23.5}. The corresponding polymer structures are schematically illustrated with light blue, red, and light green representing the PEGMA, BuMA, and DMAEMA units, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, 51(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

It is noteworthy that the polymer B19 with theoretical polymer structure PEGMA_{5.5}-*b*-DMAEMA₁₃-*b*-BuMA₁₇-*b*-DMAEMA₁₃ (ACBC) forms gels with maximum viscosity of 1730 Pa s at 55 °C, which corresponds to a cloudy gel within the experimental error. This value is of one order of magnitude higher than the rest of the polymers in this study. This is also the case when it is compared to the ones obtained by the TEGMA-based polymers in Chapter 5.1 (See Figure 5.1.8), and to all the previous studies of our group.^{2-5,12} Thus, it can be concluded that this architecture (ACBC) forms stronger inter- and intra-molecular bonds between and within the polymer chains, respectively. This is a noticeable advantage of the ACBC architecture, as it forms even stronger gels than the ABC architecture, which has been previously proven to be the best-performing.^{2,3,7}

Concerning the results for PB7, the rheological and visual results differ, but both results are reproducible. More specifically, a gel is formed visually at 41 °C, and gel syneresis is observed at 45 °C. However, in rheology, no gelation is observed, i.e. $G' < G''$ at all temperatures. Nevertheless, an increase in both moduli (storage and loss) and in viscosity is observed at around 41 °C (visual gel point). This is expected as at the thermoresponsive point, the polymer-polymer interactions are enhanced and thus the solution becomes more viscous. At this point it should be noted that physical gels are characterised as “weak gels”, and the interactions that form the gel are reversible.¹⁶ As mentioned by Kocen *et al.*, the “weak gels” can flow when the applied strain is high enough to break the intermolecular interactions between the polymer chains.¹⁷

5.2.4 Conclusions

In this study, twenty-two tetrablock terpolymers of various architectures and compositions have been synthesised via GTP. All the copolymers in this Chapter are based on PEGMA (A unit), BuMA (B unit), and DMAEMA (C unit), and their MM has been kept within the intermediate value that has been proven before to induce clear sol-gel transition. These tetrablock terpolymers can be categorised in three different families. In each family, the architecture has been varied, whereas the composition and the MM have been kept constant in order to establish clear trends. In the first family, nine tetrablock architectures with A-B-C composition at 20-40-40 w/w% have been investigated: i) two hydrophilic A blocks (ABCA, ABAC, and ACAB), ii) two hydrophobic B blocks (BABC, BACB, and ABCB), iii) two thermoresponsive C blocks (CABC, CACB, and ACBC), and one statistical terpolymer for comparison. In the second family, the same tetrablock architectures have been investigated, as

well as a triblock copolymer for comparison. The A-B-C composition of the second family has been targeted to 20-30-50 w/w%. In the third family, the best-performing architectures have been chosen, which are ABAC, BABC, CABC, ACBC, and ABC, and their composition has been targeted to intermediate hydrophobic content than in the other two families (A-B-C: 20-35-45 w/w%). Concerning the synthesis and characterisation of these terpolymers in aqueous media, the following conclusions are drawn:

1. All the syntheses were successful, as the copolymers are characterised by narrow MMD, and their MM values and compositions are within the desirable range which has been established before to produce polymers that present clear transition from a solution phase to a gel phase.
2. The polymer architecture controls the solubility of the terpolymers, which can be tuned by varying the pH.
3. The hydrophobic content affects the solubility in aqueous media, and thus in the family of highest hydrophobic content, only the polymer with CABC architecture is soluble at initial pH.
4. The self-assembly behaviour and the micellisation depend strongly on the polymer architecture and composition.
5. Generally, the pK_a is independent of the polymer architecture, apart from the statistical terpolymer and the ACBC tetrablock architecture at the highest hydrophobic content studied.
6. The composition of the terpolymers affects the pK_a , as it is observed that the higher the hydrophobic BuMA content is, the lower the pK_a .
7. The CP varies depending on the polymer architecture, with the most hydrophilic ACBC architecture to present the higher CP.
8. The polymer composition affects the CP, i.e. the CP decreases as the thermoresponsive DMAEMA content increases.
9. The thermogelling behaviour does strongly depend on the architecture of the terpolymers.
10. The composition of the terpolymers affects the gelation properties, as generally, the more hydrophilic a polymer is, the more enhanced the solubility is, and the narrower the gelation region is, i.e. it forms a gel at a smaller range of temperatures and concentrations.

11. The best-performing polymer is PB19 with theoretical polymer structure PEGMA_{5.5}-*b*-DMAEMA₁₃-*b*-BuMA₁₇-*b*-DMAEMA₁₃ (ACBC) – it forms the widest gelation area and it forms a gel at the low concentration of 10 w/w%.
12. Generally, the rheological results agree with the results from the visual tests, and any discrepancies can be explained upon consideration of the different techniques, i.e. visual observation *versus* rheology.
13. PB19 (ACBC architecture), which performs best by visual tests, it forms the strongest gels when compared to all the studies of our group.

5.2.5 References

1. Chen, J.; Liu, M.; Gong, H.; Cui, G.; Lü, S.; Gao, C.; Huang, F.; Chen, T.; Zhang, X.; Liu, Z. Synthesis of linear amphiphilic tetrablock quaterpolymers with dual stimulus response through the combination of ATRP and RAFT by a click chemistry site transformation approach. *Polym. Chem.* **2013**, *4*, 1815-1825.
2. Ward, M. A.; Georgiou, T. K. Multicompartment thermoresponsive gels: does the length of the hydrophobic side group matter? *Polym. Chem.* **2013**, *4*, 1893-1902.
3. Ward, M. A.; Georgiou, T. K. Thermoresponsive terpolymers based on methacrylate monomers: Effect of architecture and composition. *J. Polym. Sci. A Polym. Chem.* **2010**, *48*, 775-783.
4. Ward, M. A.; Georgiou, T. K. Thermoresponsive triblock copolymers based on methacrylate monomers: effect of molecular weight and composition. *Soft Matter* **2012**, *8*, 2737-2745.
5. Constantinou, A. P.; Georgiou, T. K. Thermoresponsive gels based on ABC triblock copolymers: effect of the length of the PEG side group. *Polym. Chem.* **2016**, *7*, 2045-2056.
6. Ghasdian, N.; Ward, M. A.; Georgiou, T. K. Well-defined clickable copolymers prepared via one-pot synthesis. *Chem. Commun.* **2014**, *50*, 7114-7116.
7. Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. A Comprehensive Systematic Study on Thermoresponsive Gels: Beyond the Common Architectures of Linear Terpolymers. *Polymers* **2017**, *9*, 31.
8. Popescu, M. T.; Athanasoulas, I.; Tsitsilianis, C.; Hadjiantoniou, N. A.; Patrickios, C. S. Reversible hydrogels from amphiphilic polyelectrolyte model multiblock copolymers: The importance of macromolecular topology. *Soft Matter* **2010**, *6*, 5417-5424.
9. Carroll, D. R.; Constantinou, A. P.; Stingelin, N.; Georgiou, T. K. Scalable syntheses of well-defined pentadecablock bipolymer and quinterpolymer. *Polym. Chem.* **2018**, *9*, 3450-3454.
10. Hadjiantoniou, N. A.; Triftaridou, A. I.; Kafouris, D.; Gradzielski, M.; Patrickios, C. S. Synthesis and Characterization of Amphiphilic Multiblock Copolymers: Effect of the Number of Blocks on Micellization. *Macromolecules* **2009**, *42*, 5492-5498.
11. Raduan, N. H.; Horozov, T. S.; Georgiou, T. K. "Comb-like" non-ionic polymeric macrosurfactants. *Soft Matter* **2010**, *6*, 2321-2329.
12. Ward, M. A.; Georgiou, T. K. Thermoresponsive gels based on ABA triblock copolymers: Does the asymmetry matter? *J. Polym. Sci. Part A: Polym. Chem.* **2013**, *51*, 2850-2859.
13. Simmons, M. R.; Patrickios, C. S. Synthesis and aqueous solution characterization of catalytically active block copolymers containing imidazole. *Macromolecules* **1998**, *31*, 9075-9077.
14. Georgiou, T. K.; Phylactou, L. A.; Patrickios, C. S. Synthesis, characterization, and evaluation as transfection reagents of ampholytic star copolymers: Effect of star architecture. *Biomacromolecules* **2006**, *7*, 3505-3512.
15. Georgiou, T. K.; Vamvakaki, M.; Patrickios, C. S.; Yamasaki, E. N.; Phylactou, L. A. Nanoscopic cationic methacrylate star homopolymers: Synthesis by group transfer polymerization, characterization and evaluation as transfection reagents. *Biomacromolecules* **2004**, *5*, 2221-2229.
16. Gulrez, S. K. H.; Al-Assaf, S.; O Phillips, G. Hydrogels: Methods of Preparation, Characterisation and Applications. In *Progress in Molecular and Environmental Bioengineering*; Carpi, A., Ed.; Intechopen: 2011; pp 117-150.
17. Rok Kocen and Michael Gasik and Ana Gantar, and Sa Viscoelastic behaviour of hydrogel-based composites for tissue engineering under mechanical load. *Biomedical Materials* **2017**, *12*, 025004.

5.3 DEGMA-Based Thermoresponsive Terpolymers

There is great scientific interest on the thermoresponsive behaviour of pEG-based methacrylate polymers, because pEG is a hydrophilic, biocompatible polymer and FDA approved.^{1,2} Therefore, several reviews and research papers have been published on this topic.¹⁻¹⁴ Specifically, it has been reported that the thermoresponse and hydrophilicity of a pEG methacrylate polymer depends on the length of the side group, i.e. the fewer the EG groups, the more enhanced the thermoresponse. In particular, the CPs of the homopolymers decreases from 90 °C to 26 °C, as the number of EG groups decreases from 9 to 2.⁹ The mono(ethylene glycol) methyl ether methacrylate has such reduced hydrophilicity that is not well-soluble in water.⁹

Lutz and co-workers have investigated either linear^{2-4,10,11} or star^{8,11} copolymers based on DEGMA and NEGMA in terms of their thermoresponsive behaviour. When linear random copolymers are concerned, it has been observed that as the composition of the more hydrophobic DEGMA increases, the CP decreases,^{2,4,10} as expected. These linear copolymers have been proven to be biocompatible, which renders them suitable for use in biological applications.⁴ The star copolymers have been synthesised via ATRP by randomly copolymerising DEGMA and NEGMA on a four-arm pEG macroinitiator ($MM = 10000 \text{ g mol}^{-1}$).^{8,11} Interestingly, a star copolymer consisted of DEGMA and NEGMA (DP of the arm to be around 100, and 5 mol% NEGMA) formed stable gels of maximum viscosity at 38 °C (at 15 w/w% solution in PBS).⁸ The T_{gel} of the copolymers with higher NEGMA content was higher, because of increased hydrophilicity.⁸ These star copolymers were compared to linear ones formed by randomly copolymerising DEGMA and NEGMA on a bifunctional PEG macroinitiator.¹¹ It has been observed that the star copolymers showed better gelation properties, as the linear ones did not form gel within the concentration and temperature range tested.¹¹ However, it should be noted that these studies have been carried out in a non-systematic way, i.e. both the composition and the MM were simultaneously varied.^{2,10,11} Therefore, it is important to carry out systematic studies, as it is well-established that both the MM and the composition affect the thermoresponsive and gelation properties.

As pEG-based methacrylate polymers have shown potential on forming stable gels, this Chapter focuses on synthesising and investigating polymers based on pEG methacrylate units. It is hypothesised that incorporating a hydrophobic block in the structure will promote self-assembly and enhance thermoresponse, since the linear copolymers investigated by Lutz and

co-workers did not form gels.¹¹ Therefore, in this study, three monomers have been used, the structures of which can be found in Figure 5.3.1. In order for this study of the thesis to be comparable with the previous two studies (Chapters 5.1 and 5.2), the other two monomers, PEGMA and BuMA, have been kept the same, as the hydrophilic and hydrophobic monomers, respectively. Thus, PEGMA and BuMA form the A and B blocks, respectively as in the previous Chapters. Therefore, DEGMA is used as the thermoresponsive unit (C block), instead of DMAEMA that has been studied in the previous Chapters. As has been proven before by our group, the ABC architecture demonstrates the best thermogelling properties. Consequently, this architecture has been chosen for the current study. Moreover, the MM of all the copolymers has been targeted to 8300 g mol^{-1} to be comparable with the previous studies, whereas the compositions have been systematically varied in order to investigate its effect on the thermoresponsive properties of the copolymers and to identify the optimum comonomer ratio/composition for this novel combination of monomer units. Consequently, three families of three different BuMA weight percentages have been studied: i) 25 w/w% (PC1-PC3), ii) 30 w/w% (PC4-PC9), and iii) 35 w/w% (PC10-PC12). In each family, the PEGMA and DEGMA weight percentages have been varied to tailor the hydrophobicity/solubility and thermoresponsive behaviour of the copolymers. In addition to the triblock terpolymers, one ABA triblock bipolymer (PC13), and one CBC triblock bipolymer (PC14), both with 30 w/w% BuMA content, and one statistical terpolymer (PC15) with intermediate A-B-C composition of 45-30-25 w/w% have been synthesised for comparison.

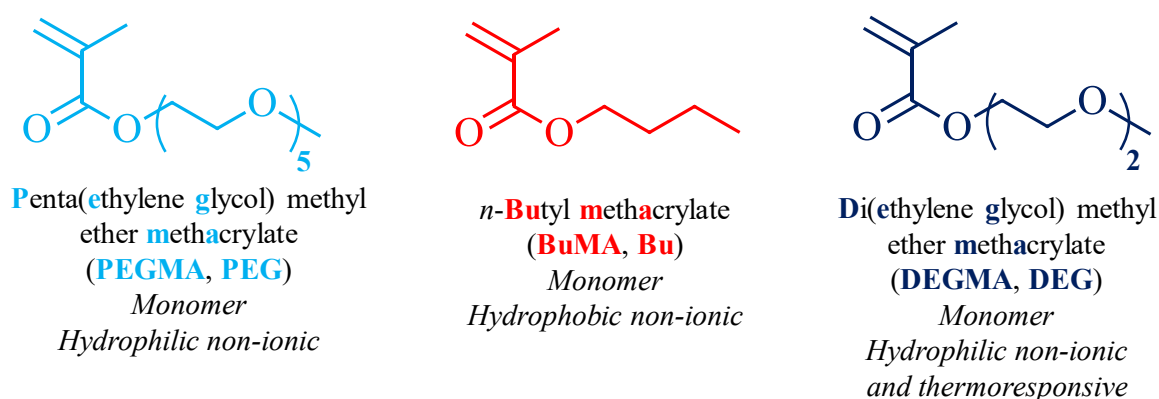


Figure 5.3.1: Chemical structures, names, and abbreviations of the monomers used in this polymer series: penta(ethylene glycol) methyl ether methacrylate (PEGMA), *n*-butyl methacrylate (BuMA), and di(ethylene glycol) methyl ether methacrylate (DEGMA).

5.3.1 Polymer Synthesis

All the block copolymers have been synthesised via sequential GTP. Figure 5.2.3 shows the synthesis of an ABC triblock terpolymer with A, B, and C being based on PEGMA, BuMA, and DEGMA, respectively. The synthetic procedure is similar to the ones discussed before, as the monomers are added sequentially in the polymerisation flask, after the addition of the catalyst (TBABB), the solvent (THF), and the initiator (MTS). The statistical terpolymer has been synthesised by adding the initiator last, as has been discussed in the previous chapters and schematically illustrated in Figure 5.1.2 (b). The polymer structures are schematically illustrated in Figure 5.3.3 with PEGMA, BuMA, and DEGMA being represented by light blue, red, and dark blue spheres, respectively.

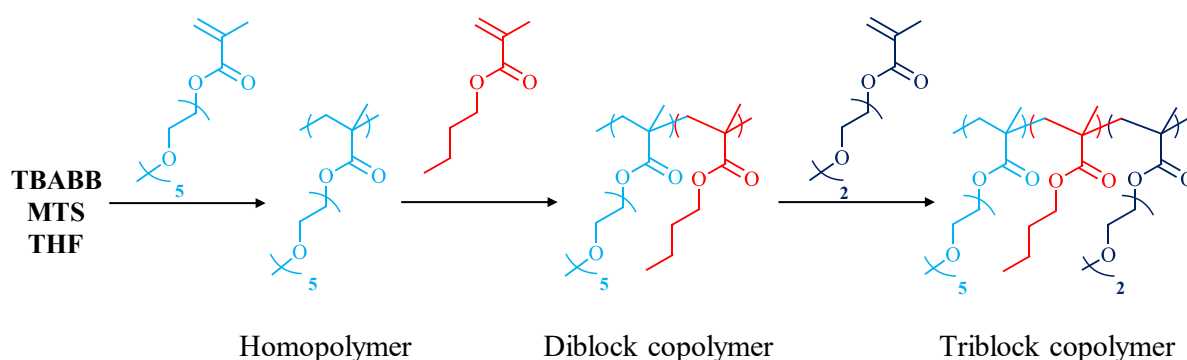


Figure 5.3.2: Chemical reaction of the GTP synthesis of an ABC triblock terpolymer which is based on PEGMA, BuMA, and DEGMA as A, B, and C units, respectively. *Note: TBABB, MTS, and THF are the catalyst, initiator, and solvent used for this PhD project.*

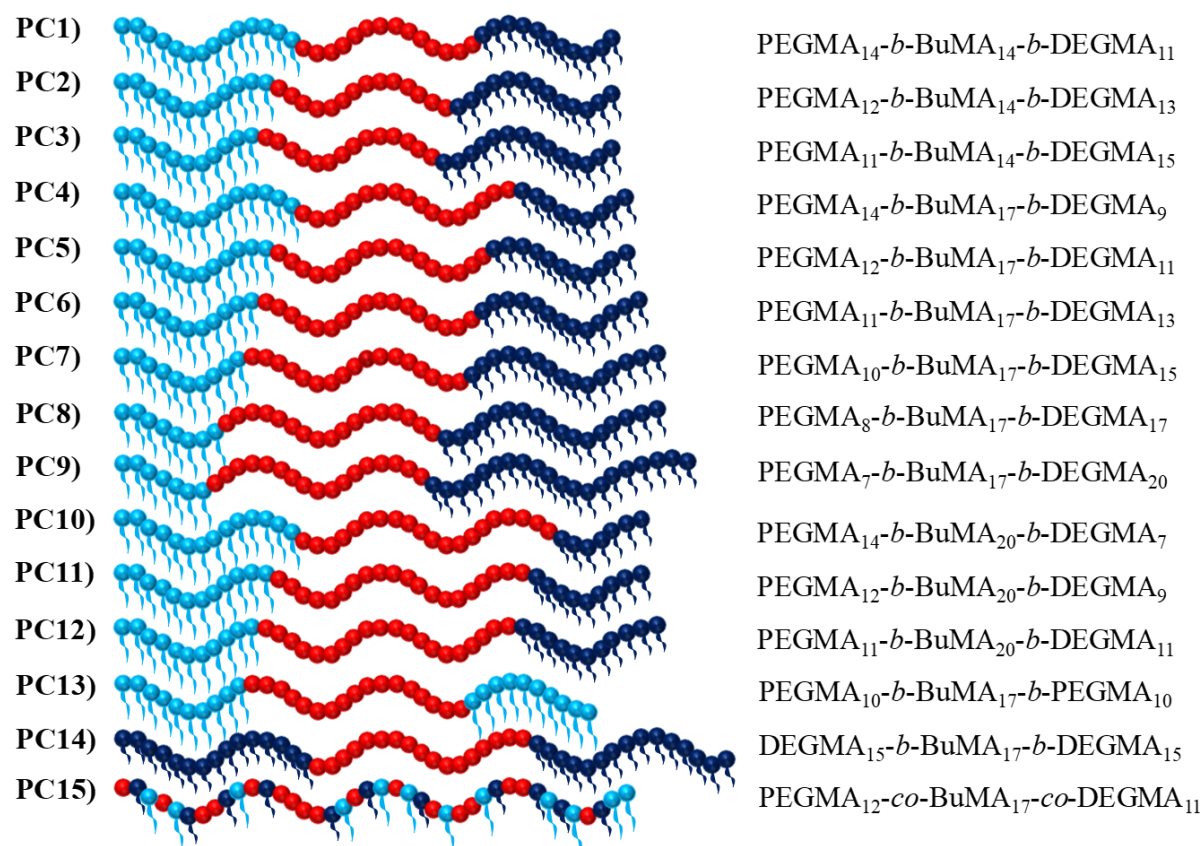


Figure 5.3.3: Schematic representation of the ABC triblock terpolymers of various compositions (PC1-PC12), the ABA triblock copolymer (PC3), and CBC triblock bipolymer (PC14), and the statistical terpolymer (PC15). Light blue, red, and dark blue spheres represent PEGMA (A unit), BuMA (B unit), and DEGMA (C unit).

5.3.2 Molecular Mass and Composition

The molecular mass characteristics of the copolymers have been determined via GPC. In the case of the block copolymers, their linear precursors have also been examined. Figure 5.3.4 shows the GPC traces of PC1, PEGMA₁₄-*b*-BuMA₁₄-*b*-DEGMA₁₁, in dark blue dashed line. Figure 5.3.4 also shows the GPC traces of the linear precursors of PC1; the GPC chromatograms of PEGMA₁₄ homopolymer and PEGMA₁₄-*b*-BuMA₁₄ diblock copolymer are given in light blue solid and red dotted lines, respectively. As can be seen in Figure 5.3.4, the peak moves to higher MM values as the polymerisation progresses, i.e. from the homopolymer to the diblock and to the final triblock copolymer, with no shoulders being present. This confirms the successful sequential GTP polymerisation. Similar chromatograms have been collected for all the copolymers and they are presented in Figure A3 in the Appendix.

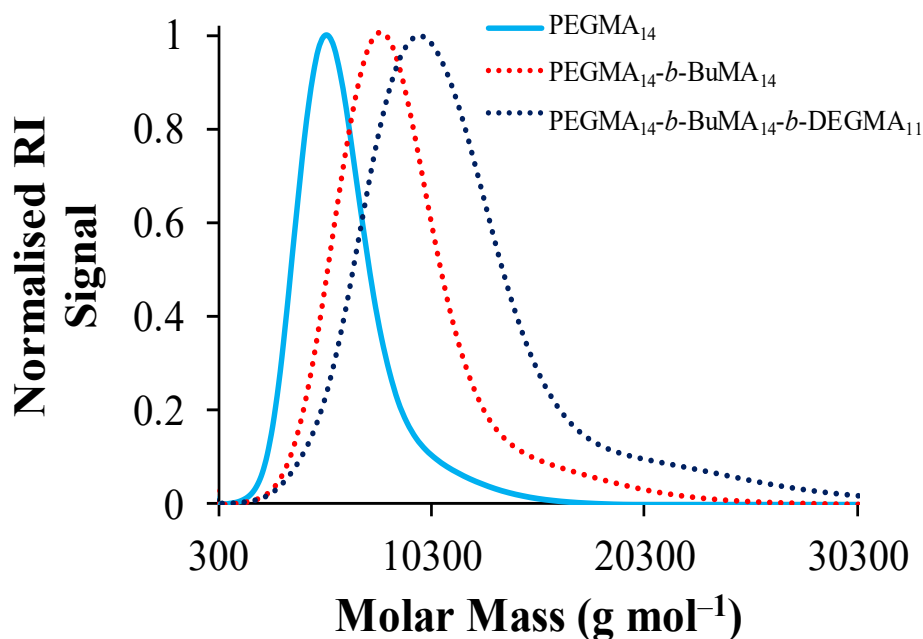


Figure 5.3.4: The GPC chromatogram of the triblock terpolymer PEGMA₁₄-*b*-BuMA₁₄-*b*-DEGMA₁₁ (PC1) is shown in dark blue dotted line. The GPC traces of its linear precursors are also given: i) PEGMA₁₄ in light blue solid line, and ii) PEGMA₁₄-*b*-BuMA₁₄ in red dotted line.

Table 5.3.1 lists the theoretical polymer structures of the final copolymers and their linear precursors (if any), their targeted and experimental MM and MMD, as resulted by GPC.

The $\bar{D}$ values of the copolymers vary between 1.15 and 1.23, and they are satisfactory narrow for the scope of this study. These values are within the range of $\bar{D}$ values reported before in GTP studies, especially when macromonomers like pEG based methacrylate monomers are used.¹⁵⁻²¹ Unlike the trends previously reported,¹⁹ and what is expected for a living polymerisation method like GTP, the $\bar{D}$ values increase as the MM increases. This can be attributed to some deactivation occurring when samples are obtained for GPC and NMR analysis, and when the next monomer addition occurs and to some tailing effect that is observed in some samples from the beginning. The latter might be due to some transfer reactions. Note that more impurities than normal are introduced with the addition of the PEGMA macromonomer, as it is unable to be distilled on account of its high MM.^{15,19}

The statistical copolymer has narrower MMD than the block copolymers, which is similar to the observations made in the previous chapters. The narrow MMD can be attributed to i) the simultaneous addition of the monomers, thus introducing less deactivation in the polymerisation flask, and ii) the polymerisation being performed in a water-bath, thus a more

controlled polymerisation was performed.

Table 5.3.1: Theoretical chemical structures, dispersity indices ($\bar{D}$), theoretical and experimental molecular masses ($MM^{\text{theor.}}$ and M_n , respectively) and compositions of the final terpolymers polymers and their precursors (if any).

No.	Theoretical Polymer Structure ^a	$MM^{\text{theor. b}}$ (g mol ⁻¹)	M_n^c (g mol ⁻¹)	$\bar{D}^c$	w/w% PEG-Bu-DEG	
					Theoretical	¹ H NMR
PC1	PEG ₁₄	4200	4900	1.13	100-00-00	100-00-00
	PEG ₁₄ - <i>b</i> -Bu ₁₄	6250	7100	1.13	67-33-00	69-31-00
	PEG ₁₄ - <i>b</i> -Bu ₁₄ - <i>b</i> -DEG ₁₁	8300	8600	1.16	50-25-25	54-25-22
PC2	PEG ₁₂	3790	5100	1.11	100-00-00	100-00-00
	PEG ₁₂ - <i>b</i> -Bu ₁₄	5840	7600	1.13	64-36-00	66-34-00
	PEG ₁₂ - <i>b</i> -Bu ₁₄ - <i>b</i> -DEG ₁₃	8300	9500	1.17	45-25-30	47-25-28
PC3	PEG ₁₁	3380	4400	1.12	100-00-00	100-00-00
	PEG ₁₁ - <i>b</i> -Bu ₁₄	5430	6700	1.12	62-38-00	64-36-00
	PEG ₁₁ - <i>b</i> -Bu ₁₄ - <i>b</i> -DEG ₁₅	8300	9200	1.16	40-25-35	45-25-30
PC4	PEGA ₁₄	4200	5900	1.11	100-00-00	100-00-00
	PEG ₁₄ - <i>b</i> -Bu ₁₇	6560	9200	1.13	62-38-00	65-35-00
	PEG ₁₄ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₉	8300	10700	1.18	50-30-20	54-28-18
PC5	PEG ₁₂	3790	6000	1.10	100-00-00	100-00-00
	PEG ₁₂ - <i>b</i> -Bu ₁₇	6250	9600	1.14	60-40-00	62-38-00
	PEG ₁₂ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₁₁	8300	12200	1.18	45-30-25	49-30-21
PC6	PEG ₁₁	3380	5200	1.11	100-00-00	100-00-00
	PEG ₁₁ - <i>b</i> -Bu ₁₇	5840	8700	1.13	57-43-00	59-41-00
	PEG ₁₁ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₁₃	8300	11500	1.18	40-30-30	41-29-30
PC7	PEG ₁₀	2970	4300	1.14	100-00-00	100-00-00
	PEG ₁₀ - <i>b</i> -Bu ₁₇	5430	7600	1.12	54-46-00	56-44-00
	PEG ₁₀ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₁₅	8300	10300	1.16	35-30-35	38-30-32
PC8	PEG ₈	2560	3700	1.14	100-00-00	100-00-00
	PEG ₈ - <i>b</i> -Bu ₁₇	5020	6500	1.14	50-50-00	53-47-00
	PEG ₈ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₁₇	8300	9600	1.17	30-30-40	32-30-38
PC9	PEG ₇	2150	3000	1.15	100-00-00	100-00-00
	PEG ₇ - <i>b</i> -Bu ₁₇	4610	5900	1.15	46-54-00	49-51-00
	PEG ₇ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₂₀	8300	9200	1.17	25-30-45	29-30-41
PC10	PEG ₁₄	4200	4700	1.15	100-00-00	100-00-00
	PEG ₁₄ - <i>b</i> -Bu ₂₀	7070	7900	1.17	59-41-00	59-41-00
	PEG ₁₄ - <i>b</i> -Bu ₂₀ - <i>b</i> -DEG ₇	8300	9200	1.19	50-35-15	50-35-15
PC11	PEG ₁₂	3790	5400	1.14	100-00-00	100-00-00
	PEG ₁₂ - <i>b</i> -Bu ₂₀	6660	9500	1.16	56-44-00	58-42-00
	PEG ₁₂ - <i>b</i> -Bu ₂₀ - <i>b</i> -DEG ₉	8300	11300	1.18	45-35-20	48-34-18
PC12	PEG ₁₁	3380	4200	1.14	100-00-00	100-00-00
	PEG ₁₁ - <i>b</i> -Bu ₂₀	6250	7700	1.16	53-47-00	55-45-00
	PEG ₁₁ - <i>b</i> -Bu ₂₀ - <i>b</i> -DEG ₁₁	8300	10000	1.19	40-35-25	41-34-25
PC13	PEG ₁₀	2970	4200	1.13	100-00-00	100-00-00
	PEG ₁₀ - <i>b</i> -Bu ₁₇	5430	7600	1.13	54-46-00	55-45-00

	PEG ₁₀ - <i>b</i> -Bu ₁₇ - <i>b</i> -PEG ₁₀	8300	11000	1.19	70-30-00	72-28-00
PC14	DEG ₁₅	2970	4900	1.10	00-00-100	00-00-100
	DEG ₁₅ - <i>b</i> -Bu ₁₇	5430	9300	1.14	54-46-00	54-46-00
	DEG ₁₅ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₁₅	8300	12500	1.23	00-30-70	00-31-69
PC15	PEG ₁₂ - <i>co</i> -Bu ₁₇ - <i>co</i> -DEG ₁₁	8300	11300	1.15	45-30-25	47-29-24

^a PEG, Bu and DEG are the abbreviations for penta(ethylene glycol) methyl ether methacrylate, *n*-butyl methacrylate and di(ethylene glycol) methyl ether methacrylate, respectively.

^b The theoretical MM is calculated using the Equation 4.1 $MM^{theor.}(g\ mol^{-1}) = (\sum_i MM_i \times DP_i) + 100$

^c The values of the number-average molar mass (M_n) and the dispersity index ($\bar{D}$) of the terpolymers and their linear precursors (if any) were determined by gel permeation chromatography, GPC. The calibration was based on well-defined linear poly(methyl methacrylate) (PMMA) standard samples with MM equal to 2, 4, 8, 20, 50, and 100 kg mol⁻¹.

Table 5.3.1 also lists the theoretical and experimental MMs. As can be seen, the experimental M_n values are slightly higher than the targeted ones; this trend has been previously reported and it is attributed to the partial deactivation of the initiator because of moisture and impurities introduced in the polymerisation flask.¹⁵⁻²¹

Table 5.3.1 also lists the theoretical and experimental compositions of the copolymers and their linear precursors (if any). The experimental values have been calculated *via* analysis of the ¹H NMR spectra. The ¹H NMR spectra of PEGMA₁₄-*b*-BuMA₁₄-*b*-DEGMA₁₁ (PC1) and its linear precursors (homopolymer and diblock) are shown in Figure 5.3.5; they are coloured in light blue, red, and dark blue, respectively. In Figure 5.3.5, it can be observed that while BuMA shows distinct peaks, the peaks of PEGMA and DEGMA appear at the same region because of their similar chemical structures. For calculating the experimental compositions, two distinct peaks are used, as discussed in the experimental part, and they correspond to the f protons (for BuMA), and to the e and l protons for PEGMA and DEGMA. In the case of the triblock terpolymers, the peaks of PEGMA and DEGMA sometimes overlap. Hence, the ratio of PEGMA/DEGMA triblock copolymers could not be calculated unless information from the previous synthetic step, i.e. diblock copolymer, was used. Thus, the integration of DEGMA has been calculated by subtracting the integration of PEGMA (as calculated from the ¹H NMR of the diblock copolymer) from the total integration (PEGMA and DEGMA, as calculated from the ¹H NMR of the triblock copolymer), after the values have been normalised. Concerning the statistical terpolymer (PC15), the peak has been integrated in two parts, as shown in Figure 5.3.5 (c). As can be seen in Table 5.3.1, the theoretical values agree with the experimental ones, within the experimental error of the technique.

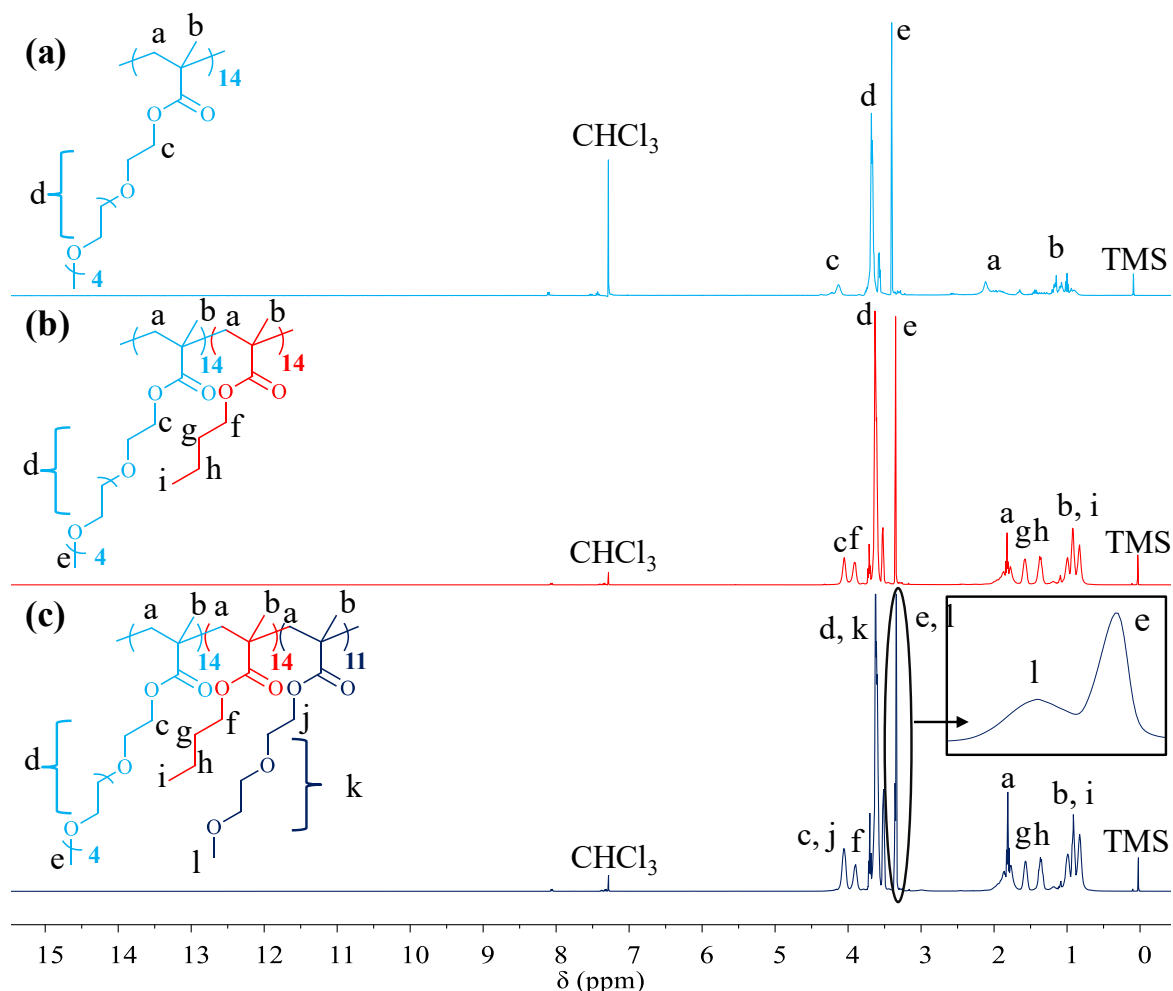


Figure 5.3.5: ^1H NMR spectra of (a) the homopolymer PEGMA₁₄, (b) the diblock copolymer PEGMA₁₄-*b*-BuMA₁₄, and (c) the final triblock terpolymer PEGMA₁₄-*b*-BuMA₁₄-*b*-DEGMA₁₁ (PC1) are presented in light blue, red, and dark blue, respectively. Also, the corresponding chemical structures are shown. In these structures, the PEGMA, BuMA, and DEGMA repeated units are coloured in light blue, red, and dark blue, respectively.

5.3.3 Aqueous Solution Properties

5.3.3.1 Hydrodynamic Diameters

The self-assembly behaviour of the polymers in water has been investigated by DLS. The theoretical and experimental hydrodynamic diameters are listed in Table 5.3.2. The theoretical values have been calculated by assuming that: i) the block copolymers form spherical micelles, and ii) the statistical copolymer coils in a random way.

Table 5.3.2: Theoretical polymer structures, architectures, and hydrophobic composition, theoretical and experimental hydrodynamic diameters (d_h), polydispersity indices (PDI), and cloud points (CPs) of 1 w/w% copolymer solutions in deionised water.

No.	Polymer Architecture	w/w% Bu	Theoretical Polymer Structure ^a	Hydrodynamic diameter (d_h , nm)			Cloud Points ± 2 °C
				Theor. ^b	Experimental ± 0.5 ^f	PDI	
PC1	ABC	25	PEG ₁₄ - <i>b</i> -Bu ₁₄ - <i>b</i> -DEG ₁₁	11.8 ^c	13.5	0.018	49
PC2	ABC	25	PEG ₁₂ - <i>b</i> -Bu ₁₄ - <i>b</i> -DEG ₁₃	12.5 ^c	15.7	0.051	49
PC3	ABC	25	PEG ₁₁ - <i>b</i> -Bu ₁₄ - <i>b</i> -DEG ₁₅	11.8 ^c	13.5	0.047	45
PC4	ABC	30	PEG ₁₄ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₉	16.0 ^c	43.8	0.192	50
PC5	ABC	30	PEG ₁₂ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₁₁	17.1 ^c	50.7	0.197	48
PC6	ABC	30	PEG ₁₁ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₁₃	16.2 ^c	21.0	0.117	47
PC7	ABC	30	PEG ₁₀ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₁₅	14.4 ^c	21	0.098	39
PC8	ABC	30	PEG ₈ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₁₇	15.1 ^c	15.7	0.060	34
PC9	ABC	30	PEG ₇ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₂₀	14.9 ^c	15.7	0.036	29
PC10	ABC	35	PEG ₁₄ - <i>b</i> -Bu ₂₀ - <i>b</i> -DEG ₇	14.3 ^c	ND ^g	ND	ND
PC11	ABC	35	PEG ₁₂ - <i>b</i> -Bu ₂₀ - <i>b</i> -DEG ₉	16.3 ^c	ND	ND	ND
PC12	ABC	35	PEG ₁₁ - <i>b</i> -Bu ₂₀ - <i>b</i> -DEG ₁₁	12.6 ^c	43.8	0.219	41
PC13	ABA	30	PEG ₁₀ - <i>b</i> -Bu ₁₇ - <i>b</i> -PEG ₁₀	12.3 ^d	24.4	0.130	60
PC14	ABA	30	DEG ₁₅ - <i>b</i> -Bu ₁₇ - <i>b</i> -DEG ₁₅	19.9 ^d	ND	ND	ND
PC15	(ABC)	30	PEG ₁₂ - <i>co</i> -Bu ₁₇ - <i>co</i> -DEG ₁₁	2.0 ^e	ND	ND	ND

^a Abbreviations: penta(ethylene glycol) methyl ether methacrylate (PEG), *n*-butyl methacrylate (Bu), and di(ethylene glycol) methyl ether methacrylate (DEG).

^b The calculations of the theoretical diameters are based on the experimental degrees of polymerisation (DPs), which were calculated by using the experimental MM after precipitation, as determined by GPC analysis, and the experimental composition, as determined by ¹H NMR analysis.

^c The diameter of the spherical micelles formed by the ABC triblock terpolymers (PC1-PC12) were calculated by using either the Equation 4.3 (d_h (nm) = (DP_B + 2 × DP_A) × 0.254, or the Equation 4.4 (d_h (nm) = (DP_B + 2 × DP_C) × 0.254), depending on whether DP_A > DP_C, or DP_A < DP_C, respectively. In all the cases, A, B, and C correspond to PEGMA, BuMA, and DEGMA, respectively.

^d The equation 4.8, d_h (nm) = [DP_A + DP_B] * 0.254 nm, was used to calculate the theoretical hydrodynamic diameter of the ABA diblock bipolymers. When the hydrophilic unit is PEGMA (PC13), the DP_{PEGMA} was used, whereas when the hydrophilic unit is DEGMA (PC14), DP_A corresponds to DP_{DEGMA}.

^e The statistical terpolymer (PC15) is assumed to adopt a random coil configuration. The end-to-end distance of the random coil was calculated by the Equation 4.2: $\langle d_g^2 \rangle^{1/2} = 2 \times [2 \times 2.20 \times (DP_A + DP_B + DP_C) / 3]^{1/2} \times 0.154$, where A, B and C correspond to PEGMA, BuMA and DEGMA, respectively.

^f The experimental hydrodynamic diameters reported are the mean diameters which correspond to the maximum of the peak by intensity in the DLS histograms.

^g Not determined (ND): the polymers were not soluble, and therefore characterisation in aqueous solutions was not feasible.

As can be seen in Table 5.3.2, four of the copolymers are insoluble in aqueous media, and thus further characterisation is not feasible. More specifically, PC14 with theoretical polymer structure $\text{DEGMA}_{15}\text{-}b\text{-BuMA}_{17}\text{-}b\text{-DEGMA}_{15}$ is not soluble, presumably because of: i) the formation of bigger micelles, and ii) the increased hydrophobicity of the structure, caused by the absence of the hydrophilic PEGMA units. This indicates the necessity of incorporating PEGMA in the structure. Also, the statistical terpolymer is not soluble, as expected, because it cannot form micelles.¹⁹⁻²¹ This is similar to the DMAEMA studies, in which the statistical terpolymer was insoluble and further protonation was required to enhance solubility.^{20,21} Concerning the copolymers of higher hydrophobic content (35 w/w%), only PC12 is soluble. This was not expected since it contains more DEGMA, and thus it is considered the most hydrophobic monomer of the three. However, further characterisation is required to understand how the polymer self-assembles in solution, since it has also some fascinating thermoresponsive properties, as it will be discussed in the following sections.

As mentioned before, it is assumed that the triblock copolymers self-assemble into spherical micelles with the classical core-shell structure. In Figure 5.3.6, the spherical micelle adopted by PC1 ($\text{PEGMA}_{14}\text{-}b\text{-BuMA}_{14}\text{-}b\text{-DEGMA}_{11}$) is schematically illustrated; the PEGMA, BuMA, and DEGMA units are coloured in light blue, red, and dark blue, respectively. As can be seen in Figure 5.3.6, the BuMA units form the core of the micelles, whereas the shell of the micelle is based on both DEGMA and PEGMA units. In the case of the triblock terpolymers, the longest hydrophilic block is the one which controls the micelle size. It is expected that all the triblock copolymers (either ter- or bipolymers) will self-assemble in a similar way as shown in Figure 5.3.6.

Unlike our previous studies on DMAEMA-based polymers, the experimental DLS results show in all the cases higher hydrodynamic diameters compared to the theoretical ones, even though the theoretical model assumes fully stretched polymer chains.¹⁹⁻²¹ This can be attributed to the theoretical calculations being based only on the methacrylate backbone and not taking into consideration the different nature/size of the side chains. In this study, the polymers have higher DP of PEGMA compared to the previous studies. PEGMA consists of a long side chain of 5 repeated ethylene glycol units, which contribute to the micelle size. If this is taken into account, then ~ 4 nm can be added to the theoretical diameter for each polymer. In this case, only five polymers will form micelles with hydrodynamic diameters higher than the theoretically predicted values. The polymers that form bigger micelles than theoretically may self-assemble in a different than the theoretically expected structure, and thus further characterisation is

needed to provide information on their configuration. Also, the DLS was performed at 25 °C, and this temperature is very close to the CP of the DEGMA homopolymer. Thus, the DEGMA may be distributed more in the core rather in the shell of the micelle. This phenomenon seems to be enhanced when: i) the BuMA content is 35 w/w% (PC12), and ii) when the DP of DEGMA is lower for polymers with 30 w/w% of BuMA.

Also, it is worth-commenting on the low PDI values; they vary between 0.018 and 0.197. This shows that the samples are monodisperse,²² as only one peak is detected by intensity, i.e. no peaks corresponding to aggregates or unimers are observed. This is not the case for the DMAEMA systems discussed in the two previous chapters, and it seems that by substituting DMAEMA with DEGMA, the micellisation is enhanced.

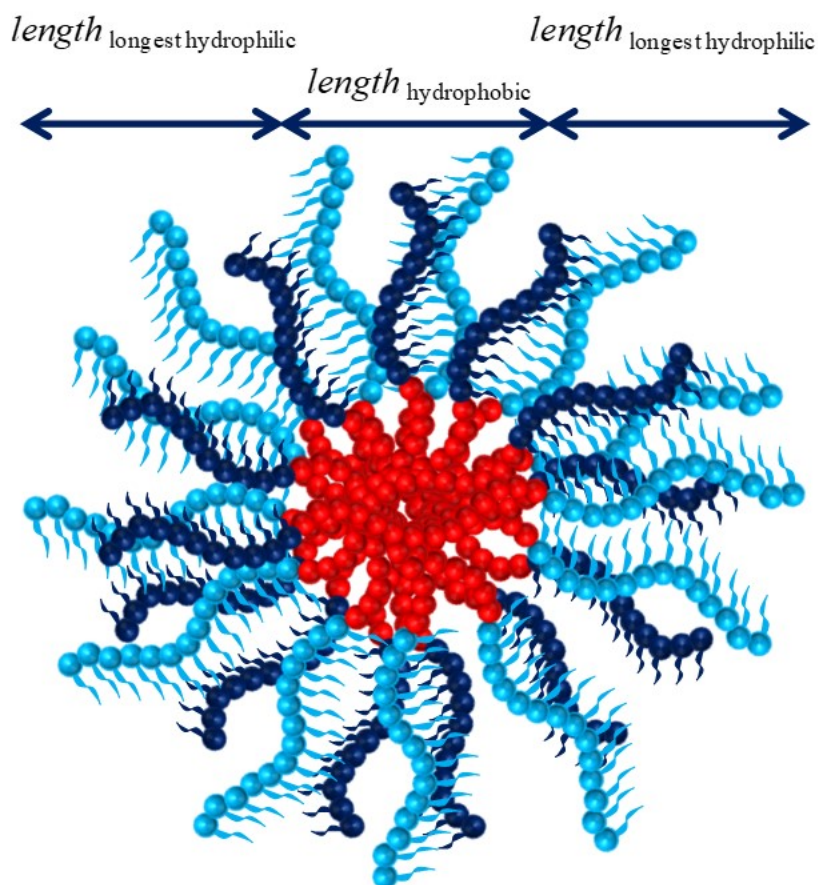


Figure 5.3.6: Schematic illustration of the spherical micelle adopted by PEGMA₁₄-*b*-BuMA₁₄-*b*-DEGMA₁₁ (PC1). The PEGMA, BuMA, and DEGMA units are shown in light blue, red, and dark blue spheres, respectively. All triblock copolymers in this study, either terpolymers (PC1-PC12) or bipolymers (PC13 and PC14), are assumed to self-assemble in the same way as shown. The longest hydrophilic block is the one which determines the micelle size.

Note: The statistical terpolymer (PC15) is assumed to adopt random coil configuration, similarly to PA7, the structure of which can be found in Figure 5.1.6.

5.3.3.2 Cloud Points

The CPs of 1 w/w% polymer solutions in DI water are listed in Table 5.3.2. It is clear from Table 5.3.2 that PEGMA₁₀-*b*-BuMA₁₇-*b*-PEGMA₁₀ presents the highest CP, which is attributed to its higher hydrophilicity. This is expected as PEGMA homopolymers with MM around 3000 g mol⁻¹ and 10000 g mol⁻¹ present a CP at 75°C and 70°C, respectively. On the other hand, the CPs of the DEGMA homopolymers with comparable MMs as the PEGMA ones change from 30°C to 27°C as the MM increases. Thus, it can be concluded that the presence of BuMA units in the polymer chain lowers the CP, as it enhances the “hydrophobic effect”, whereas the absence of DEGMA increases the CP. Interestingly, the CP of DEGMA is close to biological temperature, and thus it is an interesting candidate for producing thermoresponsive polymers.

Figure 5.3.7 shows the CPs of PEGMA_x-*b*-BuMA_y-*b*-DEGMA_z solutions; x, y, and z is the degree of polymerisation of PEGMA, BuMA, and DEGMA, respectively. Two trends are shown in Figure 5.3.7: i) the effect of the experimental DEGMA weight percentage on the CP, and ii) the effect of the experimental BuMA weight percentage; these trends are discussed in the paragraphs below.

Firstly, in terms of the effect of the hydrophobic BuMA content on the CP, it can be seen that generally the CP decreases as the BuMA content increases, as previously reported.¹⁹ There is only a small discrepancy when PC3 and PC6 are compared, but their CP are the same within the experimental error and considering PC3 has a higher MM this can explain this discrepancy.

Secondly, in Figure 5.3.7, it is clearly shown that for constant BuMA content, the CP decreases as the DEGMA/PEGMA ratio increases. This can be attributed to: i) the increase in DEGMA, which responds at lower temperatures than PEGMA, and ii) the lower content in the hydrophilic PEGMA, which increases the overall hydrophobicity of the polymers, and thus lowers the CP. At this point it should be pointed out that there are three polymers with 35wt% BuMA, CP10-CP12, but only CP12 exhibits a CP because the other two are not water-soluble. What was unexpected is that the ones with lower DEGMA/PEGMA ratio (PC10 and PC11), i.e. more hydrophilic, are the ones that are not water-soluble, while the more hydrophobic PC12 is water-soluble and presents a CP. At this stage, this behaviour cannot be explained, and further characterisation is needed to try to understand the micelles/aggregates that this polymers forms. This polymer also shows a very interesting gelling behaviour, as it will be discussed in the following sections.

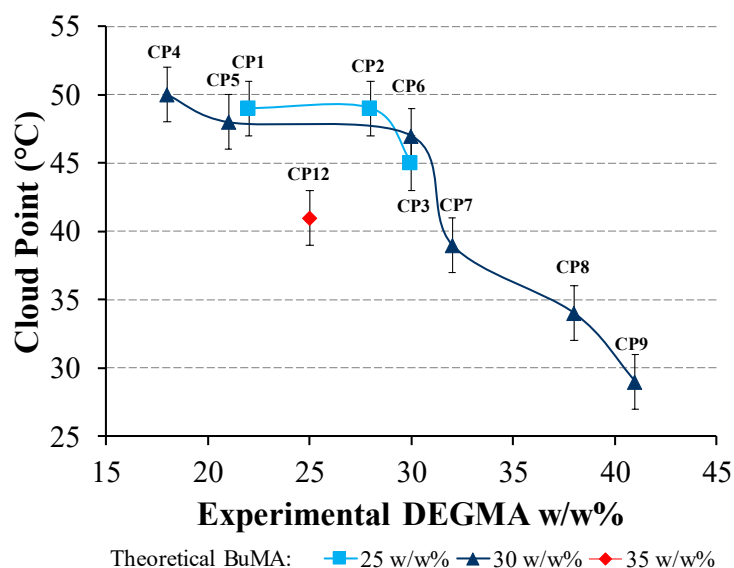


Figure 5.3.7: Effect of experimental DEGMA weight percentage on the cloud points of PEGMA_x-b-BuMA_y-b-DEGMA_z solutions (1 w/w% in deionised water); x, y, and z is the degree of polymerisation of PEGMA, BuMA, and DEGMA, respectively. The effect of the experimental BuMA weight percentage is also shown; light blue squares, dark blue triangles, and the red rhombi correspond to copolymers of 25, 30, and 35 w/w% of BuMA.

5.3.3.3 Visual Gel Points

The visual gel points of the copolymers have been investigated over a range of concentrations and temperatures. Thus, phase diagrams of eleven out of fifteen copolymers were constructed and they are presented in Figure 5.3.8. The effect of the hydrophobic BuMA content is shown from the top to the bottom: (a) hydrophobic BuMA content of 25 w/w% (top), (b) 30 w/w% BuMA content (middle), and (c) 35 w/w% BuMA content (bottom). The effect of the thermoresponsive DEGMA content is also shown from the left to the right. Also, as mentioned before, the CBC triblock bipolymer DEGMA₁₅-b-BuMA₁₇-b-DEGMA₁₅ (PC14), the statistical terpolymer PEGMA₁₂-co-BuMA₁₇-co-DEGMA₁₁ (PC15), and the ABC triblock terpolymers PEGMA₁₄-b-BuMA₂₀-b-DEGMA₇ (PC10), and PEGMA₁₂-b-BuMA₂₀-b-DEGMA₉ (PC11) are not soluble in PBS and thus the construction of meaningful phase diagrams is not feasible.

All the copolymer solutions at concentration of 1 w/w% did present a CP within the temperature range tested. Similarly to the CP values in DI water, the CPs in PBS show similar trends, i.e. the higher the content in the hydrophobic BuMA and/or the thermoresponsive DEGMA, the lower the CP. In all the cases, the CPs in PBS are lower than the CPs in DI water, because of the ionic strength effect; previously observed and reported.²³ This led to the four copolymers of the highest DEGMA content presenting CP below 40°C. Interestingly, PC7,

PEGMA₁₀-*b*-BuMA₁₇-*b*-DEGMA₁₅, presents a CP at body temperature.

The most important and interesting information which is revealed by the phase diagrams is the gelation region; this is approximately shown by a black dashed line in Figure 5.2.8. The gelation area includes the concentration and temperature values at which a stable gel (either transparent or cloudy) is formed. In the phase diagrams, the transparent and cloudy stable gels are shown in blue triangles and blue circles, respectively. As can be seen in Figure 5.2.8, six out of eleven polymers did present a clear gelation region. On the other hand, the other five polymers did not form a stable gel at the concentration and temperature ranges tested, but they did precipitate as the temperature increased.

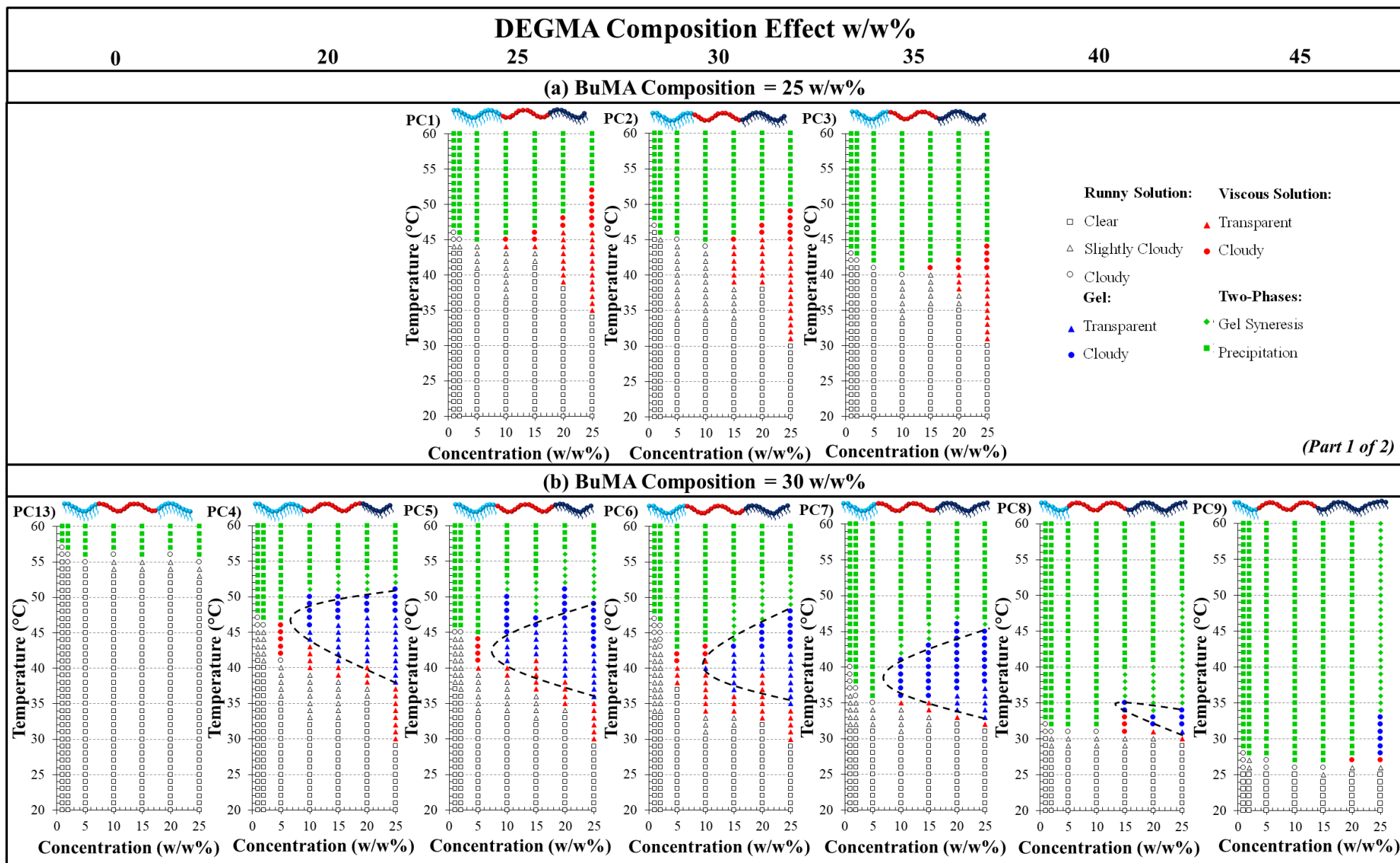
As mentioned before, five polymers did not present a gelation region. Amongst these polymers is PC13, which is the ABA triblock copolymer, based only on PEGMA. This indicates the importance of incorporating the DEGMA unit in order to induce gelation. The rest of the polymers are i) the ones of lowest hydrophobic BuMA content (PC1-PC3), and ii) PC9 with the intermediate BuMA content and the highest DEGMA content. The non-gelation of PC1-PC3 is attributed to the low hydrophobicity which disrupted the hydrophobic effect and thus the gelation. PC9 did not present a gelation region because it is too hydrophobic. Thus, it precipitated instead at the low temperatures of 27-29°C (concentration dependant). It is noteworthy, that concentrated PC9 solutions need to be handle with care, as they precipitate immediately at body temperature and/or high room temperature. These results indicate the importance of choosing the correct balance of hydrophobicity/hydrophilicity for gels to be formed.

Interestingly, PC4-PC8, which are ABC triblock terpolymers with 30 w/w% BuMA, all present a gelation region (the gelation points are shown in blue). It can be observed that the gelation region moves to lower temperatures as the thermoresponsive DEGMA content increases from PC4 to PC8; as expected. While PC4 with only 20 w/w% DEGMA gels above body temperature, only 5 w/w% increase in the DEGMA content decreases the gelation to body temperature (at the highest concentration studied in PC5). Interestingly, PC6 and PC7 with 30 and 35 w/w% DEGMA present a gelation area near body temperature. As the DEGMA content increases even more (PC8 with 40 w/w% DEGMA), the gelation region is clearly decreased. This can be attributed to the increased hydrophobicity, which favours precipitation over gelation. It is noteworthy that all the copolymers that show wide gelation area (PC4-PC7) form gels at the low concentration of 10 w/w%. Concerning the gel phase, transparent gels are

obtained in all wide gelation areas. Thus, some polymers form transparent gels at body temperature, which is desirable, as scientists can visualise the cells that have been incorporated into the structure. However, the transparent gel area becomes narrower as the DEGMA content increases. This can be explained upon consideration of the increased hydrophobicity of the structure.

The most interesting phase diagram is presented by PC12 with theoretical polymer structure PEGMA₁₁-*b*-BuMA₂₀-*b*-DEGMA₁₁, as it presents the wider gelation area. Specifically, this copolymer gels at even lower concentrations than the copolymers discussed before. Its copolymer solutions at 5 w/w% gels at body temperature, whereas gel is formed even at the low concentration of 2 w/w% at 39 °C. However, it should be noted that the gel formed by the 2 w/w% polymer solutions does not remain stable for a wide temperature range, and gel syneresis and precipitation occurs. This instability can be explained upon consideration of the low concentration, as fewer polymer chains, and thus fewer physical crosslinks form the 3-D network. Interestingly, at 10-25 w/w% a transparent stable gel is obtained at body temperature. This gel becomes cloudy, and then syneresis and precipitation are observed as the temperature increases further. Most importantly, at these concentrations, the gels are formed between 31-34 °C (concentration-dependent), which ensures that a stable gel is formed at body temperature within the experimental error of 2 °C.

It should be noted that it was expected that polymers PC10 and PC11 would have been water soluble and form gels. However, they are not water-soluble and could not be studied, while the more hydrophobic (higher DEGMA content) PC12 is water-soluble and has excellent gelling properties. In fact, PC12 out-performs all the other polymers of this study. As this was unexpected, this polymer was synthesised for a second time, and the same results were obtained, i.e. the results are reproduceable. However, in order to explain these results, more characterisation is required before and after gelation with techniques like x-ray and neutron scattering.



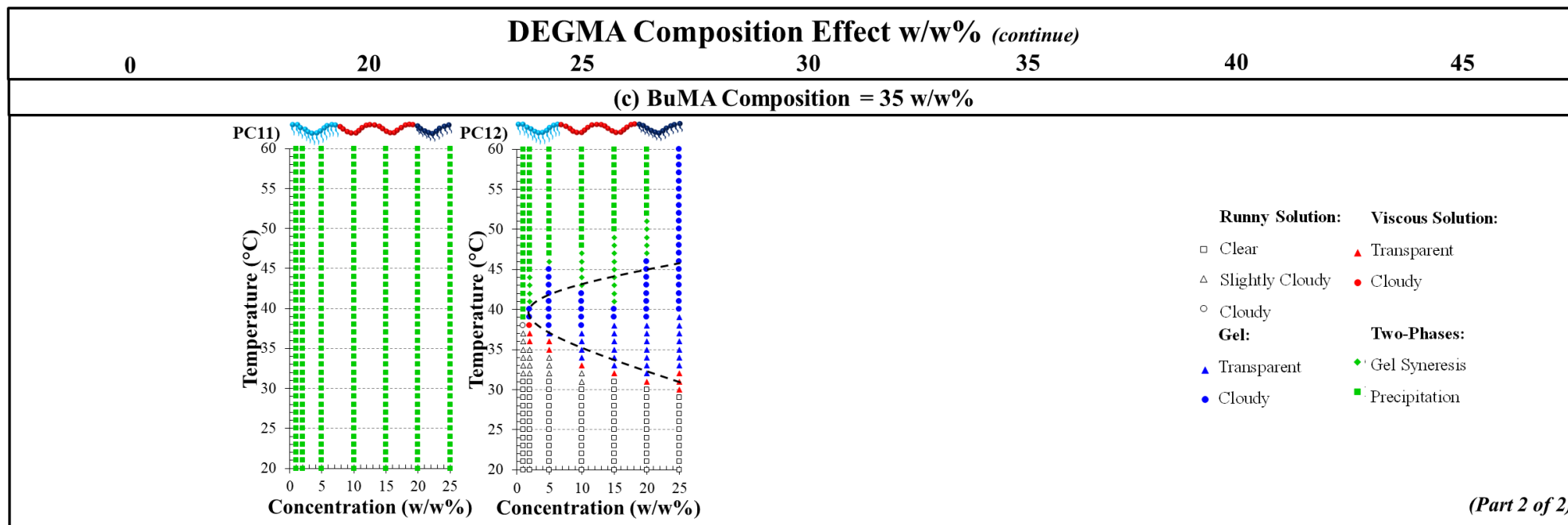


Figure 5.3.8: Phase diagrams of the PEGMA-BuMA-DEGMA triblock terpolymers (PC1-PC12) and the triblock bipolymer PEGMA-BuMA-PEGMA (PC3) in phosphate buffered saline (PBS). The effect of DEGMA composition is shown from left to right, whereas the effect of BuMA compositions is shown from the top to bottom: (a) 25 w/w% BuMA content (top), (b) 30 w/w% BuMA content (middle), and (c) 35 w/w% BuMA content (bottom). Four phases are observed: i) runny solution coloured in white (square: clear, triangle: slightly cloudy, and circle: cloudy), ii) viscous solution coloured in red (triangle and circle for transparent and cloudy, respectively), iii) the gel phase is shown in blue (triangle and circle for transparent and cloudy, respectively), the two-phase system is coloured in light green (rhombus and square for gel syneresis and precipitation, respectively). The polymer structures are schematically illustrated, in which the PEGMA, BuMA, and DEGMA units are coloured in light blue, red, and dark blue, respectively.

5.3.3.4 Gel Points by Rheology

The rheological properties of 15 w/w% copolymer solutions have also been characterised. The changes in storage and loss moduli (G' , and G'' , respectively), and in complex viscosity (η^*) over a temperature range from 20 °C to 70 °C are presented in Figure 5.3.9. Only the results corresponding to the copolymer solutions that form gel visually at 15 w/w% are shown, whereas the rest of the rheological curves can be found in Figure A7 in the Appendix. It should be noted that the solution of PC9 was unable to be tested as precipitation occurs while transferring the sample from the vial to the rheometer (precipitation occurs at 27 °C - see Figure 5.3.8 (b)).

Concerning the copolymers of 30 w/w% BuMA, the gel point by rheology (when $G' > G''$) follows the expended trend of the visual tests, i.e. it appears at lower temperatures as the content in DEGMA increases. More specifically, the gel points of PC4, PC5, PC6, and PC7 are identified at 40 °C, 39 °C, 35 °C, and 35 °C, respectively, which agree with the visual gel points of 41 °C, 42 °C, 36 °C, and 36 °C, respectively. Also, it is observed that as the temperature increases, the gel destabilises ($G' < G''$). The temperature at which the gel destabilises by rheology appears at higher values compared to the gel syneresis observed visually. This has also been observed in the case of the tetrablock terpolymers (see Section 5.2.3.5) and is attributed to the gel syneresis being subtle (i.e. subtle change in shape), and therefore the machine detects it as a stable gel, i.e. the energy is still stored in the system. Concerning PC8, which presents a very narrow gelation area (it gels only for 2 °C and then syneresis and precipitation are observed – see Figure 5.2.8 (b)), no stable gel has been detected by rheology, i.e. the storage modulus G' did not exceed the loss modulus G'' at any temperature. However, a significant increase in the magnitude was observed, with the two moduli being almost equal. The maximum viscosity was observed at 34 °C, which is the visual gelation point of PC8 at 15 w/w%.

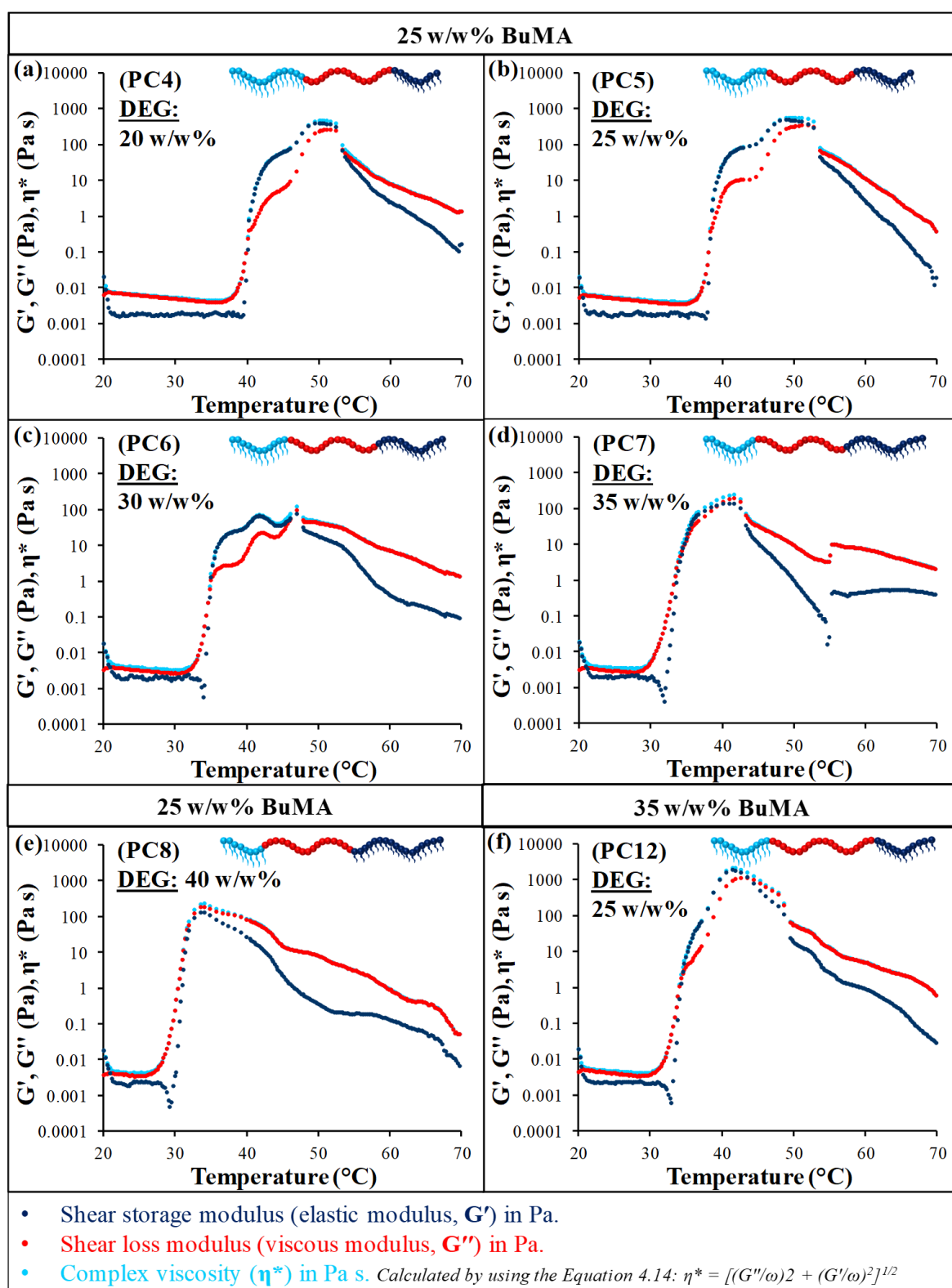


Figure 5.3.9: Rheological properties as a function of temperature for the polymer solutions which form a gel visually at 15 w/w% in phosphate buffered saline (PBS). The (a)-(e) graphs correspond to the terpolymers with 30 w/w% BuMA: (a) PC4 (PEGMA₁₄-*b*-BuMA₁₇-*b*-DEGMA₉), (b) PC5 (PEGMA₁₂-*b*-BuMA₁₇-*b*-DEGMA₁₁), (c) PC6 (PEGMA₁₁-*b*-BuMA₁₇-*b*-DEGMA₁₃), (d) PC7 (PEGMA₁₀-*b*-BuMA₁₇-*b*-DEGMA₁₅), and (e) PC8 (PEGMA₈-*b*-BuMA₁₇-*b*-DEGMA₁₇). The (f) graph corresponds to PC12 (PEGMA₁₁-*b*-BuMA₂₀-*b*-DEGMA₁₁), which has 35 w/w% BuMA. The PEGMA, BuMA, and DEGMA units on the polymer structures are coloured in light blue, red, and dark blue, respectively.

It is of great interest to compare the rheological and visual results of PC12, which is the one that shows the widest gelation area and forms transparent gels at body temperature at a range of concentrations from 5 w/w% to 25 w/w% (see Figure 5.2.8 (c)). This polymer solution at 15 w/w% it gels visually at 33 °C, and then slight syneresis is observed at 41°C, which progresses as the temperature increases, until the polymer completely precipitates out of solution (two clear phases are observed). In rheology, gelation is observed at 35 °C, which agrees with the visual observation within the experimental error. A maximum viscosity of 1986 Pa s is recorded at 42 °C, which corresponds to the area of a cloudy gel (within the experimental error). As the system is heated up to 45 °C, the loss modulus G'' becomes dominant, thus meaning that the liquid phase is prominent. As discussed before, gel syneresis is subtle at the beginning, and thus it is detected as a stable gel phase, i.e. the system is still able to store energy.

5.3.4 Conclusions

In this study, twelve ABC triblock terpolymers based on PEGMA (A unit), BuMA (B unit), and DEGMA (C unit) have been successfully synthesised using GTP. In addition to the triblock terpolymers, one ABA triblock bipolymer, one CBC triblock bipolymer, and one statistical terpolymer have also been synthesised for comparison. The MM of all the copolymers has been targeted to 8300 g mol⁻¹, whereas the composition of the triblock terpolymers has been varied to investigate its effect on the thermoresponsive properties of the copolymers. Several conclusions regarding the synthesis and the aqueous characterisation are drawn and are listed below:

1. All the copolymers have been successfully synthesised; their MM is within the desirable range for clear sol-gel transition, whereas their MMD is narrow.
2. The polymer architecture affects the solubility and thus the statistical terpolymer is insoluble.
3. The content in PEGMA is crucial for the solubility of the polymers as the CBC triblock bipolymer is not soluble, while the ABA one is.
4. The composition of the ABC triblock terpolymers controls the solubility as two of the copolymers with highest hydrophobic content are not soluble.
5. The ABC triblock terpolymers self-assemble in monodisperse micelles, the size of which is higher than the theoretically predicted values, because i) the long side chains of PEGMA are not taken into account in the theoretical calculation and/or ii) the

DEGMA block may partially contribute towards the core of the micelle especially for lower DP of DEGMA.

6. The CPs are affected by the composition of the copolymers: the CP values are reduced, as the hydrophobic BuMA content and the thermoresponsive DEGMA content increase.
7. The content in DEGMA promotes gelation, as the ABA triblock copolymer does not form gels.
8. The composition of the ABC triblock terpolymers controls the thermoresponsive properties, as by increasing the content in the thermoresponsive DEGMA and/or the hydrophobic BuMA, the polymers respond at lower temperature.
9. For copolymers with 30 w/w% BuMA, there is an intermediate composition of 25 – 35 w/w% DEGMA, which induces clear sol-gel transition at body temperature, as if the thermoresponsive content is increased further, gelation is disrupted, and precipitation occurs instead.
10. The best performing, and most promising polymer is PC12 with theoretical polymer structure $\text{PEGMA}_{11}\text{-}b\text{-BuMA}_{20}\text{-}b\text{-DEGMA}_{11}$ that forms stable transparent gels at body temperature at a wide range of concentration (5 – 25 w/w%).
11. The rheological results agree with the results from visual tests within the experimental error.

5.3.5 References

1. Lutz, J.; Akdemir, Ö; Hoth, A. Point by Point Comparison of Two Thermosensitive Polymers Exhibiting a Similar LCST : Is the Age of Poly(NIPAM) Over? *J. Am. Chem. Soc.* **2006**, *128*, 13046-13047.
2. Lutz, J.; Hoth, A. Preparation of Ideal PEG Analogues with a Tunable Thermosensitivity by Controlled Radical Copolymerization of 2-(2-Methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate. *Macromolecules* **2006**, *39*, 893-896.
3. Lutz, J.; Weichenhan, K.; Akdemir, Ö; Hoth, A. About the Phase Transitions in Aqueous Solutions of Thermoresponsive Copolymers and Hydrogels Based on 2-(2-methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate. *Macromolecules* **2007**, *40*, 2503-2508.
4. Lutz, J.; Andrieu, J.; Üzgün, S.; Rudolph, C.; Agarwal, S. Biocompatible, Thermoresponsive, and Biodegradable: Simple Preparation of "All-in-One" Biorelevant Polymers. *Macromolecules* **2007**, *40*, 8540-8543.
5. Sefcik, L. S.; Kaminski, A.; Ling, K.; Laschewsky, A.; Lutz, J.; Wischerhoff, E. Effects of PEG-Based Thermoresponsive Polymer Brushes on Fibroblast Spreading and Gene Expression. *Cellular and Molecular Bioengineering* **2013**, *6*, 287-298.
6. Chanana, M.; Jahn, S.; Georgieva, R.; Lutz, J.; Bäumler, H.; Wang, D. Fabrication of Colloidal Stable, Thermosensitive, and Biocompatible Magnetite Nanoparticles and Study of Their Reversible Agglomeration in Aqueous Milieu. *Chem. Mater.* **2009**, *21*, 1906-1914.
7. Lutz, J.; Pfeifer, S.; Zarafshani, Z. In Situ Functionalization of Thermoresponsive Polymeric Micelles using the "Click" Cycloaddition of Azides and Alkynes. *QSAR Comb. Sci.* **2007**, *26*, 1151-1158.
8. Badi, N.; Lutz, J. PEG-based thermogels: Applicability in physiological media. *J. Controlled Release* **2009**, *140*, 224-229.
9. Lutz, J. Polymerization of oligo(ethylene glycol) (meth)acrylates: Toward new generations of smart biocompatible materials. *J. Polym. Sci. A Polym. Chem.* **2008**, *46*, 3459-3470.
10. Zarafshani, Z.; Obata, T.; Lutz, J. Smart PEGylation of Trypsin. *Biomacromolecules* **2010**, *11*, 2130-2135.
11. Fechler, N.; Badi, N.; Schade, K.; Pfeifer, S.; Lutz, J. Thermogelation of PEG-Based Macromolecules of Controlled Architecture. *Macromolecules* **2009**, *42*, 33-36.
12. Kessel, S.; Schmidt, S.; Müller, R.; Wischerhoff, E.; Laschewsky, A.; Lutz, J.; Uhlig, K.; Lankenau, A.; Duschl, C.; Fery, A. Thermoresponsive PEG-Based Polymer Layers: Surface Characterization with AFM Force Measurements. *Langmuir* **2010**, *26*, 3462-3467.
13. Lutz, J. Thermo-Switchable Materials Prepared Using the OEGMA-Platform. *Adv Mater* **2011**, *23*, 2237-2243.
14. Buller, J.; Laschewsky, A.; Lutz, J.; Wischerhoff, E. Tuning the lower critical solution temperature of thermoresponsive polymers by biospecific recognition. *Polym. Chem.* **2011**, *2*, 1486-1489.
15. Ward, M. A.; Georgiou, T. K. Multicompartment thermoresponsive gels: does the length of the hydrophobic side group matter? *Polym. Chem.* **2013**, *4*, 1893-1902.
16. Ward, M. A.; Georgiou, T. K. Thermoresponsive terpolymers based on methacrylate monomers: Effect of architecture and composition. *J. Polym. Sci. A Polym. Chem.* **2010**, *48*, 775-783.
17. Ward, M. A.; Georgiou, T. K. Thermoresponsive triblock copolymers based on methacrylate monomers: effect of molecular weight and composition. *Soft Matter* **2012**, *8*, 2737-2745.
18. Ghasdian, N.; Ward, M. A.; Georgiou, T. K. Well-defined clickable copolymers prepared via one-pot synthesis. *Chem. Commun.* **2014**, *50*, 7114-7116.
19. Constantinou, A. P.; Georgiou, T. K. Thermoresponsive gels based on ABC triblock copolymers: effect of the length of the PEG side group. *Polym. Chem.* **2016**, *7*, 2045-2056.
20. Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. A Comprehensive Systematic Study on Thermoresponsive Gels: Beyond the Common Architectures of Linear Terpolymers. *Polymers* **2017**, *9*, 31.
21. Constantinou, A. P.; Sam-Soon, N.; Carroll, D. R.; Georgiou, T. K. Thermoresponsive Tetrablock Terpolymers: Effect of Architecture and Composition on Gelling Behavior. *Macromolecules* **2018**.
22. Malvern Instruments Limited Dynamic Light Scattering Common Terms Defined. http://www.biophysics.bioc.cam.ac.uk/wp-content/uploads/2011/02/DLS_Terms_defined_Malvern.pdf (accessed September, 2018).

23. de Souza, J., Carlos Perbone; Naves, A. F.; Florenzano, F. Specific thermoresponsiveness of PMMA-block-PDMAEMA to selected ions and other factors in aqueous solution. *Colloid Polym. Sci.* **2012**, 290, 1285-1291.

5.4 Mimicking Pluronic® F127

As discussed in the literature review, Pluronic® F127 is the commercially available thermoresponsive polymer. As a reminder, Pluronic® F127 consists of EG and PG, and has a total MM around 12600 g mol^{-1} , and composition of EG-PG equal to 70-30 w/w%.¹ Owing to its commercial availability, many studies have been focused on investigating this polymer and applying it either as *in vivo* injectable gel or as 3D printable material.

Several acrylate or methacrylate monomers with a pEG as a side chain are also available.² It is of great interest to try and mimic the structural characteristics of Pluronic® F127, by using acrylate or methacrylate EG and PG derivatives. In Pluronic® F127, EG and PG are polymerised to form a linear polymer chain consisting of ether bonds. However, if acrylate or methacrylate EG and PG derivatives are used, the backbone is formed by carbon-carbon bonds, whereas the ether bonds are on the side chains. This offers the advantages of having a wider variety of monomers that can be incorporated in the polymer structure. Therefore, the introduction of functional groups, i.e. groups on which peptides can be attached post-polymerisation, is easier. Introducing peptides in the polymer structure is important in TE applications.

Therefore, the aim of this Chapter is to synthesise methacrylate polymers which have EG-based and PG-based side chains. For this purpose, an *in-house* methacrylate monomer was synthesised, which has two PG groups on the side chain, namely di(propylene glycol) methyl ether methacrylate (diPGMA, diPG). The architecture investigated is ABA, to be the same as in Pluronic® F127. These synthesised ABA triblock copolymers have PEGMA as A block and diPGMA as B block. The MM and composition have been systematically varied to investigate their effect on the thermoresponsive properties of the copolymers. The MM has been targeted to 5100, 10100, and 13100 g mol^{-1} , to produce polymers with MM lower, similar, and higher than the MM of Pluronic® F127. It should be noted that the MM of 10100 g mol^{-1} has been chosen, instead of 12600 g mol^{-1} that is the MM of Pluronic® F127, to ensure that the experimental/final MM of the copolymers will be similar to the one of Pluronic® F127. The PEGMA-diPGMA composition has been varied from 80-20 w/w% (higher hydrophilic content than Pluronic® F127), to 70-30 w/w% (composition of Pluronic® F127), to 60-40 w/w% (lower hydrophilic content than Pluronic® F127).

The chemical structures, name and abbreviations of the monomers used in this study are shown in Figure 5.4.1.

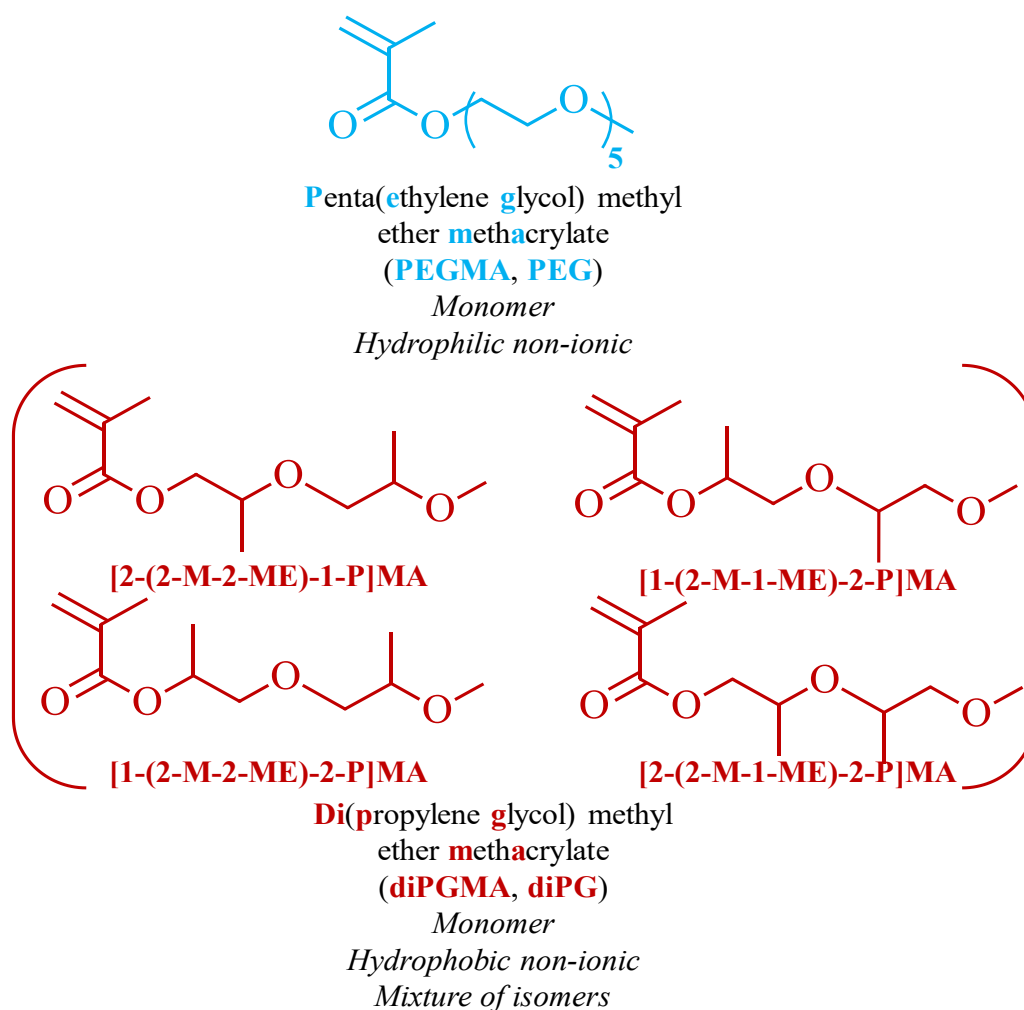


Figure 5.4.1: Chemical structures, names, and abbreviations of the monomers used in this polymer series. PEGMA and diPGMA stand for penta(ethylene glycol) methyl ether methacrylate and di(propylene glycol) methyl ether methacrylate, respectively. It should be noted that as the alcohol used for the monomer synthesis is a mixture of isomers, the four possible isomers of the monomer, shown in the Figure, are expected to be formed. The abbreviations of the four isomers are also given.

5.4.1 Monomer Synthesis

The hydrophobic monomer of this Chapter, diPGMA, has been *in-house* synthesised following a standard procedure on the synthesis of methacrylate monomers.³ Briefly, diPGMA has been synthesised via esterification of methacryloyl chloride and diPGOH (mixture of isomers). The chemical reaction can be found in Figure 5.4.2. The successful synthesis of diPGMA, and the characterisation of the monomer and its alcohol precursor are discussed in the following sections.

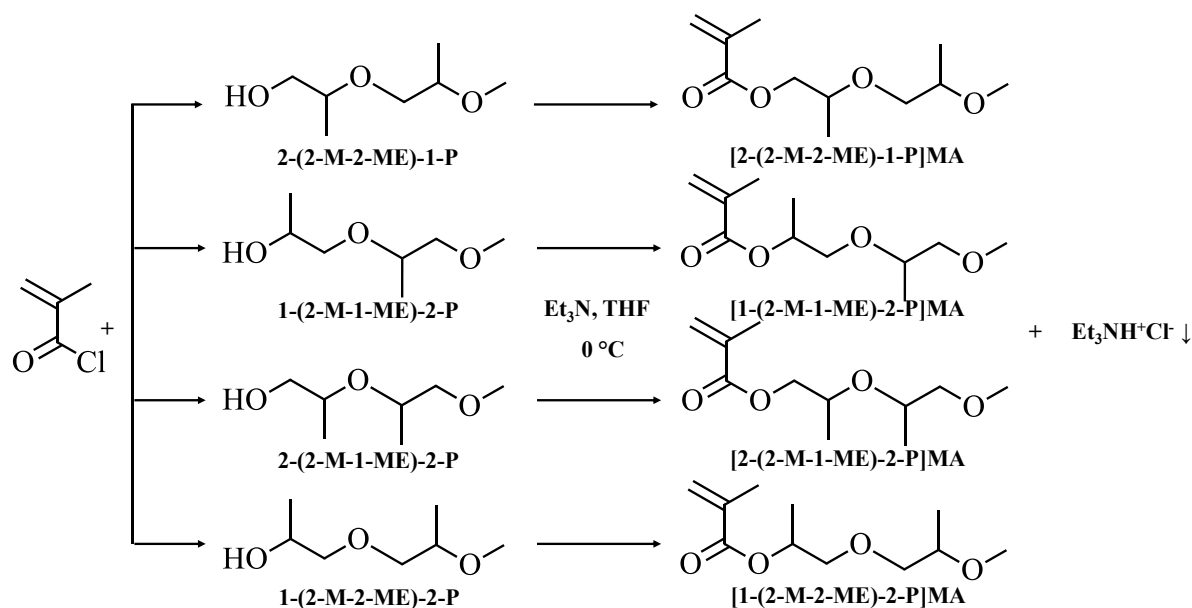


Figure 5.4.2: Chemical reaction of the synthesis of diPGMA monomer, which is catalysed by triethylamine. diPGMA was synthesised via an esterification reaction between methacryloyl chloride and di(propylene glycol) methyl ether, diPGOH. THF was used as solvent. As the diPGOH used is a mixture of isomers, four possible isomers of the monomer are expected to be formed. The four isomers of the alcohol from top to bottom are the following: i) 2-(2-methoxy-2-methylethoxy)-1-propanol [2-(2-M-2-ME)-1-P], ii) 1-(2-methoxy-1-methylethoxy)-2-propanol [1-(2-M-1-ME)-2-P], iii) 2-(2-methoxy-1-methylethoxy)-1-propanol [2-(2-M-1-ME)-1-P], and iv) 1-(2-methoxy-2-methylethoxy)-2-propanol [1-(2-M-2-ME)-2-P].

5.4.2 Monomer Characterisation

As previously mentioned, the alcohol used for the monomer synthesis, diPGOH, is a mixture of isomers. The four isomers of the alcohol are shown in Figure 5.4.2, and they are the following (from the top to the bottom): i) 2-(2-methoxy-2-methylethoxy)-1-propanol [2-(2-M-2-ME)-1-P], ii) 1-(2-methoxy-1-methylethoxy)-2-propanol [1-(2-M-1-ME)-2-P], iii) 2-(2-methoxy-1-methylethoxy)-1-propanol [2-(2-M-1-ME)-1-P], and iv) 1-(2-methoxy-2-methylethoxy)-2-propanol [1-(2-M-2-ME)-2-P]. Therefore, the monomer synthesised is a mixture of isomers. The isomers are shown in both Figure 5.4.1 and 5.4.2, and they are the following: i) 2-(2-methoxy-2-methylethoxy)-1-propyl methacrylate [2-(2-M-2-ME)-1-P]MA, ii) 1-(2-methoxy-1-methylethoxy)-2-propyl methacrylate [1-(2-M-1-ME)-2-P]MA, iii) 2-(2-methoxy-1-methylethoxy)-1-propyl methacrylate [2-(2-M-1-ME)-1-P]MA, and iv) 1-(2-methoxy-2-methylethoxy)-2-propyl methacrylate [1-(2-M-2-ME)-2-P]MA. To obtain more information on the mixture of isomers, and to confirm the successful synthesis, both the monomer and the alcohol were characterised via GC-FID, ^1H and ^{13}C NMR, FT-IR and MALDI. The results are discussed below.

5.4.2.1 Determination of Isomers

The alcohol and the *in-house* synthesised monomer have been analysed via GC-FID in order to identify the isomers present and their relative proportion. The GC-FID chromatograms are presented in Figure 5.4.3, whereas Table 5.1 lists the number of peaks identified, the detection time and the integration of each peak.

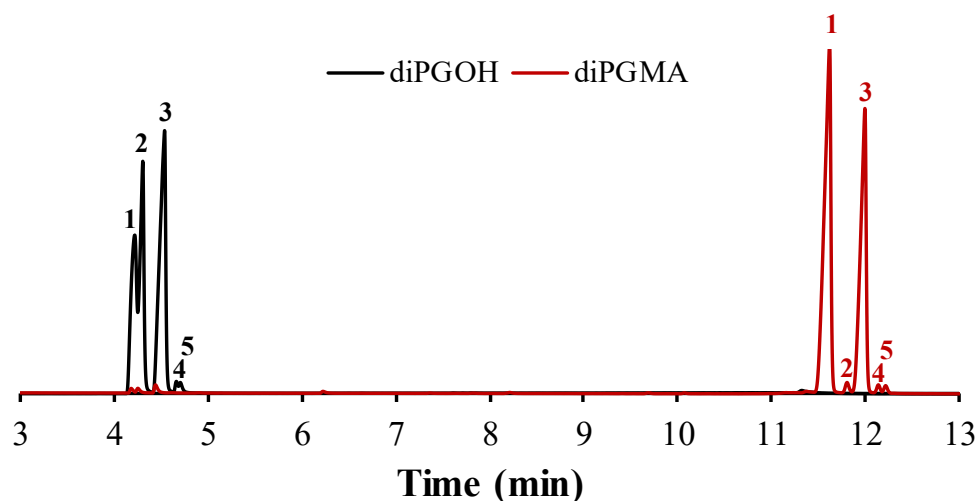


Figure 5.4.3: GC-FID chromatograms of the alcohol (diPGOH in black), and the monomer (diPGMA in dark red).

Table 5.4.1: GC-FID results for the alcohol (diPGOH) and the monomer (diPGMA): the number of peaks identified, the detection time, and the integration of each peak.

No. Peaks	Alcohol Precursor - diPGOH		Monomer - diPGMA	
	Time (min)	Area (%)	Time (min)	Area (%)
1	4.21	28.22	11.62	56.92
2	4.30	24.36	11.81	1.06
3	4.53	46.72	12.00	40.56
4	4.66	0.40	12.14	0.76
5	4.70	0.31	12.22	0.70

As can be seen in Figure 5.4.3, the peaks related to the alcohol appear around 4 – 5 mins, whereas the peaks corresponding to the monomer appear at around 11 – 12 mins. This can be explained upon considering that the alcohol, as a lower MM compound than the monomer, is more volatile, and thus it is detected at lower times compared to the monomer.

In addition, five peaks are detected in each chromatogram. As a polar column is used, it is expected that the isomer which is more polar, and thus it has a higher boiling point, it interacts more with the stationary phase, and thus it is eluted last.

For the GC analysis, the Kovats retention index, provided by the National Institute of Standards and Technology (NIST), can be used. The compound with the lowest Kovats retention index elutes first. After search in the NIST database, the Kovats retention indices for two of the alcohol isomers have been identified: i) 1-(2-M-1-ME)-2-P (second isomer in Figure 5.4.2), and ii) 1-(2-M-2-ME)-2-P (fourth isomer in Figure 5.4.2). The Kovats retention indices are: i) 1478 (capillary TC-Wax column) and 1479 (capillary DB-Wax column) for the second isomer,⁴ and ii) 1509 (capillary TC-Wax column), and 1532 (capillary DB-Wax column) for the fourth isomer.⁵ Thus, as the second isomer has a lower Kovats retention index than the fourth isomer, its peak in the GC chromatogram appears at lower retention times. However, the Kovats retention indices of the other two isomers have not been identified, and thus full identification using the Kovats retention indices and the data provided by GC-FID is not possible.

Search in the literature has revealed that diPGOH has been previously analysed by gas chromatography/mass spectrometry (GC/MS).⁶ Therefore, the previous results are used as an example for complete identification of the peaks in this study. Both peaks 1 and 2 correspond to 1-(2-M-1-ME)-2-P (second isomer in Figure 5.4.2, 28.22 w/w% and 24.36 w/w%), and they are configurational isomers, resulted by the presence of asymmetrical carbons. Peak 3 corresponds to 1-(2-M-2-ME)-2-P (fourth isomer in Figure 5.4.2, 46.72 w/w%), whereas peaks 4 and 5 correspond to the isomers 2-(2-M-2-ME)-1-P (first isomer in Figure 5.4.2, 0.40 w/w%) and 2-(2-M-1-ME)-1-P (third isomer in Figure 5.4.1, 0.31 w/w%), respectively.

Concerning the analysis of the GC chromatogram of the monomer, five peaks are also detected. However, the peaks do not have the same relative integration as in the case of the alcohol. The first peak should correspond to [1-(2-M-1-ME)-2-P]MA. As the integration of the peak is 56.92 w/w%, it is assumed that both configurational isomers appear in the same peak. Peak 3, with integration 40.56 w/w%, should correspond to [1-(2-M-2-ME)-2-P]MA, whereas peaks 4 and 5 should correspond to [2-(2-M-2-ME)-1-P]MA (0.76 w/w%) and [2-(2-M-1-ME)-1-P]MA (0.70 w/w%), respectively. Peak 2, with integration 1.06 w/w%, corresponds to either contamination or to a configurational isomer. GC-MS should be used to provide the unique fingerprint of each peak, and thus identify exactly which compound corresponds to each peak. To conclude, [1-(2-M-1-ME)-2-P]MA is the most abundant isomer, whereas [1-(2-M-2-ME)-2-P]MA is the second most abundant isomer. The other two isomers exist only in very low percentage.

5.4.2.2 Chemical Structure of the Monomer

The successful monomer synthesis and the chemical nature of the monomer has been confirmed via NMR, IR and MALDI. The results are compared to the ones corresponding to the alcohol, and they are presented and discussed below.

5.4.2.2.1 Chemical Bonds

FT-IR spectroscopy has been utilised to identify the type of chemical bonds present in the alcohol, diPGOH, and the monomer, diPGMA. The FT-IR spectra are shown in Figure 5.4.4, and the analysis is presented in Table 5.4.2.

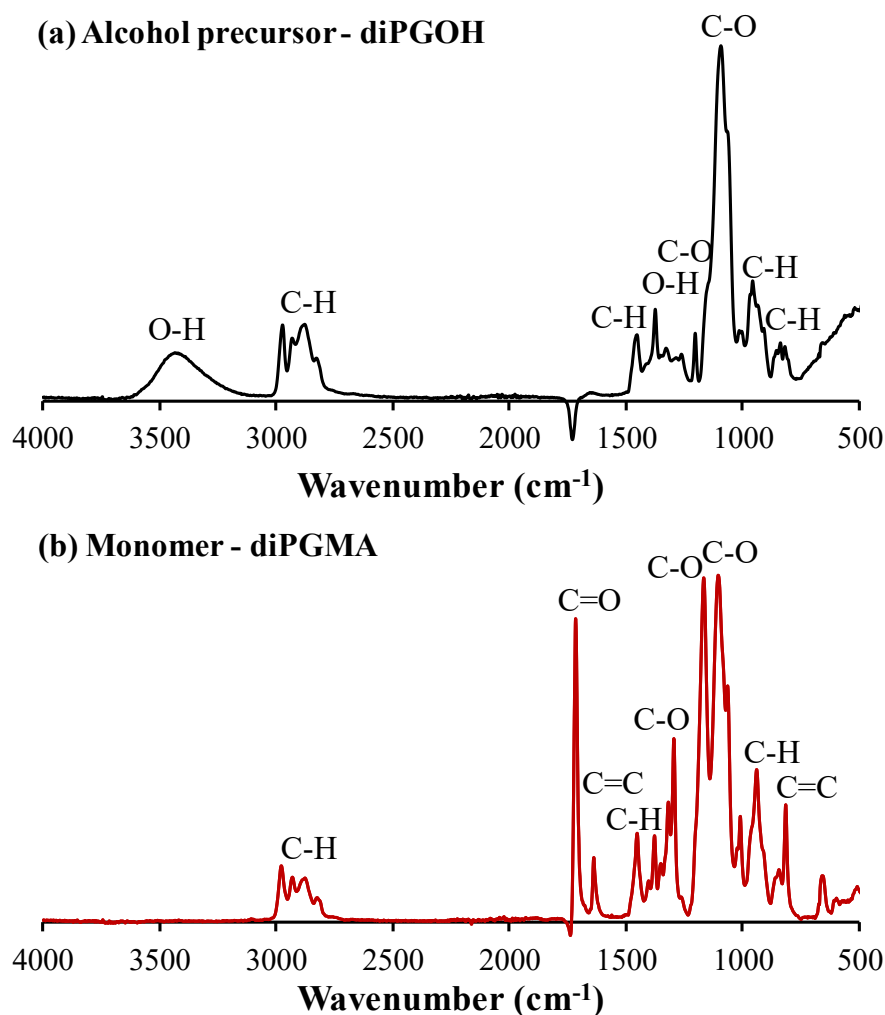


Figure 5.4.4: FT-IR spectra of (a) the alcohol (diPGOH in black), and (b) the monomer (diPGMA in dark red).

Table 5.4.2: FT-IR results for the alcohol (diPGOH) and the monomer (diPGMA): the number of peaks present, the characteristic absorptions, the functional groups, the type of vibration, and the intensity are listed.

No. Peaks	Characteristic Absorption (cm ⁻¹)	Functional Group	Type of Vibration	Intensity
Alcohol Precursor - diPG				
1	3433.1	O-H (alcohol)	Stretching	Strong, broad
2	2971.6, 2930.6, 2876.5, 2826.3	C-H (alkane)	Stretching	Strong
3	1452.2	C-H (alkane)	Bending	Medium
4	1373.8	O-H (alcohol)	Bending	Medium
5	1202.5	C-O (alcohol)	Stretching	Strong
6	1092.5	C-O (ether)	Stretching	Strong
7	956.4, 854.3	C-H (alkane)	Bending	Strong
Monomer - diPGMA				
1	2978.0, 2930.4, 2876.4, 2824.2	C-H (alkane)	Stretching	Strong
2	1715.2	C=O (ester)	Stretching	Strong
3	1637.1	C=C (Vinylidene)	Stretching	Strong
4	1451.2	C-H (alkane)	Bending	Medium
5	1293.7	C-O (ester)	Stretching	Strong
6	1165.4	C-O (ester)	Stretching	Strong
7	1103.3	C-O (ether)	Stretching	Strong
8	938.3	C-H (alkane)	Bending	Strong
9	813.7	C=C	Bending	Strong

The FT-IR results of the alcohol and the monomer are presented in both Figure 5.4.4 and Table 5.4.2. As can be seen, all the chemical bonds corresponding to the alcohol functional group are detected. The vibrations of O-H stretching and bending appear at 3433.1 cm⁻¹ and 1373.8 cm⁻¹, respectively. The C-O stretching vibration of the alcohol appears at 1202.5 cm⁻¹. It is observed that these peaks do not appear in the FT-IR spectrum of the monomer, thus confirming the consumption of the alcohol. Additional peaks are detected, which correspond to the ester functional group and the double bond, thus confirming the successful esterification reaction. More specifically, a strong peak corresponding to the C=O stretching vibration appears at 1715.2 cm⁻¹, whereas the C-O stretching vibration of the ester appears at 1293.7 cm⁻¹ and 1165.4 cm⁻¹, and the C=C stretching vibration appears at 1637.1 cm⁻¹.

5.4.2.2.2 Chemical Structure

The synthesis of the monomer is successful, as proven by both ^1H NMR and ^{13}C NMR. The ^1H NMR and ^{13}C NMR spectra of diPGMA after purification and its alcohol precursor are presented in Figure 5.4.5 and 5.4.6, respectively. The assignment of the peaks on the chemical structure is also shown.

Concerning the ^1H NMR spectrum of the alcohol (Figure 5.4.5 (a)), all the peaks corresponding to the chemical structure are detected as follows: ^1H NMR (CDCl_3) δ 0.92-0.97 [m, 6.00H, $\text{CH}_3\text{CH-}$ (100%)], δ 3.02 (s, 0.06H, $-\text{OH}$), δ 3.04 (s, 0.12H, $-\text{OH}$), δ 3.07 (s, 0.07H, $-\text{OH}$), δ 3.09-3.52 [m, 8.29H, $\text{CH}_3\text{O-}$ (100%), $-\text{OCH}(\text{CH}_3)\text{CH}_2\text{O-}$ (100%), $-\text{CH}_2\text{CH}(\text{CH}_3)\text{OH}$ (99.3%) and $\text{HOCH}_2\text{CH}(\text{CH}_3)-$ (0.7%)], δ 3.59 (s, 0.27H, $-\text{OH}$) and δ 3.72-3.81 [m, 0.95H, $\text{HOCH}(\text{CH}_3)-$ (99.3%) and HOCH_2- (0.7%)]; it should be noted that the percentages are the ones calculated by GC-FID analysis. As can be seen, all the peaks appear in the expected area, whereas the integration matches the expected number of protons. Multiple peaks are detected in the area around 3-3.5 ppm, which is attributed to the fact that the sample constitutes of four isomers, and 8 protons of the most abundant isomers and 7 protons of the least abundant isomers appear in the same area. Also, the splitting of the peaks does not match the expected/predicted theoretical one, which is result of the presence of four isomers/ four different structures in the sample.

The ^1H NMR spectrum of the monomer (Figure 5.4.5 (b)) proves the successful monomer synthesis, as the peaks corresponding to the methacrylate double bond are detected at around 5.5-6 ppm. More specifically, the following peaks are detected: ^1H NMR (CDCl_3) δ 1.02-1.05 [m, 3.04H, $-\text{OCH}_2\text{CH}(\text{CH}_3)\text{O-}$ (99%)], δ 1.16-1.18 [dd, 2.99H, $-(\text{C}=\text{O})\text{OCH}(\text{CH}_3)\text{CH}_2\text{O-}$ (97.5%) and $-(\text{C}=\text{O})\text{OCH}_2\text{CH}(\text{CH}_3)\text{O-}$ (1.5%)], δ 1.84 [s, 2.98H, $\text{CH}_3\text{C}(\text{=CH}_2)-$ (99%)], δ 3.19-3.54 [m, 8.11H, $\text{CH}_3\text{O-}$ (99%), $-\text{OCH}(\text{CH}_3)\text{CH}_2\text{O-}$ (99%), $-\text{CH}_2\text{CH}(\text{CH}_3)\text{O}(\text{C}=\text{O})-$ (97.5%) and $-(\text{C}=\text{O})\text{OCH}_2\text{CH}(\text{CH}_3)-$ (1.5%)], δ 4.96-5.06 [m, 0.95H, $-(\text{C}=\text{O})\text{OCH}(\text{CH}_3)-$ (97.5%) and $-(\text{C}=\text{O})\text{OCH}_2-$ (1.5%)], δ 5.44 [s, 1.00H, $\text{CH}_2=\text{C}(\text{CH}_3)-$ (99%)] and δ 6.00 [s, 1.00H, $\text{CH}_2=\text{C}(\text{CH}_3)-$ (99%)]; the percentages used are the ones resulted from GC-FID analysis.

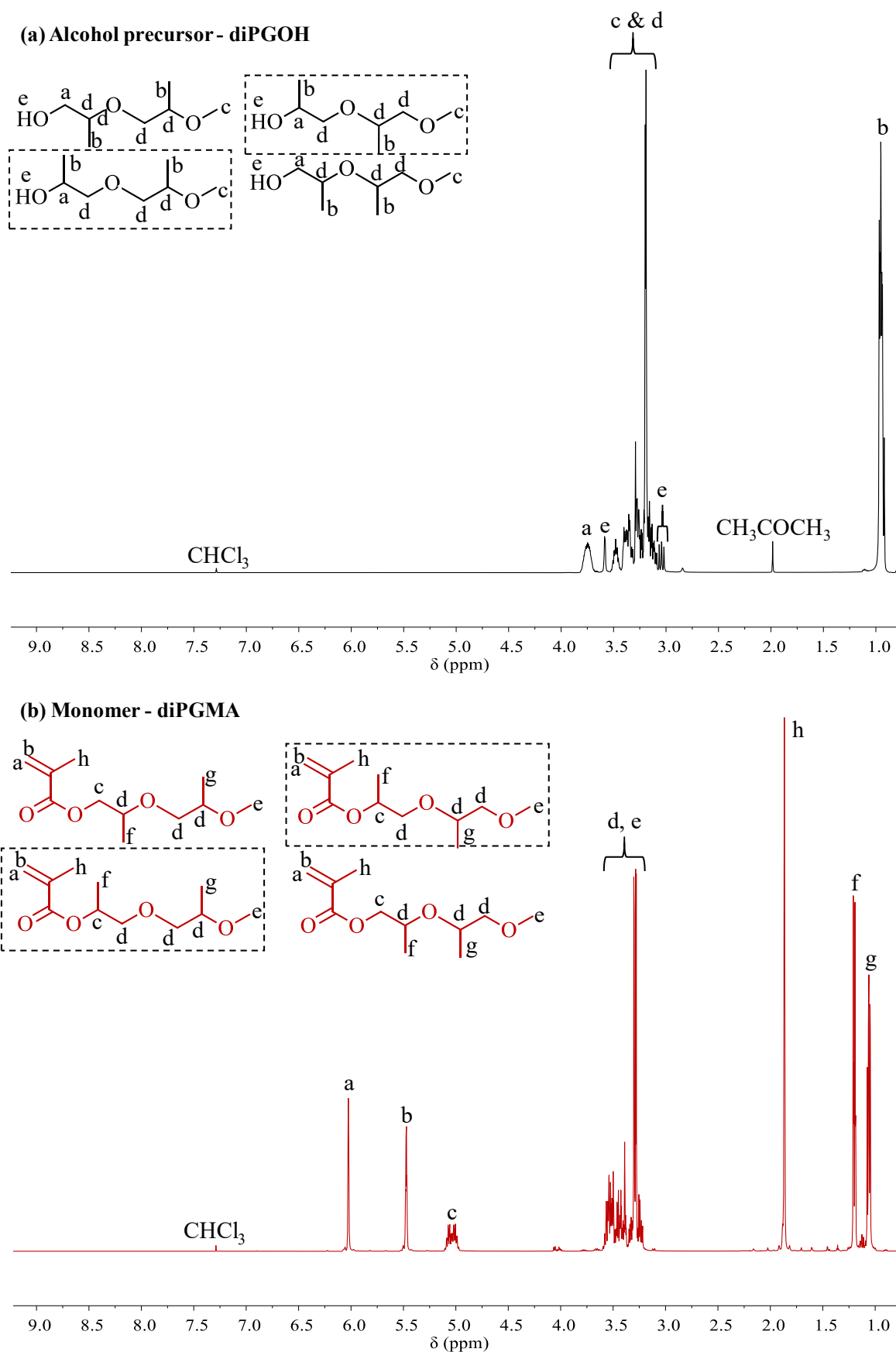
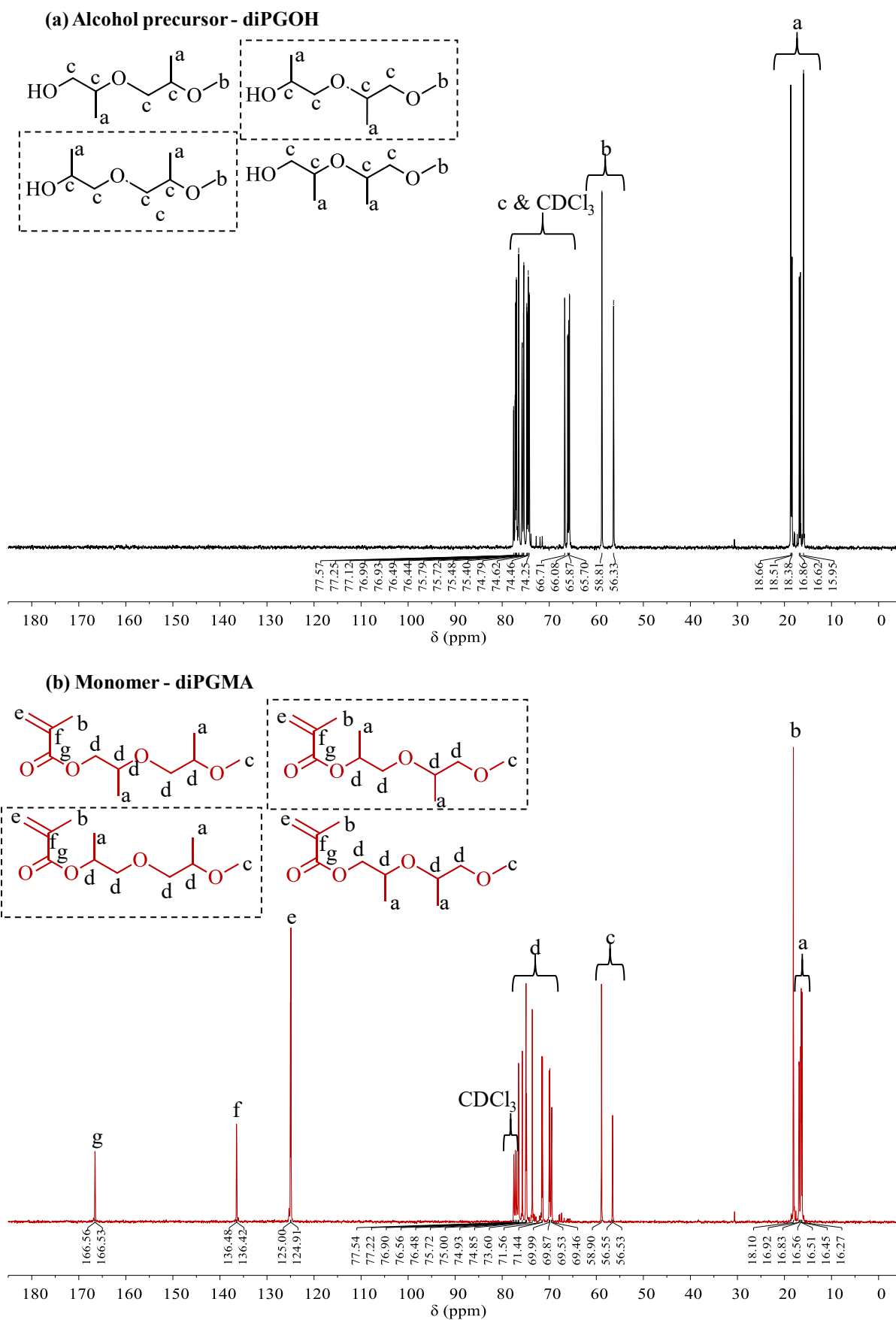


Figure 5.4.5: ^1H NMR spectra of (a) the alcohol (diPGOH in black), and (b) the monomer (diPGMA after purification in dark red). The corresponding chemical structures are also presented; the two most abundant isomers are indicated by a square.



The alcohol and the monomer diPGMA have also been analysed by ^{13}C NMR, and the corresponding spectra are presented in Figure 5.4.6 (a) and (b), respectively. As can be seen, the expected peaks are present. Most importantly, when the two ^{13}C NMR spectra are compared, three additional peaks are observed in the spectrum of the monomer, in the area at values higher than 100ppm. These peaks correspond to the carbons of the methacrylate part (double bond and ester/carbonyl bond). Generally, the number of peaks that are detected per sample is higher than the expected number for one isomer. This is because the sample consists of four different isomers, thus the different carbons present appear in the ^{13}C NMR spectra. Also, even though at the magnification used in the spectra, some peaks appear as one, two peaks very close to each other are present, e.g. for e, f and g carbons. This can be attributed to the sample consisting of four isomers, two of them are abundant at percentages of 56.92% and 40.56 %.

Even though ^1H and ^{13}C NMR analysis confirms the successful synthesis of the monomer, complete and accurate identification of each peak is not feasible. Additional characterisation is required, such as heteronuclear single quantum coherence (HSQC) NMR spectroscopy and correlated spectroscopy (COSY) NMR spectroscopy. However, one should keep in mind that as a mixture of isomers is to be analysed, complicated results are expected to be obtained, as in the case of ^1H and ^{13}C NMR, and therefore, NMR analysis upon separation of the isomers should be able to reveal the unique NMR spectra of each isomer.

5.4.3 Polymer Synthesis

All the copolymers in this study have been synthesised via sequential one-pot GTP. As the architecture was kept the same (ABA), what differs is the amount of monomer added. Figure 5.4.7 shows the synthesis of an ABA triblock copolymer with A and B blocks being based on PEGMA and diPGMA, respectively. It should be noted that in Figure 5.4.7, the most abundant isomer of diPGMA is given as an example.

As mentioned before, three different compositions and three different MMs have been targeted. Therefore, nine ABA triblock copolymers have been synthesised in total, the structures of which are schematically illustrated in Figure 5.4.8. The PEGMA and diPGMA repeated units are shown in light blue and dark red spheres, respectively. Three different families of different MMs are identified: i) PD1-PD3 with MM 5100 g mol^{-1} , ii) PD4-PD6

with MM 10100 g mol⁻¹, and iii) PD7-PD9 with MM 13100 g mol⁻¹. The PEGMA-diPGMA composition within the families has been varied, and the polymer structures are listed in an increased hydrophobic content: i) 80-20 w/w% (PD1, PD4, and PD7), ii) 70-30 w/w% (PD2, PD5, and PD8), and iii) 60-40 w/w% (PD3, PD6, and PD9).

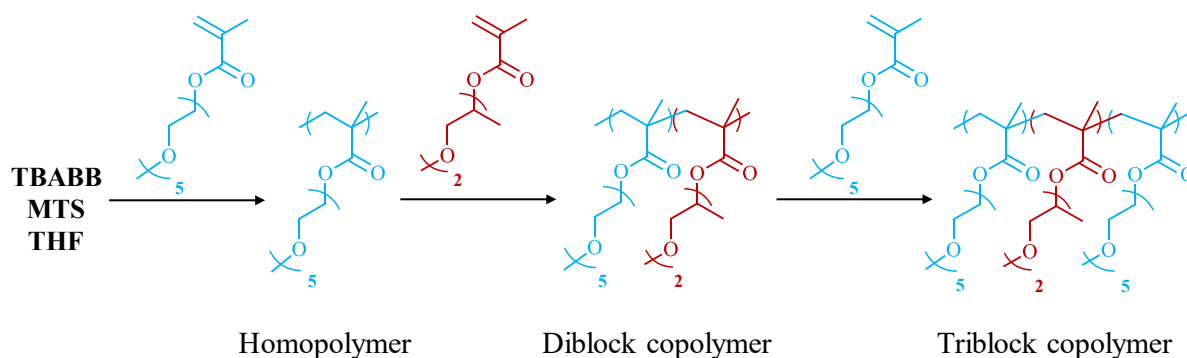


Figure 5.4.7: Chemical reaction of the GTP synthesis of an ABA triblock copolymer which is based on PEGMA, and diPGMA as A and B units, respectively. *Note:* TBABB, MTS, and THF are the catalyst, initiator, and solvent used for this PhD project. For simplicity, the most abundant isomer of diPGMA is used for this GTP reaction.

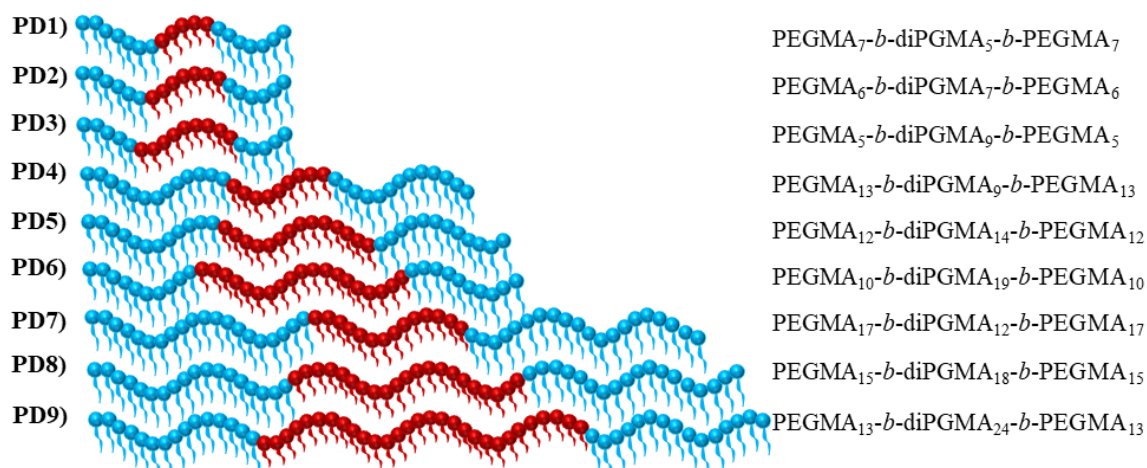


Figure 5.4.8: Schematic representation of the ABA triblock copolymers of various MMs: PD1-PD3 with MM 5100 g mol⁻¹, PD4-PD6 with MM 10100 g mol⁻¹, and PD7-PD9 with MM 13100 g mol⁻¹. Light blue, and dark red spheres represent PEGMA (A unit), and diPGMA (B unit), respectively.

5.4.4 Molecular Mass and Composition

The molar mass characteristics of the copolymers have been determined by GPC. Figure 5.4.9 illustrates the GPC chromatogram of PD1 with theoretical polymer structure PEGMA₇-b-diPGMA₅-b-PEGMA₇ (shown in dark blue dotted line). The GPC traces of its linear precursors are also presented: i) PEGMA homopolymer in light blue solid line and ii) PEGMA₇-b-diPGMA₅ in dark red dashed line. The GPC traces of the other triblock copolymers and their linear precursors can be found in Figure A4 in the Appendix.

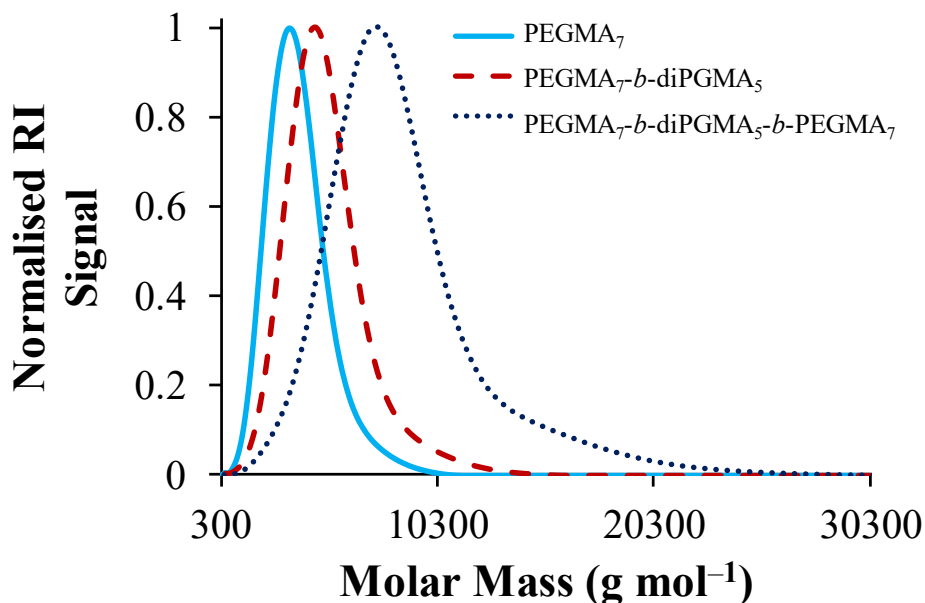


Figure 5.4.9: The GPC chromatogram of the triblock terpolymer PEGMA₇-*b*-diPGMA₅-*b*-PEGMA₇ (PD1) is shown in dark blue dotted line. The GPC traces of its linear precursors are also given: i) PEGMA₇ in light blue solid line, and ii) PEGMA₇-*b*-diPGMA₅ in red dotted line.

As can be seen in Figure 5.4.9, the peak moves to higher MMs, as the polymerisation progresses from the homopolymer to the diblock to the final triblock copolymer, thus confirming that the final triblock copolymer has been successfully obtained. Some deactivation is observed in the means of tailing at low MM values. This is attributed to the PEGMA homopolymer, the polymerisation of which slightly prevented further chain extension. As can be seen, tailing and/or shoulder is observed in the GPC traces of all the triblock copolymers, which indicates that slight deactivation during the polymerisation of PEGMA homopolymer was occurred in all the cases. This can be attributed to high amount of PEGMA used for the current synthesis, which introduces impurities, because of its inability to be distilled.

Table 5.4.3 presents the molar mass characteristics of all the copolymers, as resulted from GPC analysis. As can be seen, the experimental M_n values are slightly higher than the theoretical ones, similarly to previous GTP studies.⁷⁻¹³ This is attributed to the deactivation of the initiator and/or low MM polymer chains, caused by the presence of humidity and acidic impurities.⁷⁻¹³ Nevertheless, it can be seen that the copolymers with theoretical MM equal to 10100 g mol⁻¹ have experimental M_n values around 11100 g mol⁻¹ to 12500 g mol⁻¹. This confirms the hypothesis that the lower MM should be targeted to achieve experimentally MM similar to Pluronic® F127.

Table 5.4.3: Theoretical chemical structures, dispersity indices ($\bar{D}$), theoretical and experimental molecular masses ($MM^{theor.}$ and M_n , respectively) and compositions of the final ABA triblock copolymers and their precursors.

No.	Theoretical Polymer Structure ^a	$MM^{theor. b}$ (g mol ⁻¹)	M_n^c (g mol ⁻¹)	$\bar{D}^c$	w/w% PEG-diPG	
					Theoretical	¹ H NMR
PD1	PEG ₇	2100	2900	1.17	100-00	100-00
	PEG ₇ - <i>b</i> -diPG ₅	3100	3900	1.15	67-33	74-26
	PEG ₇ - <i>b</i> -diPG ₅ - <i>b</i> -PEG ₇	5100	6100	1.20	80-20	87-13
PD2	PEG ₆	1850	2600	1.16	100-00	100-00
	PEG ₆ - <i>b</i> -diPG ₇	3350	4400	1.14	54-46	60-40
	PEG ₆ - <i>b</i> -diPG ₇ - <i>b</i> -PEG ₆	5100	6600	1.23	70-30	74-26
PD3	PEG ₅	1600	2500	1.17	100-00	100-00
	PEG ₅ - <i>b</i> -diPG ₉	3600	5000	1.17	43-57	44-56
	PEG ₅ - <i>b</i> -diPG ₉ - <i>b</i> -PEG ₅	5100	6700	1.23	60-40	57-43
PD4	PEG ₁₃	3100	4900	1.13	100-00	100-00
	PEG ₁₃ - <i>b</i> -diPG ₉	7100	6700	1.15	67-33	69-31
	PEG ₁₃ - <i>b</i> -diPG ₉ - <i>b</i> -PEG ₁₃	10100	11100	1.29	80-20	81-19
PD5	PEG ₁₂	3600	4700	1.13	100-00	100-00
	PEG ₁₂ - <i>b</i> -diPG ₁₄	6600	8000	1.18	54-46	57-43
	PEG ₁₂ - <i>b</i> -diPG ₁₄ - <i>b</i> -PEG ₁₂	10100	12300	1.28	70-30	72-28
PD6	PEG ₁₀	4100	4400	1.11	100-00	100-00
	PEG ₁₀ - <i>b</i> -diPG ₁₉	6100	9200	1.25	43-57	46-54
	PEG ₁₀ - <i>b</i> -diPG ₁₉ - <i>b</i> -PEG ₁₀	10100	12500	1.43	60-40	63-37
PD7	PEG ₁₇	5300	6200	1.19	100-00	100-00
	PEG ₁₇ - <i>b</i> -diPG ₁₂	7900	9100	1.18	67-33	68-32
	PEG ₁₇ - <i>b</i> -diPG ₁₂ - <i>b</i> -PEG ₁₇	13100	14400	1.24	80-20	79-21
PD8	PEG ₁₅	4650	6000	1.18	100-00	100-00
	PEG ₁₅ - <i>b</i> -diPG ₁₈	8550	10600	1.20	54-46	56-44
	PEG ₁₅ - <i>b</i> -diPG ₁₈ - <i>b</i> -PEG ₁₅	13100	16000	1.29	70-30	71-29
PD9	PEG ₁₃	4000	5100	1.17	100-00	100-00
	PEG ₁₃ - <i>b</i> -diPG ₂₄	9200	11200	1.23	43-57	43-57
	PEG ₁₃ - <i>b</i> -diPG ₂₄ - <i>b</i> -PEG ₁₃	13100	15700	1.27	60-40	60-40

^a PEG, and diPG are the abbreviations for penta(ethylene glycol) methyl ether methacrylate, and di(propylene glycol) methyl ether methacrylate, respectively.

^b The theoretical MM is calculated using the Equation 4.1 $MM^{theor.} (g\ mol^{-1}) = (\sum_i MM_i \times DP_i) + 100$

^c The values of the number-average molar mass (M_n) and the dispersity index ($\bar{D}$) of the copolymers and their linear precursors (if any) were determined by gel permeation chromatography, GPC. The calibration was based on well-defined linear poly(methyl methacrylate) (PMMA) standard samples with MM equal to 2, 4, 8, 20, 50, and 100 kg mol⁻¹.

Table 5.4.3 also lists the $\bar{D}$ values, which are below 1.30, except for PD6. These values are satisfactorily low for the purpose of this study. Generally, they are higher than the previously reported GPT studies.⁷⁻¹³ However, this can be explained upon consideration of the high PEGMA content used in the current study (60 – 80 w/w%). The incorporation of high amount

of PEGMA does not only introduce impurities that affect GTP, but it also broadens the dispersity, as PEGMA is a macromonomer, i.e. it is a monomer with average MM – average number of EG units.⁷⁻¹³ Concerning PD6, with theoretical polymer structure PEGMA₁₀-*b*-diPGMA₁₉-*b*-PEGMA₁₀, it has dispersity of 1.43, caused by a problem with the addition of one of the monomers (needle was not connected well to the syringe), thus introducing extra deactivation. This deactivation can also be observed in means of a clear shoulder corresponding to the MM of PEGMA homopolymer in the corresponding GPC chromatogram (Figure A4 in the Appendix). The synthesis of PD6 should be repeated in order to obtain a better-defined polymer (lower *D* value); this was unable to be performed during this PhD project, due to time limitation. Therefore, in this PhD Chapter, the results related to PD6 should be treated with caution, as it is well-established that polymers of different compositions and/or MMs show different thermoresponsive properties.¹⁴

Table 5.4.3 also lists the theoretical and experimental compositions. The experimental compositions were determined via ¹H NMR, and the ¹H NMR spectrum of PD1 (PEGMA₇-*b*-diPGMA₅-*b*-PEGMA₇) and its linear precursors are shown in Figure 5.4.10. As can be seen in Figure 5.4.10 (a), all the peaks corresponding to the PEGMA homopolymer are present. Additional peaks are observed in the ¹H NMR spectrum of the diblock copolymer (Figure 5.4.10 (b)), which corresponds to diPGMA. Concerning the ¹H NMR of the triblock terpolymer, no additional peaks are observed, as expected, but an increase in the intensity of the PEGMA peaks is observed, thus confirming the sequential polymerisation. The absence of double bonds, which would correspond to unreacted methacrylate monomers, confirms the complete monomer consumption. The experimental compositions agree with the targeted ones.

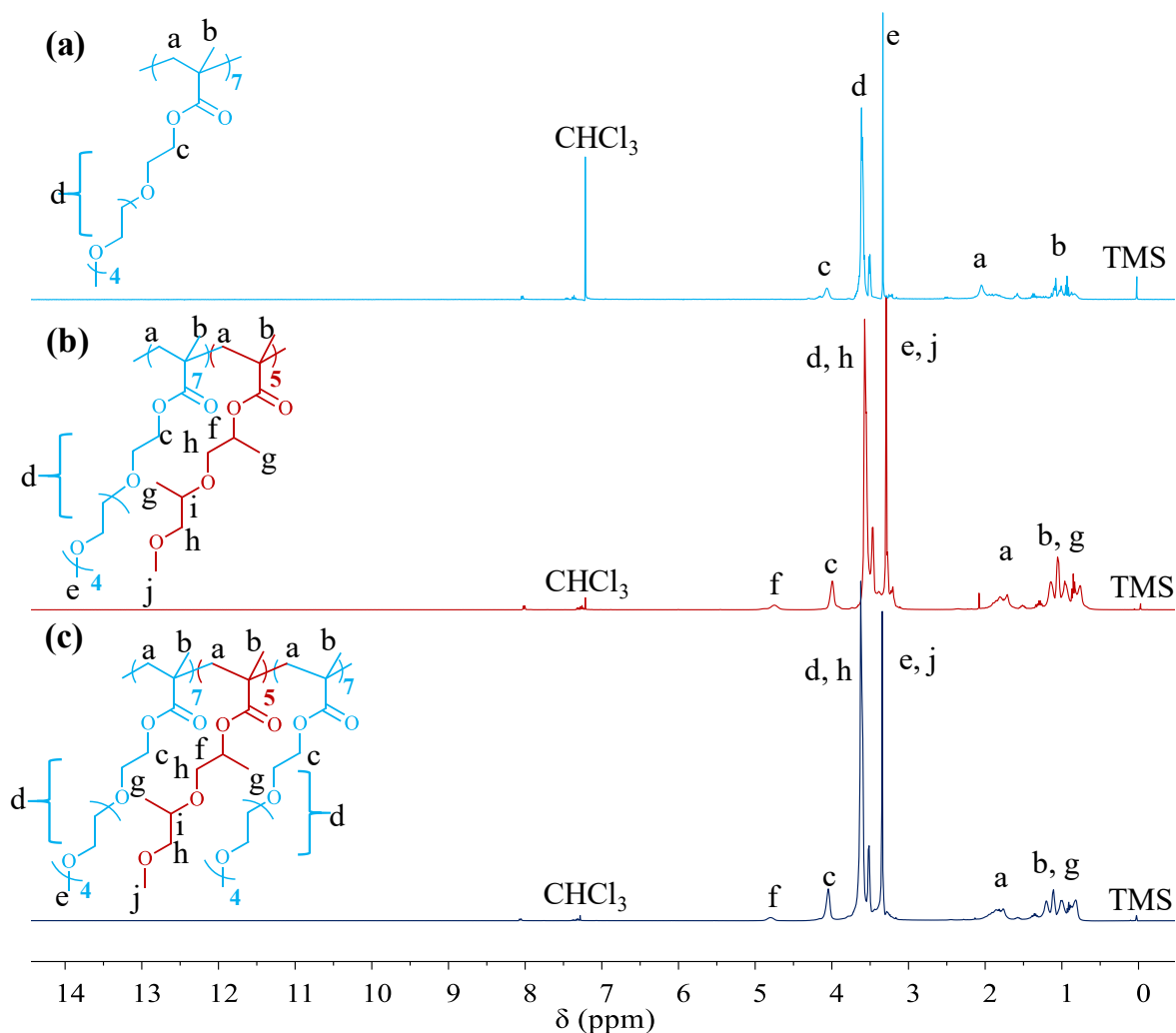


Figure 5.4.10: ^1H NMR spectra of (a) the homopolymer PEGMA₇, (b) the diblock copolymer PEGMA₇-b-diPGMA₅, and (c) the final triblock copolymer PEGMA₇-b-diPGMA₅-b-PEGMA₇ (PD1) are presented in light blue, red, and dark blue, respectively. Also, the corresponding chemical structures are shown. In these structures, the PEGMA, and diPGMA repeated units are coloured in light blue, and dark red, respectively. *Note:* the most abundant isomer of diPGMA is used in the structures shown above for simplicity.

5.4.5 Aqueous Solution Properties

5.4.5.1 Hydrodynamic Diameters

DLS has been utilised to examine the hydrodynamic diameters of the copolymers. The results are listed in Table 5.4.4, in which the theoretical values are also presented. The hydrodynamic diameter is determined as the one corresponding to the maximum of the peak by intensity. In most of the cases, only one peak is observed, which corresponds to the micelles. This result is similar to the PEGMA-BuMA-DEGMA based series in Chapter 5.3. In the case of PD1 and PD7, a low-intensity second peak is observed, which corresponds to

aggregates. Aggregate formation might have been enhanced in the case of these copolymers because of their high content in lengthy PEGMA units (80%).

It is assumed that all the ABA triblock copolymers self-assemble into spherical micelles. PEGMA and diPGMA form the shell and the core of the micelles, respectively. The theoretical configuration of the spherical micelle formed by PD1 (PEGMA₇-*b*-diPGMA₅-*b*-PEGMA₇) is shown in Figure 5.4.11. All the copolymers are expected to adopt the same configuration.

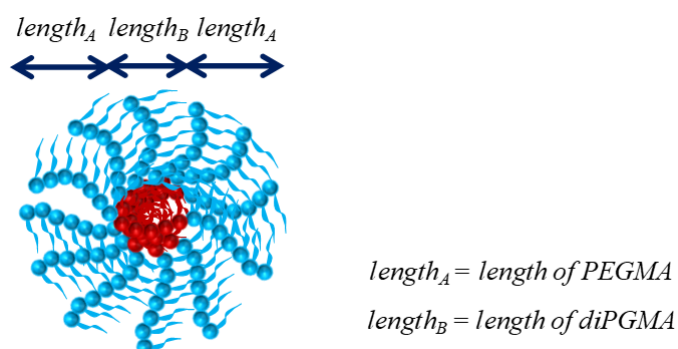


Figure 5.4.11: Schematic illustration of the spherical micelle adopted by PEGMA₇-*b*-diPGMA₅-*b*-PEGMA₇ (PD1). The PEGMA, and diPGMA repeated units are shown in light blue, and dark red, respectively. All triblock copolymers (PD1-PD9) in this study are assumed to self-assemble in the same way as shown.

When the theoretical values of the hydrodynamic diameters are compared to the experimental ones, it can be observed that the experimental ones exceed the theoretical ones. Even though the theoretical model assumes fully-stretched polymer chains (which would lead to experimental values being lower than the theoretical ones), the theoretical calculation does not take into consideration the length of the side chains of PEGMA. This observation is consistent with the observations made in Chapter 5.3. Another factor that might contribute to the bigger micelles obtained experimentally is the incomplete overlap of the hydrophobic diPGMA (the theoretical calculations assume fully overlapping of the hydrophobic part).

Table 5.4.4: Theoretical polymer structures, architectures, and hydrophobic composition, theoretical and experimental hydrodynamic diameters (d_h), polydispersity indices (PDI), and cloud points (CPs) of 1 w/w% copolymer solutions in deionised water.

No.	MM ^{theor.} (g mol ⁻¹)	diPG ^{theor.} (w/w%)	Theoretical Polymer Structure ^a	Hydrodynamic diameter (<i>d</i> _h , nm)				Cloud Points ± 2 °C
				Theor. ^b	Experimental ± 0.5 ^f			
					1 st	2 nd	PDI	
PD1	5100	20	PEG ₇ - <i>b</i> -diPG ₅ - <i>b</i> -PEG ₇	4.9	5.6	37.8	0.335	54
PD2	5100	30	PEG ₆ - <i>b</i> -diPG ₇ - <i>b</i> -PEG ₆	5.3	10.1	-	0.149	52
PD3	5100	40	PEG ₅ - <i>b</i> -diPG ₉ - <i>b</i> -PEG ₅	5.9	13.5	-	0.098	45
PD4	10100	20	PEG ₁₃ - <i>b</i> -diPG ₉ - <i>b</i> -PEG ₁₃	9.6	13.5	-	0.113	60
PD5	10100	30	PEG ₁₂ - <i>b</i> -diPG ₁₄ - <i>b</i> -PEG ₁₂	10.5	15.7	-	0.047	60
PD6	10100	40	PEG ₁₀ - <i>b</i> -diPG ₁₉ - <i>b</i> -PEG ₁₀	11.6	32.7	-	0.097	54
PD7	13100	20	PEG ₁₇ - <i>b</i> -diPG ₁₂ - <i>b</i> -PEG ₁₇	11.9	11.7	342	0.313	60
PD8	13100	30	PEG ₁₅ - <i>b</i> -diPG ₁₈ - <i>b</i> -PEG ₁₅	13.0	18.2	-	0.065	59
PD9	13100	40	PEG ₁₃ - <i>b</i> -diPG ₂₄ - <i>b</i> -PEG ₁₃	13.1	21.0	-	0.071	55

^a Abbreviations: penta(ethylene glycol) methyl ether methacrylate (PEG), and di(propylene glycol) methyl ether methacrylate (diPG).

^b The calculations of the theoretical diameters are based on the experimental degrees of polymerisation (DPs), which were calculated by using the experimental MM after precipitation, as determined by GPC analysis, and the experimental composition, as determined by ¹H NMR analysis.

^c Equation 4.8, d_h (nm) = $[DP_A + DP_B] \times 0.254$ nm, was used to calculate the theoretical hydrodynamic diameter of the ABA diblock bipolymers. A and B correspond to PEGMA and diPGMA, respectively.

^d The experimental hydrodynamic diameters reported are the mean diameters which correspond to the maximum of the peak by intensity in the DLS histograms.

5.4.5.2 Cloud Points

Table 5.4.4 lists the CP results, as obtained after visual inspection of 1 w/w% copolymer solutions in DI water. The effect of the hydrophobic content and the MM of the polymers on the CP is presented in Figure 5.4.12.

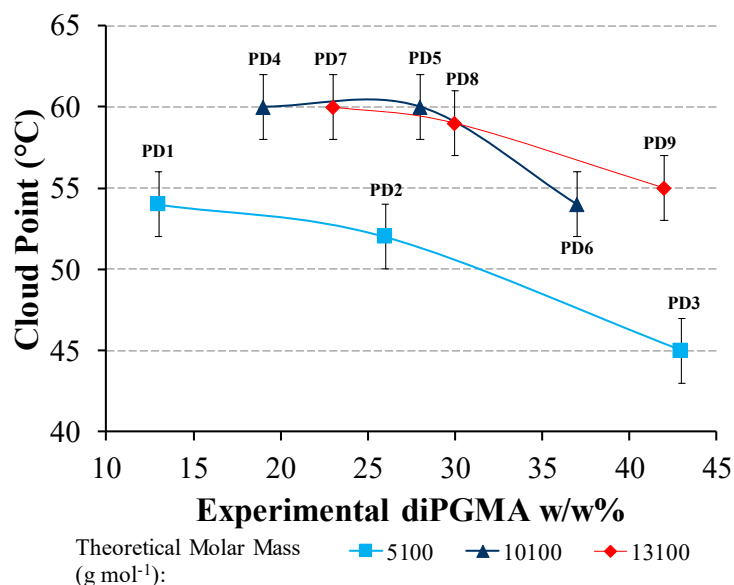


Figure 5.4.12: Effect of experimental diPGMA weight percentage on the cloud points of $\text{PEGMA}_x\text{-}b\text{-diPGMA}_y\text{-}b\text{-PEGMA}_x$ solutions (1 w/w% in deionised water); x , and y denote the degree of polymerisation of PEGMA and diPGMA, respectively. The effect of the MM is also shown; light blue squares, dark blue triangles, and the red rhombi correspond to copolymers with MM 5100, 10100, and 13100 g mol^{-1} , respectively.

As can be observed, the hydrophobic content of the copolymers influences the CP value. More specifically, when copolymers of the same target MM are compared, it is observed that as the content in diPGMA increases, the CP decreases, as expected.¹¹ This trend has been previously observed and reported,¹¹ and it is attributed to the enhancement of the “hydrophobic effect” when the hydrophobicity of the copolymers increases.

Concerning the changes in MM, two trends are observed. When the families of higher MM are compared (10100 g mol^{-1} and 13100 g mol^{-1}), no trend is observed, as the CPs of copolymers with similar diPGMA content are equal within the experimental error. On the other hand, when the CPs of these copolymers are compared to the results for the lower MM polymers (5100 g mol^{-1}), a clear trend is observed. More specifically, as the MM increases from 5100 g mol^{-1} to either 10100 g mol^{-1} or 13100 g mol^{-1} , the CP value increases. This is in contrast with the previous trends observed in DMAEMA-containing polymers, according to

which the CP decreases as the MM of the polymer increases.⁹ However, when the MM increases, the DP of the hydrophilic PEGMA also increases. Therefore, it can be concluded that the increase in hydrophilic PEGMA content, increases the hydrophilicity of the polymer's self-assembled structure (micelle), as the PEGMA is in the corona/shell, which counteracts the bigger hydrophobic core and increases the CP.

This effect is observed in the case of Pluronics®, when the MM difference is profound.¹ For example, when Pluronics® of 30 w/w% EG are compared, the CP increases from 42 °C, to 86 °C, to 90 °C, as the MM increases from 1850 g mol⁻¹, to 4950 g mol⁻¹, to 5750 g mol⁻¹, respectively (these values correspond to Pluronic® L43, P103, and P123, respectively). This is also the case when Pluronics® with 40 w/w% EG are compared. More specifically, when the MM increases from 2900 g mol⁻¹, to 4200 g mol⁻¹, to 5900 g mol⁻¹, the CP increases from 58 °C, to 74 °C, to 81 °C, respectively (for Pluronic® L64, P84, and P104, respectively).¹

5.4.5.3 Visual Gel Points

Diluted and concentrated copolymer solutions in PBS have been investigated in terms of gelation. All nine phase diagrams have been constructed and are shown in Figure 5.4.13. The effect of composition is shown from the left to the right, whereas the effect of MM is shown from the top to the bottom: (a) 5100 g mol⁻¹, (b) 10100 g mol⁻¹, and (c) 13100 g mol⁻¹.

All the diluted copolymer solutions at 1 w/w% in PBS present a CP; the values vary between 43 °C to 56 °C. When compared to the CP in DI water, the solutions become cloudy at lower temperatures, which is caused by the presence of salt in solution.¹⁵ Two trends are observed, as in the case of DI water: (a) the CP decreases as the diPGMA content increases (for polymers with similar MM), and (b) the CP increases when the increase in MM is profound (for polymer with the similar composition).

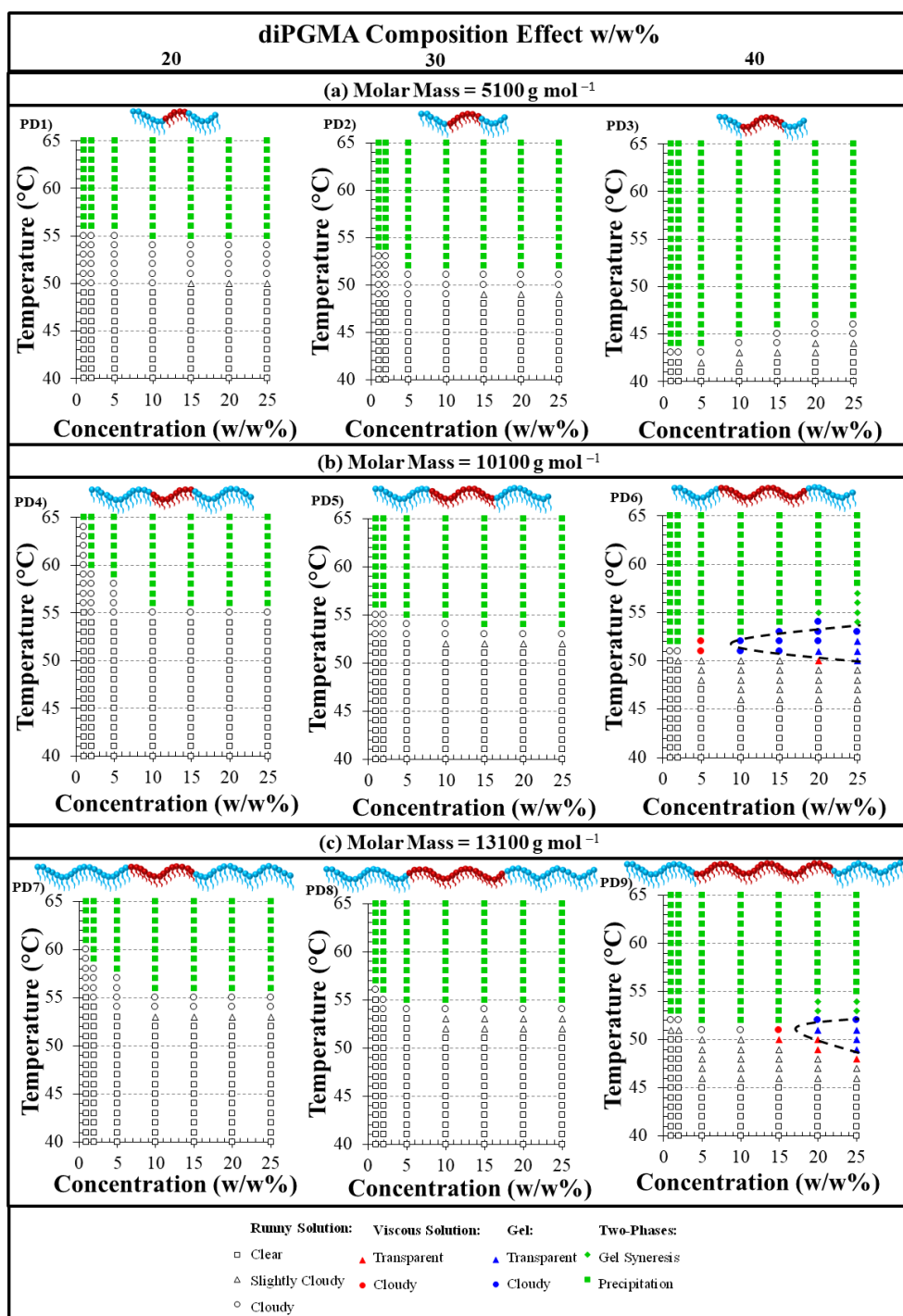


Figure 5.4.13: Phase diagrams of the PEGMA-diPGMA-PEGMA triblock copolymers (PD1-PD9) in phosphate buffered saline (PBS). The effect of diPGMA composition is shown from left to right, whereas the effect of the MM is shown from the top to bottom: (a) MM 5100 g mol⁻¹ (top), (b) MM 10100 g mol⁻¹ (middle), and (c) MM 13100 g mol⁻¹ (bottom). Four phases are observed: i) runny solution coloured in white (square: clear, triangle: slightly cloudy, and circle: cloudy), ii) viscous solution coloured in red (triangle and circle for transparent and cloudy, respectively), iii) the gel phase is shown in blue (triangle and circle for transparent and cloudy, respectively), the two-phase system is coloured in light green (rhombus and square for gel syneresis and precipitation, respectively). The polymer structures are schematically illustrated, in which the PEGMA and diPGMA repeated units are coloured in light blue, and dark red, respectively.

Concerning the concentrated polymer solutions, both a composition effect and a MM effect are observed. The effect of MM can be observed when comparing the copolymers of 40 w/w% hydrophobic diPGMA content. It is observed that PD3, which has the lowest MM (5100 g mol^{-1}), does not form gels (only precipitates as the temperature increases), while the polymers with higher MM form gels. Specifically, the PD6 and PD9, with MM 10100 g mol^{-1} and 13100 g mol^{-1} , respectively, present a narrow gelation area around $50 \text{ }^{\circ}\text{C}$ to $55 \text{ }^{\circ}\text{C}$. Interestingly, the polymer with intermediate MM (PD6) has a wider gelation region. This is similar but not completely in agreement with the earlier study of our group on ABC triblock copolymers.⁹ In the previous study of the group, by increasing the MM, the gelation was enhanced, while in this study more precipitation and syneresis are observed.⁹ This may be attributed to the difference in hydrophobic content and specifically, to the not successful synthesis of PD6. This needs to be repeated in order to confirm this trend. The rest of the copolymers, of lower hydrophobic content, do not form gels, but phase separation occurs as the temperature increases, regardless the change in MM. This shows a clear effect of composition, according to which, the increase in the hydrophilic PEGMA content disrupts the gelation. This is in agreement with the previous chapter (Chapter 5.3) and previous published studies.^{8,11}

Generally, the results are well-correlated to the thermoresponsive behaviour of PC13 (presented in Chapter 5.3), with theoretical polymer structure $\text{PEGMA}_{10}\text{-}b\text{-BuMA}_{17}\text{-}b\text{-PEGMA}_{10}$. This polymer did not form gels at any temperatures and concentrations tested, and precipitation was observed upon increasing the temperature. Therefore, it can be concluded that ABA triblock copolymers with A block being based on PEGMA, and B block being based on a hydrophobic monomer (either diPGMA or BuMA) are generally not able to form gels, which can be attributed to the increased hydrophilicity of PEGMA.

However, it is worth comparing the CP of PC13 with the CP of the copolymers in this Chapter. PC13, with PEGMA-BuMA content 70-30 w/w% and M_n 11000 g mol^{-1} , presents CP at around $56 \text{ }^{\circ}\text{C}$ to $58 \text{ }^{\circ}\text{C}$ at all the concentrations tested. On the other hand, the CP of PD2, PD5, and PD8, which have PEGMA-diPGMA content 70-30 w/w%, present lower CP values than PC13, regardless the variation in MM. More specifically, they present a CP at around $52 \text{ }^{\circ}\text{C}$ to $57 \text{ }^{\circ}\text{C}$, polymer and concentration dependant. Thus, it is concluded that the thermoresponse is enhanced as BuMA is substituted by diPGMA. These results are promising, as diPGMA is a better-performing alternative hydrophobic monomer to the commonly-used by our group BuMA.

5.4.5.4 Gel Points by Rheology

The rheological properties of the copolymer solutions at 15 w/w% in PBS were examined. The results for PD6 with theoretical chemical structure PEGMA₁₀-*b*-diPGMA₁₉-*b*-PEGMA₁₀ are presented in Figure 5.4.14. The rest of the results are presented in Figure A8 in the Appendix.

As can be seen in Figure 5.4.14, gel formation is observed upon increasing the temperature. More specifically, the gelation (instant at which $G' > G''$) is observed at 51 °C, which is the same as the temperature at which gel was observed visually. As temperature increases further above 56 °C, de-gelation is observed ($G'' > G'$), which agrees with the temperature at which precipitation occurred visually (within the experimental error).

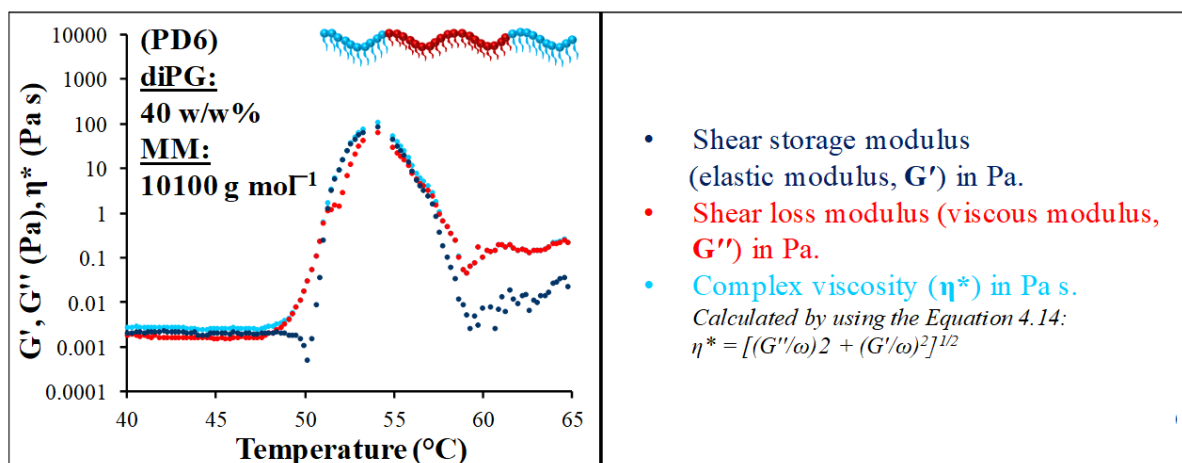


Figure 5.4.14: Rheological properties as a function of temperature for the PD6 polymer solution which form a gel visually at 15 w/w% in phosphate buffered saline (PBS). PD6 has theoretical polymer structure PEGMA₁₀-*b*-diPGMA₁₉-*b*-PEGMA₁₀, MM equal to 10100 g mol⁻¹, and 40 w/w% diPGMA content. The PEGMA and diPGMA units on the polymer structures are coloured in light blue and dark red, respectively.

5.4.6 Conclusions

In this study, nine ABA triblock copolymers with PEGMA and diPGMA as A and B blocks, respectively, have been synthesised via one-pot GTP. While PEGMA is commercially available, diPGMA has been *in-house* synthesised. The MM and composition of the copolymers have been systematically varied. Three MMs have been targeted, specifically, 5100 g mol⁻¹, 10100 g mol⁻¹, and 13100 g mol⁻¹. The hydrophobic content has also been varied from 20 w/w%, to 30 w/w%, to 40 w/w% to investigate its effect on the

thermoreponsive properties of the polymers. Concerning the monomer synthesis, polymer synthesis and characterisation in organic and aqueous solvents, the following conclusions are drawn:

1. The hydrophobic monomer of this study, diPGMA, has been successfully synthesised and characterised by GC-FID, FT-IR, and ^1H and ^{13}C NMR.
2. The copolymers have been successfully synthesised (i.e. experimental MM and compositions close to the targeted ones, and narrow MMD), except for PD6 with theoretical polymer structure $\text{PEGMA}_{10}\text{-}b\text{-diPGMA}_{19}\text{-}b\text{-PEGMA}_{10}$, the synthesis of which should be repeated.
3. All the copolymers are soluble in aqueous solvents, because of the high content in the hydrophilic PEGMA (60-80 w/w%).
4. The experimental hydrodynamic diameters are higher than the theoretical (calculated) ones, and this discrepancy can be attributed to the lengthy PEGMA chains, the size of which has not been taken into consideration in the calculations.
5. The CP is affected by the diPGMA content, i.e. the higher the content in the hydrophobic diPGMA, the lower the CP is.
6. The CP is affected by the MM of the copolymers when the difference is profound, and specifically, the CP decreases as the MM of the polymers (and thus the MM of the hydrophilic PEGMA blocks) decreases.
7. The CP in PBS follow the same trends as in DI water, however, they appear at lower values in PBS, because of the ionic strength effect.
8. Gelation is observed for two of the copolymers with high hydrophobic diPGMA content (40 w/w%), and high MM (10100 g mol^{-1} and 13100 g mol^{-1}), which is attributed to both the effect of hydrophobicity and MM.
9. The rheological results agree with the visual tests within the experimental error of the techniques.

5.4.7 References

1. Almeida, M.; Magalhães, M.; Veiga, F.; Figueiras, A. Poloxamers, poloxamines and polymeric micelles: Definition, structure and therapeutic applications in cancer. *Journal of Polymer Research* **2017**, *25*, 31.
2. Constantinou, A. P.; Georgiou, T. K. Thermoresponsive Multiblock Copolymers: Chemistry, Properties and Applications. In *Temperature-Responsive Polymers: Chemistry, Properties, and Applications*; Khutoryanskiy, V. V., Georgiou, T. K., Eds.; John Wiley & Son Ltd: UK, 2018; pp 35-65.
3. Constantinou, A. P.; Elladiou, M.; Patrickios, C. S. Regular and Inverse Polyampholyte Hydrogels: A Detailed Comparison. *Macromolecules* **2016**, *49*, 3869-3880.
4. NIST Chemistry WebBook 2-Propanol, 1-(2-methoxy-1-methylethoxy)-. <https://webbook.nist.gov/cgi/cbook.cgi?ID=C20324327&Units=CAL&Mask=2000#Gas-Chrom> (accessed 02/09, 2019).
5. NIST Chemistry WebBook 2-Propanol, 1-(2-methoxypropoxy)-. <https://webbook.nist.gov/cgi/cbook.cgi?ID=C13429077&Units=SI&Mask=2000#Gas-Chrom> (accessed 02/09, 2019).
6. United States of America, Department of Labor, Occupational Safety and Health Administration Dipropylene Glycol Methyl Ether. <https://www.osha.gov/dts/sltc/methods/organic/org101/org101.html#ref51> (accessed 02/09, 2019).
7. Ward, M. A.; Georgiou, T. K. Multicompartment thermoresponsive gels: does the length of the hydrophobic side group matter? *Polym. Chem.* **2013**, *4*, 1893-1902.
8. Ward, M. A.; Georgiou, T. K. Thermoresponsive terpolymers based on methacrylate monomers: Effect of architecture and composition. *J. Polym. Sci. A Polym. Chem.* **2010**, *48*, 775-783.
9. Ward, M. A.; Georgiou, T. K. Thermoresponsive triblock copolymers based on methacrylate monomers: effect of molecular weight and composition. *Soft Matter* **2012**, *8*, 2737-2745.
10. Ghasdian, N.; Ward, M. A.; Georgiou, T. K. Well-defined clickable copolymers prepared via one-pot synthesis. *Chem. Commun.* **2014**, *50*, 7114-7116.
11. Constantinou, A. P.; Georgiou, T. K. Thermoresponsive gels based on ABC triblock copolymers: effect of the length of the PEG side group. *Polym. Chem.* **2016**, *7*, 2045-2056.
12. Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. A Comprehensive Systematic Study on Thermoresponsive Gels: Beyond the Common Architectures of Linear Terpolymers. *Polymers* **2017**, *9*, 31.
13. Constantinou, A. P.; Sam-Soon, N.; Carroll, D. R.; Georgiou, T. K. Thermoresponsive Tetrablock Terpolymers: Effect of Architecture and Composition on Gelling Behavior. *Macromolecules* **2018**.
14. Constantinou, A. P.; Georgiou, T. K. Tuning the gelation of thermoresponsive gels. *Eur. Polym. J.* **2016**, *78*, 366-375.
15. de Souza, J., Carlos Perbone; Naves, A. F.; Florenzano, F. Specific thermoresponsiveness of PMMA-block-PDMAEMA to selected ions and other factors in aqueous solution. *Colloid Polym. Sci.* **2012**, *290*, 1285-1291.

CHAPTER 6: CONCLUSIONS AND FUTURE WORK

6.1 Conclusions

In this PhD thesis, four different studies on thermoresponsive polymers have been successfully performed. In each study, the structural characteristics of the copolymers have been systematically varied in order to investigate how these affect their thermoresponsive properties, with particular interest on their gelling behaviour.

The first two studies of this PhD thesis are based on DMAEMA-containing thermoresponsive polymers; DMAEMA is the thermoresponsive C unit. In both studies, BuMA has been used as the hydrophobic unit (B unit), whereas TEGMA and PEGMA have been used as the hydrophilic units (A units). Concerning the TEGMA-based polymers (PhD Chapter 5.1), the polymer architecture has been varied by investigating triblock terpolymers, statistical terpolymer, and diblock terpolymers. The latter is the novelty of the study, as in the best of our knowledge, this is the first systematic study in which this type of architecture has been investigated. Concerning Chapter 5.2, tetrablock copolymers have been investigated by systematically varying the tetrablock architecture and the composition for the first time.

The third and fourth PhD studies (Chapters 5.3 and 5.4) are focused on investigating non-ionic thermoresponsive units. In the third study, DEGMA has been used as the thermoresponsive unit (C unit), whereas the A and B units are based on the hydrophilic PEGMA, and the hydrophobic BuMA, respectively. The composition of the ABC triblock terpolymers has been varied, to investigate its effect on the thermoresponsive properties. In the fourth study, PEGMA is the thermoresponsive unit, whereas a novel hydrophobic monomer has been *in-house* synthesised, specifically diPGMA. Thus, ABA triblock bipolymers of varying MMs and compositions have been investigated.

Concerning the synthesis and characterisation of the copolymers in organic and aqueous solvents, the following conclusions are drawn:

1. Monomer synthesis has been successfully performed.
2. In total, fifty-eight copolymers have been synthesised via GTP, and their molar mass characteristics and compositions have been confirmed using GPC and ^1H NMR, respectively.

3. All the copolymers, except PD6 (see Chapter 5.4 for details), are characterised by narrow MMD ($\mathcal{D}$ below 1.3), which is satisfactorily low for the purpose of this study.
4. The thermoresponsive properties of the copolymers is proven to be dependent on the polymer structural characteristics.
5. Concerning the TEGMA-based copolymers, the change in the architecture has a strong effect on the solubility and self-assembly behaviour of the copolymers, and therefore, on the gelation properties. In this study, the ABC architecture is proven to show the best gelation properties with clear sol-gel transition near body temperature.
6. When the tetrablock terpolymers are concerned, both their architecture and their composition affect their properties in aqueous media. Specifically, the ACBC architecture with 30 w/w% BuMA content shows promising thermogelling properties, as it presents a wide gelation area, and the viscosity of the formed gel at 15 w/w% in PBS reaches the 1730 Pa s.
7. The composition of the DEGMA-based ABC terpolymers controls both their solubility and their thermoresponsive behaviour. When the hydrophilic content is high, the copolymers are well-soluble in aqueous media, thus no gelation is observed. On the other hand, when the hydrophobic content is high, the copolymers are either insoluble in aqueous solvents, or precipitation is favoured over gelation. Nevertheless, PEGMA₁₁-*b*-BuMA₂₀-*b*-DEGMA₁₁ is the best-performing polymer in this PhD thesis, as it forms stable transparent gels at body temperature at a wide concentration range (from 5 to 25 w/w%).
8. Both the composition and the MM of the PEGMA-diPGMA-PEGMA copolymers affect their thermoresponsive properties. However, a narrow gelation area is observed for only two of the copolymers, which are the ones with high MM and hydrophobic content, thus proving that both parameters affect the gelation.

In summary, important structure-properties relationships have been identified by investigating novel thermoresponsive polymers that will assist with the design and tailoring of thermoresponsive gels for applications in TE or/and 3-D printing.

6.2 Future Work

This PhD thesis was focused more on synthesis and characterisation of thermoresponsive polymers. Thus, future work will involve investigating the best performing polymers in applications like TE and 3-D printing.

Specifically, several copolymers revealed promising properties, with a sol-gel transition close to or just below body temperature. As thermoresponsive polymers are used in TE, the cytotoxicity of the promising copolymer solutions should be investigated in the future. Once the biocompatibility of the polymer solutions is confirmed, then *in vivo* studies should be carried out, in which the gels can be tested as drug release depots.

In terms of the last series of polymers that was fabricated, more work is required to complete the study. Specifically, PD6 is characterised by wide MMD, thus, the synthesis of the polymer should be repeated in the future. Characterisation in aqueous solutions will follow to confirm its thermogelling properties. Furthermore, PEGMA-diPGMA-PEGMA triblock copolymers of higher hydrophobic diPGMA content can be synthesised to produce polymers with better thermogelling properties, i.e. gelation at lower concentrations and temperatures. The best performing polymers from this series can then be examined in the same applications as the ones mentioned above.

Lastly, mixture of different polymer solutions could also be investigated. These can be mixtures of the best performing polymers of this study or one commercially available polymer with one of the polymers of this study.

APPENDIX

Reuse of my previously published papers – Permission:

1. Chapter 5.1, excluding Section 5.1.3.5 Gel Points by Rheology, has been already published and can be found in: “<<http://www.mdpi.com/2073-4360/9/1/31>>, Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. A Comprehensive Systematic Study on Thermoresponsive Gels: Beyond the Common Architectures of Linear Terpolymers. *Polymers* **2017**, *9*, 31.”

Email seeking Permission:

“Dear Ms Zhao,

My name is Anna Constantinou and I am a PhD candidate at Imperial College London.

I am submitting my PhD thesis by 28th of September, and I would like to ask your permission to include my paper (Manuscript ID: polymers-168154) in my thesis. My thesis will be added to Spiral, which is Imperial's institutional online repository system.

If you are happy to grant me the permissions requested, could you please fill and send me the attached document? It is Imperial's template that I should include in my thesis.

Thank you very much in advance.

Kind regards,

Anna Constantinou”

Response from the Editor:

“Dear Anna,

As an open access journal, the copyright permissions of our publications are held by the authors themselves. You can include this paper into your thesis with suitable citation and introduction. There's no need to ask for permissions from the editorial office.

Hope the information above helps!

Best,

Sigrid”

2. Part of Chapter 5.2 (results for PB11-PB25) has been already published and can be found in: “<<https://pubs.acs.org/doi/10.1021/acs.macromol.8b01251>>, Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. Thermoresponsive Tetrablock Terpolymers: Effect of Architecture and Composition on Gelling Behavior. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251”. Permission for this reuse has been granted by the ACS, and further permissions related to the material excerpted should be directed to the ACS.

Email seeking Permission:

“Dear Prof Förster,

My name is Anna Constantinou and I am a PhD candidate at Imperial College London.

I am submitting my PhD thesis by 28th of September, and I would like to ask your permission to include my recently published paper (manuscript ID: ma-2018-01251m.R1) in my thesis. My thesis will be added to Spiral, which is Imperial's institutional online repository system.

If you are happy to grant me the permissions requested, could you please fill and send me the attached document? It is Imperial's template that I should include in my thesis.

Thank you very much in advance.

Kind regards,

Anna Constantinou

Response from the ACS Customer Services & Information:

“Dear Dr. Anna Constantinou,

Thank you for contacting ACS Publications Support.

Your permission request is granted and there is no fee for this reuse. In your planned reuse, you must cite the ACS article as the source, add this direct link <<https://pubs.acs.org/doi/10.1021/acs.macromol.8b01251>>, and include a notice to readers that further permissions related to the material excerpted should be directed to the ACS.

Should you need further assistance, please let us know.

Sincerely,

Noemi D. Cabalza

ACS Customer Services & Information”

Table A1: Densities, Molecular Mass and basic chemical substances needed for the polymerisations.

Chemical Substance	Role	Density (g mL ⁻¹)	Molar Mass (g mol ⁻¹)
MTS	Initiator	0.858	174.31
DEGMA	Monomer	1.02	188.22
TEGMA	Monomer	1.027	232.27
PEGMA	Monomer	1.05	300
BuMA	Monomer	0.894	142.20
DMAEMA	Monomer	0.933	157.21
diPGMA	Monomer	0.92	216

Table A2: Calculations for the polymerisations of the copolymers presented in this PhD thesis and the temperature changes recorded during polymerisations.

Basic equations: DP=MM polymer/MM monomer DP= moles monomer/moles initiator								
Chapter 5.1: TEGMA-based								
PA1: TEGMA- <i>b</i> -BuMA- <i>b</i> -DMAEMA 25-35-40								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				59.1				
MTS				0.37	0.3	0.002	To	Tmax
TEGMA	0.25	2025	9	3.6	3.7	0.02	24.2	28.3
BuMA	0.35	2835	20	5.8	5.2	0.04	25.2	28.9
DMAEMA	0.40	3240	21	6.3	5.9	0.04	27.6	31.8
target MM		8100		Total:	15.0			
PA2: TEGMA- <i>b</i> -DMAEMA- <i>b</i> -BuMA 25-40-35								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				59.1				
MTS				0.37	0.3	0.002	To	Tmax
TEGMA	0.25	2025	9	3.6	3.7	0.02	23.3	30.6
DMAEMA	0.40	3240	21	6.3	5.9	0.04	25.4	35.7

BuMA	0.35	2835	20	5.8	5.2	0.04	28	32.4
target MM		8100		Total:	15.0			
PA3: BuMA-<i>b</i>-TEGMA-<i>b</i>-DMAEMA 35-25-40								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				59.1				
MTS				0.37	0.3	0.002	To	Tmax
BuMA	0.35	2835	20	5.8	5.2	0.04	25	33.5
TEGMA	0.25	2025	9	3.6	3.7	0.02	21.9	31.6
DMAEMA	0.40	3240	21	6.3	5.9	0.04	26.2	31.9
target MM		8100		Total:	16.03			
PA4: (TEGMA-<i>co</i>-BuMA)-<i>b</i>-DMAEMA								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				59.1				
MTS				0.37	0.3	0.002	To	Tmax
TEGMA	0.25	2025	9	3.6	3.7	0.02	24.1	38.2
BuMA	0.35	2835	20	5.8	5.2	0.04		
DMAEMA	0.40	3240	21	6.3	5.9	0.04	27.1	33.1
target MM		8100		Total:	15.0			
PA5: TEGMA-<i>b</i>-(BuMA-<i>co</i>-DMAEMA)								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				59.1				
MTS				0.37	0.3	0.002	To	Tmax
TEGMA	0.25	2025	9	3.6	3.7	0.02	24.7	28.4
BuMA	0.35	2835	20	5.8	5.2	0.04	26.4	37.2
DMAEMA	0.40	3240	21	6.3	5.9	0.04		
target MM		8100		Total:	15.0			
PA6: (TEGMA-<i>co</i>-BuMA)-<i>b</i>-DMAEMA								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				59.1				

MTS				0.37	0.3	0.002	To	Tmax
TEGMA	0.25	2025	9	3.6	3.7	0.02	24.3	33.7
BuMA	0.35	2835	20	5.8	5.2	0.04		
DMAEMA	0.40	3240	21	6.3	5.9	0.04	28.3	37.8
target MM		8100		Total:	15.0			
PA7: TEGMA-co-BuMA-co-DMAEMA								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				59.1				
MTS				0.37	0.3	0.002	To	Tmax
BuMA	0.35	2835	20	5.8	5.2	0.04	20.9	25.2
TEGMA	0.25	2025	9	3.6	3.7	0.02		
DMAEMA	0.40	3240	21	6.3	5.9	0.04		
target MM		8100		Total:	15.0			
PA8: BuMA-<i>b</i>-DMAEMA 46-54								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				58.7				
MTS				0.37	0.3	0.002	To	Tmax
BuMA	0.46	3726	26	7.6	6.8	0.048	24.6	36.0
DMAEMA	0.54	4374	28	8.6	8.0	0.05	27.6	39.9
target MM		8100		Total:	15.0			
PA9: BuMA-<i>b</i>-DMAEMA 35-65								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				29.0				
MTS				0.25	0.2	0.001	To	Tmax
BuMA	0.35	2835	20	3.8	3.4	0.024	22.5	33.2
DMAEMA	0.65	5265	34	6.9	6.4	0.041	27.1	43.5
target MM		8100		Total:	10			

Chapter 5.2: Tetrablocks								
PB1: PEGMA- <i>b</i> -BuMA- <i>b</i> -DMAEMA- <i>b</i> -PEGMA 10-40-40-10								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				63.7				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.1	750	2.5	3.2	1.7	0.006	24.2	26.2
BuMA	0.4	3000	21.1	7.4	6.7	0.05	24.2	34.6
DMAEMA	0.4	3000	19.1	7.1	6.7	0.04	28.3	38.0
PEGMA	0.1	750	2.5	3.2	1.7	0.006	28.7	29.6
target MM		7500		Total:	17.0			
PB2: PEGMA- <i>b</i> -BuMA- <i>b</i> -PEGMA- <i>b</i> -DMAEMA 10-40-10-40								
Chemical Substance	Target wt%	MM block	DP	mL	G	Moles		
THF	63.7							
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.1	750	2.5	3.2	1.7	0.006	24.4	26.8
BuMA	0.4	3000	21.1	7.4	6.6	0.05	24.2	35.9
PEGMA	0.1	750	2.5	3.2	1.7	0.006	26.1	27.7
DMAEMA	0.4	3000	19.1	7.1	6.7	0.04	25.1	34.4
target MM		7500		Total:	17.0			
PB3: PEGMA- <i>b</i> -DMAEMA- <i>b</i> -PEGMA- <i>b</i> -BuMA 10-40-10-40								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				63.7				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.1	750	2.5	3.2	1.7	0.006	24.5	26.6
DMAEMA	0.4	3000	19.1	7.1	6.7	0.004	24.3	36.8
PEGMA	0.1	750	2.5	3.2	1.7	0.006	31.3	32?
BuMA	0.4	3000	21.1	7.4	6.6	0.05	26.5	34.5
target MM		7500		Total:	17.0			
PB4: BuMA- <i>b</i> -PEGMA- <i>b</i> -DMAEMA- <i>b</i> -BuMA 20-20-40-20								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		

THF				63.7				
MTS				0.45	0.4	0.002	To	Tmax
BuMA	0.2	1500	10.5	3.7	3.3	0.02	24.2	29.2
PEGMA	0.2	1500	5.00	6.3	3.3	0.01	26.5	28.7
DMAEMA	0.4	3000	19.1	7.1	6.7	0.04	25.1	34.2
BuMA	0.2	1500	10.5	3.7	3.3	0.02	29.3	31.5
target MM		7500		Total:	17.0			
PB5: BuMA-<i>b</i>-PEGMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA 20-20-40								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				63.7				
MTS				0.5	0.4	0.002	To	Tmax
BuMA	0.2	1500	10.5	3.7	3.3	0.02	23.3	29
PEGMA	0.2	1500	5.0	6.3	3.3	0.01	26.1	28.6
BuMA	0.2	1500	10.5	3.7	3.3	0.02	25.6	29.4
DMAEMA	0.4	3000	19.1	7.1	6.6	0.04	27.1	35.2
target MM		7500		Total:	17.00			
PB6: PEGMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA-<i>b</i>-BuMA 20-20-40-20								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				63.7				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.2	1500	5.0	6.3	3.3	0.01	25.7	28.8
BuMA	0.2	1500	10.5	3.7	3.3	0.02	25.6	29.1
DMAEMA	0.4	3000	19.1	7.1	6.6	0.04	26.6	34.6
BuMA	0.2	1500	10.5	3.7	3.3	0.02	25.3	30.2
target MM		7500		Total:	17.00			
PB7: DMAEMA-<i>b</i>-PEGMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA 20-20-40-20								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				63.7				
MTS				0.45	0.4	0.002	To	Tmax

DMAEMA	0.2	1500	9.5	3.6	3.3	0.02	24.3	30.8
PEGMA	0.2	1500	5.0	6.3	3.3	0.01	26.8	29.6
BuMA	0.4	3000	21.1	7.4	6.7	0.05	25.8	35.3
DMAEMA	0.2	1500	9.5	3.6	3.3	0.02	28.4	33.1
target MM		7500		Total:	17.00			
PB8: DMAEMA-<i>b</i>-PEGMA-<i>b</i>-DMAEMA-<i>b</i>-BuMA 20-20-20-40								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				63.7				
MTS				0.45	0.4	0.002	To	Tmax
DMAEMA	0.2	1500	9.5	3.6	3.3	0.02	24.8	23.7
PEGMA	0.2	1500	5.0	6.3	3.3	0.01	26.7	--
DMAEMA	0.2	1500	9.5	3.6	3.3	0.02	25.6	30.3
BuMA	0.4	3000	21.1	7.4	6.7	0.05	26.0	33.1
target MM		7500		Total:	17.00			
PB9: PEGMA-<i>b</i>-DMAEMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA 20-20-40-20								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				63.7				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.2	1500	5.0	6.3	3.3	0.01	23.9	27.4
DMAEMA	0.2	1500	9.5	3.6	3.3	0.02	24.9	29.6
BuMA	0.4	3000	21.1	7.4	6.7	0.05	23.1	30.7
DMAEMA	0.2	1500	9.5	3.6	3.3	0.02	26.6	30.1
target MM		7500		Total:	17.00			
PB10: PEGMA-<i>co</i>-BuMA-<i>co</i>-DMAEMA								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				63.7				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.2	1500	5.0	6.3	3.3	0.01	23.1	30.7
BuMA	0.4	3000	21.1	7.4	6.7	0.05		

DMAEMA	0.4	3000	19.1	7.1	6.7	0.04		
target MM		7500		Total:	17.00			
PB11: PEGMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA-<i>b</i>-PEGMA 10-30-50-10								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.1	820	2.7	4.2	2.2	0.007	23.2	26.0
BuMA	0.3	2460	17.3	7.5	6.7	0.05	24.1	34.3
DMAEMA	0.5	4100	26.1	11.9	11.1	0.07	29.1	43.4
PEGMA	0.1	820	2.7	4.2	2.2	0.007	24.4	25.5
target MM		8200		Total:	22.67			
PB12: PEGMA-<i>b</i>-BuMA-<i>b</i>-PEGMA-<i>b</i>-DMAEMA 10-30-10-50								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.1	820	2.7	4.2	2.2	0.007	23.6	26.3
BuMA	0.3	2460	17.3	7.5	6.7	0.05	25.0	35.0
PEGMA	0.1	820	2.7	4.2	2.2	0.007	29.0	30.1
DMAEMA	0.5	4100	26.1	11.9	11.1	0.07	26.4	41.6
target MM		8200		Total:	22.7			
PB13: PEGMA-<i>b</i>-DMAEMA-<i>b</i>-PEGMA-<i>b</i>-BuMA 10-50-10-30								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.47	0.003	To	Tmax
PEGMA	0.1	820	2.7	4.2	2.22	0.007	23.6	26.5
DMAEMA	0.5	4100	26.1	11.9	11.1	0.07	24.0	41.7
PEGMA	0.1	820	2.7	4.2	2.2	0.007	28.1	28.9
BuMA	0.3	2460	17.3	7.5	6.7	0.05	25.7	33.2
target MM		8200		Total:	22.7			

PB14: BuMA- <i>b</i> -PEGMA- <i>b</i> -DMAEMA- <i>b</i> -BuMA 15-20-50-15								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax
BuMA	0.15	1230	8.65	3.7	3.3	0.02	23.6	29.4
PEGMA	0.2	1640	5.47	8.5	4.4	0.02	24.6	28.3
DMAEMA	0.5	4100	26.08	11.9	11.1	0.07	24.8	41.1
BuMA	0.15	1230	8.65	3.7	3.3	0.02	30.4	32.9
target MM		8200		Total:	22.7			
PB15: BuMA- <i>b</i> -PEGMA- <i>b</i> -BuMA- <i>b</i> -DMAEMA 15-20-15-50								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.4719	0.002707	To	Tmax
BuMA	0.15	1230	8.65	3.72	3.329912	0.023417	23.7	29.7
PEGMA	0.2	1640	5.467	8.46	4.439883	0.0148	25.3	29.0
BuMA	0.15	1230	8.65	3.72	3.329912	0.023417	24.8	28.0
DMAEMA	0.5	4100	26.08	11.90	11.09971	0.070604	24.9	37.7
target MM		8200		Total:	22.67			
PB16: PEGMA- <i>b</i> -BuMA- <i>b</i> -DMAEMA- <i>b</i> -BuMA 20-15-50-15								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.4719	0.002707	To	Tmax
PEGMA	0.20	1640	5.467	8.46	4.439883	0.0148	22.1	26.7
BuMA	0.15	1230	8.65	3.72	3.329912	0.023417	23.1	27.1
DMAEMA	0.5	4100	26.08	11.90	11.09971	0.070604	23.9	40.9
BuMA	0.15	1230	8.65	3.72	3.329912	0.023417	28.2	30.9
target MM		8200		Total:	22.67			
PB17: DMAEMA- <i>b</i> -PEGMA- <i>b</i> -BuMA- <i>b</i> -DMAEMA 25-20-30-25								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		

THF				62.3				
MTS				0.55	0.4719	0.002707	To	Tmax
DMAEMA	0.25	2050	13.04	5.95	5.549854	0.035	23.1	35.3
PEGMA	0.2	1640	5.47	8.46	4.44	0.015	24.5	29
BuMA	0.3	2460	17.30	7.45	6.66	0.047	24.9	34.2
DMAEMA	0.25	2050	13.04	5.95	5.549854	0.035	28.6	35.9
target MM		8200		Total:	22.67			
PB18: DMAEMA-<i>b</i>-PEGMA-<i>b</i>-DMAEMA-<i>b</i>-BuMA 25-20-25-30								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax
DMAEMA	0.25	2050	13.0	6.0	5.5	0.04	23.2	33
PEGMA	0.2	1640	5.5	8.5	4.4	0.01	24.5	27.9
DMAEMA	0.25	2050	13.0	6.0	5.6	0.04	24.7	32
BuMA	0.3	2460	17.3	7.5	6.7	0.05	26.7	33.8
target MM		8200		Total:	22.7			
PB19: PEGMA-<i>b</i>-DMAEMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA 20-25-30-25								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.2	1640	5.5	8.5	4.4	0.01	23.4	27.0
DMAEMA	0.25	2050	13.0	6.0	5.5	0.04	24.1	33.3
BuMA	0.3	2460	17.3	7.5	6.7	0.05	26.6	33.8
DMAEMA	0.25	2050	13.0	6.0	5.6	0.04	27.6	34.2
target MM		8200		Total:	22.7			
PB20: PEGMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA 20-30-50								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax

PEGMA	0.2	1640	5.5	8.46	4.4	0.02	20.8	26.2
BuMA	0.3	2460	17.3	7.45	6.7	0.05	23.0	32.0
DMAEMA	0.5	4100	26.1	11.90	11.1	0.07	26.2	40.7
target MM		8200		Total:	22.7			
PB21: PEGMA-<i>b</i>-BuMA-<i>b</i>-PEGMA-<i>b</i>-DMAEMA 10-35-10-45								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.1	820	2.7	4.2	2.2	0.007	25.1	27.6
BuMA	0.35	2870	20.2	8.7	7.8	0.05	26.1	37
PEGMA	0.1	820	2.7	4.2	2.2	0.007	29.9	31.3
DMAEMA	0.45	3690	23.5	10.7	10.0	0.06	27.9	38
target MM		8200		Total:	22.7			
PB22: BuMA-<i>b</i>-PEGMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA 17.5-20-17.5-45								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.47	0.003	To	Tmax
BuMA	0.175	1435	10.09	4.4	3.9	0.03	24.7	33
PEGMA	0.2	1640	5.5	8.5	4.4	0.01	29.5	32.8
BuMA	0.175	1435	10.1	4.4	3.9	0.03	28.5	34.2
DMAEMA	0.45	3690	23.5	10.7	10.0	0.06	29.7	43.5
target MM		8200		Total:	22.7			
PB23: DMAEMA-<i>b</i>-PEGMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA 22.5-20-35-22.5								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax
DMAEMA	0.225	1845	11.7	5.4	5.0	0.03	25.8	37.0
PEGMA	0.2	1640	5.5	8.5	4.4	0.02	29.0	33.5
BuMA	0.35	2870	20.2	8.7	7.8	0.06	28.6	40.0

DMAEMA	0.225	1845	11.7	5.4	5.0	0.03	31.9	36.9
target MM		8200		Total:	22.7			
PB24: PEGMA-<i>b</i>-DMAEMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA 20-22.5-35-22.5								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.2	1640	5.5	8.5	4.4	0.02	27	31.7
DMAEMA	0.225	1845	11.7	5.4	5.0	0.03	28.5	36.6
BuMA	0.35	2870	20.2	8.7	7.8	0.06	29.6	40.6
DMAEMA	0.225	1845	11.7	5.4	5.0	0.03	33.4	39.9
target MM		8200		Total:	22.7			
PB25: PEGMA-<i>b</i>-BuMA-<i>b</i>-DMAEMA 20-35-45								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.2	1640	5.5	8.5	4.4	0.02	24	29.27
BuMA	0.35	2870	20.2	8.7	7.8	0.06	26.1	38.8
DMAEMA	0.45	3690	23.5	10.7	10.0	0.06	30	43.4
target MM		8200		Total:	22.7			

Chapter 5.3: DEGMA-based								
PC1: PEGMA- <i>b</i> -BuMA- <i>b</i> -DEGMA 50-25-25								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				46.9				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.50	4100	13.7	17.3	9.1	0.03	24.5	35
BuMA	0.25	2050	14.4	5.1	4.5	0.03	27.5	33.4
DEGMA	0.25	2050	10.9	4.4	4.5	0.02	27.4	--
target MM		8200		Total:	18.5			
PC2: PEGMA- <i>b</i> -BuMA- <i>b</i> -DEGMA 45-25-30								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				47.8				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.45	3690	12.3	15.6	8.2	0.03	--	--
BuMA	0.25	2050	14.4	5.1	4.5	0.03	--	--
DEGMA	0.30	2460	13.1	5.3	5.4	0.03	--	--
target MM		8200		Total:	18.5			
PC3: PEGMA- <i>b</i> -BuMA- <i>b</i> -DEGMA 40-25-35								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				48.6				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.4	3280	10.9	13.8	7.3	0.02	25	32.7
BuMA	0.25	2050	14.4	5.1	4.5	0.03	--	--
DEGMA	0.35	2870	15.2	6.2	6.4	0.03	28.4	34
target MM		8200		Total:	18.5			
PC4: PEGMA- <i>b</i> -BuMA- <i>b</i> -DEGMA 50-30-20								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				46.8				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.5	4100	13.7	17.3	9.1	0.03	24.5	31.0

BuMA	0.3	2460	17.3	6.1	5.4	0.04	26.8	31.6
DEGMA	0.2	1640	8.7	3.6	3.6	0.02	27.9	30.3
target MM		8200		Total:	18.5			
PC5: PEGMA-<i>b</i>-BuMA-<i>b</i>-DEGMA 45-30-25								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				47.6				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.45	3690	12.3	15.6	8.2	0.03	25.5	33.2
BuMA	0.30	2460	17.3	6.1	5.4	0.04	28.3	33.4
DEGMA	0.25	2050	10.9	4.5	4.5	0.02	29.1	33.4
target MM		8200		Total:	18.5			
PC6: PEGMA-<i>b</i>-BuMA-<i>b</i>-DEGMA 40-30-30								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				48.5				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.4	3280	10.9	13.8	7.3	0.02	24.9	32.2
BuMA	0.3	2460	17.3	6.1	5.4	0.04	27.3	33.4
DEGMA	0.3	2460	13.1	5.3	5.4	0.03	28.6	34.1
target MM		8200		Total:	18.5			
PC7: PEGMA-<i>b</i>-BuMA-<i>b</i>-DEGMA 35-30-35								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				60.3				
MTS				0.55			To	Tmax
PEGMA	0.35	2870	9.6	14.8	7.8	0.003	26.8	33.8
BuMA	0.3	2460	17.3	7.4	6.7	0.03	29.9	36.6
DEGMA	0.35	2870	15.3	7.6	7.8	0.05	31.8	38.1
target MM		8200		Total:	22.7	0.04		

PC8: PEGMA- <i>b</i> -BuMA- <i>b</i> -DEGMA 30-30-40								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				61.3				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.3	2460	8.2	12.7	6.7	0.02	25.6	32.2
BuMA	0.3	2460	17.3	7.4	6.7	0.05	28.1	36.2
DEGMA	0.4	3280	17.4	8.7	8.9	0.05	30	40.5
target MM		8200		Total:	22.7			
PC9: PEGMA- <i>b</i> -BuMA- <i>b</i> -DEGMA 25-30-45								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				62.3				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.25	2050	6.8	10.6	5.6	0.02	26.5	32.0
BuMA	0.3	2460	17.3	7.4	6.7	0.05	28.9	37.4
DEGMA	0.45	3690	19.6	9.8	10.0	0.05	30.7	41.5
target MM		8200		Total:	22.7			
PC10: PEGMA- <i>b</i> -BuMA- <i>b</i> -DEGMA 50-35-15								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				57.0				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.5	4100	13.7	21.1	11.1	0.04	27.5	35.0
BuMA	0.35	2870	20.2	8.7	7.8	0.06	31	--
DEGMA	0.15	1230	6.5	3.3	3.3	0.02	32.8	35.1
target MM		8200		Total:	22.7			
PC11: PEGMA- <i>b</i> -BuMA- <i>b</i> -DEGMA 45-35-20								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				58.1				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.45	3690	12.3	19.03	10.0	0.03	28.1	36.6

BuMA	0.35	2870	20.2	8.7	7.8	0.06	31.0	38.5
DEGMA	0.2	1640	8.7	4.4	4.4	0.02	33.5	37.4
target MM		8200		Total:	22.7			
PC12: PEGMA-<i>b</i>-BuMA-<i>b</i>-DEGMA 40-35-25								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				59.1				
MTS				0.55	0.5	0.003	To	Tmax
PEGMA	0.4	3280	10.9	16.9	8.9	0.03		
BuMA	0.35	2870	20.2	8.7	7.8	0.06		
DEGMA	0.25	2050	10.9	5.4	5.6	0.03		
target MM		8200		Total:	22.7			
PC13: PEGMA-<i>b</i>-BuMA-<i>b</i>-PEGMA 35-30-35								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				43.4				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.35	2870	9.566667	12.1	6.3	0.02	26.1	31.8
BuMA	0.3	2460	17.29958	6.1	5.4	0.04	28.2	35.4
PEGMA	0.35	2870	9.566667	12.1	6.4	0.02	25	--
target MM		8200		Total:	18.5			
PC14: DEGMA-<i>b</i>-BuMA-<i>b</i>-DEGMA 35-30-35								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				55.2				
MTS				0.45	0.4	0.002	To	Tmax
DEGMA	0.35	2870	15.2	6.2	6.4	0.03	24.6	--
BuMA	0.30	2460	17.3	6.1	5.4	0.04	26.4	30.15
DEGMA	0.35	2870	15.2	6.2	6.4	0.03	27.9	32.5
target MM		8200		Total:	18.5			

PC15: DEGMA- <i>co</i> -BuMA- <i>co</i> -DEGMA 45-30-25								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				47.6				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.45	3690	12.3	15.6	8.2	0.03	--	--
BuMA	0.3	2460	17.3	6.1	5.4	0.04	--	--
DEGMA	0.25	2050	10.9	4.5	4.5	0.02	--	--
target MM		8200		Total:	18.5			

Chapter 5.4: Mimicking Pluronic® F127								
PD1: PEGMA- <i>b</i> -diPGMA- <i>b</i> -PEGMA 40-20-40								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				28.4				
MTS				0.49	0.4	0.002	To	Tmax
PEGMA	0.4	2000	6.67	9.2	4.8	0.02	--	33.6
diPGMA	0.2	1000	4.63	2.6	2.4	0.01	28.3	31.7
PEGMA	0.4	2000	6.67	9.2	4.8	0.02	27.5	33.2
target MM		5000		Total:	12.48			
PD2: PEGMA- <i>b</i> -diPGMA- <i>b</i> -PEGMA 35-30-35								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				29.4				
MTS				0.49	0.42	0.002	To	Tmax
PEGMA	0.35	1750	5.8	8.04	4.22	0.014	29.6	32.6
diPGMA	0.3	1500	6.9	4.0	3.62	0.017	26.8	32.6
PEGMA	0.35	1750	5.8	8.04	4.22	0.014	27.3	31.9
target MM		5000		Total:	12.48			
PD3: PEGMA- <i>b</i> -diPGMA- <i>b</i> -PEGMA 30-40-30								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		

THF				30.4				
MTS				0.49	0.42	0.002	To	Tmax
PEGMA	0.3	1500	5.0	6.9	3.6	0.01	25	30
diPGMA	0.4	2000	9.25	5.3	4.8	0.02	26.8	33
PEGMA	0.3	1500	5.0	6.9	3.6	0.01	27.6	30.9
target MM		5000		Total:	12.48			
PD4: PEGMA-<i>b</i>-diPGMA-<i>b</i>-PEGMA 40-20-40								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				36.3				
MTS				0.32	0.3	0.002	To	Tmax
PEGMA	0.4	4000	13.3	12.0	6.3	0.02	24.7	33.6
diPGMA	0.2	2000	9.3	3.4	3.2	0.02	27.5	30.5
PEGMA	0.4	4000	13.3	12.0	6.3	0.02	26.8	32.0
target MM		10000		Total:	16.03			
PD5: PEGMA-<i>b</i>-diPGMA-<i>b</i>-PEGMA 35-30-35								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				37.6				
MTS				0.32	0.28	0.002	To	Tmax
PEGMA	0.35	3500	11.7	10.5	5.5	0.02	24.3	30.7
diPGMA	0.3	3000	13.9	5.16	4.7	0.02	26.0	30.3
PEGMA	0.35	3500	11.7	10.50	5.5	0.02	26.4	30.3
target MM	10000			Total:	16.0			
PD6: PEGMA-<i>b</i>-diPGMA-<i>b</i>-PEGMA 30-40-30								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				38.9				
MTS				0.32	0.3	0.001575	To	Tmax
PEGMA	0.3	3000	10.0	9.0	4.7	0.02	24.3	30.4
diPGMA	0.4	4000	18.5	6.9	6.3	0.03	25.4	32
PEGMA	0.3	3000	10.0	9.0	4.7	0.02	27.9	30.5

target MM		10000		Total:	16.03			
PD7: PEGMA-<i>b</i>-diPGMA-<i>b</i>-PEGMA 40-20-40								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				66.1				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.4	5200	17.3	21.9	11.5	0.04	--	--
diPGMA	0.2	2600	12.0	6.3	5.8	0.03	--	--
PEGMA	0.4	5200	17.3	21.9	11.5	0.04	--	--
target MM		13000		Total:	29.2			
PD8: PEGMA-<i>b</i>-diPGMA-<i>b</i>-PEGMA 35-30-35								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				68.5				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.35	4550	15.2	19.2	10.1	0.03	--	--
diPGMA	0.3	3900	18.1	9.4	8.6	8.6	--	--
PEGMA	0.35	4550	15.2	19.2	10.1	10.1	--	--
target MM		13000		Total:	29.2			
PD9: PEGMA-<i>b</i>-diPGMA-<i>b</i>-PEGMA 30-40-30								
Chemical Substance	Target wt%	MM block	DP	mL	g	Moles		
THF				70.8				
MTS				0.45	0.4	0.002	To	Tmax
PEGMA	0.3	3900	13.0	16.5	8.6	0.03	--	--
diPGMA	0.4	5200	24.1	12.6	11.5	0.05	--	--
PEGMA	0.3	3900	13.0	16.5	8.6	0.03	--	--
target MM		13000		Total:	29.2			

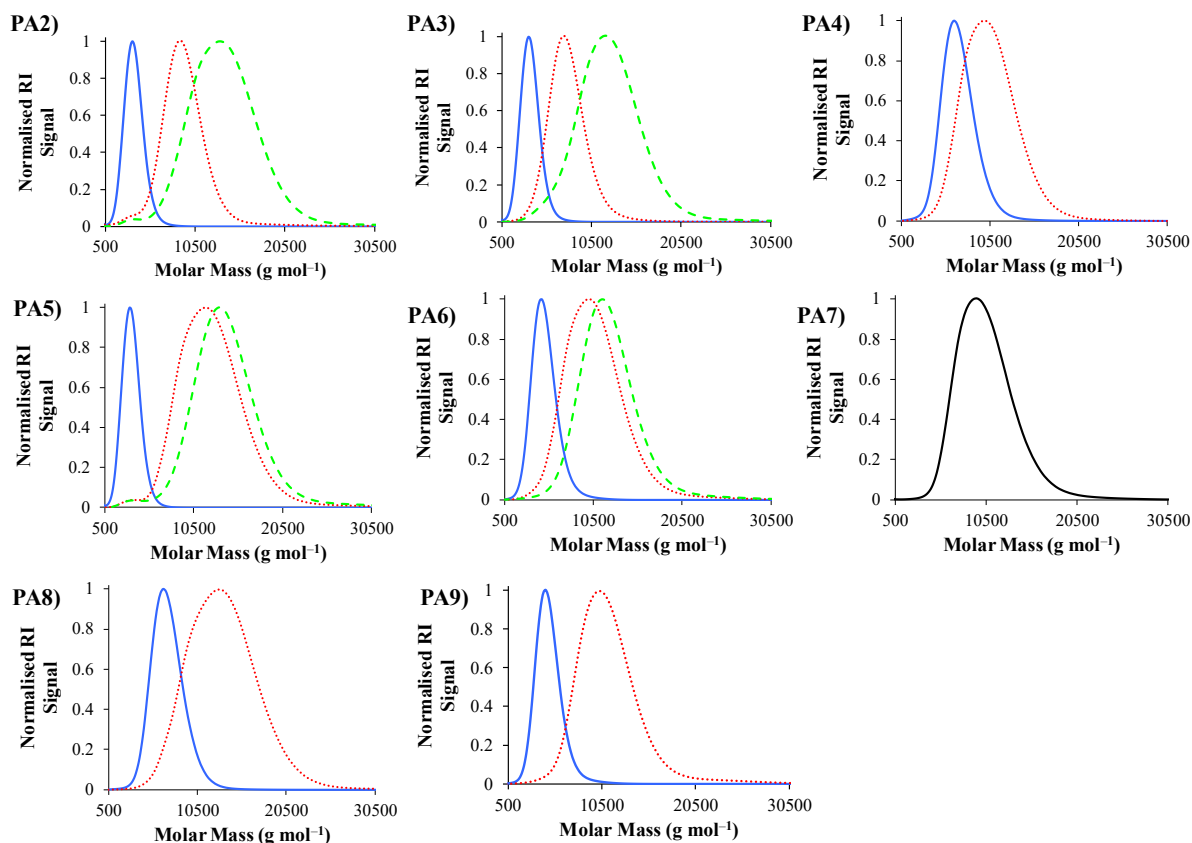


Figure A1: GPC chromatograms that are not presented in the main report: i) the triblock terpolymers (PA2 and PA3), the diblock terpolymers (PA4-PA6), the statistical terpolymer (PA7), and the diblock bipolymers (PA8 and PA9). The GPC traces of the first block, the diblock, and the triblock (if there is one) copolymers are shown in solid blue, red dotted, and green dashed lines, respectively. The GPC trace of the statistical terpolymer is coloured in black. Reproduced from “Constantinou, A. P.; Zhao, H.; McGilvery, C. M.; Porter, A. E.; Georgiou, T. K. *Polymers* **2017**, 9, 31.”

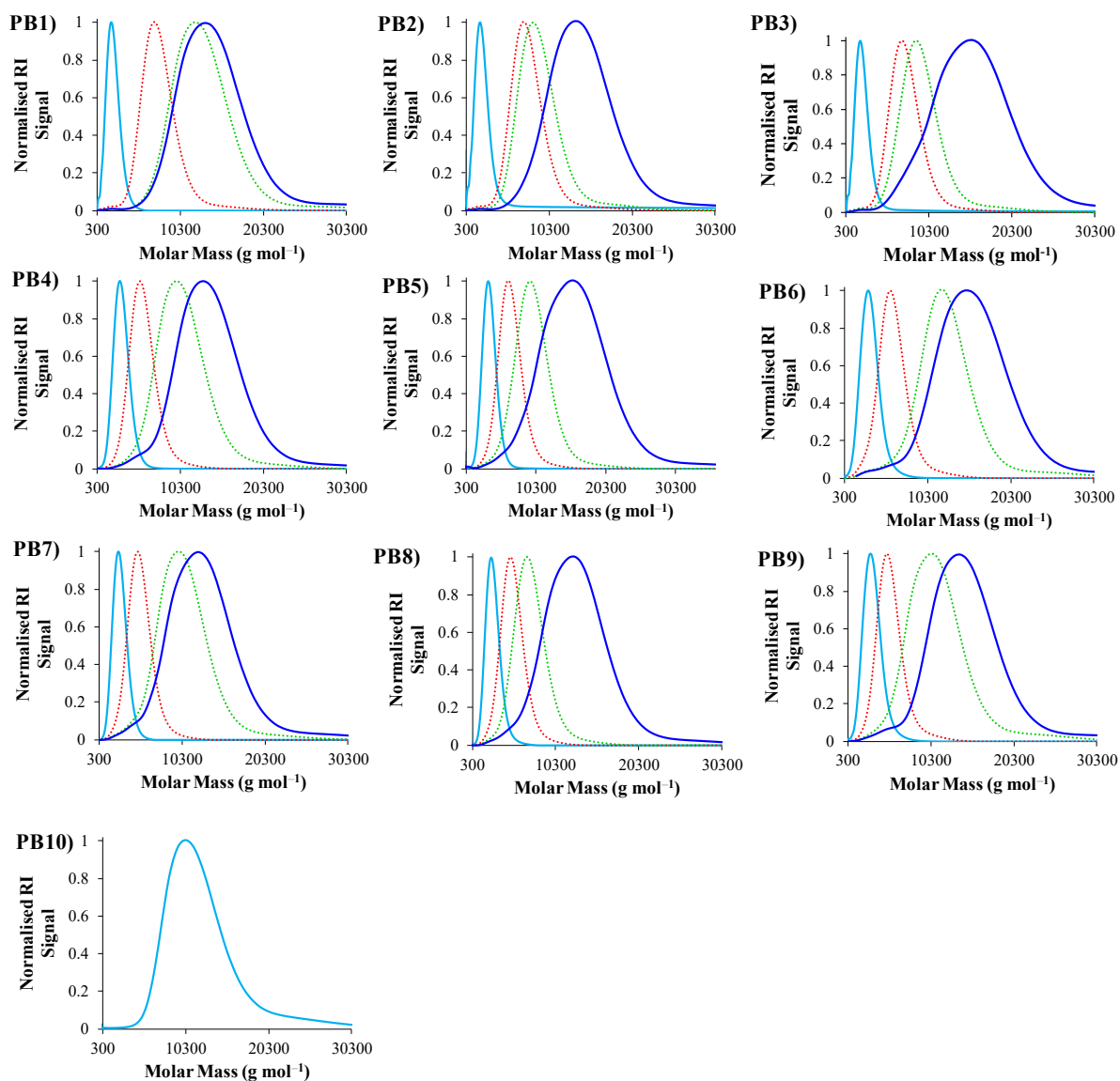


Figure A2 (a): GPC chromatograms of PB1-PB10. PB10 is a statistical terpolymer. Concerning the GPC traces of the tetrablock terpolymers, the homopolymer, diblock copolymer, triblock copolymer and the final tetrablock terpolymer are shown in light blue solid line, red dotted line, green dotted line, and blue solid line, respectively.

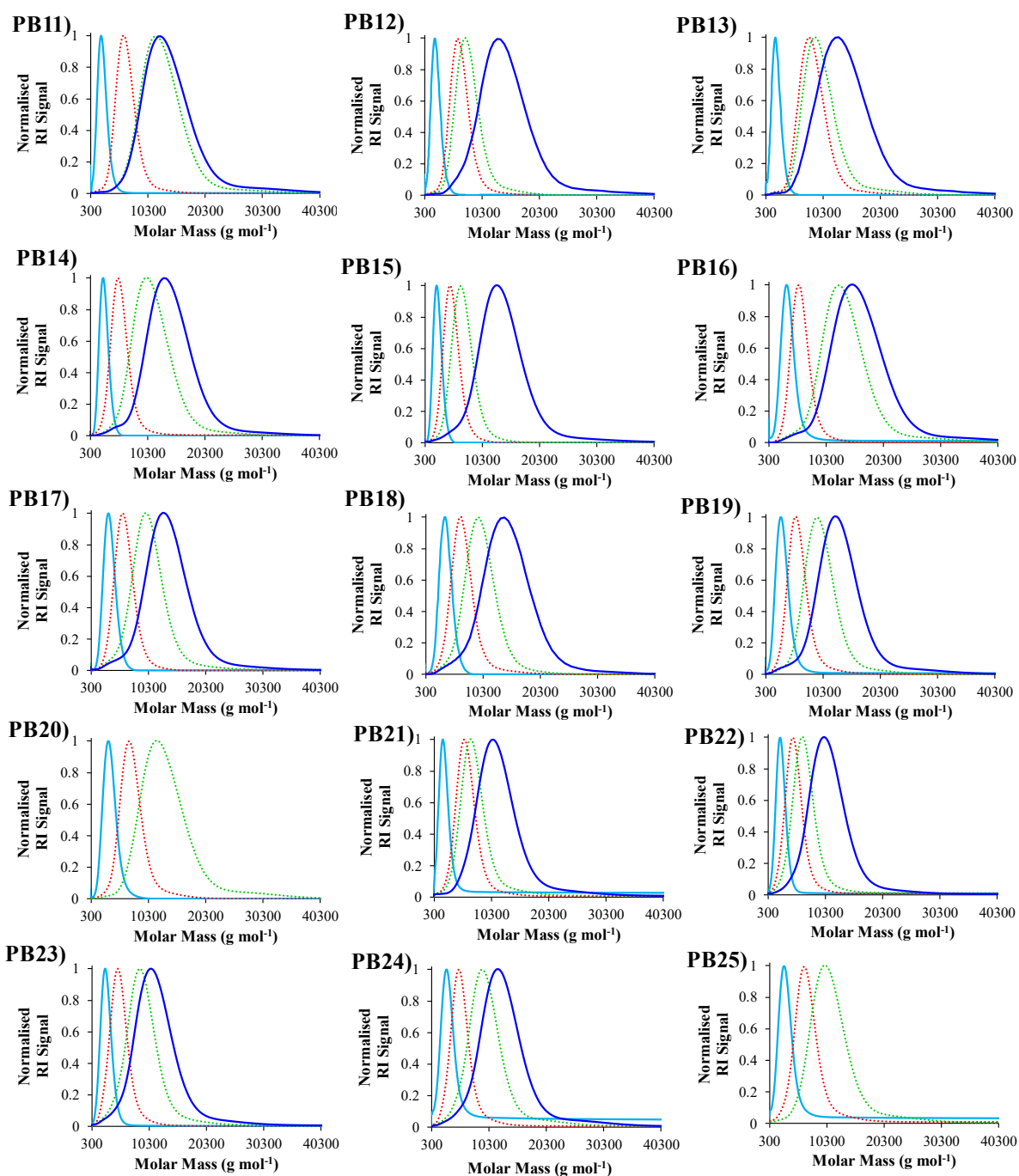


Figure A2 (b): GPC chromatograms of PB11-PB25 Concerning the GPC traces of the tetrablock terpolymers, the homopolymer, diblock copolymer, triblock copolymer and the final tetrablock terpolymer are shown in light blue solid line, red dotted line, green dotted line, and blue solid line, respectively. Concerning the triblock terpolymers (PB20 and PB25), the homopolymer, diblock copolymer, and final triblock copolymer are shown in light blue solid, dotted red, and dotted green lines, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

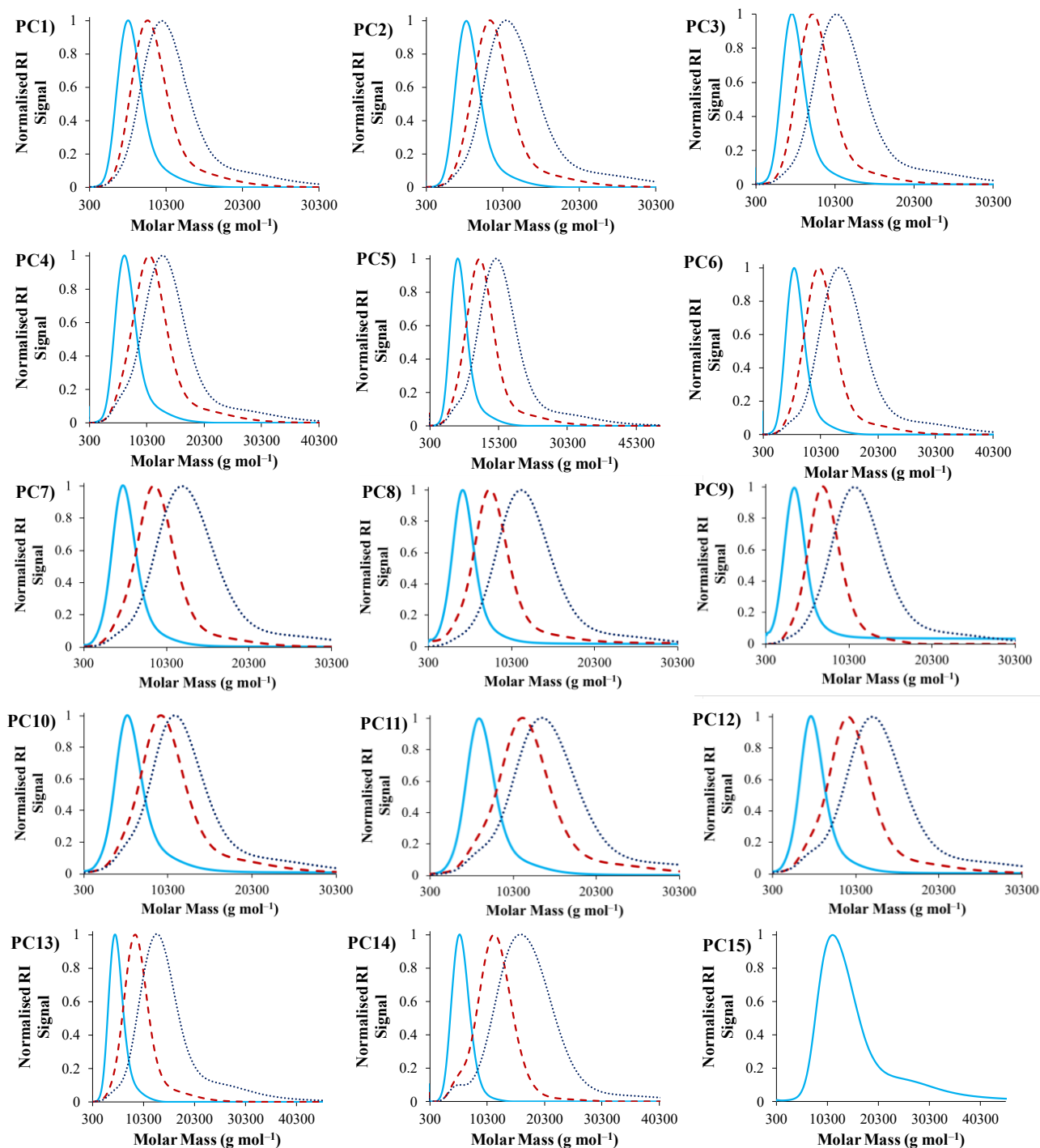


Figure A3: GPC chromatograms of PC1-PC15. Concerning the GPC traces of the triblock copolymers (PC1-PC14), the homopolymer, diblock copolymer, and final triblock copolymer are shown in light blue solid line, red dashed line, and dark blue dotted line, respectively. The GPC traces of the statistical terpolymer (PC15) is shown in light blue dotted line.

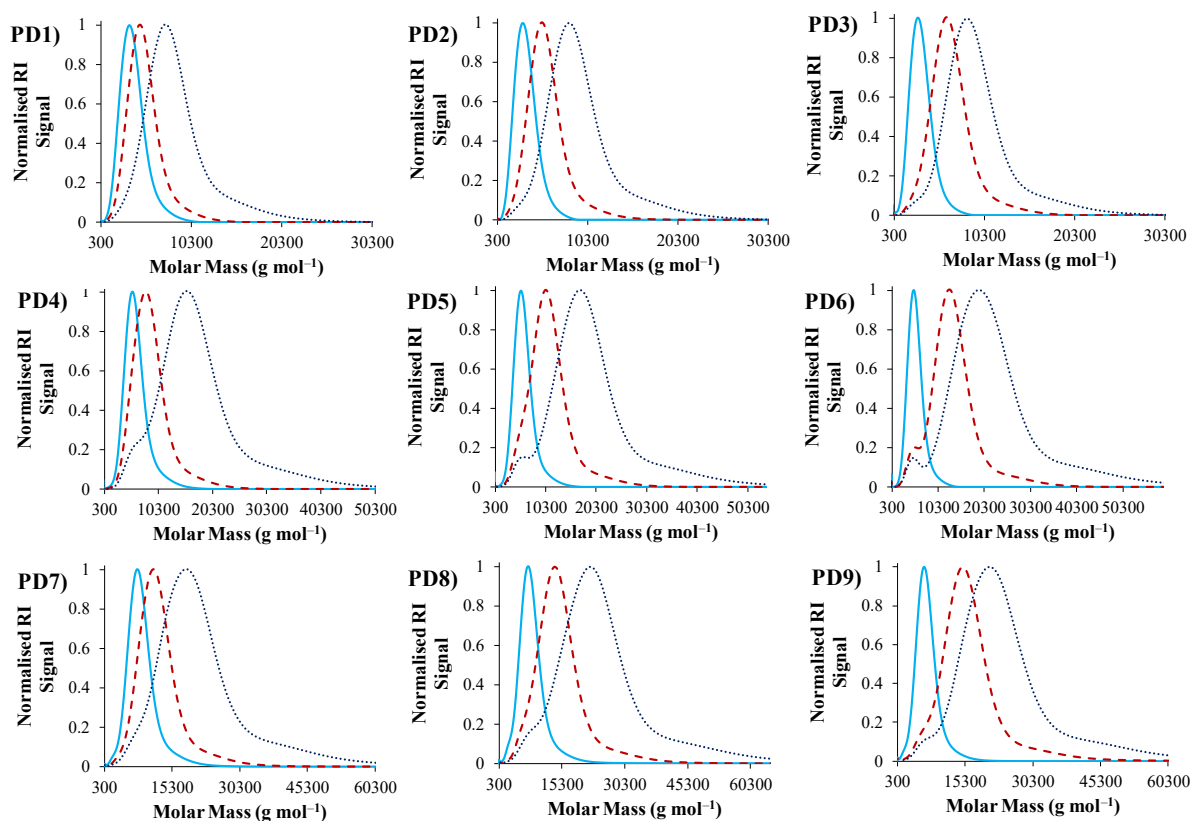


Figure A4: GPC chromatograms of PD1-PD9: the homopolymer, diblock copolymer, and final triblock copolymer are shown in light blue solid line, red dashed line, and dark blue dotted line, respectively.

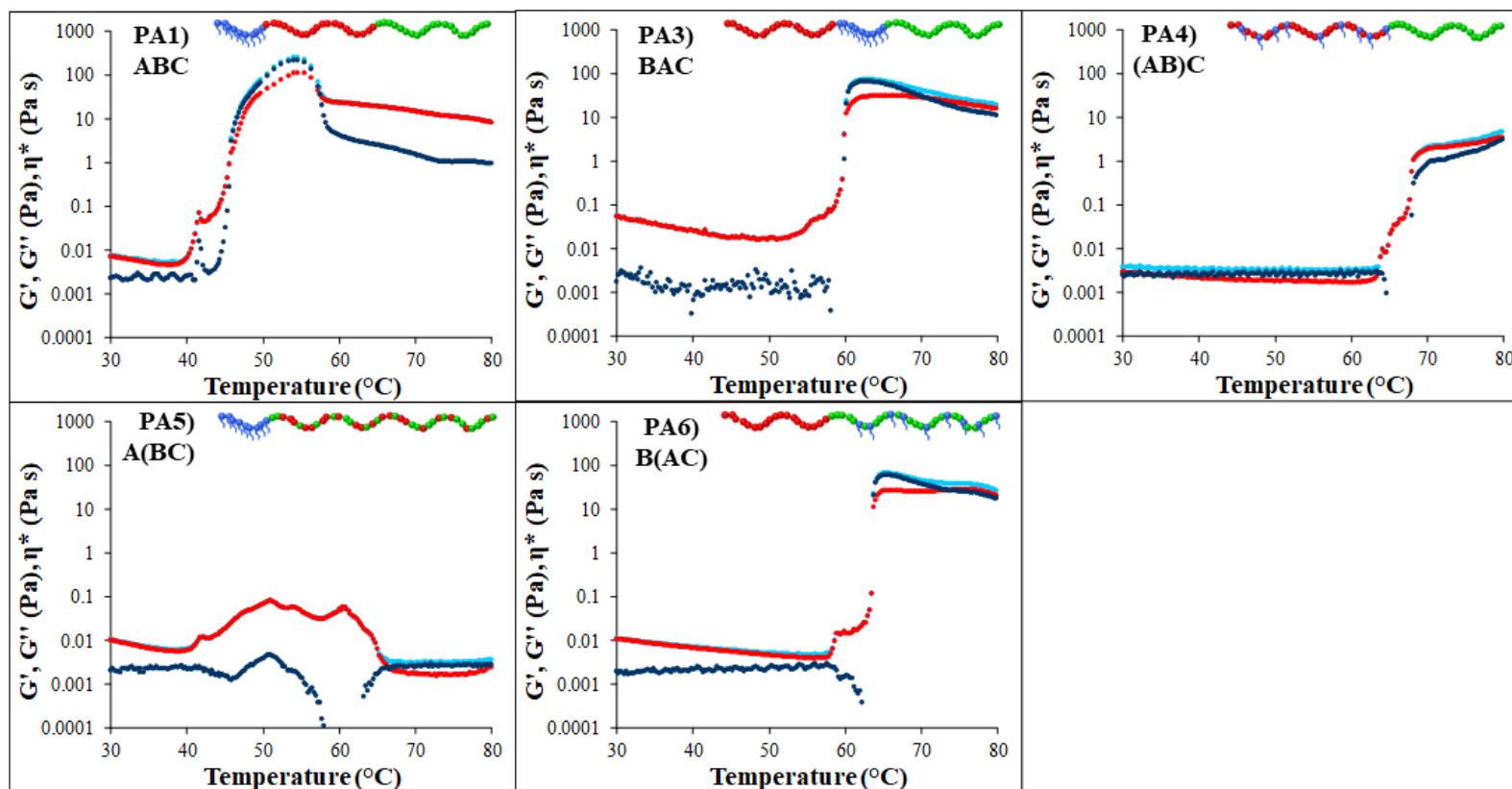


Figure A5: Rheological curves of the 15 w/w% terpolymer solutions in phosphate buffered saline (PBS) of the soluble block terpolymer solutions of Chapter 5.1, as resulted from temperature ramp measurements. The changes in the shear storage modulus (elastic modulus, G'), shear loss modulus (viscous modulus, G''), and the complex viscosity (η^*) are shown in dark blue, red, and light blue, respectively. The polymer structures are also shown with blue, red, and light green spheres representing the TEGMA, BuMA, and DMAEMA units, respectively

Architecture Effect

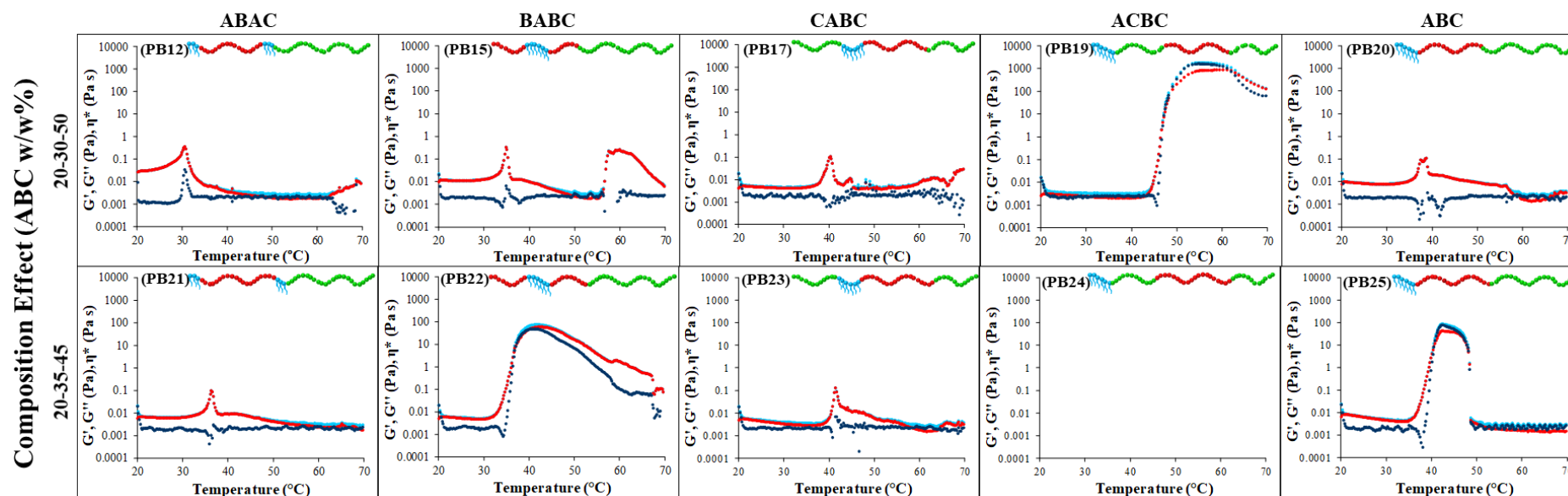


Figure A6: Rheological curves of the 15 w/w% terpolymer solutions in phosphate buffered saline (PBS) of the soluble block terpolymer solutions of Chapter 5.2, as resulted from temperature ramp measurements. The changes in the shear storage modulus (elastic modulus, G'), shear loss modulus (viscous modulus, G''), and the complex viscosity (η^*) are shown in dark blue, red, and light blue, respectively. In this Figure, the following PEGMA-BuMA-DMAEMA (ABC) compositions are presented: 20-30-50 w/w% (top), and 20-35-45 w/w% (bottom). From the most hydrophobic family, i.e. 20-40-40 w/w%, only PB7 is soluble, and the result is presented in the main report. The architecture effect is shown from the left to the right, while the composition effect is shown from the top to the bottom. The corresponding polymer structures are schematically illustrated with light blue, red, and light green representing the PEGMA, BuMA, and DMAEMA units, respectively. Reproduced, with permission, from “Constantinou, A. P.; Sam-Soon, N. F.; Carroll, D. R.; Georgiou, T. K. *Macromolecules* **2018**, *51*(18), 7019-7031, DOI: 10.1021/acs.macromol.8b01251.”

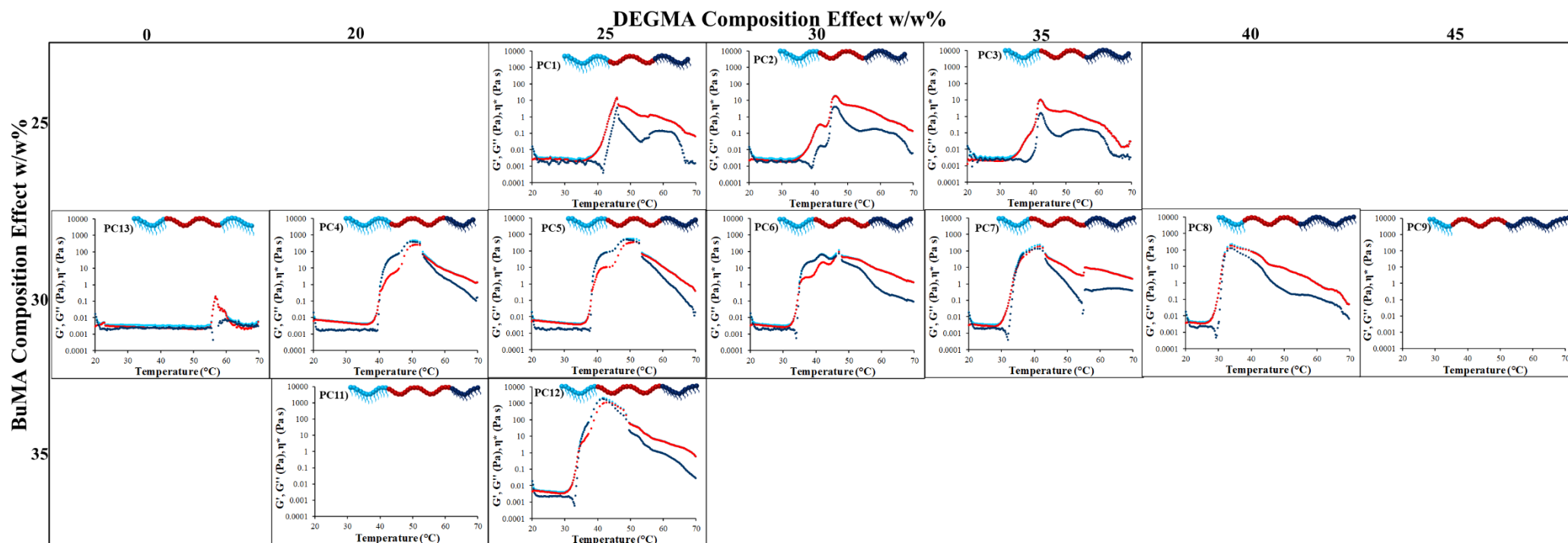


Figure A7: Rheological curves of the 15 w/w% copolymer solutions in phosphate buffered saline (PBS) of Chapter 5.3, as resulted from temperature ramp measurements. The changes in the shear storage modulus (elastic modulus, G'), shear loss modulus (viscous modulus, G''), and the complex viscosity (η^*) are shown in dark blue, red, and light blue, respectively. The effect of DEGMA composition is shown from the left to the right, while the effect of BuMA composition is shown from the top to the bottom. The corresponding polymer structures are schematically illustrated with light blue, red, and dark blue representing the PEGMA, BuMA, and DEGMA units, respectively. The rheological results of PC11 are not presented as the copolymer is insoluble in aqueous media, while PC9 was not tested as it was precipitating while transferring it to be tested.

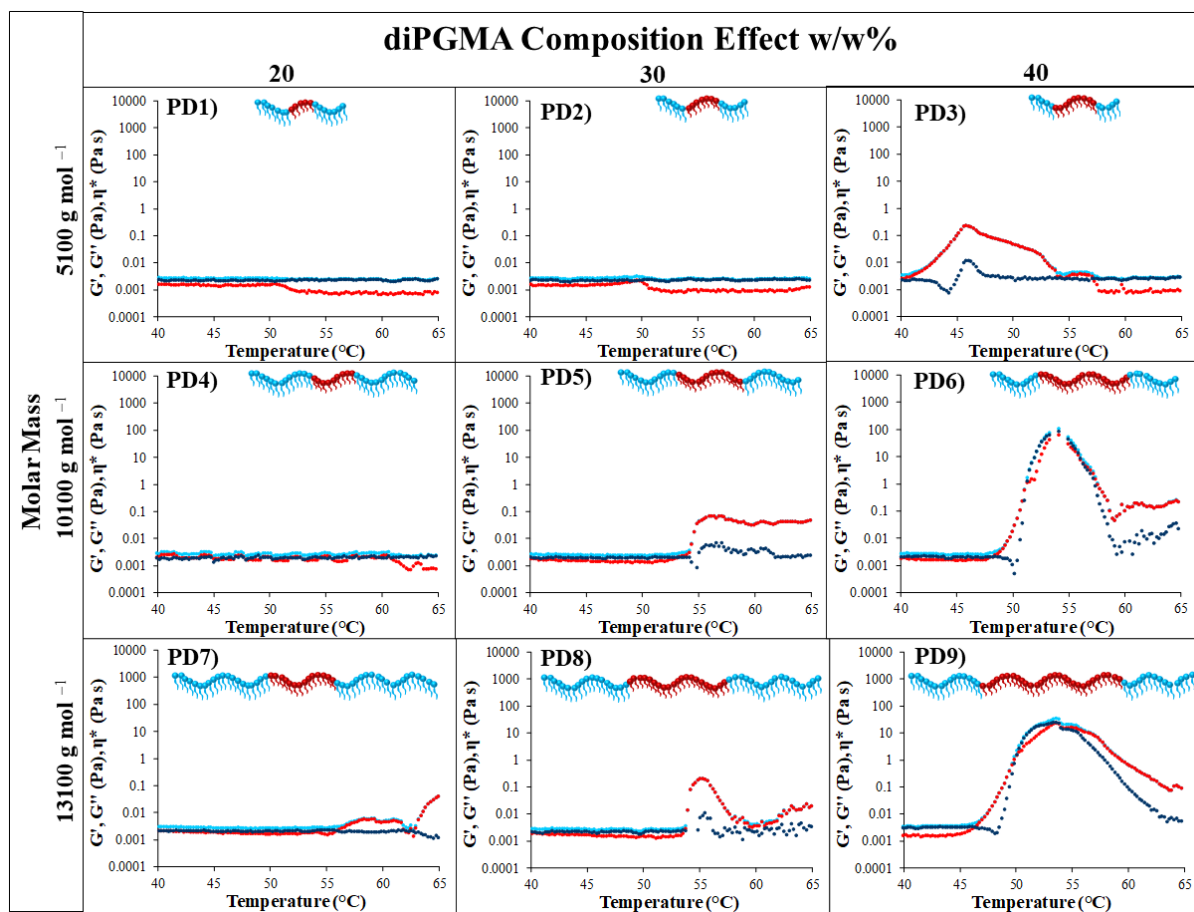


Figure A8: Rheological curves of the 15 w/w% copolymer solutions in phosphate buffered saline (PBS) of Chapter 5.4, as resulted from temperature ramp measurements. The changes in the shear storage modulus (elastic modulus, G'), shear loss modulus (viscous modulus, G''), and the complex viscosity (η^*) are shown in dark blue, red, and light blue, respectively. The effect of diPGMA composition is shown from the left to the right, while the effect of molar mass is shown from the top to the bottom. The corresponding polymer structures are schematically illustrated with light blue and dark red representing the PEGMA and diPGMA units, respectively.

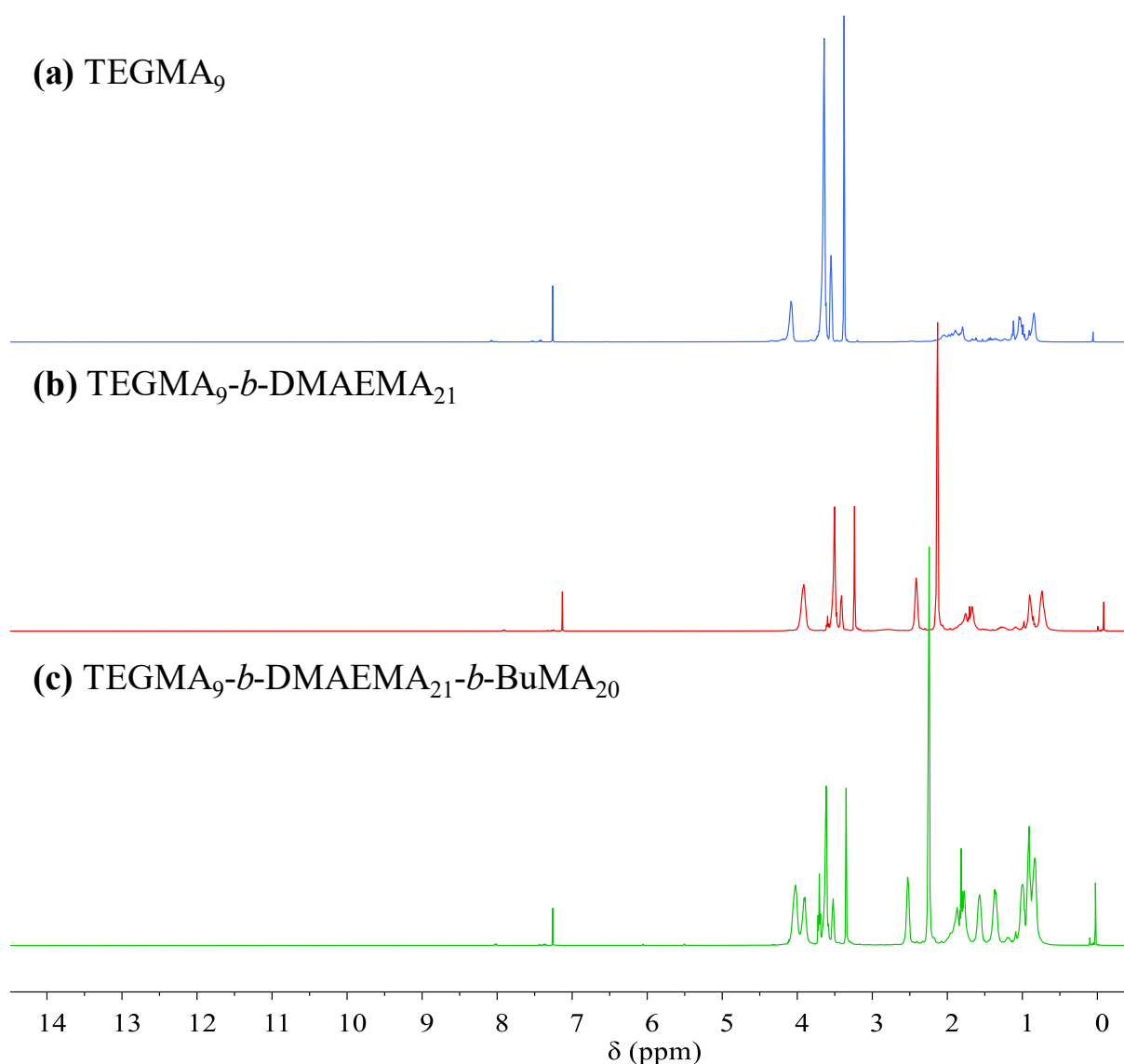


Figure A9.1: ^1H NMR spectra of (a) the TEGMA₉ homopolymer in blue, (b) the TEGMA₉-*b*-DMAEMA₂₁ diblock copolymer in red, and (c) the TEGMA₉-*b*-DMAEMA₂₁-*b*-BuMA₂₀ triblock terpolymer (Polymer A2) in light green. The analysis has been performed in a similar way as shown in Figure 5.1.5.

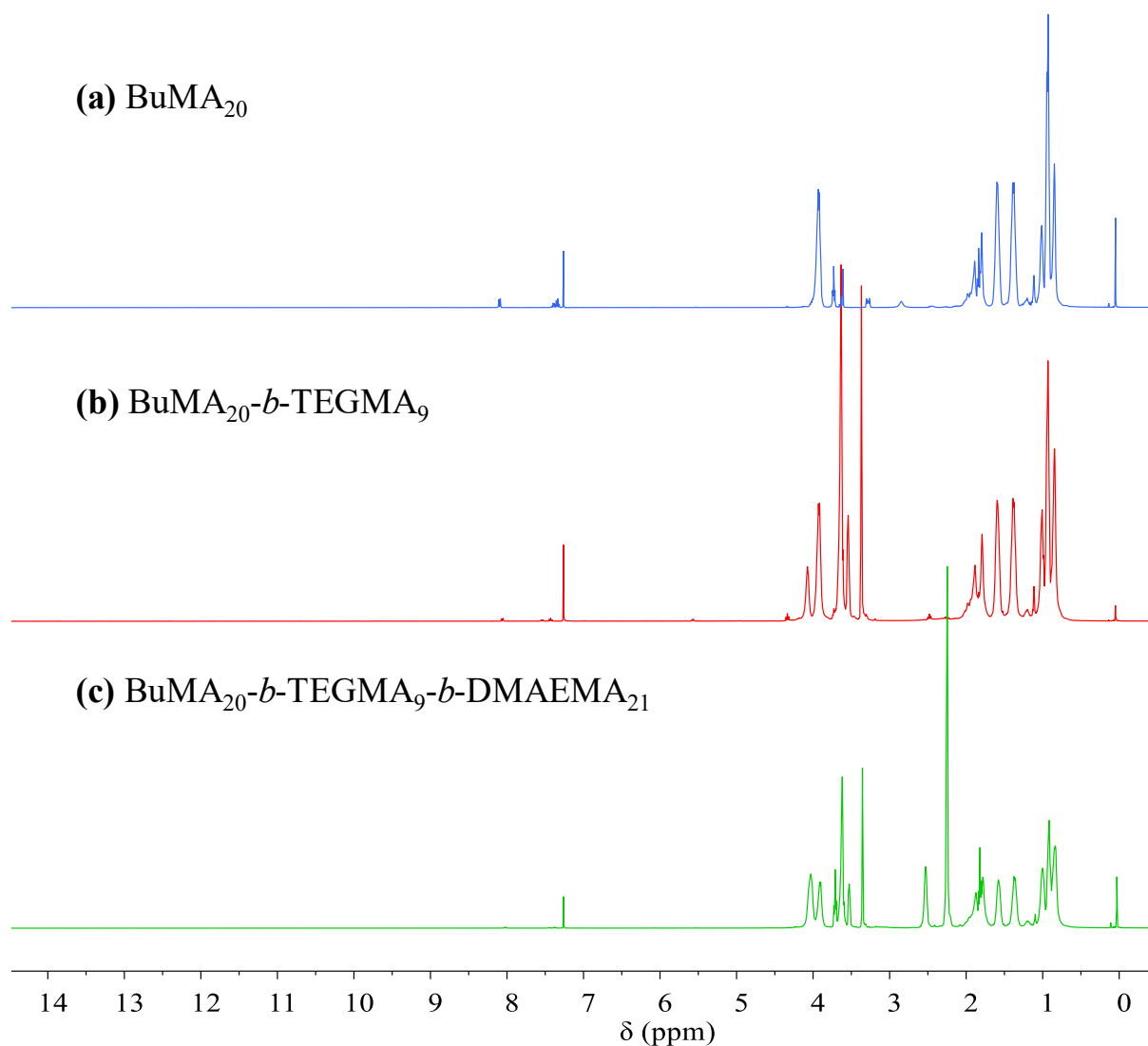


Figure A9.2: ¹H NMR spectra of (a) the BuMA₂₀ homopolymer in blue, (b) the BuMA₂₀-*b*-TEGMA₉ diblock copolymer in red, and (c) the BuMA₂₀-*b*-TEGMA₉-*b*-DMAEMA₂₁ triblock terpolymer (Polymer A3) in light green. The analysis has been performed in a similar way as shown in Figure 5.1.5.

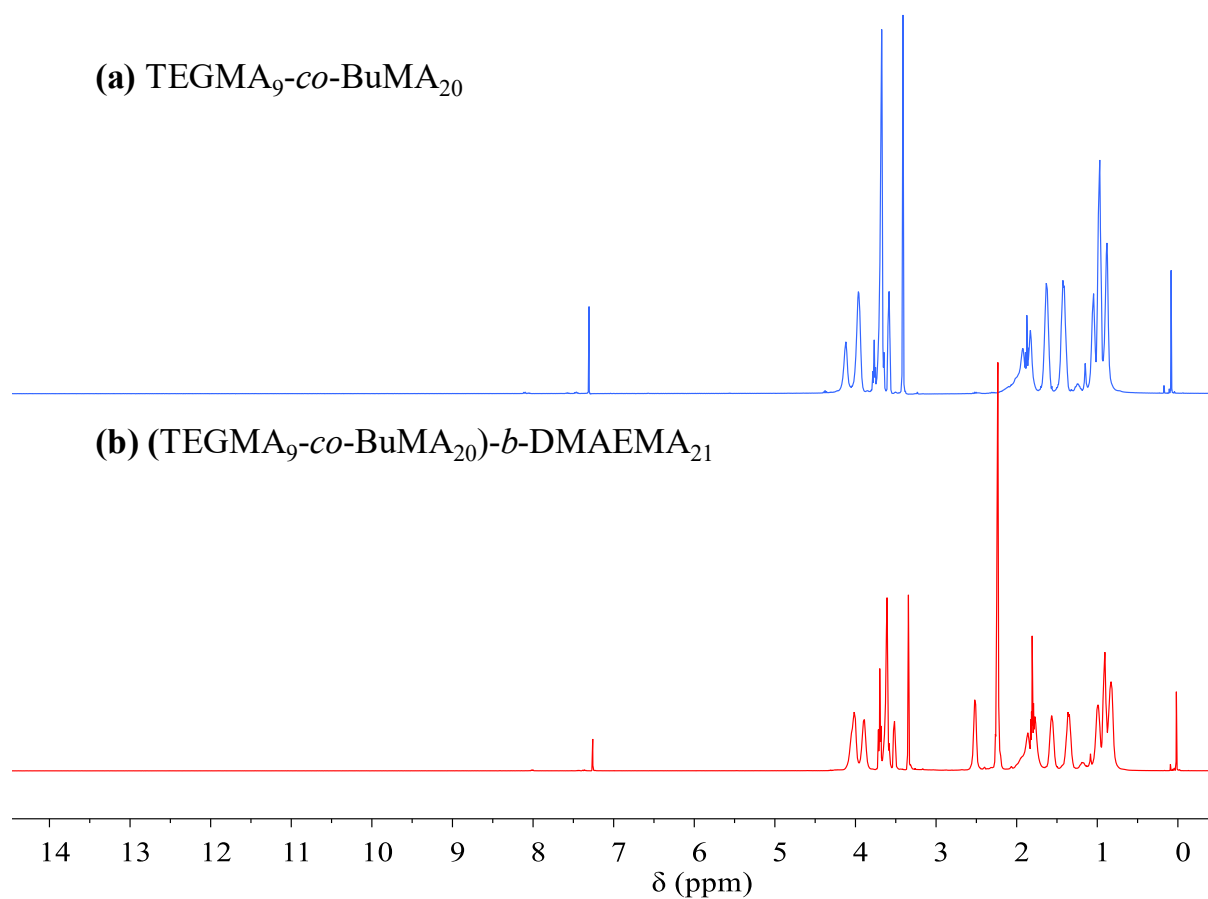


Figure A9.3: ^1H NMR spectra of (a) the TEGMA₉-*co*-BuMA₂₀ statistical copolymer in blue, and (b) the (TEGMA₉-*co*-BuMA₂₀)-*b*-DMAEMA₂₁ diblock terpolymer (Polymer A4) in red. The analysis has been performed in a similar way as shown in Figure 5.1.5.

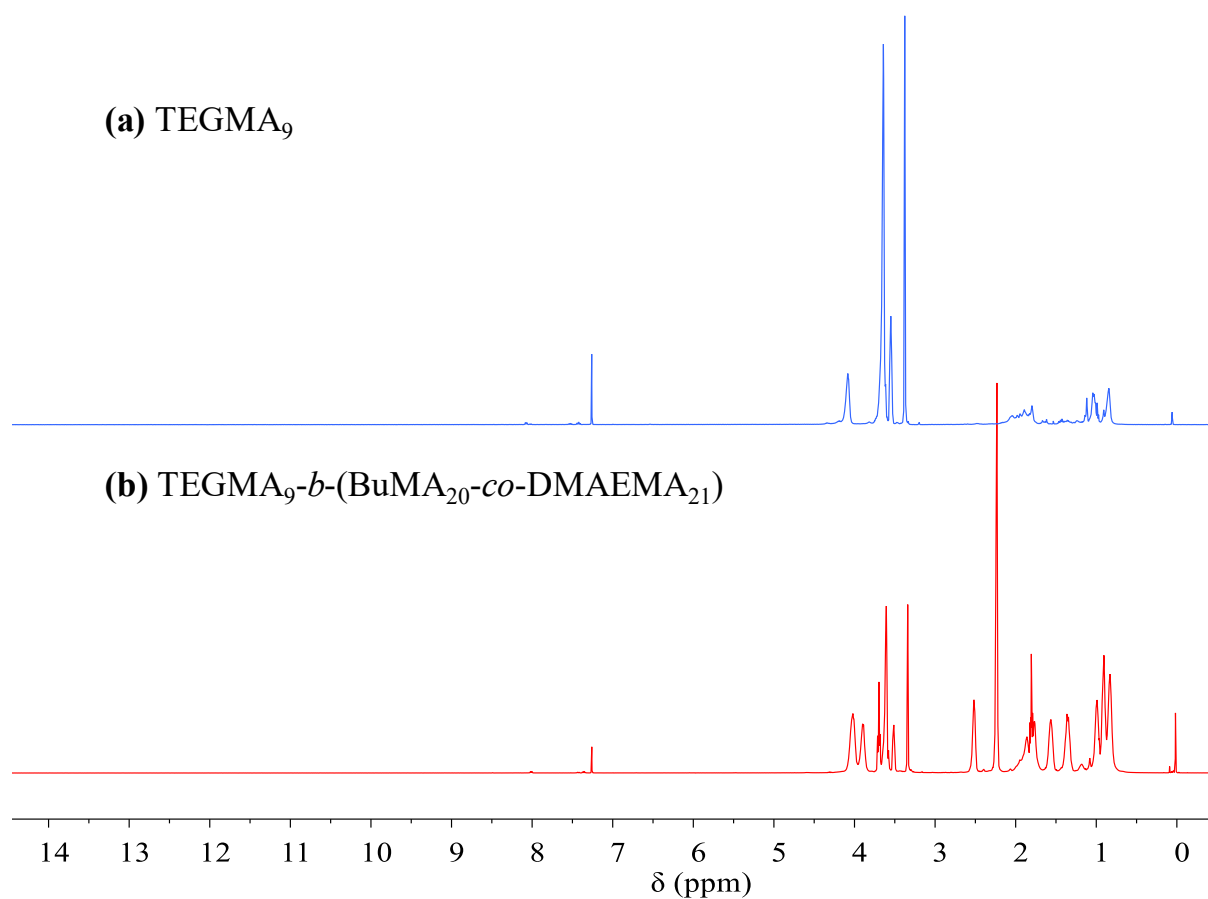


Figure A9.4: ¹H NMR spectra of (a) the TEGMA₉ homopolymer in blue, and (b) the TEGMA₉-*b*-(BuMA₂₀-*co*-DMAEMA₂₁) diblock terpolymer (Polymer A5) in red. The analysis has been performed in a similar way as shown in Figure 5.1.5.

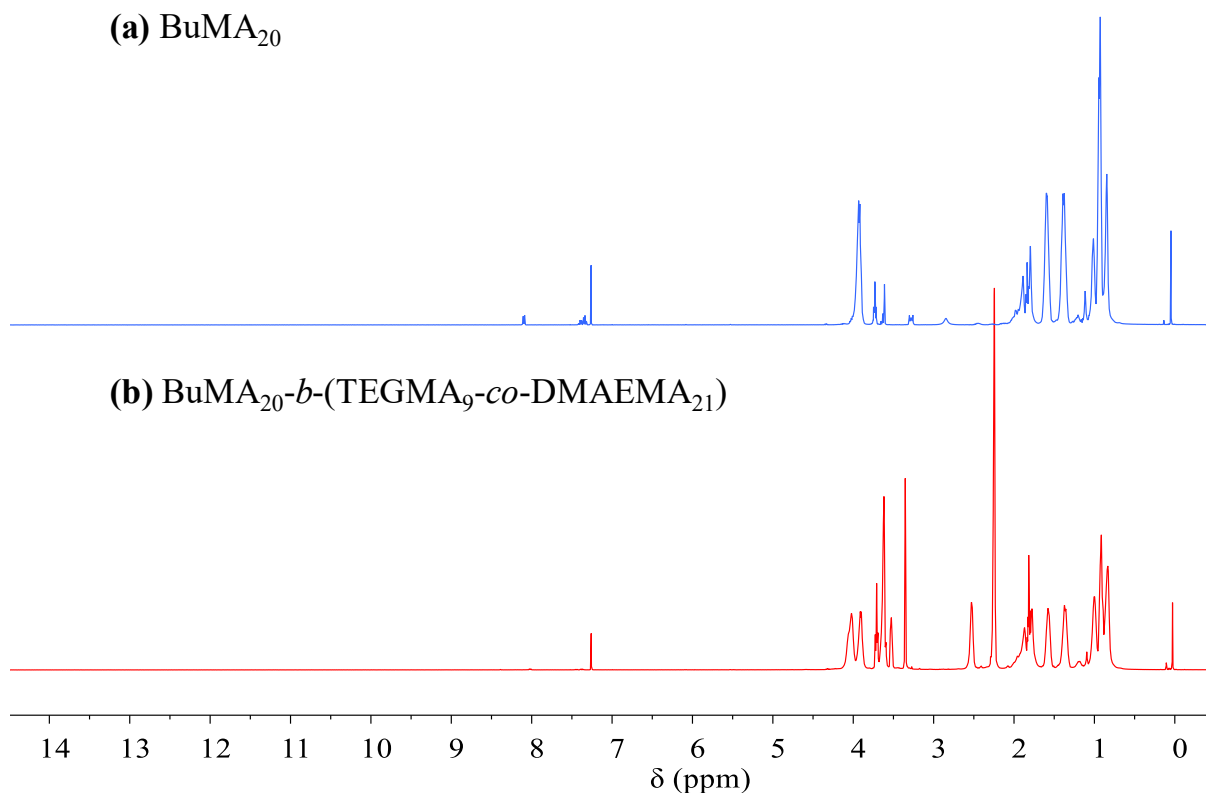


Figure A9.5: ¹H NMR spectra of (a) the BuMA₂₀ homopolymer in blue, and (b) the BuMA₂₀-*b*-(TEGMA₉-*co*-DMAEMA₂₁) diblock terpolymer (Polymer A6) in red. The analysis has been performed in a similar way as shown in Figure 5.1.5.

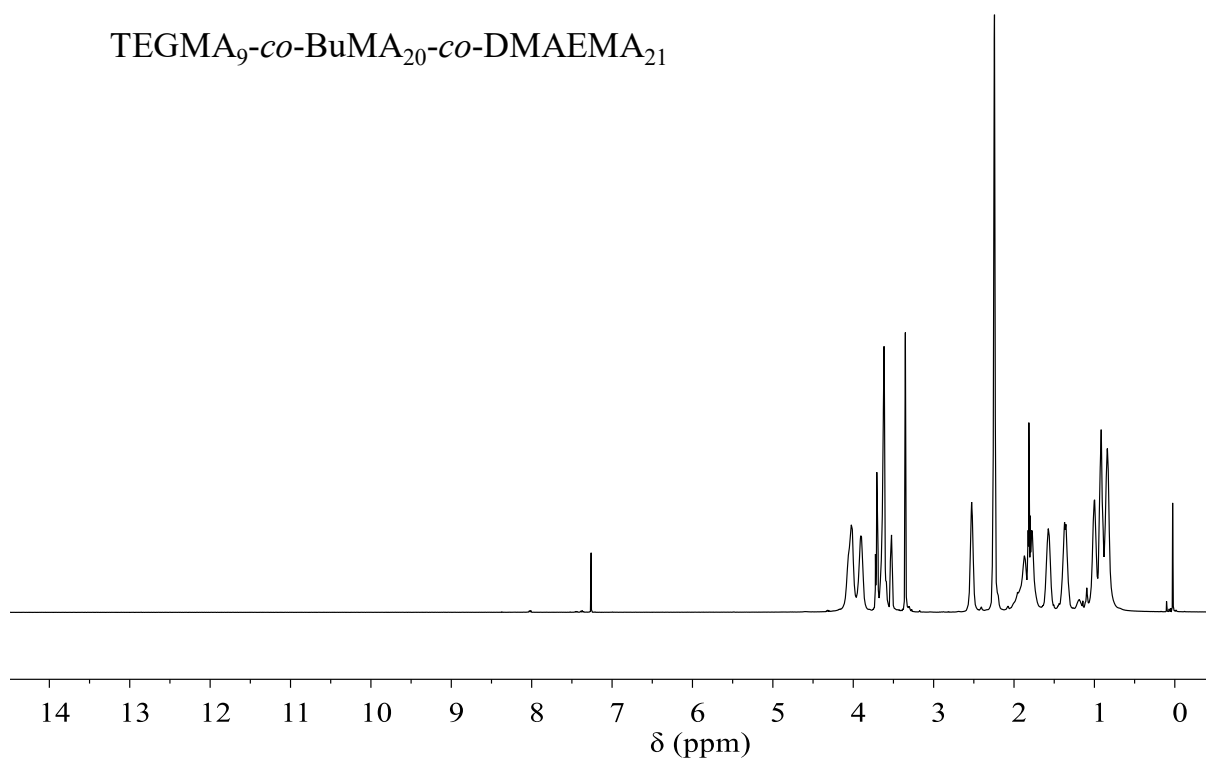


Figure A9.6: ¹H NMR spectrum of TEGMA₉-*co*-BuMA₂₀-*co*-DMAEMA₂₁ statistical terpolymer (Polymer A7) in black. The analysis has been performed in a similar way as shown in Figure 5.1.5.

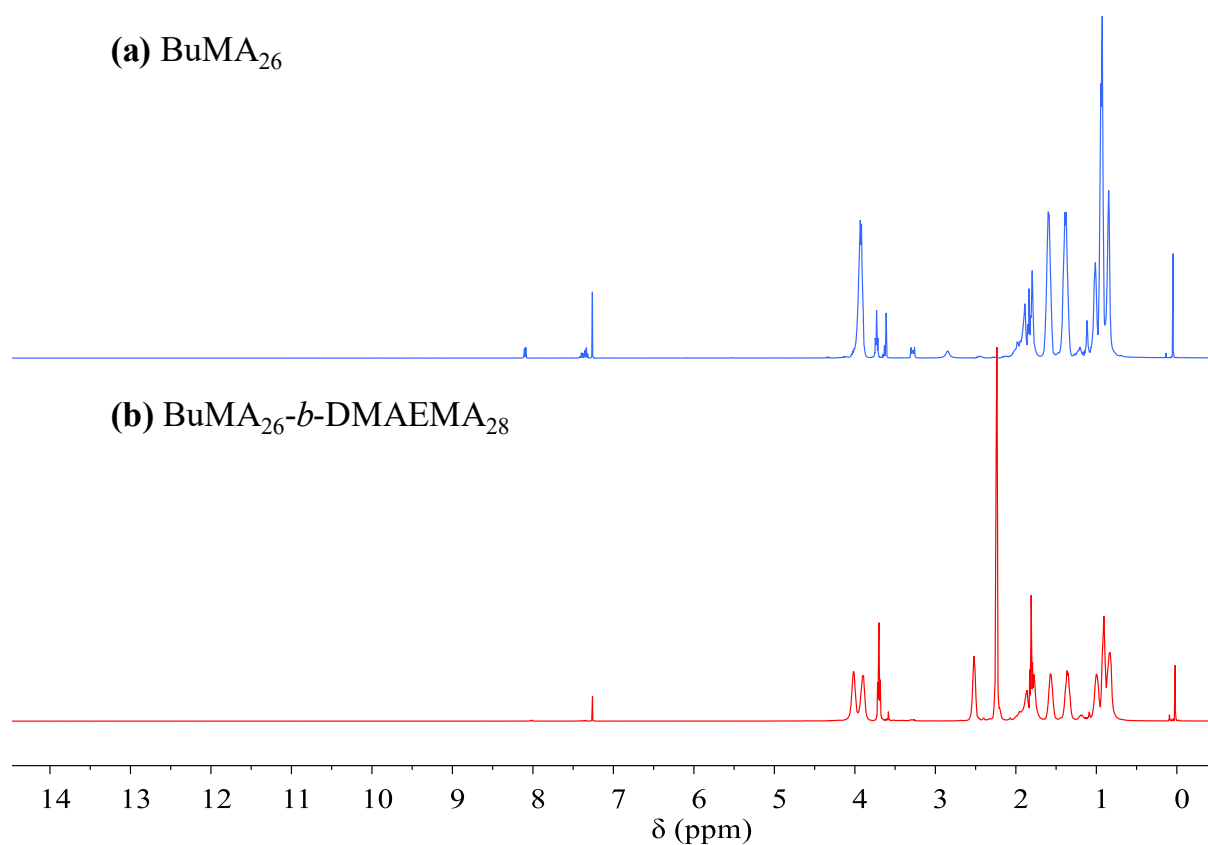


Figure A9.7: ¹H NMR spectra of (a) the BuMA₂₆ homopolymer in blue, and (b) the BuMA₂₆-*b*-DMAEMA₂₈ diblock bipolymer (Polymer A8) in red. The analysis has been performed in a similar way as shown in Figure 5.1.5.

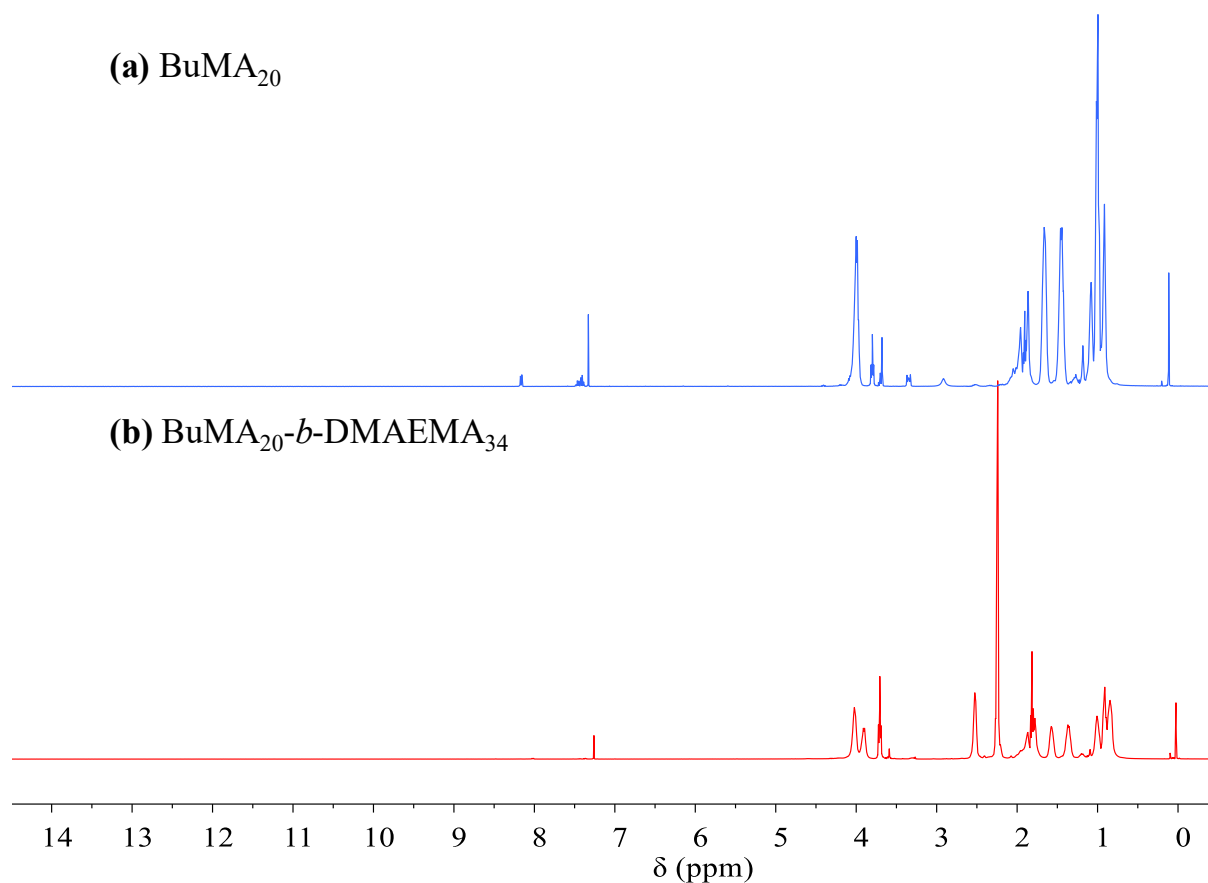


Figure A9.8: ^1H NMR spectra of (a) the BuMA₂₀ homopolymer in blue, and (b) the BuMA₂₀-*b*-DMAEMA₃₄ diblock bipolymer (Polymer A9) in red. The analysis has been performed in a similar way as shown in Figure 5.1.5.

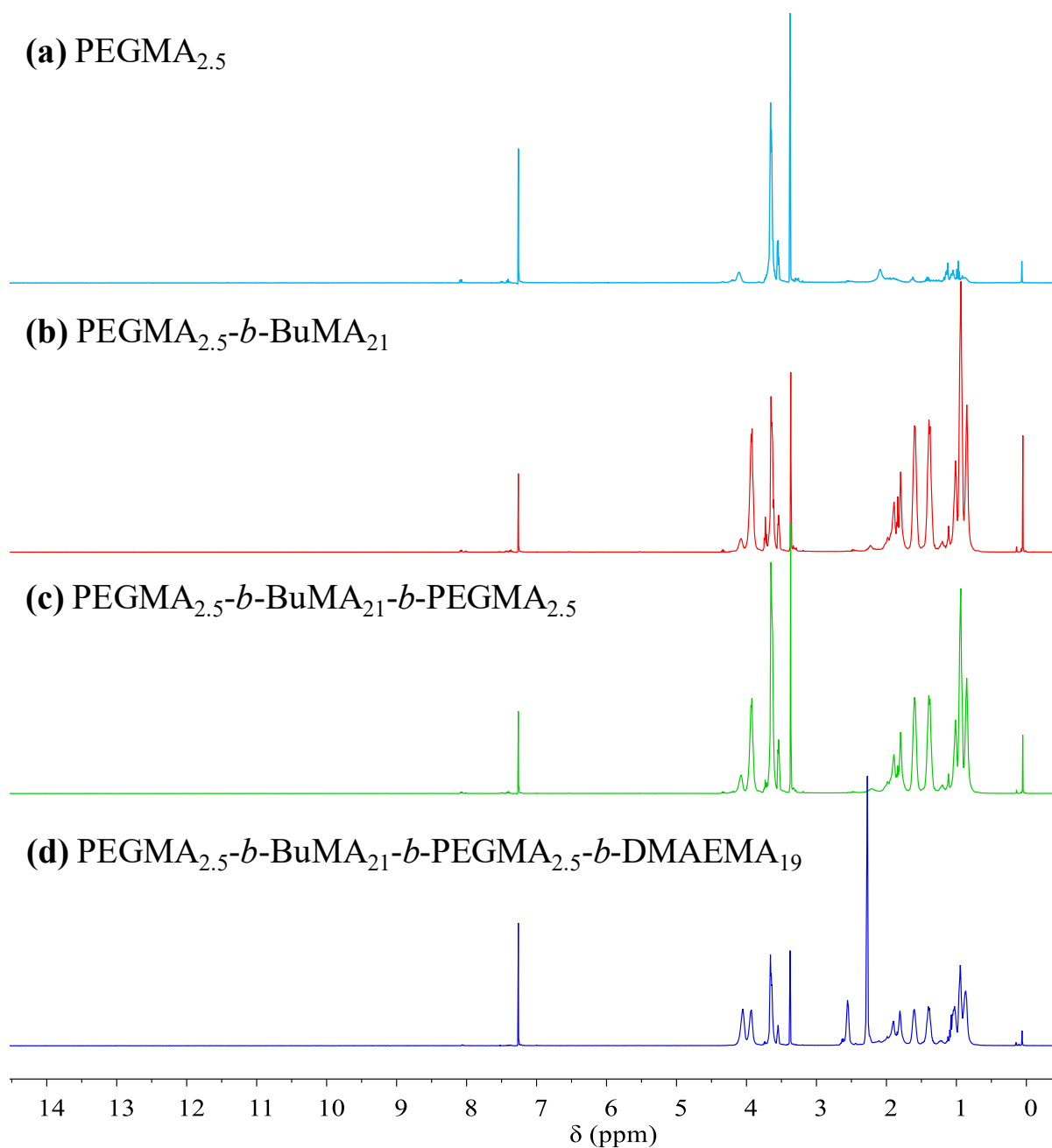


Figure A10.1: ^1H NMR spectra of (a) the PEGMA_{2.5} homopolymer in light blue, (b) the PEGMA_{2.5}-*b*-BuMA₂₁ diblock copolymer in red, (c) the PEGMA_{2.5}-*b*-BuMA₂₁-*b*-PEGMA_{2.5} triblock terpolymer in light green, and (d) the PEGMA_{2.5}-*b*-BuMA₂₁-*b*-PEGMA_{2.5}-*b*-DMAEMA₁₉ tetrablock terpolymer (Polymer B2) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

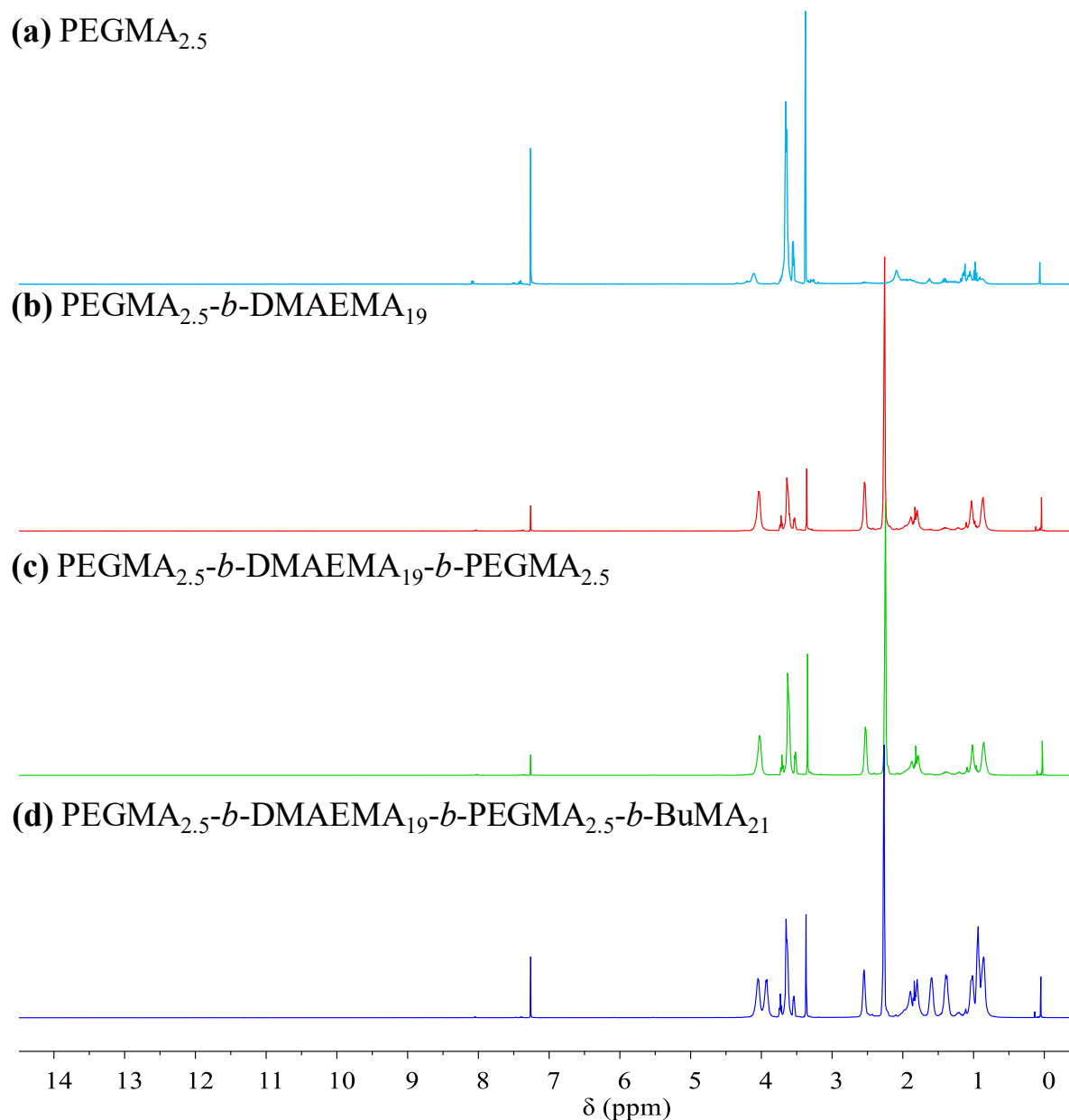


Figure A10.2: ^1H NMR spectra of (a) the PEGMA_{2.5} homopolymer in light blue, (b) the PEGMA_{2.5}-*b*-DMAEMA₁₉ diblock copolymer in red, (c) the PEGMA_{2.5}-*b*-DMAEMA₁₉-*b*-PEGMA_{2.5} triblock terpolymer in light green, and (d) the PEGMA_{2.5}-*b*-DMAEMA₁₉-*b*-PEGMA_{2.5}-*b*-BuMA₂₁ tetrablock terpolymer (Polymer B3) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

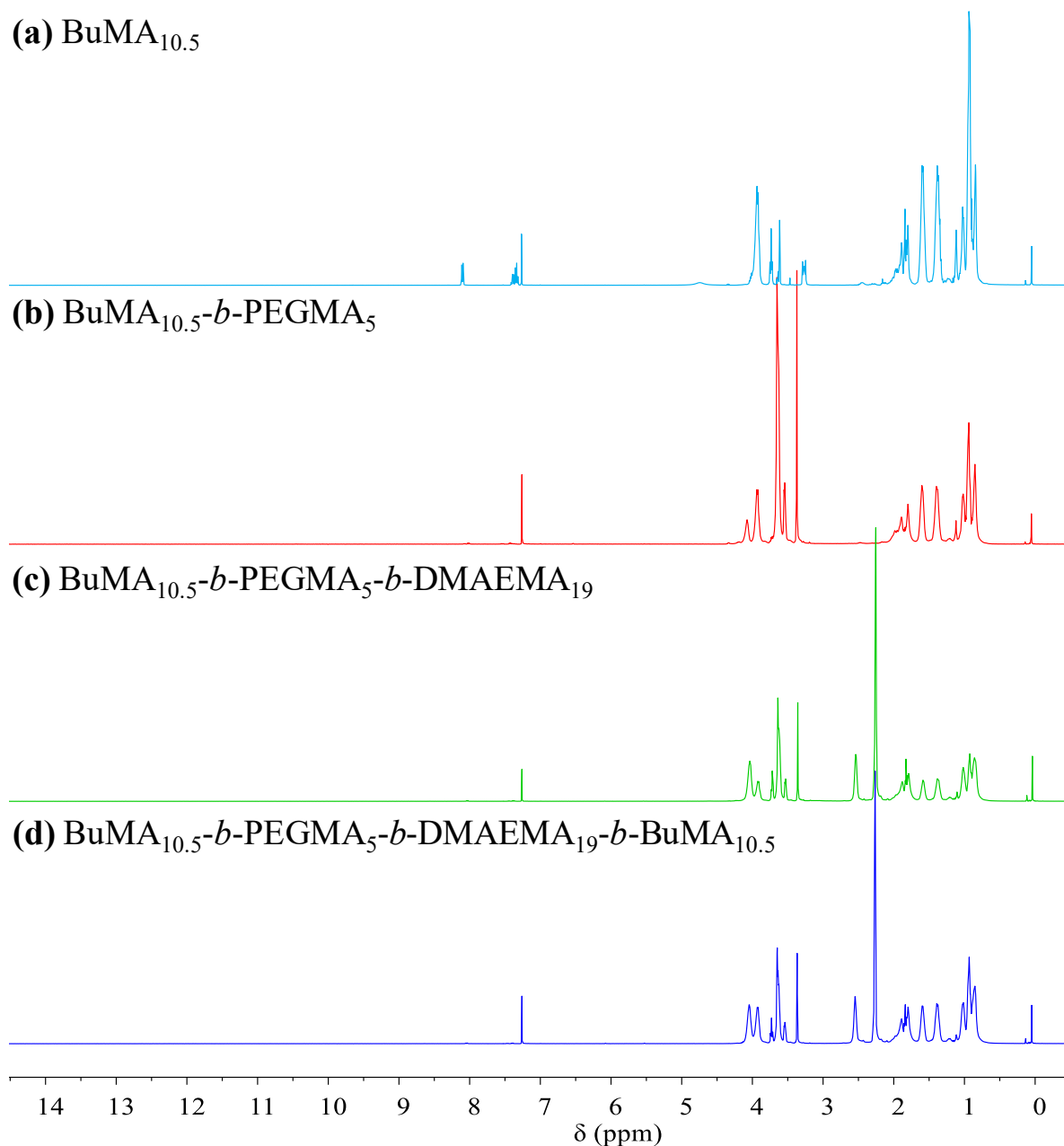


Figure A10.3: ^1H NMR spectra of (a) the $\text{BuMA}_{10.5}$ homopolymer in light blue, (b) the $\text{BuMA}_{10.5}$ -*b*-PEGMA₅ diblock copolymer in red, (c) the $\text{BuMA}_{10.5}$ -*b*-PEGMA₅-*b*-DMAEMA₁₉ triblock terpolymer in light green, and (d) the $\text{BuMA}_{10.5}$ -*b*-PEGMA₅-*b*-DMAEMA₁₉-*b*-BuMA_{10.5} tetrablock terpolymer (Polymer B4) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

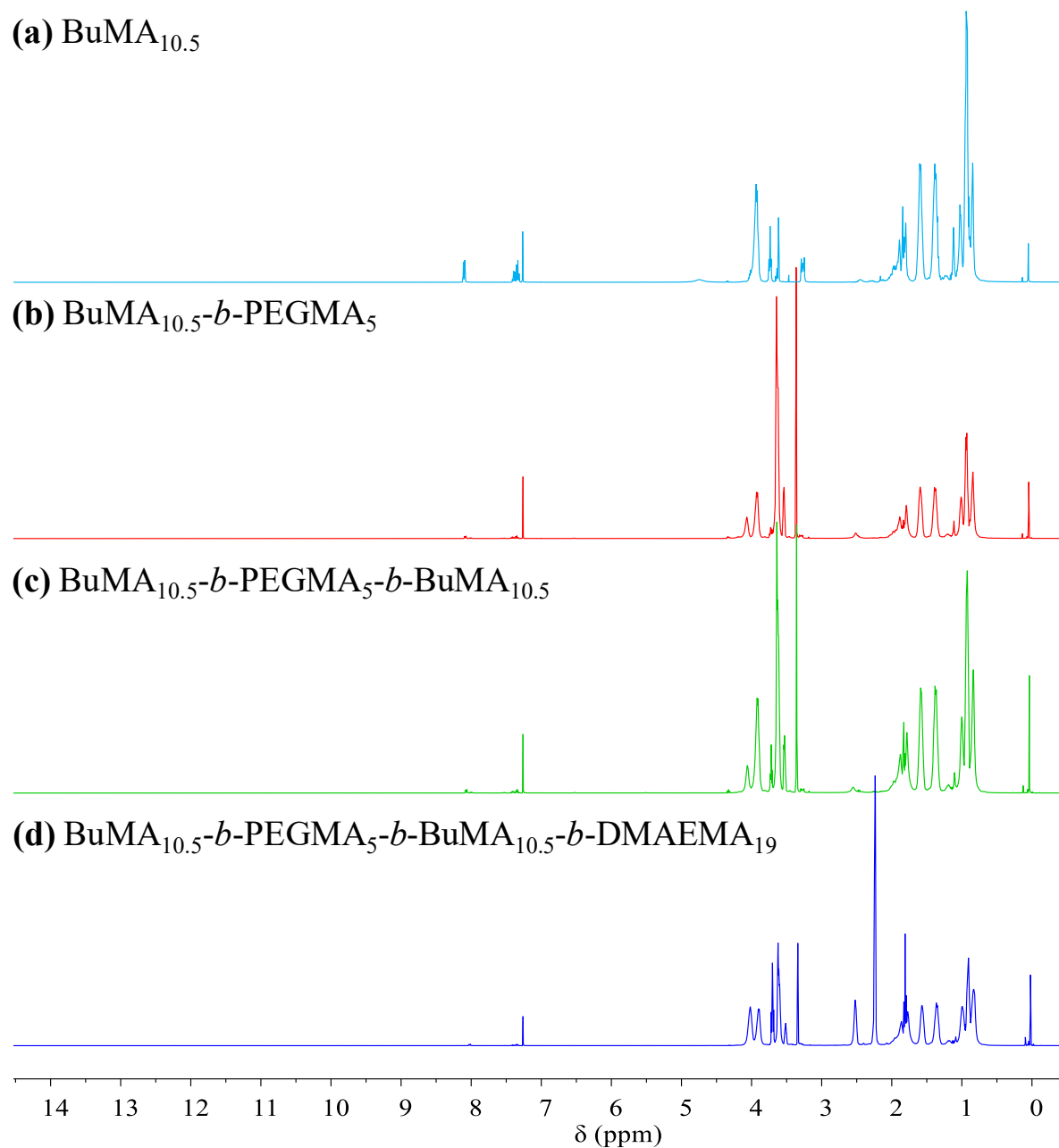


Figure A10.4: ^1H NMR spectra of (a) the BuMA_{10.5} homopolymer in light blue, (b) the BuMA_{10.5}-*b*-PEGMA₅ diblock copolymer in red, (c) the BuMA_{10.5}-*b*-PEGMA₅-*b*-BuMA_{10.5} triblock terpolymer in light green, and (d) the BuMA_{10.5}-*b*-PEGMA₅-*b*-BuMA_{10.5}-*b*-DMAEMA₁₉ tetrablock terpolymer (Polymer B5) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

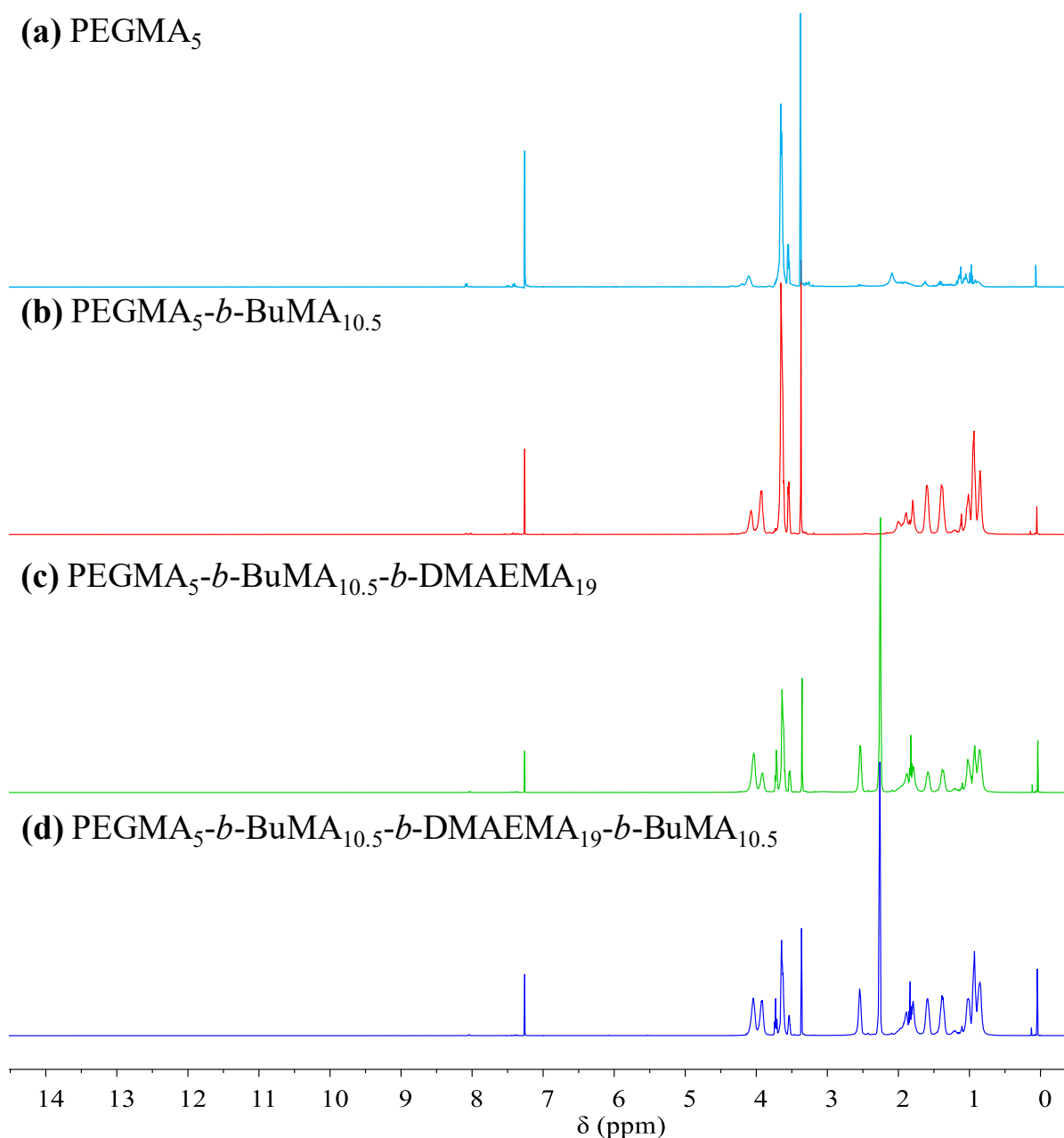


Figure A10.5: ^1H NMR spectra of (a) the PEGMA₅ homopolymer in light blue, (b) the PEGMA₅-*b*-BuMA_{10.5} diblock copolymer in red, (c) the PEGMA₅-*b*-BuMA_{10.5}-*b*-DMAEMA₁₉ triblock terpolymer in light green, and (d) the PEGMA₅-*b*-BuMA_{10.5}-*b*-DMAEMA₁₉-*b*-BuMA_{10.5} tetrablock terpolymer (Polymer B6) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

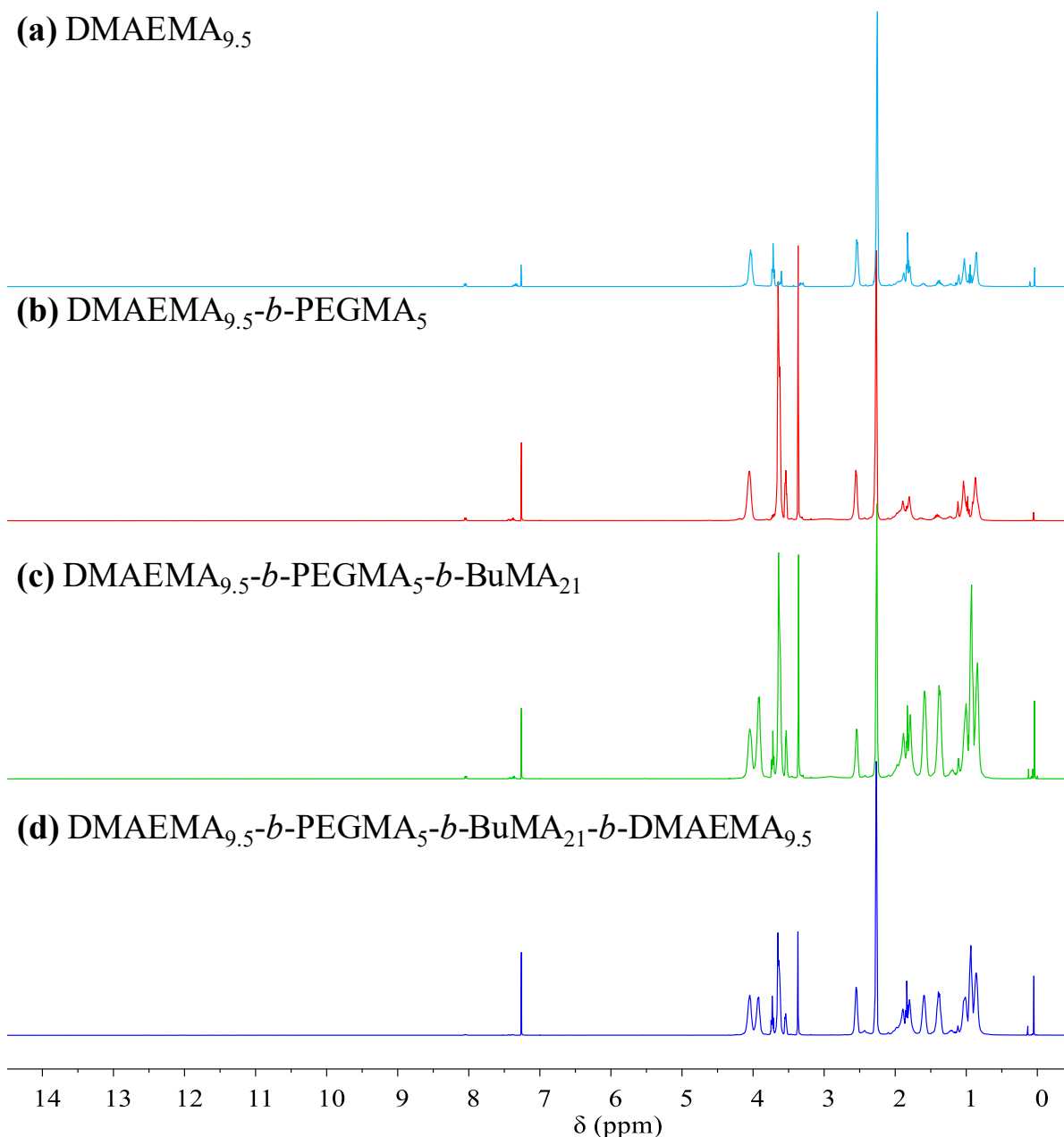


Figure A10.6: ^1H NMR spectra of (a) the DMAEMA_{9.5} homopolymer in light blue, (b) the DMAEMA_{9.5}-*b*-PEGMA₅ diblock copolymer in red, (c) the DMAEMA_{9.5}-*b*-PEGMA₅-*b*-BuMA₂₁ triblock terpolymer in light green, and (d) the DMAEMA_{9.5}-*b*-PEGMA₅-*b*-BuMA₂₁-*b*-DMAEMA_{9.5} tetrablock terpolymer (Polymer B7) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

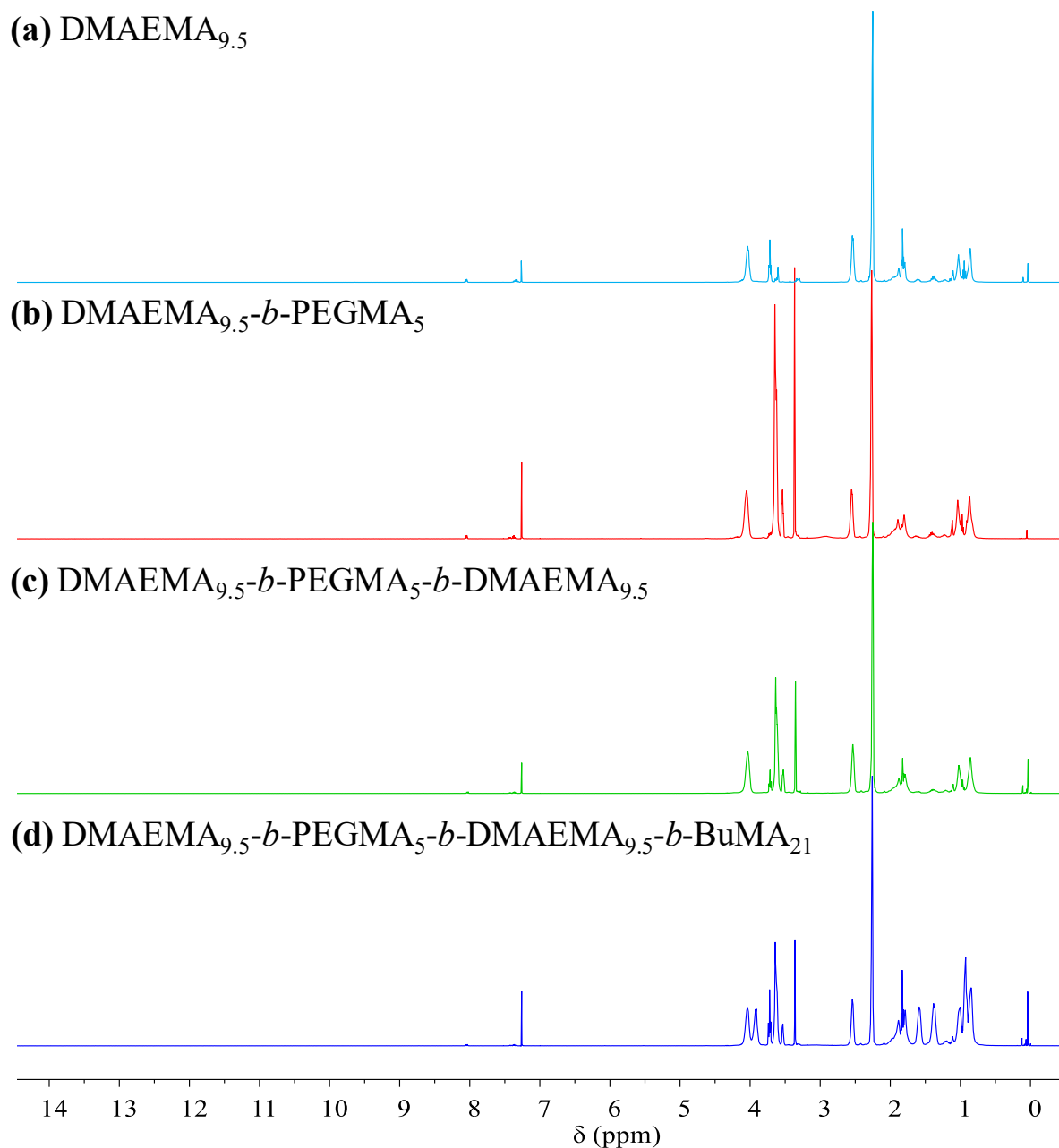


Figure A10.7: ^1H NMR spectra of (a) the DMAEMA_{9.5} homopolymer in light blue, (b) the DMAEMA_{9.5}-*b*-PEGMA₅ diblock copolymer in red, (c) the DMAEMA_{9.5}-*b*-PEGMA₅-*b*-DMAEMA_{9.5} triblock terpolymer in light green, and (d) the DMAEMA_{9.5}-*b*-PEGMA₅-*b*-DMAEMA_{9.5}-*b*-BuMA₂₁ tetrablock terpolymer (Polymer B8) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

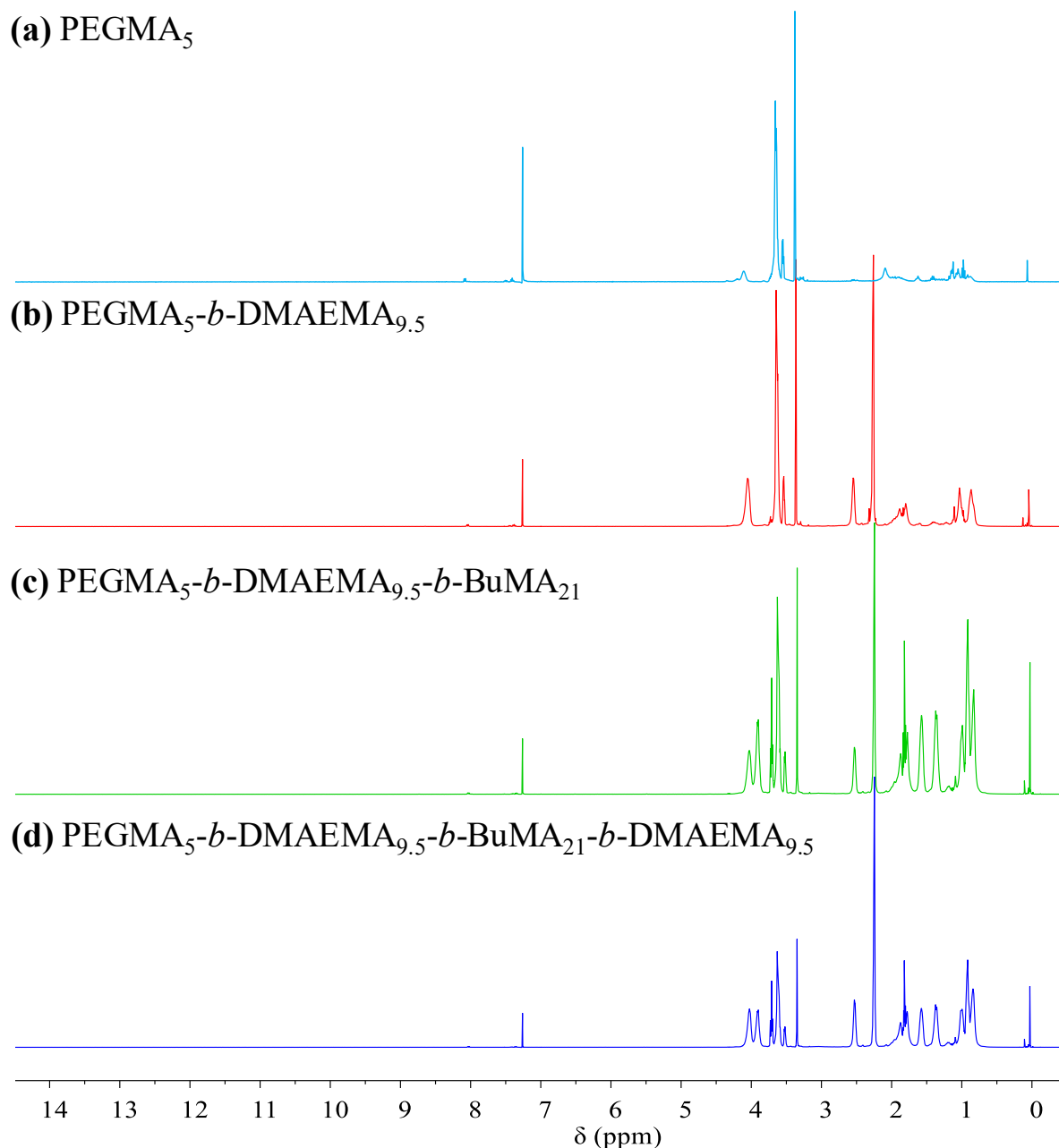


Figure A10.8: ^1H NMR spectra of (a) the PEGMA₅ homopolymer in light blue, (b) the PEGMA₅-*b*-DMAEMA_{9.5} diblock copolymer in red, (c) the PEGMA₅-*b*-DMAEMA_{9.5}-*b*-BuMA₂₁ triblock terpolymer in light green, and (d) the PEGMA₅-*b*-DMAEMA_{9.5}-*b*-BuMA₂₁-*b*-DMAEMA_{9.5} tetrablock terpolymer (Polymer B9) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

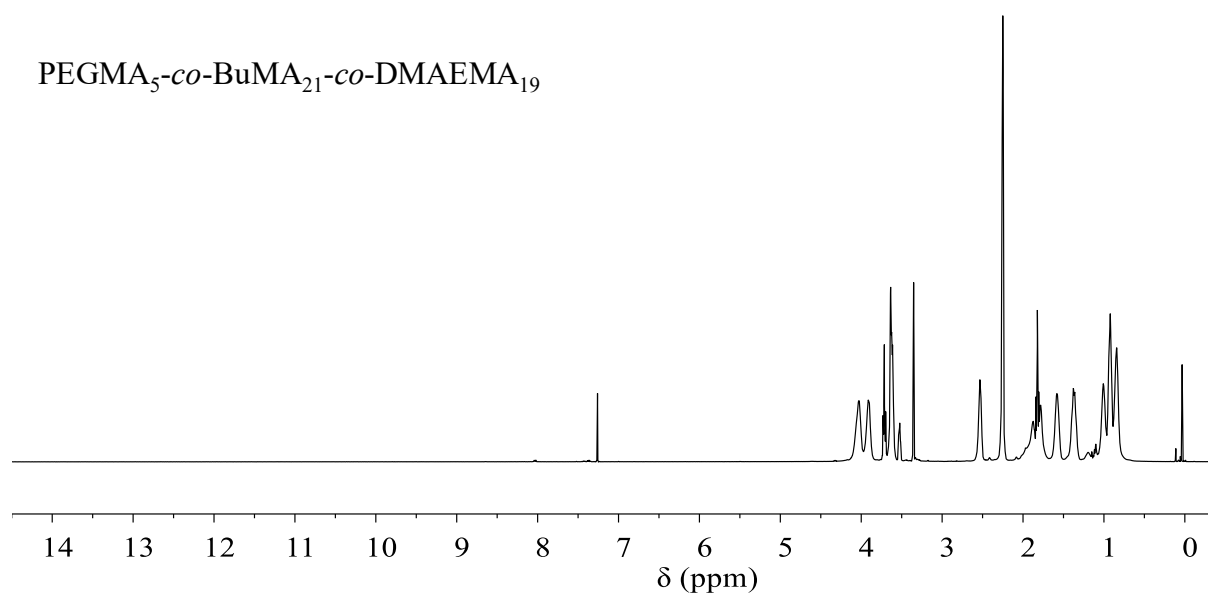


Figure A10.9: ^1H NMR spectrum of the PEGMA₅-*co*-BuMA₂₁-*co*-DMAEMA₁₉ statistical terpolymer in black (Polymer B10). The analysis has been performed in a similar way as in Figure 5.2.5.

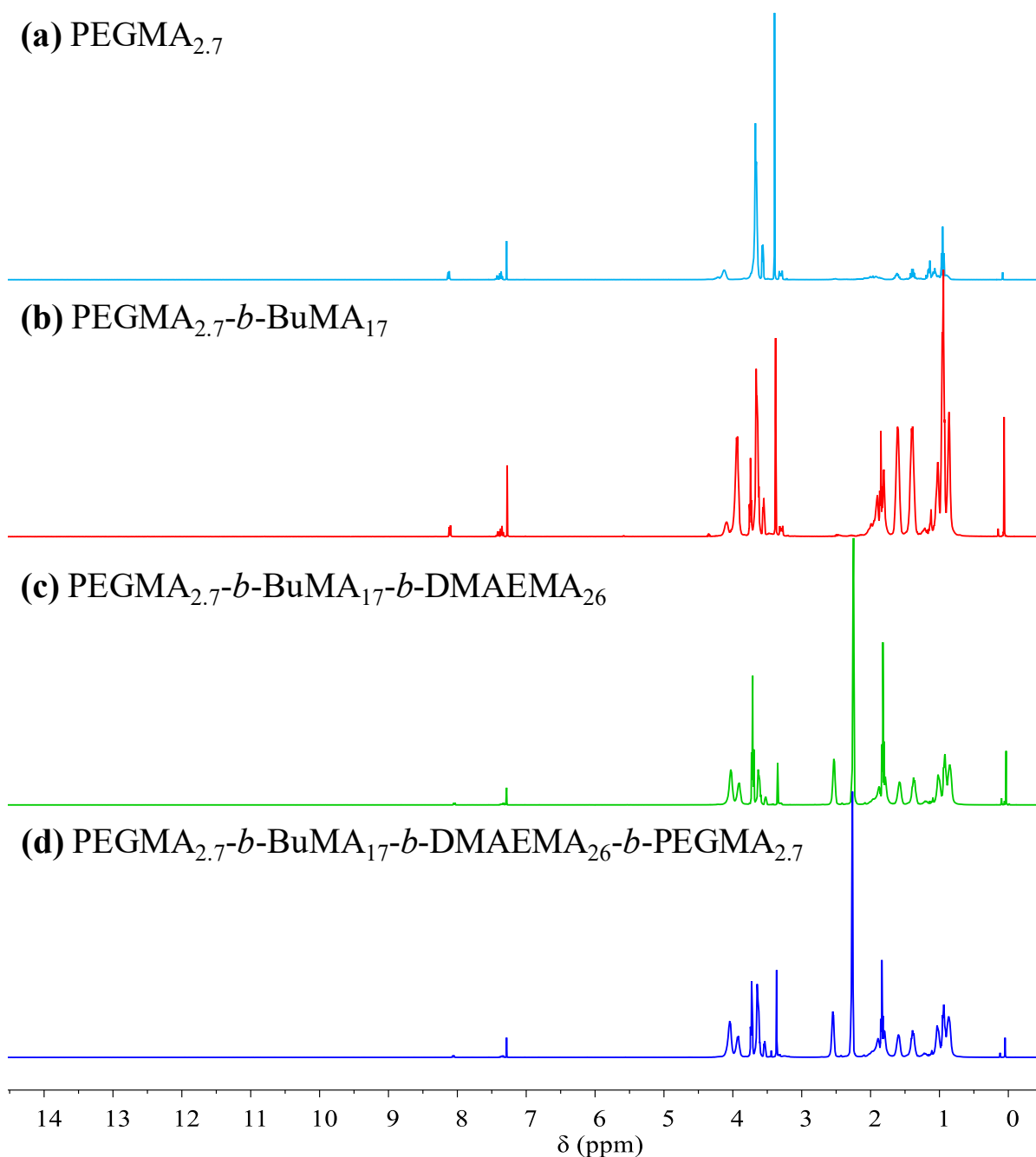


Figure A10.10: ^1H NMR spectra of (a) the PEGMA_{2.7} homopolymer in light blue, (b) the PEGMA_{2.7}-*b*-BuMA₁₇ diblock copolymer in red, (c) the PEGMA_{2.7}-*b*-BuMA₁₇-*b*-DMAEMA₂₆ triblock terpolymer in light green, and (d) the PEGMA_{2.7}-*b*-BuMA₁₇-*b*-DMAEMA₂₆-*b*-PEGMA_{2.7} tetrablock terpolymer (Polymer B11) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

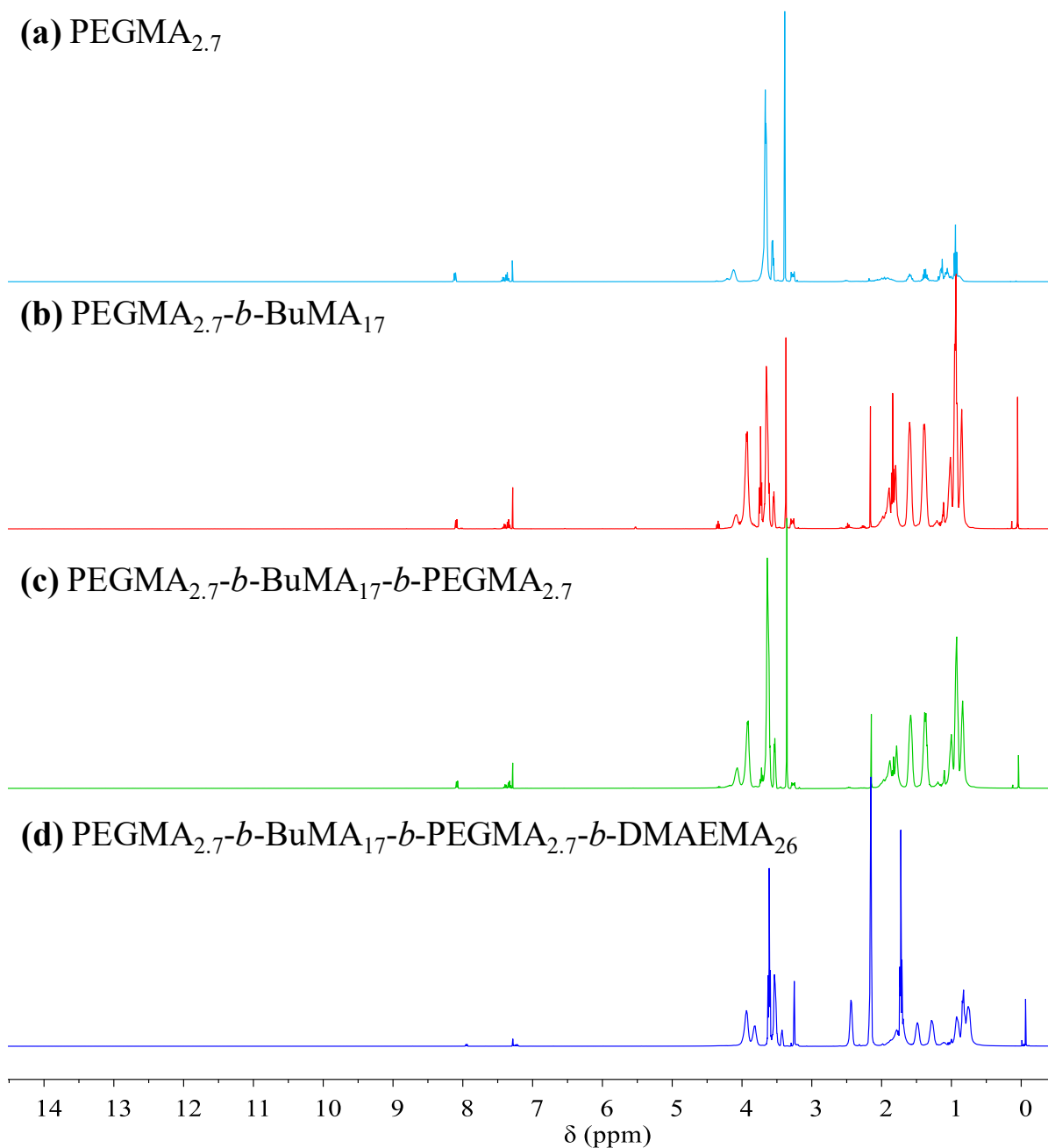


Figure A10.11: ^1H NMR spectra of (a) the PEGMA_{2.7} homopolymer in light blue, (b) the PEGMA_{2.7}-*b*-BuMA₁₇ diblock copolymer in red, (c) the PEGMA_{2.7}-*b*-BuMA₁₇-*b*-PEGMA_{2.7} triblock terpolymer in light green, and (d) the PEGMA_{2.7}-*b*-BuMA₁₇-*b*-PEGMA_{2.7}-*b*-DMAEMA₂₆ tetrablock terpolymer (Polymer B12) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

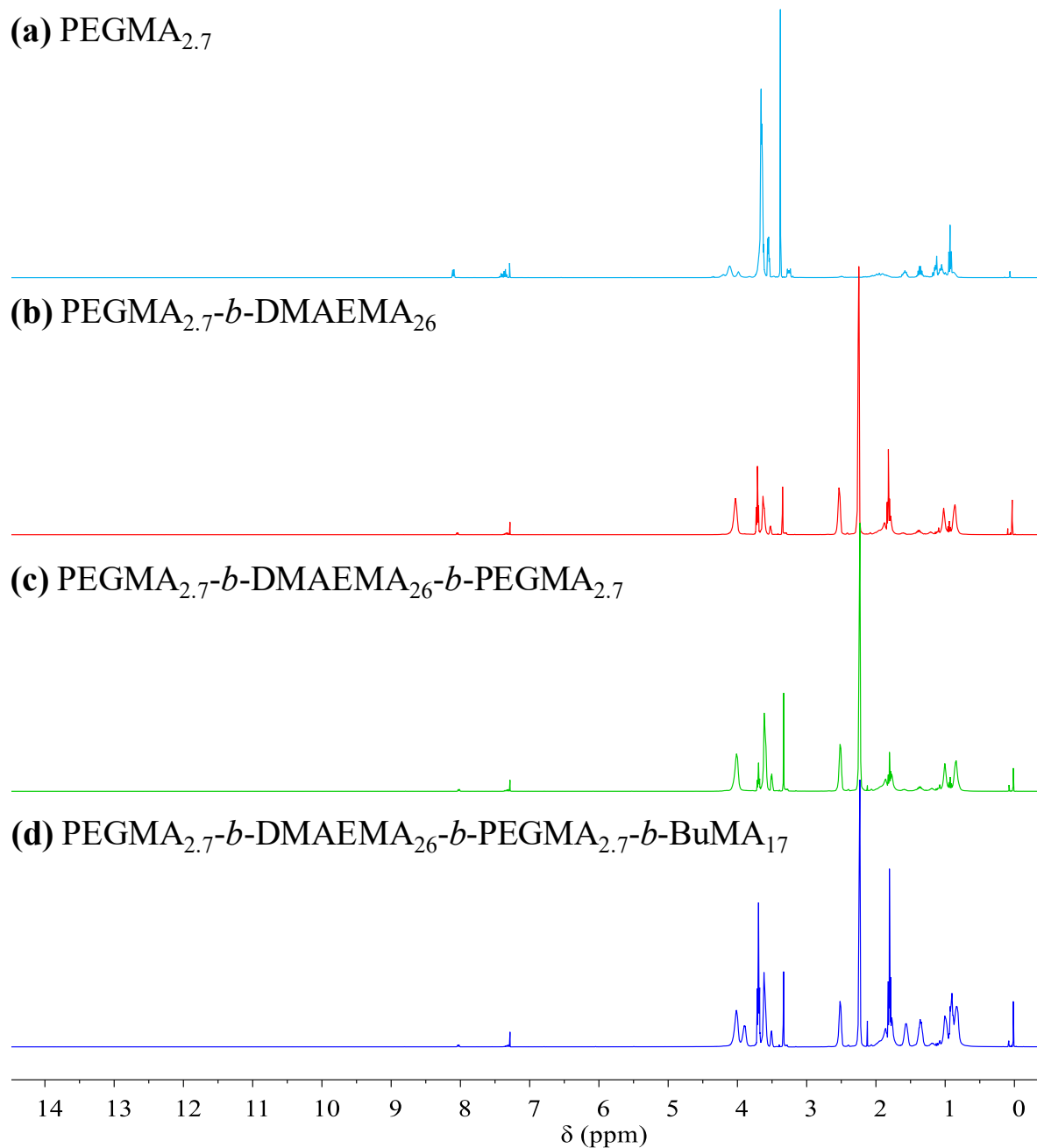


Figure A10.12: ^1H NMR spectra of (a) the PEGMA_{2.7} homopolymer in light blue, (b) the PEGMA_{2.7}-*b*-DMAEMA₂₆ diblock copolymer in red, (c) the PEGMA_{2.7}-*b*-DMAEMA₂₆-*b*-PEGMA_{2.7} triblock terpolymer in light green, and (d) the PEGMA_{2.7}-*b*-DMAEMA₂₆-*b*-PEGMA_{2.7}-*b*-BuMA₁₇ tetrablock terpolymer (Polymer B13) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

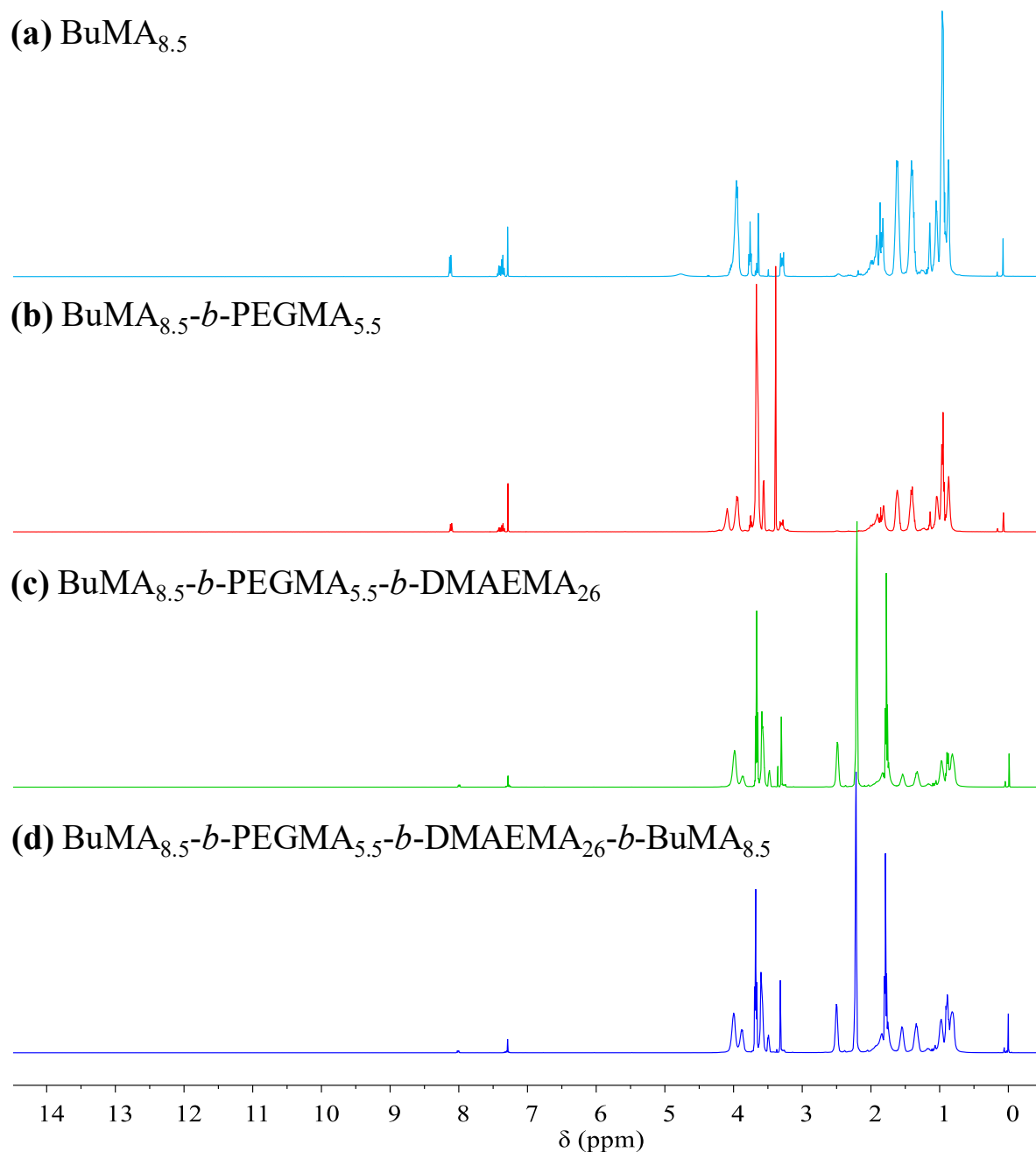


Figure A10.13: ^1H NMR spectra of (a) the $\text{BuMA}_{8.5}$ homopolymer in light blue, (b) the $\text{BuMA}_{8.5}$ -*b*-PEGMA $_{5.5}$ diblock copolymer in red, (c) the $\text{BuMA}_{8.5}$ -*b*-PEGMA $_{5.5}$ -*b*-DMAEMA $_{26}$ triblock terpolymer in light green, and (d) the $\text{BuMA}_{8.5}$ -*b*-PEGMA $_{5.5}$ -*b*-DMAEMA $_{26}$ -*b*-BuMA $_{8.5}$ tetrablock terpolymer (Polymer B14) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

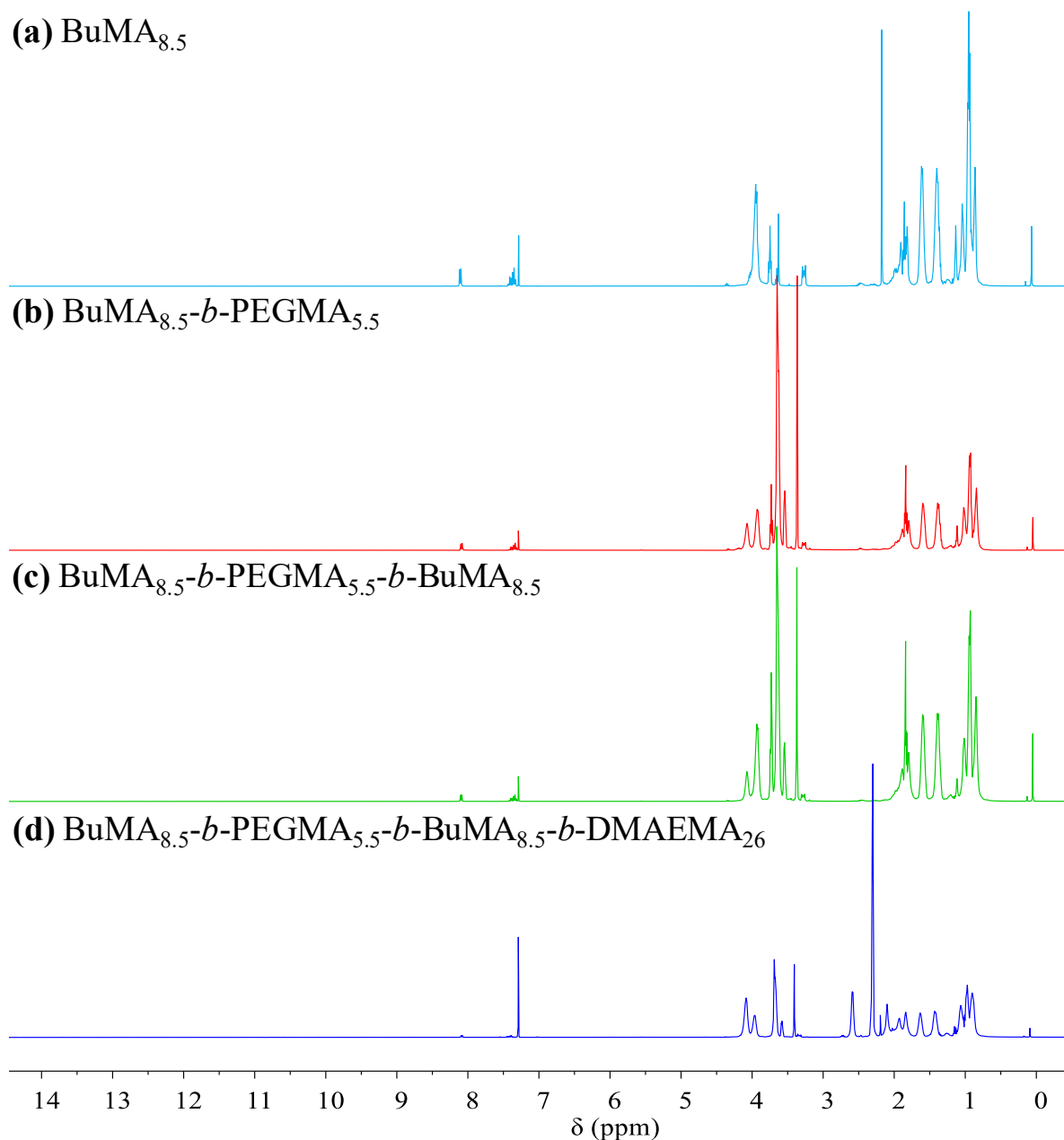


Figure A10.14: ^1H NMR spectra of (a) the $\text{BuMA}_{8.5}$ homopolymer in light blue, (b) the $\text{BuMA}_{8.5}$ -*b*-PEGMA $_{5.5}$ diblock copolymer in red, (c) the $\text{BuMA}_{10.5}$ -*b*-PEGMA $_5$ -*b*- $\text{BuMA}_{10.5}$ triblock terpolymer in light green, and (d) the $\text{BuMA}_{8.5}$ -*b*-PEGMA $_{5.5}$ -*b*- $\text{BuMA}_{8.5}$ -*b*-DMAEMA $_{26}$ tetrablock terpolymer (Polymer B15) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

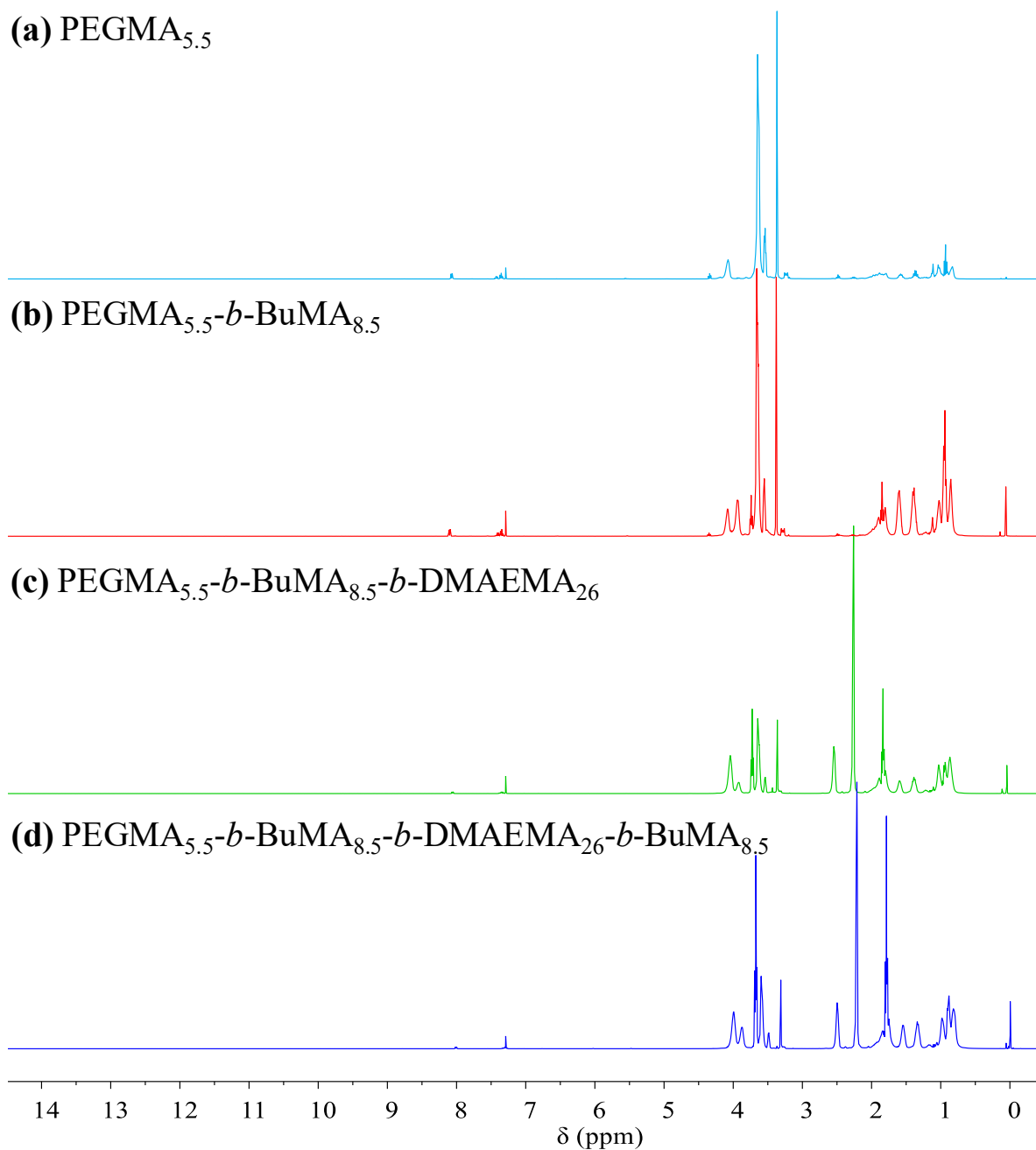


Figure A10.15: ^1H NMR spectra of (a) the PEGMA_{5.5} homopolymer in light blue, (b) the PEGMA_{5.5}-*b*-BuMA_{8.5} diblock copolymer in red, (c) the PEGMA_{5.5}-*b*-BuMA_{8.5}-*b*-DMAEMA₂₆ triblock terpolymer in light green, and (d) the PEGMA_{5.5}-*b*-BuMA_{8.5}-*b*-DMAEMA₂₆-*b*-BuMA_{8.5} tetrablock terpolymer (Polymer B16) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

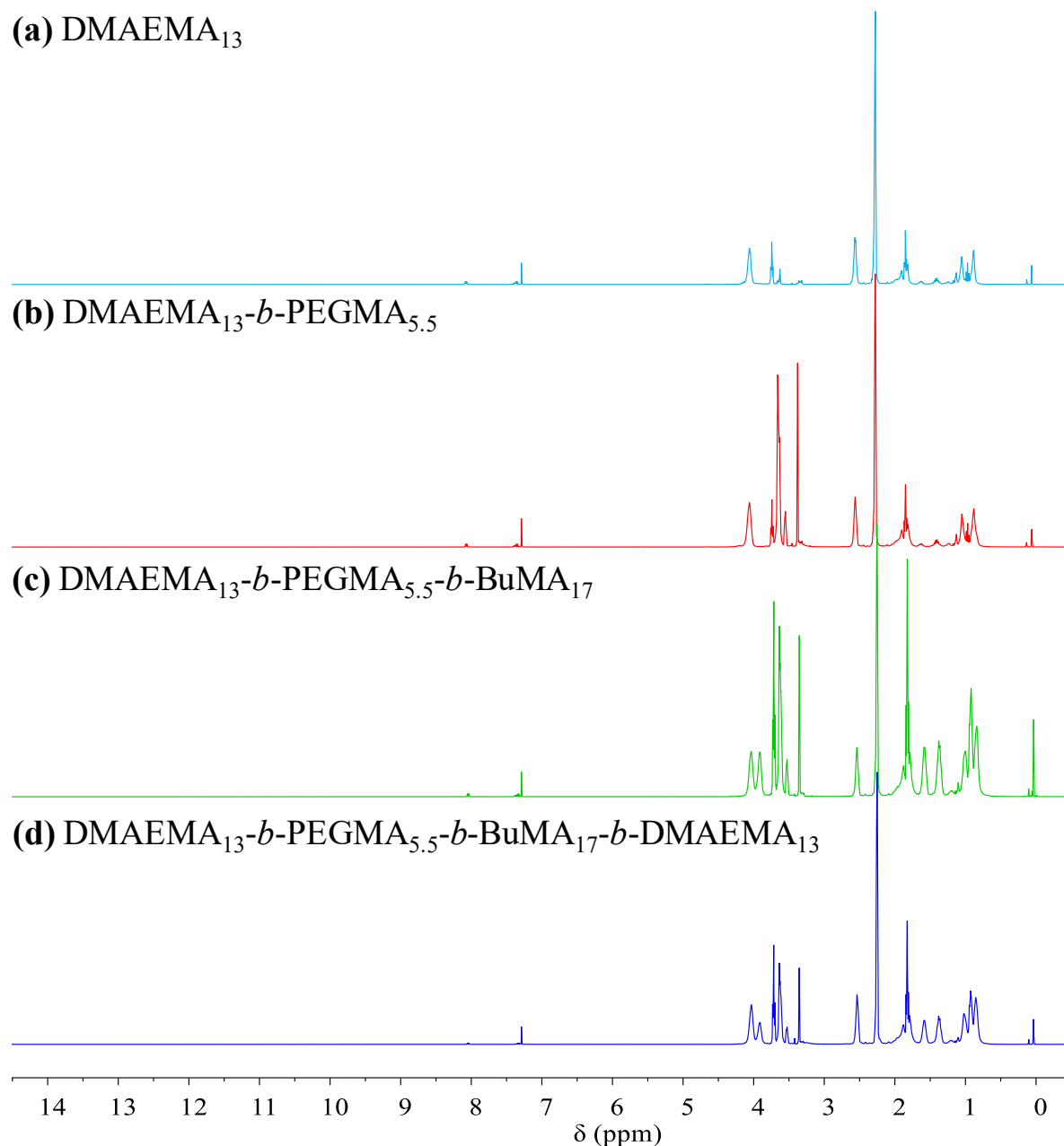


Figure A10.16: ¹H NMR spectra of (a) the DMAEMA₁₃ homopolymer in light blue, (b) the DMAEMA₁₃-*b*-PEGMA_{5.5} diblock copolymer in red, (c) the DMAEMA₁₃-*b*-PEGMA_{5.5}-*b*-BuMA₁₇ triblock terpolymer in light green, and (d) the DMAEMA₁₃-*b*-PEGMA_{5.5}-*b*-BuMA₁₇-*b*-DMAEMA₁₃ tetrablock terpolymer (Polymer B17) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

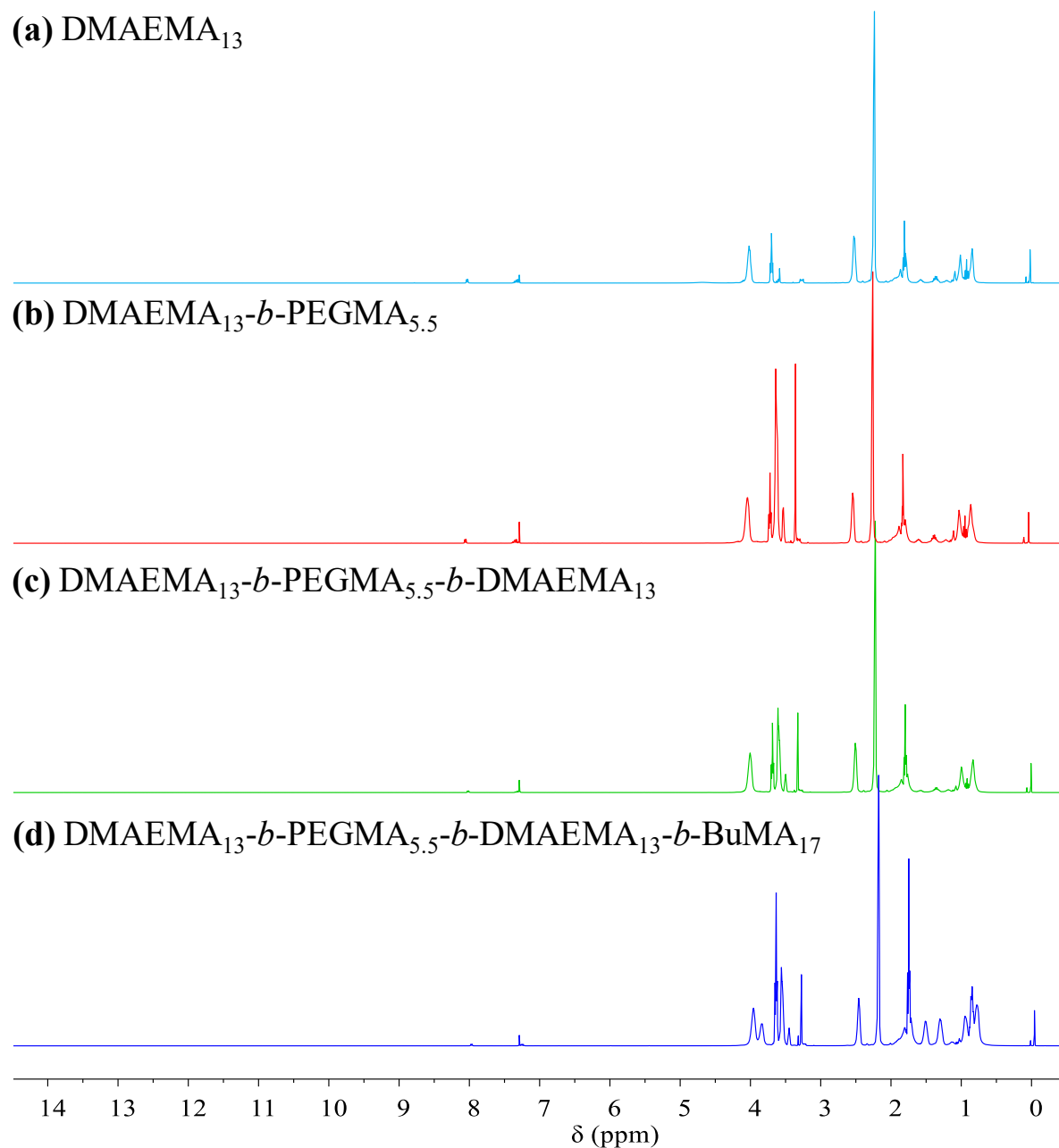


Figure A10.17: ^1H NMR spectra of (a) the DMAEMA₁₃ homopolymer in light blue, (b) the DMAEMA₁₃-*b*-PEGMA_{5.5} diblock copolymer in red, (c) the DMAEMA₁₃-*b*-PEGMA_{5.5}-*b*-DMAEMA₁₃ triblock terpolymer in light green, and (d) the DMAEMA₁₃-*b*-PEGMA_{5.5}-*b*-DMAEMA₁₃-*b*-BuMA₁₇ tetrablock terpolymer (Polymer B18) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

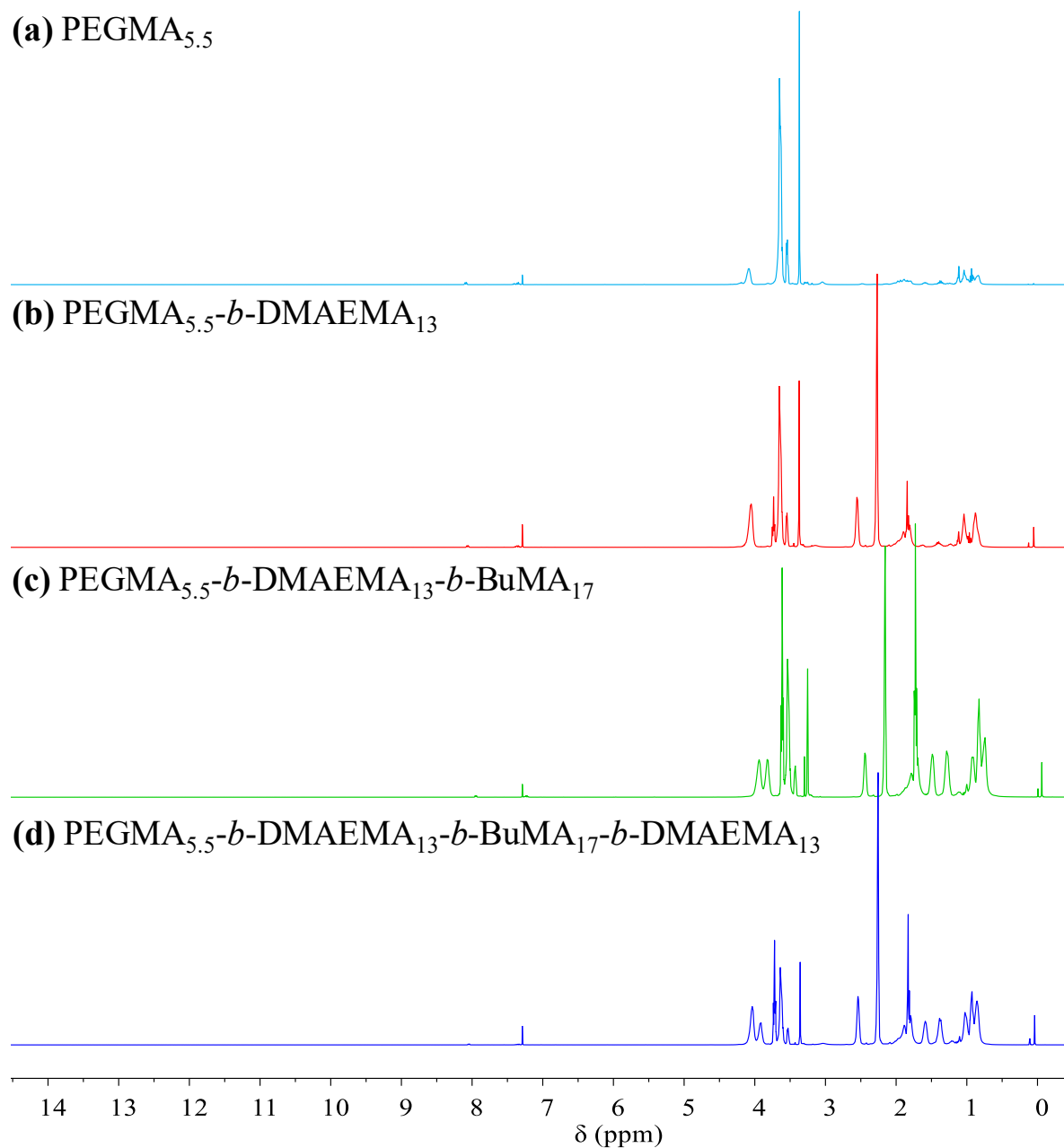


Figure A10.18: ^1H NMR spectra of (a) the PEGMA₅ homopolymer in light blue, (b) the PEGMA_{5.5}-*b*-DMAEMA₁₃ diblock copolymer in red, (c) the PEGMA_{5.5}-*b*-DMAEMA₁₃-*b*-BuMA₁₇ triblock terpolymer in light green, and (d) the PEGMA_{5.5}-*b*-DMAEMA₁₃-*b*-BuMA₁₇-*b*-DMAEMA₁₃ tetrablock terpolymer (Polymer B19) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

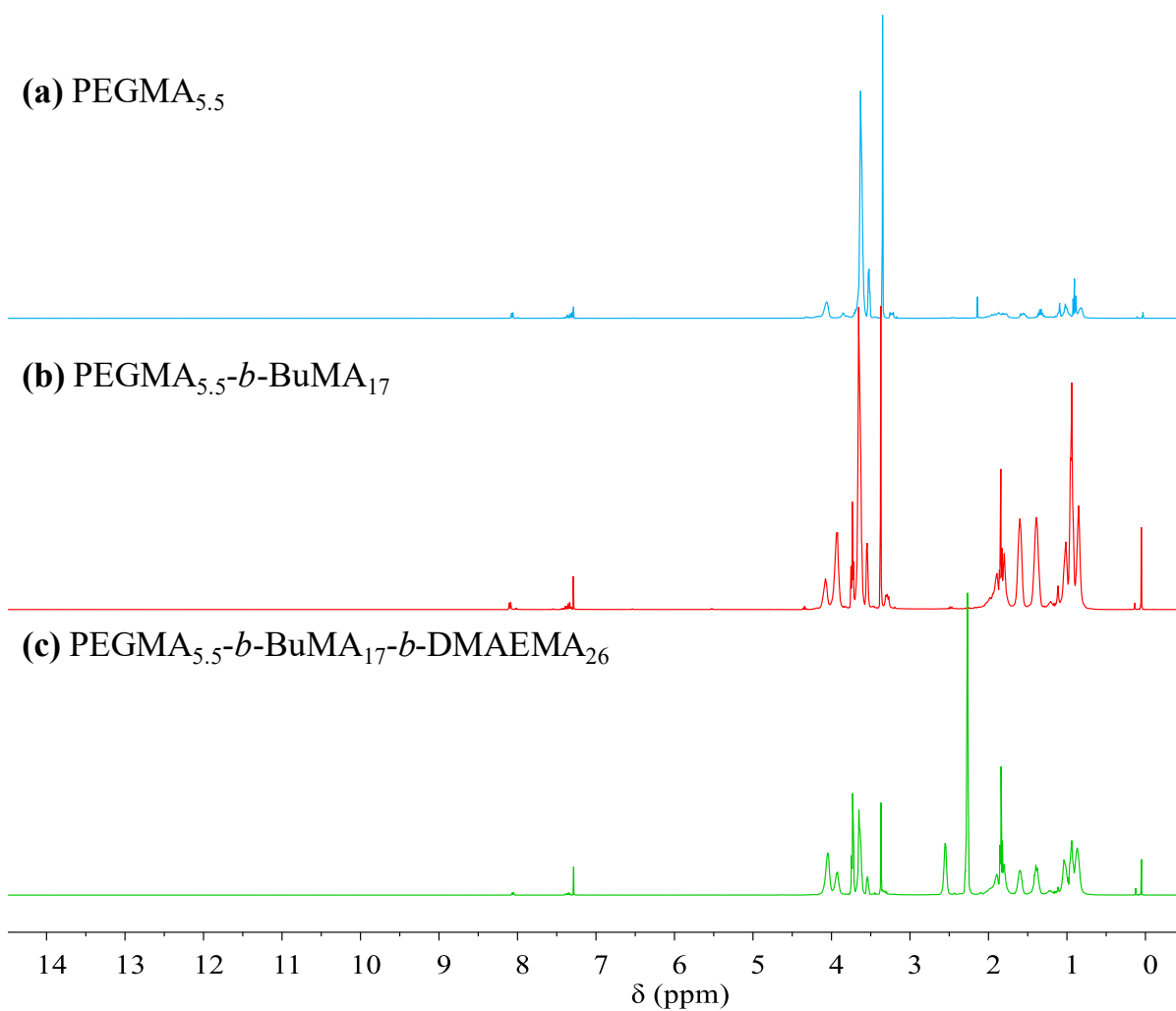


Figure A10.19: ^1H NMR spectra of (a) the PEGMA_{5.5} homopolymer in light blue, (b) the PEGMA_{5.5}-*b*-BuMA₁₇ diblock copolymer in red, (c) the PEGMA_{5.5}-*b*-BuMA₁₇-*b*-DMAEMA₂₆ triblock terpolymer in light green (Polymer B20). The analysis has been performed in a similar way as in Figure 5.2.5.

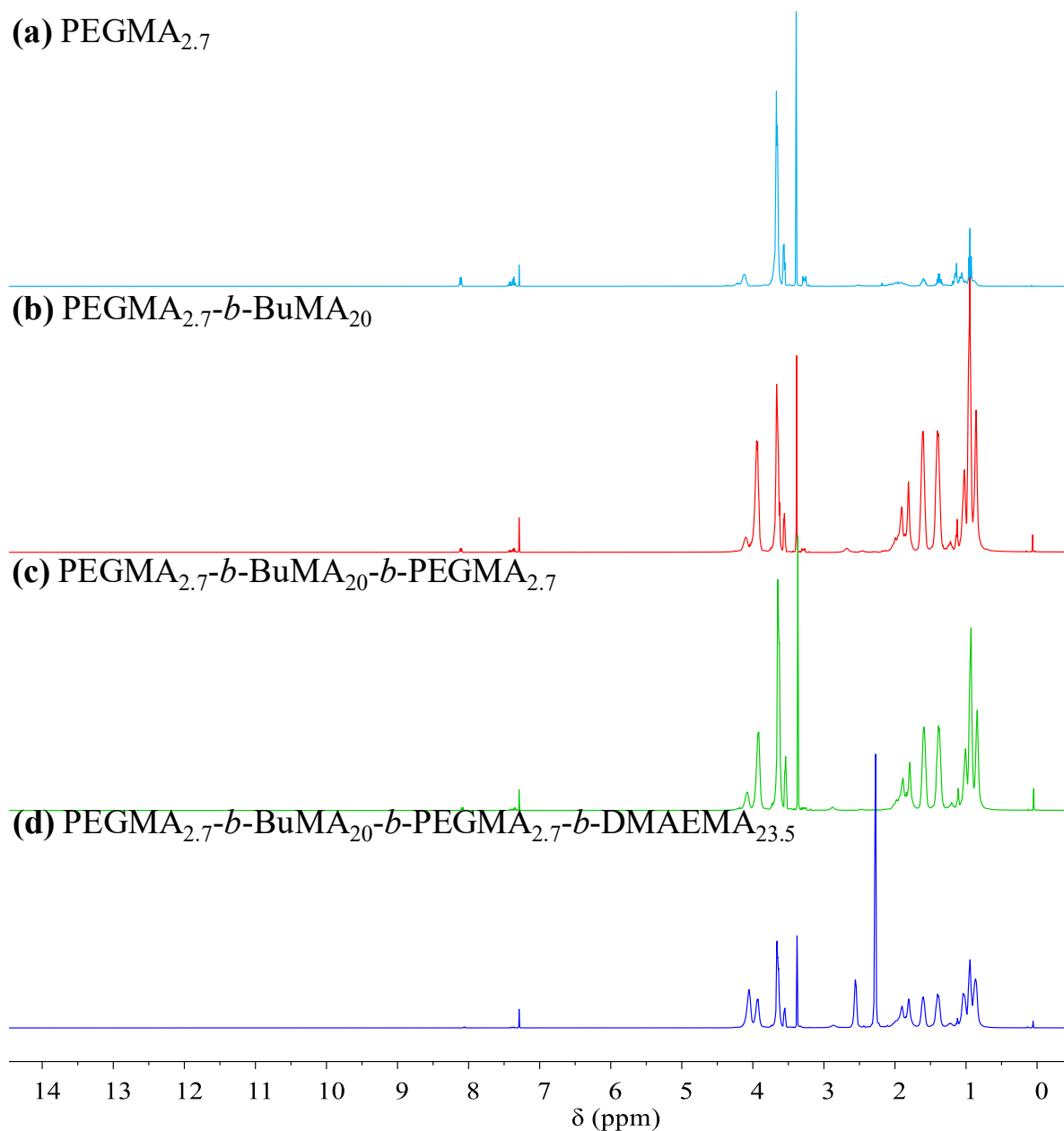


Figure A10.20: ^1H NMR spectra of (a) the PEGMA_{2.7} homopolymer in light blue, (b) the PEGMA_{2.7}-*b*-BuMA₂₀ diblock copolymer in red, (c) the PEGMA_{2.7}-*b*-BuMA₂₀-*b*-PEGMA_{2.7} triblock terpolymer in light green, and (d) the PEGMA_{2.7}-*b*-BuMA₂₀-*b*-PEGMA_{2.7}-*b*-DMAEMA_{23.5} tetrablock terpolymer (Polymer B21) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

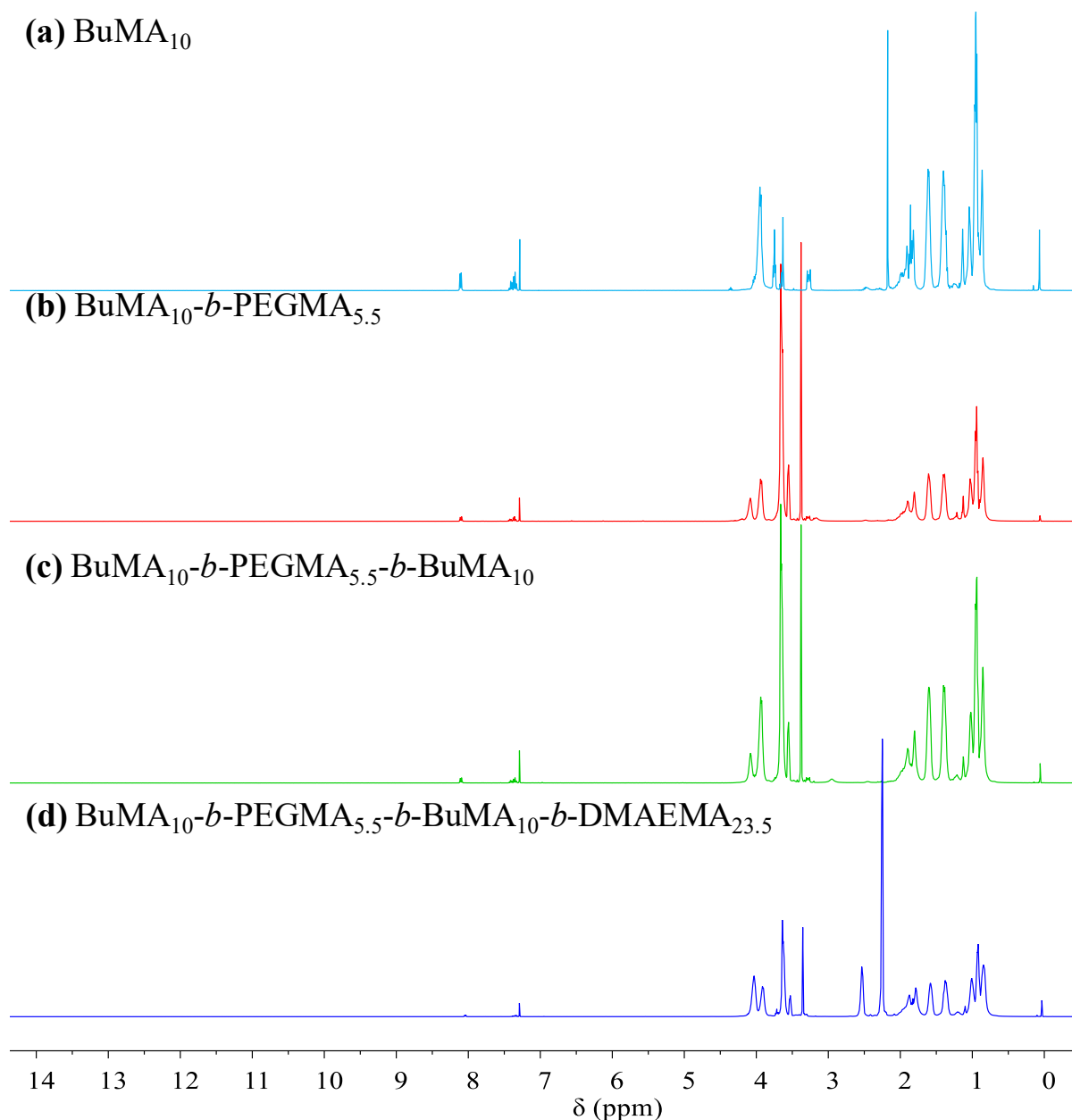


Figure A10.21: ^1H NMR spectra of (a) the BuMA₁₀ homopolymer in light blue, (b) the BuMA₁₀-*b*-PEGMA_{5.5} diblock copolymer in red, (c) the BuMA₁₀-*b*-PEGMA_{5.5}-*b*-BuMA₁₀ triblock terpolymer in light green, and (d) the BuMA₁₀-*b*-PEGMA_{5.5}-*b*-BuMA₁₀-*b*-DMAEMA_{23.5} tetrablock terpolymer (Polymer B22) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

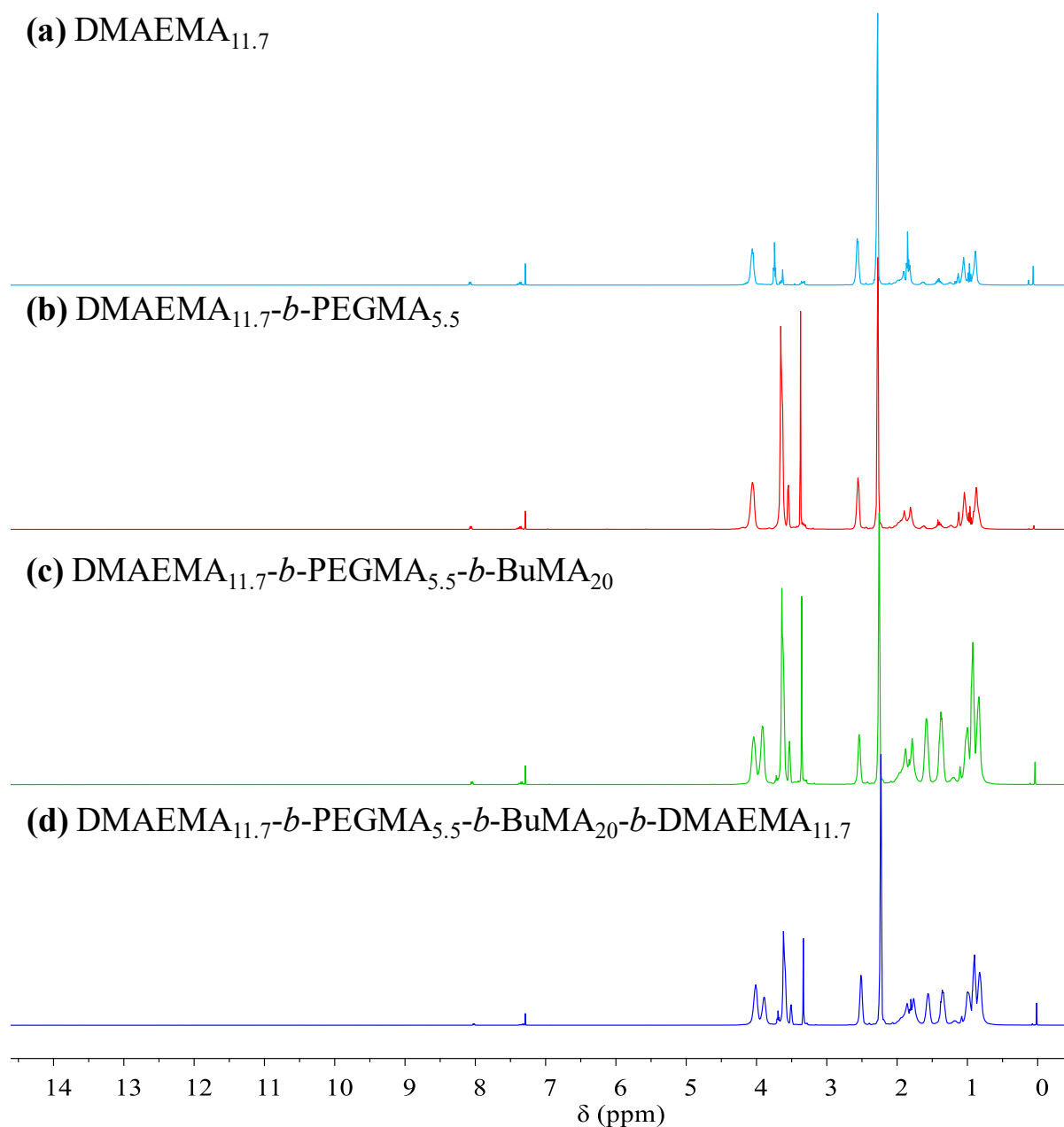


Figure A10.22: ^1H NMR spectra of (a) the DMAEMA_{11.7} homopolymer in light blue, (b) the DMAEMA_{11.7}-*b*-PEGMA_{5.5} diblock copolymer in red, (c) the DMAEMA_{11.7}-*b*-PEGMA_{5.5}-*b*-BuMA₂₀ triblock terpolymer in light green, and (d) the DMAEMA_{11.7}-*b*-PEGMA_{5.5}-*b*-BuMA₂₀-*b*-DMAEMA_{11.7} tetrablock terpolymer (Polymer B23) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

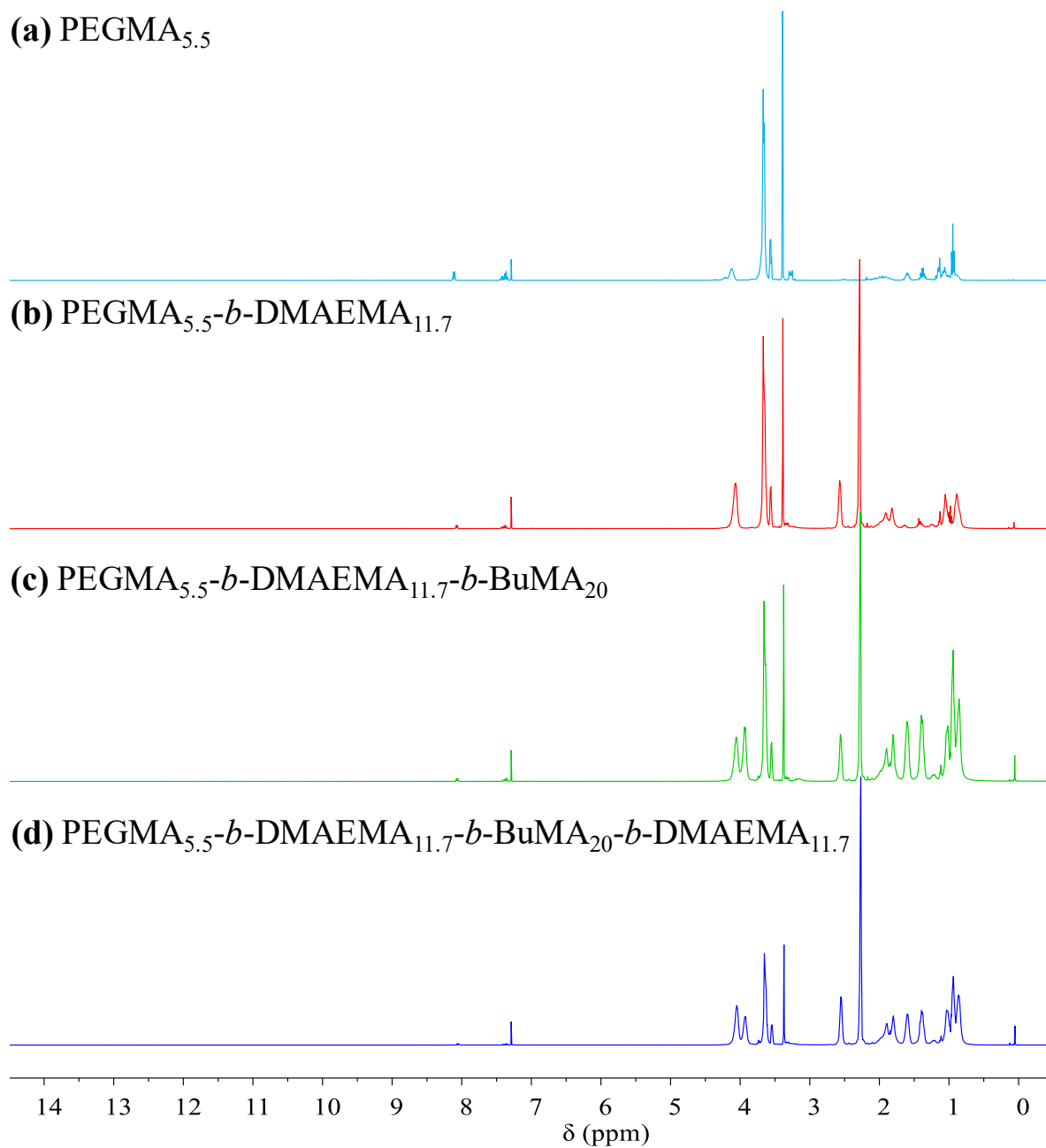


Figure A10.23: ^1H NMR spectra of (a) the PEGMA_{5.5} homopolymer in light blue, (b) the PEGMA_{5.5}-*b*-DMAEMA_{11.7} diblock copolymer in red, (c) the PEGMA_{5.5}-*b*-DMAEMA_{11.7}-*b*-BuMA₂₀ triblock terpolymer in light green, and (d) the PEGMA_{5.5}-*b*-DMAEMA_{11.7}-*b*-BuMA₂₀-*b*-DMAEMA_{11.7} tetrablock terpolymer (Polymer B24) in blue. The analysis has been performed in a similar way as in Figure 5.2.5.

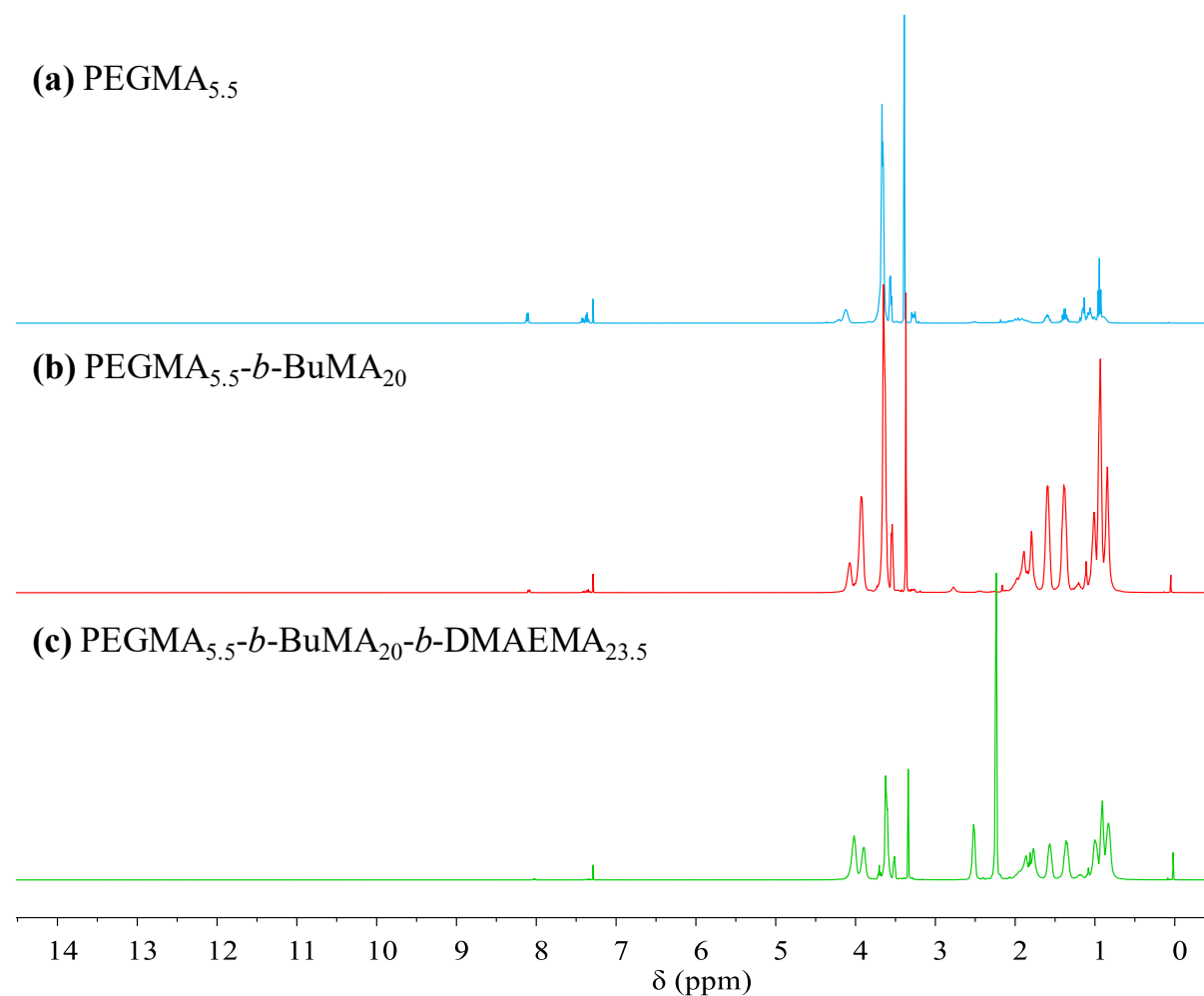


Figure A10.24: ^1H NMR spectra of (a) the PEGMA_{5.5} homopolymer in light blue, (b) the PEGMA_{5.5}-*b*-BuMA₂₀ diblock copolymer in red, (c) the PEGMA_{5.5}-*b*-BuMA₂₀-*b*-DMAEMA_{23.5} triblock terpolymer in light green (Polymer B25). The analysis has been performed in a similar way as in Figure 5.2.5.

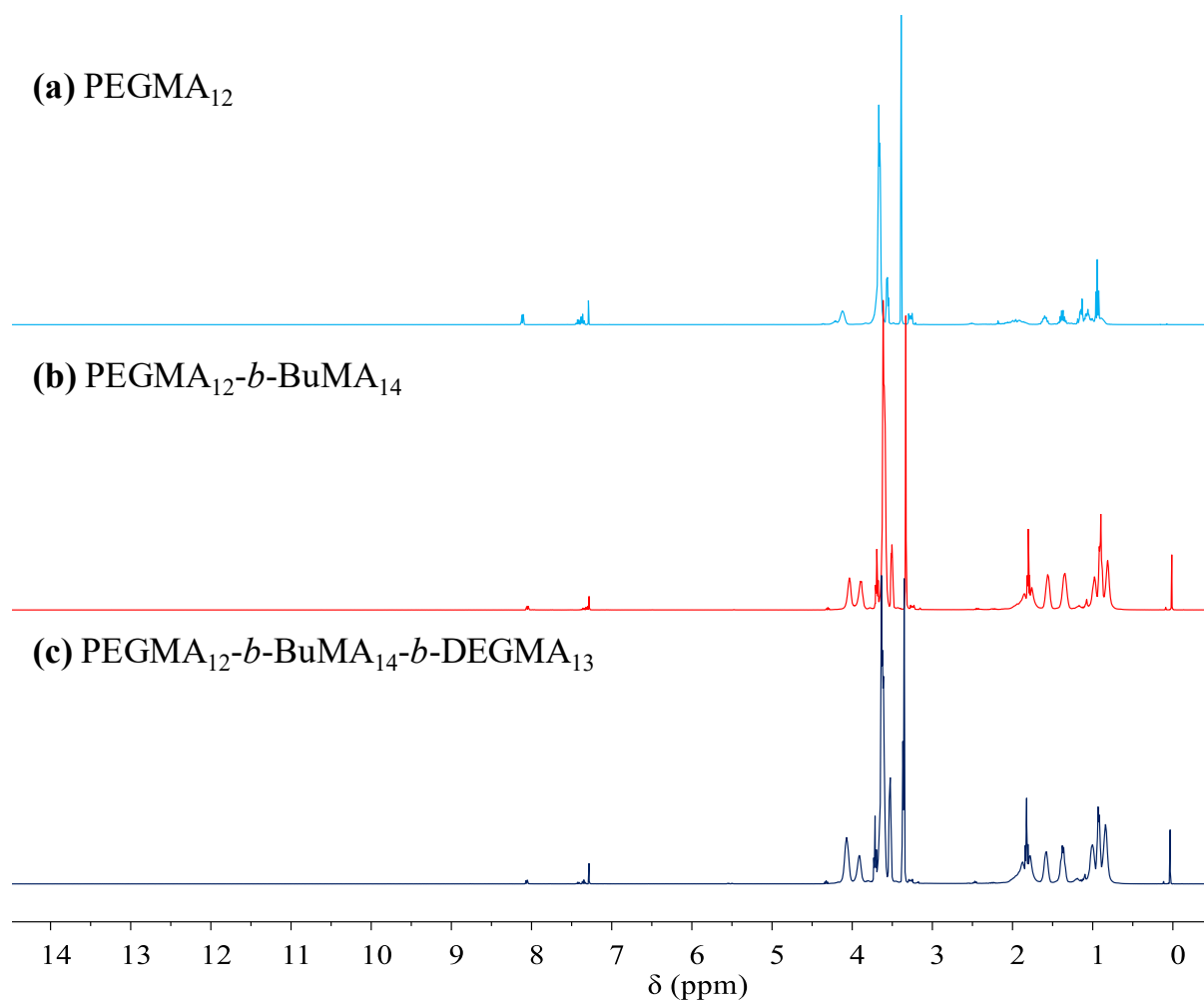


Figure A11.1: ^1H NMR spectra of (a) the homopolymer PEGMA₁₂, (b) the diblock copolymer PEGMA₁₂-*b*-BuMA₁₄, and (c) the final triblock terpolymer PEGMA₁₂-*b*-BuMA₁₄-*b*-DEGMA₁₃ (PC2) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

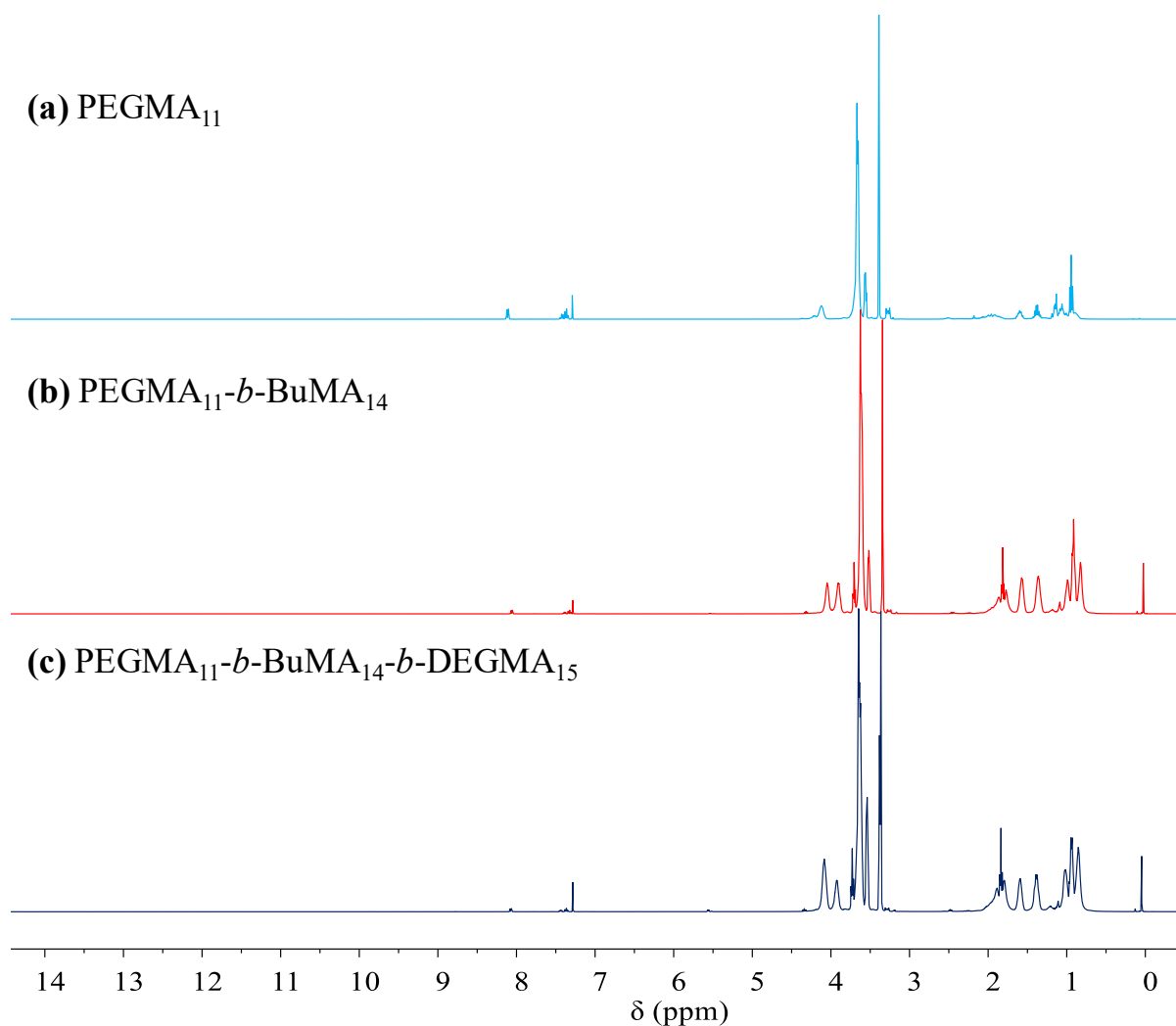


Figure A11.2: ^1H NMR spectra of (a) the homopolymer PEGMA₁₁, (b) the diblock copolymer PEGMA₁₁-*b*-BuMA₁₄, and (c) the final triblock terpolymer PEGMA₁₁-*b*-BuMA₁₄-*b*-DEGMA₁₅ (PC3) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

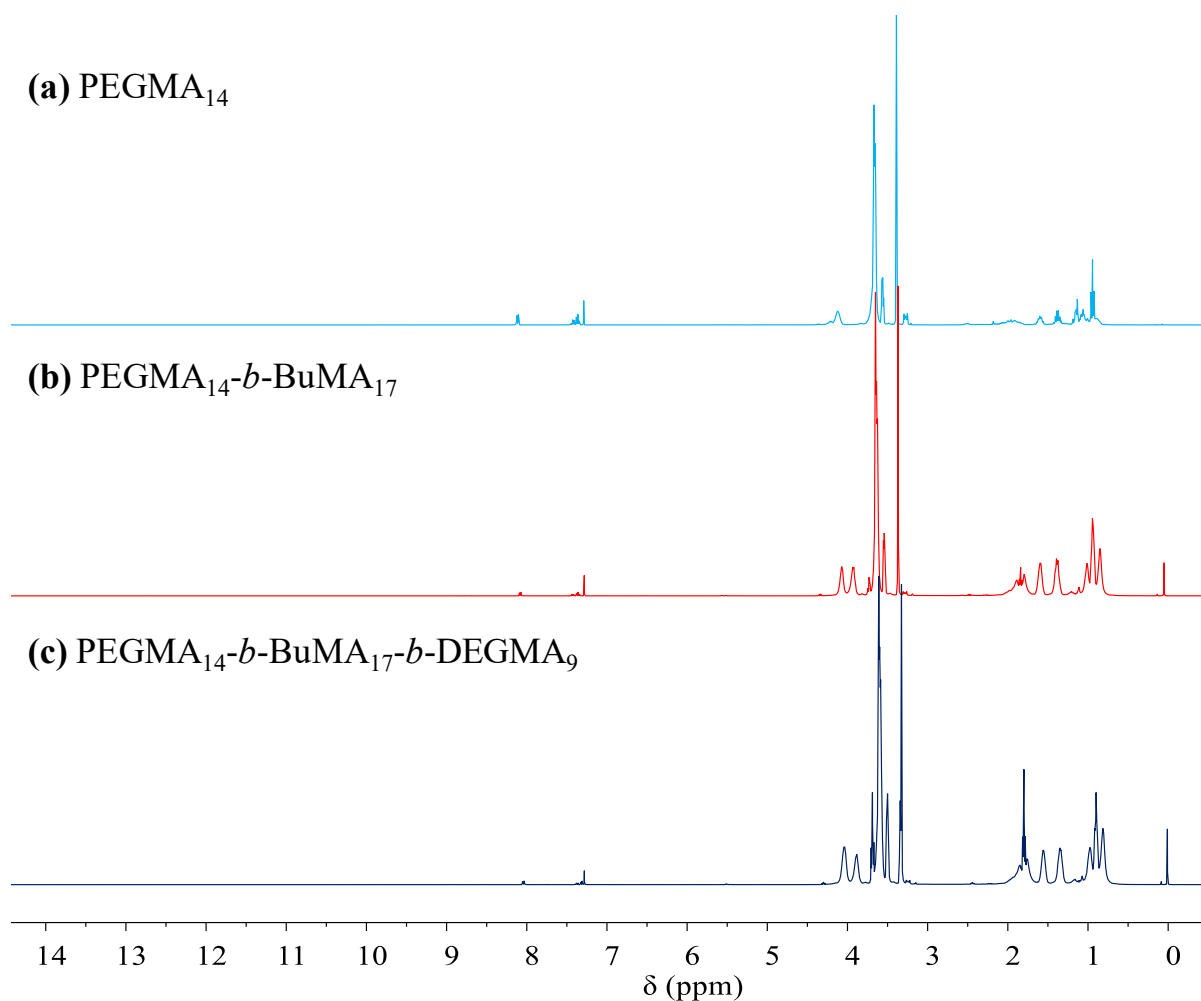


Figure A11.3: ^1H NMR spectra of (a) the homopolymer PEGMA₁₄, (b) the diblock copolymer PEGMA₁₄-*b*-BuMA₁₇, and (c) the final triblock terpolymer PEGMA₁₄-*b*-BuMA₁₇-*b*-DEGMA₉ (PC4) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

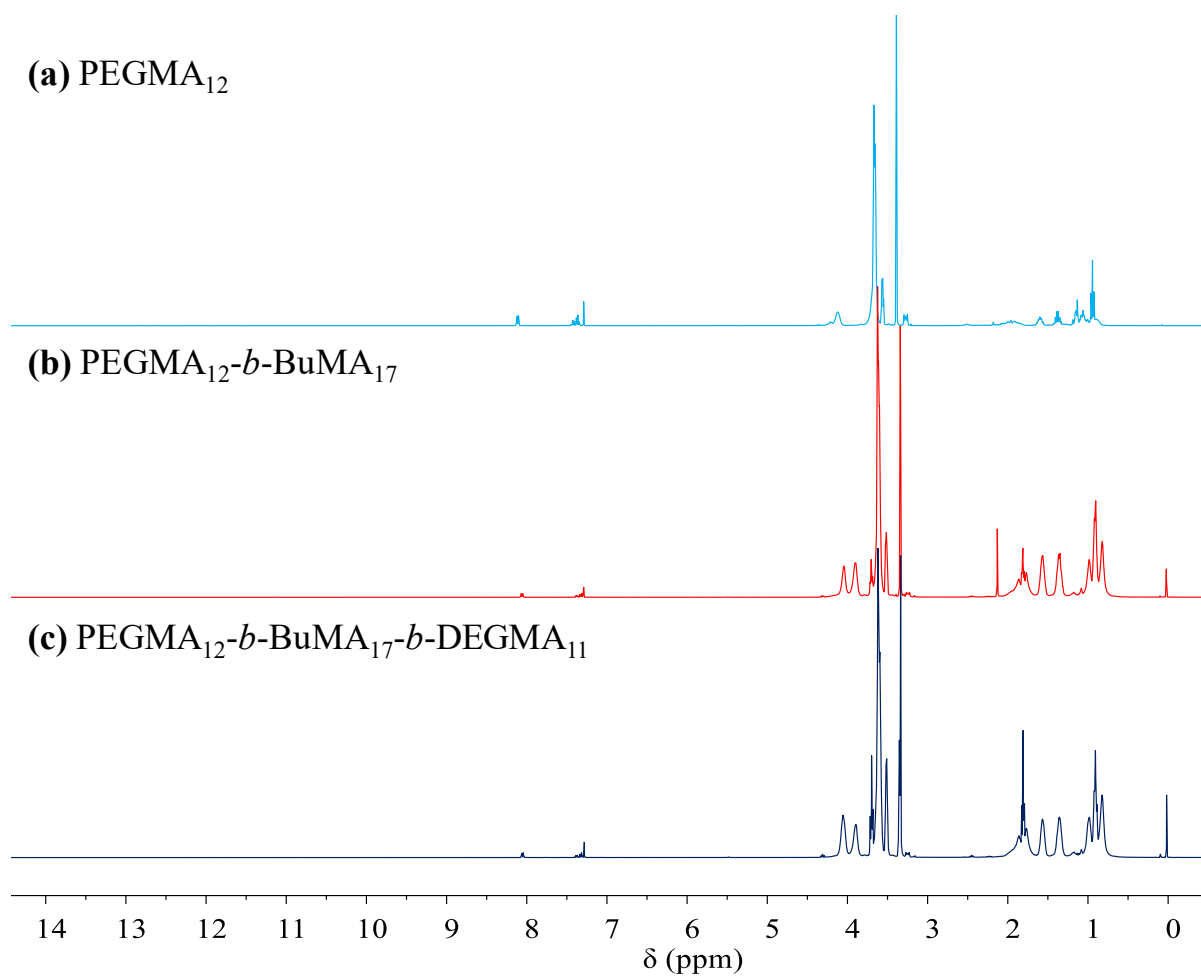


Figure A11.4: ^1H NMR spectra of (a) the homopolymer PEGMA₁₂, (b) the diblock copolymer PEGMA₁₂-*b*-BuMA₁₇, and (c) the final triblock terpolymer PEGMA₁₂-*b*-BuMA₁₇-*b*-DEGMA₁₁ (PC5) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

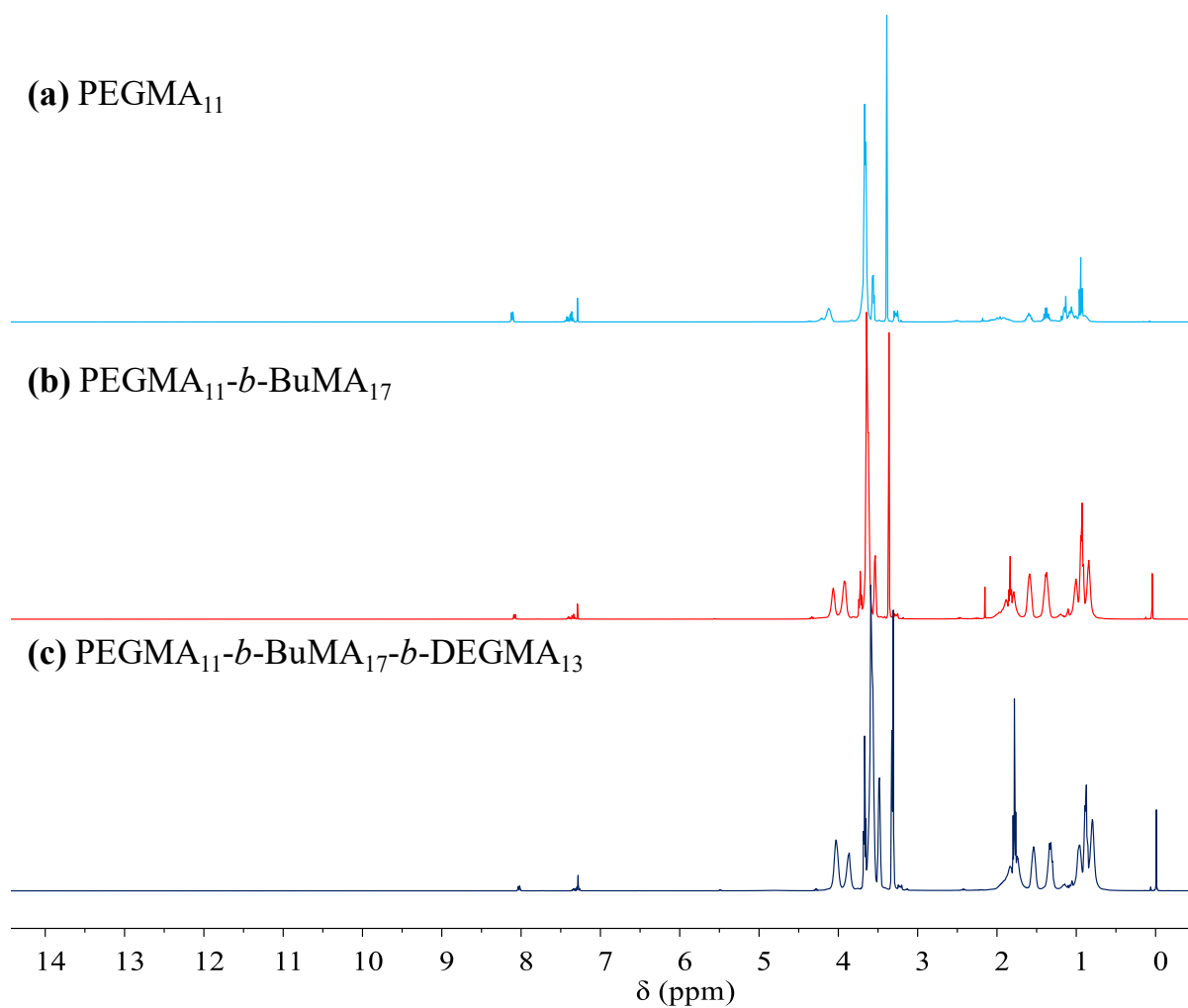


Figure A11.5: ^1H NMR spectra of (a) the homopolymer PEGMA₁₁, (b) the diblock copolymer PEGMA₁₁-*b*-BuMA₁₇, and (c) the final triblock terpolymer PEGMA₁₁-*b*-BuMA₁₇-*b*-DEGMA₁₃ (PC6) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

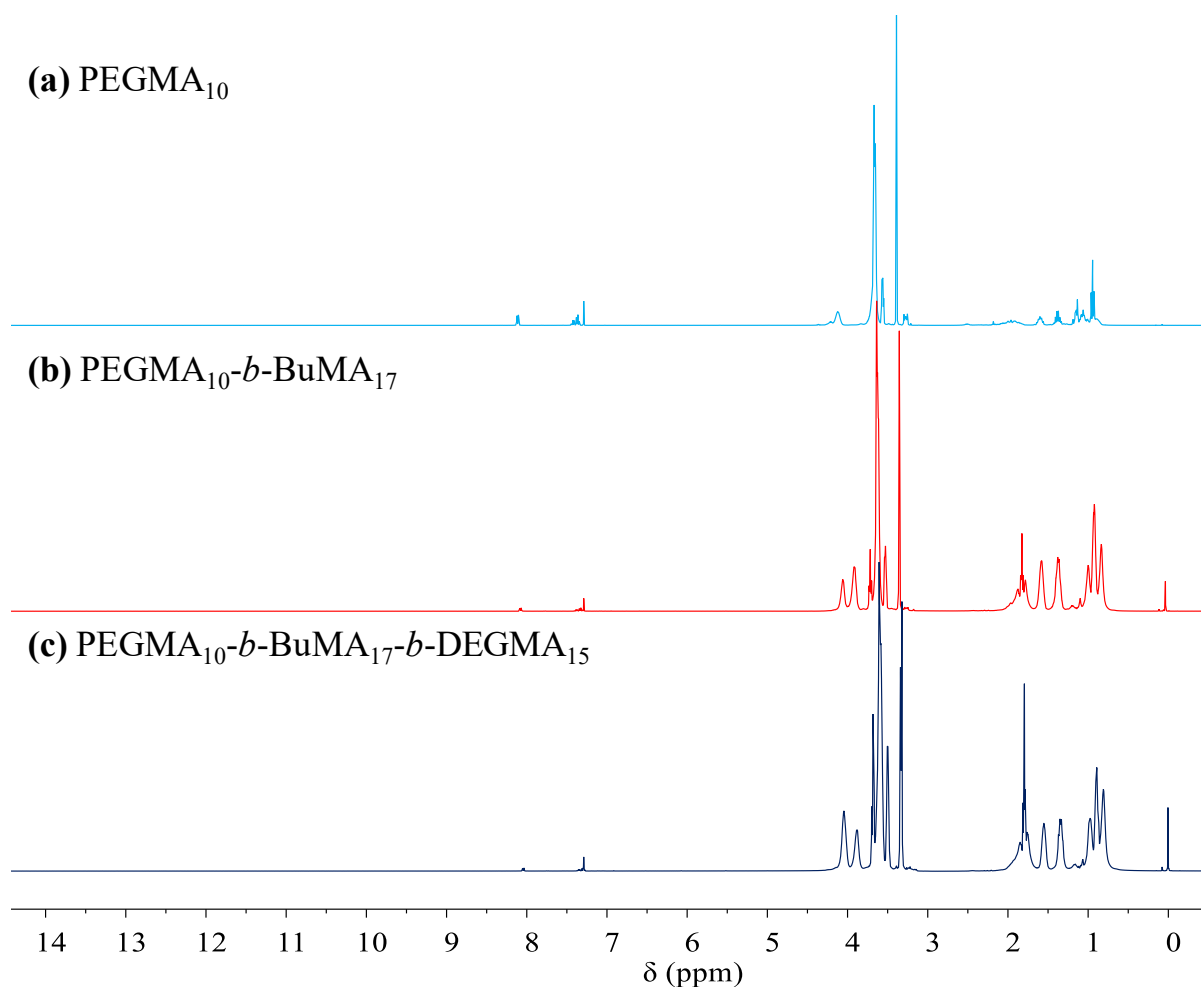


Figure A11.6: ^1H NMR spectra of (a) the homopolymer PEGMA₁₀, (b) the diblock copolymer PEGMA₁₀-*b*-BuMA₁₇, and (c) the final triblock terpolymer PEGMA₁₀-*b*-BuMA₁₇-*b*-DEGMA₁₅ (PC7) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

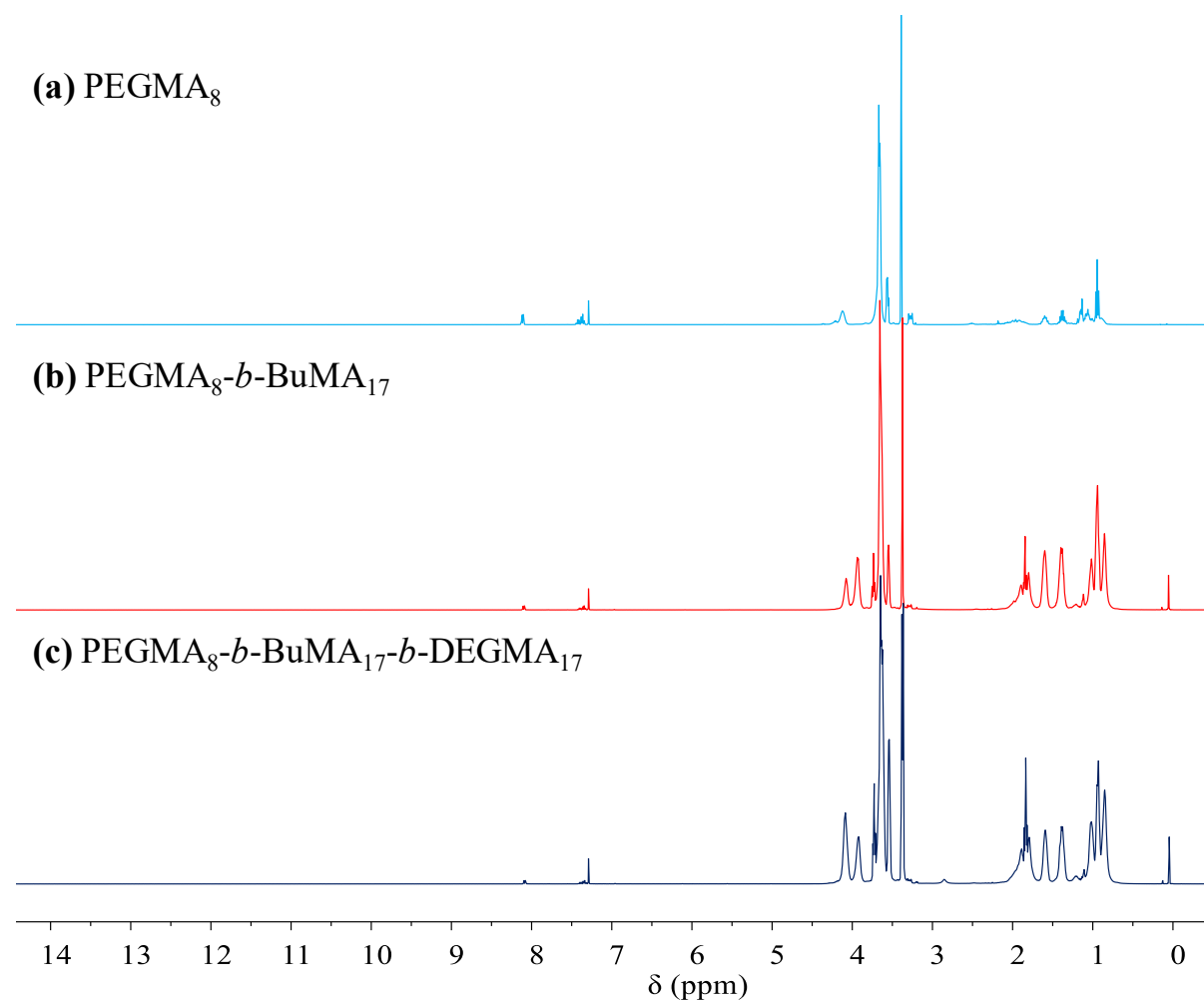


Figure A11.7: ^1H NMR spectra of (a) the homopolymer PEGMA₈, (b) the diblock copolymer PEGMA₈-*b*-BuMA₁₇, and (c) the final triblock terpolymer PEGMA₈-*b*-BuMA₁₇-*b*-DEGMA₁₇ (PC8) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

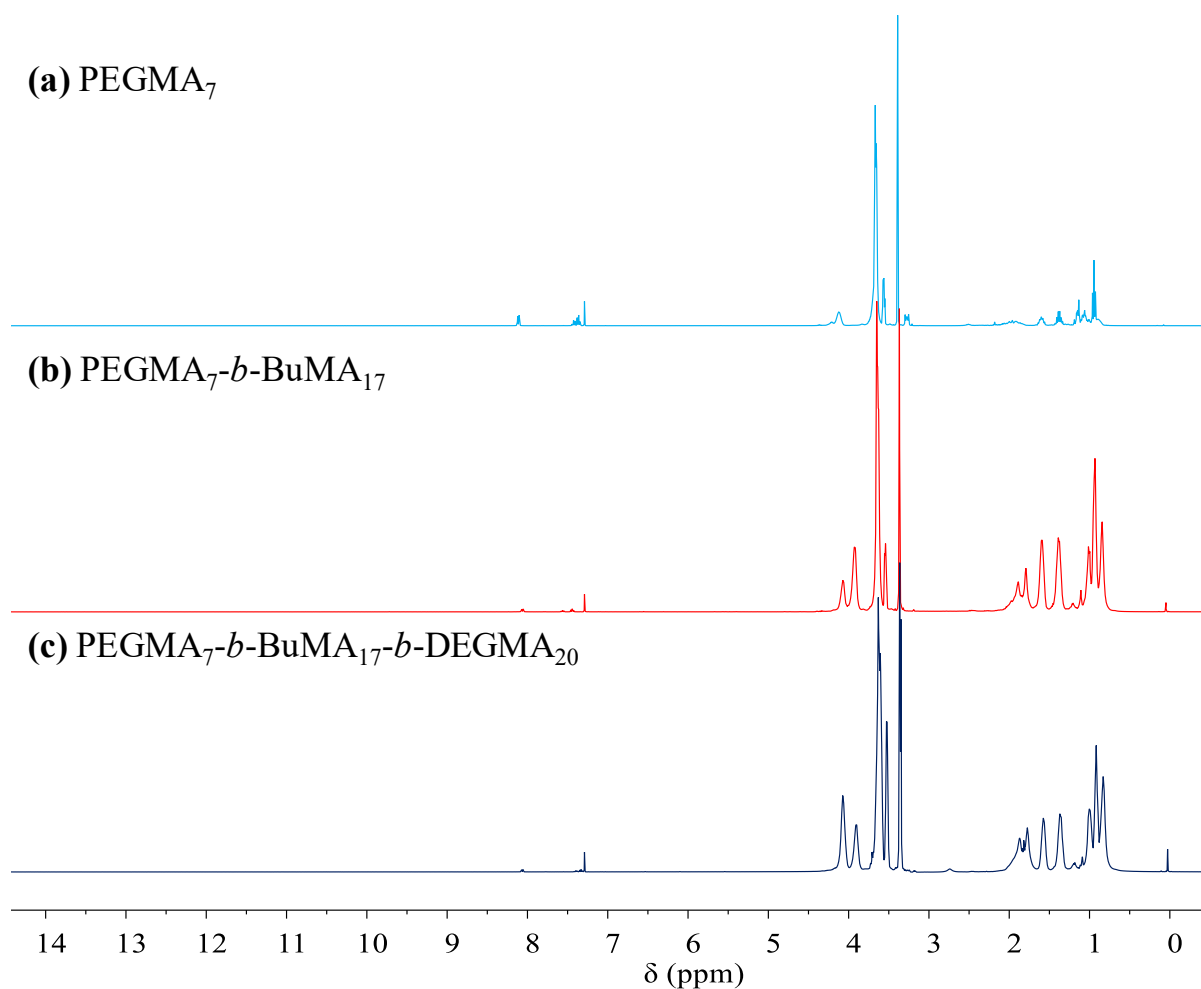


Figure A11.8: ^1H NMR spectra of (a) the homopolymer PEGMA₇, (b) the diblock copolymer PEGMA₇-*b*-BuMA₁₇, and (c) the final triblock terpolymer PEGMA₇-*b*-BuMA₁₇-*b*-DEGMA₂₀ (PC9) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

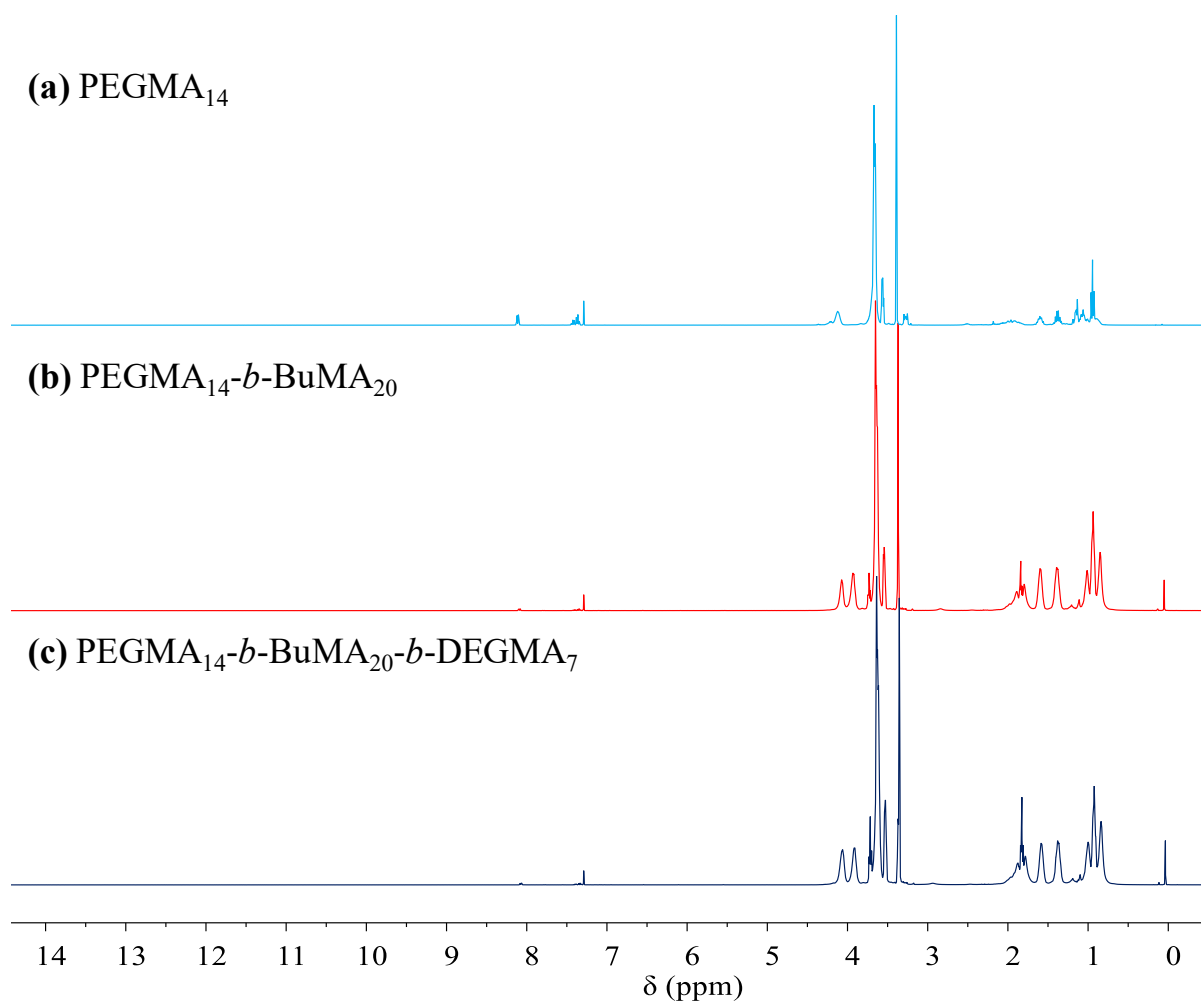


Figure A11.9: ^1H NMR spectra of (a) the homopolymer PEGMA₁₄, (b) the diblock copolymer PEGMA₁₄-*b*-BuMA₂₀, and (c) the final triblock terpolymer PEGMA₁₄-*b*-BuMA₂₀-*b*-DEGMA₇ (PC10) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

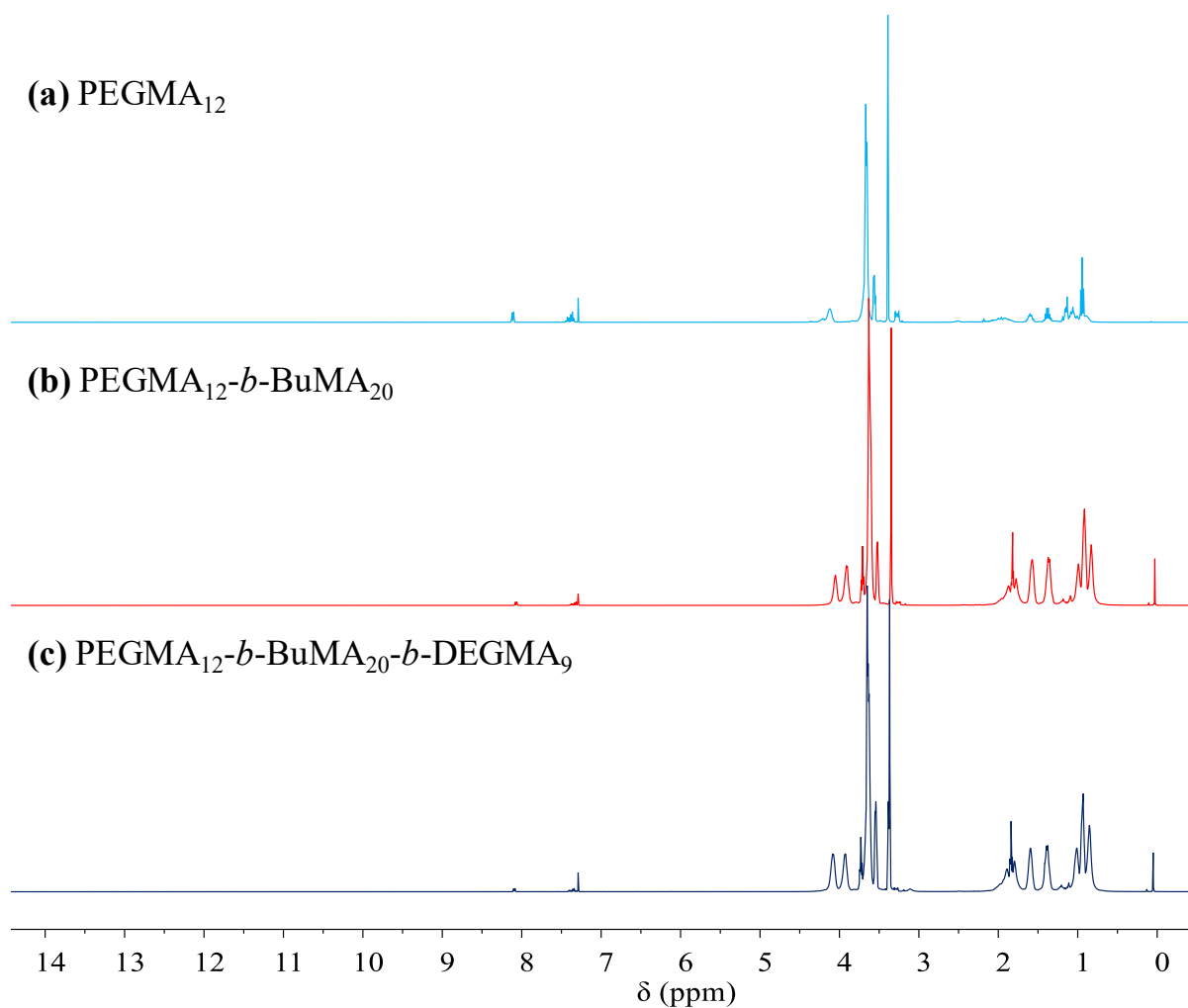


Figure A11.10: ^1H NMR spectra of (a) the homopolymer PEGMA_{12} , (b) the diblock copolymer $\text{PEGMA}_{12}\text{-}b\text{-BuMA}_{20}$, and (c) the final triblock terpolymer $\text{PEGMA}_{12}\text{-}b\text{-BuMA}_{20}\text{-}b\text{-DEGMA}_9$ (PC11) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

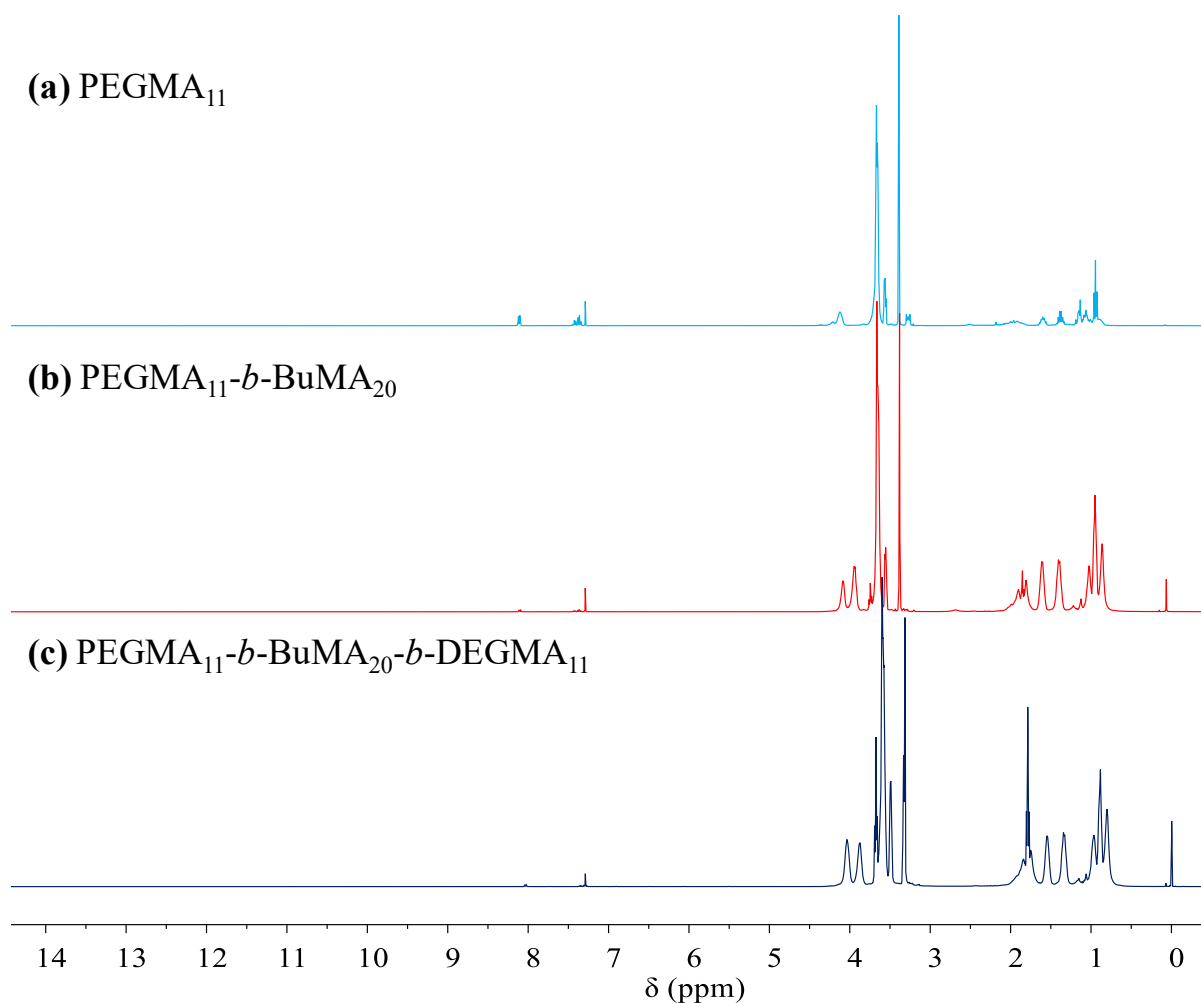


Figure A11.11: ^1H NMR spectra of (a) the homopolymer PEGMA₁₁, (b) the diblock copolymer PEGMA₁₁-*b*-BuMA₂₀, and (c) the final triblock terpolymer PEGMA₁₁-*b*-BuMA₂₀-*b*-DEGMA₁₁ (PC12) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

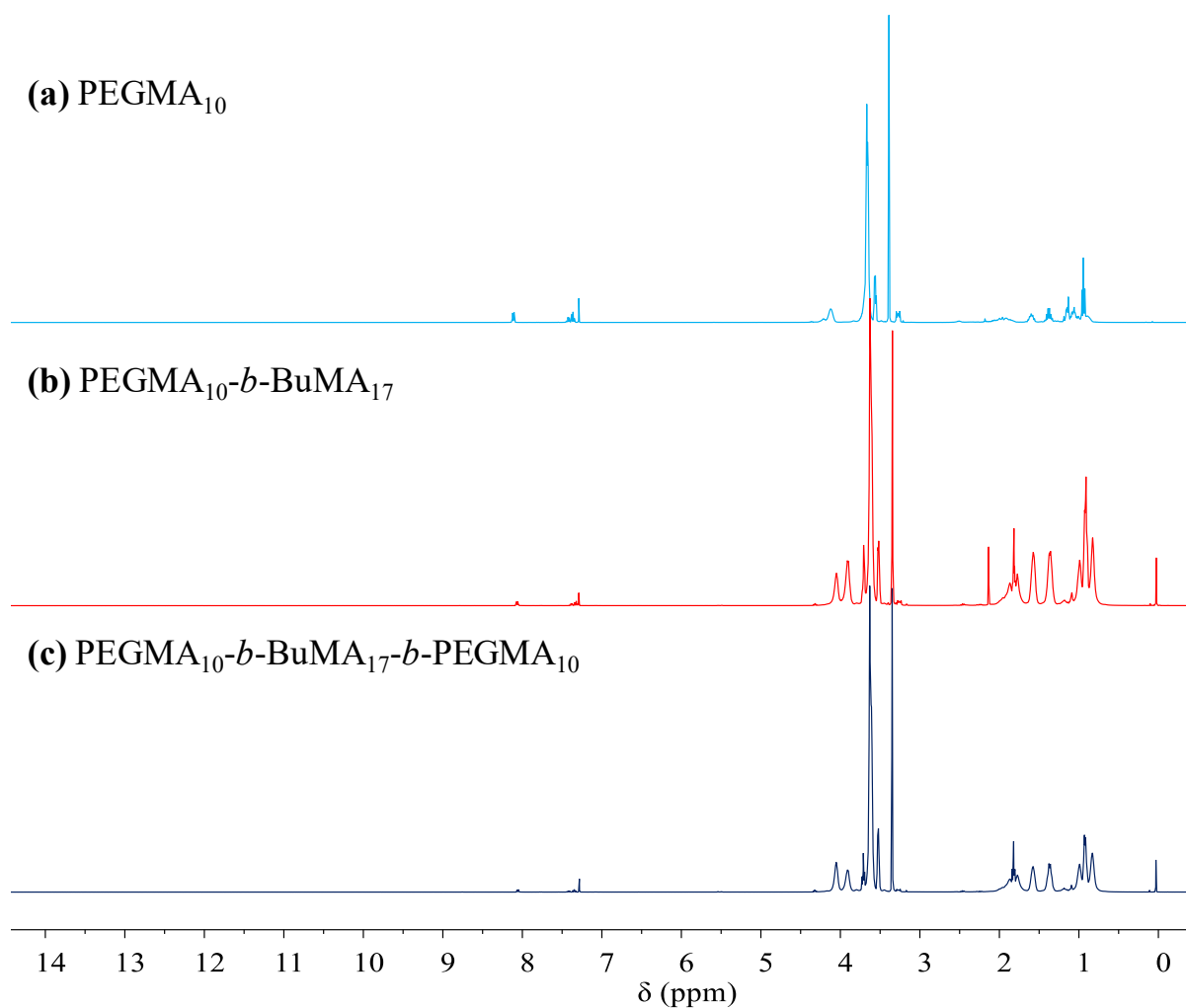


Figure A11.12: ^1H NMR spectra of (a) the homopolymer PEGMA₁₀, (b) the diblock copolymer PEGMA₁₀-*b*-BuMA₁₇, and (c) the final triblock terpolymer PEGMA₁₀-*b*-BuMA₁₇-*b*-PEGMA₁₀ (PC13) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

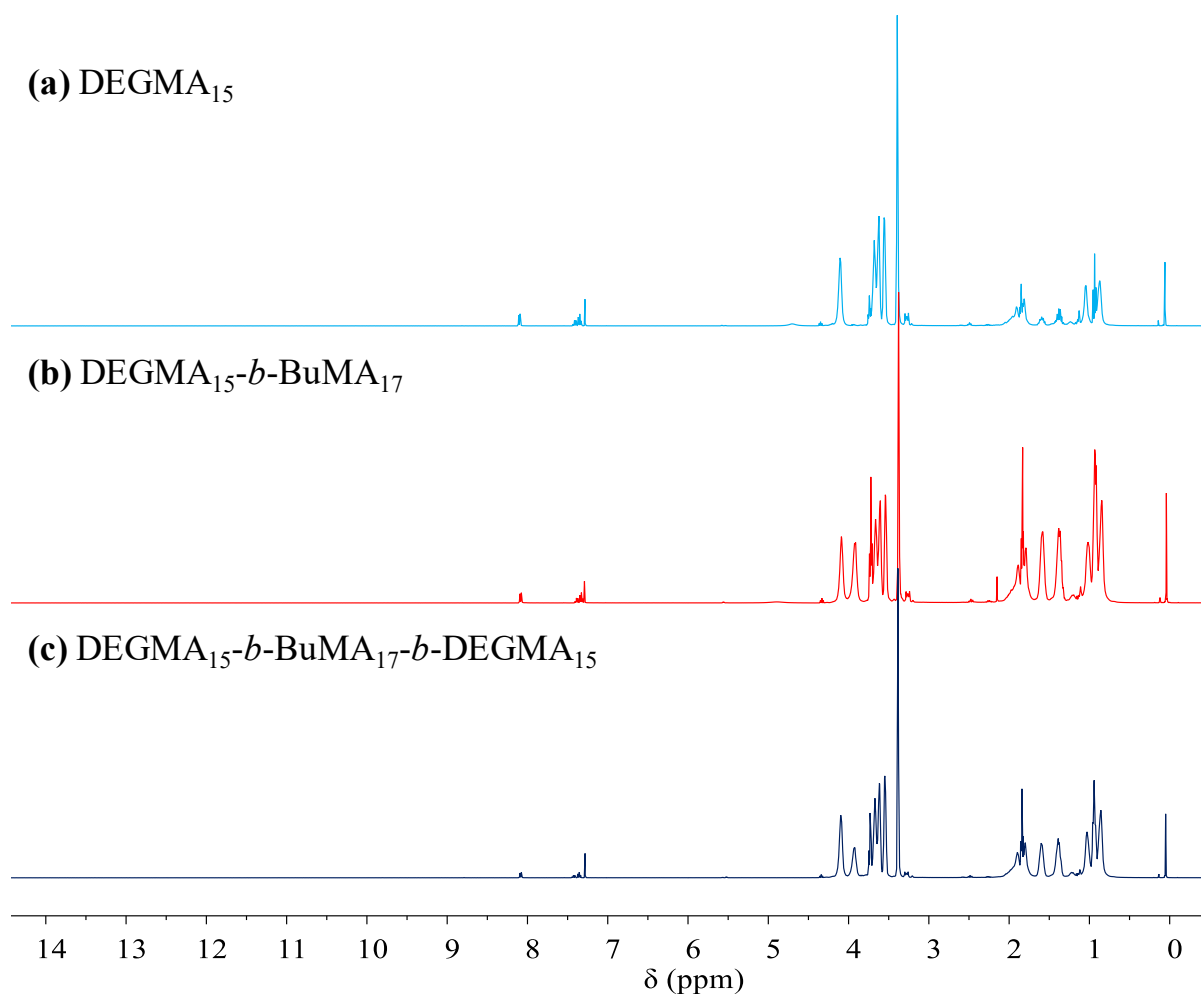


Figure A11.13: ¹H NMR spectra of (a) the homopolymer DEGMA₁₅, (b) the diblock copolymer DEGMA₁₅-*b*-BuMA₁₇, and (c) the final triblock terpolymer DEGMA₁₅-*b*-BuMA₁₇-*b*-DEGMA₁₅ (PC14) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as shown in Figure 5.3.5.

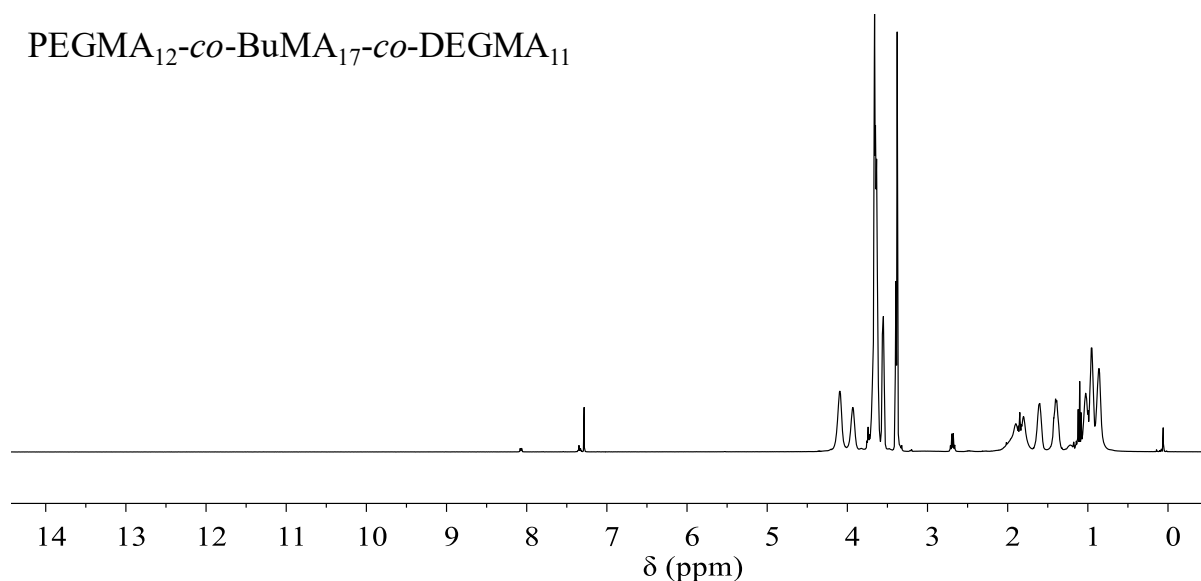


Figure A11.14: ¹H NMR spectrum of the statistical terpolymer PEGMA₁₂-*co*-BuMA₁₇-*co*-DEGMA₁₁ (PC15) in black. The analysis has been performed in a similar way as shown in Figure 5.3.5.

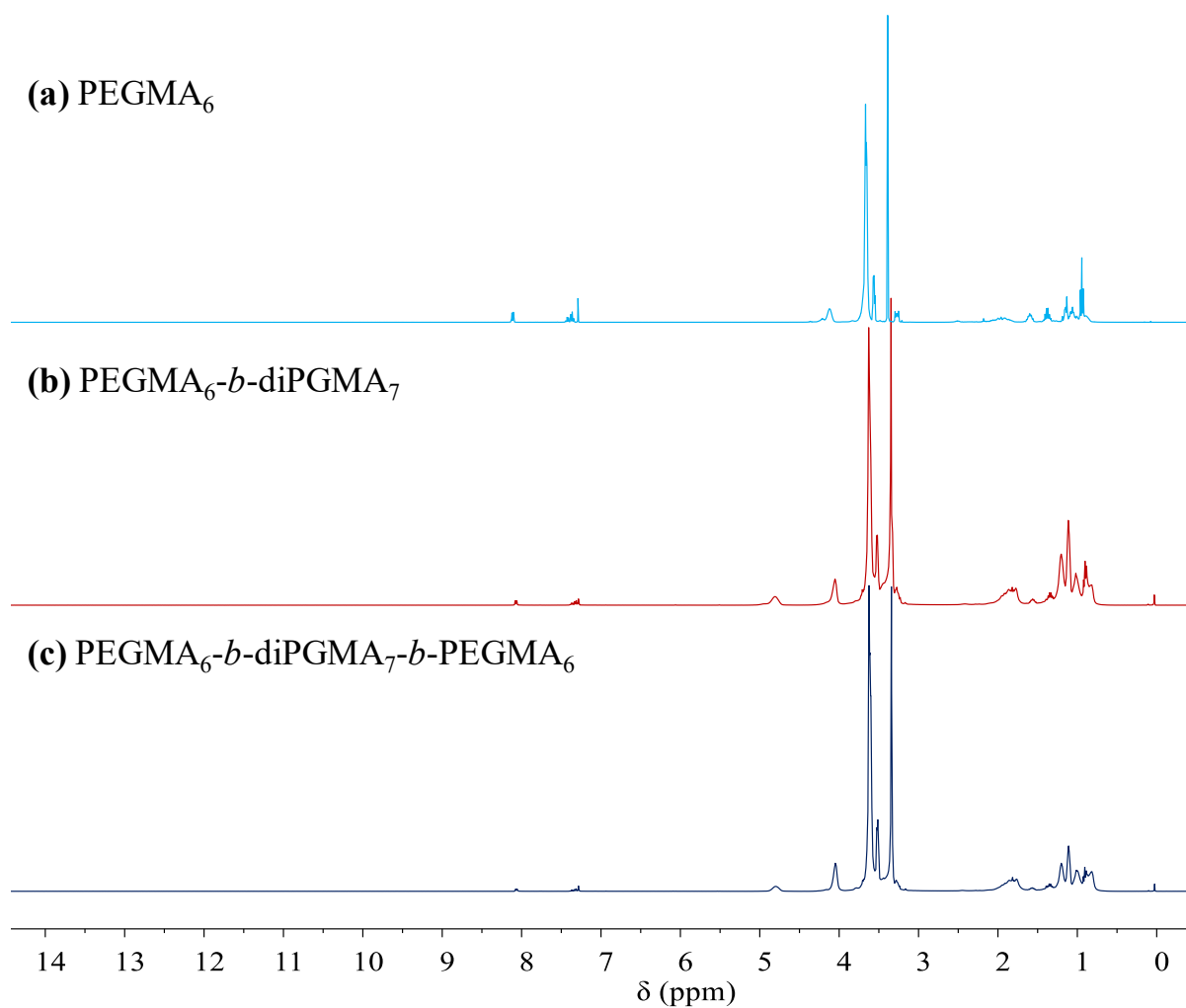


Figure A12.1: ^1H NMR spectra of (a) the homopolymer PEGMA₆, (b) the diblock copolymer PEGMA₆-*b*-diPGMA₇, and (c) the final triblock copolymer PEGMA₆-*b*-diPGMA₇-*b*-PEGMA₆ (PD2) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as indicated in Figure 5.4.10.

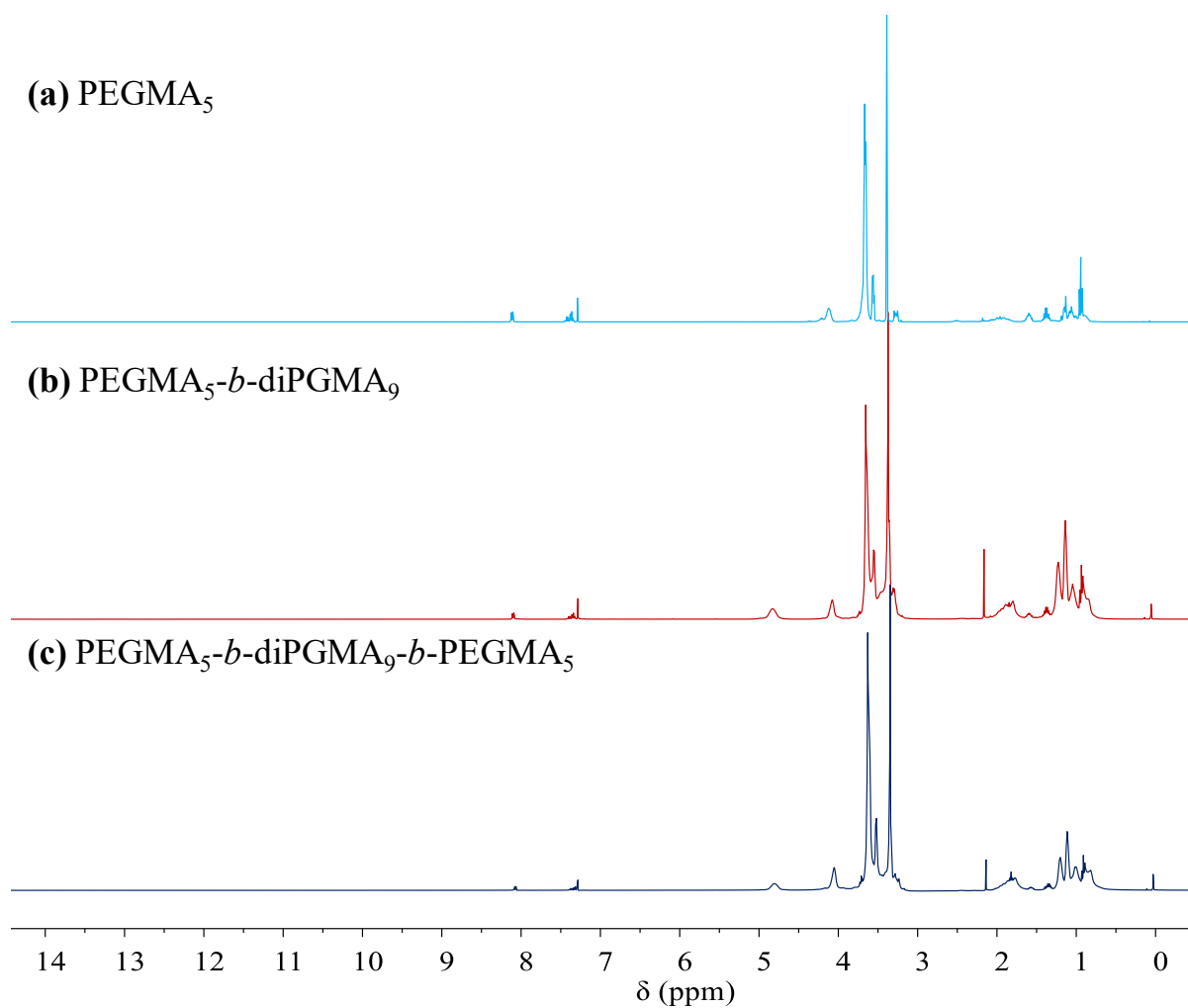


Figure A12.2: ¹H NMR spectra of (a) the homopolymer PEGMA₅, (b) the diblock copolymer PEGMA₅-*b*-diPGMA₉, and (c) the final triblock copolymer PEGMA₅-*b*-diPGMA₉-*b*-PEGMA₅ (PD3) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as indicated in Figure 5.4.10.

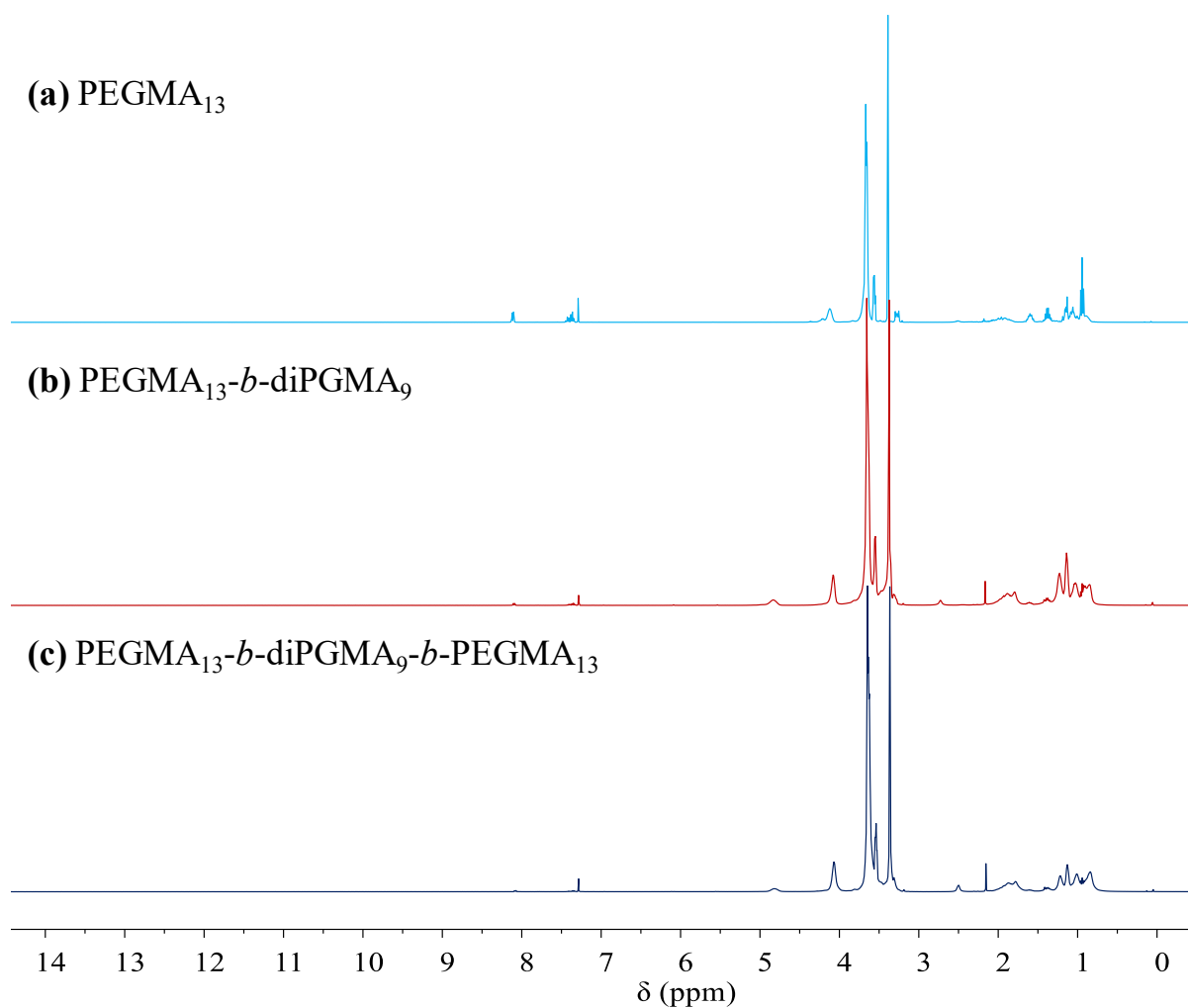


Figure A12.3: ^1H NMR spectra of (a) the homopolymer PEGMA₁₃, (b) the diblock copolymer PEGMA₁₃-*b*-diPGMA₉, and (c) the final triblock copolymer PEGMA₁₃-*b*-diPGMA₉-*b*-PEGMA₁₃ (PD4) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as indicated in Figure 5.4.10.

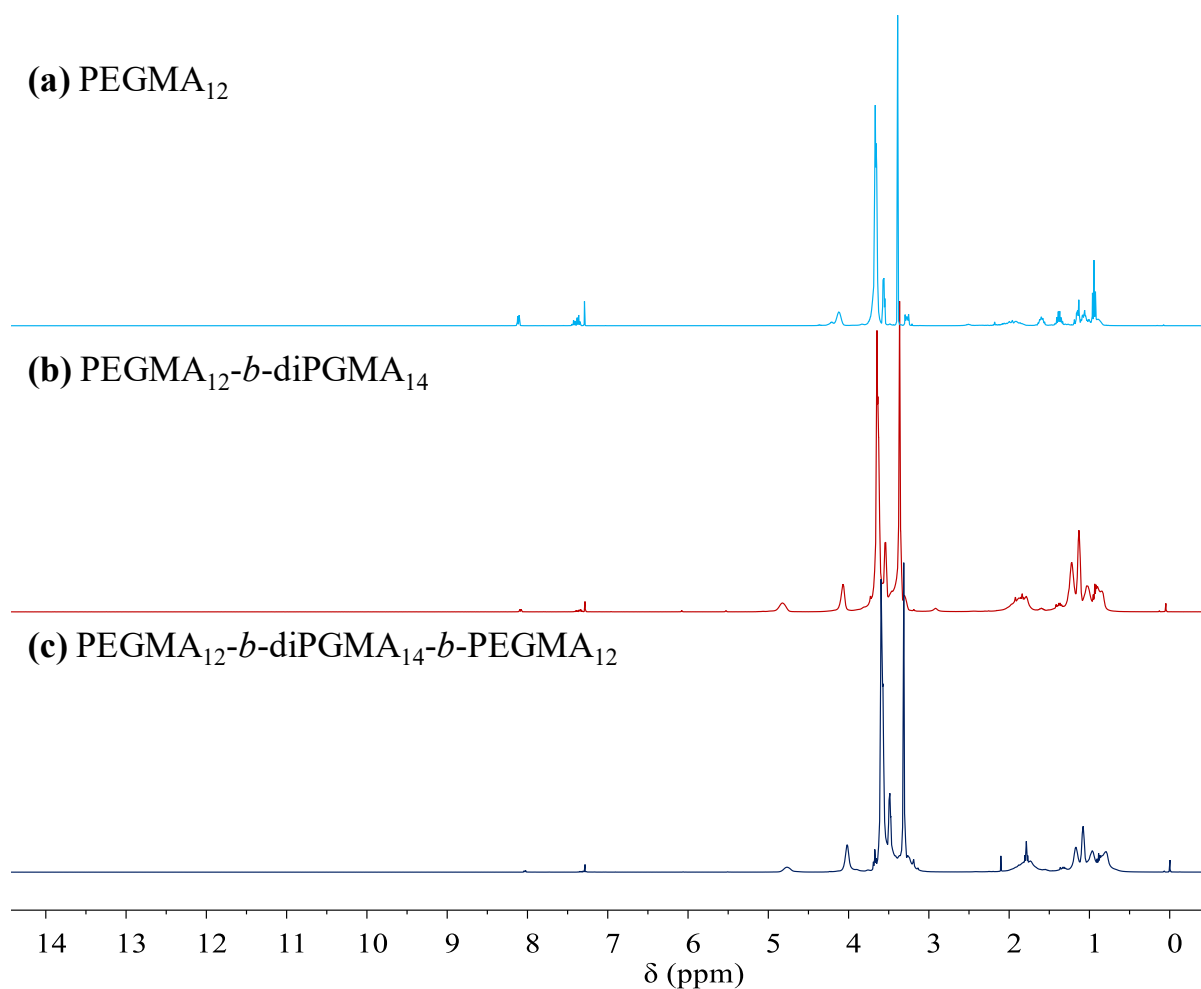


Figure A12.4: ^1H NMR spectra of (a) the homopolymer PEGMA₁₂, (b) the diblock copolymer PEGMA₁₂-*b*-diPGMA₁₄, and (c) the final triblock copolymer PEGMA₁₂-*b*-diPGMA₁₄-*b*-PEGMA₁₂ (PD5) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as indicated in Figure 5.4.10.

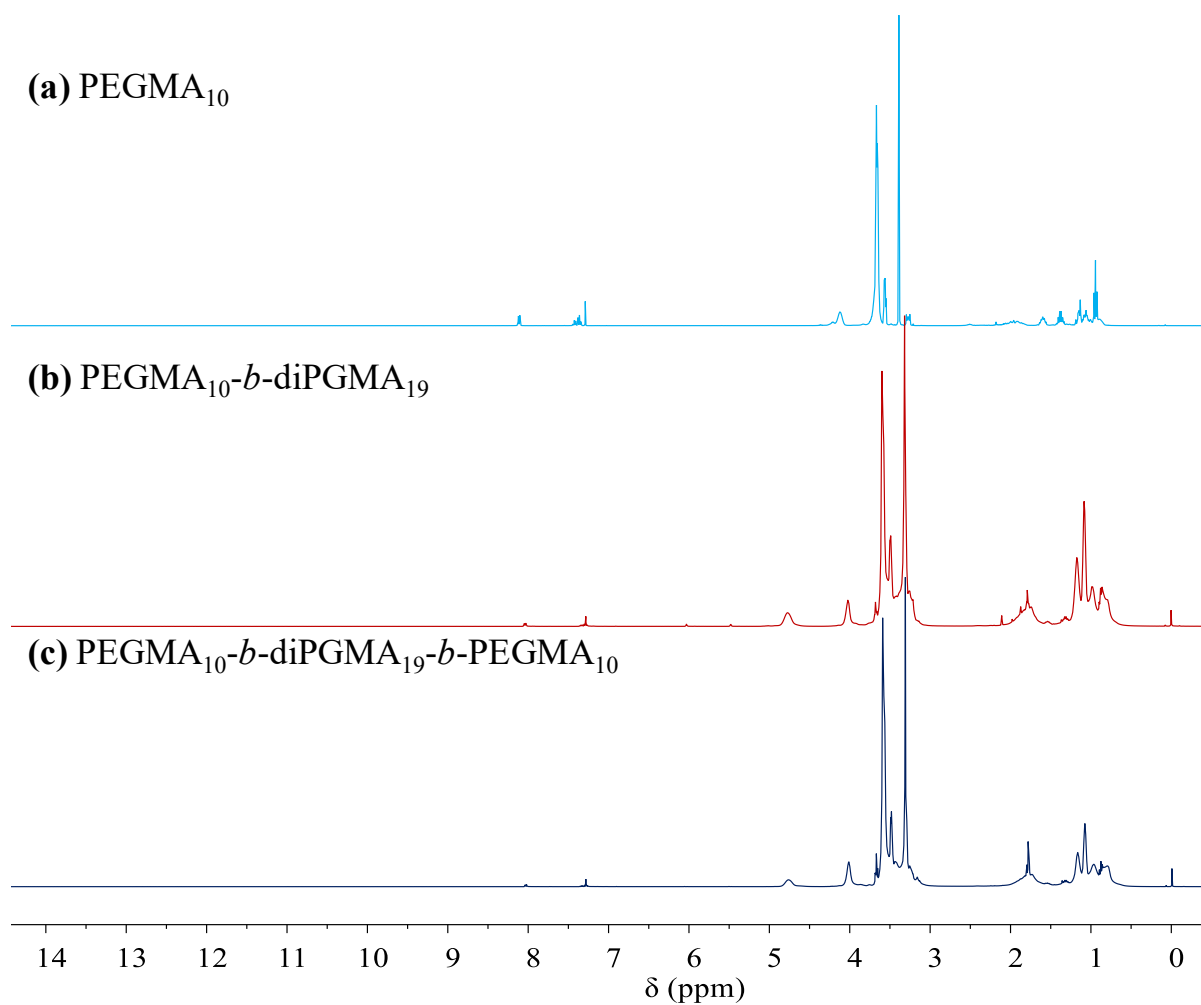


Figure A12.5: ^1H NMR spectra of (a) the homopolymer PEGMA₁₀, (b) the diblock copolymer PEGMA₁₀-*b*-diPGMA₁₉, and (c) the final triblock copolymer PEGMA₁₀-*b*-diPGMA₁₉-*b*-PEGMA₁₀ (PD6) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as indicated in Figure 5.4.10.

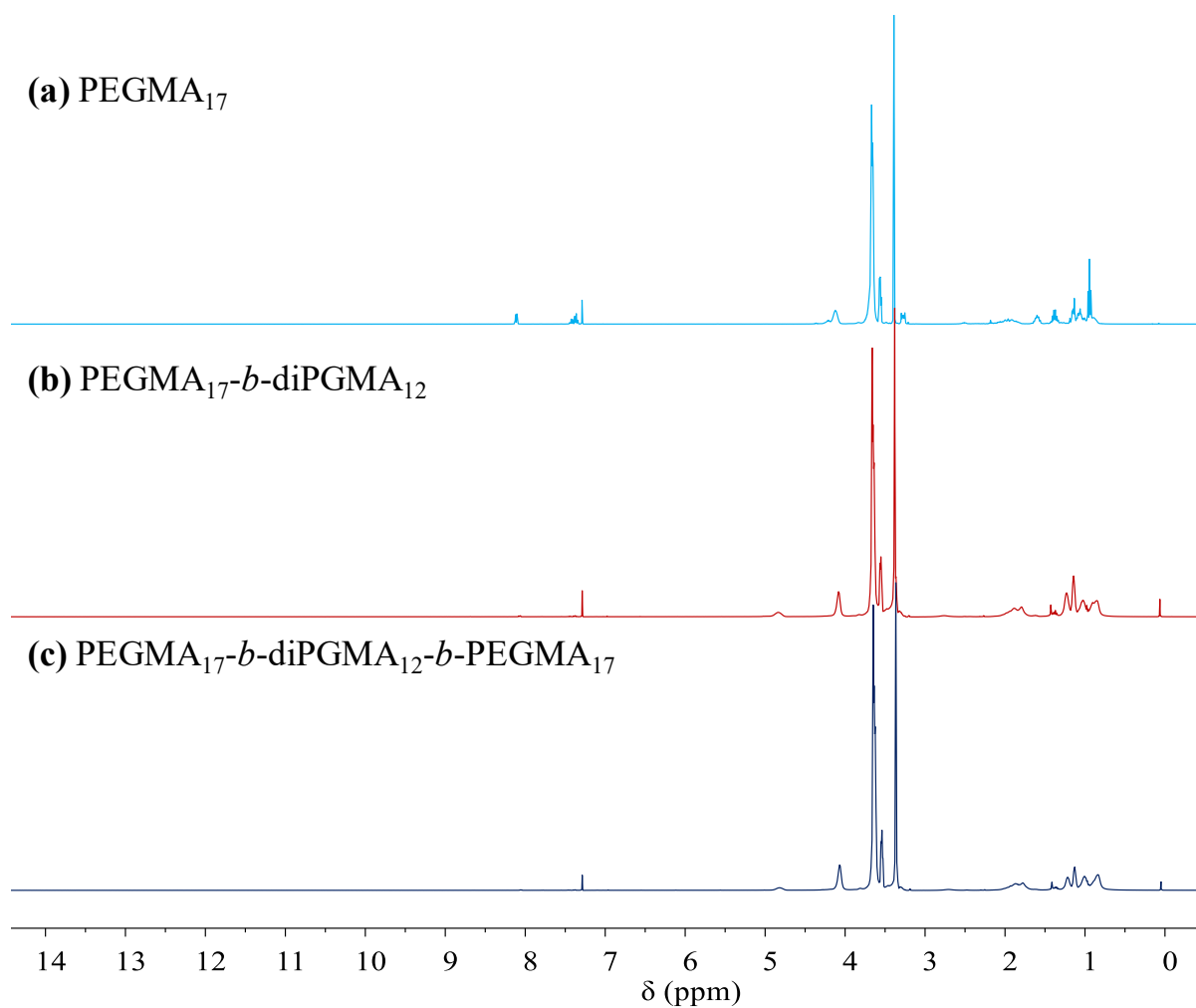


Figure A12.6: ^1H NMR spectra of (a) the homopolymer PEGMA₁₇, (b) the diblock copolymer PEGMA₁₇-*b*-diPGMA₁₂, and (c) the final triblock copolymer PEGMA₁₇-*b*-diPGMA₁₂-*b*-PEGMA₁₇ (PD7) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as indicated in Figure 5.4.10.

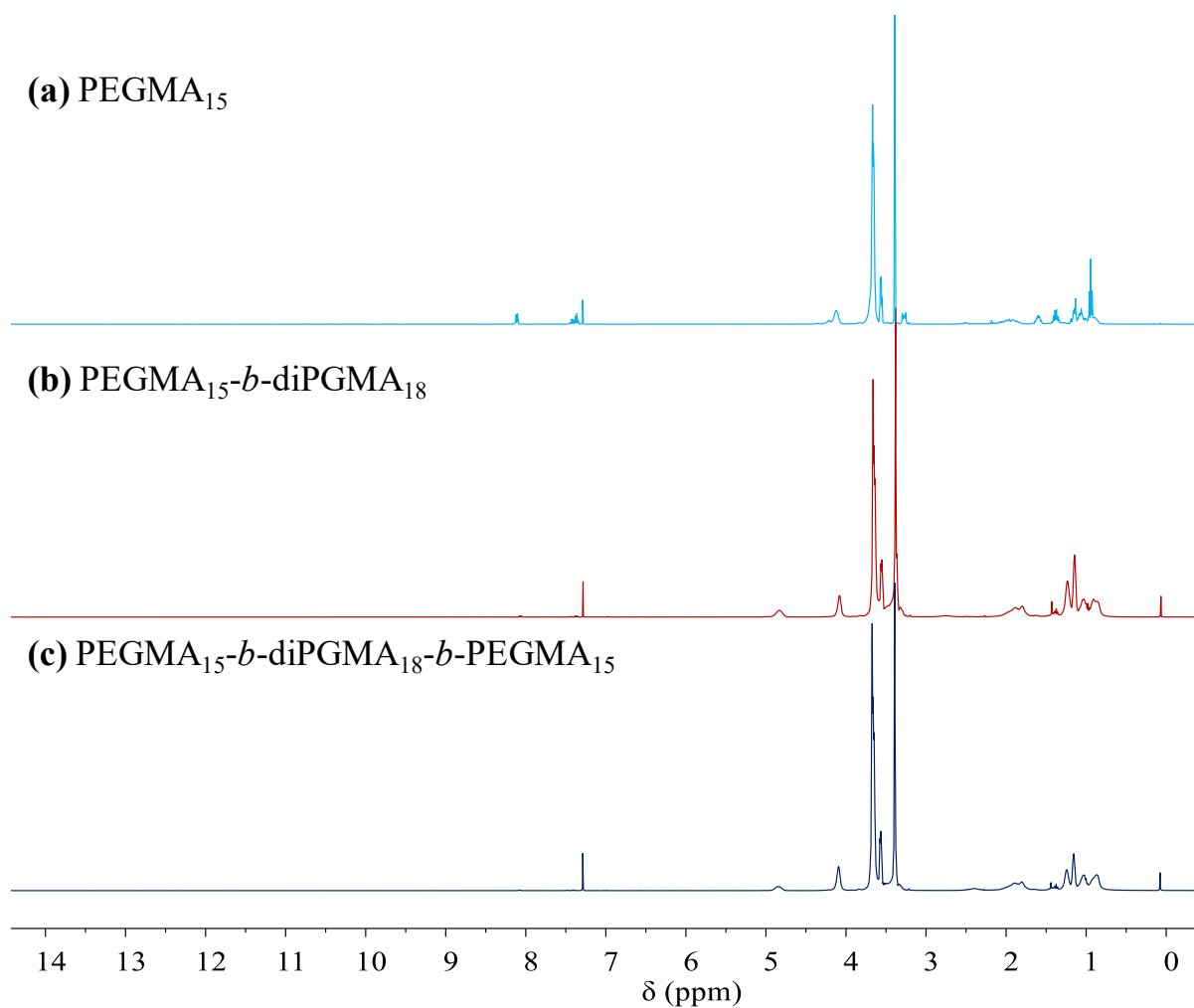


Figure A12.7: ^1H NMR spectra of (a) the homopolymer PEGMA₁₅, (b) the diblock copolymer PEGMA₁₅-*b*-diPGMA₁₈, and (c) the final triblock copolymer PEGMA₁₅-*b*-diPGMA₁₈-*b*-PEGMA₁₅ (PD8) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as indicated in Figure 5.4.10.

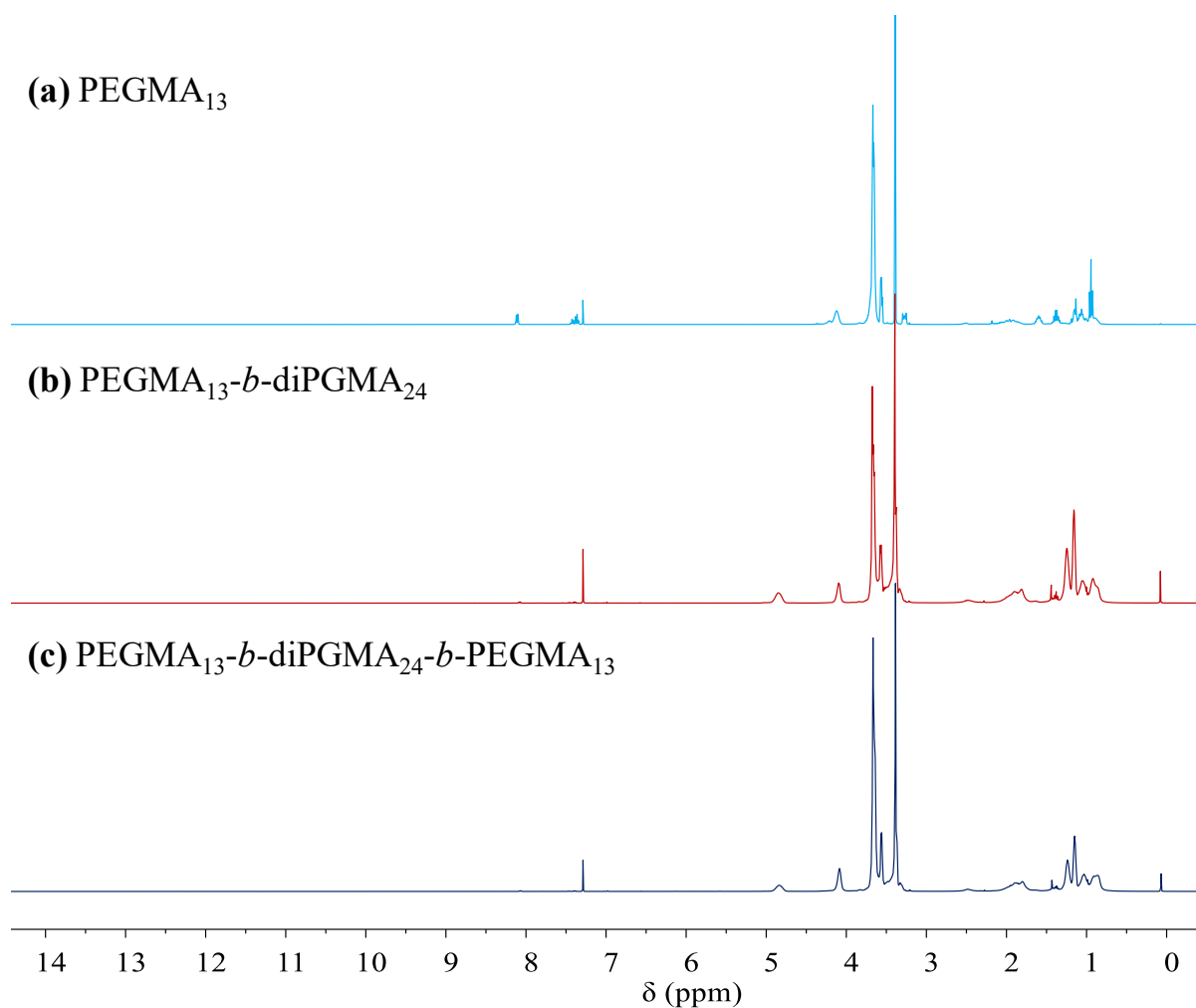


Figure A12.8: ^1H NMR spectra of (a) the homopolymer PEGMA₁₃, (b) the diblock copolymer PEGMA₁₃-*b*-diPGMA₂₄, and (c) the final triblock copolymer PEGMA₁₃-*b*-diPGMA₂₄-*b*-PEGMA₁₃ (PD9) are presented in light blue, red, and dark blue, respectively. The analysis has been performed in a similar way as indicated in Figure 5.4.10.

