STIMULI-RESPONSIVE NANOGELS
FOR ENVIRONMENTAL AND
PHARMACEUTICAL
APPLICATIONS

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degree of Doctor of Philosophy at Imperial College of Science,
Technology and Medicine
I, Marat Muratalin, hereby confirm that the work presented in this thesis is my own and the sources, where information has been derived from, were indicated.

Signed:

Date:

In the memory of my father Prof. Nurbolat Muratalin
Abstract

The term microgel has been widely used to describe particles that swell but do not dissolve in a solvent. Traditionally they can be anything from 100 nm – 100 µm in size. This project is devoted principally to investigation of the swelling/deswelling properties of largely submicron poly(N-isopropylacrylamide) [PNIPAM] microgel particles and its derivatives and also poly(2-vinylpyridine) [PVP] microgel particles. PNIPAM microgel particles are temperature-responsive because of the hydrophobic isopropyl group and the hydrophilic amide group present in its side chains. PVP microgel particles are pH-responsive due to the pyridine groups. Surfactant free emulsion polymerization (SFEP) and emulsion polymerization techniques were employed in order to copolymerize PNIPAM with acrylic acid (AA), with 3-acrylamidophenylboronic acid (3-APB) and (3-acrylamidopropyl)trimethylammonium bromide (ATMA) and with 1-vinylimidazole (VI). The resultant microgel particles exhibited multi-responsive behaviour being sensitive to changes in temperature, pH and the PNIPAM-co-3-APB-ATMA microgels were sensitive to concentration of glucose, whilst the PNIPAM-co-VI microgels were sensitive to certain metals, copper in particular. These microgel particles were characterized using dynamic light scattering (DLS), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The behaviour of the particles under various conditions of temperature, pH, glucose and metal ion are described and discussed in this work and several observations, such as swelling/deswelling transitions of PNIPAM-co-VI and PNIPAM-co-3-APB-ATMA with increase of concentration of added copper (II) and glucose respectively, were reported for the first time. The microgel containing AA exhibit characteristic temperature-sensitive behaviour with volume phase transition temperature (VPTT) being in the range of 35°-40°C and showed pH-sensitive features as the particles collapsed at low and swelled at high pHs. The PNIPAM-co-VI microgels undergo swelling before the concentration of Cu²⁺ reaches 0.3 g/L due to adsorption of the cations inside the particle which leads to charging up the internal phase of the microgel. Hence, the repulsion forces of positively charged Cu (II) ions are dominating over contraction forces of complex binding. However, at higher concentrations of copper (II) ions the binding forces of complexation between Cu²⁺ and imidazole groups of the microgels are leading to conformation of the microgel backbone, and hence weaker polymer-solvent interactions. Therefore, it is favourable that solvent would be forced out of the particle resulting into the collapse. In addition, the copper (II) uptake was calculated and the uptake was found to be well described by the Langmuir adsorption isotherm. The impact of other metal ions, such as nickel, zinc, iron and silver, was also investigated. The microgels swelled upon addition low concentrations of corresponding metal...
ions, however aggregation has been observed at higher concentrations. The microgels containing various concentration of VI were also examined on sensitivity to the temperature and pH changes. The investigation of such microgels with increasing temperature showed similar behaviour to those containing AA as the microgel particles shrunk continuously and the LCST has been shifted to higher temperatures (in the range of 35°-45°C). The particle size of these microgels was also investigated as a function of pH; the microgel particles swelled at low and collapsed at high pHs.

The particle size of the PNIPAM-co-3-APB-ATMA microgels was investigated both as a function of temperature and glucose concentration. The microgels showed typical behaviour of the PNIPAM microgels copolymerized with functional monomer, which were 3-APB and ATMA, by continuous shrinking with increasing temperature and shifted LCST towards higher temperatures. Additionally, these microgels showed swelling behaviour with the increase of glucose concentration at physiological conditions, i.e. particles swelled in the range of glucose concentration between 0.1 and 10 mmol/L at 35°C and pH 7.5. The behaviour of these microgels was also investigated at 35°C and pH 8.5 as a function of glucose concentration. Although the swelling of the particles was slightly larger at pH 8.5, considerable swelling was also observed at pH 7.5 making them the first microgel system to be glucose sensitive at physiological pH and temperature.
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2VP 2-vinylpyridine
3-APB 3-acrylamidophenylboronic acid
AA Acrylic acid
AM Allyl methacrylate
ATMA (3-acrylamidopropyl)trimethylammonium chloride
BIS N,N’-methylenebisacrylamide
DDS Drug-delivery system
DEAAm N,N-diethylacrylamide
DLS Dynamic light scattering
EP Conventional emulsion polymerization
HCl Hydrochloric acid
KPS Potassium persulfate
LCST Lower critical solution temperature
MA Methacrylic acid
MWCO Molecular weight cut-off
NaOH Sodium hydroxide
NIPAM N-isopropylacrylamide
NMR Nuclear magnetic resonance
<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>PBA</td>
<td>3-aminophenylboronic acid hemisulfate</td>
</tr>
<tr>
<td>PAA</td>
<td>Poly(Acrylic acid)</td>
</tr>
<tr>
<td>PCS</td>
<td>Photon correlation spectroscopy</td>
</tr>
<tr>
<td>PDEAAm</td>
<td>Poly(N,N-diethylacrylamide)</td>
</tr>
<tr>
<td>PEO</td>
<td>Polyethylene oxide</td>
</tr>
<tr>
<td>DVB</td>
<td>Divinylbenzene</td>
</tr>
<tr>
<td>PI</td>
<td>Polydispersity index</td>
</tr>
<tr>
<td>AI</td>
<td>Asymmetry index</td>
</tr>
<tr>
<td>PMAA</td>
<td>Poly(Methacrylic acid)</td>
</tr>
<tr>
<td>PNIPAM</td>
<td>Poly(N-isopropylacrylamide)</td>
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<tr>
<td>PNIPAM-co-AA</td>
<td>Poly(N-isopropylacrylamide) copolymerized with Acrylic acid</td>
</tr>
<tr>
<td>PNIPAM-co-VAA</td>
<td>Poly(N-isopropylacrylamide) copolymerized with Vinylacetic acid</td>
</tr>
<tr>
<td>PS</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>PVP</td>
<td>Poly(2-vinylpyridine)</td>
</tr>
<tr>
<td>VI</td>
<td>1-vinylimidazole</td>
</tr>
<tr>
<td>V50</td>
<td>2,2’-azobis[2-methylpropionamidine] dihydrochloride</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>SFEP</td>
<td>Surfactant-free emulsion polymerization</td>
</tr>
<tr>
<td>FRPP</td>
<td>Free-radical precipitation polymerization</td>
</tr>
<tr>
<td>VAA</td>
<td>Vinylacetic acid</td>
</tr>
<tr>
<td>VPTT</td>
<td>Volume phase transition temperature</td>
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Chapter 1 Introduction

1.1 Background

A gel is a solid, jelly-like material which is a three-dimensionally crosslinked network in a fluid, and, therefore, exhibits properties which are ranging from soft and weak to hard and tough. Mostly, gels consist of fluid which ensnares a solid three-dimensional crosslinked polymer network; hence such gels have a density close to that of the fluid which is composing them. The internal solid network of the gel can result from physical bonds or chemical bonds, as well as any crystallites or junctions that will remain intact within the extending fluid. Virtually any fluid can act as an extender including water (hydrogels), oil and air (aerogel) [1].

A hydrogel is a network of crosslinked polymer chains which themselves are water-soluble (hydrophilic). The crosslinks act to join the structure together. The chemical nature of the polymer network of the hydrogel dictates its behaviour. Hydrogels consisting of such materials as N-isopropylacrylamide (NIPAM) are temperature-sensitive, hence swell/shrink with the changes of temperature [2]; poly(2-vinylpyridine) and polyacrylic acid hydrogels are pH-sensitive as they respond for the changes of pH in the surrounding media [3]. Moreover, it is possible to produce hydrogels which are responsive to ionic strength, presence of certain materials and other external stimuli.
Microgels, which are essentially small particles of hydrogels, have the same polymer chemistry but their physical molecular arrangements are different. A microgel particle is usually a crosslinked latex particle which is swollen by a good solvent [4]. Microgels that have good swelling/deswelling properties and also temperature and pH sensitivity can be considered as good candidates for environmental applications. This thesis is devoted to principally poly(N-isopropylacrylamide) [PNIPAM] microgel particles and its derivatives and is based on the monomer N-isopropylacrylamide (NIPAM). Poly(2-vinylpyridine) [PVP] microgel particles have also been made. PNIPAM microgel particles are temperature-responsive because of the presence of the hydrophobic isopropyl group and the hydrophilic amide group in its side chains. PVP microgels are pH-sensitive due to the pyridine groups, which can be protonated at low pH, making the polymer water-soluble. PNIPAM and PVP microgels should therefore be temperature and pH sensitive respectively and exhibit good swelling ratios in making them suitable to be developed as functional agents for environmental applications in further work. For example, such “smart” materials could be used as water-shutoff agents to reduce the volume of water that is extracted from an oil well in addition to the oil [5]. Moreover, modified poly(N-isopropylacrylamide) [PNIPAM] microgel particles could be synthesized with other functionalities making the resultant microgels sensitive not only to temperature but to other stimuli [6], [7]. Such microgels could have the potential to be used in both environmental and
pharmaceutical applications. It is this aspect which is the main thrust of this thesis.

Conceptually, microgels could also be developed to be sensitive for certain molecules whereby they swell, or contract, in their presence. In this work microgels sensitive to copper or glucose have been prepared. Thus, the microgels have the potential to be used as sensors, extractants or as drug-delivery systems. For example, if a microgel is sensitive to glucose at physiological pH and temperature can be developed it could be used to control the release of insulin and hence be used in the treatment of diabetes, which according to the World Health Organization diabetes causes 5% of total death in the world [8]. At the present time insulin is mainly delivered by injection. So, controlled insulin release could solve such problems as repeated glucose level checking and injections several times a day, which is either painful, or not performed frequently enough, to ensure a stable glucose level in the blood.

1.2 Objectives

The main objectives of this thesis are:

1. **Synthesis and investigation of temperature- and pH-responsive microgels:**
   
   Such microgels have been investigated by many researchers in the past [9], [10], [4], [11], [12], [13], pH- and temperature-responsive microgels were employed as introductory materials in this project in
order to understand the general properties and behaviour of the microgels.

2. **Synthesis and investigation of novel metal-responsive microgels:**

This was one of two main goals of this project, namely is to prepare novel microgels with sensitivity for the presence of metals ions in solution (copper in particular) via copolymerizing N-isopropylacrylamide with 1-vinylimidazole. There were some investigations undertaken on this problem where hydrogels or macrogels of corresponding structure were made sensitive for the presence of the metals [14], [15], [16], [17], [18] and microgels that consisted of other functionalities rather 1-vinylimidazole were prepared to act as metal sensors [19], [20]; however prior to this study no research has been found where microgels of such composition were made responsive for the presence of the metals in the surrounding environment.

3. **Synthesis and investigation of glucose-responsive microgels operating at physiological pH:**

There have been a considerable number of investigations devoted to research glucose-responsive microgels but all of these investigations made the glucose-responsive microgels which operate at high pH, i.e. pH 10.5 or higher [21], [22], [23], [24], [25] rather than the physiological pH of 7.4. In this project microgels were prepared in order to be functional under physiological conditions.

### 1.3 Thesis Outline

A general overview of the properties and synthesis of microgels is described in Chapter 2, where types of microgels, their preparation, properties and applications are presented. Chapter 3 details the synthesis of microgels that
have been made during this project and the techniques used to characterise and investigate their swelling behaviour are described. Results obtained from these investigations are presented in Chapter 4, where the behaviour of certain microgel dispersions according to respective surrounding conditions is discussed and interpreted. Conclusions of the undertaken research and recommendations for future work to be done are presented in Chapter 5 of the thesis.
Chapter 2 Literature review

2.1 Microgels versus Macroscopic Hydrogels

There is often confusion between the terms “hydrogels” and “microgels”. Essentially, microgels are small particles of hydrogels. The polymer chemistry is the same, but their physical molecular arrangements are different. Microgels, as compared with hydrogels, are differentiated as discrete gel-like particles (see Figure 2-1). Microgels have such advantages as lower viscosity, very high surface area and consequently a very rapid response to an external stimulus. In contrast, hydrogels have very large dimensions with the consequential long time scales for swelling and deswelling [26]. The swelling rate will be higher for microgels due to its small size (higher surface area to volume). The grafting of N-isopropylacrylamide (NIPAM) side chains to the interstitial regions within bulk hydrogels gives rise to the creation of hydrophobic regions, aiding the expulsion of water from the network (during heating) resulting in collapse. Analogous hydrogels lacking the grafted side chains can take several weeks to undergo full deswelling, whereas the inclusion of the grafted chains results in the overall shrinking time being reduced to tens of minutes [27]. However, colloidal analogues of the same crosslinked poly(N-isopropylacrylamide) [PNIPAM] undergo a fully reversible (shrinking/swelling) conformational change virtually “instantaneously” and, therefore, offer considerable advantages over macrogel
type structures, particularly for applications such as “smart” actuators in temperature sensitive switches or valves or for controlled release [27].

![Diagram of macrogels and microgels]

**Figure 2-1:** An illustration of fundamental differences between macrogels and microgels (adapted from [28]).
2.2 Microgels

The first people to synthesize microgels were Staudinger and Husemann in 1935 [29], who made microgels consisting purely of divinylbenzene (DVB). They found that using certain amount of monomer in the reaction mixture did not result in a macrogel, but in soluble molecules. Baker described a new form of polymer, which he called “microgels” [30]. The microgel comprised cross-linked latex particles that swelled in organic solvents to form colloidally dispersed gel particles (Figure 2-2). Since then a wide range of research has been investigated and microgels can be made with a vast range of properties from different monomers according to the final requirements of the microgel. They can be responsive to temperature [31], ionic strength [32], external osmotic pressure [33] and pH [33], [34], [35]. Some of the most widespread monomers and crosslinkers are shown in Figure 2-3.

Thermo-responsive aqueous colloidal microgels have properties in common with water-soluble polymers, water-swollen macrogels, and water-insoluble latex particles. The properties of microgels are dependent upon the delicate balance of polymer/polymer versus polymer/water(solvent) interactions, as is the case for the water-soluble polymers. Like macroscopic gels, microgels are described by a swelling ratio, average crosslinking density and characteristic time constants for swelling/deswelling. Microgels can have monodisperse particle size distributions when prepared, which makes them have common
features with water-insoluble latex particles. Microgels can be characterized by several standard colloidal techniques; for example, electrophoresis, dynamic light scattering, rheology and electron microscopy.

The most widely studied water-swellable microgel system is PNIPAM [4], [36], [37]. The best example of an organic swellable microgel is polystyrene (PS) crosslinked by divinylbenzene (DVB) [38]; these particles are swollen in aromatic solvents (e.g. toluene). Ionic microgel particles are prepared containing carboxylate groups obtained from carboxylic acid (e.g. acrylic acid, methacrylic acid) or an amine type cationic group.

The microgel properties can be changed by changes in the structure of the monomers. For example, N-vinyl-butyramide is isomorphic with NIPAM and is a good monomer for microgel synthesis [39]. Such variations give an opportunity for control of the lower critical solution temperature (LCST) or volume phase transition temperature (VPTT) of the microgel. Lower critical
<table>
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<th>Monomer Name</th>
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<tr>
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</tr>
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<td>N-isopropylacrylamide (NIPAM)</td>
</tr>
<tr>
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<td>Methacrylic acid (MA)</td>
</tr>
<tr>
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<td>Styrene</td>
</tr>
<tr>
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<td>Divinylbenzene (DVB)</td>
</tr>
<tr>
<td><img src="image" alt="N,N’-methylenebisacrylamide" /></td>
<td>N,N’-methylenebisacrylamide (BIS)</td>
</tr>
<tr>
<td><img src="image" alt="Allyl methacrylate" /></td>
<td>Allyl methacrylate (AM)</td>
</tr>
<tr>
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<td>1-vinylimidazole (VI)</td>
</tr>
<tr>
<td><img src="image" alt="3-acrylamidophenylboronic acid" /></td>
<td>3-acrylamidophenylboronic acid (3-APB)</td>
</tr>
<tr>
<td><img src="image" alt="Acrylic acid" /></td>
<td>Acrylic acid (AA)</td>
</tr>
<tr>
<td><img src="image" alt="3-acrylamidopropyl trimethylammonium chloride" /></td>
<td>(3-acrylamidopropyl) trimethylammonium chloride (ATMA)</td>
</tr>
</tbody>
</table>

*Figure 2-3: Common monomers (top row), crosslinkers (middle row) and comonomers (bottom row) used to form stimuli-responsive microgels (adapted from [40]).*
solution temperature is a critical temperature below which the components of a mixture (such as polymer and water) are completely miscible for all compositions [41], and volume phase transition temperature is the critical temperature near which gel particles undergo a large-magnitude volume change, i.e. swell/deswell [42], [43].

2.2.1 NIPAM-based microgels

N-isopropylacrylamide (NIPAM) is the major building block for temperature-sensitive microgels. The monomer is available from many specialty chemical distributors. With a structure (see Figure 2-3) close to acrylamide, many of the properties of NIPAM are similar to those of acrylamide. In aqueous solution it undergoes rapid free radical polymerization in water to give high molecular weight polymers at rates similar to that of acrylamide [36]. Poly(N-isopropylacrylamide) [PNIPAM] is the most common material used and widely studied for building a temperature sensitive microgel since it was first reported in 1986 by Pelton and Chibante [44]. It has a lower critical solution temperature (LCST) of PNIPAM which is 32\(^\circ\)C [45]. The polymer is soluble in water below this temperature, and it will phase separately above this temperature. Since the LCST is near ambient and body temperatures, this has played a role in attracting researchers to study and use this material in many potential applications, including for separations and drug release [46]. PNIPAM contains hydrophobic isopropyl groups and hydrophilic amide groups, and it is this aspect which makes it extremely temperature sensitive.
N,N’-methylene-bis-acrylamide (BIS) is generally used as the crosslinker for the polymer to form microgels and it is this crosslinking which prevents it from dissolving in water at lower temperatures. The chemical structure of PNIPAM microgels is shown in Figure 2-4. The volume of the microgels can reversibly change with changes in the surrounding temperature. Below the volume phase transition temperature (VPTT), which is 32°C, the microgel is swollen due to its hydrophilicity. On the other hand, above this temperature deswelling takes place because particles become hydrophobic. PNIPAM particles are swollen at room temperature owing to hydrogen bonding between water and the amide groups. Hydrogen bonding within aqueous amide solutions has been found [47], [48] to involve the carbonyl oxygen (R-C=O…H-O-H) and the protic hydrogen (R’-N-H…OH). It is reasonable to expect that analogous bonding exists within PNIPAM particles dispersed in water at room temperature. Heating the solution above the VPTT disrupts the water-polymer hydrogen bonding, allowing inter- and intra-molecular hydrogen bonding and attractive hydrophobic interactions to dominate, inducing chain collapse (see Figure 2-5). Thermally induced deswelling occurs for both PNIPAM microgel particles and macrogels [49].

The extent of PNIPAM microgel swelling is dependent on the degree of crosslinking [26]. Kratz et al. investigated the relationship between temperature, crosslinking and swelling of the microgels [50]. Figure 2-6 shows the influence of different concentrations of crosslinker on the swelling of
Figure 2-4: The chemical structure of PNIPAM.

Figure 2-5: The interaction of microgels with water above and below the VPTT (adapted from [51]).

...microgels. As the concentration of the crosslinker, which is N,N’-methylenebisacrylamide (BIS), increases, the swelling ratio of microgels decreases. We can also see from Figure 2-6 that the VPTT of the microgels did not change with different concentration of N,N’-methylenebisacrylamide...
(BIS) in the microgel backbone. Drastic change in size occurred around 32°C for all different concentrations of N,N’-methylenebisacrylamide (BIS).

Figure 2-6 also shows that the microgels are changing in the volume over a range of temperatures starting to shrink at approximately 25°C-30°C and achieving the collapsed state at approximately 35°C-40°C suggesting that VPTT is in the range of temperature rather than at a certain temperature as LCST. This is probably due to higher degree of reactivity of BIS in comparison with reactivity of NIPAM, hence there is a core consisted mainly of BIS in the particle which will shrink at higher temperature than shell of the microgel containing less BIS and more NIPAM.

Figure 2-6: The response of microgels with different concentration of crosslinker as a function of temperature (adapted from [50]).
2.2.2 Non-NIPAM-based microgels

N,N-diethylacrylamide (DEAAm) is another potential monomer for preparing temperature-responsive microgel backbone as it exhibits a similar lower critical solution temperature (LCST) as N-isopropylacrylamide (NIPAM), which is in the range of 29\(^0\)C and 32\(^0\)C [52]. Panayiotou et al. investigated this potential monomer by comparing the morphology, phase transition, and the biocompatibility of the poly(N,N-diethylacrylamide) [PDEAAm] microgels to poly(N-isopropylacrylamide) [PNIPAM] microgels [26]. N,N’-methylenebisacrylamide (BIS) was used as the crosslinker when preparing both PDEAAm and PNIPAM microgels. They reported that the volume phase transition temperature (VPTT) of PDEAAm microgels is 31\(^0\)C, i.e. 1\(^0\)C lower than the VPTT of PNIPAM microgels. This shows that PDEAAm exhibits similar thermal response profile as PNIPAM.

Duracher reported the preparation of microgels based on N-isopropylmethacrylamide [53]. The preparation of the microgel particles were under the same conditions as for PNIPAM microgels, however the synthesis took five times longer. It had previously been reported that the propagation time for acrylamide is 22 times faster than for methacrylamide [54], hence the previous observation is understandable. Thus, these microgel particles have a significant disadvantage because the reactivity of isopropylmethacrylamide is so low. A result of this low reactivity is that BIS crosslinker is consumed much more rapidly than N-isopropylmethacrylamide, thus core-shell particles as opposed to microgels tend to be produced, where the core of the particles
consists of BIS and the shell comprised of terminally attached chains of N-isopropylmethacrylamide.

Lowe prepared microgel particles based on N-ethylacrylamide [55]. The synthesis took place at 90°C and produced monodisperse dispersions, but the VPTT was less abrupt for poly(N-ethylacrylamide) compared to PNIPAM particles behaviour and the LCST was higher.

Particularly large swelling ratios can be obtained with microgels of polymers, such as poly(2-vinylpyridine) [PVP] and poly(acrylic acid) [PAA], by using pH to induce swelling at ambient temperatures, because there is a significant disparity between the favourability of polymer-solvent interactions for charged and uncharged monomer segments and the presence of counterions within the polymer network adds a further contribution to the osmotic pressure. Whereas microgels based on PVP swell when the pH is lowered because the nitrogen atoms protonate and become positively charged, microgels based on PAA swell when the pH is raised because the carboxylic acid groups dissociate and become negatively charged.

### 2.2.3 Copolymerized hydrogels

It has been investigated by many authors [7],[51],[56],[3] that copolymerization of poly(N-isopropylacrylamide) [PNIPAM] with other monomers, which have different degrees of hydrophilicity/hydrophobicity, can shift the lower critical solution temperature (LCST) and also make hydrogels multi-responsive, i.e. a copolymer of N-isopropylacrylamide and 1-
vinylimidazole (NIPAM-VI) is likely to be responsive to both temperature, pH and the presence of metals, copper in particular [56],[57],[58]. It is thought that metal ions coordinate with several imidazole groups [59],[60], so addition of the salt containing metals such as copper, zinc, silver might cause swelling of the gel due to charging of the gel or deswelling due to the ions forming complexes with the polymer. El-Hag Ali et al. investigated the behaviour of poly(vinylpyrrolidone) hydrogels, in which vinylpyrrolidone has rather similar structure to 1-vinylimidazole, and found that presence of electron donor atoms such as N, S, O and P in the polymer backbone leads to high possibility of forming coordinate bonds with metals [3]. Hence, there is a high probability that microgels containing such electron donor atoms (Lewis base) would act in the same manner as the corresponding hydrogels. However, all the investigations, which were undertaken on this matter, were concerned only with polymers and hydrogels containing these two copolymers, and there has been no investigation on the behaviour of microgel particles with such a structure.

Moreover, copolymerization of NIPAM with glucose-sensitive agents such as 3-aminophenylboronic acid allows modifying the microgel particles to be glucose-responsive and to act as sensors and potentially as drug-delivery systems. Such modifications, whereby up to 10% of 3-aminophenylboronic acid groups were incorporated into microgel backbone, has been reported by many authors and was widely investigated [61], [21], [62]. However, such glucose-responsive microgel particles have been reported to operate in basic
solutions, i.e. pH 10.5 or higher. Therefore, the main challenge concerning these microgel dispersions is to make them functional at physiological pH, i.e. in the range of pH 7.0 and 7.5. Kabilan et al. found the ability of a phenylboronic acid derivative glucose sensor to function at physiological pH in their investigation [63]. This was achieved by copolymerizing (3-acrylamidopropyl)trimethylammonium chloride [ATMA] and 3-acrylamidophenylboronic acid (3-APB) with PNIPAM. This resulted in producing the glucose sensor operating at physiological pH. Decrease in the pKₐ of the boronic acid moiety [64], which is induced by the presence acrylamido group at the meta position of the phenyl ring in 3-APB, leads to the ability of binding the glucose at physiological pH. However, this technique was used to synthesize polymeric glucose-responsive films that were produced from analogous hydrogels. In this work this methodology was adapted to synthesize microgel dispersions which are glucose-sensitive and functional at physiological pH and temperature.

2.3 Preparations of microgels

Microgel particles are synthesized generally by the following methods: emulsion polymerization [36],[46], inverse emulsion polymerization [65], living-free radical polymerization [66],[67], or via methods involving the use of radiation [68],[69].
2.3.1 Emulsion polymerization

Emulsion polymerization can occur both in the presence of added surfactant (conventional emulsion polymerization [EP]) and without adding any surfactant (surfactant-free emulsion polymerization [SFEP]) [42]. These techniques can yield narrow particle size distributions; both methodologies typically yield nanogel particles with diameters between 100 and 1000 nm. However, EP has a significant problem associated with complete removal of residual surfactant. SFEP does not have such difficulties and is now the standard method for the synthesis of N-isopropylacrylamide-based (NIPAM-based) microgels.

NIPAM-based microgel particles are usually polymerized in the presence of an initiator (often potassium persulfate) and crosslinker (usually N,N’-methylenebisacrylamide [BIS]) at a temperature above 70°C in an inert atmosphere of nitrogen. The high reaction temperature is needed for decomposition of the $K_2S_2O_8$ to form free radicals and also to form colloidal particles as the temperature is well above the lower critical solution temperature (LCST) of the polymer [70]. Figure 2-7 shows the features of the SFEP technique.

2.3.2 Inverse emulsion polymerization

Inverse microemulsion polymerization is a new method for the synthesis of microgels which was developed by Neyret and Vincent [71]. The mixing
media consisted of anionic and cationic monomers (2-acrylamido-2-methylpropanesulfonate and 2-meth-acryloylxy-ethyl-trimethylammonium respectively) in addition to the BIS crosslinker. UV irradiation was employed to start the polymerization reaction and the product was isolated and redispersed in an aqueous electrolyte solution to yield polyampholyte microgel particles. The particles swelled in the presence of high electrolyte

Figure 2-7: The mechanism for the preparation of microgel particles by SFEP \([M \text{ represents a vinyl monomer}] \) (adapted from [28]).
concentrations as a result of screening the attractive electrostatic interactions between neighbouring chains [70]. An inverse emulsion polymerization is one of the most convenient ways to synthesize water-swellable microgels, particularly for the polymerization of a monomer that dissolves only in aqueous media. Kaneda developed a novel method for copolymerization of poly(dimethylacrylamide-co-2-acrylamide-2-methyl-1-propane-sulfonic acid) crosslinked microgel particles via inverse microemulsion polymerization which employed a non-ionic surfactant [72].

2.3.3 Radiation polymerization

Radiation polymerization has been used for the preparation of different gel-like dispersions [68]. Pulsed irradiation produced by fast electrons was used for the synthesis of nanogel particles, which are crosslinked latex particles with the diameter ranging from 100 to 1000 nm in size, from an aqueous solution of linear poly(acrylic acid) (PAA). PAA radicals are formed due to the irradiation energy, and it is these radicals which undergo a major reaction path of intramolecular recombination. Thus, nanogel particles are produced by the interlinking process within the polymer molecules [67].

In this way a novel magnetic nanogel has been synthesized using UV radiation by Sun [73]. The reaction mixture contained acrylamide monomers and Fe₃O₄ nanoparticle dispersion; N,N’-methylenebisacrylamide (BIS) was employed as the crosslinking agent. The reaction started after initiating by UV light and occurred at room temperature. Magnetic core-shell nanoparticles with Fe₃O₄

42
core and PAA shell were produced. The application of irradiation in microgel synthesis has been studied using $\gamma$ rays to synthesize biocompatible microgels based on a purified high guluronic acid-alginate copolymer. Since these microgels are aimed at medical applications, the use of $\gamma$-irradiation brings about the extra advantage of sterilization upon preparation [74], [73].

2.3.4 Living free-radical polymerization

The main advantage of conventional free-radical polymerization is that reactions can be carried out in a single step with the possibility to copolymerize a wide range of monomers under various experimental conditions [75]. Such polymerization conditions as monomer concentration, ionic strength and pH dictate the structure of the crosslinked microgels which are synthesized via a free-radical polymerization mechanism [76]. Synthesis of statistical microgels via living free-radical polymerization is much better controlled in comparison with traditional free-radical polymerization technique. In addition, living free-radical polymerization does not present the problem of divinyl monomer gelation during the synthesis of statistical and star microgels. Statistical microgels are microgels having random distribution of crosslinks and lack structural control due to the irreversible termination steps; alternatively, star microgels consist of a series of linear arms held together by a central crosslinked core, i.e. have very high degree of structural control (Figure 2-8) [67]. The method of living free-radical polymerization is reported to allow better control over the molecular-weight properties of the
polymers and has potential for the synthesis of star microgels with “tunable” physicochemical properties (see Figures 2-8 and 2-9) [69]. However, living free-radical polymerization is much more sensitive to oxygen and continuous flow of inert gas through the polymerization reactor is needed during the synthesis.

Different techniques of radical crosslinking polymerization, either conventional, or controlled, for the synthesis of various types of microgels are shown in Figure 2-9. The main difference between conventional radical polymerization and controlled radical polymerization is that functional groups/monomers are used as initiators as well during controlled radical polymerization which gives an opportunity to control the size of the microgels, amount of grafted functional groups/monomers. However, core-shell crosslinked microgels can be synthesized only via conventional radical polymerization, where core is synthesized firstly and then shell is grown from the core.
Figure 2-8: A schematic illustration of the structural differences between statistical and star microgels (adapted from [28]).

Figure 2-9: Synthetic approaches for the preparation of nano-/microgels of different morphologies and functionalities via conventional and controlled radical crosslinking polymerization (adapted from [77]).
2.4 Properties of microgels

Thermo-responsive polymers demonstrate a critical solution temperature (CST) behaviour where phase separation is induced by surpassing a certain temperature. Such polymers undergo a thermally induced, reversible phase transition. Polymers of this type are soluble in a solvent (water) at low temperatures but become insoluble as the temperature rises above the critical solution temperature (CST) [78].

The liquid-liquid phase diagram at constant pressure of binary polymer solutions is usually determined by plotting the temperature of incipient phase separation as a function of the overall polymer concentration. Although the solution is homogeneous at low temperature, a macroscopic phase separation appears when temperature exceeds the critical solution temperature (CST) or the cloud point of the mixture. The minimum in the phase diagram (known also as a cloud-point curve) is called lower critical solution temperature (LCST), since it denotes the extreme temperature at which phase separation occurs.

Figure 2-10 shows a typical curve of cloud point versus composition that one might find for a thermosensitive system. The upper part of the experimental phase diagram defines the composition of the polymer that precipitates at various temperatures (the 2 phase region). It can also be viewed as the equilibrium swelling ratio of the polymer solvent system as a function of the
temperature. In a critical solution temperature (CST) system, the right-hand branch of the curve is characterized by a positive slope, indicating that the polymer (or gel) will precipitate (collapses) as the temperature increases.

2.4.1 Swelling Behaviour of Microgels
The temperature-sensitivity of microgels depends on the properties of the polymer backbone. If most of the polymer in the microgel network shows temperature-sensitive phase behaviour in the swelling solvent, then the microgel is temperature-sensitive. Temperature-sensitive particles of the poly(N-isopropylacrylamide) [PNIPAM] gels can swell, or shrink, when temperature is changed, and undergo a large-magnitude volume change at
particular temperature, called the volume phase transition temperature (VPTT). It is the reversible breakage and formation of hydrogen bonds between water molecules and amide groups which dictate the mechanism of the volume phase transition of the PNIPAM microgels. According to this mechanism, it is possible to change the volume phase transition temperature (VPTT) of PNIPAM microgels by tuning the hydrophilic and hydrophobic balance of the monomers in the microgel. Copolymerized microgels display a range of volume phase transition temperatures (VPTTs) according to the monomers which have been copolymerized with the NIPAM. However, a greater degree of hydrophobicity does not always result in a decrease of the volume phase transition temperature (VPTT). There are other factors, e.g., the network structure of the microgels, determined by the relative reactivity of the monomer, etc., that also play an important role in determining the volume phase transition temperature (VPTT) [42].

The temperature change induces microgels’ steady-state swelling virtually instantaneously, i.e. in less than a second [36]. The characteristic swelling/deswelling relaxation time (t_c) for a spherical microgel particle depends on its radius (R) as follows:

\[ t_c = \frac{R^2}{D} \]  

*Equation 1*

where \( D \) is the diffusion coefficient of the polymer chain in the gel network [12].
The turbidity of microgel dispersions decreases dramatically due to the lower refractive index when the microgel particles swell below the lower critical solution temperature [LCST] (i.e. in a good solvent). Arguably, one of the most important properties of microgel particles is the extent of swelling. Usually measurements of hydrodynamic diameter changes are employed to determine this parameter; these measurements are obtained from photon correlation spectroscopy (PCS). It is experimentally convenient to measure swelling changes relative to the fully swollen hydrodynamic diameter, $d_0$. The extent of particle deswelling is expressed as the deswelling ratio ($\alpha$), which is simply

$$\alpha = \frac{d}{d_0} \quad \text{Equation 2}$$

where $d$ is the measured hydrodynamic diameter at a given temperature.

A description of the swelling of microgel particles in organic and aqueous systems has been made using Flory’s theory of network swelling [79]. A polymer network immersed in a good solvent imbibes solvent to balance the solvent chemical potential inside and outside the gel network; the presence of crosslinks restricts the extent of swelling. Thus, swelling continues until the sum of the elastic forces between crosslinks is equal to the osmotic force. The extent of network swelling is usually described by the polymer volume fraction ($\phi_2$) obtained at equilibrium ($\phi_2=1$ in the collapsed state) [4]. Flory’s theory leads to [80]:
\[ \varphi_2 = \left( \frac{X \varphi_1}{V_c \left( \frac{1}{2} X + \chi_{12} \right)} \right)^{3/5} \]  \hspace{1cm} \text{Equation 3}

where \( X \) is the number of crosslinks present within a collapsed network volume, \( V_c \). The superscripts 1 and 2 refer to the solvent and network polymer, respectively; \( \varphi_1 \) is the molar volume of the solvent and \( \chi_{12} \) represents the Flory-Huggins solvent-polymer interaction parameter. The term \( (X/V_c) \) represents the average density of crosslinked units in the collapsed particle [4].

The proportion of non-crosslinking polymer segments (monomer B), which are contained in the microgel particles, are much higher than proportions of difunctional crosslinking segments (monomer A); the mole fraction \( (X_A) \) of the latter being typically less than 0.1. Assuming that the molecular weights of both segments are the same and that two moles of crosslinked units are introduced by each mole of difunctional crosslinking monomer, the following equation can be derived [4]:

\[ \frac{X}{V_c} = \frac{2X_{AB}}{M_B} \]  \hspace{1cm} \text{Equation 4}

where \( M_B \) and \( \rho_B \) are the molecular weight and density of the B segments, respectively. The dependence of the volume fraction of the microgel particles on the network composition and solvency can be obtained by substituting Equation 4 into Equation 3 and assuming that \( \rho_B=1 \) [4]:

50
\[ \phi_2 = \left( \frac{2X_A V_1}{a_{12} M_B} \right)^{3/5} \]  

Equation 5

where the excluded volume parameter, \( \phi_{12} = (0.5 - \chi_{12}) \). The polymer volume fraction increases (i.e. particles shrink) with the increase of the crosslinking density or deterioration of the solvency (\( \phi_{12} \) decreases).

2.4.2 Rheological properties

The rheological behaviour of homogeneous PNIPAM microgels as a function of temperature, shear rate, and concentration has been investigated. The elastic modulus decreases by an order of magnitude between 25\(^0\)C and 50\(^0\)C, reflecting a decrease in the effective volume fraction of the dispersed gel phase [81], [65].

Due to the temperature dependence of PNIPAM microgels, rheological properties, such as viscosity, yield stress and storage modulus, decrease above the lower critical solution temperature (LCST) [82]. The key parameter of rheological behaviour for microgel particles is the volume fraction occupied by the spheres, as the rheological behaviour microgel particles is very similar to the behaviour of displayed by suspensions of hard spheres. However, if soft spheres are considered higher volume fraction are needed to describe the rheological behaviour of the dispersion as compared with hard ones, since deswelling can take place upon adding of new dry particles [83].
2.4.3 Electrical properties

PNIPAM microgel dispersions show interesting electrical properties due to the presence of covalently bonded electrically charged groups originating from the initiator. Charge contents for PNIPAM microgel synthesized with persulfate initiator reported to be 3.8 µeq./g [84]. Several efforts were made to summarize electrophoretic mobility of microgel particles as a function of temperature. Pelton et al. [85] modelled the electrophoretic mobility of temperature-dependent microgels via following equation:

\[
\mu = \frac{-\Omega e}{4\pi \eta r^2} \quad \text{Equation 6}
\]

where \(\mu\) is electrophoretic mobility of the microgels; \(\Omega\) is the number of charges per particle; \(r\) is the particle’s radius; \(e\) is the charge of an electron; \(\eta\) is the viscosity; \(\kappa\) is the Debye screening parameter. However, this equation has many assumptions such as: the charges are located only on the exterior surface of the microgel; the charge density is related to the potential by the Helmholtz equation; the surface potential is equal to the zeta potential of the microgel particle; the electrophoretic mobility is related to the zeta potential by the Smoluchowski equation. Only the number of charges per particle is needed to calculate the electrophoretic mobility if the microgel diameter as a function of temperature is measured [36]. There is an extensive database of
electrophoretic mobility of PNIPAM microgels as functions of temperature, electrolyte, and surfactant concentration [65].

2.4.4 Effect of copolymer composition on microgel properties

Microgel properties can be changed by varying the copolymer composition. Such variations give an opportunity for fine control of the volume phase transition temperature (VPTT). Copolymerizing of monomers with different stimuli response or different hydrophobicities (therefore different VPTTs) will generate microgels with certain physico-chemical characteristics. These characteristics will be a combination of properties of the comonomers used in the synthesis. Even the addition of a small amount of comonomer (typically 1 to 5% w/w monomer) can have a strong influence on the overall properties of the resultant microgel particles. For example, a pH- and temperature-sensitive microgel can be prepared by copolymerizing NIPAM with acrylic acid [86].

Recently, a new spherically shaped poly(N-isopropylacrylamide)/poly(ethylene-glycol) microgel has been synthesized [87]. In order to improve the temperature-sensitive properties without any significant impact on the mechanical properties of the PNIPAM hydrogels, the newly synthesized microgel was added to the latter hydrogel during the polymerization and gelation of the hydrogel synthesis. The modified PNIPAM hydrogel had a more compact and more constrained porous network than pure PNIPAM hydrogels without these additives (Figure 2-11). Increasing the amount of modified microgel additive led to a reduction of the pore size in the
microgel-impregnated PNIPAM hydrogels. Since the chemical nature of the microgel backbone and surrounding matrix remained the same for the microgel-impregnated hydrogels as for the normal hydrogel; hence, VPTTs of the modified hydrogels did not change. The temperature-sensitive properties of these hydrogels were significantly improved by the addition of these microgel additives in terms of swelling/deswelling ratios (shrinking rate, swelling rate), as well as oscillatory shrinking-swelling kinetics upon temperature changes around the VPTT. It is the amount of additives that dictate the level of these improvements [88].

This faster and larger magnitude of oscillating responses from microgel-impregnated PNIPAM hydrogel is advantageous for practical applications in fields such as bioengineering and biotechnology because the faster response kinetics of the oscillating shrinking-swelling property of hydrogels to small temperature cycles (e.g., cycled around the physiological temperature) should be useful [88].
2.4.5 pH sensitivity

The swelling of pH-responsive microgel particles is controlled by the internal osmotic pressure, which is attributed to mobile counterions contained within the particles, which balance the internal electrostatic repulsion. Equation 7 describes the balance between the osmotic pressure inside and outside microgels. This balance determines the swelling of microgels.

\[ \Pi_{\text{in}} + \Pi_{\text{el}} = \Pi_{\text{out}} \quad \text{Equation 7} \]

where \( \Pi_{\text{in}} \) and \( \Pi_{\text{out}} \) are, respectively, the osmotic pressure of the mobile ions inside the microgels and bulk solution, and \( \Pi_{\text{el}} \) is the elastic pressure of the polymeric network.
Microgel particles which contain a weak acid or base, and are therefore pH-responsive, have complex behaviour. Certain functional groups in the polymer backbone, e.g. -COOH or –NH₂ groups, can become ionized above or below the appropriate pKₐ or pKₐ of the groups concerned. In addition, the effect of charged groups will depend on the background ionic strength, which screens local electrostatic repulsion. Irrespective of the addition of any inert electrolyte, adjustment of pH will also lead to changes in the background ionic strength.

PNIPAM microgels become pH-sensitive when copolymerized with monomers, which contain acidic or basic groups. There has been a considerable amount of research undertaken for the microgel particles of PNIPAM copolymerized with acrylic acid [89], [7], [10], [86]. It was found that the hydrodynamic diameter (volume change) of the microgel particles increased with a corresponding rise in pH (pH>pKₐ). At a pH value above that of the pKₐ, the polymer chains of the microgel become ionized, forcing the microgel to adopt a more expanded conformation as a result of intramolecular charge repulsion. At a pH below the pKₐ, the microgel particles adopt a compact structure [10], [90]. Figure 2-12 shows a schematic representation of the shrinking and swelling of the microgel composed of PNIPAM and acrylic acid.
Figure 2-12: The shrinking and swelling behaviour of the microgel composed of PNIPAM and acrylic acid (adopted from [28]).

2.5 Microgel applications

Figure 2-13: Applications of microgels.
2.5.1 Cosmetic and pharmaceutical applications

Microgel particles have found a vast area of application as a thickeners for paints and pharmaceuticals. Carbopol is a good example of a popular microgel thickener for use in aqueous systems, in such industries as cosmetics and pharmacy. One of the alternative applications of the microgels is as a carrier system for small molecules (e.g. drugs). Other carrier systems (e.g. surfactant micelles) have found widespread usage due to their properties. Microgels though are larger and more robust, and hence more effective as microgel particles offer an even more firm and stable environment. Microgel particles have to be stable in aqueous media over pH and ionic strength ranges for applications in pharmacy and cosmetics. Acrylic acid groups impregnated into the microgel particles is a good method for stabilizing the charge in aqueous media. Nevertheless, this method does not work if the pH is low, or the electrolyte concentration is high; as under these conclusions microgel particles become destabilized. An alternative strategy for stabilizing the particles is to use sterically stabilized particles. Kaneda et al. reported about the preparation of polymer latex particles in organic solvents, which were terminally grafted with polyethylene oxide (PEO) chains via a dispersion polymerization route [12]; the main monomer has been copolymerized with a PEO-terminated macromonomer. The synthesis resulted in the preparation of polymer particles with a core-shell structure, where the shell consists of hydrated PEO chains and the core is the hydrophobic polymer. The resultant particles are expected to be stable under high ionic strength and low-pH conditions [12].
2.5.2 Drug delivery systems (DDS)

Carrier systems which offer fine release control of a quickly metabolized drugs, or have the ability to protect sensitive drugs, are of particular interest to many researchers and companies. Microgel particles can be good candidates to fulfil these requirements. They have been used to achieve both zero-order and pulsatile release patterns [22]. By showing capabilities to work in various physical formats, these “smart” materials gave an opportunity for intelligent loading and release of drugs, proteins, nanoparticles, DNA, etc. in response to temperature changes, ionic strength, pH values, and even light [91] (Figure 2-14).

The main problem of transdermal drug delivery systems is skin toxicity. Therefore, such requirements for drug molecules as being innocuous, i.e. creating neither irritation nor allergenicity, are imperative; so in some circumstances using a larger patch area can alleviate the problem. The possibility of using pH and/or temperature-sensitive microgels as transdermal drug-delivery systems has been considered due to this issue, as well as taking into consideration one of the possible practical applications of these “smart materials” in wound treatment. Microgels have the potential to enable drug release to the skin. It is these materials that can be of particular importance where the skin barrier is compromised, as in a diseased state, or in wound management. In this situation, controlled delivery to the skin of active materials can provide therapeutic levels where required and minimize systemic uptake. The microgels could be used at higher temperatures and
release more material, as can be anticipated within wound tissue. They appear to be pH intensive in the release of the compounds, and therefore any pH effects in the wound would be negligible [22].

The development of targeted drug carriers with magnetic nanogels have been investigated by many researchers in recent years [73], [92]. Magnetic nanogels are nanometre-sized particles which consist of inorganic core and polymer shell. Nanogels with ferromagnetic magnetite (Fe₃O₄) core coated with crosslinked polymer shell are materials of particular interest. Such microgels are desirable for biomedical applications such as drug delivery systems due to the low toxicity of the Fe₃O₄ core to the human body, when this core is coated by a protective polymer shell which has good hydrophilicity and biocompatibility. This shell also prevents the core from oxidation and aggregation. Taking into consideration these aspects, modified magnetic nanoparticles have the potential to be used in medical applications such as protein and enzyme immobilization, bioseparation, immunoassays, and improved magnetic resonance imaging contrast agents for cancer diagnosis [93].
Figure 2-14: A schematic representation of a post-fabrication encapsulation strategy of protein drugs based upon an “intelligent” and biodegradable microgel (adapted from [28]).

2.5.3 Surface-coating industry

Microgel particles have shown a wide range of applications in the surface-coatings industry. A rheological control for automotive surface coatings is acquired due to dispersions of microgel particles. Smooth cover which is adjusted to the surface as parallel film-forming layer of metallic flakes is another very useful property of certain microgels. Recently, environmental and safety legislations required a decrease of the volatile components of surface-coating formulations. This was achieved by increasing the total solids content and decreasing the molecular weight of the resin; however, this led to an unacceptably low viscosity. The microgel particles had the added effect of
imparting a yield stress to the dispersion and hence improving the rheology of the system [4].

2.5.4 Molecular imprinting

Molecular imprinting can be generally defined as a synthetic approach by which a molecular receptor is assembled via template-guided synthesis. Certain functional monomers interact with a template molecule; this interaction is followed by fixing the functional groups on the monomers via chemical crosslinking of these monomers. The polymer network possesses complementary binding sites if the template is removed from this network. These binding sites are able to selectively rebinding the same template or its analogue. Molecular imprinting has three different approaches: covalent [94], non-covalent [95] and semi-covalent [96]. Molecular-imprinted polymers have been produced in different configurations such as polymer monoliths, polymer beads, membranes and imprinted monolayers. However, molecular-imprinted microgels offer further advantages, the main of which is stability; therefore there is the possibility of using them for a variety of different applications compared with their insoluble analogs [97], [98]. Molecular-imprinted microgel spheres can be easily synthesized using a novel precipitation method [99]. These materials can be used for various immobilization and conjugation purposes, such as recognition components in chemical sensors and specific adsorbents in competitive drug assays [99], [100], [101].
2.5.5 Oil recovery

New environmental regulations, which impose severe limits on disposing produced water, has led to the need to reduce water production for the oil industry. One of the methods to reduce water production is to inject a polymer solution together with a crosslinker into the well-bore. However, it is very difficult to control the gelation process using this method because of its high sensitivity to physico-chemical conditions such as pH, salinity and temperature [5]. However, conceptually microgels provide a better mobility control of water than the polymer alone and have lower viscosity that makes injection process much easier [102], [103]. It is due to the ability of a stable colloid to become destabilized by heteroaggregation and the process relies on the charged microgel particles forming stable dispersions below their volume phase transition temperature (VPTT); hence, for the exploitation of the thermosensitivity of PNIPAM microgels are of particular interest. Since the temperatures in oil-bearing rock are high (above the VPTT), injected microgels shrink in response to these temperatures. Moreover, the presence of electrolytes in the pumping materials, e.g. seawater or reservoir water, enhances the flocculation of the particles [104]. These colloidal aggregates block off channels of high permeability and it is this process which enables the oil to mobilize towards areas with lower permeability; therefore, increasing the amount of oil recovered and decreasing the volume of produced water [105]. In addition such fluid media containing the microgels are used as a fluid in well drilling operations, for example as packer fluids in well completion
operations and as mobility control fluids in other enhanced oil recovery operations [103].

2.6 Summary

The general properties of microgels are described in this chapter as well as their preparation techniques and areas of application or potential application. It is worth noting that due to the presence of hydrophilic amide group and hydrophobic isopropyl group in the backbone, NIPAM-based microgel particles are extremely temperature-sensitive; therefore, this material undergoes reversible volume change transition around 32°C, which corresponds to the lower critical solution temperature (LCST) of PNIPAM, and collapses above it (the mechanism of this transition is shown in Figure 2-5 and described in Section 2.2.1). However, copolymerization of NIPAM with other monomers, for example acrylic acid or 1-vinylimidazole, leads to shifting of LCST as the microgel functional groups become more hydrophilic and to making the microgel particles to be multi-responsive, i.e. NIPAM copolymerized with 1-vinylimidazole produce particles which are responsive to both temperature, pH and the presence of metal ions due to formation of complex by imidazole groups of the microgel with the cations. For non-NIPAM based microgel particles, such as PVP, swelling/deswelling is dependent on the pH of the surrounding media but is relatively insensitive to temperature. This is due to disparity between the favourability of polymer-
solvent interactions for charged and uncharged monomer segments, for example PVP microgels swell when pH is lowered because the nitrogen atoms protonate and become positively charged. The physico-chemical properties of microgel dispersions mentioned above are described in Section 2.4 of this chapter.

There are four commonly used techniques for microgels preparations, which are emulsion polymerization, inverse emulsion polymerization, living free-radical polymerization and radiation polymerization. Emulsion polymerization, which can occur both in the presence of added surfactant or without it, is the most widely used technique for synthesizing microgel dispersions. Both techniques of polymerization was employed throughout this research, where surfactant-free emulsion polymerization was used for the synthesis of PNIPAM microgel dispersions and for preparation of microgels with copolymerized monomers backbone; whereas free-radical precipitation polymerization was employed for synthesizing microgel dispersions, which contained NIPAM and 1-vinylimidazole copolymer as the backbone. These techniques are presented in Section 2.3 in more detail.

The Chapter concludes with a brief summary of the uses and potential uses of microgels.
Chapter 3 Synthesis and Characterization of Temperature-, pH-, Metal- and Glucose-responsive Microgels

3.1 Introduction

As the literature relating to microgel dispersions of different structure and physico-chemical properties was studied, it was decided to produce NIPAM based microgel particles firstly in order to understand the polymerization procedure and generally practice the synthesis process. After that acrylic acid was incorporated into the microgel structure by copolymerizing the corresponding monomer with NIPAM; this led to producing pH- and temperature-responsive microgels. These microgel particles have a higher LCST in comparison with PNIPAM microgels and it is discussed in the next Chapter in detail. The surfactant-free emulsion polymerization technique was employed to produce the microgel dispersions mentioned above. The next step of the synthesis stage was preparation of PVP microgel particles. These particles are pH-responsive and their swelling/deswelling behaviour is opposite to that of microgels made of NIPAM and acrylic acid copolymer. Investigations of these microgel dispersions led to the idea of synthesizing microgels based on NIPAM and 1-vinylimidazole copolymer, which are also positively charged. The resultant microgel particles considered to be novel
materials which are not only pH- and temperature-sensitive but also responsive to the presence of metal ions. The last stage of the microgel dispersions synthesis was producing glucose-responsive particles with copolymer made of NIPAM, 3-acrylamidophenilboronic acid (3-APB) and (3-acrylamidopropyl)trimethylammonium chloride [ATMA] groups; 3-APB was the monomer which was synthesized beforehand. The procedure of the synthesis together with a description of the techniques that can be used to characterise them are described in this chapter.

3.2 Experimental Procedure and Materials

As previously mentioned, the main monomer which was employed to synthesize the microgels was N-isopropylacrylamide (NIPAM). Acrylic acid was put in an inhibitor remover column in order to remove the inhibitor, hydrochinonemethylether. 1-vinylimidazole was used as received and was stored at 5°C in the fridge. 3-aminophenylboronic acid (PBA) and acryloyl chloride were used to synthesize 3-acrylamidophenylboronic acid (3-APB) that was employed as glucose-sensitive monomer. (3-acrylamidopropyl)trimethylammonium chloride (ATMA) was used as an agent for modifying the pKa of boronic group. The crosslinker, which was methylene-bis-acrylamide (BIS), was also used as received and stored in the fridge. The initiator, potassium persulfate (KPS), was Analar grade material. The initiator 2,2’-azobis[2-methylpropionamidine] dihydrochloride (V50) was
stored at $5^\circ$C in the fridge as well. The cationic surfactant, cetyl trimethylammonium bromide (CTAB), was used as received. Dialysis membrane with molecular weight cut-off (MWCO) of 12-14000 Daltons was used for removing unreacted monomer.

All chemicals were purchased from Sigma Aldrich, except N-isopropylacrylamide and cetyl trimethylammonium bromide, which were purchased from Acros Organics and BDH Chemicals, respectively. Analytical grade deionized water (Imperial College London: Triple Red, resistivity > 18 MΩ cm) was used in all experimental procedures.

All glassware, which was used, had been alternately placed in a base (NaOH) and then an acid (HCl) bath overnight, rinsed with deionized water, cleaned with detergent, rinsed with deionized water again and dried in the oven before use.

Solid chemicals were weighed on a balance (±0.0001g, OHAUS GA200D). Autopipettes with the ranges 0.5-10 µL (±4.0%-0.5%), 10-100 µl (±1.6%-0.8%) and 100-1000 µL (±0.9%-0.6%) (VWR) were used for measuring small volumes of liquid chemicals.

The pH of the solutions were set using 0.1M hydrochloric acid (HCl) or 0.1M sodium hydroxide (NaOH) and measured using Orion 2 star pH meter (Thermo Scientific). pH meter was calibrated using buffer solutions of pH 4.00, pH 7.00 and pH 9.22 (BDH Chemicals).
3.3 Preparation of PNIPAM-based microgel particles

3.3.1 Synthesis of PNIPAM and P(NIPAM-co-Acrylic acid) microgels.

The first published account of PNIPAM-based microgels described a “surfactant-free emulsion polymerization” (SFEP) of aqueous NIPAM and methylene-bis-acrylamide [44]. The classical SFEP method was used to prepare the microgel dispersion of PNIPAM and P(NIPAM-co-Acrylic acid) [P(NIPAM-co-AA)]. Colloidal microgels were prepared by reaction in water at 70°C under a nitrogen atmosphere. The reaction was conducted at 70°C to enable the generation of free radicals by the decomposition of the persulfate initiator. In addition, high temperature conditions were also needed to ensure that the growing PNIPAM chains phase separated to form colloidal particles in a manner similar to the synthesis of a polystyrene latex. The crosslinker N,N’-methylenebisacrylamide (BIS) was used to prepare all microgel particles described in this work. This procedure has been used by many authors [36], [106], [44], [107]. The NIPAM and acrylic acid concentrations were varied to prepare particles with different concentrations of incorporated acrylic acid groups in the microgel backbone. Table 3-1 shows a summary of the different compositions used to prepare microgels. Microgels containing up to 30% of acrylic acid could be prepared, at higher acrylic acid concentrations no microgels formed, simply a polymer solution.

The polymerization for all samples took place in a 500 mL three-necked round bottom flask. The flask was equipped with a nitrogen inlet, a condenser and an
overhead stirrer (Figure 3-1). The reactor was put into a water bath thermostat. After the temperature was stabilized, NIPAM and acrylic acid were dissolved

Table 3-1: The amounts of NIPAM and acrylic acid used for the synthesis of microgel dispersions with different concentrations of acrylic acid.

<table>
<thead>
<tr>
<th>Acrylic acid ratio</th>
<th>0%</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of NIPAM used, g</td>
<td>3</td>
<td>2.65</td>
<td>2.3</td>
<td>1.95</td>
</tr>
<tr>
<td>Volume of acrylic acid used, mL</td>
<td>0</td>
<td>0.33</td>
<td>0.67</td>
<td>1</td>
</tr>
<tr>
<td>Resultant microgel concentration, mmol/L</td>
<td>1.3</td>
<td>1.2</td>
<td>1.0</td>
<td>0.8</td>
</tr>
</tbody>
</table>

in 350 mL analytical grade deionized water and transferred to the flask. The reaction mixture was purged of oxygen by blowing nitrogen through it for 30 min and the stirring speed was set to 300 rpm. Once thermal equilibrium was achieved, the dissolved initiator, potassium persulfate, was added to the solution to start the reaction. Finally, the solution was left to react for 12 h with continuous nitrogen bubbling and mild stirring.

3.3.2 Synthesis of P(NIPAM-co-1-vinylimidazole) microgels.

Colloidal microgel particles of P(NIPAM-co-1-vinylimidazole) [P(NIPAM-co-VI)] were prepared using the emulsion polymerization method. Four microgel dispersions with 0%, 10%, 20% and 30% w/w of 1-vinylimidazole
monomer were prepared, higher 1-vinylimidazole concentrations were attempted but did not produce stable microgels.

Figure 3-1: A schematic setting of the reactor for the microgels synthesis.

Microgel synthesis was carried out in a three-necked round bottom flask, which was put in a water bath and the reaction temperature was set to 72±1°C. The flask was purged with nitrogen. The required amount of the monomers (the amount of NIPAM and 1-vinylimidazole presented in Table 3-2), the surfactant (cetyltrimethylammonium bromide [CTAB] 0.02g) and the crosslinker (N,N’-methylenebisacrylamide [BIS] 0.5g) were dissolved in 350
mL of water and stirred for 30 min. The surfactant was used in order to stabilize latex particles during the polymerization. To start the polymerization 0.2g of 2,2’-azobis[2-methylpropionamidine] dihydrochloride (V50) initiator (a cationic initiator was purchased from Sigma Aldrich; chemical structure presented in Figure 3-2), dissolved in 10 mL of water, was added to the mixture. Positively charged initiator was used in order to initiate the polymerization as 1-vinylimidazole is positively charged, and so a cationic initiator was chosen to ensure the microgels were stable by having a high positive charge. A negatively charged initiator (potassium persulfate) was used during the polymerization of P(NIPAM-co-AA) microgels for similar reasons. After 10 min the solution had turned milky white. The reaction proceeded for 6 h under a nitrogen atmosphere and constant stirring. The pH of the resultant microgel dispersions were in the range of 6.0 and 6.4. The procedure for the synthesis of all microgel dispersions was the same and only the NIPAM and 1-vinylimidazole monomers ratio varied.

The reactor was the same as for preparation of P(NIPAM-co-AA) microgel dispersions and illustrated in Figure 3-1.

![Chemical structure of cationic initiator](image)

*Figure 3-2: The chemical structure of cationic initiator 2,2’-azobis[2-methylpropionamidine] dihydrochloride (V50).*
Table 3-2: The amounts of the monomer used for the preparation of the microgel dispersions with different concentrations of 1-vinylimidazole.

<table>
<thead>
<tr>
<th>1-vinylimidazole ratio in the copolymer</th>
<th>0%</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIPAM, g</td>
<td>5</td>
<td>4.5</td>
<td>4</td>
<td>3.5</td>
</tr>
<tr>
<td>1-vinylimidazole, mL</td>
<td>0</td>
<td>0.48</td>
<td>0.96</td>
<td>1.44</td>
</tr>
<tr>
<td>BIS, g</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Resultant microgel concentration, mmol/L</td>
<td>2.2</td>
<td>1.1</td>
<td>1.7</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Resultant P(NIPAM-co-VI) microgels were also investigated as a function of metal ions concentration. The following salts: Cu(NO₃)₂·3H₂O, FeCl₃, Ni(NO₃)₂, Co(NO₃)₂, ZnCl₂ and AgNO₃, were dissolved in 18 different concentrations ranging from 0.02 g/L and 40 g/L in order to be added to the microgel dispersions and further investigate their the impact on the particle size. The salts were purchased from Sigma Aldrich and were used as received.
3.3.3 Synthesis of P(NIPAM-co-3-acrylamidophenylboronic acid -(3-acrylamidopropyl)trimethylammonium chloride) microgels.

Firstly, 3-acrylamidophenylboronic acid (3-APB) was synthesized in order to prepare glucose-sensitive microgel dispersions. 3-aminophenylboronic acid (PBA) was used as a starting material for the synthesis via the method described by Kabilan et al. [63] although with some modification, which would be noted further. 5.16 g of NaOH was dissolved in deionised water to prepare a 50 mL aqueous solution, which was cooled down in the ice bath before adding 5.95 g of the PBA. The mixture was stirred for 1 h until the PBA totally dissolved. Then, 5.2 mL of acryloyl chloride was added dropwise to the stirring mixture over a period of 15 min. This resulted in the formation of a precipitate. The resultant mixture was removed from an ice bath and rather than letting the mixture to stir further at room temperature as was stated by method of Kabilan et al., the resultant precipitate was left in the fridge overnight to fully crystallize. A beige precipitate was recovered. Afterwards, the precipitate was filtered and washed 3 times with deionised water, before leaving it overnight for drying. Drying of the precipitate resulted in the formation of a white powder which was dissolved in 40 mL of hot 20% v/v aqueous ethanol solution in order to remove impurities. The latter solution was filtered through fluted filter paper (Whatman, Qualitative, 90 mm in diameter, obtained from Schleicher&Schuell) and the filtrate left to stand overnight in the fridge, which resulted in the formation of white crystals. The bottom of the beaker, where the filtrate was poured, was scratched in order to form irregular
surface which is needed for crystals to nucleate. Approximately 4 g of white
 crystals was produced.

The microgel dispersions of poly(N-isopropylacrylamide-co-3-
acrylamidophenylboronic acid-(3-acrylamidopropyl)trimethylammonium
chloride) [P(NIPAM-co-3-APB-ATMA)] were prepared via surfactant free
emulsion polymerization. There were two dispersions prepared which
contained various concentrations of incorporated functional monomers. 0.25 g
of the synthesized 3-APB and 0.25 g of (3-
acrylamidopropyl)trimethylammonium chloride (ATMA), which was present
to lower the pKa of the boronic acid group, were mixed with 5 g of NIPAM
and 0.3 g of the crosslinker (BIS) to prepare the microgel particles containing
5% 3-APB and 5% ATMA. These monomers were dissolved in 500 mL of
deonised water and poured into three-necked round bottom flask equipped
with a nitrogen inlet, a stirrer and a condenser (Figure 3-1) and put in a water
bath. The reaction temperature was set to 72°C; the mixture was stirred until
reaching this temperature before adding the initiator to start the
polymerization. 0.3 g of the initiator (KPS) was dissolved in 50 mL of water
and added to start the polymerization. The solution turned cloudy in a few
minutes indicating the polymer growth, and the reaction continued for 5 h.

Microgel particles containing 10% 3-APB and 10% ATMA were prepared
using the same methodology, where amounts of NIPAM and BIS were the
same but 0.5 g of 3-APB and 0.5 g of ATMA were employed for the synthesis.
3.3.4 Purification of microgels.

Dialysis was employed for purification of all the colloidal dispersion of microgels. The resultant dispersions were put into dialysis tubing with molecular weight cut-off 12-14000 Daltons; the tubing was obtained from Medicell International Ltd. The dialysis tubes were immersed in deionized water and the process took 2 weeks with daily exchange of deionized water. After this procedure the microgels were centrifuged in order to concentrate the dispersion. The microgel was put into 50 mL centrifugation tubes, after centrifuging water was removed and the sediment was redispersed in 25 mL of deionized water in order to saturate the solution.

3.4 Preparation of PVP microgels

3.4.1 Materials.

The monomer to synthesize PVP microgels was 2-vinylpyridine which was obtained from Sigma Aldrich. The crosslinker, divinylbenzene, and the initiator, which was 2,2’-azobis[2-methylpropionamide] dihydrochloride were also obtained from Sigma Aldrich. All chemicals were used as received. Analytical grade deionized water was used in all experimental procedures.

3.4.2 Microgel synthesis.

The surfactant-free emulsion polymerization method was employed to synthesize PVP microgel particles. This procedure was described by Loxley
and Vincent [90]. A three-necked round bottom flask was equipped with a nitrogen inlet, stirrer and condenser. The flask was put into the water bath and heated up to 72±1°C (Figure 3-1). The synthesis of microgel particles included copolymerization of 2-vinylpyridine with divinylbenzene. Four different samples with various crosslinker ratios were synthesized. The mass of the initiator 0.2 g was the same for all samples.

The reaction mixture was fixed to a total mass of 500 g, which mostly was deionized water. The reaction mixture was purged of oxygen by blowing nitrogen through it before the reaction was started. The mixture was stirred throughout the reaction at a speed of 300 rpm. 2-vinylpyridine with the mass of 4.950 g and divinylbenzene crosslinker with the volume of 99.8 μL were added to the reaction and left to mix for 10 min. After temperature equilibrium was achieved, 2,2’-azobis[2-methylpropionamide] (V50) initiator with the mass of 0.2 g was added to the reaction in order to start the polymerization process. The reaction occurred for 5 h and the resulting microgel was put into the dialysis tubes in order to remove unreacted monomer. The pH of the mixture was 6.0. The dialysis took place for 2 weeks with changing water 2 times per day. Other samples at different crosslinker densities were prepared with the same procedure and the amounts used to produce the microgel are shown in Table 3-3.
Table 3-3: The amounts of 2-vinylpyridine and divinylbenzene used for the synthesis of PVP microgel dispersions with different concentrations of the crosslinker.

<table>
<thead>
<tr>
<th>Mass of 2VP, g</th>
<th>4.985</th>
<th>4.975</th>
<th>4.950</th>
<th>4.900</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of DVB, μl</td>
<td>29.9</td>
<td>49.9</td>
<td>99.8</td>
<td>199.6</td>
</tr>
<tr>
<td>Crosslinker ratio</td>
<td>0.3%</td>
<td>0.5%</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>Microgel concentration, mmol/L</td>
<td>2.4</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

3.5 The Tools and Instruments for Measuring Particle Size and Analysis of the Microgels

3.5.1 Dynamic Light Scattering

A Brookhaven ZetaPALS, zeta potential and particle size analyzer, was used to determine the size of the microgels for all samples. This machine employs dynamic light scattering for measuring the sizes of microgels and uses a 35 mW helium-neon laser at 658 nm with a photomultiplier tube, which measures the intensity of light scattered at 90° to the incident beam. A digital correlator determines the autocorrelation function. The size distribution is analyzed by a non-negative least squares method. The samples were diluted with water in the ratio 1:10. The diluted sample was poured into a cuvette and loaded into the instrument. The measurement consisted of six runs for each sample, and all the...
size measurements and polydispersity of the microgels were obtained directly from the computer interfaced to the instrument (Particle Sizer for ZetaPALS software was used).

In addition, the instrument has an internal temperature controller, thus changing the temperature for samples can be adjusted by setting the parameter of the equipment. However, the temperature of the instrument could only be raised up to 55°C. Therefore, the sizes of the PNIPAM-based microgels were measured at different temperatures ranging from 25°C to 55°C.

Further details of the theory of photon correlation spectroscopy (PCS) are given in Appendix 1.

3.5.2 Freeze-drying

Due to the fact that samples have to be completely dry for SEM imaging and drying in the oven resulted in the formation of a film, for the P(NIPAM-co-VI) microgels a freeze-drying technique was employed. 10 mL of each sample were poured into glass tubes, and tubes immersed in liquid nitrogen. Frozen samples were placed in standing 50 mL centrifuging tubes (Sterilin), which were put into freeze-dryer Heto Power Dry LL1500 (Thermo Scientific) for one week.

Freeze-drying, also known as lyophilisation or cryodessication, is a process of removing water from the material by freezing it and then reducing the surrounding pressure in order to allow the frozen water to sublime from the
solid to the gas phase. Pretreatment, freezing, primary drying and secondary drying are the four stages of the complete drying process.

Pretreatment stage includes any necessary methods of treating the product prior to freezing, such as increasing the stability, increasing the surface area, decreasing high pressure vapour solvents. Arguably, the most critical part of the whole process of freeze-drying is the freezing phase as it is this stage where the product can be spoilt. Usually the process of freezing is done by placing the product into the freezing flask which is then rotated in the bath cooled by a dry ice/methanol/liquid nitrogen. It is important to cool the material below the triple point, at which liquid and solid phases can coexist, to insure that sublimation rather than melting will occur in further processes of drying. The process of primary drying induces the removal of 95% of water from the material. The pressure is lowered by applying partial vacuum and certain amount of calculated heat is supplied to start the sublimation during this process. This stage is slow and could take several days to be completed. The secondary drying phase, which is governed by adsorption isotherms of the material, is aimed to remove unfrozen water from the material. The pressure at this stage is also lowered to quicken the desorption process; however, temperatures can be risen higher than in previous stage to break any physico-chemical interactions between the frozen material and the water molecules [108].
3.5.3 Scanning Electron Microscopy

Dry samples were pasted to the carbon pads (Agar), which were stuck on the aluminium stubs (Agar) of the SEM. Samples were sputter coated (Emitech K575X) with a 10 nm film of gold (Emitech) and images obtained with a JEOL JSM 5610LV electron microscope in the Department of Materials at Imperial College London. Dr. Mahmoud Ardakani, whom I wish to thank, operated the SEM.

Images of high resolution are usually obtained using electron microscopy. Particularly, scanning electron microscopy (SEM) is the tool which gives an opportunity to reveal information about morphology, chemical composition and crystalline structure of the sample. A beam of electrons which is streamed onto a solid sample in the vacuum is used in a scanning electron microscope. The SEM images are obtained from secondary electrons which are emitted by the sample. Areas ranging from approximately 1 cm to 5 microns in width can be imaged by SEM techniques (magnifications ranging from 20X to 30000X, spatial resolution from 50 to 100 nm). A schematic structure of a scanning electron microscope is shown in Figure 3-3.

Samples have to be conductive while being studied by SEM due to the building up of negative charge by the electrons on the substrate. If this occurs, those areas deflect the electron beam as well as secondary electrons which resulting dissembling image lines and poor contrast. Therefore, all samples must be coated with a metal coating which is usually sputtered gold or chromium.
3.5.4 Transmission Electron Microscopy

A small amount of diluted solution of each sample was dropped on a copper grid Formvar/Carbon 300 Mesh Cu covered with carbon (Agar). Samples left for 24 hours to dry out at room temperature. Two samples were heated up to 60°C in the oven and a small amount of the sample was dropped on to similar grids as mentioned above. Samples were dried in the oven at 60°C for 2 h. This was made in order to obtain the images of collapsed microgel particles.
Images were obtained with a TEM JEOL 2010 200 kV and the microscope was operated by Ecaterina Ware, whom I wish to thank.

Transmission electron microscopy (TEM) is another tool used to characterize the morphology and particle size of microgel particles. Despite that fact that microgels have to be dried in order to be imaged and not necessarily represent microgel particles in swollen state on the images, transmission electron microscopy is one of the most common tools to characterise colloidal particles. In particular this technique is very useful to characterise the binding heavy metals ionic complexes to ionizable functional groups of microgels [109].

The transmission electron microscope (TEM) operates on the same basic principles as the light microscope but uses electrons instead of light. The interaction of electrons transmitted through the specimen forms an image, which is focused on the imaging device after magnification. The system of lenses in the TEM gives an opportunity to image the sample at different magnifications. A schematic structure of TEM is presented in Figure 3-4.
3.5.5 UV-VIS spectroscopy

According to the Beer-Lambert law, which states that the absorbance of the solution is directly proportional to the concentration of the absorbing species in the solution and the path length, UV-VIS can be used to determine the concentration of the absorber in the solution.
\[ A = \log_{10}(I_0/I) = \varepsilon \times c \times L \quad \text{Equation 8} \]

where \( A \) is the measured absorbance, \( I_0 \) is the intensity of the incident light at a given wavelength, \( I \) is the transmitted intensity, \( L \) the path length through the sample, and \( c \) the concentration of the absorbing species.

Due to ability of d-electrons within the transition metal ions to shift from one energy orbit to another, solutions of such metal ions are usually coloured and can absorb visible light. Thus, UV-VIS can be used for quantitative determination of solutions of such compounds. Moreover, organic compounds are also able to absorb visible light. Hence, solutions of transition metal ions with conjugative organic compounds can be investigated for quantitative purposes. However, too intense colours of solutions cannot be used for quantitative measures because wavelength of maximum adsorption is changing with intensifying of the colour of the solution. In these cases the solution needs to be diluted.
Chapter 4 Results and Discussion

4.1 P(NIPAM-co-AA)

The SFEP method was employed to synthesize microgel particles. All samples were synthesized successfully. The samples containing acrylic acid turned milky white after a few minutes when the initiator was added. The sample with 0% of acrylic acid turned cloudy immediately after adding the initiator. However, it changed to a nearly clear solution after the resultant microgel was cooled to room temperature. In contrast, mixtures which contained acrylic acid resulted in thick dispersions (i.e. highly viscous) which retained their milky white state on cooling. The resultant dispersions were dialysed in 5 litre beakers for two weeks with changing the water twice a day.

4.1.1 Response of Microgels to Temperature

The particle sizes of P(NIPAM-co-AA) microgels were determined as a function of temperature at pH 6.0. These investigations were run on a ZetaPALS instrument which has an internal heating facility. The samples were investigated in the range of temperature between 25\(^0\) and 50\(^0\)C. Figure 4-1 shows the changing size and consequent swelling/deswelling of the microgel particles. The values of diameter of the particles are presented in Table A in Appendix 2.
Figure 4-1 shows the effect of temperature on the particle size of microgels containing various concentrations of acrylic acid. Let us consider first the microgel containing no acrylic acid, i.e. a pure PNIPAM microgel. At 25°C the particle size is 425±20 nm, on increasing the temperature to 30°C the particle size, within error remaining constant, but at higher temperatures the particle size is successively reduced until 50°C, the microgel has shrunk to 285±20 nm, with the majority of the shrinkage occurring between 30°C and 40°C. These data are consistent with numerous studies on PNIPAM microgels [10], [7]. The swelling of the particles occurs because as the temperature decreases, the PNIPAM dissolves further into the water as the lower critical
solution temperature (LCST) is reported to be $32^0C$ [45]. Although swelling occurs above the LCST, it must be remembered that the LCST is the phase transition temperature for an infinite molecular weight polymer and that the solvency will be improving before the LCST is reached. Also the N,N'-methylenebisacrylamide (BIS) is more hydrophilic than NIPAM (it has no isopropyl groups), and so it may be expected to have a volume phase transition temperature (VPTT) slightly higher than $32^0C$.

![Swelling ratio of microgel particles](image)

**Figure 4-2:** Swelling ratio of the microgel particles with different concentration of acrylic acid groups as a function of temperature at $pH=6.0$ (electrolyte concentration $2*10^{-5} \text{ mol/l}$).

The effect of adding acrylic acid to the microgels is to increase their particle size. This can be seen both in the collapsed state at $50^0C$, but more particularly
in the swollen state at 25\(^{0}\)C. In order to investigate this behaviour more simply in Figure 4-2 the swelling ratio, i.e. the particle size at any given temperature divided by the collapsed particle size (i.e. 50\(^{0}\)C), is plotted. Figure 4-2 clearly shows that in swollen state the acrylic acid containing microgels are larger. This is due to the presence of COO\(^{-}\) groups in the microgel. The pH of the microgels was 5.5-6.0, well above the pK\(_a\) of acrylic acid which is 4.4 [111]. Likewise the electrolyte concentration is very low (approximately 2\(\times\)10\(^{-5}\) mol/L), thus the charges are only weakly screened by the solvent and so the charges repel each other causing the microgel to swell as shown in Figure 4.3. Not surprisingly the greatest swelling is seen in the microgel containing 30\% of acrylic acid groups.

![Diagram showing microgel swelling](image)

**Figure 4-3:** A schematic representation of microgel swelling due to negatively charged repulsion.
Careful inspection of Figure 4-2 shows that the greatest swelling of the microgel containing no acrylic acid occurs between 35\(^0\)C-30\(^0\)C, whilst for those with acrylic acid occurs between 40\(^0\)-35\(^0\)C, suggesting that the LCST of the acrylic acid containing PNIPAM microgels is increased somewhat. This is hardly surprising as the charged incorporated acrylic acid does increase the hydrophilicity of the copolymer and so it is expected to shift the LCST to higher temperatures. It is of interest to note that the LCST has been shifted to close to body temperature, i.e. approximately 37\(^0\)C; a shift of the VPTT for analogous hydrogels has also been reported recently by other authors [112]. Both the presence of counterions, which increase osmotic pressure, and increase in the average interchain distance due to Coulombic repulsion are the reasons for this shift. Other researchers observed similar behaviour of microgels with partial deprotonation of acrylic acid groups as well [113], [114], [115]. For example, Jones et al. [113] observed such behaviour of microgel particles; however, the concentration of acrylic acid groups in the microgel particles synthesized by these researchers was lower (approximately 5\%) in comparison with those samples of microgel dispersions employed in this research. Hence, similar behaviour of the microgel particles at different temperatures occurs due to increase of acrylic acid groups which have to be deprotonated.

The ZetaPALS instrument not only measures the particles size, but also provides data about polydispersity of the microgels. For all samples the
polydispersity is lower than 0.1; hence, did not change significantly with temperature, suggesting that the microgels are dispersed and not flocculated.

4.1.2 Response of the Microgels to pH

The effect of pH on size of the microgel dispersions consisting of the copolymer of NIPAM and acrylic acid particles were investigated using a ZetaPALS instrument.

![Figure 4-4: The diameter of the microgels with different concentrations of acrylic acid groups as a function of pH at 25°C.](image)

Figure 4-4 shows the results of these investigations. As can be seen from Figure 4-4, considering firstly the microgel without acrylic acid in its
structure, the particle size remains almost constant over all the range of applied pH changes. In fact there is a slight contraction of the particles below pH 4.0. This may simply be due to the error in the measurement, but may be due to some hydrolysis of the amide group of NIPAM, either following impurities in the synthesis or the monomer which was quoted as being 97% NIPAM. Thus, pure PNIPAM microgel may contain a low concentration of COO⁻ groups. However, those microgels containing added acrylic acid undergo considerable shrinking/swelling transition with the change of pH, i.e. microgels contract at lower pH levels and swell with increasing of pH.

Figure 4-4 implies that concentration of the acrylic acid has an impact on the particle size of the collapsed particle, i.e. the particle size at pH 1.0. The diameter of the collapsed particle is increasing with the increase of incorporated acrylic acid concentration. For example, the diameter of the microgels containing 30% acrylic acid is approximately 700±50 nm whereas for 10% and 0% acrylic acid it is 540±50 nm and 360±30 nm respectively. pH 1.0 is well lower the pKa of acrylic acid, therefore it is the electrolyte concentration, which is approximately 0.1 mol/l at pH 1.0, that is causing this effect by reducing the solvent quality for N-isopropylacrylamide, e.g. hydrophobic hydration around polymer side chains is weakened by the solvation of salt ions, while at the same time electrostatic repulsion is diminished.

Obviously, the concentration of the incorporated acrylic acid has an effect on the extent of swelling as well. Figure 4-5 has been plotted in order to
investigate the swelling ratio (the extent of swelling), i.e. the particle size at any given pH divided by the collapsed particle size (pH 1.0). This increase of the diameter happens due to ionization of the polymer chains of the microgel

*Figure 4-5: Swelling ratio of the microgel particles with different concentration of acrylic acid groups as a function of pH at 25°C.*

with the increase of pH; so, intramolecular charge repulsion is forcing the microgel to adopt a more expanded conformation. The concentration of the anionic carboxylate groups increases with increasing pH due to dissociation and this brings about the electrostatic repulsion which disrupts hydrogen bonding between carboxyl group of acrylic acid and amide group of NIPAM; this process is illustrated in Figure 4-6. Kratz et al. observed similar behaviour
of analogous microgel dispersions [116]; however, the highest concentration of acrylic acid in the microgel dispersions prepared by this author was 12.5%. This is possibly the first time when such a high concentration (up to 30%) of acrylic acid has been incorporated into the structure of PNIPAM based microgels. Swelling of the particles at the pH level of the blood in the human body, which is in range of 7.35 to 7.45 [117], as well as having LCST in the range of temperature of human body gives an opportunity to develop further these microgel particles as potential drug-delivery agents.

All four samples were also investigated as a function of temperature at different pH levels. The samples for each acrylic acid concentration were adjusted to range of pH from 1.0 to 10.0. The Figures 4-7 – 4-12, show the dependence of microgels to the temperature changes at different pH levels.

Figure 4-6: A schematic representation of the swelling of the microgels containing carboxylic group in the backbone induced by increasing of the effective charge density on the polymer network.
Figure 4-7: The diameter of the microgels made of 90%NIPAM/10% acrylic acid at pH 1.0 and 3.0 as a function of temperature.

Figure 4-8: The diameter of the microgels made of 90%NIPAM/10% acrylic acid at pH 6.0, 8.0 and 10.0 as a function of temperature.
Figure 4-9: The diameter of the microgels made of 80%NIPAM/20% acrylic acid at pH 1.0 and 3.0 as a function of temperature.

Figure 4-10: The diameter of the microgels made of 80%NIPAM/20% acrylic acid at pH 6.0, 8.0 and 10.0 as a function of temperature.
Figure 4-11: The diameter of the microgels made of 70%NIPAM/30% acrylic acid at pH 1.0 and 3.0 as a function of temperature.

Figure 4-12: The diameter of the microgels made of 70%NIPAM/30% acrylic acid at pH 6.0, 8.0 and 10.0 as a function of temperature.
Let us first consider microgel shrinking/swelling behaviour at low pHs, i.e. pH 1.0 and 3.0. The behaviour of the microgels as a function of temperature at these pHs is presented in Figures 4-7, 4-9 and 4-11. The behaviour of the microgel particles has a similar trend at pH 1.0, i.e. the particles appear to undergo swelling as the temperature is increased. However this is not the case. The particles are aggregating since small flocs can be seen in the samples and the polydispersity increased. A schematic representation of the aggregation process is illustrated in Figure 4-13. The hydrodynamic diameter of the particles increases approximately by a factor of 2-2.5 during the aggregation. Moreover, the size of the aggregates at pH 1.0 and 3.0 for the microgel particles containing 10% acrylic acid (Figure 4-7) is almost the same which is indicating a relatively narrow size distribution of the aggregates. Zhu reported about a limited growth of aggregation clusters of polystyrene lattices stabilized by PNIPAM [118]. It is also worth of noting that the microgel particles obtained almost the same size upon cooling these samples to room temperature as before heating, hence, the process of aggregation is reversible. However, the behaviour of the same microgels is considerably different at pH 3.0 as aggregation occurs only for microgels containing 10% acrylic acid, whilst continuous contracting is observed for microgels with 20% and 30% acrylic acid, due to stronger electrostatic repulsion forces between the particles that is preventing aggregation (Figures 4-9 and 4-11).

Now it is worth of looking at the behaviour of microgels at pH 6.0 and 8.0, which are depicted in Figures 4-8, 4-10 and 4-12. The microgels undergo a
continuous deswelling transition as the temperature increases at pH 6.0 and 8.0 and shows similar behaviour to the pure PNIPAM microgels [106], [119]. The deswelling transition of microgel particles shifts to slightly higher temperature with pH increase, as Figures 4-7 - 4-12 imply that microgel particles start to shrink before reaching 30°C in acidic and neutral solutions; this is probably due to an earlier dominance of hydrophobic attractive forces between isopropyl groups. However, such pre-shrinking behaviour is not observed in solutions with pH>7.0 due to an increasing Donnan osmotic pressure and electrostatic repulsion. The same effect has been observed by Kratz et al. [116].

![Figure 4-13: A schematic representation of the reversible formation of aggregates.](image-url)
A common feature for all microgel dispersions containing acrylic acid, which were described in this Chapter, is observed in the basic solutions, i.e. pH 10.0, as the hydrodynamic diameter of the microgels with 10%, 20% and 30% of incorporated acrylic acid is almost unchanged in the range of temperature changes between 25°C and 50°C (Figure 4-8, 4-10 and 4-12); this is probably due to dominance of electrostatic repulsion over hydrophobic attraction forces. However, the swelling ratio, i.e. \( \frac{D_{T1}}{D_{T=50}} \), is increasing with the increase of concentration of acrylic acid incorporated in the slightly basic solutions. So, the highest swelling ratio amongst the microgel dispersions investigated is observed at pH 8.0 reaching a value of 2.6 for the microgel particles with 30% of incorporated acrylic acid, for the samples with 20%, 10% and 0% incorporated acrylic acid the swelling ratios were 2.03, 1.83 and 1.49, respectively.

It is also worth of comparing the diameter of the particles of the P(NIPAM-co-AA) microgels at the collapsed state which was initiated by temperature and pH (temperature induced contraction is presented in Figure 4-1, pH induced contraction in Figure 4-4). The particles collapsed with increasing temperature and have smaller diameters in comparison with the particles deswelled by lowering the pH. For example, the microgel particles containing 10% acrylic acid have a diameter of approximately 310±50 nm at the collapsed state induced by temperature (i.e. at 50°C), whereas when collapsed by lowering the pH (i.e. pH 1.0) the same particles are approximately 540±50 nm in diameter.
Thus we conclude that at pH 1.0 the particles are not fully collapsed. Such a difference in the diameter of the collapsed particles that were deswelled via different stimuli is probably due to the chemical nature of the microgel, i.e. temperature-induced shrinking is governed by the hydrophobic isopropyl groups in the NIPAM moieties which are present in the microgel backbone with considerably higher concentrations rather than acrylic acid groups which are inducing the shrinkage at low pH. Another reason for the difference in the diameter of collapsed particles might be high electrolyte concentration at pH 1.0, which weakens the hydrophobic hydration around polymer side chains as the salt ions undergo solvation.

The analysis of the response of the microgels at different pHs implies that being both pH- and temperature-responsive with the certain concentration of acrylic acid groups in the backbone, the resultant microgel particles are dual-responsive. However, the microgels aggregated at pH 1.0 at higher temperatures. Although at pH 3.0 the microgels containing 10% acrylic acid groups aggregated, those containing 20% and 30% acrylic acid groups in the microgel backbone did not. This led to an attempt to synthesize microgel particles with increased concentration of acrylic acid but these attempts were unsuccessful as microgels could not be produced with acrylic acid concentration higher than 30%. Since the increase of acrylic acid concentration caused linear polymerization rather than the synthesis of microgels; hence, it was decided to prepare PVP microgel particles as pH-sensitive agents.
4.2 PVP

In the same way as for P(NIPAM-co-AA) microgels preparation, the surfactant free emulsion polymerization method was employed for the synthesis of poly(2-vinylpyridine) [PVP] microgels. The resultant microgel dispersions had a milky white colour due to scattering of light by the particles. During the dialysis the odour of 2-vinylpyridine (2VP) could be smelt for the first few days because of the presence of some unreacted monomer. However, this odour could no longer be detected in less than a week. The particles in the visking tubing exhibited iridescence after dialysis due to the ordering of the particles [120]. Hence, particles of the prepared microgel dispersions were highly monodisperse as polydisperse particles are not able to form ordered structures in this way.

The synthesized dispersions had the physical characteristics of water in terms of viscosity and density. Adding acid to the samples caused their transformation from white to transparent suggesting considerable swelling of the particles. Moreover, addition of the acid changed the physical characteristics of the microgel dispersions as they became noticeably more viscous. Addition of base caused a reversible transition of the microgels causing them to deswell. Although repeated addition of acid and base resulted in the coagulation of the collapsed particles due to the increased ionic strength.
4.2.1 Response of the Microgels to pH

The diameter of the PVP-based microgels was investigated as a function of pH, using the ZetaPALS instrument. pH levels were adjusted in range from 1.0 to 5.0 for measurements. The microgel particles were aggregated resulting in sedimentation at pH levels higher than 5.0; however, adding acid redispersed the sediment. This reversibility was not indefinite though as the particles started to flocculate at a total ionic strength of approximately $1.2*10^{-3}$ mol/l; similar observations were made by Loxley and Vincent [90]. The results of the investigation are shown in Figure 4-14.

![Figure 4-14: The diameter of PVP microgels with different concentrations of the crosslinker as a function of pH at 25°C.](image)

Figure 4-14: The diameter of PVP microgels with different concentrations of the crosslinker as a function of pH at 25°C.
The data from Figure 4-14 shows that the extent of swelling for the PVP microgels is governed by the crosslinking density. The microgels with the highest crosslinking ratio have the lowest swelling ratio, i.e. microgels with 2% crosslinker. The microgel dispersions with a higher degree of crosslinking have also been prepared, but their swelling ratio appeared to be very low, therefore, it was not included into the results section and was not investigated further.

The crosslinker concentration affects swelling of the microgel particles in the same way as it does for PNIPAM particles. However, the diameter of the collapsed particles is virtually unaffected by the degree of crosslinking in contrast to the PNIPAM particles, the collapsed size of which was affected by the degree of crosslinking [50]. Also, the resultant microgel particles obtained a highly swollen state at low pH levels, whereas the particles of P(NIPAM-co-AA) microgels showed the opposite tendency. This is due to the different nature of the monomers used for the synthesis of microgels. PVP microgel particles swell at low pH levels due to the repulsion of partially electronegative nitrogen atom in the pyridine ring. The electron deficient molecule of PVP keen to balance itself, therefore attracts a proton to become positively charged and that leads to the swelling of the particle at low pH; whereas, P(NIPAM-co-AA) particles undergo swelling transition due to dissociation of the carboxylic group of acrylic acid to produce a negative charge. So, the mechanism of pH-induced swelling transition of the microgel...
particles of P(NIPAM-co-AA) and PVP structure is similar, but it is the chemical structure which makes them behave in similar way at opposite pH levels of the solution.

The water uptake or swelling ratio of microgels with different concentrations of the crosslinker is shown in Figure 4-15. The swelling ratio is calculated by the following equation:

\[
\text{Swelling ratio} = \frac{\text{Diameter}_{\text{pH}}}{\text{Diameter}_{\text{pH}=5}} \quad \text{Equation 9}
\]

where Diameter\(_{\text{pH}}\) is the diameter of the swollen microgel particle at any given pH and Diameter\(_{\text{pH}=5}\) is the diameter of the collapsed microgel particle at pH 5.0.

Microgels with 0.5% crosslinker have the largest swelling ratio, although the swelling ratio of microgels with 0.3% crosslinker concentration is virtually the same; these particles swell up to 6 times in comparison to their collapsed state. 2% crosslinker microgels and 1% crosslinker microgel particles are swelling least; their swelling ratios are about 3.8 and 4.8, respectively. These microgel dispersions swell and deswell in response to pH changes of the surrounding environment. The extent of swelling of the particles in the resultant microgel dispersions is dictated by the degree of crosslinking. The higher the concentration of the crosslinker is, the lower the extent of swelling. This feature of PVP microgel particles is similar with those of PNIPAM microgel particles and its derivatives.
The PVP microgels exhibited pH-dependent behaviour as the microgel particles swelled significantly at low pH. However, being completely different in their chemical structure from PNIPAM-based microgels limited some of the properties of such microgels, such as temperature-sensitivity and ability to be functionalized by copolymerizing with other monomers. Therefore, it was decided to prepare multi-responsive microgels which would have temperature-dependent properties of PNIPAM and pH-dependent properties of PVP, and this was achieved by the copolymerization of NIPAM with 1-vinylimidazole. Moreover, such microgels could be potentially responsive to the presence of

4.3 P(NIPAM-co-VI)

Figure 4-15: Swelling ratio of PVP microgels as a function of pH at 25°C.
metal ions, copper (II) particularly due to formation of stronger complex by this metal ion with imidazole groups, as has been reported for analogous macrogels [17], [57].

An emulsion polymerization technique was employed to prepare microgel particles using P(NIPAM-co-VI). A cationic initiator was used to start the reaction. The solution turned milky white after 15 minutes following the addition of the initiator. After the polymerization was completed, dispersions were cooled to room temperature, poured into dialysis tubing and put into 5 litres beakers with DI water for dialysis. Dialysis took place for two weeks with changing the water two times per day.

4.3.1 Response of the Microgels to Temperature

Samples of the resultant microgel dispersions were investigated as a function of temperature. A new batch of the pure PNIPAM microgels was prepared using the same cationic initiator 2,2’-azobis[2-methylpropionamidine] dihydrochloride as was used for the P(NIPAM-co-VI) microgels to enable direct comparison between PNIPAM microgels and the copolymer microgels. Therefore, the hydrodynamic diameter of the pure PNIPAM microgels described in this section is slightly different compared to those described in the section 4.1.1. A ZetaPALS instrument was employed to determine the diameter of the microgels. The results are presented in Figure 4-16.
The diameter of the microgel particles with different concentration of 1-vinylimidazole groups in the microgel backbone as a function of temperature at pH 6.0 (electrolyte concentration $2 \times 10^{-5}$ mol/L).

The effect of temperature on the particle size of microgels containing various concentrations of 1-vinylimidazole (VI) is shown in Figure 4-16. Analyzing the response of the microgel without any incorporated 1-vinylimidazole (VI) monomer shows that the size of the swollen particle, which is approximately 300±20 nm at 25°C, has shrunk gradually to approximately 170±20 nm at 55°C. The majority of shrinkage occurring between 25-35°C, whereas in the range of temperature changes between 35-55°C the particle size remains almost constant. Such behaviour of pure poly(N-isopropylacrylamide) [PNIPAM] microgels is consistent with that which have been observed for analogous microgel dispersions during investigations of P(NIPAM-co-AA)
Figure 4-17: The swelling ratio of the microgel particles with different concentration of 1-vinylimidazole groups in the microgel backbone as a function of temperature at pH 6.0.

microgels, and the explanation of such behaviour is described in Section 4.1.1 of this Chapter.

Figure 4-16 also illustrates that incorporation of 1-vinylimidazole (VI) into microgel backbone has an impact on the diameter of the particles. This can be seen both at collapsed, but more obviously at swollen state at 55°C and 25°C respectively. For example, the diameter of the pure PNIPAM microgel particles is about 300±20 nm and 125±20 nm at 25°C and 55°C, respectively, whereas the diameter of the microgels containing 30% 1-vinylimidazole (VI) is approximately 1025±50 nm and 350±50 nm at the same temperatures of 25°C and 55°C. The difference in the diameter of the particles with and without
incorporated functional monomer can be more easily seen in Figure 4-17, which shows the swelling ratio of P(NIPAM-co-VI) microgels (the ratio is the particle size at any given temperature divided by the particle size at collapsed state, i.e. at 55°C). The fact that the 1-vinylimidazole copolymer microgels are larger in size in the swollen state is due to the presence of electronegative nitrogen atoms in their structure that makes the molecule electron deficient; hence, protonated molecules, which are positively charged, which repel each other causing the microgel to swell (Figure 4-18). However, since the measurements of the particle size for these microgels were obtained at pH 6.0 and pKₐ of 1-vinylimidazole groups is reported to be around 6.0 [121], half of the groups are ionised and half are non-ionised. Therefore, the effect of the 1-
vinylimidazole groups on the size of the particle should be investigated at higher pHs and this will be described in detail later.

It can be seen from Figure 4-17 that the greatest swelling ratio is shown by the microgels containing 10% 1-vinylimidazole groups. The swelling ratio has an interesting trend as it increases sharply at low concentrations of incorporated 1-vinylimidazole groups and decreases upon increasing the concentration of 1-vinylimidazole groups incorporated into the microgel. This might be due to the fact that addition of the high concentrations of functional monomer is increasing the particle size, both in the swollen and collapsed states; however, at low concentrations of added 1-vinylimidazole groups, the particle size in the swollen state increased sharply, but does not increase significantly in the collapsed state at 55°C (see data in Figure 4-16 at 55°C which shows only a weak dependence of particle size with 1-vinylimidazole groups concentration in the microgel). This may be due to the dominance of electrostatic repulsion forces over the hydrogen bonding forces at higher concentrations of 1-vinylimidazole groups in the collapsed state of the particles. Since the swelling ratio is the diameter of the particle at any given temperature, divided by the diameter of the particle in the collapsed state, i.e. 55°C, the increase of the diameter in the collapsed state at higher concentrations of added functional monomer leads to a decrease of the swelling ratio; whereas at low concentrations of added 1-vinylimidazole groups, the diameter of the particles in the collapsed state does not change significantly with 1-vinylimidazole groups concentration (see Figure 4-16), whereas the diameter of the particle in
the swollen state increases sharply and that leads to the high values of the swelling ratio (see Figure 4-17).

It is also worth of noting that microgels without any added 1-vinylimidazole groups have the greatest swelling in the range of temperature changes between 25-35°C, whilst for those with 1-vinylimidazole groups it is observed between 35-45°C. This behaviour is similar to that shown by the microgels containing acrylic acid, which were described previously, and suggesting that addition of 1-vinylimidazole group shifts the lower critical solution temperature to higher temperatures, in the same way as incorporation of acrylic acid into the microgel backbone, i.e. the more hydrophilic monomer 1-vinylimidazole shifts the LCST of PNIPAM to higher temperatures.

![Image](image_url)

*Figure 4-19: The SEM imaging of the freeze-dried microgel particles made of 90%NIPAM/10%VI.*
Figure 4-20: The SEM imaging of the freeze-dried microgel particles made of 80%NIPAM/20%VI.

Figure 4-21: The SEM imaging of the freeze-dried microgel particles made of 70%NIPAM/30%VI.
Additionally, the measurements of the diameter of the microgel particles in the swollen state obtained from DLS on a ZetaPALS instrument were confirmed by SEM imaging of freeze-dried samples of the corresponding microgels; the results of this imaging are presented in Figures 4-19, 4-20 and 4-21. By rapidly freezing the samples it is likely that the particles remain in their swollen state when examined in the SEM. The average diameter in the swollen state for the microgel particles made of 90% NIPAM/10% VI obtained from DLS is approximately 660±50 nm, and from SEM imaging it is approximately 790±130 nm; for the microgel particles made of 80% NIPAM/20% VI DLS measurements showed the average size of approximately 680±50 nm and approximately 800±160 nm from SEM; and, finally, for the microgel particles made of 70% NIPAM/30% VI it was approximately 1025±50 nm from DLS and 1200±120 nm from SEM. The difference between the hydrodynamic diameters of the particles is not that great; therefore, DLS was the technique used to routinely determine the microgel particle size.

4.3.2 Response of the Microgels to pH

The diameter of novel multi-responsive microgel particles, which were possibly prepared for the first time in this work, were investigated as a function of pH and temperature using ZetaPALS instrument. The pH was adjusted using hydrochloric acid and sodium hydroxide in the range of 2.0 to 10.0. The results of the investigation are shown in the Tables 4-1, 4-2 and 4-3.
Table 4-1: The diameter of microgels made of 90%NIPAM/10%VI as a function of temperature at different pH levels.

<table>
<thead>
<tr>
<th>Temperature, C</th>
<th>pH 2.0</th>
<th>pH 4.0</th>
<th>pH 6.0</th>
<th>pH 8.0</th>
<th>pH 10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>2970 nm</td>
<td>1440 nm</td>
<td>650 nm</td>
<td>295 nm</td>
<td>395 nm</td>
</tr>
<tr>
<td>35</td>
<td>2650 nm</td>
<td>1010 nm</td>
<td>510 nm</td>
<td>295 nm</td>
<td>365 nm</td>
</tr>
<tr>
<td>45</td>
<td>2500 nm</td>
<td>875 nm</td>
<td>220 nm</td>
<td>1140 nm*</td>
<td>1160 nm*</td>
</tr>
<tr>
<td>55</td>
<td>2305 nm</td>
<td>540 nm</td>
<td>120 nm</td>
<td>1335 nm*</td>
<td>1390 nm*</td>
</tr>
</tbody>
</table>

* – polydispersity index (PI) value is higher than 0.3. PI for all other values is lower than 0.1.

Table 4-2: The diameter of microgels made of 80%NIPAM/20%VI as a function of temperature at different pH levels.

<table>
<thead>
<tr>
<th>Temperature, C</th>
<th>pH 2.0</th>
<th>pH 4.0</th>
<th>pH 6.0</th>
<th>pH 8.0</th>
<th>pH 10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>3165 nm</td>
<td>1670 nm</td>
<td>670 nm</td>
<td>440 nm</td>
<td>355 nm</td>
</tr>
<tr>
<td>35</td>
<td>2935 nm</td>
<td>1315 nm</td>
<td>600 nm</td>
<td>320 nm</td>
<td>305 nm</td>
</tr>
<tr>
<td>45</td>
<td>2875 nm</td>
<td>985 nm</td>
<td>390 nm</td>
<td>1245 nm*</td>
<td>1220 nm*</td>
</tr>
<tr>
<td>55</td>
<td>2710 nm</td>
<td>725 nm</td>
<td>190 nm</td>
<td>1235 nm*</td>
<td>1335 nm*</td>
</tr>
</tbody>
</table>

* – polydispersity index (PI) value is higher than 0.25. PI for all other values is lower than 0.1.

Table 4-3: The diameter of microgels made of 70%NIPAM/30%VI as a function of temperature at different pH levels.

<table>
<thead>
<tr>
<th>Temperature, C</th>
<th>pH 2.0</th>
<th>pH 4.0</th>
<th>pH 6.0</th>
<th>pH 8.0</th>
<th>pH 10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>3350 nm</td>
<td>2040 nm</td>
<td>1025 nm</td>
<td>630 nm</td>
<td>500 nm</td>
</tr>
<tr>
<td>35</td>
<td>3220 nm</td>
<td>1765 nm</td>
<td>890 nm</td>
<td>500 nm</td>
<td>440 nm</td>
</tr>
<tr>
<td>45</td>
<td>3100 nm</td>
<td>1320 nm</td>
<td>415 nm</td>
<td>330 nm</td>
<td>375 nm</td>
</tr>
<tr>
<td>55</td>
<td>3090 nm</td>
<td>1145 nm</td>
<td>350 nm</td>
<td>285 nm</td>
<td>245 nm</td>
</tr>
</tbody>
</table>

PI for all values is lower than 0.1.
Let us first consider the behaviour of microgels as a function of temperature at pH 6.0 (Figure 4-16). The particles undergo continuous shrinking as was described in previous section, where behaviour of microgels was described as a function of temperature. Considering the behaviour of microgels under these conditions as the initial state of microgels, the behaviour of the microgels at various pH and temperature conditions will be described further in comparison with the initial state.

The effect of pH can be seen from the results in Tables 4-1 – 4-3. Results obtained for the microgels at acidic pH imply that the microgel particles swell as the pH is lowered. Let us describe the swelling behaviour of the microgels as a function of pH at 25°C firstly. Upon slight decrease of pH to 4.0 from pH 6.0 the diameter of the particles approximately doubles, hence the volume is increased up to 8 times for all microgels containing 1-vinylimidazole groups. Further decrease of pH to 2.0 led to even more swelling, where the extent of swelling was higher in those microgels containing higher 1-vinylimidazole groups concentrations, i.e. the diameter of the particles containing 30% 1-vinylimidazole groups increased up to approximately 3350±100 nm, whilst the diameter of the particles containing 10% 1-vinylimidazole groups increased up to 2970±100 nm. Such behaviour of the microgels can be explained by the fact that nitrogen atoms of 1-vinylimidazole group become protonated in acidic solutions, as the pKa of 1-vinylimidazole group is about 6.0 [121]; and these protonated groups repel each other. The protonation of N-atoms leads to the
electron deficiency of the molecule; hence it attracts water to become stabilized and as a result swell. Therefore, the higher the concentration of imidazole groups the higher the degree of swelling. Also it is the concentration of imidazole groups that dictates higher degree of swelling upon decreasing the pH. The degree of swelling at pH 2.0 is very high. It is worth remembering that the accuracy of photon correlation spectroscopy (PCS) decreases as the particle size increases, and so the errors at pH 2.0 will be higher. Flocculation of particles could be an alternative explanation to the increasing particle size, but the sample did not appear to be flocculated as the cloudy dispersion at pH 6.0 became transparent at pH 2.0 suggesting swelling of the particles, in the same way as was described for PVP microgels in section 4.2.

Figure 4-22: The diameter of microgel particles made of P(NIPAM-co-VI) as a function of temperature at pH 2.0.
Now it is worth of describing the behaviour of microgel particles as a function of temperature at pH 2.0 and 4.0. In order to analyse this behaviour more simply Figures 4-22 and 4-23 were plotted. Figure 4-22 shows that the particles contract slightly as the temperature is increasing at pH 2.0; however, the shrinkage of these microgels is minor due to the dominance of electrostatic repulsion forces of repelling imidazole groups over the hydrophobic forces of isopropyl groups of PNIPAM. A similar trend of the behaviour for the same microgel dispersions is observed in Figure 4-23, i.e. temperature behaviour at pH 4.0. However at this pH there is more contracting occurring with the increase of temperature for the microgel particles of all samples. This suggests
that the dominance of the electrostatic repulsion forces is no longer as strong as we are getting closer to the pK\textsubscript{a} of poly(vinylimidazole) under these conditions and hydrophobic forces of isopropyl groups are now playing a role. Even though electrostatic repulsion forces are still affecting the particle size of these microgels as the diameter of the particles at any given temperature under these conditions is significantly larger than the corresponding diameter of the same particles at initial state conditions, i.e. pH 6.0.

Careful inspection of Tables 4-1 – 4-3 shows that the microgel particles shrink in basic solution in comparison with their initial state at pH 6.0. The decreasing particle size at pH 8.0 and 10.0 is due to the fact that at initial state, i.e. pH 6.0, half of 1-vinylimidazole groups are ionised as the pK\textsubscript{a} is reported to be 6.0 [121], whereas at higher pH levels ionisation of 1-vinylimidazole groups is lower; hence, there is less electrostatic repulsion forces and the particles obtain less swollen state. Therefore, the effect of 1-vinylimidazole groups concentration on the particle size of the microgels should be described at these conditions as it was mentioned previously. In that viewpoint it is worth of observing the particle size of the microgels at pH 10.0 at 25\textdegree{}C. The particle size for microgels containing 10% and 20% 1-vinylimidazole groups is very similar, however for those containing 30% of the monomer the particle size increases slightly. The increase of the diameter with increase in the concentration of incorporated 1-vinylimidazole is due to the presence of NH\textsuperscript{+} groups in the microgel. However, the pH being 10.0 which is well above the pK\textsubscript{a}, the electrolyte concentration during the measurement is quite high 0.001
mol/l, thus the charges can be screened. That might explain the similarity of the diameter for the particles containing 10% and 20% of 1-vinylimidazole groups. This explanation is also supported by the results obtained at pH 8.0, whereby a continuous increase in the particle size is observed with the increase in the concentration of the functional monomer; since the electrolyte concentrations under these conditions is lower, screening of the charges by the solvent is weaker, so the charges repel each other causing the microgels to swell.

The other interesting observation which can be made for the microgels at pH 8.0 and 10.0 is that swelling is followed by the aggregation upon temperature increase for the microgels containing 10% and 20% 1-vinylimidazole groups; whilst no aggregation is observed for those containing 30% 1-vinylimidazole groups as this microgel particles undergo continuous shrinking with increasing temperature. This transition may be due to some residual electrostatic repulsion as a result of the high concentration of 1-vinylimidazole groups, whilst it is likely that there is less charge on the surface of the particles at lower concentration of incorporated 1-vinylimidazole which is leading to flocculation of the particles. The mechanism of the aggregation, which is presented in Figure 4-13, was described earlier in section 4.1.2 for the particles containing acrylic acid groups in their structure, which were aggregating with the increase of temperature at low pH. The mechanism of aggregation for the particles described in this section is virtually the same but
occurring at high pH since the chemical nature of the functional monomers, i.e. acrylic acid and 1-vinylimidazole groups, is the opposite.

Such behaviour of these microgel particles, which was described above, has never been observed before and this is the first time that such materials have been characterized. However, as well as being both temperature- and pH-responsive, these novel materials ought to be metal-sensitive; and this property has been explored and the results obtained described in the next section.

4.3.3 Response of the Microgels to copper (II) Ions

The main reason for copolymerization of microgel particles with the backbone of P(NIPAM-co-VI) was to determine whether these microgels are sensitive to the presence of metal ions in solution, specifically copper (II) ions. Copper (II) nitrate trihydrate was dissolved in water and 18 different concentrations, in range from 0.02 g/L to 40 g/L, of copper salt were made. Each of the resultant microgels at pH 6.0 were mixed with copper solutions obtained in ratio of 1 mL of copper solution to 10 mL of microgel. Each mixture was shaken for 2 h. After that each sample was investigated on the ZetaPALS instrument, which uses dynamic light scattering, in order to obtain the particle size.

Four different P(NIPAM-co-VI) ratio microgels made of various NIPAM/VI concentrations in the microgel backbone were investigated namely: 100%NIPAM/0%VI; 90%NIPAM/10%VI; 80%NIPAM/20%VI; 70%NIPAM/30%VI.
Figure 4-24: The diameter of microgel particles made of 100%NIPAM/0%VI as a function of added copper salt concentration at 25°C and pH 6.0.

Figure 4-25: The diameter of microgel particles made of 90%NIPAM/10%VI as a function of added copper salt (Cu(NO₃)₂·3H₂O) concentration 25°C and pH 6.0.
Figure 4-26: The diameter of microgel particles made of 80% NIPAM/20% VI as a function of added copper salt (Cu(NO$_3$)$_2$·3H$_2$O) concentration at 25°C and pH 6.0.

Figure 4-27: The diameter of microgel particles made of 70% NIPAM/30% VI as a function of added copper salt (Cu(NO$_3$)$_2$·3H$_2$O) concentration at 25°C and pH 6.0.
Figures 4-24 – 4-27 show the effect of copper (II) ions on the particle size of microgels containing various concentrations of 1-vinylimidazole groups. Let us consider first the microgel containing no 1-vinylimidazole groups, i.e. a pure PNIPAM microgel. The particle size remains almost constant throughout the whole range of concentration of the salt being added (Figure 4-24). In fact there appears to be a slight swelling observed with increasing concentration of copper (II) salt but this is likely to be due to the error in the measurement rather than actual swelling.

However, as can be seen from Figures 4-25 - 4-27 the diameter of the microgel particles containing 1-vinylimidazole (VI) groups is significantly affected by the addition of the copper (II) ions to the dispersions. There is a common trend in all three different P(NIPAM-co-VI) microgels - the particles tend to swell as the copper (II) ion concentration is increased until a certain point and then shrink dramatically. The microgels containing 10% and 20% 1-vinylimidazole groups start to shrink upon reaching the concentration 0.3 g/L of copper (II) nitrate trihydrate, whilst those containing 30% 1-vinylimidazole groups start to contract when the concentration is exceeding 0.1 g/L. Continuous contracting, which was preceded by swelling, is observed for microgels containing 10% of 1-vinylimidazole groups throughout the whole range of concentrations of the added salt, i.e. from 0.01 to 20 g/L. However, microgels containing higher amounts of 1-vinylimidazole groups, i.e. 20% and 30%, displayed aggregation of the particles at higher concentrations of salt (these data have not been shown on figures). The particles of microgels
containing 20% 1-vinylimidazole groups started to aggregate when the salt concentration was higher than 10 g/L; whilst for those containing 30% 1-vinylimidazole groups the same behaviour was observed when the concentration exceeded 3 g/L.

Figure 4-28: A schematic representation of the complex compound of the copper (II) ion with 1-vinylimidazole groups of the microgel.

The copper ion complexes with the 1-vinylimidazole groups of the microgel (Figure 4-28). It is likely that the increase of the particle size occurs due to domination of electrostatic repulsion forces induced by adsorption of the Cu$^{2+}$ cation inside the particle and, hence, charging up the internal phase of the microgel (Figure 4-29). In the same way that adding more acrylic acid groups
to PNIPAM particles increased the swollen state of the microgels and lowering the pH of these microgels (i.e. P(NIPAM-co-VI) microgels) resulted in swelling of the particles. However at higher concentrations of copper (II) ions the binding forces of complexation between Cu (II) and imidazole groups of the microgel are leading to conformation of the microgel backbone, and hence weaker polymer-solvent interactions. Therefore, it is favourable that solvent would be forced out of the particle resulting into the collapse of the latter.

![Diagram of swelling mechanism](image)

**Figure 4-29: The mechanism of the swelling of the particles due to adsorption of the copper (II) cations on the surface of the microgel particles.**

However, this collapse leads to the formation of aggregates at high concentrations of added salts essentially due to the high concentration of ions in the solution screening the electrical double layer of the collapsed microgel particles causing them to aggregate through van der Waals forces.
Transmission electron microscopy (TEM) imaging of the particles with added copper (II) ions in the solution was undertaken (Figures 4-30 - 4-32). The particles were dried at room temperature in order to remove water which prevented a significant change in the diameter of the particles, which may

Figure 4-30: The diameter of microgel particles made of 90% NIPAM/10% VI with 10 g/L of added Cu(NO$_3$)$_2$*3H$_2$O obtained by the TEM imaging.
Figure 4-31: The diameter of microgel particles made of 80% NIPAM/20% VI with 3 g/L of added Cu(NO$_3$)$_2$*3H$_2$O obtained by the TEM imaging.

Figure 4-32: The diameter of microgel particles made of 70% NIPAM/30% VI with 1 g/L of added Cu(NO$_3$)$_2$*3H$_2$O obtained by the TEM imaging.
occur if heat is applied to aid drying, as temperature increase brings about the collapse of the particles. The microgel particles, with the concentration of the salt at which the almost fully collapsed state was achieved (i.e. 10g/L for microgels with 10% and 20%, and 3g/L for 30% 1-vinylimidazole groups), were imaged using TEM and the results of this imaging confirmed those obtained from a ZetaPALS instrument which is using DLS to determine the particle size. So, for microgels containing 10% 1-vinylimidazole groups the mean diameter obtained from TEM was approximately 270±10 nm in comparison with 240±30 nm from DLS; whilst for those containing 20% 1-vinylimidazole groups it was 220±10 nm from TEM and 190±30 nm from DLS, and for 30% of 1-vinylimidazole groups the diameter was 560±10 nm from TEM and 580±30 nm from DLS. Clearly, the results obtained from both methods of measuring the particle size are very similar and are within error. These TEM images, therefore, confirm the hypothesis of complexation of copper (II) with the imidazole groups of the microgel causing microgel shrinkage.

TEM imaging which was employed for imaging of the particles and was also used to undertake electron diffraction of the particle to determine whether there was any crystalline nature to the particles. Pure PNIPAM particles were amorphous and showed no electron diffraction. Figure 4-33 presents the electron diffraction pattern of the swollen particle of the microgel containing 10% 1-vinylimidazole groups with copper (II) concentration 0.3 g/L. The ring
Figures 4-33: Diffraction pattern of microgel particles made of 90% NIPAM/10% VI in the swollen state with 0.3 g/L of added Cu(NO₃)₂·3H₂O obtained by the TEM imaging.

around the centre shows that the particle has some crystalline structure. Since a ring, rather than a spot pattern is observed implies that the particles contain polycrystalline regions. Figure 4-34 shows the diffraction pattern of the collapsed particle of the similar microgel with copper (II) concentration 10 g/L. This image shows that there is a dashed ring around the centre, implying that the particle is also crystalline and the more defined diffraction pattern implies that there are much fewer, but larger, crystalline regions in the particle. It is likely that the electron diffraction pattern is due to a uniform
Figure 4-34: Diffraction pattern of 90%NIPAM/10%VI microgel particles in the collapsed state with 10 g/L of added Cu(NO$_3$)$_2$·3H$_2$O obtained by the TEM imaging.

arrangement of copper (II) ions in the particle, but the precise nature of this is yet to be determined.

It was also decided to conduct a series of experiments where the amount of microgel was doubled compared to the amount of added copper solutions, i.e. 20 mL of microgel was added to 1 mL of copper salt solution. This was made in order to double the amount of particles which are present in the solution and check whether the assumption regarding complex structure of the copper and VI is reasonable or not. The results are presented in Figure 4-35.
As we can see from Figure 4-35, the swelling at lower concentrations of added copper is less for the samples with double the amount of the microgel particles and at higher concentrations the particle size for both of the samples is almost the same. Also, at lower concentrations of added salt the initial particles do not swell until reaching a certain point of concentration ratio; whereas the same tendency is observed for the sample with double the amount of the particles, but the concentration when swelling occurs is approximately twice as much for the samples with initial amount of particles. Moreover, swelling of the

![Figure 4-35: The diameter of microgel particles made of 90%NIPAM/10%VI as a function of added copper (II) salt concentration and the diameter of double amount of microgel particles made of 90%NIPAM/10%VI as a function of added copper (II) salt concentration at 25°C and pH 6.0.](image)

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particles in the sample with double the concentration of the microgel is lower than for the initial one until reaching the concentration of 1 g/L of Cu(NO$_3$)$_2$*3H$_2$O due to higher amount of particles on the surface of which copper (II) ions are locating; therefore less copper (II) ions on the surface of each particle leads to lower degree of swelling. However, from the point of reaching concentration of 1 g/L of the salt the diameter of the particles in both samples is virtually the same. This is likely to be due to the binding of the copper ions with imidazole groups of the microgel into complexes (Figure 4-29). This experiment demonstrates that a certain ratio of copper (II) ions to microgel is required to observe an effect and that the initial concentration of copper (II) ions is rather immaterial.

### 4.3.4 Response of the Microgels to the Presence of other Metal Ions

Addition of the other cations, such as silver, sodium, nickel, zinc and iron, was also investigated. The results are presented in Table 4-4.

Cations of these metals also affect the particle size, as particles swell in their presence. The extent of swelling increases with increasing concentration of the added salt. However, addition of a high concentration of these salts leads to aggregation of the particles, and no contraction of the particles is observed when the concentration of added salt is above 1.5 g/L apart from with copper (II); suggesting that the formation of complex for this microgel particles is occurring at higher concentrations in comparison with copper due to weaker
complex binding of the corresponding metal ions. The formation of aggregates does not preclude that the diameter of the microgel particles is decreasing; however, due to weaker binding forces and high electrolyte concentration aggregates are forming preventing accurate measurement of the collapsed particle size. It is worth noting that the size of the aggregates for corresponding microgel dispersions does not change significantly in the range of concentration between 1.5 g/L and 20 g/L (see Tables B, C and D in Appendix 3). To summarize, swelling takes place due to the charging of the microgel, when the metal ions are adsorbed inside the particle, but excess of the charge leads to the aggregation of the particles and not the shrinking as observed with copper (Tables 4-5 – 4-7). The behaviour of the microgels as a function of various salt concentration is depicted in Figure A in Appendix 4.

Table 4-4: The diameter of the particles in the microgel dispersions with different added salts at concentration of 1 g/L at 25°C and pH 6.0.

<table>
<thead>
<tr>
<th></th>
<th>90%NIPAM/10%VI</th>
<th>80%NIPAM/20%VI</th>
<th>70%NIPAM/30%VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No salt</td>
<td>590±20 nm</td>
<td>670±15 nm</td>
<td>1020±25 nm</td>
</tr>
<tr>
<td>With Copper (II)</td>
<td>665±15 nm</td>
<td>670±15 nm</td>
<td>580±20 nm</td>
</tr>
<tr>
<td>With Silver</td>
<td>840±25 nm</td>
<td>1250±40 nm</td>
<td>1560±30 nm</td>
</tr>
<tr>
<td>With Nickel (II)</td>
<td>890±25 nm</td>
<td>1170±65 nm</td>
<td>1280±40 nm</td>
</tr>
<tr>
<td>With Iron (III)</td>
<td>910±35 nm</td>
<td>1200±35 nm</td>
<td>1370±50 nm</td>
</tr>
<tr>
<td>With Zinc</td>
<td>820±30 nm</td>
<td>980±35 nm</td>
<td>1060±40 nm</td>
</tr>
</tbody>
</table>
Table 4-5: The diameter of the particles in microgels made of 90% NIPAM/10% VI with different added salts at concentrations of 0.1, 1 and 10 g/L at 25°C and pH 6.0.

<table>
<thead>
<tr>
<th></th>
<th>No salt</th>
<th>0.1 g/L</th>
<th>1 g/L</th>
<th>10 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Copper (II)</td>
<td>590±20 nm</td>
<td>980±20 nm</td>
<td>665±20 nm</td>
<td>240±20 nm</td>
</tr>
<tr>
<td>With Silver</td>
<td>590±20 nm</td>
<td>670±25 nm</td>
<td>840±25 nm</td>
<td>2880±200 nm *</td>
</tr>
<tr>
<td>With Nickel (II)</td>
<td>590±20 nm</td>
<td>685±25 nm</td>
<td>890±25 nm</td>
<td>3025±250 nm *</td>
</tr>
<tr>
<td>With Iron (III)</td>
<td>590±20 nm</td>
<td>690±35 nm</td>
<td>910±35 nm</td>
<td>2570±100 nm *</td>
</tr>
<tr>
<td>With Zinc</td>
<td>590±20 nm</td>
<td>675±30 nm</td>
<td>820±30 nm</td>
<td>2470±150 nm *</td>
</tr>
</tbody>
</table>

* - particles appeared to be aggregated, PI is higher than 0.3 for these values. PI is lower than 0.1 for all other values.

Table 4-6: The diameter of the particles in microgels made of 80% NIPAM/20% VI with different added salts at concentrations of 0.1, 1 and 10 g/L at 25°C and pH 6.0.

<table>
<thead>
<tr>
<th></th>
<th>No salt</th>
<th>0.1 g/L</th>
<th>1 g/L</th>
<th>10 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Copper (II)</td>
<td>670±15 nm</td>
<td>1675±15 nm</td>
<td>670±15 nm</td>
<td>175±15 nm</td>
</tr>
<tr>
<td>With Silver</td>
<td>670±15 nm</td>
<td>810±40 nm</td>
<td>1250±40 nm</td>
<td>3310±240 nm *</td>
</tr>
<tr>
<td>With Nickel (II)</td>
<td>670±15 nm</td>
<td>800±65 nm</td>
<td>1170±65 nm</td>
<td>3025±200 nm *</td>
</tr>
<tr>
<td>With Iron (III)</td>
<td>670±15 nm</td>
<td>800±35 nm</td>
<td>1200±35 nm</td>
<td>3870±300 nm *</td>
</tr>
<tr>
<td>With Zinc</td>
<td>670±15 nm</td>
<td>790±35 nm</td>
<td>980±35 nm</td>
<td>2960±280 nm*</td>
</tr>
</tbody>
</table>

* - particles appeared to be aggregated, PI is higher than 0.35 for these values. PI is lower than 0.1 for all other values.
Table 4-7: The diameter of the particles in microgels made of 70%NIPAM/30%VI with different added salts at concentrations of 0.1, 1 and 10 g/L at 25°C and pH 6.0.

<table>
<thead>
<tr>
<th></th>
<th>No salt</th>
<th>0.1 g/L</th>
<th>1 g/L</th>
<th>10 g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Copper (II)</td>
<td>1020±25 nm</td>
<td>2095±20 nm</td>
<td>580±20 nm</td>
<td>4880±300 nm *</td>
</tr>
<tr>
<td>With Silver</td>
<td>1020±25 nm</td>
<td>1250±30 nm</td>
<td>1565±30 nm</td>
<td>5010±350 nm *</td>
</tr>
<tr>
<td>With Nickel (II)</td>
<td>1020±25 nm</td>
<td>1035±40 nm</td>
<td>1280±40 nm</td>
<td>4790±500 nm *</td>
</tr>
<tr>
<td>With Iron (III)</td>
<td>1020±25 nm</td>
<td>880±50 nm</td>
<td>1370±50 nm</td>
<td>3565±320 nm *</td>
</tr>
<tr>
<td>With Zinc</td>
<td>1020±25 nm</td>
<td>800±40 nm</td>
<td>1065±40 nm</td>
<td>3370±410 nm *</td>
</tr>
</tbody>
</table>

* - particles appeared to be aggregated, PI is higher than 0.3 for these values. PI is lower than 0.1 for all other values.

4.3.5 Calculation of the copper Uptake by the Microgel Particles

It was then decided to investigate the degree of uptake of copper (II) by the microgels. Samples of microgels made of 90%NIPAM/10%VI, 80%NIPAM/20%VI and 70%NIPAM/30%VI with added copper salt were centrifuged and the supernatant was decanted. The supernatant was then analyzed on UV-VIS spectrometer in order to obtain the value of absorption, which then was used to calculate the copper uptake by the microgel. The calculation was made via following procedures:

\[
\frac{[Cu(NO_3)_2 \cdot 3H_2O]_X}{[Cu(NO_3)_2 \cdot 3H_2O]_i} = \frac{Absorption_X}{Absorption_i}
\]

Equation 10

where, \([Cu(NO_3)_2 \cdot 3H_2O]_X\) is the concentration of the salt in the certain solution prepared to be added to the microgel dispersion, \([Cu(NO_3)_2 \cdot 3H_2O]_i\)
$3H_2O]_t$ is concentration of the salt remaining in the certain supernatant, $Absorption_X$ is the measured absorption of the solution prepared to be added to the microgel dispersion, $Absorption_t$ is the measured absorption of the certain supernatant.

From Equation 10 the concentration of the remaining salt in the supernatant can be found as:

$$[Cu(NO_3)_2 \cdot 3H_2O]_t = \frac{[Cu(NO_3)_2 \cdot 3H_2O]_X \times Absorption_t}{Absorption_X} \quad Equation\ 11$$

So, from the values obtained via Equation 11, the concentration of the salt left in the sediment after centrifugation ($[Cu(NO_3)_2 \cdot 3H_2O]_t$) can be found as:

$$[Cu(NO_3)_2 \cdot 3H_2O]_t = [Cu(NO_3)_2 \cdot 3H_2O]_X - [Cu(NO_3)_2 \cdot 3H_2O]_t \quad Equation\ 12$$

From Equation 12 we can calculate the mass of copper which made a complex compound with the microgel particles. Three samples with 10 mL of the microgel dispersion were dried and the mass of the polymer in the solution were obtained in order to calculate the copper uptake. As the weight of the copper in the salt is 0.26 moles, the copper uptake (CU) by 1g of dry microgel was calculated as:

$$CU = \frac{[Cu(NO_3)_2 \cdot 3H_2O]_t \times 0.26}{the\ mass\ of\ dry\ microgel} \quad Equation\ 13$$
The copper uptake results of the microgel dispersions are presented in Figure 4-36, which shows that the highest copper uptake is achieved by microgel particles made of 70%NIPAM/30%VI and the lowest by microgel particles made of 90%NIPAM/10%VI, presumably because the ability to bind the copper (II) ions is decreasing with decrease in concentration of incorporated 1-vinylimidazole groups. In fact, it was not possible to measure the value of adsorption on UV-VIS spectrometer for the particles at concentration of added salt lower than 1 g/L due to high colour intensity of the supernatant solution without dilution. Since the microgel particles are swollen, i.e. consisted mainly of water, at the conditions where the concentration of the added copper (II) salt is lower than 1 g/L it was impossible to separate the microgel particles by centrifugation. Therefore, the complexation of copper (II) ions with VI-groups
Figure 4-36: Copper uptake by 1g of dry microgel as a function of 

\[ \text{Cu(NO}_3\text{)}_2*3\text{H}_2\text{O concentration left in the supernatant}. \]

of the microgel produced highly intense colour of the supernatant solution which made the values of absorbance inaccurate, and, hence, distorted the calculation of copper uptake at low Cu\(^{2+}\) concentrations leading to negative values. However, at higher concentrations of the added copper (II) salt the particles shrunk which made the separation process possible via centrifugation and absorbance values were accurate.

It was then decided to determine whether the uptake can be described by a Langmuir adsorption isotherm and if it is to determine the maximum amount adsorbed (Γ\(_m\)). The Langmuir isotherm can be written as:
\[ \Gamma = \Gamma_m \left[ \frac{K C_e}{1 + K C_e} \right] \quad \text{Equation 14} \]

where \( C_e \) is equilibrium concentration of metal ion in the solution (g/L), \( \Gamma \) is amount of copper (II) adsorbed, \( \Gamma_m \) is a maximum amount of copper (II) adsorbed, \( K \) is an adsorption equilibrium constant. Frequently a linear form of Equation 14 is used to calculate \( \Gamma_m \) and \( K \) from the slope and intercept as the plot of \( C_e/\Gamma \) and \( C_e \) is linear if the Langmuir isotherm can be applied.

\[ \frac{C_e}{\Gamma} = \frac{C_e}{\Gamma_m} + \frac{1}{K \Gamma_m} \quad \text{Equation 15} \]

The adsorption data were applied to linear form of Langmuir equation (Equation 15). Figure 4-37 is a linear plot for the microgel dispersion containing 30% imidazole groups; a reasonable straight line fit of the data is observed implying that the Langmuirian isotherm describes the copper (II) uptake by the microgel. Plots for microgels containing 10% and 20% imidazole groups are presented in Figures B and C in Appendix 5.

The calculation of the copper uptake using equations described in this section implies that the microgel dispersions containing 30% imidazole groups bind up to almost 2 g of copper (II) per gram of microgel (Figures 4-36). The curves presented in Figures 4-37 and Figures B and C in Appendix 5 can be used to determine the maximum amount of copper (II) adsorbed, i.e. copper
Figure 4-37: Linearized plot of Langmuir isotherm of Cu(NO$_3$)$_2$·3H$_2$O adsorbing onto the microgel particle made of 70% NIPAM/30% VI.

uptake, in weight terms. The results of this calculation are presented in Table 4-8.

Since the values of maximum amount of copper adsorbed ($\Gamma_m$) and adsorption equilibrium constant (K) are determined, it is possible to compare the calculated data to the Langmuir fit by inserting the values of $\Gamma_m$ and K into the Equation 14. The Figure 4-38 and Figures D and E in Appendix 6 represent adsorption isotherms of the copper ions in comparison with the Langmuir fit for microgels containing various concentrations of imidazole groups.
Table 4-8: Maximum amount of copper (II) adsorbed obtained from adsorption isotherms on weight basis.

<table>
<thead>
<tr>
<th>Microgel dispersion made of:</th>
<th>Maximum amount absorbed, Γm (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90% NIPAM/10% VI</td>
<td>0.68±0.5</td>
</tr>
<tr>
<td>80% NIPAM/20% VI</td>
<td>1.71±0.19</td>
</tr>
<tr>
<td>70% NIPAM/30% VI</td>
<td>2.25±0.49</td>
</tr>
</tbody>
</table>

Figure 4-38: Adsorption isotherm of copper (II) using microgels made of 70% NIPAM/30% VI in weight units.
4.4 P(NIPAM-co-3-APB-ATMA)

Although investigations on glucose-responsive microgels and hydrogels have been reported previously [61], [21], the resultant microgels were sensitive to glucose only at high pH conditions, i.e. pH 10 and higher which are pH conditions considerably higher than the physiological pH of 7.4. The challenge of preparation of the microgel which would be glucose-responsive and operate at physiological pH remains unsolved. Recently, Kabilan et al. [63] reported successfully synthesized polymeric films of hydrogels, which were operating at physiological pH, by synthesizing monomers of 3-acrylamidophenylboronic acid and 2-acrylamido-5-flurophenylboronic acid that were synthesized from 3-aminophenylboronic acid and 2-aminomethyl-5-flurophenylboronic acid, respectively. Matsumoto et al. [122] also reported preparation of a glucose-responsive polymer gel operating at physiological conditions; however, the functional monomer of phenylboronic acid derivative, 4-(1,6-dioxo-2,5-diaza-7-oxamyl)phenylboronic acid, that has been used in their research was rather complicated to synthesize. Therefore, it was decided to adapt the methodology of Kabilan et al. [63] that employed the preparation of 3-acrylamidophenylboronic acid (3-APB) copolymerized with (3-acrylamidopropyl)trimethylammonium chloride (ATMA) in order to synthesize PNIPAM-based glucose-responsive microgels, to determine whether these microgels are functional at physiological conditions. This route was adapted due to simplicity of the methodology and the availability of
materials for the synthesis. If successful this would be the first time that microgel particles of such chemical structure and properties have been produced and that are sensitive to glucose at physiological pH.

The classical SFEP technique was employed for the synthesis of P(NIPAM-co-3-APB-ATMA) microgel particles. An anionic initiator was used to start the reaction, addition of which made the solution turn milky and then white with a slight pink undertone. After the synthesis was completed the solution was dialysed in order to remove all the impurities.

4.4.1 The Response of the Microgels to Temperature

The particle size of the resultant microgel dispersions were investigated as a function of temperature using ZetaPALS instrument. The results of these investigations are shown in Figure 4-39.

Figure 4-39 shows the effect of temperature on the particle size of microgels with various concentrations of incorporated functional monomers that make microgels glucose-responsive. Firstly, it is worth of analysing pure PNIPAM microgels, i.e. containing no 3-acrylamidophenylboronic acid (3-APB) or (3-acrylamidopropyl)trimethylammonium chloride (ATMA). The particle size of such microgels shows typical PNIPAM microgels behaviour by the size decreasing with temperature increase, being approximately 300±15 nm and 125±15 nm at 25°C and 55°C, respectively. The majority of shrinkage occurs between 25°C and 35°C. This behaviour of analogous microgels was described previously in the earlier sections of the Chapter 4.
Figure 4.39: The diameter of P(NIPAM-co-3-APB-ATMA) microgel particles as a function of temperature at pH 7.5 (electrolyte concentration $2 \times 10^{-5}$ mol/L).

The effect of adding functional monomers to the microgels, i.e. 3-APB and ATMA, is to increase their particles size. This can be seen in the swollen state of the microgel particles, i.e. at $25^\circ$C, as the particle size increases at higher concentrations of impregnated monomers. Such an effect is due to presence of charged groups that repel from each other causing the microgel to swell. It might be expected that oppositely charged boronic acid - B(OH)$_2$O$^-$ and trimethylammonium - N(CH$_3$)$_3^+$ groups have to neutralize each other; however, experimental data does not support this thought and it is more likely that repulsion of likely charged groups takes place. Therefore, the higher the concentration of 3-APB and ATMA groups is the larger the particle size of the
microgels. However, at the collapsed state, i.e. 55°C, the particle size of the microgels containing 5% 3-APB and 5% ATMA groups and 10% 3-APB and 10% ATMA groups is slightly higher than the particle size of pure PNIPAM microgels. This likely to be due to electrostatic repulsion forces with increase of impregnated functional monomers preventing the particles from full shrinkage at 55°C.

Careful inspection of Figure 4-39 shows that the greatest swelling of the microgels containing no 3-APB and ATMA occurs between 35°C and 25°C, whilst those with 5% 3-APB, 5% ATMA and 10% 3-APB, 10% ATMA shrinking most between 40°C and 35°C, suggesting that the lower critical solution temperature of the microgels containing functional monomers is increased. This is to be expected as the charged groups would be hydrophilic and so would be expected to shift the lower critical solution temperature to higher temperatures and is similar to the effect seen in acrylic acid and 1-vinylimidazole containing microgels.

4.4.2 Response of the Microgels to the Presence of Glucose

Resultant microgel dispersions were adjusted to pH 7.5 and pH 8.5 and glucose solutions with concentrations in the range of 10^{-2} mmol/L – 10 mmol/L were added to the microgel dispersions at certain pH. The diameter of the corresponding samples was measured on ZetaPALS instrument using dynamic light scattering. The results of these measurements are presented in Tables 4-9 - 4-12.
Table 4-9: The diameter of the particles in microgels made of 90%NIPAM/5%-3-APB/5%ATMA with different glucose concentrations as a function of temperature at pH 7.5.

<table>
<thead>
<tr>
<th>Glucose concentration</th>
<th>Temperature, °C</th>
<th>0 mmol/L</th>
<th>10⁻² mmol/L</th>
<th>10⁻¹ mmol/L</th>
<th>1 mmol/L</th>
<th>5 mmol/L</th>
<th>10 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>620 nm</td>
<td>605 nm</td>
<td>535 nm</td>
<td>550 nm</td>
<td>720 nm</td>
<td>790 nm</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>480 nm</td>
<td>435 nm</td>
<td>420 nm</td>
<td>490 nm</td>
<td>530 nm</td>
<td>605 nm</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>280 nm</td>
<td>240 nm</td>
<td>235 nm</td>
<td>250 nm</td>
<td>355 nm</td>
<td>400 nm</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>180 nm</td>
<td>105 nm</td>
<td>120 nm</td>
<td>120 nm</td>
<td>230 nm</td>
<td>245 nm</td>
<td></td>
</tr>
</tbody>
</table>

Polydispersity index (PI) is lower than 0.1 for all values presented.

Table 4-10: The diameter of the particles in microgels made of 90%NIPAM/5%-3-APB/5%ATMA with different glucose concentrations as a function of temperature at pH 8.5.

<table>
<thead>
<tr>
<th>Glucose concentration</th>
<th>Temperature, °C</th>
<th>0 mmol/L</th>
<th>10⁻² mmol/L</th>
<th>10⁻¹ mmol/L</th>
<th>1 mmol/L</th>
<th>5 mmol/L</th>
<th>10 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>600 nm</td>
<td>590 nm</td>
<td>560 nm</td>
<td>560 nm</td>
<td>685 nm</td>
<td>750 nm</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>570 nm</td>
<td>465 nm</td>
<td>440 nm</td>
<td>500 nm</td>
<td>550 nm</td>
<td>635 nm</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>350 nm</td>
<td>245 nm</td>
<td>245 nm</td>
<td>260 nm</td>
<td>315 nm</td>
<td>350 nm</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>190 nm</td>
<td>120 nm</td>
<td>130 nm</td>
<td>130 nm</td>
<td>195 nm</td>
<td>210 nm</td>
<td></td>
</tr>
</tbody>
</table>

Polydispersity index (PI) is lower than 0.1 for all values presented.
Table 4-11: The diameter of the particles in microgels made of 80%NIPAM/10%3-APB/10%ATMA with different glucose concentrations as a function of temperature at pH 7.5.

<table>
<thead>
<tr>
<th>Glucose concentration</th>
<th>Temperature, °C</th>
<th>0 mmol/L</th>
<th>$10^{-2}$ mmol/L</th>
<th>$10^{-1}$ mmol/L</th>
<th>1 mmol/L</th>
<th>5 mmol/L</th>
<th>10 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>730 nm</td>
<td>740 nm</td>
<td>710 nm</td>
<td>800 nm</td>
<td>980 nm</td>
<td>1150 nm</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>590 nm</td>
<td>630 nm</td>
<td>600 nm</td>
<td>820 nm</td>
<td>900 nm</td>
<td>955 nm</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>360 nm</td>
<td>350 nm</td>
<td>345 nm</td>
<td>445 nm</td>
<td>500 nm</td>
<td>530 nm</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>190 nm</td>
<td>305 nm</td>
<td>280 nm</td>
<td>300 nm</td>
<td>380 nm</td>
<td>450 nm</td>
<td></td>
</tr>
</tbody>
</table>

Polydispersity index (PI) is lower than 0.1 for all values presented.

Table 4-12: The diameter of the particles in microgels made of 80%NIPAM/10%3-APB/10%ATMA with different glucose concentrations as a function of temperature at pH 8.5.

<table>
<thead>
<tr>
<th>Glucose concentration</th>
<th>Temperature, °C</th>
<th>0 mmol/L</th>
<th>$10^{-2}$ mmol/L</th>
<th>$10^{-1}$ mmol/L</th>
<th>1 mmol/L</th>
<th>5 mmol/L</th>
<th>10 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>885 nm</td>
<td>865 nm</td>
<td>840 nm</td>
<td>920 nm</td>
<td>1095 nm</td>
<td>1230 nm</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>730 nm</td>
<td>695 nm</td>
<td>645 nm</td>
<td>870 nm</td>
<td>995 nm</td>
<td>1065 nm</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>515 nm</td>
<td>420 nm</td>
<td>450 nm</td>
<td>500 nm</td>
<td>580 nm</td>
<td>615 nm</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>325 nm</td>
<td>170 nm</td>
<td>335 nm</td>
<td>325 nm</td>
<td>440 nm</td>
<td>480 nm</td>
<td></td>
</tr>
</tbody>
</table>

Polydispersity index (PI) is lower than 0.1 for all values presented.
Also glucose solutions of the same concentrations as described above were added to the microgels consisting of pure PNIPAM in order to be sure that glucose is only affecting the particle size for those microgels containing 3-APB and ATMA groups. The results of these investigations are presented in Figure 4-40.

Figures 4-40 – 4-42 show the effect of addition of glucose on the particle size of the microgels. Figure 4-40 shows that the microgels without any added functional monomers are not affected by the addition of glucose at both pH 7.5 and 8.5, in fact their particle size remains almost the same and any changes are within experimental error. Therefore, it can be concluded that the changing particle size of microgels containing 5% 3-APB, 5% ATMA groups and 10% 3-APB, 10% ATMA groups is governed by the functional monomers mentioned.
Figure 4-40: The diameter of microgel particles made of 100%PNIPAM/0%3-APB/0%ATMA as a function of added glucose concentration at pH 7.5 and 8.5 at 35°C.

Figure 4-41: The diameter of microgel particles made of 90%PNIPAM/5%3-APB/5%ATMA as a function of added glucose concentration at pH 7.5 and 8.5 at 35°C.
The diameter of microgel particles made of 80% PNIPAM/10% 3-APB/10% ATMA as a function of added glucose concentration at pH 7.5 and 8.5 at 35°C.

Figures 4-41 and 4-42 show the effect of glucose addition on the particle size of the microgel containing various amounts of functional monomers in their backbone at 35°C, close to physiological temperatures. The trend in behaviour of these microgels is similar as the microgels swell high concentrations of added glucose at both pH 7.5 and 8.5 above 0.1 mmol/L. The shrinkage of the microgels can be explained by the fact that a bis(bi-dentate) glucose complex is formed in the presence of low glucose concentrations due to very little competition between the free available glucose molecules for binding sites on the charged boronic acid group (see Figure 4-43). Hence each glucose
molecule is able to bind to two boronic acid groups. The formation of this complex leads to an additional crosslink which in turn leads to an effective increase in the crosslinking density. Both Tables 4.11 and 4.12 show a shrinkage of the microgel as glucose is added up to a concentration of 0.1 mmol/L. This additional crosslink also leads to stronger network elasticity of the microgel, i.e. an increase in energy due to elasticity. This will cause shrinkage of the microgel. This behaviour is particularly noticeable at 55°C, but can be seen to a lesser extent at lower temperatures. The mechanism of such behaviour is presented in Figure 4-43.

However, in the presence of high glucose concentrations, a mono(bi-dentate) glucose complex is formed. This is because there are more glucose molecules available for the binding sites on the charged boronic acid group. Hence, it is more likely that each glucose molecule will bind to only one site on the boronic acid groups. The formation of this complex leads to favourability of polymer-solvent interactions due to arising additional hydrogen bonds from the glucose molecule attached to the microgel backbone. The mechanism of such complex formation is presented in Figure 4-44.
Figure 4-43: The mechanism of formation of a bis(bi-dentate) glucose complex formed at low glucose concentration (adopted from [123]).

Figure 4-44: The mechanism of formation of a mono(bi-dentate) glucose complex formed at high glucose concentration (adopted from [123]).

Also it can be concluded from both Figures 4-41 and 4-42 that the extent of swelling of the particles is higher at pH 8.5 rather than at pH 7.5. Although the difference in the extent of swelling at high concentrations of added glucose is not very significant for microgels containing 5% 3-APB and 5% ATMA.
groups, it is considerably higher for microgels containing 10% 3-APB and 10% ATMA groups at the same condition. However, this is first time that such glucose-responsive microgels to be functional at physiological pH and at physiological temperature, i.e. pH 7.5 and 35°C, has been reported. The possibility of operating at physiological pH has been achieved by lowering the pKₐ of boronic acid group by the incorporation of the ATMA groups with the 3-APB groups onto the PNIPAM backbone of the microgels. An alternative way of looking at this is that the local pH of water in microgel has been changed by the ATMA group. Moreover, the fact that the diameter of the particles expands in the range of glucose concentrations which are in the range of glucose level in human body makes these novel materials to be good candidates for pharmaceutical applications as a drug delivery systems in diabetes treatment.

The desired blood glucose concentration is between 4-7 mmol/L [124], so ideally we would like to construct microgels which do not swell until above the glucose concentration is above 7.0 mmol/L. It is likely that this could be achieved by finer control of the crosslinker density and 3-APB and ATMA concentration.
Chapter 5 Conclusions and Future Work

The work presented in this thesis demonstrates successful preparation of microgel dispersions consisting of PNIPAM and various functional groups such as AA, VI, 3-APB and ATMA via an emulsion polymerization technique. In addition, PVP microgels were prepared via surfactant free emulsion polymerization method. The resultant microgels swelled or shrunk in response to various external stimuli, such as change in temperature, pH of the surrounding media, and the presence of copper (II) ions or glucose. Behaviour of these microgels has been described in this work and the reason for certain behaviour under corresponding conditions were given in order to explain swelling/shrinking transitions. Some general conclusions of the work which has been undertaken during this research and recommendations for future work to be done will be described in this Chapter. Each system studied will be described in turn.

5.1 General Conclusions and Suggestions on Future Work to Be Done for the P(NIPAM-co-AA) Microgels

Firstly, microgels containing 10%, 20% and 30% of acrylic acid groups were prepared. The particle size of these microgels under different conditions has been measured. The particle size of the microgels containing acrylic acid groups was affected by the incorporation of functional monomer in
comparison with the microgels without any acrylic acid in the backbone, i.e.
pure poly(N-isopropylacrylamide) microgels. The acrylic acid monomer being
a more hydrophilic monomer shifts the lower critical solution temperature of
the microgels towards the temperature of human body, which give an
opportunity to consider these materials as possible candidates in
pharmaceutical applications.

The impact of pH on these microgels has also been investigated and PNIPAM-
co-AA microgels showed similar trend in their behaviour, i.e. collapsed at
lower pH, when the hydrophobic COOH groups present, and swelling was
observed at higher pH, when the carboxylic acid group is ionized to COO\(^-\).
Both pH and temperature effects of these microgels were described in this
work and the reasons for corresponding behaviour of the microgels are
presented in this work. The diameter of the P(NIPAM-co-AA) particles is
increasing with higher concentration of acrylic acid groups due to the
electrostatic repulsion of COO\(^-\) groups. Additionally, increased hydrophilicity
of the microgel containing acrylic acid groups leads to the shift of the LCST
to higher temperatures, i.e. from 30\(^0\)-35\(^0\)C for pure PNIPAM microgels to 35\(^0\)-
40\(^0\)C for PNIPAM-co-AA microgels. Even though the behaviour of similar
microgels was reported before [113], [10], [7], this is the first time when such
high concentrations, i.e. up to 30\%, of acrylic acid groups have been
incorporated into the microgels backbone.

Overall, swelling properties of the resultant microgels and volume phase
transition temperature which is near temperature of human body give an
opportunity to modify these materials for environmental and pharmaceutical applications, e.g. as an agents for reducing waste of water during flooding process in enhanced oil recovery. For example, to inject microgels in order to reduce water production in oil industry by controlling water mobility. The P(NIPAM-co-AA) microgels aggregate at high temperature under acidic conditions [125], and since majority of oil reservoirs have acidic and high temperature conditions, such microgels could be used as water-blocking agents for high permeability zones. However, such microgels have to be investigated over a wider range of temperatures, presence of high degrees of salinity, i.e. severe physico-chemical conditions in order to work as water-shutoff agents. Moreover, additional investigations have to be undertaken to determine control of adsorbed microgels on reducing oil permeability [5]. Also the LCST shifted towards the temperature of human body makes these materials potentially useful as a sensors or controlled release agents for drug-delivery systems.

5.2 General Conclusions and Suggestions on Future Work to Be Done for the PVP Microgels

The next stage of the research was to produce PVP microgels, which were pH-responsive materials, but now since 2-vinylpyridine group ionizes at low pH the response to pH changes is the opposite way round for PVP microgels compared to P(NIPAM-co-AA) microgels. These microgels, which were
prepared with various crosslinker concentrations, have been investigated to
determine changes of their particles’ size as a function of pH. The results of
investigations showed opposite behaviour in comparison with P(NIPAM-co-
AA) microgels as the particles increased in size with a decrease of pH; however, aggregation of the particles occurred at high pH. Although decrease
of pH redispersed the resultant aggregates, repeated pH adjustments led to the
formation of irreversible aggregates due to coagulation of the particles as the
electrolyte concentration eventually become too high. The crosslinker density
governed the swelling ratio of PVP microgels and greater extent of swelling
has been observed for the microgels containing lower concentrations of the
crosslinker. The swelling/deswelling transitions were consistent with those
reported previously [13], and the changes of particle size in response to
changes in pH of surrounding media was also similar to those reported by
Cook [13].
Such properties of the resultant microgels, together with their passivity for
temperature affects make them good candidates for environmental
applications, e.g. water-shutoff agents for oil deposits with high temperature
conditions in those reservoirs containing high concentration of sulphur, i.e.
with acidic conditions as corresponding microgels expand in diameter only at
low pHs. These microgels should be investigated in wider range of crosslinker
concentrations in order to find that concentration of the crosslinker at which
microgel particles of desired size with high degree of swelling will be
produced. Also the effect of high electrolyte concentration on the diameter of
microgel particles could be investigated in order to make microgels which are stable under such conditions. These aspects are considered to be the main areas of future investigations that might be undertaken regarding corresponding microgel dispersions.

5.3 General Conclusions and Suggestions on Future Work to Be Done for the P(NIPAM-co-VI) Microgels

Since one of the two main goals of this project was to prepare novel microgels with sensitivity for the presence of metal ions, arguably the most important part of the research was to copolymerize P(NIPAM-co-VI) microgel particles. Microgels with various concentrations of imidazole groups were successfully synthesized via an emulsion polymerization technique. The effect of temperature and pH on these microgels was investigated. The behaviour of the resultant microgel particles was similar to those containing acrylic acid as functional monomer in the backbone as both swelling ratio and the diameter of the particles were affected by the concentration of impregnated 1-vinylimidazole groups. The size of the microgel particles increased with the increase in concentration of incorporated imidazole groups, however the greatest swelling ratio was observed at lower concentrations of 1-vinylimidazole groups as particle size increased both at swollen and collapsed states upon increasing 1-vinylimidazole groups concentration.
The effect of pH was similar to those observed for PVP microgels as the resultant microgels swelled in acidic solutions and reversible aggregation occurred upon increasing of pH, i.e. aggregates redispersed when pH was lowered again. Moreover, the effect of temperature under different pH conditions was also investigated and described in this work. These investigations showed that the microgel particles contract slightly at pH 2.0 with increasing temperature due to domination of electrostatic repulsion forces caused by imidazole groups over hydrophobic forces of N-isopropylacrylamide. Although the similar trend of behaviour has been observed for the same microgel particles at pH 4.0, the contraction of the particles with increasing temperature is more significant under these conditions. This implies that the dominance of the electrostatic forces is becoming weaker as pH 4.0 is close to the pKₐ of imidazole group, which is 6.0 [121]; therefore, hydrophobic forces of isopropyl groups of PNIPAM are now playing role. Continuous shrinking with increasing temperature followed by aggregation at temperatures higher than 45°C has been observed for microgels containing 10% and 20% 1-vinylimidazole groups at pH 8.0 and 10.0; whilst for those containing 30% 1-vinylimidazole groups continuous contraction occurs throughout whole temperature range. Some residual electrostatic repulsion as a result of the high concentration of imidazole groups is likely to be the reason of continuous shrinking, whilst at lower concentrations of 1-vinylimidazole groups there is less charge on the surface of the particles and that leads to flocculation of the particles.
Additionally, these microgel particles were found to be sensitive for the presence of metal ions, copper (II) in particular. The effect of the addition of various concentrations of copper (II) salt was determined. The microgel particles showed interesting behaviour under such conditions as swelling occurred at low concentrations followed by contracting at higher concentrations of added copper (II) ions. The complexation of the copper (II) with imidazole groups of the microgel occurs. Charging of the microgels by Cu$^{2+}$, which is causing the electrostatic repulsion, was thought to be the reason for swelling. In the same way that adding more acrylic acid groups to PNIPAM particles increased the swollen state of the microgels and lowering the pH of these microgels (i.e. P(NIPAM-co-VI) microgels) resulted in swelling of the particles. However at higher concentrations of copper (II) ions the binding forces of complexation between Cu (II) and imidazole groups of the microgel are leading to conformation of the microgel backbone which is weakening polymer-solvent interactions. Therefore, solvent is forced out of the particle resulting in shrinkage. Also addition of other metals, such as iron (III), nickel (II), silver and zinc, was investigated and described. The particles swelled at lower concentrations which followed by aggregation at higher concentrations of added salts, suggesting that the formation of complex for this microgel particles is occurring at higher concentrations in comparison with copper due to weaker complex binding of the corresponding metal ions. The formation of aggregates implies that the diameter of the microgel particles is decreased; however, due to a weaker binding forces and high electrolyte
concentration aggregates are forming preventing accurate measurement of the collapsed particle size

The copper (II) uptake has been calculated with the highest uptake value of almost 2 g of copper (II) by 1 g of dried microgel. Also plotting linearized Langmuir isotherm led to the possibility of comparing calculated data of the copper (II) uptake with Langmuir fit, where the calculated data appeared to fit the curve plotted via Langmuir equation reasonably well.

This was possibly the first time that such microgels have been prepared. Having such properties these novel materials can be good candidates in both environmental and possible medical applications, e.g. for waste water treatment and binding heavy metals in order to remove them from the blood system of the human body. However, a considerable amount of further research has to be undertaken on these microgels in the future, such as investigating the impact of crosslink density on swelling/shrinking properties and applying different method of polymerization of analogous microgels, that can lead to the formation of rather more monodisperse particles and finer control over the particles size of the resultant microgels.
5.4. General Conclusions and Suggestions on Future Work to Be Done for the P(NIPAM-co-3-APB-ATMA) Microgels

The other important part of this project was to find the monomer, which is sensitive to the presence of glucose and operate at physiological conditions, in order to copolymerize with NIPAM to prepare microgel particles. Finally, glucose-sensitive microgel particles, which were functional at physiological pH and temperature, i.e. pH 7.5 and 35°C, were prepared. It was achieved by adapting the method of Kabilan et al. [63], which was used to prepare glucose-responsive polymeric films. The pKₐ of the boronic group was reduced by (3acrylamidopropyl)trimethylammonium chloride that has been grafted to the microgel backbone alongside 3-APB. Resultant microgel dispersions swelled in the presence of glucose at the concentrations higher than 0.1 mmol/L at pH 7.5 and 8.5 at 35°C. Such microgel particles that operate as glucose sensors at physiological pH were prepared and described for the first time. Taking into consideration that glucose level in human body is in the range of 3.9-7.2 mmol/L [124], resultant microgels have the potential to be used in pharmaceutical applications as drug delivery systems, insulin particularly, in diabetes treatment. However, the resultant microgel particles should be investigated in wider range of incorporated functional monomers and crosslinker concentrations in order to find optimum swelling degree and optimum sensitivity to the concentration of the glucose, as well as synthesized
via different polymerization techniques that could offer better particle size values and higher degrees of swelling. Also these microgel particles should be investigated under high polyelectrolyte concentration in order to simulate conditions of the human body.

5.5 Specific suggestions on further investigations to be undertaken

In the previous sections general suggestions as to future work that could be undertaken have been given, here we shall discuss specific work that ought to be carried out. Further work should be concentrated on the novel microgels, namely:

- The P(NIPAM-co-VI) microgels
- The P(NIPAM-co-3-APB-ATMA) microgels

Apart from using different polymerization conditions and using different crosslinking densities for finer control of the microgel particle size, the impact of the metal ions, except copper (II), on the P(NIPAM-co-VI) microgel particles should be investigated more thoroughly. To accomplish these investigations microgels should be stabilized, so the aggregation of the particles would be avoided. For example, such microgel particles can be prepared in the form of core-shell microgels by addition of more NIPAM
monomer after 2 hours from the start of the synthesis of the P(NIPAM-co-VI) microgels. Hence, P(NIPAM-co-VI)-core and PNIPAM-shell microgels would be produced (Figure 5-1). The PNIPAM-shell of the resultant microgels would prevent aggregation of the particles caused by excess of the charge from the added metal ions and anionic groups of the salts present. Such modification of metal-sensitive microgels would give an opportunity to investigate them under wider range of concentration of added salts; therefore, it might lead to finding the concentration range whereby the contraction occurs in the similar way it does upon addition high concentrations of copper (II) ions.

Figure 5-1: A schematic representation of the P(NIPAM-co-VI)-core and PNIPAM-shell microgel particle.

Another area of possible investigation might be modifying the P(NIPAM-co-VI) microgel with grafting proteins onto the microgel backbone in order to make them biocompatible; hence, such materials could be used as agents for heavy metal removing (chelation therapy) from the human body via binding these metals into complexes.
Further investigations of P(NIPAM-co-3-AP-BATMA) microgels should be also conducted. Although the microgels have been shown to be sensitive to glucose at physiological pH and temperature, the studies were only conducted in water, not 0.1M NaCl, the physiological electrolyte concentration. In addition, it would be desirable to manipulate the microgels such that the particle size was constant up to a concentration of glucose of 7 mmol/L and undergo swelling above this concentration. Investigations of the impact of the concentration of added functional monomers (i.e. 3-AP and ATMA) and crosslinking density on the sensitivity and the extent of swelling of the microgel particles ought to give clearer overview and understanding of the problem. Therefore, finding optimum concentration of incorporated functional monomers (3-AP and ATMA) and optimum crosslinking density is the challenge to be resolved by further investigations. Afterwards, it would be interesting to incorporate insulin into the microgels and then to determine the release properties. Completing the investigations described in this section would lead to possibility of implementation of the resultant microgels to the potential use, and, if successfully approved, even to the industrial scale production.
References


121. Asayama, S., Sekine, T., Kawakami, H., and Nagaoka, S., *Design of aminated poly (1-vinylimidazole) for a new pH-sensitive polycation to*


Appendices

Appendix 1

Background theory of dynamic light scattering

The overall size and shape of mesoscopic particles in solution can be investigated by dynamic light scattering (DLS). In general, the terms of correlation functions of dynamic variables are always used to describe the response of the scattering molecules to the incident light [126]. The following equation is used to calculate the translation diffusion coefficient (D):

$$\Gamma = Dq^2$$  \hspace{1cm} \textit{Equation 16}

where $\Gamma$ is the decay rate, which is the inverse of the relaxation time, $q$ – the scattering vector.

The local concentration fluctuations are the reason of the scattering of light in solutions. The strength of these fluctuations is characterized by $\langle x^2 \rangle$. The concentration fluctuations are also the measure of the spontaneous local disturbances in solution, which vary in time similar to the macroscopic gradients resulting from the external stimulus. Therefore, their behaviour can be described with the macroscopic equations of hydrodynamics [127], which
result into the autocorrelation function for the concentration fluctuations of the following form [128]:

\[ G(t) = \langle \Delta x(0)\Delta x(t) \rangle = \langle \Delta x^2 \rangle \exp(-Dq^2\tau) \]  

Equation 17

where \( \langle \ldots \rangle \) denotes the averaging over a large number of separate time periods of duration \( \tau \), \( D \) and \( q \) are the diffusion coefficient and the scattering vector, respectively, given by Equation 16.

1. Photon Correlation Spectroscopy

Berne and Pecora reviewed the theory of DLS and found that the modulation of the amplitude of the electric field of the scattered wave by the local concentration fluctuations result into the same form of the field autocorrelation function as the autocorrelation function for the concentration fluctuations (Equation 9) [129]:

\[ G^1(\tau) = \langle E^*(0)E(\tau) \rangle = G^1(0)\exp(-Dq^2\tau) \]  

Equation 18

where \( E(\tau) \) is the representation of the electric field at time \( \tau \), \( E^*(0) \) is the complex conjugate of the electric field at time zero, and \( G^1(0)=\langle E^2(0)\rangle=I_0 \) is the average intensity of the scattered light.

Nowadays, the most common way to analyze data from dynamic light scattering is photon correlation spectroscopy (PCS), which have two
alternative techniques: the heterodyne spectroscopy and the homodyne spectroscopy, which is more feasible experimentally. The intensity autocorrelation function in the homodyne spectroscopy is defined by following equation:

\[ G^{(2)}(\tau) = \langle I(t)I(t+\tau) \rangle = \int_{0}^{\infty} I(t)I(t+\tau)dt \quad \text{Equation 19} \]

where \( I(t) \) is the intensity measured at time \( t \). It reaches the maximum at \( \tau=0 \) and falls to zero as \( \tau \) increases. The experimentally determined autocorrelation function \( G^{(2)}(\tau) \) can be converted using the Siegert relation to the normalized field autocorrelation function, \( g^{(1)}(\tau) \), if the scattering signal is a stationary Gaussian process, as following:

\[ G^{(2)}(\tau) = B(1 + \beta^2 |g^{(1)}(\tau)|^2) \quad \text{Equation 20} \]

where \( B \) is the experimentally determined baseline, and \( \beta \) (0<\( \beta \)<1) is an experimental constant that characterises the efficiency of optical mixing [130]. However, this consideration is valid only for ergodic systems, which have the statistically independent optical inhomogeneities resulting from the molecular motion. Light scattering measurements for non-ergodic systems are discussed in next section.
2. Data Analysis: Monodisperse Spheres

The autocorrelation function for a dilute solution of small monodisperse spherical particles is defined as follows:

\[ g^{(1)}(\tau) = \exp(-Dq^2\tau) \quad \text{Equation 21} \]

where \( \tau \) is the correlation time, \( q \) is the scattering vector, and \( D \) is the diffusion coefficient given by the Stokes-Einstein equation, which is a definition for an effective sphere of the hydrodynamic radius, \( R_h \):

\[ D = \frac{k_B T}{6\pi\eta R_h} \quad \text{Equation 22} \]

where \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature, \( \eta \) is the solvent viscosity.

3. Effect of Polydispersity

The autocorrelation function for a mixture of particles with different diameter size is related to the relaxation rates distribution, which is defined as \( G(\Gamma) \), or to the relaxation times distribution, \( A(\tau) \), via a Laplace transformation:

\[ g^{(1)}(t) = \int_0^\infty G(\Gamma) \exp(-\Gamma t) d\Gamma = \int_0^\infty A(\tau) \exp(-t/\tau) d\tau \quad \text{Equation 23} \]
where \( \Gamma = \tau^{-1} \) is the relaxation rate. \( G(\Gamma) \) or \( A(\tau) \) has to be calculated by solving the integral Equation 23, as \( g^{(1)}(t) \) is determined experimentally, in order to get the particle size distribution.

For polydisperse systems of non-interacting spherical molecules, DLS are usually interpreted using the cumulant analysis, the autocorrelation function of which is expanded in moments about the mean relaxation rate, \( \bar{\Gamma} \), as follows [129]:

\[
g^{(1)}(\tau) = \exp \left( -\bar{\Gamma} \tau \right) \left[ 1 + \frac{\mu_2}{2!} \tau^2 - \frac{\mu_3}{3!} \tau^3 + \cdots \right] \quad \text{Equation 24}
\]

where \( \bar{\Gamma} = Dq^2 \) is the relaxation rate, and \( \mu_n = \int_0^\infty (\Gamma - \bar{\Gamma})^n G(\Gamma) d\Gamma \) is the \( n \)th cumulant. Such cumulant analysis is only effective for a relatively narrow particle size distribution if two conditions are satisfied: \( \Delta\Gamma/\Gamma \leq 1 \) and no more than three terms of expansion series (Equation 24) to be considered.

The correlation time is determined as \( \tau_c = 2\pi/\bar{\Gamma} \). The diffusion coefficient is calculated from \( \bar{\Gamma} \) using Equation 16. The average hydrodynamic radius can be expressed using Equation 22:

\[
\langle R_h \rangle = \frac{k_B T}{6\pi\eta D} \quad \text{Equation 25}
\]
The reduced second cumulant $\sqrt{\mu_2/\bar{I}}$ is referred to as the polydispersity index (PI), which is the dimensionless measure of the size distribution broadness and is related to the polydispersity of the sample as follows:

$$\frac{M_w}{M_n} \approx 1 + PI$$  \hspace{1cm} \textit{Equation 26}

where $M_w$ and $M_n$ are the weight and number average molecular mass of macromolecules, respectively. The system can be considered as monodisperse if PI≤1. The asymmetry index (AI), the value of which is $\mu_3/(\mu_2\bar{I})$, is the dimensionless measure of skewness of the particle size distribution. This measure is negligible in the most cases as AI<<1.

4. Effect of concentration

For concentrated systems or macromolecular solutions with strong intermolecular interactions, the diffusion coefficient determined by DLS exhibits the concentration dependence [131],[132]. The translational diffusion coefficient contains both kinetic and thermodynamic factors [133], [134]:

$$D = b_2 x_2 \left( \frac{\partial \mu_2}{\partial x_2} \right)_{p,T}$$  \hspace{1cm} \textit{Equation 27}
where $b_2 = 1/N_A f$ is the mobility of the macromolecules (Tyrell and Harris, Diffusion in liquids, 1984), $N_A$ is the Avogadro number, and $f$ is the particle-solvent friction coefficient. The exact calculation of both mobility and $(\partial \mu_i/\partial x_i)_{p,T}$ in non-ideal solutions is an unsolved problem. However, the concentration effect on the diffusion coefficient can be derived by representing the chemical potential Equation 28 and friction coefficient, $f = f_0(1 + k_c c_2 + ...$, as a virial expansions in Equation 27:

$$
\mu_1 = \mu_1^0 - RT V_1^0 c_2 \left( \frac{1}{M_2} + A_2 c_2 + A_3 c_2^2 + \cdots \right) \quad \text{Equation 28}
$$

where $\mu_1^0$ and $V_1^0$ are the standard chemical potential and molar volume of pure solvent, respectively; $c_2$ is a series of weight fractions in polymer solutions (grams per mL); $M_2$ is the molecular mass of polymer; and $A_2$ and $A_3$ are termed the second and the third virial coefficients, the value of which is dependent on the binary and the ternary interactions, respectively. So, the concentration effect on the diffusion coefficient would be:

$$
D = \frac{RT(1+2A_2M_2c_2+\cdots)}{N_A f_0(1+k_c c_2+\cdots)} \approx \frac{k_BT}{f_0} \left( 1 + \left( 2A_2 M_2 - k_c - 2\partial_2 \right) c_2 + \cdots \right) =
$$

$$
D_0 \left( 1 + k_d c_2 + \cdots \right) \quad \text{Equation 29}
$$
where $f_0$ is the friction coefficient at infinite dilution; $D_0 = k_B T / f_0$ is the translational diffusion coefficient at infinite dilution; $k_d = 2A_2 M_2 - k_f - 2\theta_2$ is the hydrodynamic virial coefficient; and $\nu_2$ is the partial specific volume of the solute. The approximation $(1 - x)^{-1} \approx (1 + x)$ has been used in deriving Equation 29. Within the framework of the Stokes-Einstein approach (cf. Equation 22), the friction coefficient per particle at infinite dilution is $f_0 = 6\pi \eta R_h$.

Li et al. reported that hydrodynamic virial coefficient $k_d$ changes from positive to negative with the increase of salt concentration in the solution [135]. This observation indicates the changes in macromolecular interactions from repulsion to attraction due to enhanced screening of Coulombic repulsion by counterions with the increase in ionic strength.
Appendix 2

Table A: The particles’ diameter (in nm) of the samples with different concentrations of incorporated acrylic acid as a function of temperature in the range of 25\(^\circ\)-50\(^\circ\)C at pH=6.0 (electrolyte concentration 2*10\(^{-5}\) mol/L).

<table>
<thead>
<tr>
<th>NIPAM/acrylic acid ratio</th>
<th>Temperature, (^\circ)C</th>
<th>100%NIPAM/0%AA</th>
<th>90%NIPAM/10%AA</th>
<th>80%NIPAM/20%AA</th>
<th>70%NIPAM/30%AA</th>
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<tr>
<td>25</td>
<td>430</td>
<td>645</td>
<td>900</td>
<td>1170</td>
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<tr>
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<td>570</td>
<td>785</td>
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</table>

Polydispersity index (PI) is lower than 0.1 for all values presented.
### Appendix 3

**Table B:** The diameter of the particles aggregates in 90\%NIPAM/10\%VI microgel dispersions with different added salts at various concentrations at 25\(^\circ\)C and pH 6.0.

<table>
<thead>
<tr>
<th></th>
<th>1.5 g/L</th>
<th>2 g/L</th>
<th>3 g/L</th>
<th>5 g/L</th>
<th>7 g/L</th>
<th>10 g/L</th>
<th>14 g/L</th>
<th>18 g/L</th>
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</table>

Polydispersity index (PI) is higher than 0.25 for all values presented.

**Table C:** The diameter of the particles aggregates in 80\%NIPAM/20\%VI microgel dispersions with different added salts at various concentrations at 25\(^\circ\)C and pH 6.0.

<table>
<thead>
<tr>
<th></th>
<th>1.5 g/L</th>
<th>2 g/L</th>
<th>3 g/L</th>
<th>5 g/L</th>
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Polydispersity index (PI) is higher than 0.25 for all values presented.
Table D: The diameter of the particles aggregates in 70\%NIPAM/30\%VI microgel dispersions with different added salts at various concentrations at 25\degree C and pH 6.0.

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<th>5 g/L</th>
<th>7 g/L</th>
<th>10 g/L</th>
<th>14 g/L</th>
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<th>20 g/L</th>
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Polydispersity index (PI) is higher than 0.25 for all values presented.
Appendix 4

![Graph showing the diameter of 90% NIPAM/10% VI microgel particles with different added salts at concentrations of 0.1, 1 and 10 g/L at 25°C and pH 6.0.]

*Figure A: The diameter of 90%NIPAM/10%VI microgel particles with different added salts at concentrations of 0.1, 1 and 10 g/L at 25° C and pH 6.0.*
Appendix 5

**Figure B**: Linearized plot of Langmuirian isotherm of Cu(NO$_3$)$_2$$*$_3H$_2$O adsorbing onto the 90%NIPAM/10%VI microgel particle.

\[ y = 1.4632x + 2.919 \]
\[ R^2 = 0.9432 \]

**Figure C**: Linearized plot of Langmuirian isotherm of Cu(NO$_3$)$_2$$*$_3H$_2$O adsorbing onto the 80%NIPAM/20%VI microgel particle.

\[ y = 0.5836x + 3.1258 \]
\[ R^2 = 0.7784 \]
Appendix 6

Figure D: Adsorption isotherm of copper (II) using 90%NIPAM/10%VI microgels in weight units.

Figure E: Adsorption isotherm of copper (II) using 80%NIPAM/20%VI microgels in weight units.