Injectable microgel systems:

towards an injectable gel for heart tissue repair

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<tr>
<td>$^1$H NMR</td>
<td>Nuclear magnetic resonance</td>
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<td>3D</td>
<td>3 dimension</td>
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<tr>
<td>AAc</td>
<td>Acrylic acid</td>
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<td>AEM</td>
<td>2-aminoethyl methacrylate</td>
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<td>AFM</td>
<td>Atomic force microscopy</td>
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<td>APM</td>
<td>$N$-(3-aminopropyl)methacrylamide</td>
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<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium persulfate</td>
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<td>DLVO</td>
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<td>DVB</td>
<td>Divinylbenzene</td>
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<td>NVF</td>
<td>N-vinylformamide</td>
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<td>NVF</td>
<td>2-(N-Vinylformamido) ethyl ether</td>
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<td>OpePOSS</td>
<td>Octa(propylglycidyl ether) polyhedral oligomeric silsesquioxane</td>
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<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PEU</td>
<td>Poly(ester urethane)</td>
</tr>
<tr>
<td>PEUU</td>
<td>Polyester urethane urea</td>
</tr>
<tr>
<td>PGA</td>
<td>Poly(glycolic acid)</td>
</tr>
<tr>
<td>PGMA</td>
<td>Poly(glycidyl methacrylate)</td>
</tr>
<tr>
<td>PGMA-NH₂</td>
<td>Aminated poly(glycidyl methacrylate)</td>
</tr>
<tr>
<td>PGOHMA</td>
<td>Poly(glycerol methacrylate)</td>
</tr>
<tr>
<td>PLLA</td>
<td>Poly(L-lactic acid)</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methyl methacrylate)</td>
</tr>
<tr>
<td>PNIPAM</td>
<td>Poly(N-isopropylacrylamide)</td>
</tr>
<tr>
<td>PNVF</td>
<td>Poly(N-vinylformamide)</td>
</tr>
<tr>
<td>POSS</td>
<td>Polyhedral oligomeric silsequioxane</td>
</tr>
<tr>
<td>PS</td>
<td>Poly(styrene)</td>
</tr>
<tr>
<td>PU</td>
<td>Polyurethane</td>
</tr>
<tr>
<td>PVAM</td>
<td>Poly(vinylamine)</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
</tr>
<tr>
<td>PVP-PVA</td>
<td>Poly(1-vinylpyrrolidone-co-vinyl acetate)</td>
</tr>
<tr>
<td>PyC</td>
<td>Pyrene carboxylic acid</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>SX</td>
<td>Singly crosslinked</td>
</tr>
<tr>
<td>TEMED</td>
<td>$N,N,N',N'$-Tetramethylethylenediamine</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>TSC</td>
<td>Total solid content</td>
</tr>
<tr>
<td>VAM</td>
<td>Vinylamine</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
</tbody>
</table>
List of symbols

[ ] Concentration
$\Delta E$ Difference between two energy states
$A_{\text{eff}}$ Effective Hamaker constant
$AR$ Area of FTIR band region
$a$ Radius of particle
$B_0$ Magnetic field
$c$ Electrolyte concentration
$D$ Diffusion coefficient
$d$ Particle diameter
$d^*$ Approximated particle diameter
$d_{(c)}$ Collapsed particle diameter
$d_h$ Hydrodynamic diameter
$d_{h(c)}$ Hydrodynamic diameter in collapsed state
$d_{\text{opt}}$ Particle diameter determined from optical micrograph
$d_{\text{SEM}}$ Particle diameter determined from scanning electron micrograph
$E$ Applied electric fields
$f(\kappa a)$ Henry’s function
$G'$ Elastic or storage modulus
$G''$ Viscous or loss modulus
$g(\tau)$ Correlation function
$H$ Distance between particles
$H$ Planck’s constant
$h$ Planck’s constant
$I$ Nuclear spin quantum number
$i$ Degree of ionicity
$K_a$ Acid dissociation constant
$k_B$ Boltzmann constant
$k_p$ Propagation rate constant
\( kT \) \quad \text{Thermal energy of the particles}

\( M_f \) \quad \text{Mass of final sample}

\( M_i \) \quad \text{Mass of initial sample}

\( M_{\text{rep}} \) \quad \text{Repeating unit molar mass}

\( n \) \quad \text{Refractive index}

\( \text{pK}_a \) \quad \text{pH of the acid dissociation constant}

\( Q \) \quad \text{Swelling ratio}

\( q \) \quad \text{Wave vector}

\( R_{\text{NC}} \) \quad \text{Ratio of } \%N \text{ to } \%C

\( T \) \quad \text{Absolute temperature}

\( U_E \) \quad \text{Electrophoretic mobility}

\( V_{\text{att}} \) \quad \text{Attractive van der Waals force}

\( V_{\text{crit}} \) \quad \text{Potential maximum}

\( V_{\text{rep}} \) \quad \text{Electrostatic repulsion energy}

\( V_{\text{ste}} \) \quad \text{Repulsive energy due to steric stabilisation}

\( V_{\text{total}} \) \quad \text{Total interaction energy}

\( V_0 \) \quad \text{Collapsed network volume}

\( V_s \) \quad \text{Drift speed}

\( W_{\text{PVP}} \) \quad \text{weight fraction of PVP}

\( \text{wt}\% \) \quad \text{Weight percent}

\( x \) \quad \text{Number of moles of crosslinks}

\( z \) \quad \text{Charge number of electrolyte}

\( z\epsilon N \) \quad \text{Density of the fixed charges in the hydrogel layer}

\( \nu_l \) \quad \text{Solvent molar volume}

\( \chi \) \quad \text{Flory-Huggins interaction parameter}

\( \zeta \) \quad \text{Zeta potential}

\( \psi_0 \) \quad \text{Surface potential}

\( \psi_d \) \quad \text{Potential at the stern plane}

\( 1/\kappa \) \quad \text{Double layer thickness}
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\varepsilon$</td>
<td>Dielectric constant</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Viscosity</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Wavelength</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Scattering angle</td>
</tr>
<tr>
<td>$\nu$</td>
<td>Resonance frequency</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Volume fraction</td>
</tr>
<tr>
<td>$\phi_p$</td>
<td>Particle volume fraction</td>
</tr>
<tr>
<td>$\phi_{\text{eff}}$</td>
<td>Effective volume fraction</td>
</tr>
<tr>
<td>$\phi_{\text{hs}}$</td>
<td>Hard sphere volume fraction</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Phase angle</td>
</tr>
<tr>
<td>$\sigma_0$</td>
<td>Maximum stress</td>
</tr>
<tr>
<td>$\gamma_0$</td>
<td>Maximum strain</td>
</tr>
<tr>
<td>$\gamma_c$</td>
<td>Yield strain</td>
</tr>
<tr>
<td>$\gamma^*$</td>
<td>Gyromagnetic ratio</td>
</tr>
<tr>
<td>$\varepsilon_r$</td>
<td>Relative permittivity of solution</td>
</tr>
<tr>
<td>$\varepsilon_0$</td>
<td>Relative permittivity of vacuum</td>
</tr>
<tr>
<td>$\Psi_0$</td>
<td>Electrostatic potential at surface</td>
</tr>
<tr>
<td>$\Psi_{\text{DON}}$</td>
<td>Donnan potential of the hydrogel layer</td>
</tr>
<tr>
<td>$\kappa_m$</td>
<td>Debye-Huckel parameter of the layer</td>
</tr>
</tbody>
</table>
Abstract

This thesis presents an investigation of cationic microgels based on poly(N-vinylformamide-co-glycidyl methacrylate) (PNVF-GMA) and poly(N-vinylformamide-co-2-(N-vinylformamido) ethyl ether) (PNVF-NVEE). They are studied in the context of future heteroaggregated doubly crosslinked (DX) microgels for damaged heart tissue repair. The microgel particles were synthesised from PNVF-GMA, which is also a water swellable microgel. The PNVF-GMA particles had a core-shell structure in which PNVF provides the core and PGMA creates the cross-linked shell. The morphology of particles is that of a “cane-ball” like shape. There are interconnected ridges, and this unusual morphology can be controlled by the weight fraction of GMA used during preparation. The hydrolysed PNVF-GMA (H-PNVF-GMA) particles were both positively and negatively charged. Moreover, charge patch aggregation occurred at low ionic strength. However, these microgels were colloidally unstable after water rinsing due to shell fragmentation.

PNVF microgel particles containing (N-Vinylformamido) ethyl ether (NVEE) as a crosslinking agent were also studied to avoid the fragmentation of the particles. This microgel was hydrolysed in alkali conditions to provide poly(vinylamine-co-bis(ethyl vinylamine) ether) (PVAM-BEVAME), which contains primary amine groups. It is proposed from the data presented that the content of hydrolysis was very high and the particles were stable after hydrolysis owing to the stability of ether linkage in NVEE. These microgels were able to swell upon decreasing pH. The PVAM-BEVAME microgel with 9 mol% of BEVAME was then used to form doubly crosslinked (DX) microgel. To form the inter-particles crosslinking, the vinyl groups were included by functionalisation using glycidyl methacrylate (GMA) monomer. The vinyl groups of neighbouring particles were linked together via free radical reaction. The DX microgel formed under physiological temperature and showed extensive porosity. These DX microgels had good mechanical properties confirmed by high storage modulus ($G'$). Moreover, the precursor gels were injectable which is favourable for future biomaterial applications. The study provides a new family of cationic microgel that may be suitable for a future heteroaggregated DX microgel for heart tissue repair.
Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Sineenat Thaiboonrod
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Chapter 1

Introduction

1.1 Motivation

Around 40% of all human mortality is caused by heart disease; this is the main cause of death and disability in the UK. Myocardial infarction (heart attack) damages the heart muscle and cells, which has a very poor regenerative capacity. Remodelling of the damaged heart muscle causes a change in its mechanical properties, which increases the chance of subsequent heart attacks. Surgical procedures for repair or correction are invasive; the final stage is the requirement for transplantation. Because of the complications, and high mortality rates, associated with existing surgical procedures, there are many studies searching for less invasive strategies for regenerating damaged heart muscle.

Whilst there has been a sustained research focus on implanting elastomeric biomaterials to repair damaged heart muscle, much less work has been reported concerning injectable gels. The advantages of the injectable gels are that they reduce patient mortality because they are minimally-invasive. However, the mechanical properties of the injectable gel are not strong enough to provide a mechanically equivalent matrix to heart muscle; this is a major challenge to approaches involving injectable gels. This project is extended from the Saunders group’s recent work to establish injectable, gel-forming dispersions that are elastic, biocompatible and biodegradable. The new materials class envisaged here, which are covalent-linked elastomeric particle gels, offer the long-term potential to deliver a nonsurgical approach to heart muscle repair.

1.2 Aims of thesis

The aim of this project is to establish one key component for future doubly crosslinked heteroaggregated microgels from an injectable dispersion of oppositely charged components for repair of damaged heart tissue. The microgel is required to be elastic, biocompatible and biodegradable. Also, it should be able to form a gel rapidly after injection. The research aims are to:
1. Establish a method of producing pH responsive amine-functionalised microgels and characterise the properties of the microgel.
2. Investigate the formation of doubly crosslinked (DX) cationic microgels and examine the properties of DX cationic microgels.
3. Identify the conditions for preparing injectable DX microgels.

1.3 Survey of thesis

Within this thesis, theoretical aspects and literature involving the research are presented in Chapter 2. This chapter also includes literature related to colloid theory and doubly crosslinked microgels, characterisation techniques and microgel properties.

Results and discussion are presented individually in each chapter. Chapter 3 is the first experimental chapter and focuses on the synthesis and characterisation of poly(N-vinylformamide-co-glycidyl methacrylate (PNVF-xGMA) and hydrolysed PNVF-xGMA (H-PNVF-xGMA) particles (x is weight fraction of GMA). Particle swelling behaviour and particle morphology are studied.

![Diagram of H-PNVF-0.4GMA particles and SEM images of H-PNVF-0.4GMA particle showing as-made and unattached shells surrounded the cores after water rinsing at pH = 7.4](image)

Figure 1.1 (a) diagram of H-PNVF-0.4GMA particles and (b) SEM images of H-PNVF-0.4GMA particle showing as-made and unattached shells surrounded the cores after water rinsing at pH = 7.4
Chapter 4 is concerned with the formation of primary amine particles based on poly(vinylamine) (PVAM) using poly(\(N\)-vinylformamide-co-2-(\(N\)-vinylformamido) ethyl ether particles (PNVF-xNVEE) (\(x\) is mol\% of NVEE) as the precursor particles and an investigation of the effect of crosslinker (NVEE). Furthermore, alkaline hydrolysis was used to generate the primary amine microgel. Microgel functionalisation using the primary amine was demonstrated to show potential of using primary amine groups as chemical handles.

![Figure 1.2](image.png)

**Figure 1.2** (a) particle diameter with decreasing pH and (b) fluorescence micrograph of pyrene-labelling of PVAM-9BEVAME microgel

In the Chapter 5, the poly(vinylamine-co-bis(ethyl vinylamine) ether) (PVAM-9BEVAME) microgel from Chapter 4 was functionalised with glycidyl methacrylate (GMA) to create vinyl groups on the particles and allow the particles to form inter-particle linkages. This type of microgel is known as a doubly crosslinked (DX) PVAM microgel. The mechanical properties and morphology of the DX PVAM microgel are investigated.
Figure 1.3 (a) schematic of DX PVAM microgel formation and images of (b) physical gel being injected through a syringe needle (c) gel after swelling for 3 days at pH = 7.4

Finally, the conclusions of the thesis and suggestions and ideas for future work in this area are in Chapter 6.
1.4 References

Chapter 2

Literature review

2.1 Heart muscle tissue damage and treatment

Heart muscle is comprised of a group of interlacing bundles of cardiac myocytes (Muscle cells). Each area of heart muscle is supplied by blood coronary arteries.¹ Myocardial infarction or heart attack is a result of the blockage of one of the coronary arteries which supply the cardiac tissue leading to ischemia (an inadequate blood supply) of the heart segment, this damages the heart permanently.² The extent of damage relies on the size of the area supplied by the blocked artery and the time.

Tissue engineering is a technique of imitating nature based on the fact that living bodies have regenerative potential.³ It is potentially an effective method to repair large scar areas due to ischemia.⁴,⁵ The ideal materials for cardiac tissue engineering should have functional and morphological properties of native heart muscle and remain viable after implantation.⁶

The main criteria of biomaterials for myocardial tissue engineering are biocompatibility, biodegradability, and appropriate mechanical properties.³ Both natural and synthetic materials have been used in myocardial tissue engineering e.g. collagen, peptide.³,⁶

Synthetic polymers are used for tissue engineering because of their excellent processing characteristics, and biocompatibility.⁷,⁸ Moreover, they have predictable and reproducible mechanical and physical properties e.g. tensile strength, elastic modulus, and degradation rate. Examples of synthetic polymer which have been investigated for cardiac tissue engineering are poly(L-lactic acid) (PLLA)⁹, poly(ε-caprolactone) (PCL)¹⁰, and polyurethane (PU).¹¹,¹²

An injectable microgel for heart tissue repair is attractive because it could avoid the need for invasive surgery. However, the stiffness of injectable gels is usually much lower than that of human heart muscles and it is not sufficient for the application in human tissues. The injectable gels have stiffness in range 10 Pa to 20 kPa.¹³,¹⁴,¹⁵,¹⁶,¹⁷ Whereas, the stiffness of human heart muscles at the end of diastole is approximately 50 kPa in normal heart or 200 – 300 kPa in congestive heart failure (CHF) hearts.¹⁸,¹⁹,²⁰,²¹ To potentially solve this problem, new doubly crosslinked (DX)
microgel are studied here because they have improved mechanical properties and the properties are tuneable.\textsuperscript{22}

\section*{2.2 Microgel}

A microgel is a crosslinked polymer colloid particle which can swell upon changes in external triggers such as a good solvent, temperature or pH.\textsuperscript{23,24,25,26,27} Even in the collapsed state, microgel particles themselves contain a large amount of solvent.\textsuperscript{28} The most important property of microgel particles is the extent of swelling which is usually determined by changes in the hydrodynamic diameters measured using photon correlation spectroscopy (PCS).\textsuperscript{24}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{microgel.png}
\caption{Microgel particle in (a) poor solvent and (b) good solvent\textsuperscript{24}}
\end{figure}

Microgels are generally prepared by (surfactant-free) emulsion polymerisation, precipitation polymerisation or inverse emulsion polymerisation.\textsuperscript{23} However, in this work the microgel has been prepared via non-aqueous dispersion (NAD) polymerisation.

Microgels can be used in a variety of applications including surface coating, printing, water treatment, oil recovery and pharmaceutical industries.\textsuperscript{24,29} The aim of this thesis focuses on developing new microgels for future biomaterials applications.

\subsection*{2.2.1 Non-aqueous dispersion polymerisation}

Dispersion polymerisation is generally used for the preparation of non-aqueous latex dispersions, thus it is known as non-aqueous dispersion polymerisation (NAD). For the NAD procedure, a monomer is dissolved in a non-aqueous solvent including an
initiator and a stabiliser which is used to prevent particle flocculation. The solvent which is used for NAD is not a solvent for the polymer. The function of the stabiliser is to provide the molecule with an anchor chain and a stabilising chain. The anchor chains are insoluble in the medium and have strongly affinity to the polymer particles which are produced. On the other hand, the stabilising chains are soluble in the medium and strongly solvated by its molecules, giving effective steric stabilisation. The strong adsorption between the anchor chain and particle surface and the layer thickness of stabilising chain are the factors that prevent close approach of the particles to a distance in which the Van der Waals attraction is strong. This can be controlled by the length of the anchor and stabilising chains. The main criteria for dispersion polymerisation are the solubility of the monomer and initiator and the insolubility of the formed polymer in the continuous phase.\textsuperscript{30,31} The dispersion polymerisation consists of two stages: a “nucleation stage” (first stage) and a “particle growing stage” (second stage).\textsuperscript{32} Initially, the system is homogeneous. The insolubility of the resulting polymer forces precipitation when sufficient polymerisation occurs. Initially, polymer nuclei are produced and develop into polymer particles. After that, they are stabilised against aggregation by the stabiliser which is added before the reaction starts.\textsuperscript{30,31} There are some examples of dispersion polymerisation such as the polymerisation of styrene in ethanol using AIBN as an initiator and also methyl methacrylate in hydrocarbon medium with poly-(isobutylene-co-isoprene) as a steric stabiliser.\textsuperscript{34}

Dispersion polymerisation is simple, easy to scale up and gives particles with a narrow size distribution. The diameters of particles which are prepared by dispersion polymerisation are in the range of 1-15 \( \mu \text{m} \).\textsuperscript{35,36} However, the limitation of this method is the difficulty of particle size control. Two reactions run in the same conditions can give different diameters and sometimes coalescence occurs.\textsuperscript{32} This is because of the high sensitivity of the nucleation stage. Flocculation or coagulation often occurs when using crosslinker in dispersion polymerisation.\textsuperscript{37} Moreover, highly crosslinked polymer particles with monodisperse size distribution are difficult to obtain by the dispersion polymerisation method owing to the instability that occurs during the process.\textsuperscript{38,39} Tseng et al.\textsuperscript{38} prepared polystyrene with divinylbenzene (DVB) as a crosslinker and found that increasing DVB concentration resulted in coagulation of the dispersion.
Song et al.\textsuperscript{32} found a method for preparing polystyrene using two-stage dispersion polymerisation. They added comonomers after the nucleation stage of the reaction to give a narrow size distribution with control over the particle diameter. Moreover, they were able to prepare crosslinked particles containing up to 3 mol\% crosslinker.

### 2.3 Particle swelling

When placed in a good solvent collapsed microgel particles swell until a balance is established for the solvent chemical potential inside and outside the microgel network. Crosslinking within the microgel limits the extent of swelling.\textsuperscript{24} Moreover, the degree of expansion depends on the strength of the interaction of the solvent\textsuperscript{40}, the size of solvent molecules\textsuperscript{41} and the crosslink density.\textsuperscript{42} Therefore, it is important to understand the behaviour of microgel dispersions by considering the swelling of the network. Flory’s theories have been used for explain the interaction between polymer and solvent. The swelling ratio ($Q$) is given by equation 2.1\textsuperscript{23}:

$$Q = \left( \frac{d}{d_{(c)}} \right)^3 = \frac{1}{\phi}$$

(2.1)

Where $d$ is the particle diameter (micrometre) and $d_{(c)}$ is the collapsed particle diameter (micrometre). $\phi$ is the polymer volume fraction which is related to the Flory-Huggins interaction parameter (equation 2.2)\textsuperscript{43,44}:

$$\phi = \left( \frac{xv_1}{V_0((1/2)-\chi)} \right)^{3/5}$$

(2.2)

Where $x$ is number of moles of crosslinks within a collapsed network volume ($V_0$). $v_1$ is the solvent molar volume (dm$^3$ mol$^{-1}$) and $\chi$ represents the Flory-Huggins interaction parameter. For strong interaction (good solvent), swelling occurs and $\chi < 0.5$. Equation 2.2 is valid only when $\chi < 0.5$. In the case of $\chi = 0.5$ the polymer is in a theta ($\theta$) solvent.
2.3.1 pH-triggered swelling

Microgels that contain at least one comonomer, which has a charge when pH approaches the pK_a for that species, are pH responsive. The microgel swelling is dominated by the internal osmotic pressure resulting from repulsive electrostatic interactions of ionic species along the polymer chain length. The solvent is able to penetrate the polymer structure and causes swelling via the charged segments due to the repulsion. The microgel in this work contains a primary amine (NH_2), which causes the particles to swell when the decreased pH is below pK_a. As the pH decreases to lower than the pK_a, the polyelectrolyte groups within the microgel structure become dissociated (ionised) and repel one another, causing the particle to swell. The equation for the proton transfer equilibrium is:

\[
RNH_3^+ + H_2O(l) \rightleftharpoons RNH_2 + H_3O^+(aq)
\]  

(2.3)

When the pH of the dispersion decreases by addition of acid, there is an increasing concentration of H^+ groups. This causes protonation of RNH_2 groups to RNH_3^+, increasing the RNH_3^+ groups concentration and decreasing the concentration of RNH_2 groups. The RNH_3^+ and RNH_2 in equation 2.3 is acid and conjugate base, respectively.

The acid dissociation constant (K_a) is a value that shows the tendency of acids to deprotonate. The point that half of acid groups are deprotonated is identified using K_a. The pH at which this occurs is the pK_a and it is given by equation 2.4:

\[
pK_a = -\log[K_a]
\]

(2.4)

The measured pH of a system is related to the pK_a and the degree to which it has dissociated. This is given by the Henderson-Hasselbalch equation:

\[
pH = pK_a + \log\left(\frac{[RNH_2]}{[RNH_3^+]}\right)
\]

(2.5)

Where [RNH_2] and [RNH_3^+] are the concentration of RNH_2 and RNH_3^+, respectively.

The degree of ionicity (i) is a numerical measure of extent of deprotonation of acid groups and the extent of charge present. The equation to calculate i is:
\[
    i = \frac{[RNH_3^+]}{[RNH_3^+]+[RNH_2]} \quad (2.6)
\]

It is possible to calculate \(i\) using the pK\(_a\) and pH by the following equation:

\[
    i = \frac{1}{10^{pH-pK_a} + 1} \quad (2.7)
\]

For cationic materials at pH greater than the pK\(_a\), particles do not have charges so they are in a collapsed state. When the pH decreases to the pK\(_a\), charges will be created due to the ionisation and this causes the particles to swell.

### 2.3.2 Microgels for biomedical application

A hydrogel is a polymer containing a 3D network with high water content, which is highly response to pH, temperature, ionic strength and enzyme activities.\(^{51}\) They are able to shrink or swell in specific environmental conditions.\(^{29}\) This property makes them appropriate for biomedical applications. Moreover, their chemistry and properties are controllable and reproducible. They can be reproduced with specific molecular weights, block structure, degradable linkages and crosslinking and these properties determine gel formation dynamics, crosslinking density and mechanical and degradation properties.\(^{52}\)

Hydrogels are now widely used as biomaterials because their physiochemical properties such as high water content, soft and rubbery texture, low interfacial tension with water or biological fluids are generally similar to biological tissues.\(^{53}\) Poly(N-isopropylacrylamide) (PNIPAM) microgels have been investigated as drug carrier because it has a sponge-like structure with spaces filled with solvent. The drugs can be loaded by equilibrium partitioning between the solution and microgel phases.\(^{54}\)

For tissue regeneration applications, the microgel must be biocompatible, biodegradable, have mechanical properties that match the tissue and enable regeneration of the tissue.\(^{23}\) Injectable microgel dispersions have been studied for improving the degenerated interverbral disc (IVD).\(^{55,56}\) Microgel dispersions based on high concentration of methacrylic acid (MAA) were injected to the degenerated IVD at pH = 4 and then neutralised \textit{in situ}, using sodium hydroxide (NaOH) to form
a gel. Compression data showed the modulus value increased significantly compared to the control samples which contained injected PBS.

The concept of injectable microgels can be applied to drug delivery. Sivakumaran et al.\textsuperscript{57} studied injectable microgel-hydrogel composites for prolonged small-molecule drug delivery. They entrap PNIPAM microgel inside an in situ-gelling carbohydrate-based hydrogel to form a soft nanocomposite hydrogel. The composite hydrogels release the drug over a longer duration than by using the hydrogels or microgels alone. The rate and duration of release can be tuned by adjusting the affinity of each of the two soft phases for the target drug and crosslink density within the bulk hydrogel.

2.4 Colloidal stability
Microgel particles in a swollen state contain a large volume fraction of solvent and van der Waals forces are not important for this state.\textsuperscript{24} The van der Waals attractive force between the particles is a cause of aggregation for collapsed particles. To promote stability, repulsive forces are required.\textsuperscript{68}

DLVO theory describes the attractive van der Waals force and repulsive double layer interactions in terms of interparticle distance.\textsuperscript{58} DLVO is named after Derjaguin, Landau\textsuperscript{59}, and Verwey and Overbeek.\textsuperscript{60} They proposed the theory of stability of lyophobic sols. They showed that the colloidal stability is based on the balance of attractive van der Waals force ($V_{\text{att}}$) and repulsive double layer force ($V_{\text{rep}}$). When charged particles are dispersed in water, the ions will create an electric double layer around the particles.\textsuperscript{61}

2.4.1 Electrophoretic mobility and zeta potential of colloidal particles
An electric double layer around the particles is a consequence of the net positive or negative surface charge.\textsuperscript{62} Upon immersion, positive or negative charged ions in the solution will be attracted to the opposing charge on the surface. These ions adsorb within a region known as the Stern layer. The adsorbed ions are strongly attracted so that they resist thermal shear.\textsuperscript{63} The outer layer, known as the diffuse layer, is a group of counter ions attracted to the net charge of the Stern layer.\textsuperscript{62} These ions are less firmly attached. There is a boundary known as the shear plane within the diffuse
layer which separates water that moves with shear and that which does not. The electrical potential at this point is experimentally accessible, and called the zeta potential (\(\zeta\)). A schematic of the electrical double layer is shown in Figure 2.2.

![Schematic of the electrical double layer](image)

**Figure 2.2 A schematic representation of the electrical double layer of positively charged particle**

The surface potential (\(\psi_0\)) declines exponentially across the diffuse layer (as shown in Figure 2.2). The potential at the stern plane is assigned by \(\psi_d\). The \(\zeta\) value is a function of the charge on the surface of the particles.

The double layer thickness (1/\(\kappa\)) is a measure of the distance over which electrostatic interactions are significant. For a symmetrical electrolyte at 25 °C, \(\kappa\) is given by equation 2.8.\(^{58}\)

\[
\kappa = 0.329 \times 10^{10} \left( \frac{c z^2}{mol \cdot dm^{-3}} \right)^{1/2} m^{-1}
\]

(2.8)

Where \(c\) is electrolyte concentration (mol dm\(^{-3}\)), \(z\) is a charge number of electrolyte.
2.4.2 Electrostatic interaction between two spherical particles

When two spherical particles with the same charge approach, the electrical double layers of two particles overlap and a repulsive force is generated. The repulsive interaction energy \( V_{\text{rep}} \) (J) of two spheres at equal size can be calculated using equation 2.9.

\[
V_{\text{rep}} = 2 \pi \varepsilon a \psi_d^2 \exp(-\kappa H)
\]  

(2.9)

Where \( \varepsilon \) is dielectric constant of the solution, \( a \) is radius of the particles (micrometre), \( \psi_d \) is electrical potential (volts) at the Stern layer, and \( H \) is the distance between particles (micrometre).

The van der Waals energy of attraction \( V_{\text{att}} \) (J) between two spherical particles is given by:

\[
V_{\text{att}} = -\frac{A_{\text{eff}}}{12} \left( \frac{1}{h(h+2)} + \frac{1}{(h+1)^2} + 2 \ln \left( \frac{h(h+2)}{(h+1)^2} \right) \right)
\]  

(2.10)

Where \( h = H/2a \) and \( A_{\text{eff}} \) is the effective Hamaker constant which is given by

\[
A_{\text{eff}} = \left( \sqrt{A_{\text{colloid}}} - \sqrt{A_{\text{medium}}} \right)^2
\]  

(2.11)

Where \( A_{\text{colloid}} \) is the Hamaker constant of the particles and \( A_{\text{medium}} \) is that of the dispersion medium.

For conditions where \( h << 1 \):

\[
V_{\text{att}} = -\frac{A_{\text{eff}} a}{12H}
\]  

(2.12)

2.4.3 Potential energy curves

To investigate colloidal stability, the total interaction energy \( V_{\text{total}} \) between two particles is given by equation 2.13.

\[
V_{\text{total}} = V_{\text{rep}} + V_{\text{att}}
\]  

(2.13)
Figure 2.3 shows an example of a DLVO total two particle interaction energy curve. For the interaction between particles of the same materials, $V_{\text{rep}}$ is an exponential function of the distance between the particles within a range of double layer thickness ($1/\kappa$) and $V_{\text{att}}$ decreases as an inverse power of the distance between the particles. At large particle separation, repulsive forces caused by the electrical double layer are more significant because the van der Waals attractive forces have a short range. The attractive forces are dominant for small separations. Figure 2.3 shows the interaction energy curve for the colloidal dispersion. Instability of the colloidal dispersion (coagulation) occurs when the maximum potential ($V_{\text{crit}}$) is small compared with the thermal energy ($kT$) of the particles. For some systems, the attractive forces are higher than the repulsive force. This may cause flocculation (secondary minimum) which is a reversible aggregation. Flocculated dispersions can be redispersed by mechanical energy or when changing the solvent environment.

![Figure 2.3 A schematic representation of total interaction energy curve](image)

### 2.4.4 Steric stabilisation

Steric stabilisation induces the adsorption of hydrophilic polymer chains onto the surface of colloidal particles. When they are in a good solvent, the chains will remain in solution. If solvated tethered polymer chains overlap between approaching particles, this region of high polymer chain concentration will generate an osmotic pressure. The repulsive energy due to steric stabilisation ($V_{\text{ste}}$) is added to the total interaction energy of the particles (equation 2.14):
\[ V_{\text{total}} = V_{\text{rep}} + V_{\text{att}} + V_{\text{ste}} \] (2.14)

A steric stabiliser adsorbed onto colloidal dispersions prevents the particles from the aggregating because of steric interactions.

### 2.5 Doubly crosslinked (DX) microgel

A conventional microgel has crosslinking within the microgel particles (intra particles crosslink) and this is known as a singly (SX) crosslinked microgel. This type of concentrated dispersion can give a physical gel. The SX microgel can redisperse in water or good solvent. A new type of hydrogel; doubly crosslinked (DX) microgels have been studied recently.\(^{68,69,70,71}\) It is defined as a microgel which is comprised of SX microgels that acts as the crosslinker within the hydrogel.\(^{72}\) This means the DX microgels consist of *intra*-particle chains (within the SX microgels) and *inter*-particle chains (linking neighbouring particles). Generally, the *inter*-particle chains provide permanent crosslinking because they involve covalent bonds. Those DX microgel do not redisperse in water. However, if *inter*-particle chains were interrupted and the hydrogel was placed in a good solvent, the DX microgel would redisperse to give a SX microgel dispersion.\(^{72}\)

DX microgels are composed of SX microgels, therefore they have the same responsive behaviours.\(^{72}\) Most DX microgels studied have been temperature responsive. The pH responsive DX microgels have been reported recently by Saunders et al.\(^{68,69,70,71}\) SX microgels are responsive to temperature, pH, redox species and also enzymes\(^{23,25,26,27}\) so DX microgels should also have these behaviours.

The methods of characterisation for DX microgels are usually the generally same as those for hydrogels i.e. scanning electron microscopy (SEM), swelling experiment, dynamic rheology. It is crucial to characterise the SX microgels because the functionalisation of microgels can decrease the colloidal stability.\(^{72}\)

The swelling or de-swelling response time of DX microgels can be faster than that of a conventional hydrogel.\(^{73}\) The morphology and properties of DX microgels are controllable and tuneable. It is possible to use the DX microgels as biomaterials.\(^{74}\)
Liu et al.\textsuperscript{71} studied pH responsive DX microgels based on methyl methacrylic acid (MAA). They found that the storage modulus values of DX microgels had improved and the mechanical properties are tunable by varying the degree of functionalisation of the parent microgel, particle volume fraction ($\phi_p$) and pH. This microgel was further studied using 2-aminoethylmethacrylate (AEM) for vinyl-functionalisation and using N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) as a coupling chemistry.\textsuperscript{70} This method is widely used for conventional polymers and gave a high extent of functionalisation.\textsuperscript{75,76} Dynamic rheology data showed that the elastic modulus of these DX microgels is proportional to the degree of vinyl group functionalisation. The DX microgel had a microporosity morphology and high elastic modulus. Moreover, they exhibit pH dependent swelling and are injectable which is an advantage for biomaterial applications. The EDC chemistry enables incorporation of any primary amine-containing molecules within the microgels. This is potentially useful for biomaterial applications because it is possible to incorporate bioactive functional groups or chromophores into the microgels.

Milani et al.\textsuperscript{68} studied injectable pH-responsive DX microgels based on methyl methacrylate (MMA) for improving the mechanical properties of degenerated interverbral discs (IVD). The microgel is injectable and forms permanently crosslinked gels within degenerated IVDs. The degenerated IVDs show improved strain, modulus, toughness and resilience after injection in DX microgels. Moreover, the cytotoxicity experiments proved that the DX microgel based on MMA was biocompatible.\textsuperscript{68} This data confirms the possibility that DX microgels can be used for biomaterial applications. The concept of this article is applied for the work in this thesis, which aims to prepare the DX microgels for repair of damaged heart tissue. However, the microgel studied is cationic microgel and contains high primary amine content.

2.5.1 Methods of preparation for DX microgels
There are several methods to prepare DX microgels, based on the structures of the hydrogels (Figure 2.4).\textsuperscript{72} The first method is the formation of DX microgels by aggregation (Figure 2.4(a)). Hu et al.\textsuperscript{77} used an aggregation to prepare hydroxypropyl cellulose DX microgels. They first made nanoparticle gels and then covalently bonded them together. The DX microgels showed a fast swelling response. This
method was later investigated by Cho et al. They prepared thermoresponsive DX microgels from PNIPAM and allylamine. Poly(acrylic acid) (PAAc) was added to cause the microgel inter-linking, and then the microgel was heated to induce shrinkage and compaction. Glutaraldehyde crosslinking was used to form permanent DX microgels.

![Figure 2.4 Schematics of six methods for preparing DX microgels](image)

The second method demonstrated the formation of DX microgels by using SX microgels as crosslinking species. This means the SX microgels form covalent bonds between themselves. A thermoresponsive photonic hydrogel was synthesised by Cai et al. They copolymerised vinyl-functionalised microgels with acrylamide or poly(ethyleneglycol) methacrylate. The SX microgel particles were linked by linear polymer chains when they were in the swollen state. The crosslinking was initiated by light. This method is shown in Figure 2.4(b)(i). An alternative method to prepare this type of DX microgel was investigated by Xia et al. They prepared poly(N-isopropylacrylamide-co-acrylic acid) (P(NIPAM-co-AAc)) microgels and
converted some of the carboxylic acid groups to per-acid initiators by reaction of the washed microgels with H$_2$O$_2$. Linear polymer chains were also used to link the microgel particles in the collapsed state. (Figure 2.4(b)(ii))

Microgel reinforced hydrogels is the third approach. This method is based on the formation of a hydrogel network in the presence of functionalised (reactive) microgel particles (Figure 2.4(c)).$^{72,74,80,81}$ The crosslinked networks are formed by covalent linking between the functional groups on the microgel particles. Bencherif et al.$^{81}$ prepared polyethylene glycol (PEG) microgel particles and reacted the hydroxyl groups on the surface to create vinyl groups. The microgel reinforced hydrogels were produced by photocrosslinking of the vinylfunctionalised microgels with hyaluronic acid-glycidyl methacrylate macromonomer.

DX microgel crystals can be prepared by addition of small molecules. Figure 2.4(d) shows the reaction of DX microgels which were prepared by a difunctional molecule linker. Hu et al.$^{82,83}$ investigated this approach using PNIPAM-co-allylamine microgel particles. The concentrated dispersion was heated to above the volume phase transition temperature to allow the microgel to form an ordered array, and then cooled down to bring the interface close enough for inter-particle crosslinking. Glutaraldehyde was used as a small molecule linker to react with primary amine groups from the allylamine segments.

Hu et al.$^{84}$ also studied the formation of a self-crosslinked DX microgel crystal (Figure 2.4(e)). Drying poly(NIPAM-co-N-hydroxymethacrylamide) microgel dispersions at room temperature persuaded the crosslinking reactions between the microgel particles. However, this approach was very slow and it was expected to give a brittle DX microgel because the crosslink between the microgels was short.

The approach used in this thesis was developed by Saunders et al.$^{68,69,70,71}$ Inter-penetrating DX microgels involve crosslinking of vinyl-functionalised microgels (Figure 2.4(f)).$^{72}$ However, this approaches different from those mentioned above. The microgel used in this method is pH-responsive and forms a physical gel before crosslinking between the vinyl groups. Particle swelling and inter-penetration of segments at the periphery of the microgel particles cause the physical gel. The vinyl
groups can be obtained from glycidyl methacrylate functionalisation\textsuperscript{68,69,71} or 2-aminoethyl methacrylate (AEM) functionalization.\textsuperscript{70} The glycidyl methacrylate functionalisation is used to prepare DX microgels in Chapter 5 (Scheme 5.2).

2.6 Instrumentation

2.6.1 Scanning electron microscopy

Scanning electron microscopy (SEM) is a microscopy method which is used to examine the surface of a specimen with magnifications of 20-100000.\textsuperscript{85} SEM images can be created by scanning a probe, a focused electron beam, across the specimen. Then, the probe interacts with a surface layer of the specimen.\textsuperscript{86} The images obtained from SEM are easy to interpret because they show only surface characteristics (whereas the images from light microscope are mix of surface and internal characteristics).\textsuperscript{85} The surface of the specimen must be electrically conductive in order to reflect the electron beam. For non-conductive materials, such as a polymer, this requires a conductive coating to prevent charging by the electron beam.\textsuperscript{86}

A schematic diagram of a scanning electron microscope is shown in Figure 2.10. The electron gun releases electrons using a variable potential of between 1 - 40 kV.\textsuperscript{87} The electron beam is focused by a condenser lenses and objective lens into a nano-diameter spot on the sample surface. The electron beam is scanned across the surface of the specimen.\textsuperscript{88} The sample scatters the electrons and they are collected by a detector. These electrons are accelerated and passed through a photomultiplier which amplifies and forms an image.
2.6.2 Photon correlation spectroscopy

Photon correlation spectroscopy (PCS) is a light-scattering technique which is non-invasive and non-destructive and is used to study the properties of colloids and suspensions, macromolecules and polymers.\(^9\) The hydrodynamic radius of particles between 5 – 1000 nm and size distribution can be obtained by PCS. A particle suspension is probed by a laser beam. Colloidal sized particles in a liquid undergo random ("Brownian") motion.\(^9\) When the particles move, the scattered light intensity from the diffusing particles will fluctuate. The different sizes of the particles in the dispersion and the density of the dispersed liquid cause the particles to move at different speeds. Due to the variations in temporal fluctuation of intensity between the particles and the dispersed phase, it is possible to use the light scattering technique to determine the particle size.

A laser beam is passed through a particle dispersion and then the light scattered is collected by a photomultiplier at 90°. The photomultiplier is connected to a correlator which transfers the light into an electrical signal which may be read by the

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Figure 2.5 A schematic of the electron beam in a scanning electron microscope\(^8\)

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Electron gun

Condenser lens

Scanning coils

Objective lens

Specimen

Detector

Photoamplifier
computer (Figure 2.6). The hydrodynamic diameter ($d_h$) of the particle is calculated by the Stokes-Einstein equation$^91$ and the diffusion coefficient ($D$) is determined.$^{92,93,94}$

$$d_h = \frac{k_B T}{3\pi D \eta}$$  \hspace{1cm} (2.15)$$

In this equation, $k_B$ is the Boltzmann constant (J/K), $T$ is the absolute temperature (K) and $\eta$ is the viscosity of the suspending fluid (cP). The diffusion coefficient of the particles ($D$) is a measure of the time taken for the particles to diffuse through the dispersant.$^{94}$ The light intensity delays with time, $\tau$, and this is described by the correlation function ($g(\tau)$) which decays exponentially according to the following equation$^{91}$:

$$g(\tau) = \exp(-Dq^2\tau)$$  \hspace{1cm} (2.16)$$

Where $q$ is the wave vector which is defined by

$$q = \frac{4\pi n}{\lambda} \sin \frac{\theta}{2}$$  \hspace{1cm} (2.17)$$

In this equation, $n$ is the refractive index of the suspending medium, $\lambda$ is the wavelength of incident light, and $\theta$ is the scattering angle. The hydrodynamic diameter from PCS is assumed to be for monodisperse and spherical particles.$^{94}$

Figure 2.6 A schematic of a PCS measurement
2.6.3 Electrophoretic mobility measurement

The zeta potential of a microgel cannot be directly measured from the shear plane because there is no clearly defined surface. Therefore, the electrophoretic mobility of charged microgels have to be measured instead.

Typically, the particles in a colloidal suspension or emulsion have an electrical charge. This comes from the chemical groups which can ionize and generate a charged surface or the adsorption of ion by the surface itself. An electric field ($E$) is applied to the dilute dispersion and a laser is used to measure the time that particles take to travel over a fixed distance. By applying the electric field across an electrolyte solution, the charged particles move through the solution and are attracted to the electrode of opposite charge.

Electrophoretic mobility ($U_E$) is the velocity of a particle in a unit electric field. To calculate the electrophoretic mobility, the following equation was used:

\[ U_E = \frac{V_s}{E} \]  

(2.18)

Where $V_s$ is the drift speed (m s$^{-1}$) and $E$ is the applied electric field (V m$^{-1}$).

By the Henry equation (equation 2.19), it is possible to calculate the zeta potential from the electrophoretic mobility.

\[ U_E = \frac{2\varepsilon\zeta f(ka)}{3\eta} \]  

(2.19)

Where $U_E$ is the electrophoretic mobility, $\zeta$ is the zeta potential (mV), $\varepsilon$ is the dielectric constant, $\eta$ is the viscosity and $f(ka)$ is Henry’s function with a value of 1.5 if the solvent used is water.

Figure 2.7 shows a disposable capillary cell used for mobility measurement. The particle dispersion is injected into the cell which is equipped with electrodes on both sides. The particle speed when the electric field is applied is monitored with a laser.
2.6.4 Fluorescence microscopy

Fluorescence microscopy analyses the emission of light which occurs after the absorption of light that is of shorter wavelength. It is possible to see objects that are fluorescent by selectively filtering out the excitation light, without blocking the emitted fluorescence. Fluorophore molecules are used to label the object. In the ground state, the fluorophore absorbs light (photons) and the molecules rotate and vibrate. Sometimes, the absorbed energy moves an electron into a different orbital; this transition is the excited state. Methods to return the excited electron to the ground state are vibrational relaxation and fluorescent emission. The latter emits a photon of light and causes the fluorescence which can be seen through a microscope.

Figure 2.8 shows a diagram of the optics of a fluorescence microscope. Incident light passes through the fluorescence filter. The excitation filter is a low-pass or band-pass filter which selects the excitation wavelengths. Then, the second, (long-pass filter) reflects the excitation light into the objective lens. The fluorescence light returns through the objective. Some of the fluorescence lights which are of a longer wavelength are transmitted by the long-pass filter. The fluorescence wavelengths are further selected by the emission filter which selects the wavelength for observation.
2.6.5 Fourier transform infrared spectroscopy

Infrared spectroscopy is a method to study the interaction of infrared electromagnetic radiation with a sample.\textsuperscript{104} This technique is based on the vibrations of the atoms of a molecule.\textsuperscript{105} An incident infrared (IR) radiation passes through a sample. Some of the IR radiation is absorbed by the molecules of the substance and cause molecular vibrations whereas some is transmitted. This determines the fraction of incident radiation absorbed at a particular energy. The IR spectrum shows the molecular absorption and transmission, which is a molecular fingerprint of the sample. The absorption peak is related to the frequencies of the vibrations between the bonds of the atoms in materials. Each material has a unique IR spectrum since they have a different combination of atoms\textsuperscript{104,105}. The FTIR technique is suitable for a variety of materials such as solids, liquids, polymer, biological materials etc.\textsuperscript{104} Figure 2.9 shows a typical infrared band of polymer functional groups.\textsuperscript{105}
2.6.6 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) is a phenomenon whereby atomic nuclei re-emit some of the absorbed energy in the form of radio signals when placed in a magnetic field and are stimulated by radio waves of a particular frequency.\textsuperscript{106} The nuclei of atoms have magnetic properties which can be utilised to yield chemical information.\textsuperscript{107}

Atomic nuclei consist of protons and neutrons. Each of them has an intrinsic angular momentum or spins which generate a local magnetic field.\textsuperscript{106} The dipole orientation is random when there is no externally applied field. However, in a uniform magnetic field ($B_0$), the dipoles will orient themselves to align with the line of induction of the applied magnetic field. The number of orientations for the nuclei is $2I+1$, where $I$ is a nuclear spin quantum number.\textsuperscript{108} The $I$ value for $^1$H and most nuclei in organic chemistry is $\frac{1}{2}$.\textsuperscript{109} When an external magnetic field is applied to nuclei, there are only two nuclear magnetic moments; spin up and spin down, known as spin flipping.
The difference between two energy states \( \Delta E \) is related to the applied magnetic field shown in equation 2.20:\(^{108,109}\):

\[
\Delta E = h\nu
\]  
(2.20)

where \( h \) is Planck’s constant and \( \nu \) is the resonance frequency (Hz) of incident radiation which is required to flip the magnetic moment. The latter is given by the following equation\(^{106,110}\):

\[
\nu = \frac{\gamma^* B_0}{2\pi}
\]  
(2.21)

where \( \gamma^* \) is the gyromagnetic ratio. For hydrogen nuclei (proton), \( \gamma^* \) is \( 2.675 \times 10^8 \text{ s}^{-1}\text{T}^{-1} \).

Identical nuclei in different chemical surroundings absorb electromagnetic radiation at differing frequencies. The separation between absorption peaks is usually referred to as chemical shift.\(^{110}\) The area of an absorption peak is proportional to the number of equivalent nuclei. Therefore, it is possible to identify the chemical structure of a molecule based on the frequency that the nuclei absorb the radiation.

The chemical shift is defined in terms of the difference in resonance frequency \( \nu \) between the nucleus of sample (\(^1\)H) and a reference nucleus (e.g. \(^1\)H of tetramethylsilane, TMS). It is given by equation 2.22\(^{110}\):

\[
\text{Chemical shift} = \frac{10^6(\nu_{\text{sample}} - \nu_{\text{TMS}})}{\nu_{\text{TMS}}}
\]  
(2.22)

The chemical shift values are quoted in \textit{parts per million}, or ppm.

To prevent the peak from the solvent being shown in spectra, the sample must be dissolved in a solvent which contains deuterium in place of hydrogen (e.g. deuterium oxide (D\(_2\)O)).

2.6.7 Dynamic rheology
Dynamic rheology is used to measure the mechanical properties of the gels. Rheological studies involve the deformation of matter resulting from the application
of a force. An oscillating rotational shear force is applied to a sample. A rheometer measures the resistance to shear strain and can be used to determine the mechanical properties of the sample. An oscillation rheometer is sketched in Figure 2.10.

![Figure 2.10 A schematic of an oscillation rheometer geometry](image)

In an alternative configuration, the rheometer applies a strain to the sample. Then the sample responds to the applied strain and the static plate measures a stress via the torque between itself and the sample. For ideal elastic materials there is no phase shift between the stress and strain because the material will immediately resist the strain applied to it and oscillate to the same degree as the head of the rheometer. For a viscoelastic material, it cannot immediately resist the strain and there is a difference between stress and strain phases. The difference in phase between the applied stress and measured strain is known as the phase angle (δ) (shown in Figure 2.11).
For a viscoelastic material, $\delta$ will be non-zero and the properties of the material consist of both viscous and elastic parts. The elastic or storage modulus ($G'$) is a measurement of the stored energy. The energy is fully stored for elastic materials. The $G'$ value can be calculated using equation 2.23. For a viscous material the energy applied to the material is lost. The viscous or loss modulus ($G''$) is a measurement of the lost energy (dissipated). The $G''$ value can be calculated using equation 2.24.

$$G' = \frac{\sigma_0}{\gamma_0} \cos(\delta) \quad (2.23)$$

$$G'' = \frac{\sigma_0}{\gamma_0} \sin(\delta) \quad (2.24)$$

Where $\sigma_0$ is the maximum stress and $\gamma_0$ is the maximum strain.

Using the $G'$ and $G''$ values, we can calculate a tan $\delta$ value using the equation 2.25.
$$\tan \delta = \frac{G''}{G'} \quad (2.25)$$

The tan δ value is the ratio of loss modulus and elastic modulus values. It is used to identify the viscosity or elasticity of a material. For materials with more viscous behaviour, $G''$ is higher than $G'$. Whereas $G'$ is higher than $G''$ for materials with more elasticity behaviour. Tan δ is used as a way to classify fluids and gels. If tan δ is less than 1 ($G' > G''$), samples are classified as a gel.
2.7 References


96. Holmberg, K. Handbook of applied surface and colloid chemistry Vol. 1–2; John Wiley & Sons Ltd: Chichester, 2002.
100. Disposable Capillary Cell (DTS1061), Malvern, 2011.


Chapter 3

Preparation and characterisation of poly(N-vinylformamide-co-glycidyl methacrylate), (PNVF-GMA) and hydrolysed PNVF-GMA

Abstract
In this chapter, the preparation of poly(glycidyl methacrylate) (PGMA) and poly(N-vinylformamide-co-glycidyl methacrylate) (PNVF-xGMA) particles (x is the weight fraction of glycidyl methacrylate (GMA)) via one-step non-aqueous dispersion (NAD) polymerisation is reported. The microgel was then aminated and/or hydrolysed in alkali conditions to generate primary amines. The microgels were investigated using photon correlation spectroscopy (PCS) and optical microscopy for particle size, then through scanning electron microscopy (SEM) to observe their morphology. Fourier transform infrared spectroscopy (FTIR) was used to obtain the spectra of the microgel particles. The pH dependent swelling of particles was studied by measuring particle diameter against pH. Electrophoretic mobility measurements were also used. It has been found that the PNVF-xGMA has a core-shell structure in that the particle core contains mostly PNVF and the shell comprises PGMA. There is also crosslinking between PNVF and PGMA. The particles are water swellable and their morphology is “cane-ball”-like. The hydrolysed PNVF-xGMA (H-PNVF-xGMA) particles were composed of both positively and negatively charged. Moreover, charge patch aggregation occurred at low ionic strength.
3.1 Introduction and aims
Microgel mixtures which contain particles with opposite charges offer flexibility, while their heteroaggregation increases structural strength and elasticity.\textsuperscript{1} Moreover, oppositely charged microgel dispersions rapidly form gels when mixed. Anionic microgels were successfully prepared within the Saunders group.\textsuperscript{2-5} They studied the relationship between particle composition and mechanical properties of pH-responsive poly(A/MAA/X) particles where A and X are the primary co-monomer and crosslinking monomer, respectively. The primary co-monomers used were methyl methacrylate (MMA), ethyl acrylate (EA) or butyl methacrylate. The crosslinking monomers were either butanediol diacrylate (BDDA) or ethyleneglycol dimethacrylate (EGDMA). It was shown that poly(EA/MAA/X) microgels swelled more strongly than poly(MMA/MAA/X) microgels. Furthermore, greater swelling occurred for particles prepared using EGDMA than BDDA.\textsuperscript{2}

In this work, we focus on the preparation of cationic microgels. The aim is to generate a primary amine functionalised microgel. Santos et al.\textsuperscript{6} synthesised poly(methyl methacrylate)-poly-(N-isopropylacrylamide) (PMMA-PNIPAM) core-shell nanoparticles, then functionalised core-shell particles were prepared using aminoethyl methacrylate hydrochloride (AEM) and N-(3-aminopropyl)methacrylamide (APM) as cationic co-monomers to increase the surface charge density. They found that increasing the APM concentration made the dispersions unstable, which is probably due to the formation of water soluble polymers. Meunier et al.\textsuperscript{7} prepared cationic poly(N-isopropylacrylamide) (PNIPAM) copolymer latexes using methylenebisacrylamide (MBA) as the crosslinking agent in the presence of AEM. It was found that the concentration of AEM affected the polymerisation kinetics and particle nucleation. The use of high AEM content (above 1.2 mol\% with respect to the overall monomer concentration) led to large amounts of polyelectrolyte and broad particle size distributions. To avoid the ionic strength problem of the AEM, N-vinylformamide (NVF) and glycidyl methacrylate (GMA) were selected for preparation of primary amine microgels in this study. This should have enabled functionalisation of particles able to form the primary amine.

PGMA has various applications because epoxide groups in its polymer backbone can react with various functional groups.\textsuperscript{8} These functional groups can be employed
directly or via modification using cationic, anionic, chelate forming, or fluorescent routes for the immobilization of biopolymers.\textsuperscript{9,10} Furthermore, PGMA can be used for biomaterials. Gao et al.\textsuperscript{9} generated the poly(glycerol methacrylate) (PGOHMA) derivatives from PGMA. This polymer contained a polyol structure. They can attach to ligands and drugs, which is useful for drug targeting applications.

The PGMA particles studied here are broadly similar to the PGMA particles prepared by dispersion polymerisation reported by Horak et al.\textsuperscript{10} and Elmas et al.\textsuperscript{11} Horak et al. studied reactive PGMA microspheres using non-aqueous dispersion polymerisation and modified the oxirane group to create a primary amine on the PGMA structure by aminolysis and hydrolysis in acidic conditions. They found that PGMA prepared in neat alcoholic medium retained oxirane group content after the polymerisation. After modification reactions, more than 40\% of the oxirane groups were converted to amines.\textsuperscript{10} Elmas et al. prepared aminated PGMA by aminolysis in aqueous ammonia solution (25\%, w/w) and investigated the FITC labelled aminated PGMA by confocal laser scanning microscopy.\textsuperscript{11} The fluorescent images confirmed the presence of FITC in the structure of aminated particles. This indicated the presence of amine groups.

Poly(vinylamine) (PVAM) is a linear polymer containing primary amine groups bonded directly to the main chain.\textsuperscript{12} The high reactivity of amine groups provides important active sites for crosslinking and/or derivatisation.\textsuperscript{13} This polymer is interesting because of its pH-dependent polycationic nature.\textsuperscript{12} It has been used in a variety of industry; water treatment, papermaking, adhesives, and coatings applications.\textsuperscript{14} Moreover, PVAM has been used for bioapplication. Wolfert et al. demonstrated PVAM as an effective gene delivery vector due to its ability to condense DNA.\textsuperscript{15} PVAM cannot be prepared directly from vinylamine monomer because it is not stable in the free state. It is readily tautomised to acetaldehyde imine.\textsuperscript{16} The most simple and economical method to prepare PVAM is through hydrolysis the PNVF in either acidic or basidic aqueous solution. For linear polymers, alkaline hydrolysis is the most efficient hydrolysis method.\textsuperscript{14}

This chapter consists of two main parts. Firstly, the morphology and characterisation of PGMA and aminated PGMA (PGMA-NH\textsubscript{2}) were investigated. Poly(N-
vinylformamide-\textit{co}-glycidyl methacrylate) (PNVF-GMA) particles have not been well studied in the open literature. Therefore, in the second part, we studied a procedure for preparing NVF containing microgels, which were prepared by two monofunctional comonomers (NVF and GMA). The microgels can be hydrolysed to generate VAM groups. PNVF-\textit{x}GMA (\textit{x} indicates the weight fraction of GMA) showed a core-shell structure and unusual “cane-ball”-like morphology. Moreover, crosslinking between PNVF and PGMA has been proposed from the data.
3.2 Experimental details

3.2.1 PGMA and PNVF dispersion preparation

PGMA and PNVF contained only GMA monomer and NVF monomer respectively. All dispersions were prepared using a non-aqueous dispersion polymerisation method. The method used to prepare the dispersions is based on that used by Horak and Shapoval.\textsuperscript{10}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{synthesis_diagram.png}
\caption{Schematic showing the synthesis of (a) PNVF and (b) PGMA}
\end{figure}

A typical preparation for PGMA and PNVF is as follows. GMA (12 g, 0.084 mol), polyvinylpyrrolidone (PVP) (1.8 g), and azobisisobutyronitrile (AIBN) (0.2416 g, 1.47 mmol) were dissolved in 86 mL (68 g) ethanol in a 250 mL four neck reactor equipped with an overhead IKA RW 20.n stirrer and condenser. The stirring rate used was 500 rpm. The system was purged with nitrogen for 15 minutes and then heated at 70 °C for a total of 16 h. A picture of the equipment used is shown in Figure 3.2. The dispersion obtained at the end was filtrated (50 µm mesh filter) and purified by repeated centrifugation and redispersion in ethanol. To test the effect of PVP concentration, a dispersion was prepared with a larger PVP mass (3.6 g). This system is referred to as PGMA\textsubscript{HPVP}. The compositions are also given in Table 3.1.
Table 3.1 Compositions for dispersion polymerisation

<table>
<thead>
<tr>
<th>Code</th>
<th>Compositions</th>
<th>Monomer (g)</th>
<th>Other chemicals (g)</th>
<th>Reaction duration (h)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>NVF</td>
<td>GMA</td>
<td>AIBN</td>
</tr>
<tr>
<td>PNVF</td>
<td></td>
<td>6</td>
<td>-</td>
<td>0.24</td>
</tr>
<tr>
<td>PGMA</td>
<td></td>
<td>-</td>
<td>12</td>
<td>0.24</td>
</tr>
<tr>
<td>PGMA_{HPVP}</td>
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<td>-</td>
<td>12</td>
<td>0.24</td>
</tr>
<tr>
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<td>0.24</td>
</tr>
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<td>3.00</td>
<td>0.24</td>
</tr>
<tr>
<td>PNVF-0.4GMA (F)</td>
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<td>4.48</td>
<td>3.00</td>
<td>0.24</td>
</tr>
<tr>
<td>PNVF-0.75GMA</td>
<td></td>
<td>2.39</td>
<td>7.16</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Figure 3.2 A picture of the equipment used for dispersion polymerisation

The method for preparing PNVF is the same method as used for PGMA. The NVF mass used was 6 g (0.084 mol). The reaction duration used was 1 h. The dispersion obtained at the end was filtrated (50 μm mesh filter) and purified by repeated centrifugation and redispersion in ethanol.\textsuperscript{10,11,13}
3.2.2 Aminolysis of PGMA particles

1 g. of particles was added to 25 ml of 35% ammonia solution, and then heated to 60 °C while stirring overnight under a nitrogen atmosphere. Then, the particles were centrifuged and redispersed with deionised water three times.

![Figure 3.3 Schematic showing the aminolysis of PGMA](image)

3.2.3 PNVF-xGMA dispersion preparation

The non-hydrolysed copolymer particles are termed PNVF-xGMA, where x is the weight fraction of GMA used during preparation with respect to total monomer mass. Three dispersions were used for the study. They were PNVF-0.2GMA, PNVF-0.4GMA and PNVF-0.75GMA. PNVF-0.2GMA contained 80 and 20 wt% of NVF and GMA, respectively. PNVF-0.4GMA contained 60 and 40 wt% of NVF and GMA, respectively. PNVF-0.75GMA contained 25 and 75 wt% of NVF and GMA respectively. The compositions of each system are given in Table 3.1. All dispersions were prepared using a non-aqueous dispersion polymerisation method. The method used to prepare the dispersions was the same method as used for PNVF. The reaction duration used was 1 h. The dispersion obtained at the end of the reaction was filtered and purified by repeated centrifugation as described above. In addition, a PNVF-0.4GMA preparation was conducted with a continuous feed of NVF and GMA solution over a period of 1 h. This was followed by a further 1 h of reaction. This system is referred to as PNVF-0.4GMA(F).

![Figure 3.4 Schematic showing the synthesis of PNVF-PGMA](image)
3.2.4 PVAM and hydrolysed PNVF-xGMA dispersions preparation

PVAM was prepared from PNVF particles. 1 g of PNVF particles were dissolved in water containing 1 M NaOH and the solution was heated to 80 °C for 24 h. The product was then extensively dialysed against water.

![Figure 3.5 Schematic showing the hydrolysis of PNVF](image)

Hydrolysis of PNVF-xGMA dispersions was performed in alkaline conditions. 1 g of PNVF-xGMA particles was dispersed in water containing 1 M NaOH and the dispersion heated to 80 °C for 16 h. The PNVF-xGMA dispersions, subjected to hydrolysis are referred to as H-PNVF-xGMA. The dispersion obtained at the end was purified by centrifugation and redispersed in deionised water.

![Figure 3.6 Schematic showing the hydrolysis of PNVF-PGMA](image)

3.2.5 FITC labelling of particles

The particles were labelled with fluorescein 5(6)-isothiocyanate (FITC). FITC 10 mg (0.026 mmol) was dissolved in aqueous solution (containing 10 vol% acetone) at pH of 9. Then 0.5 g of particles were dispersed in the solution. The dispersion was stirred at room temperature for 6 h in the dark. The particles were repeatedly centrifuged and redispersed in water until no FITC was detected in the supernatant by UV–visible absorption at a wavelength of 500 nm.

3.2.6 Sodium fluorescein and rhodamine B labelling of H-PNVF-0.4GMA

A solution of sodium fluorescein and Rhodamine B (0.1 wt%) was added into 3 mL of H-PNVF-0.4GMA dispersion (0.1 wt% particles). The mixture was stirred in the
dark for 16 hr. Then the particles were centrifuged to remove the excess dyes and redispersed again in DI water.

3.2.7 Physical measurement

3.2.7.1 Total solid content
Measurement of the exact dispersion concentration is essential prior to functionalisation. This is to ensure that the repeat functionalisation processes are consistent. Polymer dispersion (1 g) was poured into an aluminium dish and dried at least 24 hour at room temperature in desiccators containing silica gel. The silica gel was dried in the oven at 70 °C overnight before use. The total solid content (TSC) was calculated using equation 3.1.

\[
TSC = 100 \times \frac{M_f}{M_i}
\]  

(3.1)

The parameters \( M_f \) and \( M_i \) represent the mass of the final sample and the initial sample (grams), respectively. For hydrolysed samples dispersed in water, they were dried in desiccators containing phosphorous pentoxide (P\(_2\)O\(_5\)) for 3 days. The fresh P\(_2\)O\(_5\) was changed everyday.

3.2.7.2 Optical microscopy
Images of particle dispersions were obtained via optical microscopy conducted using an Olympus BX41 microscope. Particle dispersions were diluted by water or ethanol before being observed at a magnification of 60×. At least 80 particles were measured using calibrated circles on transparent plastic in a particular range to determine the number-average diameters \( d_{opt} \). The circles used to place on the particles in particular size ranges. The particle size was obtained manually by particle sizing method. This method was considered superior to image J because of the latter’s tendency to poorly identify particles for our samples.

3.2.7.3 Fluorescence Microscopy
Fluorescence microscopy was conducted using a Nikon Eclipse 50i microscope. The samples were illuminated with a mercury lamp. For experiments involving FITC and
sodium fluorescein, the excitation and emission wavelengths had an average of 480 and 535 nm, respectively. For rhodamine B, the excitation and emission wavelengths were 540 and 605 nm, respectively.

Samples were labelled using fluorophore molecules as described in Section 3.2.5. The fluorophore absorbed photon which was supplied by a mercury lamp, which moved the electrons to an excited state. When the excited electrons returned to the ground state they emitted a large energy photon, which was observed through the fluorescence microscope.

### 3.2.7.4 Scanning electron microscopy

Scanning electron microscopy (SEM) was undertaken using a Philips FEGSEM instrument. Dispersions were diluted to a concentration 0.02 wt%, and then deposited from water or ethanol on a glass slide which was fixed to an aluminium stub. The samples were allowed to dry in the desiccators containing silica gel at room temperature and carbon sputter coated to aid charge dispersal.

The surface of the sample was scanned by the electron beam, and then the detector collected the scattered electrons. These electrons were accelerated and passed through a photomultiplier which amplified and formed an image. At least 40 particles were measured by a series of calibrated circles on transparent plastic in a particular range to determine the number-average diameters \(d_{\text{SEM}}\) as described above. A number of measured particles were fewer than that for the optical microscopy because samples was observed at higher magnification for SEM (5000× or 10000×) which showed fewer particles.

### 3.2.7.5 Photon Correlation Spectroscopy

PCS measurements were conducted using a BI-9000 Brookhaven light scattering apparatus (Brookhaven Instrument Corporation) fitted with a 20 mW He-Ne laser (632.8 nm) and the detector angle was set at 90°. The measurements were performed using dispersions containing a particle concentration of 0.01 wt% which provided a count rate of between 100 – 200 kcps (kilocounts per second), as suggested by the instrument manufacturer. The hydrodynamic diameter \(d_h\) is an average value from ten runs.
The detector measured scattered light which was generated by movement of particles. The scattered light was transmitted to the photon correlator. The correlator counted the number of photons of the scattered light and calculated the normalised autocorrelation function of the intensity using equation 2.16. The hydrodynamic diameter \((d_h)\) was calculated by the Stokes-Einstein equation (equation 2.15).

### 3.2.7.6 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) measurements were conducted using a Nicolet 5700 FTIR equipped with an attenuated total reflectance (ATR) unit. Samples were dried in oven at 80 °C for 24 h before it was ground using a mortar and pestle. Then, it was pressed against an ATR diamond crystal in order to obtain a good contact. The spectra were collected at room temperature in the range of 500 to 4000 cm\(^{-1}\) using a scan resolution of 2 cm\(^{-1}\) and a total number of 32 scans. The absorbance was corrected for the baseline.

For the ATR unit, an IR beam was directed into a crystal with a high refractive index. The IR beam reflected from the internal surface of the crystal and creates an evanescent wave. The evanescent wave extended beyond the crystal surface into the sample in contact with the crystal. The sample absorbed some of the energy of the evanescent wave and the wave was attenuated. The attenuated energy was returned to the IR beam and detector, respectively.\(^{17,18}\)

The photon energy of the IR induced vibrational excitation of covalently bound atoms. Each molecule had specific frequencies at which they rotated or vibrated depended on shape of the molecular potential energy surfaces and the masses of the atoms. Organic compounds absorbed IR radiation that corresponded in energy to these vibrations.\(^{19,20}\) Different molecules absorbed different IR radiation depended on the frequencies of the vibrations between the bonds of the atoms in sample, which is a molecular fingerprint of the sample. An evanescent wave beam passed through the sample and molecules absorbed some of the IR radiation. The spectrum showed how much energy was absorbed at each wavelength.
3.2.7.7 Electrophoretic mobility measurement

The electrophoretic mobility measurements were performed using a Zetasizernano ZS90 (Malvern Instruments Ltd.). The dispersions had the same particle concentrations as those used for PCS e.g. 0.01 wt% (and contained 0.001 M NaCl). The measurements were performed at 25 °C and the electrophoretic mobility values were averaged from three measurements (using ten sub-runs per measurement).

A laser beam passed through the capillary cell that contained the particles within sample. When an electric field \( (E) \) was applied to the dilute dispersion in the cell, particles moving caused the intensity of light detected to fluctuate with a frequency proportional to the particle speed. The time that the charged particles moved through the solution and attracted to the electrode of opposite charge was measured by a Laser Doppler Velocimetry (LDV). LDV was a technique used to study the velocity of particles within the fluid streams moving at the velocity of the fluid.\(^{21}\) Then, the detector sent the data to a processor and computer and the electrophoretic mobility was calculated by the equation 2.18 in Chapter 2, Section 2.6.3.

3.2.7.8 Elemental analysis

Samples were dried in oven at 80 °C for 24 h before it was ground using mortar. Elemental analysis (C, H, and N) was conducted using a Thermo Flash 2000 Organic Elemental Analyzer at the School of Chemistry, University of Manchester. Dry powdered samples were combusted in a furnace at about 900°C with excess oxygen which produced carbon dioxide (CO\(_2\)), water (H\(_2\)O), and nitrogen (N\(_2\)). These are separated on a gas chromatography column and then measured using a Thermal conductivity detector. The masses of combustion products were used to calculate the compositions (C, H, N) of sample.
3.3 Results and discussion

3.3.1 PGMA and PGMA-NH$_2$ particles

3.3.1.1 Morphology of the particles

Poly(glycidyl methacrylate) (PGMA) has attracted interest for various biomaterial applications such as drug delivery, DNA carriers, protein extraction, and peptide separation due to activated epoxide groups in the PGMA backbone. PGMA can be directly coupled with biomolecules via their ring opening reactions and further modified for a variety of biological applications. Aminolysis is one of the reactions, which can convert an epoxide ring to a primary amine by a ring opening reaction. This was studied prior to the NVF particles and the results are discussed in the following section. Optical images of particles are shown in Figure 3.7 for PGMA dispersed in ethanol and PGMA-NH$_2$ dispersed in water. The appearances are the same and the diameters of both PGMA and PGMA-NH$_2$ are approximately 2 µm (Table 3.2). This means that aminolysis did not effect the particle size and morphology of the particles. However, $d_{\text{SEM}}$ values are slightly smaller than $d_{\text{opt}}$ because the particles were dried for SEM. Morphology of PGMA and PGMA-NH$_2$ were characterised by SEM (Figure 3.8).

![Figure 3.7 Optical micrographs of (a) PGMA dispersed in ethanol and (b) PGMA-NH$_2$ dispersed in water](image-url)
Figure 3.8 SEM micrographs of PGMA dispersed from ethanol (a) PGMA and PGMA-NH₂ deposited from aqueous dispersions (b) and (c).

Table 3.2 Particle size for PGMA and PGMA-NH₂

<table>
<thead>
<tr>
<th>Code</th>
<th>(d_{\text{opt}}^a) (µm)</th>
<th>(d_{\text{SEM}}^b) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Water</td>
</tr>
</tbody>
</table>

*aNumber-average diameter measured by optical microscopy for particles dispersed in water or ethanol. bNumber-average diameter from SEM. The number in square brackets is the coefficient of variation (CV). CV = \[100 \times (\text{SD/mean})\]. SD is standard deviation and mean is average particle diameter.

3.3.1.2 FTIR analysis of PGMA and PGMA-NH₂

FTIR spectra of PGMA and PGMA-NH₂ are shown in Figure 3.9. The spectrum of GMA is shown for comparison. PGMA showed a band at 905 cm⁻¹ which belongs to an epoxide group of GMA⁹,¹⁰,²²,²³, while PGMA-NH₂ did not exhibit the band at this position. In addition, the carboxyl band at 1725 cm⁻¹ due to the carboxyl group of GMA part was ended.¹⁴,²⁵ Moreover, after the aminolysis process, a new primary amine band at 1640 cm⁻¹ is present⁸ (Figure 3.9(c)) but it is very weak. This suggests that the epoxide group of PGMA was hydrolysed to an amine group. Furthermore,
Elemental analysis results (Table 3.3) indicated that about 40% of the epoxide groups of PGMA reacted with the ammonia solution. (Calculation is shown in 3.3.1.4)

<table>
<thead>
<tr>
<th>Code</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
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<tr>
<td>PGMA</td>
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<td>7.2</td>
<td>0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>PGMA-NH$_2$</td>
<td>49.6</td>
<td>8.4</td>
<td>3.5</td>
<td>0.070</td>
</tr>
</tbody>
</table>

$^a$%C, %H, and %N are percentage by mass. $^b$R$_{NC}$=N/C

Figure 3.9 FTIR spectra of GMA, PGMA, PGMA-NH$_2$

PGMA-NH$_2$ particles did not dissolve or swell in any solvent. This is consistent with the work of Gao et al.$^g$ They found that polymers with a low degree of amination (25 and 40%) were insoluble in water or several organic solvents due to intermolecular/intramolecular crosslinking side reactions which could probably have occurred during hydrolysis. It is proposed that the amine groups reacted with epoxide groups during aminolysis to give highly crosslinked particles.$^{22,26}$ (see Figure 3.10).

Figure 3.10 The crosslinking of PGMA and PGMA-NH$_2$
3.3.1.3 Fluorescence labelling

Fluorescent images in Figure 3.11 were obtained by the fluorescent emission originated from FITC covalently bound to the PGMA-NH$_2$. PGMA was used as a control. FITC labelled PGMA-NH$_2$ showed a brighter colour compared to PGMA because the FITC dye reacted with the amine groups of PGMA-NH$_2$. This confirmed the presence of the primary amine in the structure of PGMA-NH$_2$ particles. The reaction between FITC and the amine group in PGMA-NH$_2$ is shown in Figure 3.12. The primary amine on PGMA-NH$_2$ reacts with the S=C=N group of FITC dyes and forms a covalent bond. Therefore, the aminated particles become fluorescent. PGMA particles exhibit a much paler colour (Figure 3.11(a)) due to the physically absorbed FITC on the particles.

![Figure 3.11 Fluorescence microscopy images of FITC labelled for (a) PGMA and (b) PGMA-NH$_2$ particles](image)

![Figure 3.12 The reaction between FITC and amine group](image)
The fluorescent intensity is a measure of the FITC concentration, which should be proportional to the primary amine content. Therefore, the fluorescent intensity could be indicative of the primary amine concentration in the particle structure. As seen in Figure 3.11(b), there was clearly a strong fluorescence emission in the shell, while the fluorescence intensity was low in the core. This may indicate the concentration of primary amine was low in the core.

### 3.3.1.4 Elemental analysis of the particles

Elmas et al. demonstrated two methods for calculating the primary amine content of aminated PGMA particles. First, by using elemental analysis, they found that approximately 48% (w/w) of epoxide groups are converted into the primary amine. Secondly, the volume fraction of the fluorescent outer shell in the whole sphere was calculated as approximately 52% (v/v) by using the radial distance of the fluorescent shell and the whole particle diameter. For our work, we calculated the percent of epoxide groups, which were converted to primary amines by using the elemental analysis of PGMA and PGMA-NH$_2$ (Table 3.3).

![Schematic showing aminolysis of PGMA used for calculation the percent of aminolysis](image)

**Figure 3.13** Schematic showing aminolysis of PGMA used for calculation the percent of aminolysis

Based on the theoretical nitrogen content after complete aminolysis of all epoxide groups, reported by Elmas et al. which is 8.8%, the following equation was used to estimate percent of aminolysis from microanalysis data:

$$\%\text{Aminolysis} = \frac{\%N}{8.8} \times 100$$

(3.2)

The nitrogen content, %N, is from the elemental analysis data of PGMA-NH$_2$ (Table 3.3). About 40 mol% of epoxide groups reacted with ammonia and converted to primary amines.

There is no evidence of swelling for PGMA-NH$_2$ particles because of crosslinking of PGMA and PGMA-NH$_2$ (Figure 3.10). Therefore, a different approach for preparation of cationic microgel contains primary amine is needed.
3.3.2 PNVF-xGMA particles
These particles use an alternative approach by replacing some GMA with hydrophilic NVF which are able to be converted to primary amines by alkali hydrolysis.

3.3.2.1 Particle characterisation
PNVF-xGMA particle dispersions were initially studied using optical microscopy. They showed low polydispersity for PNVF and PNVF-xGMA prepared by batch NAD polymerisation (Table 3.4). This is because of a short nucleation period and rapid attainment of a constant particle number. Whereas the polydispersity was larger for PNVF-0.4GMA, which was prepared by a feed method since the nucleation stage occurred continuously throughout the feed. The particles did not dissolve in water or ethanol. Optical micrographs of particles dispersed in ethanol or water are shown in Figure 3.14. PNVF-xGMA particles dispersed in water were larger than in ethanol since water is a good solvent for PNVF, which is a major component of the copolymer particles. This means PNVF-xGMA particles swell in water and are therefore microgels. The larger particle size in water can be seen from both hydrodynamic diameter ($d_h$) and $d_{opt}$ values in Table 3.4. The $d_h$ values were obtained by PCS and sizing by optical micrographs were measured in the fluid state; whereas, the particles were dried for SEM. This can cause microgel particle shrinkage, altering the particle size. This is why the $d_{SEM}$ values are smaller.

Table 3.4 Particles and dispersion properties

<table>
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<tr>
<th>Code</th>
<th>$W_{PVP}$</th>
<th>$d_{opt}$ (µm)</th>
<th>$d_h$ (µm)</th>
<th>$d_{SEM}$ (µm)</th>
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<td>Water</td>
<td>Ethanol</td>
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<tr>
<td>PNVF</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>PNVF-0.2GMA</td>
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<td>0.90 [8]</td>
<td>0.80</td>
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<td>1.30</td>
</tr>
<tr>
<td>PNVF-0.4GMA(F)</td>
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<td>0.80 [19]</td>
<td>0.90 [22]</td>
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<td>PNVF-0.75GMA</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PGMA</td>
<td>0.15</td>
<td>1.90 [9]</td>
<td>2.10 [6]</td>
<td>-</td>
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<tr>
<td>PGMA_HPVP</td>
<td>0.30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$Weight fraction of PVP used (calculated based on monomer mass). $^b$Number-average diameter measured by optical microscopy for particles dispersed in water or ethanol. The number in parentheses is the coefficient of variation. $^c$Hydrodynamic diameters of the particles dispersed in water or ethanol. $^d$Number-average diameter from SEM. The number in square brackets is the
coefficient of variation (CV). CV = \[100 \times (\text{SD}/\text{mean})\], SD is standard deviation and mean is average particle diameter.

The N:C ratio ($R_{NC}$) from elemental analysis data (Table 3.5) was used to determine the particle composition. They all showed experimental ratios, that are close to the theoretical values, which were calculated from the compositions used to prepare the particles.

Figure 3.14 Optical micrographs of particles dispersed in ethanol or water
Using optical microscopy it was not possible to observe the particle morphology. Detailed resolution of the particle morphology was further achieved by SEM. This shows particles varying in diameter from 0.8 – 1.90 μm for PNVF-xGMA. The variation of diameter depended on the weight fraction of PVP used (\( W_{\text{PVP}} \)). The \( W_{\text{PVP}} \) values were calculated based on the total monomer mass. The particle size decreased with increasing \( W_{\text{PVP}} \) (Figure 3.15). The function of PVP is to stabilise the growing particles in the dispersion during NAD polymerisation.\(^3\) An increased surfactant concentration stabilises the growing particle nuclei earlier, which leads to increasingly smaller particles. The same effect of \( W_{\text{PVP}} \) on particle size was also found for the PGMA preparation; PGMA with a higher PVP concentration (PGMA\( _{\text{HPVP}} \)) showed a smaller particle size compared to PGMA (Figure 3.16(f) and (g)).

---

**Table 3.5 Elemental analysis data for PNVF, PGMA and PNVF-xGMA\(^a\)**

<table>
<thead>
<tr>
<th>Code</th>
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<th>%N</th>
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<th>( R_{\text{NC}}^{c} ) (theory)</th>
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<td>PGMA</td>
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<td>0.2</td>
<td>0.003</td>
<td>0</td>
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<td>PNVF</td>
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<td>10.6</td>
<td>0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>PNVF-0.4GMA(F)</td>
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<td>7.3</td>
<td>9.4</td>
<td>0.19</td>
<td>0.22</td>
</tr>
</tbody>
</table>

\(^a\)%C, %H, and %N are percentage by mass. \(^b\)\( R_{\text{NC}} = \frac{\%N}{\%C} \). \(^c\)Theoretical ratio of %N and %C are calculated based on numbers of N and C in the molecular structure of PGMA, PNVF and PNVF-xGMA.
3.3.2.2 Morphology of the particles
PNVF-xGMA particles showed a morphology with interconnected ridges on the surface (Figures 3.16(b)-(c)). The width of the ridges for the PNVF-xGMA particles increased with increasing $x$ (Figure 3.16(b)-(d)). The interconnected ridges were also found for PGMA particles but they were broader and less pronounced (Figure 3.16(f) and (g)). This morphology was also found for particles dispersed in water (Figure 3.17(b)-(d)). In contrast, the PNVF particles had a smooth surface (Figure 3.16(a)). We propose that PNVF-xGMA had a core-shell morphology because the morphologies for all the PNVF-xGMA particles were similar to the morphology of PGMA, but they were very different from that of PNVF (Figure 3.16(a)). This suggests that the surface of the PNVF-xGMA particles was rich in PGMA.
Figure 3.16 SEM micrographs of particles deposited from ethanol
Figure 3.17 SEM micrographs of particles deposited from water

The core-shell morphology, proposed for PNVF-xGMA, is consistent with the difference of the propagation rate constants between the two monomers (NVF and GMA). A propagation rate constant ($k_p$) of NVF$^{31}$ is at least $4 \times 10^3$ L·mol$^{-1}$·s$^{-1}$ and NVF is a highly reactive monomer. The $k_p$ for GMA has not been reported, to the author best knowledge, but the $k_p$ for methyl methacrylate (MMA) is about$^{32} 10^3$ L·mol$^{-1}$·s$^{-1}$. This means PNVF polymerises faster than PGMA and they should form the core of the particles. Then, PGMA should have formed the shell because it is slower polymerising and would have been swept up the PNVF particles. However, PGMA is not likely to have penetrated the PNVF core. PNVF and PGMA are
chemically different polymers; they are not likely to be compatible. PNVF is hydrophilic and PGMA is hydrophobic. Therefore, the PGMA phase should minimise its interfacial contact area with a PNVF core. NVF was, however, able to dissolve PGMA and could have reacted with a PGMA phase present as a shell on these particles.

Several studies showed the unusual morphology pattern on the surface of particles. Okubo et al.\textsuperscript{33} studied the morphology and the formation mechanism of golf ball-like polystyrene/polybutyl acrylate composite particles. Hwangbo et al.\textsuperscript{34} introduced a simple fabrication method for uniform golf-ball-shaped microparticles with an internal porous structure. Zhao et al.\textsuperscript{35} prepared highly folded crosslinked polymeric microparticles via a one-pot suspension polymerisation method under high-speed homogenisation. The wrinkles of particles result from the evaporation of solvent in the crosslinked microparticles. However, the morphologies in this work (Figure 3.16 - 3.17) are different from “golf-ball”-like morphologies and “wrinkled” morphologies. The particles in our work consist of interconnected ridges and look similar to a cane-ball (see Figure 3.18) in appearance and they are termed “cane-ball”-like morphologies. These characteristics have not been reported elsewhere.

![Image of a cane ball](image)

Figure 3.18 Image of a cane ball

We have compared our particles to golf-ball-like particles\textsuperscript{33,34} and wrinkled particles\textsuperscript{35}, which have been reported. The morphologies of cane-ball-like particles have features comparable to those reports, but they also are visually distinct due to the ridge shapes. We proposed that the ridges came from a separate PGMA-rich phase that forms on the surface of the particles. The morphology is most probably the consequence of non-uniform formation of the PGMA phase instead of just
wrinkling. The wrinkling mechanism is not supported for our particles because PNVF particles surface did not show the cane-ball-like morphology (Figure 3.16(a)). Moreover, high vacuum during SEM characterization also may have caused the partial particle shrinkage and contributed to the cane-ball morphologies.

### 3.3.2.3 FTIR analysis

Figure 3.19 shows FTIR spectra for the particles. The band at 3250 cm\(^{-1}\) in Figure 3.17(a) is the result of the amide group of NVF. PGMA, PNVF-0.2GMA, and PNVF-0.4GMA present the band at 905 cm\(^{-1}\) due to the stretching vibration of the epoxide group.\(^{10,36}\) This indicates that most of the epoxide remains unreacted upon formation of PNVF-xGMA. The band at 1725 cm\(^{-1}\) is due to RCOO groups within PGMA segments.\(^{24,25}\) An amide band appeared at 1650 cm\(^{-1}\) owing to the amide group (-NH(C=O)) from NVF segment.\(^{13}\) The amide I (1650 cm\(^{-1}\)) and amide II (1530 cm\(^{-1}\)) bands stay in the same places for each of the spectra. The N-C-H bending of NVF was responsible for the band at 1385 cm\(^{-1}.\)\(^{37}\) Thus, the FTIR spectra confirm the identities of the particles prepared.

![Figure 3.19 FTIR spectra of PGMA, PNVF-0.2GMA, PNVF-0.4GMA and PNVF](image)

### 3.3.2.4 Crosslinking of PNVF-0.4GMA

It was found that PNVF-xGMA could not dissolve in water and ethanol. Therefore, crosslinking between PNVF and PGMA was investigated. PNVF is soluble in water and PGMA is soluble in tetrahydrofuran (THF), respectively. PNVF-0.4GMA particles were dispersed in THF for 24 hours and then in water for a further 24 hours. In the case that the particles comprised independent phases of PNVF and PGMA,
they should have dissolved completely after these solubility tests. However, optical micrographs (Figure 3.20(a) and (b)) and SEM images (Figure 3.20(c)) show that PNVF-0.4GMA particles failed to dissolve after this treatment. This indicates that crosslinking occurred for the PNVF-0.4GMA particles. In addition, SEM for the PNVF-0.2GMA particles deposited from water (Figure 3.20(d)) showed intact particles, implying crosslinking for that system.

Crosslinking in PGMA has been reported in the work of Horak and Shapoval work.10 They stated that crosslinking was due to the ring-opening of oxirane groups during the NAD polymerisation in dimethylformamide/methanol medium. Insolubility of their products in common solvents, such as acetone or chloroform confirmed the crosslinking reaction in dimethylformamide/methanol medium. Moreover, the crosslinking between epoxide groups and polyhedral oligomeric silsequioxane (POSS) has been reported by Mu and Zheng.36 They prepared poly(N-
isopropylacrylamide) (PNIPAM) networks with POSS using octa(propylglycidyl ether) polyhedral oligomeric silsesquioxane (OpePOSS) as a nanocrosslinking agent. The crosslinking developed through the amide groups of NIPAM segments and epoxide groups of POSS (Figure 3.21). We propose a portion of the PNVF segments crosslink with the PGMA segments in the shell of the PNVF-0.4GMA particles based on Mu and Zheng’s work.$^{36}$

![Figure 3.21 The crosslinking of PNIPAM with OpePOSS$^{36}$](image)

The FTIR spectra for PNVF-0.2GMA and PNVF-0.4GMA were compared with those of physical blends of PNVF and PGMA (PNVF/\(x\)GMA) with the same respective mole ratios to quantify the crosslinking. Comparison of the FTIR spectra in Figure 3.22 exhibits a decreased absorbance of the epoxide band for the PNVF-\(x\)GMA particles compared to the PNVF/\(x\)PGMA homopolymer blends.
Figure 3.22 FTIR spectra of PNVF-xGMA and blends of PNVF and PGMA at the same mole ratios as PNVF-xGMA

From the FTIR spectra, the conversion (mol%) for the reaction of the epoxide groups and the NH groups of the amide was determined using equation 3.3. The ratios of the area of the epoxide band region \( AR_{905} \) to the area for the N–C–H band \( AR_{1385} \) were used in equation 3.3:

\[
\text{Conversion for reaction} = 100 \left( 1 - \frac{AR_{905,P}}{AR_{1385,P}} \right) \left( 1 - \frac{AR_{905,B}}{AR_{1385,B}} \right)
\]  

(3.3)

Where \( AR \) represents the peak area for the spectra and P and B refer to PNVF-xGMA particles and PNVF/PGMA blend, respectively. This equation has been adapted from Barbey and Klok.\textsuperscript{38} They measured the conversion of epoxide groups when reacted with amines as a function of reaction time by monitoring the peak area at 908 cm\(^{-1}\), which is characteristic for the epoxide group, and calculated the ratio of this area relative to that of the ester carbonyl peak at 1730 cm\(^{-1}\). For the calculated values of conversion for the reaction for the epoxide groups of PNVF-0.2GMA and PNVF-0.4GMA in our work, we assumed that the loss of epoxide groups is only due to reaction with the NH groups. The calculated values of conversion for the reaction for the epoxide groups of PNVF-0.2GMA and PNVF-0.4GMA were 62.7% and 60.7%, respectively. It follows that about 60 mol% of the epoxide groups reacted with NVF groups. This suggests a crosslinked PGMA-NVF copolymer and is consistent with the results of the solubility tests given above.
Figure 3.23 shows the proposed crosslinking reaction between GMA and NVF segments. Unreacted epoxide groups were present in the FTIR spectra (905 cm\(^{-1}\), Figure 3.19(b)). This may be due to steric hindrance not allowing PNVF and PGMA chains to closely approach. Furthermore, the proposed crosslinking reaction is supported by the RCOO (1725 cm\(^{-1}\)), amide I (1650 cm\(^{-1}\)), and amide II (1530 cm\(^{-1}\)) bands present in Figure 3.19.

![Diagram of proposed crosslinking reaction of PNVF and PGMA](image-url)
3.3.3 Hydrolysis of PNVF-xGMA

3.3.3.1 Morphology of the particles

The hydrolysed PNVF-0.4GMA (H-PNVF-0.4GMA) particles were examined by SEM. The SEM images before (Figure 3.16(c)) and after hydrolysis (Figure 3.24(a)) showed large differences in morphology. After hydrolysis, particle shells were fractured and the core copolymer was probably released from the crack during the washing steps. The average diameter of H-PNVF-0.4GMA (1.21 µm) is noticeably larger than PNVF-0.4GMA particles displayed in Figure 3.16(c) (1.0 µm). A distinct morphological change can be seen when the particles were rinsed with water and the pH and ionic strength reduced (Figure 3.24(b)). The core particles were usually unchanged, whereas the external shell seems to have detached. A different polymer phase to the shell made the particles appear darker because the SEM contrast is sensitive to electron density. This new phase is proposed to be incompletely hydrolysed PNVF, namely PNVF-VAM. A core-shell morphology for PNVF-xGMA was supported by the H-PNVF-0.4GMA SEM micrograph (Figure 3.24(b)). The shells are detachable, and leave the core particles, which have the typical diameter 1.53 µm. Moreover, surrounding of core are the detached nano-sized particles that came from the shell fracture. Based on the SEM images of particles during redispersion and purification, we propose that the core of particle expanded and caused fragmentation of the fractured, hydrolysed, shell (Figure 3.24(a)). This is a result from stress imbalance between the core and shell.\(^{39}\)

![Figure 3.24 SEM images of H-PNVF-0.4GMA (a) as made particles after hydrolysis at pH = 11.3 showing a large crack on the shell (b) particles with unattached shells and enclosed cores after water rinsing at pH = 7.4](image-url)
3.3.3.2 Crosslinking of H-PNVF-0.4GMA

A simple dissolution experiment was used to examine the crosslinking of the core of H-PNVF-0.4GMA which were found by SEM (Figure 3.24(b)). A drop of water was placed on the settled particles, causing them to redisperse (Figure 3.24(b)), and they were allowed to dry out overnight before being examined by SEM. The image (Figure3.25(a)) shows some of the particles relocated from the detached nanoparticles shell layer and gaps in the deposited nanoparticle layer (black arrows)). The particles lifted away from aggregated shell particles and redeposited in the middle of the SEM stub (Figure 3.25(b)). From SEM images of the aggregated H-PNVF-0.4GMA at higher magnification (Figure 3.25(c) and (d)), the core particles had swollen additionally and were still enclosed by some nano-particles on the shell. Some of the particles, which remained on the stub (Fig. 3.25(a) (red arrows), were flattened suggesting light crosslinking of the core particles. The undamaged core particles (Figure 3.25(b)-(d)) indicated that self-crosslinking occurred inside the core. Gu et al.40 studied free radical polymerisation kinetics of NVF in bulk and in aqueous solution.40 They found a “gel-effect” for both bulk and solution polymerisation at high monomer concentration due to chain entanglement. Based on this knowledge, self-crosslinking in our work may possibly come from branching at some stage in PNVF polymerisation at locally high concentrations (e.g. within monomer swollen particles).31,40
Figure 3.25 SEM images of H-PNVF-0.4GMA particles after redispersion in water and droplet evaporation. The particles were then rehydrated by placing a drop of water on the SEM stub. (a) some of the cores lifted off the surface (arrows indicate the lift-off points) (b) particles redeposited at the centre of the stub (c) and (d) redeposited particles at high magnification showing the shell sticking on the core particles

Hydrolysed PNVF-0.2GMA (H-PNVF-0.2GMA) particles presented some important distinctions compared to H-PNVF-0.4GMA. Before hydrolysis, PNVF-0.2GMA deposited in water showed a “cane-ball”-like morphology (Figure 3.26(a)). Compared to the particles before hydrolysis (Figure 3.26(a)), fragmentation of PGMA shells occurred (Figure 3.26(b)) upon hydrolysis and this released the PNVF-VAM. This was due to the inadequately crosslinked core and shell. This demonstrates that to prohibit particle dissolution by hydrolysis, a minimum mole fraction of PGMA of at least 0.4 was needed.
3.3.3.3 FTIR analysis

Changes in the chemical structure of the particles after hydrolysis were examined by FTIR. The spectra of H-PNVF-0.4GMA (Figure 3.27) confirm the VAM (amine group) has been created after hydrolysis in alkaline condition. Hydrolysis eliminates the band at 1725 cm$^{-1}$ which is assigned to the C=O stretching of the ester groups and also the epoxide band at 905 cm$^{-1}$. This reveals that the OH-groups can penetrate the PGMA domains. However, the amide I (1660 cm$^{-1}$) can be found for H-PNVF-0.4GMA, which implies that unreacted PNVF exists. A new band at 1560 cm$^{-1}$ is present in the spectrum of H-PNVF-0.4GMA that was not evident in the PNVF spectrum. We suggest that this band belongs to the a primary amine group (RNH$_2$)$^{13}$. The intensity of this band means that considerable PVAM was present in the purified particles. The broad peak at 3250 cm$^{-1}$ in the H-PNVF-0.4GMA spectrum (Figure 3.27(a)) indicates a mixture of PVAM and PNVF. Based on comparison of the FTIR spectra of H-PNVF-0.4GMA, PNVF-0.4GMA, and PVAM (Figure 3.27), it follows that hydrolysis was not complete and H-PNVF-0.4GMA contained mainly PNVF.
3.3.3.4 Fluorescence labelling

Hydrolysis of PNVF-0.4GMA causes particle swelling, whereas the crosslinking prevents particle dissolution. To further probe the morphologies of the particles, we studied the particles by labelled them using FITC (structure shown in Figure 3.12), sodium fluorescein (Figure 3.28(a)) and rhodamine B (Figure 3.28(b)). Fluorescence microscopy images of FITC labelled particles of PNVF-0.4GMA and H-PNVF-0.4GMA at pH = 6 - 7 are shown in Figure 3.29(a) and 3.29(c), respectively. The H-PNVF-0.4GMA particles had a lower brightness at the particle cores (Figure 3.29(c)). This means that an increase FITC concentration was present at the particle shell compared to the core, and indicates greater amine content in the particle shell than the core. The FITC images support a core-shell structure.

Figure 3.28 The structure of (a) Sodium fluorescein and (b) Rhodamine B
Figure 3.29 (a) Fluorescence images of FITC labelled PNVF-0.4GMA particles (b) optical micrograph of H-PNVF-0.4GMA (c) Fluorescence image of FITC labelled H-PNVF-0.4GMA particles (d) Fluorescence image of sodium fluorescein labelled H-PNVF-0.4GMA particles and (e) Fluorescence image of rhodamine B labelled H-PNVF-0.4GMA particles. The pH of the particles was 6 – 7.
The charge distribution was studied by labelling particles using sodium fluorescein or rhodamine B for 16 h (Figure 3.29(d) and (e), respectively). We found the particles were larger than those labelled by FITC (Figure 3.29(a)). This showed the particles were swollen in these chromophores. Sodium fluorescein is an anionic fluorophore and it was found at the edge as well as in the core of the particles (Figure 3.29(d)), which is comparable to FITC. This signifies that positive charges exist in the core and shells. In contrast, rhodamine B is a cationic fluorophore (contain positively charge). It was less able to pass through to the core so the large intensity was present at the edge of particles (Figure 3.29(e)). This suggested negatively charged areas of the shell. From these fluorescent images, we hypothesised that the particle shells contained both positive and negative charges. The electrophoretic mobility data in Figure 3.30 confirms this proposal. The periphery is mostly negatively charged at pH of 6 – 7. Meanwhile the FITC and sodium fluorescein labelled particles demonstrated positive charges in the core. These investigations indicate that the H-PNVF-0.4GMA core-shell particles contain positive and negative charges (polyampholytes) at pH 6 - 7.

3.3.3.5 Study of electrophoretic mobility and particle size after rinsing with water

Changes in size and electrophoretic mobility of PNVF-0.4GMA after the hydrolysis process were studied. Relationships between size (swelling) and electrophoretic mobility and pH are shown in Figure 3.30. The H-PNVF-0.4GMA particles were centrifuged and redispersed in 1, 0.1, and 0.01 M NaOH solution respectively, and finally in water. The electrophoretic mobility value of PNVF-0.4GMA is small and negative (-0.09×10^{-8} m^2 s^{-1} V^{-1}) (shown in solid filled marker) then, after hydrolysis, the electrophoretic mobility increased to around 4.62×10^{-8} m^2 s^{-1} V^{-1} at pH = 14. When particles were redispersed in lower NaOH concentration solutions and in water, the electrophoretic mobility values turned highly negative (-0.73×10^{-8} to -1.81×10^{-8} m^2 s^{-1} V^{-1}) at pH ≤ 13.5. At high pH, a very high electrophoretic mobility (4.62×10^{-8} m^2 s^{-1} V^{-1}) is proposed to be a result of PVAM-rich polymer at the particle surface.
Figure 3.30 The particle diameter and electrophoretic mobility of H-PNVF-0.4GMA vs. pH

Considering the size of the particles (Figure 3.30), the maximum diameter was seen at pH 9 - 11, which is close to the pKₐ of PVAM, which is 10.⁴¹ At pH lower than 9 - 11, the particle size decreased. Both particle swelling and changes of electrophoretic mobility from positive to negative were not reversible. This is because of the pH-triggered swelling and loss of PVAM segments in the core. These results correspond to the shell fragmentation at pH 7.4, as shown by SEM images in Figure 3.24(b). At pH < 10, we proposed that the shell fragmentation occurred and the core PVAM copolymer was released and this decreased the swelling pressure and particle size.

Particle aggregation occurred during purification by redispersion in water (Figure 3.31). This is attributed to a charge-patch aggregation mechanism, which occurred when the hydrolysed PNVF-0.4GMA particles contained both positively and negatively charged patches. This mechanism has been reported for related particles at low ionic strengths.⁴²,⁴³ This heteroaggregate-like pattern was also consistent with Figure 3.25 (c) and (d). pH-triggered aggregation was due to this mechanism. As water rinsing progressed the pH decreased and aggregation became more pronounced, as shown in Figure 3.31.
A charge-patch aggregation mechanism is shown in Figure 3.32. After hydrolysis, the PVAM core swell causes the PGMA rich shell fragment. This allows the release of some of the PVAM through fractures. The anionic hydrolysed PGMA-rich nanoparticles adhere to the surface of the locally cationic PNVF-VAM core particles.
3.3.3.6 Elemental analysis

Elemental analysis was used to characterise the particles after hydrolysis and redispersion in water. The data are shown in Table 3.6. The hydrolysed particles show a reduction of the N:C ratio (R_NC), compared to the parent particles. The R_NC for H-PNVF-0.4GMA was 0.17; whereas, the value from theory (calculation) was 0.27. This value was calculated based on the completely hydrolysed PVAM-0.4GMA. The decrease in the R_NC is probably a result of loss of core polymer which is PNVF-VAM copolymer. By comparison, PNVF particles were hydrolysed for 24 and 48 h, and we found that the R_NC were 0.53 and 0.54, respectively. These values indicate that the percentage conversions of PNVF to PVAM are 80 and 84%, respectively. This implies that the effectiveness of hydrolysis for PNVF-0.4GMA was much less than 80%. Hydrolysis efficiency of H-PNVF-0.4GMA was low because PNVF-0.4GMA particles was not soluble and remained as particles; whereas, PNVF dissolved completely in aqueous NaOH solution. NaOH penetration was not through for PNVF-0.4GMA. Because of the loss of polymer in the core segment during particle purification, the elemental analysis data could not be used to calculate the degree of hydrolysis.
Table 3.6 Elemental analysis data of PNVF-0.4GMA, H-PNVF-0.4GMA, PNVF and PVAM (24h and 48 h reaction)\(^a\)

<table>
<thead>
<tr>
<th>Code</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
<th>(R_{NC})^(b)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>10.6</td>
<td>0.214</td>
</tr>
<tr>
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</tr>
<tr>
<td>PVAM (24 h reaction)</td>
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<td>24.1</td>
<td>0.530</td>
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<tr>
<td>PVAM (48 h reaction)</td>
<td>45.2</td>
<td>11.0</td>
<td>24.3</td>
<td>0.538</td>
</tr>
</tbody>
</table>

\(^a\)%C, %H, and %N are percentage by mass. \(^b\)\(R_{NC} = \frac{%N}{%C}\)
3.4 Conclusions

Microgel particles containing primary amine groups have been successfully prepared. In the first step, the parent microgel PNVF-xGMA was prepared via non-aqueous dispersion (NAD) polymerisation. This microgel swelled in water, which was confirmed by optical micrographs and particle size by photon correlation spectroscopy (PCS). The particles sizes were in the range 0.8 - 1.8 µm. The size can be controlled by varying the weight fraction of GMA. SEM images showed a unique “cane-ball”-like morphology with interconnected ridges on the surface, which has not been reported before. PNVF-xGMA had a core-shell structure; the PNVF is mostly in the core whereas the shell is PGMA. This was proposed by the SEM images of the particles. The crosslinked shell is due to the reaction between epoxide and amide groups. Alkaline hydrolysis for PNVF-xGMA converted PNVF core to PVAM. The existence of amine groups were confirmed by FTIR. However, the amide band was still present and this implied incomplete hydrolysis. The core-shell structure and charge distribution were examined using different fluorescently labelled particles. FITC labelling at the periphery demonstrated the presence of amine groups and verified the core-shell structure. From the scanning electron micrographs, core swelling and shell fragmentation appeared after purification and redispersion in water. This caused PGMA to detach and the release of PNVF-VAM from the core. The charge on the shell became negative overall after hydrolysis and lead to charge-patch aggregation.
3.5 References


Chapter 4

Preparation and characterisation of poly(vinylamine) microgels: pH-responsive and high primary amine content particles

Abstract
Whilst anionic pH-responsive microgels are more developed it has proven especially challenging to prepare cationic pH-responsive microgels with high primary amine contents. In this chapter, we establish a simple and scalable, NAD polymerisation method for preparing new cationic pH-responsive microgels with high primary amine contents. Initially, poly(N-vinylformamide-co-2-(N-vinylformamido) ethyl ether particles (PNVF-xNVEE) were prepared using a range of x values (x is the mol% of NVEE used). These microgels were water swellable and colloidally stable and represent a new family of microgels. Then, the PNVF-xNVEE microgels were hydrolysed in isopropanol or water to provide poly(vinylamine-co-bis(ethyl vinylamine) ether) (PVAM-xBEVAME) microgels. Aqueous hydrolysis was found to give the most efficient hydrolysis. SEM images showed that the PVAM-xBEVAME microgel particles were cluster particles. The PVAM-xBEVAME particles exhibited pH-triggered increases in the electrophoretic mobility and hydrodynamic diameter. The primary amines within the microgels were used as chemical handles to attach pyrene carboxylic acid via N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide (EDC) chemistry. Because these new microgels are structurally associated with polyethylenimine (PEI) and have high content of primary amine groups, they potentially have extensive biomaterial applications in the future.
4.1 Introduction and aims

In this chapter, we study poly(vinylamine) (PVAM) microgels. Because PVAM has the highest nitrogen content of all the amine based polycationics\textsuperscript{1}, the high reactivity of these primary amines make it a very attractive polymer. However, the synthesis of microgels particles which have high primary amine contents has remained challenging. In this chapter, we introduce a method for preparing cationic pH-responsive microgels with high primary amine contents. The PNVF-xGMA microgel particles in Chapter 3 offer a route to primary amine functionalised microgels. However, the particle shells fragment when hydrolysed and charge patch aggregation occurred after purification. Based on Mohammadi and Berkland’s work\textsuperscript{2}, we hypothesised that particle stability could be improved by preparing NAD polymerisation of NVF with a crosslinker which has similar structure to NVF and also does not cleave when hydrolysed. The non-degradable crosslinker used in this work is 2-\textit{(N-Vinylformamido) ethyl ether} (NVEE). This crosslinker contains an ether linkage which survives after hydrolysis. Moreover, here we demonstrate the possibility of using \textit{N}-\textit{(3-dimethylaminopropyl)}-\textit{N’-ethylcarbodiimide} (EDC) chemistry to covalently link the primary groups onto the PVAM microgels. EDC chemistry was selected since it is commonly used for conjugation of biomaterials.\textsuperscript{3} Here we replaced the GMA with NVEE.

pH-responsive microgels are particles which swell when the pH approaches the pK\textsubscript{a} of the particles.\textsuperscript{4-7} Most pH-responsive microgel research involves anionic microgels (the microgel contains negative charges) based on carboxylic acid groups, which swell in alkali conditions.\textsuperscript{8-11} Acid swellable microgels prepared from aromatic amines have been studied. Dupin et al.\textsuperscript{12} studied the kinetics of acid swelling of 2-vinylpyridine (2VP) microgels in the presence of divinylbenzene (DVB) crosslinker and used a cationic surfactant and emulsion polymerisation. They found that the critical pH for the latex-to-microgel swelling transition of poly(2-vinylpyridine) (P2VP) particles was in the range of 3.85 to 4.55, depending on the degree of crosslinking. Dynamic light scattering (DLS) studies confirmed the swollen microgels became highly cationic at low pH. However, microgels with a high primary amine content could be better for biomaterial applications because the amine groups are versatile for chemical substitution e.g. conjugation with peptides.\textsuperscript{13}
In addition, microgel particles containing high primary amines are structurally comparable to PEI which has been commonly used for DNA delivery.\textsuperscript{14}

pH-responsive microgels, which contain primary amines, have been investigated by a number of groups. Microgels containing N-isopropylacrylamide (NIPAM) and NVF were reported by Pelton et al.\textsuperscript{15-17} The microgel was prepared by precipitation polymerisation and then hydrolysed to convert the NVF to VAM. However, the particles reported did not have a VAM content higher than 25%\textsuperscript{17}. Also, the particles that contained high amounts of VAM were colloidally unstable.\textsuperscript{15} Gan and Lyon\textsuperscript{18} also prepared PNIPAM core/shell microgels with 1 mol\% of 2-aminoethyl methacrylate (AEM) based on monomer using precipitation polymerisation. The latter was thermoresponsive and contained primary amine groups in either the core or shell. Recently, Han et al.\textsuperscript{19} introduced a method for preparing PVAM hydrogel shells, which were hollow-structured microcapsules. They found that the hydrogel shells had the ability of loading active ingredients and releasing them through pH changes\textsuperscript{19}. A couple of groups have reported PVAM capsules.\textsuperscript{20,21} PVAM hydrogel capsules which consist of water-filled cores surrounded by hydrogel shells have been studied by Kim et al.\textsuperscript{20} Shi and Berkland\textsuperscript{21} reported the synthesis and characterisation of pH-sensitive PVAM micro and nanocapsules that could degrade more quickly at reduced pH. Their further study showed that they can improve protein stability during nanogel fabrication by free radical copolymerisation of NVF and crosslinker, 2-bis[2,2′-di(N-vinylformamido)ethoxy]propane (BDEP).\textsuperscript{22} This may offer selective protein delivery to acidic tissues or intracellular organelles.\textsuperscript{22}

Berkland’s research group have reported the preparation of the 2-(N-vinylformamido)ethyl ether (NVEE) crosslinker.\textsuperscript{2} This was appropriate for the hydrolysis of PNVF to PVAM because NVEE is an alkali-stable crosslinker. In this chapter, we use NVEE (Figure 4.1) to prepare PNVF-\(\alpha\)NVEE microgel particles. Successful hydrolysis of the particles to colloidally stable PVAM-rich microgels resulted due to the NVEE crosslinker. Figure 4.2 shows the procedure used to prepare high primary amine content microgel particles. This method has not been reported elsewhere.
Figure 4.1 Structure of 2-[(N-vinylformamido)ethyl ether

![Structure of 2-[(N-vinylformamido)ethyl ether]

Figure 4.2 Procedure used for prepare PNVF-xNVEE and PVAM-xBEVAME

The procedure used in this chapter was developed in Chapter 3 and involved the preparation of PNVF-xGMA particles via NAD polymerisation. Hydrolysis was used to convert NVF to VAM microgels. As reported in Chapter 3, hydrolysed PNVF-GMA microgels fragmented during hydrolysis and released PVAM and the particles were not cationic at neutral pH. In this chapter, we used NAD to prepare PNVF-xNVEE which was then hydrolysed to PVAM-xBEVAME (Figure 4.2). The PVAM-xBEVAME microgels had a cluster-like morphology. These types of morphologies have been reported earlier.\textsuperscript{23,24,25} Li and Stover\textsuperscript{23} studied polymerisation of divinylbenzene (DVB) and found surface roughness of the “popcorn”-like particles prepared at 15-30 vol% of DVB. Also, the popcorn-like morphology has been reported for poly(methyl methacrylate) (PMMA) particles (Figure 4.3).\textsuperscript{24} However, here we provide the first report of pH-responsive microgel particles with cluster-like morphologies. Particles with surface roughness provide enhanced potential for biomaterial applications because they can enhance tissue growth.\textsuperscript{26}
PVAM-xBEVAME microgels were initially studied and the compositional variations occurring, due to hydrolysis, investigated. SEM was used to characterise the morphologies of the microgel particles. From this data, we suggest a mechanism for particle formation. The pH-responsive properties of the PVAM-xBEVAME particles were then measured using fluorescence microscopy, PCS and electrophoretic mobility measurements. Finally, the ability to use the primary amine groups to functionalise the microgel particles was demonstrated by using EDC chemistry. Considering that PVAM-xBEVAME particles are similar in structure to PEI, which is popular in delivery studies, the new microgels presented here have good potential for biomaterials application. The approach used for this study is shown in Figure 4.4.

Figure 4.3 SEM images of PMMA particles prepared by dispersion polymerisation at different monomer conversions.\textsuperscript{24}
Figure 4.4 Synthesis of PNVF-xNVEE microgels and their conversion to PVAM-xBEVAME microgels using alkaline hydrolysis
4.2 Experimental details

4.2.1 Preparation of NVEE

Based on the work of Mohammadi et al., the preparation for NVEE was as follows (Figure 4.5). NVF (98%) (7.1 g), potassium tert-butoxide (95%) (11.97 g), and dicyclohexyl-18-crown-6 (98%) (0.99 g) were dissolved in 100 mL THF in a 250 mL reactor equipped with an overhead IKA RW 20.n stirrer. The solution was stirred vigorously at room temperature for 45 min and then cooled in an ice bath for 20 minutes before bis(2-bromoethyl)ether (BBE, 95%) (9.28 g) was added dropwise during mixing in an ice bath at 0 °C. All reagents were purchased from Aldrich and used as received. The mixture was then stirred at room temperature for 72 h. In the next step, potassium bromide salt was removed by filtration, and then the reaction mixture was concentrated by removing half of the solvent using rotary evaporation and then diluted with 100 mL of water. The product was obtained by extraction with chloroform five times (40 mL × 5). The combined layers were washed twice with brine and dried over anhydrous sodium sulphate (40 g.) for 24 h. Finally, the product was recovered after concentration using rotary evaporation.

Figure 4.5 Schematic showing synthesis of NVEE

4.2.2 PNVF Dispersion Preparation

PNVF particles were prepared by using the procedure described in Chapter 3.2.1.

4.2.3 PNVF-xNVEE microgel Preparation

Three PNVF-xNVEE microgels (x is the mol% of the crosslinker (NVEE) used) were used for the study. These included PNVF-4NVEE, PNVF-9NVEE and PNVF-13NVEE. PNVF-4NVEE contained 96 and 4 mol% of NVF and NVEE, respectively. PNVF-9NVEE contained 91 and 9 mol% of NVF and NVEE, respectively. PNVF-13NVEE contained 87 and 13 mol% of NVF and NVEE,
respectively. The mol% was calculated based on preparation masses used. The compositions of each system are given in Table 4.1. All dispersions were prepared using NAD. The methods used to prepare and purify the dispersions were the same method as used for PNVF (described in chapter 3.2.1).

Table 4.1 Compositions for dispersion polymerisation

<table>
<thead>
<tr>
<th>Code</th>
<th>NVF (g)</th>
<th>NVEE (g)</th>
<th>AIBN (g)</th>
<th>PVP-PVA (g)</th>
<th>EtOH (g)</th>
<th>IPA (g)</th>
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<tr>
<td>PNVF-4NVEE</td>
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<td>0.85</td>
<td>0.24</td>
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<td>68</td>
<td>-</td>
</tr>
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<td>PNVF-9NVEE</td>
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<td>0.24</td>
<td>1.8</td>
<td>68</td>
<td>-</td>
</tr>
<tr>
<td>PNVF-9NVEE(I)</td>
<td>6</td>
<td>1.79</td>
<td>0.24</td>
<td>1.8</td>
<td>-</td>
<td>68</td>
</tr>
<tr>
<td>PNVF-13NVEE</td>
<td>6</td>
<td>2.68</td>
<td>0.24</td>
<td>1.8</td>
<td>68</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 4.6 Schematic showing the synthesis of PNVF-xNVEE

4.2.4 PVAM-xBEVAME microgel preparation
PNVF-xNVEE was converted to PVAM-9BEVAME by alkali hydrolysis (Figure 4.7). PNVF-xNVEE particles (1 g) were dispersed in water containing 1 M aqueous NaOH and heated whilst stirring to 80 °C for 16 h under a nitrogen atmosphere. For PNVF-9NVEE and PNVF-13NVEE, the hydrolysed dispersion obtained at the end was purified by centrifugation and redispersion in deionised water. In the case of PNVF-4NVEE, the hydrolysis product was extensively dialysed against water.
4.2.5 PVAM-9BEVAME microgel preparation by hydrolysis in isopropanol
PNVF-9NVEE particles (1 g) were dispersed in potassium hydroxide/isopropanol (KOH/IPA) (5 wt%) and heated to 80 °C whilst stirring for 16 h under nitrogen atmosphere. The hydrolysed dispersion obtained at the end was purified by centrifugation and redispersion in deionised water (Figure 4.8).

4.2.6 FITC labelling of PNVF-4NVEE and PVAM-4BEVAME microgel
20 µL of 0.1% FITC solution was added to 200 µL of 0.1 wt% dispersion. The mixed dispersion was then put on a rotational frame overnight.

4.2.7 Pyrene labelling of PVAM-9BEVAME microgel
PVAM-9BEVAE dispersion (5 mL of 0.1 wt% dispersion) was adjusted to pH 6.8 using 1M HCl. Then, N-(3-dimethylaminopropyl)-N”-ethylcarbodiimide (EDC) (5.1 mg, 0.033 mmol), and N-hydroxysuccinimide (NHS) (3.9 mg, 0.034 mmol) were
added. The dispersion was stirred at room temperature for 15 minutes before adding pyrene carboxylic acid (PyC) (4.1 mg, 0.017 mmol). The reaction was allowed to proceed for 18 h at room temperature before purification by centrifugation and redispersion in 0.15M PBS, 0.15M NaCl solution and then water respectively. A control dispersion was prepared via the same method without EDC and NHS. To remove the physically absorbed PyC, cetyltrimethylammonium bromide (CTAB) was added to the dispersion and it was stirred overnight at room temperature before being repeatedly centrifuged and redispersed in water.

![Chemical structures]

**Figure 4.9** The structures of (a) pyrene carboxylic acid (b) EDC and (c) NHS

### 4.2.8 Physical measurement

#### 4.2.8.1 Total solid content
Polymer dispersion (1 g) was poured into an aluminium dish and dried for at least 24 hr at room temperature in desiccators containing silica gel. The total solid content (TSC) was calculated using equation 3.1 (shown in Chapter 3, Section 3.2.7.1). For hydrolysed samples dispersed in water, they were dried in desiccators containing phosphorous pentoxide (P₂O₅) for 3 days. The fresh P₂O₅ was changed everyday.

#### 4.2.8.2 Optical microscopy
Images of particle dispersions were obtained via optical microscopy conducted using an Olympus BX41 microscope. As described in Chapter 3 Section 3.2.7.2, at least 80 particles were measured to determine the number-average diameters (d_{opt}). The particle swelling ratio (Q) was calculated using equation 2.1. Collapsed particle diameter, d_{(c)} was obtained using the particles dispersed in ethanol, which is a poor solvent for PNVF and PVAM.
4.2.8.3 Fluorescence Microscopy
Fluorescence microscopy was conducted using a Nikon Eclipse 50i microscope. The samples were illuminated with a mercury lamp. PyC labelled microgels were examined using a DAPI filter which allowed transmission of light at 475 nm. FITC labelled microgels were examined using excitation and emission wavelengths of 480 and 535 nm, respectively. The experimental method was described in Chapter 3, Section 3.2.7.3.

4.2.8.4 Scanning electron microscopy
SEM was undertaken using a Philips FEGSEM instrument. The particle dispersions and particle size measurement were prepared using the processes described in Chapter 3, Section 3.2.7.4.

4.2.8.5 Photon correlation spectroscopy
PCS measurements were conducted using a BI-9000 Brookhaven light scattering apparatus (Brookhaven Instrument Corporation) fitted with a 20 mW He-Ne laser (632.8 nm) and the detector angle was set at a 90° scattering angle. The sample preparation was described in Chapter 3, Section 3.2.7.5. The hydrodynamic diameter of particle ($d_h$) is an average value from ten runs.

4.2.8.6 Fourier transform infrared spectroscopy
FTIR measurements were conducted using a Nicolet 5700 FTIR equipped with an attenuated total reflectance (ATR) unit. The sample preparation and measurement was described in Chapter 3, Section 3.2.7.6.

4.2.8.7 Electrophoretic mobility measurements
The electrophoretic mobility measurements ($U_E$) were performed using a Zetasizernano ZS90 (Malvern Instruments Ltd.). Measurement and sample preparation were described in Chapter 3, Section 3.2.7.7.

4.2.8.8 Proton nuclear magnetic resonance spectroscopy
$^1$H NMR spectroscopy was undertaken using a Bruker 300 MHz instrument. The samples were dissolved at low concentration in deuterated chloroform (CDCl$_3$). The magnetic field was applied through the sample and caused the dipole moment of nuclei within the sample to reorient. Identical nuclei in different chemical
surroundings absorb electromagnetic radiation at differing frequencies. The separation between absorption peaks in NMR spectrum is usually referred to as chemical shift which was used to identify the chemical structure of a molecule (as described in Chapter 2, Section 2.6.6). Moreover, the area under each peak of spectrum is quantitative proportional to the number of protons in the molecule.

4.2.8.9 Elemental analysis

Elemental analysis (C, H, and N) was conducted using a Thermo Flash 2000 Organic Elemental Analyzer at the School of Chemistry, University of Manchester. The measurement and sample preparation were described in Chapter 3, Section 3.2.7.8.
4.3 Results and discussion

4.3.1 NVEE characterisation

NVEE is a difunctional monomer which was prepared from NVF. The synthetic method is shown in Figure 4.5. The NVEE was selected as crosslinker in our work because it can be alkali-hydrolysed without cleaving the ether linkage between crosslinking junctions.

4.3.1.1 NMR analysis

Figure 4.10 exhibits the $^1$H NMR spectra of NVEE and NVF. The peak positions are similar to those reported previously by Shi et al. For NVF, there are two hydrogen atoms on the amide ($e_{cis}$ and $e_{tr}$), also the vinyl hydrogen which is the nearest to the nitrogen ($c_{cis}$ and $c_{tr}$). These have trans and cis isomers. The trans-to-cis ratio of NVF was 3:1 from spectral integrations. This ratio is consistent with White’s report. In regards of NVEE, there are two hydrogens attached to amide ($e_{cis}$ and $e_{tr}$) and vinyl hydrogen which is closest to nitrogen atom ($c_{cis}$ and $c_{tr}$). The ratio of the combined areas for $e_{tr}/e_{cis}$ and $c_{tr}/c_{cis}$ are 1 to 2. Thus, the $^1$H NMR data indicates that the proportion of trans-to-cis species for NVEE is 1:2. The ratio of trans to cis was much smaller for NVEE compared to NVF. It was hypothesised that the cis isomer was favoured since it maximises separation of the unpaired oxygen electrons (from C=O) and the hydrogen atoms (labelled as f) in the CH$_2$ groups from the ether linkage.
Figure 4.10 $^1$H NMR spectrum of (a) NVF and (b) NVEE. The asterisk indicates a solvent (CHCl$_3$) peak.

4.3.1.2 Elemental analysis

The elemental analysis data for NVEE are shown in Table 4.2. The measured %C, %H and %N values are very close to the expected values from stoichiometry. This indicates the high purity of NVEE.
Table 4.2 Elemental analysis data for NVEEa

<table>
<thead>
<tr>
<th>Code</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected valuesb</td>
<td>56.6</td>
<td>7.6</td>
<td>13.2</td>
</tr>
<tr>
<td>Measured values</td>
<td>56.2</td>
<td>8.2</td>
<td>13.4</td>
</tr>
</tbody>
</table>

a% C, % H, and % N are percentage by mass. bExpected values were calculated based on the numbers of C, H, and N in structure of NVEE.

4.3.1.3 FTIR analysis

The FTIR spectra of NVEE and NVF are shown in Figure 4.11. The spectra for NVEE and NVF show similar bands at 1632 cm\(^{-1}\) and 1680 cm\(^{-1}\) which are indicative of vinyl groups\(^{29}\) and \(\text{–NHC(=O)H}\) stretch\(^{21}\), respectively. For the NVF spectrum, the band at 3260 cm\(^{-1}\) indicates N-H stretching vibration of the formamide group. The band at 1510 cm\(^{-1}\) is demonstrative of an amide II group, and the band at 1380 cm\(^{-1}\) is due to N-C-H bending\(^{30}\). These bands were absent in the NVEE spectrum, which confirmed our product did not have residual NVF. In addition to the characteristic groups identified for NVEE, including the new band at 1090 cm\(^{-1}\) which is ascribed to an ether group (CH\(_2\)-O-CH\(_2\))\(^{31}\), the absence of this band for NVF differentiates NVEE from NVF.

![Figure 4.11 FTIR spectra of NVF and NVEE](image)
4.3.2 PNVF-xNVEE particles

4.3.2.1 Particle characterisation

PNVF-xNVEE dispersions were prepared in ethanol (x = 4, 9 and 13) as well as IPA (PNVF-9NVEE(I)) using batch NAD polymerisation. We investigated the use of IPA as the solvent for preparation of PNVF-9NVEE particles base on Takemoto’s work.\(^{32}\) The particles were observed using optical microscopy and they were colloidally stable. The \(d_h\) values of the particles are in the range of 0.60 to 1.10 \(\mu m\) and the \(d_{opt}\) are in the range of 0.70 to 1.20 \(\mu m\) (Table 4.3). Figure 4.12 shows the optical micrographs of the particles dispersed in ethanol, water, and IPA. All of the particles dispersed in ethanol or IPA seem well defined (Figure 4.12(a), (c), (e) and (g)). Compared to the particles dispersed in water (Figure 4.12 (b), (d), (f) and (h)), they were more diffuse. This characteristic is evidence of swollen particles in water. Moreover, this was confirmed by the values of swelling ratio (\(Q\)) of the particles dispersed in water (Table 4.3). The \(Q\) values were greater than 1.0 for the particles dispersed in water. Figure 4.13 shows additional proof for particle swelling in water.

A PNVF-4NVEE white paste was collected from ethanol by centrifugation (Figure 4.13(a)) and a small volume of water added. It formed a clear physical gel quickly (Figure 4.13(b)). This result and \(Q\) values show that PNVF-xNVEE is a new group of water swellable microgels.

**Table 4.3 Particle properties of PNVF-xNVEE**

<table>
<thead>
<tr>
<th>Code</th>
<th>(d_{opt}^a) ((\mu m))</th>
<th>(d_h^b) ((\mu m))</th>
<th>(Q^c)</th>
<th>(d_{SEM}^d) ((\mu m))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Water</td>
<td>Ethanol</td>
<td>Water</td>
</tr>
<tr>
<td>PNVF</td>
<td>-</td>
<td>-</td>
<td>0.80</td>
<td>-</td>
</tr>
<tr>
<td>PNVF-9NVEE</td>
<td>0.90 [7]</td>
<td>1.10 [11]</td>
<td>0.70</td>
<td>1.30</td>
</tr>
<tr>
<td>PNVF-9NVEE(I)</td>
<td>0.70 [14]</td>
<td>0.80 [14]</td>
<td>0.60</td>
<td>0.90</td>
</tr>
<tr>
<td>PNVF-13NVEE</td>
<td>0.80 [9]</td>
<td>0.90 [14]</td>
<td>0.80</td>
<td>1.10</td>
</tr>
</tbody>
</table>

\(^a\)Number-average diameter measured by optical microscopy for particles dispersed in water or ethanol. The number in square brackets is the coefficient of variation. \(^b\)Hydrodynamic diameters of the particles dispersed in water or ethanol. \(^c\)Nominal swelling ratio at pH = 7 calculated using equation 2.1. \(^d\)Number-average diameter from SEM. The number in square brackets is the coefficient of variation (CV). CV = [100*(SD/mean)], SD is standard deviation and mean is average particle diameter.
Generally, the degree of swelling for microgel particles decreases with increasing the concentration of crosslinker.\textsuperscript{7} The maximum $Q$ was expected for PNVF-4NVEE. However, the data in Table 4.3 shows a maximum $Q$ of 7.0 for PNVF-9NVEE, whereas the $Q$ value of PNVF-4NVEE was only 3.0. The small $Q$ for PNVF-4NVEE was probably due to fragmentation of lightly crosslinked shells of microgel particles and they detach from the core. This causes the hydrodynamic diameter of microgels to decrease.\textsuperscript{33} Consideration of the lower $Q$ and the physical gel formed in Figure 4.13 for PNVF-4NVEE confirms particle swelling and means that partial fragmentation of the shell occurred when PNVF-4NVEE was dispersed in water. Self-rupturing particles are attractive for potential application in drug delivery.\textsuperscript{34}
Figure 4. Optical micrographs of PNVF-\(x\)NVEE particles dispersed in ethanol, IPA or water.
4.3.2.2 Morphology of the particles

SEM images of PNVF and PNVF-\text{xNVEE} particles deposited from ethanol are shown in Figure 4.14 and 4.16, respectively. Deformation and coalescence of PNVF particles can be seen from Figure 4.14. Flattening of the particles can be seen because PNVF is a hydrophilic polymer.\textsuperscript{35} The deformation was supported by optical micrographs of deposited particles (Figure 4.15). The PNVF particles had begun to spread within 5 min of deposition onto a microscope slide in the air and they coalesced. If the deposited particles were placed over phosphorous pentoxide (\(P_2O_5\)), this prevented the particles spreading. This confirmed that the spreading was caused by water vapour absorption. This form of solvent vapour annealing is a new effect for microgels\textsuperscript{36} and can occur at ambient temperatures. SEM images of PNVF-4NVEE particles deposited from ethanol also showed coalescence when in contact with neighbouring particles (Figure 4.16(a) and (b)). The diameter measured by SEM (\(D_{\text{SEM}}\)) of PNVF-4NVEE was the largest compared to PNVF-9NVEE, PNVF-9NVEE(I) and PNVF-13NVEE (Table 4.3). The smaller particle size of those particles indicates fewer spreading particles. PNVF-9NVEE (Figure 4.16(c) and (d)), PNVF-9NVEE(I) (Figure 4.16(e) and (f)) and PNVF-13NVEE (Figure 4.16(g) and (h)) particles were closely packed and did not coalesce. The SEM images and the diameter of the particles (Table 4.3) show that 9 mol\% of NVEE was sufficient to provide the high crosslinking density for the shell of PNVF-\text{xNVEE}, which resisted inter-particle coalescence and film formation over the duration of the drying process.

Figure 4.13 Gel formation (a) shows PNVF-4NVEE particles dispersed in ethanol and centrifuged particles. (b) shows PNVF-4NVEE particles dispersed in water
Figure 4.14 SEM micrographs of PNVF particles deposited from ethanol

Figure 4.15 Optical micrographs of PNVF particles (a) deposited from ethanol and (b) 5 minutes after ethanol had evaporated. The particles had begun to spread and coalesce in air.
Figure 4.16 SEM images of PNVF-xNVEE deposited from ethanol or IPA.
SEM images of PNVF-xNVEE particles deposited from water are shown in Figure 4.17. Particles spreading was noticeable for PNVF-4NVEE deposited from water (Figure 4.17(a) and (b)). The spreading of microgel particles which were swollen before deposition causes particle deformation. This effect has been frequently reported in SEM studies.\textsuperscript{37,38} Horigome et al.\textsuperscript{37} studied the drying mechanism of PNIPAM crosslinked $N,N'$-methylenebis(acrylamide) (BIS) microgel dispersions using digital camera, optical microscope and SEM. They found that when 0.1 and 0.01 wt% microgel dispersions were dried, the microgels were fused to each other and the spherical shapes of the microgels could not be seen clearly (see Figure 4.18).
Figure 4.17 SEM images of PNVF-xNVEE particles deposited from water.
Saunders and Vincent studied the effect of temperature and added free polymer on the swelling of PNIPAM microgel particles crosslinked with \(N,N'\)-methylene bisacrylamide (BA).\(^{38}\) They found that the particles appear to have formed oblate spheroids upon drying (Figure 4.19).

\[
\text{Figure 4.19 SEM image of PNIPAM/9.0BA}^{59}
\]

In our work, a lot of PNVF-4NVEE particles seemed to be missing and circular voids were found (Figure 4.17(a) and (b)). Those particles had flowed to a height lower than that of the neighbouring material. The latter was debris from the fragmented of PNVF-4NVEE shells. The voids in Figure 4.17(a) are a mark of swollen PNVF-4NVEE cores before they were entirely dried out. The approximate diameter of the voids was 1.80 µm and that is comparable to the \(d_h\) value for microgel particles in water (1.40 µm) (Table 4.3). Some of the fully spread particles were remarkable (arrow in Figure 4.17(a)). PNVF-9NVEE, PNVF-9NVEE(I) and PNVF-13NVEE particles deposited from water had low- to moderate-polydispersities (Table 4.3). SEM images show that the particles can oppose
coal coalescence when they were deposited in water (Figure 4.17(c)-(h)). This confirms that at least 9 mol% of NVEE crosslinking was sufficient to provide intact particles. PNVF-9NVEE at higher magnification (inset of Figure 4.17(d)) demonstrated that the surface of particles had a cluster-like morphology. The higher magnification images for PNVF-9NVEE(I) (inset of Figure 4.17(f)) revealed that the particles were in fact composed of aggregates of smaller particles. Some of these smaller particles separate from the particle during particle deposition. These particles also belong to the family of particles referred to as “popcorn”\textsuperscript{23} or “cluster particles”. Compared to PNVF-13NVEE (Figure 4.17(g) and (h)), the exterior region of the PNVF-13NVEE particles seemed to have flowed onto the substrate surrounding the core of the particles. This is compatible with a relatively lightly crosslinked PNVF-rich shell. A cluster-like morphology was not noticeable for PNVF-13NVEE.

4.3.2.3 FTIR analysis

FTIR spectra of PNVF-\textit{x}NVEE and PNVF particles are shown in Figure 4.20. The spectra of PNVF-\textit{x}NVEE microgels had several features in common with PNVF. The band at 3260 cm\textsuperscript{-1} is consequence of N-H bending of formamide group. The amide I band at 1650 cm\textsuperscript{-1} is a result of –NHC(=O)H stretch\textsuperscript{21} from NVF. Band at 1540 cm\textsuperscript{-1} is due to the amide II.\textsuperscript{39} Moreover, the presence of the band at 1380 cm\textsuperscript{-1} which is ascribed to the N-C-H bending\textsuperscript{30} mode of the formamide group in PNVF-\textit{x}NVEE, supports the view that NVF is main component of the microgel particles.

![Figure 4.20 FTIR spectra of PNVF and PNVF-\textit{x}NVEE](image-url)
4.3.3 PVAM-xBEVAME particles

4.3.3.1 Particle characterisation

Alkaline hydrolysis was used to prepare PVAM-xBEVAME from PNVF-xNVEE (Figure 4.7-4.8). Optical micrographs of PVAM-xBEVAME are shown in Figure 4.21. The PVAM-4BEVAME particles swelled strongly in water and could not be seen clearly by optical microscopy since the refractive index of the swollen microgels was very close to that of water. Therefore, the particles were labelled with FITC to be visualised by fluorescence microscope (Figure 4.21(a)). The FITC labelled PNVF-4NVEE particles appeared more sharp and distinct when compared with PVAM-4BEVAME (Figure 4.21(b)). The latter particles seemed smaller and had reduced brightness. However, PCS data (Table 4.4) showed that PVAM-4BEVAME microgels had the largest particle size. The reason why they looked smaller compared to the other microgels in Figure 4.21 is that the shells were swollen greatly and were unable to be seen by fluorescence microscopy. PCS determines the hydrodynamic diameter which is more sensitive to the peripheries of swollen particles. For PVAM-9BEVAME and PVAM-13BEVAME systems, the optical images showed microgel particles clearly (Figure 4.21(c) and (d)). This indicates less swelling occurred for those systems.

<table>
<thead>
<tr>
<th>Code</th>
<th>$d_h^a$ / µm</th>
<th>$Q^b$</th>
<th>$d_{SEM}^c$ / µm</th>
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</thead>
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<td>Ethanol (redispersed)</td>
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</tr>
<tr>
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<td>38</td>
</tr>
<tr>
<td>PVAM-9BEVAME</td>
<td>1.30</td>
<td>1.040</td>
<td>7.8</td>
</tr>
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<td>PVAM-9BEVAME(I)</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
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</tbody>
</table>

\(^a\)Hydrodynamic diameters of the particles dispersed in water or ethanol. \(^b\)Nominal swelling ratio at pH = 7 calculated using equation 2.1. \(^c\)Number-average diameter from SEM. The number in square brackets is the coefficient of variation (CV). CV = [100*(SD/mean)], SD is standard deviation and mean is average particle diameter.
Figure 4.21 PVAM-xBEVME and PNVF-4NVEE microgel particles dispersed in water (a) and (b) show fluorescence micrographs of FITC-labelled particles. (c) and (d) show optical micrographs. The pH in all cases was 7.

Based on related work on macroscopic PVAM hydrogel preparation\textsuperscript{32} we investigated the use of isopropanol (IPA) as the solvent for hydrolysis. An advantage of using isopropanol for preparation and hydrolysis was that the solvent did not have to be changed for each step. However, the dispersions initially aggregated upon hydrolysis in isopropanol. The extent of aggregation gradually diminished with extended hydrolysis duration over a 5 day period (Figure 4.22). This behaviour contrasted to the aqueous hydrolysis where the dispersions remained colloidally stable. Although we cannot be certain of the origin of this effect it could be due in part to lower electrostatic interactions in isopropanol compared to water.
Table 4.4 shows the particle size of PVAM-xBEVAME particles. The hydrodynamic diameter ($d_h$) was measured by PCS at pH = 7 for PVAM-xBEVAME dispersed in water. The $d_h$ values were in range of 1.31 to 1.72 µm which are greater than those for non-hydrolysed PNVF-xNVEE microgels (Table 4.3). To study the reversibility of particle swelling for PVAM-xBEVAME, they were redispersed in ethanol after being dispersed in water. It was found that $d_h$ decreased but they were still larger for both PVAM-9BEVAME and PVAM-13BEVAME when compared to PNVF-xNVEE particles measured in ethanol (Table 4.3). This demonstrates partial
irreversibility of particle swelling after changing solvent from a good to a poor solvent. Entire hydrolysis leads to significant mass loss owing to the release of formate (Figure 4.4). After hydrolysis, PVAM-\(x\)BEVAME lost the intra-segment interactions that had been present with non-hydrolysed PNVF-\(x\)NVEE particles, which may have prevented subsequent full collapse when redispersed in ethanol. Additional proof of shell fragmentation for PVAM-4BEVAME is the \(d_h\) measured in ethanol (0.51 µm, Table 4.4) was much smaller than for PNVF-4NVEE particles (0.96 µm, Table 4.3).

Swelling ratios of PVAM-\(x\)BEVAME were calculated using equation 2.1. However, it was complicated since they were made in the swollen state, unlike conventional microgels. Also, a mass loss occurred during hydrolysis. Nominal \(Q\) values for PVAM-9BEVAME and PVAM-13BEVAME were approximated via the \(d_h\) value of the parent PNVF-\(x\)NVEE in ethanol (Table 4.3) as values for \(d_{h(c)}\) in equation 2.1. These types of values are not exact since they did not consider the mass loss due to hydrolysis mentioned earlier. For \(Q\) value of PVAM-4BEVAME, a different method was used since the system had major shell fragmentation. The \(Q\) value was calculated via the \(d_h\) value of that microgel dispersed in ethanol (0.51 µm, Table 4.4). This method also gives an underestimate of the true value for the \(Q\) value because of the probability of partial irreversibility of microgel swelling in ethanol. All of the \(Q\) values measured at pH = 7 for PVAM-\(x\)BEVAME (Table 4.4) were larger than those for PNVF-\(x\)NVEE (Table 4.3) which is a consequence of ionised PVAM\(^+\) groups. The \(Q\) values for PVAM-\(x\)BEVAME microgel declined with increasing the crosslinker concentration as expected.

**4.3.3.2 Morphology of the particles**

SEM was used for further investigation of PVAM-\(x\)BEVAME. Those SEM images are shown in Figure 4.23. The morphology of PVAM-\(x\)BEVAME changed significantly when compared with the parent microgels (PNVF-\(x\)NVEE) (Figure 4.16 and 4.17). The particle surfaces were increasingly evident, which was probably because of the mass loss that occurred during hydrolysis. PVAM-4BEVAME displayed a high population of narrowly dispersed particles (Figure 4.23(a)). At high magnification, it showed a cluster-like morphology including dispersed nanometer-sized particles (Figure 4.23(b)). The cluster-like morphology is the cores of the
microgel particles. Moreover, some of them had partly fragmented (inset of Figure 4.23(b)), which is the result of inadequate crosslinking. Increase in the crosslinker effect to the particle morphology. For PVAM-9BEVAME (Figure 4.23(c) and (d)) and PVAM-9BEVAME(I) (Figure 4.23(e) and (f)), the particles had an undamaged cluster-like morphology. A nodule on the particle surface has been noticed for PNIPAM microgel.\textsuperscript{40} Kratz et al.\textsuperscript{40} reported atomic force microscopy (AFM) images of collapsed PNIPAM microgels (Figure 4.24). They reported roughness of the particle interface. This roughness also manifests in the “raspberry”-like pattern (a grain structured surface), which may arise from the collapse of dangling polymer chains on the particle surface. However, the surface morphology in our work is much more distinct.
Figure 4.23 SEM images of PVAM-xBEVAME particles deposited from water
At 13 mol% of crosslinker which is the maximum value for our work, PVAM-13BEVAME was more uniform (Figure 4.23(g) and (h)) and also appeared similar to that of the parent PNVF-13NVEE microgels (Figure 4.16(g) and (h)). This demonstrates that the concentration of crosslinker (BEVAME) determines the PVAM-xBEVAME particles morphologies and they can be tuneable. Moreover, we found that film formation of close packed particles did not appear for PVAM-9BEVAME and PVAM-13BEVAME which shows that the crosslinking which provided by NVEE remained after hydrolysis for these systems. This confirms the stability of the ether linkage in NVEE.

4.3.3.3 FTIR analysis
Alkaline hydrolysis of PNVF-xNVEE dispersions caused major changes in the spectra (Figure 4.25). All of the spectra for PVAM-xBEVAME are comparable to PVAM. New bands at 1590 cm\(^{-1}\) and in the region of 3260 cm\(^{-1}\) are the result of RNH\(_2\).\(^{21,41}\) However, these changes were least pronounced for PVAM-9BEVAME(I). For that system a major Amide I band at 1650 cm\(^{-1}\) was present. From these data it appears that the extent of conversion was greatest for PVAM-9BEVAME and least for PVAM-9BEVAME(I). Consequently, the FTIR
spectra support the view that hydrolysis of the PNVF-xNVEE particles to PVAM-xBEVAME was successful in water; however, it was incomplete when IPA was used. The FTIR spectra of PVAM-4BEVAME and PVAM-13BEVAME show the same features as present for PVAM-BEVAME. High extents of conversion are indicated.

Figure 4.25 FTIR spectra of PVAM-xBEVAME, PVAM and PNVF-9NVEE

4.3.3.4 Elemental analysis
The degree of hydrolysis for PVAM-xBEVAME was quantified by the N:C ratio ($R_{NC}$) from elemental analysis data (Table 4.5). In Chapter 3 using PNVF-xGMA microgels the $R_{NC}$ values decreased upon hydrolysis which was due to escape of PVAM. In contrast to that system, the $R_{NC}$ values for PVAM-xBEVAME increased compared to PNVF-xNVEE. This is a significant sign of effective hydrolysis. From the calculation using equation 4.2, the percentage conversions to PVAM-9BEVAME and PVAM-9BEVAME(I) microgels based on these values were 99% and 49%, respectively. These values agree with the trends established from the FTIR data (Figure 4.25) and show that hydrolysis was effectively complete for microgels hydrolysed in water. We attribute this to the fact that those particles were swollen, which facilitated penetration by OH$^-$ groups. In contrast the microgels were collapsed (deswollen) in IPA and this restricted OH$^-$ access. PVAM-9BEVAME and PVAM-13BEVAME showed efficient hydrolysis, whereas the percent of hydrolysis for PVAM-4BEVAME (72%) is much lower than PVAM-9BEVAME and PVAM-13BEVAME. We note that the PNVF-4NVEE particles were the largest of the
particles prepared (Table 4.3). This should result in a larger penetration distance for OH− required to completely convert these particles compared to the smaller PNVF-9NVEE and PNVF-13NVEE particles. A lower extent of conversion would seem reasonable on the basis of initial particle size. However, the data indicate very high proportions of primary amine groups were generated for each of the microgels; also the dispersions were colloidally stable. The $R_{SC}$ were used for calculate the mol% VAM (equation 4.1). It was found that the mol% VAM was about 90% for PVAM-9BEVAME and PVAM-13BEVAME. The PVAM-xBEVAME has been prepared in this work had the highest amount of primary amine groups for any colloidally stable microgel reported.

$$\% \text{Hydrolysis} = 100 \left[ \left( \frac{7x + 3}{x + 1} \right) - \left( \frac{1.1662}{\% N/\% C} \right) \right]$$

(4.1)

<table>
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<th>Code</th>
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<th>%H</th>
<th>%N</th>
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<td>10.3</td>
<td>20.3</td>
<td>0.47</td>
<td>96</td>
<td>84</td>
</tr>
</tbody>
</table>

*a* %C, %H, and %N are percentage by mass. *$R_{SC} = \% N/\% C$.* *%Hydrolysis calculated using equation 4.2.* *$X_{VAM}/\text{mol\%}$ of VAM calculated from the product of % Hydrolysis and x.

4.3.3.5 Study of pH-triggered swelling and electrophoretic mobility

The pH-triggered swelling of the PVAM-xBEVAME microgels can be studied via their hydrodynamic diameters ($d_h$) (Figure 4.26(a)). At $pH = 12$, the microgels were swollen which was partially resulting from the hydrophilic character of PVAM. PVAM-4BEVAME shows the greatest pH-triggered increase in $d_h$ upon declining the pH from 12 to 4. Compared to PVAM-9BEVAME and PVAM-13BEVAME, the pH-triggered swelling was weaker, this could be a consequence of the higher
concentration of crosslinking segments there. The pH-triggered swelling had occurred when the pH had lowered to 10 in all cases, that is the pK_a of PVAM^{12}. The nominal Q value was the most beneficial parameter used to compare the swelling (Figure 4.26(b)). These Q values were calculated using the d_h(c) values used in Table 4.4. The strong pH-triggered swelling when pH decreased was present for PVAM-xBEVAME, also the degree of swelling is tuneable by means of x.

![Figure 4.26 pH-responsive behaviour of PVAM-xBEVAME microgels (a) shows the hydrodynamic diameters and (b) shows Q values vs. pH](image)

The trends demonstrated in this work for pH responsive microgel are similar to those reported by Shi and Berkland.\textsuperscript{21} They prepared hollow PVAM capsules and found that the size of PVAM capsules changed in accordance with pH.\textsuperscript{21} They reported that PVAM capsules consist of pendant amine groups which are cationic or neutral, dependent on their pK_a and solution pH. When the particles were put into a medium
of pH ≤ pKₐ, the protonated amine groups were ionised and neutralised by counterions in solution contributing to an osmotic pressure, which swells the particle against its restoring elastic constant. Consequently, a decreased pH increases the extent of protonation and related counterion content of PVAM capsules, causing the particles to swell further (Figure 4.27).

![Figure 4.27 Size of PVAM capsules decreased as pH increased](image)

The electrophoretic mobility of the microgels at different pH are shown in Figure 4.28. All of them were positive and the electrophoretic mobility values increased as the pH reduced for each of the PVAM-xBEVAME. This was due to an increase in positive charge and mobile ion concentration within the particles, which was the reason for the pH-triggered swelling displayed in Figure 4.26. At pH ≤ 10, the positive electrophoretic mobility is an additional indication of efficient hydrolysis for PVAM-xBEVAME microgels since the pKₐ of PVAM is 10. At pH ≤ 7, the positive charge density at the boundary of the particles is highest for the minimum crosslinker concentration used for prepare the microgels. Figure 4.26(b) shows that PVAM-13BEVAME had a minimum swelling and displayed the least electrophoretic mobility increase with declining pH. These type of tendencies are an indication of a more strongly crosslinked shell. A high crosslink density level might obstruct protonation as confined polycationic chains are known to have decreased apparent pKₐ values.
4.3.3.6 Study of PVAM-9BEVAME microgel functionalisation using primary amines

The potential for the primary amines of the microgels to work as chemical handles was illustrated by covalently linking pyrene carboxylic acid (PyC) onto the PVAM-9BEVAME microgels using \(N-(3\text{-dimethylamino})\text{-propyl})-N'\text{-ethylcarbodiimide} (EDC) and \(N\text{-hydroxysuccinimide} (NHS) chemistry. EDC was used to couple carboxylic acid groups of pyrene carboxylic acid (PyC) to primary amines of the microgel. Including NHS during the reaction increases the reaction efficiency\(^{45}\). The mixing of PyC and microgel without EDC and NHS was used as a control experiment. The microgels were rinsed thoroughly with 0.15 M NaCl solution, then CTAB solution, and finally water to remove PyC. Figure 4.29 shows the fluorescence micrographs of the microgels after this experiment. The particles in which EDC and NHS were used evidently presented as fluorescently labelled. Compared to the control samples, no particles were imaged (Figure 4.29(c)). The optical micrographs confirm the existence of the particles (Figure 4.29(d)). The data from these experiments confirm effective functionalisation of PVAM-9BEVAME microgels via primary amine groups. The chemical reaction for this experiment is shown in Figure 4.30.
Figure 4.29 Pyrene-labelling of PVAM-9BEVAME microgels. (a and b) show fluorescence and optical micrographs of microgels mixed with PyC in presence of EDC/NHS (c and d) show micrographs of microgels mixed with PyC without EDC/NHS

Figure 4.30 Reaction for pyrene-labelling of PVAM-9BEVAME microgels in the (a) presence or (b) absence of EDC/NHS
4.4 Conclusions
The properties of a new family of cationic pH-responsive microgels that contain very high primary amine contents were examined in this chapter. The study has revealed that NVEE, which is convenient to prepare, enables the formation of colloidally stable PNVF-\(x\)NVEE microgels which swell in water. It was found that the shells of the PNVF-\(x\)NVEE microgels fragmented when the value for \(x\) was 4 mol\%. At 9 mol\% (or higher) of crosslinker used, the microgels did not coalesce or fragment when swollen. Moreover, the particles were able to be hydrolysed to generate cationic, pH-responsive PVAM-\(x\)BEVAME particles. The data showed that the aqueous hydrolysis was most efficient for the system, compared to hydrolysis in IPA. This is because the particles remained swollen in water during the process. The PVAM-4BEVAME and PVAM-9BEVAME particles had a cluster-like morphology. The particle morphology is controlled by \(x\) and is tuneable. The conversion was 99\% determined by elemental analysis data for PVAM-9BEVAME. The PVAM-\(x\)BEVAME microgels exhibited a pH-triggered increase in electrophoretic mobility and also hydrodynamic diameter when the pH was decreased. We demonstrated the versatility of these new microgels using the primary amine groups as chemical handles to covalently link PyC, which is a RCOO\(^-\) functionalised dye, to the particles. The PVAM-9BEVAME system was selected as the best system in terms of conversion, pH response and polydispersity. This study has given a new two-step method for preparing a new family of colloidally stable dispersions of high primary amine content microgels that have considerable potential application. This pH-responsive microgel with cluster-like morphology may be applied for biomaterials application because the rough surface is beneficial for support tissue growth.
4.5 References


Chapter 5

Preparation and characterisation of doubly crosslinked poly(vinyl amine) microgels

Abstract
Doubly crosslinked microgels (DX microgels) are constructed from covalently-linked singly crosslinked microgel particles. Whilst anionic DX microgels have been studied in our group\(^1\), there are no reports of cationic DX microgels elsewhere. This is the first study for DX poly(vinylamine) (PVAM) microgels. They can be prepared from singly (SX) crosslinked PVAM microgel particles which are cationic and contain high primary amine contents. Glycidyl methacrylate (GMA) monomer was used to functionalise the SX PVAM microgel to create pendant vinyl groups. Then, the DX microgels were formed by covalently linking GMA groups of neighbouring microgels using ammonium persulfate (APS). The morphologies of DX PVAM microgels were identified by optical microscopy and SEM and showed an extended interconnected porosity. The porosity of the microgels was tuneable through the microgel particle concentration. The mechanical properties of DX PVAM microgels were studied by dynamic rheology. It was found that the microgel with the best mechanical properties had a storage modulus \(G'\) of 41 kPa and yield strain \(\gamma_c\) of 46% indicating a good elasticity and ductility. In addition, the DX PVAM gels were prepared at 37 °C and can be injected. Furthermore, after a swelling experiment for 3 days at pH 7.4, the mechanical properties of the gel were close to those before swelling. The high primary amine contents (which facilitates function) of the injectable DX PVAM microgels coupled with their preparation at 37 °C mean that they are potentially useful as biomaterials.
5.1 Introduction and aims

Microgels are crosslinked polymer colloid particles that swell when dispersed in a good solvent, or when charge groups dissociate. Most microgel particles that have been previously studied were singly crosslinked microgel particles (SX microgels) which had only intra-particle covalent crosslinking. Doubly crosslinked (DX) microgels are covalently inter-linked microgel particles, which have permanent crosslinking. DX PVAM microgels do not redisperse in water. In this chapter, we demonstrate the preparation of DX PVAM microgels which contain high primary amine contents. DX microgels have first reported by Hu et al. They prepared nanoparticles using N-isopropylacrylamide and acrylic acid. After addition of epichlorohydrin, inter-crosslinking between nanoparticles was formed by the reaction of epichlorohydrin and the carboxyl group which was provided by acrylic acid. The DX microgel in this chapter was built upon the microgel in Chapter 4 to establish a system capable of being an injectable tissue scaffold. Here, we study the gel, but not its biocompatibility.

DX microgels have been also studied in the Saunders group. DX microgels have been prepared using glycidyl methacrylate (GMA) functionalised SX poly(methyl methacrylate-co-methacrylic acid-co-ethyleneglycol dimethacrylate) (PMMA-MAA-GMA) microgels. A physical gel was formed by pH-triggered swelling of concentrated dispersions. Then, a free radical reaction using ammonium persulfate (APS) as the initiator was used to covalently link vinyl groups (which were provided by GMA of neighbouring microgels) to form DX microgels. This microgel was demonstrated to improve the mechanical properties of degenerated intervertebral discs. The DX microgels that have been studied until now were anionic microgels based on MAA. It should be possible to prepare DX microgels using a cationic SX microgel system. In Chapter 4, we demonstrated a method to prepare high primary-amine content SX poly(vinylamine) (PVAM) microgels which will be used to prepared DX PVAM microgel in this chapter (Figure 5.1). PVAM structure is similar to polyethylene imine (PEI) which has been widely studied for delivery applications. Therefore, it should be possible for DX PVAM microgels to be used in biomaterials application. This is tested to a limited extent in this chapter.
In this chapter, we prepare the doubly crosslinked microgel (DX microgels) by using poly(vinylamine-co-bis(ethyl vinylamine) ether) (PVAM-9BEVAME) microgels from Chapter 4 as a precursor. We refer to PVAM-9BEVAME microgel as SX-PVAM because of the copolymerisation of di-vinyl crosslinking monomer within their structure during particle formation (Figure 5.1). Physically gelled dispersions of SX PVAM microgel particles was prepared by alkali hydrolysis of poly(N-vinylformamide-co-2-(N-vinylformamido) ethyl ether (PNVF-9NVEE) (we term them SX PNVF in this chapter). Then, GMA functionalisation was used to create the vinyl group on the SX PVAM to give SX PVAM-GMA particles. Those dispersions were concentrated to form a physical gel. The free-radical reaction then occurred using APS and heating to create DX PVAM. The reactions were formed from polymer bridges by connecting the microgel particles. The mechanism is shown in Figure 5.2.
The SX PVAM-GMA particles, used to prepare the DX PVAM, were characterised. The size of these microgels particles was investigated by optical microscopy, SEM and PCS. SEM was also used to characterise the morphologies of the microgel particles. The optical microscopy data was used to estimate the effective volume fraction occupied by the microgel particles ($\phi_{\text{eff}}$) within the gels using equation 5.7. The mechanical properties of the DX PVAM gels were measured by a dynamic rheology measurement. We also studied the injectability of the DX PVAM gel and the swelling behaviour at $\text{pH} = 7.4$, which is body pH. The potential application of the DX microgels studied here range from composite hydrogels$^6$ and catalysis$^7$ to biomaterials.$^4$ However, we need to be concerned about the cytotoxicity of related primary amines. Seow et al.$^8$ reported a simple method using hydrogen peroxide ($\text{H}_2\text{O}_2$) to oxidize the amine groups for polyethyleneimine (PEI)/DNA complexes. They found that this method can reduce the surface charge and did not damage the DNA. Moreover, the oxidised complexes were not toxic to cells. The $\text{H}_2\text{O}_2$ could be removed using sodium pyruvate before cell culture. This method could, in principle,
be applied to our DX PVAM microgel because PEI and PVAM have similar structures.
5.2 Experimental details

5.2.1 SX PNVF microgel preparation
The methods used for prepare and purify the SX PVAM microgels dispersions were described in section 4.2.3. SX PNVF particles containing 9 mol% NVEE were prepared by NAD in latex form.

5.2.2 SX PVAM microgel preparation
The preparation of the SX PVAM microgels was described in section 4.2.4. A microgel dispersion was produced by redispersing the particles in water. SX PNVF particles were converted to SX PVAM microgel by alkali hydrolysis. The hydrolysed dispersions (SX PVAM) were purified using dialysis against PBS, 0.15M NaCl and water, respectively.

5.2.3 SX PVAM-GMA microgel preparation
The SX PVAM microgel was vinyl-functionalised using GMA. 50 g. of SX PVAM at $\phi_p = 0.015$ containing 0.19 g. of added GMA was heated to 50 °C at pH = 9 with mechanical stirring for 24 h. The GMA-functionalised dispersion was washed three times with chloroform to remove unreacted GMA.

5.2.4 SX PVAM and SX PVAM-GMA physical gel preparations
SX PVAM and SX PVAM-GMA microgels were concentrated from a dilute dispersion ($\phi_p$ of 0.015) to $\phi_p$ at 0.05, 0.1, 0.12, 0.15 and 0.17 using rotary evaporation at 32 °C.

5.2.5 DX PVAM microgel preparation
DX microgels were prepared from SX PVAM-GMA. Aqueous ammonium persulfate (APS) (0.31 mL, 1wt%) was added to SX PVAM-GMA (10 g., 1.5 wt% particle) at pH=9 with vigorous mixing for about 5 min before rota-evaporation was conducted at 32 °C to concentrate the microgel to $\phi_p = 0.05, 0.1, 0.12, 0.15$ and 0.17. Then, the physical gel was placed into an O-ring (internal diameter = 19 mm, thickness = 2.2 mm) between two clean microscope slides, carefully sealed and heated in an oven at 50 °C for 24 hours.
In the case of the injectable DX PVAM system, 0.40 mL of \( N,N,N',N' \)-Tetramethylethylenediamine (TEMED) was added with the APS solution and rotary evaporation was conducted at 30 °C to give \( \phi_p = 0.15 \). Then, the physical gel was injected into the sealed O-ring as described above.

5.2.6 Physical Measurements

5.2.6.1 Optical microscopy
Images of particle dispersions were obtained via optical microscopy conducted using an Olympus BX41 microscope. At least 80 particles were measured to determine the number-average diameters \( (d_{opt}) \) by using the same method as Chapter 3, Section 3.2.7.2. The particle swelling ratio \( (Q) \) was calculated using equation 2.1. The collapsed particle diameter, \( d_{(c)} \) was calculated using equation 5.2.

5.2.6.2 Scanning electron microscopy
SEM measurements were obtained using a Philips FEGSEM instrument. The gels were freeze-dried using liquid nitrogen and coated with platinum. In the case of dilute dispersions, the particles were deposited from aqueous dispersions. Then, they were allowed to dry in the desiccators containing silica gel at room temperature and coated with carbon. The particles were measured to determine the number-average SEM diameters \( (d_{SEM}) \) using method described in Chapter 3, Section 3.2.7.4.

5.2.6.3 Photon correlation spectroscopy
PCS measurements were conducted using a BI-9000 Brookhaven light scattering apparatus (Brookhaven Instrument Corporation) fitted with a 20 mW He-Ne laser (632.8 nm) and the detector angle was set at a 90° scattering angle. The sample preparation was described in Chapter 3, Section 3.2.7.5. The hydrodynamic diameter of particle \( (d_h) \) is an average value from ten runs.

5.2.6.4 Fourier transform infrared spectroscopy
FTIR measurements were conducted using a Nicolet 5700 FTIR equipped with an attenuated total reflectance (ATR) unit. The sample preparation and measurement was described in Chapter 3, Section 3.2.7.6.
5.2.6.5 **Electrophoretic mobility measurements**

The electrophoretic mobility measurements were performed using a Zetasizernano ZS90 (Malvern Instruments Ltd.). Measurement and sample preparation were described in Chapter 3, Section 3.2.7.7.

5.2.6.6 **Elemental analysis**

Elemental analysis (C, H, and N) was conducted using a Thermo Flash 2000 Organic Elemental Analyzer at the School of Chemistry, University of Manchester. The measurement and sample preparation were described in Chapter 3, Section 3.2.7.8.

5.2.6.7 **Dynamic rheology measurements**

Dynamic rheology measurements were performed using a TA Instruments AR G2 rheometer. A 20 mm diameter plate geometry was used, and the gap size was 2000 µm. Frequency sweeps were undertaken at a strain of 0.1 %, at a range of 0.1 – 10 Hz. For the strain sweep measurements a frequency of 1 Hz was used at range 0.1 – 1000 %. The tan δ values were calculated from Equation 5.1,

\[
\tan \delta = \frac{G''}{G'}
\]

(5.1)

where \( G'' \) is the loss modulus and \( G' \) is the storage modulus. \( G' \) is a measure of how elastic a material is. Elastic materials deform reveally when strain is applied. \( G'' \) is a measure of how viscous a material is. Viscous materials have a lag in deformation as stress is applied and dissipate energy.
5.3 Results and discussion

5.3.1 GMA functionalised microgel

5.3.1.1 Particle characterisation
Optical microscopy, SEM and PCS were used to measure the particle size of the SX PVAM-GMA. Number-average diameters determined by optical microscopy ($d_{\text{opt}}$) of both SX PVAM–GMA (Figure 5.3(a)) and SX PVAM (Figure 5.3(b)) particles were 1.50 µm (Table 5.1). The SX PVAM-GMA dispersion remained colloidally stable after functionalisation. Figure 5.1(c and d) show SEM images for particles deposited from water. The number-average diameters determined by SEM ($d_{\text{SEM}}$) for SX PVAM-GMA and SX PVAM were close to 1.0 µm. $D_{\text{SEM}}$ values are slightly smaller than $d_{\text{opt}}$ because the particles collapsed during SEM preparation. A high vacuum was used during analysis. The SX PVAM and SX PVAM-GMA particles were spherical and monodisperse as shown Figure 5.3.

![Image](image-url)

Figure 5.3 Optical and SEM micrographs of microgels. (a) and (b) show optical micrographs of SX PVAM-GMA and SX PVAM particles dispersed in water at pH = 7. (c) and (d) show SEM micrographs of SX PVAM-GMA and SX PVAM deposited from water at pH = 9.
Table 5.1 Microgel particles properties of SX PVAM and SX PVAM-GMA

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<th>$d_h$ / µm</th>
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</thead>
<tbody>
<tr>
<td>SX PVAM</td>
<td>1.50 [13]</td>
<td>1.00 [7]</td>
<td>1.30</td>
<td>0.60</td>
<td>10.9</td>
</tr>
<tr>
<td>SX PVAM-GMA</td>
<td>1.50 [12]</td>
<td>1.10 [10]</td>
<td>1.40</td>
<td>0.60</td>
<td>10.0</td>
</tr>
</tbody>
</table>

*a* Number-average diameter determined from optical micrographs at pH = 7. *b* Number-average diameters determined from SEM images. The number in square brackets is the coefficient of variation (CV). CV = [100*(SD/mean)]. SD is standard deviation and mean is average particle diameter. *c* Hydrodynamic diameter measured at pH = 7. *d* Collapsed hydrodynamic diameter values calculated using equation 5.3. *e* Particle swelling ratio calculated at pH = 7 from equation 2.1.

5.3.1.2 Study of pH-triggered swelling and electrophoretic mobility of GM-PVAM-9BEVAME

Figure 5.4 shows the hydrodynamic diameter ($d_h$) of SX PVAM-GMA and SX PVAM at different pH. The SX PVAM-GMA particles demonstrated notable pH-triggered swelling when the pH approached to 10, which is the $pK_a$ of PVAM, from higher values. This was a consequence of protonation of the primary amine groups. The same tendency was also found for SX PVAM particles.
The particle swelling ratio \((Q)\), which is a crucial parameter for microgels, was calculated using equation 2.1. The \(d(c)\) of SX PNVF-GMA and SX PVAM particles were measured using the particles dispersed in ethanol, which is a non-solvent for PVAM. The accurate value of \(d(c)\) for SX PVAM and SX PVAM-GMA cannot be obtained because the SX PVAM-GMA and SX PVAM particles did not completely de-swell in ethanol. The hydrolysis of SX PNVF to prepare SX PVAM (Figure 5.1) lost about 37% of its mass because formate was released. This prevented the full collapse of the microgel in ethanol. Therefore, \(d(c)\) for SX PVAM-GMA and SX PVAM cannot be measured directly.

Figure 5.4 pH-responsive behaviour of SX PVAM and SX PVAM-GMA microgels (a) shows the hydrodynamic diameters \((d_h)\) and (b) shows swelling ratio \((Q)\) values vs. pH
Equation 5.2 corrected for the respective particle mass losses due to hydrolysis in order to approximate a hydrodynamic diameter, $d_{h(c)}$ (we term as $d^*_{h(c)}$) value for SX PVAM and SX PVAM-GMA particles. The $d_{h(c)}$ value for SX PNVF ($d_{h(c,PNVF)} = 0.66 \, \mu m$) and the repeating unit molar mass ($M_{\text{rep}}$) (g mol$^{-1}$) of the microgels (Table 5.1) were used for the following equation:

$$d^*_{h(c)} = d_{h(c,PNVF)} \left( \frac{M_{\text{rep}}}{M_{\text{rep(PNVF)}}} \right)^{1/3}$$

(5.2)

To derive the equation 5.2, the number of repeating units per particle was assumed not to change as a result of hydrolysis and the density of polymer was 1.0 g/cm$^3$. The calculated value for $M_{\text{rep(PNVF)}}$ was 84 g/mol based on its composition. The $d^*_{h(c)}$ values correspond to the fully collapsed particles dispersed in ethanol. The values of $d_{h(c,PNVF)}$ and $d^*_{h(c)}$ obtained from equation 5.2 were used to calculated $Q$ for SX PVAM-GMA and SX PVAM by equation 2.1 and using $d_{h(c)} = d^*_{h(c)}$. The variation of $Q$ with pH was shown in Figure 5.2(b). At pH < 7, the microgel particles exhibited swelling due to protonation of BEVAME, which is the product of NVEE after hydrolysis (Figure 5.1).

The electrophoretic mobility ($U_E$) values vs. pH for SX PVAM and SX PVAM-GMA are shown in Figure 5.5. Both SX PVAM-GMA and SX PVAM microgel had electrophoretic mobility values that were positive over the pH range 4 to 12. The electrophoretics mobilities values were higher at decreased pH because of increased protonation of the microgel particles. However, at pH 7 to 10 significant differences in the relative changes were noticeable. The electrophoretic mobilities are not only impacted by structure, but also depend on a variety of factors. Ohshima et al. studied the electrophoretic mobility of latex particles which were prepared from styrene and 2-ethylhexyl methacrylate monomer covered with a temperature-sensitive poly(N-isopropylacrylamide) hydrogel layer. They proposed the following equation to express the electrophoretic mobility of a colloidal particle covered with a layer of polyelectrolytes:

$$U_E = \frac{\varepsilon \varepsilon_0 \psi_0 l \kappa_n + \psi_{DON} l \lambda \kappa_n}{\eta} + \frac{z e N}{\eta \lambda^2}$$

(5.3)
Where $\varepsilon_r$ and $\varepsilon_0$ are the relative permittivity of solution and a vacuum, respectively.

The $\eta$ value is viscosity, $\Psi_0$ is the electrostatic potential at surface between the hydrogel layer and the surrounding solution and $\Psi_{\text{DON}}$ is the Donnan potential of the hydrogel layer. $\kappa_m$ was interpreted as the Debye-Huckel parameter of the layer. The parameter $\lambda$ has dimensions length, and $zeN$ represents the density of the fixed charges in the hydrogel layer.

![Figure 5.5 Electrophoretic mobilities ($U_E$) of SX PVAM and SX PVAM-GMA vs. pH](image)

5.3.1.3 FTIR analysis

FTIR spectroscopy was also used to investigate the microgel compositions (Figure 5.6). The RNH$_2$ groups in SX PVAM exhibited bands at 1590, 3275 and 3350 cm$^{-1}$, which contrasted to those of SX PNVF. Regarding the GMA spectrum, a distinct band for vinyl groups$^{12}$ was seen at 1637 cm$^{-1}$, the band overlapped a strong band in the SX PVAM spectra so there is not a band for C=C groups evident in SX PVAM-GMA. However, the weak band presented at 1715 cm$^{-1}$ for SX PVAM-GMA spectra was a consequence of ester groups of GMA. This supports the incorporation of GMA. Another evidence is the absence of the epoxide band at 905 cm$^{-1}$ in the SX PVAM-GMA spectrum indicating the reaction of GMA incorporation and the removal of unreacted GMA by the washing treatment.
5.3.1.4 Elemental analysis

Before considering the DX PVAM microgels, we need to characterise the vinyl-functionalised SX PVAM-GMA microgel composition because it is new. The SX PVAM-GMA can be prepared in a single step from SX PVAM or in two steps beginning with SX PNVF (Figure 5.1). Table 5.2 showed the compositions for SX PVAM and SX PVAM-GMA and the structures shown in Scheme 5.1. The ratio of the N:C ($R_{NC}$) from elemental analysis data (Table 5.2) were used to determine the $x$, $y$ and $z$ values.

The following equation was derived from the composition and structure of SX PNVF (PNVF$_{1-x}$-NVEE$_x$) and was employed to identify the value for mole fraction of NVEE incorporated ($x$).

$$x = \frac{1.1662 - 3R_{NC}}{7R_{NC} - 1.1662} \quad (5.4)$$

The $x$ value from the calculation is 0.09 so the composition of SX PNVF was PNVF$_{0.91}$-NVEE$_{0.09}$. This value agrees with the stoichiometric ratios of NVF and NVEE used for preparing the copolymer.

SX PVAM was hydrolysed to SX microgel. The degree of hydrolysis was estimated through the elemental analysis data shown in Table 5.2 and the structure for SX PVAM (Figure 5.1). The ratio of VAM to BEVAME units was assumed to equal that
of NVF to NVEE before hydrolysis. The following equation was derived from the structure for PNVF-NVEE and the general formulation \((\text{PVAM}_{0.91}-\text{BEVAME}_{0.09})_y-(\text{PNVF}_{0.91}-\text{NVEE}_{0.09})_{1-y}\). It was used to determine the degree of hydrolysis \((100y)\).

\[
y = \frac{3.595R_{NC} - 1.2653}{1.085R_{NC}}
\]

The \(y\) value from the calculation is 0.84 which means that SX PVAM preparation generated very high conversion with about 84 mol% of the NVF being hydrolysed to VAM. Therefore the composition of SX PVAM was \((\text{PVAM}_{0.91}-\text{BEVAME}_{0.09})_{0.84}-(\text{PNVF}_{0.91}-\text{NVEE}_{0.09})_{0.16}\).

To calculate the amount of GMA functionalisation \((z)\) for SX PVAM-GMA, the following equation was used.

\[
z = \frac{0.2344}{R_{NC}} - 0.497
\]

This equation was derived from the structure of SX PVAM-GMA (Scheme 5.1) and the \(x\) and \(y\) values determined above, \([(\text{PVAM}_{1-z}(\text{VAM-GMA})_z)_{0.91}-\text{BEVAME}_{0.09}]_{0.84} [(\text{PNVF}_{0.91}-\text{NVEE}_{0.09})_{0.16}\). The \(z\) value from the calculation is 0.17 which equates to an overall GMA mole fraction of 0.13 (this comes from 0.17 x 0.91 x 0.84). This value is similar to that reported for anionic PMMA-MAA-GMA microgel.\(^1\)\(^4\) Liu et al. reported the extent of GMA functionalisation of 1.8 mol% for poly(MMA/MAA/EGDMA) (methyl methacrylate, methacrylic acid and ethyleneglycol dimethacrylate) and they found that this was sufficient to form DX gels.\(^1\) This DX microgels was further studied for improving the mechanical properties of degenerated interverbal discs.\(^4\)
Table 5.2 Compositions of the microgels\textsuperscript{a}

<table>
<thead>
<tr>
<th>Code</th>
<th>Compositions</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
<th>(R_{NC})</th>
<th>Mol. % Hydr\textsuperscript{c}</th>
<th>Mol. % GMA\textsuperscript{d}</th>
<th>(M_{rep}) (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SX PNVF</td>
<td>PNVF\textsubscript{0.91}-NVEE\textsubscript{0.09}</td>
<td>48.6</td>
<td>7.8</td>
<td>17.1</td>
<td>0.35</td>
<td>0</td>
<td>0</td>
<td>84</td>
</tr>
<tr>
<td>SX PVAM</td>
<td>[PVAM\textsubscript{0.91}-BEVAME\textsubscript{0.09}\textsubscript{0.84}[PNVF\textsubscript{0.91}-NVEE\textsubscript{0.09}]\textsubscript{0.16}</td>
<td>40.4</td>
<td>9.0</td>
<td>19.0</td>
<td>0.47</td>
<td>84.0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>SX PVAM-GMA</td>
<td>[PVAM\textsubscript{0.76}(VAM-GMA)\textsubscript{0.15}[BEVAME\textsubscript{0.09}\textsubscript{0.84}][PNVF\textsubscript{0.91}-NVEE\textsubscript{0.09}]\textsubscript{0.16}</td>
<td>43.9</td>
<td>8.8</td>
<td>15.4</td>
<td>0.35</td>
<td>84.0</td>
<td>13.0</td>
<td>78</td>
</tr>
<tr>
<td>DX PVAM</td>
<td>-</td>
<td>42.6</td>
<td>8.64</td>
<td>14.9</td>
<td>0.35</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{a}%C, %H, and %N are percentage by mass. \textsuperscript{b}\(R_{NC}=%N/%C\). The extent of hydrolysis was 100\%. \textsuperscript{c}Extent of GMA functionalisation. \textsuperscript{d}Calculated molar mass of repeat unit using the values for \(x\), \(y\) and \(z\).

5.3.2 DX PVAM microgel

5.3.2.1 Particle characterisation

Microgels are polymer colloid particles which are able to swell in water. However, in some cases the particles do not contain water and not swell; they are defined as collapsed particles or hard spheres. The SX PVAM-GMA dispersions at a hard sphere volume fraction (\(\phi_{hs}\)) > 0.05 in the presence of added APS were needed for preparing the DX PVAM microgels. They formed physical gels due to the non-covalent interactions between the concentrated microgel particles. Rotary evaporation was an effective technique for making shear-thinning physical gels at high \(\phi_{hs}\). DX PVAM microgels were produced from SX PVAM-GMA physical gels by free-radical reaction (Figure 5.1 and 5.2).

A method was used for examining the existence of inter-particle crosslinking of DX PVAM microgels that involved placing a gel in 0.1 M NaCl aqueous solution at pH = 7 for 24 h. (Figure 5.7). A SX PVAM-GMA physical gel was used as a control, we found that it redispersed whilst the DX PVAM microgel did not redisperse. This is the first time that a high primary amine content DX microgel has been reported. We noticed the yellow colour for DX PVAM and SX PVAM-GMA (Figure 5.7) and the colour was apparent for SX PVAM-GMA or when SX PVAM with APS was heated.
The yellow of heated SX PVAM with APS might be due to the oxidation of VAM groups by persulfate to give imine groups.\textsuperscript{14} In the matter of SX PVAM-GMA, the imine groups could be formed by the reaction of C=O groups with primary amines.\textsuperscript{15}

![Figure 5.7 DX PVAM microgel dissolution test](image)

5.3.2.2 Morphology of the particles

The morphologies of freeze dried DX PVAM microgels were studied using SEM (Figure 5.8(a) and (b)). SX PVAM-GMA microgel was used as a control sample (Figure 5.8(c) and (d)). A small piece of DX microgel was put into liquid nitrogen for 3 - 5 minutes until it freeze dried. Then, it was connected to freeze dryer until it dried. Sample was coated by carbon prior SEM characterisation. The SEM images for DX PVAM and SX PVAM-GMA gel show similar morphologies. They exhibit distinct porous morphologies and had interconnected, 3-dimensional porosity and the pores were micrometer sized. This morphology is different to morphologies of anionic DX microgels containing methyl methacrylate (MMA), methacrylic acid (MAA), ethyleneglycol dimethacrylate (EGD) and glycidyl methacrylate (GM), (poly(MMA/MAA/EGD)-GM) which showed distinct homogeneous morphologies probably due to the much smaller particle size (nano-scale) of the microgels.\textsuperscript{4}
higher magnification, polymer bridges between the particles can be seen within the gels and this connected each particle together. The bridges for DX PVAM microgel were covalently linked and resisted redispersion when placed in water.

Figure 5.8 SEM images of freeze dried (a) and (b) DX PVAM microgel. (c) and (d) SX PVAM-GMA at $\phi_s = 0.10$

Optical micrographs of the gel morphology are shown in Figure 5.9. This is the first time that the morphology of hydrated DX microgel was examined by optical microscopy. As mentioned above that the polymer bridges between particles can be found (Figure 5.8), the optical micrographs also exhibit the local ordered packing (Figure 5.9). A square-like lattice was shown on the top of four particles in inset of Figure 5.9(a) and (c). Figure 5.9(b) presented a Fast Fourier Transform (FFT) of the particle image, which displays a central amorphous ring surrounded by points in a square-like arrangement. This is indicative of square symmetry in the x-y plane. Furthermore, these kinds of features were visible from optical images and FFT for SX PVAM (Figure 5.9(d)). Slightly stretched in y direction can be seen. Face-centred cubic lattices have been stated for microgels$^{16}$ which enable it to form body centred tetragonol lattices (BCT) when subjected to an external field as well.$^{17}$ However, the optical micrographs cannot enable a clear assignment of the 3-dimension unit cell.
Figure 5.9 Morphologies of DX PVAM and SX PVAM gels in the hydrated state. Optical micrographs for DX PVAM (a) and SX PVAM (c). (b) and (d) show FFT images from (a) and (c), respectively. The arrows indicate highlight some of the bright points present.

The effective volume fraction occupied by the microgels within DX PVAM microgel, $\phi_{\text{eff}}$, can be estimated because particle sizes could be measured from the optical micrographs. The $\phi_{\text{eff}}$ value for the DX PVAM gels was calculated from the following equation:

$$\phi_{\text{eff}} = Q\phi_{hs}$$  \hspace{1cm} (5.7)

The method to calculate $Q$ from the gel optical micrographs is the same as used above for PCS data (equation 2.1 and 5.2). The collapsed diameter of the microgel particles was determined from optical microscopy, $d^{*}_{(c,\text{Opt})}$. A particle size of collapsed
PNVF particles dispersed in ethanol based on optical microscopy, $d_{(c,PNVF,Opt)}$ was 0.78 \( \mu \text{m} \). This value was used to calculate $d_{(c,\text{opt})}$ via an equation corresponding to equation 5.2. The calculated $d_{(c,\text{opt})}$ values for SX PVAM-GMA and SX PVAM particles were 0.76 and 0.70 \( \mu \text{m} \), respectively. The $d_{(c,\text{opt})}$ was used to calculate $Q$ values from $d_{\text{opt}}$ values using equation 2.1. Then, $\phi_{\text{eff}}$ was calculated using equation 5.7 to get $\phi_{hs}$, which were shown in Figure 5.10(b).

Figure 5.10 Particle size and swelling dependence on polymer volume fraction for DX PVAM microgels. (a) shows the variation of the particle size and particle swelling ratio with $\phi_{hs}$. (b) shows the calculated variation of the effective polymer volume fraction with $\phi_{hs}$.

Figure 5.10(a) shows that the microgel particle diameter decreased from the dilute dispersion value when incorporated into the gels. Osmotic deswelling of polyelectrolyte microgel particles can be produced by the external electrolyte.\(^\text{18}\) For the present system at $\phi_{hs} = 0.05$, the highest ionic strength owing to PVAM microgel particles can be estimated as 0.4 M which could have been an increasing function of $\phi_{hs}$. Thus, the decrease in particle diameter, $d_{\text{opt}}$ for the microgels within the gels is due to osmotic deswelling caused by the high ionic strength of the external water phase based on the mobile ions that came from the microgel particles.

Particle aggregation within the gels can occur before DX formation due to a high ionic strength with low $Q$ values (Figure 5.10(a)). Then, particle-particle contacts would form a space-filling network and the gels seemed homogeneous by observed vision (Figure 5.7). However, SEM (Figure 5.8) and optical micrographs (Figure 5.9) for DX PVAM and SX PVAM showed that particles contained particle networks bridges. In addition, $\phi_{\text{eff}}$ values in Figure 5.10(b) were lower than values for a cubic lattice (0.52) or randomly close packed lattice (0.64). The reason is the microgel particles were not completely swollen in the gels. They formed a space-
filling network with the porosity between interconnected particle chains. The $\phi_{\text{eff}}$ values increased with $\phi_{hs}$ for the gels (Figure 5.10(b)), so the effective porosity of the DX PVAM gels ($= 1 - \phi_{\text{eff}}$) can be vary from 76 to 93 vol. % via $\phi_{hs}$.

5.3.2.3 FTIR analysis

The FTIR spectra of SX PVAM-GMA and DX PVAM microgels (Figure 5.11) were similar, confirming that the primary amine DX PVAM microgel maintained its structure. This was supported by elemental analysis data (Table 5.2), the $R_{NC}$ value for DX PVAM was the same as that for SX PVAM-GMA. Thus, DX formation did not significantly change the microgel composition.

![FTIR spectra of DX PVAM and SX PVAM-GMA](image)

**Figure 5.11** FTIR spectra of DX PVAM and SX PVAM-GMA

5.3.2.4 Mechanical properties of DX PVAM microgel

Dynamic rheology was used to investigate the mechanical properties of the microgels. Figure 5.12 (a-b) shows frequency sweep rheological data for DX PVAM, SX PVAM and SX PVAM-GMA microgels. The DX VPAM microgel exhibited low frequency dependencies for both storage modulus ($G'$) and tan $\delta$ ($= G''/G'$, $G''$ is loss modulus). This implies the most elastically ideal network for DX PVAM microgels in our works.\textsuperscript{19} We propose that double crosslinking decreased the proportion of elastically ineffective structures, for instance loops and dangling chains. This hypothesis is supported by the remarkably lower tan $\delta$ for DX PVAM compared to SX PVAM (Table 5.3). The SX PVAM was not able to create covalent inter-particle linkages.
Figure 5.12 Rheological properties of DX PVAM, SX PVAM-GMA and SX PVAM at \( \phi_{hs} = 0.10 \). Frequency-sweep ((a) and (b)) and strain-sweep (c).
Table 5.3 Mechanical properties for gels at \( \phi_{hs} = 0.10 \)

<table>
<thead>
<tr>
<th>Code</th>
<th>( G'^a / \text{Pa} )</th>
<th>( \tan \delta^b )</th>
<th>( \gamma_c / %^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DX PVAM</td>
<td>7400</td>
<td>0.065</td>
<td>29.5</td>
</tr>
<tr>
<td>SX PVAM-GMA</td>
<td>1770</td>
<td>0.090</td>
<td>100.0</td>
</tr>
<tr>
<td>SX PVAM</td>
<td>1350</td>
<td>0.125</td>
<td>57.2</td>
</tr>
</tbody>
</table>

\(^a\)Storage modulus measured at 10 Hz. \(^b\)\( \tan \delta \) measured at 10 Hz. \(^c\)Yield strain.

Table 5.3(a) shows the \( G' \) value for DX PVAM was higher than the value for SX PVAM-GMA because of further elastically effective chains from covalently crosslinked GMA groups. GMA groups of each PVAM-GMA microgel particle within the DX PVAM microgel were a covalent crosslinking centre. Moreover, we found that the value of \( G' \) for SX PVAM-GMA was notably greater than the value for SX PVAM. This could possibly be a result of some covalent inter-particle crosslinking for SX PVAM-GMA during the heating process since air was not excluded. It was also found that there was a contribution to the elasticity for both the SX PVAM-GMA and SX PVAM gels from reversible particle-particle connections which were present. The \( \tan \delta \) values for the DX PVAM were equal or lower than 0.065 (Table 5.3) and this confirmed the gel state of DX PVAM (as described earlier) which was defined as \( \tan \delta < 1 \). Regarding the \( \tan \delta (G''/G') \) of DX PVAM was 0.065, this implied that the energy from the applied shear that was stored elastically within the DX PVAM microgel network was approximately 95%.

Figure 5.12(c) shows strain-sweep data for the DX PVAM, SX PVAM and SX PVAM-GMA microgels. For strain-sweep measurement, the gel broke down at high strain e.g. greater or equal to the critical yield strains (\( \gamma_c \)). The \( G' \) values decreased at higher strain than 10%, demonstrating the beginning of shear-induced network failure. In all case, the \( G' \) data crossed \( G'' \) data at the highest \( G'' \) value. At the cross-over points \( G' = G'' \) (\( \tan \delta = 1.0 \)), those strain (\( \gamma \)) values are the critical yield strains (\( \gamma_c \)). More energy was lost via dissipation compared to energy storage at higher strain values. The physical gels (SX PVAM and SX PVAM-GMA) showed the highest for \( \gamma_c \) and decreased to 30% for the DX PVAM microgel (Table 5.3). SX PVAM-GMA gel exhibited a high \( \gamma_c \) value of 100%, which can be resulting from a low level of inter-particle crosslinking. This could have occurred within the system at some stage during rotary evaporation.
From Figure 5.12(c), it can be seen that there were noticeable differences for the $G''$ data for the DX PVAM and SX PVAM. The maximum of $G''$ for DX PVAM was broad. This might be a result of the short inter-particle linkages which were broken over a wide range of strains. Whereas the $G''$ maxima for SX PVAM and SX PVAM-GMA were more narrow and similar. This indicates equivalent viscoelastic behaviours for those systems. The strain-induced network failure mechanism was affected by double crosslinking.

5.3.2.5 Effect of polymer volume fraction on mechanical properties

The effect of $\phi_{hs}$ and $\phi_{eff}$ on the dynamic rheological properties was investigated to study the source of the DX PVAM elasticity and also to establish the possibility of tuning the mechanical properties. Figure 5.13(a) show the frequency-sweep $G'$ data. $G'$ data exhibited low frequency dependencies. The tan $\delta$ at different $\phi_{hs}$ for both systems are shown in Figure 5.13(b). They show tan $\delta$ decreased with increasing $\phi_{hs}$ and the minimum for the DX microgel containing the maximum microgel content ($\phi_{hs} = 0.17$). Higher tan $\delta$ values and frequency dependence also can be found for SX PVAM in Figure 5.14. The low tan $\delta$ values and low frequency dependence for the DX PVAM gels indicate that double crosslinking generated a network with relatively elastic loops and chains.

Figure 5.13 Rheological properties of DX PVAM. (a) frequency dependence for $G'$. (b) the variation of tan $\delta$ with $\phi_{hs}$ at different frequencies (shown in the legend in Hz)
Figure 5.14 Rheological properties of SX PVAM. (a) frequency dependence for $G'$. (b) the variation of $\tan \delta$ with $\phi_{hs}$ at different frequencies (shown in the legend in Hz).

The strain-sweep rheology at different $\phi_{hs}$ has been investigated for the DX PVAM (Figure 5.15(a)) and SX PVAM gels (Figure 5.15(b)). At about 10% strain for the gels at $\phi_{hs} \geq 0.10$, $G'$ data exhibits strain induced decreases as a result of network disruption. Only one distinct maximum for each DX PVAM data can be seen and this is in according with there was one typical network breaking process that occurred over a range of strain. Most of the bond breaking was based on the three-dimensional particle network morphologies seen in the images shown in Figures 5.8 and 5.9.
From the strain-sweep data (Figure 5.15), we can determine the values and the data are plotted as a function of both $\phi_{hs}$ and $\phi_{eff}$ in Figure 5.16(a). The $\gamma_c$ gradually decreased with increasing $\phi_{hs}$ or $\phi_{eff}$ and then increased again at the highest $\phi_{hs}$ or $\phi_{eff}$ values. We can explain the gradual decrease in $\gamma_c$ with regards to an increasingly crosslinked network with short elastically effective chains. The unexpected $\gamma_c$ at the highest $\phi_{hs}$ or $\phi_{eff}$ values for DX PVAM system was higher than the SX that for PVAM microgel (Figure 5.16(b)). A more narrow distribution of linkage lengths results in relatively high yield strains for conventional hydrogels. An increase in
total order occurred with the $\phi_{hs} = 0.17$ was hypothesised that because of a higher $\phi_{eff}$ which made the elastically effective chain lengths become more uniform. Moreover, this system had the lowest frequency dependent tan $\delta$ for all of the DX PVAM microgels studied (Figure 5.13(b)), which means a more interconnected network.

Figure 5.16 Strain-sweep data shows the variation of the yield strain ($\gamma_c$) with $\phi_{hs}$ and $\phi_{eff}$ of (a) DX PVAM and (b) SX PVAM

5.3.2.6 Injectable DX PVAM gels swollen at physiological pH

The main objective of this work is to prepare the injectable gels for soft tissue repair. Therefore, the key physical properties of gels for biomaterials are injectability through a syringe needle and then forming a gel at body temperature (37°C). In this part, the DX PVAM gels ($\phi_{hs} = 0.15$) were prepared by using TEMED as an accelerator which allows them to be crosslinked at 37°C. In this work, we showed that a DX PVAM precursor physical gel (containing APS and TEMED) was sufficiently shear-thinning to be injectable by using a syringe (Figure 5.17). The DX PVAM gel was formed at 37°C.

Figure 5.17 A picture of gel (a) being injected through an 18 gauge needle (b) DX PVAM gel before swelling test and (c) gel after swelling for 3 days at pH = 7.4.
The swelling experiment was studied by placing the DX PVAM microgels in phosphate buffer saline solution (pH = 7.4) for 3 days. The measured $\phi_{hs}$ value before swelling was 0.16 and then it decreased to 0.11 after swelling. A sol fraction of 0.06 was determined gravimetrically; this implied that 94 wt% of the gel was still intact. Thus, the decrease in $\phi_{hs}$ was mainly caused by gel expansion. The particle determined diameter from the optical micrographs ($d_{opt}$) of the swollen gel was 0.79 $\mu$m (CV = 11). The method mentioned earlier was used to calculate a $Q$ value, of 1.4. It was found that the $d_{opt}$ and $Q$ values were similar to those measured for the DX PVAM prepared with no TEMED without swelling (Figure 5.8(a)). This means the expansion of the DX PVAM gel when placed in buffer was attributing to particle-network relaxation rather than microgel particle swelling. In addition, a $\phi_{eff}$ value of $(0.11 \times 1.4 =) 0.15$ was calculated; this demonstrated that the DX PVAM gel maintained about 85 vol% of porosity. High porosity is useful for scaffold performance.\textsuperscript{21}

To determine the mechanical properties of the swollen DX PVAM gel, frequency-sweep and strain-sweep dynamic rheology were studied (Figure 5.18). The $G'$, $\tan \delta$ and $\gamma_c$ values for the gels were 20.1 kPa, 0.055 and 19% respectively which are similar to those for DX PVAM microgel prepared without TEMED before swelling ($\phi_{hs} = 0.15$). This indicates that the DX PVAM microgels can be injectable and cured at body temperature and maintain their mechanical properties under physiological ionic strength and pH conditions.
Figure 5.18 Rheological properties of DX PVAM swollen gel (a) frequency-sweep data (b) the variation of $G'$ and $G''$ with strain

(a) $G', G''$ vs. Frequency / Hz

(b) $G', G''$ vs. Strain / %
5.4 Conclusions
Cationic DX microgel particles containing high primary amine content microgel particles have been reported in this chapter. The gel morphology consists of interconnected, space-filling networks of partially swollen microgel particles. The DX PVAM microgel showed the significantly higher $G'$ compared to SX PVAM microgel. The microgel prepared using $\phi_{hs} = 0.17$ showed the best mechanical properties for DX PVAM microgel. It had $G' = 41$ kPa and $\gamma_c = 46\%$ indicating good stiffness and ductility. The high primary amine content in the microgel means it is suitable for functionalization. In Chapter 4, we demonstrated the microgel functionalisation via primary amine groups using chromophore and carbodiimide chemistry. This ease of functionalisation shows that there are many applications for these DX PVAM microgel covered from composites, catalysis, membranes and biomaterials. The objective of this work was to create an injectable microgel for use as a biomaterial. The precursor physical gels had the important characteristics for injectable gels which are injectability through a needle and the ability to form a gel at body temperature (37 °C). They were also highly porous. This implies that these DX PVAM microgels could be suitable for minimally-invasive biomaterial use. The injectable anionic DX microgels have been studied in our group and can support load for damaged intervertebral discs. This supports the possibility that DX PVAM microgel may be useful for biomaterials. However, cytotoxicity which can be expected for primary amine systems may need to be reduced. Methods to reduce cytotoxicity for related primary amine-based systems have been reported by Zhang et al. and Seow et al.
5.5 References


Chapter 6

6.1 Conclusions

The aims of this thesis were to investigate heteroaggregated doubly crosslinked microgels by mixing anionic and cationic microgels together. The technique used to prepare doubly crosslinked anionic microgel has been established by Saunders’s group. This technique was applied to prepare doubly crosslinked cationic microgel in this study. The first aim for the work was to try to prepare a cationic microgel which contains a primary amine. This could be used for prepare doubly crosslinked microgel and this applies to a potential use as a gel to repair the damaged heart tissue. However, the limitation of the time allowed us to study only cationic microgel and their gel properties, not biocompatibility.

The investigation starts from studying the morphology and properties of PGMA and PGMA-NH₂ particles. The particles were prepared using non-aqueous dispersion polymerisation. The aminolysis was used to generate the primary amine by epoxide ring opening of PGMA. The work in Chapter 3 showed that the PGMA-NH₂ did not swell in water or any solvent and the morphology is the same as parent particle (PGMA). The elemental analysis showed that 40 mol% of epoxide group was converted to primary amines. The reason why the particles did not swell is a high degree of crosslinking between amine groups of PGMA-NH₂ and epoxide groups of PGMA occurred during hydrolysis. Therefore, the alkali hydrolysis PNVF-xGMA (x is the weight of GMA monomer) has been studied to establish the primary amine microgel which is a pH-triggered swelling microgel. The morphology and properties of PNVF-xGMA particles was investigated prior to hydrolysis. The PNVF-xGMA swelled in water, confirmed by hydrodynamic diameter and optical microscopy. The particle size can be controlled by varying the weight fraction of GMA. By altering the fraction of stabiliser (PVP) the resultant particles appear to change in size. An increasing surfactant concentration causes increasingly smaller particles because the function of PVP is to stabilise the growing particle nuclei earlier. Core-shell morphology which the surface is PGMA rich has been proposed because the SEM images are similar in morphology to PGMA, but they are very different from that of PNVF. SEM images showed a unique “cane-ball”-like morphology with interconnected ridges on the surface. This is a new type morphology which has not
been reported elsewhere. Moreover, there are crosslinks between the PGMA shell and the PNVF core due to the reaction between epoxide and amide groups.

Further study is the alkali hydrolysis for PNVF-xGMA to convert PNVF core to PVAM. The H-PNVF-xGMA particles consisted of both negative and positive charges. After hydrolysis, the particles carry a positive charge which is the result of PVAM-rich polymer at the particle surface. After that the electrophoretic mobility decreased to negative values when the particles were redispersed in NaOH solution and water. This is consistent with the particle diameter, the maximum diameter can be seen at pH = 11 and then decreased at lower pH. We proposed that this is due to the shell fragmentation and the PVAM in the core was released. Moreover, charge patch aggregation occurred at low ionic strength.

Regarding the shell fragmentation of PNVF-xGMA in Chapter 3, the new approach to prepare cationic microgel contained primary amine was studied in Chapter 4. PNVF has been using NVEE as a crosslinker because it has ether linkage which is resistant to the alkali hydrolysis. The PNVF-xNVEE particles swell in water and the maximum degree of swelling was found for PNVF-9NVEE which was unexpected. The maximum degree of swelling was expected for PNVF-4NVEE, however, it did not occur. It was proposed that the lightly crosslinked shells of microgel particles fragment and they detached from the core. It was found that at least 9 mol% of NVEE was sufficient to provide the high crosslinking density for the shell of PNVF-xNVEE which resist inter-particle coalescence and film formation at the time of the drying process. PVAM containing BEVAME crosslinker (PVAM-xBEVAME) was generated by alkali hydrolysis. PVAM-4BEVAME microgel is the most swelling among all systems, however shell fragmentation can be also found by SEM. The morphology of PVAM-9BEVAME and PVAM-13BEVAME microgels was undamaged and it is controlled by x and is tuneable. FTIR spectra determined the incomplete hydrolysis. Hydrolysis conversion was 99% for PVAM-9BEVAME, this was calculated based on elemental analysis data. The pH-triggered swelling of the microgel was presented when pH approached to 10 which is the pK_a of PVAM.

To demonstrate the versatility of these new microgels, the PVAM-9BEVAME was selected to functionalise with RCOO^- functionalised dye. The fluorescence
micrographs confirmed the effective functionalisation of PVAM-9BEVAME microgels via primary amine groups.

The PVAM-9BEVAME system was selected as the best system in term of conversion, pH response and polydispersity to prepare the doubly crosslinked (DX) microgel in Chapter 5. This is the first time that DX microgels can be prepared from cationic, high primary amine content microgel particles. The technology which has been used to prepare DX anionic microgels in our group\(^1\) was applied to prepare DX PVAM microgel in this chapter. The DX microgel contained interparticle linkages between the particles so it did not redisperse in 0.1 M NaCl. The gel morphology inheres in inter-connected, space-filling networks of partially swollen microgel particles. DX PVAM microgel improved the \(G'\) and the best mechanical properties were \(G' = 41\) kPa and \(\gamma = 46\) implying good stiffness and ductility. The precursor physical microgel is injectable through a syringe needle and the DX gel was able to form at body temperature (37 °C). The DX PVAM microgel also maintained the porous morphology at physiological pH and ionic strength. The potential biomaterial application for these DX PVAM microgels is to use them as minimally-invasive biomaterial for damaged heart tissue repair. This idea is based on the injectable anionic DX microgels that have been shown to restore load for damaged interverbral discs.\(^1\)
6.2 Suggestions and future works

This work is the first time that cationic microgel contained high primary amine content and DX PVAM microgel have been prepared. As mentioned earlier, the aim of this microgel is for biomaterial application. There are a few points that may need to be overcome before the cationic microgel can be used as biomaterials. Biological and biodegradation testing are required. The ability of the gels to support growth of cells will be investigated using two and three-dimensional cell culture. Also, the cytotoxicity due to primary amine needs to be reduced.

The method we suppose that can reduce the toxicity to the cells is to oxidise the primary amine by using hydrogen peroxide (H$_2$O$_2$). This strategy has been applied to reduce surface charge of polyethyleneimine (PEI)/DNA complexes but still leaves remaining cationic charges. PEI has high amine content and cationic charge density which is comparable to PVAM. Therefore, we assume that this technique should be effective for PVAM microgel in this work. This method is simple and only requires mixing of solutions at room temperature. However, excess H$_2$O$_2$ need to be removed with sodium pyruvate before cell culture.

Further study for the cationic microgel is the investigation of heteroaggregation of mixed vinyl functionalised microgels with the anionic microgel to form heteroaggregated DX gel. The rate at which heteroaggregate formation occurs and investigation of gel structure may be required. Dynamic light scattering (DLS), SEM, electrophoretic mobility analysis are beneficial techniques to characterise the heteroaggregated microgel. There are three different types of stimuli that have been used to control the heteroaggregation; temperature, pH, light. In order to ensure mixing microgels with cells, biocompatibility testing and the effect of microgel composition on biocompatibility study are needed.
6.3 References


One-Step Preparation of Uniform Cane-Ball Shaped Water-Swellable Microgels Containing Poly(N-vinyl formamide)

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ABSTRACT: In this study we report the preparation of a new family of core−shell microgels that are water-swellable and have a morphology that is controllable by particle composition. Here, nearly monodisperse core−shell PNVF-xGMA [poly(N-vinylformamide-co-glycidyl methacrylate)] particles (where x is the weight fraction of GMA used) were prepared via nonaqueous dispersion (NAD) polymerization in one step. The shells were PGMA-rich and were cross-linked by reaction of epoxide groups (from GMA) with amide groups (from NVF). The core of the particles was PNVF-rich. A bifunctional cross-linking monomer was not required to prepare these new microgels. The particles had a remarkable “cane-ball”-like morphology with interconnected ridges, and this could be controlled by the value for x. The particle size was tunable over the range 0.8−1.8 μm. Alkaline hydrolysis was used to hydrolyze the PNVF segments to poly(vinylamine), PVAM. The high swelling pressure of the cationic cores caused shell fragmentation and release of some of the core polymer when the hydrolyzed particles were dispersed in pure water. The extent to which this occurred was controllable by x. Remarkably, the PGMA-rich shells could be detached from the hydrolyzed particles by dispersion in water followed by drying. The hydrolyzed PNVF-0.4GMA particles contained both positively and negatively charged regions and the dispersions appeared to exhibit charge-patch aggregation at low ionic strengths. The new cross-linking strategy used here to prepare the PNVF-xGMA particles should be generally applicable for amide-containing monomers and may enable the preparation of a range of new water-swellable microgels.

INTRODUCTION

Microgels are cross-linked polymer colloid particles that swell in a good solvent.1−4 Stimulus-responsive microgels have particle sizes and polymer volume fractions that can be controlled by the local environment. Microgels have been already used in surface coatings.5 More recently, microgels have received considerable attention in the context of future high-performance applications. These include self-healing surfaces,6 glucose release,7 structural support for degenerated soft tissue,8 and quantum dot−microgel composites for biological imaging.9 The majority of the studies involving water-swellable microgels have used microgels based on (poly)N-isopropylacrylamide [(P)NIPAM].10−14 A bifunctional cross-linking monomer is usually added during the preparation of PNIPAM microgels.4 However, this is not essential because NIPAM can undergo interchain self-cross-linking by chain transfer.15 New water-swellable microgels that are NIPAM-free are of considerable interest for biomedical applications. There may be some cytotoxicity concerns associated with PNIPAM.16 Nonaqueous dispersion (NAD) polymerization, although a well established method for homopolymer particle preparation,17−20 has not been widely used to prepare water-swellable microgels. Here we used NAD polymerization to prepare a new family of core−shell microgels that are cross-linked in both the core and shell. This study investigated two hypotheses: first, that it is possible to use a reaction between comonomers containing amide and epoxide functionalities to prepare cross-linked microgel particles, and second, that core−shell microgels can be prepared in one step by NAD polymerization if the comonomers are chemically dissimilar and at least one is
water-soluble. We also investigated the effects of hydrolysis on the particles because of the potential to generate microgels containing primary amines. These types of microgels are of considerable interest for potential biomaterial applications.\(^{21}\)

*N-Vinylformamide, NVF, is an isomer of acrylamide and was developed to enable preparation of poly(vinylamine), PVAM. For linear polymers, alkaline hydrolysis is the most efficient hydrolysis method.\(^{22}\) However, the conversion of NVF-containing hydrogels to PVAM can be less than 100%.\(^{23}\) NVF, a nonconjugated monomer, is highly reactive and can form gels during polymerization.\(^{24}\) NVF is known to undergo chain transfer\(^{25}\) and this is believed to be involved in gel formation.\(^{24}\) This raises the possibility of self-cross-linking if PNVF can be formed at high monomer concentration during particle formation. Particles containing NVF have been studied by a number of groups. Shi and Berkland\(^{26}\) investigated PNVF and PVAM nanocapsules prepared from silica particles as a template. Pelton and co-workers\(^{27,28}\) conducted an extensive study of the preparation of PNIPAM-VAM \([i.e., \text{poly(NIPAM-co-VAM)}]\) microgels by precipitation polymerization. They first prepared PNIPAM-NVF microgels and used acid hydrolysis to obtain amine-functionalized microgel particles. The yield of NVF incorporation and conversion to PVAM was limited to about 50%. PVAM microgel has been reported that was completely hydrolyzed;\(^{29}\) however, the dispersions were not colloidally stable. It has proven difficult to prepare PVAM microgels that remain colloidally stable after hydrolysis. Colloidal stable microgel dispersions containing high mole fractions of primary amines have not yet been reported to our knowledge. One obstacle to this goal is that conventional cross-linkers such as \(N,N'\)-methylenebis(acrylamide) are not stable to hydrolysis. This has necessitated synthesis of nondegradable cross-linking monomers.\(^{30}\) We investigate here an alternative, simple, and versatile cross-linking strategy for preparing NVF-containing microgels that relies on reaction between the two monofunctional comonomers. The microgels can be hydrolyzed to generate VAM groups.

An unexpected result from this study is that the new PNVF-xGMA \([\text{poly(N-vinylformamide-co-glycidyl methacrylate)}]\) microgel particles had an unusual “cane-ball”-like surface morphology \((x \text{ indicates the weight fraction of GMA). Morphologically controlled microparticles are attracting considerable interest in the biomaterial area. This is because surface texture can be used to favorably control biologic–particle interactions.\(^{31}\) An excellent summary of the wide variety of surface morphologies reported has been given by Fujibayashi and Okubo.\(^{32}\) Recent examples of morphological controlled particles include “golf-ball”-shaped\(^{33}\) and highly folded microparticles.\(^{34}\)

In the present study we prepared core–shell PNVF-xGMA microgel particles by NAD polymerization. Our approach (Scheme 1) greatly extends two earlier reports which suggested that amides could cross-link epoxide groups.\(^{35,36}\) The present work represents the first report of polymer particles prepared from only NVF and GMA, that is, PNVF-xGMA. Men’skhova et al.\(^{37}\) prepared poly(methylmethacrylate) \((\text{PMMA})\) particles containing NVF and GMA using surfactant-free emulsion polymerization. Uyama et al.\(^{38,39}\) were the first to prepare PNVF particles by NAD polymerization. Horak and Shapoval\(^{45}\) performed seminal studies on PGMA particles prepared by NAD polymerization. They noted that cross-linking occurred between the GMA and amide groups of dimethylformamide \((\text{DMF})\). Importantly, no cross-linking occurred if ethanol was used as a solvent. Here we use a polymerizable \((\text{and hydrolyzable)}\) amide \((i.e., \text{NVF})\) in place of DMF.

PNVF polymerizes at a faster rate than GMA. We propose here that this produces a PNVF core (Scheme 1). PGMA nanoparticles form at a slower rate and adsorb to the PNVF-rich cores. The PGMA nanoparticles swell with NVF and this gives rise to cross-linking of the PGMA-rich shell by NVF. Subsequently, alkaline hydrolysis in aqueous medium was used to hydrolyze NVF segments in the core to PVAM. Conversion of NVF to VAM was not complete, and because of this, the hydrolyzed PNVF-xGMA particles are referred to as H-PNVF-xGMA. The formation of a partially hydrolyzed PNVF-VAM core and its subsequent swelling triggered fracture of the PGMA-rich shell. This is shown to enable detachment of the PGMA-rich shell from the core. The new, one-step, method for preparing core–shell microgels established here should be applicable to any NAD polymerization involving GMA and an amide-containing comonomer that is soluble in ethanol.

**EXPERIMENTAL SECTION**

**Reagents.** NVF (98%), GMA (97%), azaoisobutyronitrile \((\text{AIBN}, 98\%)\), and ethanol (99.5%) were purchased from Aldrich and used as received. Poly(vinylpyrrolidone) \((\text{PVP}, \text{Aldrich})\) had a weight-average molecular mass of 40 kg/mol and was also used as received.

**PGMA and PNVF Dispersion Preparation.** The method used to prepare PGMA particles was based on that reported by Horak and Shapoval.\(^{35}\) GMA \((12 \text{ g}, 0.084 \text{ mol})\), PVP \((1.8 \text{ g})\), and AIBN \((0.2416 \text{ g}, 1.47 \text{ mmol})\) were dissolved in ethanol \((86\text{ mL})\) and agitated for 1 h to ensure complete dissolution. The resulting solution was then degassed and placed in a temperature-controlled water bath. Monomer solutions were prepared fresh each day. GMA \((12 \text{ g}, 0.084 \text{ mol})\) and NVF \((12 \text{ g}, 0.084 \text{ mol})\) were dissolved in ethanol \((86\text{ mL})\) with thorough agitation to ensure complete dissolution. The resulting solution was degassed and placed in a water bath. It was then allowed to polymerize for 1 h.

**The shell is PGMA-rich and contains nanoparticles, which are proposed to overlap and inter-cross-link. PVP is poly(vinylpyrrolidone).**
mL) and then placed in a reactor equipped with an overhead IKA RW 20.n stirrer and nitrogen supply. The stirring rate used was 300 rpm. The system was purged with nitrogen and then heated at 70 °C for a total of 16 h. At the end of the reaction, the dispersion was filtered (50 μm mesh filter) and the particles were purified by repeated centrifugation and redispersion in ethanol. To test the effect of PVP concentration, a dispersion was prepared with a larger PVP mass (3.6 g). This system is referred to as PGMA-PVP.

PNVF particles were prepared via the same method as used for PGMA, with the exception that the NVF mass used was 6 g (0.084 mol). The reaction duration used was 1 h. The dispersion was purified by repeated centrifugation and redispersion with ethanol.

**PNVF-xGMA Dispersion Preparation.** The nonhydrolyzed copolymer particles are termed PNVF-xGMA, where x is the weight fraction of GMA used during polymerization with respect to total monomer mass. The following gives an example of the method used to prepare PNVF-0.4GMA particles. NVF (4.48, 0.063 mol), GMA (2.99 g, 0.021 mol), PVP (1.8 g), and AIBN (0.24 g, 1.47 mmol) were added to ethanol (86 mL). The solution was purged with nitrogen and then heated at 70 °C for 1 h unless otherwise stated. The other PNVF-xGMA dispersions were prepared in the same manner and the same mole ratio of monomer to initiator. The dispersions were purified by centrifugation as described above. In addition, a PNVF-0.4GMA preparation was conducted with a continuous feed of NVF and GMA solution over a period of 1 h. This was followed by a further 1 h of reaction. This system is referred to as PNVF-0.4GMA(F).

**Preparation of PVAM and Hydrolyzed PNVF-xGMA Dispersions.** PVAM was prepared from PNVF particles. This involved dissolving the PNVF particles in water containing 1 M NaOH and heating the solution to 80 °C for 24 or 48 h. The product was extensively dialyzed against water. Hydrolysis of the PNVF-xGMA dispersions was performed in the same manner with a heating period of about 16 h. In the case of PNVF-0.4GMA, the particles remained colloidal stable and did not dissolve during hydrolysis. The PNVF-xGMA dispersions subjected to hydrolysis are referred to as H-PNVF-xGMA. The dispersions were purified by centrifugation as described in the text.

**FITC Labeling of Particles.** The particles were labeled with fluorescein 5(6)-isothiocyanate (FITC). FITC (10 mg, 0.026 mmol) was dissolved in 10 mL of water/acetone solution (10 vol % acetone) at pH 9. Then PNVF-0.4GMA or H-PNVF-0.4GMA particles (0.5 g) were added. The dispersion was stirred at room temperature (RT) for 6 h in the absence of light. The particles were repeatedly centrifuged and redispersed in water until FITC could no longer be detected in the supernatant by UV−visible absorption at a wavelength of 500 nm.

**Physical Measurements.** Microanalysis (C, H, and N) was conducted at the School of Chemistry, University of Manchester. (All microanalysis data are shown in Table S1, Supporting Information.) Scanning electron microscopy (SEM) measurements were obtained on a Philips FEGSEM instrument. Samples were dried at room temperature. At least 40 particles were measured to determine the number-average SEM diameters, D_{SEM}. A Malvern Zetasizer was used to measure the ζ potentials for the particles. Photon correlation spectroscopy (PCS) measurements were performed on a BI-9000 Brookhaven light scattering apparatus (Brookhaven Instrument Corp.) fitted with a 20 mW HeNe laser, and the detector was set at a scattering angle of 90°. Optical microscopy was conducted on an Olympus BX41 microscope. At least 80 particles were measured to determine the number-average diameters (D_{opt}). Fluorescence microscopy was conducted on a Nikon Eclipse 50i microscope. For experiments involving rhodamine B, the excitation and emission wavelengths were 540 and 605 nm, respectively. For FITC and sodium fluorescein, the excitation and emission wavelengths had an average of 480 and 535 nm, respectively. Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) measurements were conducted on a Nicolet 5700 FTIR instrument.

**RESULTS AND DISCUSSION**

**PNVF-xGMA Particles.** A range of PNVF-xGMA systems were investigated in this study (see Table 1). The particles could be dispersed in ethanol or water and did not dissolve (see Figure 1). We used the ratio of % N to % C data determined by microanalysis to assess particle composition. The experimental ratios for PNVF, PNVF-0.2GMA, PNVF-0.4GMA, and PNVF-0.4GMA(F) are close to the theoretical values based on the compositions used to prepare the particles (Table 1). The hydrodynamic diameters (d) for PNVF-0.4GMA were larger (Table 1) than the D_{SEM} and D_{opt} values. This is expected because the measured hydrodynamic diameter is weighted in favor of larger particles. Importantly, the values of d for PNVF-xGMA (x = 0.2 and 0.4) are consistently larger for the particles dispersed in water (a good solvent for PNVF) than ethanol (a poor solvent). The same trend is apparent for the D_{opt} values.

### Table 1. Characterization Data for the Particles Investigated

<table>
<thead>
<tr>
<th>code</th>
<th>W_{PVAM}</th>
<th>D_{SEM}</th>
<th>D_{opt}</th>
<th>% N/% C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNVF-0.2GMA</td>
<td>0.27</td>
<td>0.80 (8)</td>
<td>1.25 (9)</td>
<td>1.15 (10)</td>
</tr>
<tr>
<td>PNVF-0.4GMA</td>
<td>0.24</td>
<td>1.00 (12)</td>
<td>1.54 (13)</td>
<td>1.27 (0.05)</td>
</tr>
<tr>
<td>PNVF-0.4GMA(F)</td>
<td>0.24</td>
<td>0.90 (81)</td>
<td>1.12 (0.07)</td>
<td>0.88 (0.09)</td>
</tr>
<tr>
<td>PNVF-0.75GMA</td>
<td>0.19</td>
<td>1.85 (5)</td>
<td>0.38</td>
<td>0.39</td>
</tr>
<tr>
<td>PNVF</td>
<td>0.30</td>
<td>0.20 (56)</td>
<td>0.003</td>
<td>0</td>
</tr>
<tr>
<td>PGMA</td>
<td>0.15</td>
<td>2.00 (3)</td>
<td>1.90 (9)</td>
<td>0</td>
</tr>
<tr>
<td>PGMA/HPVP</td>
<td>0.30</td>
<td>1.15 (6)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

“Weight fraction of PVP used with respect to total monomer mass. “Number-average diameter from SEM. The coefficient of variation is shown in parentheses. “Number-average diameter measured by optical microscopy for particles dispersed in water or ethanol. The number in parentheses is the coefficient of variation. “Hydrodynamic diameters of the particles dispersed in water or ethanol. The number in parentheses is the polydispersity index value. “Experimental and theoretical ratio of % N and % C from microanalysis. The full set of microanalytical data is shown in Table S1, Supporting Information.”
These PNVF-xGMA particles swell in a good solvent for the major constituent (NVF). Consequently, these are new microgel particles. The particles had low polydispersities; for NAD polymerization, this indicates a short nucleation period and rapid attainment of a constant particle number. The polydispersity increased when the feed method was used to prepare PNVF-GMA(F) (Table 1). In that case, the nucleation stage occurred throughout the feed.

We qualitatively assessed the internal porosity of the PNVF-0.4GMA particles using FITC labeling. The procedure involved alkaline conditions that would have resulted in the formation of some RNH₂ groups. Those groups would, in turn, have reacted with FITC. The fluorescence images (Figure 1c) show that PNVF-0.4GMA particles have a uniform intensity. It follows that FITC was able to completely penetrate the interiors of the microgel particles.

The average \( D_{\text{SEM}} \) of the PNVF-xGMA dispersions could be tuned over the range 0.8–1.85 \( \mu \text{m} \) by the weight fraction of PVP used with respect to total monomer mass (\( W_{\text{PVP}} \)) (see Figure S1, Supporting Information). This is because of PVP stabilization of growing particles during NAD polymerization. A higher surfactant concentration stabilizes the growing particle nuclei at an earlier stage which results in a greater number of smaller particles. PGMA was also prepared using a higher \( W_{\text{PVP}} \) value (PGMAHPVP) and the value for \( D_{\text{SEM}} \) was 1.15 \( \mu \text{m} \), which is significantly smaller than the value of 2.0 \( \mu \text{m} \) for PGMA (Table 1).

SEM images (Figure 2) of PNVF-0.2GMA, PNVF-0.4GMA, and PNVF-0.75GMA particles showed a surprisingly rich morphology with interconnected ridges. This morphology is different than "golf-ball"-like morphologies³³,⁴¹ and "wrinkled" morphologies³⁴ reported elsewhere. Because we could not find a polymer particle morphology that was similar to these in the literature, we term them "cane-ball"-like morphologies. This is because they consist of interconnected ridges and bear a resemblance to cane balls in appearance. However, we do not
believe the ridges are interwoven. This remarkable morphology was apparent for PNVF-0.4GMA prepared by both batch (Figure 2d–f) and feed (Figure S2e,f, Supporting Information) methods. It was also present for PNVF-0.4GMA particles heated for 16 h (Figure S2d, Supporting Information). This shows the morphology is not due to unreacted NVF. The PGMA particles (Figure 2j–l) also have interconnected ridges, but they were broad and less pronounced. PGMAHPVP also had PGMA particles (Figure 2j) shows the morphology is not due to unreacted NVF. The PGMA particles reported elsewhere had PGMAHPVP also had interwoven ridges apparent (see Figure S2g,h, Supporting Information). This differs from the PGMA particles reported elsewhere that appeared to have featureless surfaces from SEM.35,40 For the PNVF-xGMA particles, the ridges increased in width and became less pronounced with increasing x (Figure 2). In contrast, the PNVF particles had a smooth surface (Figure S2a,b, Supporting Information). The fact that the morphologies for all the PNVF-xGMA particles are similar to that of PGMA, but strikingly different than that of PNVF, strongly suggests that the surface of the PNVF-xGMA particles is rich in PGMA. We suggest a core–shell morphology. On the basis of the composition for PNVF-0.4GMA, if a perfectly phase-separated, uniform, PNVF core/PGMA shell morphology were present, the ratio of the average shell thickness to total particle diameter would be about 0.1.

The cane-ball-like morphologies (Figure 2) appear to be a new polymer particle morphology and are worthy of further comment. They do have features similar to those reported for golf-ball-like particles33,41 in that troughs are present, but they are visually different because of the ridge patterns. In the present case these morphologies form spontaneously in one step. The cane-ball-like morphologies can also be compared to wrinkling on microparticles.34 If the wrinkling were operative, then the surface of PNVF would have a wrinkled appearance because PNVF is soluble in NVF. But this was not the case as the surface was smooth (Figure S2b, Supporting Information). The strongly pronounced ridges for the PNVF-0.2GMA particle surfaces (Figure 2a–c) are strongly suggestive of a separate PGMA-rich phase that forms on the surface of the particles. (See also the SEM image for PNVF-0.4GMA particles polymerized for 16 h in Figure S2d, Supporting Information.) The morphology is most likely the result of uneven growth of the PGMA phase rather than wrinkling.34 However, we cannot rule out partial shrinkage under the high vacuum of the SEM contributing to the appearance of the particles. We stress that the images shown in Figure 2 are representative of a large number of images and samples and are reproducible.

NVF is a highly reactive monomer with a propagation rate constant \((k_p)^{25}\) of at least \(4 \times 10^3 \text{ L.mol}^{-1}\text{s}^{-1}\). To our knowledge the equivalent rate constant for GMA has not been reported. The \(k_p\) for methyl methacrylate (MMA) is about42 \(10^2 \text{ L.mol}^{-1}\text{s}^{-1}\). It is very likely that NVF polymerizes faster than PGMA. In that case, PNVF should form the core of the particles. We propose that the more slowly polymerizing PGMA is swept up by the larger PNVF particles and becomes a shell and does not significantly penetrate the PNVF core. PNVF and PGMA are dissimilar polymers; PNVF is hydrophilic and PGMA is hydrophobic. The PGMA phase should minimize its interfacial contact area with a PNVF core. However, PNVF does dissolve in NVF, which provides a means by which NVF could react with a PGMA phase present as a shell on these particles.

The PNVF-0.2GMA and PNVF-0.4GMA particles did not appear to dissolve in water or ethanol (Figure 1). In an effort to force particle dissolution (and test for cross-linking), the PNVF-0.4GMA particles were dispersed in a good solvent for PGMA (tetrahydrofuran, THF) for 24 h and then a good solvent for PNVF (water) for a further 24 h. It should be noted that PGMA and PNVF particles dissolved in THF and water, respectively. Optical microscopy images and SEM showed that the PNVF-0.4GMA particles did not dissolve after this treatment (see Figure S3, Supporting Information). If the particles contained separate, solvent-accessible domains of linear PGMA and PNVF, then complete dissolution would have occurred. This demonstrates that cross-linking is effective for the PNVF-0.4GMA particles. SEM for the PNVF-0.2GMA particles deposited from water (Figure S6a, Supporting Information) also showed intact particles, suggesting effective cross-linking for that system.

FTIR spectra for the particles are shown in Figure 3. The band at 3250 cm\(^{-1}\) in Figure 3a is attributed to the amide group of NVF. The band at 905 cm\(^{-1}\) in Figure 3b is the stretching vibration of the epoxide group35,36 and is present for PGMA, PNVF-0.2GMA, and PNVF-0.4GMA. This band is relatively small for PVF-0.2GMA. The band at 1725 cm\(^{-1}\) is due to COO groups within PGMA segments. The amide I (1650 cm\(^{-1}\)) and amide II (1530 cm\(^{-1}\)) bands are in the same positions for each of the spectra. The band at 1385 cm\(^{-1}\) is ascribed to the N–C–H bending mode35 of NVF.

Horak and Shapoval35 reported that PGMA particles were cross-linked when DMF was used as the solvent for NAD polymerization. Recently, Mu and Zheng42 reported that the amide groups of NIPAM segments formed cross-links with
epoxide groups of a polyhedral oligomeric silsequioxane (POSS). On the basis of their work and our particle dissolution tests described above, we propose that a portion of the PNVF segments cross-link with the PGMA segments in the shell of the PNVF-0.4GMA particles. To quantify this effect, the FTIR spectra for PNVF-0.2GMA and PNVF-0.4GMA were compared with those of physical blends of PNVF and PGMA with the same respective mole ratios (see Figure S4, Supporting Information). Comparison of the spectra clearly shows a decreased absorbance of the epoxide band for the PNVF-xGMA particles compared to the PNVF/xPGMA homopolymer blends. The ratios of the area for the epoxide band ($A_{905}$) to the area for the N–C–H band ($A_{1385}$) were used to estimate the percentage of conversion ($C$) for reaction of the epoxide groups with the NH groups of the amide:

\[
C = 100 \left( 1 - \frac{A_{905}(P)}{A_{1385}(P)} \right)
\]

For eq 1, the subscripts P and B refer to the PNVF-xGMA particles and PNVF/PGMA blend, respectively. This analysis assumes that the loss of epoxide groups is only due to reaction with the NH groups. Barbey and Klok\textsuperscript{44} used a related equation to assess conversion of epoxide groups when reacted with amines. The calculated values of $C$ for the epoxide groups of PNVF-0.2GMA and PNVF-0.4GMA were 62.7% and 60.7%, respectively. It follows that the PNVF-xGMA particle shells consist of PGMA chains where up to about 60% of the epoxide groups react with NVF segments. This implies a highly cross-linked PGMA-NVF copolymer and is consistent with the solubility tests described above.

On the basis of the above, we propose a cross-linking reaction between GMA and NVF segments in Scheme 2. This scheme is related to that suggested by Mu and Zheng\textsuperscript{56} for their PNIPAM/POSS system. Scheme 2 is consistent with unreacted epoxide groups found in the FTIR spectra (905 cm\textsuperscript{-1}, Figure 3b). These unreacted groups could result from steric hindrance preventing close approach of segments from neighboring PNVF and PGMA chains. Furthermore, the RCOO (1725 cm\textsuperscript{-1}), amide I (1650 cm\textsuperscript{-1}), and amide II (1530 cm\textsuperscript{-1}) bands present in Figure 3 are consistent with Scheme 2.

**Scheme 2. Proposed Cross-Linking of PNVF and PGMA**

Hydrolysis of PNVF-xGMA Particles. We investigated the effect of alkaline hydrolysis on the PNVF-xGMA particles. The dispersions retained their colloidal stability after hydrolysis. However, they had a tendency to swell and also to aggregate during redispersion with water. We probed the changes in size and \(\zeta\) potential upon hydrolysis of PNVF-0.4GMA. The hydrolyzed particles (H-PNVF-0.4GMA) were successively centrifuged and redispersed in aqueous NaOH with concentrations of 1, 0.1, and 0.01 M and then water. Redispersion using water commenced at pH = 10.9 (see Figure 4a,b). The initial small, negative \(\zeta\) potential for PNVF-0.4GMA of about \(-1\) mV changed to 59 mV after hydrolysis (pH = 14) and then became strongly negative (at pH \(\leq 13.5\)) with redispersion in lower NaOH concentration solutions. The large positive \(\zeta\) potential (59 mV) at such a high pH was unexpected and reproducible. It may be due to PVAM-rich polymer at the particle surface.

The diameter passed through a maximum and then decreased (Figure 4a) at pH values less than 11. This is close to the apparent $pK_a$ of 10 reported for PVAM.\textsuperscript{49} Comparison of the diameter and \(\zeta\) potential values for PNVF-0.4GMA at the start (pH = 7.3) and the end (pH = 7.1) of the hydrolysis/redispersion procedure (Figure 4a,b) shows that an irreversible change occurred. The particles swelled and became more negatively charged. We propose that this is due to pH-triggered swelling of PVAM segments in the core of the particles. The maximum in Figure 4a requires further comment. SEM evidence is presented below (Figure 5c) for shell fragmentation at a pH of 7.4. Because of this, it is suggested that at pH values less than 10 the particle shell fragmented to such an extent that increased release of core PVAM-rich copolymer reduced the swelling pressure and particle size (Figure 4a).

Hydrolysis, and redispersion in water, resulted in a decrease of the % N/% C ratio. The experimental ratio for H-PNVF-0.4GMA was 0.17, compared to a theoretical value of 0.27 for fully hydrolyzed PVAM-0.4GMA. The reduction in % N/% C due must be due to loss of NVF and/or VAM-containing polymer from the particles. For comparison, we hydrolyzed PNVF particles for 24 and 48 h under the same conditions used to hydrolyze PNVF-0.4GMA. The % N/% C values were 0.53 and 0.54, respectively. These values correspond to 80 and 84 mol % conversion of PVF to PVAM, respectively. The hydrolysis efficiency for PNVF-0.4GMA was probably much less than 80 mol % (24 h of heating) because, unlike PNVF, the PNVF-0.4GMA particles did not dissolve and remained as particles. Because of the copolymer release that occurred during H-PNVF-0.4GMA particle purification, microanalysis could not be used to estimate the extent of hydrolysis.

The particles swelled strongly as a consequence of hydrolysis but did not fully dissolve, and this is best seen after FITC treatment (compare Figures 4d and 1c). There is a reduced intensity at the core of the particles in Figure 4d. This suggests that more FITC is located at the particle peripheries than in the cores. This implies a higher amine content in the particle shells at this pH (6–7) than in the core and is consistent with a core–shell structure. To further investigate the charge distribution in the particles, they were exposed to sodium fluorescein or rhodamine B for ca. 16 h (see Figure 4e,f). The particles were more swollen for these chromophores compared to FITC. This could be due to differences in chromophore concentration and binding mechanism, which is covalent for FITC. This is in the case of sodium fluorescein (Figure 4e), the anionic fluorophore is present at the periphery and also in the center of the particles (which is similar to FITC). This indicates that positive charge is present in both core and shells. Rhodamine B (a cationic fluorophore) was less able to penetrate the core, and high intensity was mostly located at the periphery (Figure 4f).

This may be due to negatively
charged regions of the shell. We speculate that these particles have both positive and negative charge within the shell. The $\zeta$ potential data (Figure 4b) show that the periphery is predominantly negatively charged at pH of 6−7. The experiments with FITC and sodium fluorescein indicate a positively charged core. These qualitative tests indicate that the core−shell microgel particles have positive and negative charge (polyampholytes) at pH values of 6−7.

We investigated the hydrolysis of PNVF-0.4GMA particles using SEM. Images were taken before (Figure 5a) and after hydrolysis (Figure 5b) and also after redispersion in water (Figure 5c). The arrows in Figure 5b show that the particle shells were cracked. These cracks may provide a pathway for loss of core copolymer during the washing steps (Figure 4a). The average $D_{\text{opt}}$ of these particles (Figure 5b, 1.21 $\mu$m) is significantly larger than that of the PNVF-0.4GMA particles (1.0 $\mu$m) shown in Figure 5a. When the particles were washed with water and the pH and ionic strength decreased, a profound morphological change occurred (see Figure 5c). The outer shell appears to have detached, leaving the core particles intact. The darker particles originate from a different polymer phase to the shell because SEM contrast is sensitive to electron density. This new phase must be partially hydrolyzed PNVF, that is, PNVF-VAM. This sequence of SEM micrographs provides additional evidence for a core−shell morphology for PNVF-xGMA. The shell is detachable. The core particles have an average $D_{\text{SEM}}$ of 1.53 $\mu$m. Detached nanoparticles from the shell neatly surround the cores (Figure 5c). On the basis of these images, we can suggest that during the water redispersion stage of particle purification the core expanded (Figure 4a), causing fragmentation of the cracked, hydrolyzed, shell (Figure 5b). Core−shell microgels are known to develop significant stress imbalances at the boundaries between the core and shell.46 However, the present behavior indicates very strong stress imbalances. These morphological changes are both remarkable and reproducible.

We investigated whether the cores observed in SEM of the H-PNVF-0.4GMA particles (Figure 5c) were cross-linked by a simple dissolution test. This involved placing a drop of water in the particles' sample dish. The test was conducted under aerobic conditions to ensure that the hydrolysis was complete. After the particles were washed with water and the pH and ionic strength decreased, a profound morphological change occurred (see Figure 5c). The outer shell appears to have detached, leaving the core particles intact. The darker particles originate from a different polymer phase to the shell because SEM contrast is sensitive to electron density. This new phase must be partially hydrolyzed PNVF, that is, PNVF-VAM. This sequence of SEM micrographs provides additional evidence for a core−shell morphology for PNVF-xGMA. The shell is detachable. The core particles have an average $D_{\text{SEM}}$ of 1.53 $\mu$m. Detached nanoparticles from the shell neatly surround the cores (Figure 5c). On the basis of these images, we can suggest that during the water redispersion stage of particle purification the core expanded (Figure 4a), causing fragmentation of the cracked, hydrolyzed, shell (Figure 5b). Core−shell microgels are known to develop significant stress imbalances at the boundaries between the core and shell.46 However, the present behavior indicates very strong stress imbalances. These morphological changes are both remarkable and reproducible.

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We investigated whether the cores observed in SEM of the H-PNVF-0.4GMA particles (Figure 5c) were cross-linked by a simple dissolution test. This involved placing a drop of water
on the deposited particles from Figure 5c, allowing the droplet to evaporate overnight, and then examining the sample by SEM (see Figure 6). Some of the cores lifted away from the detached nanoparticle shell layer. The holes in the deposited nanoparticle layer are shown with arrows in Figure 6a. These particles redeposited in the center of the SEM stub as a large aggregate (see Figure S5, Supporting Information). The SEM images (Figure 6b,c) show that these core particles had swollen further (prior to final redeposition on the stub) and contained some residual nanoparticles on the shell. The SEM value was about 1.95 μm. The fact that these particles were still intact demonstrates that the core particles were (lightly) cross-linked. (The number density of intraparticle cross-links was probably low because there are indications from the top left of Figure 6a that not all of the particle cores remained intact.) The observation of intact particles shows that a significant extent of self-cross-linking occurred for these PNVF-VAM microgel cores. Self-cross-linking may originate from branching that is known to occur during PNVF polymerization at high concentrations.24,25

PNVF-0.2GMA particles showed some important differences from PNVF-0.4GMA when subjected to hydrolysis. When PNVF-0.2GMA particles were deposited from water (prior to hydrolysis) most of the particles remained intact and a cane-ball-like morphology was evident (Figure S6a, Supporting Information). When they were hydrolyzed, the PGMA shell fragmented and the core PNVF-VAM copolymer was completely released (see Figure S6b, Supporting Information). For this system the core was not cross-linked sufficiently to enable the core particles to remain intact. This shows that a...
minimum value for $x$ of at least 0.4 is required to prevent core dissolution as a result of hydrolysis.

FTIR spectra were obtained for purified H-PNVF-0.4GMA (Figure 7). PVAM has a strong band at 1560 cm$^{-1}$ and similar features to those reported for PVAM capsules.\[26\] Compared to the spectrum for PNVF-0.4GMA, hydrolysis removes the RCOO band at 1725 cm$^{-1}$ and also the epoxide band at 905 cm$^{-1}$. This shows that the OH$^-$ groups were able to penetrate all of the PGMA domains. In Figure 7b, the amide I is present for H-PNVF-0.4GMA (1660 cm$^{-1}$). This shows that unreacted PNVF is present. However, the band moved to a slightly higher wavenumber (cf. 1650 cm$^{-1}$ for PNVF-0.4GMA). This may indicate a change in local environment. There is also new band at 1560 cm$^{-1}$ for H-PNVF-0.4GMA. Because this feature is present in the spectrum of PVAM and was not present in the spectrum of PNVF, we ascribe this band to RNH$_2$. The strength of this band suggests that significant PVAM remained in the purified particles. The features at about 3250 cm$^{-1}$ in the spectrum for H-PNVF-0.4GMA (Figure 7a) are consistent with a combination of PVAM and PNVF. Consideration of these data implies that H-PNVF-0.4GMA contained mostly NVF.

We summarize our interpretation of the unusual behaviors observed in this study in Scheme 3. PNVF-0.4GMA is a core-shell microgel with PGMA-rich shell and a PNVF-rich core. Both the shell and core are cross-linked. The shell is mostly nanoparticulate in nature and highly cross-linked. It may be a thickness of more than one PGMA nanoparticle monolayer. The core is lightly cross-linked. Hydrolysis converts a significant proportion of PNVF to PVAM, and this leads to monolayer. The core is lightly cross-linked. Hydrolysis converts nanoparticulate in nature and highly cross-linked. It may be of core dissolution as a result of hydrolysis.

**CONCLUSIONS**

In this study we have investigated a new family of low polydispersity, water-swellable core–shell microgels. PNVF-xGMA were prepared in one step by NAD polymerization. The particles have controllable size over the range 0.8–1.8 μm. The PNVF-xGMA particles spontaneously formed core–shell morphologies and have a remarkable cane-ball-like morphology. This was controllable by $x$, the weight fraction of GMA. Particles with novel morphologies are potential useful for enhanced movement in applied fields and also potentially in biomaterials research. The PNVF-0.4GMA particles swelled in water, exhibiting microgel behavior. A cross-linking mechanism for the shell involving reaction of amide and epoxide groups was proposed. This cross-linking approach should be generally applicable and enable new core–shell microgels to be prepared from GMA and other amide-containing monomers. Alkaline treatment hydrolyzed NVF segments in the core to VAM. Purification resulted in core swelling and shell fragmentation, which released some PNVF-VAM from the core. The hydrolyzed PGMA-rich shells could be detached. The core particles were lightly cross-linked microgels, based on PNVF-VAM. Hydrolysis caused the shells to become negatively charged and charge-patch aggregation occurred. Because the shells can be detached on demand, these particles provide a new approach for architectural control for microgels. The new, reactive, core–shell microgels presented here for the first time have the most remarkable morphologies and geometric versatility of any microgels so far reported to our knowledge.

**ASSOCIATED CONTENT**

Supporting Information

Six figures showing variation of diameter with weight fraction of added PVP, optical and SEM image for PNVF-0.4GMA particles after washing with good solvents, SEM image of crushed PNVF-0.4GMA($P$) particles, FTIR spectra for PNVF-xGMA and PNVF/xPGMA blends ($x = 0.2$ and 0.4), SEM image of H-PNVF-0.4GMA aggregate, and SEM images of PNVF-0.2GMA before and after hydrolysis and one table listing microanalysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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**REFERENCES**

(28) Xu, J.; Pelton, R. J. Colloid Interface Sci. 2004, 276, 113.
(37) Men’shikova, A. Y.; Inkin, K. S.; Evseeva, T. G.; Skurkis, Y. O.; Shabsel’s, B. M.; Shevchenko, N. N.; Ivanchev, S. S. Colloid J. 2011, 73, 76.
Poly(vinylamine) microgels: pH-responsive particles with high primary amine contents†

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Introduction

Microgels are crosslinked polymer colloid particles that swell in a good solvent.1–4 pH-responsive microgels swell when the pH approaches the pK_a of the constituent polybase or polyacid chains.5 They have potential application in the areas of rheological modifiers, surface coatings,6 photonic materials,7 drug delivery8 and regenerative medicine.9 The overwhelming majority of research conducted on pH-responsive microgels has involved anionic, alkali-swellable, microgels containing carboxylic acid groups.10–14 Acid-swelling microgels15–22 and gel bead particles23 containing primary amines are versatile systems for functionalization. Unfortunately, a method to prepare colloidally stable dispersions of microgels containing high primary amine contents has not been reported. Acid-swelling microgels based on secondary and tertiary amines have been well studied,24,25 but they have limited potential for functionalization. Poly(vinylamine) (PVAM) is a very attractive design target for microgels containing primary amines because it contains the highest nitrogen content of all polycations26 and has a very wide-range of functionalization reactions.27 Unfortunately, the synthesis of dispersions of colloidally stable PVAM-rich microgels or, indeed, any other high content primary amine microgel has remained elusive. PVAM cannot be synthesized from vinylamine.27 PVAM is commonly introduced post-polymerisation, e.g. by alkali-hydrolysis of the corresponding poly(N-vinylformamide) (PNVF) material to reveal the primary amine groups.27 The harsh hydrolysis conditions required has obstructed attempts to prepare colloidally stable microgel dispersions with high primary amine contents. PVAM microgels are an important omission from the literature that has potential to provide a plethora of new functionalized microgels and microgel-based materials and hybrids. The motivation for this study was the need to establish a method for preparing colloidally stable pH-responsive PVAM microgel dispersions. Here, we introduce a new, scalable, two-step method to synthesize PVAM microgels and investigate the properties of the new microgels.

Our previous work involving poly(N-vinylformamide-co-glycidyl methacrylate) microgel particles offered a route to microgels...
containing PVAM. However, the particles severely fragmented during alkali-hydrolysis due to cleavage of the crosslinks. Cross-link degradation is a well known general obstacle that frustrates synthesis of colloidal stable PVAM microgels. Based on a report from the Kansas laboratory we hypothesised that improved chemical stability to alkali-hydrolysis could be achievable by synthesizing of NVF particles containing a crosslinking monomer that was structurally related to NVF. We selected 2-(N-vinylformamido)ethyl ether (NVEE) because it was expected to have a similar reactivity ratio to NVF and also contained an alkali-stable ether linkage that is resistant to cleavage. An aim of this study was to demonstrate the feasibility of functionalizing primary amine groups of the PVAM microgels. We used N-[3-dimethylaminopropyl]-N'-ethylcarbodiimide (EDC) assisted conjugation for this purpose because it is widely used for functionalization of polymers (and biomaterials) containing primary amines.

PVAM readily adsorbs onto anionic surfaces and has been prepared as composite nanoparticles. Well-defined amphiphilic core–shell particles containing a PVAM shell have been reported. Recently, PVAM hydrogel shells and hydrogel capsules have been reported. Shi et al. studied the preparation of silica/PNVF clusters and also PVAM micro and nanogel capsules. In contrast to those studies, the PVAM microgel particles introduced here are not hollow. Therefore, microgel particles contain a much higher total primary amine contents (and functionalization capacity) than capsules. Microgels have an inherently higher elastic modulus than capsules because they have a higher number of elastically effective chains per particle. Our two-step synthetic method used to prepare colloidal stable pH-responsive PVAM microgel dispersions is depicted in Scheme 1. We used non-aqueous dispersion polymerization (NAD) to prepare new PNVF-xNVEE microgels (x is the mol% of NVEE employed) which were then hydrolysed to give poly(vinylamine-co-bis(ethyl vinylamine)ether) (PVAM-xBEVAME) microgels.

A finding of this study (from SEM images) was that PVAM-9BEVAME microgels had a cluster-like morphology. Cluster particles or popcorn-shaped particles have been reported earlier. Li and Stover reported popcorn-shaped particles for poly(divinylbenzene) particles prepared by NAD. The mechanism of their formation was attributed to adsorption of primary particles by larger monomer-swollen particles that were prepared early in the polymerization. Here, we provide the first report of pH-responsive microgel particles with cluster-like morphologies to our knowledge.

The structure of this paper is as follows. We first study a series of new water-swellable PNVF-xNVEE microgels and then hydrolyse them to pH-responsive PVAM-xBEVAME microgels. The morphologies of the microgel particles were characterised using SEM. Their compositions were probed using FTIR and quantified using elemental analysis. The pH-responsive properties of the PVAM-xBEVAME particles were measured using hydrodynamic diameter and electrophoretic mobility measurements. Finally, the ability to use the primary amine groups within the microgels for functionalization is

![Scheme 1](image_url)
demonstrated. The new two-step method to synthesize PVAM microgels (Scheme 1) introduced here is scalable. The pH-responsive PVAM microgels introduced here should open up a wide range of potential applications from advanced surface coatings, hybrid particles, rheological modifiers and delivery.

**Experimental section**

**Materials**

NVF (98%), azoisobutyronitrile (AIBN, 98%), potassium-tert-butoxide (95%), bis(2-bromoethyl)ether (BPE, 95%), dicyclohexyl-18-crown-6 (98%), anhydrous THF (99.9%), 1-pyrene carboxylic acid (PyC, 97%), fluorescein 5(6)-isothiocyanate (FITC, >90%) and ethanol (99.9%) were purchased from Aldrich and used as received. EDC (97%), N-Hydroxysuccinimide (NHS, 98%), cetyltrimethylammonium bromide (CTAB, 98%) and poly(1-vinylpyrrolidone-co-vinyl acetate) (PVP-co-PVA) were purchased from Aldrich and used as received. PVP-co-PVA had a weight-average molecular weight of 50 000 g mol⁻¹ and a VA content of 43 mol%. The linear PVAM used here was prepared earlier by alkalihydrolysis of PVNF particles and is described elsewhere. High purity water that was distilled and deionised was used.

**Crosslinking monomer synthesis**

NVF was synthesised using a modification to a method reported earlier87 (see Scheme S1). A mixture of NVF (7.1 g, 100 mmol), potassium-tert-butoxide (12.0 g, 105 mmol) and dicyclohexyl-18-crown-6 (1.00 g, 2.65 mmol) in THF was stirred vigorously and cooled to 0 °C. BBE (9.30 g, 40 mmol) was added dropwise to the mixture. The mixture was allowed to warm to room temperature and stirred for another 72 h. KBr was removed by filtration and the reaction mixture concentrated using rotary evaporation and diluted with water (100 mL). The product was extracted using chloroform washing and the extracts were combined and washed with brine and then dried over anhydrous sodium sulfate. NVEE was recovered from the chloroform solution as a liquid (5.69 g, 53% yield). The compositional purity of NVEE was confirmed by elemental analysis, ¹H NMR and FTIR spectroscopy (ESI, Fig. S1 and S2†). Based on ¹H NMR spectroscopy data (Fig. S1†) NVEE was a mixture of trans and cis isomers in the ratio of 1:2.

**PNVF latex preparation**

PNVF particles were prepared by NAD in latex form, i.e., as non-swollen particles dispersed in ethanol. NVF (6 g, 85.5 mmol), AIBN (0.240 g, 1.45 mmol) and PVP-co-PVA (1.8 g) were added to ethanol (68 g) in a 4-neck round bottomed flask equipped with an overhead stirrer, nitrogen supply and a reflux condenser. The solution was purged using nitrogen and heated to 70 °C whilst being stirred vigorously. The polymerization time was 1 h. After cooling, the dispersion was purified by three centrifugation/redispersion cycles.

**PNVF-xNVEE microgel synthesis**

PNVF-xNVEE microgels were prepared in latex form (i.e., as collapsed particles) using NAD using the procedure described above for PNVF. The value for x represents the mol% of NVEE used during preparation with respect to monomer. NVF (6 g, 85.5 mmol), AIBN (0.240 g, 1.45 mmol), PVP-co-PVA (1.8 g) and the appropriate mass of NVEE were added to ethanol (68 g) and the solution purged using nitrogen. The NVEE masses used were 0.845 g (3.91 mmol), 1.79 g (8.28 mmol) and 2.68 g (12.4 mmol), respectively, for PNVF-xNVEE where x = 4, 9 and 13. After cooling, the dispersion was purified by centrifugation using ethanol three times. The yield of recovered PNVF-9NVEE particles was 4.2 g.

**PVAM-xBEVAME microgel synthesis**

Alkali-hydrolysis was used to convert NVF and NVEE into, respectively, VAM and BEVAME (see Scheme 1). PNVF-xNVEE particles (1 g) were redispersed in aqueous NaOH solution (1 M) and heated, with stirring, at 80 °C under a nitrogen atmosphere for 16 h. For PNVF-xNVEE (x = 9 and 13) the hydrolysed dispersions were purified using repeated centrifugation and redispersion in water. In the case of PNVF-4NVEE the hydrolys product was extensively dialysed against water. The yield of recovered PVAM-9BEVAME particles after purification by centrifugation was 0.33 g.

**FITC-labelled PVNE-4NVEE and PVAM-4BEVAME microgel synthesis**

FITC solution (20 µL, 2.6 mM) was added to the microgel dispersion (200 µL, 0.1 wt% dispersion). The dispersion was subjected to end-over-end mixing overnight.

**Pyrene-labelled PVAM-9BEVAME microgel synthesis**

The pH of a PVAM-9BEVAME dispersion (5 mL of 0.1 wt% dispersion in water) was adjusted to 6.8 using aqueous HCl (1 M). EDC (5.1 mg, 0.033 mmol) and NHS (3.9 mg, 0.034 mmol) were then added. The dispersion was stirred at room temperature before adding PyC (4.1 mg, 0.017 mmol) and the reaction was allowed to proceed for 18 h at room temperature. The product was purified by centrifugation and redispersion in PBS solution (0.15 M), NaCl solution (0.15 M) and then water. Physically absorbed PyC was removed by adding CTAB (0.1 M) to the dispersion and this was stirred overnight at room temperature before being repeatedly centrifuged and redispersed in water. A control dispersion was prepared and purified using the same method without added EDC and NHS.

**Physical measurements**

Elemental analysis (C, H, and N) was performed at the School of Chemistry, University of Manchester. Photon correlation spectroscopy (PCS) measurements were performed using a BI-9000 Brookhaven light scattering apparatus (Brookhaven Instrument Cooperation), fitted with a 20 mW HeNe laser and the detector was set at a scattering angle of 90°. (Measurements were also conducted at 30° and showed that the data were not angle dependent.) The particle volume swelling ratio (Q) was obtained using the hydrodynamic diameter measured by PCS at a given pH (dH) and that measured for the particles in the collapsed
A Malvern Zetasizer was used to measure the electrophoretic mobilities of the particles in the presence of 0.001 M NaCl. SEM measurements were obtained using a Philips FEGSEM instrument. Samples were dried at room temperature. Optical microscopy was conducted with an Olympus BX41 microscope. Fluorescence microscopy was conducted on a Nikon Eclipse 50i microscope. Experiments involving PyC used a DAPI filter which allowed transmission of light at 475 nm. Experiments involving FITC used excitation and emission wavelengths of 480 and 535 nm, respectively. Attenuated total reflectance (ATR) Fourier transform infrared (FTIR) measurements were conducted using a Nicolet 5700 FTIR instrument.

Results and discussion
PNVF-xNVEE microparticle swelling and morphology
PNVF-xNVEE latex dispersions (x = 4, 9 and 13) were prepared using NAD and had $d_{h(b)}$ values of 0.66 to 0.96 μm (Table 1). PNVF latex was also prepared for comparison. The dispersions were colloidally stable. Optical micrographs are shown in Fig. 1(a)–(d). For all of the dispersions examined in ethanol the particles appeared well defined (e.g., Fig. 1(a)). When dispersed in water (Fig. 1(b)–(d)) they were more diffuse, which is an indication of swollen particles. This was confirmed by the values of Q for the particles dispersed in water (Table 1) which were much greater than 1.0. Additional evidence for particle swelling in water can be seen from the tubes shown in Fig. 1(a) and (b). PNVF-4NVEE particles were collected from ethanol as a white paste using centrifugation (Fig. 1(a)). When a small quantity of water was added a transparent physical gel rapidly formed (inset of Fig. 1(b)). PNVF-xNVEE is a new family of water-swellable microparticles.

The extent of swelling for microparticle usually decreases with increasing crosslinker concentration. The data shown in Table 1 indicate a maximum Q of 7.0 for PNVF-9NVEE. The value for Q was only 3.0 for PNVF-4NVEE. The $d_h$ value for the micropel was not as large as expected. When the shells of lightly crosslinked micropel fragment and detach from the cores the hydrodynamic diameter of micropels can decrease. The lower Q than expected for PNVF-xNVEE combined with the inset of Fig. 1(b) (which confirms particle swelling) suggests that partial fragmentation of the shells occurred when PNVF-xNVEE particles were dispersed in water. Self-rupturing particles are interesting candidates for potential application in drug delivery.

Fig. 2 shows SEM images for PNVF and the PNVF-xNVEE latex particles deposited from ethanol. Deformation (flattening) and coalescence of PNVF particles can be seen from Fig. 2(a) and (b). PNVF is a hydrophilic polymer. Optical microscopy showed that within 5 min of deposition of the PNVF particles onto a microscope slide in air they had begun to spread and coalesce (see Fig. S3†). Particle spreading could be prevented if the deposited particles were stored over P2O5 which confirmed that spreading was due to absorption of water vapour. This effect, which is new for micropels, is a form of solvent vapour annealing. SEM images showed that deposited PNVF-4NVEE particles (Fig. 2(d)–(f)) also coalesced when in contact with neighbouring particles (Fig. 2(d)). Well-separated particles (Fig. 2(e)) had the largest h(c) values of 0.66 to 0.96 (Table 1). By contrast, deposited PNVF-9NVEE (Fig. 2(g)–(i)) and PNVF-13NVEE (Fig. 2(j)–(l)) particles that were closely packed did not coalesce. They also had smaller $D_{SEM}$ Values (Table 1), which is indicative of less spreading. The SEM data show that 9 mol% of NVEE was sufficient to give the shells of PNVF-xNVEE particles sufficiently high crosslinker densities to resist inter-particle coalescence and film formation during drying.

Fig. 3 shows SEM images for PNVF-xNVEE particles deposited from water. The PNVF-xNVEE particles (Fig. 3(a)–(c)) spread considerably when deposited from water. Particle deformation due to spreading of micropel that were swollen prior to deposition has been frequently observed using SEM. Here, many of the PNVF-xNVEE particles appeared to be absent (Fig. 3(a)) and circular voids were present. Those particles had flowed to a height lower than that of the surrounding material. The latter was debris from the fragmented PNVF-xNVEE shells. Dispersed PNVF-xNVEE micropel particle cores were observed in water by both optical microscopy (Fig. 1(b)) and fluorescence microscopy (Fig. 4(b)). The voids shown in Fig. 3(a) are an imprint of the swollen PNVF-xNVEE particle cores before the dispersion was completely dried. The average diameter of the circular voids was 1.8 μm (coefficient of variation of 7%), which is comparable to the $d_h$ value for the micropel particles in water (1.38 μm, Table 1). Some flattened (fully spread) particles were evident – see the arrows in Fig. 3(a) and the expanded views (Fig. 3(b) and (c)).

More robust micropels were obtained using the higher x values. The SEM images for PNVF-9NVEE (Fig. 3(d)–(f)) and PNVF-13NVEE (Fig. 3(g)–(i)) particles deposited from water showed that they had low to moderate polydispersities. The micropels resisted coalescence when deposited from water (Fig. 3(d) and (g)) confirming that NVEE crosslinking was effective when x was 9 mol% or more. The higher magnification

Table 1

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<th>Code</th>
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<th>Water $d_h$/μm</th>
<th>$Q^2$</th>
<th>$D_{SEM}$/μm</th>
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* Hydrodynamic diameter. $^b$ Swelling ratio determined from the hydrodynamic diameters and eqn (1). $^c$ Number-average diameter measured from SEM. The coefficient of variation is shown in brackets. $^d$ Ratio of nitrogen and carbon contents determined by elemental analysis (see Table S1). $^e$ These values were affected by spreading (flattening) of the particles (see text).
image for PNVF-9NVEE (Fig. 3(f)) revealed that the particle surfaces had a cluster-like morphology. A representative higher magnification image for PNVF-13NVEE microgels is shown in Fig. 3(i). The outer region of the particles (indicated by the dotted circle) appeared to have flowed and deposited onto the substrate surrounding the core of the particles. This is consistent with a relatively lightly crosslinked PNVF-rich shell. A cluster-like morphology was not apparent for PNVF-13NVEE.

The widely accepted mechanisms for NAD are those proposed by Antl et al.\textsuperscript{49} as well as Barrett and Thomas.\textsuperscript{50} Accordingly, the mechanism for PNVF-xNVEE formation involves nucleation of primary polymer particles through aggregation of growing polymer chains that become insoluble in ethanol. The structures of NVEE and NVF (Scheme 1) show that NVEE is the more hydrophobic monomer. Therefore, we propose that NVEE-rich oligomers precipitated earlier during particle formation and the NVEE concentration was greatest in the core of the PNVF-xNVEE particles. A cluster-like morphology was not apparent for PNVF-13NVEE.

PVAM-xBEVAME microgel morphologies, compositions and pH-responsive behaviours

PVAM-xBEVAME microgels were prepared by alkali-hydrolysis of PNVF-xNVEE (Scheme 1). The effective refractive index of the swollen PVAM-4BEVAME microgels was too close to that of water for the particles to be imaged using optical microscopy. Reaction of PVAM-4BEVAME microgel with FITC was used to visualize the particles using fluorescence microscopy (Fig. 4(a)). PNVF-4NVEE was used as a control system (Fig. 4(b)). The FITC-labelled PNVF-4NVEE particles appear sharp and distinct (Fig. 4(b)). The FITC-labelled PVAM-4VAME particles (Fig. 4(a)) appeared smaller and less bright. PCS data (Table 2) showed that the PVAM-4BEVAME microgels had the largest particle size. The reason they appeared smallest (Fig. 4(a)) in comparison to the other microgels is that the shells were highly swollen and could not be visualised by fluorescence microscopy. PCS measures the hydrodynamic diameter and is more sensitive to the peripheries of swollen particles. By contrast to PVAM-4BEVAME, optical images with microgel particles evident were readily obtained for PVAM-9BEVAME and PVAM-13BEVAME (Fig. 4(c) and (d)). These images imply less swelling occurred for those systems.

PCS data for the PVAM-xBEVAME microgels measured in water at pH = 7 are shown in Table 2 and the $d_h$ values were in the range of 1.31–1.72 μm. These values are all larger than those for the respective non-hydrolysed PNVF-xNVEE microgels (Table 1). To probe the reversibility of particle swelling the PVAM-xBEVAME particles were redispersed in ethanol after having been first dispersed in water. In each case $d_h$ decreased. However, the $d_h$ values measured in ethanol (Table 2) were larger for both PVAM-9BEVAME and PVAM-13BEVAME compared to those for the respective PNVF-xNVEE particles measured in ethanol (Table 1). This shows partial irreversibility of particle swelling when the solvent was changed from a good to a poor
Complete hydrolysis of PNVF-xNVEE to PVAM-xBEVAME results in considerable mass loss because of the liberation of formate (Scheme 1). The mass loss was calculated to be in the range of 35–39 wt% for 100% hydrolysis depending on x. The intra-segment interactions that existed within the PNVF-xNVEE particles at the time of particle formation in ethanol will not be identical to those for the respective PVAM-xBEVAME particles when redispersed in ethanol. A significant proportion of the conformation changes required for reversible particle swelling must no longer have been possible after hydrolysis. By contrast, the value for $d_h$ measured in ethanol for redispersed PVAM-4BEVAME particles (0.51 μm, Table 2) was much smaller than that for PNVF-4NVEE (0.96 μm, Table 1). This is further evidence of shell fragmentation for that system.

The calculation of $Q$ values for PVAM-xBEVAME microgels was challenging because they were created in the swollen state, unlike conventional microgels. Furthermore, a mass loss due to hydrolysis and partial fragmentation was involved. Nominal $Q$ values for PVAM-9BEVAME and PVAM-13BEVAME were estimated by using the $d_h$ value for the respective parent PNVF-xNVEE latex in ethanol (Table 1) as the value for $d_{h(c)}$ (eqn (1)). These values should underestimate the true $Q$ values because they did not take into account the mass loss due to hydrolysis described above. A different approach was used to estimate $Q$ for PVAM-4BEVAME because that system underwent significant shell fragmentation. In that case the $d_h$ value in ethanol for the as-made PNVF-4NVEE particles (Table 1) was not a meaningful value of $d_{h(c)}$ for the PVAM-4BEVAME microgel. For PVAM-4BEVAME the $Q$ values...
were estimated using the $d_h$ value for that microgel redispersed in ethanol (0.51 mm, Table 2). The $Q$ values calculated in this way would also underestimate the true values because of the likelihood of partial irreversibility of microgel swelling in ethanol. The $Q$ values at pH = 7 for the PVAM-$x$BEVAME microgels (Table 2) were all larger than those for the respective PNVF-$x$NVEE microgels (Table 1) due to ionised PVAM$^+$ groups. At pH = 7 each microgel contained at least 84 vol% water. The value for $Q$ decreased with increasing $x$ for the PVAM-$x$BEVAME microgels, as expected.

The PVAM-$x$BEVAME microgels were also investigated by SEM (Fig. 5). Major morphological changes occurred compared to the parent microgels (Fig. 3). The surface textures were more pronounced, presumably as a result of the mass loss that occurred due to hydrolysis discussed above. PVAM-4BEVAME showed a high population of narrowly dispersed particles (Fig. 5(a)). Higher magnification images showed a cluster-like morphology for those particles (Fig. 5(b) and (c)) as well as dispersed nanometer-sized particles (arrow in Fig. 5(b)). The former are the cores of the microgel particles. Some of the cluster-like particles had partially disintegrated (Fig. 5(c)). This is attributed to insufficient crosslinking. By contrast, the PVAM-9BEVAME particles (Fig. 5(d)–(f)) showed remarkably well-developed, intact, cluster-like morphologies (Fig. 5(e) and (f)). A nodule type morphology has been previously reported for conventional poly(N-isopropylacrylamide) microgels.\textsuperscript{31} However, the present surface morphology is much more pronounced.

For the highest $x$ value used (PVAM-13BEVAME) the particle morphology was more uniform (Fig. 5(i)) and closer to that of the parent PNVF-13NVEE particles (Fig. 3(i)). This shows that the value for $x$ controls the morphologies of the PVAM-$x$BEVAME particles and demonstrates tunability. We note that film formation of close packed PVAM-9BEVAME and PVAM-13BEVAME particles did not occur (Fig. 5(d) and (g)) which demonstrates that the crosslinking initially provided by NVEE survived hydrolysis for these systems. This confirms the stability of the ether linkage in NVEE.

Taken together, all the PCS, fluorescence microscopy and SEM data discussed above strongly support the view that PNVF-$x$NVEE and PVAM-$x$BEVAME particle shells had lower crosslinker concentrations than the cores. We interpret the morphological changes for the PVAM-$x$BEVAME particles (Fig. 5) with decreasing $x$ in terms of increasing nanometer-scale fragmentation as a consequence of hydrolysis-triggered mass loss. As $x$ decreased the consequence of the intra-particle crosslinker concentration decrease was most significant for the...
particle shells. This caused fragmentation and release of increasing numbers of nanoparticles (see arrow in Fig. 5(b)). Some of this shell fragmentation may also have occurred prior to hydrolysis. Although we cannot be certain it is possible that the pronounced cluster morphology for PVAM-9BEVAME particles (Fig. 5(f)) occurred because the shell crosslinker concentration permitted localised nanometer-scale shell fragmentation. However, the extent of fragmentation was much less than that which occurred for PVAM-4BEVAME.

The compositional changes that accompanied hydrolysis of the PNVF-xNVEE microgels were probed using FTIR spectroscopy. The spectra for the PNVF-xNVEE microgels had a number of features in common with that of PNVF (see Fig. 6 and S4†). Those spectra all had bands that are due to amide I (1650 cm\(^{-1}\)) and amide II (1540 cm\(^{-1}\)) and are consistent with spectra for PNVF reported earlier.\(^{39}\) Alkali hydrolysis of the PNVF-xNVEE dispersions caused major changes in the spectra (see Fig. 6). The spectra for all of the PVAM-xBEVAME particles are very similar to that for PVAM. New bands appeared at 1590 cm\(^{-1}\) and in the vicinity of 3260 cm\(^{-1}\) due to RNH\(_2\).\(^{12,39,53}\) The FTIR spectra show that alkali-hydrolysis of the PNVF-xNVEE particles to PVAM-xBEVAME was successful.

The %N/%C ratio from elemental analysis data was used to quantify the extent of hydrolysis for PVAM-xBEVAME (see Table 2). The %N/%C values all increased (Table 2) compared to those of the parent microgels (Table 1), which is an important indicator of successful hydrolysis. The calculated percentage hydrolysis values range from 72% to 99% and agree with the trends established from the FTIR data (Fig. 6). Hydrolysis was effectively complete for PVAM-9BEVAME and PVAM-13BEVAME microgels. It is not clear why the conversion was lowest for PVAM-4BEVAME (72%). Nevertheless, the data show that very high proportions of primary amine groups were produced for each of the microgels and these dispersions were also colloidally stable. The mol% VAM in each microgel was calculated from the %N/%C values and values of up to 90% were determined (see Table 2). The PVAM-xBEVAME microgels reported here contained the highest proportion of primary amine groups for any colloidally stable microgel dispersion reported to our knowledge.

All of the PVAM-xBEVAME microgels exhibited strong pH-triggered swelling as judged by hydrodynamic diameter measurements (Fig. 7(a)). The microgels were swollen at pH = 12 which was partly due to the hydrophilic nature of PVAM. The pH-triggered increase in \(d_h\) upon decreasing the pH from 12 to 4 was greatest for PVAM-4BEVAME. For PVAM-9BEVAME and PVAM-13BEVAME the pH-triggered swelling was less strong, which is due to the higher concentration of crosslinking units present. In each case considerable pH-triggered swelling had occurred when the pH had decreased to 10, which is the pK\(_a\) of PVAM.\(^{48}\) The trends established here for these pH-responsive microgels are comparable to those reported by Shi and Berkland for hollow PVAM capsules which were prepared by a template-and-etch route.\(^{39}\) The swelling is best compared in terms of the nominal Q values and these data are shown in Fig. 7(b). The values used for \(d_h(x)\) were the same as used in Table 2 and were discussed above. The data reported in Fig. 7(b) show the expected trend that Q decreased with increasing x across the whole pH range. This can also be seen from plots of \(d_h\) and Q data as a function of x (see Fig. S5(a) and (b)|). This is the first time this trend has been reported for PVAM microgels. PVAM-xBEVAME microgels show

\[
\begin{array}{lcccccc}
\text{Code} & \text{Water} & \text{Ethanol (redispersed)} & Q^f & D_{SEM}/\mu m & \%N/%C^e & %\text{Hyd.}^e & X_{VAM}/\text{mol}%^e \\
\hline
\text{PVAM} & -- & -- & -- & -- & 0.54^e & 83^e & 83^e \\
\text{PVAM-4BEVAME} & 1.72 & 0.51 & 38 & 0.84[12] & 0.48 & 72 & 69 \\
\text{PVAM-9BEVAME} & 1.31 & 0.04 & 7.8 & 0.50[15] & 0.50 & 99 & 90 \\
\text{PVAM-13BEVAME} & 1.46 & 1.10 & 6.1 & 0.69[14] & 0.47 & 96 & 84 \\
\end{array}
\]

\(^{a}\) Hydrodynamic diameter measured in water (pH = 7) or when redispersed in ethanol. \(^{b}\) Nominal volume swelling ratio at pH = 7 (see text). \(^{c}\) Number-average diameter measured from SEM images. The coefficient of variation is shown in the brackets. \(^{d}\) Ratio of nitrogen and carbon contents determined by elemental analysis. All elemental analysis data are shown in Table S1.\(^{1}\) \(^{e}\) % Hydrolysis calculated using: %\text{Hyd.} = 100 \([(7x + 3)/(x + 1)) – (1.1662(%N/%C)]\), mol% of VAM calculated from the product of %\text{Hyd.} and x. \(^{f}\) From ref. 19.
strong pH-triggered swelling when the pH decreases and the extent of swelling is tuneable using $x$.

The electrophoretic mobilities of the dispersions were measured as a function of pH (Fig. 7(b)). The mobilities were all positive and increased as the pH decreased for each of the PVAM- $x$BEVAME microgels. It is the increase in positive charge, and hence increased mobile ion concentration within the particles, that was responsible for the pH-triggered swelling shown in Fig. 7(a) and (b). Positive electrophoretic mobilities at all pH values of less than or equal to 10 is a further indication of successful hydrolysis for our PVAM-$x$BEVAME microgels because the $pK_a$ of PVAM is $\leq 10$. The data from Fig. 7 were plotted as a function of $x$ (see Fig. S5(c)). The electrophoretic mobilities at pH = 4 and 7 increased substantially with decreasing $x$. At pH less than or equal to about 7 the positive charge density at the periphery of the particles is highest for the microgels prepared using the least crosslinker concentrations. PVAM-4BEVAME cores must have had a periphery composed of dangling, PVAM chains. By contrast, the PVAM-13BEVAME particles swelled the least, and showed the smallest electrophoretic mobility increase, with decreasing pH (Fig. 7(a)). These trends are indicative of a more highly crosslinked shell. A high crosslink density may oppose protonation as confined polycationic chains are known to have decreased apparent $pK_a$ values.

Demonstration of PVAM-9BEVAME microgel functionalization using primary amines

To demonstrate the potential for the primary amines of the microgels to act as chemical handles we covalently linked PyC onto the PVAM-9BEVAME microgels using EDC chemistry. As a control experiment PyC was mixed with the microgel without

Fig. 5 PVAM-$x$BEVAME microgels deposited from water. The arrow in (b) identifies nanometer-sized particle fragments. The identities of the systems are given in the figures.

Fig. 6 FTIR spectra of PNVF-9NVEE and PVAM-$x$BEVAME microgels. The spectrum for linear PVAM is shown for comparison. The spectra for PNVF and the other PNVF-$x$NVEE microgels are shown in Fig. S4.
EDC and NHS. To remove PyC the microgels were washed extensively with aqueous NaCl (0.15 M) solution, CTAB solution and then water. The fluorescence and optical micrographs obtained after this procedure are shown in Fig. 8. The fluorescently labelled particles were clearly observed for the particles where EDC/NHS was used (Fig. 8(a)). However, no particles were imaged for the control sample (no EDC/NHS, (Fig. 8(c)). The optical micrographs confirmed the presence of particles in each case (Fig. 8(b) and (d)). These data demonstrate successful functionalization of PVAM-9BEVAME microgels using primary amine groups.

Conclusions

This study has established two new families of microgels; PNVF-xNVEE and PVAM-xBEVAME. PVAME-xBEVMAE are a new family of colliquially stable pH-responsive microgels that contain very high primary amine contents and were prepared using a scalable two-step method. All of the data obtained in this study suggest that the cores of the PVAM-xBEVAME microgel particles are more highly crosslinked than the shells. It was found that the shells of the PNVF-4NVEE microgels fragmented when the value for x was 4 mol%. A value for x of 9 mol% (or higher) provided microgels PNVF-xNVEE microgels that were not susceptible to major inter-particle coalescence or fragmentation when hydrolysed. The PVAM-4BEVAME and PVAM-9BEVAME particles had a cluster-like morphology. This potentially useful particle morphology is controlled by x and is tuneable. Particles with built-in surface texture are of considerable academic interest.

40–42,56 The PVAM-xBEVAME microgels were strongly pH-responsive and their hydrodynamic diameters and electrophoretic mobilities increased substantially when the pH was decreased. The particles had hydrolysis extents of 72 to 99%. We demonstrated the chemical versatility of the new PVAM-9BEVAME microgels by using the primary amine groups as chemical handles to covalently link a RCOOH-functionalised dye. This study has provided a new method for preparing colloquially stable dispersions of high primary amine content microgels. PVAM is structurally related to polyethyleneimine (PEI) which has been widely used for DNA delivery.57 Our new microgels should have potential application in a number of areas from advanced surface coatings, rheological modifiers, hybrid particles and delivery.

Acknowledgements

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References

Hydrogels are covalently crosslinked swellable polymers and continue to attract an increasing amount of interest especially in catalysis, sensing and biomaterials applications.1–3 As their structures become increasingly complex, so too does the range of properties available. Their mechanical properties can be greatly improved by preparing composite hydrogels containing inorganic nanoparticles,4 incorporating uniform elastically effective chain lengths5 or through double network formation.6 The majority of the hydrogel research conducted to date has focused on the bottom up formation of hydrogels, i.e., co-polymerisation of monomers. There has been much less work involving construction of hydrogels from pre-formed colloidal sized gel particles (e.g., microgels). However, the latter provide a unique and exciting opportunity to manipulate the macroscopic properties of hydrogels at the colloidal size scale. Moreover, minimal chemistry is involved in transforming the fluid form of a microgel dispersion to a covalent gel, which renders this general approach potentially attractive for biomaterial use.7 Work at Manchester established a method for covalently interlinking microgel particles to form doubly crosslinked (DX) microgels,8 which is a new approach for gel formation. Microgels are crosslinked polymer colloid particles that swell when the pH approaches the pKₐ of the polyacid or polybase chains9 or when dispersed in a good solvent.10–12 Here, we refer to the precursor microgel particles as singly crosslinked (SX) because of the copolymerisation of di-vinyl crosslinking monomer within their structure during particle formation (Scheme 1(a)). The motivations for the present study were to demonstrate that the DX microgel formation process previously established for anionic SX microgels8 was generally applicable as well as to demonstrate that new DX PVAM microgels could be prepared at 37 °C and remain intact when swollen under physiological conditions. Here, we selected a new microgel system that had a high primary amine content they are well suited to functionalisation and should have potential applications in areas including catalysis, composite hydrogels and biomaterials.

The earliest report of a DX microgel was by Hu et al. Their approach relied upon an addition reaction of the microgel particles with epichlorohydrin within a concentrated, crystalline, SX poly(N-isopropylacrylamide-co-acrylic acid) gel. Their covalent gels consisted of inter-linked microgels and had photonic properties.14 An aggregation based DX microgel was later reported by Cho et al.15 where a linear polymer was added to the microgels prior to crosslinking. Other microgel composites have also attracted interest.16

Doubly crosslinked poly(vinyl amine) microgels: hydrogels of covalently inter-linked cationic microgel particles†

Sineenat Thaiboonrod, Amir H. Milani and Brian R. Saunders*

Doubly crosslinked (DX) microgels are macroscopic hydrogels comprised of covalently inter-linked singly crosslinked (colloidal) microgel particles. In this study we demonstrate for the first time that DX microgels can be prepared from concentrated dispersions of singly crosslinked (SX) poly(vinyl amine) (PVAM) microgel particles. The latter were of micrometer size, cationic and contained high primary amine contents. The DX PVAM morphologies contained extensive inter-connected porosity as determined by optical microscopy and SEM. The effective porosity ranged from 76 to 93 vol% and was tuneable through microgel particle concentration. The mechanical properties of the DX PVAM microgels were investigated using dynamic rheology. The best DX PVAM microgel had a storage modulus (G′) of 41 kPa and yield strain of 46%, which are a good combination of elasticity and ductility. This gel had an internal porosity of 76 vol%. The dependence of G′ on the effective volume fraction (φ_eff) for the DX PVAM microgels was tuneable and followed the equation: G′ ∝ exp(bφ_eff), with b = 16.4. The latter value indicated low particle softness. The DX PVAM gels were also injectable and could be prepared at 37 °C. Furthermore, the gel mechanical properties after swelling for 3 days at physiological pH and ionic strength were similar to those before swelling. Because these injectable DX PVAM microgels have high primary amine contents they are well suited to functionalisation and should have potential applications in areas including catalysis, composite hydrogels and biomaterials.

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3tb21579b
In 2011 our group reported a simple preparation of DX microgels using glycidyl methacrylate (GMA) functionalised SX poly(methyl methacrylate-co-methacrylic acid-co-ethylene glycol dimethacrylate) microgels. The latter are abbreviated here as PMMA-MAA-GMA. pH-triggered swelling of concentrated dispersions was used to prepare physical gels that consisted of inter-penetrating microgel particles. Free-radical reaction using added initiator (ammonium persulfate, APS) was used to covalently link GMA groups of neighbouring microgels to form DX microgels. The precursor dispersions were injectable and a biocompatible version was used to demonstrate load support for degenerated intervertebral discs. Until now, the number of these DX microgels reported to date was limited to two microgel types and both were based on anionic MAA-containing microgel particles. If the DX microgel approach is generally applicable, then it should be possible to prepare DX microgels using a cationic SX microgel system. Our recent discovery of a method to prepare high primary-amine content SX poly(vinyl amine) (PVAM) microgels provides the possibility for preparing DX PVAM microgels. The latter is the subject of this study. PVAM and related systems have attracted considerable research interest. The poly(N-vinyl formamide-co-2-(N-vinylformamido)ethyl ether) microgels (abbreviated as SX PNVF) were prepared by non-aqueous dispersion polymerisation. The SX PNVF microgel was hydrolysed to poly(VAM-co-bis(ethyl vinyl amine)ether) (abbreviated as SX PVAM) using alkaline hydrolysis. Although a very high extent of hydrolysis occurred, the process was not fully complete and a minor proportion of SX PNVF remained (Scheme 1(a)). After GMA functionalisation, the dispersion of SX PVAM-GMA particles was concentrated to form a shear-thinning physical gel and then heated in the presence of APS to give a DX PVAM microgel. The DX PVAM microgels were connected by polymer bridges and this is depicted in Scheme 1(b).

PVAM contains the highest primary amine content of all synthetic polymers and has a wide range of potential functionalisation reactions. However, it cannot be synthesised from vinyl amine. PVAM is usually produced by post-polymerisation hydrolysis, e.g. by alkali-hydrolysis of the corresponding poly(N-vinylformamide) (PNVF). Unfortunately, it has been difficult to prepare macroscopic PVAM hydrogels because of the necessity to subject the precursor PNVF hydrogels to alkaline hydrolysis. This can result in hydrolysis (and hence the PVAM phase) being confined to the outer surface of gel particles. The assembly of a macroscopic hydrogel from PNVF microgel particles that have been fully hydrolysed to PVAM is an attractive alternative to overcome this problem and was an aim of the present study. Accordingly, the present study fills an important gap for hydrogels. Because PVAM is structurally related to polyethylene imine (PEI), which has been widely investigated for delivery applications, there should be potential future biomaterials applications for DX PVAM microgels.

Here, we prepare physically gelled dispersions of SX PVAM microgel particles and show that it is possible to convert them into DX PVAM microgels. The method used is shown in Scheme 1. Physically gelled microgel dispersions have attracted considerable research interest. The poly(N-vinyl formamide-co-2-(N-vinylformamido)ethyl ether) microgels (abbreviated as SX PNVF) were prepared by non-aqueous dispersion polymerisation. The SX PNVF microgel was hydrolysed to poly(VAM-co-bis(ethyl vinyl amine)ether) (abbreviated as SX PVAM) using alkaline hydrolysis. Although a very high extent of hydrolysis occurred, the process was not fully complete and a minor proportion of SX PNVF remained (Scheme 1(a)). After GMA functionalisation, the dispersion of SX PVAM-GMA particles was concentrated to form a shear-thinning physical gel and then heated in the presence of APS to give a DX PVAM microgel. The DX PVAM microgels were connected by polymer bridges and this is depicted in Scheme 1(b).

The study begins by characterisation of the SX PVAM-GMA particles used to prepare the DX PVAM microgels (Scheme 1). The size of these microgel particles was larger than the previously studied SX PMMA-MAA-GMA microgel particles by a factor of 5, which provided the opportunity to directly study the morphology of hydrated DX microgels. The optical microscopy
data enabled estimation of the effective volume fraction occupied by the microgel particles \( (\phi_{\text{eff}}) \) within the gels. We also measured the mechanical properties of the DX PVAM microgels. A simple model was fitted to the relationship between the storage modulus \( (G') \) and \( \phi_{\text{eff}} \). The injectability of the DX PVAM gels was demonstrated and the swelling behaviour at \( pH = 7.4 \) was studied. We also demonstrate that DX PVAM gels can be prepared at 37 °C. The data imply that our DX microgel preparation method is general. The new gels studied here should have potential applications which range from composite hydrogels\(^2\) and catalysis\(^3\) to biomaterials.\(^7\) Whilst polymers with very high primary amine contents can be cytotoxic, the literature shows a number of strategies that could be used to render PVAM gels cytocompatible.\(^7,9,50\)

**Experimental**

**Reagents**

APS (purity greater than 98%), GMA (97%), \( N,N,N',N'\)-tetramethylthelyenediamine (TEMED, 99%) and ethanol (99.9%) were purchased from Aldrich and used as received. The synthesis and characterisation of NVE (2-(N-vinylformamidino)ethyl ether) has been described previously.\(^5\) High purity water that was distilled and deionised was used.

**Preparation of SX PNVF and SX PVAM microgel**

The preparation of the SX PNVF and SX PVAM microgels was described in detail earlier.\(^4\) SX PNVF particles containing 9 mol\% NVE were prepared in latex form by non-aqueous dispersion polymerisation in ethanol. The particles were redispersed in water to give a microgel dispersion. SX PVAM microgel was prepared by alkaline hydrolysis of SX PNVF particles in aqueous NaOH solution (1 M) at 80 °C under a nitrogen atmosphere for 16 h. The hydrolysed dispersions (SX PVAM) were purified using sequential dialysis against phosphate buffered saline, aqueous 0.15 M NaCl and water, respectively.

**Preparation of SX PVAM-GMA microgel**

The SX PVAM microgel was vinyl-functionalised using GMA. SX PVAM (50 g, \( \phi_{\text{hs}} = 0.015 \)) containing 0.19 g of added GMA was heated to 50 °C at a pH of 9 with mechanical stirring for 24 h. Note that \( \phi_{\text{hs}} \) is the hard sphere volume fraction. The GMA-functionalised dispersion was washed three times with chloroform to remove unreacted GMA.

**Preparation of SX PVAM and SX PVAM-GMA physical gels**

SX PVAM or SX PVAM-GMA microgel was concentrated from a dilute dispersion \( (\phi_{\text{hs}} \text{ of } 0.015) \) to the required \( \phi_{\text{hs}} \) value using rotary evaporation at 32 °C. Unless otherwise stated the preparation pH was 9.0 and the final \( \phi_{\text{hs}} \) value was 0.10.

**Preparation of DX PVAM microgel**

DX microgels were prepared from SX PVAM-GMA. Briefly, aqueous APS solution (0.31 mL, 1 wt\%) was added to SX PVAM-GMA (10 g, 1.5 wt\%) at pH = 9 with vigorous mixing for about 5 min before rotary-evaporation was used at 32 °C to concentrate the dispersion. Then, the physical gel (shear-thinning) was placed in an O-ring (internal diameter = 19 mm, thickness = 2.2 mm) between two clean microscope slides, sealed and heated in an oven at 50 °C for 24 h. The \( \phi_{\text{hs}} \) value used was 0.10 unless otherwise stated.

In the case of the injectable DX PVAM system the same procedure was used as described above. However, in this case 0.40 mL of TEMED was added with the APS solution and rotary evaporation was conducted at 30 °C to give \( \phi_{\text{hs}} \) of 0.15. The physical gel was injected into the sealed O-ring/microscope slide arrangement discussed above and then cured at 37 °C for 24 h.

**Physical measurements**

Optical microscopy was conducted with an Olympus BX41 microscope. Fast Fourier Transformation (FFT) of the images was obtained using Image J (National Institute of Health). Photon correlation spectroscopy (PCS) measurements were performed using a BI-9000 Brookhaven light scattering instrument (Brookhaven Instrument Cooperation), fitted with a 20 mW HeNe laser and the detector was set at a scattering angle of 90°. SEM measurements were obtained using a Philips FEG-SEM instrument. The gels were freeze-dried using liquid nitrogen and coated with platinum. In the case of dilute dispersions, the particles were deposited from aqueous dispersions. Dynamic rheology measurements were performed using a TA Instruments AR G2 temperature-controlled rheometer with an environmental chamber. A 20 mm diameter plate geometry with a solvent trap was used. The gap was 2000 μm. For the strain-amplitude measurements a frequency of 1 Hz was used. A strain of 0.1% was used for the frequency-sweep measurements.

**Results and discussion**

**GMA functionalised microgel composition and properties**

Because the vinyl-functionalised SX PVAM-GMA microgel is new it was important to establish its composition and properties prior to considering the DX PVAM microgels. The SX PVAM-GMA microgel was prepared in two steps starting from SX PNVF, or in one step from SX PVAM (Scheme 1(a)). The compositions for SX PVAM-GMA and SX PVAM are shown in Table 1 and the general structures appear in Scheme 1(a). The values for \( x, y \) and \( z \) were determined from elemental analysis data (Table S1, ESI†) using the ratios of the %N to %C values, \( i.e. \), \( R_{\text{NC}} \). The procedures and equations used are described in the ESI. The SX PNVF microgel contained \( x = 0.09 \), as calculated from elemental analysis data [ESI]. The preparation of SX PVAM resulted in high conversion with approximately 84 mol% of the NVF segments being hydrolysed to VAM \( (i.e., y = 0.84) \) (Table 1). The SX PVAM-GMA microgel had a calculated GMA content of 13 mol%. This value is within the range of vinyl contents reported for anionic vinyl-functionalised microgels.\(^7,8\)

The compositions of the microgels were also studied using FTIR spectroscopy (Fig. S1, ESI†). The spectrum for SX PVAM showed bands due to \( \text{RNH}_2 \) at\(^31\) 1590, 3350 and 3275 cm\(^{-1}\) and
contrasted to that for SX PNVE. Although a unique band for C=\-C groups\textsuperscript{32,33} was present in the spectrum of GMA at 1637 cm\textsuperscript{-1} (Fig. S1, ESI\textsuperscript{†}), the band overlapped a strong band in the SX PVAM spectra and prevented a unique FTIR assessment for vinyl groups in SX PVAM-GMA. A weak band was evident at 1715 cm\textsuperscript{-1} in the spectrum for SX PVAM-GMA, which was due to ester groups of GMA (Fig. S1, ESI\textsuperscript{†}) and supports incorporation of GMA. Furthermore, the epoxide band at\textsuperscript{34} 905 cm\textsuperscript{-1} was absent in the SX PVAM-GMA FTIR spectrum implying reaction of those groups upon GMA incorporation and subsequent removal of unreacted GMA by the washing procedure employed.

The sizes of the SX PVAM-GMA and SX PVAM microgels were measured using optical microscopy, SEM as well as PCS. Both SX PVAM-GMA (Fig. 1(a)) and SX PVAM (Fig. S2(a), ESI\textsuperscript{†}) particles had number-average diameters of 1.48–1.52 \(\mu\)m as determined by optical microscopy (\(D_{N\text{Opt}}\), Table 2). Fig. 1(b) shows a representative SEM image for deposited SX PVAM-GMA particles. They were spherical and monodisperse. (An SEM image for the precursor SX PVAM microgel particles is shown in Fig. S2(b), ESI\textsuperscript{†}) The number-average diameters determined by SEM (\(D_{N\text{SEM}}\)) for SX PVAM-GMA and SX PVAM were close to 1.0 \(\mu\)m. These values were smaller than the respective \(D_{N\text{Opt}}\) values due to particle collapse that occurred during SEM sample preparation and measurement where high vacuums were used.

The variation of \(d_h\) with pH for SX PVAM-GMA is shown in Fig. 2(a). (Data for the pre-cursor SX PVAM particles are shown in Fig. S3(a), ESI\textsuperscript{†}) The \(pK_a\) for PVAM has been reported as\textsuperscript{35} 10.0. The SX PVAM-GMA particles showed significant pH-triggered swelling as the pH approached 10 from higher values, which is due to protonation of the primary amine groups. The particle volume swelling ratio, \(Q_p\), is an important parameter for microgels and can be calculated using

\[
Q_p = \left( \frac{d_h}{d_{h(c)}} \right)^3
\]

where \(d_{h(c)}\) is the hydrodynamic diameter of the collapsed particles. The SX PVAM-GMA and SX PVAM particles were prepared in aqueous solution and did not fully de-swell when re-dispersed in ethanol (a non-solvent for PVAM) which prevented direct measurements of \(d_{h(c)}\) for both systems. Moreover, the SX PVAM microgel was prepared by hydrolysis of SX PNVE (Scheme 1(a)) and lost up to 37 wt\% of its mass due to formate liberation.\textsuperscript{36} This process interfered with the ability of PVAM microgel to fully collapse in ethanol.\textsuperscript{37} Consequently, a direct measure of \(d_{h(c)}\) for the SX PVAM-GMA and SX PVAM particles was not possible.

The \(d_{h(c)}\) value for SX PNVE (\(d_{h(c,PNVE)} = 0.66 \mu\m\)) of the as-made particles in latex form dispersed in ethanol\textsuperscript{37} was used to estimate \(d_{h(c)}\) values for SX PVAM and SX PVAM-GMA. This was an appropriate value to use because ethanol is a poor solvent for PNVE and the SX PNVE particles were not in contact with water prior to the PCS data in ethanol being measured. We term the estimated \(d_{h(c)}\) value as \(d_{h(c)}^\ast\). Values for \(d_{h(c)}^\ast\) for SX PVAM-GMA and SX PVAM were estimated from eqn (2). This equation corrected for the respective particle mass losses due to hydrolysis (based on their compositions) and used their repeat unit molar masses (\(M_{\text{rep}}\), Table 1).

\[
d_{h(c)}^\ast = d_{h(c,PNVE)} \left( \frac{M_{\text{rep}}}{M_{\text{rep,PNVF}}} \right)^{1/3}
\]

Table 1 Compositions for the microgels

<table>
<thead>
<tr>
<th>Sample</th>
<th>Composition\textsuperscript{a}</th>
<th>Mol% Hydr.\textsuperscript{b}</th>
<th>Mol% GMA\textsuperscript{c}</th>
<th>(M_{\text{rep}}/(g\ mol^{-1})\textsuperscript{d})</th>
<th>(R_{NC}\textsuperscript{e})</th>
</tr>
</thead>
<tbody>
<tr>
<td>SX PVAM</td>
<td>[PVAM\textsubscript{0.91}−BEV\textsubscript{0.09}]\textsubscript{0.84}−[PNVF\textsubscript{0.91}−NVE\textsubscript{0.09}]\textsubscript{0.16}</td>
<td>84.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SX PVAM-GMA</td>
<td>[PVAM\textsubscript{0.76}−(VAM-GMA)\textsubscript{0.15}−BEV\textsubscript{0.09}]\textsubscript{0.84}−[PNVF\textsubscript{0.91}−NVE\textsubscript{0.09}]\textsubscript{0.16}</td>
<td>84.0</td>
<td>13.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Calculated from elemental analysis data using equations shown in the ESI. \textsuperscript{b} The extent of hydrolysis was 100\%. \textsuperscript{c} Extent of GMA functionalisation. \textsuperscript{d} Calculated molar mass of repeat unit using the values for \(x, y, z\). \textsuperscript{e} \(R_{NC} = \%N/%C\), where the latter values were obtained by elemental analysis (see Table S1, ESI). The error for these values was ±0.001.

Fig. 1 Optical microscopy and SEM images for various microgels. Optical microscopy (a) and SEM (b) images for SX PVAM-GMA particles. The microgel particles (white spheres) were dispersed in water at pH = 7 for (a) and were deposited from aqueous dispersion at pH = 9 for (b).

Fig. 2 pH-dependent properties for SX PVAM-GMA microgel particles. (a) Shows the variations of the hydrodynamic diameter and electrophoretic mobility with pH. (b) Shows the pH-dependence of the particle swelling ratio (see text).
Eqn (2) was derived assuming the number of repeat units per particle did not change due to hydrolysis and that the polymer density was 1.0 g cm\(^{-3}\). The calculated value for \(M_{\text{rep}}(\text{PNVF})\) was 84 g mol\(^{-1}\) based on its composition (see above). The \(d_{b(c)}^0\) values correspond to the fully collapsed particles dispersed in ethanol and appear in Table 2. The values for \(d_{b(c,\text{PNVF})}\) and \(d_{b(c)}^0\) enabled values of \(Q_p\) for SX PVAM-GMA (and SX PVAM) to be calculated from eqn (1) using \(d_{b(c)} = d_{b(c)}^0\). Fig. 2(b) shows the variation of \(Q_p\) with pH. The microgel particle swelling that occurred at pH values less than 7 may have been due to protonation of BEV. The latter is the hydrolysis product of NVE (Scheme 1(a)).

The electrophoretic mobility (\(\mu\)) vs. pH data are also shown in Fig. 2(a). The mobility increased with decreasing pH, as expected, which was due to increased protonation of the microgel particles. Although there was general agreement between the trends for \(d_h\) and \(\mu\) with pH, major differences in the relative changes were apparent in the pH region of 7 to 10. Exact agreement between the changes in hydrodynamic diameter (a whole particle measurement) and electrophoretic mobility (an outer shell sensitive parameter) is often not obtained for microgels. This is due in part to the multiple parameters that contribute to the mobility of charged microgels. Importantly, the present data show that the SX PVAM-GMA microgel particles were positively charged over the pH range of 4 to 12. Comparable data were obtained for the SX PVAM microgels (see Fig. S3(a)). It is highly likely that the DX PVAM microgels were positively charged because their preparation only involved free-radical crosslinking of a minor proportion of GMA groups (Scheme 1).

### DX PVAM microgel morphology

The preparation of DX PVAM microgels required use of relatively high \(\phi_{hs}\) (\(\geq 0.05\)) SX PVAM-GMA dispersions in the presence of added APS. Those dispersions formed reversible physical gels unless covalently interlinked (below). Rotary evaporation was an effective method for providing shear-thinning (injectable) physical gels with high \(\phi_{hs}\) values and DX PVAM microgels were prepared from concentrated SX PVAM-GMA physical gels by free-radical reaction (Scheme 1).

The presence of interparticle crosslinking for DX PVAM microgels was tested by placing a gel in aqueous 0.1 M NaCl solution (pH = 7) for 24 h (Fig. 3(a)). A SX PVAM-GMA physical gel (no APS added) was used as a control. The latter re-dispersed; whereas, the DX PVAM microgel did not. This test demonstrated that a DX microgel had been successfully prepared.

The DX and SX PVAM-GMA microgels had a yellow colour (Fig. 3(a)). The colour was evident for SX PVAM-GMA or PVAM gels heated with APS. For the latter, the yellow colour could be due to the oxidation of VAM groups by persulfate to give imine groups. In the case of GMA, the formation of imine groups by reaction of C==O groups with primary amines is possible. The FTIR spectrum for DX PVAM (Fig. S4, ESI†) was similar to that for SX PVAM-GMA (Fig. S1, ESI†) confirming that the DX PVAM microgel was primary-amine rich and maintained its structural integrity upon double crosslinking. This was supported by elemental analysis data (Table S1, ESI†) which showed the same R\(_{NC}\) value as SX PVAM-GMA.

We investigated the morphologies of freeze-dried DX PVAM microgels using SEM (see Fig. 3(b) and (c)). The SEM images clearly show space-filling, highly porous, particulate morphologies. The inter-connected, 3-dimensional, porosity present was of the order of micrometers in size. (SEM images for SX PVAM-GMA showed similar morphologies – see Fig. S5, ESI†) This is different to the more homogeneous morphologies observed for anionic DX microgels. The higher magnification image (Fig. 3(c)) shows that the particles within the gels were

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**Table 2** Particle characterisation data for the microgels investigated in this work\(^a\)

<table>
<thead>
<tr>
<th>Sample</th>
<th>(D_n(\text{Gel})^b/\mu\text{m})</th>
<th>(D_n(\text{SEM})^b/\mu\text{m})</th>
<th>(d_h^d/\mu\text{m})</th>
<th>(d_h^c*/\mu\text{m})</th>
<th>(Q_p^f)</th>
<th>(\mu^j\times 10^{-6}\text{ m}^2\text{ V}^{-1}\text{ s}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>SX PVAM</td>
<td>1.52 ± 0.13</td>
<td>0.98 ± 0.07</td>
<td>1.31 ± 0.038</td>
<td>0.59 ± 0.017</td>
<td>10.9 ± 0.9</td>
<td>3.60 ± 0.2</td>
</tr>
<tr>
<td>SX PVAM-GMA</td>
<td>1.48 ± 0.12</td>
<td>1.08 ± 0.10</td>
<td>1.38 ± 0.040</td>
<td>0.64 ± 0.019</td>
<td>10.0 ± 0.8</td>
<td>3.33 ± 0.2</td>
</tr>
</tbody>
</table>

\(a\) The numbers after the ± symbols for optical microscopy and SEM are the standard deviations. \(b\) Number-average diameter determined from optical micrographs at pH = 7. \(c\) Number-average diameters determined from SEM images. \(d\) Hydrodynamic diameter measured at pH = 7. \(e\) Collapsed hydrodynamic diameters calculated using eqn (2). \(f\) Particle swelling ratio calculated at pH = 7 from eqn (1). \(j\) Electrophoretic mobility measured at pH = 7.

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![Fig. 3](image-url) DX PVAM microgel dissolution test and morphology (a) shows images for DX PVAM microgel and physical gel of SX PVAM-GMA particles. Both samples were heated at 50 °C for 24 h prior to the dissolution experiment. (b) and (c) Show SEM images for DX PVAM. The value for \(\phi_{hs}\) was 0.10.
connected to each other via polymer bridges. The bridges are proposed to act as elastically effective links that distribute load. The bridges for the DX PVAM microgel contained covalent linkages and opposed re-dispersion when placed in water (Fig. 3(a)).

The morphologies of the gels were also examined using optical microscopy (Fig. 4). These optical images are the first reported that show the morphology of a DX microgel in the hydrated state. The polymer bridges between neighbouring particles identified by SEM (above) can also be seen from the higher magnification optical image (Fig. 4(b)). The optical images also show evidence of local ordered packing. The face of a square-like lattice is sketched on top of four particles in Fig. 4(b). A FFT of the optical image is shown in Fig. 4(c), which reveals a central amorphous ring surrounded by points in a square-like arrangement (indicated with arrows). The latter is suggestive of square symmetry in the x-y plane. (Similar features were also apparent from optical images and FFT for SX PVAM – see Fig. S6, ESI†) This demonstrates that the occasionally locally ordered lattices were present globally. The square-like arrangements were slightly stretched in the y direction. Microgels have been reported to have face-centred cubic lattices and can also form body centred tetragonal lattices (BCT) when subjected to an external field. Unfortunately, our optical images do not permit a clear assignment of the 3-dimensional unit cell.

In this study DX PVAM gels were prepared using a range of φhs values. Because particle sizes could be measured from the optical micrographs they provided an opportunity to estimate ϕeff for the gels. This was achieved by calculating Qp and applying the following equation.

\[ \phi_{eff} = \frac{Q_p}{\phi_{hs}} \] (3)

We applied the same approach to calculate Qp from the gel optical micrographs as used above for the PCS data (eqn (1) and (2)). Accordingly, a value for the collapsed diameter of the microgel particles determined from optical microscopy, \(D_n^{*}(\text{OPT})\), was required. Optical micrographs for the precursor PNVF particles dispersed in ethanol (see Fig. S7†) yielded the collapsed particle size determined by optical microscopy, \(D_n(\text{PNVF,OPT}) = 0.78\ \mu m\). The latter enabled \(D_n^{*}(\text{OPT})\) Values to be calculated using an equation equivalent to eqn (2). The calculated \(D_n^{*}(\text{OPT})\) values for SX PVAM-GMA and SX PVAM particles were 0.76 and 0.70 \(\mu m\), respectively. The former value was used to calculate \(Q_p\) values from measured \(D_n^{*}(\text{OPT})\) values (Fig. 5(a)) using an equation equivalent to eqn (1). Hence, \(\phi_{eff}\) was then calculated for each \(\phi_{hs}\) value using eqn (3). The latter data appear in Fig. 5(b). The same analysis methodology was applied for the PVAM gels and the \(D_n^{*}(\text{OPT}), Q_p \) and \(\phi_{eff}\) data are shown in Fig. S8.†

The microgel particle size decreased strongly from the dilute dispersion value when incorporated into the gels (Fig. 5(a) and S8(a)†). External electrolyte is well known to cause osmotic deswelling of polyelectrolyte microgel particles. For the present systems the maximum ionic strength due to PVAM microgel particles at \(\phi_{hs} = 0.05\) can be estimated as 0.4 M and this would have been an increasing function of \(\phi_{hs}\). Therefore, the decrease in \(D_n^{*}(\text{OPT})\) for the microgels within the gels is attributed to osmotic deswelling caused by the high ionic strength of the external water phase due to the mobile ions that originated from the microgel particles.

A high ionic strength coupled with low \(Q_p\) values (Fig. 5(a)) could have led to some particle aggregation within the gels prior to DX formation. Particle–particle contacts would then form a space-filling network. Although the gels appeared visually homogeneous (Fig. 3(a)), particle networks with bridges between particles was apparent from SEM and optical microscopy images for the DX PVAM and SX PVAM gels (Fig. 3(c), 4(b), S5(b) and S6(b)†). Furthermore, the \(\phi_{eff}\) values (Fig. 5(b)) were low compared to values for a cubic lattice (0.52) or randomly close packed lattice (0.64). This is because the microgel particles were

**Fig. 4** Morphologies of DX PVAM gel in the hydrated state. Optical micrographs are shown in (a) and (b). (c) Shows an FFT image from (a). The arrows highlight some of the bright points present \((\phi_{hs} = 0.10)\).

**Fig. 5** Particle size and swelling dependences on polymer volume fraction for DX PVAM microgels. (a) Shows the variation of the particle size and particle swelling ratio with \(\phi_{hs}\). (b) Shows the calculated variation of the effective polymer volume fraction with \(\phi_{hs}\).
not fully swollen in the gels. Rather, they formed space-filling networks with porosity between interconnected particle chains. The $\phi_{\text{eff}}$ values increased linearly with $\phi_{\text{hs}}$ for the gels (Fig. 5(b)).

Consequently, the effective porosity of the DX PVAM gels ($= 1 - \phi_{\text{eff}}$) could be tuned from 76 to 93 vol% simply using $\phi_{\text{hs}}$.

**DX PVAM microgel mechanical properties**

In this study the DX PVAM microgels were prepared and studied using a pH of 9.0. The gels would have been positively charged based on the $\mu$ values for the SX PVAM-GMA particles (Fig. 2(a)). The mechanical properties were probed using dynamic rheology. Frequency-sweep rheological data are shown for the DX PVAM microgel in Fig. 6(a) and (b). Data for the SX PVAM and SX PVAM-GMA physical gels are shown for comparison. All of these systems were heated at 50 °C for 24 h. The DX PVAM microgel showed low frequency dependences for both $G'$ (storage modulus) and tan $\delta$ ($= G''/G'$, $G''$ is the loss modulus). Nearly frequency-independent $G'$ values have been reported for a range of colloidal gels.\(^\text{43}\) For the present systems, this implies the DX PVAM system behaved as the most elastically ideal network.\(^\text{44}\) We propose that double crosslinking decreased the proportion of elastically ineffective structures, such as loops and dangling chains. This conjecture is supported by the significantly lower tan $\delta$ value for DX PVAM compared to SX PVAM (Table 3). The latter system was not capable of forming covalent interparticle linkages.

The $G'$ value for DX PVAM was a factor of 4.2 greater than the value for SX PVAM-GMA (Table 3). This increase is due to additional elastically effective chains from covalently cross-linked GMA groups. Each PVAM-GMA microgel particle within the DX PVAM microgel acts as a covalent crosslinking centre via the GMA groups. The value of $G'$ for SX PVAM-GMA was significantly higher than the value for SX PVAM (Table 3). This increase may be due to some covalent inter-particle crosslinking for SX PVAM-GMA because air was not excluded during the heating process. In addition, there was a contribution to the elasticity for both the SX PVAM-GMA and SX PVAM gels from reversible particle–particle connections that were present. The tan $\delta$ values for the DX PVAM system were typically less than or equal to 0.065, and the lowest of the gels (Table 3 and Fig. 6(b)). About 95% of the energy from the applied shear was stored elastically within the DX PVAM microgel network.

Strain-sweep data were also obtained for the DX microgel and the two physical gels (see Fig. 6(c)). At strains greater than about 10% the $G'$ values decreased, which is an indication of the onset of strain-induced network failure. In each case the $G'$ data crossed the $G''$ data at the maximum $G''$ value. At the cross-over points $G' = G''$ (i.e., tan $\delta = 1.0$) and those $\gamma$ values are the critical yield strains ($\gamma_c$). At higher $\gamma$ values more energy was lost through dissipation than energy storage. The value for $\gamma_c$ was highest for the physical gels (SX PVAM and SX PVAM-GMA) and decreased to 30% for the DX microgel (Table 3). The high $\gamma_c$ value of 100% for SX PVAM-GMA physical gel was unexpected. This may be due to a low level of inter-particle crosslinking that could have occurred within that system during rotary evaporation.

It can be seen from Fig. 6(c) that there were significant differences for the $G''$ data for the DX PVAM and SX PVAM systems. The DX PVAM $G''$ maximum has a larger breadth. This is proposed to be due to the presence of a range of relatively short inter-particle linkages that were broken over a wide range of strains. By contrast the $G''$ maxima for the SX PVAM and SX PVAM-GMA systems were more narrow and similar, implying equivalent viscoelastic behaviours for those systems. Double crosslinking significantly changed the strain-induced network failure mechanism.

**Effect of polymer volume fraction on DX PVAM microgel mechanical properties**

In order to learn more about the origins of the DX PVAM elasticity and establish potential for tuning the mechanical properties we investigated the effect of $\phi_{\text{hs}}$ and $\phi_{\text{eff}}$ on the dynamic rheological properties. The frequency sweep $G'$ data are shown in Fig. 7. The $G'$ data showed low frequency dependencies. Fig. 7(b) shows the variation of tan $\delta$ with $\phi_{\text{hs}}$ for both systems. The frequency dependence for tan $\delta$ (i.e., range of the tan $\delta$ values at each $\phi_{\text{hs}}$) decreased with increasing $\phi_{\text{hs}}$. This was lowest for the DX microgel containing the highest microgel content, i.e., $\phi_{\text{hs}} = 0.17$. The SX PVAM gels were also studied and they showed generally higher tan $\delta$ values and frequency dependence (see Fig. S9f) for a given $\phi_{\text{hs}}$ value. The

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**Table 3 Mechanical properties for a range of gels\(^a\)**

<table>
<thead>
<tr>
<th>Sample</th>
<th>$G''/\text{Pa}$</th>
<th>tan $\delta$</th>
<th>$\gamma_c$/%(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DX PVAM</td>
<td>7400 ± 230</td>
<td>0.065 ± 0.015</td>
<td>29.5 ± 1.4</td>
</tr>
<tr>
<td>SX PVAM-GMA</td>
<td>1770 ± 55</td>
<td>0.090 ± 0.020</td>
<td>100.0 ± 4.8</td>
</tr>
<tr>
<td>SX PVAM</td>
<td>1350 ± 42</td>
<td>0.125 ± 0.028</td>
<td>57.2 ± 2.7</td>
</tr>
</tbody>
</table>

\(^a\) Data obtained for $\phi_{\text{hs}} = 0.10$. \(^b\) Storage modulus measured at 10 Hz. \(^c\) Data measured at 10 Hz. \(^d\) Yield strain.
combination of the low tan δ values and low frequency dependence for the DX PVAM gels imply that double crosslinking produced a network with relatively few elastically ineffective loops and trains.

Fig. 7(c) shows the variation of $G'$ plotted as a function of $\phi_{hs}$ and $\phi_{eff}$ for DX PVAM microgel. Both sets of data show exponential relationships for $G'$. Although an exponential dependence for $G'$ on $\phi_{eff}$ does have a theoretical basis, this is less clear for our DX PVAM microgels in the case of $\phi_{hs}$. Those data and the exponential fit for $\phi_{hs}$ are shown here because they provide a practical tool to tune the elasticity for future DX PVAM gels. The $G'$ value was tuneable over more than an order of magnitude (2–41 kPa).

Zong et al. studied the mechanical properties for colloidal glasses of binary mixtures of poly(styrene-co-NIPAM) microgels. From their work, eqn (4) was applied to the rheological data for our DX PVAM microgels.

\[
G = a \exp(b\phi_{eff})
\]  

The parameter $b$ is a measure of particle softness and $a$ is a pre-exponential constant. A relationship of this general type has also been predicted for particle–particle suspensions using mode coupling theory. Here, the data for DX PVAM microgel (Fig. 7(c)) gave a $b$ parameter of 16.4. This value is comparable to the value of 13.8 reported for binary microgel mixtures. Our DX PVAM gels can be considered as a gel consisting of attractive particle–particle contacts which are strengthened by covalent crosslinking. Our $b$ value is lower than the theoretical limit of 26 for hard-sphere systems and higher than the value of $b = 9$ for silica with long poly(dimethylsiloxane) grafts. Accordingly, the PVAM microgel particles have low softness within the DX PVAM gels. This is congruent with their relatively low $Q_p$ values of about 1.4 (Fig. 5(a)). We note that the data for the SX PVAM gel did not follow an exponential relationship (Fig. S9(c)†), which indicates major differences in the elasticity mechanisms that were operative for each system. These differences are due to double crosslinking within DX PVAM.

The strain-sweep rheological data for the DX PVAM gels obtained using a range of $\phi_{hs}$ values are shown in Fig. 8. (Strain-sweep data for the SX PVAM gels are shown in Fig. S10†) The $G'$ data show strain induced decreases due to network disruption which began at a strain of about 10% for the gels prepared with $\phi_{hs}$ values greater than or equal to 0.10. There was no evidence for more than one distinct maximum within each DX PVAM data set and this is consistent with the view that there was one general network breaking process that occurred over a wide range of strain values. This was most likely bond breaking based on the three-dimensional particle network morphologies observed from the images shown in Fig. 3 and 4.

The strain-sweep data enabled determination of $\gamma_c$ values and the data are plotted as a function of both $\phi_{hs}$ and $\phi_{eff}$ in Fig. 8(c). A gradual decrease for $\gamma_c$ with increasing $\phi_{hs}$ or $\phi_{eff}$ is evident which is followed by an unexpected (but reproducible) increase. The gradual decrease in $\gamma_c$ is explainable in terms of an increasingly highly crosslinked network with shorter elastically effective chains. However, the increase for $\gamma_c$ (to 46%) at the highest $\phi_{hs}$ or $\phi_{eff}$ values requires further comment. This system had a higher $\gamma_c$ value than the SX PVAM microgel equivalent (Fig. S10(c), ESI†). For conventional hydrogels a more narrow distribution of linkage lengths results in relatively high yield strains. We speculate that an increase in overall order occurred for this system ($\phi_{hs} = 0.17$) due to a higher $\phi_{eff}$ which caused the elastically effective chain lengths to become more uniform. We note that this system also had the lowest frequency dependent tan δ values for all the DX PVAM microgels studied (Fig. 7(b)), implying a more interconnected network. FFT analysis of those gels did show square-like lattice symmetry. Unfortunately, it was not possible to be certain from the optical images whether the relative proportion of ordered phase had increased for $\phi_{hs} = 0.17$. 

![Fig. 7](image_url)  

**Fig. 7** DX PVAM frequency sweep data. (a) Shows the frequency dependence for $G'$. (b) Shows the variation of tan δ with $\phi_{hs}$ at different frequencies which are shown in the legend in Hz. (c) Shows the variation of $G'$ with $\phi_{hs}$ and $\phi_{eff}$ plotted in semi-logarithmic form.

![Fig. 8](image_url)  

**Fig. 8** DX PVAM strain-sweep data. (a) and (b) Show the variation of $G'$ and $G''$ with strain at different $\phi_{hs}$ values (legend). (c) Shows the variation of the yield strain with $\phi_{hs}$ and $\phi_{eff}$.
Injectable DX PVAM gels swollen at physiological pH

One potential application for future DX PVAM microgels is an injectable biomaterial for soft tissue repair. Two desirable properties for gels as biomaterials are the ability to be injected through a syringe needle and then to form a gel at 37 °C. The DX PVAM gels studied in this section were prepared using added TEMED in order for them to be crosslinked at 37 °C. Here, we demonstrated that a representative DX PVAM precursor physical gel was sufficiently shear-thinning to be injectable through a narrow gauge syringe (Fig. 9(a)). The digital photograph shows a shear-thinning SX VAM–GMA physical gel (containing APS and TEMED) being injected through a syringe needle. The DX PVAM gel was subsequently formed at 37 °C (Fig. 9(a)).

The DX PVAM microgel was allowed to swell in pH = 7.4 phosphate buffered saline (PBS) solution for 3 days. The measured \( \phi_{hs} \) values were 0.16 and 0.11, respectively, before and after swelling. A sol fraction of 0.06 was determined gravimetrically which meant that 94 wt% of the gel remained intact. Therefore, the decrease in \( \phi_{hs} \) was primarily due to gel expansion. Optical micrographs taken from the swollen gel enabled a value for \( D_{ho}^{(opt)} \) of 0.79 \( \mu \)m to be determined (CV = 11%). Following the method described above a \( Q_p \) value of 1.4 was calculated. The \( D_{ho}^{(opt)} \) and \( Q_p \) values were not significantly different to those measured for the DX PVAM prepared in the absence of TEMED without swelling (Fig. 5(a)). Accordingly, the expansion of the DX PVAM gel when placed in buffer was due to particle-network relaxation (“breathing in”) and not microgel particle swelling. Furthermore, a \( \phi_{ext} \) value of (0.11 \* 1.4 = ) 0.15 was calculated. This shows that the DX PVAM gel retained a high effective porosity (ca. 85 vol%) under physiological conditions. High porosity is one criterion for potentially beneficial scaffold performance.46

The mechanical properties of the swollen DX PVAM gel were measured using frequency-sweep and strain-sweep dynamic rheology (see Fig. 9(b) and (c)). The \( G' \), \( \tan \delta \) and \( \gamma_e \) values for the gel were 20.1 kPa, 0.055 and 19%, respectively. These values are comparable to the values of the DX PVAM microgel prepared without TEMED before swelling (cf. Fig. 7, \( \phi_{hs} = 0.15 \)). These data demonstrate that the DX PVAM microgels can be formulated as injectable gels that can be cured at physiological temperature and maintain their mechanical properties under physiological ionic strength and pH conditions. Future work will investigate their pH-dependent swelling.

Conclusions

This study has shown for the first time that DX microgels can be prepared from cationic, high primary amine content microgel particles. Accordingly, our double crosslinking strategy for preparing hydrogels from microgel particles appears to be generally applicable. We have shown that for DX PVAM microgels the dependence of the elastic modulus follows \( G' \sim \exp(bf_{ext}) \) and \( b = 16.4 \). The \( b \) value implies that the particles within the gel had low softness and this was attributed to a relatively low extent of microgel particle swelling. The morphology of the gels consisted of inter-connected, space-filling networks of partially swollen microgel particles. Both \( G' \) and the porosity were tuneable using \( \phi_{hs} \). The new DX PVAM microgel with the best mechanical properties had \( G' = 41 \) kPa and \( \gamma_e = 46 \), implying good stiffness and ductility. That system was prepared using \( \phi_{hs} = 0.17 \) and had an effective porosity of 76 vol% from Fig. 5(b). The microgel used here had a very high content of primary amine groups and, accordingly, is well suited to functionalisation.46 We demonstrated microgel functionalisation here by epoxide reaction and also earlier using a chromophore and carbodiimide chemistry.44 Accordingly, there are many potential applications for future versions of these new DX PVAM microgel hydrogels, spanning the range from composites, catalysis, membranes and biomaterials. Factors favouring the latter are that the precursor physical gels can be injected through a narrow-gauge needle (Fig. 9(a), inset) and crosslinked at 37 °C. Furthermore, the DX PVAM maintained a highly porous morphology at physiological pH and ionic strength. For one potential biomaterial application we note that injectable anionic DX microgels have been shown to restore load for damaged intervertebral discs.7 This approach could be available for a future version of the DX PVAM microgels. Of course, it will be necessary to reduce the cytotoxicity often associated with primary amines and a range of suitable strategies that have been successfully used for related primary amine-based systems have been established.28,29

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References
