Microgel functionalisation using click chemistry

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Robert Farley

School of Materials

The University of Manchester
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<td>$^1$H NMR</td>
<td>Proton nuclear magnetic resonance</td>
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<td>AIBA</td>
<td>$\alpha,\alpha'$-Azodiisobutyramidine dihydrochloride</td>
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<td>AEA</td>
<td>2-Azido-1-ethylamine</td>
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<tr>
<td>ATRP</td>
<td>Atom transfer radical polymerisation</td>
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<td>Au/Pd</td>
<td>Gold/palladium</td>
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<td>AZPMa</td>
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<td>AZPOI</td>
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<td>BDD</td>
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<td>CuAAC</td>
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<td>Copper (I) bromide</td>
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<td>DLS</td>
<td>Dynamic light scattering</td>
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<td>DLVO</td>
<td>Derjaguin Landau Verwey Overbeek</td>
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<td>DMF</td>
<td>Dimethylformamide</td>
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<td>DN</td>
<td>Double network</td>
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<td>DVB</td>
<td>Divinylbenzene</td>
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<td>DX</td>
<td>Doubly crosslinked</td>
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<td>EA</td>
<td>Ethyl acrylate</td>
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<td>FFT</td>
<td>Fast Fourier transform</td>
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<td>FTIR</td>
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<td>GMA</td>
<td>Glycidyl methacrylate</td>
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<td>KOH</td>
<td>Potassium hydroxide</td>
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<tr>
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<td>VP</td>
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**List of Symbols**

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Abstract

This thesis is titled “Microgel functionalisation using click chemistry” and is presented in 2015 to The University of Manchester for the degree of Doctor of Philosophy in the School of Materials, Faculty of Engineering and Physical Sciences, by Robert Farley.

The research described in this thesis aimed to assess the suitability of the copper(I) catalysed azide-alkyne cycloaddition (CuAAC), a click chemistry reaction, in preparing microgel particles with specific functionalities. A series of acetylene-functionalised microgels were synthesised by the co-polymerisation of propargyl acrylate (PA) with ethyl acrylate (EA), N-isopropylacrylamide (NIPAm) and 2-vinyl pyridine (VP). The CuAAC reaction was employed to introduce primary amine functionality to the microgels via their reaction with 2-azido-1-ethyamine (AEA). The reaction proved to be high yielding and the degree of primary amine functionalisation was only limited by the concentration of available acetylene groups in each microgel. However, this was not sufficient to produce the desired pH-responsive microgels and the incorporation of PA into the VP based system severely restricted the swelling capacity of the poly(VP-PA) microgel.

An alternative route to high primary amine content microgels was presented by the synthesis and emulsion polymerisation of the azide-bearing monomer 3-azidopropyl methacrylate (AZPMa). A CuAAC reaction between the poly(AZPMa) microgel and propargylamine (PAm) resulted in a pH-responsive microgel with a very high primary amine content. This poly(AZPMa-PAm) microgel demonstrated pH-triggered swelling from 225 nm at pH 9 and above to over 370 nm at pH 7 and below. The use of the CuAAC in precisely controlling the compositions of microgels with specific functionalities was examined via the incorporation of incremental amounts of PA into a poly(VP-co-PA) microgel.

This concept was further examined via the CuAAC reaction between a poly(VP-co-PA) microgel and AZPMa. The high efficiency of the CuAAC reaction enabled highly precise control over the extent of functionalisation. The pH-responsive ‘clicked’ microgels demonstrated strong pH-triggered swelling and formed physical gel networks below pH 4. Double-crosslinked (DX) microgels were manufactured by polymerising the pendant alkene groups of neighbouring particles, creating covalent inter-particle linkages. The DX microgels had significantly greater mechanical properties than singly crosslinked (SX) physical gel precursors. DX gel storage modulus (G’) increased linearly with the degree of alkene group functionality.

The CuAAC reaction proved to be highly efficiently in introducing desired functionalities to microgel systems. Considerations for the use of the reaction were that high concentrations of ionisable primary amine groups were necessary to produce a pH-responsive microgel and the incorporation of either PA or AZPMa into pH and temperature responsive systems resulted in microgels with reduced swelling capacities. The reaction may therefore be of most use at low levels of functionalisation, or in microgel or hydrogel systems in which a swelling response is not of paramount importance.
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.

Robert Farley
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Finally, an enormous thank you to my family, in particular my parents, for their limitless support and patience. This would not have been possible without your help and I consider myself to be very fortunate.
1. Introduction

1.1 Motivation

Smart materials demonstrate a change in properties in response to an external stimulus such as temperature, pH or light. Control over the responsive behaviour of stimuli-responsive materials has attracted considerable attention for use in biomedical applications, amongst others. Polymeric smart materials are well suited to biomedical applications because they can undergo significant conformational changes in response to relatively minor alternations in environment conditions.\(^1\)

One such class of polymeric smart materials are microgels, which are colloidal particles consisting of lightly crosslinked polymer chains swollen by solvent.\(^2\) Microgels can exhibit dramatic changes in particle size by swelling, or de-swelling, in response to a stimulus. Enzymatic activity is another potential stimulus that could be used to elicit a response from polymeric biomaterials, including microgels. This could facilitate interactions between the biological environment and a synthetic material with beneficial results, such as targeted drug release, regulated by the highly selective nature of enzymatic activity.\(^5\)

The control of microgel composition is crucial in designing microgels with both desirable responsive swelling behaviour and the potential for further useful functionalisation. A group of reactions that could be extremely useful in microgel functionalisation are those of click chemistry. Click chemistry reactions are characterised by their high yields, specificity and tolerance to a range reaction conditions.\(^6\) One reaction in particular has become synonymous with click chemistry, the Huisgen 1,3-dipolar azide alkyne cycloaddition (CuAAC). This highly selective reaction takes place between azide and alkyne functional groups and allows for an array of functionalisation opportunities. Responsive behaviour can be engendered in a microgel by the incorporation of a specific functional group using the click chemistry reaction.

Primary amine functionalised microgels have great potential as colloidal microgels that could demonstrate a strong swelling response. They also offer the potential for functionalisation with peptides required to prepare enzyme responsive microgels, or to be used in other biomedical applications.\(^7\) However,
primary microgels have been difficult to develop. The CuAAC reaction offers an alternative route towards the preparation of this useful class of microgel. This could be achieved via the reaction between an alkyne-functionalised microgel with a bi-functional molecule containing both azide and primary amine groups, or vice versa.

1.2 Aims of thesis

This thesis aims to assess the merits of the CuAAC reaction in microgel functionalisation in two ways. Firstly, the use of the reaction in preparing primary amine functionalised microgels is examined. For the reaction to be considered as truly successful in this regard it must be able to provide adequate and efficient primary amine functionalisation, and this functionalisation must also result in significant pH-responsive behaviour. Secondly, the ability of the CuAAC reaction to provide fine control over microgel composition is studied using the incremental functionalisation of alkyne groups. The effect of microgel composition on gel mechanical properties was observed, using covalent gels prepared by the polymerisation of these pendant double bonds.

1.3 Survey of thesis

This thesis consists of three research chapters preceded by a literature review. The literature review forms Chapter 2 of the thesis and serves to introduce the concepts that feature in the research. The topics covered include an overview of microgels and polymer gel networks, an introduction to colloidal stability, emulsion polymerisation, the CuAAC reaction and its use in polymer and colloid functionalisation and the experimental techniques that are employed in this work.

Chapter 3 details a general method of preparing primary amine functionalised microgels via the CuAAC reaction. Three alkyne-functionalised microgel systems are prepared by the emulsion co-polymerisation of each of the monomers ethyl acrylate (EA), N-isopropylacrylamide (NIPAm), and 2-vinyl pyridine (VP) with the alkyne-bearing monomer propargyl acrylate (PA). The CuAAC reaction is employed to introduce primary amine functionalisation to the microgels via their reaction with 2-azidoethylamine (AEA).
Figure 1.1: An illustration of the preparation of a primary amine functionalised microgel through the CuAAC reaction between an alkyne-bearing microgel with 2-azido-1-ethylamine (AEA).

The ability of PA to co-polymerise with EA, NIPAm and VP is discussed, along with the effectiveness of the CuAAC reaction in providing primary amine functionalisation, and the properties of both the alkyne and amine bearing microgels.

In Chapter 4, an azide-bearing monomer, 3-azidopropyl methacrylate (AZPMA), is synthesised and used to prepare azide-functionalised microgels. Two microgel systems are prepared: a lightly crosslinked poly(AZPMA) microgel and a poly(VP-co-AZPMA) microgel with a lower concentration of AZPMA.

Figure 1.2: The CuAAC functionalisation of an azide-bearing microgel with either propargylamine (PAm) to produce a primary amine functionalised microgel, or propargyl acrylate (PA) to prepare an alkeine-functionalised microgel.
The poly(AZPMa) microgel is used to prepare microgels with a high primary amine concentration via the incorporation of propargylamine using click chemistry. The resultant microgel demonstrates pH-responsive swelling behaviour; made possible by the high concentration of primary amine groups. Incremental amounts of PA are clicked onto the poly(VP-co-AZPMa) microgel in order to assess the efficiency of the reaction and its ability to finely control microgel composition. The CuAAC reaction offers positive results in this endeavour and control of vinyl group concentration is possible without altering the properties of the predominantly VP-based particles.

This concept is further examined in Chapter 5, which discusses an effort to use the CuAAC functionalisation of an alkyne-functionalised poly(VP-co-PA) microgel with AZPMa to prepare a series of microgels with controlled alkyne group functionalisation. The pH-responsive behaviour of the microgels, coupled with their pendant vinyl group functionalisation, allows the formation of polymer gel networks containing both intra-particle and inter-particle crosslinking. These so-called double crosslinked microgels show greater mechanical properties than singly crosslinked microgels networks. The relationship between alkyne group concentration and gel mechanical properties is discussed, as is the ability of the CuAAC reaction to both control microgel composition and influence gel mechanical properties.

Figure 1.3: Preparation of a double crosslinked (DX) microgel through the cationic swelling of alkyne functionalised VP-based microgel prepared via CuAAC and the polymerisation of the pendant alkyne groups of neighbouring particles.
The conclusions of the thesis are outlined in Chapter 6. This chapter assesses the compatibility of the CuAAC reaction with microgel functionalisation in terms of reaction yield, its ability to prepare pH responsive microgels, and its success in closely controlling microgel composition. Areas in which future may be suitable and productive are also suggested.
2. Literature Review

2.1 Polymer microgels

2.1.1 Introduction to microgels

Microgels are defined as crosslinked colloidal polymer particles that swell in a good solvent, or in response to stimuli\(^4,8,9\). Each microgel particle is essentially a solvent-containing network of polymer chains, held in place by covalent crosslinks that provide structural integrity\(^3\). This structure is illustrated in Figure 2.1\(^3\), which shows that solvent can penetrate throughout the microgel particle interior rather than the sphere of polymer that is more representative of a latex particle.

![Figure 2.1: Structures of a) a collapsed latex particle and b) a swollen microgel particle. Image b) reproduced from reference\(^3\).](image)

The size range of microgel particles is generally considered to be the same as the colloidal size range; from 1 nm to 10 μm when in the swollen state\(^8\). A feature of colloidal materials is their stability in dispersion and this equally applies to microgel particles. The ability to manipulate the swelling and de-swelling of microgels through external stimuli such as temperature and pH has generated great interest over the last 30 years and seen microgels emerge as functional colloidal materials. This development was triggered by the successful preparation of temperature responsive poly(N-isopropylacrylamide) (poly(NIPAm)) microgel particles by Pelton and Chibante\(^10\), which remains the most studied water-based microgel system\(^8\).

The useful structural and responsive properties of microgels have attracted interest for their use in biomedical applications. Microgel biocompatibility is aided
by high degrees of hydration when swollen, while attributes such as ease of
preparation and functionalisation, colloidal stability and precise control over
particle size potentially offer improvements on existing drug delivery dispersions
featuring drugs in particulate form. However, microgel biocompatibility also
strongly depends on the monomers used in their preparation and many monomers
are unsuitable for biological applications.

2.1.2 Microgel swelling ratio

Perhaps the most important property of a microgel is the maximum degree to
which it can swell. This is calculated using the swollen and collapsed particle
diameters. The microgel swelling ratio (Q) is defined in equation 2.1 and gives a
measure of the volume increase of a swollen particle compared to its collapsed
state,

\[ Q = \left( \frac{d_h}{d_c} \right)^3 \]  (2.1)

where \( d_h \) is the diameter of the swollen particle and \( d_c \) is the diameter of the
collapsed particle.

The change in size of microgels from the collapsed state to the swollen state is
sometimes described as the latex-to-microgel transition. It is not uncommon for
swollen microgels to increase in volume by as much a factor of 30 compared to
their collapsed volumes. In practice, calculation of the collapsed particle diameter
can be difficult as even collapsed particles are likely to contain some solvent.
Options for obtaining this value include measuring the size of collapsed particles
using electron microscopy or measuring the diameter of the particles in a non-
solvent.

2.1.3 Responsive microgels

Stimuli used to induce microgel swelling or de-swelling, or elicit another response,
include temperature, pH, light and enzymatic action. Microgel swelling or de-
swelling occurs when a stimulus causes an imbalance in osmotic pressure between
the inside and outside of a particle. The particle size of the microgel changes until
equilibrium is achieved and the osmotic pressure difference inside and outside of
the particle becomes equal to zero\textsuperscript{2}. The osmotic pressure inside the particle is defined by Flory theory and consists of a mixing component ($\pi_m$) and an elastic component ($\pi_e$)\textsuperscript{15,16}.

\[
\pi_m = -\frac{N_A k T}{V_s} \left[ \phi + \ln(1 - \phi) + \chi \phi^2 \right] \tag{2.2}\textsuperscript{15}
\]

\[
\pi_e = \frac{N_c k T}{V_0} \left[ \left( \frac{\phi}{2\phi_0} \right) - \left( \frac{\phi}{\phi_0} \right)^{1/3} \right] \tag{2.3}\textsuperscript{15}
\]

In these equations $N_A$ represents the Avogadro number, $k$ is the Boltzmann constant and $T$ is the temperature. $V_s$ is the molar volume of the solvent, $\phi$ is the volume fraction of polymer in the swollen gel and $\chi$ is the Flory polymer-solvent interaction parameter. $N_c$ is the effective number of network chains, $V_0$ and $\phi_0$ are the volume fraction of polymer and microgel volume of particles in the unswollen state respectively\textsuperscript{15}.

In the case of microgels that contain ionisable groups, the contribution of electrostatic repulsion between charged polymer chains must also be taken into account. The effects of charged groups in the polymer network and counterions that cause an associated increase in osmotic pressure both contribute to an expansion of the polymer network and microgel swelling\textsuperscript{15}. These contributions are closely related. The osmotic pressure due to counterions ($\pi_i$), the Donnan effect, is expressed in the following equation, in which $f$ represents the number of counterions per network chain\textsuperscript{15,17}.

\[
\pi_i = \frac{f N_c k T}{V_0} \frac{\phi}{\phi_0} \tag{2.4}\textsuperscript{15}
\]

Combining equations 2.2 - 2.4 forms the equilibrium condition for microgel particles: $\pi_{\text{total}} = \pi_m + \pi_e + \pi_i = 0$\textsuperscript{15}. The derivation of this equation is the basis of the microgel swelling ratio, previously defined in equation 2.1, which is valid assuming that particle swelling is isotropic\textsuperscript{15}.

The most studied responsive microgel system is the temperature-responsive poly(NIPAm) system, which undergoes thermally induced de-swelling when the temperature of the dispersion exceeds the Lower Critical Solution Temperature (LCST) of poly(NIPAm) (32°C in water)\textsuperscript{4}. Below the LCST, water acts as a good
solvent and hydrogen bonding between the amide groups of NIPAm and water maintains microgel swelling. Increasing the temperature disrupts these hydrogen bonds. Above 32 °C the polymer-polymer hydrophobic interactions become dominant over polymer-solvent interactions, resulting in particle collapse. In effect, a temperature above the LCST of poly(NIPAm) causes a change in the solubility of the polymer in water and creates an osmotic pressure difference that is only resolved when water is driven out of the particles and osmotic equilibrium is achieved by particle collapse.

pH responsive microgels contain co-monomers with functional groups that are either charged or uncharged depending on the pH. When the pH of the system exceeds the pKₐ of the specific functional group these groups become charged. This causes in electrostatic repulsion between polymer chains. The osmotic pressure within particles is reduced and microgel swelling occurs in order to resolve this pressure difference. Many examples of both anionic and cationic microgels have been reported. Anionic microgels based on the polymerisation of carboxylic acid containing monomers have been studied by Rodriguez et al. and Lally et al., among others. These microgels contained a significant proportion of methacrylic acid, which caused a strong particle swelling response when the pH exceeded values of approximately 6.0. Above this pH the carboxylate groups of the methacrylic acid repeat units became negatively charged ions. This resulted in the electrostatic repulsion of polymer chains and particle swelling. For individual systems, the increase in particle diameter was as much as from approximately 100 nm when collapsed to over 300 nm when swollen. This was consistent with a particle swelling ratio (Q), which is indicative of the increase in particle volume upon swelling, of over 30.

Cationic microgels have been reported by Dupin et al. and Amalvy et al. The poly(vinyl pyridine) microgels reported by Dupin et al. demonstrated extensive and sharp cationic swelling of up to three orders of magnitude in particle diameter below a pKa of 4.1. The collapsed and swollen sizes of the microgels were also controlled using a variation of the amounts of surfactant and crosslinking monomer used in microgel preparation. Amalvy et al. synthesised a new class of pH
responsive microgels based on the monomers 2-(diethylamino)ethyl methacrylate and 2-(disopropylamino) ethyl methacrylate. These tertiary amine microgels exhibited swelling below neutral pH for the former monomer and slightly below pH 7 for the latter (due to its more hydrophobic structure). The particle diameters of both increased approximately three times from the collapsed to swollen states. A representation of cationic microgel swelling is displayed in Figure 2.2.

![Diagram of cationic microgel swelling](image)

**Figure 2.2: Cationic swelling and latex-to-microgel transition of a tertiary amine methacrylate microgel.** Reproduced from reference 12.

The work in this thesis initially centres on the preparation of cationic primary amine-functionalised microgels, which could offer a wide range of possible further functionalisation options including with peptides to potentially yield enzyme responsive microgels. Temperature, pH and enzyme responsive microgels and hydrogels all have potential in the biomedical applications of drug delivery and tissue engineering. The following examples illustrate some of the useful properties and potential applications of polymeric biomaterials.

Poly(ethylene glycol) (PEG) grafted chitosan hydrogels undergo a temperature responsive sol-gel transition at body temperature and have been proposed for *in vivo* drug release applications and as tissue engineering as potential scaffolds. A microgel system featuring poly(methacrylic acid) was able to deliver the cationic drug procaine hydrochloride via pH-responsive action. Poly(N-isopropylacrylamide-co-acrylic acid) microgels have been examined for drug
delivery application. A system devised by Das et al. underwent pH induced swelling from pH 4 to pH 7 and through the bioconjugation of receptor specific molecules onto the microgel surfaces were able to specifically target cancer cells using the release of an anti-cancer drug\textsuperscript{30}.

2.1.4 Polymer gel networks

Polymer networks have attracted considerable attention for biomedical applications, in particular polymer hydrogels. These are networks of hydrophilic, water soluble polymers that are crosslinked to form water-insoluble networks that contain large degrees of hydration\textsuperscript{31}. This high hydration, allied with the ability to biodegrade and the demonstration of useful responses to stimuli, has seen hydrogels applied in the fields of drug delivery\textsuperscript{32} and scaffolds for coronary artery\textsuperscript{33}, bone\textsuperscript{34}, cartilage\textsuperscript{35} and muscle tissue engineering\textsuperscript{36}.

2.1.5 Double crosslinked microgels

pH-responsive microgels can form macroscopic gel networks at sufficiently high particle concentrations. This occurs when the polymer chains of neighbouring swollen microgel particle peripheries entangle, causing the formation of a physical gel\textsuperscript{37, 38}. Permanent covalent crosslinks can be introduced to gel networks, either through the polymerisation of pendant vinyl groups\textsuperscript{19, 37, 38} or through reactions between other functional groups\textsuperscript{39, 40}, resulting in ‘double-crosslinked’ microgels. Inter-particle covalent bonds endow double-crosslinked microgel networks with significantly greater mechanical properties than physical polymer gel networks.

A similar class of hydrogel can be obtained by polymerising one polymer network within another swollen polymer hydrogel or microgel network to form a ‘double network hydrogel’\textsuperscript{41, 42}. In this case the interpenetrating secondary polymer network adds mechanical strength to the lightly crosslinked and ductile original swollen polymer network\textsuperscript{43}. Both double-crosslinked and double network microgels and hydrogels could prove to be highly useful in biomedical restorative load bearing and low friction applications such as the restoration of dehydrated or damaged inter-vertebral discs (IVDs)\textsuperscript{26}, or as artificial cartilage\textsuperscript{41}, due to their superior
mechanical properties in comparison to singly crosslinked hydrogels, high degrees of hydration and biocompatibility.

2.2 Stability of colloidal dispersions

2.2.1 Colloidal stability and DLVO theory

Colloids are defined as materials that have at least one dimension in the size range of 1 nm - 10 μm. Within this classification falls a range of materials including emulsions such as milk, or paint, and microgel particles. Colloidal materials are characterised by their large surface area to volume ratios and dispersed, non-aggregating state. DLVO theory, pioneered by Derjaguin, Landau, Verwey and Overbeek, is used to explain the stability of colloidal dispersions. This theory can be applied to particle-particle and particle-surface interactions and reasons that the tendency of systems to aggregate is determined by the balance of the attractive and repulsive forces that occur between particles (or particles and surfaces). The strength of these interactions varies with the separation distance between the two species.

Dispersed particles are constantly moving in Brownian motion. As two particles are brought into close proximity two opposing interactions occur: an attractive interaction \( V_{\text{att}} \), based on van der Waal’s forces, and a repulsive interaction \( V_{\text{rep}} \), based on repulsive electrostatic interactions. The intensity of these interactions varies with separation distance and so the tendency of a system to aggregate is determined by the position of the sum of the interactions \( V_{\text{tot}} \) at a given distance.

Calculation of the attractive and repulsive forces can be modelled using equations. The attractive van der Waal’s interactions between two spherical particles can approximated using the following equation derived by Hamaker.

\[
V_{\text{att}}(h) = -\frac{A(r_1r_2)}{6h(r_1+r_2)}
\]  (2.5)

In this equation, \( r_1 \) and \( r_2 \) is the particle radii, \( h \) is the particle separation distance and \( A \) is the Hamaker constant. If the particles are of equal radius, the equation can be simplified further.
The Hamaker constant is a material specific property that gives a measure of its polarisability and the strength of its van der Waals forces. Swollen microgel particles contain a large volume fraction of water and so there is a lower chemical difference between the swollen particles and the dispersive phase. This reduces the scale of the attractive interactions between microgel particles, which contributes to their colloidal stability.

Colloidal particles dispersed in an aqueous environment develop surface charges due to mechanisms including the preferential adsorption of ions from the solution and the ionisation of surface functional groups. A surrounding electrical double layer is formed around the particles in order to balance the net surface charge. Repulsive interactions occur between colloidal particles as a result of the overlapping of the electrical double layers of particles in close proximity. The electrical double layer consists of two regions: a compact inner layer, the Stern layer, and a diffuse layer. In the Stern layer counter-ions are effectively bound to the particle surface. The thickness of the Stern layer is approximately one ionic radius. Counter-ions have mobility in the diffuse layer, which comprises two parts. The boundary between ions in the diffuse layer that move with the particle surface and those that do not is referred to as the shear plane, or slipping plane. The configuration of the electrical double layer is illustrated in Figure 2.3.
Figure 2.3: Representation of the electrical layer formed around a spherical particle in an aqueous dispersion, featuring the positions of the Stern layer, shear plane and diffuse layer.

Electrical potential decreases away from the particle surface approximately linearly through the Stern layer and exponentially in the diffuse layer. The zeta potential ($\zeta$) is the electrical potential at the shear plane. At the outer boundary of the diffuse layer the electrical potential is equal to zero. Therefore the electrical double layer is measured from the particle surface to the boundary of the diffuse layer with the ‘bulk’ solution.

The electrostatic repulsion between two identical spherical particles with overlapping electrical double layers can be modelled using the following equations\(^{45, 47, 52, 53}\).

$$\kappa = \left( \frac{2e^2z^2c}{\varepsilon_0\varepsilon k_B T} \right)^{1/2} \quad (2.7)^{47}$$

$\kappa$ represents the Debye-Hückel parameter, $c$ is the bulk ionic concentration, $z$ is the ionic valence, $e$ is the charge of an electron, $\varepsilon$ is the dielectric constant of the
dispersive phase, \( \varepsilon_0 \) is the permittivity of vacuum, \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature.

\[
\gamma = \frac{\left( \exp\left(\frac{ze\phi_S}{2k_BT}\right) + 1 \right)}{\left( \exp\left(\frac{ze\phi_S}{2k_BT}\right) - 1 \right)}
\]

(2.8)

\( \gamma \) is the reduced surface potential, \( \phi_S \) is the Stern layer potential.

\[
V_{\text{rep}}(h) = \left[ \frac{64\pi r c k_B T \gamma^2}{\kappa^2} \right] \exp\left( \frac{e^{-\kappa h}}{\kappa} \right)
\]

(2.9)

\( V_{\text{rep}}(h) \) is the repulsive interaction energy of the electrical double layer at separation distance \( h \).

The total interaction energy (\( V_{\text{tot}} \)) is the sum of the attractive and repulsive interactions and determines therefore whether the system tends towards stability or aggregation at a given separation distance.

\[
V_{\text{tot}}(h) = V_{\text{att}}(h) + V_{\text{rep}}(h)
\]

(2.10)

Figure 2.4 plots the interaction energies for a colloidal system using equations 2.5 – 2.10.

Figure 2.4: Variation of attractive (\( V_{\text{att}} \)), repulsive (\( V_{\text{rep}} \)) and total (\( V_{\text{tot}} \)) interactive energy with particle separation distance, plotted using equations 2.5 – 2.10. Particle radius = 150 nm, Hamaker constant = 1 \( \times \) 10\(^{-20} \) J, Stern layer potential = 4 mV, ionic concentration = 10 mM and temperature = 298K, dispersive phase is water. a) Separation distance = 0 – 20 nm, b) separation distance = 0 – 50 nm.
A primary minimum is displayed at very short particle separation distances in Figure 2.4a). In this region, the van der Waal’s attractive interactions are stronger than the electrical double layer repulsive interactions and particles will become aggregated. However, the double layer repulsive interactions provide an energy barrier to aggregation that must be overcome as particles move into closer proximity for permanent aggregation to occur. This feature aids the stability of colloidal dispersions. The repulsive interactions are dominant at close range, up to approximately 1 nm in the system shown in Figure 2.4, but are only significant when the double layers of particles overlap. The repulsive interactions rapidly diminish in intensity to zero at larger particle separations. Figure 2.4 b) shows at separations larger than 20 nm the double layer repulsive interactions become non-existent. Another notable feature of Figure 2.4b) is the secondary minimum of the total interaction energy curve indicated at a separation distance of approximately 16 nm. In this region the van der Waal’s attractive interactions are dominant and particles may become flocculated, but will not be irreversibly aggregated.

2.2.2 Stabilisation of colloidal particles

Two primary strategies are employed to improve the stability of colloidal dispersions: steric and electrostatic stabilisation. Both of these techniques are reflected in the interaction energy diagram of Figure 2.4. In the case of steric stabilisation, polymer chains are incorporated into particle surfaces, extending out from the surfaces, which prevent particles from coming into close proximity with one another. This reduces the intensity of short range attractive interactions and the likelihood of particle flocculation or aggregation. An example of the purely steric stabilisation of polystyrene latex particles was demonstrated by Weiss et al. and involved the poly(ethylene oxide) based surfactants Tween 60 and Tween 80. In the swollen microgel state, the polymer chains that comprise the particle are extended and provide steric stabilisation. This was illustrated in the structure of the swollen microgel particle depicted in Figure 2.1.

Electrostatic stabilisation involves the presence of ionic species at particle surfaces. These charged groups repel each other when particles come into proximity, thus ensuring that repulsive electrostatic interactions are dominant over
attractive interactions. The adsorption of cationic polyelectrolytes onto polystyrene latex particles was investigated by Fuchs and Killman. Ionic surfactants and residual initiator fragments in the particle peripheries also contribute to electrostatic stabilisation. At high ionic strengths electrostatic stabilisation can be screened, leading to particle flocculation and aggregation. Additional steric stabilisation contributions are necessary under these conditions. In microgel particles, both steric and electrostatic contributions to colloidal stability are often present. This is termed electrosteric stabilisation. Swollen microgel particles are also highly stable because they contain a large volume fraction of solvent, therefore the difference in effective Hamaker constant between the swollen particle and the solvent is low and so are the attractive van der Waals forces between the particles.

### 2.3 Microgel preparation methods

Methods of preparing polymer particles include emulsion, suspension, precipitation and dispersion polymerisation. Classification of these methods is based on the solubility of the components of the polymerisation mixture, polymerisation kinetics, the mechanism of particle formation and the size range of the polymer particles produced by the process. The most significant difference between emulsion and suspension polymerisation is in the size of the particles produced by each method. Emulsion polymerisation produces colloidal particles less than 1 μm in diameter in size, while suspension polymerisation produces particles much greater than 1 μm.

#### 2.3.1 Emulsion polymerisation

Emulsion polymerisation is a widely used method for the preparation of polymer latexes and microgel particles. It produces uniform particles in a size range of 50 - 1000 nm. Latexes are defined as “colloidal dispersions of polymer particles in an aqueous medium” and have been used in applications varying from paints and coatings to adhesives and drug delivery systems. The basic procedure of emulsion polymerisation is that a monomer or monomer mixture is polymerised in an aqueous solution, with the latex product formed via a free chain polymerisation mechanism that is initiated by a free radical species.

There are four key components to the emulsion polymerisation process:
monomer(s), water, surfactant and initiator. However, surfactant-free emulsion polymerisation is also practised and chain transfer agents are often employed in order to control the molar mass of the polymer particles\textsuperscript{58}. In the case of surfactant-free emulsion polymerisation, colloidal stability is assisted by the presence of charged initiator fragments at the particle surface\textsuperscript{8}. Water acts as the continuous phase for the reaction and provides a medium for the transfer of monomer from droplets to particles, whilst also facilitating heat transfer and maintaining a low viscosity\textsuperscript{58}. Surfactants are amphiphilic molecules that contain polar and non-polar regions. They are typically characterised as having polar ‘head groups’ and non-polar ‘tails’. The role of a surfactant is to assist colloidal stability by adsorbing at the particle-water interface, thus lowering the free energy of the system. Surfactants also provide particle nucleation sites through the initial formation of monomer-swollen micelles\textsuperscript{58}. Initiators degrade to form the free radical species that trigger the polymerisation process.

**2.3.2 Process of emulsion polymerisation**

The sequence of events that occur to produce polymer particles during emulsion polymerisation is as follows. Upon the combination of water, surfactant and monomer, a large number of surfactant micelles form (5-10 nm in diameter; $10^{19} - 10^{21}$ dm\(^{-3}\) in number) due to stirring action\textsuperscript{59}. These micelles are swollen through the absorption of monomer. The surfactant molecules are arranged with hydrophilic heads at the interface of micelle surface with water and hydrophobic tails extending into the hydrophobic micelle cores. The majority of the monomer exists in monomer droplets coated with adsorbed surfactant. These monomer droplets are significantly larger in size (1-10 μm in diameter), but fewer in number ($10^{12} - 10^{14}$ dm\(^{-3}\)) than the surfactant micelles\textsuperscript{59}. Upon the addition of initiator, free radical species form in the aqueous phase and react with dissolved monomer molecules (representing a small fraction of the total monomer). This generates oligoradicals\textsuperscript{58}.

At this point particle nucleation begins. This can occur in three ways; through micellar, homogenous, or droplet based nucleation. Micellar nucleation occurs when initiator radicals or oligoradicals, formed due to the interaction of
initiator radicals with dissolved monomer in the aqueous phase, diffuse into the surfactant micelles and initiate polymerisation of the absorbed monomer\textsuperscript{58}. This generates propagating polymer particles. In homogenous nucleation, oligoradicals are formed in the aqueous phase and propagate via the addition of monomer molecules until they become insoluble in water and precipitate into primary polymer particles\textsuperscript{58}. These particles adsorb surfactant and absorb monomer, allowing particle growth to continue whilst simultaneously maintaining colloidal stability. Droplet nucleation occurs when radicals and oligoradicals diffuse nucleate polymer particles by diffusing from the aqueous phase directly into monomer droplets, whereby the propagation of polymer particles occurs from within the monomer droplet\textsuperscript{58}.

Once polymer particles have been nucleated they become swollen with monomer, which diffuses out of the large monomer droplets and through the aqueous phase. Polymerisation of this monomer, and particle growth, continue until all of the surfactant micelles and monomer droplets have been consumed\textsuperscript{58}. As the nucleated polymer particles grow, surfactant is transferred from the surface of monomer droplets, which shrink as polymerisation continues, to adsorb onto the surfaces of the growing polymer particles\textsuperscript{58}. The surfactant of any uninitiated micelles also adsorb onto growing particles. This maintains the colloidal stability of the nascent polymer particles. The formation and growth of polymer particles via micellar nucleation is illustrated in Figure 2.5\textsuperscript{59}.

The processes of emulsion polymerisation can be categorised into three intervals. Interval I encompasses the events that occur up to and including the initial formation of polymer particles. By the end of Interval I all of the uninitiated micelles have been consumed and the number density of growing polymer particles is constant. During Interval II the growth rate of the particles (i.e. rate of polymerisation) remains constant as the growing particles are adequately supplied with monomer to continue their propagation\textsuperscript{58}. The disappearance of the monomer droplets signifies the start of Interval III, in which the rate of polymerisation decreases. The polymer particles can continue to undergo polymerisation during this stage until all of the absorbed monomer is consumed\textsuperscript{58}.
Variations of emulsion polymerisation include miniemulsion and inverse emulsion polymerisation. In miniemulsion polymerisation particle nucleation and growth take place exclusively within monomer droplets, while inverse emulsion polymerisation features an oil-in-water polymerisation rather than a water-in-oil polymerisation\textsuperscript{58}. Alternative, and similar, polymerisation methods used in preparing colloidal polymer particles are suspension, dispersion and precipitation polymerisation\textsuperscript{57, 60}. Four key criteria are used to distinguish between these polymerisation methods: “the initial state of the polymerisation mixture, the polymerisation kinetics, the mechanism of particle formation and the shape and size of the polymer particles produced”\textsuperscript{57}. Emulsion and suspension polymerisation are distinguished by the size of the particles that are prepared by each method. Dispersions featuring particles smaller than 1 μm are referred to as emulsions, while those containing particles greater than 1 μm in size are termed suspensions\textsuperscript{57}. 

\textbf{Figure 2.5: “A schematic of micellar nucleation”}. Reproduced from reference\textsuperscript{59}. 
2.3.3 Free-radical polymerisation

Emulsion polymerisations proceed by a free-radical polymerisation mechanism, which is a form of chain polymerisation in which chain growth occurs through the addition of monomer units onto the terminal free radical ‘active centres’ of the polymer chains\(^6\). Free-radical polymerisation is also the mechanism by which solution, dispersion, and precipitation polymerisation occur. The mechanism comprises three distinct stages: initiation, propagation and termination.

i) Initiation

The initiation stage is characterised by the formation of free radical species from an initiator and the subsequent addition of reactive free radicals to monomer molecules to form active centres\(^6\). Free radicals can be created from the initiator either by the homolytic scission of a single or double bond, or through single electron transfer to, or from, the molecule\(^6\). The thermolysis of the \(\alpha,\alpha’\)-azodisobutyramidine dihydrochloride (AIBA) initiator is depicted in Figure 2.6. Other initiators commonly used in emulsion polymerisation include benzoyl peroxide and azobisisobutyronitrile (AIBN), which undergo homolysis, and ammonium persulphate, which can create free radicals via a thermolysis reaction or a redox reaction\(^6\).

![Figure 2.6: Thermolysis of AIBA initiator resulting in the formation of free radical fragments.](image)

Following initiator degradation, active centres are generated on monomer units when initiator fragments containing free radicals attack the \(\pi\)-bond of the monomer molecule\(^6\).
ii) Propagation

During the propagation stage polymer chains grow rapidly through the sequential addition of monomer molecules to the terminal active centres of the polymer chains.

The propagation process is extremely rapid and occurs at the rate of approximately one monomer unit addition per millisecond. A terminal active centre remains present on each polymer chain following the addition of a monomer molecule.

iii) Termination

The termination of polymer chain growth involves a reaction between two growing polymer chains. There are two methods of termination: combination and disproportionation. Termination by combination occurs when two growing polymer chains react together to form a single chain.
In the disproportionation termination route, a hydrogen atom is abstracted from one growing polymer chain to another\textsuperscript{61}. It differs from combination in that two polymer molecules are generated, one saturated and one unsaturated, with initiator fragments at one end only. Following the combination route one saturated chain is formed with initiator fragments present at both chain ends\textsuperscript{61}.

\textbf{Figure 2.9: Termination by combination.} Reproduced from reference\textsuperscript{61}.

\textbf{Figure 2.10: Termination by disproportionation.} Reproduced from reference\textsuperscript{61}.
2.4 Click chemistry

2.4.1 Introduction to click chemistry

Click chemistry reactions are a group of highly reliable reactions that result in the formation of carbon-heteroatom bonds (C-X-C)\(^6\). Since the inception of the click chemistry strategy, the reactions have been used in an array of applications including extensively in polymer modification\(^6\).\(^3\), \(^4\). The click chemistry strategy was pioneered by Kolb, Finn and Sharpless and intended to aid the rapid discovery of new molecules with specific desirable functionalities\(^6\).\(^2\). The ethos of this work was a move away from carbonyl-based reactions to carbon-heteroatoms\(^6\).\(^5\).\(^2\). The click chemistry groups of reactions rely on energetic “spring-loaded” reactants and are specific and high yielding\(^5\).\(^2\).

The click chemistry philosophy outlined by Sharpless et al. aimed to provide a template for the rapid and straightforward discovery of useful molecules, particularly drug molecules that can interact with larger biological systems\(^6\).\(^2\). The criteria for a true click chemistry reaction is one that is “modular, gives very high yields, is stereospecific, creates only inoffensive by-products, is easy to perform and requires only a benign or easily removable solvent”\(^5\).\(^2\). Of these reactions, the copper (I) catalysed Huisgen 1,3-dipolar cycloaddition (CuAAC) reaction between alkynes and terminal azides has become synonymous with click chemistry due to the near perfect conversion of reactants, the rapid reaction rate under copper catalysis, the facile synthesis of alkyne and azide species and their tolerance to a range of functionalities and reaction conditions including temperature and pH\(^5\).\(^5\).

In addition to a significant increase in reaction rate, copper catalysis also ensures an exclusive formation of the 1,4-triazole species, rather than the mixture of 1,4- and 1,5-triazole stereoisomers that occurs when the reaction is thermally induced\(^6\). This is an important result given that many click reactions aim to produce new drug molecules. Products of the CuAAC reaction have a wide range of applications in medicinal chemistry, molecular biology and materials science\(^3\), \(^4\). Although initially conceived as a pathway to rapid drug discovery, click chemistry has also found significant applications in the field of polymer chemistry\(^3\), \(^4\).
2.4.2 Reactants, catalysts and ligands of the CuAAC reaction

There are five components of the reaction to consider: the terminal alkyne and azide reactants, a copper catalyst, a ligand and the solvent for the reaction. The reaction is extremely versatile and a broad range of catalysts, ligands and solvents are suitable. A simple overview of the CuAAC reaction, featuring the terminal azide and alkyne functionalities and the 1,4 triazole reaction product, is displayed in Figure 2.11.

Figure 2.11: An overview of the copper catalysed azide alkyne dipolar cycloaddition reaction (CuAAC) between terminal alkyne and azide species that results in the formation of a 1,4 triazole product.

The alkyne, or acetylene, functional group consists of a two carbon atoms linked by a triple bond, while the azide species is the N$_3$ group with structure shown above in Figure 2.11. A wide range of molecules with terminal azide and alkyne groups can be synthesised and the reaction is frequently used to introduce specific functionalities to molecules, particles and surfaces due to its highly specificity and efficiency.$^6$

Most commonly, Cu(I) salts are used to catalyse the reaction, either directly (for example Cu(I)Br or Cu(I)I) or through the reduction of a Cu(II) salt (such as Cu(II)SO$_4$ in combination with sodium ascorbate)$^{66}$. Although the reaction is usually referred to as copper catalysed, other transition metal catalysts are effective and include ruthenium, nickel, platinum and palladium species$^{66, 67}$. The reaction can be performed without the use of a ligand, however the use of one protects the Cu(I) ion from being oxidised$^{68}$ and accelerates the rate of reaction$^{69}$. Polydentate amine ligands are most commonly used$^{68}$. Matyjaszewski et al. studied the effects of ligands on the copper catalysed ATRP reaction and surmised that tridentate amine ligands, such as $N,N,N',N',N''$-pentamethyldiethylenetriamine (PMDETA), provide a
significantly faster reaction rate the tetradentate amines\textsuperscript{67, 68}. The versatility of the CuAAC reaction is reflected by its tolerance to a wide range of solvents. These include hexane, toluene, alcoholic solvents, halogenated solvents, DMF, DMSO, THF and diethyl ether\textsuperscript{66}.

\subsection*{2.4.3 Proposed mechanism of the CuAAC reaction}

Mechanisms for the CuAAC reaction based on reaction kinetics and computational studies have been proposed, although the exact natures of intermediary complexes are yet to be fully determined\textsuperscript{63, 65, 68}. Initially, the proposed mechanisms for the stepwise cycloaddition featured a mononuclear copper intermediary complex\textsuperscript{63, 65}. However, further examination and evidence has deduced that a dinuclear copper intermediary complex, or even polynuclear complexes\textsuperscript{70}, to be more likely and represent a better explanation of the dramatic rate acceleration demonstrated if the reaction is copper catalysed\textsuperscript{65, 71, 72}. A proposed dinuclear copper intermediary mechanism is displayed in Figure 2.12\textsuperscript{72}.

![Diagram](image_url)

\textbf{Figure 2.12: Proposed catalytic mechanism for the copper catalysed azide-alkyne cycloaddition reaction featuring two copper atoms.} Reproduced from reference\textsuperscript{72}.

Although the proposed mechanism has progressed over time, the key concepts have remained fairly constant. The first step of the cycle involves the formation of a
copper-alkyne intermediate through the π coordination of the alkyne species with a copper atom\textsuperscript{63, 65}. This lowers the pK\textsubscript{a} of the intermediate sufficiently for the terminal alkyne proton to be deprotonated in an aqueous medium without the presence of an additional base and results in a σ-alkynyl-Cu\textsuperscript{i} intermediate (a copper(I) acetylide)\textsuperscript{68, 71}. This is depicted in Step A of the cycle in Figure 2.12. The product of this step features one copper atom (a) σ-bound to the complex and a second copper atom (b) bound through weaker π-interactions\textsuperscript{72}.

The next stage in the cycle (Step B) involves the activation of the azide species via coordination with the Cu(I) acetylide species in a ligand exchange sequence\textsuperscript{63, 72}. This is followed by the key bond forming step in the cycle, the generation of a copper metallacycle (Step C)\textsuperscript{63}. This formation of a copper intermediate considerably lowers the activation energy for this bond forming reaction compared to the uncatalysed reaction\textsuperscript{63}. Finally, triazole ring contraction and protonation in Steps D and E respectively complete the catalysed cycloaddition\textsuperscript{63}.

The formation of an initial copper(I) acetylide intermediate and an overall stepwise cycloaddition have been agreed upon\textsuperscript{63, 65, 73}. However, highly similar but alternative precise structures and interactions of the copper metallacycle intermediates have been proposed\textsuperscript{65, 70, 71, 73}. Regardless of these differences, it has been concluded that the presence of a second copper centre in a dinuclear metallacycle results in the increased stabilisation and higher reactivity of the intermediate\textsuperscript{70, 71}. This also supports the second order reaction rate observed for the copper catalysed reaction\textsuperscript{70, 71}.

### 2.4.4 Polymer based applications of the CuAAC reaction

Click chemistry reactions have been used to functionalise polymeric and other materials in a number of ways. Nanoparticle modification by click chemistry is a popular area of research\textsuperscript{74-78} and enzyme responsive nanoparticles, among others, have been prepared in this way. Wesler et al. incorporated azide and alkyne bearing monomers into polymeric nanoparticles using an inverse microemulsion polymerisation method\textsuperscript{76}. The alkyne-functionalisation nanoparticles were ‘clicked’ with a pH sensitive azide-functionalised peptide fluorophore, yielding pH
responsive nanoparticles sensors, while the azide-functionalised nanoparticles were clicked a fluorogenic peptide substrate that was sensitive to the action of the protease subtilisin. This manufactured enzyme-responsive nanosensor particles. Proteolytic cleavage of the peptide substrate by subtilisin ended the quenching of the fluorophore component of the nanoparticle and resulted in fluorescent emission. This system could be adapted with other ‘clickable’ substrates for use in diagnostic systems.

Kar et al. demonstrated that the CuAAC reaction could be used to attach a high density of polypeptide chains onto the surface of silica nanoparticles using a polymer grafting technique. In this study, the click reaction was of particular use in greatly improving the graft density of polypeptide chains in comparison to solely grafting based methods. The attachment of poly-L-lycine and poly-L-leucine chains to the silica nanoparticles garnered them with strong antimicrobial properties against both gram-positive and gram-negative bacteria. This methodology may be useful in developing bactericidal coatings and antimicrobial paints.

Lu et al. reported a method of functionalising polymeric nanoparticles with the RGD peptide motif, an important amino acid sequence in cell attachment, using the CuAAC reaction. In this study an amphiphilic co-polymer was prepared by coupling hydrophobic poly(2-methyl-2-carboxytrimethylene carbonate-co-L-lactide) backbones and hydrophilic poly(ethylene glycol) azide-modified chains, which self assembled into nanoparticles in solution with azide groups presented at the particle surfaces. Alkyne-modified peptides containing the RGD sequence were clicked onto the particles, which then effectively attached to rabbit corneal cells. This work may be useful in the delivery of drug molecules to damaged corneas, which are difficult to treat due to the secretion of tears and the low permeability of the epithelial layer.

The CuAAC reaction has also been applied to microgel particles. Meng et al. reported the of azide and alkyne bearing poly(NIPAm) based microgels via a one-pot preparation method. Azide and alkyne functionality was achieved by the azidation of the co-monomer acrylic acid or the incorporation of the acetylene monomer propargyl acrylate for the respective species. A successful click chemistry
reaction was demonstrated by the attachment of complementary fluorophores to the microgels\textsuperscript{60}.

Aside from colloidal particles, click chemistry has also been used to create novel polymer architectures such as star and dendritic polymers, micelles and multifunctional surfaces\textsuperscript{81, 82}. Malkoch et al. synthesised hydrogel networks with impressive mechanical properties by introducing crosslinking between azide and alkyne poly(ethylene glycol) derivatives\textsuperscript{39}.

The 1,2,3-triazole product of the CuAAC reaction is well suited to bioconjugation techniques due to its stability under biological conditions and high aqueous solubility\textsuperscript{64}. This has seen CuAAC applied in the labelling and modification of biomolecules including DNA\textsuperscript{83}, polysaccharides\textsuperscript{84}, viruses\textsuperscript{85} and glycoproteins\textsuperscript{64, 86}. It is worth noting that for in vivo techniques, copper catalysed click chemistry is avoided in favour of strain promoted cycloaddition due to the toxic effects of copper on biological systems\textsuperscript{86, 87}. Adequate copper removal is an important challenge to overcome for those products of the CuAAC reaction intended towards use in biomedical applications. The issue has proved to be problematic in developing hydrogels for biomedical applications, despite the high reaction efficiency offered by the method\textsuperscript{64}. 
2.5 Characterisation methods

Over the course of this project a number of characterisation techniques were employed in order to accurately determine the compositions, properties and morphologies of the microgels prepared and investigated. The fundamental principles of these methods are outlined here.

2.5.1 Photon Correlation Spectroscopy (PCS)

PCS is a form of dynamic light scattering (DLS) used to measure the average size and size distribution of particles in a dilute dispersion. A simplified schematic of the measurement set-up is displayed in Figure 2.13.

![Figure 2.13: Simplified schematic of a PCS sample measurement, featuring the incident laser source, scattered laser beam and scattering angle θ.]

Particles in suspension are constantly moving in Brownian motion, which causes local fluctuations in particle concentration and differences in light scattering. PCS detects fluctuations in the intensity of the scattered light from a laser source, by the particles over time, and thus determines how quickly the particles are moving in suspension. The correlation function $G(\tau)$ relates the change in the intensity of scattered light over a time period $\tau$.

$$G(\tau) = e^{\frac{-\tau}{Dq^2}}$$  \hspace{1cm} (2.11)
D represents the diffusion coefficient, \( q \) is the scattering vector (a function of the scattering angle \( \theta \)), \( n \) is the refractive index of the liquid and \( \lambda_0 \) is the incident wavelength.

\[
q = \frac{4\pi n}{\lambda_0} \sin \left( \frac{\theta}{2} \right)
\]  

(2.12)

In this way, PCS does not directly measure the average particle size. Instead, the diffusion coefficient is determined and related to particle size through the Stokes-Einstein equation, as the speed at which the particles move is inversely proportional to their size.

\[
D = \frac{kT}{3\pi \eta d_h}
\]  

(2.13)

In equation 2.13, \( k \) represents the Boltzmann constant, \( T \) is the temperature, \( \eta \) is the viscosity of the suspension liquid, and \( d_h \) is the hydrodynamic particle diameter.

PCS can be used to measure particles ranging from approximately 5 nm to 3 \( \mu \)m in size, although with decreasing accuracy for particle sizes larger than 1 \( \mu \)m, and requires only a short measurement time and minimal sample preparation\(^8^9\). Average particle diameters calculated by PCS apply to spherical, non-interacting particles\(^8^9\). The particles studied in this thesis range from approximately 100 – 1300 nm in size and are sterically stabilised to avoid aggregation. In this way, the criteria for accurate particle size measurement using PCS are met. The particle diameter measured by PCS is the hydrodynamic diameter, which represents the diameter of the effective sphere that displaces liquid as it moves through the dispersion.

2.5.2 Zeta potential measurement

The zeta potential is a measure of the electrostatic potential that resides in the electrical double layer surrounding the surfaces of particles in an aqueous dispersion\(^4^9\). As described previously, the electrical double layer balances the surface charge of dispersed particles and consists of two regions: an inner Stern layer where oppositely charged counter ions are effectively bound to the surface of the material in dispersion and an outer diffuse layer in which ions are associated with the surface but have mobility\(^4^9, 5^0\). Electrostatic potential cannot be measured
directly at the particle surface, but can be measured at the interface between the Stern layer and the diffuse layer of the electrical double layer. This boundary is referred to as the slipping plane, or shear plane\textsuperscript{50, 51}. This value is obtained by zeta potential measurements and is a property of the surface charge of the particle\textsuperscript{49}.

The measurement instrument uses electrodes to apply an electric field to the sample. Particles move in response by electrophoresis and this movement is measured by laser Doppler velocimetry\textsuperscript{49}. Particles with negatively charged surfaces will migrate towards the anode and vice versa\textsuperscript{50}. The zeta potential (\(\zeta\)) can then be determined using the Henry equation, which relates the electrophoretic mobility (\(\nu_c\)), the dielectric constant (\(\varepsilon\)), the absolute zero-shear viscosity of the dispersion (\(\eta\)), the Henry function (\(f_\kappa\alpha\)) and the ratio of the particle radius to the Debye length (\(\kappa\alpha\))\textsuperscript{49}. The Henry function is either taken as 3/2 according to the Schmoluchowski approximation, or 1 using the Huckel approximation\textsuperscript{91}.

\[
\nu_c = \frac{2\varepsilon \zeta f(\kappa\alpha)}{3\eta} \quad (2.14)
\]

Microgel particles in dispersion may be viewed using Ohshima’s electrokinetic model as being covered by an ion-penetrable polyelectrolyte layer\textsuperscript{92-94}. This model refers to latex particles with a core-shell structure and, when applied to microgel structures, implies a conformation consisting of a relatively densely crosslinked, ion-impenetrable core and a lightly crosslinked polyelectrolyte surface layer that contains a higher proportion of ionic groups\textsuperscript{94}. This model differentiates microgels as ‘soft’ particles as opposed to hard polymer spheres and has been demonstrated to fit the temperature-responsive electrophoretic mobility behaviour of poly(NIPAm) microgel particles\textsuperscript{94}.

Zeta potential values are dependent upon the ionic strength, pH and temperature of the aqueous medium\textsuperscript{50}. Values greater than 30 mV or less than -30 mV are indicative of strong cationic and anionic behaviour respectively, while values between -10 and 10 mV are considered to be neutral\textsuperscript{49} and can lead to flocculation or aggregation if appropriate stabilisation measures are not taken. Particles with strongly ionic surfaces are less likely to flocculate or aggregate.
according to DLVO theory and are therefore considered advantageous towards colloidal stability.

2.5.3 Fourier Transform Infra Red (FTIR) spectroscopy

FTIR spectroscopy is a form of vibrational spectroscopy used to characterise materials by identifying the types of bonds present in the material. Many functional groups have specific absorption frequencies within the infra-red range of the electromagnetic spectrum. When an oscillating electromagnetic field is applied it causes changes in the vibrational states of atoms within the molecule at these absorption frequencies. These changes in vibrational state are also referred to as bond deformations. They occur when the vibrational frequency of the chemical bond is equal to the frequency of the applied electromagnetic field\(^{61}\). Planck’s equation defines this frequency as \(\nu = \Delta E/h\), where \(\Delta E\) is the difference in energy between the upper and lower vibrational energy levels of the bond deformation and \(h\) is Planck’s constant\(^{61}\). Types of bond deformations include stretching, bending, scissoring, rocking and wagging\(^{95}\).

Applying oscillating infra-red radiation across a frequency range and measuring the absorptions that occur at different frequencies creates a spectrum that shows which bonds and functional groups are present within the sample. Each absorption peak within the spectrum corresponds to a specific bond deformation, allowing analysis of the molecular structure of the sample. FTIR spectra are typically reported in terms of absorptions across a wavelength spectrum.

The azide and alkyne functional groups involved in the CuAAC reaction have specific absorption frequencies. The azide species produces a very sharp and strong absorption peak at approximately 2100 cm\(^{-1}\)\(^{96}\), while terminal alkynes produce a broader, less intense peak at approximately 3300 cm\(^{-1}\)\(^{97}\). The precise position of these peaks depends upon the surrounding molecular environment. The presence of these peaks in reactants, and their disappearance following consumption of the azide and alkyne species during the CuAAC reaction, are important markers for the success of the CuAAC reaction. This makes FTIR spectroscopy an important analysis method for the work undertaken in this thesis.
2.5.4 Nuclear Magnetic Resonance (NMR) spectroscopy

In the nuclei of many atoms, magnetic moments are generated by the spin of the positive charge around the nuclear axis\(^{61}\). Magnetic moments are the product of the angular momentum of the proton and the magnetogyric ratio, which is characteristic to the specific atomic nucleus, and are either positive or negative depending on the direction of the nuclear rotation\(^{98}\). Nuclei that contain magnetic moments also have nuclear spin quantum numbers (I) that are either integral or half-integral\(^{61}\). The spin number of the proton (\(^{1}\)H) is ½ and can only exist in the spin state of ½ or -½. When an external magnetic field is applied, the nuclear spins can be aligned in 2I + 1 orientations, with each orientation pertaining to a specific energy\(^{61}\).

In NMR spectroscopy, the externally applied magnetic field promotes nuclei from low energy to high energy spin states. This is illustrated in Figure 2.14, which shows the two possible spin states, or eigenstates, for atomic nuclei where I = ½.

![Figure 2.14: The orientation of energy spin states before and after the application of an external magnetic field (B\(_0\)). \(\Delta E\) is the difference in energy between and high (excited) and low energy (ground) spin states.](image)

The value of \(\Delta E\) is a function of the magnetogyric ratio (\(\gamma\)), the strength of the external magnetic field (\(B_0\)) and Plank’s constant (\(h\)).

\[
\Delta E = \gamma B_0 h
\]  \hspace{1cm} (2.15)\(^{98}\)
The resonance frequency ($\nu$) of the radiation needed to flip the magnetic moment of a nucleus from being aligned against the external magnetic field at the nucleus (B) to being aligned with it is given by equation 2.16$^{61}$.

$$\nu = \gamma B / 2\pi$$  \hspace{1cm} (2.16)$^{61}$

The electronic environment of an atomic nucleus within a molecule causes the strength of the magnetic field at the nucleus (B) to be slightly different to the external strength of the field ($B_0$). The greatest contributor to this effect is that of electrons shielding the nucleus from the external field by creating a local magnetic field that opposes the external field$^{61}$. Chemical groups that donate electrons to the nucleus increase this shielding effect, while groups that withdraw electrons, such as any group containing $\pi$-bonds, de-shield the nucleus$^{61}$. This means that the local electronic environment causes differences in the strength of the magnetic field at the nucleus, therefore nuclei will absorb radiation at specific frequencies and can be identified. Identification occurs either by using a constant external magnetic field strength and measuring the absorption of radiation over a resonance frequency range, or by using a constant frequency and measuring the absorption of radiation as the external field strength is varied$^{61}$.

The absorptions of a sample are displayed in terms of chemical shifts compared to those of a reference standard. Tetramethysilane is most commonly used for this purpose as its 12 protons give a sharp absorbance signal that does not overlap with the absorbencies of most samples$^{61,98}$. The resonance frequency range of protons and atomic nuclei is in the radio waves region of the electromagnetic spectrum ($3.10^6$ – $3.10^8$ Hz); this is a longer wavelength compared to the infra red region in which FTIR spectroscopy operates$^{98}$. 
2.5.5 Scanning Electron Microscopy (SEM)

As conventional optical microscopy is limited to a magnification of approximately 1000x, electron microscopy is employed to provide the enhanced resolution and higher magnifications that are necessary for the examination of materials at the lower end of the colloidal size range. Magnifications up to 1,000,000x are possible using SEM and information on particle and surface topography and chemical composition can be obtained with relatively simple sample preparation. SEM works using a finely focused electron beam that scans the sample in a raster pattern. Electrons are emitted from the sample, and detected, and this signal is scanned onto a cathode ray tube in an identical pattern to the incident beam to display the image. Magnification is controlled by varying the area of the sample scanned by the incident electron beam; the smaller the area scanned the greater the magnification will be. The arrangement of the instrument is displayed in Figure 2.15.

![Figure 2.15: “Principle of the scanning electron microscope”](image)

The incident electron beam is generated from an electron gun, or tip, at the top of the microscope column. Three types of electron gun are commonly used: tungsten hairpin filaments, lanthanum hexaboride filaments and field emission guns.
(FEGs). The filaments are heated to over 2500 °C, causing the emission of electrons from their tips. Lanthanum hexaboride filaments offer a larger maximum beam current (and therefore a brighter beam) and longer lifetimes than tungsten filaments, but at greater expense. Field emission guns are not heated and instead utilise a very high electric field applied to a finely pointed tip to cause the quantum tunneling of electrons. This produces the brightest beam but requires a higher vacuum to maintain the tip. A vacuum is necessary in electron microscopes to prevent the electron beam from being knocked out of alignment by gas molecules.

Two or more sets of electromagnetic lenses in the microscope column focus the electron beam into a fine point of 0.1 – 10 nm in diameter, which then scans the sample. The first set are the condenser lenses and the second set are the objective lenses. Interaction of the beam with the sample causes the emission of secondary electrons, Auger electrons, backscattered electrons, low-loss electrons and x-rays. Secondary electrons are those that are knocked out of their orbits by the incident electron beam and are responsible for the majority of the spatial resolution as they can only escape from near the surface layer of the sample. Backscattered electrons approach are scattered from the nuclei of atoms and re-emerge from the sample. They are fewer in number than and provide less resolution than secondary electrons because they originate from deeper in the sample, but can relay information on sample composition as atoms of higher atomic number give brighter contrast. These emissions are detected and scanned onto the cathode ray tube to produce the images. Scanning electron microscopy is particularly useful when a wide range of magnification (e.g. for large samples) and depth of field (for rough surface features) are required as the the magnification can easily be adjusted without the need for significant refocusing.
2.5.6 Dynamic rheology

Rheology is the study of the deformation and flow of materials in response to the application of force\(^{103, 104}\). Dynamic rheology is used to give information on the mechanical properties of materials and their classification. Liquids and gases flow in response to applied stress and strain, whereas solids deform and can regain their original dimensions when the force is removed\(^{103}\). Elastic solids and viscous liquids operate under Hooke’s law (equation 2.17) and Newton’s law (equation 2.18) respectively.

\[
\sigma = \frac{F}{A} \tag{2.17}
\]

\[
\sigma = \eta \dot{\gamma} \tag{2.18}
\]

Hooke’s law defines shear stress (\(\sigma\)) in terms of the applied force (\(F\)) and the area of the sample (\(A\)). Newton’s law relates viscosity (\(\eta\)), a measure of a material’s resistance to flow, to shear stress (\(\sigma\)) and shear rate (\(\dot{\gamma}\)).

For Newtonian liquids, the viscosity is constant and independent of shear rate. However, in non-Newtonian liquids the viscosity is not constant and depends upon the shear rate or the time of shearing\(^{105}\). Non-Newtonian fluids can, for example, be shear thickening or shear thinning. Most materials display viscoelastic properties, meaning that they simultaneously display the behavioural responses of both elastic solids and viscous liquids to an extent\(^{105}\). Dispersions of polymer particles and polymer gels display viscoelastic properties and can be characterised using dynamic rheology\(^{106}\).

In dynamic rheology measurements an oscillating stress or strain is applied to the sample and its response is measured by the instrument. The components of the instrument are illustrated in Figure 2.16. The sample is placed between two parallel plates. The bottom surface is fixed but the top surface, part of the rheometer geometry, moves under torque which introduces an oscillatory shear stress or strain to the sample. A variety of geometries are employed to interact with different types of material.
Figure 2.16: Simplified schematic of a dynamic oscillatory rheometer featuring a parallel plate geometry.

When an oscillating stress is applied to a linear viscoelastic material, an oscillating strain develops in the material in response after an initial lag period. The response of the sample is measured by the rotation of the top plate of the rheometer. Oscillating rheometers operate frequency and strain sweeps. In a frequency sweep the strain applied to the sample is constant and the frequency is varied; in a strain sweep the frequency is constant and the strain is varied. An oscillatory strain develops in the sample in response to an applied oscillatory stress and vice versa. The following set of equations describes the operation of a strain sweep.

The applied strain and stress response oscillate with time and can therefore be depicted as two wave-forms. This is depicted in Figure 2.17. From this construction, the complex modulus ($G^*$) at a constant given radial frequency ($\omega = 2\pi f$) can be defined in terms of the maximum stress ($\sigma_0$) and the maximum strain ($\gamma_0$). 

$$G^*(\omega) = \frac{\sigma_0}{\gamma_0}$$ (2.19)

In an entirely elastic material there is no phase difference and the strain response is in phase with the applied stress. However, in viscoelastic materials the strain response is out of phase with the applied stress by a phase angle ($\delta$), which represents the phase difference in radians between the maximum values of the stress and strain waves. $G^*$ and $\delta$ are characteristic properties of a
The oscillatory stress ($\sigma(t)$) and oscillatory strain over time ($\gamma(t)$) can be defined by the following equations, where $i = \sqrt{-1}$.

\[
\sigma(t) = \sigma_0 \exp(i\gamma t) \quad (2.20)
\]
\[
\gamma(t) = \gamma_0 \exp(i\sigma t + \delta) \quad (2.21)
\]

Rearranging these equations leads to the determination of the storage modulus ($G'$) and the loss modulus ($G''$) of a material.

\[
G^* = G' + iG'' = \frac{\sigma_0}{\gamma_0} (\cos\delta + i\sin\delta) \quad (2.22)
\]
\[
G' = \left( \frac{\sigma_0}{\gamma_0} \right) \cos\delta \quad (2.23)
\]
\[
G'' = \left( \frac{\sigma_0}{\gamma_0} \right) \sin\delta \quad (2.24)
\]

Figure 2.17: The oscillatory strain response of a viscoelastic material to an applied oscillatory stress. The phase angle $\delta$ represents the lag time between the applied stress and the strain response.

The storage modulus ($G'$) represents the in-phase elastic solid response of a viscoelastic material, while the loss modulus ($G''$) represents the out-of-phase viscous liquid component. The storage modulus is therefore indicative of the mechanical energy that is stored and recovered per oscillation and the loss modulus.
is the energy that is dissipated, mainly as heat, as the sample flows\textsuperscript{106}. Another value that is used in the rheological characterisation of materials is that of $\tan(\delta)$, which is defined as the ratio of the loss modulus to the storage modulus.

$$\tan(\delta) = \frac{G''}{G'}$$

(2.25)

Values of $\tan(\delta)$ close to zero are associated with elastic solid behaviour, while a value close to one is indicative of highly viscous behaviour.
3. Primary amine functionalisation of microgel particles using the CuAAC reaction.

3.1 Introduction

Microgels are defined as colloidal polymer particles that swell in a good solvent\(^8\). They can also be induced to swell as a result of electrostatic repulsion caused by pH manipulation to produce oppositely charged functional groups within the network, or by an entropically driven process in response to a change in temperature\(^8\). In the case of pH-responsive microgels at least one co-monomer will become charged when the pH approaches the pK\(_a\) for that species. This results in swelling due to electrostatic repulsion between polymer chains\(^8\).

The synthesis of microgel particles containing high primary amine contents is an area of interest due to the versatile nature of primary amine reactions. Successful preparation would allow a wide range of particle functionalisation possibilities\(^23\). Of particular significance is the potential ability to manufacture peptide functionalised microgels by the grafting of peptides onto primary amine groups. Various options for the grafting of peptides onto primary amine functionalised materials have been discussed\(^107-112\). This could then allow enzymatic action to be used as the stimulus for particle swelling, which would be of particular use for drug delivery applications whereby an encapsulated payload can be released upon the action of a predetermined enzyme. In this case, enzymatic cleavage of the peptides leaves charged fragments exposed, which generates microgel swelling through electrostatic repulsion. The highly selective nature of enzymes mean that this approach could be of use in treating diseases including cancers and chronic wounds, which often express specific proteases\(^14\). This approach also potentially offers an improvement on current strategies by maximising the concentration of the medicinal agent at the target site, thus reducing the systemic concentration and the chance of negative side effects\(^112\). This could be a significant improvement on current cancer treatment strategies, where the systemic side effects of chemotherapy treatment are significant\(^112\).

Some success has been reported in the area of peptide functionalised microgels. McDonald et al. reported poly(ethylene glycol) acrylamide microparticles...
functionalised with branched peptide actuators that were triggered to swell upon the action of the enzymes thermolysin and chymotrypsin\textsuperscript{14}. Hydrolysis of the peptide actuators caused a change in their charge balance from zwitterionic to cationic and initiated particle swelling.

The formulation of peptide functionalised microgels that could deliver a significant swelling response upon enzymatic actions requires the prior preparation of a microgel with high primary amine contents capable of significant pH responsive behaviour. Attempts at synthesising colloidally stable primary amine microgels have proved challenging, particularly in polymerising the simplest amine monomer vinylamine\textsuperscript{23}. Xu et al. demonstrated a copolymerisation of $N$-vinylformamide (NVF) with styrene and $N$-isopropylacrylamide (NIPAm) by a semi-batch process. This prepared particles with shells rich in NVF, which were converted to yield primary amine groups by acid hydrolysis\textsuperscript{113}. The main limitation of this method was that the yields of NVF incorporation and amide hydrolysis were less than optimal. The maximum NVF concentration obtained in the poly(NIPAm-NVF) microgel was 10.8 mol\% and the yield of the hydrolysis reaction was approximately 39\%. This process resulted in a relatively low concentration of primary amine group present in the final microgel although the high concentration of NIPAm did confer the microgel with temperature-responsive properties\textsuperscript{113}.

Thaiboonrod et al. used a two-step process involving the non-aqueous dispersion polymerisation of poly($N$-vinyformamide-co-2-$($N$-vinylformamido)$)$ethyl ether (PNVF-xNVEE) particles that were then acid hydrolysed to give colloidally stable, strongly pH-responsive poly(vinylamine-co-bis(ethylvinylamine)ether) particles (PVAM-BEVAE)\textsuperscript{7}. The extent of hydrolysis of the series of microgels was measured as 72 – 99\% and led to microgels with very high amounts of primary amine functionalisation. The incorporation of primary amines was demonstrated through the covalent linking of a carboxylate dye\textsuperscript{7}.

The approach discussed in this chapter aims to circumvent the problems associated with the synthesis of poly(vinylamine) by utilising an entirely different pathway for primary amine functionalisation. The copper (I) catalysed Huisgen 1,3-dipolarylcarboxyloaddition (CuAAC), commonly referred to as click chemistry, takes
advantage of the highly efficient reaction between azide and alkyne species to yield 1,2,3-triazoles. If a microgel can be polymerised with significant amounts of an acetylene-containing monomer, such as propargyl acrylate (PA), then it should be feasible to attach any azide-containing molecule to the microgel, including the bi-functional 2-azido-1-ethylamine (AEA) shown below in Figure 3.1.

![Chemical structures](image)

**Figure 3.1: Structures of propargyl acrylate (PA) and 2-azido-1-ethylamine (AEA).**

Successful attachment of AEA to the acetylene microgels through the click chemistry reaction would leave primary amine groups exposed for further functionalisation. The specific advantages of click chemistry reactions are their high selectivity and efficiencies, tolerance to a range of solvents and ease of preparation. The work of Jung et al. demonstrated that the incorporation of AEA into PHEMA-alkyne linear polymer via the CuAAC reaction resulted in a dually-responsive polymer with an LCST that was tuneable by the alteration of either solution temperature or pH. pH-responsiveness is an inherent property of primary amines; pH triggered swelling is expected for microgels with high primary amine contents as a decrease in pH below the pKa of the primary amine groups causes their protonation and electrostatic repulsion forces positively charged chains away from one another. The pKₐ of PVAM is approximately 10. Particle swelling below this pH was reported in the PVAM-BEVAME microgels prepared by Thaliboonrod et al. and PVAM nanocapsules studied by Shi and Berkland.

There are several examples of the functionalisation of microgels with acetylene groups, primarily for use in click chemistry applications. Meng et al. demonstrated that terminal acetylene functionalisation was achievable via a one-pot, two-stage co-polymerisation of NIPAm with propargyl acrylate and gycidyl
methacrylate\textsuperscript{80}. The amount of propargyl acrylate incorporated into this microgel was 5 mol\%. The CuAAC click chemistry reaction was then employed to click a fluorescent azido dye onto the particles and reaction success was confirmed by fluorescence microscopy images\textsuperscript{80}. The versatility of the preparation method was proven by preparing azido-containing microgels and clicking them with fluorescent alkyne dyes, with the same positive outcome observed\textsuperscript{80}. Evans and Lovell also reported the successful surface functionalisation of colloidal polymer particles by attaching propargylamine to the surface of poly(MMA-MAA) particles, which then reacted with an azido-containing pro-fluorophore\textsuperscript{118}.

The strategy for preparing primary amine functionalised microgels described in this chapter is outlined in Scheme 3.1 and is split into three experimental sections. These are the synthesis of AEA from 2-chloroethylamine hydrochloride and sodium azide, the synthesis of an acetylene-containing microgels from the co-polymerisation of PA with one of three different structural monomers, and the incorporation of primary amine functionality using the CuAAC reaction between the microgels and AEA. Three different structural monomers were investigated in order to evaluate the suitability of the alkyne-bearing monomer propargyl acrylate (PA) in generating pH-responsive microgels. Ethyl acrylate (EA) has been employed in the preparation of pH-responsive microgels by co-polymerisation with methacrylic acid, with a view to biomedical applications\textsuperscript{20,22}. Microgels based on 2-vinylpyridine (VP) are strongly pH-responsive\textsuperscript{18} and incorporation of PA into this system offered insights into the effects of the co-polymerisation of PA on microgel swelling behaviour. NIPAm microgels have been also been widely studied and successful primary amine functionalisation of this system could offer a dually responsive microgel with enhanced biologically interactive behaviour\textsuperscript{119}. The structures of the monomers EA, VP and NIPAm are shown in Scheme 3.1 b).
Scheme 3.1: Overall reaction scheme for the preparation of primary amine functionalised microgels using click chemistry. a) Synthesis of AEA, b) Preparation of acetylene-containing microgels by emulsion polymerisation, c) Attachment of primary amine functional groups using CuAAC. X = EA, or NIPAm, or VP (EA shown as example.)
The extents of the acetylene and primary amine functionalisation of the microgels were determined using FTIR spectroscopy and elemental analysis. The properties of the primary amine functionalised microgels were investigated using PCS, SEM and zeta potential measurements and compared to those of the precursor ‘unclicked’ acetylene-functionalised microgels and microgels of the three primary structural monomers prepared without PA. The temperature-responsive behaviour of the NIPAm family of microgels was also investigated using particle size and zeta potential measurements.
3.2 Experimental Methods

3.2.1 Materials

All materials were purchased from Sigma Aldrich with the exception of divinylbenzene (DVB, 85%), which was purchased from VWR. EA (99%), NIPAm (97%), VP (97%) and PA (97%) were purified by passing chloroform solutions of the monomers through columns filled with neutral alumina followed by solvent removal under reduced pressure. Aliquat 336, poly(ethylene glycol methyl ether methacrylate (PEGMA, number average molecular weight = 2000 g/mol), α,α’-azodiisobutyramidine dihydrochloride (AlBA, 97%), 1,4-butanediol diacrylate (BDD), N,N’-methylenebis(acrylamide) (BA, 99%), divinylbenzene (DVB, 85%), sodium azide (>99%), 2-chloroethylamine hydrochloride (99%), copper(I) bromide (≥ 98%) and N,N,N’,N”,N’’-pentamethyldiethylenetriamine (PMDETA, 99%) were used as received.

3.2.2 Synthesis of 2-azido-1-ethylamine

In accordance with the established methods\textsuperscript{114}, 2-chloroethylamine hydrochloride (5.0 g, 43 mmol) and sodium azide (8.0 g, 123 mmol) were dissolved in 50 mL DI water and heated while stirring to 80 °C for 15 hours. The solution was made alkaline with potassium hydroxide (15 g), extracted with diethyl ether (4 x 20 mL) and dried over magnesium sulphate. Removal of the solvent by rotary evaporation yielded a volatile oil.

3.2.3 Microgel preparation

The cationic one-pot batch emulsion polymerisation method devised by Dupin et al.\textsuperscript{18} was adapted for the co-polymerisation with PA. EA, VP and NIPAm were each used as primary structural monomers to formulate three distinct microgel systems. The monomers were passed through a column packed with basic alumina to remove their inhibitors. BDD, DVB and BA were employed respectively for each system as crosslinking monomers. Each structural monomer was polymerised with and without 28 mol% PA. This concentration was chosen as it has produced pH responsive microgels in previous acrylate based studies and aimed to limit potential crosslinking of the acetylene groups of PA during the emulsion polymerisation.
process\textsuperscript{20,38,97}.

The preparation procedure was as follows: Aliquat 336 surfactant (1.5 g, 10 wt%) and PEGMA (3.0 g) were combined with 120 mL deionised water and degassed for twenty minutes in a 250mL 5-neck reaction vessel. The mixture was stirred at 250 rpm and heated to 60 °C. The monomers (15 g total) were added before the introduction of an AIBA initiator solution (0.15 g in 15 mL water). Polymerisation was allowed to progress for 24 hours before quenching in an ice/water bath. The microgels were purified by extensive dialysis. The preparation compositions of all of the microgels studied in this chapter are listed in Table 3.1.

Table 3.1: Preparation compositions and notations of the EA, NIPAm and VP microgel systems prepared by batch emulsion polymerisation.

<table>
<thead>
<tr>
<th>Microgel preparation composition by mol fraction</th>
<th>Microgel notation</th>
<th>Mass of EA/VP/NIPAm / g</th>
<th>Mass of PA / g</th>
<th>Mass of crosslinker / g</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEA\textsubscript{0.99}-BDD\textsubscript{0.01}</td>
<td>PEA</td>
<td>14.37</td>
<td>-</td>
<td>0.29</td>
</tr>
<tr>
<td>PEA\textsubscript{0.71}-PA\textsubscript{0.28}-BDD\textsubscript{0.01}</td>
<td>PEA-PA</td>
<td>10.35</td>
<td>4.50</td>
<td>0.29</td>
</tr>
<tr>
<td>PNIPAm\textsubscript{0.99}-BA\textsubscript{0.01}</td>
<td>PNIPAm</td>
<td>14.79</td>
<td>-</td>
<td>0.21</td>
</tr>
<tr>
<td>PNIPAm\textsubscript{0.71}-PA\textsubscript{0.28}-BA\textsubscript{0.01}</td>
<td>PNIPAm-PA</td>
<td>10.73</td>
<td>4.18</td>
<td>0.21</td>
</tr>
<tr>
<td>PVP\textsubscript{0.99}-DVB\textsubscript{0.01}</td>
<td>PVP</td>
<td>14.79</td>
<td>-</td>
<td>0.19</td>
</tr>
<tr>
<td>PVP\textsubscript{0.71}-PA\textsubscript{0.28}-DVB\textsubscript{0.01}</td>
<td>PVP-PA</td>
<td>10.54</td>
<td>4.36</td>
<td>0.19</td>
</tr>
</tbody>
</table>

To measure the solids contents of the dialysed microgels, samples were placed into aluminium weighing dishes (with pre-recorded masses) and dried in an 80 °C oven for 24 hours. Solids contents were calculated as the percentage of the final dry sample mass to the initial dispersion sample mass. Microgel solids contents were used to calculate the overall percentage monomer conversion in each microgel preparation, including the contribution of the PEGMA stabiliser. These values are recorded in Table 3.2.
Table 3.2: Microgel solids contents and percentage monomer conversion of the EA, NIPAm and VP microgel systems prepared by batch emulsion polymerisation.

<table>
<thead>
<tr>
<th>Microgel notation</th>
<th>Microgel % solids content</th>
<th>% Monomer conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEA</td>
<td>5.1</td>
<td>44.3</td>
</tr>
<tr>
<td>PEA-PA</td>
<td>4.9</td>
<td>42.6</td>
</tr>
<tr>
<td>PNIPAm</td>
<td>8.2</td>
<td>71.3</td>
</tr>
<tr>
<td>PNIPAm-PA</td>
<td>6.1</td>
<td>53.0</td>
</tr>
<tr>
<td>PVP</td>
<td>9.2</td>
<td>80.0</td>
</tr>
<tr>
<td>PVP-PA</td>
<td>7.5</td>
<td>65.2</td>
</tr>
</tbody>
</table>

3.2.4 CuAAC reaction

Microgels containing 0.2 g total solids (maximum 0.510 mmol PA\textsuperscript{1}) were re-dispersed in DMF (10 mL) and placed to a 25 mL Schlenk flask. PMDETA (0.0174 g, 0.101 mmol) and AEA (0.0876 g, 1.02 mmol) were added and the dispersion was allowed to rest for several hours to allow for the penetration of AEA into the swollen microgel particles. The dispersion was then subjected to two freeze-pump-thaw cycles. Cu(I)Br (solid, 0.0144 g, 0.101 mmol) was introduced to the system under argon and the flask was stirred for 18 hours. To remove the catalyst, the resultant microgels were treated with DOWEX resin and extensively dialysed against DI water.

3.2.5 Fourier transform infra red spectroscopy

Measurements were undertaken on a Nicolet 5700 ATR FTIR instrument. Pure liquids were measured neat. Microgels were freeze dried prior to FTIR analysis. The number of scans per sample was 64 and the resolution was 2.0 cm\textsuperscript{-1}

\textsuperscript{1} Assuming full incorporation of PA into each microgel system.
3.2.6 **Nuclear magnetic resonance (NMR) spectroscopy**

A Bruker 400 Hz instrument was used for $^1$H NMR measurements. The solvent for all measurements was deuterated chloroform and the sample concentration was 0.1 wt%.

3.2.7 **Elemental analysis**

Elemental analysis was performed using a Thermo Scientific Flash 2000 Organic Elemental Analyzer using acetonilide and cyclohexane 2,4 dinitrophenylhydrazone as calibration standards. Microgels were freeze dried prior to analysis.

3.2.8 **Proton correlation spectroscopy (PCS)**

PCS particle size measurements were made using a Brookhaven BI-9000 light scattering apparatus with a 20mW HeNe laser and a scattering angle of 90°. For pH sensitive measurements, dilute aqueous HCl and NaOH solutions were used to adjust sample pH. Sample concentration was altered to meet a count rate specification of 100 – 200 kcps.

3.2.9 **Zeta potential measurements**

A Malvern zetasizer was used to measure the electrophoretic mobilities of the microgel particles in the presence of 0.001M aqueous NaNO₃. Electrophoretic mobilities were converted to zeta potentials using the Smoluchowski equation, given below¹²⁰,¹²¹.

\[
\nu_E = 4\pi\varepsilon_0 \varepsilon_r \frac{\zeta}{6\pi \mu} (1 + \kappa a)
\]  

(3.1)¹²¹

In the Smoluchowski equation, $\nu_E$ represents the particle mobility, $\varepsilon_0$ is the relative dielectric constant, $\varepsilon_r$ is the electrical permittivity of a vacuum, $\zeta$ is the zeta potential, $\mu$ is the solution viscosity, $a$ is the particle radius and $\kappa$ is the Debye-Hückel parameter. For pH sensitive measurements, dilute aqueous HCl (0.1 M) and NaOH (0.1 M) solutions were used to decrease and increase sample pH respectively.
3.2.10 Scanning electron microscopy (SEM)

SEM images were recorded using a Philips XL30 FEGSEM instrument with an accelerating voltage set of 5.0 kV. Samples were deposited onto clean glass slides and coated with carbon. Scale bars are given on the images. A minimum of 50 particles were measured to give an average particle size for each microgel.
3.3 Results and Discussion

3.3.1 Characterisation of AEA by $^1$H NMR spectroscopy and FTIR spectroscopy

The purity of synthesised AEA was ascertained using FTIR and $^1$H NMR spectroscopy. The FTIR spectrum of AEA is displayed in Figure 3.2.

![FTIR spectra of synthesised AEA.](image)

The spectrum included a sharp peak at 2100 cm$^{-1}$ belonging to the azide functional group. The peaks between 1550 – 1650 cm$^{-1}$ and 660-900 cm$^{-1}$ were representative of the primary amine functional group.$^7, 122$

The $^1$H NMR spectrum of AEA (Figure 3.3) consisted of triplet peaks at 3.39 and 2.89 ppm and a singlet peak at 1.38 ppm. The ratio of the integrals of these peaks was 1.0:1.0:1.1. These values corresponded with those reported by Jung et al.$^{114}$ The groups of smaller peaks at approximately 1.2 and 3.5 ppm represented impurities including diethyl ether that were not completely removed following the extraction and solvent removal processes.
3.3.2 Characterisation of alkyne-containing microgels

3.2.2.1 FTIR characterisation

Alkyne-functionalised microgels were prepared by emulsion polymerisation using an adaptation of the method described by Dupin et al. \(^{18}\) (see experimental section). Successful incorporation of PA into the microgels was determined by FTIR spectroscopy and elemental analysis. It was not possible to use NMR spectroscopy to analyse the composition of the microgels because crosslinking within the particles prevented them from dissolving or strongly swelling in deuterated solvents. The FTIR spectra of the ‘homopolymer’ and PA alkyne-functionalised microgels are displayed in Figure 3.4.

By comparing the spectra of the PEA, PNIPAm and PVP microgels prepared with and without PA it was possible to show that acetylene incorporation occurred to a varying extent within microgels prepared by co-polymerisation with PA. The most direct indication of the acetylene group is a broad absorption peak at approximately 3300 cm\(^{-1}\). This peak was present in the spectrum of PA monomer and was also clearly visible in the spectrum of the PEA-PA microgel (Figure 3.4a)). However, the acetylene group absorbance band is not particularly strong and can easily be masked by other functional groups, especially if the amount of acetylene group present is low.

Figure 3.3: \(^1\)H NMR spectrum of synthesised AEA.
Figure 3.4 FTIR spectra of parent microgels, acetylene microgels and acetylene monomer PA for the a) PEA and PEA-PA, b) PNIPAm and PNIPAm-PA, and c) PVP and PVP-PA microgel systems.

The acetylene peak was not clearly visible in the spectra of the PNIPAm-PA or PVP-PA microgels. In these cases, it was necessary to examine peaks corresponding to other functional groups specific to PA in order to determine the composition of the
microgels. The structures of the monomers NIPAm, VP and PA were displayed previously in Scheme 3.1 b). Of these monomers, only PA contains an ester group. In the FTIR spectrum of PA the C=O stretch bond deformation of this ester group gave a much sharper absorbance peak at 1730 cm\(^{-1}\) than the acetylene peak at 3300 cm\(^{-1}\). This particular C=O absorbance peak was absent in the spectra of PNIPAm and PVP but appeared in both PNIPAm-PA and PVP-PA, indicating that PA was present in both cases. The C=O absorbance peak was much weaker in the PNIPAm-PA spectrum compared to the PVP-PA spectrum, suggesting that PA incorporation into the NIPAm based system was poor. As both PA and EA contain ester groups it was not possible to use the C=O stretch peak as evidence of PA incorporation into the PEA-PA microgel; however the visible alkyne band at 3300 cm\(^{-1}\) was a more direct indicator of PA inclusion in any case.
3.3.2.2 Elemental analysis

Elemental analysis was used as a complementary technique to FTIR spectroscopy to quantitatively measure the elemental compositions of the microgels. The ratio of % nitrogen to % carbon ($R_{N/C}$) was calculated from the experimentally measured concentrations of nitrogen and carbon in each microgel system. This value was used to calculate the concentration of alkyne groups in the acetylene functionalised microgels, and the concentration of primary amine functionalisation in the AEA-clicked microgels. The incorporation of PA into the PVP and PNIPAm microgel systems should result in a lower experimentally measured $R_{N/C}$ value compared to the PVP and PNIPAm microgels as PA does not contain nitrogen, while both VP and NIPAm do. In contrast, the incorporation of AEA into the clicked microgels should result in an increase in the $R_{N/C}$ value of AEA is very nitrogen rich.

Quantitative determination of the elemental compositions of the microgels required an assumption to be made. This was that the only contributions to the percentages of carbon, nitrogen and oxygen in the theoretical microgels were from the co-monomers. The concentration of the crosslinker was assumed to be too low to have any significant effect on the composition. The contribution of the surfactant, PEGMA stabiliser and crosslinking monomer was empirically taken into account by using a correcting factor, which was calculated as the difference between the experimentally measured $R_{N/C}$ value ($R_{N/C(\text{exp})}$) for each of the ‘homopolymer’ microgels (PEA, PVP and PNIPAm) and the theoretical composition of the ‘homopolymer’ microgel based on the structure of the structural monomer (PEA, PVP, or NIPAm) only.

This was an imperfect method because in reality the PEGMA stabiliser would be incorporated into the polymer repeat unit and it assumes that the concentrations of PEGMA, Aliquat336, crosslinking monomer and residual initiator fragments to be equal in the ‘homopolymer’ and alkyne-functionalised microgels for each of the EA, VP and NIPAm systems. However, the method did offer realistic compositional determinations that were used to corroborate the findings of the FTIR spectra. The experimentally measured compositions of each of the ‘homopolymer’ and alkyne-functionalised microgels are listed in full in Table 3.3.
The equation used to calculate the $R_{N/C}$ correcting factor ($\Delta R_{N/C}$) is displayed in equation 3.2

$$\Delta R_{N/C} = R_{N/C(hp, theory)} - R_{N/C(hp, exp)} \tag{3.2}$$

where $R_{N/C(hp, theory)}$ is the theoretical $R_{N/C}$ value for each of the ‘homopolymer’ microgels based on their monomer structure and $R_{N/C(hp, exp)}$ is the experimentally measured $R_{N/C}$ value of the ‘homopolymer’ microgel.

The $\Delta R_{N/C}$ correcting factor was applied to experimentally determined $R_{N/C}$ values of each of the alkyne-functionalised microgels to give a ‘corrected’ experimental $R_{N/C}$ ratio ($R_{N/C(corr)}$).

$$R_{N/C(corr)} = R_{N/C(exp)} + \Delta R_{N/C} \tag{3.3}$$

The $R_{N/C(corr)}$ value was employed to calculate the concentration of PA in each of the alkyne-functionalised microgels using structure based equations generated from the compositions of the microgel repeat unit.

Table 3.3: Elemental compositions of parent and acetylene-containing microgels and their experimental nitrogen-over-carbon ratios ($R_{N/C(exp)}$), measured by elemental analysis.

<table>
<thead>
<tr>
<th>Microgel notation</th>
<th>% Carbon</th>
<th>% Hydrogen</th>
<th>% Nitrogen</th>
<th>$R_{N/C(exp)}$</th>
<th>$\Delta R_{N/C}^a$</th>
<th>$R_{N/C(corr)}^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEA</td>
<td>58.77</td>
<td>8.46</td>
<td>0</td>
<td>0.000</td>
<td>0.0175</td>
<td>0.0175</td>
</tr>
<tr>
<td>PEA-PA</td>
<td>59.48</td>
<td>7.38</td>
<td>0</td>
<td>0.000</td>
<td>0.0175</td>
<td>0.0175</td>
</tr>
<tr>
<td>PNIPAm</td>
<td>60.00</td>
<td>9.97</td>
<td>10.70</td>
<td>0.178</td>
<td>0.016</td>
<td>0.194</td>
</tr>
<tr>
<td>PNIPAm-PA</td>
<td>61.00</td>
<td>9.90</td>
<td>10.13</td>
<td>0.166</td>
<td>0.016</td>
<td>0.182</td>
</tr>
<tr>
<td>PVP</td>
<td>75.66</td>
<td>7.63</td>
<td>11.23</td>
<td>0.148</td>
<td>0.019</td>
<td>0.169</td>
</tr>
<tr>
<td>PV-PA</td>
<td>73.33</td>
<td>6.85</td>
<td>8.90</td>
<td>0.121</td>
<td>0.019</td>
<td>0.140</td>
</tr>
</tbody>
</table>

$^a$$\Delta R_{N/C}$ represents the difference the experimentally determined and theoretical $R_{N/C}$ values for the parent homopolymer microgels, $^b$ $R_{N/C(corr)}$ is the corrected experimentally measured $R_{N/C}$ value.
3.3.2.3 Composition determination of the PEA-PA microgel

The structures of the monomer EA and the polymer repeat unit of the PEA-PA microgel are shown in Figure 3.5.

![Figure 3.5: Structures of the monomer EA and the polymer repeat unit of the PEA-PA microgel.](image)

As neither EA nor PA contain nitrogen, it was not possible to use the $\text{R}_{\text{N/C (exp)}}$ values of the PEA and PEA-PA microgels to directly determine the extent of PA incorporation into the PEA-PA microgel. A simplified approach was employed. It was assumed that the incorporation of PA was complete due to the strong evidence for acetylene incorporation in the FTIR spectrum of the PEA-PA microgel (Figure 3.4a)). This assumption gave the provisional composition of the PEA-PA microgel to be $\text{PEA-PA}_{0.28}$, which was equivalent to the preparation composition of the microgel. This value was subsequently verified by measuring the increase in $\text{R}_{\text{N/C}}$ that occurred following the CuAAC reaction of the PEA-PA microgel with the nitrogen-rich AEA. This is discussed accordingly later in section 3.3.6.4.

3.3.2.4 Composition determinations of PVP-PA microgel

The structures of the monomer VP and the PVP-PA microgel polymer repeat unit are displayed in Figure 3.6.

![Figure 3.6: Structures of the monomer VP and the polymer repeat unit of the PVP-PA microgel.](image)
Based on these structures, the following equations allowed calculation of the extent of PA incorporation into the PVP-PA microgel.

\[
\% C = \left( \frac{12.011 \times 100}{MW} \right) \left[ 7(1 - x) + 6x \right]
\]  

\text{(3.4)}

MW is the molecular weight of the polymer repeat unit of the PVP-PA microgel shown in Figure 3.6.

\[
\% N = \left( \frac{14.007 \times 100}{MW} \right) (1 - x)
\]  

\text{(3.5)}

Combining equations 3.4 and 3.5 led to equation 3.6:

\[
x = \frac{1.1662 - 7R_{N/C(\text{corr})}}{1.1662 - R_{N/C(\text{corr})}}
\]  

\text{(3.6)}

Inserting the value of \(R_{N/C(\text{corr})}\) (0.140, Table 3.3) into this equation found the composition of the microgel to be PVP-PA_{0.18}, i.e. 18 mol % PA. This was lower than the 28 mol % PA concentration used the preparation of the microgel, so the incorporation of PA was incomplete for the VP system.

\subsection{Composition determination of PNIPAm-PA microgel}

The process of using structure-based equations and assumptions to determine the composition of the VP microgels was repeated for the NIPAm series of microgels. The compositions of the repeat units of the PNIPAm and PNIPAm-PA microgels are shown in Figure 3.7.

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{figure3.7}
\caption{Figure 3.7: Structures of the monomer NIPAm and the polymer repeat unit of the PNIPAm-PA microgel.}
\end{figure}

These compositions gave rise to equation 3.7.

\[
x = 1 - 5.145R_{N/C(\text{corr})}
\]  

\text{(3.7)}
Inserting the $R_{N/C(\text{corr})}$ value (0.182, Table 3.3) found that the incorporation of PA into the PNIPAM-PA microgel was 6.3 mol%. This low value correlated well with the weak C=O absorbance peak shown in the FTIR spectrum of the PNIPAm-PA microgel (Figure 3.4b)).

Of the three acetylene-functionalised microgels, full incorporation of PA only occurred in the EA based microgel. The mol% PA in PEA-PA was assumed to be 28% and this value correlated with the significant acetylene peak visible in the FTIR spectra of PEA-PA (Figure 3.4a)), which was similar in intensity to the acetylene peak of PA monomer. The mol% PA in PVP-PA was determined to be 18%, which was significantly lower than the 30 mol% PA monomer present during polymerisation. Figure 3.8 illustrates the decrease in $R_{N/C}$ caused by incorporated PA into the nitrogen-containing VP and NIPAm systems.

![Graph of $R_{N/C}$ vs mol% PA](image)

**Figure 3.8:** a) Measured elemental analysis $R_{N/C(\text{exp})}$ values for acetylene-containing microgels and the parent EA, NIPAm and VP microgels; variation in $R_{N/C(\text{corr})}$ ratios with the theoretical concentration of incorporated PA for the b) PVP-PA and c) PNIPAm-PA microgels.

85
Figure 3.8a) shows that, as expected, the %N/%C ratio for PVP-PA is lower than that of PVP. The fact that the ester C=O stretch peak was present in the FTIR spectrum of PVP-PA but absent in PVP (Figure 3.4c)) also confirmed PA incorporation. It was not possible to compare the ester peaks of the VP and NIPAm based acetylene microgels with the EA system to estimate PA incorporation because both EA and PA contain ester groups and FTIR spectroscopy is usually a qualitative, rather than quantitative, method of analysing the composition of a material (although quantitative analysis is possible in applicable cases\textsuperscript{123}). The extent of PA incorporation into PNIPAm-PA was found to be just 6.3 mol%; a figure supported by the small ester peak in the spectrum of PNIPAm-PA in Figure 3.4b) and the relatively small decrease in $R_{N/C(\text{corr})}$ indicated in Figure 3.8a).

Figures 3.8 b) and c) demonstrated the sensitivity of $R_{N/C(\text{corr})}$ values to PA incorporation in the VP and NIPAm microgel systems. Even full PA incorporation of 28 mol% would only have caused a small decrease in $R_{N/C(\text{corr})}$, from approximately 0.15 to 0.10 for the VP system and 0.17 to 0.12 for the NIPAm system. The experimentally determined levels of PA incorporation are indicated in these figures as 18 mol% PA in the PVP-PA microgel and 6.3 mol% in the PNIPAm-PA microgel.

### 3.3.3 Solubility of PA with the primary structural monomers

A substantial acetylene concentration was necessary in order to achieve the high level of primary amine functionalisation desired by this research. The levels of PA incorporated into the NIPAm system and to a lesser extent the VP system were significantly lower than the amount of PA present during polymerisation. The underlying causes of this issue were investigated. Meng et al. reported that PA has a relatively slow propagation rate\textsuperscript{80, 97}. However, the reaction conditions were adequate for near full incorporation of PA into the PEA-PA microgel and so other causes of incomplete PA incorporation were examined. Reactivity ratios and polarity have an effect on polymerisation. The Alfrey and Price values for reactivity and polarity for butyl acrylate (a similar structure to PA) are 0.40 and 0.35 respectively\textsuperscript{124}. The values listed for NIPAm are 0.40 and 0.47\textsuperscript{125}, which are similar to those of butyl acrylate and this implies that the reactivity and polarity of PA are not limiting enough to hinder its co-polymerisation with NIPAm and VP. Therefore,
an alternative explanation for incomplete PA incorporation was required.

It is known that for an emulsion polymerisation of co-monomers to be successful it must be possible for growing polymer particles to be penetrated and swollen by monomer\textsuperscript{58}. It follows, therefore, that for a successful co-polymerisation the co-monomers must be soluble in the polymers of the main structural monomers and vice versa. This idea was explored by testing the solubility of the linear polymers of EA, NIPAm and VP in PA. Small amounts of each polymer were placed in PA and agitated. The results of this test are shown in Figure 3.9.

![Figure 3.9: Photos depicting the solubility of linear a) poly(EA), b) poly(VP) and c) poly(NIPAm) in PA.](image)

The photos displayed in Figure 3.9 were taken following 5 minutes of vigorous agitation after introducing samples of poly(EA), poly(VP) and poly(NIPAm) to 1 ml PA. Poly(EA) dissolved readily in PA but poly(VP) and poly(NIPAm) were noticeably less soluble. The solubility of poly(VP) was initially poor upon introducing poly(vinyl pyridine) to PA, but improved after 5 minutes of vigorous mixing. The photo displayed in Figure 3.9b) was taken after the period of mixing. Poly(NIPAm) remained clearly insoluble in PA, even after vigorous agitation. The resultant mixture of poly(NIPAm) and PA after agitation is displayed in Figure 3.9c). The insolubility of poly(NIPAm) in PA presents a barrier to the effective co-polymerisation of the two monomers, as PA monomer would not be able to swell the poly(NIPAm)-rich particles during their growth. In contrast, the good solubility of poly(EA) in PA implies that PA would be able to swell particles mainly comprising poly(EA) and provide a constant supply of monomer for particle growth, resulting in a complete incorporation of PA. The images in Figure 3.9 strongly supported the
trend that PA incorporation into the three microgels is greatest in PEA, intermediate in PVP and poor in PNIPAm.

3.3.4 Particle size characterisation of alkyne-functionalised microgels

For the CuAAC reaction to be successful and result in complete AEA functionalisation of the PA-containing microgels it would be necessary for the acetylene-functionalised microgels to be swellable in DMF or another suitable solvent to allow penetration of AEA, the catalyst and the ligand into the particle interiors. The size of the microgels in water (at pH 7) and DMF were measured using PCS and are recorded in Table 3.4.

Table 3.4: Microgel hydrodynamic diameters ($d_h$) in water at pH 7, collapsed diameters ($d_c$), swollen diameters in DMF and swelling ratios in DMF (Q).

<table>
<thead>
<tr>
<th>Microgel</th>
<th>$d_h$ in water / nm</th>
<th>$d_c$ a / nm</th>
<th>$d_h$ in DMF / nm</th>
<th>Q in DMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEA</td>
<td>102</td>
<td>102</td>
<td>213</td>
<td>9.1</td>
</tr>
<tr>
<td>PEA-PA</td>
<td>133</td>
<td>128</td>
<td>157</td>
<td>1.6</td>
</tr>
<tr>
<td>PNIPAm</td>
<td>338</td>
<td>125°</td>
<td>417</td>
<td>37</td>
</tr>
<tr>
<td>PNIPAm-PA</td>
<td>333</td>
<td>193°</td>
<td>505</td>
<td>18</td>
</tr>
<tr>
<td>PVP</td>
<td>370</td>
<td>370</td>
<td>664</td>
<td>4.5</td>
</tr>
<tr>
<td>PVP-PA</td>
<td>352</td>
<td>353</td>
<td>512</td>
<td>2.8</td>
</tr>
</tbody>
</table>

a Collapsed diameters were selected as the smallest observed particle diameter for the EA and VP based systems. The $d_c$ values of the NIPAm microgels were measured at 50 °C due to their temperature-responsive swelling behaviour. All other PCS measurements were performed at 25 °C.

The swelling ratio of each microgel system was analysed by calculating the particle volume swelling ratio, Q, in DMF for each microgel. This parameter is given by equation 3.8 below. $d_h$ and $d_c$ represent the hydrodynamic diameter of a swollen particle and the diameter of a collapsed particle respectively.
\[ Q = \left( \frac{d_h}{d_c} \right)^3 \]  \hspace{1cm} (3.8)

The three microgel systems had distinct average particle sizes. The PEA particles had an average size of 102 nm. Co-polymerisation with PA resulted in an increased particle size of 133 nm in the PEA-PA microgel. The sizes of these systems were comparable to the microgel particles prepared by Liu et al. (75 nm in water at pH 7)\textsuperscript{37} and Lally et al.\textsuperscript{126}, which were also acrylate based microgels.

At pH 7 the VP series of microgels all had a similar average particle size of 350-370 nm. The values in Table 3.4 show that all of the PA-functionalised microgels swelled in DMF. This was necessary for the CuAAC reaction to modify the interiors of the microgels. However, the PEA-PA and PVP-PA microgels had significantly lower Q values than the ‘parent’ PEA and PVP microgels. This suggested that PA incorporation into these systems restricted the swelling potential of the microgels.
3.3.5 SEM characterisation of parent and alkyne-functionalised microgels

SEM images of each microgel are displayed in Figure 3.10 and the number-average particle diameters measured from the images are listed in Table 3.5.

Figure 3.10: SEM images of parent and acetylene-containing microgels for the EA, NIPAm and VP systems. a) PEA, b) PEA-PA, c) PNIPAm, d) PNIPAm-PA, e) PVP, f) PVP-PA.

Dispersed spherical particles were visible for each microgel and the number-average diameters measured using SEM corresponded well with the $d_h$ values measured in water using PCS for the EA and VP microgel systems (Table 3.5). The $d_{SEM}$ values for the NIPAm system microgels were significantly lower than the $d_h$ values at 25 °C. For the PNIPAm and PNIPAm-PA microgels these values were 245 and 200 nm respectively measured by SEM and 338 and 333 nm, respectively,
measured using PCS at 25 °C and pH 7. However, this disparity can be attributed to the fact that NIPAm polymers are temperature responsive and sensitive to the effects of the electron beam. They also deform upon deposition onto SEM stubs\textsuperscript{127}. A better comparison for these microgels may be the sizes of the NIPAm based particles measured at temperatures greater than 25 °C. This temperature-responsiveness of the NIPAm based microgels is discussed in Section 3.3.6.4.

Table 3.5: Average microgel diameters measured by PCS and SEM.

<table>
<thead>
<tr>
<th>Microgel</th>
<th>(d_{h(PCS)}/\text{nm}) \textsuperscript{a}</th>
<th>(d_{c(SEM)}/\text{nm}) \textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEA</td>
<td>102</td>
<td>107</td>
</tr>
<tr>
<td>PEA-PA</td>
<td>133</td>
<td>123</td>
</tr>
<tr>
<td>PNIPAm</td>
<td>338</td>
<td>245</td>
</tr>
<tr>
<td>PNIPAm-PA</td>
<td>333</td>
<td>200</td>
</tr>
<tr>
<td>PVP</td>
<td>370</td>
<td>372</td>
</tr>
<tr>
<td>PVP-PA</td>
<td>352</td>
<td>339</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Average microgel hydrodynamic diameter measured at pH 7 at 25 °C using PCS, \textsuperscript{b} number average collapsed particle diameter measured from SEM images (minimum of 50 particles)
3.3.6 Characterisation of ‘clicked’ primary amine functionalised microgels

3.3.6.1 FTIR spectroscopy

AEA was ‘clicked’ onto the acetylene-containing microgels using the procedure described in the experimental section. Following purification by dialysis in deionised water, the microgels were characterised using FTIR spectroscopy, PCS, zeta potential and SEM measurements. The FTIR spectra of the AEA-clicked microgels are displayed in Figure 3.11.

Figure 3.11: FTIR spectra of AEA-clicked microgels, the precursor acetylene-functionalised microgels and AEA for the a) EA, b) NIPAm and c) VP based microgel systems.
The primary indicators for the success of the CuAAC reaction were the disappearance of the acetylene band at 3280 cm\(^{-1}\) and the appearance of primary amine peaks at 1590 cm\(^{-1}\). It was important that the azide peak at 2100 cm\(^{-1}\) is not present in the clicked spectra as all AEA should have either reacted with the acetylene groups of the microgels or have been removed during purification. The EA based system showed the clearest indication of PA inclusion as it contained the largest amount of PA. The spectrum of PEA-PA-AEA did not contain acetylene peak at 3280 cm\(^{-1}\) but did feature a primary amine ‘shoulder’ at 1590 cm\(^{-1}\). The fact that there was not an azide peak at 2100 cm\(^{-1}\) in the spectrum indicated that there was not any unreacted AEA present in the purified dispersion. These were strong indications that the CuAAC reaction occurred to a high level of efficiency for the PEA-PA-AEA system.

As the acetylene bands of the PNIPAm-PA and PVP-PA microgels were masked or not strong enough to be visible it was not possible to use this functional group to determine the success of the CuAAC reaction for these microgel systems. The primary amine band at 1590 cm\(^{-1}\) was partially obscured by other peaks in the spectra of the PNIPAm-PA-AEA and PVP-PA-AEA microgel, which made analysis of the success of the CuAAC reaction difficult using FTIR spectroscopy. Due to this, quantitative elemental analysis was a better measure of reaction success for these two systems.
3.3.6.2 Elemental analysis characterisation of clicked microgels

Elemental analysis was carried out on the AEA-clicked microgels to quantify the degree of primary amine functionalisation and the efficiency of the CuAAC reaction. The elemental compositions of the clicked microgels are listed in Table 3.6.

**Table 3.6: Elemental compositions of precursor acetylene-containing microgels, AEA-clicked primary amine functionalised microgels and their experimental and corrected nitrogen-over-carbon ratios, measured by elemental analysis.**

<table>
<thead>
<tr>
<th>Microgel notation</th>
<th>% Carbon</th>
<th>% Hydrogen</th>
<th>% Nitrogen</th>
<th>$R_{N/C}(\text{exp})$</th>
<th>$\Delta R_{N/C}$</th>
<th>$R_{N/C}(\text{corr})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEA-PA</td>
<td>59.48</td>
<td>7.38</td>
<td>0</td>
<td>0.000</td>
<td>0.0175</td>
<td>0.0175</td>
</tr>
<tr>
<td>PEA-PA-AEA</td>
<td>51.20</td>
<td>6.94</td>
<td>11.39</td>
<td>0.222</td>
<td>0.0175</td>
<td>0.240</td>
</tr>
<tr>
<td>PNIPAm-PA</td>
<td>61.00</td>
<td>9.90</td>
<td>10.13</td>
<td>0.166</td>
<td>0.016</td>
<td>0.182</td>
</tr>
<tr>
<td>PNIPAm-PA-AEA</td>
<td>57.23</td>
<td>8.78</td>
<td>12.08</td>
<td>0.211</td>
<td>0.016</td>
<td>0.0227</td>
</tr>
<tr>
<td>PV-PA</td>
<td>73.33</td>
<td>6.85</td>
<td>8.90</td>
<td>0.121</td>
<td>0.019</td>
<td>0.0140</td>
</tr>
<tr>
<td>PVP-PA-AEA</td>
<td>62.56</td>
<td>7.05</td>
<td>14.73</td>
<td>0.235</td>
<td>0.019</td>
<td>0.254</td>
</tr>
</tbody>
</table>

Calculation of the degree of primary amine functionalisation in the PEA-PA-AEA microgel was more straightforward than the determination of the extent of acetylene functionalisation in the PEA-PA microgel because the addition of a significant nitrogen concentration from AEA introduced a $R_{N/C}$ ratio that was be used to calculate the mol% of AEA present in the clicked microgel. A precondition for the previously outlined method, however, is the calculation of a $\Delta R_{N/C}$ value between the theoretical and experimental $R_{N/C}$ values of the homopolymer microgel (PEA). This was not possible for the PEA microgel as it did not contain a nitrogen content, so the average $\Delta R_{N/C}$ values of the PVP and PNIPAm microgels were used in that case.

The significant rise in the $R_{N/C}(\text{exp})$, displayed in Figure 3.12, clearly showed that primary amine groups were present in the clicked microgel. The $R_{N/C}(\text{exp})$ value was particularly compelling evidence as there was no nitrogen present in the
 precursor PEA-PA and the only place the significant nitrogen content could have come from was through incorporation of AEA.

**Figure 3.12:** Variation in $R_{N/C(\text{exp})}$, measured using elemental analysis, for the parent EA, NIPAm and VP microgels, the acetylene-containing PA microgels and the primary amine functionalised microgels clicked with AEA. Error bars = 0.002.

### 3.3.6.3 Composition determination of the PEA-PA-AEA microgel

The structure of the polymer repeat unit of the PEA-PA-AEA microgel is shown in Figure 3.13.

**Figure 3.13:** Structure of the polymer repeat unit of the PEA-PA-AEA microgel.

The following equations detail the calculation of the mol% of AEA in this microgel. Due to the format of the CuAAC reaction there was also a constraint that $x + y = 0.28$, as this is the maximum mol fraction of reactive acetylene species present in the PEA-PA microgel. The acetylene groups must either react with AEA, resulting in primary amine functionality, or be left unchanged. These assumptions led to
equation 3.9, where \( x \) is the mol fraction of unreacted PA in the PEA-PA-AEA microgel.

\[
x = \frac{1.3061 - 5.84 R_{N/C(corr)}}{4.6648 - 2 R_{N/C(corr)}}
\]  

(3.9)

Using the corrected \( R_{N/C(corr)} \) value of 0.240 (Table 3.6), the equation yielded that \( x = -0.02 \). This can be viewed as \( x = 0 \) allowing for a small experimental error due to the lack of a true \( R_{N/C} \) value for the PEA microgel. Therefore, \( y \) and the degree of primary amine functionalisation were equal to 28 mol\% and the CuAAC reaction was deemed to be 100 % efficient. These results supported the validity of the assumption that the mol\% of PA in the PEA-PA microgel was also 28%.

3.3.6.4 Composition determination of the PVP-PA-AEA microgel

The degree of primary amine functionalisation in the PVP-(PA-AEA) microgel was calculated similarly. The structural formula of the microgel is shown in Figure 3.14.

\[
(C_7H_7N)(1-x-y)(C_6H_6O_2)x(C_8H_{12}N_4O_2)y
\]

Figure 3.14: Structure of the polymer repeat unit of the PVP-PA-AEA microgel.

A constraint of this structure was that \( x + y = 0.18 \). From this structure, the following equation was derived.

\[
x = \frac{1.796 - 7.18 R_{N/C(corr)}}{4.665 - 2 R_{N/C(corr)}}
\]  

(3.10)

Using the \( R_{N/C(corr)} \) value for this microgel (0.255, Table 3.6) gave \( x = -0.008 \), which, allowing for a small error, was taken as \( x = 0 \), and \( y = 0.18 \). This gave a final microgel composition of \( \text{PVP-} \text{(PA-AEA)}_{0.18} \) and again implied 100% efficiency for the click reaction.
3.3.6.5 Composition determination of the PNIPAm-PA-AEA microgel

The structure of the polymer repeat unit of the PNIPAm-PA-AEA microgel is displayed in Figure 3.15.

![Figure 3.15: Structure and composition of the polymer repeat unit of the PNIPAm-PA-AEA microgel.](image)

\[
(C_4H_{11}NO)_{(1-x-y)}(C_6H_6O_2)_x(C_8H_{12}N_4O_2)_y
\]

Composition determination of the PNIPAm-PA-AEA microgel yielded a structure of PNIPAM-(PA-AEA)$_{0.063}$; implying that all of the available acetylene groups had been consumed during the CuAAC reaction and that the degree of AEA functionalisation was 6.3 mol%. Figure 3.12 showed that the click reaction resulted in increases in $R_{N/C(exp)}$ ratios relative to the amount of PA present in the unclicked microgels for the VP and NIPAm based systems. These results were in agreement with those of the EA system and reinforce that conclusion that the CuAAC reaction is highly efficient. The degree of primary amine functionalisation was limited only by the amount of PA present in the precursor microgels.
3.3.7 Physical characterisation of primary amine functionalised microgels

3.3.7.1 Particle size characterisation

It has been established that it is possible to functionalise EA, NIPAm and VP microgels with primary amine groups. These clicked microgels should be pH responsive as the amine groups become protonated and repel each other below the \( pK_a \) of the primary amine groups, leading to particle swelling. It is logical that the EA based system should show a stronger response to pH than the NIPAm microgel as it contains a higher mol\% of primary amines. The VP based microgel should already be pH responsive due to the structure of VP, although introducing primary amines to the microgel should increase the magnitude of the response compared to the PVP-PA microgel. This hypothesis was tested using size measurements across a pH range of 3 to 12 and the results are displayed in Figure 3.16.

It was clear from the Figure 3.16 that the pH triggered swelling did not occur to the desired extent for any of the primary amine functionalised microgel systems. The EA based system showed an increase of approximately 30 nm in average particle size between the ‘unclicked’ PEA-PA and the clicked PEA-(PA-AEA) microgels, but this size increase remained constant across the pH range and can be attributed to the increase in material from the incorporation of AEA. In comparison, the microgel swelling ratio \((Q)\), defined in equation 3.8, for an anionic EA based system containing 30 mol\% RCOO\(^{-}\) groups was 30 \((37)\) and it follows a cationic system with 28 mol\% charged groups should have a similar value. However, the maximum \(Q\) value for the PEA-(PA-AEA) microgel was 2.0. To examine the cause of this lack of pH response, the swelling ratios of the EA based microgels were calculated in water and DMF. Between pH 4 - 10 the average \(Q\) of PEA-(PA-AEA) particles was 1.7, which is not significantly different to the value in DMF. This indicates that swelling caused by repulsion between protonated \(\text{NH}_3^{+}\) groups was occurring across this pH range in water but was restricted. There is also evidence of partial particle collapse at pH 12 due to the deprotonation of \(\text{NH}_3^{+}\) groups leading to a decrease in \(d_h\) and average particle size. This partial collapse was expected. Full
collapse of the latter particles was not expected because the structure of AEA is nitrogen rich and hydrophilic by nature.

Figure 3.16: Variation of microgel hydrodynamic dynamic diameters (a), (c), (e)) and microgel swelling ratios, Q, (b), (d), (f)) with pH for all of the microgels in the EA, NIPAm and VP based systems. Measurements were performed at 25 °C.
A restriction in particle swelling could be caused by crosslinking between acetylene groups during emulsion polymerisation or during the CuAAC reaction. This has been reported as an issue for PA based microgels\textsuperscript{97, 129-131} and acetylene compounds used in CuAAC reactions\textsuperscript{64}. During the CuAAC reaction, Glaser\textsuperscript{132} and Straus\textsuperscript{133} reactions could potentially have occurred between the acetylene groups of microgel particles. The Glaser reaction is an oxidative coupling reaction and requires the presence of an amine species and oxygen, however the Straus reaction can take place under inert conditions\textsuperscript{134}.

Possible acetylene group crosslinking, occurring either during the preparation of the acetylene-functionalised microgels or the CuAAC reaction, also supports the dramatic decrease in swelling ability of the PVP-PA microgel compared to the PVP microgel. It was shown in Figure 3.16 that the PVP-PA microgel swelled significantly less than PVP microgel particles at low pH, with a maximum value of just 537 nm at pH 2. This restriction was clearly shown by the Q values for the respective microgels, which were of the order of 10 times lower for the PVP-PA microgel below pH 4. A decrease in swelling potential was expected for the PVP-PA microgel compared to PVP because incorporating PA reduced the number density of pyridine residues whose protonation led to swelling, but the restriction shown was severe. Acetylene crosslinking that occurred during the emulsion polymerisation would also restrict the swelling of the clicked primary amine microgels. It is logical that as the PEA-(PA-AEA) microgel contained the greatest PA concentration out of the three systems, it also contained a greater degree of crosslinking. This effect could have been responsible for the lack of pH response in the EA based system, while the hydrophobic nature of PA could also have contributed\textsuperscript{20}.

The NIPAm system showed few differences in $d_h$ between the three microgels across the pH range. This was consistent as there was little difference in structure between the microgels, with only 6.3 mol\% charged groups incorporated into the PNIPAm-(PA-AEA) microgel, so significant swelling was not expected. The $d_h$ and $Q_p$ values were higher for the PNIPAm-(PA-AEA) microgel than the PNIPAm-PA microgel between pH 4 – 7, indicating that some electrostatic swelling did take
The VP based microgels displayed interesting pH-responsive behaviour. The PVP microgel, which had the same composition as one of the VP microgels prepared by Dupin et al., showed the same swelling behaviour\textsuperscript{18}. Above the pK\textsubscript{a} of 4.10 the average size was 370 nm; decreasing the pH below this value caused the particles to swell rapidly to over 1100 nm by pH 3. Introducing PA into the PVP system had a significant effect on its swelling behaviour, with particle swelling below pH 4 severely restricted. It was noted that the pK\textsubscript{a} of VP based microgels decreased upon an increase in crosslinker concentration\textsuperscript{18}, so it is likely that the addition of PA would have an effect on the pK\textsubscript{a} of PVP-PA relative to PVP. When AEA is clicked onto the PVP-PA microgel swelling was, however, able to occur and began at a higher pH. The particle sizes of the PVP-PA-AEA microgel were the largest above pH 4. It can be summarised that the addition of AEA partially restored the ability of the PVP-PA microgel to swell that was restricted due to the dilution of positive charges at low pH by incorporating PA and the physical restrictions of self-crosslinking between acetylene groups.
3.3.7.2 Zeta potential of microgel systems

The effect of pH on the zeta potential of the microgels was also investigated and the results of these measurements are displayed in Figure 3.17.

**Figure 3.17: Variation of zeta potential with pH for all of the microgels in the a) EA, b) NIPAm and c) VP based systems.** Error bars were smaller than the data points in this figure.

Both the pH-triggered swelling response of the parent PVP microgel and the lack of charge and pH response of the PNIPAm microgel corresponded well with previous reports and the previously discussed particle size measurements\(^{18, 119, 135, 136}\). These features are also represented in Figure 3.17. The PVP microgel showed a strongly positive zeta potential at pH 4, below the pK\(_a\) of poly(2-vinylpyridine)\(^{18}\) (Figure 3.17a)), while the PNIPAm microgel displayed zeta potential values close to zero between pH 4 – 10.
A notable feature of Figure 3.17 is that below pH 10, the zeta potential values of the primary amine functionalised microgels were greater than those of the precursor alkyne functionalised and ‘homopolymer’ microgels in all of the microgel systems. The pKₐ of primary amine groups in previously discussed poly(vinylamine) based microgel systems was reported to be approximately 10⁷. It is suggested that below pH 10 the primary amine groups of the AEA-clicked microgels became protonated, contributing to the greater positive zeta potential values observed for the primary amine functionalised microgels at pH 7 and pH 4 compared to the alkyne functionalised and homopolymer microgels.

However, despite the presence of ionisable primary amine groups in the AEA-clicked microgels, strong pH-triggered swelling was only displayed by the PVP-PA-AEA microgel and that was largely attributed to its high concentration of pyridine residues. A possible explanation for this is that zeta potential values relate to potential measured at the Stern layer and give a measure of the surface charge of a particle. Therefore, zeta potential values do not represent the charge profile of the entire particle and do not necessarily correlate into particle swelling unless the ionisable groups are also distributed throughout the bulk of the particle.

The fact that the positive zeta potential values of the AEA-clicked microgels did not correspond to particle swelling at pH 7 and below indicated that the primary amine groups were not evenly distributed throughout the particles, and instead were present in greatest concentration towards the particle surfaces. This was supported by the results of the PNIPAm-PA-AEA microgel, which despite only containing 6.3 mol% primary amine groups demonstrated significant positive zeta potential values at pH 4 and 7. This concentration of positively charged groups close to the particle surface was not significant enough to cause particle swelling because the bulk of each particle remained unchanged.

A similar effect was observed for the PVP-PA-AEA microgel, which contained approximately 18 mol% primary amine groups. The parent PVP microgel displayed negative zeta potentials above pH 4. However, the PVP-PA-AEA microgel showed strongly positive zeta potentials at both pH 4 and pH 7. It is likely that the primary amine groups, largely present close to the particle surface, were protonated at pH
7, while at pH 4 both the primary amine and pyridine moieties were positively charged. This would also account for the small increase in the particle size of the PVP-PA-AEA microgel measured at pH 7 (displayed in Figure 3.16) that was not reflected in either the PVP or PVP-PA microgel. The concentration of primary amine groups towards the particle exteriors would have been determined by the distribution of PA in the alkyne-functionalised microgels. These findings suggest that the compositions of the alkyne-functionalised microgels were not homogenous.

Figure 3.17 also indicates that isoelectric points occurred for all of the microgels. Negative zeta potentials were recorded at pH 12 for every microgel, despite the lack of obvious anionic functional groups in any of the microgel structures. Similar results have been reported for poly(NIPAm)\textsuperscript{136} and poly(2-vinylpyridine)\textsuperscript{18} microgel systems, with weakly anionic character displayed at basic pH in both cases. The formation of carboxylate ions at high pH, via the hydrolysis of amide or ester groups, has been suggested as an explanation for this behaviour\textsuperscript{136,138}. Amide groups were present in the polymer repeat units of the NIPAm based microgels, while ester groups were present in EA and PA, as well as in the PEGMA steric stabiliser employed in each microgel system. The anionic character displayed at high pH was not significant enough to result in microgel swelling, with the exception of the NIPAm based microgels at pH 12. This suggested that the overall proportion of ester groups hydrolysed at high pH was low and potentially limited to surface groups or extended PEGMA chains. Particle aggregation did not occur at the isoelectric points of the microgel dispersions; this can be attributed to the steric stabilisation of the microgels achieved by the incorporation of PEGMA.
3.3.7.3 SEM characterisation of ‘AEA-clicked’ microgels

SEM images of the AEA-clicked primary amine microgels are displayed in Figure 3.18.

Figure 3.18: SEM images of AEA-clicked microgels. a) PEA-PA-AEA, b) PNIPAm-PA-AEA, c) PVP-PA-AEA. The widths of the inset images are equal to 500 nm.

SEM images of the ‘AEA-clicked’ microgels corresponded well with the sizing and morphology results of the precursor acetylene-functionalised microgels. The average size of the PEA-PA-AEA microgel measured by SEM was very similar to the hydrodynamic diameter measured by PCS (166 nm by SEM compared to 160 nm by PCS). A size of 245 nm was recorded for the PNIPAm-PA-AEA microgel using SEM images. This represented a similar size to the PNIPAm microgel measured using SEM (249 nm). As discussed previously, lower particle sizes were recorded for the NIPAm based microgels using SEM images compared to hydrodynamic diameters measured by PCS and so this was an expected result. The size of the PVP-PA-AEA microgel measured as 403 nm by SEM was comparable to the 370 nm size recorded at pH 12 by PCS. Although the measurement of particles using SEM only recorded
the size of a minute fraction of the total number of particles in dispersion, it was important that the values recorded by SEM and PCS were comparable in order to confirm that the CuAAC reaction did not adversely affect colloidal stability. There was no significant evidence that microgel colloidal stability was compromised by the CuAAC reaction due to general agreement of SEM and PCS measurements, with the exception of the NIPAm based microgels explained, and the consistency of particle sizes between the acetylene-functionalised microgels and the AEA-clicked microgels (discussed previously).

### 3.3.8 Temperature-responsive behaviour of NIPAm based microgels

The effects of incorporating PA and AEA into PNIPAm microgels were investigated using temperature sensitive size and zeta potential measurements. The temperature-triggered collapse of PNIPAm particles above an LCST has been well reported\textsuperscript{127, 139-141}. This was evident in Figure 3.19 a) as the particle size of the PNIPAm microgels decreased from 343 to 125 nm across the temperature range. However, the extent of particle collapse was lower in the PNIPAm-PA and PNIPAm-PA-AEA microgels, with the average particle diameter decreasing to approximately 200 nm for both systems.

![Figure 3.19](image)

**Figure 3.19:** a) Variation of microgel size with temperature showing temperature triggered collapse and b) variation of zeta potential with temperature for the NIPAm based microgel system.
There were also distinct differences between all three microgels when zeta potential ($\zeta$) was measured as a function of temperature (Figure 3.19 b)). The $\zeta$ values increased in the order PNIPAm < PNIPAm-PA < PNIPAm-PA-AEA across the temperature range, implying that the microgels had different volume surface charge densities in their outer layers. Despite the relatively low levels of PA and AEA incorporated into their respective microgels (6.3 mol%), the $\zeta$ values were significantly different at lower temperatures for all three microgels, with PNP-PA-AEA having the highest values. This strongly suggested that the PA and PA-AEA groups were predominantly located near the surface of the microgels, resulting in significantly different surface charge densities. This observation implied that PA groups were accessible to reactants during the CuAAC reaction.
3.4 Conclusions

The results discussed in this chapter showed that it was possible to prepare acetylene-bearing microgels via a one-pot emulsion polymerisation method with different structural monomers. The extent of PA incorporation into these microgels was strongly influenced by the choice of structural co-monomer and the solubility of PA with the growing polymer particles. PA was very compatible with EA and co-polymerised with VP but was less compatible with NIPAm.

The presence of PA in the PVP-PA microgel (18 mol%) and PNIPAm-PA microgel (6 mol%) had a significant effect on the swelling capacity of the microgels compared to the PVP and PNIPAm ‘homopolymer’ microgels. Successful CuAAC click chemistry reactions were performed on microgel particles swollen in DMF. The reactions were close to 100% efficient and primary amine functionalisation was only limited by the amount of PA in the precursor microgel. pH-responsive behaviour did not occur in the PEA-PA-AEA microgel, despite the presence of 28 mol% ionisable primary amine groups. Possible explanations for this lack of responsive behaviour included an inadequate level of primary amine functionalisation or possible coupling reactions between acetylene groups, either during polymerisation or the CuAAC reaction, resulting in additional crosslinking and hindered swelling behaviour. Primary amine functionalisation in the PVP-PA-AEA microgel appeared to improve the swelling behaviour of the PVP-PA microgel, but not to the same level as the strongly responsive PVP microgel.
4. The preparation of azide-bearing microgels and their functionalisation using the CuAAC reaction.

4.1 Introduction

In Chapter 3 it was shown that it is possible to prepare acetylene-functionalised microgels by a batch emulsion polymerisation process. These microgels were functionalised via click chemistry in a highly efficient manner without compromising colloidal stability. Ultimately the potential for pH responsive microgel behaviour offered by significant primary amine functionalisation was not fulfilled by the PEA-PA-AEA microgel discussed in Chapter 3, which contained 28 mol% primary amine groups. Possible explanations for this included the self-crosslinking of PA during polymerisation\textsuperscript{97}, or an inadequate density of charged groups.

Considering that the complementary CuAAC reaction occurs regardless of whether a material contains terminal alkyne or azide moieties, an alternative pathway is presented in this chapter. A microgel prepared or functionalised with a significant azide concentration would in theory not be subject to the restrictive behaviour of acetylene functionalisation. There is an understandable wariness about the use of azides in some reactions due to the instability of those of low molecular weight, particularly at high concentrations and elevated temperatures\textsuperscript{63, 142-145}. However, with appropriate care, this is not an insurmountable barrier to azide-functionalised microgel preparation. Such a microgel would have an array of functionalisation possibilities. In an inverse approach to the method studied in Chapter 3, any molecule containing acetylene functionalisation could be clicked onto the azide-containing microgel particles.

There are a number of ways in which to incorporate azide functionality into polymeric materials. Methods have been reported in which the halogen end groups of polymers prepared via ATRP were replaced with azide moieties using a nucleophilic substitution mechanism\textsuperscript{144, 146, 147}. In microgel microgel systems, conversion of the epoxy groups of particles prepared using glycidyl methacrylate (GMA) has been demonstrated by the Lyon group in a one-step multi feed polymerisation process\textsuperscript{80}. In this study, NIPAm was co-polymerised with acrylic acid (AAC), GMA and \textit{N},\textit{N}'-methylene bisacrylamide (BMA) crosslinker\textsuperscript{80}. The
introduction of sodium azide, after the co-polymerisation had been allowed to proceed for 60 minutes, caused the azidation of the epoxy groups of GMA. The process was deemed an improvement on previous two-step polymerisation procedures and bypassed the complications involved in directly synthesising and isolating the azide-containing monomer 3-azido-2-hydroxypropyl methacrylate.\textsuperscript{80, 148, 149} The presence of azide functionality was then verified by FTIR spectroscopy and the clickable nature of the microgels was demonstrated through the attachment of the acetylene fluorophore propargyl thiourea.\textsuperscript{80}

The azidation of epoxy groups was similarly utilised by Slater et al. in functionalising monodisperse porous poly(glycidyl methacrylate-co-ethylene dimethacrylate) polymer beads.\textsuperscript{142} These beads had particular applications as stationary phases for the separation of peptides and proteins by hydrodynamic chromatography.\textsuperscript{142} The beads were able to immobilise alkyne-modified soy trypsin inhibitor. This demonstrated the selectivity and effectiveness of the CuAAC reaction in the context of affinity chromatography.\textsuperscript{142}

Another pathway for the azide functionalisation of microgels, which at the time of writing had not been reported, is the co-polymerisation of an azide-containing monomer to directly prepare azide functionalised microgel particles. One such monomer that has been synthesised and used in click chemistry and ATRP to prepare azide functionalised polymers is 3-azidopropyl methacrylate (AZPMa). This monomer was synthesised by Sumerlin et al. and polymerised using ATRP. This produced a polymer with a controlled molecular weight and molecular weight distribution, as well the potential further functionalisation using click chemistry.\textsuperscript{143} AZPMa has also been used to create amphiphilic block co-polymers using ATRP and click chemistry\textsuperscript{150} and in the preparation of bioconjugable nanoparticles of 5-20 nm in size via a co-polymerisation and CuAAC method.\textsuperscript{151}

This chapter aimed to investigate the preparation of azide-functionalised microgels using the azide monomer AZPMa, to prepare high primary amine content microgels using the CuAAC reaction with propargylamine and to compare the properties of these clicked primary amine microgels with the previously studied PA systems. The efficiency of the CuAAC reaction was also assessed by clicking
incremental amounts of PA onto azide-functionalised vinyl pyridine based microgels. The experimental processes that were used to prepare these microgels are described and illustrated in the following experimental methods section.
4.2 Experimental methods

4.2.1 Safety considerations for the use of azide compounds

The use of azide species must be treated with caution, carefully planning and strict control measures at all times. Azides containing olefinic bonds are significantly less stable than saturated azides\textsuperscript{62, 145} and AZPMa has been reported to be shock sensitive at temperatures exceeding 75 °C. It has, however, been used in quantities less than 5 mL in polymerisation, click chemistry and ATRP reactions at temperatures of 50 °C or greater\textsuperscript{143, 150, 151}. Methods for the emulsion polymerisation and co-polymerisation of AZPMa were adapted to adhere to these reaction criteria and trial preparations using small ratios of AZPMa were undertaken before higher content reactions were attempted. Although the general emulsion polymerisation procedure was similar to that of the microgels described in Chapter 3, changes were necessary. Firstly, the reaction was scaled down and the total monomer masses for any AZPMa preparation did not exceed 5 g. The poly(3-azidopropyl methacrylate) (PAZPMa) microgel was only prepared after trialling lower concentration reactions and the total monomer mass was limited to 1.5 g for its preparation. The temperature for the polymerisation was set at 50 °C and not the 60 °C used in Chapter 3; and the reaction length was reduced from 24 hours to 8 hours to allow for supervision of the process.

4.2.2 Materials

3-chloro-1-propanol (98%), methacryloyl chloride (≥97%), hydroquinone (≥99%) and propargylamine were purchased from Sigma Aldrich and used as received (PAm, 98%). VP, PA, DVB, AIBA, sodium azide, Cu(I)Br and PMDETA were purchased and treated as described previously in section 3.2.1.

4.2.3 Synthesis of 3-azidopropanol

3-azidopropanol (AZPOl) was synthesised following the method outlined by Sumerlin et al.\textsuperscript{143}. The preparation process is summarised in Scheme 4.1. 3-chloropropanol (30 mL, 33.9 g, 0.36 mol) was combined with sodium azide (47 g, 0.72 mol), tetrabutylammonium hydrogen sulphate (1 g, 2.95 mmol) and water (40 mL) in a 250 mL flask. Under stirring, the reaction mixture was heated to 80 °C for
24 hours. Stirring was continued for an additional 14 hours after the slurry was cooled to room temperature. Following an ether extraction the resulting solution was dried over sodium sulphate, with the solvent then removed by rotary evaporation at 20 °C. Vacuum distillation was employed to obtain the product 3-azidopropanol (AZPOI); the purity of which was confirmed using \(^1\)H NMR spectroscopy.

**Scheme 4.1: The synthetic routes used to prepare 3-azido-1-propanol (AZPOI)**

### 4.2.4 Synthesis of 3-azidopropyl methacrylate

The preparation of 3-azidopropyl methacrylate (AZPMa) from AZPOI is summarised in Scheme 4.2.

**Scheme 4.2: The synthetic route used to prepare the azide-containing monomer 3-azidopropyl methacrylate (AZPMa) from AZPOI.**

AZPOI (23.5 mL, 0.253 mol) was introduced to a mixture of triethylamine (45 mL, 0.323 mol, dried over sodium sulphate), hydroquinone (0.1 g) and dichloromethane (100 mL, dried over sodium sulphate) in a 250 mL flask under stirring, which was placed in an ice/water bath until a constant temperature of 0 °C was maintained. At this temperature, 29 mL (0.3 mol) of methacryloyl chloride was added dropwise and slowly over a period of 20 minutes. Stirring was allowed to proceed at 0 °C for 1 h and then for an additional 14 hours at room temperature. A further 100 ml of dichloromethane was added to the mixture, which was then extracted with aqueous HCl (0.3 M), followed by water, aqueous NaOH (10 wt%) and water once more. Each extraction step was performed twice. The resultant
solution was dried over sodium sulphate following the addition of hydroquinone (0.1 g) and rotary evaporation at 20 °C was used to obtain the product AZPMa.

4.2.5 Microgel preparation

The reaction compositions of each microgel prepared in this chapter are summarised in Table 4.1 and the process is illustrated in Scheme 4.3.

Scheme 4.3: Preparation of azide-functionalised microgels via the emulsion polymerisation of AZPMa and co-polymerisation of AZPMa with VP.

Microgel preparation followed a similar batch emulsion polymerisation method to the one outlined in Chapter 3, but was operated a smaller scale with altered reaction conditions to accommodate the use of the monomer AZPMa. The ratios of monomer, crosslinker, surfactant and stabiliser remained the same as used in previous preparations.

An example procedure for the preparation of the PAZPMa microgel follows. Aliquat 336 surfactant (0.15 g) and PEGMA (0.3 g, 50 wt% solution) were dissolved in 13.5 mL deionised water and the mixture was transferred to a 25 ml round bottomed flask equipped with a magnetic stirrer. AZPMa (1.480 g) and BDD (0.0202 g) were introduced to the vessel and the mixture was degassed with nitrogen for 15
minutes. The temperature of the water bath was raised to 50 °C, under stirring at 250 rpm, and the mixture was allowed to equilibrate at this temperature for 20 minutes. An AIBA initiator solution (0.015 g AIBA, 1.5 g deionised water) was added to the reaction mixture and polymerisation was allowed to proceed for 8 hours. The subsequent microgel dispersion was quenched in an ice/water bath and purified by a repeated cycle of centrifugation followed by re-dispersion in deionised water. A sample of the PVP microgel prepared in Chapter 3 was used to aid the determination of the composition of the PVP-AZPMa microgel prepared in this chapter by elemental analysis.

**Table 4.1: Preparation compositions and notations of the AZPMa-containing microgels, prepared by batch emulsion polymerisation.**

<table>
<thead>
<tr>
<th>Microgel preparation composition by mol fraction</th>
<th>Microgel notation</th>
<th>Mass of AZPMa / g</th>
<th>Mass of co-monomer / g</th>
<th>Mass of crosslinker / g</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAZPMa$<em>{0.99}$-BDD$</em>{0.01}$</td>
<td>PAZPMa</td>
<td>1.482</td>
<td>-</td>
<td>0.018</td>
</tr>
<tr>
<td>PVP$<em>{0.84}$-AZPMa$</em>{0.15}$-DVB$_{0.01}$</td>
<td>PVP-AZPMa</td>
<td>1.104</td>
<td>3.839</td>
<td>0.0566</td>
</tr>
</tbody>
</table>

**Table 4.2: Solids contents and percentage monomer conversion of the PAZPMa and PVP-AZPMa microgel systems.**

<table>
<thead>
<tr>
<th>Microgel notation</th>
<th>Microgel % solids content</th>
<th>% Monomer conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAZPMa</td>
<td>5.9</td>
<td>51.3</td>
</tr>
<tr>
<td>PVP-AZPMa</td>
<td>8.1</td>
<td>70.4</td>
</tr>
</tbody>
</table>

4.2.6 CuAAC reaction

The CuAAC reaction was carried out using the same general procedure as outlined in Chapter 3. However, in this case the PAZPMa microgel was clicked with propargylamine (PAm) and the PVP-AZPMa microgel was clicked with propargyl acrylate (PA) ranging in increments of 2, 4 and 8 mol%. The abbreviations TA-1, TA-2 and TA-3 are used respectively to denote the PA clicked microgels, which contain
‘triazole acrylate’ pendant segments. The reaction quantities used to prepare each of the clicked microgels described in this chapter are listed in Table 4.3.

Table 4.3: Reaction quantities used for the preparation of microgels ‘clicked’ with PAm or PA.

<table>
<thead>
<tr>
<th>Microgel notation</th>
<th>Mass of microgel solids / g</th>
<th>Mass of alkyne reagent a / g</th>
<th>Mass of PMDETA / g</th>
<th>Mass of Cu(I)Br / g</th>
<th>Theoretical clicked microgel composition b</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAZPMa-PAm</td>
<td>0.5</td>
<td>0.326</td>
<td>0.0511</td>
<td>0.0423</td>
<td>P(AZPMa-PAm)0.99</td>
</tr>
<tr>
<td>TA-1</td>
<td>0.5</td>
<td>0.00957</td>
<td>0.00602</td>
<td>0.00498</td>
<td>PVP-AZPMa-TA0.02</td>
</tr>
<tr>
<td>TA-2</td>
<td>0.5</td>
<td>0.0192</td>
<td>0.00602</td>
<td>0.00498</td>
<td>PVP-AZPMa-TA0.04</td>
</tr>
<tr>
<td>TA-3</td>
<td>0.5</td>
<td>0.0383</td>
<td>0.00602</td>
<td>0.00498</td>
<td>PVP-AZPMa-TA0.08</td>
</tr>
</tbody>
</table>

a The alkyne reagent was either PAm with the PAZPMa microgel of varying increments of PA for with PVP-AZPMa microgel, b theoretical clicked microgel composition if the CuAAC is assumed to be 100 % efficient.

The procedure used to functionalise the PAZPMa microgel with primary amines using a CuAAC reaction with PAm follows. The solids content of the PAZPMa microgel was calculated and a dispersion containing 0.5 g solids (2.96 mmol) was centrifuged and redispersed in DMF (10 mL), then transferred to a 25 mL Schlenk flask with a magnetic stirrer bead. PAm (0.326 g, 5.95 mmol) and PMDETA (0.0511 g, 0.296 mmol) were added to the dispersion, which was allowed to equilibrate for 3 hours to allow PAm to penetrate the swollen microgel particles. The dispersion was then subjected to two freeze-pump-thaw cycles. Cu(I)Br (solid, 0.0423 g, 0.296 mmol) was added to the flask under argon and the dispersion was stirred for 18 hours. The resultant microgel dispersion was purified by a cycle of centrifugation followed by re-dispersion in fresh deionised water. The CuAAC reaction used to prepare the alkyne functionalised PVP-AZPMa-TA x microgels differed only in the amount of PA clicked onto each microgel. These quantities are listed in Table 4.3.
The CuAAC reactions used for the preparation of these respective microgels are illustrated in Schemes 4.4 and 4.5.

**Scheme 4.4:** CuAAC reaction of the PAZPMa microgels with propargylamine (PAm), resulting in high primary amine content microgels.

**Scheme 4.5:** CuAAC reaction of the PVP-AZPMa microgel with propargyl acrylate (PA), producing alkyne-functionalised microgels.

### 4.2.7 Characterisation methods

$^1$H NMR spectroscopy was used to characterise the synthesised AZPMa. Microgel composition and properties were analysed using FTIR spectroscopy, elemental analysis, PCS, zeta potential measurements and SEM imaging. The conditions used for these instruments were described in Chapter 3 and remained unchanged, with the exception that Au/Pd coating was used to prepare microgel samples for SEM.
4.3 Results and discussion

The monomer AZPMa was synthesised according to Schemes 4.1 and 4.2. The purity of the product was analysed using $^1$H NMR spectroscopy.

4.3.1 $^1$H NMR spectroscopy characterisation of 3-azidopropanol (AZPOl) and 3-azidopropyl methacrylate (AZPMa)

The $^1$H NMR spectra of the precursor reactant AZPOl and the monomer AZPMa are displayed in Figure 4.1.

Figure 4.1: $^1$H NMR spectra of a) AZPOl and b) AZPMa.

AZPMa and its precursor AZPOl were synthesised according to the method outlined by Sumerlin et al.\textsuperscript{143} and analysed by $^1$H NMR spectroscopy in order to ascertain their purity. The peaks at 6.11 (1H), 5.58 (1H), 4.24 (2H), 3.52 (2H) and 1.91-2.02 (5H, overlapping) correspond exactly in position and integration with the values
reported in the literature\textsuperscript{143, 150}. The spectrum of the AZPOI also corresponded with reported results\textsuperscript{143, 150}. The clusters of smaller peaks visible in both spectra relate to impurities such as dichloromethane and diethyl ether that were not completely removed by the purification process. The purification methods were not performed at elevated temperatures due to the potentially volatile nature of the azide material.

4.3.2 Characterisation of azide-containing microgels

The azide-bearing monomer AZPMa was used to prepare poly(azidopropyl methacrylate) (PAZPMa) and poly(vinyl pyridine-co-azidopropyl methacrylate) (PVP-AZPMa) particles via emulsion polymerisation. The AZPMa concentration for each system was chosen with different functionalisation routes in mind. The PAZPMa microgel contained a significantly greater azide concentration than the 28 mol% acetylene functionalisation attempted in Chapter 3 in order to promote a higher maximum concentration of ionic groups following a click reaction with propargylamine. This approach aimed to alleviate the lack of a swelling response encountered with the acetylene-containing microgels reported in Chapter 3, and to introduce significant pH-triggered particle swelling to the ‘clicked’ primary amine functionalised system.

A lower concentration of 15 mol% AZPMa was used in the preparation of PVP-AZPMa microgel particles. This lower azide concentration was favoured for two reasons. Firstly, to maximise the strong pH-triggered swelling response afforded by incorporating a high concentration of pyridine residues. The CuAAC reaction was then used to introduce additional functionality to this microgel. Secondly, the efficiency of the CuAAC reaction was assessed by clicking smaller, discrete levels of functionalisation onto the microgel and this sensitive approach required a lower concentration of azide groups to be available for reaction. Many click approaches focus on generating complete functionalisation by using an excess of the azide or alkyne moiety. This method allowed for an investigation of the ability of CuAAC to precisely control the level of microgel functionalisation. The co-polymerisation of AZPMa with VP also enabled a comparison between the use of azide and acetylene...
monomers with different monomer systems in the emulsion polymerisations used to prepare polymer microgels.

4.3.2.1 FTIR characterisation of azide-containing microgels

The extent of azide functionality in PAZPMa and PVP-AZPMa microgels was investigated using FTIR spectroscopy and elemental analysis. The FTIR spectra of the azide functionalised microgels and the monomer AZPMa are displayed in Figure 4.2.

![FTIR spectra of the azide monomer AZPMa and the azide-functionalised microgels PAZPMa and PVP-AZPMa.](image)

**Figure 4.2:** FTIR spectra of the azide monomer AZPMa and the azide-functionalised microgels PAZPMa and PVP-AZPMa.

The most prominent feature of the spectra was that azide functionality introduced a sharp peak at 2095 cm\(^{-1}\) in both microgels. This provided a very clear indication of azide incorporation in the microgel systems\(^{143,152}\). In addition, the C=O stretch bond deformation of the ester group of AZPMa was represented by a characteristic absorbance peak at 1735 cm\(^{-1}\). This peak was not present in the structure of the PVP ‘homopolymer’ microgel discussed in Chapter 3 and was another clear indication of AZPMa incorporation into the PVP-AZPMa microgel.
4.3.2.2 Elemental Analysis of azide-containing microgels

Elemental analysis measurements were used to determine the composition of the azide functionalised microgels by mol %. The elemental compositions of the azide microgels are listed in Table 4.4.

Table 4.4: Elemental compositions of AZPMa monomer, the PVP microgel, the azide-containing microgels and their experimental nitrogen-to-carbon ratios (R\text{N/C}\text{(exp)}), measured by elemental analysis.

<table>
<thead>
<tr>
<th>Monomer/microgel notation</th>
<th>% Carbon</th>
<th>% Hydrogen</th>
<th>% Nitrogen</th>
<th>R\text{N/C}\text{(exp)}</th>
<th>ΔR\text{N/C}</th>
<th>R\text{N/C}\text{(corr)}</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZPMa</td>
<td>48.5</td>
<td>6.5</td>
<td>21.7</td>
<td>0.447</td>
<td>0.0503</td>
<td>-</td>
</tr>
<tr>
<td>PVP</td>
<td>75.0</td>
<td>7.4</td>
<td>11.8</td>
<td>0.158</td>
<td>0.009</td>
<td>0.167</td>
</tr>
<tr>
<td>PAZPMa</td>
<td>52.2</td>
<td>7.3</td>
<td>20.8</td>
<td>0.399</td>
<td>0.101</td>
<td>0.500</td>
</tr>
<tr>
<td>PVP-AZPMa</td>
<td>71.2</td>
<td>7.4</td>
<td>13.9</td>
<td>0.195</td>
<td>0.009</td>
<td>0.204</td>
</tr>
</tbody>
</table>

ΔR\text{N/C} represents the difference the experimentally determined and theoretical R\text{N/C} values for the parent ‘homopolymer’ microgels.

The process of deriving structure based equations to determine microgel composition and degree of functionalisation was described in Chapter 3. The main assumption of the method was that the contributions of the surfactant, stabiliser and crosslinking monomer could be accounted for by comparing the R\text{N/C}\text{(exp)} to the theoretical value of the ‘homopolymer’ microgel. The difference between these values, the ΔR\text{N/C} value, was applied to each microgel within a particular system to negate the effect of surfactant, stabiliser and crosslinking monomer on the R\text{N/C}\text{(exp)} value. This assumed that these contributions were equal for all of the microgels within a particular microgel system. The corrected experimentally measured R\text{N/C} values (R\text{N/C}\text{(corr)}), allowed the degree of functionalisation to be calculated using algebraic equations based on the structure of the polymer repeat unit for each microgel.
4.3.2.3 Composition determination of the PAZPMa microgel

Although the PAZPMa microgel could be considered a homopolymer microgel due to the very low concentration of the crosslinker BDD (1 mol%), compositional determination by elemental analysis was required to compensate for the contributions of the stabiliser PEGMA, surfactant Aliquat 336 and any residual initiator fragments. This would then allow for an estimation of the efficiency of the CuAAC reaction following the incorporation of PAm. However, microanalysis of this microgel was complicated by the fact that the nitrogen content of AZPMa exceeded that of the calibration standards of the elemental analysis instrument, acetanilide and cyclohexanone 2,4 dinitrophenyl hydrazone, by approximately 5%. Therefore a greater capacity for error was expected for this microgel system and this was indeed the case. Elemental analysis of synthesised AZPMa (values listed in Table 4.4) reported a lower $R_{N/C}$ value than expected for pure AZPMa, despite the apparent high purity expressed by $^1$H NMR spectroscopy.

The $R_{N/C(\text{exp})}$ value of the PAZPMa microgel was also significantly less than that of the synthesised AZPMa monomer due to the incorporation of low nitrogen content stabiliser, surfactant, and crosslinking monomer into a nitrogen-rich system. The $\Delta R_{N/C}$ value, i.e. the difference between theoretically pure AZPMa and the experimentally measured $R_{N/C(\text{exp})}$ value of the PAZPMa microgel, was much greater than those reported for the microgel systems studied in Chapter 3. This made it difficult to calculate the degree of azide functionality with the same certainty as for the determination of acetylene functionality for the microgels reported in Chapter 3.

Nonetheless, the azide functionality of the PAZPMa microgel was considered to be very high due to the high measured nitrogen content of the microgel and the striking similarity of its FTIR spectrum to that of the monomer AZPMa in Figure 4.2. If the $\Delta R_{N/C}$ correcting factor was to be applied for the PAZPMa microgel, it would naturally determine the extent of azide functionalisation to be 100%. Ignoring the correcting factor entirely, the azide functionalisation can be determined as 79.6% by comparing the $R_{N/C(\text{exp})}$ value of the PAZPMa microgel (0.399, Table 4.4) to the theoretical $R_{N/C(\text{thr})}$ value of pure AZPMa (0.500). These two scenarios represent a
simplistic approach based on the limitations of elemental analysis for this particular microgel system. However, in either case it can be concluded that the degree of azide functionality for the PAZPMa microgel was likely to be at 80% or greater.

4.3.2.4 Composition determination of PVP-AZPMa microgel

Due to the fact that a lower concentration of AZPMa was used in the preparation of the PVP-AZPMa microgel, there were no such problems associated with determining the composition of the microgel using elemental analysis data. The chemical composition of the polymer repeat unit of the PVP-AZPMa microgel is displayed in Figure 4.3.

\[
(C_7H_7N)_1(1-x)(C_7H_{11}N_2O_2)x
\]

**Figure 4.3: Structure of the repeat unit of the PVP-AZPMa microgel.**

The percentages of carbon and nitrogen present in the microgel were calculated using the following equations.

\[
\%C = \left(\frac{12.011 + 100}{MW}\right) \times 7
\]  

(4.1)

\[
\%N = \left(\frac{14.007 + 100}{MW}\right) \times (1 + 2x)
\]  

(4.2)

MW is the molecular weight of the polymer repeat unit of the PVP-AZPMa microgel shown in Figure 4.3. The mol fraction of AZPMa present (x) in the microgel can be expressed in terms of its nitrogen-to-carbon ratio (R\text{N/C}) using the following equation.

\[
x = \left(\frac{7R_{N/C(corr)} - 1.1662}{2.2334}\right)
\]  

(4.3)

The R\text{N/C(exp)} value was corrected using the ΔR\text{N/C} value of the PVP microgel prepared in Chapter 3. Inserting the corrected R\text{N/C(corr)} value of 0.204 (Table 4.4) into
equation 4.3 determined the concentration of AZPMa in the PVP-AZPMa microgel to be 11.7 mol%.

This concentration was less than the 15 mol% AZPMa used in the emulsion copolymerisation of the microgel. However, this method assumed that the AZPMa used in the microgel preparation was 100% pure. In reality the purity of AZPMa was likely to be closer to 90% based on the $^1$H NMR spectrum of AZPMa (Figure 4.1b)). The reported volatility of AZPMa$^{143}$ meant that purification methods were limited. The values of AZPMa purity and incorporation into the PVP-AZPMa microgel were therefore considered reasonable. The preparation of a microgel containing approximately 12 mol% azide groups provided a good platform to investigate the controlled functionalised of an azide-bearing microgel using click chemistry.

4.3.4 Characterisation of ‘clicked’ microgels

4.3.4.1 FTIR characterisation of the PAZPMa-PAm high primary amine content microgel.

The FTIR spectrum of the primary amine functionalised PAZPMa-PAm microgel was recorded and compared to those of the component reactants: the monomer AZPMa and the bifunctional acetylene and primary amine bearing molecule PAm. These spectra are displayed in Figure 4.4.

As shown in Figure 4.2 previously, the FTIR spectra of the AZPMa functionalised microgels both contained an intense absorbance peak at 2095 cm$^{-1}$ relating to the azide functional group. The spectrum of the monomer AZPMa in Figure 4.4 included a stark azide peak at 2095 cm$^{-1}$. This azide absorbance peak was also present in the spectrum of the ‘unclicked’ PAZPMa microgel in Figure 4.2. The strongest indicator of the success of the click reaction was the disappearance of this prominent azide peak in the spectra of the PAm-clicked PAZPMa-PAm microgel. The absence of such a stark peak at 2095 cm$^{-1}$ in the spectrum of PAZPMa-PAm represented clearer evidence for CuAAC reaction success than the disappearance of the weakly absorbing acetylene band in the spectra of the PA functionalised microgels studied in Chapter 3.
Further evidence for the attachment of primary amine groups to the clicked microgels was offered by the presence of a absorbance band at approximately 1595 cm\(^{-1}\) (Figure 4.4), which related to the N-H bend deformation of the primary amine group\(^{153}\). Contributions from the newly formed triazole ring may also have been represented in this region\(^{154}\). This band appeared as a shoulder to the right of the C=O stretch peaks of the methacrylate ester groups of the clicked microgel and was also present in the spectrum of PAm, but was absent in the spectrum AZPMA. The broad, weakly absorbing peak at 3400 cm\(^{-1}\) in the spectrum of the clicked microgel was also indicative of a primary amine absorption peak\(^{152}\). The complete loss of the azide peak in the spectrum of the PAZPMA-PAM microgel suggested that all of the available azide groups present in the precursor PAZPMA microgel had reacted. Given that the CuAAC reaction was performed with an excess of PAM (a 1:2 molar ratio of azide to acetylene functionalities) this result was to be expected, especially taking into account the typically high yield of the CuAAC reaction\(^6,66,155,156\).
4.3.4.2 FTIR characterisation of ‘PA-clicked’ microgels

A more sensitive approach towards microgel modification was investigated by ‘clicking’ incremental amounts of PA onto the PVP-AZPMa microgel in order to ascertain whether the high efficiency of the CuAAC reaction could be used to closely control microgel composition. This was easily investigated using FTIR spectroscopy, since the incorporation of discrete portions of PA into the PVP-AZPMa microgel resulted in an a stepwise reduction in the intensity of the azide absorbance peak as the increments of PA were increased and more azide groups were consumed to form triazole rings.

This was more visible in the reaction of an azide-functionalised microgel than an acetylene-functionalised microgel because the absorbance of the azide group in the spectra of the microgels co-polymerised with AZPMa was markedly more intense than that of the acetylene group of the microgels co-polymerised with PA in Chapter 3. Also, the azide absorbance peak of the PAZPMa and PVP-AZPMa microgels and was not masked the contributions of other functional groups (as seen in the spectrum of the PVP-PA microgel in Figure 3.3b). The FTIR spectra of the PA-clicked VP based microgels are displayed in Figure 4.5.

![FTIR spectra](image)

**Figure 4.5:** FTIR spectra of the PA-clicked TA-1 – TA-3 microgels and the precursor PVP-AZPMa (TA-0) microgel. The wavelength of the absorbency of the azide group at 2094 cm\(^{-1}\) is also shown.
The ‘unclicked’ PVP-AZPMa microgel clearly displayed the most intense azide absorbency peak at 2095 cm\(^{-1}\). The amount of PA clicked on to this microgel increased from 2 mol% in the TA-1 microgel, to 4 mol% in the TA-2 microgel and finally to 8 mol% in the TA-3 microgel. Corresponding decreases in the size of azide peak of the clicked microgels were visible as more azide groups are consumed following reaction with an increased amount of PA. There was a substantial reduction in the intensity of the azide absorbance peak between the PVP-AZPMa and TA-2 spectra. In the TA-3 spectrum the azide peak was almost entirely extinguished.

These results indicated that the amount of alkyne functionalisation, from the reaction of PA, increased from the PVP-AZPMa to the TA-2 and TA-3 microgels and this was been controlled using the CuAAC reaction. However, there was not an obvious decrease in the intensity of the azide peak in the spectrum of the TA-1 microgel. This microgel was prepared using 2 mol% PA and although this was a low level of functionalisation a small decrease in the intensity of the azide peak was expected for an efficient CuAAC reaction. As FTIR spectroscopy is not primarily a quantitative measurement technique elemental analysis was also required to determine the exact extent of alkyne group concentration in the clicked microgels.
4.3.4.3 Elemental analysis of clicked microgels

The elemental compositions of the clicked microgels were used to determine the efficiency of the CuAAC reaction and to quantify their compositions. These compositions, as measured by elemental analysis, are listed in Table 4.5.

Table 4.5: Elemental compositions of microgels clicked with PAm or PA.

<table>
<thead>
<tr>
<th>Microgel notation</th>
<th>% Carbon</th>
<th>% Hydrogen</th>
<th>% Nitrogen</th>
<th>R_{N/C}(exp)</th>
<th>ΔR_{N/C}</th>
<th>R_{N/C}(corr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAZPMa</td>
<td>52.2</td>
<td>7.32</td>
<td>20.8</td>
<td>0.399</td>
<td>0.101</td>
<td>0.500</td>
</tr>
<tr>
<td>PAZPMa-PAm</td>
<td>45.9</td>
<td>7.20</td>
<td>17.4</td>
<td>0.379</td>
<td>0.101</td>
<td>0.480</td>
</tr>
<tr>
<td>PVP-AZPMa</td>
<td>71.2</td>
<td>7.39</td>
<td>13.9</td>
<td>0.195</td>
<td>0.009</td>
<td>0.204</td>
</tr>
<tr>
<td>TA-1</td>
<td>69.8</td>
<td>6.71</td>
<td>13.8</td>
<td>0.197</td>
<td>0.009</td>
<td>0.206</td>
</tr>
<tr>
<td>TA-2</td>
<td>68.13</td>
<td>6.58</td>
<td>12.9</td>
<td>0.190</td>
<td>0.009</td>
<td>0.199</td>
</tr>
<tr>
<td>TA-3</td>
<td>66.84</td>
<td>6.68</td>
<td>12.1</td>
<td>0.181</td>
<td>0.009</td>
<td>0.190</td>
</tr>
</tbody>
</table>

The experimentally determined nitrogen to carbon ratios (R_{N/C}(exp)), ΔR_{N/C} and R_{N/C}(corr) values used to quantify the composition of the clicked microgels are also listed.

4.3.4.4 Composition determination of the primary amine functionalised PAZPMa-PAm microgel

Successful addition of PAm into the PAZPMa microgel via the CuAAC reaction would produce a microgel with a very high primary amine content. The structural formula of the repeat unit of the clicked microgel, assuming that the reaction is not 100% efficient, is \((C_7H_{11}N_3O_2)_{1-x}(C_{10}H_{16}N_4O_2)x\). This structure is illustrated in Figure 4.6.

\[
(C_7H_{11}N_3O_2)_{1-x}(C_{10}H_{16}N_4O_2)x
\]

Figure 4.6: Structure of the polymer repeat unit of the PAZPMa-PAm microgel.
Based on the structure of the polymer repeat unit of the microgel, the following equations were derived concerning the carbon and nitrogen contents of the microgel.

\[
\%C = \frac{12.011 \times 100}{MW} (7 + 3x) \tag{4.4}
\]

\[
\%N = \frac{14.007 \times 100}{MW} (3 + x) \tag{4.5}
\]

MW is the molecular weight of the PAZPMa-PAm repeat unit structure shown in Figure 4.6. The molar concentration of x, which represented the mol fraction of primary amine groups present in the microgel, was calculated using equation 4.6.

\[
x = \frac{3.4986 - 7R_{N/C(corr)}}{3R_{N/C} - 1.1662} (3 + x) \tag{4.6}
\]

Using the corrected nitrogen to carbon ratio \(R_{N/C(corr)}\) of 0.480 (Table 4.4), the mol fraction of clicked primary amine groups was determined to be 49.5 mol%. This was significantly lower than the azide functionalisation of the PAZPMa microgel and, if accurate, reflected an inefficient CuAAC reaction. This result contrasted markedly with the FTIR spectrum of the PAZPMa-PAm microgel in Figure 4.4, which showed no evidence of the intensely absorbing azide peak at 2095 cm\(^{-1}\) and strongly implied that all of the available azide groups in the PAZPMa microgel had been consumed. Given that the theoretical 100% functionalisation of poly(AZPMa) with PAm would only decrease the \(R_{N/C(thr)}\) value from 0.500 to 0.466, due to the presence of nitrogen in both species, there was a greater capacity for error associated with elemental analysis results in this case; especially considering that the high levels of nitrogen present in the PAZPMa microgel exceeded those of the calibration standards of the analysis instrument. This made accurate quantification of the extent of primary amine functionalisation difficult; however there was compelling evidence in the FTIR spectra of the PAZPMa and PAZPMA-PAm microgels to suggest that it was extremely high and that the modification of the microgel was complete.
4.3.4.5 Composition determination of ‘PA-clicked’ alkyne-functionalised microgels

The structural formula of the PA clicked VP-based microgels is illustrated in Figure 4.7.

![Structural formula of PA clicked microgels](image)

(C$_{7}$H$_{2}$N)$_{1-x-y}$([C$_{7}$H$_{11}$N$_{3}$O$_{2}$]$_{x}$[C$_{13}$H$_{17}$N$_{4}$O$_{4}$]$_{y}$)

**Figure 4.7: Structure of the polymer repeat unit of the PA-clicked ‘TA’ microgels.**

The elemental compositions of carbon and nitrogen and the mol fraction of clicked segments in the repeat unit were expressed with the following equations, using the constraint that $x + y = 0.117$ (which was the previously calculated mol fraction of azide groups in the PVP-AZPMa microgel available in the CuAAC reaction).

\[
\%C = \frac{12.011 + 100}{MW}(7 + 6y) \quad (4.7)
\]

\[
\%N = \frac{14.007 + 100}{MW}(1 + 2x + 2y) \quad (4.8)
\]

MW is the molecular weight of the TA microgel repeat unit structure shown in Figure 4.7.

\[
x = \frac{1.439 - 7R_{N/C}(corr)}{6R_{N/C}} \quad (4.9)
\]

The experimentally calculated extents of PA functionalisation for this series of microgels are listed in Table 4.6 and compared to the maximum theoretical reaction yield, which gave an indication of CuAAC reaction efficiency.
Table 4.6: Calculated efficiencies of the CuAAC reaction and the experimentally determined degree of PA functionalisation for each ‘PA-clicked’ microgel using elemental analysis data.

<table>
<thead>
<tr>
<th>Code</th>
<th>Composition</th>
<th>$R_{N/C}^{(corr)}$</th>
<th>$\alpha_{\text{thr}}^a$</th>
<th>$\alpha_{\text{exp}}^b$</th>
<th>% Click $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP-AZPMa</td>
<td>PVP-AZPMa$_{0.117}$</td>
<td>0.204</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>TA-1</td>
<td>PVP-AZPMa$<em>{0.097}$-TA$</em>{0.02}$</td>
<td>0.208</td>
<td>0.020</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TA-2</td>
<td>PVP-AZPMa$<em>{0.077}$-TA$</em>{0.04}$</td>
<td>0.199</td>
<td>0.040</td>
<td>0.0385</td>
<td>96</td>
</tr>
<tr>
<td>TA-3</td>
<td>PVP-AZPMa$<em>{0.037}$-TA$</em>{0.08}$</td>
<td>0.190</td>
<td>0.080</td>
<td>0.0956</td>
<td>120</td>
</tr>
</tbody>
</table>

$^a \alpha_{\text{thr}}$ is the theoretical maximum mol fraction of alkene groups assuming a completely efficient CuAAC reaction. $^b \alpha_{\text{exp}}$ is the experimentally determined mol fraction of alkene groups derived from elemental analysis data. $^c$ % Click is the percentage efficiency of the CuAAC reaction ($\left(\frac{\alpha_{\text{thr}}}{\alpha_{\text{exp}}}\right) \times 100$).

Elemental analysis of the PA-clicked microgels affirmed the findings of the FTIR spectra for the series of microgels. The results strongly suggested that the CuAAC reaction was not successful for TA-1 microgel. A successful CuAAC reaction would have given the microgel a 2 mol% alkyne group functionalisation but there was not a decrease in the intensity of the azide absorbance peak in the FTIR spectrum of the microgel (Figure 4.5) and the value of $R_{N/C}^{(corr)}$ for did not decrease following the CuAAC reaction, in fact it increased marginally. Experimental error was most likely the cause of this rather anomalous result. However the $R_{N/C}^{(corr)}$ ratios of the TA-2 and TA-3 microgels, which were prepared using 4 and 8 mol% PA respectively, did decrease as expected following the CuAAC reaction with PA. The final compositions of these microgels were calculating as containing 3.85 and 9.56 mol% pendant alkyne groups respectively through reaction with PA. The efficiency of the CuAAC reaction was likely very high, especially as the efficiency of the reaction appeared to exceed 100% for the TA-3 microgel. These values agreed with the corresponding loss of the azide absorbance peaks in the FTIR spectra of the microgels following the click reaction in Figure 4.5. The small azide peak remaining in the spectrum of
the TA-3 microgel was consistent with the concentration of azide groups that would be present following consumption of 9.5 out of the 11.7 mol% azide groups in the PVP-AZPMa microgel. Objectively, these results suggested that the CuAAC reaction can be applied to efficiently tune microgel composition, although better experimental procedures were necessary for finer control over all microgel systems.

4.3.5 Physical characterisation of microgels

4.3.5.1 Particle size characterisation of azide and primary amine functionalised microgels

Microgels with a significant degree of primary amine functionalisation were expected to display pH-triggered swelling due to the protonation of the amine groups under acidic conditions. This behaviour was not observed for the primary amine microgels studied in Chapter 3, which had a maximum of 28 mol % primary amine groups. However, Tiwari et al. demonstrated that pH responsive microgels can be prepared from propargyl acrylate microgels using the CuAAC reaction\textsuperscript{56}. The addition of 2-azido-\textit{N},\textit{N}-dimethylethylamine, a tertiary amine, resulted in a maximum particle swelling from 202 nm, in the collapsed state at pH 10 to 292 nm when swollen at pH 3 \textsuperscript{56}. These clicked microgels had a degree of functionalisation of 70 to 90% and so it was highly likely that the microgels prepared in Chapter 3 did not contain a large enough proportion of primary amine groups to elicit a swelling response.

It has also been reported that acetylene group crosslinking can occur during the emulsion polymerisation of microgels based upon propargyl acrylate\textsuperscript{56, 97, 131} and this could result in a restriction in the maximum possible extent of swelling of microgels prepared using click chemistry, due to physical crosslinking restrictions and a minor reduction in the number of acetylene groups available for reaction. The PAZPMa microgel prepared in this chapter was not subject to possible acetylene crosslinking because the alkyne species was introduced during the CuAAC reaction and was therefore not a part of the polymerisation process. As the FTIR spectrum of the PAZPMa-PAm microgel indicated that complete primary amine functionalisation was achieved, this method represented an opportunity to examine the limits of swelling of primary amine microgels manufactured by click chemistry. The
hydrodynamic diameters of the ‘clicked’ primary amine functionalised PAZPMa-PAm microgel and the precursor PAZPMa based microgel at variable pH are displayed in Figure 4.8. The microgel swelling ratio, $Q$, is defined again in equation 4.10.

$$Q = \left( \frac{d_h}{d_c} \right)^3$$

In equation 4.10 $d_h$ is the hydrodynamic diameter of a microgel particle and $d_c$ is the collapsed particle diameter.

**Figure 4.8:** a) Variation in particle hydrodynamic diameter ($d_h$) with pH for the ‘unclicked’ PAZPMa microgel and the ‘clicked’ primary amine functionalised PAZPMa-PAm microgel. b) Variation in swelling ratio ($Q$) with pH for the PAZPMa-PAm microgel.

It was clear from Figure 4.8 that pH-triggered swelling occurred for the PAZPMa-PAm microgel. As expected, the precursor PAZPMa microgel did not exhibit any pH responsive behaviour and its average hydrodynamic diameter remained constant at approximately 135 nm across the pH range. The PAZPMA-PAm microgel exhibited significant swelling at pH 7 and below. The average particle diameters measured below pH 7 were over 130 nm greater than those measured at pH 9 and above. The average particle size increased from 227 nm at pH to a maximum of 373 nm when swollen at pH 5. The microgel appeared to be fully swollen at pH 7 and no significant further swelling was observed at pH 5 or pH 3. The $pK_a$ of
propargylamine is 8.15\(^{157}\) so the transition of the microgel from to a fully swollen state between pH 9 and pH 7 correlated well.

The swelling ratios (Q) of the clicked microgel reached a maximum of 4.80 for the PAZPMa-PAm respectively under acidic conditions. The swelling ratio was calculated using the collapsed particle diameter. Due to the hydrophilic structure of the primary amine clicked microgels it can be reasoned that the microgel particles were not fully collapsed at high pH and contain significant amounts of solvent. This accounted for the significantly greater sizes of the clicked microgels compared to the ‘unclicked’ microgels at basic pH. The PAZPMa microgels had an average particle size of approximately 130 nm across the pH range. However, this size increased to a minimum size of 225 nm for the PAZPMa-PAm microgel in the collapsed state. Part of this increase in size can be explained by the increase in material instigated by the addition of PAm to the clicked microgels\(^{56}\), however this alone is not enough to cause a size increase of 60-90 nm. It is reasoned that the ability of the ‘clicked’ microgels to form stronger polymer-solvent interactions, due to the presence of large concentrations of hydrophilic primary amine groups (and triazole rings), has contributed to the increase in particles observed at basic pH compared to the ‘unclicked’ precursor microgel.

### 4.3.5.2 Zeta potential of AZPMa and primary amine functionalised PAZPMa-PAm microgels

The zeta potentials of the PAZPMa and PAZPMa-PAm microgels were also measured at variable pH. These results are displayed in Figure 4.9. The zeta potential measurements corroborated the pH-triggered swelling observed at pH 7 and below for the PAZPMa-PAm microgel. At pH 9 and above there was little difference between the zeta potentials of the ‘unclicked’ and ‘clicked’ microgels. However, below the pKa of propargylamine (8.15), the primary amine groups of the clicked microgels became protonated and this was reflected by the strongly positive zeta potentials values of the ‘clicked’ microgels. The zeta potential values of the PAZPMa microgel at acidic pH were significantly less positive. This was clearly visible in the results; zeta potentials greater than 30 mV are present for the
PAZPMa-PAm microgel at pH 7 and below, whereas the maximum zeta potential of the PAZPMa microgel was 12.6 mV at pH 3.

**Figure 4.9**: Variation in zeta potential with pH for the ‘unclicked’ PAZPMa microgel and the ‘PAm-clicked’ PAZPMa-PAm microgel.

The PAZPMa-PAm system represented a microgel that contained close to a maximum level of primary amine functionalisation. The swelling of this microgel could not have been hindered by additional crosslinking or a loss of possible functionalisation sites due to acetylene side reactions and so represented the limit of ionisable primary amine groups in a microgel prepared using click chemistry. The swelling ability of the PAZPMa-PAm microgel was highly similar to those of the secondary amine functionalised microgels prepared by Tiwari et al.\(^{56}\) by ‘clicking’ poly(propargyl acrylate) microgels with 2-Azido-\(N,N\)-dimethylethylamine. These particles increased in diameter from 202 nm at pH 10 to a maximum of 293 nm at pH 3. When combined, these results suggest that alkyne self-crosslinking either does not occur to a significant extent during emulsion polymerisation at 60 °C, or, if indeed present, is not a significant hindrance to particle swelling. It was also worth noting that high levels of amine functionalisation (greater than 50 mol% per polymer repeat unit) were necessary to elicit a pH-triggered swelling response. Therefore, it was likely that the clicked PEA-PA-AEA system, studied in Chapter 3, did not contain the requisite charge density necessary to result in pH induced particle swelling, even with 28 mol% primary amine groups present. One way to
potentially further improve the swelling response of the PAZPMa-PAm microgel would be to lower the concentration of crosslinking monomer to below 1 mol%. This concentration of crosslinking monomer was kept constant in this study to allow for a comparison to the acetylene functionalised microgels studied in Chapter 3, but adjusting the degree of crosslinking monomer can have a significant effect on the swelling ratio of pH-responsive microgels\textsuperscript{4, 18, 158}. The swelling ratio observed for a microgel system based on poly(acrylic acid) increased from approximately 2.5 to 12.5 upon deceasing the concentration of crosslinking monomer from 1 mol% to 0.1 mol% respectively\textsuperscript{158}. The swelling capacity of PVP microgels has also shown to be dependent on the concentration of crosslinking monomer, with a strong reduction in swelling capacity observed in particles containing greater than 1 mol% crosslinking monomer\textsuperscript{159}.

The increase in particle size upon swelling displayed by the PAZPMa-PAm microgel was nonetheless certainly comparable to the high primary amine content particles prepared by Thaiboonrod et al. using the hydrolysis of poly(N-vinylformamide-co-2-(N-vinylformamido) ethyl ether microgels to poly(vinylamine-co-bis(ethyl vinlyamine)ether)(PVAM-xBEVAME) microgel dispersions\textsuperscript{7}. These microgels contained a mol % of polyvinylamine (and therefore primary amines) of 69-90 % and swelling was influenced by the amount of crosslinker present, with a maximum swelling of approximately 0.8 μm at pH 12 to 1.8 μm at pH 3 \textsuperscript{7}. The resultant microgel responses were similar to those reported by Shi and Berkland for PVAM microcapsules (0.8 μm at pH 11 to 1.25 μm at pH 7)\textsuperscript{117}. The pKa of polyvinylamine is approximately 10 and so transition to the fully swollen state occurred at a lower pH for this propargylamine system.

Despite reasonable pH-responsive behaviour, it was not feasible for the PAZPMa-PAm microgel to be further investigated for use as an enzyme responsive material due to the problems associated with synthesising and polymerising AZPMa in significant quantities. However, this system did provide a greater understanding of the use of click chemistry in the bulk modification of microgels in preparing pH responsive materials.
4.3.5.3 Particle size characterisation of the PVP-AZPMa series of microgels

The pH-responsive behaviour of the PVP-AZPMa series of microgels was investigated using PCS, with the results shown in Figure 4.10.

Figure 4.10: Variation in hydrodynamic diameter with pH for the ‘unclicked’ PVP-AZPMa and ‘PA-clicked’ microgels.

Cationic swelling was exhibited by all of the PVP-AZPMa and ‘PA-clicked’ microgels. However, the maximum extent of particle swelling was lower for all systems compared to that of PVP microgel particles. In addition, there were differences in swelling responses between the ‘unclicked’ and ‘clicked’ microgels. At pH 7 and above the average size of of the microgels was approximately 340 nm. This was similar to the 370 nm size of the PVP particles based on those of Dupin et al.\(^\text{18}\) and studied in Chapter 3. The swelling response of these microgels broadly followed that of the major constituent of the microgels, poly(vinyl pyridine). The maximum size of the particles in the fully swollen state was also lower, at approximately 790 nm compared to 1200 nm for PVP particles, due to the reduction in the density of ionisable groups caused by the incorporation of AZPMa. The presence of hydrophobic domains within microgel particles, via the incorporation of hydrophobic monomers, has also been reported as a cause of limited microgel swelling\(^\text{20}\). In this instance, hydrophobic domains act as associative crosslinking and restricted the swelling capacity of microgels containing a significant concentration
of ionisable groups. The microgel swelling ratio (Q) decreased further following the CuAAC reaction between the PVP-AZPMa microgel and increments of PA. A Q value of 14.5 was recorded for the PVP-AZPMa microgel at pH 3 but this decreased to 7.0 for the T-2 microgel and 5.9 for the T-3 microgel, which were found to contain 3.85 and 9.56 mol% of reacted PA respectively. In comparison, the Q value of the PVP microgel discussed in Chapter 3 was 36.3 at pH 3.

These findings suggested that the incorporation of moderate amounts of hydrophobic monomer into the PVP microgel system had a significant effect on the swelling capacity of the microgels, which was further restricted by the incorporation of hydrophobic material via the CuAAC reaction. The results were consistent with those of a study by Loxley and Vincent, in which the swelling capacity of PVP microgels was shown to be strongly reduced by the incorporation of increasing amounts of styrene co-monomer. In addition, this implied that crosslinking reactions between the acetylene groups of PA were not the primary contributing factor to the severely restricted swelling capacity of the PVP-PA microgel containing 18 mol% PA discussed previously in Chapter 3. The swelling capacity of pH-responsive microgels is a crucial property, therefore microgel composition and the effects of hydrophobic co-monomers such as PA should be carefully considered when preparing responsive microgels using the CuAAC reaction.

4.3.5.4 Zeta potential measurements of the PVP-AZPMa and ‘PA-clicked’ microgels

The zeta potentials of the ‘PA-clicked’ microgels and the precursor PVP-AZPMa microgel were measured across the pH range. The results are displayed in Figure 4.11.

A broadly similar trend was observed for all of the microgels. Microgel zeta potentials became markedly more positive below pH 4. The results followed the expected pattern of the major component of the microgels, PVP. The pKₐ of pure PVP is 4.1. Protonation pyridine groups resulted in strongly positive zeta potentials at low pH. The results of the PVP-AZPMa microgel matched those recorded by Dupin et al. for PVP microgels, so the addition of approximately 12
mol% AZPMa did not appear to have had a significant effect on the surface charge profile of the microgel.

Figure 4.11: Zeta potential measurements of the PVP-AZPMa and ‘PA-clicked’ microgels measured at variable pH.

The incorporation of incremental amounts of PA to microgel through the CuAAC reaction resulted in lower zeta potentials across the pH range. The zeta potential of the PVP-AZPMa microgel was significantly more positive at pH 4 (24.4 mV) than those of the TA-2 and TA-3 microgels. This was attributed to a dilution of the density of ionisable groups in the ‘PA-clicked’ microgels and a shift in pKₐ caused by the introduction of hydrophobic material.
4.3.5.5 SEM characterisation of the ‘unclicked’ PVP-AZPMa microgel and the ‘PA-clicked’ microgels

SEM images of the unclicked PVP-AZPMa and PA-clicked microgels are displayed in Figure 4.12.

Figure 4.12: SEM images of unclicked PVP-AZPMa and clicked TA-1 – TA-3 microgels. a) PVP-AZPMa, b) TA-1, c) TA-2, d) TA-3.

SEM images of the PVP-AZPMa microgels and PA-clicked microgels recorded spherical, uniform particles. There were no significant differences in size or morphology between the clicked and unclicked microgels, or in the particle sizes measured by PCS. The average size of the PVP-AZPma microgel measured by SEM was 324 nm; which correlated with the 340 nm value measured by PCS at pH 7. The lack of difference between the microgels was to be expected due to the relatively low levels of functionalisation and chemical alteration caused by the CuAAC reaction; however it is beneficial that the reaction did not appear to have any detrimental effect on particle size, morphology or stability.
Table 4.7: Average particle diameters of the PVP-AZPMa and TA-1 – TA-3 microgels measured using SEM and PCS.

<table>
<thead>
<tr>
<th>Microgel code</th>
<th>$d_h(\text{PCS})^a$</th>
<th>$d_c(\text{SEM})^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVP-AZPMa</td>
<td>340</td>
<td>324</td>
</tr>
<tr>
<td>TA-1</td>
<td>348</td>
<td>308</td>
</tr>
<tr>
<td>TA-2</td>
<td>325</td>
<td>307</td>
</tr>
<tr>
<td>TA-3</td>
<td>384</td>
<td>313</td>
</tr>
</tbody>
</table>

$^a$ $d_h(\text{PCS})$ is the average hydrodynamic particle diameter measured using PCS, $^b$ $d_c(\text{SEM})$ is the average collapsed particle diameter measured using SEM.
4.4 Conclusions

The work of this chapter has outlined a strategy to prepare azide functionalised microgels of varying concentration using the one-pot, batch emulsion polymerisation and co-polymerisation of the azide monomer AZPMa. Building on chapter 3, high primary amine microgels were prepared using CuAAC reaction between the azide functionalised microgels and PAm that could not be subject to swelling restrictions by crosslinking between acetylene groups, as has been reported for PA based microgels.131

It was possible to prepare pH responsive microgels when the concentration of primary amines in the clicked microgels exceeded 50 mol%. This microgel was fully swollen at pH 7 and below. When combined with the work of Tiwari et al. and the results of Chapter 3, it was suggested that high concentrations of amine groups were necessary in order to elicit a significant swelling response in microgels prepared using click chemistry and that the swelling capability of microgels prepared using PA was not limited as much by the possible crosslinking of acetylene groups during polymerisation as by the overall concentration of ionisable groups.

Whilst significant, the swelling response demonstrated by these primary amine microgels may not be enough for them to be used in biological applications. Naturally the azide functionalised microgels prepared in this chapter would not be well suited to this application in any event due to the hazards associated with materials containing a high azide concentration and the synthesis of AZPMa. However these experiments did provide a greater understanding of the limits of click chemistry in preparing pH responsive microgels by bulk polymer modification and the significance of acetylene crosslinking during polymerisations. It was speculated that the overall swelling response of microgels prepared using either the CuAAC reaction between PA microgels with AEA or AZPMa microgels with PAm would be similar and the former reaction route may be useful in preparing microgels for enzyme responsive applications.

The efficiency of the CuAAC reactions was also explored by clicking incremental portions of PA onto the PVP-AZPMa microgel. FTIR and elemental analysis of these microgels was simple to achieve and showed that control over
microgel compositions was possible using CuAAC. It was found that incorporating approximately 12 mol% AZPMA into the PVP microgel system reduced the swelling capacity of the resultant microgel. The swelling capacity was further restricted by the introduction of up to 9.56 mol% PA via the CuAAC reaction. However, control over microgel composition was demonstrated. This concept is explored further in Chapter 5, which aims to introduce tuneable mechanical properties to double crosslinked microgels using the control over microgel composition afforded by the high efficiency of the CuAAC reaction.
5. Double crosslinked microgels prepared via CuAAC

5.1 Introduction

In Chapters 3 and 4 it was demonstrated that the CuAAC reaction can be used to functionalise microgel particles with a high degree of efficiency and without compromising colloidal stability. Methods for the one-pot preparation of microgels containing a significant proportion of both azide and alkyne species were also described. Whilst the efficiency and versatility of the click reaction were proven to be very successful in generating polymer particles with useful functionalities, challenges in maximizing the beneficial effects of the clicked moieties arose from the inherent properties of the component functional groups. Any demonstrable responsive behaviour of polymer microgels ultimately arises from the sum of all of the functionalities represented within the particle and not just a single species, amongst other factors. Considering this, a strong element of design control is necessary in creating polymer architectures with maximised desirable properties.

Two emerging and similar classes of polymer gels with impressive mechanical properties are double network (DN) hydrogels and double crosslinked (DX) microgels. DN gels consist of two interpenetrating polymer components, a densely crosslinked polyelectrolyte polymer within a ductile and loosely crosslinked neutral polymer, while DX microgels contain inter-particle covalent bonds between the entangled peripheries of swollen particles in addition to intra-particle crosslinking. These gels have considerably improved the mechanical properties of traditional polymer hydrogel networks and offer improvements in the biomedical applications of artificial load bearing tissue and muscle tissue engineering.

There are several routes towards preparing mechanically strong gel networks. The method pioneered by Gong et al. for DN gel formation involved the preparation of the polyelectrolyte poly(2-acrylamido-2-methylpropanesulphonic acid) then immersing the gel in a monomer solution of poly(acrylamide) and polymerising an interpenetrating second polymer network within the swollen polyelectrolyte gel. Malkoch et al. demonstrated that doubly crosslinked poly(ethylene glycol) (PEG) hydrogel networks with tuneable mechanical properties could be manufactured using click chemistry through the reaction of diacetylene...
and tetraazide-functionalised PEG derivatives. This introduced crosslinking into the hydrogel network, with PEG chosen as the main structural component of the gels due to its high hydrophilicity and biocompatibility. An alternative method was presented by McCann et al., which utilised the imine bond-forming reaction between the aldehyde groups of partially oxidised dextran and the primary amine groups of poly(vinylamine-co-bis(ethyl vinylamine) ether) cationic microgel particles. Network degradation was induced through the acidic cleavage of the imine bonds at low pH.

The Saunders group has been successful in preparing double crosslinked microgel systems based on particles of methacrylic acid copolymerised with either methyl methacrylate or ethyl acrylate as a structural monomer and ethyleneglycol dimethacrylate or butanediol diacrylate as a crosslinker. These particles were functionalised with glycigyl methacrylate (GMA) in order to introduce exposed glycidyl groups to the microgel periphery. Lowering the pH induced particle swelling and triggered the formation of a physical gel as the peripheries of swollen microgel particles overlapped. This brought the pendant glycidyl groups of neighbouring particles into close proximity with one another. Heating the gel in the presence of ammonium persulphate (APS) initiator caused the formation of a permanent, covalently bonded double crosslinked microgel. These gels have been developed towards use in the specific biomedical application of restoring structure and function of load bearing tissue, such as damaged or degenerated intervertebral discs.

In a variation of this theme, colloidal graphene oxide was introduced to anionic poly[(ethyl acrylate)-co-(methacrylic acid)] and significantly improved the mechanical properties of the double crosslinked microgel network. A cationic DX gel network based on the GMA functionalisation of poly(vinyl amine) microgel particles has also been reported with interesting porosity features and may also have biomedical applications.

A specific advantage of the click chemistry approach towards alkyne functionalisation of microgel particles and subsequent DX microgel formation is that the high efficiency of the reaction allows precise quantitative control over the
extent of functionalisation and therefore should enable enhanced tuneability of the mechanical properties of the DX gels formed. Although click chemistry has been used previously as a tool for microgel and nanoparticle functionalisation\textsuperscript{77, 80}, the alkyne functionalisation of microgel particles using click chemistry has not been reported. The aim of this chapter is to expand the range of DX microgels from anionic acrylate based systems to include cationic vinyl pyridine DX gels and investigate whether the mechanical properties of the gel can be controlled in a facile manner through click chemistry functionalisation.

In this chapter, a variation of the acetylene-bearing PVP-PA microgel studied discussed in Chapter 3 was clicked with varying amounts of the monomer AZPMa, which was synthesised and characterised in Chapter 4. The compositions of the clicked microgels were determined using FTIR spectroscopy and elemental analysis techniques to quantify the extent of alkyne functionalisation. The pH-dependent properties of the microgels were then investigated using PCS and zeta potential measurements, before the morphologies and mechanical properties of physical and doubly crosslinked gels were examined using SEM and dynamic rheology respectively. The overall synthetic scheme of this work is presented in Scheme 5.1 and comprises the preparation of PVP-PA microgels by emulsion polymerisation, their alkyne functionalisation using click chemistry and the formation of physical and DX gels under acidic conditions.
Scheme 5.1: Experimental scheme for the preparation of DX covalent gels. a) The preparation of PVP-PA particles via emulsion polymerisation, b) the alkyne-functionalisation of PVP-PA particles via CuAAC reaction with AZPMA, c) the formation of DX microgels through particle concentration, pH decrease and heating with AIBA initiator.
5.2 Experimental methods

5.2.1 Materials

All materials were purchased from Sigma Aldrich with the exception of DVB, which was purchased from VWR. VP (97% purity) and PA (97%) were purified by passing chloroform solutions of the monomers through columns filled with neutral alumina, followed by solvent removal under reduced pressure. Aliquat 336, PEGMA, AIBA (97%), DVB (85%) and PMDETA (99%) were used as received.

5.2.2 Synthesis of AZPMa

The synthesis of AZPMa was carried out as per the method described in Chapter 4. AZPMa was stored at low temperature under an argon atmosphere with hydroquinone present to prevent polymerisation.

5.2.3 Preparation of alkyne-functionalised PVP-PA microgel

The PVP-PA microgel, henceforth referred to as P-0, was synthesised by emulsion polymerisation using a modification of the method previously employed\textsuperscript{166}. The PA concentration used in this study was significantly lower than the PVP-PA microgel prepared in Chapter 3 in order to maximise the pH-triggered microgel swelling provided by a high density of pyridine residues. The method used was as follows. The surfactant Aliquat 336 (1.5 g) and stabiliser PEGMA (3 g, 50 wt% solution) were dissolved in deionised water (120 ml). This mixture was transferred to a reaction vessel fitted with an overhead stirrer set at 250 rpm, heated to 60 °C and degassed with nitrogen for 20 minutes. A co-monomer mixture of VP (10.72 g, 0.102 mol.), PA (2.18 g, 0.020 mol.) and DVB (0.096 g, 0.74 mmol) was introduced, followed by the addition of AIBA initiator (0.15 g in 15 mL of water). Polymerisation was continued for 24 hours. The resultant microgel was purified via a repeated cycle of centrifugation at 6000 rpm for 30 minutes followed by re-dispersion in deionised water.

The solids content of the PVP-PA microgel was calculated as 7.8 % and the overall monomer conversion was 67.8%.
5.2.4 Functionalisation of PVP-PA microgels with AZPMA by CuAAC

The P-0 microgel was characterised using FTIR spectroscopy and elemental analysis in order to quantify the degree of acetylene functionalisation. Incrementally increasing portions of AZPMA were clicked onto batches of the P-0 microgel. This produced a series of microgels with varying alkene functionalisation. The compositions of these microgels and their notations are listed in Table 5.1.

Table 5.1: Notations and compositions of microgels with varying extents of alkene group functionalisation.

<table>
<thead>
<tr>
<th>Microgel code</th>
<th>Composition by mol fraction a</th>
<th>( \alpha_{(\text{thr})} ) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-0</td>
<td>PVP-PA_{0.07}</td>
<td>-</td>
</tr>
<tr>
<td>P-1</td>
<td>PVP-PA_{0.05}-TMa_{0.02}</td>
<td>0.020</td>
</tr>
<tr>
<td>P-2</td>
<td>PVP-PA_{0.04}-TMa_{0.03}</td>
<td>0.040</td>
</tr>
<tr>
<td>P-3</td>
<td>PVP-PA_{0.02}-TMa_{0.05}</td>
<td>0.060</td>
</tr>
<tr>
<td>P-4</td>
<td>PVP-TMa_{0.07}</td>
<td>0.073</td>
</tr>
</tbody>
</table>

a The term TMa is used to denote the structure of the moiety formed by the reaction of the acetylene group of PA and the azide group of AZPMA. This structure is highlighted in Scheme 5.1c). b \( \alpha_{(\text{thr})} \) represents the theoretical alkene functionalisation by mol fraction of the clicked microgels, assuming 100% CuAAC reaction efficiency.

The parameters of the CuAAC reaction used to prepare each alkene functionalised microgel differed only in the mass of AZPMA used in each case. The method used to prepare the P-1 microgel is now described. The P-0 microgel (1 g solids) was redispersed in DMF (20 mL) following centrifugation and transferred to a 50 ml Schlenk flask. AZPMA (0.032 g, 0.19 mmol) and PMDETA (0.0165 g, 0.095 mmol) were added and the solution was subjected to two freeze-pump-thaw cycles before an Ar atmosphere was introduced. The Cu(I)Br catalyst (0.0136 g, 0.095 mmol) was added and the solution stirred for 16 h under an argon atmosphere at room temperature. The resultant microgel dispersion was purified by repeated
centrifugation and re-dispersion in water.

### 5.2.5 Formation of DX gels

Dispersions of microgels P-0 – P-4 (pH ~ 6) were concentrated to 10 wt% using centrifugation following calculation of their total solids contents. AIBA initiator (9.0 mg, 0.033 mmol) was added to each dispersion (1.5 g solids), which were then thoroughly mixed using a vortex mixer. The pH of the dispersion was lowered to 3.0 by addition of aqueous HCl (4 M). This triggered physical gel formation. The physical gels were placed in O-rings with 20 mm diameters and 2 mm wall thicknesses and sealed between glass plates, which were clamped together using clips. These constructions were placed in an oven at 50 °C for 12 h.

### 5.2.6 Physical measurements

PCS, zeta potential, FTIR spectroscopy, SEM and elemental analysis measurements were carried out as described in Chapter 3.

#### 5.2.6.1 Dynamic rheology

Dynamic rheology measurements were carried out on a TA Instruments AR G2 temperature-controlled rheometer fitted with a 20 mm diameter parallel plate geometry. The gap between plates was set at 1800 μm. A frequency of 1 Hz was used for the strain sweep measurements and a strain of 0.1 % was used for the frequency sweep measurements. The measurement temperature was 25 °C was for all samples.
5.3 Results and discussion

5.3.1 Microgel compositional characterisation by FTIR spectroscopy

The acetylene-functionalised PVP-PA microgel and the series of ‘AZPMa-clicked’ microgels were characterised using FTIR spectroscopy. A selection of relevant spectra is displayed in Figure 5.1.

Figure 5.1: FTIR spectra of selected microgels and monomers including those of the monomers PA and AZPMa, the PVP microgel, the acetylene-functionalised P-0 microgel and the AZPMA-clicked P-4 microgel.

The incorporation of PA into the PVP-PA microgel was once again indicated by the presence of the ester C=O stretch absorption peak at 1730 cm\(^{-1}\). This bond is present in the ester group of PA but absent in the structure of VP and can therefore be used indirectly to confirm the presence of PA (and therefore acetylene group functionalisation) in the P-0 microgel.

Two features denoted the presence of alkene group functionality in the spectra of the ‘AZPMA-clicked’ microgels. The most direct marker was the presence of the C=C stretch absorption peak at 1635 cm\(^{-1}\). However, this was an extremely weakly absorbing peak, even in the spectra of the pure monomers AZPMA and PA,
and so it did not make a significant contribution to the spectra of the clicked microgels. Figure 5.2 focuses on the relevant region of the absorbance spectrum of the AZPMa-clicked microgels.

![FTIR spectra of clicked microgels P-1 – P-4 focusing on the region of the spectra from 1500 – 1800 cm\(^{-1}\) containing C=O and C=C bond absorbance peaks.](image)

There was a suggestion of a very weakly absorbing, broad peak at 1635 cm\(^{-1}\) in the spectra the of P-3 and P-4 microgels (the clicked microgels containing the highest alkene group concentrations). Given the very low intensity of the peak in the pure monomer it was not reasonable to expect this peak to be any larger, when maximum feasible alkene group concentration cannot have exceeded 7.3 mol\%. This absorption peak alone did not constitute compelling evidence of alkene group functionalisation and so other indicators were examined elsewhere in the spectra.

An additional indicator of the incorporation of AZPMa into the ‘clicked’ microgels was increase in the area and intensity of the C=O stretch peak between 1715 and 1730 cm\(^{-1}\). This was used as a marker for AZPMa incorporation because the structures of both AZPMa and PA (shown previously in Scheme 5.1) contain C=O bonds in ester groups. Therefore, as increasing increments of AZPMa were
incorporated into the PVP-PA microgel through the CuAAC reaction, the intensity of the C=O absorbance peak also increased. This increase appeared to occur in equal and sequential portions as equal increments of AZPMa were incorporated in the P1 – P-4 microgels.

There were also differences in the C=O peak position corresponding to the amounts of PA and AZPMa present. The stretching frequency of the C=O bond of PA was 1735 cm\(^{-1}\) when present in the PVP-PA microgel, while a lower frequency of 1715 cm\(^{-1}\) was evident for the C=O bond of AZPMa. This difference was attributed to the differing distances of each species to the triazole ring of the clicked microgel. As increasing amounts of AZPMa were incorporated in the P-1 – P-4 microgel, the median position of the C=O absorption peak shifted from 1730 cm\(^{-1}\) towards 1715 cm\(^{-1}\) (Figure 5.2).

It was also important that the azide group absorption peak at approximately 2100 cm\(^{-1}\) was not present in the spectra of any of the ‘AZPMa-clicked’ microgels. This confirmed that unreacted AZPMa was not present in the ‘clicked’ microgel dispersions. FTIR is primarily used as qualitative analysis technique. Therefore it was necessary to corroborate the observations using FTIR spectroscopy with elemental analysis data in order to accurately determine the final microgel compositions of the alkene-functionalised microgels.
5.3.2 Microgel compositional characterisation by elemental analysis

The efficiency of the CuAAC reaction and the degree of alkene functionalisation of the clicked microgels were calculated using elemental analysis data and compared to theoretical compositions of the microgels. The experimentally determined microgel compositions were calculated using the method previously discussed Chapters 3 and 4. The basis of the method was that the addition of the nitrogen-rich AZPMa to the clicked microgels caused an increase in the ratio of nitrogen to carbon (R\textsubscript{N/C}). ‘Clicking’ a larger increment of AZPMa onto each microgel resulted in a corresponding increase in the nitrogen-to-carbon ratio. The experimentally determined elemental compositions of all of the microgels examined in this chapter are listed in Table 5.2.

Table 5.2: Elemental compositions of the PVP microgel \textsuperscript{a}, the acetylene-functionalised PVP-PA microgel (P-0) and the ‘AZPMa-clicked’ alkene-functionalised microgels (P-1 – P-4), measured by elemental analysis.

<table>
<thead>
<tr>
<th>Code</th>
<th>Composition</th>
<th>% Carbon</th>
<th>% Hydrogen</th>
<th>% Nitrogen</th>
<th>R\textsubscript{N/C}(exp) \textsuperscript{b}</th>
</tr>
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<tbody>
<tr>
<td>PVP \textsuperscript{a}</td>
<td>PVP</td>
<td>75.0</td>
<td>7.4</td>
<td>11.83</td>
<td>0.158</td>
</tr>
<tr>
<td>P-0</td>
<td>PVP-PA\textsubscript{0.07}</td>
<td>75.6</td>
<td>7.1</td>
<td>11.1</td>
<td>0.146</td>
</tr>
<tr>
<td>P-1</td>
<td>PVP-PA\textsubscript{0.05-TMA\textsubscript{0.02}}</td>
<td>73.3</td>
<td>6.9</td>
<td>11.2</td>
<td>0.152</td>
</tr>
<tr>
<td>P-2</td>
<td>PVP-PA\textsubscript{0.04-TMA\textsubscript{0.03}}</td>
<td>73.0</td>
<td>6.7</td>
<td>11.5</td>
<td>0.157</td>
</tr>
<tr>
<td>P-3</td>
<td>PVP-PA\textsubscript{0.02-TMA\textsubscript{0.05}}</td>
<td>71.6</td>
<td>6.7</td>
<td>11.8</td>
<td>0.165</td>
</tr>
<tr>
<td>P-4</td>
<td>PVP-TMA\textsubscript{0.07}</td>
<td>68.2</td>
<td>6.5</td>
<td>11.8</td>
<td>0.173</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The PVP microgel was previously discussed in Chapter 3 and its elemental composition was re-measured for this work; \textsuperscript{b} R\textsubscript{N/C}(exp) is the nitrogen experimentally determined nitrogen-to-carbon ratio.
5.3.2.1 Microgel composition determination

The structures of the primary monomers and polymers, shown below, were used to calculate theoretical values of x and y in the PVP-PA<sub>x</sub> and PVP-PA<sub>x</sub>-TMa<sub>y</sub> microgels. These are displayed in Figure 5.3.

![Diagram of monomer and polymer structures](image)

**Figure 5.3: Structures of VP, the polymer repeat unit of the alkyne-functionalised PVP-PA microgel (P-0), and the polymer repeat unit of the ‘AZPMA-clicked’, alkene-functionalised microgels (P-1 – P-4).**

The experimental value of the nitrogen to carbon ratio of the PVP microgel (R<sub>N/C</sub>(thr)) were found to slightly underestimate the theoretical value (R<sub>N/C</sub>(exp)). This was likely due of the presence of low nitrogen content crosslinker, initiator fragments and surfactant in the final microgel dispersions. For the simplest structural polymer PVP, discussed in Chapter 3, the following equation applies

\[ \Delta R_{N/C} = R_{N/C\,(hp,\,theory)} - R_{N/C\,(hp,\,exp)} \]  

(5.1)

where R<sub>N/C\,(hp,\,theory)</sub> is the theoretical R<sub>N/C</sub> value for each of the ‘homopolymer’ microgels based on their monomer structure and R<sub>N/C\,(hp,\,exp)</sub> is the experimentally measured R<sub>N/C</sub> value of the ‘homopolymer’ microgel.

Equation 5.1 established a correcting factor (\(\Delta R_{N/C}\)) that was applied to the experimental data to negate the effects of the nitrogen-poor crosslinker, surfactant and initiator on the experimentally measured nitrogen-to-carbon ratio (R<sub>N/C\,(exp)</sub>). These contributions were assumed to be present in equal measure in all of the microgel formulations. Using a R<sub>N/C\,(hp,\,thr)</sub> value of 0.167 (from the structure of VP, Figure 5.2) and a R<sub>N/C\,(exp)</sub> value of 0.157 (Table 5.3), \(\Delta R_{N/C}\) was calculated as 0.009.
5.3.2.2 Composition determinations of PVP-PA microgel

Based on the structures of the co-polymers of the PVP-PA_x microgel, the following equations allowed for the determination of the mol fraction of acetylene group functionalisation (x).

\[
\%C = \left(12.011 \times \frac{100}{MW}\right) \left[7(1 - x) + 6x\right] \quad (5.2)
\]

\[
\%N = \left(14.007 \times \frac{100}{MW}\right) (1 - x) \quad (5.3)
\]

MW is the molecular weight of the PVP-PA microgel repeat unit shown in Figure 5.3. Combining Equations 5.2 and 5.3 led to equation 5.4:

\[
x = \frac{1.1662 - 7R_{N/C(corr)}}{1.1662 - R_{N/C(corr)}} \quad (5.4)
\]

By inserting the value of \(R_{N/C(corr)}\) into equation 5.4 the mol fraction of acetylene group functionalisation in the PVP-PA microgel was calculated as 0.073. This value represented an incorporation of approximately half of the 15 mol% preparation concentration of PA and was a similar level of PA incorporation to that of the PVP-PA microgel discussed in Chapter 3.

5.3.2.3 Compositional determination of ‘clicked’ PVP-PA_x-TMα_y microgels

The polymeric structure of a PVP-PA_x-TMα_y series of microgels was shown in Figure 5.3. Based on this structure, the mol fraction of AZPMa incorporated into each ‘clicked’ microgel (and also therefore the mol fraction of alkene group functionalisation) was calculated using the following equations.

\[
\%C = \left(12.011 \times \frac{100}{MW}\right) \left[7(1 - x - y) + 6x + 13y\right] \quad (5.5)
\]

\[
\%N = \left(14.007 \times \frac{100}{MW}\right) \left[(1 - x - y) + 3y\right] \quad (5.6)
\]

\[
x + y = 0.073 \quad (5.7)
\]

MW is the molecular weight of the PVP-PA-TMA microgel repeat unit shown in Figure 5.3.

\[
x = \frac{6.9273R_{N/C} - 1.0814}{3.4986 - 7R_{N/C(corr)}} \quad (5.8)
\]
In equation 5.5 $R_{N/C(\text{corr})}$ values were applied. The experimentally determined degrees of alkene group functionalisation of each ‘AZPM-clicked’ microgel are recorded in Table 5.3. These values were compared to the theoretical compositions of the ‘clicked’ microgels, based on a perfectly efficient CuAAC reaction, and used to calculate the efficiency of the functionalisation reaction for each microgel.

**Table 5.3: Calculated efficiencies of the CuAAC reaction and the experimentally determined degree of alkene functionalisation for each clicked microgel using elemental analysis data.**

<table>
<thead>
<tr>
<th>Microgel</th>
<th>Composition</th>
<th>$R_{N/C(\text{exp})}^a$</th>
<th>$\alpha_{\text{thr}}^b$</th>
<th>$\alpha_{\text{exp}}^c$</th>
<th>% Click $^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-0</td>
<td>PVP-PA$_{0.07}$</td>
<td>0.147</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>P-1</td>
<td>PVP-PA$<em>{0.05}$-TM$</em>{0.02}$</td>
<td>0.152</td>
<td>0.020</td>
<td>0.016</td>
<td>79.5</td>
</tr>
<tr>
<td>P-2</td>
<td>PVP-PA$<em>{0.04}$-TM$</em>{0.03}$</td>
<td>0.157</td>
<td>0.040</td>
<td>0.029</td>
<td>73.0</td>
</tr>
<tr>
<td>P-3</td>
<td>PVP-PA$<em>{0.02}$-TM$</em>{0.05}$</td>
<td>0.165</td>
<td>0.060</td>
<td>0.053</td>
<td>89.5</td>
</tr>
<tr>
<td>P-4</td>
<td>PVP-TM$_{0.07}$</td>
<td>0.170</td>
<td>0.073</td>
<td>0.070</td>
<td>96.0</td>
</tr>
</tbody>
</table>

$^a$ $R_{N/C(\text{exp})}$ is the ratio of nitrogen to carbon determined by elemental analysis. $^b$ $\alpha_{\text{thr}}$ the theoretical maximum mol fraction of alkene groups assuming complete conversion of AZPM. $^c$ $\alpha_{\text{exp}}$ is the experimentally determined mol fraction of alkene groups derived from elemental analysis data. $^d$ % Click is the percentage efficiency of the CuAAC reaction ($\left(\frac{\alpha_{\text{thr}}}{\alpha_{\text{exp}}}\right)^\ast 100$).

5.3.2.4 Composition determination of the ‘AZPM-clicked’ microgels and CuAAC reaction efficiencies

To analyse the success and efficiency of the CuAAC reaction, the experimentally determined $R_{N/C(\text{exp})}$ values and degree of AZPM functionalisation ($\alpha_{\text{exp}}$) were compared with the theoretical extent of AZPM functionalisation ($\alpha_{\text{thr}}$) based on the masses of reactants used. These data are plotted in Figure 5.4. Strong linear correlation ($R^2 > 0.98$) and gradients close to unity in both cases were indicative of the high efficiency of the CuAAC reaction for each ‘clicked’ microgel.
The CuAAC reaction efficiencies ranged from 73 to 96% (Table 5.3). Although slightly lower than the efficiencies reported in Chapter 3, these results were arguably more impressive because they demonstrated a very high degree of control over final microgel composition. By contrast, the highly yielding functionalisation reactions of Chapter 3 took advantage of an excess of AEA and a larger reaction scale. The high reaction efficiencies demonstrated by these microgels inferred that the particles were sufficiently swollen in DMF to allow for penetration of the microgel interiors by the reactants, catalyst and ligand. This offered further evidence that polymer microgels were well suited to CuAAC functionalisation.

Figure 5.4: a) Relationship between the theoretical degree of alkene functionalisation ($\alpha_{\text{thr}}$) and the experimentally measured nitrogen-to-carbon ratios ($R_{N/C}^{\text{exp}}$); b) a comparison of the theoretical ($\alpha_{\text{thr}}$) and experimentally determined ($\alpha_{\text{exp}}$) degrees of alkene group functionalisation.
5.3.3 Physical characterisation of microgels

5.3.3.1 Particle sizing measurements

The swelling behaviour of the parent and clicked microgels was investigated using PCS. The results of these measurements are displayed in Figure 5.5.

![Graph showing hydrodynamic diameters of microgels at different pH values.

Figure 5.5: Hydrodynamic diameters of the ‘AZPMA-clicked’ P-1 – P-4 microgels and the precursor P-0 microgel measured at variable pH.

All of the VP based microgels demonstrated strong swelling behaviour from approximately 340 nm at pH 4 to over 1200 nm at pH 3. This enabled physical gel formation at high concentrations and low pH. The lower concentration of PA used to prepare the PVP-PA (P-0) microgel examined in this chapter compared to the PVP-PA microgel discussed in Chapter 3 was deliberately designed to ensure that the P-0 microgel would demonstrate sufficient swelling behaviour at low pH to induce physical gel formation. It was shown in Chapter 3 that the incorporation of significant amounts of PA into a PVP microgel severely limited its swelling capacity compared to a PVP ‘homopolymer’ microgel. Halving the PA concentration used in the emulsion polymerisation had a dramatic effect and produced a microgel with swelling behaviour almost identical to the PVP microgel prepared with similar amounts of crosslinker by Dupin et al. In effect the incorporation of this lower amount of PA had no influence on swelling behaviour, in stark contrast with the previously studied PVP-PA microgel. It is hypothesised that the lower concentration
of PA in the P-0 microgel resulted in a greater swelling response due to a reduction in concentration of a hydrophobic species, a lower probably of crosslinking reactions between acetylene groups, and an increase in the number and density of ionisable pyridine residues in the P-0 microgel.

Not only were the swelling responses of the precursor P-0 and AZPMa-clicked microgels extremely similar to that of the PVP microgels studied by Dupin et al.\textsuperscript{18}, but Figure 5.5 also suggested that the incorporation of varying amounts of AZPMa into the ‘clicked’ microgels did not significantly influence the average size of the microgels at any measured pH. This was an important result because it implied that any difference in rheological properties between DX gels prepared from the ‘clicked’ microgels would be due to the reaction between alkene groups and not due to the effects of variations in particle size.
5.3.3.2 Zeta potential measurements

The zeta potentials of the precursor P-0 and AZPMa-clicked P-1 - P-4 microgels were measured at various pH values. These results are displayed in Figure 5.6.

![Zeta potential vs pH graph](image)

**Figure 5.6: Zeta potentials of ‘AZPMa-clicked’ microgels and the precursor P-0 microgel measured at variable pH.**

Protonation of the pyridine residues present in all of the microgels resulted in strongly cationic character at acidic pHs, with zeta potentials in the range of 25 - 35 mV observed for all samples below pH 4. At pH 10 all of the samples had negative zeta potential values. This was attributed to hydrolysis effects\(^{136, 138}\).

There were no significant differences between the zeta potential values of the acetylene-functionalised P-0 microgel, the ‘clicked’ alkene-functionalised P-1 – P-4 microgels and the PVP microgels studied by Dupin et al\(^{18}\). This was attributed to the fact that all of these microgels largely comprised vinyl pyridine repeat units and the levels of additional functionality in the P-0 and P-1 – P-4 microgels were relatively low (less than 10 mol%). However, it was necessary to note that the incorporation of low levels of PA and AZPMa did not have a significant effect on the charge behaviour of the microgels. This was beneficial in preparing physical and doubly crosslinked gels whose behaviour can be examined purely in terms of the number of alkene groups present and not by the influence of other functional groups.
5.3.3.3 SEM characterisation of microgel particles

SEM images of the PVP-PA (P-0) and ‘AZPMa-clicked’ P1 – P-4 microgels are displayed in Figure 5.7.

Figure 5.7: SEM images of selected microgel systems. a) P-0, b) P-1, c) P-2, d) P-3, e) P-4.

SEM images of microgels P-0 - P-4 recorded spherical, monodisperse particles. The images suggest that the low level of chemical modification caused by CuAAC functionalisation did not have any noticeable on particle size or morphology. The number average diameters measured by SEM (d_{\text{c(SEM)}}) for each, listed in Table 5.4, correlated well with the hydrodynamic diameters measured by PCS (d_{\text{h(PCS)}}).
Table 5.4: Average particle sizes of the ‘unclicked’ P-0 microgel and the ‘APMA-clicked’ P-1 – P-4 microgels, measured by PCS and SEM.

<table>
<thead>
<tr>
<th>Microgel</th>
<th>$d_{h(PCS)}^a$</th>
<th>$d_{c(SEM)}^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-0</td>
<td>371</td>
<td>316</td>
</tr>
<tr>
<td>P-1</td>
<td>355</td>
<td>309</td>
</tr>
<tr>
<td>P-2</td>
<td>343</td>
<td>326</td>
</tr>
<tr>
<td>P-3</td>
<td>340</td>
<td>326</td>
</tr>
<tr>
<td>P-4</td>
<td>337</td>
<td>330</td>
</tr>
</tbody>
</table>

$^a$ Average hydrodynamic particle diameter measured at pH 7 using PCS, $^b$ number average collapsed particle diameter measured using SEM images (minimum of 50 particles).

The $d_h$ values were slightly larger than the $d_{SEM}$ values, but this was to be expected as PCS measures the effective hydrodynamic diameter and calculates the average diameter using a weight average, whereas SEM diameters are calculated as a number average using the dehydrated diameter of the particle$^{167}$. The CuAAC reaction did not appear to have caused any significant aggregation, evidenced by the agreement between diameters using SEM and PCS.
5.3.4 Comparison of the morphologies of microgels containing varying amounts of PA

The morphologies of P-0 and P-2 microgel were compared to the PVP-PA$_{0.18}$ and PVP-AZPMa$_{0.12}$ microgels studied in Chapters 3 and 4 respectively. SEM images of these microgel systems are displayed in Figure 5.8.

![SEM images](image)

Figure 5.8: Comparison of the morphologies of microgels particles containing varying levels of PA or AZPMa, using SEM images. a) PVP-PA$_{0.18}$, b) PVP-PA$_{0.073}$ (P-0), c) PVP-PA$_{0.033}$-TMa$_{0.04}$ (P-2) and d) PVP-AZPMa$_{0.12}$. The widths of insets b)-d) are equal to 500 nm.

SEM images hinted that the extent of PA concentration in VP based microgels influenced particle morphology. A PA concentration of 18 mol% gave rise to particles with uneven surfaces (Figure 5.8a)), while lower PA concentrations (5.8b) & c)) or azide functionality (5.8d)) led to smooth, spherical particles. The uneven surface morphology of the PVP-PA$_{0.18}$ microgel may have been caused by an unequal distribution of PA throughout the particles, with a higher concentration of PA towards the exterior of the particles. This could have increased the likelihood of acetylene group crosslinking, hydrophobic interactions and a lower charge density at the particle surface. These factors could have contributed to the restricted swelling behaviour of this microgel described in Chapter 3.
5.3.5  Physical and covalent gel characterisation

5.3.5.1  Overview of SX and DX gel formation

Microgels of 10 wt% concentration were induced to form physical (SX) and covalently double-crosslinked (DX) gels using the method outlined in Scheme 5.1c). Photographs illustrating the appearance of typical microgel samples at each stage of this process are displayed in Figure 5.9. A discussion of the conformational changes that occur during the formation of DX gels follows.

Figure 5.9: Photos of a) 10 wt% dispersed P-0 microgel particles at pH 6 and b) 10 wt% P-0 SX gel at pH 3 and c) a P-2 DX gel sample.

As the pH is lowered below the pK_a of the microgels, the dispersed particles swell and particle exteriors come into contact with one another. The milky white dispersion shown in Figure 5.9 rapidly changed to the gel of Figure 5.9b) as polymer chains of neighbouring particles were entangled. Particle swelling also brought the alkene groups of adjacent particles into close proximity with each other. At this point the only covalent bonds in the gel were intra-particle bonds formed by the crosslinker DVB. A physical singly-crosslinked (SX) gel can be induced back into a dispersion state by either increasing the pH or diluting the gel.

It was found that an approximate minimum particle concentration of 5 wt% was required to form a physical gel. A simple estimate of the particle centre-to-centre separation can be obtained by converting the microgel polymer weight fraction to a volume fraction, using the density of poly(2-vinylpyridine) (0.975 g/cm^3 at 25 °C, as supplied by Sigma Aldrich). The maximum microgel swelling ratio (Q) is inversely proportional to the polymer volume fraction of a microgel dispersion. A
polymer volume fraction of 0.051 yields a maximum swelling ratio of 19.6, therefore the maximum size that a P-0 microgel (370 nm diameter in a de-swollen state) can swell to in this system is approximately 1000 nm.

At this size the particle peripheries begin to entangle, resulting in the formation of a physical gel. The dimensions of the unit cell of the packed swollen particles can be estimated using the particle diameter, assuming a monodisperse particle diameter. Using a simplistic 2D representation of a face-centred cubic (FCC) unit cell, for example, the particle surface-to-surface surface is equal to the particle diameter and the diagonal across the face of the unit cell is equal to $4r$, where $r$ is the particle radius. This is shown in Figure 5.10, and although an over-simplification it is sufficient for this discussion.

![Structure of a FCC unit cell.](image)

Figure 5.10: Structure of a FCC unit cell.

The length of each side of the FCC unit cell is equal to $2r\sqrt{2}$. In this example for the described P-0 microgel, the particle centre-to-centre separation distance is equal to approximately 1000 nm and an estimate for the length of each side of the unit cell is 1400 nm. Increasing the dispersion concentration limits the maximum swelling ratio of the microgel particles and results in smaller particle surface-to-surface separation distances.

Double-crosslinked (DX) covalent gels were prepared by introducing initiator (AIBA) to ‘AZPMa-clicked’ SX gels and heating. The sample shown in Figure 5.9c) was a DX P-2 gel. Free radical polymerisation occurred between the alkene groups of neighbouring particles, resulting in permanent covalent crosslinking throughout the gel. These bonds prevent the gel from re-dispersing even when placed in water and exposed to osmotic action.
5.3.5.2 Morphologies of physical (SX) and covalent (DX) microgels studied using SEM

SEM images of a SX physical microgel and a DX double-crosslinked microgel are displayed in Figure 5.11.

![SEM images of SX and DX microgels](image)

Figure 5.11: SEM images of a) physical (SX) P-2 gel; and b) DX P-2 gel. The particle concentration of both gels was 10 wt%.

The SEM images of Figure 5.11 gave an interesting insight into the morphologies of SX and DX gels. Spherical microgel particles were evident in both the SX and DX microgels, which indicated that the particles maintained their structural integrity and morphology upon inter-particle entanglement and the formation of a macroscopic gel. The average diameter of particles within the gels was approximately 530 nm. The particle concentration of 10 wt% means that it was not physically possible for the particles to swell to the same level as in a dilute dispersion at pH 3, although it was possible that some de-swelling may have taken place during sample preparation.
A feature of particular interest was the ordered arrangement of particles over large regions in the DX gel (Figure 5.1b)i) and b)ii)), whereas the SX gel appeared to be more amorphous (Figure 5.1 ai) and aii)). Close examination of the images revealed that the SX gel also contained some seemingly ordered regions and the DX conversely also contains amorphous sections. Ordered sections were found in the images of Figure 5.1 (a)iii) and b)ii)). The nature of the packing arrangements of the gels was probed further using Fast Fourier Transform images.

5.3.5.3 Fast Fourier Transform (FFT) images of SX and DX gels

FFT images were obtained from SEM images and used to ascertain whether the dominant morphologies of the SX and DX gels were amorphous or ordered. 2-dimensional FFT converts the spatial information of an image into a mathematical frequency domain that tracks the rate at which pixel intensities change across the image\textsuperscript{168}. This produces a frequency plot of pixels reflecting the order and orientation of features within the analysed image. The FFTs of an SX and DX SEM image are shown in Figure 5.12.

![FFT images of SX and DX gels](image)

Figure 5.12: 2D FFT images taken from SEM micrographs of a) SX P-2 gel and b) DX P-2 gel.

The FFT of a physical SX gel displayed amorphous halos (image a)), whereas the covalent DX gel displayed apparent hexagonal symmetry (image b)), indicative of crystalline order. The face centred cubic lattice arrangement has previously been reported for colloidal microgel poly(NIPAm) particles\textsuperscript{169, 170}. Crystalline order has also been reported in polyvinylamine DX gels\textsuperscript{115}.

As SEM can only examine small surface sections of the macroscopic gels, the
extent to which the covalent double-crosslinking procedure influenced the arrangement of particles within the gel is uncertain. However, it is reasonable to suggest that the low size polydispersity of the microgel particles contributed to crystalline order and that the covalent bonds formed between particles may help maintain macroscopic order. The ‘fixed’ arrangement of particles within the DX gel, combined with its transparency (displayed in Figure 5.9a), pH responsiveness and particle spacings comparable with the wavelength of light, may allow DX microgels to be used in photonics applications. This is an emerging field for colloidal microgel particles. Poly(NIPAm) colloidal particles have applications as photonic materials in the photopatterning of colloidal crystals directed by a photothermally triggered crystallisation procedure, due to their thermally responsive behaviour\textsuperscript{169}, and responsive colloidal materials have also been applied in glucose sensing applications\textsuperscript{171}.

5.3.5.4 Rheological investigation of gels with latent acetylene functionalisation

It has been reported that acetylene functional groups can undergo free-radical polymerisation\textsuperscript{129, 131} and this was a plausible explanation for the severely limited swelling behaviour of the microgels containing significant amounts of PA studied in Chapter 3. As microgels P-0 – P-4 contained latent acetylene functionalisation, there was a possibility that these groups could have been susceptible to self-crosslinking reactions during the DX gel formation procedure, in which SX gels were heated in the presence of an initiator. If this were to occur to a significant extent it would result in DX gel formation through inter-particle crosslinking and could have an adverse impact on the ease of control over gel mechanical properties.

A control experiment compared the modulus of the P-0 gel sample to that of PVP gel (that lacked acetylene or alkene functionalisation). Both samples were subjected to the DX gel preparation procedure described in the experimental section (5.2.4). The results of this experiment are depicted in Figure 5.13.
Figure 5.13: Control rheology measurements of the PVP and PVP-PA0.073 (P-0) microgels subjected to the DX gel preparation procedure. a) Frequency sweep measurement; b) Strain sweep measurements.

As the $G'$ values of the two gels were almost identical it was safe to assume that if any inter-particle acetylene crosslinking occurred, its effects were negligible and did not contribute to the mechanical properties of the P-0 gel. It was therefore also appropriate to describe the P-0 gel as a physical, non-covalent gel even after being subjected to the DX gel forming procedure. It has previously been reported that “even small amounts of PA can result in crosslinked particles” during emulsion polymerisation\textsuperscript{131}. However, it did not appear that crosslinking between acetylene groups occurred during the DX gel formation procedure. A lower reaction temperature, shorter reaction time, incomplete particle entanglements and relatively low concentrations of PA were all feasible explanations for this behaviour. The presence of latent acetylene functionality in the P-1 – P-3 microgels also meant that additional functionalities could theoretically be ‘clicked onto’ the microgels, which offered the possibility of further responsive behaviour.

5.3.5.5 Rheological investigation into the effect of microgel concentration on DX gel mechanical properties

FTIR spectroscopy and elemental analysis measurements established that it was possible to precisely control the degree of alkene functionalisation of a VP based microgel. Having demonstrated control over microgel composition, the ability to manipulate the mechanical properties of the DX microgels was also investigated. The effect of microgel concentration on the mechanical properties of the P-2 DX gel
was probed using dynamic rheology measurements. The P-2 microgel was chosen for this study due to its previously examined ordered morphology (Figures 5.8 and 5.12) and optical clarity (Figure 5.9c)).

DX P-2 gels with concentrations ranging from 5 to 20 wt% solids were subjected to frequency-sweep dynamic rheology measurements. Figure 5.14 shows that the storage moduli ($G'$) of the gels had very low frequency dependencies, indicated by the stability of the data across the frequency range. The gels demonstrated solid-like viscoelastic behaviour, which is a common property of doubly-crosslinked gels\textsuperscript{42, 115}.

![Figure 5.14: Dependency of the storage modulus ($G'$) of the P-2 DX microgel with microgel concentration. Microgel solids weight percentage shown in key.](image)

As expected, the storage modulus ($G'$) of the P-2 DX gel increased with the particle concentration. This was examined more directly in Figure 5.15, which suggested a linear relationship between particle concentration and storage modulus. The strong linear correlation between $G'$ and particle concentration was in contrast with the reported behaviour of acrylate based DX microgel systems, which demonstrated exponential increases of $G'$ with particle concentration\textsuperscript{26}. The cause of this disparity was not obvious; however a possible explanation is that the extremely high swelling ratios of this VP based system forced particle peripheries to entangle at a comparatively lower particle concentration, resulting in more efficient inter-particle
covalent bonding and a mechanically stronger gel at lower concentrations. This therefore gave rise to a linear increase in storage modulus at the particle concentrations studied.

Figure 5.15: Variation of storage modulus (G') with particle concentration (wt%) measured at a frequency of 10 Hz.

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

tan \( \delta \) is defined as the ratio of the loss modulus (\( G'' \)) to the storage modulus (\( G' \)) and gives an indication of the elasticity of a material. Values of tan \( \delta \) greater than unity are associated with viscoelastic solids, while those less than unity are characterized as displaying greater solid-like behaviour. Materials with tan \( \delta \) values close to zero are therefore described as highly elastic. The low tan \( \delta \) values displayed in Figure 5.16 (values are less than 0.10 for all particle concentrations across the frequency range) indicated that the gels were predominantly elastic.

For the most part, the gels exhibited frequency dependent tan \( \delta \) values. The 5, 7.5 and 15 wt% gels displayed a decrease in tan \( \delta \) values over an increasing frequency range, while the 20 wt% gel demonstrated frequency independent behaviour by contrast. Interestingly, the 10 wt% gel showed almost no dependence of tan \( \delta \) with frequency, with values measured as very close to zero across the frequency range. It has been shown that frequency independent tan \( \delta \) values are specifically characteristic of systems at the critical gel point\textsuperscript{172} and it is therefore logical that the 10 wt% gel displayed critical gel behaviour over the 0.1 – 10 Hz frequency period.
5.3.5.6 Rheological investigation of the effect of alkene group concentration on the mechanical properties of DX microgels

With the relationship between particle concentration and mechanical properties having been verified, the final part of the investigation was to establish the effect of alkene group concentration on DX microgel mechanical properties. If, as was logically expected, increasing the number of inter-particle covalent linkages resulted in a corresponding increase in modulus, then close control over the degree of alkene group functionalisation may enable the tuning of DX gel mechanical properties. For this purpose, DX gels of the P-1 - P-4 microgels and an SX gel of the P-0 microgel were prepared at a particle concentration of 10 wt% and their rheological properties were examined using oscillating frequency rheology measurements. This particle concentration was chosen due to the critical gel behaviour of the P-2 gel displayed at the concentration of 10 wt%. The rheology measurements for each sample are displayed in Figure 5.17a). The G’ values measured for each sample at 10 Hz are plotted in Figure 5.17b).
Figure 5.17: Dependency of storage modulus ($G'$) of DX microgels on the degree of alkene functionalisation. a) Frequency-sweep dynamic rheology, b) Variation of $G'$ measured at 10 Hz frequency with the experimentally calculated degree of alkene group functionalisation ($\alpha_{\text{exp}}$).

The frequency-sweep data of Figure 5.17 a) confirmed the expected relationship between alkene functionalisation and storage modulus. $G'$ values rose from approximately 11 kPa at 2 mol % alkene functionalisation to over 28 kPa at 7 mol%. Figure 5.17 b) suggested that the dependency of $G'$ to the experimentally determined level of alkene group functionalisation ($\alpha_{\text{exp}}$) was approximately linear. This was an interesting result as it posited that mechanical properties could indeed be precisely tuned by altering the degree of alkene functionalisation, which itself
was easily controlled due to the high efficiency of the CuAAC reaction. From the data, it appeared that 2 mol % alkene functionalisation was sufficient to form a DX microgel network as the storage modulus value for the P-1 microgel (~11 kPa) was double the value of the P-0 gel (~5.6 kPa).

Another observation to make on this figure was that the G' values of all of the gels in Figure 5.17 a) showed very little variation with frequency. As this behaviour was also a feature of the SX P-0 gel, which contains no alkene functionalisation, it was inferred that the frequency independence of the gels was a consequence of the close packing of particles within the gels and not was caused by inter-particle covalent bonding. The tan δ values of these gels during across the frequency sweep were also recorded and are displayed in Figure 5.18.

![Graph](image_url)

**Figure 5.18: Variation of tan δ gels with frequency for the P-0 SX gel and P1-P4 DX.**

The data showed that the P-1 – P-4 DX gels displayed low frequency dependencies for tan δ, although higher tan δ values were recorded for the P-4 DX gel. This may have been caused by the formation of elastically ineffective chains between particles due to the higher level of microgel functionalisation. These chains may have restricted the full entanglement of particles required for covalent inter-linking.
5.5 Conclusions

The work in this chapter has demonstrated that the high efficiency of the CuAAC reaction provided the ability to precisely tune the compositions and functionalities of microgel particles. The controlled and incremental changes in the degree of alkene functionalisation of PVP-PA-PMA particles resulted in significant increases in the mechanical properties of doubly crosslinked microgels prepared via the pH triggered swelling of microgel dispersions and the formation of inter-particle covalent bonds through the polymerisation of the alkene groups of neighbouring particles. The mechanical properties of the DX gels increased linearly with the degree of alkene group functionality. This suggests that the strength of the gels can be predicted and tuned.

The CuAAC reaction did not compromise colloidal stability and the emulsion polymerisation preparation route produced particles with a narrow particle size distribution. The incorporation of a smaller amount of PA into the PVP-based microgels did not hinder particle swelling. Furthermore, acetylene crosslinking during DX gel formation did not take place and the presence of latent acetylene groups in the majority of the gels studied provided an opportunity for further functionalisation. SEM images of the DX gel revealed an ordered arrangement of particles in the DX gels. This was likely aided by the narrow particle size distribution and may lead to photonic applications. This study represents an efficient method for the precise control of microgel composition and may have further applications for microgel and hydrogel functionalisation.
6. Conclusions

6.1 Summary of conclusions

This research aimed to assess the suitability of the CuAAC click chemistry reaction for use in microgel functionalisation. The investigation incorporated two main facets: the preparation of primary amine functionalised microgels by the bulk modification of alkyne and azide-functionalised microgels, and the smaller-scale controlled functionalisation of microgels leading to the preparation of polymer gel networks in which gel mechanical properties were influenced by microgel composition.

Microgels with significant primary amine functionalisation have potential in biomedical applications including drug delivery. However, the preparation of pH-responsive primary amine microgels has been limited. The CuAAC reaction offered an alternative preparation route using the incorporation of primary amine functionalisation into an alkyne or azide-bearing microgel. A general method towards preparing alkyne-functionalised microgels and using the CuAAC reaction to introduce primary amine functionalisation was outlined in Chapter 3. It was possible to prepare alkyne-bearing microgels by the emulsion polymerisation of EA, NIPAm and VP with PA. Of the initial 28 mol% PA used in each preparation, PA incorporation was successful in the EA based microgel (28 mol%), but less complete in the VP based microgel (18 mol%), and very limited in the NIPAm system (6 mol%).

The CuAAC reaction proved to be very successful in modifying the alkyne-bearing microgels. All of the available alkyne groups were consumed in each alkyne-functionalised microgel and replaced with primary amine functionality. Importantly, each microgel was sufficiently swollen in the solvent (DMF) to allow for the penetration of AEA and the subsequent modification of the microgel interiors. In this respect, the CuAAC was extremely effective and high yielding in introducing the required functionality to each microgel. However, the addition of up to 28 mol% primary amine groups did not result in pH-responsive swelling behaviour in the EA based system. Possible explanations for the lack of swelling behaviour exhibited by the primary amine functionalised microgel included an insufficient number of
ionisable groups and additional restrictive crosslinking occurring between alkyne groups, either during emulsion polymerisation or the CuAAC reaction. Crosslinking between alkyne groups could also have contributed the severely compromised swelling behaviour of the PVP-PA microgel compared to the strong swelling behaviour of the PVP microgel, although it was likely that the dilution of the density of ionisable groups in the polymer structure of the microgel and the hydrophobicity of the incorporated PA were also responsible for this effect.

The validity of these possible explanations and an alternative route towards the preparation pH-responsive microgels were examined in Chapter 4. The synthesis and emulsion polymerisation of the azide-functionalised monomer AZPMA prepared a novel microgel with a high azide concentration. Modification of this microgel with PAm using the CuAAC reaction resulted in a microgel with a higher primary amine concentration than those prepared in Chapter 3. This approach also avoided the possible complication of acetylene crosslinking during polymerisation and the CuAAC reaction. The swelling response of the PAZPMA-PAm microgel corresponded to a fivefold increase in particle volume.

The efficiency of the CuAAC reaction was analysed in Chapter 4 using the incremental functionalisation of a PVP-AZPMA microgel with the alkyne-bearing monomer PA. The reaction of the azide functionality of the microgel during the CuAAC reaction was assessed using FTIR spectroscopy and elemental analysis. Reaction efficiencies close to 100% were observed for the 4 and 8 mol% ‘PA-clicked’ microgels, but the reaction did not occur for the 2 mol% functionalised microgel. This represented a promising level of control over microgel composition. It was also observed that the presence of 11.7 mol% AZPMA resulted in a lower swelling capacity of the PVP-AZPMA microgel compared to a PVP microgel. The swelling ratio of the PVP-AZPMA microgel decreased further following modification with PA, further demonstrating the effects of hydrophobic structures on microgel responsiveness.

The preparation of pH-responsive microgels via click chemistry was associated with some limitations. It was suggested that a very high level of primary amine group functionalisation is necessary in order for the microgel to demonstrate
significant pH-responsive behaviour. This, in itself, is not unrealistic to achieve using the highly efficient CuAAC. The limitations lie with microgels bearing high concentrations of alkyne or azide functionality. The acetylene-bearing monomer propargyl acrylate offers the most reliable route towards this aim and has been prepared in latex and microgel form\textsuperscript{56, 97, 118, 131}, but the use of significant concentrations of hydrophobic co-monomers severely limits the swelling capacity of pH-responsive microgels\textsuperscript{159}. Possible acetylene side reactions may also produce additional covalent crosslinking, resulting in hindered microgel swelling and pH-responsive behaviour\textsuperscript{56, 97}.

The alternative azide-functionalised microgel preparation route did result in a pH-responsive microgel but safety concerns around the synthesis, purification and storage of the azide-bearing monomer AZPMA, and with polymer particles with a high azide concentration, limit this route to a carefully controlled, small-scale study that is not recommended for future use. A suggested route towards optimising the conditions for pH-responsive microgel via CuAAC is outlined in Section 6.2. Nonetheless, the CuAAC reaction was highly successful in introducing the desired primary amine functionality to colloidal microgel particles.

The effect of microgel composition on the mechanical properties of double-crosslinked microgels was explored in Chapter 5. Expanding on the work of Chapter 4, varied concentrations of alkene groups were introduced to a PVP-PA microgel using the CuAAC reaction. Lower concentrations of PA and crosslinking monomer compared to the PVP-PA microgel studied in Chapter 3 were employed in order to maximise the extent of pH-triggered particle swelling. It was found that the high efficiency of the CuAAC reaction allowed for fine control over the concentration of alkene groups in the ‘clicked’ microgels. The polymerisation of adjacent alkene groups between entangled swollen particles prepared a series of double-crosslinked microgels. The moduli of the double-crosslinked microgels were sensitive to both particle and alkene group concentration. Increasing both of these parameters resulted in double-crosslinked microgels with higher moduli. These findings suggest that CuAAC functionalisation may be of particular use in applications in which defined and consistent properties are a requirement. The
latent acetylene functionalisation of the majority of the clicked microgels in this study also offers opportunity for further useful functionalisation.

In summary, this research has proved that the CuAAC reaction is applicable to microgel functionalisation. The microgels prepared in this work were sufficiently swollen in solvent to allow for the penetration of CuAAC reactants, catalysts and ligands required to modify the interiors of the particles. Both large and small-scale microgel functionalisations were achieved and the reaction was highly efficient in delivering desired functionalities. A high concentration of ionisable primary amine groups was required in order to produce a microgel with desirable responsive swelling behaviour. The use of co-monomers to introduce additional functionality into other responsive microgel systems, such as VP and NIPAm systems can affect their swelling capacities due to a dilution of the number and density of ionisable groups and hydrophobic effects. This represents an important consideration for the design of pH and temperature responsive microgel systems using the CuAAC reaction. The microgels and methods discussed in this work may have useful applications in the biomedical, photonics and water purification applications.

6.2 Future works

This final section discusses potential improvements to the methods described in this work and possible applications for microgels prepared using CuAAC functionalisation. The methods used to develop primary amine functionalised microgels were not immediately conducive towards biomedical applications, although a modification of the method may allow such a microgel to be prepared. A recommendation for future work in this area is the preparation of a poly(PA) microgel using a broadly similar method to the emulsion polymerisation procedure described in Chapter 3. The concentration of crosslinking monomer should be reduced to less than 0.1 mol%, or removed entirely, so as to avoid limiting microgel swelling. An alternative surfactant more suited to the potential applications should also be considered. It may also be beneficial to prepare microgel dispersions without the use of a surfactant. Methods for the emulsion polymerisation of PA have been identified\textsuperscript{56, 118}. A poly(PA) microgel could be modified using AEA to
prepare a very high primary amine content microgel. Particle size and zeta potential measurements would then determine whether this microgel has the required pH-responsive properties for potential biomedical applications.

A limitation of the CuAAC reaction not discussed previously is the issue of the adequate removal of the copper catalyst. This is a particular problem for potential biological applications of any CuAAC product due to the cytotoxicity of copper\textsuperscript{87, 173}. No effort was made to determine the concentration of residual copper species present in the washed ‘clicked’ microgels. Methods employed in the removal of copper catalyst from CuAAC and ATRP products include passage through alumina or silica columns, precipitation in non-solvents and treatment with resins\textsuperscript{174, 175}. From a visual perspective, centrifugation appeared to remove the majority of the copper through the change in colour of the clicked dispersions from vivid blue to white. This was illustrated in the purified dispersion shown in Figure 5.9. However, it must be acknowledged that this did not constitute robust evidence of complete copper removal and further investigation in this area is necessary. The potential biomedical applications of pH-responsive microgels prepared by click chemistry have been discussed; however the biocompatibility of triazoles has yet to be evaluated in detail\textsuperscript{64}. The toxicity of particles with significant primary amine contents would also need to be considered.

It was shown that the incorporation of moderate levels of the co-monomers PA and AZPMA had a significant effect on the swelling capacity of the responsive VP and NIPAm microgel systems. This represents a potential limitation for the use of the CuAAC reaction in microgel systems where moderate or high levels of functionalisation are required. Microgel composition, including the concentration of crosslinking monomer, is crucial in designing swellable particle systems. However, microgels represent a subset of colloidal polymer particles and other applications for the CuAAC reaction are available in this area. For example, colloidal polymer particles with significant primary amine contents may have useful applications in water purification\textsuperscript{176, 177} and microgels functionalised using click chemistry may be beneficial in diagnostic technologies\textsuperscript{178}. The high efficiency of the CuAAC reaction and the ability to introduce multiple useful functionalities to colloidal polymer particles may be
particularly useful in microgel and hydrogel applications in which a swelling response is not the primary objective.
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Appendix

Published works


A general method for functionalisation of microgel particles with primary amines using click chemistry

Robert Farley, Brian R. Saunders

Biomaterials Research Group, Manchester Materials Science Centre, School of Materials, The University of Manchester, Grosvenor Street, Manchester M1 7HS, UK

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In this study we introduce a general method for functionalising microgel particles with primary amine groups using a one-step copper catalysed azide-alkyne cycloaddition (CuAAC) reaction. Three different families of microgels containing copolymerised propargyl acrylate (PA) were prepared and then reacted with 2-azido-1-ethylamine (AEA) using CuAAC. The microgels contained poly(ethyl acrylate) (PEA), poly(2-vinylpyridine) (PVP) or poly(N-isopropylacrylamide) (PNP). The functionalisation of the microgels containing PA (i.e., PX-PA) by AEA to give primary amine functionalised particles (PX-PA-AEA) was assessed by elemental analysis and FTIR. The reaction of AEA with PA was quantitative for each of the PX-PA-AEA microgels (X = EA, VP and NP). The PX-PA-AEA systems generally showed larger pH-triggered swelling and zeta potentials than the non-clicked PX-PA particles. The results also showed that PA restricted swelling of the PX-PA and PX-PA-AEA particles by acting as a crosslinker. Of the three microgel systems studied, PVP-PA-AEA had the best combination of high AEA incorporation and pH-triggered swelling.

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1. Introduction

The long-standing interest in microgels in the literature continues to increase [1–4]. Microgels are crosslinked polymer colloids that swell in a good solvent or when the pH approaches the \( pK_a \) of the polycation or polyanion chains from which they are composed. Microgels can be designed to be thermally responsive [5–9], pH-responsive [10,11] or light responsive [12]. They have excellent potential for construction of advanced responsive materials and biomaterials [13]. However, the preparation of future advanced materials from microgels requires new methods for functionalising the particles in a controlled manner. Ideally, functionalisation should occur quantitatively and without sacrificing colloidal stability of the dispersions. Of special interest is the challenge of preparing microgel particles containing high concentrations of primary amines. Microgels containing high primary amine contents would themselves provide useful building blocks for construction of complex assemblies because of the versatile nature of primary amine reactions [14]. Unfortunately, microgels containing high primary amine contents have been challenging to prepare as stable dispersions [15]. Copper catalysed azide-alkyne cycloaddition (CuAAC) [16,17] has proven to be a very effective functionalisation method for polymers and nanoparticles. CuAAC has high efficiency, high tolerance to functional groups and solvents, requires moderate reaction temperatures and is effective for a range of interfaces [18–21]. The reaction yields little or no by-products. We were inspired by the work of Jung et al. [22] which showed that it was possible to functionalise linear alkyn-containing polymers with primary amines using CuAAC. Here, we sought to establish a general method that would allow a range of alkyn-containing microgels to be functionalised with an azide-functionalised primary amine in one step. In doing so we test the general principle that CuAAC reactions provide quantitative reaction for a range of interfaces [20]. In the present case the interfaces consist of swollen polymer chains within microgel particles. In addition to demonstrating the generality of CuAAC reactions for three different microgel types, this study establishes a new general method for preparing microgels functionalised with primary amines.

CuAAC has been successfully used to prepare a wide range of materials with precise design requirements. Budhathoki-Uprety used CuAAC to construct perfectly alternating functional polymers [23]. CuAAC has been used to prepare new functional monomers [24], functionalise surfaces [25] and construct novel delivery
systems [26]. CuAAC is a spring-loaded reaction [27] that is well suited to nanoparticle functionalisation [28]. Gokmen et al. [29] demonstrated post-functionalisation of polymerised high–internal phase emulsion beads using CuAAC. A key aspect for the success of the reaction in the later study was good accessibility to the grafted azide groups by the alkyne–based chromophore. CuAAC has also been studied in the context of core–shell PS particles [30]. The latter were prepared with an uncrosslinked poly(methyl methacrylate–co-propargyl acrylate) shell and functionalised with azide–terminated PEGs. That study noted that high levels of propargyl acrylate (PA) could cause shell crosslinking. Evanoff et al. prepared poly(PA) particles by emulsion polymerisation and used CuAAC to functionalise crystalline colloidal arrays [31]. CuAAC has also been used to functionalise shell-crosslinked nanoparticles [32].

CuAAC offers excellent potential to functionalise microgels by bespoke placement of azide or alkyne groups. However, there have been very few publications involving CuAAC for microgels. In a seminal study Meng et al. [33] copolymerised PA within the shell of poly(N-isopropylacrylamide–co-acrylic acid) (PNP-AA) microgels and then functionalised the microgels with azide dyes. The level of PA incorporated within those microgel particles was low (nominal PA content was 5 mol.% based on monomer) and the PA was confined to the shell because a seed–feed preparation method was used. Kupal et al. [34] prepared core–shell microgels using assemblage of azido and alkyn derivatives of poly(vinyl alcohol). They used residual surface alkylene groups to graft azido-hyaluronic acid and showed that the microgels could target adenocarcinoma colon cells. Here, we employ a PA-based microgel route to prepare three new families of PA-containing microgels. The PA incorporation was between 6.3 and 28 mol.% and is relatively high compared to other work [33].

Scheme 1 shows the PX, PX-PA and PX-PA-AEA systems investigated and their preparation. Here, X = ethylacrylate (EA), 2-vinylpyridine (VP) or N-isopropylacrylamide (NP). The poly(ethyl acrylate)/PA microgel (PEA–PA) was not intrinsically pH-responsive. PEA is hydrophobic and the linear polymer has a glass transition temperature of about $-24$ °C [35]. The poly(2-vinylpyridine)/PA (PVP–PA) microgel is pH-responsive. Linear PVP is a weak polybase and is soluble in the protonated form at pH values greater than about 4.0 [36]. The poly(N-isopropylacrylamide)/PA (PNP–PA) microgel was temperature-responsive and PNP has a lower critical solution temperature of about 32 °C [37]. Those particles were mostly hydrophilic at room temperature. Consequently, the three families of microgels have very different swelling behaviours. However, they were all prepared using the same preparation conditions. The crosslinking monomers selected for each microgel (Scheme 1) are well known to provide efficient crosslinking for each system. For the CuAAC reactions, a single (common) method was used which incorporated 2-azido-1-ethylamine (AEA) via the PA groups and formed PX-PA-AEA (Scheme 1).

There are several differences for our method for CuAAC of microgels compared to other reports [33]. Firstly, the PX-PA microgels were prepared in a single step. Furthermore, in contrast to the approach by Meng et al. [33] we used a solvent exchange method (via centrifugation) to transfer the particles from water to DMF for the CuAAC reaction (Scheme 1). The PX-PA-AEA particles were then dialysed against water to transfer them back to that phase. This process prevented the particles becoming dry at any stage and preserved dispersion stability. PEA-PA and PVP-PA as well as all of the PX-PA-AEA microgels have not been reported before to the best of our knowledge.

The study begins with the characterisation and analysis of the compositions of PX (X = EA, VP and NP), PX-PA and PX-PA-AEA. We show that incorporation of AEA (and hence primary amines) into the latter was quantitative and determined only by the extent of PA incorporation. The study also investigates the pH-responsive behaviours of the PX-PA-AEA microgels as well as the temperature responsive behaviour of PNP-PA-AEA. It is noted that the PX-PA-AEA systems are well suited to further functionalisation reactions, as shown for other primary amine-rich microgels [15]. This work introduces six microgels (PX-PA and PX-PA-AEA, where X = EA, VP and NP) that have higher PA contents than other microgels [33], were prepared using simple methods and have versatile functionalisation potential.

2. Experimental

2.1. Reagents

EA (99%), NIPAm (97%) and 2VP (97%) were purchased from Aldrich and inhibitor removed by passing chloroform solutions through a neutral alumina column followed by removal of the solvent at reduced pressure. Aliquat 336 and 1,6-azidoisobutyr-amidine dihydrochloride (AlBA, 97%), 1, 4-butanedioi diacrylate (BDD, 90%), N,N'-methylenebis(acrylamide) (BA, 99%) and p-dimethylbenzene (DVB, 85%) were also purchased from Aldrich and used as received. Polyethylene glycol methacrylate (PEGMA) with a number-average molecular weight of 2000 g/mol, NaN₃ (99.5%), and 2-chloroethylamine hydrochloride (99%) were purchased from Aldrich and used as received. The following linear polymers were purchased from Aldrich and used for solubility tests: poly(2-vinylpyridine) (PVP, $M_{n} = 35,000$ g/mol, PD = 1.07), poly(ethy acrylate) (PEA, $M_{n} = 95,000$ g/mol) and poly(N-isopropylacrylamide) (PNP, $19,000-30,000$ g/mol). For PEA, the toluene was removed by rotary evaporation prior to use. High purity water that was distilled and deionised was used.

2.2. Synthesis of 2-azido-1-ethylamine

AEA was synthesised according to the method described by Jung et al. [22]. 2-Chloroethylamine hydrochloride (5 g, 43 mmol) was ground and dissolved in deionised water (50 ml). Sodium azide (8 g, 123 mmol) was added and the mixture was heated at 80 °C for 18 h. The solution was then made alkaline with potassium hydroxide (15 g), extracted using diethyl ether and dried over magnesium sulphate. Finally the solution was concentrated using rotary evaporation to give an amber coloured, viscous oil. AEA was characterised using $^1$H NMR and FTIR spectroscopy (See Fig. S1, Supporting information). The integrations for the peaks gave the correct proton ratios and the FTIR spectra (Fig. 2(a)) showed a strong azide band at 2100 cm$^{-1}$.

2.3. Microgel synthesis

The PX and PX-PA microgels were prepared by a batch emul-sion (X = EA and VP) or precipitation (X = NP) process and followed the method of Dupin et al. [10]. The masses of the monomers used are given in Table S1. In all cases the mass of PEGMA, Aliquat and AlBA corresponded to, respectively, 10, 1.0 and 1.0 wt.% with respect to the total monomer mass. Briefly, Aliquat 336 (1.50 g) and PEGMA (3.0 g of 50 wt.% aqueous solution) were dissolved in water (120 ml) in a round bottom flask and the monomer mixture added. For example, for preparation of PEA-PA, EA (10.35 g), PA (4.5 g) and BDD (0.15 g) were used. The solution was degassed with argon and then heated with overhead, mechanical, stirring to 60 °C. AIBA (0.15 g) was added to begin the polymerisation. This was continued for 24 h, after which time the dispersion was extensively dialysed against pH = 3 water. The crosslinking monomers were present at nominal concentration of 1 mol.% with respect to monomer and are not identified in the
The PX-PA microgel (0.2 g of particles) was transferred to DMF from water using centrifugation and re-dispersion. The number of moles of PA present was typically 0.55 mmol. After degassing the dispersion thoroughly, AEA (0.163 g, 1.33 mmol), N,N,N',N'-pentamethyldiethylenetriamine (PMDETA, 0.0165 g, 0.095 mmol) and CuBr (0.0136 g, 0.095 mmol) were added with stirring. The dispersion remained collooidally stable and the reaction was allowed to proceed for 24 h at room temperature. The product was then extensively dialysed against pH = 3 water to replace the DMF solvent with water. PX-PA-AEA (X = EA, VP or NP) refer to poly(EA-co-(PA-AEA)-co-BDD), poly(VP-co-(PA-AEA)-co-DVB) or poly(NP-co-(PA-AEA)-co-BA) microgels, respectively.

**2.4. CuAAC click reaction of microgels with AEA**

The PX-PA microgel (0.2 g of particles) was transferred to DMF from water using centrifugation and re-dispersion. The number of moles of PA present was typically 0.55 mmol. After degassing the dispersion thoroughly, AEA (0.163 g, 1.33 mmol), N,N,N',N'-pentamethyldiethylenetriamine (PMDETA, 0.0165 g, 0.095 mmol) and CuBr (0.0136 g, 0.095 mmol) were added with stirring. The dispersion remained collooidally stable and the reaction was allowed to proceed for 24 h at room temperature. The product was then extensively dialysed against pH = 3 water to replace the DMF solvent with water. PX-PA-AEA (X = EA, VP or NP) refer to poly(EA-co-(PA-AEA)-co-BDD), poly(VP-co-(PA-AEA)-co-DVB) or poly(NP-co-(PA-AEA)-co-BA) microgels, respectively.
2.5. Physical measurements

Elemental analysis (C, H, and N) was performed at the School of Chemistry, University of Manchester using a Thermo Scientific Flash 2000 Organic Elemental Analyzer with the calibration standards acetanilide and cyclohexanone 2,4 dinitrophenylhydrazone. The samples were freeze-dried prior to measurement and the data are from single measurements. We have found elemental analysis to be a very reproducible technique and the errors estimated from multiple analysis of related polymers within our group are shown in Table S2. Fourier transform infrared (FTIR) measurements were conducted on a Nicolet 5700 ATR FTIR instrument. Microgel samples were freeze-dried to allow analysis, while PA and AEA were measured as pure liquids. The number of scans per sample was 64 and the resolution 2.0 cm\(^{-1}\). 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°. The extent of particle swelling was characterised in terms of the particle volume swelling ratio, \(Q_p\). SEM images were
obtained using a Philips XL30 FEG-SEM with an accelerating voltage of 5 kV. Samples were dried onto glass slides and coated with Au/Pd.

\[ Q_p = \left( \frac{d_0}{d_{h(coll)}} \right)^3 \]  

(1)

For equation (1) the parameters \( d_0 \) and \( d_{h(coll)} \) are the hydrodynamic diameters measured in the swollen and collapsed states, respectively. SEM measurements were obtained using a Philips FEGSEM instrument. A Malvern Zetasizer was used to measure the electrophoretic mobilities of the particles in the presence of aqueous 0.001 M NaNO₃. The mobilities were converted to zeta potentials (\( \zeta \)) using the Smoluchowski equation [38].

3. Results and discussion

3.1. Microgel composition

The microgel compositions were characterised using elemental analysis and FTIR. The experimental ratio of the % nitrogen to % carbon values (\( R_{N/C}(exp) \)) was a useful gauge for the extent of PA inclusion (for PX-PA) or AEA functionalisation (for PX-PA-AEA). Because AEA is nitrogen rich, its inclusion within PX-PA-AEA should increase \( R_{N/C}(exp) \) for each system (Scheme 1). (All the elemental data obtained appear in Table S2.) Fig. 1 and Table 1 show the values for \( R_{N/C}(exp) \) for each microgel. Compared to the respective \( R_{N/C}(exp) \) Values for PVP and PNP, those for PVP-PA and PNP-PA decreased because PA does not contain nitrogen. (PEA-PA and PEA monomers did not contain nitrogen.) Moreover, the \( R_{N/C}(exp) \) value increased upon AEA functionalisation for each microgel (Fig. 1). The increase of \( R_{N/C}(exp) \) was strongest for PEA-PA-AEA. These data qualitatively support the view that (a) PA was incorporated within the PX-PA microgels as a consequence of emulsion polymerisation and (b) AEA was subsequently incorporated into the PX-PA microgels (to give PX-PA-AEA) as a result of CuAAC.

The values for \( R_{N/C}(exp) \) were used to calculate the compositions of the microgels. A description of the method used is given in the Supporting information. For PNP-PA and PVP-PA the compositions were determined from elemental analysis data (Table S2). For the PEA-PA microgel a nominal composition of PEA-PA,0.28 based on the masses of co-monomers used for preparation was assumed (Table 1). This appears to have been a good assumption 100% inclusion of AEA (i.e., PNP-PA-AEA,0.28) was determined – see Supplementary information. Furthermore, quantitative inclusion of AEA into the other two microgels was apparent from the respective analyses. Our analyses did not consider competing reactions involving PA, such as crosslinking (discussed below). However, the extent of PA crosslinking seems to have been relatively low compared to the total PA contents because quantitative AEA inclusion was determined. We note that less than 1 mol.% of cross-links can strongly restrict microgel swelling [2]. PA crosslinking is considered further below. All microgel compositions are shown in Table 1. We conclude that the AEA incorporation within PX-PA-AEA particles was determined by the PA content. This is consistent with the high efficiency reported for CuAAC click reactions [20,21,27].

The compositions for PX-PA (Table 1) show that PA incorporation decreased in the order PEA > PVP > PNP. This trend was unexpected. To the best of our knowledge the Alfrey and Price Q and \( E \) values (which are measures of reactivity and polarity, respectively [35]) for PA have not been reported. However, butyl acrylate has a similar structure to PA. The Q and \( E \) values for butyl acrylate (0.40 and 0.35 [35]) and NP [0.40 and 0.47 [39]) are very similar implying similar reactivity ratios and a statistical copolymer would be expected if all other factors were equal. It follows that differences in co-monomer reactivity ratios are not responsible for the low inclusion of PA within the PNP-PA microgel. A different explanation is required to account for the compositions in Table 1.

Swelling of primary particles by monomer is an important part of incorporation of co-monomers by emulsion polymerisation [40]. We tested the solubility of the linear polymers PNP, PP, and PEA in PA. It was found that PEA was readily soluble in PA. For PVP, the polymer also dissolved in PA, although this was slower than for PEA. Interestingly, PNP was insoluble in PA. We propose that the reason for the low PA incorporation within PNP microgels (and hence compositional difference) is incompatibility between PNP and PA. PA would not have been able to swell PNP-rich particles during growth. This contrasts to PEA, where good solubility of PA enhanced swelling of the PEA particles and incorporation. The less facile dissolution of PVP in PA is also consistent with the differences in PA incorporation evident (Table 1).

FTIR spectra for the microgels are shown in Fig. 2. The PEA-PA and PEA-PA-AEA microgels contained the highest PA and AEA contents based on elemental analysis data (28 mol.% (Table 1). The alkyn band at [31] 3280 cm⁻¹ is clearly evident in the spectrum for PA (Fig. 2(a)) supporting a high extent of PA incorporation. The alkyn band at [31] 3280 cm⁻¹ was not apparent in the spectrum for PEA-PA-AEA, i.e., after CuAAC reaction of PEA-PA with AEA. Furthermore, the azide band (2100 cm⁻¹) from AEA (Fig. 2(a)) was not present in the spectrum.

Table 1: Characterisation data for the microgels.

<table>
<thead>
<tr>
<th>Code</th>
<th>Composition ae</th>
<th>( R_{N/C}(exp) )</th>
<th>( d_{h(coll)} ) in nm</th>
<th>( d_{h(coll)} ) in DMF</th>
<th>( Q_p ) in DMF</th>
<th>( Q_p ) in water</th>
<th>( \zeta ) in mVf</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEA</td>
<td>PEA</td>
<td>0</td>
<td>107 [16]</td>
<td>213 ± 11</td>
<td>102 ± 5</td>
<td>9.1 ± 1.4</td>
<td>1.0 ± 0.15</td>
</tr>
<tr>
<td>PEA-PA</td>
<td>PEA-PA,0.28d</td>
<td>0</td>
<td>125 [12]</td>
<td>157 ± 8</td>
<td>133 ± 7</td>
<td>1.6 ± 0.2</td>
<td>1.0 ± 0.15</td>
</tr>
<tr>
<td>PEA-PA-AEA</td>
<td>PEA-PA,0.28</td>
<td>0.222</td>
<td>166 [10]</td>
<td>160 ± 8</td>
<td>160 ± 8</td>
<td>1.7 ± 0.3</td>
<td>23.6</td>
</tr>
<tr>
<td>PVP</td>
<td>PVP</td>
<td>0.148</td>
<td>372 [24]</td>
<td>664 ± 33</td>
<td>1225 ± 61</td>
<td>4.5 ± 0.7</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>PVP-PA</td>
<td>PVP-PA,0.18e</td>
<td>0.121</td>
<td>339 [11]</td>
<td>512 ± 26</td>
<td>537 ± 27</td>
<td>2.8 ± 0.4</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>PVP-PA-AEA</td>
<td>PVP-PA,0.18</td>
<td>0.235</td>
<td>403 [24]</td>
<td>1020 ± 51</td>
<td>1020 ± 51</td>
<td>23 ± 3</td>
<td>20.6</td>
</tr>
<tr>
<td>PNP</td>
<td>PNP</td>
<td>0.178</td>
<td>245 [7.5]</td>
<td>417 ± 21</td>
<td>338 ± 17</td>
<td>37 ± 6h</td>
<td>20 ± 2h</td>
</tr>
<tr>
<td>PNP-PA</td>
<td>PNP-PA,0.06h</td>
<td>0.166</td>
<td>200 [7.6]</td>
<td>505 ± 25</td>
<td>333 ± 17</td>
<td>18 ± 3h</td>
<td>5.1 ± 0.8h</td>
</tr>
<tr>
<td>PNP-PA-AEA</td>
<td>PNP-PA-AEA,0.06</td>
<td>0.211</td>
<td>249 [7.8]</td>
<td>375 ± 19</td>
<td>7.7 ± 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a The compositions were calculated from the \( R_{N/C}(exp) \) values unless otherwise stated – see Supplementary information for method used.
b Values measured at pH = 4.
c Zeta potential measured at pH = 4.
d Compositions assumed based on conditions used for preparation.
e Estimated using the PEA-PA \( d_h \) value for collapsed diameter.
f Measured at pH = 2.
g Calculated using \( d_h \) values measured at pH = 2.0 and 12.0.
h Estimated at pH = 4. For these calculations the hydrodynamic diameter for each respective microgel measured at 50 °C was used as the collapsed diameter.
for PEA-PA-AEA. These spectral changes for PEA-PA-AEA are indicative of a successful CuAAC reaction [29]. There is also evidence of a broad RNH$_2$ band [15,41] at about 1590 cm$^{-1}$. This feature is also present in the FTIR spectrum for AEA. It can also be seen that the broadening of the features in the 3300 cm$^{-1}$ region of the FTIR for PEA-PA-AEA match those of AEA and are absent from the spectrum for PEA-PA. The latter is also indicative of RNH$_2$ [41–43]. Consequently, the FTIR spectra confirm that primary amine groups were incorporated (Scheme 1(a)) and are in qualitative agreement with the compositions for the EA-based microgels given in Table 1.

The spectrum for PNP-PA (Fig. 2(b)) shows a weak alkyne band at 3280 cm$^{-1}$, which is consistent with the lower extent of incorporation (18 mol.%) compared to that for PEA-PA (28 mol.%). The band was superimposed on a broad RNH$_2$ band. For this microgel support for incorporation of PA can be found from the strong band due to the ester groups present at 1725 cm$^{-1}$. The latter band was not present in the spectrum for PVP. The band was also present in the spectrum for PVP-PA-AEA (Fig. 2(b)). The absence of the alkyne and azide bands in the spectrum for PVP-PA-AEA confirms that efficient CuAAC reaction occurred. There is also evidence of a broad band at about 1590 cm$^{-1}$ due to RNH$_2$ with sharper bands due to PVP superimposed on it. Furthermore, the broad absorption due to RNH$_2$ groups is also present in the spectrum in the 3300 cm$^{-1}$ region. The spectrum for PVP-PA-AEA is also consistent with significant primary amine group incorporation.

Fig. 2(c) shows the spectra for the PNP microgel systems. For PNP-PA the alkyne band was obscured by a broad band due to N–H groups at about 3300 cm$^{-1}$. However, the ester band at 1725 cm$^{-1}$ was clearly present; although, at a lower relative absorbance compared to the same band in the spectrum for PVP-PA (Fig. 2(b)). This confirms a lower PA incorporation within this microgel (6.3 mol.%). The spectrum for PNP-PA-AEA does not show evidence of an azide band, as expected. However, the FTIR spectrum does not show any clear RNH$_2$ bands because of the strong amide I band [44] in the 1650 cm$^{-1}$ region and low AEA content.

The FTIR spectra for all of the microgels are consistent with the compositions determined from elemental analysis (Table 1). The CuAAC reactions for each of the microgels shown in Scheme 1 was quantitative. This shows that our simple approach to functionalise the microgels using CuAAC is generally applicable and only limited by the ability to incorporate PA into the PX-PA microgel particles during particle growth.

3.2. Microgel particle characterisation and swelling

SEM images for the microgels are shown in Fig. 3. The particles were well dispersed in each case as evidenced by separated particles. There was some evidence of a nodular surface morphology for the PX-PA and PX-PA-AEA (X = EA and VP) particles. The number-average diameters measured from SEM ($d_{SEM}$) for the PEA-based microgels were all close to the respective $d_h$ values measured in water (Table 1), showing that the particles existed as isolated particles in dispersion. For the PVP-based microgels the $d_{SEM}$ values are in the range of 339–403 nm and are in the same range as the $d_h$ values measured for these microgels in water at pH = 12 (Fig. 4(c)), i.e., in the collapsed state. This also supports the view that the particles were well dispersed. A comparison between the $d_{SEM}$ and $d_h$ values for the PNP-based microgels is more difficult because PNP microgels are well known to deform greatly upon deposition onto...
Values for \(d_h\) at 50 °C were measured (Fig. 6) and the values for PNP-PA and PNP-PA-AEA were about 195 nm. The latter value is close to the respective \(d_{SEM}\) values and confirms that particles existed as isolated particles in dispersion. Thus, the data strongly support the view that our CuAAC method (Scheme 1) did not result in significant aggregation, which is essential for a microgel functionalisation strategy to be useful.

The high CuAAC efficiencies for the microgels established above imply complete access of the click reagents (PMDETA, CuBr and AEA) to all of the PA within the PX-PA particles. This implies that the microgel particles were swollen. We selected DMF as the dispersion solvent for the click reactions because (a) it has been used for CuAAC of AEA \[22\], (b) it was miscible with water and (c) the PX-PA microgel particles were swollen by that solvent. The latter is evident from the \(d_h\) values for PEA-PA, PNP-PA and PVP-PA measured in DMF (Table 1). It can be seen that the \(d_h\) values for PEA-PA and PNP-PA dispersed in DMF were larger than the respective values measured in water. This could be seen visually for the PEA-PA dispersions in water and DMF at the same particle concentration (See Scheme 1(a)) where the turbidity was less for the DMF dispersion – due to swollen particles scattering less light. In the case of PVP-PA the \(d_h\) value of 512 nm measured for the particles in DMF (Table 1) was much higher than the collapsed value in water at pH = 12 (of 362 nm from Fig. 4(c)) and those microgel particles were also swollen in DMF.

A noticeable consequence of incorporating PA into the microgels was that the extent of particle swelling decreased. This can be seen from comparing the \(Q_p\) values for the PX or PX-PA (X = EA and NP) particles in DMF (Table 1). In each case the \(Q_p\) values were less for the PX-PA particles. The \(Q_p\) values for the PNP and PNP-PA microgels also follow this trend. However, those values are approximate because the diameters measured for each respectively microgel at 50 °C was used for \(d_{col}(coll)\) in equation (1). PNP is known to retain water at 50 °C \[46,47\]. Indeed, Destribats et al. \[48\] assumed that their PNP microgels contained 29 wt.% of water at 50 °C. This means that the true \(Q_p\) values will be larger than those shown in Table 1. The \(Q_p\) values used here for those systems are minimum values. Nevertheless, our comparison of the \(Q_p\) values calculated in this way within the PNP microgel series (PNP, PNP-PA and PNP-PA-AEA) is valid because the relatively low levels of PA and PA-AEA incorporated (ca. 6.3 mol.%) are unlikely to have significantly altered the water contents at 50 °C.

PA is soluble in DMF which suggests that the decreased swelling for the microgels containing PA discussed above is not due to PA-solvent incompatibility. PA is known to act as a crosslinking during emulsion polymerisation \[30\]. Accordingly, the decreased swelling for the PX-PA particles is attributed to crosslinking by PA units during particle formation. This is supported by a comparison of the \(Q_p\) values measured in DMF (Table 1) for PEA (9.1 ± 1.4) and PEA-PA microgel (16 ± 0.2). We tested this further by preparing a
PEA-PA microgel without any added BDD crosslinker, i.e., BDD-free PEA-PA. The hydrodynamic diameters for the latter particles in water and DMF were, respectively, 144 ± 7 and 163 ± 8 nm. The value for Qz was 1.5 ± 0.2. This Qz value is not significantly different to the value obtained for PEA-PA microgels prepared using BDD (Table 1). This result demonstrates that the crosslinking for the PEA-PA microgels prepared using BDD was dominated by PA. It follows that PA can be used to generate highly functionalised microgels where PA has two roles, i.e., as a crosslinker and a CuAAC site. This is a new observation for microgels.

It is instructive to compare the Qz values for the PX-PA-AEA microgels at pH = 4 to those for the parent PX-PA microgels. The Qz values increased in each case due to inclusion of AEA. Given the pK\textsubscript{a} of primary amines should be about [49] 10 we can conclude that the increase in Qz is because of electrostatic repulsion between neighbouring AEA groups within the microgel particles. The \( \zeta \) values in Table 1 support this view because at low pH the values were highest for the PX-PA-AEA microgels. These data support primary amine group incorporation within PX-PA-AEA.

3.3. pH-dependent microgel particle properties

The trends for the \( d_{h} \) values described above at pH = 4 were maintained for the PEA-based microgels across the entire pH range (Fig. 4(a) and (b)). The Qz values for the PEA-PA-AEA microgel particles in the pH range of 4–10 had an average value of 1.7 which is not significantly different to the Qz value for the PEA-PA particles dispersed in DMF (1.6, Table 1). Although repulsion between RNH\textsubscript{3}+ groups was able to swell the particles in water, the maximum Qz values are much lower than would normally be expected for microgel particles containing 28 mol.% of charged groups. For example anionic EA-based microgels containing about 30 mol.% of deprotonated RCOO\textsuperscript{-} groups had Qz values of around [50] 30. It is likely that PA crosslinks contribute to the strongly restricted swelling for PEA-PA-AEA. There was some evidence of a decrease in \( d_{h} \) (and Qz) at a pH value of 12 (Fig. 4(a) and (b)), which would be expected due to deprotonation of the primary amine groups. However, complete collapse of the particles at a pH of 12 is not expected because the nitrogen-rich structure of AEA is hydrophilic.

The PVP-PA and PVP-PA-AEA microgels contained lower PA and PA-AEA contents (cf. PEA-PA and PEA-PA-AEA). They also contained high proportions of a pH-responsive monomer (VP). PVP has a pK\textsubscript{a} of about [10] 4.9. The PVP microgel showed strong pH-triggered swelling (Fig. 4(c) and (d)) when the pH decreased to 4 or less, and the data are consistent with previous reports [10]. Importantly, the PVP-PA microgel showed a much lower swelling response (cf. PVP) which is attributed to the relatively high PA content (18 mol.%). Interestingly, after functionalisation with AEA the strong low pH swelling behaviour of PVP-PA-AEA returned and was comparable to that for the parent PVP particles. However, there was also evidence of increased swelling at pH = 7 and 10. We speculate that this ability to restore the original particle swelling behaviour is due to a relatively low level of additional crosslinking provided by the PA groups (cf. PEA-PA). The suppressed swelling for PVP-PA compared to PVP at low pH would have been the result of a dilution of the positive charged groups with non-charged PA. The loss of positive charge was restored upon functionalisation of the PA groups with AEA. This interpretation is supported by the higher Qz values for PVP-PA-AEA at pH = 4 to 10 (Fig. 4(d)) compared to PVP-PA. However, the swelling from the AEA groups alone was not sufficient to strongly swell the PVP-PA-AEA particles at pH values greater than the pK\textsubscript{a} of PVP (ca. 4.9 for linear PVP [10]). This is probably due to the hydrophobicity of the non-charged VP units that opposed particle swelling in that pH range.

The variable pH data for the PNP, PNP-PA and PNP-PA-AEA microgels (Fig. 4(e) and (f)) were different to the other systems because there were few differences between the three microgels for this series. This is attributed to the content of PA and PA-AEA being the lowest of the three microgel systems (6.3 mol.%). However, the \( d_{h} \) and Qz values at pH = 4 and 7 were higher for PNP-PA-AEA compared to PNP-PA. The supporting the view that electrostatic swelling occurred. Surprisingly there was a significant increase in swelling at pH = 12 for all three microgels. This is proposed to be due to hydrolysis and is discussed further below.

The effect of pH on the \( \zeta \) values were also measured (Fig. 5). For all three microgel systems there was a strong pH dependence of \( \zeta \) with its value being highest at low pH. Moreover, the \( \zeta \) values at pH = 4 and 7 for all of the PX-PA-AEA microgels were significantly larger than those for the respective PX-PA microgels. This suggests that there was a higher positive charge density for the AEA-containing microgels in that pH range. This finding suggests that each of the PX-PA-AEA microgels had comparable outer shell structures which contained primary amine groups. However, it is also possible that protonated 1,2,3-triazole species also contributed to the positive charge in the above pH range because these species are known to have a pK\textsubscript{a} around [51] 9.

An important observation concerning the \( \zeta \) data is that relative changes for these values did not always match the respective changes for \( d_{h} \). For example, the \( \zeta \) values for PVP-PA and PVP/PEA-AEA (Fig. 4(b)) show similar strong decreases with increasing pH and have comparable values. However, the changes in \( d_{h} \) (and Qz) for PVP-PA-AEA are more pronounced than those for PVP-PA (Fig. 4(c) and (d)). This is because \( \zeta \) and \( d_{h} \) values measure outer layer and whole particle properties, respectively. The charge density changes that occur within outer layer of microgels do not always map onto changes in charge density occurring within the particle interior [11]. We consider the \( \zeta \) values as a guide to the changes in swelling and charge densities that occur in the outer...
shell of the microgel particles or surface of the microgels in the collapsed, latex, form. The $\zeta$ data for all of the systems show an isoelectric point (iep). The PEGMa provided steric stabilisation and prevented aggregation of these dispersions even when the pH passed through the ieps. The ieps indicate that negatively charged species were present. Amines have a $pK_a$ in the vicinity of [52] 7. Furthermore, they are known to undergo hydrolysis, along with esters, under alkaline conditions. This is a common explanation for the appearance of ieps within amide-stabilised latexes and microgels [52,53]. It is the acquisition of negative charge in the shell of the PNP-based microgels that may explain the moderate swelling observed for these microgels at pH = 12 (Fig. 4(e) and (f)). We note that there was no evidence of aggregation for any of these dispersions.

To further probe the effect of incorporating PA and PA-AEA within the PNP microgels variable temperature $d_h$ and $\zeta$ measurements were conducted (Fig. 6). The data show that the similarity of the hydrodynamic diameters for PEA-PA and PEA-PA-AEA remained over the entire temperature range studied (25–50 °C). By contrast, the particle size changes were much higher for PNP. This can be seen from Fig. S2 where $Q_p$ values are plotted as a function of temperature. This is further support that PA acted as a crosslinker. PA is hydrophobic and would be expected to suppress swelling and this would have contributed to the low $Q_p$ values. However, the fact that there was only a small increase in $Q_p$ occurred upon AEA incorporation suggests that the primary cause for lack of swelling was the covalent crosslinking involving PA.

The variable temperature $\zeta$ data (Fig. 6(b)) show clear differences between the three PNP-based microgel systems. The $\zeta$ values at temperatures of 25–35 °C increased in the order PNP < PNP-PA < PNP-PA-AEA. This implies that there were significant differences in the volume surface charge density in the outer layer of these particles. The charge density was highest for PNP-PA-AEA. The $\zeta$ values were similar at 50 °C because the particles were mostly collapsed and had similar surface charge densities. These three microgels would be expected to have possessed similar charge densities when prepared because they were prepared using similar monomer-to-initiator ratios.

A final point can be made from a comparison of the $d_h$ and $\zeta$ data (Fig. 6) for PNP-PA and PNP-PA-AEA. The equivalence of the $d_h$ values for these two microgels and the dissimilarity of the $\zeta$ data at lower temperatures, coupled with the relatively low levels for their incorporation (6.3 mol.%), imply that the PA and PA-AEA groups were preferentially located in the outer shell of the microgels. This is potentially useful for future microgel functionalisation using CuAAC chemistry because it facilitates their accessibility to reactants.

4. Conclusions

In this study we have investigated the ability to functionalise three different types of microgels with primary amine groups using CuAAC chemistry. Incorporation of AEA into the microgels was quantitative for each microgel system which confirms the principle of achieving high yield CuAAC reactions applies to swollen microgel particles. The limiting factor controlling AEA incorporation was the PA content in the precursor microgel particles. The highest AEA content incorporated was for PEA-PA-AEA which contained 28 mol.% of AEA. Using this approach we prepared three new examples of microgels containing primary amine groups: PX-PA-AEA ($X =$ EA, VP and NP). The extent of incorporation of AEA into PNP-PA-AEA was the lowest (6.3 mol.%) and this was attributed to the inability of PA to swell PNP. The PCS and zeta potential data for that system imply that the PA-AEA units were located mostly in the outer shell of the PNP/microgel-AEA particles. PA was found to act as a crosslinker and it was possible to use that co-monomer as the only crosslinker for PEA microgel preparation. All of the PX-PA-AEA microgels exhibited pH-triggered swelling in water due to the primary amine groups at pH values less than or equal to 7. The pH-responsive swelling was strongest for PVP-PA-AEA and this system appeared to provide a good compromise between crosslinking due to PA and swelling by AEA incorporation. This study has established a general method that can be used to efficiently functionalise microgels with azide-containing molecules by CuAAC. Our CuAAC method for microgels did not cause aggregation and appears versatile. In addition, the three new PX-PA-AEA microgels (X = EA, VP and NP) studied here offer additional functionalisation opportunities through the wide range of reactions available for primary amines [14].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.polymer.2013.12.022.

References

Using click chemistry to dial up the modulus of doubly crosslinked microgels through precise control of microgel building block functionalisation†

Robert Farley, a Silvia Halacheva, b Jane Bramhill c and Brian R. Saunders a

Doubly crosslinked microgels (DX MGs) are hydrogels constructed by covalently interlinked vinyl-functionalised microgel particles. Until now it has not been possible to precisely control the extent of vinyl functionalisation of the microgel (MG) particles which act as the colloidal building blocks for hydrogel assembly. Furthermore, the range of DX MGs prepared to date has been modest. This study addresses both of these challenges by constructing a new class of DX MG using MG particles that were vinyl functionalised by copper catalysed azide–alkyne cycloaddition (CuAAC). Here, poly(2-vinylpyridine-co-propargyl acrylate) (PVP-PA) MG particles were prepared and vinyl functionalised by CuAAC using azidopropyl methacrylate (AZPMA) to give PVP-PA y-PMA x MGs. Values for the extent of vinyl functionalisation (y) were varied from 0 to 7.0 mol% in a precisely controlled manner. Concentrated dispersions were transformed from fluids to physical gels at pH values of less than or equal to 3.0 and covalently inter-linked to give PVP-PA y-PMA x DX MGs by free-radical coupling of the vinyl groups of neighbouring particles. The storage modulus of the DX MGs varied linearly with both MG concentration and the value for y. Not only did the new DX MGs studied here enable precise control of MG functionalisation and DX MG mechanical properties, they also showed evidence of colloidal crystallinity which may lead to future photonic gel applications. Our CuAAC-based approach should be versatile and is expected to enable a range of new DX MGs to be prepared.

Introduction

Hydrogels continue to attract major attention in the literature with advances in structure–property relationships increasing the opportunities for application. 1–3 Not only have the mechanical properties such as ductility and toughness improved greatly 1,2 but the ability to design next generation gels with increasingly sophisticated structure–function relationships has rapidly evolved. 4–6 The latter has been enabled by a range of new chemistries which are well suited to hydrogel construction, such as highly efficient copper catalysed azide–alkyne cycloaddition (CuAAC). 7,8 Hydrogels with tunable nano-morphologies are a subgroup of next generation hydrogels that hold great promise. A new approach for construction of hydrogels using covalent inter-linking of swellable microgel (MG) particles has recently emerged. 9 MG particles are crosslinked polymer colloid particles that swell when the pH approaches the pK a of the constituent polymer. 10 MG particles are singly crosslinked (SX) via intra-particle crosslinking and are termed SX MGs. Concentrated dispersions of swollen SX MGs can be covalently inter-linked to form doubly crosslinked MGs (DX MGs). The MG particles are colloidal building blocks for DX MG hydrogels. A key requirement for enabling DX MG construction from MGs is vinyl functionalisation of the MG particle peripheries. However, until now this approach has required use of relatively low efficiency functionalisation chemistry, 9,12 which has been limited to MG particles containing carboxylic acids 9 or primary amines. 12 Building on CuAAC chemistry and our earlier study with MG particles containing propargyl acrylate (PA) 13 we aimed to establish a new family of DX MGs with well-controlled vinyl functionalisation. We hypothesised that the DX MG modulus could be tuned precisely by vinyl group functionalisation. Because the CuAAC approach is versatile 8 this study should expand the range of DX MGs that can be prepared and investigated.
The overwhelming majority of hydrogel research involves network construction using small molecules, i.e., monomers.\textsuperscript{14,15} By contrast DX MGs contain pre-formed colloidal scale gel particles which enable design of gel nanomorphology on the length scale of ~50 to 1000 nm and may provide new photonic and biomaterial hydrogels. A key challenge for achieving improved DX MGs is the ability to vary the vinyl functionalising extent of MG particles in a precise manner that is also versatile. The present study addresses these challenges by establishing a new method for the vinyl functionalisation of MG particles and using this to construct new DX MGs. For this study we used MGs based on poly(2-vinyl pyridine) (PVP), which exhibit strong pH-triggered swelling. PVP particles swell when the pH decreases to below the pK\textsubscript{a}, which is about 4.9.\textsuperscript{16} Whilst PVP MGs have been studied by a number of groups\textsuperscript{16–18} they have not been reported in the context of DX MG formation until now.

Click chemistry was pioneered by Sharpless et al.\textsuperscript{19} and has proven to be an exceptionally efficient and versatile functionalisation approach for polymers, particles, beads\textsuperscript{20} and surfaces.\textsuperscript{8,21} CuAAC has also been used to prepare a new range of hydrogels by playing an integral role in the network formation process.\textsuperscript{4,22} By contrast to those studies the present work uses CuAAC to functionalise pre-formed MG particles, which are subsequently inter-linked to form hydrogels. Whilst CuAAC has also been explored in the context of MG particle functionalisation,\textsuperscript{13,23,24} it has not yet been reported in the context of vinyl functionalisation of MGs to the best of our knowledge.

Previously,\textsuperscript{13} we investigated PVP-PA MG particles and showed that they could be functionalised with primary amines via CuAAC using 2-azido-1-ethylamine. The MG particle functionalisation was highly efficient and the MG particles retained their strong pH-dependent swelling. Building on our earlier study\textsuperscript{13} we hypothesised that high efficiency vinyl functionalisation of PVP-PA MGs could be achieved using a methacrylated azide. For this study we used azido propyl methacrylate (AZPMA) – see Scheme 1a. In our approach PVP-PA MG particles containing a small proportion of crosslinker (divinyl benzene) were functionalised with AZPMA via CuAAC. Concentrated PVP-PA\textsubscript{x}–PMA\textsubscript{y} MG dispersions containing \(\alpha,\alpha’\)-azodiisobutyramidine dihydrochloride (AIBA) were subsequently swollen at pH = 3 to form physical gels and then covalently crosslinked using free-radical coupling to give PVP-PA\textsubscript{x}–PMA\textsubscript{y} DX MGs (Scheme 1b).

In this report we first determine the compositions of PVP-PA\textsubscript{x}–PMA\textsubscript{y} MG particles using a combination of elemental analysis and FTIR to quantify the extents of vinyl functionalisation. Photon correlation spectroscopy and electrophoretic mobility measurements are also used to probe the pH-dependence of the hydrodynamic diameter and zeta potential of the MG particles. The morphologies of the DX MGs is then investigated using SEM and the mechanical properties studied using dynamic rheology. The data show that the modulus of the DX MGs can be precisely controlled by MG concentration and the extent of PMA incorporation. The results show that CuAAC is a highly efficient and versatile method for obtaining DX MGs with modulus values that can be controlled precisely. The results of this study should enable a wide range of new DX MGs to be prepared.

Scheme 1 Depiction of synthesis of PVP-based DX MGs from vinyl functionalised MGs. (a) Vinyl functionalisation via CuAAC was conducted using PVP-PA MG particles synthesised by emulsion polymerisation. (b) DX MGs were formed after pH-triggered swelling of the PVP-PA\textsubscript{x}–PMA\textsubscript{y} MG particles caused overlap of the peripheries which enabled covalent inter-linking of the MG particles by free-radical coupling.
Experimental

Materials

2-Vinyl pyridine (VP, 97%), propargyl acrylate (PA, 98%), divinylbenzene (DVB, 80%), α,ω-azidodiisobutryramidine dihydrochloride (AIBA, 97%), 3-chloro-1-propanol (98%), sodium azide (≥99%), methacryloyl chloride (≥97%), hydroquinone, anhydrous dichloromethane (≥99.8%), triethylamine (≥99%) and tetrabutylammonium hydrogen sulphate (97%) were all purchased from Sigma-Aldrich and used as received unless otherwise stated. PVP MG was prepared following the method reported previously.13 VP and PA were purified by passing them through columns packed with basic alumina. High purity distilled, deionised water was used for all experiments.

Synthesis of 3-azidopropyl methacrylate

Azidopropyl methacrylate (AZPMa) was synthesised in accordance with the literature.25 Briefly, 3-chloro-1-propanol (33.9 g, 0.36 mol) was introduced to a mixture of sodium azide (47 g, 0.72 mol), tetrabutylammonium hydrogen sulphate (1.0 g, 2.95 mmol) and water (40 mL). The slurry was heated to 80 °C for 24 h whilst stirring. After cooling to room temperature, stirring was continued for a further 14 h. Following an ether extraction the resulting solution was dried over anhydrous sodium sulfate and the solvent was removed using rotary evaporation to yield AZPMa. The 1H NMR spectrum for AZPMa used for P-3 was 0.096 g (0.57 mmol). An additional 100 mL of dichloromethane was added before the mixture was extracted with aqueous HCl (4 M), followed by water, then aqueous NaOH (2.5 M) and a further water extraction step. Each extraction step listed was conducted twice. Hydroquinone (0.1 g) was added to the resultant solution, which was then dried. The solvent was removed using rotary evaporation to yield AZPMa. The 1H NMR spectrum for AZPMa is shown in Fig. S1 (ESI†) and is consistent with high purity.

Synthesis of PVP-PA MG

The PVP-PA MG was synthesised by emulsion polymerisation using a modification of the method previously employed.13 The PA concentration used in this study was much lower than in previous work.13 Briefly, Aliquat 336 surfactant (1.5 g) and PEGMA2000 (1.5 g) were dissolved in deionised water (120 mL). The solution was transferred to a reaction vessel fitted with an overhead stirrer, heated to 60 °C and degassed with nitrogen. A co-monomer solution of VP (10.72 g, 0.102 mol), PA (2.18 g, 0.020 mol) and DVB (0.096 g, 0.74 mmol) was introduced with stirring at 250 rpm followed by addition of AIBA initiator (0.15 g in 15 mL of water). The emulsion polymerisation was continued for 24 h, before heating was stopped and the dispersion purified by repetitive centrifugation and redispersion in water. Elemental analysis showed that the MG particles contained 7.3 mol% of PA (see Table 1 and Table S1 (ESI†)).

Synthesis of PVP-PAx-PMAy microgels using CuAAC

The method used for preparing each PVP-PAx-PMAy MG (Scheme 1) differed only in the amount of AZPMa added. The following gives an example for the preparation of P-1 (Table 1). A concentrated PVP-PA MG dispersion containing 1.0 g of polymer was redispersed in DMF (20 mL) and transferred to a 50 mL Schlenk flask. AZPMa (0.032 g, 0.19 mmol) and PMDETA (0.0165 g, 0.095 mmol) were added and the solution subjected to successive freeze–pump–thaw cycles before an Ar atmosphere was introduced. CuBr (0.0136 g, 0.095 mmol) was added and the solution stirred for 16 h under Ar at room temperature. The resultant MG dispersion was purified by extensive centrifugation and redispersion in water. The method was repeated to prepare P-2 to P-4 (Table 1) using proportional scaled quantities of AZPMa. For example, the mass of AZPMa used for P-3 was 0.096 g (0.57 mmol).

Synthesis of DX MG gels

The MG dispersions (pH ~ 6) were concentrated to 10 wt% using centrifugation (6000 rpm). AIBA (9.0 mg, 0.033 mmol) was added to the MG dispersion (1.5 g) which was then thoroughly mixed using a vortex mixer. The pH of the dispersion was decreased to 3.0 by addition of aqueous HCl (4 M) to trigger physical gel formation. The physical gels were placed in O-rings (20 mm diameter, 2 mm wall thickness) between...
glass plates, which were then sealed. The assembly was placed in an oven at 50 °C for 12 h.

**Physical measurements**

Elemental analysis of microgels (C, H and N) was performed with a Thermo Scientific Flash 2000 Elemental Analyzer instrument. The standards used for calibration were acetonilide and 2,4 dinitrophenylhydrazone and MG samples were freeze-dried prior to analysis. Proton nuclear magnetic resonance (1H NMR) spectroscopy measurements were conducted on a Bruker 400 Hz instrument with deuterated chloroform used as the solvent. FTIR spectroscopy analysis was carried out using a Nicolet 5700 ATR FTIR apparatus. MG samples were freeze-dried prior to FTIR analysis. Photon correlation spectroscopy (PCS) measurements were performed using a Brookhaven BI-9000 light scattering instrument containing a 20 mW HeNe laser using a scattering angle of 90°. The electrophoretic mobilities of MG particles were recorded in the presence of aqueous NaNO₃ (0.001 M) using a Malvern Zetasizer. The mobilities of MG particles were recorded in the presence of aqueous NaNO₃ (0.001 M) using a Malvern Zetasizer. The mobilities were converted to zeta potentials (ζ) using the Smoluchowski equation. SEM images were obtained using a Philips XL30 FEGSEM apparatus. Dilute MG dispersions were dried onto glass slides at room temperature. The hydrogel samples were freeze-dried for SEM analysis. All samples were coated with Au or Pd. A minimum of 100 particles were measured to calculate the number-average particle diameter. 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. A strain of 1% was used for the frequency-sweep measurements.

**Results and discussion**

**Preparation of microgel particles with precise extents of vinyl functionalisation**

Scheme 1 shows the reaction scheme use to prepare the PVP-PAₓ₀.₀₇ MG particles. Our earlier work established that PVP-PA MGs containing high PA contents (∼18 mol%) contained intra-particle crosslinking due to PA coupling. In this study we wanted to promote pH-triggered particle swelling to maximise contact between neighbouring particles in the physical gel state and subsequent crosslinking of peripheral vinyl groups to form DX MGs. Consequently, we used a much lower PA content (∼7 mol%). As will be shown below this MG composition provided strong pH-triggered swelling and enabled formation of a range of DX MGs.

Elemental analysis is a powerful method for determining the extent of functionalisation of PA groups within PVP-PA MGs. The high sensitivity of this approach relies on the rapid change of the ratio of the %N to %C values with MG functionalisation. Experimental values for the %N to %C ratio (\(R_{N/C}^{exp} = %N/%C\)) were calculated and are plotted against the theoretical extent of vinyl functionalisation (i.e., \(y_{thr}\)) in Fig. 1a. A linear increase of \(R_{N/C}^{exp}\) with \(y_{thr}\) is evident which is strong support for the success of CuAAC for vinyl functionalisation (Scheme 1).

Building on earlier work we used values for \(R_{N/C}^{exp}\) to calculate experimental \(y\) values (\(y_{exp}\)). The method, general formulae and equations employed are described in the ESI. Fig. 1b shows that a linear relationship between \(y_{exp}\) and \(y_{thr}\) occurred with a gradient close to unity. The efficiency of the click reactions (%Click = 100 × \(y_{exp}/y_{thr}\)) was calculated (Table 1) and found to be between 73 and 96%. These high values imply that the MG particles were sufficiently swollen by DMF to allow extensive permeation of the MG interior by the reactants (AZPMA, CuBr and PMDETA). Moreover, these data indicate high click conversion efficiencies were achieved. This finding agrees with our earlier work for related MGs.

To further probe the compositions of the PVP-PAₓ₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋₇₋}_
1635 cm\(^{-1}\) in the spectra for the P-1 to P-4 copolymers (see inset of Fig. S3†) and was most pronounced for P-4, as expected. This band provides direct confirmation of vinyl functionalisation of the PVP-PA\(_x\)-PMA\(_y\) MG particles.

The C=O bands in the 1715–1735 cm\(^{-1}\) region of the PVP-PA\(_x\)-PMA\(_y\) spectra were sensitive to the \(y\) value. Expanded views of the FTIR spectra are shown in Fig. 2a. The C=O bands for the PA and PMA species had different stretching frequencies; i.e., 1715 cm\(^{-1}\) for PMA and 1735 cm\(^{-1}\) for PA. These differences are ascribed to different distances of the C=O groups to the triazole ring for each moiety (structures shown in Scheme 1). It can be seen from Fig. 2a that the relative absorbance of the C=O bond due to PMA (C=O(PMA)) increased as \(y\) \(_{\text{exp}}\) increased from 0 (for P-0) to 0.07 (for P-4). We explored the latter trend by calculating \(A_{\text{Corr}}^N(1715)\) which is the absorbance of the 1715 cm\(^{-1}\) band due to PMA (\(A_{1715}^\text{PMA}\)) normalised to that of the 1590 cm\(^{-1}\) band due to the C=N of the pyridine ring (\(A_{1590}^N\)).

\[
A_{\text{Corr}}^N(1715) = \frac{A_{1715}^\text{PMA}}{A_{1590}^N} \quad (1)
\]

The second term on the right hand side is a correction for the P-0 signal which contributes to the signal for the PMA C=O in the other spectra. The values for eqn (1) are plotted as a function of \(y\) \(_{\text{exp}}\) in Fig. 2b. Good linearity is evident which supports the view that the PMA content increased in proportion with \(y_{\text{exp}}\). These data support the elemental analysis performed above and confirm our proposal that efficient CuAAC occurred during the vinyl functionalisation of the PVA-PA MG particles by AZPMA.

The P-4 MG particles contained the highest PMA content and the spectrum for the C=O region was most suitable for deconvolution (Fig. 2c). Deconvolution of the bands due to PMA and PA gave a PMA mole fraction of 0.57, which corresponds to \(y = 0.083\). By comparison the \(y_{\text{exp}}\) value was 0.070 (Table 1) with an error of ±0.007 (Table S1, ESI†). The difference between these values is not considered significant. These data, taken together with the linearity from Fig. 2b, support the elemental analysis data (Fig. 1). Accordingly, we propose that it is straightforward to dial up the vinyl functionalisation extent of MG particles using CuAAC. More generally, based on the present study and our previous work using different azide species, the approach used here should enable further or alternative functionalisation of these PA-containing MG particles in a controllable manner.

**Vinyl-functionalised microgel particle properties**

The PVP-PA\(_x\)-PMA\(_y\) MG particles were characterised using SEM (Fig. 3a, b and Fig. S4†) and had coefficients of variation (Table 1) less than 10%. The MG particles were monodisperse. At pH values greater than or equal to 4.0 the MG particles were in the collapsed state with the average hydrodynamic diameters for the PVP-PA\(_x\)-PMA\(_y\) MG particles in the range 340–355 nm. It can be seen from Table 1 that there was reasonable agreement between hydrodynamic diameters at pH = 7 (\(d_{h(7)}\)) and number-average SEM diameters with the former only slightly larger than the latter. We conclude that our CuAAC method for vinyl functionalising PVP-PA MG particles did not compromise colloidal stability, which is highly desirable for a functionalisation approach.
In all cases the MG particles exhibited strong swelling with high volume-swelling ratios ($Q$) in the range 45 to 80 (Table 1). These $Q$ values are much higher than the $Q$ value of 3.3 reported for the PVP-PA MG particles studied earlier. The latter system contained more than twice the PA content compared to the PVP-PA$_{0.07}$ MGs employed here. The high $Q$ values obtained in this study support the view that intra-particle crosslinking due to PA coupling and/or hydrophobic association, was not significant. The concentrated PVP-PA$_x$–PMA$_y$ dispersions were well suited to pH-triggered physical gel formation, and hence inter-MG linking to form DX MGs, because PMA functionalisation did not restrict particle swelling at low pH (Fig. 3c).

The pH-dependent zeta potential ($\zeta$) data (Fig. 3d) show that the MG particles were positively charged at pH values less than or equal to 7.0 and generally agree with earlier reports for PVP-based MG particles. We note that microgel electrophoretic data are often reported in terms of electrophoretic mobility and this distinction originates in large part from the pioneering work of Ohshima et al., which has recently been reviewed elsewhere. CuAAC did not significantly affect the $\zeta$ values. Furthermore, the latter values were not significantly affected by $y_{exp}$. This result is not surprising because at low pH the MG particles had a very high positive volume–charge density and functionalisation should not extinguish charge because it provides charged triazole groups. It also follows from these data that DX MGs prepared in this study from the PVP-PA$_x$–PMA$_y$ MGs (below) were cationic.

**Synthesis and morphology of doubly crosslinked microgels**

The DX MG formation process involved triggered physical gel formation (Fig. 4a) and covalent interlinking of the particles to form a permanent gel (Scheme 1). Physical gels formed when the MG concentration was greater than or equal to 5 wt% and pH less than 4.0. In the physically gel state the MG particle peripheries overlap and covalent crosslinking can occur. Generally, the DX MGs had good transparency (Fig. 4d), which is due to the high extents of swelling of the constituent MG particles.

We probed the morphologies of freeze-dried SX MG physical gels and DX MG covalent gels using SEM (Fig. 4b, c, e and f). SEM images for P-2 gels before (b and c) and after (e and f) DX MG formation are shown. MG particles are clearly evident which shows that they maintained their integrity upon macroscopic gel formation. The SEM images showed the MG particle packing was mostly disordered for the physical gel (Fig. 4b) and disordered for the mostly ordered DX MG (Fig. 4e). Interestingly, a mostly crystalline arrangement was evident for the DX MG (Fig. 4e). However, close examination of the SEM images (insets for Fig. 4b and e) showed there were some domains with the opposing morphologies present for each system, i.e. ordered assembly for the mostly disordered physical gel (Fig. 4b) and disordered for the mostly ordered DX MG (Fig. 4e).

Fast Fourier Transform (FFT) images (see insets of Fig. 4b and e) confirmed that amorphous and crystalline morphologies were dominant for the physical SX MGs and covalent DX MGs, respectively. The physical gel showed amorphous
halos; whereas, the DX MG showed an hexagonal array of points (these differences were repeatable for different domains within the samples). It is interesting to note that recently reported polyvinylamine DX MGs were also reported to have crystalline order. It is suggested that crystalline order is favoured for DX MGs comprised of MG particles with low size polydispersity. It is the control (and locking in) of ordered particle arrangements with spacings comparable to the wavelength of light that may allow DX MGs to be prepared for new photonic applications. Furthermore, if the interstitial sites apparent in Fig. 4e and f act as pores these new DX MGs may have potential application as membranes. These aspects will be explored in future work.

**Dialing up doubly crosslinked microgel modulus**

Having established that functionalisation of the MG particles could be precisely controlled we next sought to investigate the ability to control DX MG mechanical properties. We first examined the effect of MG concentration ($C_{MG}$) on the dynamic rheological properties. The P-2 system was selected for this study because of its good combination of optical clarity (Fig. 4d) and ordered morphology (Fig. 4f).
Frequency-sweep dynamic rheology measurements were obtained for P-2 gels prepared using a range of $C_{\text{MG}}$ values and are shown in Fig. 5a and b. The $G'$ (storage modulus) values had very low frequency dependence and the gels exhibited solid-like viscoelastic behaviour. The latter is a common feature reported for DX MGs.¹²,³⁰ The tan $\delta$ values ($= G''/G'$) were less than 0.10 (Fig. 5b) which shows that the gels were mostly elastic. Whilst most of the DX MGs had frequency dependent tan $\delta$ values, the data obtained using $C_{\text{MG}} = 10\%$ had negligible frequency dependence. Winter and Chambon³¹ established that systems at the critical gel points have frequency independent tan $\delta$ values. It follows that the DX MG prepared using $C_{\text{MG}} = 10\%$ exhibited critical behaviour over the frequency region studied.

The $G'$ values measured at 10 Hz were considered plateau values and are plotted as a function of $C_{\text{MG}}$ in this study (Fig. 5c). Effective volume fractions occupied by MG particles may be reported for concentrated MG dispersions. Such values are often determined using low concentration viscosity measurements.³²–³⁴ Extrapolation of those values to higher concentrations often results in effective volume fraction values greater than unity and such values offer limited insight beyond showing that the MG particles have deformed. We therefore used $C_{\text{MG}}$ values in this work and note that it is highly likely that the MG particles were deformed in the physical and covalent gels. Interestingly, the $G'$ vs. $C_{\text{MG}}$ data showed a linear increase with $C_{\text{MG}}$ (Fig. 5c). This behaviour contrasts to acrylate-based DX MGs which have been reported to give exponential dependences of $G'$ with MG particle concentration.⁹ Whilst we cannot be certain of the cause of these differences the linear behaviour observed here may be aided by the very strong swelling nature of the MG particles as evidenced by high $Q$ values measured using PCS (Table 1). We conjecture that strong MG particle swelling forced the MG peripheries close together and, in turn, favoured efficient inter-MG covalent bonding (Scheme 1).

Fig. 5d shows the tan $\delta$ values measured as a function of $C_{\text{MG}}$. Data for the parent non-inter-linked SX MG are also shown at $C_{\text{MG}} = 10\%$. DX MG formation decreased the tan $\delta$ value, which has been reported for acrylate based DX MGs.³⁵ This decrease is attributed to formation of elastically effective linkages between neighbouring particles. The tan $\delta$ values are in the range 0.01 to 0.02 for the gels containing $C_{\text{MG}} < 20\%$. These values imply that 98–99% of the mechanical energy used to deform the gel matrix was stored for these systems. The tan $\delta$ value was significantly larger (0.09 at a frequency of 10 Hz) for the $C_{\text{MG}} = 20\%$ system, which suggests a higher proportion of inelastic linkages were present.

In the final part of the study we investigated the effect of vinyl functionalisation on the mechanical properties of the DX MGs using a $C_{\text{MG}}$ value of 10 wt%. The latter value was chosen because of the critical behaviour exhibited by P-2 gel discussed above. Fig. 6a shows that the $G'$ values were almost frequency independent. This behaviour was also observed for P-0, which
was a SX MG physical gel (not doubly crosslinked). Consequently, the low frequency dependence for the $G'$ values is attributed to the close packed nature of the MG particles within the gels (Fig. 4b, c, e and f), rather than the inter-MG linking.

Low frequency dependences for tan $\delta$ were also observed for most of the gels (Fig. 6b). However, the P-0 system (not doubly crosslinked) showed tan $\delta$ values that increased with frequency. The P-4 system showed surprisingly high tan $\delta$ values which had the same tendency to decrease with increasing frequency that was apparent for most of the other DX MGs. It appears from the data shown in Fig. 5b and 6b that DX MGs which are highly functionalised or have high MG concentrations favour formation of elastically ineffective chains that give rise to frequency dependent tan $\delta$ values. Such chains may result from trapping of MG particles in structural arrangements that do not permit sufficient movement for inter-linking to occur.

As shown in Fig. 6c a linear relationship was evident between $G'$ and $y_{\text{exp}}$. This result is important for two reasons. It is the first demonstration of the ability to precisely tune the modulus of DX MGs via vinyl functionalisation control. Secondly, the data imply that CuAAC can be used to precisely vary the extent of functionalisation by an azide species within a hydrogel using CuAAC. Part of the reason this approach was successful for the present gels is that the functionalisation occurred with high surface area-to-volume ratio MG particles which were then assembled to form a hydrogel. Uniquely, our DX MG approach means that large scale (slow) diffusion of reactants is not required for uniform functionalisation of a macroscopic hydrogel on the length scale of micrometres.

Fig. 6c also shows that the tan $\delta$ values were only weakly dependent on $y_{\text{exp}}$. The tan $\delta$ values were all smaller than that for P-0, which is due to the latter being a SX MG physical gel.

An interesting question that can be addressed using the data presented here concerns the proportion of PMA groups that form elastically effective chains. To address this question we first calculate the number of elastically effective chains per particle ($\nu_{\text{eff}(P)}$), then the number of PMA groups per particle ($N_{\text{PMA}(P)}$) and finally the number of elastically effective chains per PMA group ($N_{\text{eff}(P)}$). Using the affine approximation the number-density of elastically effective chains ($\nu_{\text{eff}}$) is related to the modulus ($G$) by:

$$G = \nu_{\text{eff}} kT$$

where $k$ and $T$ are the Boltzmann constant and temperature, respectively. We assume that $G$ and $G'$ measured at 10 Hz are equivalent. The $G'$ values have contributions from particle–particle interactions and also inter-MG linking. Non-covalent particle–particle interactions were corrected by subtracting the $G'$ value for the non-interlinked SX MG physical gel (P-0) from the $G'$ values for the DX MGs using the data shown in Fig. 6c. All of the gels had the same $C_{\text{MG}}$ value of 10 wt%. The corrected $G'$ values were then used to calculate $\nu_{\text{eff}}$ from eqn (2) – see Table 2.

Calculation of the value for $\nu_{\text{eff}(P)}$ required the mass of a particle ($M_p$) which was calculated from the diameters of the collapsed particles obtained from SEM (Table 1). The $M_p$ and
### Table 2: Estimation of the number of elastically effective chains per PMA unit

<table>
<thead>
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<th>Code</th>
<th>$M_p^{\text{eff}}/10^{-5}$ g</th>
<th>$\nu_{\text{eff}}^{\text{exp}}/10^{3}$ m$^{-3}$</th>
<th>$N_{\text{PMA}}^{\text{eff}}/10^{3}$</th>
<th>$N_{\text{eff}/PMA}$</th>
<th>$a$</th>
<th>$b$</th>
<th>$c$</th>
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<td>—</td>
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<td>15</td>
<td>0.070</td>
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</tr>
</tbody>
</table>

*Particle mass. *Number density of elastically effective chains. *Number of elastically effective chains per particle. *Number of PMA groups per particle. *Number of elastically effective chains per PMA group.

The $\nu_{\text{eff}}^{\text{exp}}$ values appear in Table 2. The $\nu_{\text{eff}}^{\text{exp}}$ values are of the order of $10^4$ to $10^6$, and are comparable to those calculated by Roeder et al. for their vinyl-functionalised hematite particle gels. To calculate the value for $n_{\text{PMA}}^{\text{eff}}$, we used the composition of the parent P-0 particles as well as the particle mass to estimate the number of PA molecules per particle. Use of the $\nu_{\text{exp}}$ values (Table 1) then enabled calculation of $n_{\text{PMA}}^{\text{eff}}$ (Table 2). $N_{\text{eff}/PMA}$ values were calculated from the ratios of $\nu_{\text{eff}}^{\text{exp}}$ to $n_{\text{PMA}}^{\text{eff}}$ (Table 2) and are plotted in Fig. 6d.

The data shown in Fig. 6d provide an estimate of the proportion of vinyl groups that are involved in DX MG formation for the first time. The average value for $N_{\text{eff}/PMA}$ was 0.080 (i.e., 8%) and there was no significant difference for the gels (Fig. 6d). The relatively low proportion of PMA groups that formed elastically effective chains is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains result from inter-MG linking at the particle peripheries. Hence, a low proportion of PMA groups will be available for elastically effective chains at the particle peripheries. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firstly, the PMA groups were probably distributed throughout each MG particle. Secondly, the elastically effective chains per particle is reasonable for two reasons. Firste

## Conclusions

In this study we have used CuAAC to vinyl functionalise cationic PVP-PA MG particles and create new DX MGs using a synthetic method that did not compromise colloidal stability. CuAAC efficiencies were calculated to be in the range of 79 to 96% and enabled precise control of the vinyl functionalisation over the range of 0 to 7.0 mol%. The mechanical properties were probed by dynamic rheology and the modulus varied linearly with $C_{MGO}$ over the range of about 11 to 50 kPa. Furthermore, the modulus also varied linearly with $y_{\exp}$ over the range of 13 to 29 kPa for DX MGs prepared $C_{MGO} = 10$ wt% Analysis of the data revealed that ~8% of the PMA groups formed elastically effective chains and this appeared to be independent of $y_{\exp}$. The former value was attributed to the location of the PMA groups at the periphery of the MG particles being key to DX MG formation. The study presented here provides the first example of a VP-based DX MG and also demonstrates the ability of CuAAC to enable unprecedented control of hydrogel functionalisation. Not only does this work pave the way for improvements in hydrogel functionalisation and also new tools for precisely “dialing up” gel modulus but it also should increase the number of gels that can be prepared by the DX MG route. This expansion in the number of DX MG types should be possible by inclusion of PA during MG synthesis and utilisation of our CuAAC approach. Furthermore, the presence of latent functionality of the unreacted acetylene groups in the MG particles (Scheme 1) offers excellent potential for additional functionalisation of the MG particles prior to DX MG formation, which could enable construction of next generation functional hydrogels. Future control of the ordering of the MG particles within VP-based DX MGs may also provide a new generation of photonic gels as well as membranes.

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## References