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End-group ionisation enables the use of poly(N-(2-methacryloyloxy)ethyl pyrrolidone) as a steric stabiliser block for polymerisation-induced self-assembly in aqueous media

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A series of near-monodisperse poly(N-2-(methacryloyloxy)ethyl pyrrolidone) (PNMEP) homopolymers was prepared via reversible addition-fragmentation chain transfer (RAFT) solution polymerisation of NMEP in ethanol at 70 °C using a carboxylic acid-functional RAFT agent. The mean degree of polymerisation (DP) was varied between 19 and 89 and acid titration indicated end-group pK_a values of between 5.07 and 5.44. Turbidimetry studies indicated that homopolymer cloud points were significantly higher at pH 7 (anionic carboxylate) than at pH 3 (neutral carboxylic acid). Moreover, this enhanced hydrophilic character was sufficient to enable PNMEP to be used as a steric stabiliser for polymerisation-induced self-assembly (PISA) syntheses conducted in aqueous media. Thus, a $PNMEP_{42}$ precursor was chain-extended via RAFT aqueous dispersion polymerisation of 2-hydroxypropyl methacrylate (HPMA) at 44 °C. A series of $PNMEP_{42}$-$PHPMA_x$ diblock copolymers were synthesised at 20% w/w solids using this protocol, with the target PHPMA DP being varied between 150 and 400. High conversions were achieved and a linear increase in M_n with increasing PHPMA DP was observed. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) studies confirmed that a spherical morphology was obtained in all cases. The nanoparticles flocculated either below pH 4.5 (as a result of protonation) or on addition of 60 mM KCl (owing to charge screening). Thus the anionic end-groups on the PNMEP stabilizer chains make an important contribution to the overall colloidal stability. Similarly, a $PNMEP_{53}$ macro-CTA was chain-extended via RAFT aqueous emulsion polymerisation of 2-ethoxyethyl methacrylate (EEMA) at 44 °C. Again, a neutral solution pH was critical for the synthesis of colloidally stable nanoparticles at 20% w/w solids. High conversions were achieved as the target PEEMA DP was varied between 100 and 600 and a linear evolution in molecular weight with PEEMA DP was confirmed by chloroform GPC studies. DLS experiments indicated a monotonic increase in nanoparticle diameter with PEEMA DP and a spherical morphology was confirmed in each case by TEM studies. In summary, PNMEP can be used as a water-soluble steric stabiliser for aqueous PISA syntheses of diblock copolymer nanoparticles provided that it contains an anionic carboxylate end-group to enhance its hydrophilic character.

Introduction

Poly(*N*-vinylpyrrolidone) (PNVP) is a highly polar water-soluble polymer that is typically prepared by conventional free radical polymerisation: it has been utilised in a wide range of commercial applications, including laundry, personal care and cosmetics.[1] More specifically, PNVP acts as an anti-dye transfer agent in laundry formulations, a film-forming agent in hair sprays, and as a binder for various types of cosmetics.[2] Furthermore, it is sufficiently biocompatible to be used as an excipient for drug formulations and for the manufacture of soft contact lenses.[3–5]

NVP is a less-activated monomer (LAM), so its reversible addition-fragmentation chain transfer (RAFT) polymerisation requires a dithiocarbamate or xanthate chain transfer agent (CTA) for optimal control.[6–10] Moreover, NVP copolymerises well with only a rather limited range of similarly reactive monomers, such as vinyl acetate or acrylics.[11–14] In contrast, 2-(*N*-methacryloyloxy)ethyl pyrrolidone (NMEP) can be copolymerised with a wider range of monomers, including methacrylics and styrene.[15–19] Therefore, the synthesis of well-defined pyrrolidone-functional polymers by RAFT polymerisation is rather more straightforward using NMEP (or 2-(*N*-acryloyloxy)ethyl pyrrolidone[20,21]) rather than NVP.

Polymerisation-induced self-assembly (PISA) enables the rational design of block copolymer nanoparticles, including spheres, worms and vesicles.[22–25] In PISA, a soluble macromolecular chain transfer agent (macro-CTA) acts as a steric stabiliser block and is chain-extended with a solvent-miscible monomer. The growing second block becomes insoluble at some critical DP, leading to *in situ* self-assembly. For example, Cunningham and co-workers reported the RAFT dispersion polymerisation of benzyl methacrylate in ethanol

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using PNMEP as a steric stabiliser block.[16] Alternatively, PNMEP can be utilised as a core-forming block for RAFT dispersion polymerisation formulations conducted in *n*-dodecane.[17] Turbidimetry studies confirm that PNMEP exhibits inverse temperature solubility in aqueous solution.[15,26] Such behaviour has been used to prepare sterically-stabilised block copolymer nanoparticles with weakly hydrophobic PNMEP cores in water at 70 °C that subsequently dissolve to afford molecularly-dissolved copolymer chains on cooling to ambient temperature. This strategy has enabled the synthesis of relatively high molecular weight PNMEP via RAFT aqueous dispersion polymerisation.[15] However, it also suggests that PNMEP does not have sufficient hydrophilic character to be used as a steric stabiliser block for PISA formulations conducted in aqueous media. In the present study, we show that this apparent limitation can be overcome by the judicious use of an appropriate RAFT agent.

Thermoresponsive polymers exhibiting lower critical solution temperature (LCST) behaviour have received substantial interest, particularly in the context of drug delivery.[27–33] Undoubtedly, the most studied example is poly(*N*-isopropylacrylamide) (PNIPAM), which exhibits a cloud point (CP) at around 32 °C.[34,35] This critical temperature can be raised via statistical copolymerisation of NIPAM with various hydrophilic monomers[36,37] or by employing hydrophilic end-groups.[38,39] Pyrrolidone-functional polymers are usually biocompatible and therefore offer an alternative to PNIPAM for potential bioapplications. In particular, PNMEP exhibits inverse solubility behaviour as already noted above. [26,40] At around ambient temperature, the pyrrolidone ring forms hydrogen bonds with water that confer aqueous solubility, despite the relatively hydrophobic methacrylic backbone. However, such hydrogen bonding is disrupted on heating to a sufficiently high temperature, causing PNMEP to precipitate from aqueous solution.

The effect of polymer structure on the LCST behaviour of PNMEP was explored by Sun *et al*.[26] They compared its LCST behaviour with that of poly(*N*-(3-acryloyloxypropyl)pyrrolidone) (PNAPP) and poly(*N*-(3-methacryloyloxypropyl)pyrrolidone) (PNMPP). As expected, PNMEP exhibited a very similar CP to that of its isomeric acrylic analogue (PNAPP). However, addition of just one methylene group to the backbone (i.e. PNMEP vs. PNMPP) led to a 37 °C reduction in CP for polymers of comparable mean degree of polymerisation (DP).

Herein we demonstrate that ionisation of a single terminal carboxylic acid (derived from a RAFT chain transfer agent) renders PNMEP sufficiently hydrophilic to serve as a suitable steric stabiliser block for PISA syntheses conducted in aqueous media. This is demonstrated for both the RAFT aqueous dispersion polymerisation of 2-hydroxypropyl methacrylate (HPMA) and the RAFT aqueous emulsion polymerisation of 2-ethoxyethyl methacrylate (EEMA).

Experimental

Materials

N-(2-(Methacryloyloxy)ethyl pyrrolidone (NMEP; 98% purity) was kindly provided by Ashland Inc. (USA) and was used without further purification. 2-Hydroxypropyl methacrylate (HPMA) was provided by GEO Specialty Chemicals (Hythe, UK). 2-Ethoxyethyl methacrylate (EEMA), ethanol (≥99.8%) and NaOH were purchased from Sigma Aldrich UK. 4,4'-Azobis(4-cyanopentanoic acid) (ACVA; 99%) was purchased from Alfa Aesar (Heysham, UK). 2,2'-Azobis(2-(2-imidazolin-2-yl)propane)dihydrochloride (VA-044) was purchased from Wako Pure Chemical Industries (Japan). d_4-Methanol and d_6-dimethyl sulfoxide were purchased from Goss Scientific Instruments Ltd. (Cheshire, UK), while 4-cyano-4-(2-phenylethanesulfanylthiocarbonyl)sulfanyl-pentanoic acid (PETTC) RAFT agent was synthesised as previously reported.[41] Deionised water was used for all experiments.

Synthesis of a PNMEP$_x$ macro-CTA by RAFT Solution Polymerisation at 70 °C. A typical protocol for a PNMEP$_{53}$ macro-CTA is described below. Macro-CTAs of other degrees of polymerisation (19 – 89) were also synthesised by varying the NMEP/PETTC molar ratio.

NMEP (10.99 g, 55.7 mmol), PETTC RAFT agent (0.27 g, 0.80 mmol; target DP = 70), ACVA (44.6 mg, 0.59 mmol; PETTC/ACVA molar ratio = 5.0) and ethanol (16.96 g, 40% w/w solids) were weighed into a 50 mL round-bottom flask and degassed with stirring in ice for 30 min. The reaction flask was allowed to proceed for 210 min in an oil bath set to 70 °C resulting in a monomer conversion of 69% judged by ^{1}H NMR. The crude polymer was precipitated into diethyl ether to remove residual monomer before freeze-drying in the minimum amount of water to afford a dry yellow powder. The mean DP was judged to be 53 by comparing the integrals of the aromatic in PETTC at 7-8 ppm to the methylene carbonyl proton signal at 2.5 ppm. GPC analysis using dimethyl formamide (DMF) eluent indicated an M_n of 7 700 g mol^{-1} and M_w/M_n of 1.26 against a series of ten near-monodisperse poly(methyl methacrylate) calibrants.

Polymerisation-Induced Self-Assembly Synthesis of PNMEP$_x$-PHPMA$_y$ Diblock Copolymer Particles via RAFT Aqueous Dispersion Polymerisation of HPMA at 44 °C. A typical protocol for the synthesis of PNMEP$_{42}$-PHPMA$_{300}$ is described as follows: PNMEP$_{42}$ macro-CTA (0.06 g, 6.96 µmol), HPMA (0.30 g, 2.09 mmol), VA-044 (0.40 mg, 1.39 µmol added as a 10 mg/g VA-044 in water stock solution: PNMEP$_{42}$ macro-CTA/VA-044 molar ratio = 5.0) and water (1.45 g) were added to a 14 mL vial and the solution pH was adjusted from 3.8 to pH 7 with NaOH. The reaction vial was sealed and degassed under N_2 for 30 minutes before placing in a pre-heated oil bath set at 44 °C for 16 h. The polymerisation was quenched by exposing to air. The polymer nanoparticles were characterised by ^{1}H NMR in MeOD, DLS and TEM with 0.01% w/w aqueous dispersions made using pH 7 water. DMF GPC analysis indicated M_n = 46 300 g mol^{-1} and M_w/M_n = 1.44. ^{1}H NMR indicated >99% monomer conversions in all cases. Other diblock compositions

were synthesised by adjusting the amount of monomer to target HPMA DP of 150 – 400.

Polymerisation-Induced Self-Assembly Synthesis of $PNMEP_x$-$PEEMA_y$ Diblock Copolymer Particles via RAFT Aqueous Dispersion Polymerisation of EEMA at 44 °C. The pH of the reaction mixtures was adjusted prior to addition of EEMA monomer. A typical protocol for the synthesis of $PNMEP_{53}$-$PEEMA_{100}$ is described: $PNMEP_{53}$ macro-CTA (0.12 g, 11.10 µmol), VA-044 (0.07 mg, 2.22 µmol added as a 10 mg/g VA-044 in water stock solution: $PNMEP_{53}$ macro-CTA/VA-044 molar ratio = 5.0) and water (1.19 g) were added to a 14 mL vial and the pH was adjusted to 7 using NaOH. EEMA (0.18 g, 1.11 mmol) was then added to the vial which was then sealed and degassed using N_2 for 30 minutes. The reaction was placed into a pre-heated oil bath set to 44 °C for 5 h. After quenching the polymerisation by exposure to air, portions of the resulting copolymer nanoparticles were freeze-dried prior to GPC and ^{1}H NMR analysis in chloroform and DMSO respectively. 0.01 %w/w dispersions for DLS and TEM analysis were made using pH 7 water. Chloroform GPC analysis indicated Mn = 13 800 g mol^{-1} and M_w/M_n = 1.31. ^{1}H NMR indicated >99% monomer conversions in all cases. Other diblock compositions were synthesised by adjusting the amount of monomer to target EEMA DP of 100 – 600.

Copolymer Characterisation

^{1}H NMR spectroscopy. d_4-Methanol was used to record the ^{1}H NMR spectra of the $PNMEP_x$ macro-CTAs and the $PNMEP_{42}$-$PHPMA_x$ diblock copolymers. The $PNMEP_{53}$-$PEEMA_x$ diblock copolymers were freeze-dried prior to recording the ^{1}H NMR spectra in deuterated DMSO. The spectrometer used was a 400 MHz Bruker Advance 400 spectrometer.

Gel permeation chromatography (GPC). The molecular weights of both the PNMEP homopolymers and $PNMEP_{42}$-$PHPMA_x$ copolymers were obtained using a DMF GPC at 60 °C containing 10 mmol LiBr at a flow rate of 1.0 mL min^{-1}. For $PNMEP_{53}$-$PEEMA_x$ copolymers, the molecular weight was obtained using chloroform GPC containing 0.25% TEA by volume at 35 °C. Two Polymer Laboratories PL gel 5 µm Mixed C columns were connected in series to a Varian 390 multidetector suite (refractive index detector) and a Varian 290 LC pump injection module. For the PNMEP homopolymers a Polymer Laboratories PL gel 5 µm Mixed C column was connected in series to a Polymer Laboratories PL gel 5 µm Mixed E column to aid resolution of the lower molecular weight polymers. Ten near-monodisperse poly(methyl methacrylate) standards (PMMA; M_n = 625 – 618 000 g mol^{-1}) were used for calibration and the data was analysed using Varian Cirrus GPC software.

Dynamic light scattering (DLS). A Malvern Zetasizer NanoZS instrument was used to determine the intensity-average hydrodynamic diameter of the copolymer nanoparticles at 20 °C at a scattering angle of 173 °C. Dispersions of 0.01% w/w in pH 7 water were analysed using disposable plastic cuvettes. The data was averaged over three consecutive runs for each sample. Aqueous electrophoresis measurements were also conducted at 0.01% w/w but were diluted using 1 mM KCl. The pH was adjusted HCl and KOH as required.

Transmission electron microscopy (TEM). Copper/palladium grids were surface-coated in-house to produce a thin film of amorphous carbon before plasma glow-discharged for 40 seconds producing a hydrophilic surface. 0.01% w/w aqueous copolymer dispersions at pH 7 were dropped on to the grids for 60 seconds, blotted to remove the excess solution before being negatively stained uranyl formate solution (0.75% w/v) for 20 seconds. The excess stain was removed by blotting and the grid was further dried using a vacuum hose. A FEI Tecnai Spirit microscope fitted with a Gatan 1kMS600CW CCD camera operating at 80 kV was used to image the grids.

Cloud point (CP) determination. Spectra were recorded for the $PNMEP_x$ homopolymers of varying DPs using aqueous solutions of 1.0% w/w using a Varian Cary 300 Bio UV-visible spectrometer. Samples were heated at a rate of 1.0 °C per min with spectra recorded every 1.0 °C between 25 and 90 °C. The solution pH was adjusted using NaOH and HCl. The point of inflection on a plot of transmittance (recorded at an arbitrary wavelength of 600 nm) vs temperature was used to determine the CP.[42]

Results and Discussion

End-group ionisation effects have been recently reported for various aqueous PISA formulations by Lovett and co-workers[43,44] and Penfold *et al.*[45,46] In each case, ionisation (or protonation) of a terminal carboxylic acid (or tertiary amine) group led to a change in the diblock copolymer morphology owing to greater solvation of the steric stabiliser block, which leads to a subtle shift in the fractional packing parameter. In contrast, we show herein that end-group ionisation is *an essential prerequisite to ensure colloidal stability* when using PNMEP as a moderately hydrophilic steric stabiliser block.

A similar phenomenon was previously reported by Weaver *et al.* for relatively short poly(2-hydroxyethyl methacrylate) (PHEMA) chains.[29] PHEMA had been classified as water-insoluble[47,48] but by targeting relatively low molecular weights (DP < 45), LCST behaviour was observed at pH 6.5, while no LCST behaviour occurred under the same conditions for PHEMA oligomers (e.g. DP = 20). By reducing the solution pH to 2.2, the terminal morpholine end-groups became protonated, thus increasing the hydrophilic character of the PHEMA chains and ensuring their aqueous solubility at all temperatures for DPs of up to 50.

In principle, higher molecular weight should reduce the CP as the entropy of mixing becomes less favourable.[49,50] However,

such molecular weight-dependent LCST behaviour is not necessarily characteristic of all water-soluble polymers. In particular, end-group effects can play an important role: hydrophilic end-groups typically increase the CP, whereas hydrophobic end-groups usually reduce it.[51,52,53] For example, Luan *et al.* removed the hydrophobic dithiobenzoate group from a series of PNIPAM-poly(*N*-(2-hydroxypropyl) methacrylamide) diblock copolymers by aminolysis to produce thiol-capped chains.[54] This derivatisation raised the CP of the thiol-functional copolymers by up to 23 °C relative to the precursor copolymer. Moreover, the increase in CP observed for lower molecular weight copolymers suggests that end-group effects are particularly strong in this regime.[54]

Summers *et al.* investigated the effect of converting a pyridyl disulfide end-group into a thioglycerol group.[38] Higher cloud points were observed for both a di(ethylene glycol) methyl ether methacrylate / oligo(ethylene glycol) methyl ether methacrylate statistical copolymer and also for a PNIPAM homopolymer. No cloud point was observed for relatively short PNIPAM chains, which again suggests that end-groups can have a substantial influence in the lower molecular weight limit.

In the present study, a series of PNMEP homopolymers of varying mean DP were prepared by RAFT ethanolic solution polymerisation and their LCST behaviour in aqueous solution was investigated. These PNMEP precursors were then chain-extended using either 2-hydroxypropyl methacrylate (HPMA) via RAFT aqueous dispersion polymerisation or 2-ethoxyethyl methacrylate (EEMA) via RAFT aqueous emulsion polymerisation.

Synthesis of PNMEP Homopolymers via RAFT Solution Polymerisation in Ethanol.

A series of PNMEP homopolymers were prepared by RAFT solution polymerisation of NMEP in ethanol using a PETTC RAFT agent at 70 °C, see Scheme 1. Depending on the target DP, these polymerisations were allowed to proceed for between 100 min and 4 h prior to quenching by cooling to 20 °C followed by exposure to air.

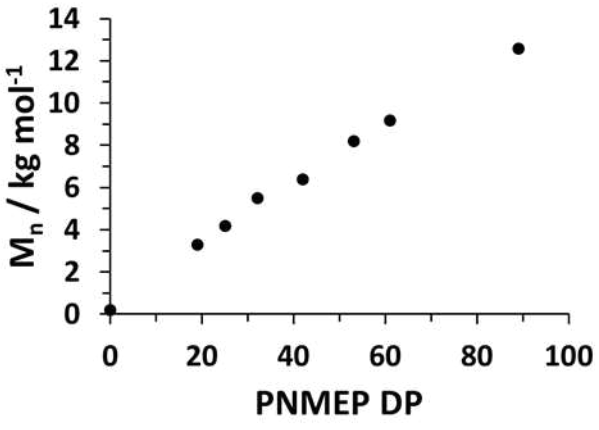
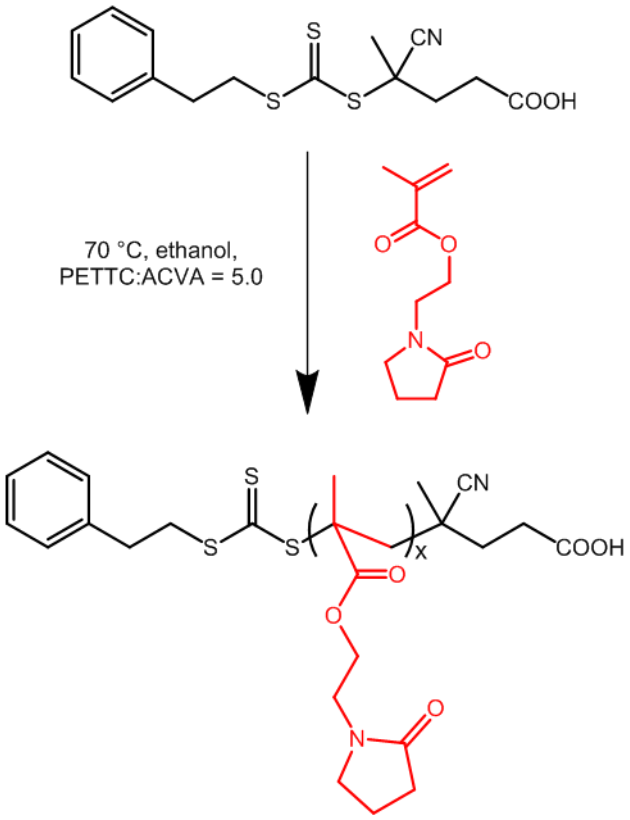


Figure 1. Relationship between number-average molecular weight (Mn) as determined by DMF GPC and mean DP determined by end-group using 1H NMR for a series of seven PNMEP homopolymers prepared by RAFT solution polymerisation of NMEP in ethanol at 70 °C.



Scheme 1. Synthesis of PNMEPx homopolymer precursors by RAFT solution polymerisation of NMEP in ethanol using PETTC at 70 °C.

^{1}H NMR spectroscopy studies indicated 70-90% conversion and, after purification by precipitation, mean DPs ranged between 19 and 89. DMF GPC analysis confirmed the expected linear increase in molecular weight with PNMEP DP (see Figure 1 and Table 1) The mean DP for each of the seven PNMEP homopolymers was also determined using UV spectroscopy; these data are reasonably consistent with that determined by end-group analysis using ^{1}H NMR. The relationship between GPC molecular weight and the mean DP as determined by UV spectroscopy is shown in Figure S1.

Aqueous Solution Properties of PNMEP Homopolymers.

PETTC confers a terminal carboxylic acid group on each PNMEP chain (see Scheme 1). The pK_a was determined for each homopolymer by acid titration, see Figure 2. The pK_a increased with increasing PNMEP DP. This end-group is 50% ionised at a solution pH of 5.07-5.44 for mean PNMEP DPs of between 19 and 89.

Cloud points were determined for 1.0% w/w aqueous solutions of a series of seven PNMEP homopolymers at either pH 3 or pH 7 by turbidimetry at an arbitrary fixed wavelength of 600 nm. Cloud points observed at pH 7 were always significantly higher compared to those determined at pH 3. This thermal transition occurred over a broader temperature range for relatively short PNMEP chains (DP < 50) at pH 7. Moreover, no cloud point was observed for PNMEP DPs above 50 on heating up to 90 °C at this

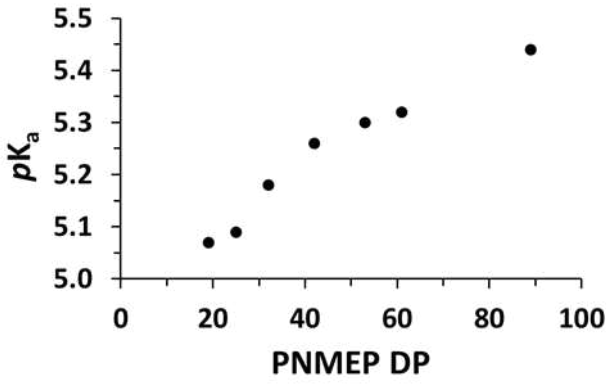


Figure 2. Effect of varying the mean degree of polymerisation on the pK_a of the carboxylic acid end-group as determined by acid titration for a series of near-monodisperse $PNMEP_x$ homopolymers prepared by RAFT solution polymerisation of NMEP in ethanol at 70 °C using the PETTC RAFT agent (see Scheme 1).

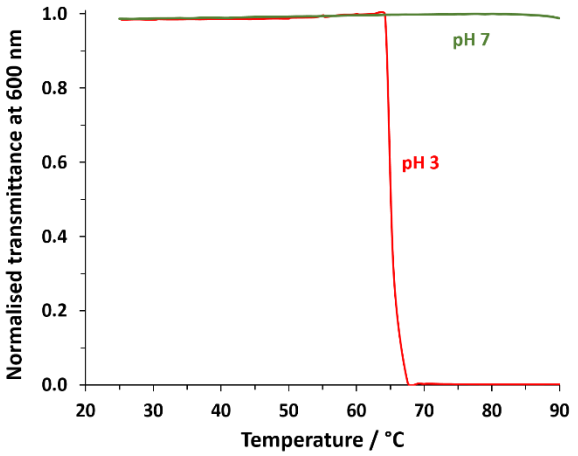


Figure 3. Normalised transmittance at 600 nm against temperature for 1.0% w/w aqueous solutions of a PNMEP53 homopolymer at either pH 3 or pH 7. A CP of approximately 64 °C was obtained at pH 3 (red curve) but no CP was observed for the same homopolymer at pH 7.

pH, which corresponds to the upper limit temperature for our instrument set-up. 1.0% w/w aqueous solutions of $PNMEP_x$ were placed in an oil bath and heated up to 100 °C. Each solution remained transparent indicating that LCST behaviour was eliminated for DPs greater than 50 at a solution pH of 7.

At pH 3, a solution of $PNMEP_{53}$ homopolymer exhibited a cloud point of 64 °C, so ionisation of the terminal carboxylic acid group raises the CP of $PNMEP_{53}$ chains by 36 °C in this case.

Interestingly, higher CP values were observed for increasing PNMEP DP, contrary to normal behaviour.[15,49,50] However, Miasnikova *et al.* observed a similar trend when examining end-group effects on the LCST behaviour of methoxy-capped poly(diethylene glycol acrylate) (PDEGA) homopolymers.[55] More specifically, a series of PDEGA homopolymers were synthesised using a bistrithiocarbonate RAFT agent that conferred tert-butyl benzoate groups on both chain-ends. These hydrophobic *tert*-butyl groups were selectively removed in a post-polymerisation step to generate hydrophilic benzoic acid end-groups. Higher cloud points were observed with increasing DP for both the precursor and the benzoic acid-terminated PDEGA homopolymers.[55] Thus, for the *tert*-butyl-terminated homopolymers, the cloud points increased monotonically from

9.0 °C (DP = 28) to 41.2 °C (DP = 513), while for the benzoic acid-terminated PDEGA homopolymers, the corresponding cloud points ranged from 16.8 °C to 42.0 °C. Furthermore, Miasnikova *et al.* observed an *increase* in cloud point when using a more hydrophilic end-group for PDEGA homopolymers of the same DP.[55] This effect is analogous to that obtained when adjusting the solution pH for an aqueous solution of a given PNMEP homopolymer from pH 3 to 7 (see Figure 4a). Miasnikova *et al.* tentatively suggested that their unexpected observations might be due to conformational effects of the hydrophobic acrylic backbone.[55] In the context of the present study, it is perhaps worth emphasising that the same remarkable phenomenon is observed for a water-soluble *methacrylic* polymer, which has significantly lower chain mobility (the glass transition temperature of PNMEP is above ambient temperature). However, it is noteworthy that normal LCST behaviour (i.e. a systematic *reduction* in cloud point with increasing DP) was observed by Cunningham and co-workers for a series of PNMEP hompolymers prepared using a non-ionic dithiobenzoate end-group, which suggests that end-groups play an important role in determining cloud point.[15]

At pH 7, thermal transitions were relatively broad for shorter PNMEPs (DP < 53), whereas homopolymers with higher DPs exhibited relatively sharp CP values. Similar effects were also observed by Miasnikova *et al.*,[55] who suggested that dispersity effects (expected to be stronger for lower molecular weights) might lead to broader transitions for lower DPs. Alternatively, the sharper transitions observed for higher DPs might be the result of cooperative collapse.[55] As discussed above, lower molecular weight polymers are usually more sensitive to end-group effects. Given that the RAFT agent used to prepare these PNMEP homopolymers contains a hydrophobic aromatic ring, this could lead to lower CP values in this case. Relatively fast thermal transitions were observed at pH 3 (Figure 3) while the broader transitions observed at pH 7 (Figure 4b).

When determining CP values, non-zero final transmittances were observed for all $PNMEP_x$ homopolymers at pH 7 (see Figure 4b). Thus, the data obtained at pH 7 are best regarded as estimates. In contrast, these 1.0% w/w aqueous solutions became completely opaque (zero transmittance) above the CP for turbidity studies conducted at pH 3.

It is clear from these turbidimetry studies that ionisation of the terminal carboxylic acid group raises the CP of PNMEP homopolymers. In principle, this significant increase in hydrophilic character should enable RAFT aqueous dispersion polymerisation syntheses to be conducted, provided that the polymerisation temperature remains well below the CP of the steric stabiliser block.

Table 1. Summary of molecular weight and CP data for a series of PNMEP homopolymers prepared by RAFT ethanolic solution polymerisation as determined by DMF GPC and turbidimetry respectively. The pK_a of each homopolymer was determined by acid titration.

Reaction time / min	PNMEP DP[a]	Conversion[b] / %	M_n[c] / g mol^{-1}	M_w/M_n[c]	CP / °C		pK_a
					pH 3	pH 7	
100	19	77	3 300	1.13	38	59	5.07
210	25	73	4 200	1.14	50	68	5.09
200	32	72	5 500	1.15	61	81	5.18
210	42	74	6 400	1.15	62	80	5.26
210	53	69	8 200	1.19	64	> 90	5.30
195	61	90	9 200	1.20	62	> 90	5.32
240	89	81	12 600	1.21	64	> 90	5.44

[a]PNMEP DP as determined by end-group analysis using ^{1}H NMR. [b] Conversion determined by ^{1}H NMR in d_4-methanol. [c] Determined by DMF GPC (relative to poly(methyl methacrylate) calibration standards).

Synthesis of PNMEP$_{42}$-PHPMA$_x$ Diblock Copolymer Nanoparticles via RAFT Aqueous Dispersion Polymerisation of HPMA.

PNMEP$_{42}$ homopolymer was prepared by RAFT solution polymerisation of NMEP in ethanol using PETTC at 70 °C prior to purification by precipitation into excess diethyl ether. ^{1}H NMR analysis indicated 74% NMEP conversion and a mean DP of 42 after purification (based on end-group analysis of the aromatic

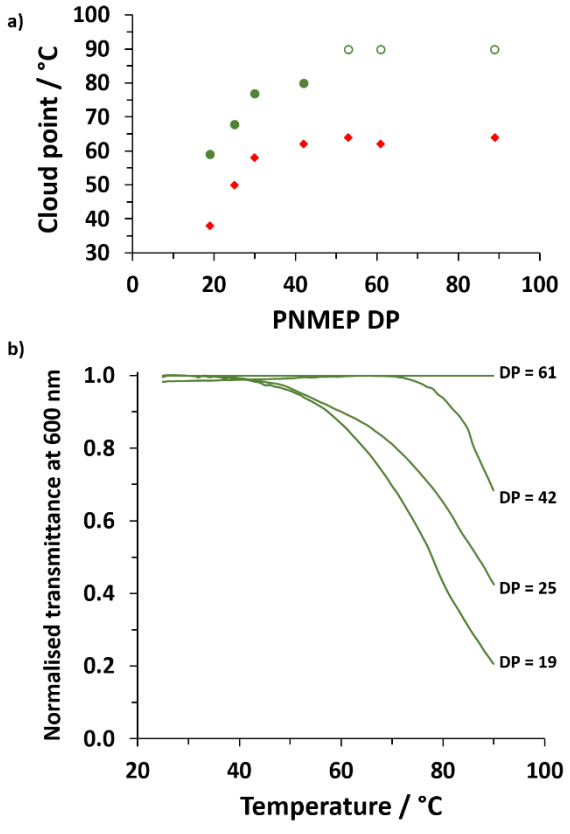
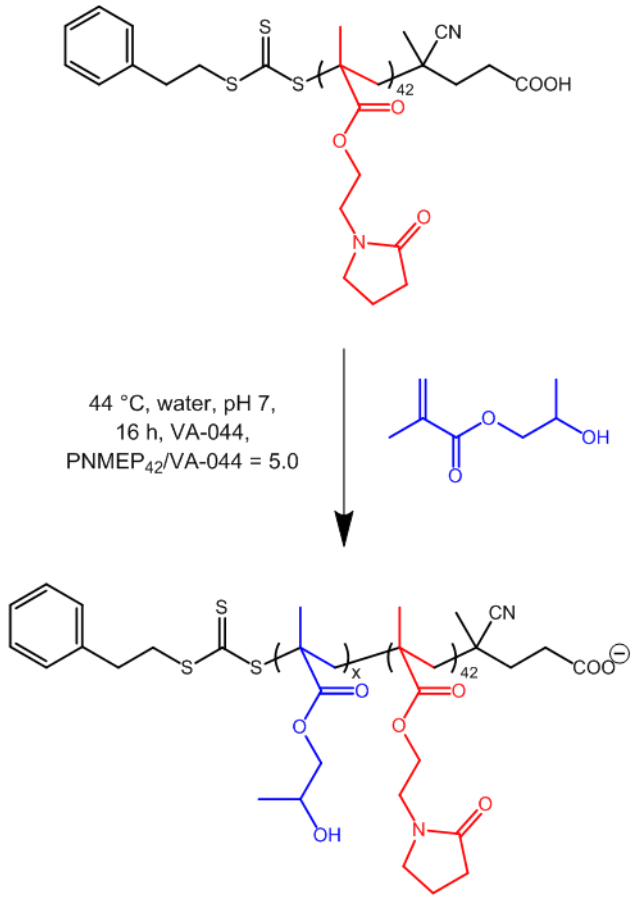


Figure 4. (a) Relationship between CP (°C) determined for 1.0% w/w aqueous solution and PNMEP homopolymer DP at pH 3 (◆) and pH 7 (●). The (○) symbol indicates that no CP was observed using turbidimetry on heating up to 90 °C (which corresponds to the upper limit temperature for the instrument set-up). (b) Normalised transmittance at 600 nm for a series of seven PNMEP homopolymers with mean DPs of 19 to 89.



Scheme 2. Synthesis of PNMEP$_{42}$-PHPMA$_x$ diblock copolymer nanoparticles at pH 7 by RAFT aqueous dispersion polymerisation of HPMA at 44 °C.

Table 2. Summary of target diblock copolymer compositions, HPMA conversions determined by ^{1}H NMR spectroscopy, number-average molecular weights (M_n) and dispersities (M_w/M_n) determined by DMF GPC analysis, and z-average particle diameter and dispersities (PDI) determined by DLS analysis of dilute aqueous dispersions at pH 7.

Target copolymer composition	Conversion[a] / %	GPC[b]		DLS[c]	
		M_n / g mol^{-1}	M_w/M_n	Z-average diameter / nm	PDI
$PNMEP_{42}$ precursor	74	6 100	1.22	/	/
$PNMEP_{42}$-$PHPMA_{150}$	> 99	26 600	1.30	25	0.32
$PNMEP_{42}$-$PHPMA_{200}$	> 99	33 300	1.32	35	0.15
$PNMEP_{42}$-$PHPMA_{250}$	> 99	39 300	1.43	53	0.07
$PNMEP_{42}$-$PHPMA_{300}$	> 99	46 300	1.44	59	0.06
$PNMEP_{42}$-$PHPMA_{350}$	> 99	53 100	1.44	64	0.08
$PNMEP_{42}$-$PHPMA_{400}$	> 99	56 600	1.59	81	0.23

[a] Determined by ^{1}H NMR spectroscopy in d_4-methanol. [b] DMF eluent, refractive index detector (relative to poly(methyl methacrylate) calibration standards. [c] Nanoparticle dispersions were diluted to 0.01% w/w using pH 7 water.

groups). DMF GPC analysis indicated an M_n of 6 100 g mol^{-1} and an M_w/M_n of 1.22, which suggests good RAFT control. This water-soluble precursor was subsequently chain-extended via RAFT aqueous dispersion polymerisation of HPMA at 44 °C targeting a copolymer concentration of 20% w/w, see Scheme 2. A series of $PNMEP_{42}$-$PHPMA_x$ diblock copolymer nanoparticles were prepared by varying the target DP of PHPMA from 150 to 400. It is well-known that RAFT polymerisation becomes problematic above pH 7 because this leads to loss of RAFT end-groups via hydrolysis[56,57] thus, initial aqueous PISA syntheses were performed at pH 7. In each case the reaction solution was adjusted to pH 7.0 ± 0.1 before the HPMA polymerisation was allowed to proceed overnight. More than 99% conversion was achieved for all syntheses as judged by ^{1}H NMR spectroscopy. DMF GPC was used to analyse all diblock copolymers (see Figure 5). Increasing the target DP of the PHPMA core-forming block led to progressively broader molecular weight distributions (higher M_w/M_n values) as RAFT control was gradually lost although the GPC traces remained unimodal. Relatively high blocking efficiencies were achieved but a minor fraction of the $PNMEP_{42}$ precursor chains could not be chain-extended. Nevertheless, the GPC molecular weight increased linearly with the mean PHPMA DPs determined from ^{1}H NMR analysis.

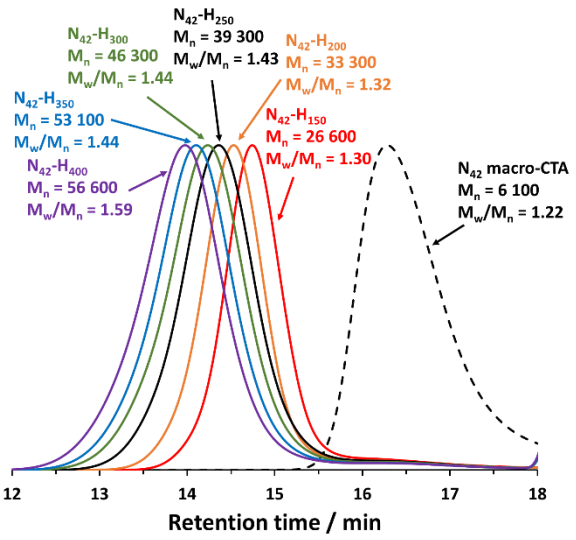


Figure 5. DMF GPC chromatograms recorded for a series of $PNMEP_{42}$-$PHPMA_x$ diblock copolymers and the corresponding $PNMEP_{42}$ precursor (refractive index detector; calibration using a series of near-monodisperse poly(methyl methacrylate) standards). (For brevity, 'N' denotes PNMEP and 'H' denotes PHPMA).

Transmission electron microscopy (TEM) was used in conjunction with dynamic light scattering (DLS) to analyse the $PNMEP_{42}$–$PHPMA_x$ diblock copolymer nanoparticles. DLS indicated the formation of relatively small nanoparticles, with narrow, monomodal size distributions being obtained in some cases, see Table 2. TEM studies of the dried nanoparticles confirmed that spherical morphologies were obtained regardless of the target PHPMA DP, see Figure 6. Presumably, the anionic surface charge prevents the formation of so-called higher order morphologies such as worms or vesicles. Similar examples of kinetically-trapped spheres have been previously reported for aqueous PISA syntheses by Semsarilar and co-workers.[58]

As a control experiment, the synthesis of $PNMEP_{42}$-$PHPMA_{200}$ diblock copolymers via aqueous dispersion polymerisation was attempted using the same conditions as stated above but at a solution pH of 3. The pK_a of $PNMEP_{42}$ is 5.26, hence the degree of ionisation of the carboxylate end-group was relatively low. Macroscopic phase separation was observed with a white upper layer of PHPMA homopolymer and a yellow layer of PNMEP-PHPMA (where the PHPMA block was relatively short (see Figure S2).

In view of the unsuccessful PISA syntheses conducted at pH 3, the anionic carboxylate end-groups on the PNMEP stabiliser

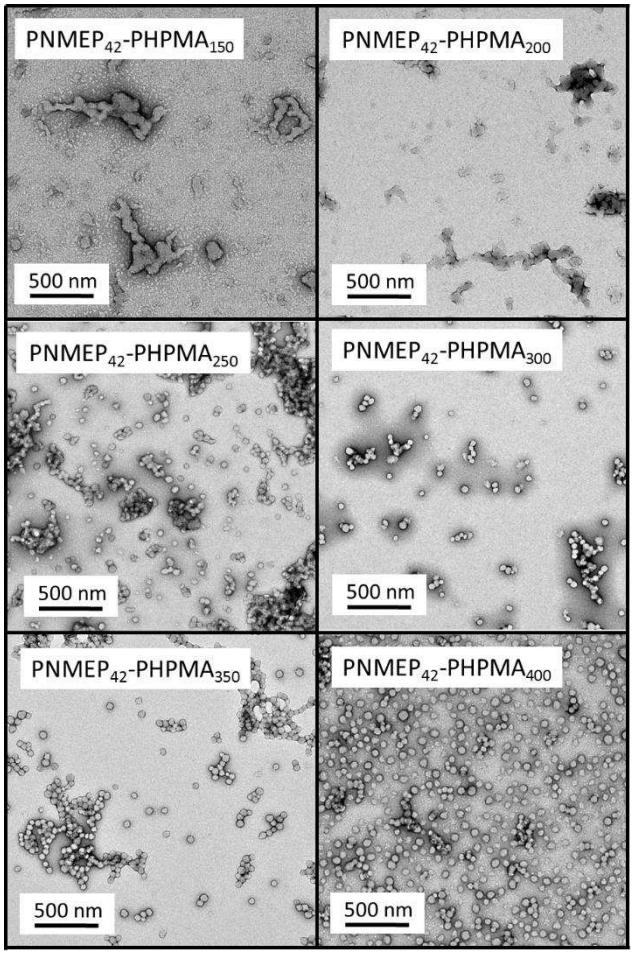


Figure 6. Representative TEM images recorded for dried dilute aqueous dispersions of $PNMEP_{42}$-$PHPMA_x$ diblock copolymer nanoparticles prepared at pH 7 showing well-defined spherical nanoparticles regardless of the DP of the PHPMA core-forming block.

chains were considered likely to play an important role in conferring colloidal stability at pH 7. This hypothesis was examined by systematically varying the pH of a 0.1% w/w aqueous dispersion of $PNMEP_{42}$-$PHPMA_{300}$ nanoparticles while conducting DLS and electrophoresis studies at 20 °C to examine the extent of nanoparticle aggregation, see Figure 7a. The z-average particle diameter remained constant at approximately 58 nm from pH 5 to pH 8, with corresponding zeta potentials of -20 to -30 mV over this range. However, the apparent particle diameter increased significantly to 260 nm at pH 4.8, which is below the pK_a of the $PNMEP_{42}$ macro-CTA (pK_a = 5.26). This indicates that incipient flocculation of these nanoparticles occurs as the degree of ionisation of their end-groups falls below 50%. Zeta potentials became progressively less negative at lower pH, stabilising at approximately -3.0 mV below pH 4. Moreover, the flocculated nanoparticles could not be redispersed on adjusting the solution pH back to pH 7. This is in contrast to the reversible aggregation-redispersion behaviour reported by Lovett and co-workers[43,44] and Penfold et al.[45,46] for closely related nanoparticles. Clearly, anionic surface charge makes an important contribution to the colloidal stability of these $PNMEP_{42}$-$PHPMA_{300}$ nanoparticles.

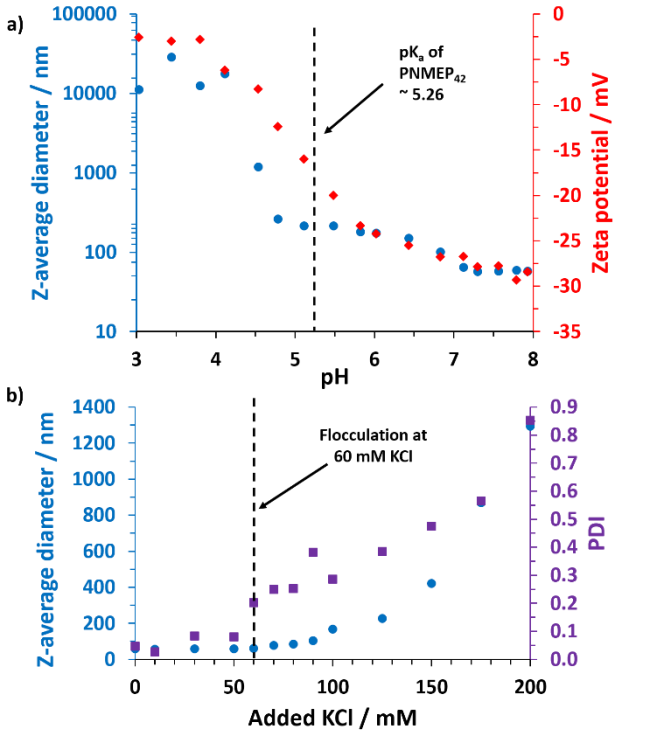


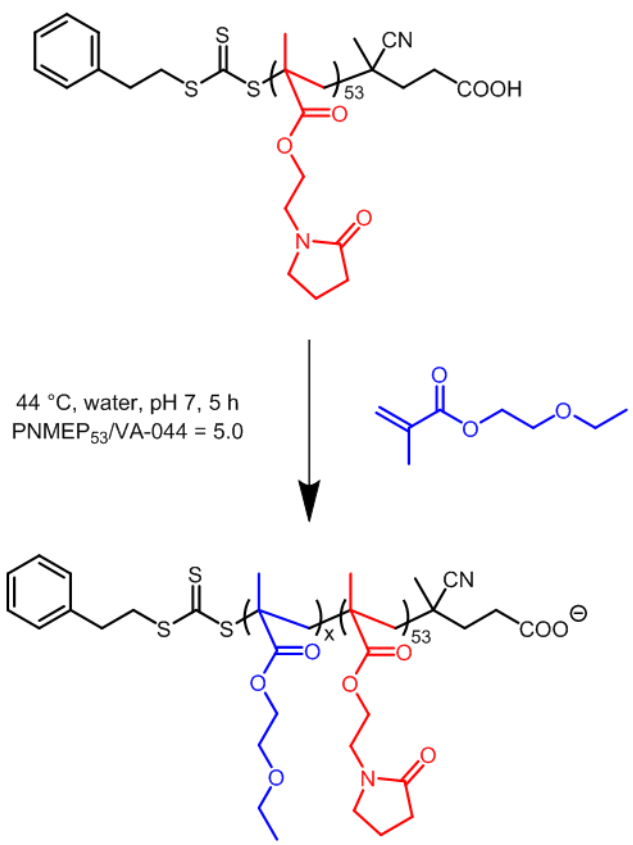
Figure 7. (a) Zeta potential (♦) and z-average diameter (●) vs. solution pH and (b) z-average diameter and polydispersity (■) vs. added KCl at pH 7 recorded for $PNMEP_{42}$-$PHPMA_{300}$ nanoparticles.

As further proof that the colloidal stability of these nanoparticles is at least partly governed by their anionic end-groups, the effect of addition of an electrolyte was explored. It is well known that charge-stabilised nanoparticles are readily flocculated on addition of salt, whereas sterically-stabilised nanoparticles usually remain colloidally stable under such conditions.[59]

Thus, 0.1% w/w aqueous dispersions of $PNMEP_{42}$-$PHPMA_{300}$ nanoparticles were exposed to 10-200 mM KCl at pH 7. The DLS size distribution became significantly broader (i.e. higher polydispersities, PDI) above 60 mM KCl, indicating incipient flocculation. However, the z-average particle diameter did not increase significantly until the KCl concentration exceeded 100 mM. Nevertheless, it is clear that the colloidal stability of such dispersions is compromised in the presence of sufficient added salt.

Synthesis of $PNMEP_{53}$-$PEEMA_x$ Diblock Copolymer Particles via RAFT Aqueous Emulsion Polymerisation of EEMA.

A $PNMEP_{53}$ macro-CTA was prepared by RAFT solution polymerisation of NMEP at 70 °C in ethanol prior to purification by precipitation into diethyl ether. ^{1}H NMR spectroscopy indicated a mean DP of 53 via end-group analysis after purification. Chloroform GPC analysis indicated an M_n of 6,100 g mol^{-1} and an M_w/M_n of 1.24. This eluent was chosen because it is also a good solvent for the diblock copolymer PNMEP-PEEMA (see below).



Scheme 3. Synthesis of $PNMEP_{42}$-$PEEMA_x$ diblock copolymers by RAFT aqueous emulsion polymerisation of EEMA at 44 °C at pH 7.

The $PNMEP_{53}$ precursor was subsequently chain-extended via RAFT aqueous emulsion polymerisation of EEMA at 20% w/w solids while varying the target PEEMA DP from 100 to 600. All reaction solutions were adjusted to pH 7.0 ± 0.1 using NaOH before the EEMA polymerisation was allowed to proceed for 5 h at 44 °C. More than 99% conversion was achieved in all cases as judged by ^{1}H NMR spectroscopy. Chloroform GPC analyses indicated that the molecular weight increased linearly with DP (Figure 8a) and molecular weight distributions became significantly broader on increasing the target DP of the PEEMA core-forming block (Figure 8b).

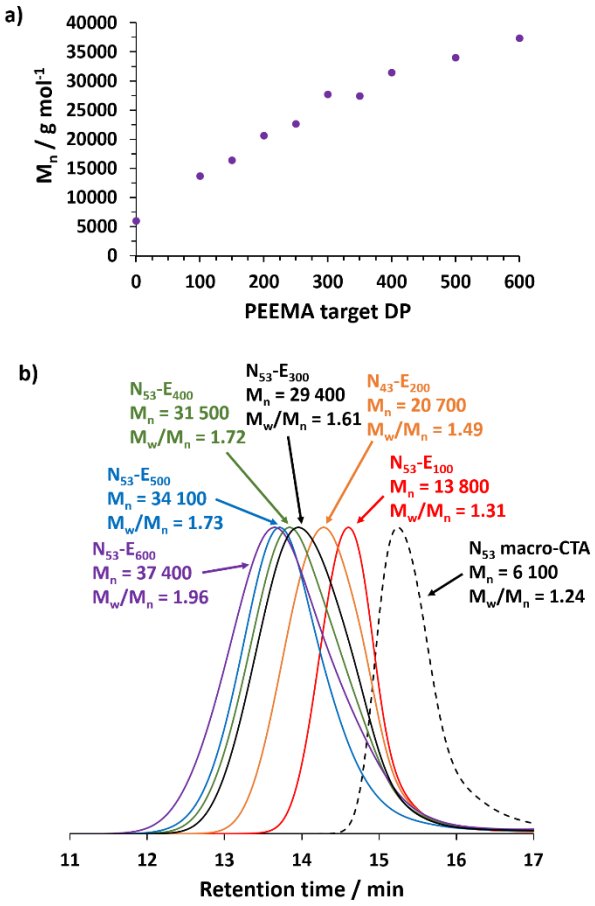


Figure 8. (a) Linear increase in molecular weight (M_n) with increasing PEEMA target DP as determined by chloroform GPC. (b) Overlaid GPC chromatograms obtained for a series of $PNMEP_{53}$-$PEEMA_x$ diblock copolymers prepared by RAFT aqueous emulsion polymerisation of EEMA at 44 °C, and the corresponding $PNMEP_{53}$ precursor (refractive index detector; calibration using a series of near-monodisperse poly(methyl methacrylate) calibration standards). (For brevity, 'N' denoted PNMEP and 'E' denotes PEEMA).

All nanoparticles exhibited an exclusively spherical morphology as judged by TEM analysis (Figure 9). DLS studies indicated that the z-average particle diameter increased with target PEEMA DP and particle size distributions were relatively narrow (PDI < 0.10 in most cases).

Table 3. Summary of target $PNMEP_{53}$-$PEEMA_x$ diblock copolymer compositions, monomer conversions by ^{1}H NMR analysis, molecular weights (M_n) and dispersities (M_w/M_n) determined by chloroform GPC, and z-average particle diameter and dispersities (PDI) determined by DLS analysis of dilute aqueous dispersions at pH 7.

Target copolymer composition	Conversion[a] / %	GPC[b]		DLS[c]	
		M_n / g mol^{-1}	M_w/M_n	z-average diameter / nm	PDI
$PNMEP_{53}$ precursor	69	6 100	1.24	n.d.	n.d.
$PNMEP_{53}$-$PEEMA_{100}$	> 99	13 800	1.31	35	0.14
$PNMEP_{53}$-$PEEMA_{200}$	> 99	20 700	1.49	42	0.09
$PNMEP_{53}$-$PEEMA_{300}$	> 99	27 800	1.63	49	0.10
$PNMEP_{53}$-$PEEMA_{400}$	> 99	31 500	1.72	55	0.09
$PNMEP_{53}$-$PEEMA_{500}$	> 99	34 100	1.73	53	0.10
$PNMEP_{53}$-$PEEMA_{600}$	> 99	37 400	1.96	66	0.08

[a] Determined by end-group analysis by ^{1}H NMR in d-chloroform [b] Determined by chloroform GPC analysis (relative to poly(methyl methacrylate) calibration standards). [c] Nanoparticle dispersions were diluted to 0.01% w/w using water (pH 7).

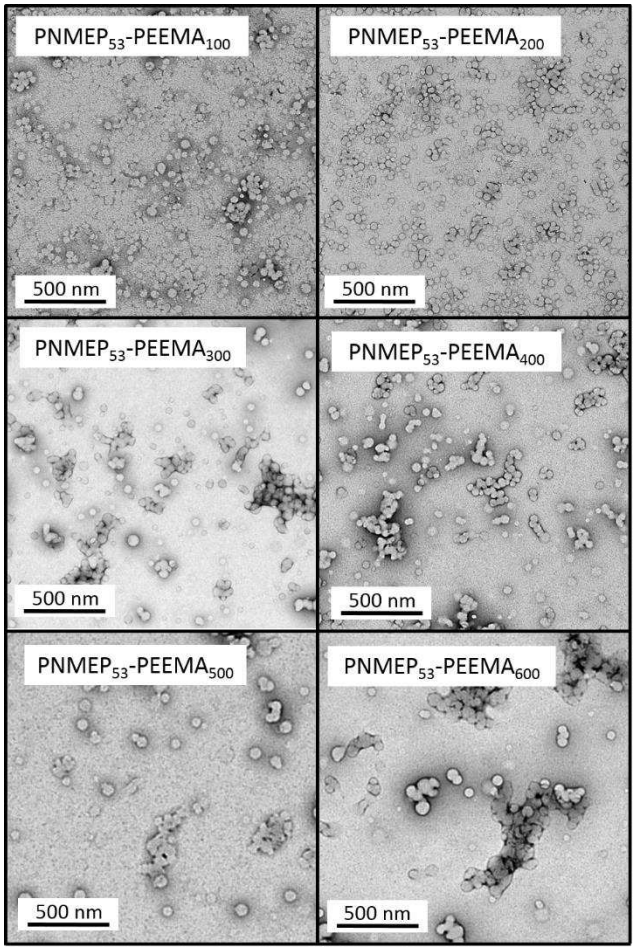


Figure 9. Representative TEM images showing well-defined spherical morphologies for $PNMEP_{53}$-$PEEMA_x$ diblock copolymer nanoparticles prepared at pH 7.

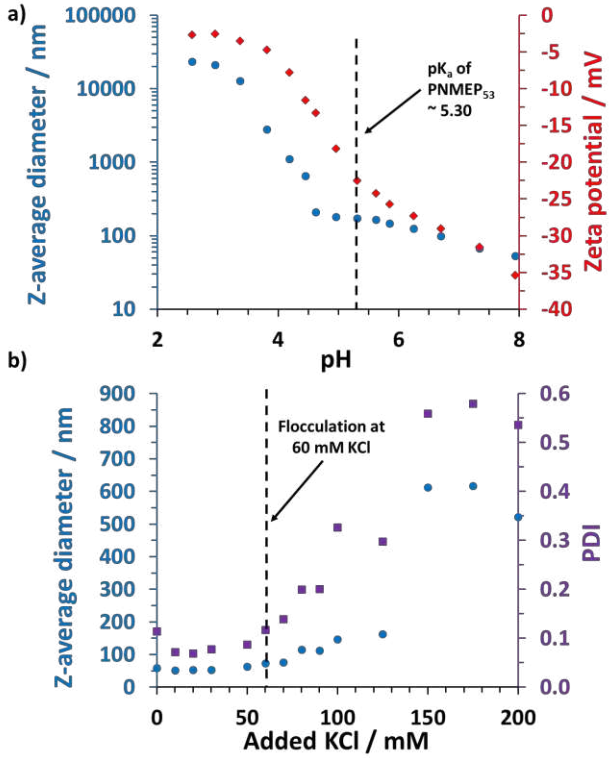


Figure 10. (a) Zeta potential (♦) and z-average diameter (●) vs. pH and b) z-average diameter and DLS polydispersity (■) vs. KCl concentration observed for a 0.01% w/w aqueous dispersion of $PNMEP_{53}$-$PEEMA_{500}$ diblock copolymer nanoparticles in the presence of 1 mM KCl as background electrolyte. The aqueous dispersion pH was adjusted using either 0.05 M or 0.01 M HCl.

As discussed above, ionisation of the majority of the carboxylic acid end-groups is essential if the PNMEP precursor is to be used as a steric stabiliser block for aqueous PISA syntheses. The anionic surface charge leads to colloidally stable $PNMEP_{53}$-$PEEMA_{500}$ diblock copolymer nanoparticles at pH 7, which can be destabilised by lowering the aqueous dispersion pH. This eventually leads to flocculation at 20 °C, as judged by DLS and aqueous electrophoresis studies, see Figure 10. A monotonic reduction in zeta potential from approximately –36 mV at pH 8 to –13 mV at pH 4.62 was observed. Incipient flocculation occurred at the latter pH, which corresponds to protonation of most of the anionic carboxylate groups as the pK_a of the $PNMEP_{53}$ homopolymer is 5.30. The zeta potential became progressively less negative on further lowering the dispersion pH, reaching a constant value of approximately –3 mV below pH 3.

To provide further evidence that the colloidal stability of these nanoparticles is indeed critically dependent on their anionic end-groups, their sensitivity to added electrolyte was examined. Thus aqueous dispersions of $PNMEP_{53}$-$PEEMA_{500}$ diblock copolymer nanoparticles were diluted to 0.1% w/w using 10–200 mM KCl at pH 7. Particle size distributions became much broader for KCl concentrations above 60 mM, indicating incipient flocculation. However, the z-average particle diameter only increased substantially above 125 mM KCl

Conclusions

A series of seven near-monodisperse $PNMEP_x$ homopolymers with mean DPs ranging from 19 to 89 were prepared via RAFT solution polymerisation of NMEP in ethanol at 70 °C using a carboxylic acid-based RAFT agent. Critical solution temperatures were determined for each $PNMEP_x$ homopolymer at pH 3 (where the carboxylic acid end-groups are present in their neutral form) and at pH 7 (where most of the carboxylic acid end-groups are ionised). In each case, end-group ionisation increased the hydrophilic character of each homopolymer significantly: no cloud point was observed on heating up to 90 °C for DPs greater than 50. The pK_a for each homopolymer was determined via acid titration and ranged from 5.07 to 5.44 for all DPs.

A $PNMEP_{42}$ macro-CTA was chain-extended via RAFT aqueous dispersion polymerisation of HPMA at 44 °C. A series of $PNMEP_{42}$-$PHPMA_x$ diblock copolymer nanoparticles were prepared at 20% w/w solids with the PHPMA DP systematically varied between 150 and 400. High monomer conversions were achieved in all cases and a linear correlation between GPC M_n and PHPMA DP was observed. TEM studies confirmed that the sole morphology was kinetically-trapped spheres and DLS studies indicated a monotonic increase in the hydrodynamic particle diameter with PHPMA DP. These nanoparticles became

flocculated either below pH 4.5 (as a result of protonation of the anionic carboxylate end-groups) or on addition of 60 mM KCl (owing to charge screening).

PNMEP was also used as a steric stabiliser to prepare a series of diblock copolymer nanoparticles at pH 7 via RAFT aqueous emulsion polymerisation of EEMA at 44 °C. More specifically, a $PNMEP_{53}$ precursor was chain-extended with the target PEEMA DP being varied between 100 and 600. High EEMA conversions were achieved in all cases and GPC studies confirmed a linear increase in molecular weight with PEEMA DP. A strong correlation between the mean nanoparticle diameter and the PEEMA DP was observed and TEM studies indicated kinetically-trapped spheres as the sole morphology. Like the $PNMEP_{42}$-$PHPMA_{300}$ nanoparticles, these $PNMEP_{53}$-$PEEMA_{500}$ nanoparticles became flocculated either on addition of 60 mM KCl or by adjusting the solution pH to below pH 4.5. Overall, such experiments confirm that the colloidal stability of these sterically-stabilised nanoparticles is critically dependent on the anionic charge located at the end of each stabiliser chain.

In summary, despite the inverse temperature solubility behaviour exhibited by PNMEP in aqueous solution, conferring appropriate hydrophilic end-groups enables use of this water-soluble polymer as a steric stabiliser for the synthesis of block copolymer nanoparticles via polymerisation-induced self-assembly (PISA) in aqueous solution at around neutral pH.

Conflicts of interest

There are no conflicts to declare.

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