

Chapter 2

Synthesis and Characterisation of *Hyp*-polydendron Materials and Subsequent Nanoparticle Formation

2.1 Introduction

The synthesis of high generation dendrimers continues to be a non-cost effective and difficult process, as previously discussed, although the possibility of producing larger materials whilst maintaining such high surface functionality as present in dendrimers is highly desirable.

The aim of this work was to synthesise first and second generation dendron initiators for ATRP, investigate whether these dendron initiators would polymerise under ATRP conditions and to assess how this new functionality might affect the behaviour of branched polymers produced using a simple divinyl monomer incorporation strategy. Three ATRP initiators were chosen for this study, shown in Fig. 2.1. The first and second generation dendron initiators, **G1** and **G2** respectively, were synthesised and compared to a commercially available non-dendron initiator, ethyl bromo isobutyrate, **EBiB**.

These initiators were used to polymerise the monomer 2-hydroxypropyl methacrylate (HPMA) to give linear and branched poly(2-hydroxypropyl methacrylate) (pHPMA), targeting three different number average degrees of polymerisation (DP_n); 20, 50 and 100 monomer units.

The subsequent linear and branched polymers, bearing each initiating group at one end of every polymer chain were to be studied, to assess their behaviour in solvent and aqueous environments to ascertain their ability to form nanoparticles. The ability to form nanoparticles from such materials could give polymeric nanoparticles which contain a high level of functionality introduced via the dendron moieties at one polymer chain end, which has important implications in various applications such as developing biological systems toward drug delivery [1, 2].

Publication arising from this chapter: “Hyperbranched polydendrons: a new controlled macromolecular architecture with self-assembly in water and organic solvents”, Fiona L. Hatton, Pierre Chambon, Tom O. McDonald, Andrew Owen and Steven P. Rannard, *Chem. Sci.*, **2014**, 5, 1844–1853.

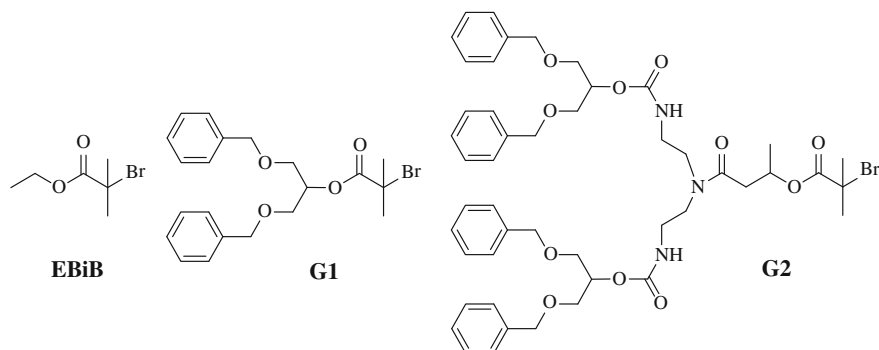


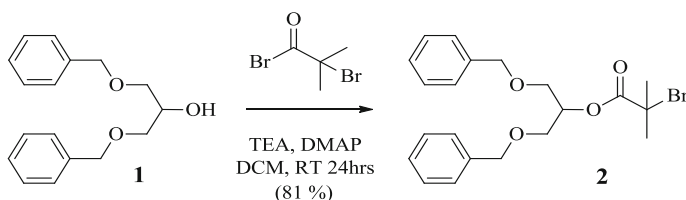
Fig. 2.1 ATRP initiators chosen for the study

2.2 Hydrophobic Dendron Initiator Synthesis

The target surface group for the two dendron initiators was 1,3-dibenzyloxy-2-propanol (DBOP), **1**, shown in Scheme 2.1. This surface group was targeted as it provides a hydrophobic surface functionality which provides a good control for comparison with hydrophilic surface functionalities. The benzyl groups were also useful as they are easily distinguished by ^1H NMR spectroscopy as the shifts corresponding to the aromatic protons on the benzyl groups have significantly different chemical shifts to any others present in the targeted polymers.

2.2.1 Generation 1 DBOP Dendron (G1)

Due to the structure of the parent alcohol, **1**, a simple esterification reaction with α -bromoisobutyryl bromide yielded the **G1** dendron initiator, **2**, outlined in Scheme 2.1. This esterification reaction is well reported in the literature for the preparation of various functional initiators for ATRP [3–5].



Scheme 2.1 Synthesis of the **G1** DBOP dendron initiator, **2**

The 4-dimethylaminopyridine (DMAP) acted as a catalyst, whereby the nitrogen in the pyridine ring attacks the acyl bromide giving a brief pyridinium intermediate, which is more reactive than the initial acyl bromide therefore aiding the reaction. Triethylamine (TEA) was used to neutralise HBr (a side product of the reaction), this could be seen as a white precipitate which was observed showing formation of the $\text{Et}_3\text{NH}^+\text{Br}^-$ salt, indicative of reaction. The **G1** dendron initiator, **2**, was analysed by ^1H and ^{13}C NMR spectroscopy, electrospray (ES) mass spectrometry and elemental microanalysis, all of which are in agreement with the desired product. Figure 2.2 shows the ^1H NMR spectrum with each peak assigned. The residual solvent peak for CHCl_3 , which appears at 7.26 ppm, is overlaid by the aromatic proton peaks (7.20–7.35 ppm) present in the molecule therefore the integration for the aromatic protons is slightly higher than expected by theory.

The ^{13}C NMR spectrum is shown in Fig. 2.3 with peaks assigned; the peak corresponding to C9 in the molecule has the same chemical shift as the solvent, and cannot be identified amongst the CDCl_3 peaks. The mass spectrum is shown in Fig. 2.4 with important peaks highlighted. It is worth noting that those peaks corresponding to the molecular ion exemplify the isotope pattern expected for bromine perfectly.

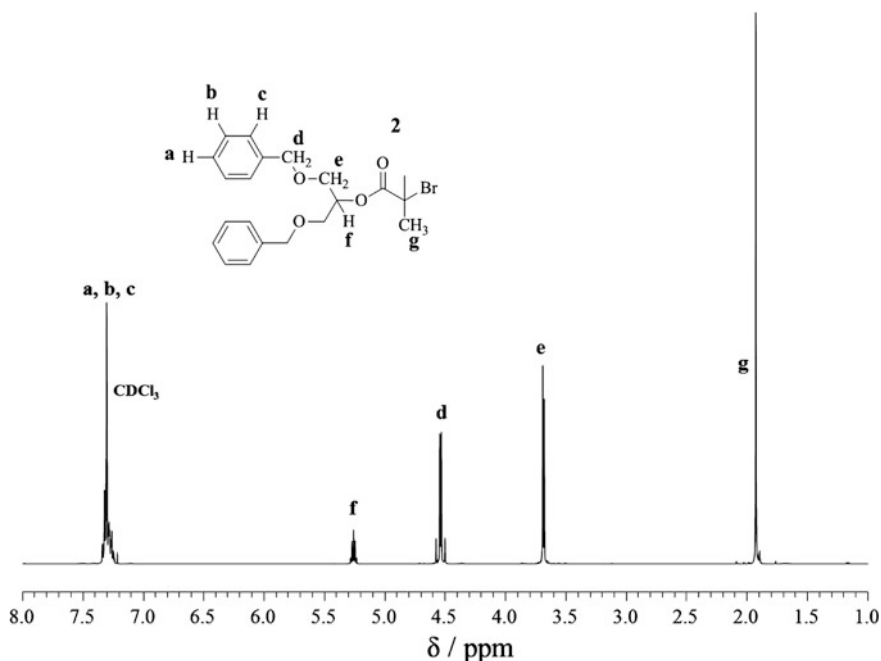


Fig. 2.2 ^1H NMR (CDCl_3 , 400 MHz) of **G1** DBOP initiator, **2**

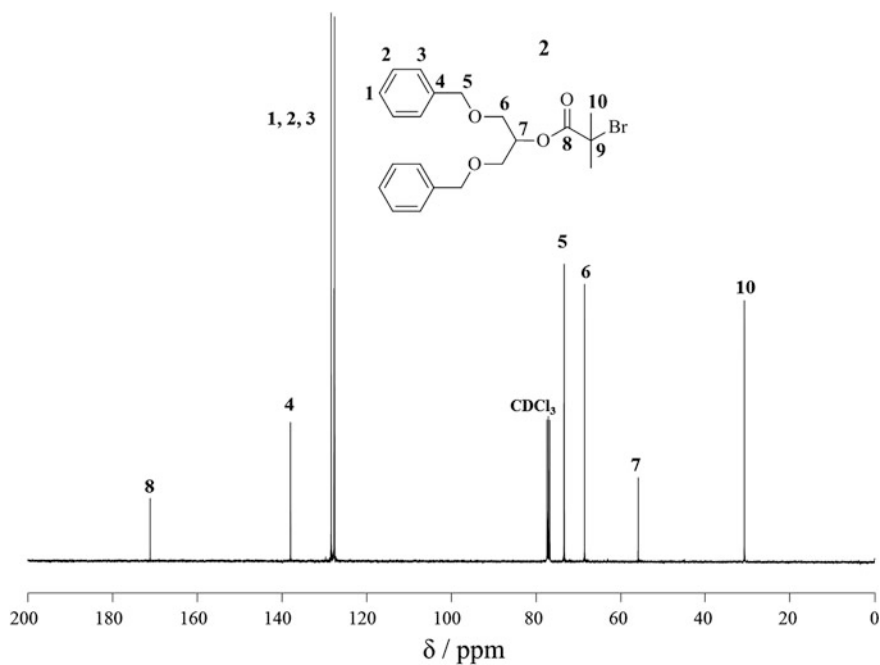


Fig. 2.3 ^{13}C NMR (CDCl₃, 100 MHz) of **G1** DBOP initiator, **2**

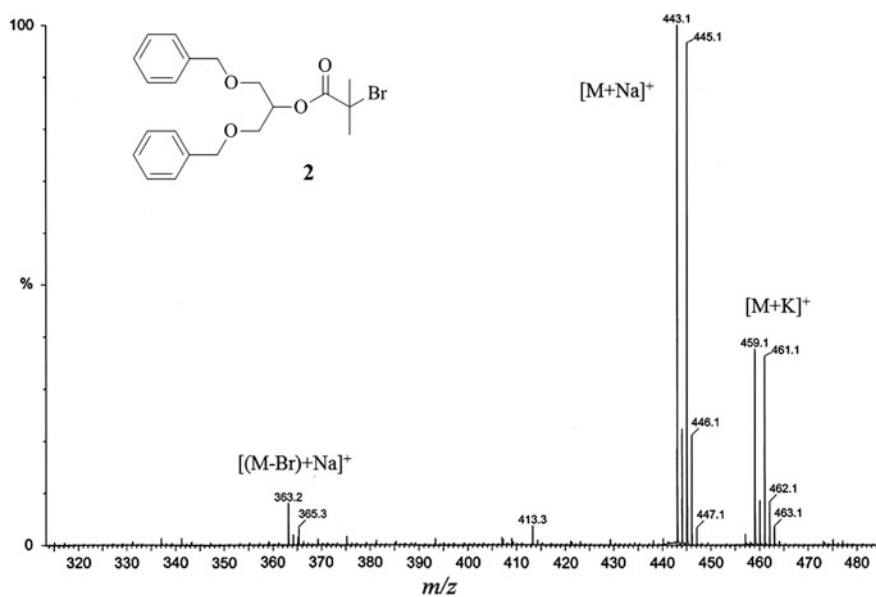


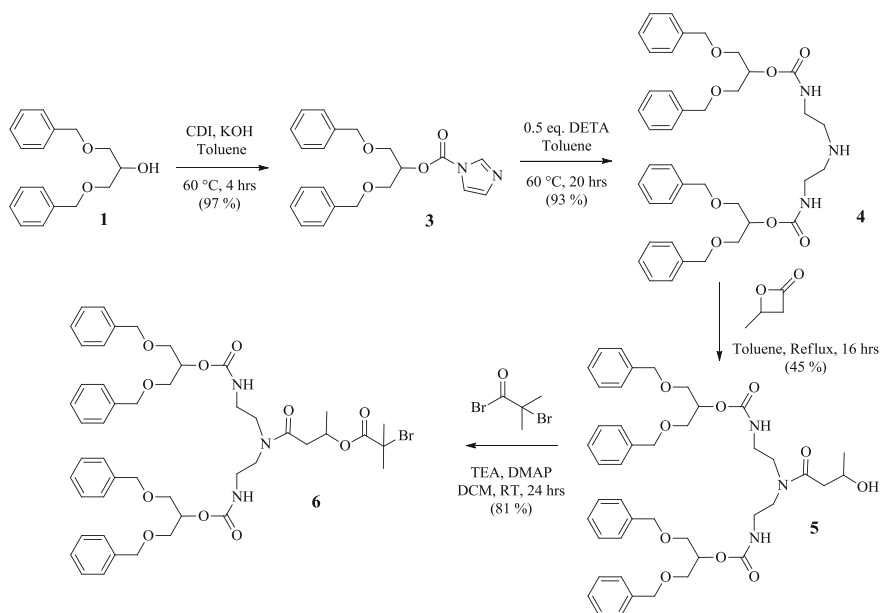
Fig. 2.4 Mass spectrum (ES-MS) of **G1** DBOP initiator, **2**

2.2.2 Generation 2 DBOP Dendron (G2)

The second generation initiator, **6**, was synthesised via a convergent strategy utilising the selectivity of 1,1'-carbonyldiimidazole (CDI) chemistry [6, 7], shown in Scheme 2.2.

The intermediary products from each step (**3**, **4** and **5**) were purified and analysed by ^1H and ^{13}C NMR spectroscopy, mass spectrometry and elemental microanalysis (see Appendix, Figs. A.1, A.2, A.3, A.4, A.5, A.6, A.7, A.8 and A.9). The first step involved reaction of the parent alcohol, DBOP, which bears the functionality of the resulting dendron surface group, with CDI. This generated the DBOP imidazole carboxylic ester, **3**, in high yields (97 %). The ^1H NMR spectrum of **3** (see Appendix, Fig. A.1) shows clearly that the imidazole ring is present, as 3 resonances were observed for the attached imidazole ring, whereas free imidazole would give only 2 resonances in the aromatic region. The ^{13}C NMR spectrum, see Appendix Fig. A.2, also confirms the correct structure due to the peak at 148.8 ppm, corresponding to the carbonyl carbon (C8) which is not present in the initial alcohol.

This imidazole carboxylic ester intermediate, **3**, was subsequently reacted with diethylenetriamine (DETA) [8]. Due to the selective nature of CDI chemistry only the primary amine functionalities of DETA reacted with **3**, whereas the secondary amine remained intact, leaving a reactive functional group on the molecule allowing for further reaction [6]. This coupling of two 1st generation dendrons, **3**, gave rise



Scheme 2.2 Synthesis of G2 dendron initiator, **6**

to the second generation dendron or **G2** dendron, **4**. The ^1H NMR spectrum (Appendix Fig. A.4) shows new proton environments corresponding to the CH_2s between the urethane bond and secondary amine at 2.60 and 3.15 ppm, with the integrations of the other protons being concordant with the rest of the molecule. ^{13}C NMR spectrum and ES mass spectrometry analysis (Appendix Figs. A.5 and A.6) also confirm the structure of **4**.

The ring-opening reaction of **4** with β -butyrolactone [9] afforded molecule **5** after purification by column chromatography. One side product of the reaction was the ring opening of β -butyrolactone with the urethane nitrogen, which had the same molecular weight as the desired product, **5**, therefore indistinguishable by mass spectrometry, however, the ^1H NMR spectroscopy analysis elucidated each structure. The ^1H NMR spectrum (Appendix Fig. A.7), ^{13}C NMR spectrum (Appendix Fig. A.8) and ES mass spectrometry analysis (Appendix Fig. A.9) all support the proposed structure of **5**. The yield of **5** was relatively low (45 %) this was believed to be due to the side product formed that reduced the yield of the desired product.

The **G2** dendron, **5**, was converted to the **G2** dendron initiator, **6**, by esterification of the secondary alcohol with α -bromoisobutyryl bromide [3–5], to give the target second generation dendron initiator (**G2** dendron initiator), **6**, in good yields (81 %). The ^1H NMR spectrum, shown in Fig. 2.5 with peaks assigned, is strongly indicative of the correct product. It is worth noting however, that as the generation number increases, and therefore the number of protons in the same or similar

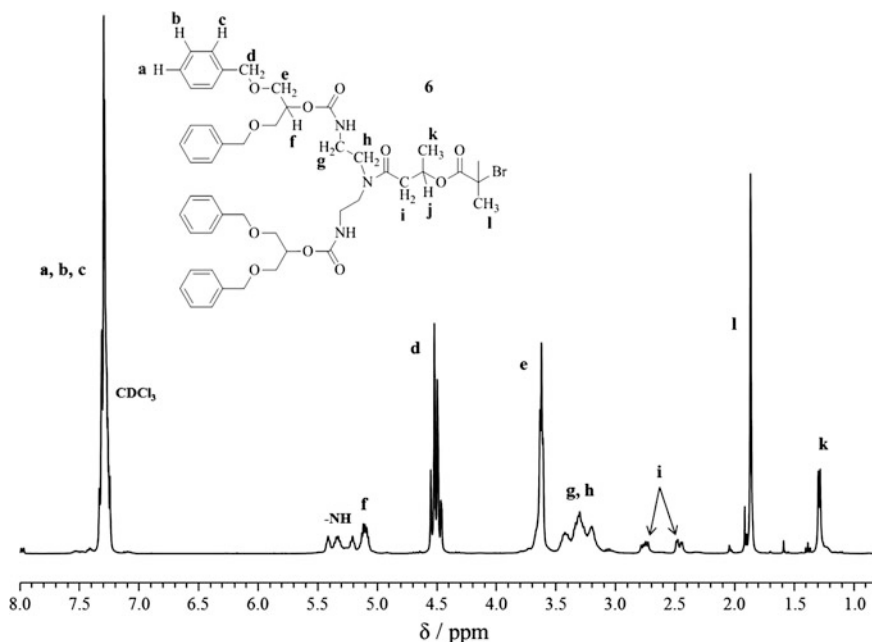


Fig. 2.5 ^1H NMR (CDCl_3 , 400 MHz) of **G2** DBOP initiator, **6**

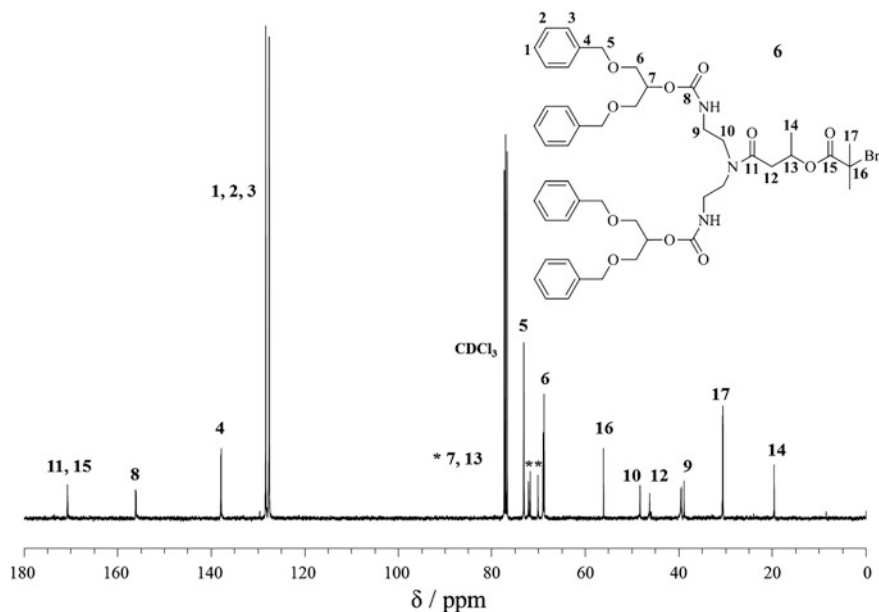


Fig. 2.6 ^{13}C NMR (CDCl_3 , 100 MHz) of **G2** DBOP initiator, **6**

environment increases, the peaks corresponding to those protons become broader and less well defined. This is due to the peak in the NMR spectrum being a representation of the average of those protons, and so as the number of protons in such environments increases, each of their environments becomes slightly different. This is more evident in higher generation dendrons and dendrimers. ^{13}C NMR spectroscopy (Fig. 2.6) and ES mass spectrometry (Fig. 2.7) also confirm the structure of the **G2** DBOP initiator, **6**. The expected bromine isotope pattern is also observed for this molecule again confirming the correct structure.

2.3 Polymer Synthesis

EBiB, **G1** and **G2** initiators were then used to synthesise either linear or branched pHPMA via ATRP [10, 11]. For the linear polymers, the initiator functionality was present at one chain end. When a divinyl monomer, ethylene glycol dimethacrylate (EGDMA), was introduced highly branched polymer architectures were formed [12–14], as illustrated in Scheme 2.3.

The globular structures of the branched polymers were composed of a core of pHPMA with the initiator decorating the surface and, when a dendron initiator was used, termed a “hyperbranched polydendron”—a *hyp*-polydendron. This term was chosen to represent the hyperbranched structure which contained multiple dendrons in one macromolecule, therefore a polydendron. When the initiator used was a

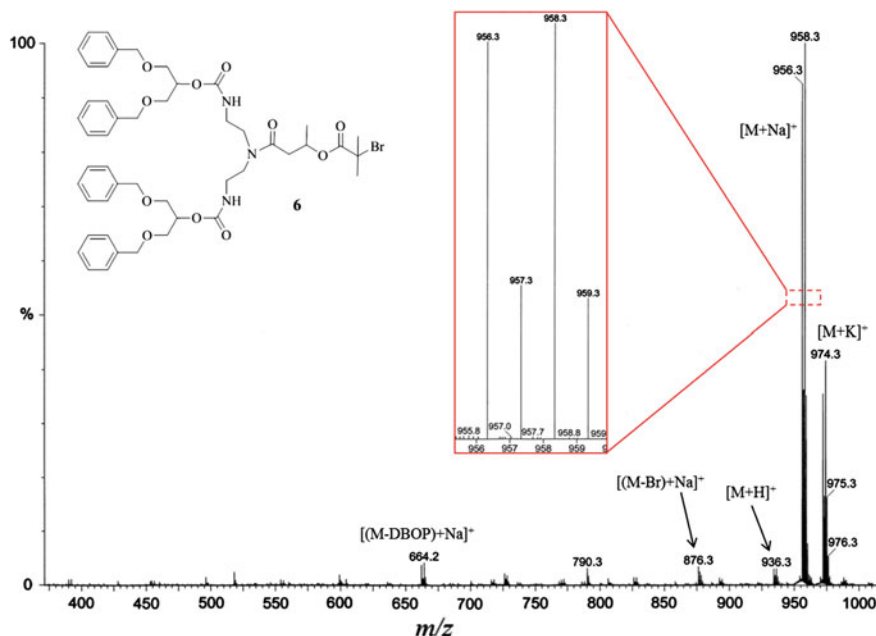
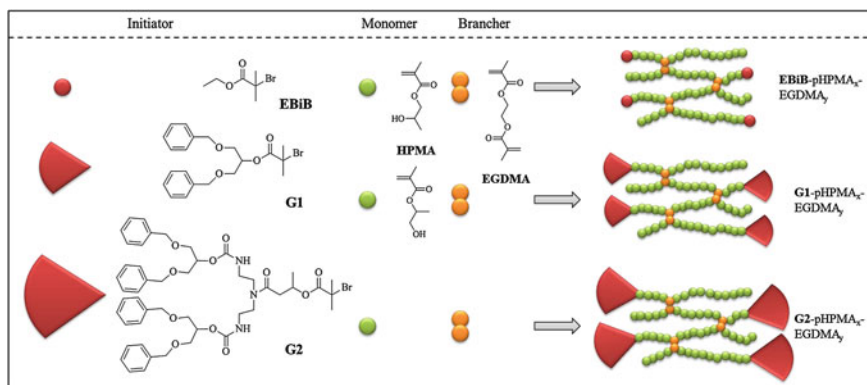


Fig. 2.7 Mass spectrum (ES-MS) of **G2** dendron initiator, **6**, including the $[M + Na]^+$ peak enlarged to see the bromine isotope pattern



Scheme 2.3 Idealised schematic representation of initiators, monomer and brancher used, and their corresponding branched polymers (both of HPMA's isomers have been depicted)

non-dendron initiator the resulting hyperbranched polymers were termed *hyp*-polymers. Three different number average degrees of polymerisation (DP_n) were targeted (20, 50 and 100 monomer units) for each polymer. Analysis by gel permeation chromatography (GPC) and ^1H NMR spectroscopy analysis for each polymer is collated in Table 2.1.

Table 2.1 ^1H NMR and GPC data for all polymers synthesised

Initiator	Target polymer composition	GPC (THF)				^1H NMR ^b
		M_n (g mol ⁻¹)	M_w (g mol ⁻¹)	$\bar{D}$	DP_n	DP_n
E	HPMA ₂₀	5,900 ^a	8,000 ^a	1.37 ^a	39	–
E	HPMA ₅₀	11,300	14,000	1.24	77	–
E	HPMA ₁₀₀	20,700	25,800	1.25	142	–
E	HPMA ₂₀ -EGDMA _{0.8}	24,800	165,900	6.70	–	–
E	HPMA ₅₀ -EGDMA _{0.8}	147,000	928,500	6.31	–	–
E	HPMA ₁₀₀ -EGDMA _{0.8}	214,200	2,328,000	10.9	–	–
G1	HPMA ₂₀	5,900	7,800	1.32	38	24
G1	HPMA ₅₀	9,800	13,000	1.33	65	55
G1	HPMA ₁₀₀	20,400	25,600	1.26	138	120
G1	HPMA ₂₀ -EGDMA _{0.8}	52,800	545,000	10.3	–	21
G1	HPMA ₅₀ -EGDMA _{0.8}	47,200	1,169,000	24.7	–	57
G1	HPMA ₁₀₀ -EGDMA _{0.8}	69,300	1,354,500	19.5	–	119
G2	HPMA ₂₀	5,700	7,300	1.28	33	21
G2	HPMA ₅₀	14,400	20,300	1.41	94	49
G2	HPMA ₁₀₀	23,300	32,700	1.40	155	101
G2	HPMA ₂₀ -EGDMA _{0.8}	153,000	1,565,000	10.2	–	21
G2	HPMA ₅₀ -EGDMA _{0.8}	68,400	661,000	9.67	–	55
G2	HPMA ₁₀₀ -EGDMA _{0.8}	164,200	2,227,500	13.6	–	107

^aCalculated using a different GPC (DMF eluent at 60 °C)^bDetermined by ^1H NMR analysis in d_6 -DMSO

2.3.1 Linear Polymers

The polymerisations of HPMA with targeted DP_{20} , DP_{50} and DP_{100} were carried out at 30 °C in methanol with CuCl:bipyridyl (bpy) (1:2), as the catalytic system [15, 16]. Each linear chain bore the functionality of the initiator only once. The DP_n calculated by ^1H NMR spectroscopy was less than the value obtained by GPC for all linear polymers. This discrepancy was probably due to the initiator efficiency being less than 100 % for each dendron. The DP_n by ^1H NMR includes the unreacted initiator as well as the polymer chain end groups, therefore leading to calculations of polymer chains that are shorter than the GPC analysis, and this effect of reduced initiator efficiency has been previously reported [17, 18]. The DP_n of the **EBiB** initiated polymers could not be calculated as the initiator peaks overlapped with the polymer peaks and could not be adequately distinguished.

In all polymerisations, unreacted initiator remained in the reaction medium after the polymerisations had achieved complete conversion. This could be seen in the refractive index (RI) GPC chromatograms as a small peak corresponding to the initiator eluting just before the solvent front, shown in Fig. 2.8a.

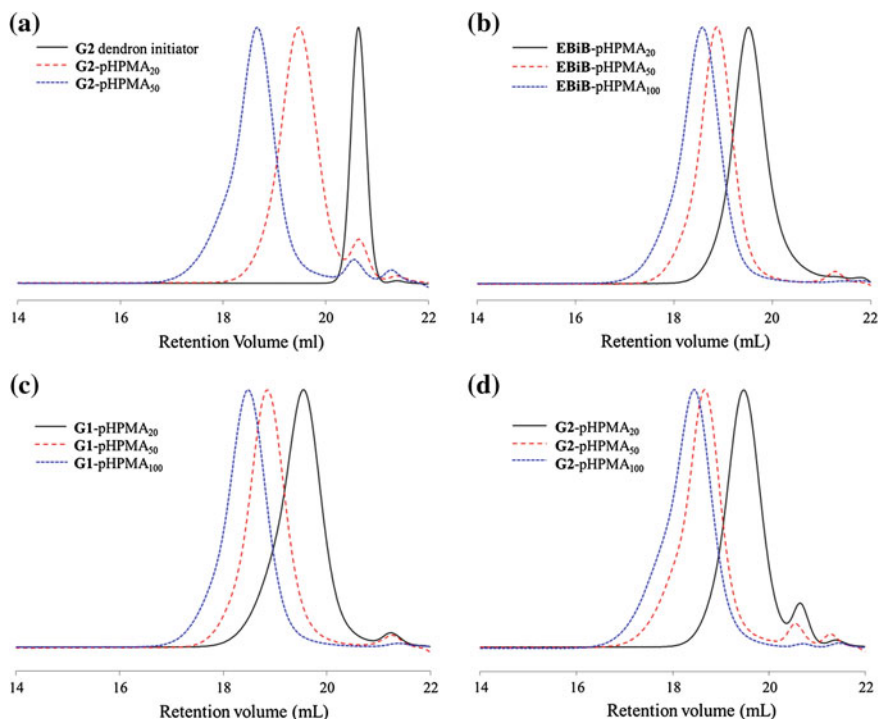


Fig. 2.8 GPC RI chromatogram overlays of **a** G2 dendron initiator with G2-pHPMA₂₀ and G2-pHPMA₅₀, **b** EBiB-pHPMA₂₀, EBiB-pHPMA₅₀ and EBiB-pHPMA₁₀₀, **c** G1-pHPMA₂₀, G1-pHPMA₅₀ and G1-pHPMA₁₀₀ and **d** G2-pHPMA₂₀, G2-pHPMA₅₀ and G2-pHPMA₁₀₀

The GPC RI overlays for each linear polymer (DP₂₀, DP₅₀ and DP₁₀₀) synthesised with each initiator are shown in Fig. 2.8b for the EBiB initiated, Fig. 2.8c for G1 dendron initiated and Fig. 2.8d for G2 dendron initiated. The GPC chromatograms show in each case that the linear and linear-dendritic polymers have a monomodal distribution, and the higher the targeted DP_n the lower the retention volume, indicating that the higher the targeted DP_n the higher the molecular weights obtained. In some of the linear samples a slight shoulder is visible on the high molecular weight side of the peak. This could either be due to coupling of chain ends at high conversions [19] or the fact that the HPMA monomer contains a small amount of a dimethacrylate impurity as a result of the synthetic route utilised in the production of the monomer [20], which can also result in the coupling of chains.

2.3.2 *Hyp*-polymer and *Hyp*-polydendron Synthesis

Incorporation of a difunctional monomer into the polymerisation allows for large branched polymer structures to be obtained [10]. The ratio of initiator to brancher

was crucial to the polymerisation success as, in theory, a molar ratio of 1:1 or more will cause cross-linking of polymer chains and ultimately macroscopic gelation [21, 22]. Although theoretically a ratio of 1:0.95, initiator:brancher, would give the large branched structures desired, it was found that due to the initiator efficiencies being lower than 100 %, at this level of brancher the polymers formed a cross-linked insoluble network, therefore the ratio of initiator to brancher was kept lower at 1:0.8. This was also affected by the concentration of the polymerisation, as the more dilute the polymerisation the more likely intramolecular looping will occur rather than the desired intermolecular branching. Therefore concentrations were maintained constant across all polymerisations at 50 v/v% with respect to the monomer.

The GPC overlays of **EBiB** initiated polymers (Fig. 2.9a, b) highlight the difference between the linear and *hyp*-polymer samples via the RI (Fig. 2.9a) and right angle light scattering (RALS) (Fig. 2.9b) detector chromatograms. The GPC chromatograms indicate that introduction of the difunctional monomer, EGDMA, affords high molecular weight branched polymers, eluting at lower retention volumes. Due to the statistical nature of branching in ATRP there is a broad distribution of materials present, ranging from linear chains to highly branched macromolecules [10]. The RI detector response is dependent upon the concentration of the polymer species present, whereas the RALS detector response is dependent upon the size of the polymeric species present. Therefore, although a small RI response is detected for the *hyp*-polymers around an elution volume of 12 mL (for **EBiB**-pHPMA₁₀₀-EGDMA_{0.8}), there is a huge RALS detector response due to the

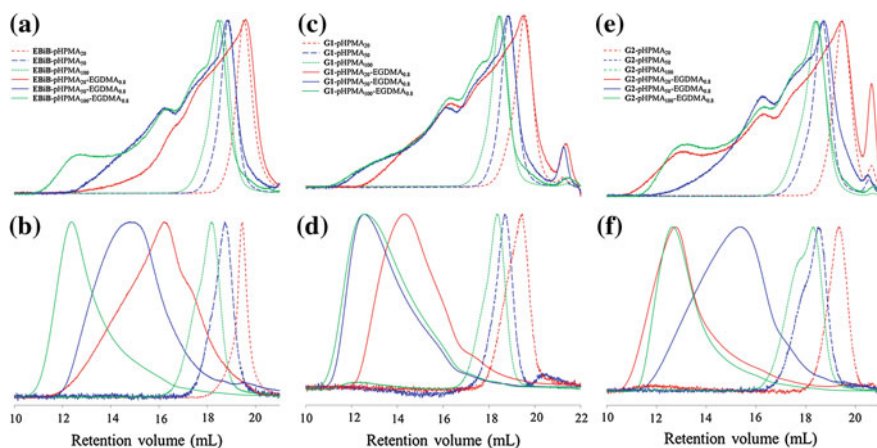


Fig. 2.9 GPC chromatograms overlays of **a** RI chromatograms and **b** RALS chromatograms for **EBiB**-pHPMA₂₀-EGDMA_{0.8}, **EBiB**-pHPMA₅₀-EGDMA_{0.8}, **EBiB**-pHPMA₁₀₀-EGDMA_{0.8} and the linear equivalents with dotted lines. **c** RI chromatograms and **d** RALS chromatograms for **G1**-pHPMA₂₀-EGDMA_{0.8}, **G1**-pHPMA₅₀-EGDMA_{0.8}, **G1**-pHPMA₁₀₀-EGDMA_{0.8} and the linear equivalents with dotted lines. **e** RI chromatograms and **f** RALS chromatograms for **G2**-pHPMA₂₀-EGDMA_{0.8}, **G2**-pHPMA₅₀-EGDMA_{0.8}, **G2**-pHPMA₁₀₀-EGDMA_{0.8} and the linear equivalents with dotted lines

presence of highly branched polymeric species which scatter considerably more light than the small polymeric species. It is worth noting, however, that in Figs. 2.8 and 2.9 the height of each peak has been normalised so this does not reflect the true differences in detector response, however, if the differences in detector responses needed to be compared it would be the area under the curve which would allow a true comparison.

Figure 2.9 shows the GPC overlays for the **G1** dendron initiated (Fig. 2.9c, d) and **G2** dendron initiated (Fig. 2.9e, f) linear-dendritic and *hyp*-polydendrons, with RI (Fig. 2.9c, e) and RALS (Fig. 2.9d, f) detector chromatograms.

These linear dendritic and *hyp*-polydendron samples follow the same trend as the **EBiB** initiated polymers; those with the inclusion of EGDMA all elute at a much lower retention volume than their linear equivalents. For example, the **G1**-pHPMA₅₀-EGDMA_{0.8} sample begins to elute at an approximate retention volume of 11 mL, whilst the **G1**-pHPMA₅₀ only begins to elute at approximately 17.5 mL. Each of the **G2** initiated *hyp*-polydendrons elute between 10 and 12 mL, whilst their linear-dendritic equivalents elute between 16 and 18 mL.

The linear polymers and branched polymers were analysed by ¹H NMR spectroscopy, as discussed previously, Fig. 2.10 shows each linear DP₅₀ polymer ¹H NMR spectrum overlaid, with the major peaks present in each polymer assigned. The aromatic protons attributed to the two dendron initiators are also highlighted. The DP₂₀ and DP₁₀₀ linear polymer ¹H NMR spectra are shown in the Appendix; Figs. A.10 and A.12 respectively.

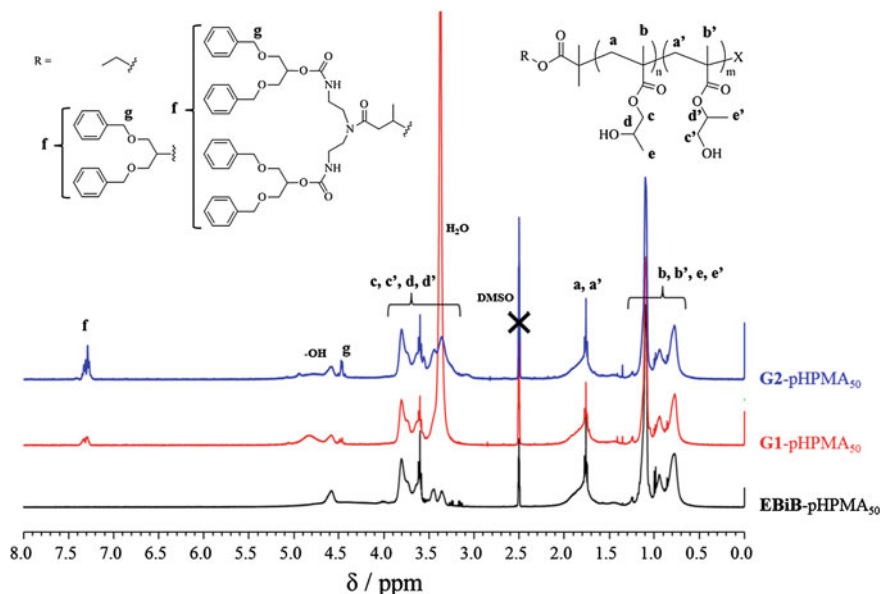


Fig. 2.10 ¹H NMR (*d*₆-DMSO, 400 MHz) spectra overlay for **EBiB**-pHPMA₅₀, **G1**-pHPMA₅₀ and **G2**-pHPMA₅₀ with major peaks assigned

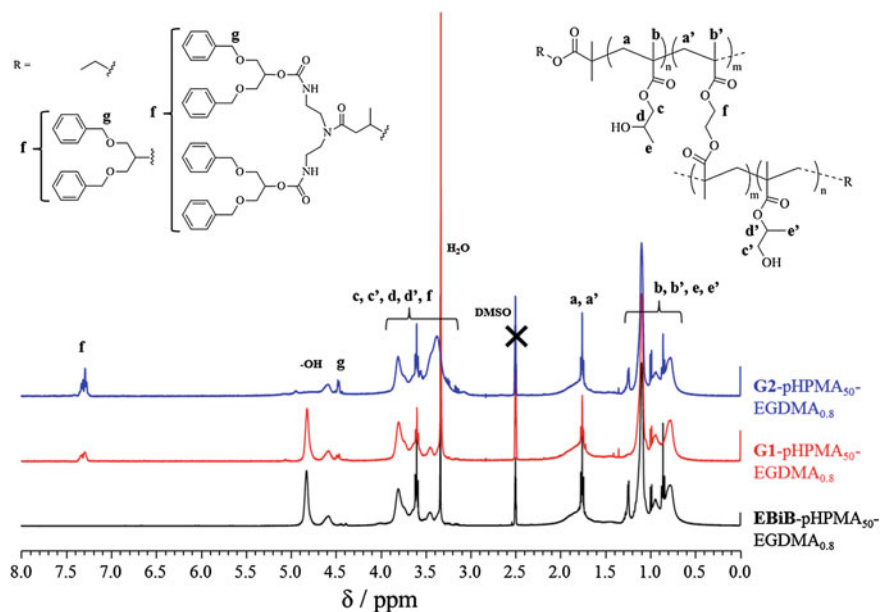


Fig. 2.11 ¹H NMR (*d*₆-DMSO, 400 MHz) spectra overlay for **EBiB-pHPMA₅₀-EGDMA_{0.8}**, **G1-pHPMA₅₀-EGDMA_{0.8}** and **G2-pHPMA₅₀-EGDMA_{0.8}** with major peaks assigned

The DP₅₀ *hyp*-polymer and *hyp*-polydendrons' ¹H NMR spectra are overlaid in Fig. 2.11 with major peaks assigned. It is worth noting that the only difference between the linear and branched equivalent polymers is the presence of EGDMA in the branched polymers, which contains protons with similar environments to the HPMA monomer and therefore cannot be distinguished in the ¹H NMR spectra. The DP₂₀ and DP₁₀₀ *hyp*-polymer and *hyp*-polydendron ¹H NMR spectra are shown in the Appendix in Figs. A.11 and A.13.

2.4 Kinetic Experiments

Kinetic experiments were undertaken for each of the linear and branched polymerisations that targeted a DP_n of 50 monomer units to confirm that each polymerisation followed first order kinetics with respect to the monomer concentration, and to follow the evolution of molecular weight with respect to monomer conversion.

2.4.1 Linear Polymerisation Kinetics

The kinetic plots for each linear polymerisation, Fig. 2.12, show that each polymerisation reached high conversion within 6–7 h (Fig. 2.12a, c, e) and followed first order kinetics as expected [17]. The evolution of M_n with conversion was linear and was close to that expected from the targeted M_n (Fig. 2.12b, d, f).

Figure 2.13 shows the ^1H NMR spectra of the **G1**-pHPMA₅₀ polymerisation at various time points throughout the polymerisation. As the reaction proceeds, the vinyl peaks at 5.65 and 6.05 ppm decrease as the monomer is consumed. The aromatic peaks attributed to the initiator are observed at 7.26–7.38 ppm which remain constant throughout the polymerisation and were therefore used as a reference to calculate conversion. The conversion for the **EBiB** initiated polymerisations used the pendant CH_3 of the monomer as a reference at 1.2 ppm as this

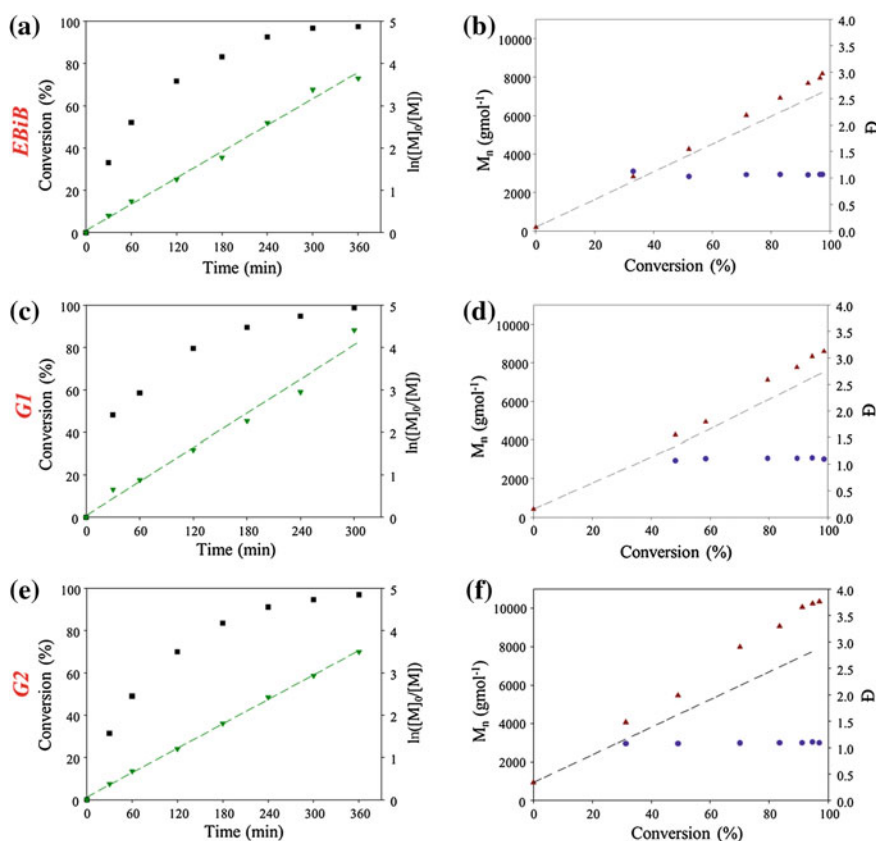


Fig. 2.12 Kinetic plots for linear DP₅₀ polymers. **a** and **b** **EBiB**-pHPMA₅₀, **c** and **d** **G1**-pHPMA₅₀, **e** and **f** **G2**-pHPMA₅₀. Conversion (black squares), $\ln([M]_0/[M])$ (green down triangles), M_n (red up triangles), Đ (blue circles) and theoretical M_n (black dotted lines)

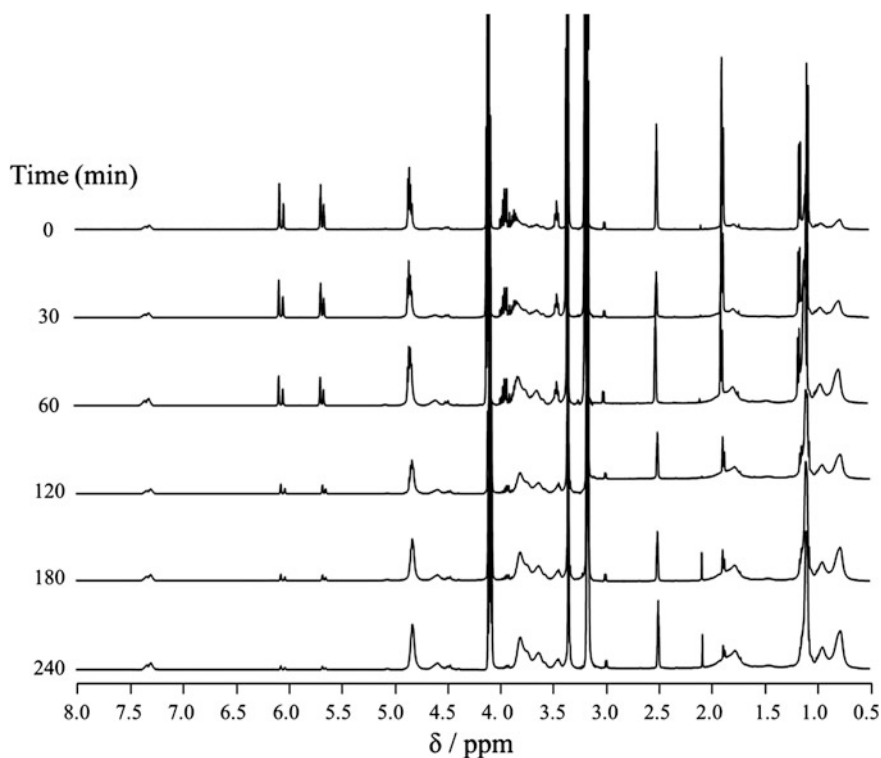


Fig. 2.13 ^1H NMR (d_6 -DMSO, 400 MHz) overlay of kinetic samples for **G1-HPMA₅₀** polymerisation

signal remains constant in the monomeric and polymeric form of HPMA, when compared to the vinyl peaks (5.65 and 6.05 ppm).

2.4.2 Branched Polymerisation Kinetics

The kinetic plots for each branched polymerisation with targeted DP_{50} , shown in Fig. 2.14, also show that these polymerisations followed first order kinetics, and reached high conversion within 8–9 h (Fig. 2.14a, c, e). The M_n and M_w increased linearly at the beginning of the polymerisations, until the conversion reached 80–90 %, where the M_w increases steeply indicating large branched polymers are being formed at high conversion (Fig. 2.14b, d, f). This steep increase in M_w is indicative of the linear chains joining to form a high M_w branched structure via intermolecular reaction.

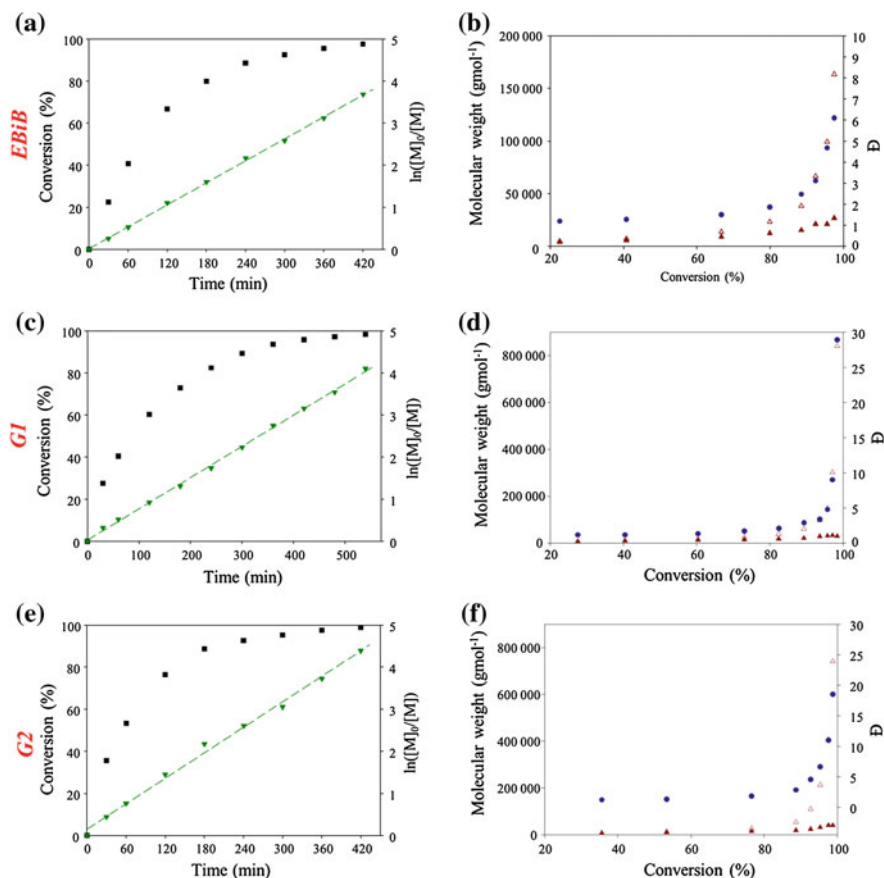


Fig. 2.14 Kinetic plots for linear DP₅₀ polymers. **a** and **b** EBiB-pHPMA₅₀-EGDMA_{0.8}, **c** and **d** G1-pHPMA₅₀-EGDMA_{0.8}, **e** and **f** G2-pHPMA₅₀-EGDMA_{0.8}. Conversion (black squares), $\ln([M]_0/[M])$ (green down triangles), M_n (red filled up triangles), M_w (red open up triangles), $\bar{D}$ (blue circles)

2.5 Solvent Driven Self-assembly of Hydrophobic Polymers

In order to assess whether these *hyp*-polydendrons could be used to produce nanoparticles, a nanoprecipitation approach was utilised [23]. While conventional nanoprecipitation typically uses water as the anti-solvent [24, 25], here the hydrophobic nature of both the initiator and polymer warranted use of two organic solvents. This approach is highlighted in Fig. 2.15. It is worth noting that the vials used were kept sealed at all times except when anti-solvent addition was occurring. Anti-solvent was added using a syringe pump set at 0.5 mL/min through the inlet needle, an outlet needle was used to prevent a build-up of pressure.

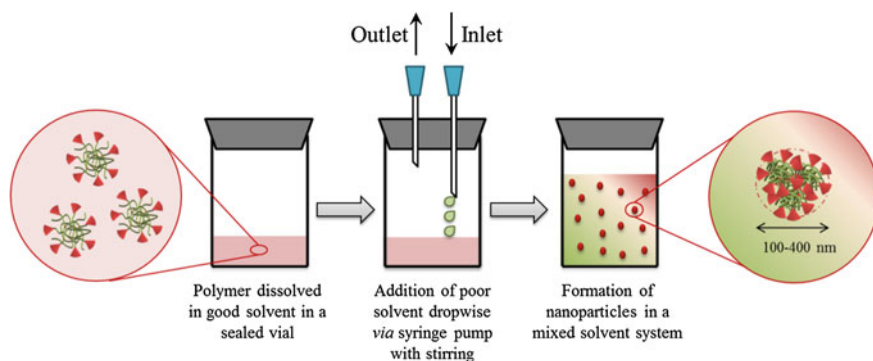


Fig. 2.15 Graphical representation of how organic nanoparticles are formed via the use of addition of an anti-solvent to polymer dissolved in a good solvent

Acetone was used as the good solvent for both the pHPMA core and the initiator groups at the end of each polymer chain. Nanoprecipitation was induced by dropwise addition of hexane (an anti-solvent for the pHPMA core but a good solvent for the initiator groups, henceforth described as an anti-solvent). Therefore theoretically, the initiator groups around the surface of each branched macromolecule should stabilise the particle with increased amounts of anti-solvent introduced into the system. A further reason for using acetone/hexane was that their refractive indices and viscosities are both very similar. An initial concentration of 5 mg/mL polymer in acetone was used, unless stated otherwise, therefore depending upon how much hexane has been added (hexane fraction, Φ_{hex}) the final polymer concentration would vary.

2.5.1 Nanoparticle Formation Utilising Hyp-polymers and Hyp-polydendrons

The formation of nanoparticles was followed primarily by dynamic light scattering (DLS). A clear trend was observed for the branched polymers (Figs. 2.16a, 2.18a and 2.20b): with the addition of a low fraction of hexane, the solvated branched polymers appear to decrease in z-average diameter (D_z) slightly, until enough hexane was added to force the branched dendritic polymers to aggregate into larger spherical particles. These particles have low polydispersities (PDI), below 0.2, and appear to shrink slightly with the addition of more anti-solvent until they increase in size due to precipitation. Figures 2.16b, 2.18b and 2.20b each highlights various stages of nanoparticle formation in the acetone/hexane solvent mixtures of **EBiB**-pHPMA₅₀-EGDMA_{0.8}, **G1**-pHPMA₅₀-EGDMA_{0.8} and **G2**-pHPMA₅₀-EGDMA_{0.8} respectively via scanning electron microscopy (SEM).

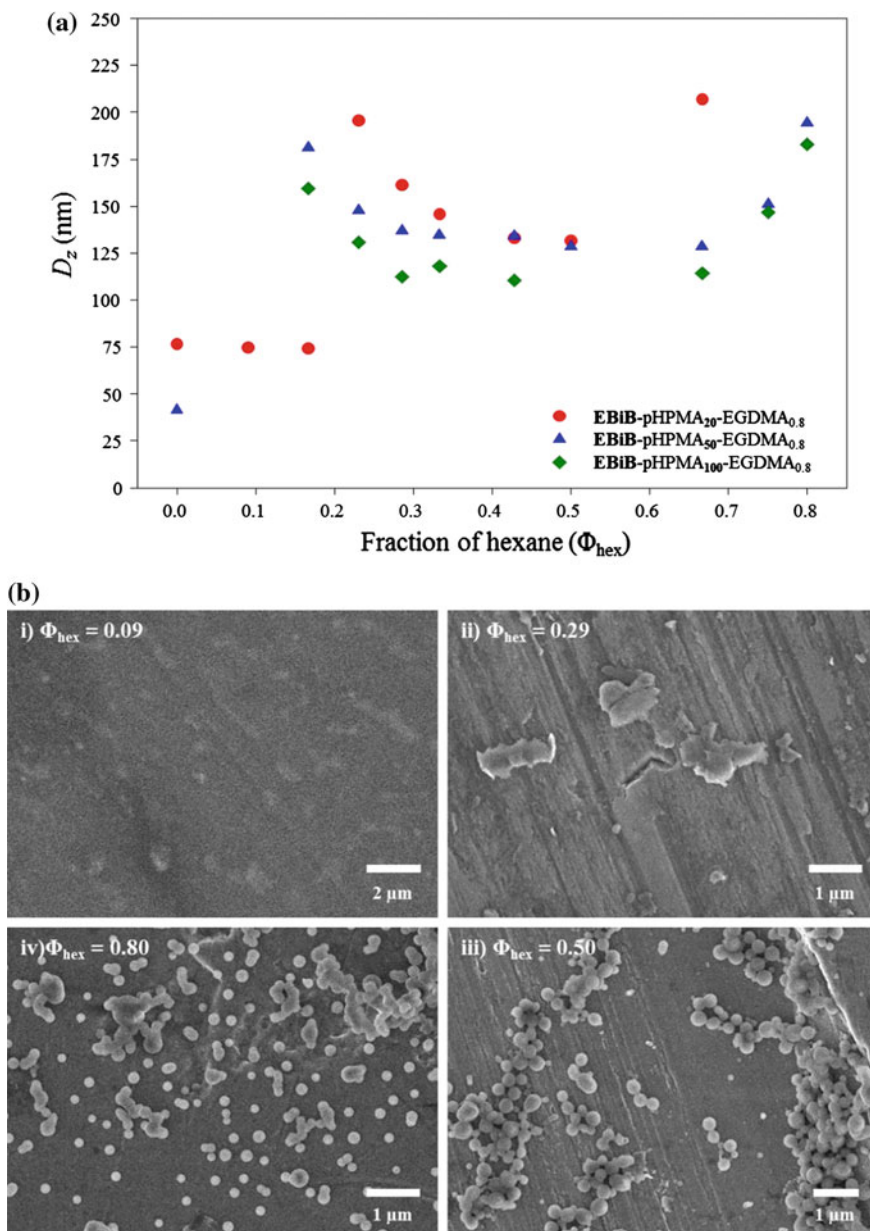


Fig. 2.16 The formation of nanoparticles followed via **a** DLS of each **EBiB** initiated branched polymer DP_{20} (red circles) DP_{50} (blue triangles) DP_{100} (green diamonds) and **b** SEM images of the formation of nanoparticles with **EBiB**-pHPMA₅₀-EGDMA_{0.8} at various hexane solvent fractions; (i) 0.09, (ii) 0.29, (iii) 0.50 and (iv) 0.80

Figure 2.16a shows the trend of formation for each of the **EBiB** initiated *hyp*-polymers (see Table 2.2 for the D_z and PDI values). At low hexane fractions the polymers were fully solvated by the good solvent, acetone, and may be described as individual objects. This is why in some cases (**EBiB**-pHPMA₅₀-EGDMA_{0.8} at $\Phi_{\text{hex}} = 0.09$ and **EBiB**-pHPMA₁₀₀-EGDMA_{0.8} at $\Phi_{\text{hex}} = 0$ and 0.09) the samples were not suitable for measurement by DLS as the sample was too polydisperse (therefore there are no values in Table 2.2 for these measurements), and in other case the PDI is quite high (**EBiB**-pHPMA₂₀-EGDMA_{0.8} at $\Phi_{\text{hex}} = 0, 0.09$ and 0.17 have PDI values of 0.493, 0.496 and 0.408). This is due to the fact that when fully solvated the DLS is measuring the distribution of polymeric species present in the sample—ranging from linear chains to highly branched materials. The polymers self-assembled into nanoparticles at $\Phi_{\text{hex}} = 0.17$ (for DP₅₀ and DP₁₀₀) or 0.23 (DP₂₀ sample) which were of a uniform size and had low polydispersities (<0.1). Upon further addition of hexane the D_z of these nanoparticles decreased until a hexane fraction around 0.5, most probably due to the nanoparticles being highly swollen with good solvent at low hexane fractions. The addition of hexane lowers the good solvent fraction causing the nanoparticles to de-swell or compress. The **EBiB**-pHPMA₂₀-EGDMA_{0.8} sample showed a decrease in D_z from 196 to 132 nm between Φ_{hex} 0.23–0.50, the **EBiB**-pHPMA₅₀-EGDMA_{0.8} nanoparticles showed a decrease in D_z from 181 to 128 nm between Φ_{hex} 0.17–0.67 and the **EBiB**-pHPMA₁₀₀-EGDMA_{0.8} sample showed a decrease in D_z from 160 to 111 nm between Φ_{hex} 0.17–0.43. When the hexane fraction was increased above those hexane fractions, up to 0.80, the nanoparticles increased in size, then ultimately precipitated after an excess of hexane was added. After self-assembly, the corresponding nanoparticle D_z followed a general trend of DP₂₀ > DP₅₀ > DP₁₀₀.

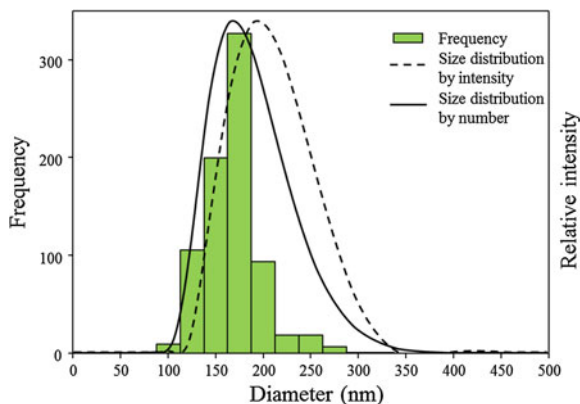
The formation of nanoparticles using **EBiB**-pHPMA₅₀-EGDMA_{0.8} was followed by SEM (Fig. 2.16b) by taking a sample at various hexane fractions (0.09, 0.29, 0.50 and 0.80) and dropping the samples onto an SEM stub, whereby rapid solvent evaporation occurred leaving a dry nanoparticulate polymer sample. At the lowest hexane fraction of 0.09 (Fig. 2.16b-i) the polymer was still fully solvated by the good solvent and no self-assembly has occurred, therefore it was expected that upon drying a polymer film would form, the SEM shows no hierarchical structure and small deposits of polymer are barely distinguishable. Once self-assembly had occurred ($\Phi_{\text{hex}} = 0.29$) it was expected that some structure would be seen by SEM, however, possibly due to the effect of drying none could be seen (Fig. 2.16b-ii). It was proposed that upon drying, due to the larger fraction of good solvent that the nanoparticles became aggregated upon drying to form uneven polymer deposits on the surface of the stub. When hexane fractions of 0.50 and 0.80 were imaged via SEM (Fig. 2.16b-iii and iv) it was clear that a level of anti-solvent had been reached that allowed the nanoparticle to retain their structure upon solvent evaporation. Both SEM images show spherical nanoparticles which have diameters that are in accordance with the D_z by DLS. The SEM images for the **EBiB**-pHPMA₅₀-EGDMA_{0.8} sample with $\Phi_{\text{hex}} = 0.80$ (Fig. 2.16b-iv) were studied in more detail by measuring the particle size from the SEM images and calculating a number average from the SEM analysis which could be compared to the number average as

Table 2.2 D_z and Pdl measurements by DLS corresponding to Figs. 2.16, 2.18 and 2.20 of each *hyp*-polymer or *hyp*-polydendron at various hexane fractions

Fraction of hexane present (Φ_{hex})	EBiB (Fig. 2.16)				GI (Fig. 2.18)				G2 (Fig. 2.20)			
	pHPMA ₂₀ - EGDMA _{0.80}		pHPMA ₅₀ - EGDMA _{0.80}		pHPMA ₁₀₀ - EGDMA _{0.80}		pHPMA ₂₀ - EGDMA _{0.80}		pHPMA ₅₀ - EGDMA _{0.80}		pHPMA ₁₀₀ - EGDMA _{0.80}	
	D_z (nm)	Pdl	D_z (nm)	Pdl	D_z (nm)	Pdl	D_z (nm)	Pdl	D_z (nm)	Pdl	D_z (nm)	Pdl
0	76	0.493	42	0.396	–	–	35	0.271	46	0.369	–	–
0.09	75	0.469	–	–	–	–	32	0.309	42	0.361	–	–
0.17	74	0.408	181	0.055	160	0.02	30	0.294	40	0.325	180	0.227
0.23	196	0.003	148	0.005	131	0.036	204	0.094	163	0.021	146	0.031
0.29	161	0.008	137	0.014	112	0.066	169	0.012	141	0.027	140	0.049
0.33	146	0.018	135	0.038	118	0.083	173	0.032	149	0.075	135	0.084
0.43	133	0.038	134	0.058	111	0.091	168	0.067	140	0.056	129	0.054
0.50	132	0.037	129	0.043	–	–	167	0.016	136	0.072	135	0.093
0.67	207	0.016	128	0.037	114	0.043	172	0.073	133	0.060	129	0.055
0.75	*	*	151	0.020	147	0.011	176	0.010	161	0.033	171	0.052
0.80	*	*	195	0.019	183	0.019	210	0.025	201	0.013	190	0.014

Sample not suitable for measurement by DLS due to ‘-’ Lack of scattering or ‘*’ Sample precipitated
 D_z z-average diameter

Fig. 2.17 Histogram analysis of nanoparticles via SEM imaging (784 particles from 2 images) overlaid with the DLS size distribution by intensity and number traces for **EBiB**-pHPMA₅₀-EGDMA_{0.8}, $\Phi_{\text{hex}} = 0.80$



measured by DLS. Figure 2.17 shows the histogram analysis of two SEM images, totalling 784 particles. The number average diameter (D_n) measured by DLS for this sample was 179 nm, whilst the D_z was 195 nm. The estimated mean calculated from the histogram was 156 nm. This was slightly lower than observed via DLS measurements, however, DLS was measuring the diameter of the particles dispersed in the organic solvent mixture, whilst SEM imaging was conducted on a dry sample. Therefore some shrinkage upon solvent evaporation was to be expected.

The same experiment was conducted with the **G1** dendron initiated *hyp*-polydendron materials (see Fig. 2.18a and Table 2.2). When there was only good solvent present DLS analysis was of solvated polymer samples which contained a distribution of linear to highly branched materials and therefore had high PDI values. For example, the PDI values for the **G1**-pHPMA₅₀-EGDMA_{0.8} sample at hexane fractions of 0, 0.09 and 0.17 were 0.369, 0.361 and 0.325 respectively. This was indicative of multiple populations of sizes being present in the sample measured via DLS. There are no values present in the table for **G1**-pHPMA₁₀₀-EGDMA_{0.8} with $\Phi = 0$ and 0.09 due to the samples being too polydisperse for measurement, which predominantly occurred with low hexane fractions, as with the **EBiB** series. Once a hexane fraction of 0.23 had been reached the solvated polymers underwent self-assembly to produce nanoparticles of uniform size and low polydispersities (<0.1). After self-assembly had occurred, further addition of hexane caused the nanoparticles to decrease in size until a hexane fraction around 0.50–0.67 was reached. **G1**-pHPMA₂₀-EGDMA_{0.8} decreased in D_z from 204 to 167 nm (from $\Phi_{\text{hex}} = 0.23$ to 0.50), **G1**-pHPMA₅₀-EGDMA_{0.8} decreased in D_z from 162 to 133 nm (from $\Phi_{\text{hex}} = 0.23$ to 0.67) and **G1**-pHPMA₁₀₀-EGDMA_{0.8} decreased in D_z from 180 to 130 nm (from $\Phi_{\text{hex}} = 0.17$ to 0.67). This series of materials also followed the trend observed with the **EBiB** series of branched polymers whereby the nanoparticles formed with the DP₂₀ sample were larger than those with the DP₅₀, and the nanoparticle formed with the DP₅₀ sample were larger than those with the DP₁₀₀ sample ($\text{DP}_{20} > \text{DP}_{50} > \text{DP}_{100}$).

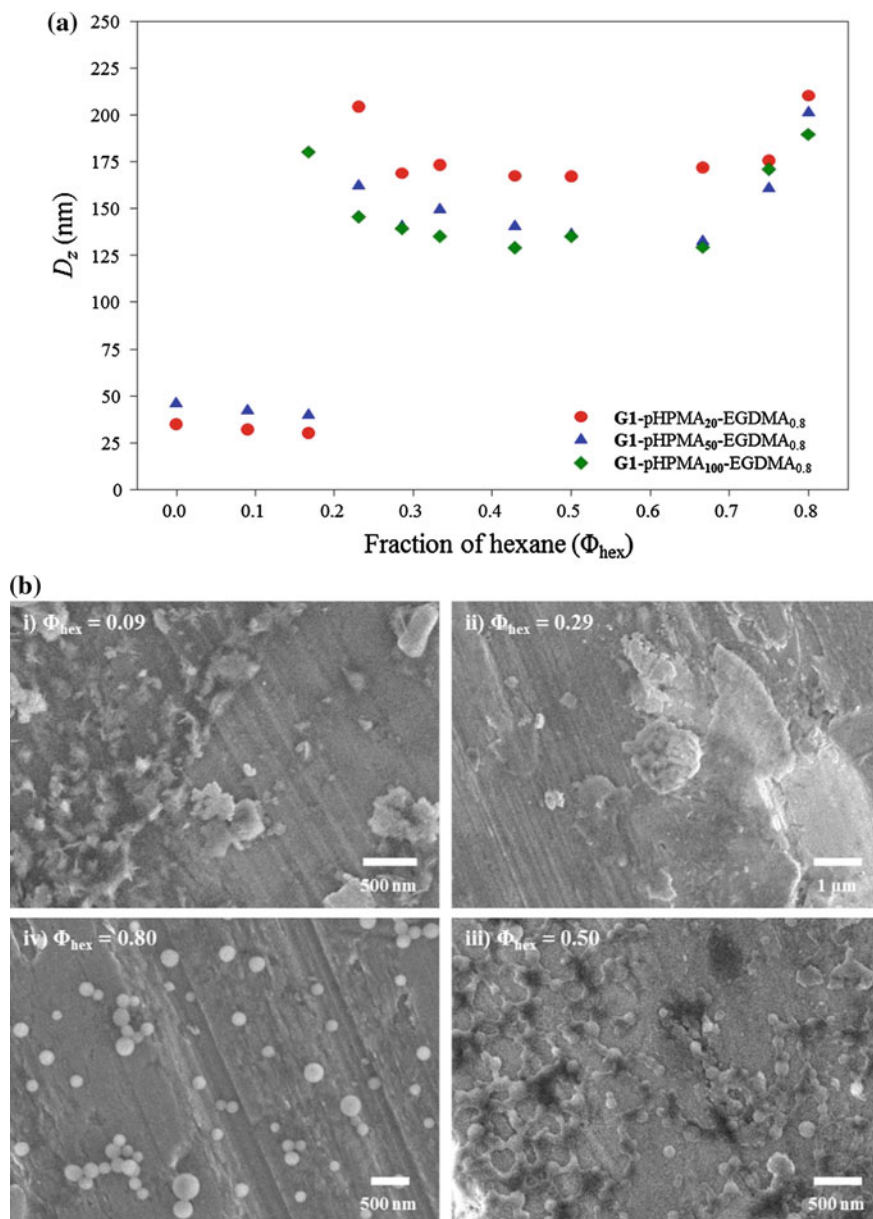


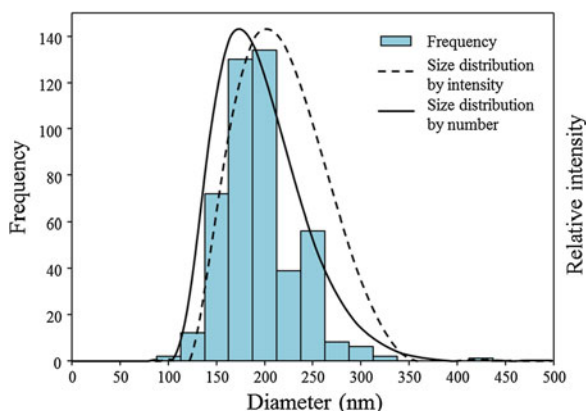
Fig. 2.18 The formation of nanoparticles followed via **a** DLS of each **G1** dendron initiated *hyp*-polydendron DP₂₀ (red circles) DP₅₀ (blue triangles) DP₁₀₀ (green diamonds) and **b** SEM images of the formation of nanoparticles with **G1**-pHPMA₅₀-EGDMA_{0.8} at various hexane solvent fractions; (i) 0.09, (ii) 0.29, (iii) 0.50 and (iv) 0.80

SEM investigations of the formation of nanoparticles using the **G1**-pHPMA₅₀-EGDMA_{0.8} *hyp*-polydendron were also studied at hexane fractions 0.09, 0.29, 0.5 and 0.8 (Fig. 2.18b). It was immediately obvious that at the two lowest hexane fractions (0.09 and 0.29, Fig. 2.18b-i, ii) no hierarchical structure was observed, even though self-assembly was measured by DLS at a hexane fraction of 0.23. The cause for this at $\Phi_{\text{hex}} = 0.09$ (Fig. 2.18b-i) was again most probably due to the fact that the polymer was well solvated by the good solvent, acetone, and therefore upon drying an uneven polymer film was formed. At $\Phi_{\text{hex}} = 0.29$ there was still a large good solvent fraction present, therefore, possibly the nanoparticle structure was lost upon solvent evaporation. Figure 2.18b-iii shows the sample at a hexane fraction of 0.5, where spherical particles were observed, however, they appear to have dried in a manner to suggest that they adhere to one another and appear slightly different from those observed at the same solvent fraction for the equivalent **EBiB** initiated branched polymer (Fig. 2.16b-iii). This may be due to the fact that the dendron moiety at the chain end is in fact soluble in the anti-solvent, hexane, for the polymer core, therefore this may affect the properties of the nanoparticles upon drying.

This was not the case for the corresponding **G2** sample (Fig. 2.20b-iii) which could suggest that it is dependent upon which area of the stub is being imaged. This phenomena was not observed for the sample taken at $\Phi_{\text{hex}} = 0.80$ where discrete spherical nanoparticles are observed (Fig. 2.18b-iv). SEM analysis of three images of the **G1**-pHPMA₅₀-EGDMA_{0.8} samples at $\Phi_{\text{hex}} = 0.80$ (Fig. 2.18b-iv) was conducted totalling 462 particles. Figure 2.19 shows the histogram analysis of measuring the diameters of these nanoparticles via SEM and DLS. The D_n and D_z of this sample as measured by DLS were 186 and 201 nm respectively. The mean diameter calculated from the SEM images was 182 nm, which is incredibly close to the number mean diameter calculated via DLS.

The formation of nanoparticles using the **G2** dendron initiated *hyp*-polydendrons, followed by DLS, (see Fig. 2.20a and Table 2.2) also followed the same trends as the **EBiB** *hyp*-polymers and the **G1** *hyp*-polydendron materials. As mentioned previously, at low hexane fractions the DLS measurements gave smaller D_z and higher PDI values. For example, at $\Phi_{\text{hex}} = 0$ and 0.09 all of the samples, and at $\Phi_{\text{hex}} = 0.17$ the

Fig. 2.19 Histogram analysis of nanoparticles via SEM imaging (462 particles from 3 images) overlaid with the DLS size distribution by intensity and number traces for **G1**-pHPMA₅₀-EGDMA_{0.8}, $\Phi_{\text{hex}} = 0.80$



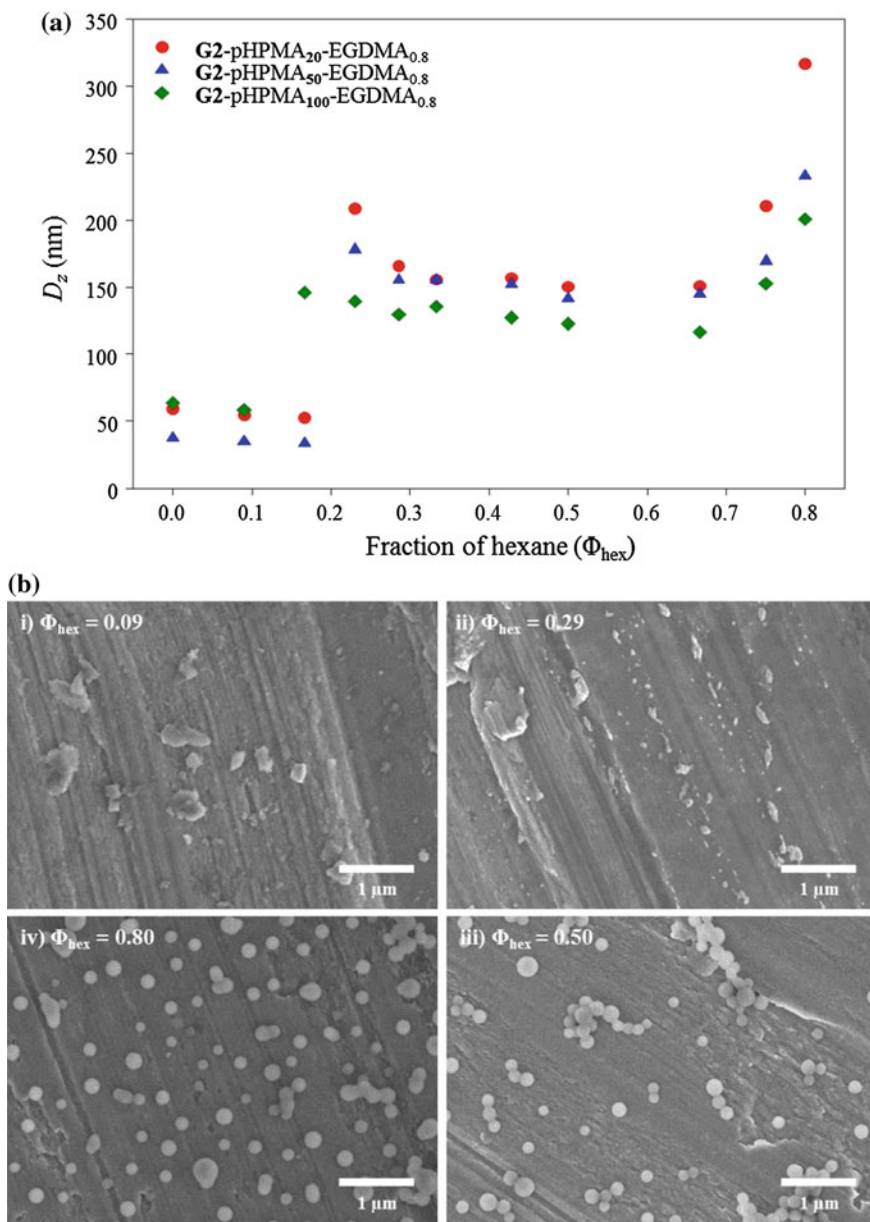


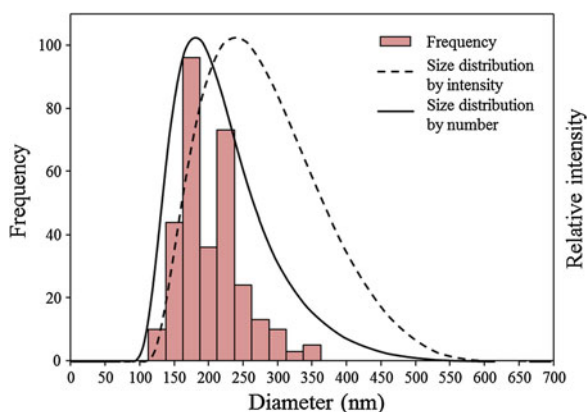
Fig. 2.20 The formation of nanoparticles followed via **a** DLS of each **G2** dendron initiated *hyp*-polydendron DP₂₀ (red circles) DP₅₀ (blue triangles) DP₁₀₀ (green diamonds) and **b** SEM images of the formation of nanoparticles with **G2-pHPMA₅₀-EGDMA_{0.8}** at various hexane solvent fractions; (i) 0.09, (ii) 0.29, (iii) 0.50 and (iv) 0.80

DP₂₀ and DP₅₀ samples had D_z less than 65 nm and high PDI values (>0.34). Interestingly, in the case of both the DP₂₀ and DP₅₀ *hyp*-polydendron samples when the hexane fraction was increased from 0 to 0.17 the corresponding PDI values decreased, as did the D_z . This suggests that as more anti-solvent is added the fully solvated polymer chains are minimising their interactions with the hexane by collapsing slightly and subsequently reducing in size and polydispersity. Once a hexane fraction of 0.17 (DP₁₀₀) or 0.23 (DP₂₀ and DP₅₀) was reached the *hyp*-polydendrons self-assembled into nanoparticles with low PDI values (<0.1). As the hexane fraction increased to around 0.50 the resulting nanoparticle decreased in D_z (from 209 to 150 nm, from $\Phi_{\text{hex}} = 0.23$ to 0.5 for **G2**-pHPMA₂₀-EGDMA_{0.8}, from 178 to 142 nm, from $\Phi_{\text{hex}} = 0.23$ to 0.5 for **G2**-pHPMA₅₀-EGDMA_{0.8} and from 146 to 116 nm, from $\Phi_{\text{hex}} = 0.17$ to 0.67 for **G2**-pHPMA₁₀₀-EGDMA_{0.8}).

With increasing hexane fraction after this point the D_z increased in size further to 317, 233 and 201 nm for the DP₂₀, DP₅₀ and DP₁₀₀ *hyp*-polydendrons, respectively. This was thought to be due to the nanoparticles aggregating, and with an excess of hexane, the samples would ultimately precipitate. It is worth noting that once again the order of size of the nanoparticles formed followed the same trend as the **EBiB** *hyp*-polymers and **G1** *hyp*-polydendrons, where DP₂₀ > DP₅₀ > DP₁₀₀.

SEM images for various hexane fractions corresponding to the **G2**-pHPMA₅₀-EGDMA_{0.8} sample are shown in Fig. 2.20b. Here the lowest hexane fraction studied ($\Phi_{\text{hex}} = 0.09$, Fig. 2.20b-i) showed only polymer deposits on the SEM stub due to the polymer being solvated by the good solvent, therefore upon drying an uneven polymer film was formed. Although self-assembly was observed at $\Phi_{\text{hex}} = 0.23$, there was minimal structure present in the corresponding SEM image (Fig. 2.20b-ii), again most probably due to the large good solvent fraction present affecting the drying of the sample on the SEM stub. At hexane fractions of 0.50 and 0.80 (Fig. 2.20iii, iv) discrete spherical polymeric nanoparticles were observed with diameters that closely correlated with the D_z measured by DLS. Analysis of the SEM images for the **G2**-pHPMA₅₀-EGDMA_{0.8} nanoparticle sample with $\Phi_{\text{hex}} = 0.80$ was conducted by measuring the diameter of nanoparticles from two images, totalling 314 particles, see Fig. 2.21.

Fig. 2.21 Histogram analysis of nanoparticles via SEM imaging (314 particles from 2 images) overlaid with the DLS size distribution by intensity and number traces for **G2**-pHPMA₅₀-EGDMA_{0.8}, $\Phi_{\text{hex}} = 0.80$



The D_z measured by DLS was 234 nm, whilst the D_n was 203 nm. The mean diameter calculated from the histogram analysis was 190 nm, which was smaller than that measured via DLS most likely due to the SEM analysis performed on dry particles whilst DLS measurements occur when the particles are in the organic solvent mixture.

This mechanism of *hyp*-polymer or *hyp*-polydendron self-assembly is outlined graphically in Fig. 2.22, and has only been observed when using the branched polymers. When the linear polymers and linear-dendritic polymers are subjected to the same treatment the results were quite different.

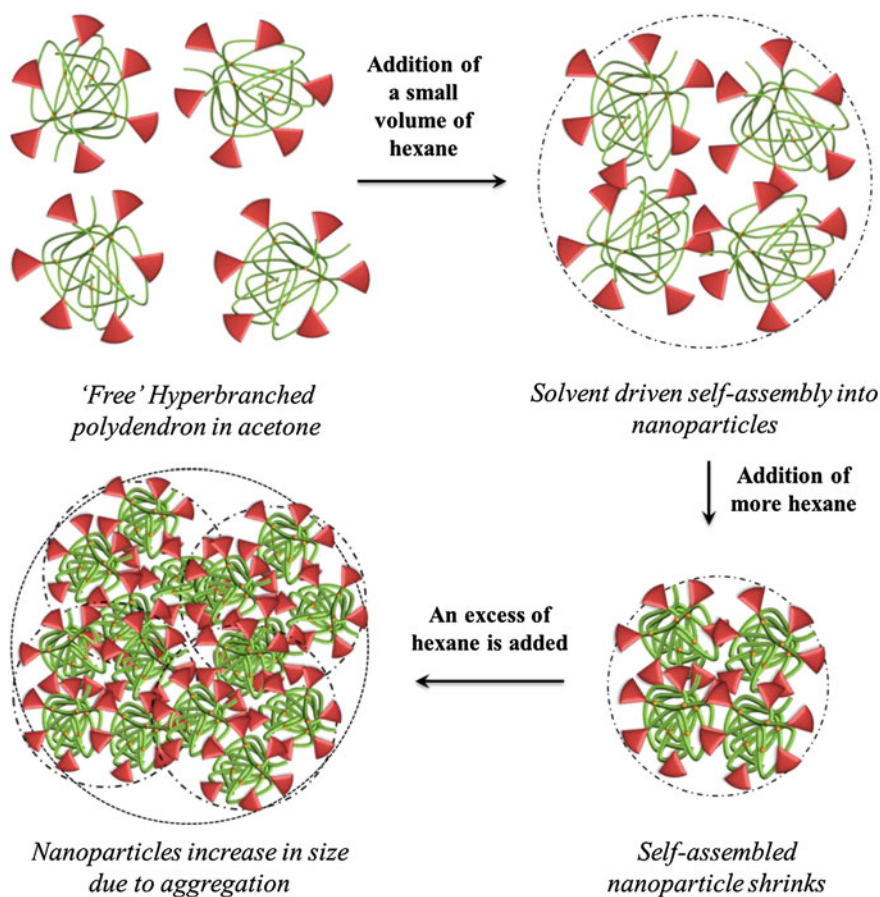


Fig. 2.22 Proposed mechanism of nanoparticle formation in organic solvent mixtures

2.5.2 Nanoparticle Formation Utilising Linear Polymers and Linear-Dendritic Polymers

DLS investigations of the self-assembly of the linear and linear-dendritic polymer analogues of those discussed in Sect. 2.5.1 showed that they did not follow the same trend. In fact it was difficult to find a trend which was applicable to all the linear polymers when they are subjected to the same treatment (dissolving the polymers in acetone (5 mg/mL) and adding various volumes of hexane in an attempt to induce self-assembly). They required a larger fraction of anti-solvent to be added before any form of nanoprecipitation was observed and thus be measured by DLS. Once formed these particles were larger in size (between 500 and 900 nm in diameter, D_z) and had broader polydispersities (approx. 0.5).

The DLS measurements of nanoparticles formed by addition of hexane to polymers dissolved in acetone for each of the **EBiB** initiated linear polymers is shown in Fig. 2.23a and Table 2.3. The DLS measurements at hexane fractions lower than $\Phi_{\text{hex}} = 0.29$ all failed on the DLS quality control criteria due to very high polydispersity, or the linear chains which are solvated were too small to be detected

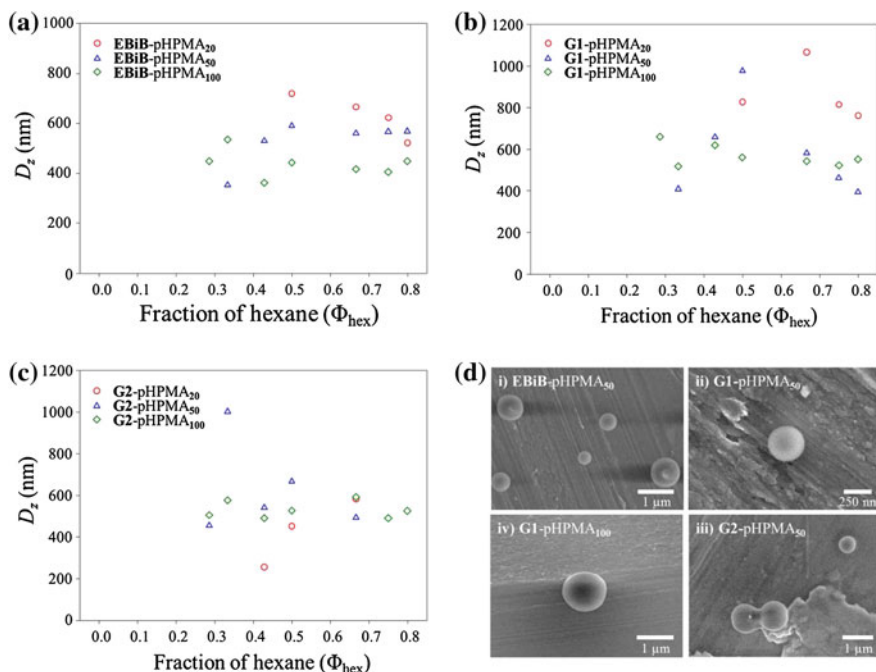


Fig. 2.23 Solvent manipulation as followed by DLS of **a** **EBiB** linear polymers **b** **G1** linear-dendritic polymers, **c** **G2** linear-dendritic polymers. DP₂₀ (red open circles), DP₅₀ (blue open triangles) DP₁₀₀ (green open diamonds). **d** SEM images of nanoparticle samples with a hexane fraction $\Phi_{\text{hex}} = 0.80$ for (i) **EBiB**-pHPMA₅₀, (ii) **G1**-pHPMA₅₀, (iii) **G2**-pHPMA₅₀ and (iv) **G1**-pHPMA₁₀₀

Table 2.3 D_z and PdI measurements by DLS corresponding to Fig. 2.23a for **EBiB** linear polymers

Fraction of hexane added (Φ_{hex})	EBiB-pHPMA₂₀		EBiB-pHPMA₅₀		EBiB-pHPMA₁₀₀	
	D_z (nm)	PdI	D_z (nm)	PdI	D_z (nm)	PdI
0.29	–	–	–	–	447	0.224
0.33	–	–	352	0.210	534	0.331
0.43	–	–	529	0.173	361	0.117
0.50	719	0.519	590	0.075	443	0.051
0.67	665	0.095	559	0.046	416	0.053
0.75	622	0.139	566	0.091	405	0.067
0.80	522	0.076	568	0.076	448	0.103

 D_z z-average diameter

accurately. Once enough hexane had been added, nanoprecipitates were formed. This was $\Phi_{\text{hex}} = 0.5$ for **EBiB-pHPMA₂₀** (719 nm), $\Phi_{\text{hex}} = 0.33$ for **EBiB-pHPMA₅₀** (352 nm) and $\Phi_{\text{hex}} = 0.29$ for **EBiB-pHPMA₁₀₀** (447 nm), which were larger in size than the branched equivalents (see Sect. 2.5.1). The nanoparticles formed were between 350–720 nm in D_z and their formation did not appear to follow a trend. The DP₂₀ polymer sample decreased in size with increasing hexane fraction (to 522 nm at $\Phi_{\text{hex}} = 0.80$), whilst the DP₅₀ increased in size up to $\Phi_{\text{hex}} = 0.50$ where it reached a plateau to $\Phi_{\text{hex}} = 0.80$ (568 nm). The DP₁₀₀ appeared to vary with increasing hexane fraction but is the same size at $\Phi_{\text{hex}} = 0.80$ (448 nm) as at $\Phi_{\text{hex}} = 0.29$ (447 nm).

The **G1** dendron initiated linear-dendritic polymers (Fig. 2.23b and Table 2.4) also formed nanoparticles at hexane fractions above $\Phi_{\text{hex}} = 0.29$. The **G1-pHPMA₂₀** sample did not give a reliable DLS measurement until $\Phi_{\text{hex}} = 0.50$ (827 nm), **G1-pHPMA₅₀** formed nanoparticles at $\Phi_{\text{hex}} = 0.33$ (408 nm) and the **G1-pHPMA₁₀₀** at $\Phi_{\text{hex}} = 0.29$ (660 nm). The increasing hexane fractions appears to have similar effects upon **G1-pHPMA₂₀** and **G1-pHPMA₅₀** whereby with

Table 2.4 D_z and PdI measurements by DLS corresponding to Fig. 2.23b for **G1** linear-dendritic polymers

Fraction of hexane added (Φ_{hex})	G1-pHPMA₂₀		G1-pHPMA₅₀		G1-pHPMA₁₀₀	
	D_z (nm)	PdI	D_z (nm)	PdI	D_z (nm)	PdI
0.29	–	–	–	–	660	0.511
0.33	–	–	408	0.212	517	0.258
0.43	–	–	658	0.25	618	0.116
0.50	827	0.226	977	0.228	560	0.073
0.67	1066	0.203	581	0.148	543	0.11
0.75	814	0.186	460	0.136	522	0.09
0.80	761	0.167	394	0.048	552	0.207

 D_z z-average diameter

increasing hexane fraction, the nanoprecipitates increased in size then subsequently decreased in size. **G1**-pHPMA₂₀ reached a maximum D_z at $\Phi_{\text{hex}} = 0.67$ of 1066 nm, then decreased to 761 nm at $\Phi_{\text{hex}} = 0.80$. **G1**-pHPMA₅₀ reached a maximum D_z at $\Phi_{\text{hex}} = 0.50$ of 977 nm, then decreased to 394 nm at $\Phi_{\text{hex}} = 0.80$. This trend was not observed with the **G1**-pHPMA₁₀₀ sample which instead varied slightly with increasing hexane fraction and at $\Phi_{\text{hex}} = 0.80$ was 552 nm.

Figure 2.23c and Table 2.5 show the resulting DLS measurements for the same experiment using the **G2** dendron initiated linear dendritic polymers. In this case again the DLS readings were not reliable until solvent fractions of $\Phi_{\text{hex}} = 0.43$ was reached for **G2**-pHPMA₂₀ (255 nm) and $\Phi_{\text{hex}} = 0.29$ for **G2**-pHPMA₅₀ (454 nm) and **G2**-pHPMA₁₀₀ (504 nm). The DP₂₀ linear-dendritic polymer samples increased in D_z between solvent fractions 0.43–0.67 (from 255 to 581 nm) then, at higher hexane fractions, precipitate was observed in the sample, which therefore could not be measured by DLS as the result would be inaccurate due to the sedimentation of material. The **G2**-pHPMA₅₀ polymer sample followed a similar trend as that observed for **G1**-pHPMA₂₀ and **G1**-pHPMA₅₀, where the nanoparticles formed initially increased in size up to 1001 nm ($\Phi_{\text{hex}} = 0.33$) then decreased to 493 nm ($\Phi_{\text{hex}} = 0.67$). For this polymer at a hexane fraction of 0.75 the DLS measurement appeared reliable, however, the size obtained (1754 nm) is usually not reliably measured using this technique and with increasing the hexane fraction again to 0.80 the sample precipitated, therefore the size measured at $\Phi_{\text{hex}} = 0.75$ must have been at the onset of aggregation. The **G2**-pHPMA₁₀₀ linear-dendritic polymer remained around the same D_z throughout the experiment and the final size at $\Phi_{\text{hex}} = 0.80$ was 525 nm.

In each case the DP₂₀ polymers required a much higher hexane fraction ($\Phi_{\text{hex}} = 0.5$ for the **EBiB**-pHPMA₂₀ and **G1**-pHPMA₂₀ polymers and $\Phi_{\text{hex}} = 0.43$ for the **G2**-pHPMA₂₀ sample). The DP₅₀ polymers required a lower hexane fraction to induce self-assembly; $\Phi_{\text{hex}} = 0.33$ for the **EBiB**-pHPMA₅₀ and **G1**-pHPMA₅₀ polymers and $\Phi_{\text{hex}} = 0.29$ for the **G2**-pHPMA₅₀ sample, and then all the DP₁₀₀ required a hexane fraction of 0.29 to self-assemble.

Table 2.5 D_z and PdI measurements by DLS corresponding to Fig. 2.23c for **G2** linear-dendritic polymers

Fraction of hexane added (Φ_{hex})	G2 -pHPMA ₂₀		G2 -pHPMA ₅₀		G2 -pHPMA ₁₀₀	
	D_z (nm)	PdI	D_z (nm)	PdI	D_z (nm)	PdI
0.29	–	–	454	0.203	504	0.315
0.33	–	–	1001	0.438	575	0.148
0.43	255	0.233	540	0.119	490	0.052
0.50	451	0.401	666	0.165	525	0.083
0.67	581	0.138	493	0.104	592	0.208
0.75	–	–	1754 ^a	0.294	489	0.077
0.80	–	–	–	–	525	0.179

^aToo high for accurate measurement by DLS

D_z z-average diameter

SEM images of some linear samples are shown in Fig. 2.23d; (i) **EBiB**-pHPMA₅₀, (ii) **G1**-pHPMA₅₀, (iii) **G1**-pHPMA₁₀₀ and (iv) **G2**-pHPMA₅₀. They show that spherical discrete particles are formed, although the sizes observed by SEM would vary somewhat with those observed by DLS. This could be due to the linear nanoprecipitates aggregating as the solvent evaporated when being prepared on the SEM stub.

2.5.3 Comparison of Nanoparticle Formation of Linear Versus Branched

A comparison of the linear and branched nanoparticles formed via this organic solvent nanoprecipitation approach showed a huge difference in the mechanism of formation and the characteristics of the nanoparticles. Whilst all the branched samples follow the same trend with addition of an anti-solvent to the polymer dissolved in a good solvent, the behaviour of the linear equivalents was quite erratic. These trends have been discussed in detail previously (see Sects. 2.5.1 and 2.5.2), however, it is the differences between the linear and branched samples which were most interesting. Figure 2.24 exemplifies the huge difference in nanoparticles size and shows the DP₁₀₀ branched and linear polymers plotted on the same graph. The linear equivalents were approximately 3–4 times larger than the branched polymers and generally had higher polydispersities.

Variables which may affect the sizes of the resulting nanoparticles such as the rate of anti-solvent addition, the temperature and the age of the sample were

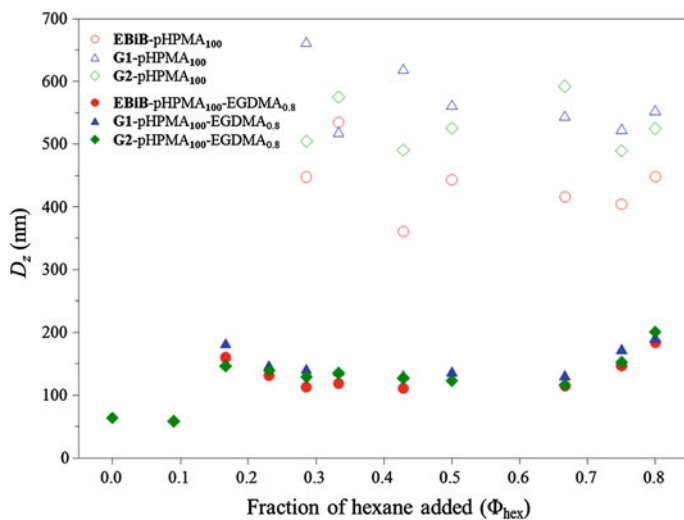


Fig. 2.24 DLS measurements of nanoparticles formed using DP₁₀₀ polymers, highlighting the difference between linear polymers (*open symbols*) and branched polymers (*closed symbols*)

investigated. The rate of hexane addition of 0.5 mL/min was chosen, a temperature difference between 16 and 20 °C had no significant effects on nanoparticle sizes or PDIs. The only factor affecting the samples was the age of the sample; the nanoparticles appeared to precipitate over time. This was probably due to solvent evaporation even though the samples were sealed, as the acetone evaporates the polymers aggregate more, this is observed by the increase of nanoparticle size and eventually the precipitation of the polymers.

2.5.4 Controlling Nanoparticle Size

To determine whether particle size could be tailored to a certain degree, nanoparticles of **G1**-pHPMA₅₀-EGDMA_{0.8} were studied by DLS at various initial concentrations; 0.5, 5.0 and 20 mg/mL. Figure 2.25 shows that at each concentration studied the same trend was observed, as previously discussed, suggesting that the mechanism of nanoparticle formation remains the same.

Increasing the initial concentration of polymer dissolved in acetone increased the subsequent size of the nanoparticles formed. This suggests the self-assembly of numerous branched molecules into a nanoparticle, as at higher concentrations, more molecules are available in a confined area to form the nanoparticle. In the more dilute system there were fewer molecules available in the same area to form the nanoparticle, resulting in smaller sizes. The D_z and PDI values for these samples are shown in the Appendix, Table A.1.

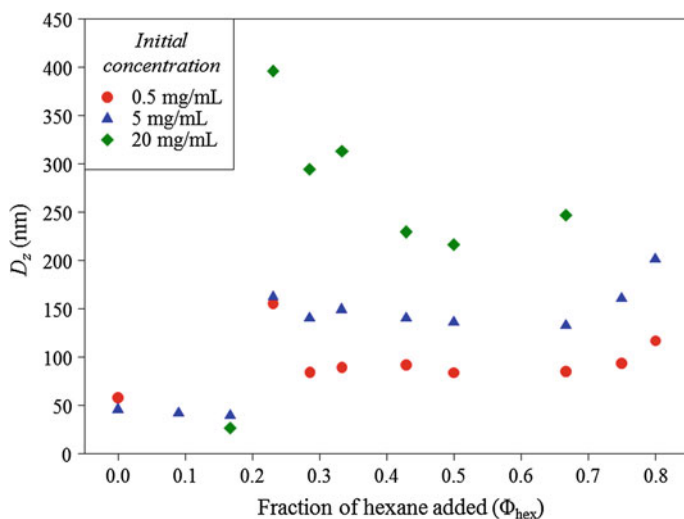


Fig. 2.25 Formation of nanoparticles with varying the initial concentration of **G1**-pHPMA₅₀-EGDMA_{0.8} in acetone at 20 mg/mL (red circles), 5.0 mg/mL (blue triangles) and 0.5 mg/mL (green diamonds)

2.5.5 Dilution Experiments

To understand the formation of the nanoparticles dilution experiments were performed. The first, where a nanoparticle formulation was diluted with acetone (a good solvent for both the initiator surface groups and the polymer core) and the second was the dilution of a nanoparticle formulation without altering the solvent system.

2.5.5.1 Dilution with Good Solvent, Acetone

The nanoparticle formulation used for this dilution experiment was **G1**-pHPMA₅₀-EGDMA_{0.8} nanoparticles at $\Phi_{\text{hex}} = 0.67$ with an initial concentration of 5 mg/mL. Figure 2.26 shows the DLS measurements of the formation of the corresponding nanoparticles (red dotted line) and with increasing the solvent fraction of acetone present (blue dotted line). With addition of acetone the nanoparticles become slightly more solvated by a slight increase in size at the anti-solvent fraction of $\Phi_{\text{hex}} = 0.5$, then decreased in size due to complete solvation, returning to their original state of freely dissolved molecules. The D_z and PdI values corresponding to Fig. 2.26 are shown in the Appendix in Table A.2.

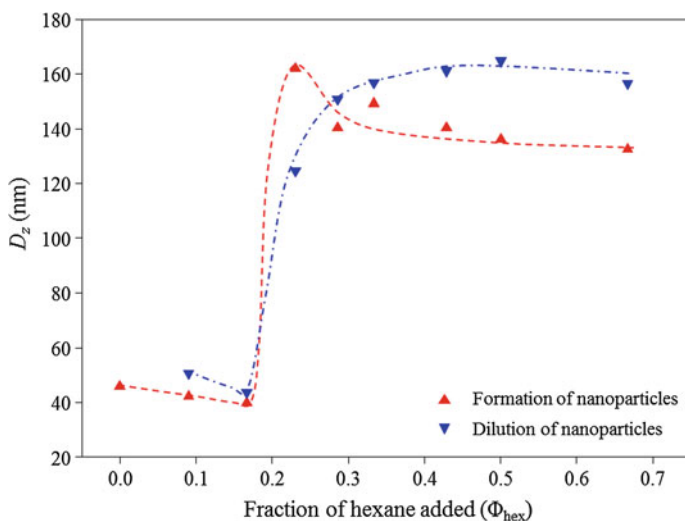


Fig. 2.26 Formation of **G1**-pHPMA₅₀-EGDMA_{0.8} nanoparticles (red up triangles) and dilution with good solvent, acetone (blue down triangles). Trend lines added for ease of viewing only

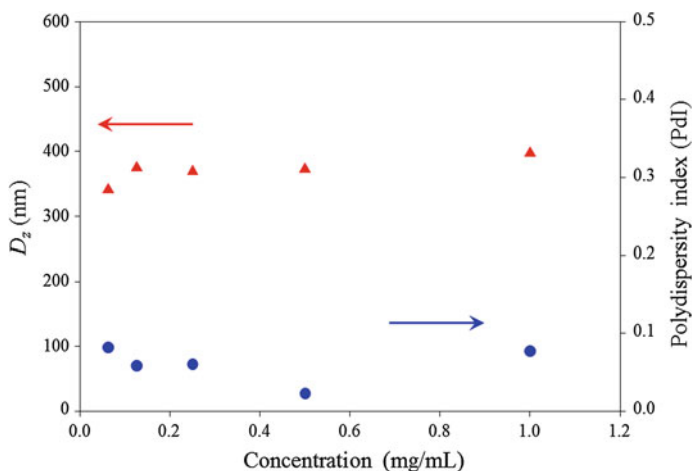


Fig. 2.27 Dilution experiment of **G1-pHPMA₅₀-EGDMA_{0.8}** nanoparticles at a hexane fraction of 0.80 and initial concentration of 5 mg/mL, diluting with the same solvent system. D_z (red triangles) and Pdl (blue circles)

2.5.5.2 Dilution with the Same Solvent System

To investigate the nanoparticles' stability, a nanoparticle sample of **G1-pHPMA₅₀-EGDMA_{0.80}** at an initial concentration 5.0 mg/mL and solvent fraction of hexane $\Phi_{\text{hex}} = 0.80$ was diluted with the same solvent system ($\Phi_{\text{hex}} = 0.8$, $\Phi_{\text{ace}} = 0.20$). Figure 2.27 shows with increasing dilution the size of the nanoparticles is stable until the sample is too dilute for accurate DLS measurement. Therefore once formed the nanoparticles are stable to dilution. The D_z and Pdl values corresponding to Fig. 2.27 are shown in Table A.3, in the Appendix.

2.6 Aqueous Nanoprecipitation of Hydrophobic Polymers

The overall aim of the project was to produce aqueous nanoparticles for drug delivery, therefore, even though at this stage of the project each polymer synthesised was hydrophobic, they were nanoprecipitated into water, firstly to see if they would produce stable nanoparticles, and secondly, if so, to study their properties. Nanoprecipitation of hydrophobic polymers into water has been reported and is a facile method of producing polymeric nanoparticles [26–28]. The polymers were dissolved in a good solvent, tetrahydrofuran (THF), at two different initial concentrations (5 and 10 mg/mL) and underwent rapid addition to water [11]. The THF was allowed to evaporate overnight to give a final concentration of polymer in water. The final concentration could be altered by either changing the initial

polymer concentration within the good solvent, changing the volume of polymer/THF added or by changing the volume of water used. The relationship between initial concentration, final concentration and dilution factor can be described by Eq. (2.1). Throughout this thesis the initial and final concentration of samples prepared by aqueous nanoprecipitation will be described as i_x - f_y , where x represents the initial concentration and y the final concentration in mg/mL, whilst the dilution factor will be referred to as df .

$$\text{initial concentration } (i_x) \times \text{dilution factor } (df) = \text{final concentration } (f_y) \quad (2.1)$$

2.6.1 Aqueous Nanoprecipitations with *Hyp*-polymers and *Hyp*-polydendrons

The aqueous nanoprecipitation process is represented graphically in Fig. 2.28 below with a *hyp*-polydendron depicted. Table 2.6 highlights the different concentrations and dilution factors used and the resulting nanoparticle D_z , PDI and zeta potential values after evaporation of the volatile good solvent (THF). The zeta potential is a measure of the surface charge of the particles and is quoted in mV.

The data in Table 2.6 shows that when the initial concentration was increased in conjunction with a constant dilution factor (df), hence an increase in the final concentration, the resultant nanoparticle size increased. This effect is evident when analysing the size distribution by intensity traces for each sample. Figure 2.29a–c show these traces for the nanoparticle dispersions formulated using polymers **EBiB**-pHPMA₂₀-EGDMA_{0.8}, **EBiB**-pHPMA₅₀-EGDMA_{0.8} and **EBiB**-pHPMA₁₀₀-EGDMA_{0.8} respectively. When the initial concentration was increased but the df

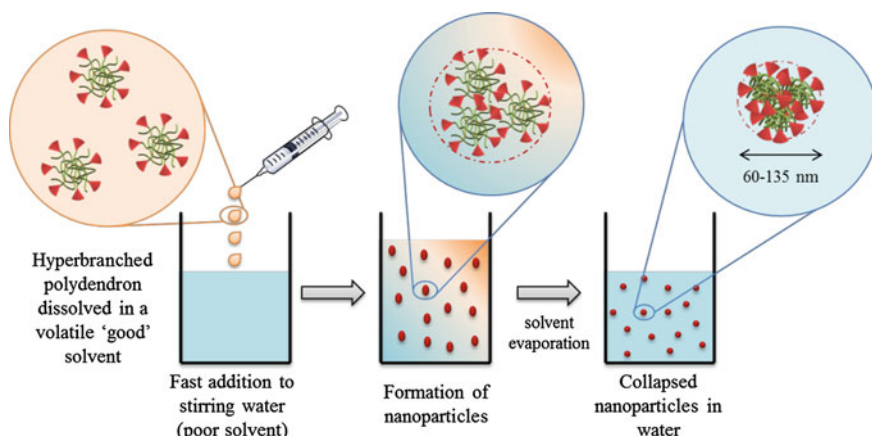


Fig. 2.28 Graphical representation of aqueous nanoparticle formation via a nanoprecipitation approach

Table 2.6 D_z , PdI and zeta potential measurements for all *hyp*-polymer and *hyp*-polydendron aqueous nanoprecipitations at various initial and final concentrations

<i>Nanoprecipitation conditions</i>			DP₂₀			DP₅₀			DP₁₀₀		
i_x (mg/mL)	f_y (mg/mL)	df	D_z (nm)	PdI	Zeta potential (mV)	D_z (nm)	PdI	Zeta potential (mV)	D_z (nm)	PdI	Zeta potential (mV)
EBIB	10	2	0.2	0.127	-51.7	83	0.111	-60.6	101	0.121	-43.2
	10	0.1	0.01	0.135	-38.5	76	0.114	-34.8	103	0.112	-28.1
	5	1	0.2	0.278	-71.6	66	0.190	-57.0	78	0.125	-44.5
	5	0.05	0.01	0.182	-31.3	62	0.216	-30.2	79	0.140	-23.7
G1	10	2	0.2	0.108	-61.3	86	0.110	-42.1	96	0.079	-47.3
	10	0.1	0.01	0.112	-47.4	91	0.101	-37.8	110	0.106	-41.0
	5	1	0.2	0.117	-59.6	64	0.130	-64.5	70	0.070	-54.3
	5	0.05	0.01	0.108	-37.1	67	0.160	-24.6	73	0.114	-26.1
G2	10	2	0.2	0.080	-46.4	106	0.083	-38.0	109	0.113	-50.2
	10	0.1	0.01	0.086	-46.1	134	0.064	-34.3	111	0.127	-41.3
	5	1	0.2	0.076	-42.6	81	0.083	-52.5	81	0.119	-62.1
	5	0.05	0.01	0.084	-30.0	93	0.071	-37.6	83	0.172	-31.0

D_z z-Average diameter, i_x Initial concentration, f_y Final concentration, df Dilution factor

remains constant (e.g. $i_{10}f_2$ and i_5f_1), the size of the nanoparticle formed was increased. In Fig. 2.29a–c those sample pairs which show this are $i_{10}f_2$ versus i_5f_1 (black solid line vs. blue dotted line) and $i_{10}f_{0.1}$ versus $i_5f_{0.05}$ (red dashed line vs. green long-dashed line). Compared to when the same initial concentration but different dilution factors were used (e.g. $i_{10}f_2$ and $i_{10}f_{0.1}$), the sizes observed were very similar in this case, even though the final concentration produced varied by 20-fold. The samples which matched this trend in Fig. 2.29a–c were $i_{10}f_2$ and $i_{10}f_{0.1}$ (black solid line vs. red dashed line) and i_5f_1 and $i_5f_{0.05}$ (blue dotted line vs. green long-dashed line).

These described trends were also observed with the **G1** dendron (Fig. 2.30) and **G2** dendron (Fig. 2.31) initiated *hyp*-polydendrons to varying degrees. For example, nanoparticle dispersions formed using **G1**-pHPMA₅₀-EGDMA_{0.8} (Fig. 2.30b, Table 2.6) have very similar sizes for the same initial concentrations; $i_{10}f_2$ and $i_{10}f_{0.1}$ (86 and 91 nm) and i_5f_1 and $i_5f_{0.05}$ samples (64 and 67 nm). Whilst the

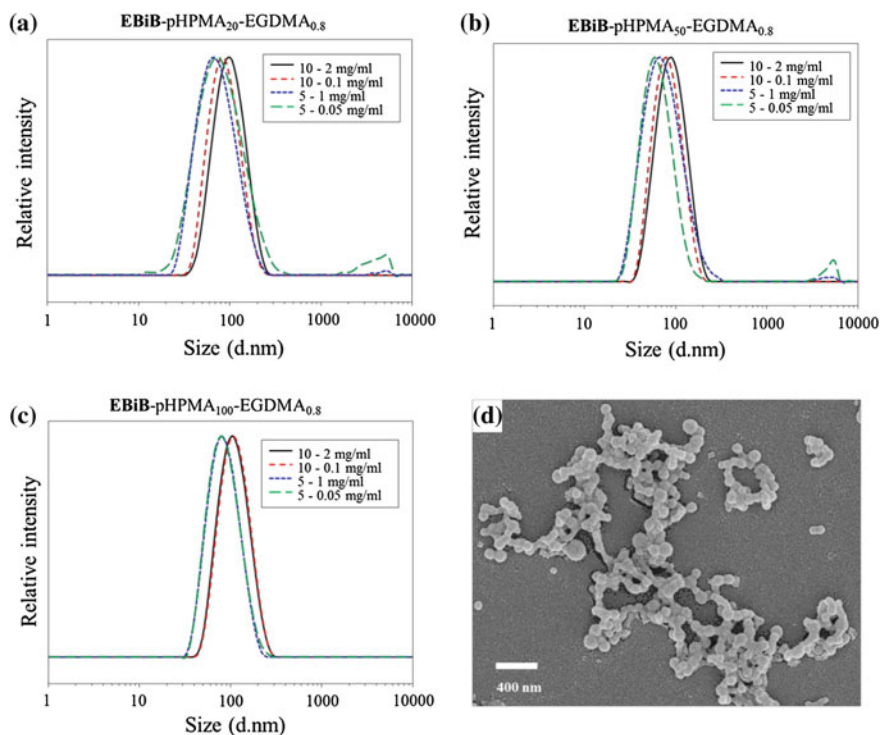


Fig. 2.29 DLS size distribution by intensity traces of aqueous nanoparticle dispersions of: **a** EBiB-pHPMA₂₀-EGDMA_{0.8}, **b** EBiB-pHPMA₅₀-EGDMA_{0.8} and **c** EBiB-pHPMA₁₀₀-EGDMA_{0.8} with various initial and final concentrations, indicated in the legends, and **d** SEM image of the EBiB-pHPMA₅₀-EGDMA_{0.8} (i_5f_1) diluted to 0.1 mg/mL for imaging

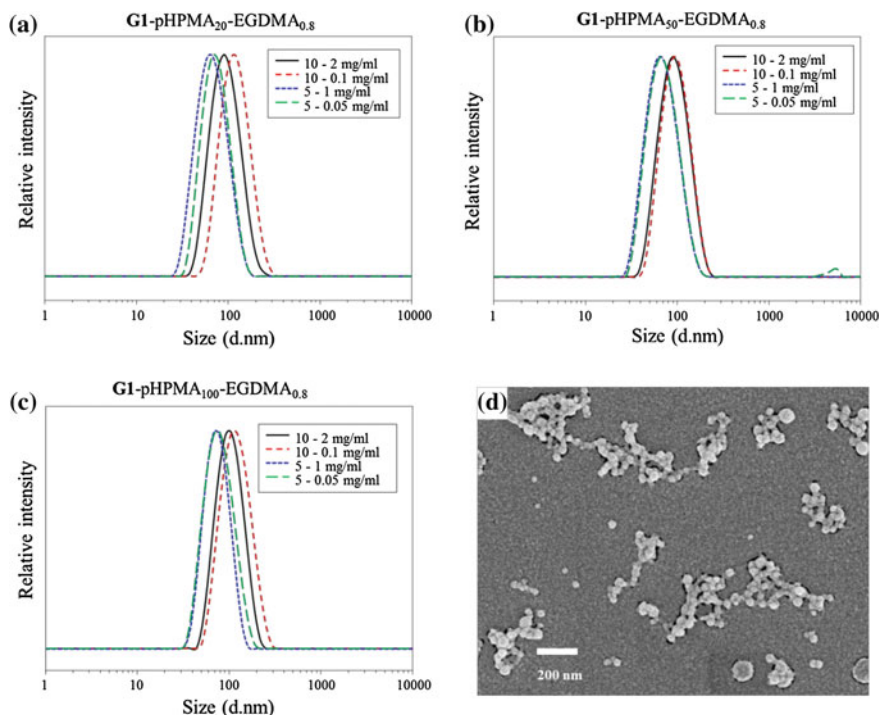


Fig. 2.30 DLS size distribution by intensity traces of aqueous nanoparticle dispersions of; **a** G1-HPMA₂₀-EGDMA_{0.8}, **b** G1-HPMA₅₀-EGDMA_{0.8} and **c** G1-HPMA₁₀₀-EGDMA_{0.8} with various initial and final concentrations, indicated in the legends, and **d** SEM image of the G1-HPMA₅₀-EGDMA_{0.8} (*i*₅-*f*₁) diluted to 0.1 mg/mL for imaging

samples which have the same dilution factor but different initial and final concentrations (*i*₁₀-*f*₂ and *i*₅-*f*₁; 86 and 64 nm, and *i*₁₀-*f*_{0.1} and *i*₅-*f*_{0.05}; 91 and 67 nm) show that with a higher initial concentration the resulting nanoparticle *D_z* was larger.

Again these trends are well exemplified by both G2-HPMA₂₀-EGDMA_{0.8} (Fig. 2.31a, Table 2.6) and G2-HPMA₁₀₀-EGDMA_{0.8} (Fig. 2.31c, Table 2.6). The G2-HPMA₅₀-EGDMA_{0.8} nanoparticles (Fig. 2.31b, Table 2.6) produced show an increase in size with increasing the initial concentration and maintaining *df*, however, when using the same initial concentration and different *df* the resulting particle also vary in size.

The presence of spherical nanoparticles was confirmed by the SEM images of nanoparticles formed using an initial concentration of 5 mg/mL in THF and a final concentration of 1 mg/mL in water with; EBiB-HPMA₅₀-EGDMA_{0.8} (Fig. 2.29d), G1-HPMA₅₀-EGDMA_{0.8} (Fig. 2.30d) and G2-HPMA₅₀-EGDMA_{0.8} (Fig. 2.31d).

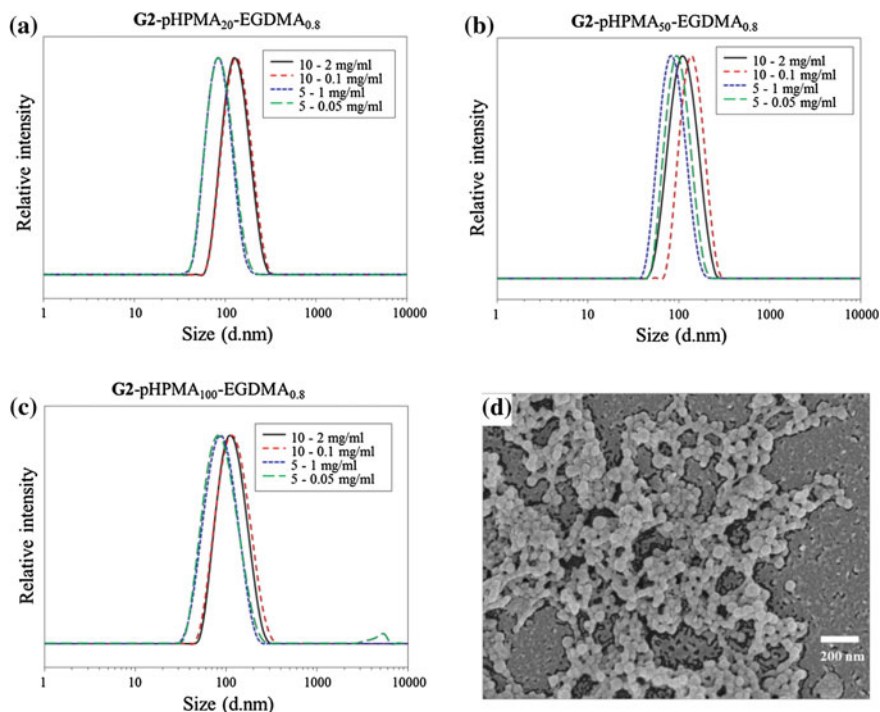


Fig. 2.31 DLS size distribution by intensity traces of aqueous nanoparticle dispersions of; **a** G2-pHPMA₂₀-EGDMA_{0.8}, **b** G2-pHPMA₅₀-EGDMA_{0.8} and **c** G2-pHPMA₁₀₀-EGDMA_{0.8} with various initial and final concentrations, indicated in the legends, and **d** SEM image of the G2-pHPMA₅₀-EGDMA_{0.8} (*i*₅-*f*₁) diluted to 0.1 mg/mL for imaging

Table 2.7 DLS measurements for linear DP₅₀ polymers

Sample name	Nanoprecipitation conditions			D_z (nm)	PDI	Zeta potential (mV)
	i_x (mg/mL)	f_y (mg/mL)	df			
EBiB-pHPMA ₅₀	5	1	0.2	331	0.166	-56.7
	5	0.05	0.01	186	0.077	-50.2
G1-pHPMA ₅₀	5	1	0.2	228	0.147	-62.6
	5	0.05	0.01	193	0.156	-57
G2-pHPMA ₅₀	5	1	0.2	142	0.107	-62
	5	0.05	0.01	138	0.123	-54.6

D_z z-average diameter, i_x Initial concentration, f_y Final concentration, df Dilution factor

2.6.2 Aqueous Nanoprecipitations with Linear and Linear-Dendritic Polymers

The linear and linear-dendritic polymers were subjected to a similar aqueous nanoprecipitation approach using the **EBiB**-pHPMA₅₀, **G1**-pHPMA₅₀ and **G2**-pHPMA₅₀. DLS measurements of the linear nanoprecipitates are shown in Table 2.7, whilst the DLS size by intensity plots are shown in Fig. 2.32a–c.

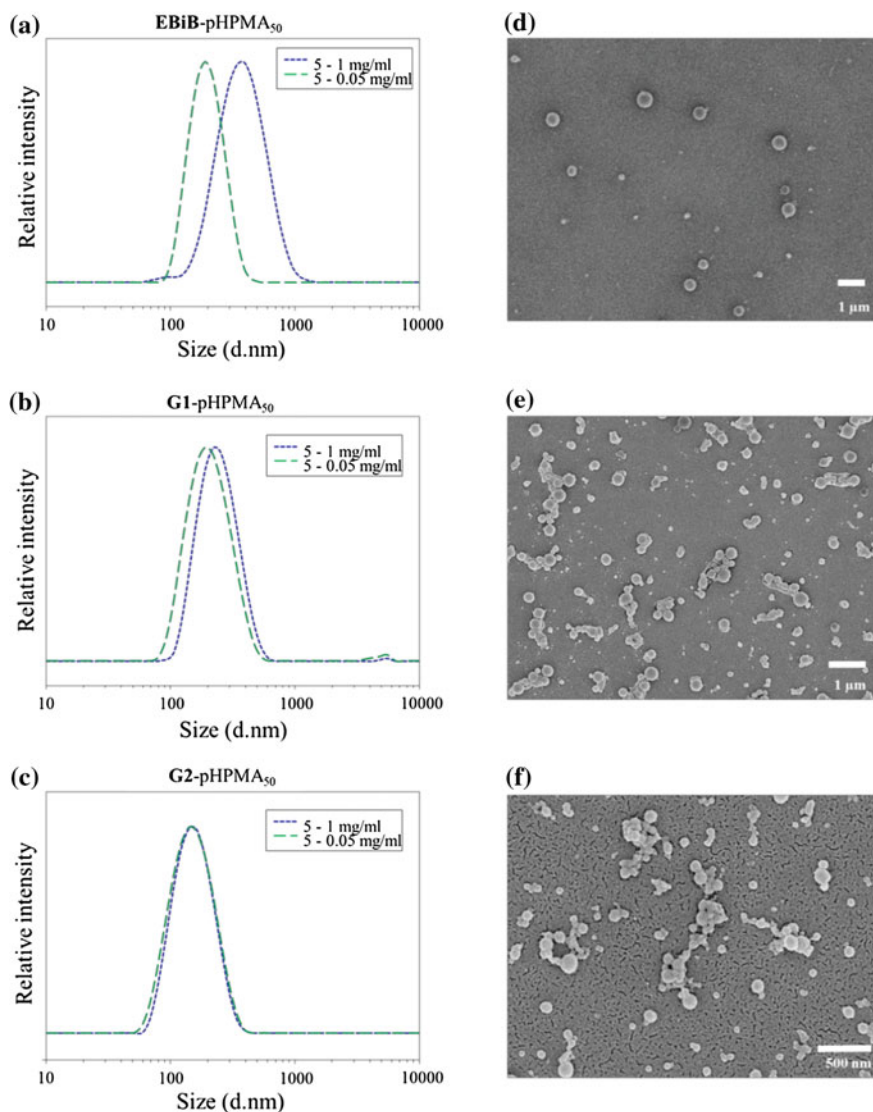


Fig. 2.32 DLS size distribution by intensity traces of aqueous nanoparticle dispersions (*i5-f1*) and SEM images of; **EBiB**-pHPMA₅₀ (a and d), **G1**-pHPMA₅₀ (b and e) and **G2**-pHPMA₅₀ (c and f)

Table 2.8 Nanoprecipitation samples prepared (i_5-f_I) for stability over time study with **EBiB**, **G1** and **G2** initiated linear and branched DP₅₀ polymers

Sample name	Original		+23 months	
	D_z (nm)	PdI	D_z (nm)	PdI
EBiB -pHPMA ₅₀	844	0.238	–	–
G1 -pHPMA ₅₀	562	0.278	612	0.517
G2 -pHPMA ₅₀	315	0.322	–	–
EBiB -pHPMA ₅₀ -EGDMA _{0.8}	68	0.125	88	0.438
G1 -pHPMA ₅₀ -EGDMA _{0.8}	84	0.063	83	0.105
G2 -pHPMA ₅₀ -EGDMA _{0.8}	63	0.076	72	0.251

‘–’ Not suitable for measurement by DLS due to precipitation

D_z z-average diameter

The nanoprecipitates formed from the equivalent linear polymers to the previously discussed branched polymers are generally larger in size. For example, the i_5-f_I sample for **EBiB**-pHPMA₅₀ was 331 nm in diameter, whilst the **EBiB**-pHPMA₅₀-EGDMA_{0.8} had a diameter of 66 nm. The **G1**-pHPMA₅₀ and **G1**-pHPMA₅₀-EGDMA_{0.8} nanoprecipitates formed were 228 and 64 nm in diameter, respectively, with the i_5-f_I samples. This is also true for the **G2** dendron initiated polymers; **G2**-pHPMA₅₀ had a diameter of 142 nm and the **G1**-pHPMA₅₀-EGDMA_{0.8} equivalent had a diameter of 81 nm.

Figure 2.32d–f show SEM images of the **EBiB**-pHPMA₅₀, **G1**-pHPMA₅₀ and **G2**-pHPMA₅₀, for each i_5-f_I sample which show spherical particles that correlate with the D_z measured by DLS.

2.6.3 Colloidal Stability of Aqueous Nanoparticles

The nanoprecipitates produced in this chapter were originally not expected to be stable when dispersed in water due to the fact that every component of the polymeric materials were hydrophobic in nature. The stability of the particles was studied over time by storing the samples at ambient temperature out of the light. Samples prepared at i_5-f_I were used for the study and a separate batch was prepared using the linear and branched DP₅₀ polymers (**EBiB**-pHPMA₅₀, **EBiB**-pHPMA₅₀-EGDMA_{0.8}, **G1**-pHPMA₅₀, **G1**-pHPMA₅₀-EGDMA_{0.8}, **G2**-pHPMA₅₀ and **G2**-pHPMA₅₀-EGDMA_{0.8}). Table 2.8 shows the D_z and PdIs for the original sample and repeat measurement after 23 months. Figure 2.33 shows the DLS size distribution by intensity traces for the original measurement and repeat measurement after 23 months.

It is evident from these measurements that the *hyp*-polymer and *hyp*-polydendrons were much more stable over time than their linear polymer equivalents. Two of the linear nanoparticle samples had fully precipitated; **EBiB**-pHPMA₅₀ and **G2**-pHPMA₅₀. The only linear sample which could be measured by DLS after

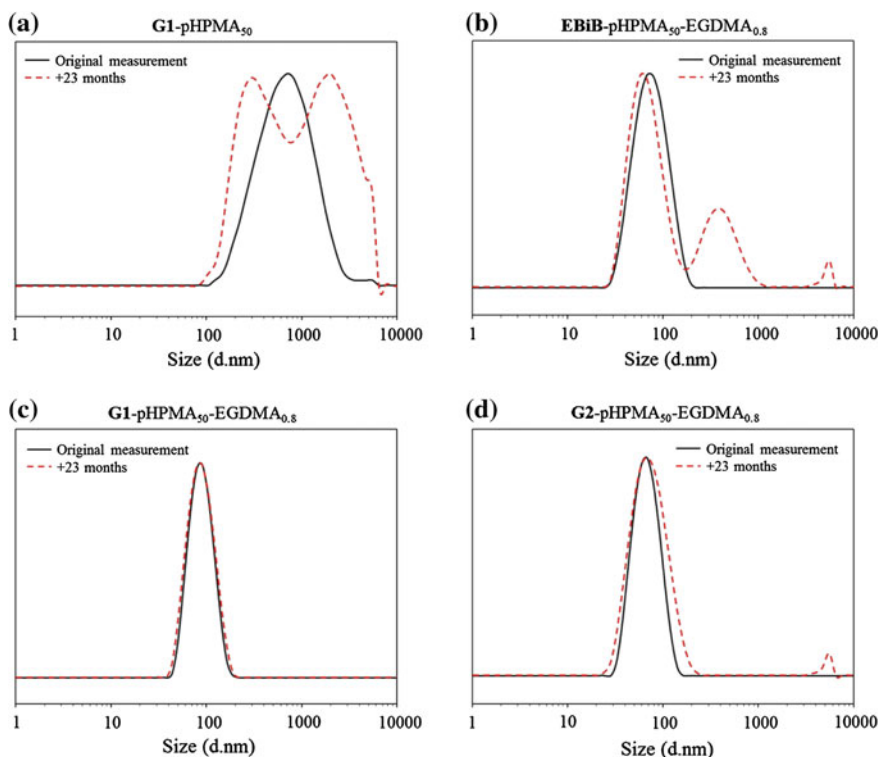


Fig. 2.33 DLS size by intensity traces for **a** G1-pHPMA₅₀, **b** EBiB-pHPMA₅₀-EGDMA_{0.8}, **c** G1-pHPMA₅₀-EGDMA_{0.8} and **d** G2-pHPMA₅₀-EGDMA_{0.8} original measurements and measurements after 23 months

23 months was the G1-pHPMA₅₀ sample (see Fig. 2.33a), which had increased in size from 562 to 612 nm and the PdI had almost doubled, from 0.278 to 0.517. All of the branched polymer samples were suitable for DLS measurement and had not increased in size significantly over 23 months. The EBiB-pHPMA₅₀-EGDMA_{0.8} had increased the most from 68 to 88 nm, with an increase in PdI from 0.125 to 0.438. This was attributed to aggregation of the nanoparticles, which is evident from the two new populations present in the size distribution by intensity traces (see Fig. 2.33b). The G1-pHPMA₅₀-EGDMA_{0.8} sample remained the same size over 23 months of storage and the PdI only increased slightly from 0.063 to 0.105, see Fig. 2.33c for the DLS size distribution by intensity traces. The G2-pHPMA₅₀-EGDMA_{0.8} nanoparticle dispersion had only increased from 63 to 72 nm, however an increase in PdI from 0.076 to 0.251 was observed and an extra population was observed in the size distribution by intensity trace after 23 months indicating some aggregation had occurred.

Although the nanoparticles formed using the *hyp*-polymer and *hyp*-polydendron remained stable over time, the zeta potentials measured for each sample were very

negative (<-20 mV) which indicates that the nanoparticles were charge stabilised. The negative charges at the surface of each particle repel one another therefore preventing flocculation or aggregation which can lead to sedimentation and precipitation. Charge stabilised colloids can easily be de-stabilised by addition of electrolytes, such as NaCl, due to the screening of colloidal charges by the ions present [29]. This screening of charges stops the negatively charged particles from repelling one another and they can aggregate and ultimately precipitate. Therefore it is desirable to produce sterically stabilised nanoparticles when designing them for use in biological systems due to the presence of various salts present in the human body [30].

2.7 Conclusion

The synthesis of dendron initiators for ATRP was achieved, with successful synthesis of linear and branched vinyl polymers using the monomer HPMA and divinyl monomer EGDMA. These polymerisations proceeded via 1st order kinetics, as expected, and produced monomodal distributions of polymer chains when a linear polymer was targeted and a much more broad disperse polymer species when the divinyl monomer was incorporated and a branched polymer was targeted. The resulting branched polymers and *hyp*-polydendrons were exposed to two different nanoprecipitation approaches to produce nanoparticles. Solvent manipulation to form nanoparticles in mixtures of good and anti-solvents (acetone and hexane) showed a huge difference in the formation of particles when comparing the *hyp*-polymers and *hyp*-polydendrons to their linear polymer equivalents. When aqueous nanoprecipitation was conducted, surprisingly these hydrophobic polymers produced nanoparticles which were stable over time due to charge stabilisation. The *hyp*-polymers and *hyp*-polydendrons were much more stable over time than their linear polymer equivalents.

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