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Unimolecular branched block copolymer nanoparticles in methanol for preparation of poorly water-soluble drug nanoparticles

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Abstract

Spherical unimolecular amphiphilic branched A-B block copolymer nanoparticles in methanol are fabricated via thermal annealing using the methanolic upper critical solution temperature (UCST) of the hydrophobic block segment. This finding is then used to produce aqueous poorly water-soluble drug nanoparticle suspension with a mass:drug ratio of 1:1 and 100% nanoparticle yield. The drug nanoparticles in the suspension are stabilized by multiple polymer nanoparticles.

The synthesis of polymer nanoparticles has attracted significant attention due to potential applications from drug/nucleic acid delivery systems [1,2] to templating agents for nanoporous microelectronic materials.[3] A multitude of approaches have been reported to generate polymer nanoparticles with controlled size, shape and chemical functionality including dendrimer synthesis (size ranging from 1 to 10 nm), microemulsion polymerization techniques (particles from 20 to 50 nm) and emulsion/evaporation techniques.[4-6] Indirect formation of nanostructures by cooperative self-assembly of pre-synthesized macromolecular building blocks has also been reported, with cross-linking of self-assembled structures utilized to generate persistent particles.[7-9] In general, self-assembly and subsequent crosslinking is relatively time-consuming, requires careful control of concentrations during the reactions and presents difficulties for scale-up. We have reported a direct monomer-to-particle synthetic strategy based on branched amphiphilic A-B block copolymers. [10, 11] Using the atom transfer radical polymerization (ATRP) technique to form soluble high molecular weight species, well-defined spherical polymer nanoparticles could be obtained after a dialysis process of the polymer solution in tetrahydrofuran (THF) against water. This synthetic approach differs significantly from the reported arm-first or core-first core-crosslinked star-polymer synthesis where the core is effectively a highly cross-linked microgel formed by the addition of a large volume of a cross-linker such as divinylbenzene at the end of the polymerisation. [12,13] It was possible to further prepare amphiphilic materials with defined nanoparticle shape in a one-pot, concerted growth process rather than joining of pre-formed spheres.[10,11] Apart from the direct synthetic method, the branched polymer nanoparticles have several additional advantages over conventional micelles and dendrimers; the high stability which means that they would retain their sizes and spherical shapes when applied in vivo, and would not disassemble when environmental conditions changes (compared to conventional micelles), would also allow larger loading capacity of guest compounds (compared to highly-crosslinked nano/micro gels) due to light crosslinking, and the high potential to be scaled-up.

For biomedical or environmental applications, aqueous formulations are often required for hydrophobic organic compounds. Particularly for poorly water-soluble drugs, where a high percentage of developed drug compounds are poorly water-soluble, the low water solubility can lead to low therapeutic efficacy and sometimes the drug candidate compounds would have to be abandoned.[14] One promising way to address this issue is the preparation of aqueous drug nanoparticle dispersions. The nanoformulations can enhance the dissolution rate or can be used directly as evidenced by the nanodrugs on the market.[14] Both top-down (milling, homogenization) and bottom-up methods (e.g., micelles, dendrimers, emulsion evaporation) have been widely reported.[14, 15] An excellent formulation method should have the characteristics of mild processing conditions, use of intoxic solvent, small nanoparticles, high loading of active drugs, etc.

Very recently, we have reported the use of branched copolymer nanoparticles poly(N-isopropyl acrylamide)-*b*-poly

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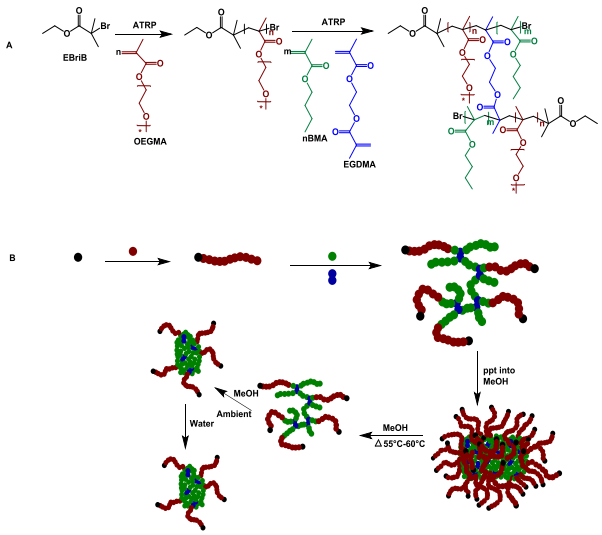
(ethylene glycol) (PNIPAM-PEG) for preparation of aqueous drug nanoparticle dispersions via an emulsion freeze-drying approach.[16] The hydrophobic drugs were dissolved in water-immiscible organic solvent which was then formulated to form oil-in-water emulsions before applying the emulsion freeze-drying procedure. There are a few issues unfavoured in that technique: (i) the volatile organic solvent may be toxic; (ii) many drugs are only soluble in polar organic solvent (e.g., alcoholic solvent, acetone) and cannot be further processed to form emulsions; (iii) use of energy-intensive freeze-drying technique. Herein, we describe the synthesis of branched copolymer of POEGMA-PnBMA (a more hydrophobic core) from oligo(ethylene glycol mono-methyl ether) methacrylate (OEGMA) and n-butyl methacrylate (n-BMA). Importantly, we report for the first time the formation of such polymer nanoparticles by thermal annealing of its methanolic solution. Instead of a two-step procedure (preparation of THF solution followed by the time-consuming dialysis process in water) reported previously,[10,11] the simple thermal annealing method allows the quick formation of polymer nanoparticles in water (usually within one hour). The finding is then employed for the preparation of drug nanoparticle suspensions by a simple solvent evaporation approach.



Scheme 1. Schematic synthesis of p(OEGMA-b-(nBMA-co-EGDMA)) amphiphilic branched A-B block copolymers. A) ATRP sequential polymerization of OEGMA and a mixed monomer nBMA/EGDMA feedstock; B) Branched A-B block copolymers precipitate in methanol and subsequently dissolve on heating through the UCST.

The synthesis of the amphiphilic A-B branched block copolymers (and their linear analogues) consisted of a one-pot, Cu(I)Br/2,2-bypridine catalysed, ATRP polymerisation in isopropanol (IPA), using an ethyl α-bromoisobutyrate (EBriB) initiator to polymerise OEGMA (Mn = 300 g/mol) to a relatively low degree of polymerization (DP). Addition of a mixture of n-butyl methacrylate (nBMA) and ethylene glycol dimethacrylate (EGDMA) to the growing p(OEGMA) chain allowed propagation of hydrophobic p(nBMA) with simultaneous branching to other propagating chains (Scheme 1A). Careful manipulation of the EGDMA/EBriB ratio and monomer concentrations prevents gelation and ensures, on average, less than one branching EGDMA per primary polymer chain.[17] The synthesized branched polymers were characterized by ^{1}H NMR and gel permeation chromatography (GPC), as shown in Figure S1 and S2 for p(OEGMA$_{36}$-b-(nBMA$_{60}$-co-EGDMA$_{0.9}$)). The original feeding ratio of OEGMA:nBMA was 40:60. The component ratio in the polymer was calculated from the ^{1}H NMR spectrum (Figure S1). Due to the high molecular weights of each block copolymer linear chain (e.g., around 18000 gmol^{-1}), it was difficult to find the remaining initiator peaks and obtain the accurate integral value to calculate the ^{1}H NMR Mw. But the integral values of methylene groups from PnBMA and methyl groups form POEGMA could be used to calculate the ratio of each blocks, which gave a ratio of OEGMA:nBMA = 36:57. This is consistent with the conversions of 90 % and 98% for OEGMA and nBMA after polymerisation, respectively. During the GPC analysis, very high operation pressure was noticed and it was likely that most of the branched polymer could 'stick' to the stationary phase and be trapped in the GPC column.[10] Although larger molecules are usually easily eluted out in the GPC analysis, the high molecular weight and larger size of the branched copolymer may interact strongly with the stationary phase, accumulate, and partially block the pores in the column, as evidenced by the high back pressure. This resulted in the elution of small amount of poorly-branched copolymers, which could be detected by the GPC (Figure S2). This data may be not accurate, but it still indicates the formation of branched structures (relatively big polydispersity and Mw).

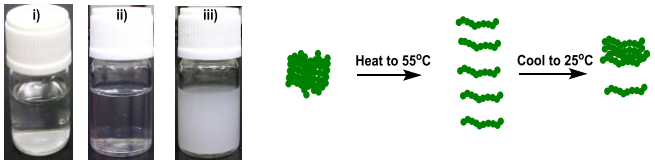


Figure 1. P(nBMA) at different temperatures in MeOH – (i) at 25°C prior to heating(precipitate at bottom) (ii) 55-60°C, and (iii) after cooling to 25°C.

Although p(nBMA) is considered to be hydrophobic, the polar solvent IPA is the theta solvent for the polymer,[18] allowing successful ATRP of nBMA in IPA and H_2O/IPA mixtures.[19] Methanol is normally a poor solvent for p(nBMA) and often used during workup at ambient or chilled temperature to precipitate p(nBMA) after synthesis. However, during a study of p(nBMA) (Mn= 13570 g mol-1), the precipitated polymer in methanol was heated to temperatures approaching the boiling point of MeOH. Surprisingly, it was noticed that the polymer dissolved fully to produce a clear solution which precipitated again when the solution returned to ambient temperature (Figure 1). This behaviour could be utilized to conduct Cu-catalyzed ATRP of nBMA within methanol under different temperatures to generate linear homopolymers with a wide range of molecular weights whilst maintaining very low

dispersities and avoiding termination reactions that would lead to increasing molecular weight at high monomer conversions.[20] It should be pointed out that only linear polymers were produced and no nanoparticle study was carried out in the previous study.[20] Thus, the novelty of this study in preparing the branched block copolymer and utilizing it to form polymer nanoparticles is still high.

The upper critical solution temperature (UCST) behaviour of p(OEGMA) in octan-1-ol was used to create micelles, prior to crosslinking of linear di-block copolymers containing OEGMA, N-isopropyl acrylamide, pentafluorophenyl acrylate and N,N-diethylacrylamide.[21,22] Similarly, statistical copolymers of N-phenyl maleimide and n-octadecyl vinyl ether were shown to exhibit UCST behaviour in N,N-dimethylformamide and a range of alcohols such as 1-butanol and 1-hexanol[23] whilst p(BMA) and p(BMA) A-B block copolymers with PEG were reported to exhibit lower critical solution temperature (LCST) behaviour in ionic liquids.[24]

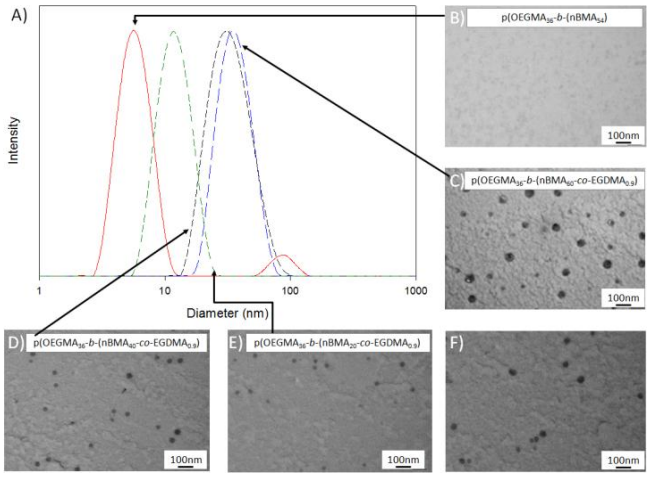


Figure 2. Characterisation of p(OEGMA$_{36}$-b-(nBMA-co-EGDMA$_{0.9}$)) branched A-B block copolymer nanoparticles and corresponding linear analogue after annealing the solutions through the methanolic UCST. A) Dynamic light scattering of branched copolymer nanoparticles: blue large dashed line, p(BMA) block DP = 60; black short dashed line, p(BMA) block DP = 40; green triple dashed line, p(BMA) block DP=20; red solid line, linear p(OEGMA$_{36}$-b-nBMA$_{54}$) control. TEM images of B) linear p(OEGMA$_{36}$-b-nBMA$_{54}$), and branched copolymers where C) p(BMA) block DP = 60; D) p(BMA) block DP = 40; E) p(BMA) block DP = 20 and F) branched copolymer nanoparticles of p(OEGMA$_{36}$-b-(nBMA$_{60}$-co-EGDMA$_{0.9}$)) after annealing in methanol and dialysis into water.

The dissolution of p(nBMA) in warm methanol offers an alternative route to nanoparticle formation after synthesis of the branched A-B block copolymers. The synthesized branched block POEGMA-PnBMA was added to methanol at ambient temperature (10mg/10ml, polymer/methanol) and heated with stirring to 55-60°C for 10 minutes and subsequently allowed to cool (Scheme 1B). At ambient temperature a clear solution was seen with no obvious precipitate. Methanol is a good solvent for the p(OEGMA) block and dissolves the branched p(BMA-EGDMA) core block at elevated temperature. Therefore, we postulated the formation of discreet, unimolecular nanoparticles during cooling of the dilute MeOH solution and collapse of the insoluble branched block segments (Scheme 1B).

In this work, a range of polymers were synthesized with the same degree of polymerization (DP) of p(OEGMA) and decreasing block length of the branched p(nBMA), *i.e.,* p(OEGMA$_{36}$-b-(nBMA$_{60}$-co-EGDMA$_{0.9}$)), p(OEGMA$_{36}$-b-(nBMA$_{40}$-co-EGDMA$_{0.9}$)), p(OEGMA$_{36}$-b-(nBMA$_{20}$-co-EGDMA$_{0.9}$)). A linear A-B block copolymer analogue, p(OEGMA$_{36}$-b-nBMA$_{54}$), was also synthesized and processed in the same manner. After the thermal annealing procedure, transmission electron microscopy (TEM) and dynamic laser scattering (DLS) analysis of the polymer solutions in methanol was conducted. The presence of nanoparticles is clearly demonstrated in Figure 2. In methanol, the Z-average nanoparticle diameters varied from 37 nm to 33 nm to 25 nm in relation to the decreasing p(nBMA) chain length (DP= 60, 40 and 20 monomer units respectively) for the branched copolymers. However, for the linear block copolymer, there were no particles larger than 10 nm, assumed to be the single linear block copolymers with solvated p(OEGMA) segments. Accordingly, by TEM, irregular films were generated by the annealed and dried MeOH solutions of linear p(OEGMA$_{36}$-b-nBMA$_{54}$) (Figure 2B) whilst spherical nanoparticles were observed for three branched block copolymers with different chain lengths of p(nBMA), showing slightly larger sizes 48nm, 40nm and 28nm, respectively (Figure 2C-E). This is likely due to spreading of the low Tg p(OEGMA corona and p(nBMA) cores. The MeOH suspension of p(OEGMA$_{36}$-b-(nBMA$_{60}$-co-EGDMA$_{0.9}$)) nanoparticles was dialysed to transfer the nanoparticles from MeOH to H_2O. After dialysis for 1 day, aqueous nanodispersion was obtained and analyzed by DLS and TEM with almost identical sizes seen in water: TEM diameter = 45 nm and DLS Z-average = 32 nm (Figure 2F). This indicates that, as an alternative way to prepare polymer nanoparticles, the thermal annealing method can produce polymer nanoparticles of similar sizes, but more efficient as it takes much less preparation time.

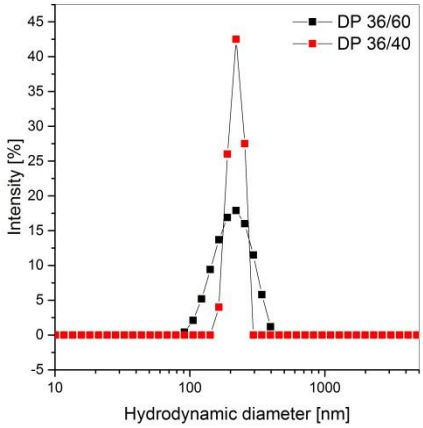
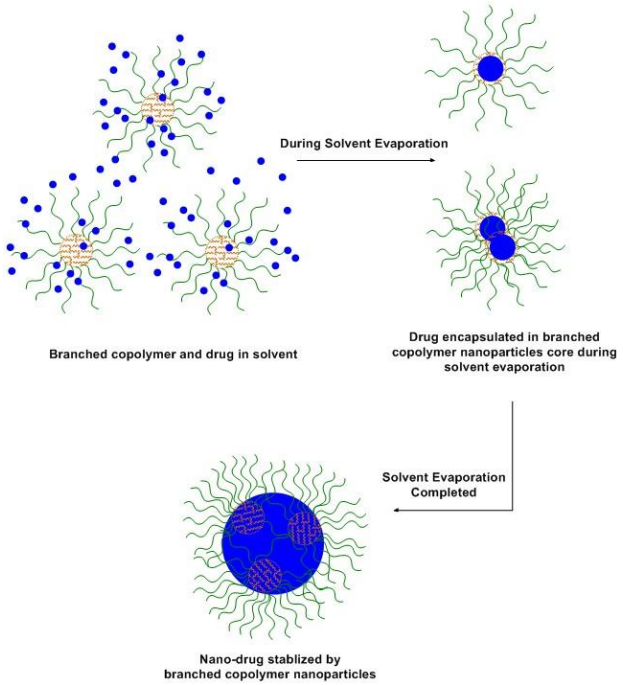


Figure 3. DLS profiles of ketoprofen nanoparticles formed by direct solvent evaporation of p(OEGMA-b-nBMA) (DPs of 36/40 and 36/60) and ketoprofen (mass ratio 1:1) in MeOH/H_2O (1:1 v/v).

Linear and block copolymers have been used to produce hydrophobic drug nanoparticles, usually be solvent displacement or nanoprecipitation.[14] Commonly, the polymers would be dissolved in a water-miscible solvent (e.g., acetone, THF) and the resulting solution is added dropwise into water. After evaporation of the volatile organic solvent, drug-encapsulated polymer nanoparticles can be obtained. Whether drug nanoparticles in water can be formed may depend on the type of polymer, hydrophobic drug/compound, type of solvent, concentration of solutions. The preparation conditions need to be optimized to form nanoparticles with desirable sizes (e.g., < 200 nm) and high yield. Here, we demonstrate how the P(OEGMA)-P(nBMA) nanoparticles can be used to facilitate the formation of poorly water-soluble drug nanoparticles, using ketoprofen as the model drug. P(OEGMA)-P(nBMA) (the as-synthesized polymer, not the nanoparticles after dialysis against water) and ketoprofen were dissolved in warm methanol at 55°C, cooled down to room temperature. At this stage, ketoprofen was still dissolved but with polymer nanoparticles (induced by thermal annealing) in methanol, whilst both the drug and polymer would be molecularly dissolved in the previous nanoprecipitation or solvent evaporation approach. After adding equal volume of water to the methanol suspension, methanol was allowed to evaporate at room temperature in a fume cupboard to produce aqueous ketoprofen nanoparticle suspension directly.

At a ketoprofen:POEGMA-PnBMA ratio of 1:1, a nanoparticle yield of 100% was achieved. As measured by DLS, the Z-average nanoparticle sizes by intensity were 198 nm and 214 nm for p($OEGMA_{36}$-b-($nBMA_{60}$-co-$EGDMA_{0.9}$) and p($OEGMA_{36}$-b-($nBMA_{40}$-co-$EGDMA_{0.9}$), respectively. The DLS profiles showed quite narrow particle size distributions for both aqueous ketoprofen nanoparticle suspensions (Figure 3). It was also found that when the polymer ratio was higher, *e.g.,* polymer:drug = 3:1, the nanoparticle yields of 100% were also achieved. This is understandable because more polymer can better stabilize the hydrophobic drug nanoparticles. We also tried the ratio of polymer:drug = 1:3. The nanoparticle yields of 81% and 85% for ketoprofen were obtained for p(OEGMA36-b-(nBMA40-co-EGDMA0.9) and p(OEGMA36-b-(nBMA60-co-EGDMA0.9), respectively. The nanoparticle yield was calculated as the percentage of ketoprofen nanoparticles in the suspension after centrifugation (see the Experimental in the Supporting Information), stabilized by the branched copolymer nanoparticles, based on the amount of ketoprofen used in the preparation. It should be noted that at the centrifugation speed of 3000 rpm, all the polymer nanoparticles still remained in the suspension and provided stabilization for the ketoprofen nanoparticles. Based on the amount of ketoprofen used, ketoprofen loading efficiency should be the same as the ketoprofen nanoparticle yields. However, the loading percentages of drug nanoparticle in the formulation (i.e., taking both drug and polymer nanoparticles into account) should be 50 % for drug:polymer = 1:1 at 100 % nanoparticle yield, 63.75 % for 85 % nanoparticle yield and 60.75 % for 81% nanoparticle yield both at drug:polymer =3:1.

The branched block copolymers form unimolecular nanoparticles in methanol and water, as discussed and demonstrated in Figure 2. However, the polymer-stablized ketoprofen nanoparticles are multimolecular. This may be explained by Scheme 2.



Scheme 2. Formation of ketoprofen nanoparticles assisted by branched block copolymers via solvent evaporation

The block copolymer POEGMA-PnBMA forms clear solution as swollen nanoparticles in methanol at 55 °C and nanoparticle suspension with dense hydrophobic core at room temperature. During the preparation, POEGMA-PnBMA and the drug compound are dissolved in methanol to form clear solution at 55°C. When cooling to room temperature, the polymer nanoparticles with dense PnBMA core with some ketoprofen molecules are formed while the rest of ketoprofen is still soluble in methanol. When adding water to the methanol solution, with the evaporation of methanol, the increasing hydrophilic environment drives the hydrophobic drug molecules moving towards the hydrophobic core (which may be just on or close to the core due to the dense nature in methanol at room temperature), with the whole structure stabilized by the outer hydrophilic PEG chains. With further evaporation, more drug can be loaded into the polymer nanoparticles core, and the nanoparticles core becomes more hydrophobic and solid. This makes the outer PEG chains difficult to stabilize each nanoparticles, which resulted in the aggregation of several nanoparticles and resulted in bigger nanoparticles (Scheme 2). It is clear that although the block copolymer nanoparticles are unimolecular, the drug-polymer nanoparticles are multimolecular. To be more precise, the hydrophobic drug nanoparticles are stabilized by multiple polymer nanoparticles. This explanation was supported by the control experiment of

evaporating methanol from the methanol solution (without adding water) directly. Low nanoparticle yields were recorded (Table S1), which could be the result of low capacity and drive for the drug molecules to move into the dense PnBMA core. The drug molecules could just form large particles outside the POEGMA-PnBMA nanoparticles.

In summary, a branched A-B block copolymer comprising p(OEGMA) and branched p(BMA) block segments has been synthesized and employed to form nanoparticles in methanol via a thermal annealing process using the methanolic UCST of the hydrophobic block p(BMA). The z-average diameter of the particles (25-37nm) is controlled by the number average degree of polymerisation of the hydrophobic block p(BMA). These polymer nanoparticles are utilized to produce aqueous drug nanoparticle dispersion by a simple solvent evaporation approach. By design and careful selection of block monomers and block chain length, one may be able to synthesize and optimize the block copolymer structure for a target organic compound. The combination of solvent evaporation and branched block polymer can be a very effective method in organic nanoparticles and nanomedicine research.

Notes and references

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Supporting Information

Unimolecular branched block copolymer nanoparticles in methanol for preparation of poorly water-soluble drug nanoparticles

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Experimental Details

Chemicals and reagents

Deionized water was prepared using an AquaMAX-Basic 321 DI water purification system. Ketoprofen ≥ 98% (TLC), copper (I) bromide (CuBr, 98%), copper (I) chloride (CuCl, 99+%), 2,2-dipyridyl (bpy, 99+%), ethyl 2-bromoisobutyrate (EBriB, 98%), Azobis-(isobutyronitrile) (AIBN), and 1-dodecanethiol (DDT)were purchased from Sigma-Aldrich and used as received. Olig(ethylene glycol) methyl ether methacrylate (OEGMA, Mn = 300 g/mol), ethylene glycol dimethacrylate (EGDMA, 98%) were obtained from Sigma-Aldrich, and passed through an aluminium oxide (Al_2O_3) column to remove the inhibitor before use. Tetrahydrofuran (THF) from Aldrich was purified by refluxing with sodium overnight under nitrogen to remove water, then distilled prior to use. All other solvents were purchased from Sigma-Aldrich and used as received.

Synthesis of branched diblock copolymer P(OEGMA)-P(nBMA) by ATRP using EBriB (ethyl 2-bromoisobutyrate) as the initiator.

Typically, synthesis of $P(OEGMA)_{36}$-$P(nBMA)_{40}$ could be described as follows: into a Schlenk flask (50 ml) was added OEGMA (2.04 g, 6.8 mmol), CuBr (24.5 mg, 0.17 mmol), bipyridine (bpy) (53.1 mg, 0.34 mmol) and solvent (isopropanol/water (92.5/7.5 v/v; 4.2 ml) and anisole (0.3 ml; internal standard for ^{1}H NMR). The reaction mixture was bubbled with nitrogen for 40 minutes to completely remove the oxygen. A small sample was taken for ^{1}H NMR analysis, followed by the addition of EBriB (25.0 µL, 33.2 mg, 0.17 mmol) using a microsyringe. The polymerization was carried out at ambient temperature (~20 ^{o}C) under N_2. Samples were taken periodically from the reaction mixture during polymerization for 1H NMR analysis to measure the monomer conversion. In a second 50 ml Schlenk flask, CuCl (16.9 mg, 0.17 mmol), 2,2-bypridine (53.1 mg, 0.34 mmol), n-butyl methacrylate (n-BMA) (0.965 g, 6.8 mmol), EGDMA (29.2mg, 0.15 mmol) and 5.8 ml of the water-alcohol solvent mixture were added, and this mixture was bubbled with N_2 for 1 h. After the conversion of OEGMA reached around 90%, the mixture from the second flask was added into the first flask rapidly using a syringe and taking care not to admit any air into the vessel. A

sample was taken immediately after the addition of the n-BMA monomer solution for 1H NMR analysis. The block copolymerization reaction was carried at ambient temperature and samples were taken periodically from the reaction mixture for 1H NMR analysis. After the monomer conversion reached above 95%, the polymerization was stopped by adding THF into the reaction mixture and exposing the sample. The mixture was passed through an Al_2O_3 column to remove the copper species and the branched copolymer was obtained after reprecipitation in cold hexane three times and drying in a vacuum oven overnight.

Nanoparticle formulation by solvent evaporation

40 mg of POEGMA-*b*-nBMA (DP 36/60 and 36/40) and 40 mg drug (ketoprofen, anthracene and OR) were stirred in 5 ml methanol for 20 min at room temperature (20 °C). The samples were then heated to 56˚C and stirred at 5000 rpm for further 20 min. After cooling to room temperature, 5 ml deionized water was added. It was noticed that the solution with POEGMA-b-nBMA (DP 36/40) turned opaque while the solution with POEGMA-b-nBMA (DP 36/60) was still clear. Another procedure was to directly process the methanol solution at room temperature. Methanol was evaporated off at room temperature in the fume cupboard. After evaporation of methanol, the samples with added water produced aqueous suspensions, which were analysed directly. The dry samples formed from methanol solution after evaporation were dissolved in 5 ml water to form suspensions for measurement.

Characterisation

The ^{1}H NMR spectra were recorded using an Agilent VNMRS600 instrument at 600 MHz. Samples were analyzed at room temperature and $CDCl_3$ was used as the solvent for all the samples. Gel Permeation Chromatograph (GPC) results were obtained using a Viscotek system employing OmniSEC 4.2 software, TDA Model 302 (right-angle & low-angled light scattering, refractive index, ultra-violet and viscometer detectors all in a temperature controlled oven (set at 35 °C)) coupled to a gpcMAX integrated solvent and

sample delivery module (degasser, pump and autosampler). The system was fitted with a ViscoGEL HHR-H guard column and two ViscoGEL GMHHR-M columns (also stored in the detector oven at 35 °C), at a flow rate of 1 ml/min, using tetrahydrofuran (THF), (GPR, stabilized with BHT (VWR, UK)) as the eluent and with an injection volume of 100 µl.

Particle sizes and Zeta-potentials were measured by dynamic laser scattering (DLS) analysis on a Malvern Zetasizer Nanoseries at 25 °C from Malvern Instruments. The measurements were performed on aqueous nanoparticles suspensions with a concentration of ~ 0.2 mg/ml. Microparticles or aggregates were removed by centrifugation with an Eppendorf Centrifuge 5415 D at 3000 rpm for 3 minutes and one minute at 3600 rpm to ensure that larger particles precipitate.

The branched copolymer nanoparticles were analyzed using a Tecnai Spirit transmission electron microscope (TEM) (Tecnai G2 12) with an accelerating voltage of 100 KV. Sample solutions were dropped onto 400 mesh copper TEM grids, and dried overnight at room temperature. Imaging at multiple spots was undertaken over several areas of the TEM grid to ensure that the analysis was representative of the sample.

Determination of Nanoparticle yield

Evaporated samples were dispersed into DI water. Microparticles and aggregates were precipitated by centrifugation for 3 minute at 3000 rpm and a further minute at 3600 rpm on an Eppendorf Centrifuge 5415 D. After the processing, the hydrophobic drug compound is transferred into drug nanoparticles (in the suspension after centrifugation) and large microparticles/aggregates (precipitated after centrifugation). The nanoparticles yield is the percentage of the drug compound transformed into nanoparticles, based on the total amount of the hydrophobic drug used. The nanoparticle yield can be calculated as below:

$$Yield = \frac{m_{NP}}{m_T} \times 100 = \frac{m_S}{m_S + m_P} \times 100$$

$$m_T = m_S + m_P$$

Where m_{NP} is the mass of nanoparticles, m_T the total mass of added drug compound, m_S the mass of particles in the suspension after centrifuging, and m_P the mass of precipitated particles after centrifuging.

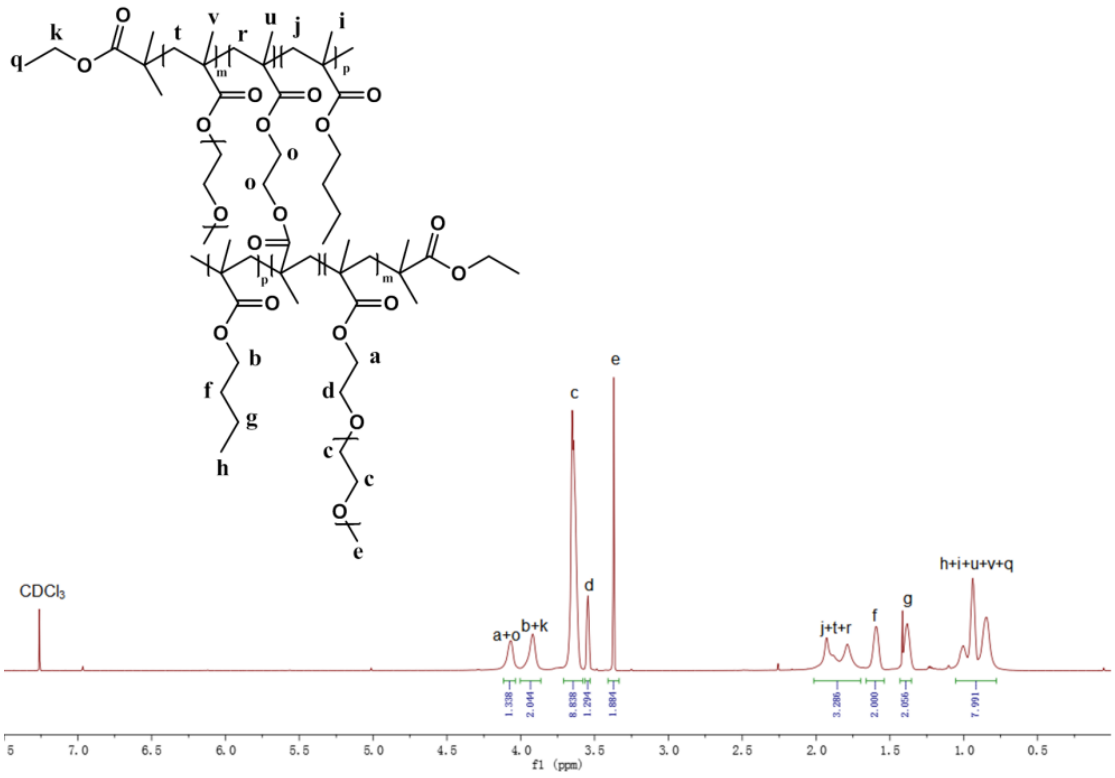

Figure S1. The ^{1}HNMR spectrum for the sample p(OEGMA$_{36}$-b-(nBMA$_{60}$-co-EGDMA$_{0.9}$))

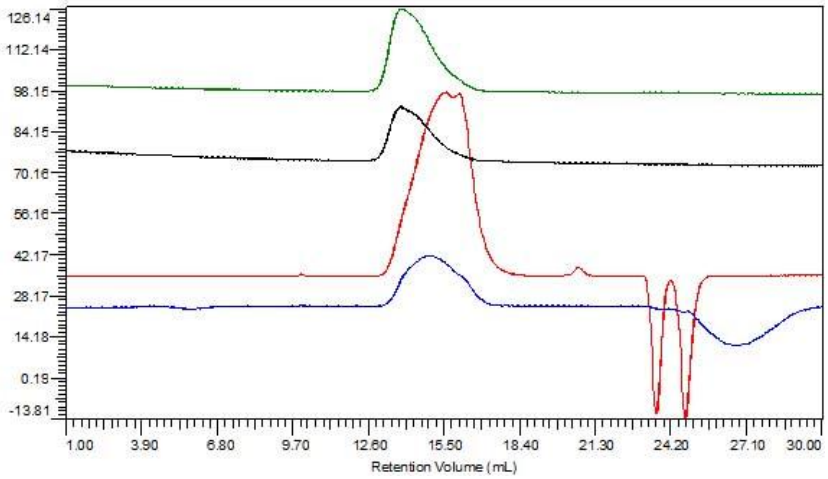

Figure S2. The GPC chromatogram for the sample p(OEGMA$_{36}$-b-(nBMA$_{60}$-co-EGDMA$_{0.9}$)), column set HHR-H and 2xGMHHR-M, eluent 1 ml/min THF, 100 µl injection (RI (red), DP (blue), RALS (green), LALS (black)). Mw: 286,030, Mn: 94,598, PDI: 3.10.

Table S1. DLS data of the nanosuspensions formed by evaporating methanol solution at room temperature and re-dispersion in water

Sample name	DP OEGMA/nBMA	Drug	Drug:Polymer Ratio	Z-Average [nm]	Number % [nm]	PDI	Yield [%]
1*	36/60	Ketoprofen	1:1	-	-	-	-
2	36/40	Ketoprofen	1:1	80 ± 2	34 ± 5	0.38	

* no measurement possible due to sample agglomerating.