Nanoparticle forming polyelectrolyte complexes derived from well-defined block copolymers

Sara E. Bakhtiari, Fanny Joubert, George Pasparakis, Steve Brocchini, Gareth R. Williams

PII: S0014-3057(23)00160-X
DOI: https://doi.org/10.1016/j.eurpolymj.2023.111977
Reference: EPJ 111977

To appear in: European Polymer Journal

Received Date: 18 January 2023
Revised Date: 28 February 2023
Accepted Date: 4 March 2023

Please cite this article as: Bakhtiari, S.E., Joubert, F., Pasparakis, G., Brocchini, S., Williams, G.R., Nanoparticle forming polyelectrolyte complexes derived from well-defined block copolymers, European Polymer Journal (2023), doi: https://doi.org/10.1016/j.eurpolymj.2023.111977

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2023 The Author(s). Published by Elsevier Ltd.
Nanoparticle forming polyelectrolyte complexes derived from well-defined block copolymers

Sara E. Bakhtiari a, Fanny Joubert a, George Pasparakis a,b, Steve Brocchini a and Gareth R. Williams a

a UCL School of Pharmacy, 29-39 Brunswick Square, WC1N 1AX London, United Kingdom
b Department of Chemical Engineering, University of Patras, Panepistimioupoli Patron 26504, Greece

Corresponding Author: Professor Gareth Williams (g.williams@ucl.ac.uk)
Abstract

Polymers can be used in nanoparticle associated formulations to encapsulate cytotoxic drugs (e.g., paclitaxel). Polyelectrolyte complexes (PECs) that form drug associated colloids also have potential to form particulate associated formulations. We used RAFT polymerisation to prepare small families of narrow molecular weight distributed (i) methacrylate block co-polymers comprised of oligomeric ethylene glycol, poly(ethylene glycol) methyl ether methacrylate (PEGMA), and dimethyl amino pendant chains, 2-(dimethylamino) ethyl methacrylate (DMAEMA), and (ii) poly(methacrylic acid), PMAA. These polymers were examined for their ability to form PECs capable of drug encapsulation. Optimal control in RAFT polymerisation was confirmed by the linear increase of molecular weight and the narrow dispersity of the polymers (<1.2) as determined by $^1$H nuclear magnetic resonance and gel permeation chromatography. Dynamic light scattering and transmission electron microscopy showed formation of well-defined monodispersed nanoparticles with a hydrodynamic diameter of 25 ± 3 nm upon self-assembly of poly(PEGMA$_{0.23}$-b-DMAEMA$_{0.77}$)$_{99}$ and PMAA$_{75}$. These PECs are highly haemocompatible. Thin film hydration was used to encapsulate two hydrophobic drugs, paclitaxel and carmofur, into spherical nanoparticles. The results show that carmofur was encapsulated markedly more effectively than paclitaxel (72 vs 1.5%).

Keywords: RAFT polymerisation; polymer; PEC nanoparticles; cytotoxic drug; drug encapsulation
Introduction

Polyelectrolyte complexes (PECs) can be made by mixing two solutions of oppositely charged polymers (polyelectrolytes) that can interact to give nanoparticles with a narrow size distribution, which have potential for use in the formulation sciences [1]. Complexation of polyelectrolytes into PECs in aqueous media is rapid and spontaneous [2]. If one polyelectrolyte is used in excess the resulting PEC will display a net charge associated with the excess polyelectrolyte [3]. Of particular interest is the potential of PECs to encapsulate a drug within a colloidal particle that may display optimal charge and size to reduce systemic distribution of cytotoxic drugs, improve solubility and stability of poorly soluble actives, and avoid their rapid clearance from the blood compartment. Targeted drug delivery can also be achieved, for instance by using PECs with pH-tunable solubility. The PECs sense changes in the pH of their environment and respond by altering their structure, e.g., swelling or dissociation, and thereby freeing a drug cargo [4].

Homo- and block copolymers composed of hydrophilic or hydrophobic blocks and with at least one charged block can form PECs. For example, mixing phosphate buffered solutions of poly(ethylene glycol)-poly(L-lysine) block copolymer, PEG-b-P(Lys), and poly(ethylene glycol)-poly(α,β-aspartic acid) block copolymer, PEG-b-P(Asp) were found to give a monodispersed neutral spherical PEC [5]. The driving force for block copolymer derived PEC formation is due to the electrostatic interaction between the charged segments [6, 7]. This results in phase separation and immediate rearrangement into stable PECs, for instance following mixing solutions of poly(acrylic acid) and poly(dimethylaminoethyl methacrylate)-co-poly(glyceryl methacrylate). The neutral block of the copolymer formed the shell of the PECs [8]. The difference in the solubility of each segment of the block copolymers can also influence the resulting PEC, causing polymer micelle formation with the hydrophilic non-charged blocks comprising the outer region of the colloid in aqueous media [9].

Here, we report the fabrication of PEC nanoparticles of less than 100 nm derived from a homo-polymer and a block-copolymer, each with a narrow polydispersity index (PDI < 1.2). Both were generated by reversible addition-fragmentation chain-transfer (RAFT) polymerization, which allows preparation of a wide range of functional polymers of various architectures with narrow molecular weight distributions [10-12].

A hydrophilic block copolymer designed to have a positive charge at physiological pH was prepared with different block sizes from poly(ethylene glycol) methyl ether methacrylate 1 and dimethylaminoethyl methacrylate 4, giving poly(PEGMA-b-DMAEMA) 5 (Scheme 1a). A PEG-based block was incorporated into the copolymer as PEGylation is known to prolong circulation times and improve therapeutic efficacy by preventing opsonization and recognition by the immune system [13]. A complexing homopolymer, poly(methacrylic acid) 7 (PMAA) was also prepared (Scheme 1b), with a degree of polymerization (DP) to match the DMAEMA block. PMAA has a negative charge at physiological pH and should complex to the positively charged dimethyl amino block in poly(PEGMA-
b-DMAEMA) 5 to form PECs designed for drug encapsulation. These polymers were used to fabricate PEC derived nanoparticles and to examine their capacity to encapsulate paclitaxel and carmofur.

Scheme 1. (a) RAFT polymerisation of PEGMA 1 with the RAFT chain transfer agent (CTA) 2 gives the poly(PEGMA) block 3, which was then used as a macromonomer in polymerisation with DMAEMA 4 to give the desired block copolymer poly(PEGMA-b-DMAEMA) 5; (b) RAFT polymerisation of methacrylic acid 6 gave poly(methacrylic acid) 7 (PMAA).

Experimental section

Materials

Poly(ethylene glycol) methyl ether methacrylate (PEGMA, $M_n = 300$ g mol$^{-1}$), methacrylic acid (MAA), 2-(dimethylamino) ethyl methacrylate (DMAEMA), 4-cyano-(phenyl-carbonothioylthio) pentanoic acid (CTA), and azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich. 1,4-Dioxane and diethyl ether were supplied by Honeywell Specialty Chemicals. Hexane was purchased from Fisher Chemicals. Methanol (MeOH, 100%) was purchased from VWR Chemicals. Deuterated dimethyl sulfoxide ($d_6$-DMSO) was supplied by Cambridge isotope Laboratories. Paclitaxel was purchased from Fluorochem. Carmofur was obtained from ChemCruz (Santa Cruz Biotechnology, UK).

Polymer Synthesis

Synthesis of poly(PEGMA)

For the synthesis of poly(PEGMA) with DP of 20, PEGMA (1.00 g, 3.30 mmol, 20 eq.), 4-cyano-(phenyl-carbonothioylthio) pentanoic acid (46.60 mg, 0.16 mmol, 1 eq.) and AIBN (2.74 mg, 0.016
mmol, 0.1 eq.) were dissolved in 1,4-dioxane (7 ml) in a 25 ml single neck round bottom flask. The mass ratio of CTA to initiator used in the synthesis of all the polymers was 10:1. Details are given in Table S1. The reaction mixture was sealed with a rubber septum and purged using argon for 30 min; the flask was then heated at 70 °C for 17 h under magnetic stirring inside a fume cupboard with the sash in the down position. The reaction was stopped by exposing the solution to air via a needle and the polymer was precipitated twice under mild stirring using 70 ml of cooled hexane (10× the volume of dioxane). The precipitated sample was washed with acetone (6 ml) and dried using a rotatory evaporator under vacuum. The same procedure was repeated with 10, 30, 50, 60, and 70 equivalents of PEGMA.

**Synthesis of poly(PEGMA-b-DMAEMA)**

Poly(PEGMA) (0.20 g, 0.028 mmol, 1 eq.), 2-(dimethylamino) ethyl methacrylate (DMAEMA) (0.34 g, 2.14 mmol, 77 eq.) and AIBN (0.46 mg, 0.0028 mmol, 0.1 eq.) were dissolved in 1,4-dioxane (3.78 ml) in a 10 ml single neck round bottom flask. Details can be found in Table S2. The reaction mixture was sealed with a rubber septum and purged using argon for 30 min; the flask was then heated at 70 °C for 17 h under magnetic stirring. Experiments were performed in a fume hood with the sash down. The reaction was stopped by exposing the solution to air via a needle and the polymer was precipitated using 38 ml of cooled hexane (10× the volume of dioxane) under mild stirring. The precipitated sample was washed with acetone (4 ml) and dried using a rotatory evaporator under vacuum. The same procedure was repeated to prepare copolymers with total DP of 100 by copolymerising DMAEMA with the different poly(PEGMA) blocks. This was done by using the correct amount of macroRAFT agents (PEGMA homopolymers) and DMAEMA monomers.

**Synthesis of PMAA**

The synthesis of PMAA with DP of 85 is described as representative example. Methacrylic acid (1.00 g, 11.60 mmol, 85 eq.), 4-cyano-(phenyl-carbonothioylthio) pentanoic acid (38 mg, 0.14 mmol, 1 eq.) and AIBN (2.24 mg, 0.014 mmol, 0.1 eq.) were dissolved in methanol (7 ml) in a 25 ml single neck round bottom flask. Details can be found in Table S3. The reaction mixture was sealed with a rubber septum and purged using argon for 30 min; the flask was then heated at 70 °C for 17 h under magnetic stirring. The reaction was stopped by exposing the solution in open air via a needle and the polymer was precipitated twice in 70 ml cooled diethyl ether (10× the volume of methanol) with mild stirring. The obtained polymer was then washed with methanol (6 ml) and dried using rotatory evaporator and vacuum. The same procedure was repeated for synthesis of methacrylic acid polymers with degrees of polymerisation of 18, 28, 41, 50, 58, and 76.
Polymer characterisation

Nuclear magnetic resonance (NMR)

Solution state $^1$H NMR spectra were recorded in $d_6$-DMSO using Bruker Avance 400 MHz NMR spectrometer, and were analysed using the Topspin software. The average length of polymer molecules and the average molecular weight of the polymers and copolymers were calculated by end-group analysis. Briefly, the proton signals for the end-group and the repeating units were identified, integrated, and calibrated. The degree of polymerisation was calculated by dividing the normalised integrated value by the number of protons in the repeating unit.

Gel permeation chromatography (GPC)

GPC was conducted with DMF containing 5 mM NH$_4$BF$_4$ as the mobile phase, at 70 °C with a flow rate of 1.00 ml min$^{-1}$. Polymer aliquots (100 μl, 5 mg ml$^{-1}$ in DMF) were filtered through a nylon membrane with 0.22 μm pore size and were injected in a Malvern Viscotek system equipped with a refractive index (RI) detector. Poly(methyl methacrylate) (PMMA) standards were used for calibration and the OMNISEC software was used to determine the average molecular weight ($M_n$) and dispersity (D). Four samples each containing two PMMA homopolymers with different molecular weights were used to calibrate the instrument. Molecular weights of the PMMA calibrants were as follows; sample 1 (26550 Da and 569000 Da), sample 2 (4770 Da and 98550 Da), sample 3 (10280 Da and 223900 Da) and sample 4 (960 Da and 72000 Da).

Nanoparticle preparation

Solutions of 1 mg ml$^{-1}$ of poly(PEGMA-b-DMAEMA) and PMAA with similar chain lengths of the charged segments were prepared in water. Nanoparticles of 1:1 polycation to polyanion molar ratio were prepared by mixing solutions of the cationic copolymer and anionic homopolymer. For instance, 1 ml of a polycation solution (poly(PEGMA$_{0.23}$-b-DMAEMA$_{0.77}$)$_{99}$) and 0.364 ml of a polyanion solution (PMAA$_{75}$) were mixed together to prepare PEC nanoparticles. The mixture was then filtered using 0.45 μm filters. The impact of different molar ratios (0.25, 0.5, 1, 2, and 4) of the cationic copolymer CP2 to the anionic homopolymer P2 (C/A) on the formation of PECs and their hydrodynamic size and zeta potential was also investigated.

Nanoparticle characterisation

Dynamic light scattering (DLS)

DLS size measurements and zeta-potentials were obtained using a Malvern Zetasizer Nano-ZS instrument. One ml of the nanoparticle solution was pipetted into a 1.6 ml disposable cuvette for size measurements, and transferred into a folded capillary cell for zeta potential measurements.
Transmission electron microscopy (TEM)

TEM images were recorded using a Philips/FEI CM120 Bio Twin transmission microscope. Nanoparticle samples in water (1 mg ml\(^{-1}\)) were directly dispensed onto TEM grids and left to dry, in some cases also with staining using an aqueous 3% phosphotungstic acid (PTA) solution.

pH Responsiveness of PEC nanoparticles

pH responsivity of the PEC nanoparticles was investigated by observing the changes in their hydrodynamic size in different pH environments (7.4, 6.5, and 5.5) at 37 °C, using DLS.

Haemolysis assay

The haemolytic activity of the polymers and nanoparticles was assessed using freshly obtained blood from adult female Wistar rats. All animal handling was performed by licensed researchers. Red blood cells (RBC) were separated from the plasma by centrifugation and washed with Dulbecco’s phosphate buffered saline (DPBS, Sigma Aldrich) three times. The RBC suspension was diluted with DPBS to a total volume of 50 ml. The RBC suspension (180 μl) was then added to the wells of a clear 96-well plate (Corning). Polymer solutions and nanoparticle suspensions (20 μl) in DPBS at four different concentrations (1 mg ml\(^{-1}\), 500 μg ml\(^{-1}\), 100 μg ml\(^{-1}\), and 20 μg ml\(^{-1}\)) were added to the wells to give a final polymer or nanoparticle concentration of 100, 50, 10, and 2 μg ml\(^{-1}\). For negative and positive control measurements, DPBS and a solution of Triton-X in DPBS (10% v/v) were used. The plates were incubated at 37 °C for 1 hour. Next, the plates were centrifuged and 100 μl of the supernatant from each well was removed and deposited into a clean microplate. The procedure was carried out three times, and in each independent plate three wells were used for each concentration. Absorbance was read at 540 nm using a SpectraMax M2e microplate reader (Molecular Devices). The extent of haemolysis was calculated using the following equation:

\[
\text{Haemolysis (\%)} = \frac{\text{sample absorbance}}{\text{positive control absorbance}} \times 100
\]  

Drug encapsulation

A 0.2 mg ml\(^{-1}\) solution of drug in methanol was prepared. 10 ml of this stock solution was added to a vial containing 10 mg of the cationic copolymer or the anionic homopolymer to prepare 1 mg ml\(^{-1}\) solutions in separate vials. The appropriate volume of PMAA or PMAA/drug solution was added to 1 ml of the copolymer solution to give PECs with 1:1 molar ratio of polycation to polyanion. Methanol was evaporated using a rotatory evaporator until a thin film was formed in the vial followed by addition of water. The samples were then sonicated for 2 minutes and passed through a 0.45 μm filter. The filtered samples were centrifuged in Vivaspin 6 centrifugal concentrators with MWCO of 3000 at 10000 rpm for 15-20 minutes at room temperature. Following centrifugation, the supernatant was collected and the entrapment efficiency (EE%) and drug loading capacity (DLC%) of the concentrated nanoparticles were determined using an Agilent Cary 60 UV-Vis spectrophotometer and calculated.
using equations (2) and (3). UV absorbance of paclitaxel and carmofur was measured at 227 and 246 nm, respectively.

\[
E_{ncapsulation\ Efficiency}\ (\%) = \frac{\text{mass\ of\ encapsulated\ drug}}{\text{mass\ of\ drug\ used}} \times 100 \quad (2)
\]

\[
D_{rug\ Loading\ Capacity}\ (\%) = \frac{\text{mass\ of\ drug\ loaded}}{\text{mass\ of\ polymers\ and\ drug}} \times 100 \quad (3)
\]

Results and discussion

Polymer synthesis

RAFT polymerisation allows for the synthesis of polymers and copolymers with target DPs and narrow dispersity. Poly(PEGMA) 3 was first synthesised by RAFT polymerisation using 4-cyano-(phenyl-carbonothioylthio) pentanoic acid 2 as the chain transfer agent (CTA). This thiocarbonylthio CTA has previously been reported to be suitable for RAFT polymerisation of methacrylic monomers, providing good control over their polymerisation [14-17]. Poly(PEGMA) 3 was then used as a macro-CTA to prepare the desired poly(PEGMA-b-DMAEMA) block copolymers 5. Several samples of Poly(PEGMA) were prepared with target degree of polymerisation (DP) was 10, 20, 30, 50, 60, and 70. Polymer DP was estimated using 1H-NMR spectroscopy, by comparing the integral of the peaks assigned to the aromatic CH protons of the CTA at δH ~ 7.48, 7.65, 7.83 ppm and the methylene -C(=O)-OCH₂- group of the PEGMA at δH ~ 4.03. Monomer conversions of the PEGMA polymers were determined from the 1H-NMR spectrum of the crude non-purified material (Figure S1) and were in the range of 78-86%. This was calculated by comparing the integrals of the NMR peaks at δH ~ 4.03 and 4.21 ppm in the crude samples, assigned to the methylene protons of the polymer and monomer respectively. The GPC elutograms for the PEGMA polymers are given in Figure 1a. The dispersity and the number average molecular weight of the polymers are inversely proportional (Figure 1b). This is because small changes in the chain length of the polymers are more noticeable in polymers with lower degrees of polymerisation (and molecular weights).
Figure 1. (a) GPC elutogram of PEGMA polymers synthesised via RAFT polymerisation with DP = 13, 23, 35, 52, 60, and 70. Monomer conversions of the PEGMA polymers were in the range of 78-86%; (b) dispersity (D) vs number average molecular weight ($M_n$).

Several samples of the poly(PEGMA-b-DMAEMA) block copolymers 5 with a similar DP (overall DP of 100) were then prepared using the small family of poly(PEGMA)s as macro-CTAs (Table 1). Our target was to form hydrophilic pH-responsive block copolymers with a total DP of approximately 100 repeat units, to ensure the molecular weight of the polymer is well below the renal threshold and allow for rapid elimination of polymer after PEC dissociation. The peak at $\delta_{H} \sim 3.99$ ppm is the –CH$_2$O- group of both poly(PEGMA) and poly(DMAEMA). The integration of this peak was compared with the signa integration of the aromatic protons of the CTA at $\delta_{H} \sim 7.48, 7.65, 7.83$ ppm to estimate the degree of polymerisation (Figure 2a, Table 1). Monomer conversions for DMAEMA were in the range of 66-97%. The weight distributions of poly(PEGMA)$_{23}$ and poly(PEGMA$_{0.23}$-b-DMAEMA$_{0.77}$)$_{99}$ are presented in Figure 2b. The GPC traces show the copolymers were retained in the column for a shorter time compared to the PEGMA homopolymers. The chromatogram of the block copolymer shows a small shoulder, which is the result of the presence of a negligible amount of unreacted PEGMA polymers.

Figure 2. (a) $^1$H NMR spectra for poly(PEGMA)$_{23}$ and poly(PEGMA$_{0.23}$-b-DMAEMA$_{0.77}$)$_{99}$; (b) GPC traces for the PEGMA homopolymer and the copolymer with $M_n$ 7200 and 19100 Da, respectively.
Synthesis of random and block copolymers of PEGMA and DMAEMA has previously been reported. The resultant copolymers have been used to complex with plasmid DNA for effective gene transfection [18, 19]. Linear block copolymers of DMAEMA and poly(2-hydroxyethyl methacrylate (PHEMA), an uncharged non-toxic hydrophilic polymer, synthesised via sequential RAFT polymerisation, were reported to provide better accessibility for charge-to-charge interaction with DNA compared to random copolymers [20]. Likewise, in this study, the aim was to synthesise block copolymers that allow charge-to-charge interaction and complexion with an oppositely charged polymer.

Samples of poly(methacrylic acid) (PMAA) with similar chain length as the DMAEMA blocks were prepared (Figure 3). High monomer conversions (84-90%; see Figure S2) and low dispersity (1.05-1.11) were obtained for the RAFT polymerisation of methacrylic acid. Comparable results have been reported previously in the literature [21-23]. The chain lengths of the polymers were estimated by comparing the integrals of the peak of CTA to that of the -CH3 group of the polymers at δH ∼ 0.94. A summary of the results is listed in Table 2.

### Table 1. Summary of the copolymers synthesised. Dispersity (Ɖ) was determined by GPC. PMMA standards were used to calibrate GPC instrument.

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>DP_{PEGMA}</th>
<th>DP_{DMAEMA}</th>
<th>DP_{total}</th>
<th>M_n NMR (Da)</th>
<th>M_n GPC (Da)</th>
<th>Ɖ_{GPC}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP1</td>
<td>13</td>
<td>85</td>
<td>98</td>
<td>17300</td>
<td>20700</td>
<td>1.20</td>
</tr>
<tr>
<td>CP2</td>
<td>23</td>
<td>76</td>
<td>99</td>
<td>19100</td>
<td>20800</td>
<td>1.19</td>
</tr>
<tr>
<td>CP3</td>
<td>35</td>
<td>77</td>
<td>112</td>
<td>22600</td>
<td>23700</td>
<td>1.10</td>
</tr>
<tr>
<td>CP4</td>
<td>52</td>
<td>58</td>
<td>110</td>
<td>24700</td>
<td>27800</td>
<td>1.15</td>
</tr>
<tr>
<td>CP5</td>
<td>60</td>
<td>50</td>
<td>110</td>
<td>25900</td>
<td>26500</td>
<td>1.13</td>
</tr>
<tr>
<td>CP6</td>
<td>70</td>
<td>41</td>
<td>111</td>
<td>27400</td>
<td>30400</td>
<td>1.14</td>
</tr>
</tbody>
</table>
Figure 3. (a) $^1$H NMR spectrum for PMAA$_{75}$ with $M_n$ of 6700 Da; (b) GPC chromatogram of methacrylic acid polymers synthesised via RAFT polymerisation with DP = 23, 31, 46, 52, 63, 75, and 88; (c) number average molecular weight ($M_n$) versus dispersity (D).

Table 2. Summary of the methacrylic acid polymers prepared in this work.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>DP</th>
<th>$M_n$ NMR (Da)</th>
<th>$M_n$ GPC (Da)</th>
<th>D$_{GPC}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>88</td>
<td>7900</td>
<td>15500</td>
<td>1.05</td>
</tr>
<tr>
<td>P2</td>
<td>75</td>
<td>6700</td>
<td>12800</td>
<td>1.05</td>
</tr>
<tr>
<td>P3</td>
<td>63</td>
<td>5700</td>
<td>11900</td>
<td>1.05</td>
</tr>
<tr>
<td>P4</td>
<td>52</td>
<td>4800</td>
<td>10700</td>
<td>1.07</td>
</tr>
<tr>
<td>P5</td>
<td>46</td>
<td>4200</td>
<td>10200</td>
<td>1.08</td>
</tr>
<tr>
<td>P6</td>
<td>31</td>
<td>2900</td>
<td>7200</td>
<td>1.11</td>
</tr>
<tr>
<td>P7</td>
<td>23</td>
<td>2300</td>
<td>5300</td>
<td>1.15</td>
</tr>
</tbody>
</table>
PEC preparation

The association of poly(PEGMA-b-DMAEMA) block copolymers 5 (CP1-CP6) to PMAA homopolymers 7 (P1-P6) to fabricate PEC nanoparticles was examined. The PMAA samples was matched to have similar DP to that of DMAEMA block of the copolymers. The DLS derived size and zeta potential measurements of the six different nanoparticle combinations prepared by using 1:1 molar ratios of the copolymer and PMAA are listed in Table 3. The sizes and zeta potentials of the polymers alone in solution were also determined (see Table S4).

NP1, formulated using the lowest fraction of PEGMA, was prepared by mixing aqueous solutions of poly(PEGMA<sub>0.13</sub>-b-DMAEMA<sub>0.87</sub>)<sub>98</sub> and PMAA<sub>88</sub>. The resultant nanoparticles had a hydrodynamic diameter of 104 ± 13 nm and a PDI of 0.25 ± 0.09. Copolymer CP2 was used in combination with PMAA with degrees of polymerisation of 75 (P2) and 31 (P6). Nanoparticles made by mixing solutions of CP2 and P2 in water have polyelectrolyte elements with similar chain lengths. These nanoparticles (NP2) had hydrodynamic diameter of 25 ± 3 nm and PDI of 0.08 ± 0.01, and ζ-potential of -23.2 ± 1.4 mV (Figure 4a). In contrast the mean hydrodynamic diameter of NP3, made from mixing CP2 and P6 with a 1:1 molar ratio, was 91 ± 19 nm with PDI of 0.38 ± 0.14 and ζ-potential of -5.4 ± 1.2 mV. Therefore, the DP of PMAA was selected to match the DP of the DMAEMA block as closely as possible. NP4, NP5, and NP6 were prepared using copolymers with longer PEGMA blocks and shorter polyelectrolyte blocks. These PECs were polydisperse, with PDI values above 0.29.

Use of PEG in low molar fraction in a polymer mixture has been reported to avoid steric hindrance between PEG strands, allowing the formation of stable nanoparticles [24]. Therefore, the smaller size NP2 nanoparticles formed in water could be due to the longer chain length of the ionic segments of the PEC nanoparticles and the lower weight fraction of PEGMA in the mixture compared to the other copolymers. A 1:1 molar mixture of CP2 and P2 was chosen as the optimal NP formulation given the PEC’s small hydrodynamic size and PDI.
Table 3. Formulation details and DLS measurements (size, PDI, and zeta potential) for the different sets of PEC nanoparticles prepared with a 1:1 molar ratio of polycation to polyanion.

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Cationic Copolymer</th>
<th>Anionic Homopolymer</th>
<th>Hydrodynamic Diameter (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP1</td>
<td>CP1</td>
<td>P1</td>
<td>104</td>
<td>0.25</td>
<td>-16.9</td>
</tr>
<tr>
<td>NP2</td>
<td>CP2</td>
<td>P2</td>
<td>25</td>
<td>0.08</td>
<td>-23.2</td>
</tr>
<tr>
<td>NP3</td>
<td>CP2</td>
<td>P6</td>
<td>91</td>
<td>0.38</td>
<td>-5.4</td>
</tr>
<tr>
<td>NP4</td>
<td>CP4</td>
<td>P3</td>
<td>51</td>
<td>0.32</td>
<td>-8.5</td>
</tr>
<tr>
<td>NP5</td>
<td>CP5</td>
<td>P4</td>
<td>61</td>
<td>0.29</td>
<td>-18.6</td>
</tr>
<tr>
<td>NP6</td>
<td>CP6</td>
<td>P5</td>
<td>57</td>
<td>0.49</td>
<td>-15.3</td>
</tr>
</tbody>
</table>

TEM images of NP2 (Figure 4b) were broadly in agreement with the DLS data (Figure 4a; Table 3). According to DLS measurements, a filtered sample of 1 mg ml\(^{-1}\) copolymer CP2 in water had a mean diameter of 117 nm and PDI of 0.864. The mean hydrodynamic diameter of 1 mg ml\(^{-1}\) anionic polymer P2 was 550 nm and the PDI was 0.618. Mixing solutions of the cationic poly(PEGMA\(_{0.23}\)-b-DMAEMA\(_{0.77}\))\(_{99}\) CP2 and the anionic homopolymer PMAA\(_{75}\) P2 resulted in formation of nanoparticles with 25 ± 3 nm and 20 ± 3 nm size according to DLS and TEM images, respectively (Figure 4). The values obtained from DLS measurements are larger than the mean size observed in TEM. This is likely to be because DLS provides the hydrodynamic diameter of NPs in suspension while TEM provides images in their dried state, where there is no associated water and also which can induce shrinkage of the particles [25].

Figure 4. (a) DLS data for PEC NP2 (D\(_h\) = 25 ± 3 nm; PDI = 0.08); and (b) TEM image of NP2, showing a mean size of 20 ± 3 nm.
The molar ratio of polycation to polyanion can impact the size and zeta potential of PECs significantly [26]. Figure 5 displays the effect of five different polycation to polyanion molar ratios (1:4, 1:2, 1:1, 2:1, and 4:1) on hydrodynamic diameter and zeta potential of the resultant PECs. At a mixing ratio of 1:1, the concentration of free polymer in solution is lowest which results in the formation of nanoparticles with the smallest hydrodynamic diameter and narrowest PDI. Multiple size populations were detected when nanoparticles were prepared using other mixing ratios.

Figure 5. DLS (a) size and PDI values and (b) zeta potential measurements of nanoparticles prepared by mixing different molar ratios of cationic copolymer CP2 to the anionic homopolymer P2 at 25 °C.

The impact of polymer concentration on the hydrodynamic diameter and zeta potential of the nanoparticles was investigated by preparing nanoparticles at five different total polymer concentrations (0.2, 0.5, 1, 2, 4 mg ml⁻¹) while maintaining a constant polycation to polyanion molar ratio of 1:1 at 25 and 37 °C (Figure 6). The cationic copolymer poly(PEGMA₀.2₃-b-DMAEMA₀.7₇)₉₉ CP2 and the anionic homopolymer PMAA₇₅ P2 were used to prepare these nanoparticles.
The mean diameters of the polymers on their own are higher than those of the PECs at all five concentrations. This is likely due to the polymers being in coil form. There is also the possibility that polymer molecules could become entangled and form aggregates. At 25 °C, the mean diameter of the PEC nanoparticles remained the same at all polymer concentrations (Figure 6a) and no clear trend was seen in the zeta potential data (Figure 6b). An increase in the average diameter of the polymers was observed when the temperature was raised to 37 °C. An increase of the average end-to-end length per polymer coil is expected in good solvents as is the case here: water is a good solvent for all the examined polymers. The average diameter of the PECs, however, remained unchanged (Figure 6c). This is because the PECs are tightly bound due to the interactions between the oppositely charged polymers. The PECs also had considerably lower dispersity compared to the polymers. Again, no clear trend was seen in the zeta potential values at 37 °C (Figure 6d).

**Figure 6.** Effect of polymer concentration on (a) the mean diameter and (b) the zeta potential of polymers (poly(PEGMA<sub>0.23</sub>-b-DMAEMA<sub>0.77</sub>)<sub>99</sub> CP2 and PMAA<sub>75</sub> P2 and PECs prepared at 25 °C as well as (c) the mean diameter and (d) the zeta potential at 37 °C. The error bars represent standard deviation (n = 3).
**pH responsiveness of PEC nanoparticles**

The changes in hydrodynamic size of PEC nanoparticles in PBS at different pH values was monitored (Figure 7). PBS was used as the solvent to help maintain a constant pH during the experiments. PBS pH 7.4 was used to mimic the body’s physiological pH and PBS pH 6.5 and 5.5 were used to mimic the more acidic environment of cancer tumours. The size of the PECs remained unchanged for 48 hours in PBS at pH 7.4. At a slightly reduced pH (at pH 6.5), their size remained unchanged after 24 hours but showed an increase in size and distribution at the 48-h time point. The size and size distribution of the PECs increased more rapidly in PBS at pH 5.5 as the nanoparticles became unstable. This instability is likely due to the dissociation of PECs into their individual components, and subsequent formation of polymer aggregates. At a decreased pH, the negative charge of the anionic homopolymer ($pK_a = 4.65$) is reduced while the cationic copolymer ($pK_a = 8.44$) remains positively charged, which leads to reduced interaction between the polyelectrolytes within the core of the PECs. According to the Henderson-Hasselbalch equation, the percentage ionisation of MAA polymers reduces to 87.6% at pH 5.5 (cf. 99.8% at pH 7.4). Similar behaviour was observed in a PEG-based micellar drug delivery system by Xiao et al. [27]. The instability of the micelles at low pH was attributed to partial PEG chain shedding and formation of aggregates. These results suggest that the PECs are stable in blood at physiological conditions, whereas they become unstable in slightly acidic environments. This means that they could potentially be used for anti-cancer drug delivery, given the acidic nature of the tumour microenvironment.

![Figure 7](image)

**Figure 7.** Changes in hydrodynamic diameter of NP2 PEC nanoparticles in PBS solutions at pH (a) 7.4; (b) 6.5; and (c) 5.5.

The changes in hydrodynamic diameter of PEC nanoparticles in PBS at different pH was further investigated by DLS at different temperatures (25, 37, 45, and 60 °C). The size and PDI of nanoparticles increased with rising temperature, possibly due to dissociation of the PECs and the increase variability
in particle size (Figure 8). The effect of temperature on the size of the PECs was more evident in slightly acidic solutions. The instability caused by the increased temperature and the reduced pH is likely due to the increased thermal energy and change in the solubility of polymers in solution as well as decreased interaction between the polyelectrolytes making up the PECs.

**Figure 8.** The hydrodynamic diameter of NP2 PECs at different pH values (7.4, 6.5, and 5.5) and varied temperatures, recorded immediately after fabrication.

**Haemolytic activity**

Prior to testing the encapsulation abilities of the PECs, their blood compatibility was assessed. Haemolysis can lead to life threatening conditions such as anaemia and renal failure, therefore blood compatibility is a vital requirement for drug delivery systems developed for intravenous administration [28]. Red blood cells (RBCs) were exposed to different concentrations of polymers and PECs. RBCs were also incubated with PBS and Triton X as negative control and positive control, respectively. Incubation of red blood cells with different concentrations of polymers and PECs did not result in any observable haemolysis (Figure 9). Triton X, on the other hand, caused 100% haemolysis. It can be concluded that the PECs are safe to be used for intravenous administration of drugs.
Drug encapsulation

To assess the ability of the optimal formulation (NP2) to encapsulate hydrophobic anticancer drugs, a hydrophobic dye was first used in a preliminary study. Phthalocyanine is an organic dye with a strong absorption in the far-red region (670 nm) and is often used as a photosensitizer in photodynamic therapy studies [29]. The PECs were able to encapsulate the hydrophobic dye with encapsulation efficiency (EE) of 71 ± 2 % and loading capacity of 11 ± 2 % when a 0.2 mg ml\(^{-1}\) phthalocyanine solution was used for the encapsulation process. The individual polymers however were unable to encapsulate the dye, resulting in the precipitation of the dye in the vials.

We next looked at loading two exemplar drugs into the PECs. The first, paclitaxel, is a poorly water-soluble anti-neoplastic drug that has long been used clinically and in recent years has been reformulated in macromolecule derived nanoparticles (e.g. albumin, PLGA-PEG) and are used for treatment of various types of cancer [30]. The second, carmofur, is a derivative of 5-fluorouracil that is clinically registered to treat breast and colorectal cancer. Carmofur is poorly water-soluble and is reported to cause leukoencephalopathy. We characterised the encapsulation of paclitaxel and carmofur in our poly(PEGMA-b-DMAEMA)/PMAA PECs.

Paclitaxel stock solutions (0.2 mg ml\(^{-1}\)) were used to prepare paclitaxel loaded PECs with total polymer concentration of 1 mg ml\(^{-1}\). An EE of 1.5% and drug loading capacity of 0.3% were recorded. Increasing the concentration of paclitaxel resulted in lower EE% and LC%. However, increasing the total polymer concentration in solution from 1 to 2 mg ml\(^{-1}\), while maintaining the 0.2 mg/ml concentration of paclitaxel, resulted in a slight increase in EE (to 2.0 %). Even though the solubility of paclitaxel in aqueous media was improved when encapsulated in the PECs, the results clearly indicate the inability of the nanoparticles to encapsulate paclitaxel.
In contrast, an EE of 72% and loading capacity of 12.6% were recorded when carmofur was incorporated into the PECs. It was possible to load more carmofur into the PEC nanoparticles, likely due to the carbonyl group and the fatty acid moiety of carmofur, which allows carmofur to be loaded into the core of the nanoparticles and also exist in the hydrophilic segment of the PECs. In contrast, the hydrophobic paclitaxel can only reside in the hydrophobic core of the particles. Given the promising encapsulation efficiency, a preliminary study was performed to look at the release of carmofur. These assays showed a burst release (>80%) within the first hour at pH 7.4 and 5.5, indicating that the PECs are able to effectively disassemble and free their drug cargo in the tumor microenvironment.

Conclusions

In this study, RAFT polymerisation was used to synthesise polymers and copolymers with pre-determined degrees of polymerisation. The synthesised polymers and copolymers have a narrow distribution of molecular weights with dispersity ranging from 1.05 to 1.20. This proves that polymers of similar chain length were synthesised following RAFT polymerisation. Polyelectrolyte complex nanoparticles with a narrow size distribution were formed upon mixing aqueous solutions of poly(PEGMA-b-DMAEMA) and PMAA. These were found to be non-hemolytic and to effectively encapsulate the anticancer drug carmofur. The PECs are also sensitive to temperature and pH, breaking up more rapidly at reduced pH values or increased temperatures.

Declaration of competing interest

The authors declare no conflict of interest.

Funding

This work was part-supported by the Engineering & Physical Sciences Research Council (grant EP/M014649/1).

Abbreviations

The following abbreviations are used in this manuscript:

RAFT: Reversible Addition Fragmentation chain Transfer
PEGMA: Poly(ethylene glycol) methyl ether methacrylate
DMAEMA: 2-(dimethylamino) ethyl methacrylate
MAA: Methacrylic acid
DP: Degree of Polymerisation
PEC: Polyelectrolyte Complex
NP: Nanoparticle
PDI: Polydispersity Index
Data availability

The raw data required to reproduce these findings are available from the authors on request. The processed data required to reproduce these findings are available from the authors on request.

References


- Narrow molecular weight poly(methacrylic acid) and block co-polymers synthesized.
- These self-assembled into small, monodisperse, polyelectrolyte complexes (PECs)
- The PECs are highly haemocompatible, and can be loaded with a drug cargo.
- The PECs are responsive to temperature and pH.
SEB: conceptualisation, investigation, verification, formal analysis, data curation, writing – original draft, writing – review & editing, visualisation. FJ: investigation, verification, writing – review & editing. GP: conceptualisation, writing – review & editing, supervision, project administration, funding acquisition. SB: conceptualisation, writing – review & editing, supervision, project administration, funding acquisition. GRW: conceptualisation, data curation, writing – review & editing, supervision, project administration, funding acquisition.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: