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RESEARCH ARTICLE

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How does the hydrophobic content of methacrylate ABA triblock copolymers affect polymersome formation?

Xinmo Zhang[1] | **Claudia Contini**[2] | **Anna P. Constantinou**[2] | **James J. Doutch**[3] | **Theoni K. Georgiou**[1]

[1]Department of Materials, Royal School of Mines, Exhibition Road, London, SW7 2AZ, UK

[2]Department of Chemistry, Molecular Sciences Research Hub, Imperial College London, 82 Wood Lane, London, W12 0BZ, UK

[3]ISIS Neutron and Muon Source, STFC, Rutherford Appleton Laboratory, Didcot, UK

Correspondence
Theoni K. Georgiou, Department of Materials, Royal School of Mines, Exhibition Road, Imperial College London, SW7 2AZ London, UK.
Email: t.georgiou@imperial.ac.uk

Abstract

Polymersomes are exciting self-assembled structures with great potential in pharmaceutical applications. A systematic investigation of a novel series of methacrylate-based polymersomes is reported in this study. Five well-defined ABA triblock copolymers with A being based on tri(ethylene glycol) methyl methacrylate and B being based on 2-(diethylamino)ethyl methacrylate (DMAEMA) were synthesized using a living polymerization method. The effect of the composition of the ABA triblock copolymers on the thickness of the hydrophobic membrane of the polymersomes and the release of a model drug is demonstrated.

KEYWORDS

2-(diethylamino)ethyl methacrylate, ABA triblock copolymers, poly(ethylene glycol) methyl methacrylate, polymersomes, self-assembly

1 | INTRODUCTION

Polymersomes are hollow polymeric vesicles made from amphiphilic copolymers.[1–5] The amphiphilic membrane of polymersomes is cell-mimicking, and has enhanced stability, durability and more tunable functionalization[6] compared to liposomes,[7] thus triggering intensive interest during the past decades.[4] Due to the amphiphilic nature of polymersomes, both hydrophilic and hydrophobic molecules can be encapsulated, absorbed, and dispersed in them, leading to their wide use in drug delivery, medical imaging, and other pharmaceutical applications.[8–16] The hydrophobic fraction of the amphiphilic copolymers can directly affect the formation of the polymeric structures. Spherical micelles, cylindrical micelles, and polymersomes could be formed depending on the hydrophilic/hydrophobic ratio. In the case of polymersomes, it can also affect the size, membrane thickness, and capacity to encapsulate drugs within the polymersomes.[17] Thus, investigating the effect of the block copolymer composition (hydrophobic/hydrophilic ratio) on their ability to self-assemble remains critical.

Polymersomes can be formed from diblock, triblock, and multiblock copolymers with various membrane conformations: AB, ABA, ABC, and so on., where A, C stand for hydrophilic blocks while B stands for the hydrophobic block.[5,18] One example of polymersomes formed by ABA block copolymers structure is: poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-block-poly(2-methyloxazoline) (PMOXA-*b*-PDMS-*b*-PMOXA). These commercially available copolymers were used to produce nano-reactors, which

Xinmo Zhang and Claudia Contini have contributed equally to this article.

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were investigated in terms of their ability to incorporate membrane proteins.[19–21] Other ABA polymers used in forming polymeric vesicles are poly(ethylene glycol)-*block*-poly(propylene sulfide)-*block*-poly(ethylene glycol) (PEG-*b*-PPS-*b*-PEG),[22] and poly(glycidyl methacrylate)-*block*-poly(dimethylsiloxane)-*block*-poly(glycidyl methacrylate) (PGMA-*b*-PDMS-*b*-PGMA) which were synthesized via a combination of reaction and polymerization methods.[23]

The focus of the present study is on methacrylate-based polymers because they can be fabricated using living chain growth polymerizations that allow the control of their molar mass, composition and architecture as well as easy functionalization of the polymers using fewer steps and, in some cases, one pot reactions. Functionalization could be crucial for attaching targeting groups useful for biomedical and imaging applications. In the case of methacrylates polymers, this can occur not only at the end of the polymer chain but also within the polymer chain if desirable.

Thus, this study focuses on ABA methacrylate-based copolymers and in particular pH responsive methacrylate polymers. Very few studies are reported on polymersomes or polymer vesicles based on fully on methacrylate monomers, most of which are on diblock copolymers.[24–26] In fact, to the best of our knowledge, only one study has been reported on ABA methacrylate polymer vesicles and this was based on a nonionic hydrophobic B block and pH responsive hydrophilic A blocks.[27] In the present study, the hydrophobic block is also the pH responsive block while the hydrophilic A blocks are nonionic. Specifically the pH responsive hydrophobic block was based on 2-(diethylamino) ethyl methacrylate (DEAEMA).[28] Several polymersomes or polymer vesicles studies used DEAEMA containing polymers.[25,29–39] These studies were either on AB diblocks[25,30,32,34–38] or ABC block copolymers.[29,31,33,39] Interestingly in some cases the hydrophobic B was a statistical copolymer where DEAEMA was copolymerized with another hydrophobic monomer.[30,32,34,36–39] Thus, to the best of our knowledge this is the first ABA triblock copolymer study where the B block is the CO_2 and pH responsive DEAEMA.

2 | RESULTS AND DISCUSSION

The polymers were synthesized by group transfer polymerization (GTP), a living polymerization method ideal for the fabrication of methacrylate block polymers on large scales.[40–42] The two monomers used were: a PEG-based monomer, specifically, the triethylene glycol methyl ether methacrylate (TEGMA) and the pH responsive DEAEMA[28] which is normally hydrophobic at physiological conditions (pH 7.4). The hydrophilic and nonionic monomer TEGMA forms the A block while the hydrophobic DEAEMA is the B block and thus, these ABA amphiphilic polymers can self-assemble into polymersomes or micelles.

A series of well-defined ABA triblock copolymers with a wide range of hydrophobic ratios were synthesized and the composition effect (hydrophobic /hydrophilic ratio) on the size and thickness of the formed polymersomes was investigated. Specifically, the molar mass of the polymers was kept constant while the ratio of the TEGMA (A block) and DEAEMA (B block) was varied. Note that the hydrophobic content was kept below 70 wt% as above this percentage the polymer would have been insoluble in aqueous media.

The results of the GTP syntheses are summarized in Table 1. In particular, the number average molar mass (M_n), dispersity ($Đ$) and composition of the synthesized polymers as obtained from gel permeation chromatography (GPC) and proton nuclear magnetic resonance (^{1}HNMR), are given. Note that the $MM_{theoretical}$, M_n, M_p, and $Đ$ of the polymer precursors synthesized from each addition during polymerization are listed in detail in Supporting Information in Table S1.

In total, five ABA polymers were synthesized, with the $Đ$ smaller than 1.32, which is adequate for polymers made via GTP when using a PEG-based methacrylate macromonomer.[28,43–49] A narrow $Đ$ is critical in this study to be able to carry out a systematic investigation.[50] The illustration of the sequential polymerization reaction is shown in Figure 1.

From P1 to P5, there is a clear trend of decreasing hydrophobic content while the molar masses (MM's) are

TABLE 1 Number average molar mass, M_ns, dispersity indices, and composition of the synthesized polymers

				ABA (wt/wt%)	
No)	**Theoretical polymer structure**	**M_n (g Mol^{-1})[a]**	**$Đ$[a]**	**Theoretical**	**Experimental[b]**
P1	$TEGMA_6$-*b*-$DEAEMA_{34}$-*b*-$TEGMA_6$	13,800	1.20	15-70-15	16-70-14
P2	$TEGMA_8$-*b*-$DEAEMA_{29}$-*b*-$TEGMA_8$	11,900	1.19	20-60-20	20-62-18
P3	$TEGMA_{10}$-*b*-$DEAEMA_{24}$-*b*-$TEGMA_{10}$	12,200	1.26	25-50-25	26-50-24
P4	$TEGMA_{12}$-*b*-$DEAEMA_{19}$-*b*-$TEGMA_{12}$	13,300	1.31	30-40-30	31-40-29
P5	$TEGMA_{14}$-*b*-$DEAEMA_{15}$-*b*-$TEGMA_{14}$	10,500	1.23	35-30-35	40-32-27

[a]These values were determined by GPC analysis before precipitation. The GPC system was in THF and was calibrated using poly(methyl methacrylate) standards.
[b]These values were determined by ^{1}H NMR analysis after precipitation.

kept in an acceptable range. All polymers were dissolved in deionized water with a concentration of 1 wt/wt% to determine the pK_a values. The pK_a for all polymers within experimental error is 7.2 as expected and observed before for DEAEMA containing polymers.[28]

The film rehydration method was used to produce polymersomes in phosphate buffered saline (PBS). It was observed that TEGMA-*b*-DEAEMA-*b*-TEGMA vesicles are colloidally stable in this buffer when prepared by film rehydration in PBS buffer. As shown in the dynamic light scattering (DLS) measurements in Figure 2, the formation of self-assembled colloidal aggregates is already visible after the first day of film rehydration under continuous stirring (Figure 2(A)) and stay stable over 2 months (Figure 2(B)). The DLS analysis revealed the presence of two intensity-weighted peaks at ~10 and ~200 nm. The former is a minor peak and it is ascribed to the presence of small aggregates, that is, core-shell micelles in solution; while the latter is the dominant peak and characterizes the presence of polymersomes. The average hydrodynamic diameter of vesicles in Figure 2 is approximately 170, 270, 130, 270, and 200 nm for P1–P5, respectively.

Transmission electron microscopy (TEM) studies confirmed the presence of vesicular structures in these copolymer assemblies as shown in Figure 3(A). The polymer vesicle wall thickness was determined for all TEGMA-*b*-DEAEMA-*b*-TEGMA vesicles measuring in total 10 spots on several vesicles (schematic representation Figure 3 (B)). Interestingly, this analysis revealed a membrane thickness reduction by changing from P1 to P5, that is, reducing the hydrophobic DEAEMA content. Specifically, the membrane thickness decreases from 4.3 to 1.4 nm (Figure 3(C), see supporting information). This trend has been confirmed by comparing these membrane thicknesses measurements with the theoretical random coil-like conformation of the employed TEGMA-*b*-DEAEMA-*b*-TEGMA copolymers and shown in Figure 3(C).

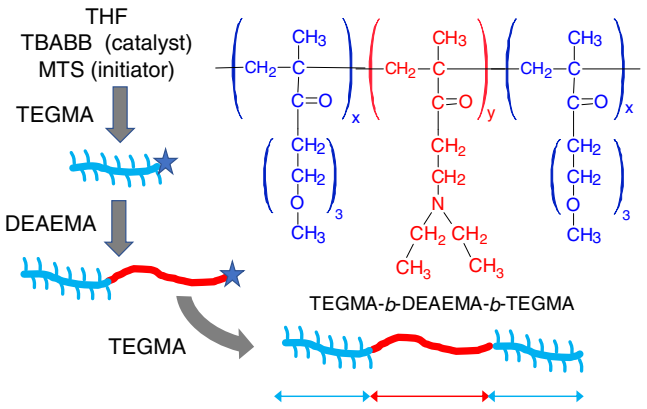


FIGURE 1 Schematic illustration of the synthesis of the TEGMA-*b*-DEAEMA-*b*-TEGMA copolymers. The TEGMA and DEAEMA blocks are denoted with blue and red, respectively. Note that the block length was systematically varied as illustrated by the blue and red arrows. THF, MTS, and TBABB stand for tetrahydrofuran, methyl trimethylsilyl dimethylketene acetal and tetrabutylammonium bibenzoate, respectively

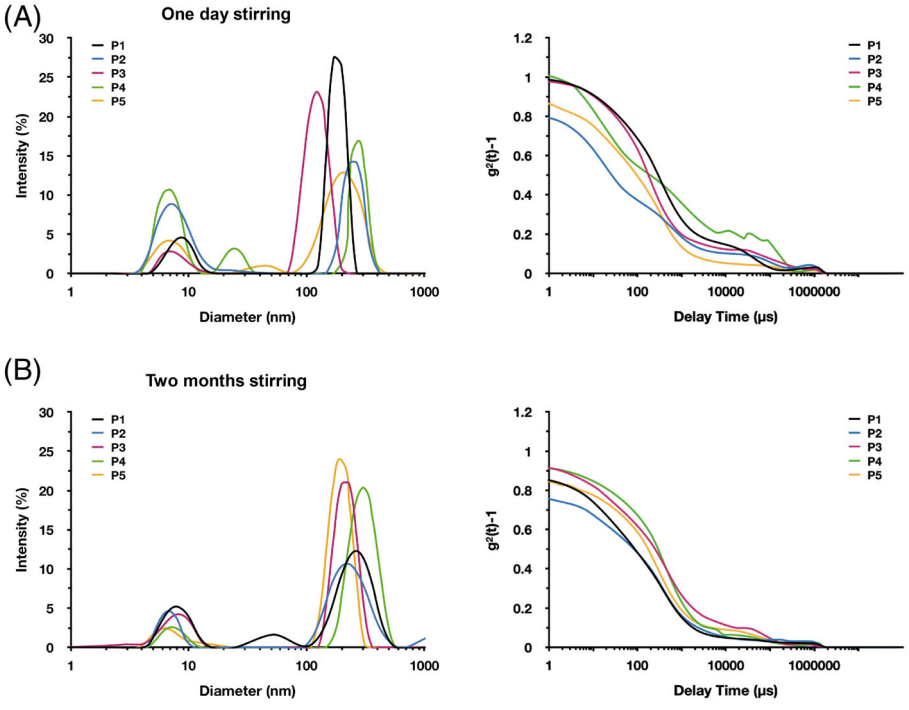


FIGURE 2 Size distribution and autocorrelation profiles of self-assembled colloidal aggregates formed by TEGMA-*b*-DEAEMA-*b*-TEGMA copolymer at different molecular weights (i.e., P1, P2, P3, P4, P5) in aqueous solution after (A) 1-day and (B) 2-months of stirring

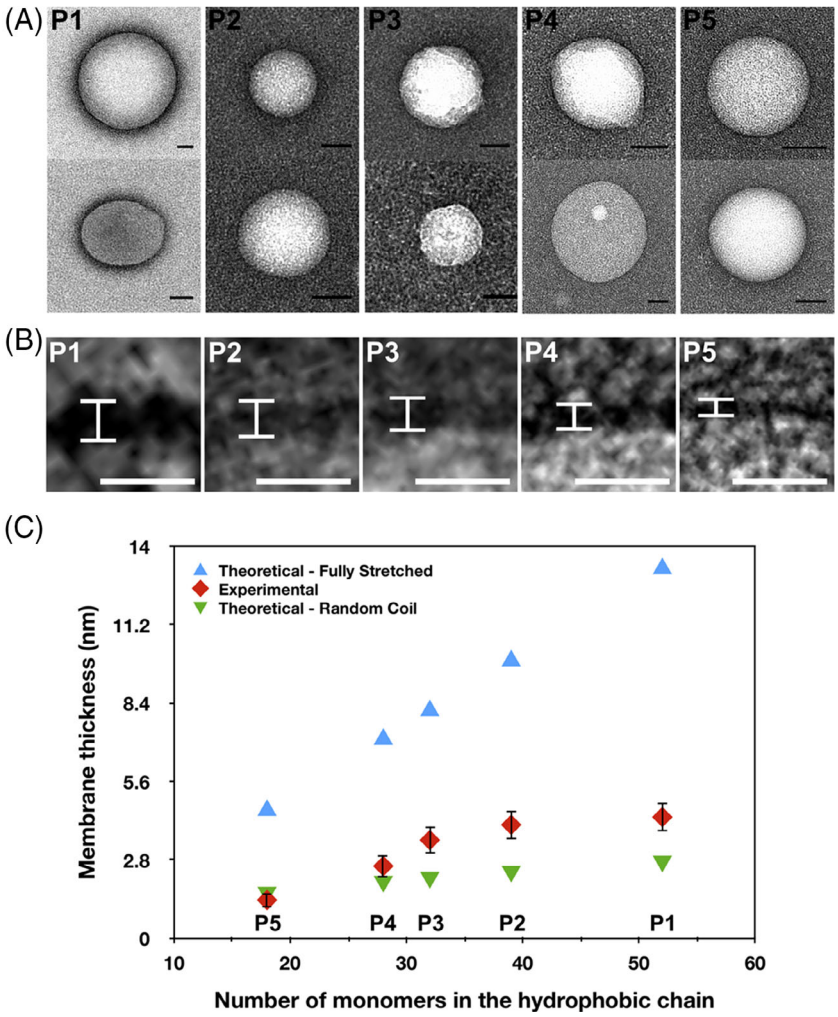


FIGURE 3 (A) TEM images of the TEGMA-*b*-DEAEMA-*b*-TEGMA polymersomes formed by film rehydration and (B) image analysis used to determine the difference in membrane thickness changing from P1 to P5. (C) The measured membrane thickness (blue) is plotted versus the theoretical coil-like conformation (red) of TEGMA-*b*-DEAEMA-*b*-TEGMA copolymers. Scale bars are 50 and 10 nm in panel A and B, respectively. TEM, transmission electron microscopy

Specifically, two models were used to calculate the theoretical membrane thickness. One model assumed that the hydrophobic block is in a random coil configuration and the other that the hydrophobic block is in its fully extended configuration (not realistic). As expected, the random coil conformation is closer to what is observed by TEM. In particular, the thickness of the membrane is slightly higher than the theoretical diameter of the random coil configuration of the DEAEMA block. This is expected as the presence of the TEGMA blocks will constrain the DEAEMA block from fully collapsing. Thus, the above observation suggests that the TEGMA-*b*-DEAEMA-*b*-TEGMA chains are interdigitated within the vesicle wall and are placed perpendicular to the membrane such that the hydrophilic TEGMA blocks are expressed at the outer and inner surface of the polymersomes, respectively. To confirm that the polymer vesicles formed by these ABA triblock copolymers are indeed hollow (i.e., polymersomes) and not just hard spheres one polymer, P2 was chosen for small angle neutron scattering characterization (SANS). The fitting was performed by assuming both micelles and polymersomes were present (see supporting information). The average diameter of the P2-based polymersomes according to SANS was 134 nm and the thickness of the membrane 8.8 nm that are in good agreement with the TEM data.

Finally, all polymers were evaluated in terms of their ability to deliver sodium fluorescein. The model drug release was evaluated over time (Figure 4). It was observed that the release increased by increasing the hydrophobic content (B block) of the ABA triblock copolymers. Thus, with decreasing the thickness of the polymersome membrane the sodium fluorescein release increased. This was expected as the thicker the membrane the diffusion through the membrane would be slower. It also proves that the release could be tuned by tuning the hydrophobic/hydrophilic ratio of the ABA triblock copolymers.

3 | CONCLUSION

In summary, a systematic study on polymersomes based on ABA triblock methacrylate copolymers is reported.

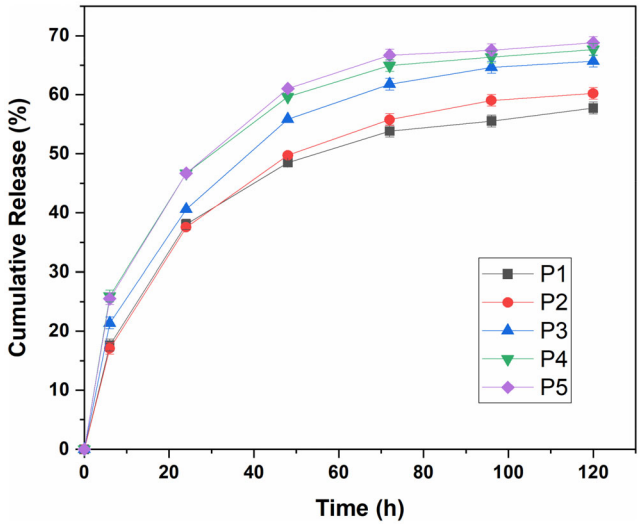


FIGURE 4 Release of sodium fluorescein over time from polymersomes formed P1 (black), P2 (red), P3 (blue), P4 (green), and P5 (purple)

The hydrophobic/hydrophilic bloc ratio affected the membrane thickness of the polymersomes and it was concluded that by decreasing the hydrophobic/hydrophilic ratio the membrane thickness is decreased. Furthermore, it was demonstrated that by tuning the hydrophobic/hydrophilic block ratio, the release of a model drug can also be tuned. These findings could be beneficial for the designing of future polymersome based drug delivery systems.

4 | MATERIALS AND METHODS

4.1 | Materials

The polymers studied in this paper were synthesized in the lab using commercially available monomers, initiator, and solvents. The catalyst (tetrabutylammonium bibenzoate, TBABB) was synthesized according to Dicker et al. using tetrabutyl ammonium hydroxide (40% in water) and benzoic acid that were purchased by Fisher Scientific UK Ltd.[51] The TEGMA monomer (MM = 232.27 g mol^{-1}, 94%) as well as the initiator, methyl trimethylsilyl dimethylketene acetal, MTS, 95%) were purchased from Sigma Aldrich Co Ltd., Irvine, UK. The DEAEMA monomer (MM = 185.27 g mol^{-1}, 98.5%) was purchased from Tokyo Chemical Industry UK Ltd. The polymerization solvent, tetrahydrofuran THF (HPLC grade, ≥99.9%) was also purchased by Sigma Aldrich Co Ltd., UK. Hexane (>95%) that was used for the polymer precipitations was purchased from Fisher Scientific UK Ltd., Loughborough, UK. Other chemicals that were used in monomer and solvent purification, polymerization and following characterization include calcium hydride (CaH_2, ≥90%), basic aluminum oxide (Al_2O_3·KOH), the free radical inhibitor 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), sodium hydroxide pellets (NaOH, 97%), concentrated hydrochloric acid (HCl, ACS reagent, 37%), hydrochloric acid solution (1 M), triethylamine (Et_3N, HPLC grade, used as a co-solvent in GPC), phosphate buffer solution (PBS) (NaCl (0.137 M), KCl (0.0027 M), Na_2HPO4 (0.01 M), and KH_2PO4 (0.018 M)) and deuterated chloroform ($CDCl_3$, 99.8%, used as a solvent to dissolve chemicals for NMR) and were also purchased by Sigma Aldrich Co Ltd., Irvine, UK. In addition, polytetrafluoroethylene (PTFE) syringe filters (0.45 μm in pore size, 13 mm in diameter) were purchased from WR International Ltd., Lutterworth, UK. PTFE syringe filters (0.45 μm in pore size, 25 mm in diameter) and Nylon syringe filters (0.45 μm in pore size, 25 mm in diameter) were purchased from Fisher Scientific UK Ltd., Loughborough, UK. Poly(methyl methacrylate) (PMMA) standard samples for GPC calibration were purchased from Fluka, Sigma Aldrich Co Ltd., Irvine, UK.

4.2 | Methods

4.2.1 | Polymer synthesis (GTP)

All the synthesized polymers in this manuscript were prepared by GTP similarly to previous published studies.[28,46,52–54] The general procedure was identical for all ABA triblock polymers. One example, specifically the synthesis of $TEGMA_{16}$-*b*-$DEAEMA_{10}$-*b*-$TEGMA_{16}$ triblock copolymer is given below to illustrate the general procedure of GTP.

A round bottom 250 ml flask was hydrophobized with dichlorodimethylsilane for 4 h. Then all the glassware (syringes, needles, Schlenk tubes and round bottom flask) was kept at 120°C in an oven over night before use. In the 250 ml flask TBABB (catalyst, ~10 mg) was added and then the flask was sealed with a rubber septum and purged with argon gas. Freshly purified THF was obtained from the solvent purification system and injected into the flask, followed by injection of 0.35 ml MTS (0.3 mg, 1.7 mmol) initiator. Then the first monomer TEGMA (6 ml, 6.2 g, 26.7 mmol) was added in the reaction flask. The temperature was monitored using a thermo couple, and a polymerization exotherm was reported as expected. After approximately 15 min when the temperature has returned close to two 2 samples were taken from the polymerization flask for GPC and ^{1}H

NMR analysis. Consequently, the second monomer, 3.36 ml of DEAEMA (3.1 g, 16.7 mmol) was injected into the flask. An exotherm was reported again and when the temperature depleted two more aliquots were taken for GPC and ^{1}H NMR analysis. Finally, the same amount of TEGMA monomer (6 ml, 6.2 g, 26.7 mmol) was added again using a syringe. 1 ml of methanol was added to quench the reaction and more aliquots were taken for GPC and ^{1}H NMR analysis.

All polymers were then precipitated in cool hexane and dried in a vacuum oven at room temperature for a week.

4.2.2 | Gel permeation chromatography

The MMs and the MMDs of all the ABA triblock copolymers and their linear precursors (homopolymer and diblock copolymer) were determined by GPC using an Agilent, SECcurity GPC system, with a polymer standard service (PSS) SDV analytical linear M column (SDA083005LIM). THF containing 5 vol% triethylamine was used as the mobile phase and was pumped with a flow rate of 1 ml min^{-1} using a "1260 ISO" isocratic pump. An Agilent 1260 RI detector was used to measure the refractive index (RI) signal The calibration curve was based on six narrow MM linear PMMA standard samples (2, 4, 8, 20, 50, 100 kg mol^{-1}).

4.2.3 | Proton nuclear magnetic resonance spectroscopy (^{1}H NMR)

All the ^{1}H NMR spectra of the final triblock copolymers and their linear precursors in $CDCl_3$ were obtained using a 400 MHz Avance Bruker NMR spectrometer instrument. The experimental compositions were then calculated as the integral ratio of the two peaks corresponding to two repeated units in the ^{1}H NMR spectra. Specifically, for DEAEMA the peak appearing at 2.5 ppm, which corresponds to the 10 ethyl protons next to the amine group was used while for the PEGMA units the peak at 3.35 ppm which corresponds to the three methoxy protons of PEGMA used.

4.2.4 | Potentiometric titration

Polymers solutions of all the triblock copolymers 1 wt/wt % in deionized water were prepared. Then 1 M hydrochloride acid was added dropwise until the pH reached 2. Then the polymer solutions were titrated from pH 2 to 12 using a standard 0.25 M NaOH aqueous solution. The pH change was monitored by using a portable HI98103 pH checker from Hanna instruments. The pK_a was determined as the pH at 50% protonation of DEAEMA units.[54–56]

4.2.5 | Production of polymersomes

The polymeric films of the triblock copolymer were prepared by dissolving the polymers in THF. The solvent was then evaporated using a rotary evaporator to give a thin-polymer film, which was then dried under vacuum overnight at room temperature. The dried polymeric films were finally hydrated in a PBS solution to results in a final polymer concentration of 4 mg/ml. The pH of the solutions was 8.1, 8.2, 8.3, 8.2, and 8.1 for P1–P5, respectively.

4.2.6 | Dynamic light scattering

The DLS analysis of the polymeric dispersions were analyzed using a Malvern Panalytical Zetasizer Ultra at a controlled temperature of 25°C. For the analysis, the samples were diluted with PBS buffer at a final concentration of polymer of 0.5 mg/ml into a final volume of 500 µl. The samples were analyzed using a polystyrene cuvette (Malvern Panalytical DTS0012). All the DLS data were processed using Dispersion Technology Software (Malvern Panalytical). The size distribution data are shown considering the intensity-weighted particle signal.

4.2.7 | Transmission electron microscopy

A 2% uranyl acetate (UA) solution was used as a negative staining agent. 5 µl of polymeric dispersion was deposited onto a glow-discharged 200 square mesh copper grid (Agar scientific). After 1 min, the grid was blotted with filter paper and quickly immersed in a 50 µl water drop to prevent crystals formation before blotting it again. Then, the grid was immersed into the UA staining solution for 20 s and blotted again. Grids were imaged using a JEOL JEM-2100F fitted with a Gatan Orius SC 1000 camera (2 × 4k).

4.2.8 | Sodium fluorescein (model drug) release study

The polymer thin film was prepared as described above. Then it was rehydrated with a 0.08 mg/ml sodium fluorescein solution in PBS. The polymer concentration was

10 mg/ml. After 1-day stirring to form the polymersomes, 2.6 ml of solution was syringed into a prehydrated 3000 MWCO dialysis tube. The dialysis tube was placed in a beaker with 578 ml of PBS. The sample was left to dialysed over 24 h to remove the excess sodium fluorescein. Then the dialysis tube was transfer to a 50 ml glass vial with 40 ml PBS. A sample of was taken out at regular intervals and the concentration of fluorescein was determined using Uv–vis.

4.2.9 | Small angle neutron scattering

A polymersome solution of P2 prepared with the film rehydration method in deuterated PBS was analyzed with SANS (after a week of preparation). The experiment was performed at the time-of-flight SANS instrument ZOOM at the ISIS pulsed neutron source at the Rutherford Appleton Laboratory (Didcot, UK), using source to sample and sample to detector distance of 4 m, and wavelengths 1.5–16.5 Å. The data were reduced using MantidPlot, and the resulted SANS curves were fitted using the SasView (version 5) software. The fitting took into account the presence of both micelles (sphere model) and the presence of polymersomes (vesicles model).

ORCID

Anna P. Constantinou https://orcid.org/0000-0002-1606-8515
Theoni K. Georgiou https://orcid.org/0000-0003-4474-6931

REFERENCES

[1] D. E. Discher, F. Ahmed, *Ann. Rev. Biomed. Eng.* **2006**, *8*, 323.
[2] B. M. Discher, Y. Y. Won, D. S. Ege, J. C. M. Lee, F. S. Bates, D. E. Discher, D. A. Hammer, *Science* **1999**, *284*, 1143.
[3] C. Nardin, T. Hirt, J. Leukel, W. Meier, *Langmuir* **2000**, *16*, 1035.
[4] S. Matoori, J.-C. Leroux, *Mater. Horiz.* **2020**, *7*, 1297.
[5] J. Lefley, C. Waldron, C. R. Becer, *Polym. Chem.* **2020**, *11*, 7124.
[6] C. Contini, R. Pearson, L. Wang, L. Messager, J. Gaitzsch, L. Rizzello, L. Ruiz-Perez, G. Battaglia, *iScience* **2018**, *7*, 132.
[7] E. Rideau, R. Dimova, P. Schwille, F. R. Wurm, K. Landfester, *Chem. Soc. Rev.* **2018**, *47*, 8572.
[8] A. Joseph, C. Contini, D. Cecchin, S. Nyberg, L. Ruiz-Perez, J. Gaitzsch, G. Fullstone, X. Tian, J. Azizi, J. Preston, G. Volpe, G. Battaglia, *Sci. Adv.* **2017**, *3*, e1700362.
[9] K. Letchford, H. Burt, *Eur. J. Pharm. Biopharm.* **2007**, *65*, 259.
[10] Z. Fu, M. A. Ochsner, H. P. M. De Hoog, N. Tomczak, M. Nallani, *Chem. Commun.* **2011**, *47*, 2862.
[11] J. Du, Y. Li, J. Wang, C. Wang, D. Liu, G. Wang, S. Liu, *ACS Appl. Mater. Interfaces* **2020**, *12*, 26966.
[12] N. Ben-Haim, P. Broz, S. Marsch, W. Meier, P. Hunziker, *Nano Lett.* **2008**, *8*, 1368.
[13] L. Ruiz-Pérez, J. Madsen, E. Themistou, J. Gaitzsch, L. Messager, S. P. Armes, G. Battaglia, *Polym. Chem.* **2015**, *6*, 2065.
[14] A. J. Parnell, N. Tzokova, P. D. Topham, D. J. Adams, S. Adams, C. M. Fernyhough, A. J. Ryan, R. A. L. Jones, *Faraday Discuss.* **2009**, *143*, 29.
[15] C. J. Mable, I. Canton, O. O. Mykhaylyk, B. Ustbas Gul, P. Chambon, E. Themistou, S. P. Armes, *Chem. Sci.* **2019**, *10*, 4811.
[16] J. Madsen, I. Canton, N. J. Warren, E. Themistou, A. Blanazs, B. Ustbas, X. Tian, R. Pearson, G. Battaglia, A. L. Lewis, S. P. Armes, *J. Am. Chem. Soc.* **2013**, *135*, 14863.
[17] Y. Hu, L. Qiu, *Methods Mol. Biol.* **2019**, *2000*, 247.
[18] C. LoPresti, H. Lomas, M. Massignani, T. Smart, G. Battaglia, *J. Mater. Chem.* **2009**, *19*, 3576.
[19] X. Zhang, M. Lomora, T. Einfalt, W. Meier, N. Klein, D. Schneider, C. G. Palivan, *Biomaterials* **2016**, *89*, 79.
[20] S. T. Poschenrieder, S. K. Schiebel, K. Castiglione, *Eng. Life Sci.* **2017**, *17*, 58.
[21] A. Ranquin, W. Versées, W. Meier, J. Steyaert, P. Van Gelder, *Nano Lett.* **2005**, *5*, 2220.
[22] A. Napoli, M. J. Boerakker, N. Tirelli, R. J. M. Nolte, N. A. J. M. Sommerdijk, J. A. Hubbell, *Langmuir* **2004**, *20*, 3487.
[23] S. L. Banerjee, S. Samanta, S. Sarkar, N. K. Singha, *J. Mater. Chem. B* **2020**, *8*, 226.
[24] W. Agut, A. Brûlet, C. Schatz, D. Taton, S. Lecommandoux, *Langmuir* **2010**, *26*, 10546.
[25] J. Zhang, L. Wu, F. Meng, Z. Wang, C. Deng, H. Liu, Z. Zhong, *Langmuir* **2012**, *28*, 2056.
[26] H. Lomas, A. P. R. Johnston, G. K. Such, Z. Zhu, K. Liang, M. P. Van Koeverden, S. Alongkornchotikul, F. Caruso, *Small* **2011**, *7*, 2109.
[27] E. Rakhmatullina, W. Meier, *Langmuir* **2008**, *24*, 6254.
[28] A. P. Constantinou, T. Lan, D. R. Carroll, T. K. Georgiou, *Eur. Polym. J.* **2020**, *130*, 109655.
[29] R. Cheng, G. Liu, S. Ma, S. Li, F. Meng, H. Liu, Z. Zhong, *J. Controlled Release* **2011**, *152*, 136.
[30] W. Chen, J. Du, *Sci. Rep.* **2013**, *3*, 2162.
[31] X. Wang, H. Sun, F. Meng, R. Cheng, C. Deng, Z. Zhong, *Biomacromolecules* **2013**, *14*, 2873.
[32] W. Wang, H. Liu, M. Mu, H. Yin, Y. Feng, *Polym. Chem.* **2015**, *6*, 2900.
[33] A. Feng, J. Liang, J. Ji, J. Dou, S. Wang, J. Yuan, *Sci. Rep.* **2016**, *6*, 23624.
[34] F. Wang, J. Gao, J. Xiao, J. Du, *Nano Lett.* **2018**, *18*, 5562.
[35] J. Tian, B. Huang, C. Xiao, P. Vana, *Polym. Chem.* **2019**, *10*, 1610.
[36] D. Zhang, Y. Fan, H. Chen, S. Trépout, M.-H. Li, *Angew. Chem. Int. Ed.* **2019**, *58*, 10260.
[37] J. Xiao, J. Du, *J. Am. Chem. Soc.* **2020**, *142*, 6569.
[38] P. Wei, M. Sun, B. Yang, J. Xiao, J. Du, *J. Controlled Release* **2020**, *322*, 81.
[39] D. Zhou, Z. Fei, L. Jin, P. Zhou, C. Li, X. Liu, C. Zhao, *J. Mater. Chem. B* **2021**, *9*, 801.
[40] D. R. Carroll, A. P. Constantinou, N. Stingelin, T. K. Georgiou, *Polym. Chem.* **2018**, *9*, 3450.
[41] O. W. Webster, *Adv. Polym. Sci.* **2004**, *167*, 1.
[42] O. W. Webster, W. R. Hertler, D. Y. Sogah, W. B. Farnham, T. V. RajanBabu, *J. Am. Chem. Soc.* **1983**, *105*), 5706.
[43] A. P. Constantinou, U. Marie-Sainte, L. Peng, D. R. Carroll, C. M. McGilvery, I. E. Dunlop, T. K. Georgiou, *Polym. Chem.* **2019**, *10*, 4637.

[44] A. P. Constantinou, N. F. Sam-Soon, D. R. Carroll, T. K. Georgiou, *Macromolecules* **2018**, *51*, 7019.
[45] A. P. Constantinou, H. Zhao, C. M. McGilvery, A. E. Porter, T. K. Georgiou, *Polymers* **2017**, *9*, 31.
[46] A. P. Constantinou, T. K. Georgiou, *Polym. Chem.* **2016**, *7*, 2045.
[47] S. N. Georgiades, M. Vamvakaki, C. S. Patrickios, *Macromolecules* **2002**, *35*, 4903.
[48] S. C. Hadjiyannakou, M. Vamvakaki, C. S. Patrickios, *Polymer* **2004**, *45*, 3681.
[49] M. S. Kyriacou, S. C. Hadjiyannakou, M. Vamvakaki, C. S. Patrickios, *Macromolecules* **2004**, *37*, 7181.
[50] R. Whitfield, N. P. Truong, D. Messmer, K. Parkatzidis, M. Rolland, A. Anastasaki, *Chem. Sci.* **2019**, *10*, 8724.
[51] I. B. Dicker, G. M. Cohen, W. B. Farnham, W. R. Hertler, E. D. Laganis, D. Y. Sogah, *Macromolecules* **1990**, *23*, 4034.
[52] N. Ghasdian, D. M. A. Buzza, P. D. I. Fletcher, T. K. Georgiou, *Macromol. Rapid Commun.* **2015**, *36*, 528.
[53] M. A. Ward, T. K. Georgiou, *J. Polym. Sci., Part A: Polym. Chem.* **2013**, *51*, 2850.
[54] M. A. Ward, T. K. Georgiou, *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 775.
[55] A. I. Triftaridou, M. Vamvakaki, C. S. Patrickios, *Polymer* **2002**, *43*, 2921.
[56] N. A. Hadjiantoniou, T. Krasia-Christoforou, E. Loizou, L. Porcar, C. S. Patrickios, *Macromolecules* **2010**, *43*, 2713.

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