Controlling pH Responsive Polymersome Assembly

R. Pearson[*], N. J. Warren[*,**], S.P.Armes[**], A.L.Lewis[***] and G. Battaglia[*]

[c] Department of Biomedical Science, University of Sheffield, Firth Court, Western Bank, Sheffield, S102TN, UK, g.battaglia@sheffield.ac.uk
[**]Dept of Chemistry, University of Sheffield, Brook Hill, Sheffield, S37HF, UK
[***] Biocompatables UK Ltd,Champham House,Farnham Business Park, Weydon Lane, Farnham, Surry GU9 8QL, UK.

ABSTRACT

Vesicles are formed when a membrane curves to produce a spherical structure, which in turn entraps an aqueous volume within its lumen. When these structures are formed using a polymers, a polymeric vesicle or "Polymersome" is formed. The biomimetic nature of these assemblies has attracted much attention in the field of biomedicine over the last decade, especially with regards to therapeutic or diagnostic molecule delivery. Many physical and chemical properties dictate the efficacy of a delivery system, one such parameter is size. In this study we propose a simple method of controlling polymersome size by varying the formation temperature. Dynamic light scattering data shows a strong trend of decreasing average particle size with increasing temperature. Transmission electron microscope images support this observation and show several non-ergonomic structures at lower temperatures.

Keywords: polymersome, size, pH responsive, diblock.

1 INTRODUCTION

Nature often utilises membranes to compartmentalise and transport cargo between two sites within the body, in both intracellular and extracellular environments [1]. A common form of membrane transport is the vesicle. These spherical structures are most commonly used for trafficking molecules between cells or to different parts of the same cell. This has driven research into the use of vesicle structures to deliver exogenous payloads for therapies and diagnostics, firstly with lipid based approaches and more recently fully synthetic polymer vesicles, or "polymersomes"[2].

Poly (2-methacryloyoxy- ethyl phosphorylcholine) – b – poly (2- diisopropylamino) ethyl methacylate) (PMPC-PDPA) is a pH responsive, vesicle forming diblock copolymer. In mildly acidic conditions (pH <6.4) MPC and DPA are zwitterionic and cationic respectively, causing the polymer to be molecularly dissolved. When the pH is raised to neutral conditions the DPA block becomes deprotonated and attempts to drive the molecule out of solution via the hydrophobic effect. However, the MPC block retains its hydrophilicity in this environment and the amphiphilic polymer forms stable macromolecular assemblies. By exploiting this reversible hydrophilic/amphiphilic nature of the polymer we are able to generate polymersomes (Figures 1 and 2). The hydrophobic and hydrophilic environments of the polymer membrane and the aqueous lumen provide the ability to trap compounds for therapeutic or diagnositc applications. This potential of combined delivery, along with the manipulative nature of a fully synthetic system makes polymersomes highly desireable systems for targetted drug delivery in nanomedicine.

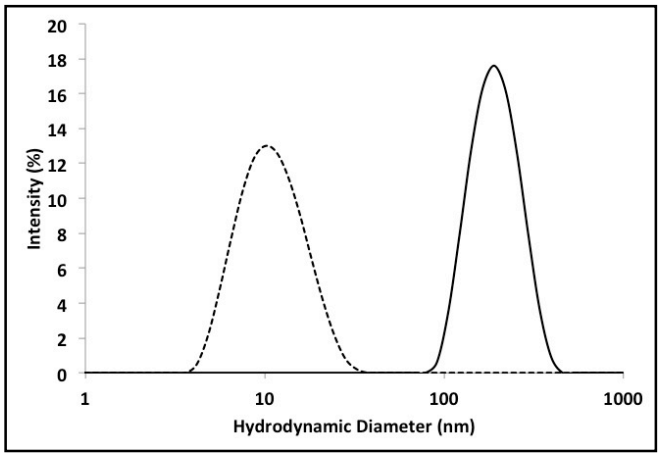

Figure 1: Dynamic light scattering data of PMPC-PDPA in its fully protonated (pH 7.4) and fully deprotonated (pH 5) configurations. Size determination is obtained using the Stokes-Einstein equation and assumes samples are hard spheres. This causes peak broadening when analysing the molecularly dissolved random coils at pH 5.

PMPC-b-PDPA polymersomes have been used to deliver various molecules to a range of cell types with no cytotoxicity [3]. It has also been shown that polymersome size as well as surface chemistry influences the rate of particle uptake into cells [4].

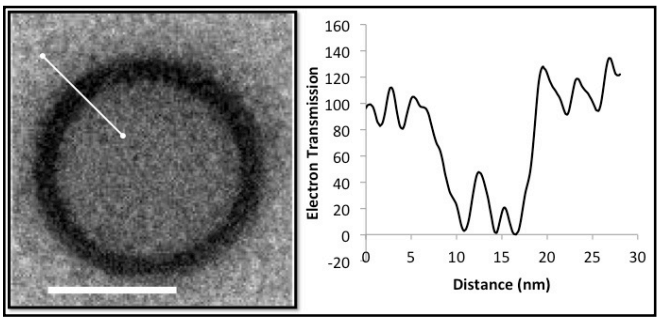

Figure 2: A transmission electron micrograph of a positively stained PMPC-PDPA polymersome. The line profile shows the grey scale across the polymersome membrane, the thickness is measured at around 7nm. The scale bar corresponds to 50nm.

Currently there are many standard methods of polymersome production, such as film rehydration, solvent exchange, microfluidics and electroformation [4],[5],[6],[7]. These are capable of producing a range of sizes from <100nm to >1µM. However, there are limitations such as non-spherical and multilamellar vesicles with the rehydration method and residual solvents present from solvent exchange approaches, which may have a toxic effect. Alongside this, methods to obtain the desired particle size and polydispersity are often conducted after polymersome formation, including sonication and extrusion or size exclusion chromatography. These methods often prove inefficient due to the relatively strong polymeric membrane [8] or large dilutions required. Photolithography [9] and inkjet printing approaches [10] have managed to produce more uniform size distributions, but may have difficulties when scaling up production.

In this work, we propose a simple method of controlling polymersome size by altering the formation temperature. By exploiting the responsive nature of PMPC-b-PDPA, the polymer can undergo a reversible transition from unimers to polymersomes as a function of pH. Samples underwent a pH switch from acidic (pH5) to neutral (pH7.5) across a range of temperatures and were analysed using electron microscopy and light scattering techniques.

2 MATERIALS AND METHODS

Poly (2-methacryloyoxy- ethyl phosphorylcholine) – b – poly (2- diisopropylamino) ethyl methacylate) (PMPC-PDPA) was synthesised by atom transfer radical polymerisation (ATRP) as described previously [11]. Target block lengths of 25 and 70 for MPC and DPA respectively were confirmed by ^{1}H NMR. The polymer was freeze dried for ease of weighing and storage. Polymer was weighed out and dissolved in a combination of 0.1M Phosphate Buffered Saline (PBS) and 1MHCl until a pH5 solution of dissolved polymer at a concentration of 5mg/mL was produced. Polymersomes were formed by mixing equal volumes of polymer solution and NaOH together to cause a jump from pH5 to pH 7.5. The concentration of NaOH required was calculated and tested before use. Separate samples of polymer solution and NaOH were brought to the correct temperature using a water bath before mixing and leaving to stir for 1 hour. Afterwards, samples were analysed by Dynamic Light Scattering (DLS) and images were taken using Transmission Electron Microscopy (TEM). Transmission Electron Microscopy (TEM) was conducted using a FEI Tecnai G2 Spirit microscope. An electron beam of 120keV was formed by thermionic emission from a tungsten filament. Images were taken using a Gatan 1k CCD camera and contrast gained using Phosphotungstic Acid (PTA) for 5 seconds exposure. Samples for analysis by Dynamic Light Scattering (DLS) were taken as 100µl volumes at a concentration of 2.5mg/ml. These were then diluted down to a concentration of 0.25mg/ml by the addition of 900µl of filtered PBS, which had been passed through a 0.2µm filter (VWR UK). DLS was conducted using a Malvern Zetasizer NanoZS (Malvern UK) at a scattering angle of 173°.

3 RESULTS AND DISCUSSION

Basic collision theory states that increasing the temperature of a reaction increases the kinetic energy of the molecules involved. This causes more collisions per second and reduces the time required for the reaction to reach completion. With respect to PMPC-PDPA polymersome formation, sufficient amphiphilic chains must interact and arrange in a highly organised manner. The formation of sub 50nm at the higher temperatures (Figure 3) investigated suggests the production of micelles, particles formed of a monolayer thus comprising of an entirely hydrophobic core. The high curvature required to produce a micelle is theoretically unfavourable for membrane forming polymers, as described by the molecular packing parameter [12],[13]. However, this molecular frustration may be more favourable than the entropic penalty of exposing the protected hydrophobic core. The results correlate with this theory and suggest that kinetically trapped micelles are being produced at higher formation rates.

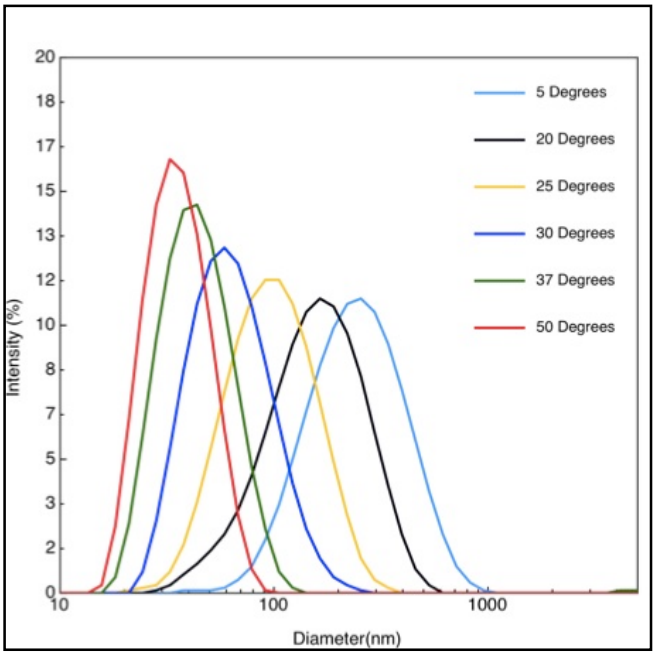

Figure 3: Size distribution profiles of samples at different formation temperatures. Polymer concentration and formation times were kept constant across temperatures and samples were left to equilibrate to room temperature before analysis. Data shows a large difference in average size and polydispersity between 5°C and 50°C

Transmission electron microscopy images support these findings (Figure 4), showing large numbers of smaller particles with the highly uniform size distribution indicative of micelles. Lower temperature samples show non-equilibrium assemblies such as highly entangled tube structures (Figure 4 A) or non-spherical membrane formations (Figure 4 B and C). These may be due to insufficient time of formation. However, a study on the same polymer system conducted by Shen et al shows the rate of formation to occur on a millisecond timescale [14]. This could mean that this temperature range may include parameters such as the glass transition temperature (T_g) which could affect the ease of membrane formation.

In this study, we have shown the effects of temperature on the formation of PMPC-PDPA polymersomes. The average size, polydispersity and shape of the structures generated are highly dependent on the reaction temperature. Further work is required to generate a more detailed relationship between reaction temperature and the size and number of polymersomes, most likely by quantifying the encapsulation of a hydrophilic compound.

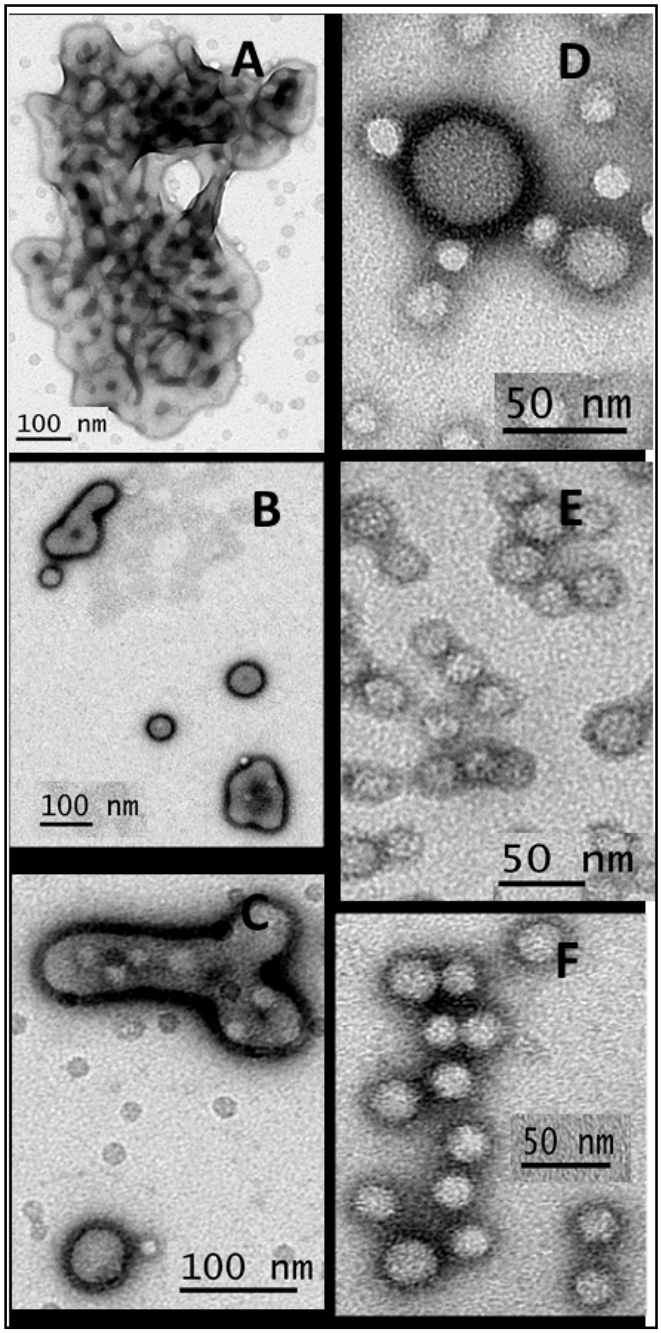

Figure 4: Typical structures seen by TEM at different formation temperatures. **A – F** correspond to 5°C, 20°C , 25°C, 30°C, 37°C and 50°C respectively.

4 REFERENCES

1. Sudhof, T. C. The synaptic vesicle cycle. Annual Review of Neuroscience, 27, 509-547, 2004.
2. Discher, D. E.; Eisenberg, A. Polymer vesicles. Science, 297 (5583), 967-973,2002.
3. Lomas, H.; Massignani, M.; Abdullah, K. A.; Canton, I.; Lo Presti, C.; MacNeil, S.; Du, J. Z.; Blanazs, A.; Madsen, J.; Armes, S. P.; Lewis, A. L.; Battaglia, G. Non-cytotoxic polymer vesicles for rapid and efficient intracellular delivery. Faraday Discussions, 139, 143-159, 2008.
4. Massignani, M.; LoPresti, C.; Blanazs, A.; Madsen, J.; Armes, S. P.; Lewis, A. L.; Battaglia, G. Controlling Cellular Uptake by Surface Chemistry, Size, and Surface Topology at the Nanoscale. Small, 5 (21), 2424-2432, 2009.
5. Lee, J. C. M.; Bermudez, H.; Discher, B. M.; Sheehan, M. A.; Won, Y. Y.; Bates, F. S.; Discher, D. E. Preparation, stability, and in vitro performance of vesicles made with diblock copolymers. Biotechnology and Bioengineering, 73 (2), 135-145, 2001.
6. Thiele, J.; Steinhauser, D.; Pfohl, T.; Forster, S. Preparation of Monodisperse Block Copolymer Vesicles via Flow Focusing in Microfluidics. Langmuir, 26 (9), 6860-6863, 2010.
7. Brown, L.; McArthur, S.L.; Wright, P.C.; Lewis, A.; Battaglia, G. Polymersome production on a microfluidic platform using pH sensitive block copolymers. Lab on a Chip, 10 (15), 1922-1928, 2010.
8. Hammer, D. A.; Discher, D. E.; Bates, F. S.; Discher, B. M.; Lee, C. M.; Bermudez, H.; Won, Y. Y. Polymersomes: Tough vesicles made from block copolymers. Abstracts of Papers of the American Chemical Society, 220, 212-COLL, 2000.
9. Howse, J.R.; Jones, R.A.L.; Battaglia, G.; Ducker, R.E.; Leggett, G.J.; Ryan, A.J. Templated formation of giant polymer vesicles with controlled size distributions. Nature Materials, 8 (6), 507-511, 2009.
10. Hauschild, S.; Lipprandt, U.; Rumplecker, A.; Borchert, U.; Rank, A.; Schubert, R.; Forster, S. Direct preparation and loading of lipid and polymer vesicles using inkjets. Small ,1 (12),1177-1180, 2005.
11. Du, J. Z.; Tang, Y. P.; Lewis, A. L.; Armes, S. P. pH-sensitive vesicles based on a biocompatible zwitterionic diblock copolymer. Journal of the American Chemical Society, 127 (51), 17982-17983, 2005.
12. Israelachvili, J. N.; Mitchell, D. J.; Ninham, B. W. Theory of Self-Assembly of Hydrocarbon Amphiphiles Into Micelles and Bilayers. Journal of the Chemical Society-Faraday Transactions Ii, 72, 1525-1568, 1976.
13. Nagarajan, R.; Ruckenstein, E. Theory of Surfactant Self-Assembly - A Predictive Molecular Thermodynamic Approach. Langmuir, 7 (12), 2934-2969, 1991.
14. Shen, L.; Du, J. Z.; Armes, S. P.; Liu, S. Y. Kinetics of pH-induced formation and dissociation of polymeric vesicles assembled from a water-soluble zwitterionic diblock copolymer. Langmuir, 24 (18), 10019-10025, 2008.