Cryo-Electron Microscopy Study of Asymmetric PEG-*b*-PMMA Block-copolymers

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Abstract - Asymmetric poly(ethylene glycol-*b*-methyl methacrylate) block copolymers have been synthesized by Atom Transfer Radical Polymerisation (ATRP) and the aggregates formed in solution investigated by Cryo-Transmission Electron Microscopy (Cryo-TEM). A morphology diagram has been constructed based on the obtained images and reveals that rod-like morphologies make a transition to vesicle based morphologies with increasing water content of the solvent system.

1. Introduction

In addition to phospholipids and synthetic surfactants, it has been shown that amphiphilic block copolymers have the ability to self assemble into multiple morphologies, including vesicles, which are of obvious commercial and biological interest for encapsulation technologies [1-3].

With a view to producing an amphiphiilc block copolymer, an α-hydroxy poly(ethylene glycol) ($Mn=2\times10^3$ g/mol) was transformed to a suitable initiator for ATRP. The resultant macroinitiator was used to polymerise methyl methacrylate to form an asymmetric amphiphilic PEG-b-PMMA block copolymer.

The self-assembly properties of this polymer was investigated by dissolution in an organic solvent, (Tetrahydrofuran, THF) followed by the slow addition of water, to induce partial precipitation of the hydrophobic block. Solutions were analysed by negatively stained TEM and Cryo-TEM, performed on a JEM 2011-LaB_6 microscope operating at 200KV, equipped with a Cryo-holder (Gatan Inc, Oxford, UK). Images were aquired on a 2048x2048 pixels CCD camera at 15 μm/pixel resolution (Gatan Inc. UK) and a nominal magnification of 10,000 - 20,000x and a typical underfocus of 2-3 μm. Samples were screened at a range of initial concentrations (10, 5 and 1 g L^{-1}) with solvent compositions ranging (10% - 80% water).

2. Results and Discussion

The Cryo-TEM images indicate that the PEG-*b*-PMMA system undergoes rod to vesicle transitions, with increasing water content of the solvent (Figure 1). However a number of intermediate complex morphologies were also observed including “pretzel”, “bi-continuous” and mixed vesicle–tubule structures.

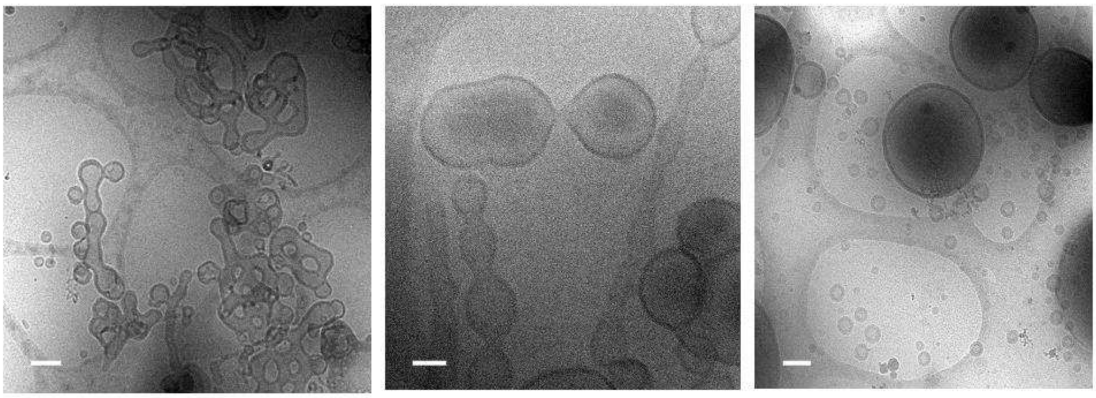

Figure 1 *– Cryo-TEM images of PEG-b-PMMA at (a) 30% water (0.7 g/L), (b)40% water (3 g/L) and at (c) 60% water (2 g/L). 10,000 - 20,000x magnification, scale bars 0.2 μm*

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The use of Cryo-TEM ensures that the true morphologies, present in solution are preserved, without drying effects, which are a disadvantage of negative staining techniques. A full characterisation of the self-assembled block-copolymer morphologies was achieved by Cryo-TEM at two initial concentrations and a range of solvent conditions. The observed morphologies are the result of thermodynamic minimum energy states, defined by the dimensions and chemistry of the amphiphiles, as the hydrophobic chains attempt to minimize unfavorable water contact. The observed structures were used to construct a morphology diagram (Figure 2) with respect to solvent and polymer concentration.

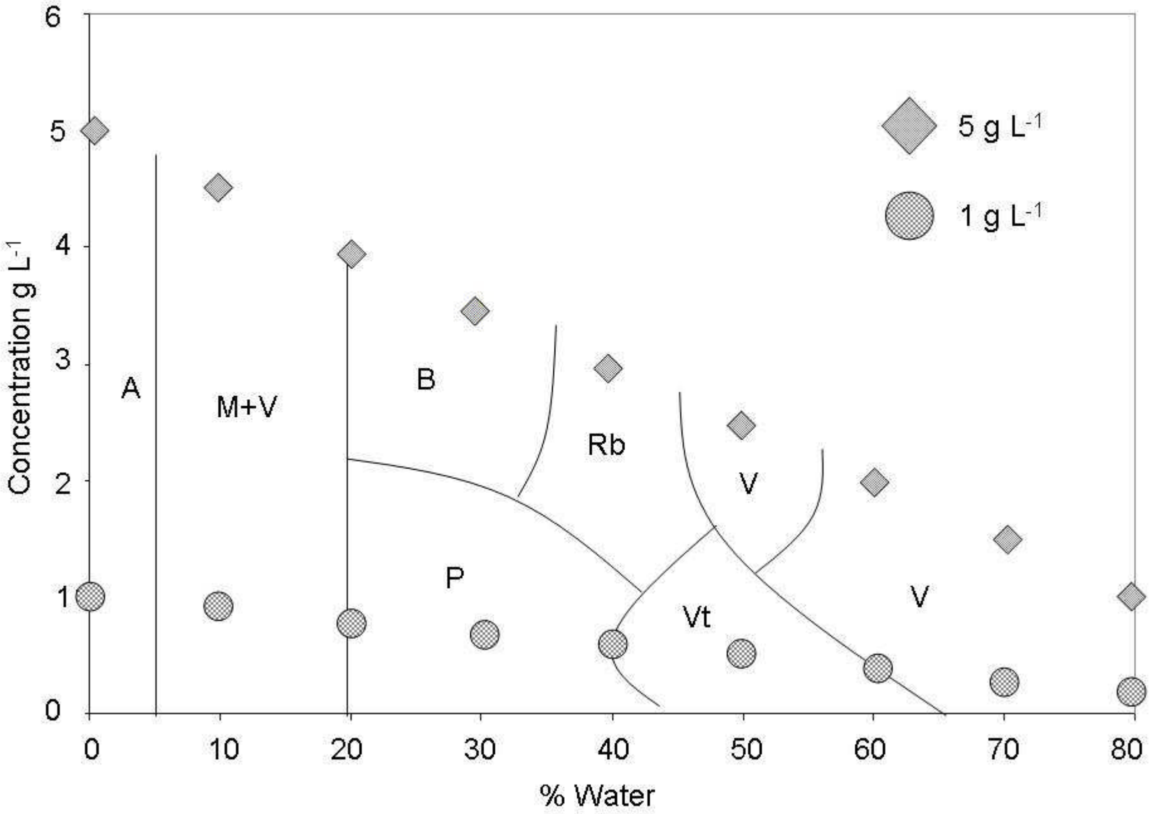

Figure 2. *Morphology diagram of dilute solutions of PEG-b-PMMA*

*A= Soluble, M= Micelles, **B**=Bi-continuous phase, **P**= Pretzels, R**b** = Budding rods, **Vt**= Vesicles with tubules, **V**= Vesicles*

The morphology diagram indicates different transitional structures for the two studied initial concentrations (1 g L^{-1} in the region 20 – 50% water and also notes that there is a general trend to reach vesicle morphologies earlier for more concentrated polymer solutions *i.e.* 45 – 50% water at 5 g/L versus 55 – 60% water at 1 g/L.

3. Conclusions

In summary, an asymmetric PEG-*b*-PMMA block copolymer has been synthesised and shown to yield a variety of morphologies in dilute solution. By the construction of a morphology diagram from Cryo-TEM images, this system has displayed a range of structures with varying initial polymer concentrations and/or solvent composition. Cryo-TEM has been used with great effect to preserve the nano-aggregates in their hydrated state, without significant drying effects, demonstrating its versatility as a technique in both biological and soft matter systems.

4. References

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