

Conformation of pH responsive alternating copolymers and self-assembly in nanoarchitectures

A.S.W. Chan, M.N. Groves, X. Li and C. Malardier-Jugroot*

* Department of Chemistry & Chemical Engineering,
Royal Military College of Canada, Kingston, ON, Canada,
anita.chan@rmc.ca, cecile.malardier-jugroot@rmc.ca

ABSTRACT

In this paper, we discuss the formation of distinct nanoarchitectures from Poly(isobutylene-*alt*-maleic acid) (IMA) copolymer chains in water, and the dependence of pH on the self-assembly process. An experimental approach was chosen to investigate and characterize the association of IMA chains at different pH values. A change in structure at different environments was observed using Dynamic Light Scattering (DLS), Small Angle Neutron Scattering (SANS) and Transmission Electron Microscopy (TEM). DLS results reveal an association occurring at neutral pH whereas the copolymer was unable to form any observable structure at low and high pH. From the fitting of SANS pattern, it is suggested that IMA copolymer at pH 7 forms double layer sheets in which the outer surfaces are hydrophilic and the center gap is hydrophobic. In addition, TEM images of a dried sample showed sheet-like structures associated in layers.

Keywords: poly(isobutyl-*alt*-maleic anhydride), alternating copolymer, self-assembled nanoarchitecture

1 INTRODUCTION

The most widely used materials for the production of self-assembled nanomaterials are amphiphilic blockcopolymers. Example applications are drug delivery [1] and nanowire templates [2]. The association, shapes and properties of block copolymers have been extensively characterized [1]. On the other hand, the association of alternating copolymers has been rarely studied, and are predicted to have several major advantages over their block counterparts [3]. Nanoscale structures formed by alternating copolymers have proven very efficient for the solubilization of small hydrophobic molecules in water for applications in storage, delivery and nanoelectronics [4]. These copolymers can be structurally very close to biological systems with hydrophilic monomers alternating with hydrophobic monomers along the chain giving rise to complex and well-defined nanoarchitectures [5]. In order to design new nanomaterials, it is therefore essential to understand the fundamental properties of the polymer assembly and the factors influencing it.

Our research focuses on the characterization of the association and the properties in aqueous solution of

nanostructures composed of amphiphilic alternating copolymers. They are composed of a pH sensitive hydrophilic group, maleic anhydride alternating with a hydrophobic group. We will present the characterization of the self-assembly mechanism of poly(isobutyl-*alt*-maleic anhydride) (IMA) by a combination of experimental methods. It has been well observed that IMA forms stable linear chains [6] at neutral pH only due to strong electrostatic interactions between the sodium cations and the hydrophilic group of the copolymer. These linear chains (Figure 1) allows for the formation of nanoarchitecture by minimizing the entropic cost of association [3].

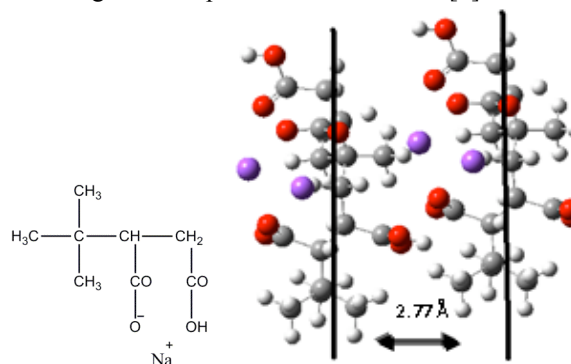


Figure 1. (Left) Representation of IMA monomer at pH 7, (Right) Representation of the interaction between two copolymer chains at pH 7.

Different structures were observed using Dynamic light scattering (DLS) at different pH environments. Small Angle Neutron Scattering (SANS) and Transmission Electron Microscopy (TEM) were used to characterize the nanoarchitecture at neutral pH, where the association among the polymer chains was predicted to occur [6].

2 METHODS

2.1 Sample Preparation

The IMA polymer was purchased from Aldrich Co. and has molecular weights of 6,000 (~85% purity). Similar to our previous work on poly(isobutylene-*alt*-maleic anhydride) in aqueous solution [6], low concentrated (0.05% wt in Milli-Q water) amphiphilic copolymer nanoarchitecture solutions of IMA were prepared and then sonicated for a few hours (6 hours to 1 day). The pH of the

solution was adjusted accordingly by stoichiometric addition of sodium hydroxide. The solutions were centrifuged at 14,500 rpm for 10 minutes prior to analysis.

High concentrated samples (2% wt in ultra pure water) were produced by adding sodium hydroxide to the IMA solution to increase the pH to 12, and then by adding accordingly hydrogen chloride to adjust to neutral pH. This method allows for a higher concentration due to a higher solubility of IMA at high pH; however the concentration of salt in the solution and its ionic strength are much greater. The solutions were centrifuged at 14,500 rpm for 10 minutes prior to analysis.

2.2 Nanoarchitecture Characterization

The radii of gyration of the copolymer nanoarchitectures as a function of pH were measured in solution using DLS (Brookhaven BI-200SM Research Light Scattering) equipped with a 632.8nm He-Ne laser. The measurements were taken at a 90° angle. The samples were filtered using Titan 2 HPLC Filters with 0.45µm regenerated cellulose membranes.

The transmission electron microscopy (TEM) images were carried out on at Brockhouse Institute for Materials Research Canadian Centre for Electron Microscopy at McMaster University in Hamilton, Ontario. The images of the nanostructure sample after drying were obtained using FEI Titan 80-300 Cubed High-Resolution Transmission Electron Microscope equipped with Gatan 866 model spectrometer optics.

SANS experiments were carried out on the NG3 30-m Small Angle Neutron Scattering Instrument at the NIST Center for Neutron Research in Gaithersburg, Maryland. The incident wavelength was 6 Å. Three sample-to-detector distances, 1.00, 6.00 and 13 m, were employed, covering a q -range of 0.005 to 0.40 Å⁻¹. The samples were loaded in quartz cells. The sample aperture was 10*10 mm². The experiment was carried out using hydrogenated polymer in deuterated water as the solvent to increase the contrast. The scattering pattern was reduced and analysed using the Igor Macros package [7]. The averaged data were corrected for empty cell and background. Rigid Cylinder and Diluted Lamellar form factors were chosen for the fitting of the scattering patterns. The scattering intensity is described by eq 1 for rigid cylinders [8]:

$$I(q) = \phi P(q) \quad (1)$$

$$P(q) = \frac{scale}{V_{cyl}} \int_0^{\pi} f^2(q, \alpha) \sin \alpha d\alpha$$

$$f(q, \alpha) = \frac{2(\rho_{cyl} - \rho_{solv})V_{cyl}j_0(qH \cos \alpha)(J_1(qr \sin \alpha))}{(qr \sin \alpha)}$$

$$V_{cyl} = \pi r^2 L \text{ and } j_0(x) = \frac{\sin(x)}{x}$$

where ϕ is the particle volume fraction. $J_1(x)$ is the first order Bessel function. Alpha is defined as the angle

between the cylinder axis and the scattering vector, q . The integral over alpha averages the form factor over all possible orientations of the cylinder with respect to q . The returned value is in units of [cm⁻¹], on absolute scale.

For diluted Lamellar structures, the scattering intensity is given by eq 2 [9-10]:

$$I(q) = \frac{2\pi P(q)}{\delta q^2} \quad (2)$$

$$P(q) = \frac{2\Delta\rho^2}{q^2 \left[1 - \cos \left[\frac{q\delta}{2} \right] e^{-\frac{q^2\sigma^2}{2}} \right]}$$

where δ is the bilayer thickness and σ = variation in bilayer thickness = δ *polydispersity

3 RESULTS AND DISCUSSION

The pH values were chosen relative to the degree of hydrolysis of the maleic acid groups in the copolymer after titration analysis. In an aqueous solution of poly(isobutylene-*alt*-maleic anhydride) at pH 3, the maleic anhydride ring is hydrolyzed to give two acid groups. With the increase of pH, one acid group is hydrolysed and a sodium counterion stabilizes the site at pH 7. The second acid group is also hydrolysed when the pH of the solution is further increased to pH 12.

Theoretical analysis was performed to determine the most stable structure of the copolymer at different pH value to predict the structure effect of the pH on the polymer [7]. The fully optimized structures reveal a linear conformation at neutral pH due to the electrostatic interaction between the hydrophilic regions of the copolymer and the sodium counterion. From a rigid 2-D scan using AMBER and another optimization at DFT-6-31++G(d,p), the association of two chains revealed that two IMA chains are about 2.77Å apart (Figure 1). This linearity is only observable at neutral pH; hence we expect to see an observable structure at pH 7. At low and high pH, the copolymer chain takes a bent shape. As a result, associations are less likely to form due to the large entropic cost that would derive from this association.

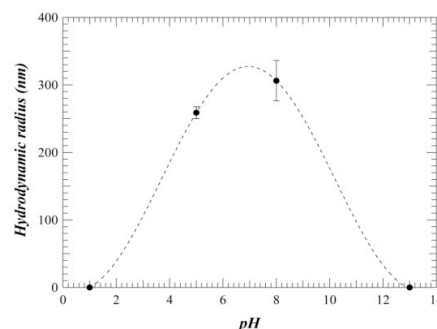


Figure 2. The points represent values of the hydrodynamic radius of 0.05 wt% IMA at varying pH obtained from DLS. Values at pH 1 and pH 13 are below the resolution of the instrument. N.B.: the dotted line is an eye guide and not an actual fit.

Following the characterization using theoretical methods, the hydrodynamic radius was measured using DLS (Figure 2). The resolution of the instrument was between 20 nm to a few hundreds of nm. The results confirmed the association at neutral pH obtained from the theoretical models. Indeed, an association occurs at neutral pH and allows the polymer chain to form stable structures. At low or high pH values, the polymer chain is unable to form a structure large enough to be detected by DLS. The values for pH 1 and pH 13 are not zero, but they are below the resolution of the instrument. It can be concluded that the linear association predicted by the simulations would form larger nanostructures. The association can only occur at neutral pH. A similar pH dependence for the association of amphiphilic copolymers was observed for SMA [3] which revealed a self-assembly into nanotubes [11].

In order to analyze the association of the chains, the copolymer was characterized using HR-TEM images taken of a 2%wt IMA sample after drying (Figure 3). This image shows the formation of liner structures of width 5 Å. The obtained d-spacing is 2.86 Å which is comparable to the value obtained from the simulations. The image shows very uniform and well-ordered structures which are unusual in polymer sample since the entropic cost associated with such linear structures would be very high. In this case, the copolymer chains are able to remain linear even after the removal of water which drives the self-assembly. This linearity is an optimistic sign that IMA nanostructures are indeed an excellent template to synthesize well ordered nanostructures.

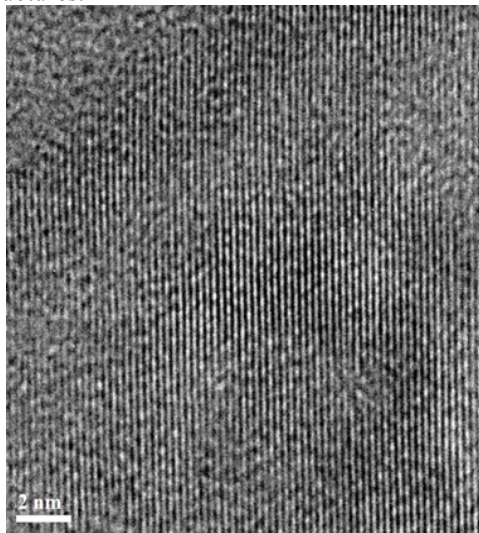


Figure 3. HR-TEM image after drying at scale 2 nm. The high resolution image shows lattice fringes. The measure the d-spacing of uniform structure is $\sim 2.9 \text{ \AA}$.

Finally, SANS, a key technique for the study in complex fluids and polymers, provides detailed information on the structure of the nanostructure *in situ*. The experiment was performed in D_2O using hydrogenated IMA to maximize the contrast between the solvent and the polymer. The scattering curve corresponds to the IMA structure,

IMA-IMA association, and chain interactions. The scattering-length density (SLD) contrast is caused by the D_2O -IMA scattering length contrast (Table 1) [12]. The scattering profile obtained for a 2 wt % solution at pH 7 is shown in Figure 5.

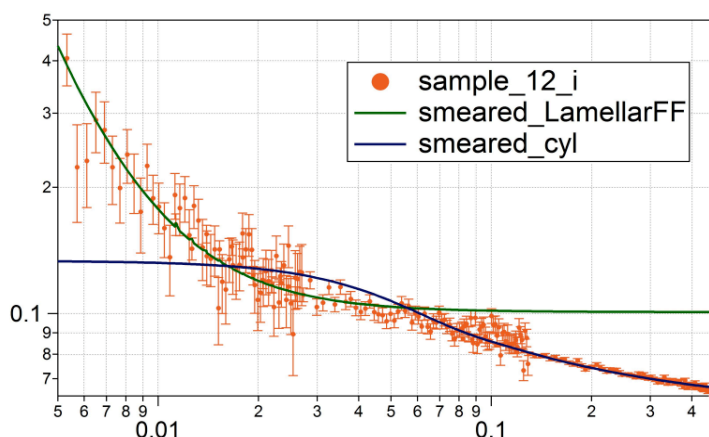


Figure 5. Neutron scattering pattern of 2% wt IMA solution at pH 7. The pattern was fitted using two different form factors: rigid cylinder for high Q values and diluted Lamellar at low Q values. There is a noticeable break between the form factors.

	IMA	Solvent
Structure	$ \begin{array}{c} \text{CH}_3 \\ \\ \text{H}_3\text{C}-\text{C}-\text{CH}-\text{CH}_2 \\ \quad \quad \\ \text{CH}_3 \quad \text{CO} \quad \text{CO} \\ \quad \quad \quad \\ \quad \quad \text{O} \quad \text{OH} \\ \quad \quad + \\ \quad \quad \text{Na} \end{array} $	$ \begin{array}{c} \text{D} \quad \text{D} \\ \diagdown \quad \diagup \\ \text{O} \end{array} $
SLD (\AA^{-2})	9.14e-07	6.38e-06

Table 1. Structure and Scattering-Length Density (SLD) of IMA and D_2O

At low Q range ($0.005\text{-}0.01 \text{ \AA}^{-1}$), there is a decaying slope. At mid Q range (0.055 \AA^{-1}), a noticeable break appears. This break is not the result of the connectivity of data between two detector positions. As the data is split between three detector positions (1, 6 and 13m from the sample), if the data was not properly reduced, breaks would appear at Q range of 0.02 and 0.1 \AA^{-1} . As this break is at not around these points, it was concluded that is part of the scattering pattern.

Several different models were fitted to this data set, and a sum of two different models was required to characterize the scattering pattern for the entire range. The Dilute Lamellar Form Factor [9-10] and the Cylinder Form Factor [8] were used to describe the pattern at low ($0.005\text{-}0.055 \text{ \AA}^{-1}$) and at high ($0.055\text{-}0.500 \text{ \AA}^{-1}$) Q ranges respectively. With the use of this particular method, we are able to describe the structure at all level of resolution of the copolymer sample such as the uniform linear copolymer chains and the

formation of nanoscale sheets that were observed using HR- and Cryo-TEM respectively.

At high Q range, the Cylinder Form Factor was chosen because of our assumption that the copolymer would behave like a rigid linear chain [6]. The resulting fit yields a radius of $2.063 \pm 0.002 \text{ \AA}$ and a length of $87.37 \pm 0.04 \text{ \AA}$. The scales of these values indicate that the model is representing a single copolymer chain. The height of the chain found using this model is similar to that found in the simulation (4.2 \AA). The model did indeed fit the range of the scattering pattern in which would describe the linear chains.

At low Q range, where the scattering pattern describes the larger system, the above model would not accurately fit. Referring to the HR-TEM image (figure 3) which showed that individual linear copolymer chains would interact with each other and form a sheet, we were led to select the dilute Lamellar Form Factor which resulted in a better fit. Indeed, while keeping the SLD of the solvent and the copolymer (Table 1) fixed, the thickness of the sheets was determined to be $2.096 \pm 0.005 \text{ \AA}$. This value is similar to the width of one IMA copolymer chain, which suggests that the model is describing only one bi-layer of the amphiphilic sheet. It was well observed that the isobutylene groups are very hydrophobic. The self-assembly in water suggests that the sheets would form with the hydrophilic groups pointing outward whereas the hydrophobic region would face inwards and interact with the hydrophobic region of another sheet (Figure 6). This behaviour is analogous to a phospholipids' bi-layer sheet.

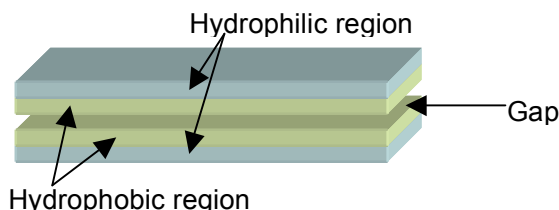


Figure 6. Representation of the copolymer bi-layer.

4 CONCLUSION

An experimental approach was chosen to investigate and characterize the association of poly(isobutylene-*alt*-maleic acid) chains at different pH values. A change in structure at different pHs was observed using DLS and SANS. It is suggested that IMA copolymers at pH 7 form double layer sheets composed of well-order uniform copolymer chains; the surfaces are hydrophilic and the center gap is hydrophobic. In addition, TEM images of a dried sample showed layers of sheet-like structure. HR-TEM images show a uniform formation of well-ordered wire structures 2.8 \AA apart which confirms the results of the scattering pattern fitting.

These results are analogous the theoretical predictions [6]. IMA is able to self-assemble into observable nanoarchitectures in water at neutral pH by adopting a linear structure, which would decrease the entropic cost of

association. This linearity was only observable at neutral pH; which was confirmed by experimental characterization; hence we expect to see an observable structure at pH 7. At low and high pH, the copolymer chain takes a bent shape. As a result, associations are less likely to form due to the large entropic cost that would derive from this association.

We also observed the formation of IMA polydispersed spheres at low pH when the second preparation method was used: with an increase amount of salt. It is suggested that IMA can self-assemble into different nanoarchitectures at neutral or low pH. It is possible that, with enough ionic interactions present, the copolymer chains can reach the entropic barrier and will remain linear, thus leading to different forms of nanoarchitectures. In the case of high pH, that barrier will not be attained; hence, no observable formation of a nanostructure would occur.

The complexity of poly(isobutylene-*alt*-maleic acid) nanostructure is vastly interesting. Unlike other polymer, its most energetically favored structure is linear once an energy barrier is reached. Once that is done, it is able to form different multi-scale nanostructures depending on the pH. Controlling the formation of these structures would have great implications to the application of bio-compatible nanosystems,

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