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Self-assembly microstructures of amphiphilic polyborate in aqueous solutions

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ABSTRACT

A new type of amphiphilic homo-polyborate was synthesized with the surface tension (γ_{CMC}) in deionized water 31.2 mN m^{-1} (pH = 7, 25 °C), which is similar to low-molecule surfactants. Self-assembly behaviors of the polyborate in aqueous solutions were investigated by TEM. Self-aggregation morphologies of the polyborate can be controlled by the concentration of NaCl. With the increase of the concentration of NaCl, micromorphologies from the amphiphilic polyborate transformed from spherical polymeric vesicles to tree-like or even vertebra-like morphologies. It was also found that, the polymeric vesicles show a strong stability against ethanol addition, even when the concentration of ethanol increases to a volume ratio of 33%, they still maintain good micromorphologies. A possible mechanism of vesicle formation was proposed based on packing parameter theory.

1. Introduction

Amphiphilic molecules in solution tend to self-assemble to produce diverse microstructures such as micelle, vesicle, which have attracted considerable attention over the past decades because of their scientific interests and potential applications in drug delivery, biomembrane mimic, nanoreactors [1–6]. Among the various morphologies, the closed bilayer membranes, polymeric vesicles (also called polymersomes), attract much attention due to their more stability and higher membrane toughness than regular low-molecule lipidsomes [3–6]. Up to now, polymeric vesicles have been prepared generally from a variety of some amphiphilic block copolymers containing covalently linked hydrophobic block and hydrophilic block. Vesicle morphologies formed with block copolymers can be controlled in aqueous solution depending on the fraction of hydrophobic/hydrophilic block, as well as solvent properties, such as the composition, pH, or ionic strength [7,8]. A variety of amphiphilic block copolymers have been synthesized to explore the interesting phenomenon, for example, poly(styrene-*b*-4-vinylpridine) (PS$_{80}$-*b*-P4VP$_{110}$) [9], polymethylene-*b*-poly(dimethyl siloxane)-*b*-polymethylene (PM-*b*-PDMS-*b*-PM) [10], ethyl cellulose-graft-polystyrene (EC-*g*-PS) [11]. In general, input of external energy during these vesicles preparation is required, so that several conventional methods have been developed, such as ultrasonic, dialysis, extrusion, etc [12–16].

During the past ten years, growing interests have been paid on investigating the spontaneous formation of amphiphilie's vesicles due to their easy process and quite stability when compared with "conventional" vesicles [16]. By far, the most spontaneous vesicle-forming systems reported in the literatures are based on aqueous mixtures of oppositely charged amphiphiles [17]. Recently, copolymers with di/triblock of PEO/PPO [18], PEO/PBO [19,20] and some complexes of block ionomers and surfactants [21] were also found to possess the properties of spontaneous vesicle formation, and great attention has been paid on these. In fact, until now, few about self-assembling character of homopolymers in aqueous solution have been reported. As a consequence, spontaneous vesicles made from homopolymers have been rather scarce.

In the present work, a novel amphiphilic linear homopolymer surfactant containing boron was synthesized. The prepared polyborate consists of a hydrophobic C$_{12}$ segment which is attached to a hydrocarbon chain by a hydrophilic link containing a borate unit (the polyborate **4a**, see Scheme 1). The target amphiphilic polyborate has an excellent surface activity in aqueous solution similar to low-molecule surfactants, and shows the ability to spontaneously self-assemble to form polymeric vesicles in aqueous solutions. By transmission electron microscopy (TEM), the transition between the assembled geometries triggered by different concentration of NaCl and ethanol was also observed. The interesting phenomenon is that the morphologies of the vesicles change spherical vesicles to tree-like and vertebra-like structure, which, to our knowledge, has not been reported in the literatures.

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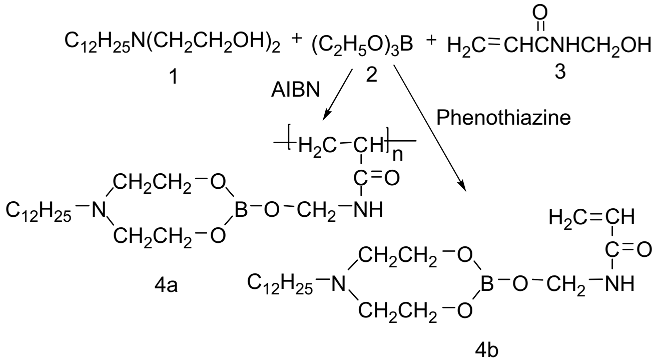


Scheme 1. Synthesis of polyborate and its monomer.

2. Experiment section

2.1. Materials and process

The polyborate **4a** and its monomer **4b** were synthesized according to the following procedures. N,N-dihydroxyethyl dodecylamine **1** and triethyl borate **2** were dissolved in benzene. The mixture was stirring for 1 h at 60 °C under reduced pressure to remove the produced ethanol by $ZnCl_2$ powder. After N-hydroxymethyl acrylamide **3** and azoisobutyronitrile (AIBN) (molar ratio: [1]/[2]/[3]/[AIBN] = 1:1:1:0.006) added, the mixture was kept for additional 1.5 h, and then was maintained at 75 °C for 2 h under normal pressure without stirring. The obtained polymer, isolated by precipitation into acetone, followed by filtration to remove unreacted material and oligopolymers, is white or light-yellow powder with the yield 78%. With phenothiazine to replace AIBN, we obtained viscous liquid monomer **4b** in the similar way (see Scheme 1). Molecular weight of **4a**, measured with SEC apparatus (using Plgel MIXED-C 79911GP-MXC column and calibrated with polystyrene standards, the effluent is DMF, Agilent 1100), is 28,400 $g\ mol^{-1}$ with polydiversity index M_w/M_n 1.16. The ^1H NMR and FT-IR analysis of **4a** and **4b** was reported in our previous study [22]. Other reagents used are all analytical grades and were used as received.

2.2. Surface activity

Surface tension in deionized water at 25 °C (pH = 7) was determined by surface tensionmetry (Du Nouy ring, JYW-200). The measurement was calibrated with respect to the surface tension of the pure water ($\gamma_{CMC} = 72.0\ mN\ m^{-1}$).

2.3. Transmission electron microscopy (TEM)

A droplet of wt. 0.1% aqueous samples in solution was placed onto a carbon-coated copper grid. Filter paper was applied to suck away the excess liquid. The specimens were examined on a JEOL JEM-2100 electron microscope at room temperature.

3. Results and discussion

3.1. Surface activity

In order to examine the surface activity of the polyborate, surface tensions were measured in deionized water. The plot of surface tension versus concentration of the polyborate **4a** and its monomer **4b** is shown in Fig. 1. The breakpoint of the plot generally determines the CMC (Critical Micelle Concentration) value of the surfactant, and the surface tension values vary slightly after CMC or remain constant afterwards. The surface tension values of the polyborate and its monomer in aqueous solutions are listed in Table 1.

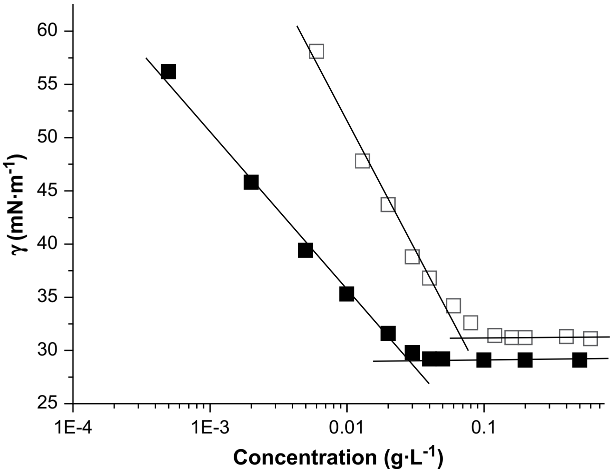


Fig. 1. Surface tension (γ) plots of polyborate **4a** (□) and monomer **4b** (■) in deionized water (pH = 7.0, 25 °C).

As can be seen from Table 1, the CMC of polyborate **4a** in water was 68.4 $mg\ L^{-1}$, and the surface tension γ_{CMC} was 31.2 $mN\ m^{-1}$ (pH = 7), while the CMC of monomer **4b** was about 28.3 $mg\ L^{-1}$ and the surface tension γ_{CMC} 29.2 $mN\ m^{-1}$. The γ_{CMC} values suggest that the surface activity of the polyborate is as good as the monomer. The subsequent measurements also showed that the NaCl addition has no significant influence on the surface tension of the polyborate **4a** and its monomer **4b** due to their nonionic properties.

3.2. Self-assembly property

The borate samples were dissolved in freshly distilled water. After the solution was kept at room temperature for 24 h, self-assembly morphology of the polyborate and monomer was investigated by TEM directly. As indicated by TEM measurements, no vesicles and other aggregates from monomer **4b** in aqueous solutions were observed, whereas, after addition of NaCl (0.5 M), thread-like micelle morphology appears (shown in Fig. 2a) in this solution.

In previous work [22], we reported that the polyborate **4a** has a tendency to produce spherical polymeric vesicles spontaneously in pure water with diameters of ~20 nm, though they were associated seriously. When NaCl concentration in aqueous solution increases to 0.1 M, **4a** exhibited spontaneous formation of ordered polymersomes with uniform diameters (150–250 nm, see Fig. 2b) without any normal mechanical process, such as extrude, dialysis, sonication. Self-assembly microstuctures generally are dynamic aggregates. Upon higher ionic strength, the molecules' polar group will undergo "dehydration" [19]. The caused increasing hydrophobic effect will reduce their exchange kinetics [17] and drive

Table 1
Values of CMC, γ_{CMC}, the maximum interfacial excess concentration Γ_m, the minimum area per molecule *A*, and the packing parameters *P* of **4a** and **4b** in aqueous solution.

	Solution	CMC/mg L^{-1}	γ_{CMC}/mN m^{-1}	Γ_m/μmol m^{-2}	*A*/nm^2	*P*
Polyborate, **4a**	Water	68.4	31.2	4.85	0.343	0.604
	0.1 M NaCl	67.0	31.1	4.78	0.348	0.596
	0.5 M NaCl	64.0	30.9	4.84	0.344	0.602
Monomer, **4b**	Water	28.3	29.2	2.66	0.625	0.332
	0.1 M NaCl	27.7	29.2	2.60	0.640	0.324
	0.5 M NaCl	26.5	29.0	2.67	0.623	0.334

polyborate molecules to rearrange to form more stable bilayer vesicles. It should be noted that fusion or fission phenomena among these polymersomes were observed, just as the areas designated by white arrows in Fig. 2b.

Further experiments showed self-assembly morphology of **4a** in aqueous solution changed greatly with the increase of NaCl concentration. In 0.5 M NaCl, polymersomes from **4a** were tree-like aggregate structure (Fig. 2c and enlarged 2d). A bilayer is composed of two monolayers. The bilayer curvature free energy, E, can be given by Helfrich-type model [23]:

$$E/A = \frac{1}{2}K\left[(c+c_o)^2+(c-c_i)^2\right] \tag{1}$$

where A is per unit area for bilayers, K the bilayer bending modulus, c the vesicle curvature ($c = 1/R$, R is the vesicle radius), c_o the outer monolayer spontaneous curvature, and c_i the inner monolayer spontaneous curvature. For single component bilayers, c_i necessarily equals c_o. Then we can imagine the free energy E/A will be reduced by decreasing c. When $c = 0$, the bilayers has the lowest E/A and become "flat" with zero spontaneous curvature. High degree of fusion among vesicles raised by increasing NaCl concentration enable polymeric bilayers broken and lower their curvature and then form a long-strip tree-like microstructures. As NaCl concentration in aqueous solution increased to 0.9 M, further fusion of these aggregates was strengthened to make polymeric bilayer curvature low enough to produce flat bilayer structures leading to the formation of a vertebra-like morphology composed of many rectangular aggregates (Fig. 2e and f).

It is well known that ethanol leads to the destruction of surfactant aggregates in that it weakens the hydrophobic property

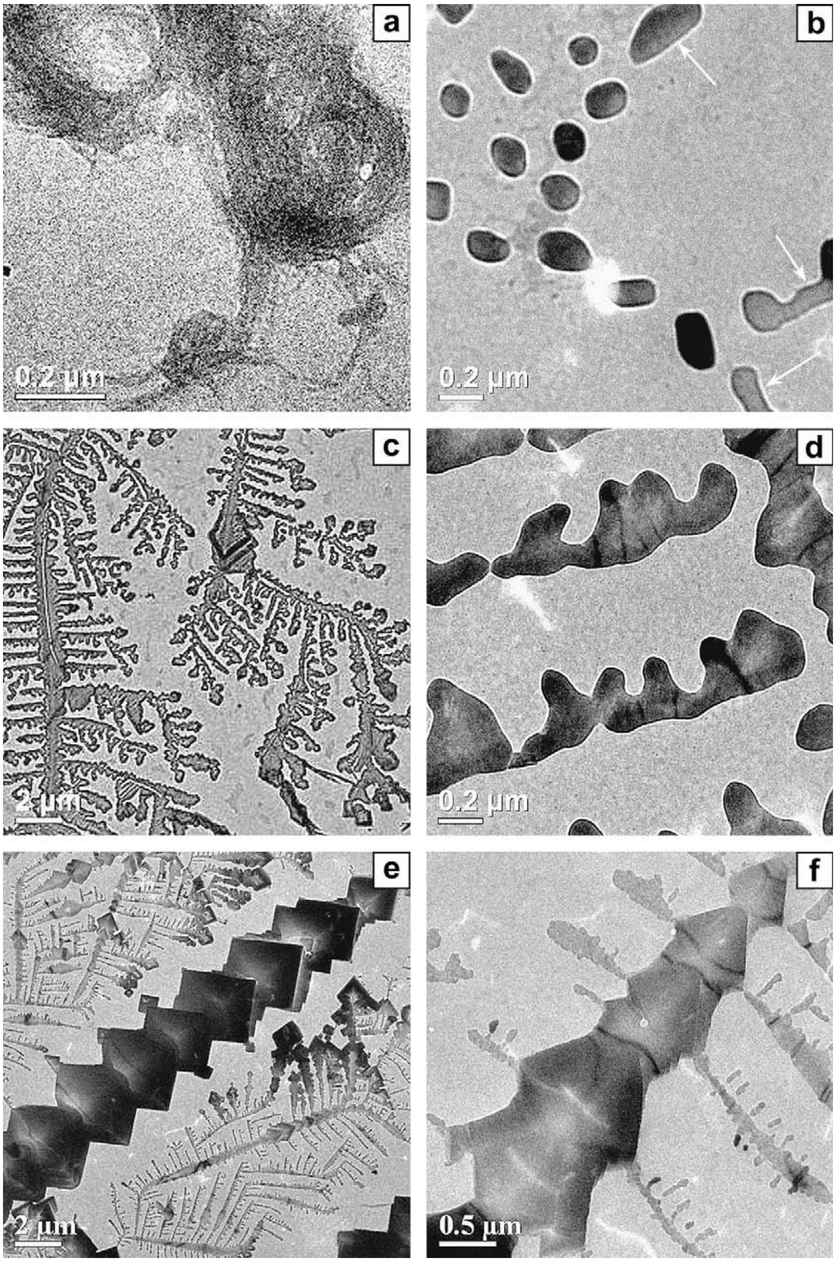


Fig. 2. TEM image of **4b** micelles in (a) 0.5 M NaCl, and TEM image of **4a** aggregates in (b) 0.1 M NaCl, in (c,d) 0.5 M NaCl, in (e,f) 0.9 M NaCl. Vesicle formation coexists with fusion and fission (arrows).

of surfactants [24]. Effects of ethanol addition on morphologies of **4a** aggregates in NaCl aqueous solution were analyzed by TEM, which were shown in Fig. 3. When the volume of ethanol reaches 33% of the total solution volume (V_{ethanol} is equal to 33%), the polyborate **4a** was still found to be able to form spherical polymersomes spontaneously in 0.1 M NaCl (Fig. 3a). It indicates that the formed vesicular aggregates from the polyborate **4a** have a strong resistance to large concentration of ethanol as a result of high toughness and low mobility of polymer bilayers [3,25], which exhibit potential applications in drug-release or nanoreactors. However, when Fig. 3a is compared with 2b, we can see that ethanol has a great impact on the self-assembly of the polyborate **4a** evidently because the polymersomes sizes were decreased after adding ethanol. At higher NaCl concentration ($V_{\text{ethanol}} = 33\%$), **4a** aggregates morphology changes greatly: in 0.5 M NaCl, the aggregates transformed from tree-like morphology into tubular morphology while in 0.9 M NaCl the vertebra-like morphology was also impaired greatly (Fig. 3c). It is interesting that a number of spherical polymersomes appeared again and aligned in a highly ordered array with increasing ethanol concentration in 0.9 M NaCl, though the polydiversity increased (Fig. 3d). This implied there should exist some interactions among them. These phenomena also indicate that the complicated microsturctures of tree-like and vertebra-like morphologies (in Fig. 2c and e) originate from the self-assembly of the polyborate and its fusion process. As a whole, aggregates morphology of **4a** in aqueous solution could be expected to be adjusted by addition of NaCl and ethanol.

3.3. Mechanism of vesicle formation

The concept of molecular packing parameter introduced by Israelachvili, Mitchell and Ninham has been widely applied to explain self-assembly phenomenon of amphiphilic molecules [12,26,27]. The molecular packing parameter is defined as $P = v_c/a_0 l_c$, where v_c is the volume of the hydrophobic tail of amphiphilic molecules, l_c is the length of the hydrophobic tail, and a_0 is the equilibrium head group area of the molecule at the aggregate surface. Generally, v_c and l_c can be calculated by using Eq. (2) and Eq. (3) [28] respectively, and a_0 can be obtained using Gibbs Equation, Eq. (4) and Eq. (5) [29].

$$v_c = (27.4 + 29.6n) \times 10^{-3}\ \text{nm}^3 \tag{2}$$

$$l_c = (0.15 + 0.1265n)\ \text{nm} \tag{3}$$

$$\Gamma_m = \left(\frac{1}{2.303RT}\right)\left(\frac{\partial\gamma}{\partial \log C}\right)_T \tag{4}$$

$$A = \frac{1}{N_A \Gamma_m} \tag{5}$$

where n is carbon number of hydrophobic chain, Γ_m is the maximum interfacial excess concentration in moles per square meter, A is the minimum area per molecule, $\partial\gamma/\partial\log C$ is the slope of the surface tension of amphiphilic molecules, and N_A is Avogadro's constant. The maximum interfacial excess concentration is obtained from the slope of the surface tension versus the logarithm of the concentration. Packing parameter P can be calculated by approximating the a_0 by the A obtained from the surface tension.

The well-known relation between the molecular packing parameter and the aggregate shape are as follows: $0 \le P \le 1/3$ corresponds to sphere micelles (cone-shaped amiphiphilies molecules), $1/3 < P \le 1/2$ corresponds to rod/thread-like micelles (truncated cone), $1/2 < P \le 1$ to bilayer or vesicle (larger truncated cone).

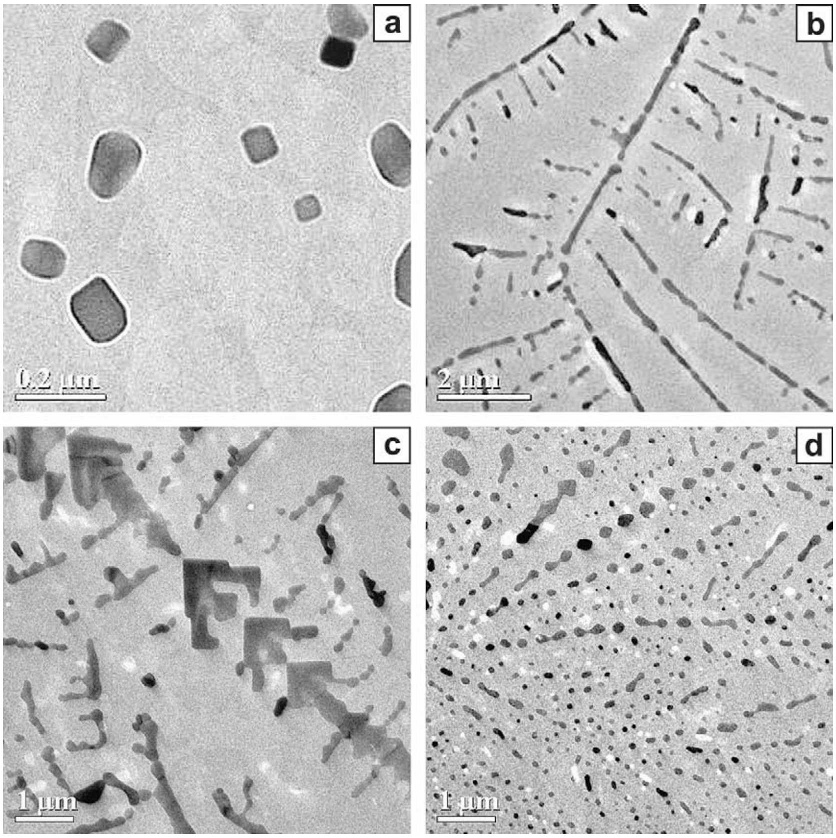
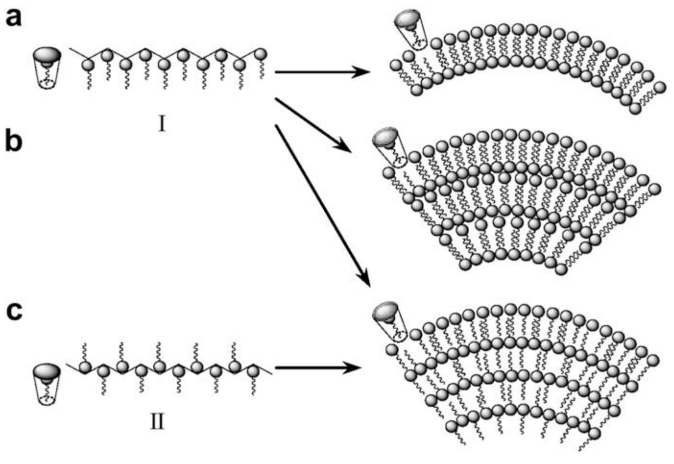


Fig. 3. TEM images of **4a** at $V_{\text{ethanol}} = 33\%$ in (a) 0.1 M, (b) 0.5 M and (c) 0.9 M NaCl; and TEM image at $V_{\text{ethanol}} = 66\%$ in (d) 0.5 M NaCl.



Scheme 2. (a) Unilamellar vesicle formation by structure I of **4a**, (b) multilamellar vesicle formation by structure I of **4a**, and (c) multilamellar vesicle formation combined by structure I and II of **4a**.

Vesicle formation of the polyborate **4a** could be explained by packing parameter theory. Here, the polyborate **4a** was considered as monomer **4b** congeries formed in some ways, in other words, we regarded the polyborate **4a** solution as an analogic monomer solution to carry out the calculations. Then, depending on l_c, v_c and a_0 of single molecules (segment) by measurement of surface tension isotherms of the amphiphiles in aqueous solution, we calculated the packing parameter ($P = v_c/a_0l_c$), which are listed in Table 1.

The addition of NaCl almost does not influence the values of P, Γ_m and A (Table 1). Nevertheless, the A of polyborate **4a** at interfaces in both water and NaCl aqueous solution are much lower than monomer **4b**. This would be attributed to the covalent link between monomer segments along the polyborate hydrocarbon chain. As a result, p values of the polyborate **4a** increase significantly to about 0.6 in contrast to about 0.32 of the monomer **4b** in both solutions. Thus, according to the packing parameter theory, the monomer **4b** molecules would show cone-shape in aqueous solution, and it is likely that no bilayer aggregates or vesicles but only thread-like micelles would appear theoretically, as was confirmed by TEM. High concentration of NaCl is beneficial to self-assembly of monomer **4b** consequently. On the other hand, since P values of the polyborate **4a** $> 1/2$, monomer segment of **4a** main chain in both water and NaCl solution would be larger truncated-cone shape and is consequently apt to form bilayers or vesicles. Generally the walls in such aggregates are formed with the hydrophobic chain of amphiphilic polymers [20]. A possible mechanism of **4a** vesicle formation in aqueous solution is illustrated in Scheme 2. The hydrophobic chains of **4a** are expected to be distributed at the same side (see structure I in Scheme 2) or contrary side (see structure II in Scheme 2) along the polymer backbones. As a result, the macromolecules possessing these structures would be located, according to the Scheme 2, to construct the outer and internal layer of asymmetry bilayers respectively with a nonzero spontaneous curvature, and then was assembled with each other to form unilamellar or multilamellar vesicles. The increased membrane thickness leads to low permeability and high stability of the polymersomes. After NaCl addition, the dehydration effect and fusion process will make vesicles more stable or lower the bilayer curvature to form the tree-like or vertebra-like morphologies.

4. Conclusions

In summary, a novel amiphiphilic polyborate in aqueous solution was synthesized, which has 28,400 g mol^{-1} molecule weight and high surface activity close to its monomer. It is more interesting that TEM measurement indicates that the obtained polyborate could spontaneously self-assemble to form spherical polymersomes of low polydiversity and the transition to tree-like and even vertebra-like structures in aqueous solutions was found with increasing NaCl concentration. Ethanol generally is liable to disrupt amphiphilies microstructures, however, polyborate at V_{ethanol} $\% = 33\%$ still can form stable polymersomes in 0.1 M NaCl. The packing parameter theory is helpful to give an illustration of the formation mechanism of the polyborate vesicles. After ethanol addition, the tree-like and vertebra-like structures from polybotate were found to be disrupted to form tubular structure or make spherical polymersomes reemerged again in solution. The self-assembly morphologies from polyborate are tunable by addition of NaCl and ethanol, which may provide some possibilities for drug-release vehicles and nanoreactor.

Acknowledgments

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