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Vesicles made of PS-PI cyclic diblock copolymers: *In situ* freeze-drying cryo-TEM and dynamic light scattering experiments

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We have studied the morphology of self-assembled micelles made of linear and cyclic poly(styrene-*b*-isoprene) PS-*b*-PI block copolymers dispersed in selective solvents of the PI block (n-heptane, n-decane). Up to a copolymer concentration of 5 mg mL⁻¹, the micelles made from linear block copolymer chains adopt a spherical shape. Those arising from cyclic copolymer chains having exactly the same molar mass and volume fraction self-assemble into (i) planar sunflower-shaped particles at low concentration ($c < 0.1$ mg mL⁻¹), (ii) giant wormlike micelles at intermediate concentration ($0.1 \text{ mg mL}^{-1} < c < 2$ mg mL⁻¹) and (iii) vesicles at higher concentration ($2 \text{ mg mL}^{-1} < c < 5 \text{ mg mL}^{-1}$). Those results were obtained using dynamic light scattering and *in situ* freeze-drying cryo-transmission electron microscopy. In this contribution, we discuss the effects of concentration and temperature on the morphology of the self-assembled particles made from both linear and cyclic PS-PI copolymers, and highlight the surprising vesicle formation in cyclic block copolymer solutions.

1. Introduction

A substantial challenge in modern colloid chemistry is to find ways to control the size and morphology of colloids at the nanometric scale. In this context, the aggregates or self-assembled structures made from block copolymers have proven to be valuable tools. Micelles, microemulsions, vesicles and a huge variety of microphase textures may be used as structural templates for new materials. Diblock and triblock copolymers can form micelles when dissolved in a selective solvent (in which only one of the blocks is soluble). The increasing number of industrial applications for these systems attracted a large interest in both applied science and basic research.¹⁻⁸ For instance, copolymer micelles have been used as drug carriers in delivery systems,^{9,10} stabilizers in organic reactions¹¹ as well as nanotemplates for nanotechnologies.^{7,8}

Most of the published work deals with amphiphilic block copolymers, where the micelles are formed in water because one of the blocks is hydrophilic and the other hydrophobic. The size and thereby the aggregation number of those micelles are rather independent of the polymer concentration, but may change with the temperature or the copolymer composition (or volume fraction).^{1,12} They usually have a spherical shape, with a very compact core and a more flexible corona. However, it was also shown in numerous studies that block copolymers can self-assemble into a large variety of objects such as rod-like¹ or wormlike⁶ micelles, cylinders,⁵ vesicles,^{11–13} hollow spheres,⁷ branched tubules,⁹ *etc.* Commercial applications of those different nanostructures call for a high degree of control on the morphology of the resulting materials. As has been demonstrated by Eisenberg and coworkers,^{12–16} as well as by Bates and coworkers^{17–22} during the past decade, several parameters can be modified and controlled in order to tailor the micellar morphology: temperature, copolymer composition or concentration and sample preparation.

Micelles made from diblock copolymers can also be prepared in organic solvents.^{23–29} In this case, the copolymer may have chemically distinct hydrophobic blocks. The most common block copolymers are constituted by an aromatic block attached to an aliphatic one. Copolymers consisting of polystyrene (PS) and polyisoprene (PI) blocks, as well as the corresponding self-assembled micelles are well documented in the literature.^{2,23–26} Micelles made of highly asymmetric PS–PI copolymer chains are usually spherical.² The size and shape of the individual micelles do not change when the concentration of the copolymer within the micellar disordered regime increases. The core can be very compact and, depending on the solvent quality (or the insolubility of the inner block), these micelles can be ‘frozen’, and not exchange any unimer,¹⁰ or mobile,³⁰ thus implying that the PS core is swollen.

The copolymer architecture plays a very important role in the micellar morphology.³¹ The case of cyclic copolymers has recently been investigated.^{29,31–36} In previous works,^{34–36} we have shown that the micelles made from asymmetric linear PS–PI (at a PS volume fraction of $\Phi_{\text{PS}} = 0.78$) adopt a ‘classical’ spherical shape and keep the same morphology over the range of considered concentrations. In contrast, the micelles formed from asymmetric cyclic copolymer chains exhibit different sizes and shapes. They evolve from small individual ‘sunflower’ micelles at low concentration to giant wormlike micelles at higher concentration. In those studies, we used dynamic light scattering (DLS), small-angle X-ray scattering (SAXS), transmission electron microscopy (TEM) and atomic force microscopy (AFM) to characterize the micellar morphology of both linear and cyclic copolymers systems having the same degree of polymerization (DP), *i.e.* 290 for PS and 110 for PI (corresponding to a volume fraction $\Phi_{\text{PS}} = 0.78$) and dispersed in heptane.

In the present paper, we shall describe the effect of concentration in both systems using DLS in conjunction with *in situ* freeze-drying cryo-TEM in two selective solvents, namely n-heptane and n-decane. In particular, we document a new result: when dispersed in heptane at relatively high concentration (2–5 mg mL^{−1}), the cyclic PS–PI copolymer chains form vesicles whereas the analogous linear block copolymer chains self-assemble into spherical micelles.

2. Experimental section

2.1. Sample preparation

Linear diblock copolymers (DP_{PS} = 290 and DP_{PI} = 110) with a total molecular weight of 37.10³ g mol^{−1} (determined by SEC-GPC analysis) were prepared using living anionic polymerization, as described elsewhere.³⁷ Cyclic poly(styrene-isoprene) was prepared by direct coupling of α -isopropylidene-1,1-dihydroxymethyl- ω -diethylacetal-heterodifunctional linear poly(styrene-*b*-isoprene). The fact that the cyclic copolymers are prepared by closure of the linear diblocks ensures that PS and PI blocks have exactly the same DP in linear and cyclic copolymers. Cyclic A–B copolymers have been also obtained by closing A–B–A triblock chains, as has been described by Hogen-Esch *et al.*^{38,39} or, more recently, by Hadjichristidis *et al.*²⁹

Copolymer solutions in heptane and decane were prepared according to two methods. The ‘direct dissolution’ method consisted of preparing solutions by directly dissolving proper amounts of copolymer to achieve predetermined concentrations. The solutions were continuously stirred for 72 h at 50 °C, under inert atmosphere. In the ‘dilution’ method, a mother solution was prepared at 5.0 mg mL^{−1} in the same conditions and subsequently diluted with pure solvent in order to achieve a

range of lower concentrations. All solvents were HPLC grade. They were distilled and filtered through a 0.2 µm PTFE membrane prior use.

In the following, micelles made of the linear PS–PI block copolymer will be referred to as ‘L-mic’ whereas those made of cyclic copolymers will be called ‘C-mic’.

2.2. Dynamic light scattering

The DLS measurements⁴⁰ were performed using an ALV Laser Goniometer, which consists of a cylindrical 22 mW HeNe linear polarized laser with a 632.8 nm wavelength and an ALV-5000/EPP Multiple Tau Digital Correlator with a 125 ns initial sampling time. The samples were kept at a constant temperature of 25.0 °C throughout the experiments. The accessible scattering angle of this equipment ranges from 12° up to 155°. All samples were systematically studied at 90° and some of them were studied at different scattering angles. The solutions were put in ordinary glass cells, 10 mm in diameter. The minimum sample volume required for an experiment was 1 mL. The data acquisition was done with the ALV-Correlator Control Software and the counting time varied for each sample from 300 s up to 15 min, depending on the sample, its concentration and the required time to build up fairly good correlation functions.

The autocorrelation functions of the scattered intensity were analyzed by means of the cumulant method to yield the effective diffusion coefficient as a function of the scattering angle. The analysis of the cumulant expansion of the correlation function is performed by fitting a polynomial up to third order to the function $\ln[g_2(t)-1]$. The polynomial coefficients are converted into the coefficients of the cumulant expansion of the field correlation function:

$$\ln(g_2(t) - 1) = -\bar{\Gamma}t + \frac{\mu^2}{2!}t^2 - \frac{\mu^3}{3!}t^3 \quad (1)$$

The mean apparent hydrodynamic radius R_h is calculated from the time of 1st to 3rd order fitting according to the Stokes–Einstein equation.

$$R_h = \frac{kT}{6\pi\eta\bar{\Gamma}}q^2 \quad (2)$$

The polydispersity index I_p was calculated from the second moment μ_2 of 2nd and 3rd order fitting:

$$I_p = \frac{\mu^2}{\bar{\Gamma}^2} \quad (3)$$

In addition, the constrained regularization method CONTIN⁴¹ was used to obtain the distribution $A(t)$ of decay times. For the latter, a statistical parameter ‘probability to reject’ is calculated for each solution, and the suggested one is that closest to 0.5.

$$\left(\frac{G^{(2)}(q, t)}{B} - 1 \right)^{1/2} = \int_0^\infty A(\tau) e^{-(t/\tau)} d\tau \quad (4)$$

These methods are now routinely used to analyze the DLS data for polymer and colloidal systems and allows the determination of the relaxation modes which characterize the dynamics.

2.3. *In situ* freeze drying cryo-TEM

TEM observations were performed using the same solutions as those used for the DLS experiments. However, they generally had to be diluted with pure solvent to achieve concentrations compatible with cryo-TEM sample preparation. Typically, copolymer solutions prepared in heptane or in decane at concentrations of 0.1 mg mL^{−1} and 0.5 mg mL^{−1} were diluted 10× and those prepared at 2 mg mL^{−1} and 5 mg mL^{−1} were diluted 100×. In recent years, cryo-TEM has been successfully used to observe various polymer nanoparticles dispersed in water^{42–45} and, in very few cases, in organic solvents.⁴⁶ According to the cryo-TEM procedure,⁴⁷ our specimens were prepared by

quenching thin films of the suspensions formed on NetMesh (Pelco, USA) 'lacey' carbon films, using a guillotine-type device. However, as most organic solvents are known to dissolve in liquid ethane, fast-freezing was performed in liquid nitrogen. The specimens were then mounted onto a Gatan 626 specimen holder cooled with liquid nitrogen, transferred into the microscope and observed at -180°C . As the freezing speed was not sufficiently high in liquid nitrogen, heptane appeared as crystalline lamellae and diffraction effects dramatically affected the contrast of the embedded objects. Therefore, the crystalline solvent was freeze-dried in the microscope by warming the sample to -115°C . Around this temperature, frozen heptane completely sublimated and many embedded objects were revealed,^{34–36} the smaller ones clinging to the edges of the supporting 'lacey' carbon membrane while the larger ones entangled across the holes in the film. Samples prepared from copolymers solutions in decane were warmed to about -70°C . The heating was immediately stopped after evaporation and micrographs were recorded after a return to complete sample stability, at a temperature of -180°C . All samples were observed under low dose conditions, using a Philips CM200 'Cryo' microscope operated at 80 kV. The micrographs were recorded on Kodak S0163 films.

3. Results and discussion

3.1. Dynamic light scattering

The DLS autocorrelation functions were measured for self-assembled L-mic and C-mic particles dispersed in PI-selective solvents (heptane or decane) at several concentrations, using the direct dissolution method. Typical results are illustrated in Figs. 1a and b. One observes that the autocorrelation functions measured for L-mic are mono-exponential (single relaxation time) and do not depend on the concentration from $c = 0.5 \text{ mg mL}^{-1}$ to 5.0 mg mL^{-1} (Fig. 1a). In contrast, those measured for C-mic present a multimodal distribution of relaxation times, as has been already reported.³⁴ In addition, the relaxation times deduced from the cumulant analysis increase with concentration. The variation of the corresponding diffusion coefficient D , calculated from $(\Gamma/q^2)_{q \rightarrow 0}$, as a function of the concentration is plotted in Fig. 2. In the case of linear block copolymer, the diffusion coefficient $D(c)$ and therefore the size are obviously independent of c while they rapidly decrease with concentration in the case of C-mic with a negative value of k_D in $D(c) = D_0(c)(1 + k_D c)$. This result indicates that the hydrodynamic size of C-mic particles is an increasing function of the concentration ($R_H \approx D^{-1}$) whereas that of L-mic is roughly constant.

Using the Stokes–Einstein relation (see eqn. (2)), R_H can be extracted from the relaxation time of frequency of the DLS autocorrelation functions. The hydrodynamic diameters $D_H = 2R_H$ of L-mic and C-mic are presented in Table 1 as a function of concentration. One observes that the size of C-mic strongly depends on the copolymer concentration, while this parameter is roughly constant for L-mic in the same conditions. Accordingly, L-mic have roughly a constant size of about 50 nm in both heptane and decane for all concentrations (0.01 up to 5 mg mL^{-1}) and temperature (see further in the discussion below). The dense core contains PS blocks while the more diffuse corona is made of PI. As one may expect, the excluded volume repulsion between the PI shells prevents an increase of the micelle size and at the same time promotes an increase in the number of micelles. The difference in size in heptane and in decane systems is directly related to the solubility of the inner PS block that decreases with the number of carbon atoms in the alkane chain used as a selective solvent. Indeed, as it has been shown by Quintana *et al.*^{48,49} n-alkanes become worse solvents for polystyrene as the n-alkane carbon number increases. This behavior is remarkable for n-heptane and suggested less repulsive polystyrene/solvent interactions than higher n-alkanes. For that reason a slightly larger size of the micelles in heptane as compared to decane is systematically observed (see Table 1).

However, as seen in Table 1, the cyclization of the linear PS–PI leads to a different behavior: C-mic are larger in heptane than in decane and their size rapidly increases with the concentration. One may explain this result as follows: C-mic are not spherical but elongated objects where the PS core is more exposed or available for potential PS–PS interactions/attractions. Therefore, increasing the polymer concentration increases the probability that two particles interact by strong core–core (PS–PS) interactions. This explains the rapid increase of the C-mic size with the concentration: from 96.6 nm to 644.5 nm in heptane and from 38.2 nm to 180.0 nm in decane. The difference in size in heptane and decane for cyclic systems could also be explained on the basis of the solubility of the

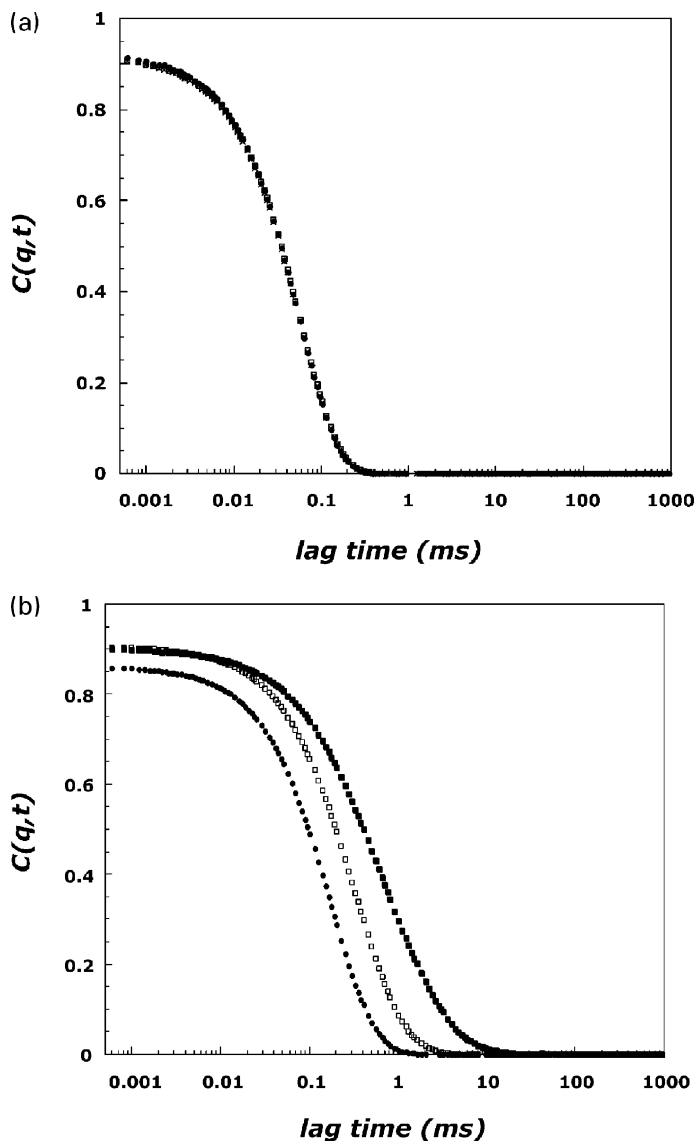


Fig. 1 DLS correlation functions measured at 25 °C: (a) micelles made of linear PS-PI at 0.5 mg mL⁻¹ (open squares), 2 mg mL⁻¹ (filled circles) and 5 mg mL⁻¹ (crosses) in heptane; (b) micelles made of cyclic PS-PI at 0.5 mg mL⁻¹ (filled circles), 2 mg mL⁻¹ (open squares) and 5 mg mL⁻¹ (filled squares) in heptane. The solutions were prepared using the direct dissolution method.

inner PS block that decreases with the number of carbon atoms in the alkane chain used as a selective solvent. PS is less soluble in decane than in heptane leading to a more compact morphology in decane than in heptane and therefore a smaller size. For that reason, the dependence of C-mic size on copolymer concentration is less pronounced in decane than in heptane. At concentrations lower than 1 mg mL⁻¹, however, the size of C-mic is smaller than L-mic in decane. This result is still intriguing.

The concentration is not the only parameter that affects the size of C-mic. By decreasing the temperature to 10 °C, we observed an important increase in the size for C-mic, while that of L-mic did not depend on temperature. This is illustrated in Fig. 3, where we show the effect of

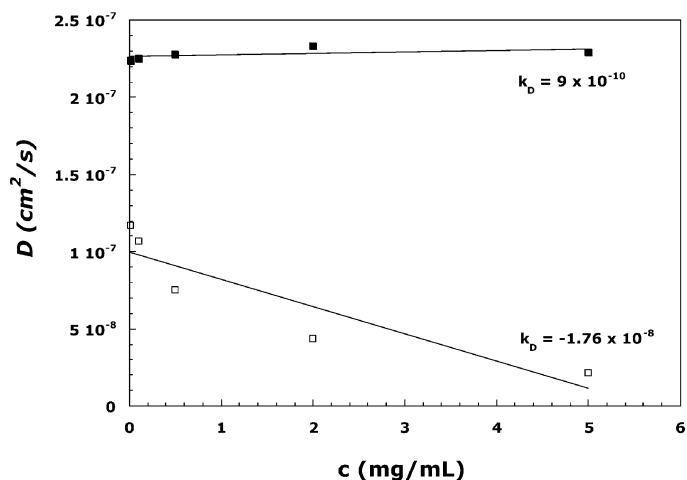


Fig. 2 Diffusion coefficient for cyclic PS-PI (open squares) and linear PS-PI (filled squares) as a function of copolymer concentration in heptane.

concentration on the R_H of L-mic and C-mic formed in decane at two different temperatures. It is important to note that the size of C-mic does not change with temperature at low copolymer concentration, but drastically increases at higher concentration. For instance, at $c = 5 \text{ mg mL}^{-1}$, $R_H = 90 \text{ nm}$ and 173 nm at 25°C and 10°C respectively. On the other hand, Table 1 and Fig. 3 show that the L-mic size does not depend either on the copolymer concentration or on the temperature.

C-mic exhibit another distinct feature: in DLS, the angular dependency is more pronounced than in the case of L-mic. While the relaxation times for L-mic do not depend on the scattering angle, those measured for C-mic strongly depend on the scattering angle. This behavior is illustrated on Figs. 4a and b for linear and cyclic cases respectively where the distribution of the hydrodynamic radius is given using Contin analysis. This polydispersity in size and certainly in morphology becomes progressively important as the copolymer concentration increases. These results confirm that: (i) C-mic are not spherical but elongated objects—in this case, the angular dependency could be driven by the anisotropy of these nanoparticles coupled with their internal motion; (ii) C-mic are polydisperse since the relative contribution of smaller and bigger objects to the total scattering strongly depends on the scattering angle. This would also contribute to the origin of the angular dependency of the relaxation times and therefore to the deduced R_H values. The second result is supported by the polydispersity index deduced from the 2nd order cumulant fit of the autocorrelation functions. Fig. 5a shows that the polydispersity of C-mic increases as a function of the copolymer concentration, while it is constant for L-mic. In other words, while L-mic have a

Table 1 Hydrodynamic diameter calculated from DLS correlation functions 2nd order cumulant fit analysis of micelles made of linear and cyclic PS-PI

$c/\text{mg mL}^{-1}$	Linear PS-PI L-mic hydrodynamic diameter/nm ^a		Cyclic PS-PI C-mic hydrodynamic diameter/nm ^a	
	Decane	Heptane	Decane	Heptane
0.01	51.0	51.3	38.2	96.6
0.10	50.4	52.1	40.1	106.3
0.50	50.4	50.7	45.1	150.3
1.00	50.2	50.8	51.5	180.5
2.00	48.0	49.6	70.3	276.8
3.00	48.6	49.5	85.9	412.3
5.00	48.9	49.8	180.0	644.5

^a Calculated from the diffusion coefficient at 25°C .

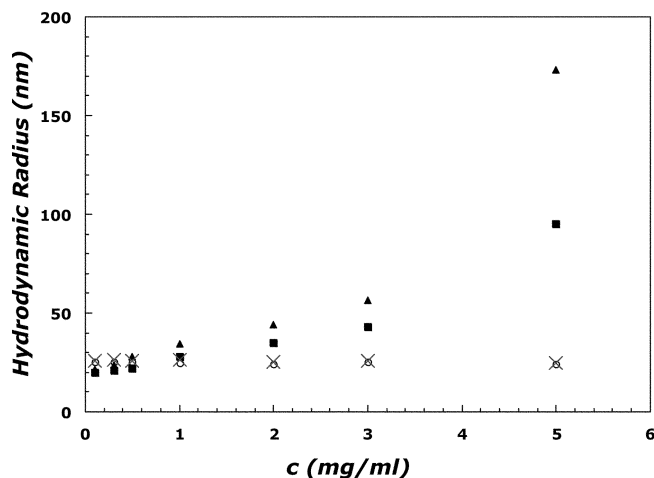


Fig. 3 Hydrodynamic radius as a function of copolymer concentration in decane for cyclic PS-PI at 10 °C (filled triangles), cyclic PS-PI at 25 °C (filled squares), linear PS-PI at 10 °C (empty circles) and linear PS-PI at 25 °C (crosses); scattering angle: 90°.

constant and monodisperse size in all the concentration ranges investigated, C-mic are growing in size, changing their morphology and becoming more polydisperse as the copolymer concentration increases. This is also illustrated in Fig. 5b.

Fig. 6 shows DLS autocorrelation functions for 3 sets of C-mic solutions prepared by both the direct dissolution and dilution methods (described in the Experimental Section). These samples, having the same final copolymer chain concentration, have distinct scattering properties. The micelles in samples prepared by dilution have the same size as in the mother solution, suggesting that the morphology of the micelles, once formed, is kept. As shown in the following section, these results were confirmed by TEM. Under such circumstances, it is worth noting that the only way to study the effect of concentration on C-mic is to prepare each sample by direct dissolution since the dilution would affect the number of particles and not their morphology. Interestingly, low concentration solutions obtained by the dilution method proved to be very useful in studying the shape of individual ‘basic’ micelles.

At higher concentrations (2–5 mg mL⁻¹) and particularly for C-mic in heptane, both elastic and quasi-elastic light scattering results indicated the presence of multimodal distributions in size and relaxation times. These features are typical of aggregate formation. Therefore, a series of direct observations using *in situ* freeze-drying cryo-TEM was carried out in order to shed light on the structure of the self-assembled aggregates. Some results obtained using AFM³⁵ and TEM^{34,36} have already been reported.

3.2. *In situ* freeze-drying cryo-TEM

3.2.1. Solutions in heptane. Cryo-TEM observations were performed to visualize and compare the morphology of L-mic and C-mic. As described in the Experimental Section, solutions prepared by direct dissolution were subsequently diluted with pure solvent in order to achieve concentrations that were compatible with cryo-TEM. DLS experiments have shown that the morphology and size of the micelles were unaffected by the dilution (Fig. 6). As a consequence, the objects observed by cryo-TEM should be similar to those present in the mother solution prepared by direct dissolution. This was also supported by AFM imaging results.³⁵

Fig. 7 shows micelles resulting from linear block copolymer chains dispersed in heptane. The micelles are seen spread on the surface of an extended area of carbon film after *in situ* freeze-drying of the embedding heptane. The observation does not provide information on the dimension parallel to the electron beam, *i.e.* on the thickness of the micelles spread on the carbon. However, the examination of many micelles clinging to the edges of the holes in the film, where there is no reason

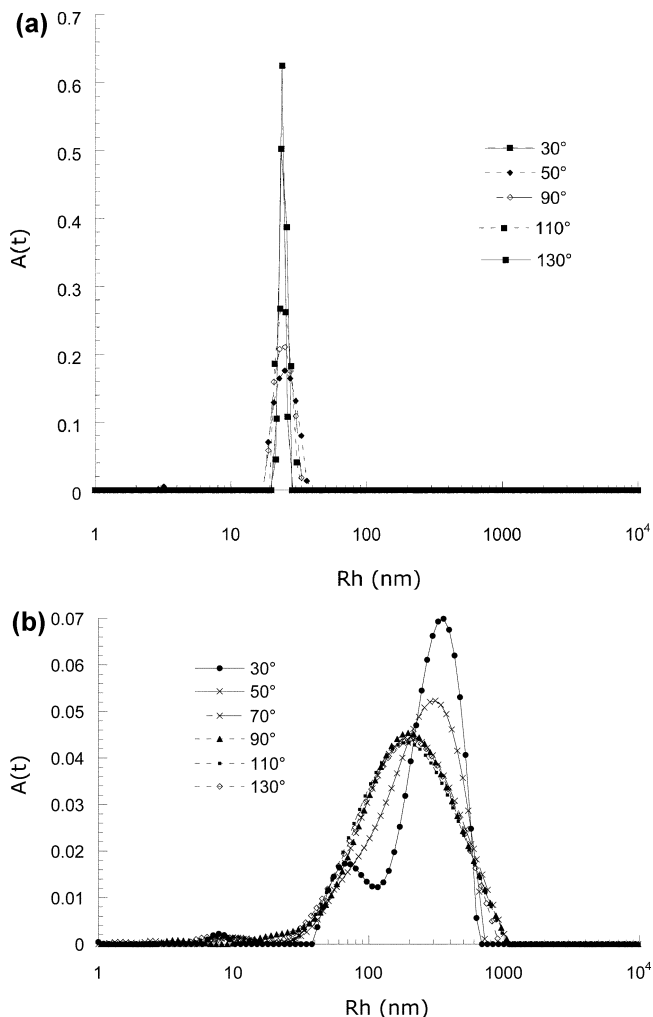


Fig. 4 CONTIN analysis from the DLS correlation functions of PS₂₉₀-PI₁₁₀ at 5 mg mL⁻¹ in heptane at different angles for (a) linear copolymer; (b) cyclic copolymer.

for the objects to adopt a preferential orientation, convinced us that the micelles were single and monodisperse spherical objects, with a diameter of about 35 nm. Their size and morphology are independent of the initial micellar concentration [(a) 0.5 mg mL⁻¹; (b) 5 mg mL⁻¹]. The slight difference in size observed between cryo-TEM and DLS data, as well as the reason why the PI corona could not be clearly resolved in TEM images, have been discussed in previous papers.^{34–36} They were attributed to the low volume fraction of PI ($\Phi_{PI} = 0.22$) and probable shrinkage of the PI corona during the freeze-drying of the embedding solvent.

The morphologies developed by C-mic are readily illustrated in Fig. 8. They are clearly different from those observed for L-mic. In Figs. 8a and b, one can see a mixture of different objects, either spread on the surface of an extended carbon area (Fig. 8a) or clinging to the lacey carbon network (Figs. 8b): seemingly spherical particles about 20 nm in diameter, 70–100 nm-long rodlike and 250–300 nm-long wormlike objects, as well as toric micelles with a circumference of 200–300 nm. The last two classes of objects have a similar cross-sectional width, 20 nm, which is also the diameter of the individual particles. In these images, the darker parts on the micelles may correspond to thicker sections that point towards the viewer. During freeze-drying, the temperature did not exceed

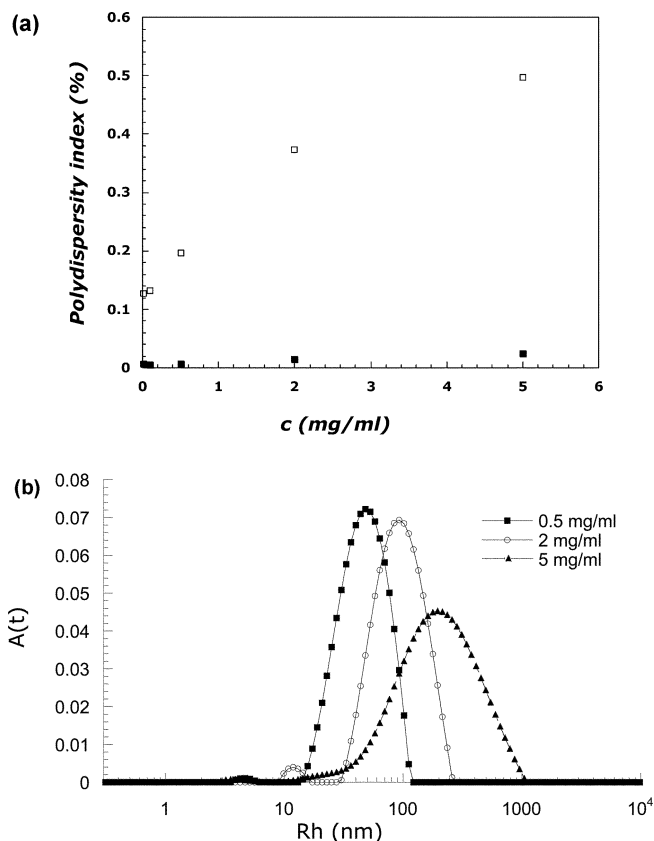


Fig. 5 (a) Polydispersity index calculated from the second moment μ_2 of 2nd order cumulant analysis according to relations (1)–(3) (see text) for cyclic PS-PI (open squares) and linear PS-PI (filled squares) solutions in heptane; (b) CONTIN analysis from the DLS correlation functions of cyclic PS₂₉₀-PI₁₁₀ in heptane (angle 90°) at 0.5 mg mL⁻¹ (filled squares), 2 mg mL⁻¹ (open circles) and at 5 mg mL⁻¹ (filled triangles).

–115 °C, which is much lower than the respective glass transition temperatures (T_g) of PS and PI. It is unlikely that the micelles could extensively deform on the supporting carbon membrane during freeze-drying. Thus, they certainly retained the 3D conformation they had in the thin liquid film of embedding heptane.

The circular 20 nm objects seen in Fig. 8a may correspond to individual sunflower micelles with a core formed by the PS half-loops and the corona by the PI half-loops. The smaller diameter of these micelles compared to that of the spherical L-mic is explained by the cyclic conformation of the copolymer chain, the looping of each block leading to shorter cores and coronas (see Scheme 1). Two opposing constraints control the shape of individual C-mic at low concentration in a PI-selective solvent: (i) the more diffuse PI corona ‘protects’ the dense PS core, favoring a spherical distribution of the molecules; (ii) the cyclic conformation of the PI loops extending out in the solvent promotes a lenticular sunflower-like arrangement. When the concentration increases, the sunflower-like C-mic pack unidirectionally to form more favorable cylindrical wormlike micelles.^{34–36} The PS core is more efficiently protected, the effect of the opposing constraints being mostly felt at the ends of the wormlike micelles.

The wormlike C-mic formed in 0.5 mg mL⁻¹ solutions appear to be tortuous, entangled and substantially longer ($> 1 \mu\text{m}$) than those observed in 0.1 mg mL solutions (Fig. 8c). However, they still have a similar and regular cross-sectional diameter of 20 nm. Looking carefully at different TEM images (Fig. 8c and d), so-called ‘Y-junctions’ can also be seen. The existence of such Y-junctions has been predicted^{50,51} and recently observed in surfactant/oil/water²² and PB-PEO/

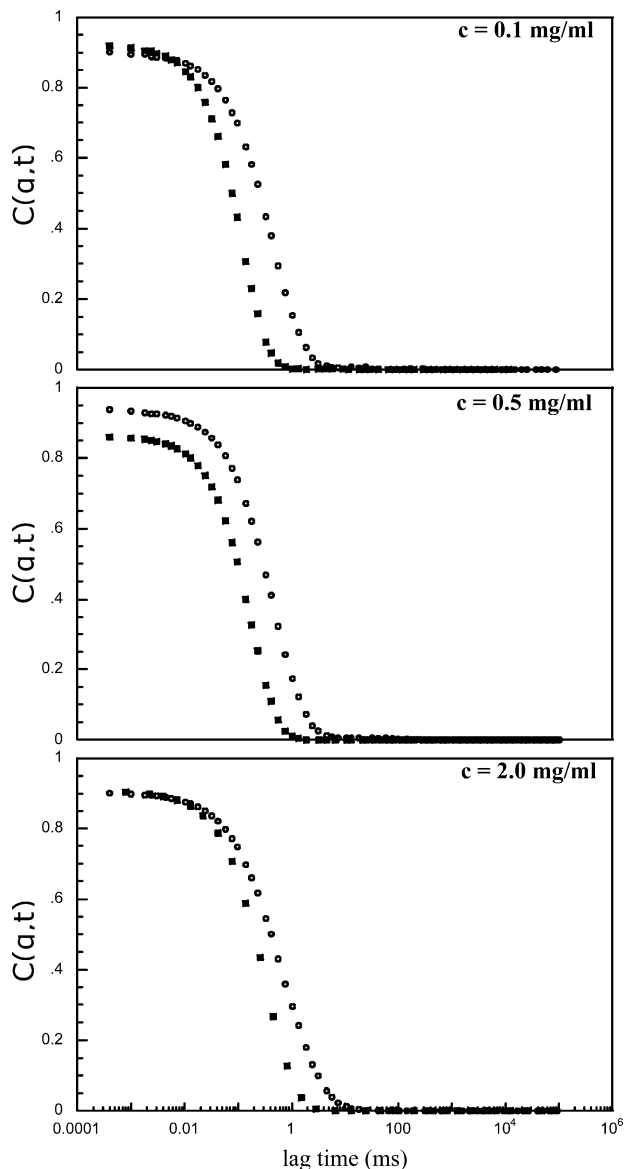


Fig. 6 DLS autocorrelation functions of micelles made from cyclic PS-PI in heptane at three different concentrations. We illustrate here the two methods of sample preparation: direct dissolution of the required amount of copolymer in heptane (filled squares) and dilution from a 5 mg mL^{-1} stock solution (open circles).

water^{52,53} systems. It has been demonstrated that these Y-junctions were stable above a critical molecular weight. In the 2 mg mL^{-1} preparation, among a majority of endless wormlike micelles, new circular objects are observed (Fig. 8d) with a diameter of a few hundreds nm. Tilt experiments in the angular range of $-30^\circ/+30^\circ$ (not shown) convinced us that these particles were spheroidal vesicles with a regular wall thickness of 20 nm. The walls appear to be darker because of the larger volume of material crossed by the electrons. In conventional TEM images recorded from similar suspensions air-dried on continuous carbon films, the vesicles appeared as deflated balloons (not shown). This means that while the rigid vesicles retained their spherical shape during the freeze-

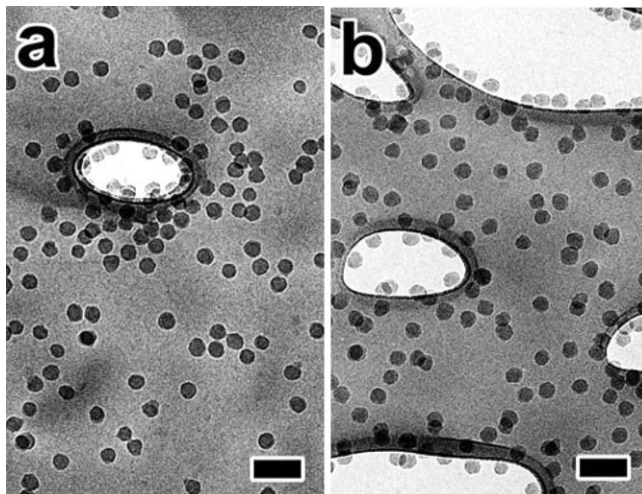


Fig. 7 Influence of the initial copolymer concentration on the morphology of micelles from linear PS-PI dispersed in heptane: (a) 0.5 mg mL^{-1} ; (b) 5 mg mL^{-1} . The concentrations indicated are those at which the solutions were prepared. They were diluted $10\times$ and $100\times$ respectively before sample preparation. The embedding heptane was freeze-dried in the microscope. The gray areas correspond to extended surfaces of supporting carbon while the white areas are holes in the membranes (scale bars: 100 nm).

drying of heptane in vacuum (at -115°C), they were softer at room temperature and did not withstand the surface tension forces during the air-drying of heptane.

Similar vesicles with a diameter ranging from 100 nm to 400 nm and wormlike micelles were observed in the samples that were prepared at 5 mg mL^{-1} although it was difficult to assess the

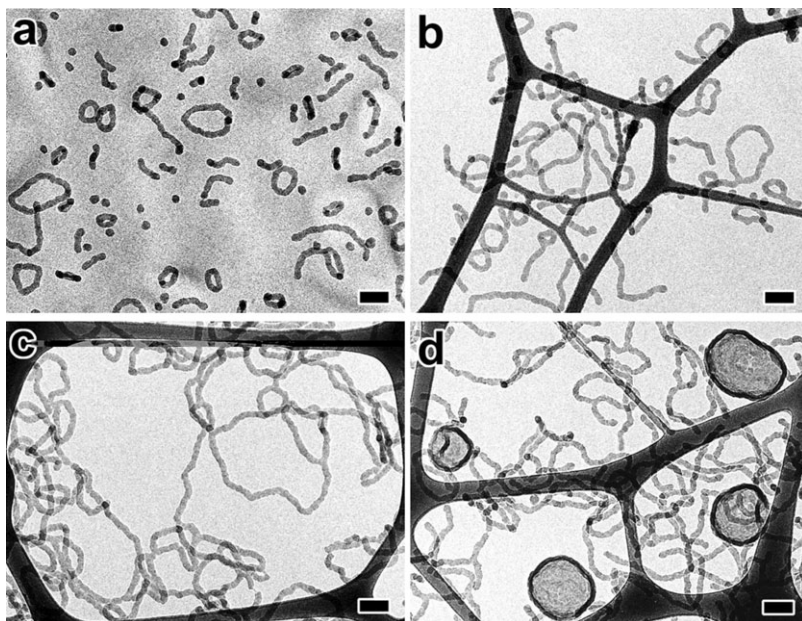
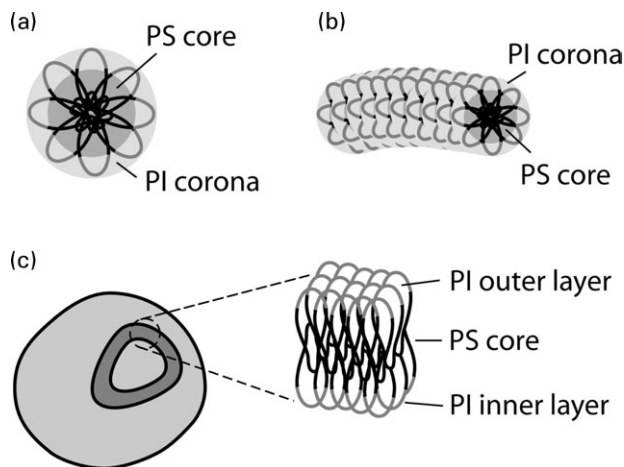


Fig. 8 Influence of the initial copolymer concentration on the morphology of micelles from cyclic PS-PI dispersed in heptane: (a), (b) 0.1 mg mL^{-1} ; (c) 0.5 mg mL^{-1} ; (d) 2 mg mL^{-1} . The concentrations indicated are those at which the solutions were prepared. They were diluted $10\times$ (a)–(c) and $100\times$ (d) before sample preparation. The embedding heptane was freeze-dried in the microscope. The gray area in (a) is an extended surface of supporting carbon while the dark areas correspond to the ‘lacey’ carbon network (scale bars: 100 nm).



Scheme. 1 (a) Sunflower micelles formed by cyclic PS–PI copolymer chains at low concentration. They are composed of a dense PS core and a more diffuse corona of PI half loops. (b) Formation of wormlike assemblies by cylindrical stacking of sunflower micelles. (c) Vesicular micelle whose envelope is a bilayer assembly with a PS core and PI outer and inner surfaces. In (b) and (c), for clarity, some molecules are schematized as opaque cycles.

change in vesicles/cylinders proportion by looking at the cryo-TEM images. Although the existence of bilayer vesicles has been reported in a number of diblock copolymer systems,^{17,21,54} it is the first time that observations of vesicles made from cyclic copolymers are reported. Considering the principles that control the shape of spherical/sunflower and wormlike molecular assemblies, it is possible to describe how the cyclic molecules self-assemble to form surfaces. The wall of the vesicles is a bilayer formed by a core layer of densely packed PS half-loops while the inner and outer surfaces are made of PI half-loops (Scheme 1). With this organization, the initial sunflower organization of the micelles is totally lost. Local organizations of the molecules in the vesicle bilayer wall may be expected in order to accommodate the fact that the cyclic conformation of individual copolymer chains favors planar configurations. Sectors may form in which PI half-loops organize in a parallel fashion. This may account for the corrugated aspect of the vesicle surface, although a freeze-drying artifact cannot be ruled out.

In addition to well-defined vesicles mixed with cylinders (Fig. 8d), a number of hybrid assemblies, part-vesicular, part-wormlike, were also observed, as illustrated in Fig. 9. In Fig. 9a, a star-like object is shown formed by nine cylinders protruding out of a central 100 nm wide discoid bilayer. Some of the cylinders also clearly exhibit Y-junctions. We can assume that the thickness of the central bilayer is equal to the cross-sectional diameter of the arms, *i.e.* 20 nm, as they both exhibit a similar contrast in the image. Micelles with resembling morphologies were reported in different polymer systems.^{14,15,54} Figs. 9b–d show octopus-looking assemblies that likely correspond to incompletely formed vesicles. The central ‘dome’ clearly looks like the skullcap of a vesicle out of which extends a varying number of wormlike ‘arms’. Fig. 9e also shows a peanut-shaped vesicle that may have been formed by the merging of two incomplete vesicles.

The images in Fig. 9 provide some hints as to how the wormlike micelles assemble and form vesicles. When the local concentration of wormlike micelles reach a critical value, the fusion into a small lamellar bilayer such as that seen in Fig. 9a is initiated. Then, the junction zones between the small bilayer and the wormlike ‘arms’ zip, increasing the surface curvature and expanding the surface of the bilayer until a closed vesicle is formed (Figs. 9b and c; Scheme 2). The size of the final vesicle would thus be defined by the number of copolymer chains within the initially connected cylinders. Although we have not studied C-mic solutions prepared at concentrations above 5 mg mL⁻¹, a concentration range where only vesicles would be observed is likely to exist.

3.2.2. Solutions in decane. Self-assembled L-mic and C-mic were also prepared in *n*-decane. The evolution of their morphology with initial concentration is shown in Fig. 10. L-mic prepared at 0.1

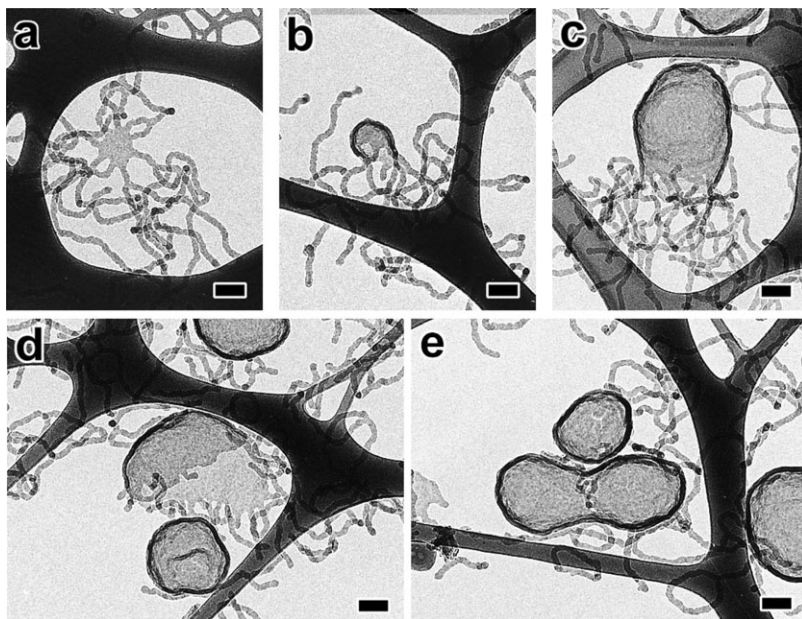
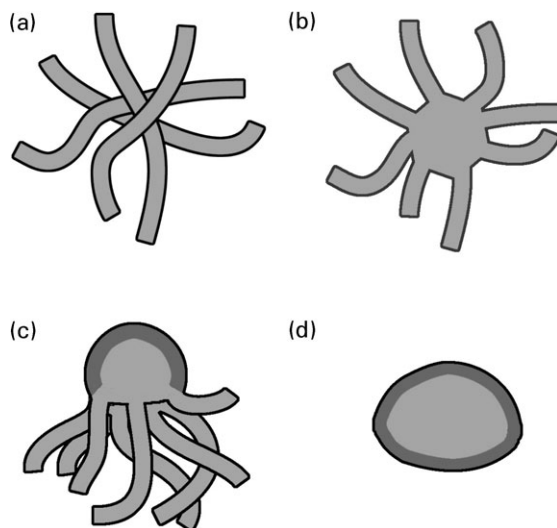


Fig. 9 Examples of incomplete vesicles observed in solutions of cyclic PS-PI copolymer in heptane: (a), (b) 2 mg mL^{-1} ; (c)–(e) 5 mg mL^{-1} . The concentrations indicated are those at which the solutions were prepared. They were diluted $100\times$ before sample preparation. The embedding heptane was freeze-dried in the microscope. The dark areas correspond to the ‘lacey’ carbon network (scale bars: 100 nm).

mg mL^{-1} (Fig. 10a) are similar in shape (spheres) and diameter (35 nm) to those made at the same concentration in heptane (Fig. 7a). However, among a majority of spheres, some extended wormlike micelles were observed in the sample prepared at 35 mg mL^{-1} and diluted $1000\times$ for observation



Scheme. 2 Formation of a cyclic copolymer vesicle from a group of wormlike micelles: (a) A critical concentration of wormlike micelles is locally reached; (b) The micelles fuse and form a small bilayer; (c) The bilayer extends as the surface curvature increases by zipping the bilayer/cylinders junctions zones; (d) A closed vesicle is formed.

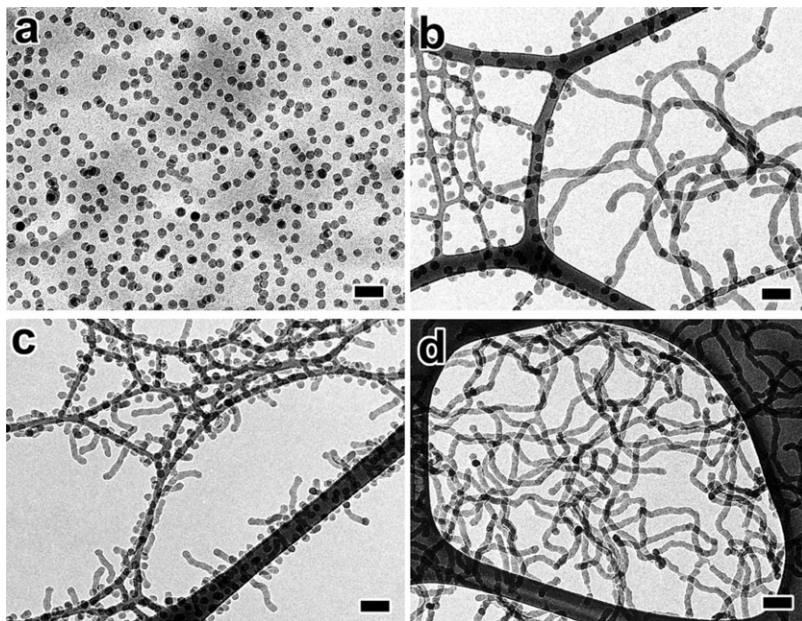


Fig. 10 Influence of the initial copolymer concentration on the morphology of PS-PI micelles dispersed in *n*-decane: (a) linear, 0.1 mg mL^{-1} ; (b) linear, 35 mg mL^{-1} ; (c) cyclic, 0.1 mg mL^{-1} ; (d) cyclic, 8 mg mL^{-1} . The concentrations indicated are those at which the solutions were prepared. They were diluted $10 \times$ (a,c), $100 \times$ (d) and $1000 \times$ (b) before sample preparation. The embedding heptane was freeze-dried in the microscope. The dark areas correspond to the 'lacey' carbon network (scale bars: 100 nm).

purpose (Fig. 10b). The width of the cylinders equals the diameter of the spheres: 35 nm . Y-junctions are also clearly observed along the wormlike micelles.

The C-mic solutions prepared in *n*-decane at 0.1 mg mL^{-1} contains a mixture of spheroidal particles that likely correspond to the individual sunflower micelles, and $100\text{--}150 \text{ nm}$ -long rodlike cylinders. The diameter of the sunflower micelles is equal to the cross-sectional width of the rods, *i.e.* 20 nm (Fig. 10c). Qualitatively, the rod-like micelles appear to be less polydisperse than the short wormlike micelles formed in heptane at the same concentration (Fig. 8a). At 8 mg mL^{-1} , only 20 nm -wide tortuous giant wormlike micelles are observed (Fig. 10d). Again, they seem to be somewhat less tortuous than those prepared in heptane (Fig. 8c and d). The major difference when comparing C-mic solutions in heptane and decane is that no vesicles were observed in decane at a concentration of 8 mg mL^{-1} while they were detected in heptane at 2 mg mL^{-1} . This may illustrate a shift of the phase diagram due to the difference of solubility of the PS block in both solvents. As already explained from the DLS data presented in section 3.1, the formation of vesicles is promoted in heptane in the $2\text{--}5 \text{ mg mL}^{-1}$ concentration range since PS is less soluble in this decane than in heptane. However, as we did not observe C-mic solutions in decane at concentrations higher than 8 mg mL^{-1} , the existence of vesicles in this solvent cannot be ruled out.

4. Conclusion

DLS and cryo-TEM have been efficiently used in conjunction to study the effect of cyclization of linear PS-PI diblock copolymer chains on their self-assembling properties in two PI-selective solvents. Despite the necessary adaptation of the 'conventional' cryo-TEM procedure to deal with the inevitable crystallization of the embedding organic solvent, *in situ* freeze-drying cryo-TEM provided high contrast images of the molecular assemblies. The micelles prepared from linear and cyclic copolymers with identical molecular weights showed clear morphological differences. L-mic kept the same aggregation number in the investigated concentration range while C-mic showed a large distribution in size (and therefore aggregation number) with a polydispersity that increased

with concentration. Finally, a dilution of the solutions initially prepared at definite concentrations did not affect the shape and size of the micelles, which is consistent with previous results obtained from AFM images.³⁵

Several conclusions can be drawn from the present results: (i) Within the investigated range of concentrations ($0.001 \text{ mg mL}^{-1} < c < 5 \text{ mg mL}^{-1}$), L-mic are spherical in heptane and decane and their sizes are slightly smaller in decane than in heptane and do not depend on the concentration or the temperature ($10^\circ\text{C} < T < 25^\circ\text{C}$). However, wormlike micelles were seen to co-exist with spherical micelles at a higher concentration in decane (35 mg mL^{-1}). (ii) At very low concentration in heptane, C-mic are more or less planar sunflower micelles which, as concentration increases, unidirectionally self-assemble into giant wormlike micelles by PS-PS core attraction. Above 2 mg mL^{-1} , vesicles build up from the fusion of giant wormlike micelles. (iii) Similar features are observed for C-mic in decane except that vesicles are not detected up to 8 mg mL^{-1} . We assume that vesicle formation is not promoted in decane in this concentration range since n-alkanes become worse solvents for polystyrene as the n-alkane carbon number increases. (iv) The morphologies of C-mic vary with temperature ($10^\circ\text{C} < T < 25^\circ\text{C}$) in heptane and decane.

The DLS and cryo-TEM results presented in this work clearly show that the morphology of self-assembled micelles made from cyclic PS-PI copolymer (sunflowers, giant wormlike micelles with or without Y-junctions, vesicles) and their behavior as a function of temperature and concentration are very different from those of spherical micelles made from linear PS-PI chains. Since both cyclic and linear copolymers have similar molecular mass and degree of polymerization, these differences are only induced by the cyclization of the copolymer chain.

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