

Preparation of Shell Cross-Linked Nano-Objects from Hybrid-Peptide Block Copolymers

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Supramolecular structures formed by self-assembly of diblock copolymers in solution are stable over restricted environmental conditions: concentration, temperature, pH, or ion strength among others. To enlarge their domain of application, it appears necessary to develop stabilization strategies. We report here different strategies to stabilize the shell of micelles formed by self-assembly of amphiphilic polydiene-*b*-polypeptide diblock copolymers. For this purpose, covalent bonds can be formed between either amine or carboxylic acid groups distributed along the soluble peptide block and a cross-linking agent that contains respectively aldehyde or amine functions. Shell stabilization affords systems with unique properties that combine three main advantages: shape persistence, control of the porosity, and stimuli-responsive behavior. The covalent capture of such macromolecular objects has been studied by light scattering, AFM, and conductimetry measurements.

Introduction

Diblock copolymers comprise two chemically different homopolymers linked together covalently. Due to the chemical incompatibility between the blocks, self-assembly occurs when the copolymer is dissolved in a good solvent for only one of the segments. A variety of morphologies can be formed, the most commonly reported being micelles,¹ vesicles,² or cylinders³. Three major contributions control the properties (size and shape) of micellar self-assembly: the stretching of the chains forming the core, the interfacial tension between the core and the solvent, and the interchain repulsion in the corona.⁴ With perfect control of these parameters and an adequate design of the block copolymers, the desired structures can thus be targeted. The interest in these morphologies resides essentially in their efficiency for a variety of applications in different fields such as nanotechnology,⁵ electronics,⁶ or in the biomedical domain as containers for biochemically sensitive medicines^{7,8} or for drug delivery purposes.^{9,10}

However, diblock copolymer assemblies have found a limited use in industrial applications where demanding environmental conditions are required including high concentration, high temperature, or extreme pH conditions and/or surfactant concentrations. High concentration regimes, for instance, can also induce thermodynamic transitions or even provoke disruption of the assemblies. In those cases, self-assembled structures obtained from block copolymers have poor stability. Therefore, several groups have recently

focused their interest on the design of novel strategies to increase the stability of such nanostructures. These normally concern cross-linking methodologies where the core or shell is covalently bonded. Ishizu et al.¹¹ were the first applying this concept on core–shell micelles and later Liu et al.¹² prepared block copolymer fibers this way. Chemical capture increases the range of concentration and temperature where self-assembled aggregates can be used. No critical micellar concentration (CMC) or temperature (CMT) transitions are identified, so high dilutions or heating are now not limiting factors in the performance of the systems. Several other reasons also justify the use of these stabilization techniques. For example, in shell-cross-linked systems, an interesting side effect has to be considered: not only the stabilization of the aggregates but also the permeability of the shell can be controlled by careful adjustment of the degree of cross-linking.¹³ The control of permeability may offer unexplored alternatives for the preparation of novel controlled drug delivery systems. Moreover, a cross-linked micelle becomes a spherically shaped molecule that can be considered as a new building unit for further self-assembly or reaction: for instance, nanofibers can be end-functionalized yielding to “supersurfactant”¹⁴ or nanofiber multiblocks.¹⁵

The enhancement of the stability of different kind of nanostructures such as spherical micelles,¹⁶ vesicles,¹⁷ or fibers¹⁸ concerns the activity of several groups. From these studies, a variety of cross-linking strategies have been reported and reviewed recently.¹⁹ Focusing on spherical morphologies, Wooley's group has been the first working on the so-called shell cross-linked needle-like nanoparticles SCKs. They synthesized an amphiphilic diblock copolymer poly(styrene-*b*-(4-vinylpyridine)) (PS-*b*-P4VP) containing

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styrenyl side groups attached to the P4VP block. Photolytic degradation of a water-soluble radical initiator starts the cross-linking reaction affording stable micelles.¹⁶ Maskos et al.²⁰ proposed an alternative methodology and prepared heterotelechelic amphiphilic block copolymers with a polymerizable group at the end-chain placed at the core of the micelle. After dissolution in water, the block containing the polymerizable function could be cross-linked, “locking” therefore the structure. Other groups such as Liu et al. described the synthesis of stable micelles with cross-linked cores (to form “nanospheres”)²¹ and shells²² using in both cases 2-cinnamoyl functions of the poly(2-cinnamoyl ethyl methacrylate) (PCEMA) block to photocrosslink the objects. In the previous commented systems, self-assembly of diblock copolymer in solution was followed by a chemical reaction to capture covalently the structure. An alternative to these approaches concerns the use of self-assembly of block copolymers in bulk to organize them in a first step. Then a cross-linking reaction can take place in confined domains of the phase separated structures. After dissolution of the films, persistent nanostructures can be obtained, even in nonselective solvents.^{23,24}

With this background, our group has focused on the development and analysis of the self-assembly properties of amphiphilic diblock copolymers containing a peptide block, i.e., poly(L-glutamic acid) or poly(L-lysine) and a non-saturated hydrophobic block (polyisoprene or polybutadiene).^{25,26} Micelles and vesicles were formed in solution when dissolving these diblock copolymers in aqueous media. In addition, the micelles possess stimuli-responsive properties: their hydrodynamic radius can be modified reversibly by small changes in pH or ionic strength. This expansion–contraction process has been related to a secondary structure transition of the polypeptide chains from a compact α -helix to an extended random coil conformation.

As an attempt to gain sophistication and make these micelles suitable for a broader range of applications, we report in this contribution the design of efficient strategies for the shell-stabilization of such micellar structures. Shape fixation will generate “nano-objects” that exhibit stimuli-sensitive behavior and might resemble the complexity and the performance of proteins. Finally, the stimuli-responsiveness effect combined with control over porosity by varying the cross-linking density can produce “smart delivery systems” that could form open structures at one pH (extended peptide chain) and closed ones at another pH (compact α -helical conformation) that are stable even when mixed with surfactants or under extreme conditions and where the drug release could be precisely controlled.

Materials and Methods

Materials. 2,2'-(Ethylenedioxy)diethylamine (98%), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide, glutaric dialdehyde (50% in water), and sodium hydroxide (NaOH) (Aldrich, >98%) were obtained from Sigma-Aldrich and were used as received. Tetrahydrofuran (THF) (J. T. Baker >99%) was distilled under benzophenone and stored under vacuum.

Measurements. ¹H NMR spectra of the diblock copolymers were recorded at room temperature on a Bruker Avance 400 MHz spectrometer using the residual proton resonance of the deuterated solvent as an internal standard.

Conductometric titrations were performed using a WTW Cond315i. All of the experiments were carried out in a titration vessel with 10 mL of 0.5% of block copolymer solution subjected to constant stirring and at 25.0 ± 0.1 °C regulated with a thermostated circulating oil bath. A standard 0.1M NaOH aqueous solution was used as titrant. One minute of lag time was allowed between each dosage, to ensure that the acid–base reaction has reached equilibrium.

Dynamic light scattering (DLS) and static light scattering (SLS) experiments were performed using an ALV Laser goniometer, which consisted of a 22 mW HeNe linear polarized laser with a wavelength of 632.8 nm and an ALV-5000/EPP Multiple Tau Digital Correlator with 125 ns initial sampling time. The samples were kept at a constant temperature of 25.0 ± 0.1 °C during all of the experiments. The accessible scattering angle range is from 10° up to 150°. However, most of the measurements were carried out at 90°. The solutions were introduced into 10 mm diameter glass cells. The minimum sample volume required for the 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 600 s. Millipore water was thoroughly filtered through 0.1 μ m filters and directly employed for the preparation of the solutions.

Adsorbed structures at the solid/solution interface were examined using a NanoScope IIIa Multimode atomic force microscope (Digital Instruments, CA) in Contact Mode, equipped with a standard fluid cell. Standard cantilevers were used with sharpened Si₃N₄ tips. These were irradiated with ultraviolet light for 30 min prior to use. The solution was held in a Plexiglas fluid cell, sealed by a silicone O-ring and resting on a muscovite mica substrate. The cell and the cantilever were cleaned by sonication for 30 min in deionized water at 40 °C and then dried using filtered nitrogen prior to use. The mica substrate was cleaved using adhesive tape and immediately used, to avoid contamination by ambient dust particles or volatile molecules. The cell was completely filled with about 0.5 mL of micellar solution at a concentration of 0.5 mg/mL. Before imaging the surfaces, the polymer was allowed to adsorb onto mica during at least 12 h.

Procedures. *General Procedure for Shell Cross-Linking in PB-b-PGA Diblock Copolymers (Calculations Made for Target Cross-Linking of 50%).* The diblock copolymer, PB₄₈-b-PGA₁₁₄ (M_w , 22 800 g/mol; M_w/M_n , 1.42), was dissolved in water at pH 12 with a concentration of 0.5 mg/mL to form the micellar structure. Micelles reached thermodynamic equilibrium after 3 days of vigorous stirring at 35 °C as confirmed by dynamic light scattering measurements (DLS). Once the micelles reached this equilibrium, 2,2'-(ethylenedioxy)diethylamine (0.25 equiv. per carboxylic group) was added to the solution under stirring. Finally, the carboxylic activating agent, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (2 equiv. per carboxylic group), was added. The reaction was allowed to proceed overnight.

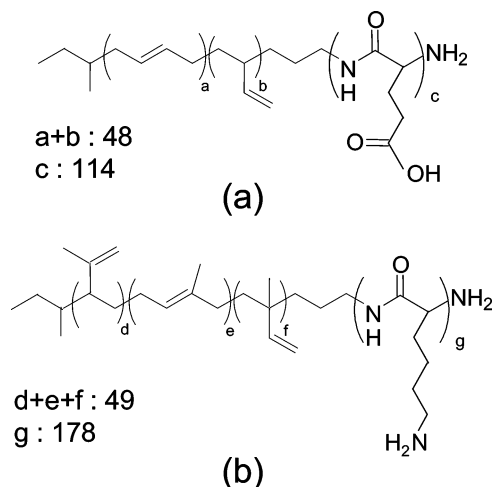


Figure 1. Chemical structures of the diblock copolymers under study: (a) PB₄₈-b-PGA₁₁₄, (b) PI₄₉-b-PLys₁₇₈.

General Procedure for Shell Cross-Linking in PI-b-PLys Diblock Copolymers (Target Cross-Linking of 50%). PI₄₈-b-PLys₁₇₈ (M_w , 39 000 g/mol; M_n/M_w , 1.23) micelles were formed by addition of the diblock copolymer in neutral aqueous solution. After 3 days of stirring at RT ($\sim 25^\circ\text{C}$), the micelles reached their equilibrium. Then, glutaric dialdehyde (0.25 eq. per amine group) was added to the micellar solution. The reaction continued overnight.

Results and Discussion

The synthesis of the PB_{*n*}-b-PGA_{*m*} and PI_{*n*}-b-PLys_{*m*} diblock copolymers recently reported has been reproduced for the preparation of PB₄₈-b-PGA₁₁₄ and PI₄₉-b-PLys₁₇₈.^{25,26} Briefly, anionic polymerization of butadiene or isoprene monomers using *sec*-butyllithium as initiator was terminated with a silyl protected chloramine compound. Acidic treatment was performed to remove the silyl groups. The resulting amine functional polymer was then used as a macroinitiator for the preparation of the second block via ring-opening polymerization of α -amino acid *N*-carboxyanhydrides. Final removal of the peptide side-chain protective groups under basic or acidic conditions for L-glutamic acid and L-lysine²⁷ respectively, lead to the final amphiphilic diblock copolymer. Dissolution in water produced different morphologies depending on the relative hydrophobic/hydrophilic block length. In agreement with geometrical considerations, spherical micelles were obtained for block copolymers with a large contribution of the hydrophilic block relative to the hydrophobic block, e.g., PB₄₈-b-PGA₁₁₄ and PI₄₉-b-PLys₁₇₈. In the present work, the stabilization of these micellar systems have been explored. Chemical structures of these block copolymers are schematically represented in Figure 1.

Preparation of the Micelles. Micelles were prepared by dissolution of the diblock copolymers (10 mg) in 20 mL of Millipore water previously filtered through a 0.10 μm cellulose membrane filter. The diblock copolymers containing PGA were dissolved at pH ~ 12 , and PLys diblock copolymer micelles were prepared directly at neutral pH. After 3 days of vigorous stirring at room temperature or eventually heating to 35°C , the micellar assemblies were at

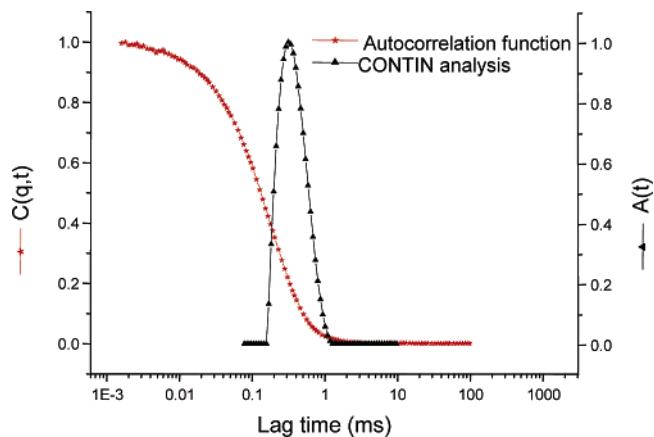


Figure 2. DLS autocorrelation function and its CONTIN analysis (determined at 90° scattering angle) of the micelles formed by PI₄₉-b-PLys₁₇₈ in aqueous media.

the equilibrium as revealed by dynamic light scattering experiments. The autocorrelation functions obtained from DLS measurements were analyzed using the CONTIN analysis²⁸ and were performed at a scattering angle of 90° . As an illustrative example, the autocorrelation function and its CONTIN analysis for the PB₄₈-b-PGA₁₁₄ diblock copolymer micelles obtained after 3 days at a concentration of 0.5 mg/mL and basic pH are shown in Figure 2. These preparation conditions were applied for all of the cross-linking reactions performed subsequently. The CONTIN analysis shows only one population with a narrow size distribution for both systems and hydrodynamic radii $R_H \sim 26$ and $R_H \sim 44$ nm were respectively obtained for PB₄₈-b-PGA₁₁₄ and PI₄₉-b-PLys₁₇₈.

Cross-Linking Reactions. As mentioned in the Introduction, a variety of methods can be employed in order to covalently stabilize the micellar assemblies, involving either the core or the corona. PB and PI are hydrophobic blocks and therefore form the core of the micelles when dissolved in polar media such as water. As unsaturated polymers, they can be cross-linked via a radical mechanism using the vinyl double bonds distributed along the chain. In a recent communication, our group has reported the first core-stabilization experiments performed in the vesicular systems PB₄₀-b-PGA₁₀₀.²⁹ This method required the use of an UV initiator and an UV source to irradiate the micelles once they reached the equilibrium in solution. It was demonstrated that 1 h of UV irradiation was enough to cross-link the micelles. In the present contribution, a shell cross-linking methodology was used to stabilize the aggregates formed in solution. Poly(L-lysine) (PLys) and poly(L-glutamic acid) (PGA) polypeptides are hydrophilic blocks and are distributed at the periphery of the micellar assemblies when dissolved in water. Along the peptide block, the amine (PLys) and carboxylic functions (PGA) can be used to stabilize the assembly by simple chemical reactions with a complementary functional group. The two main strategies adopted are schematically presented in Figure 3. The first system A (PB₄₈-b-PGA₁₁₄) forms micelles that were cross-linked based on a previously reported methodology by Wooley et al.³⁰ For that purpose, we used 2,2'-(ethylenedioxy)diethylamine (EDEA) as a cross-linking agent to convert carboxylic groups into amide bonds.

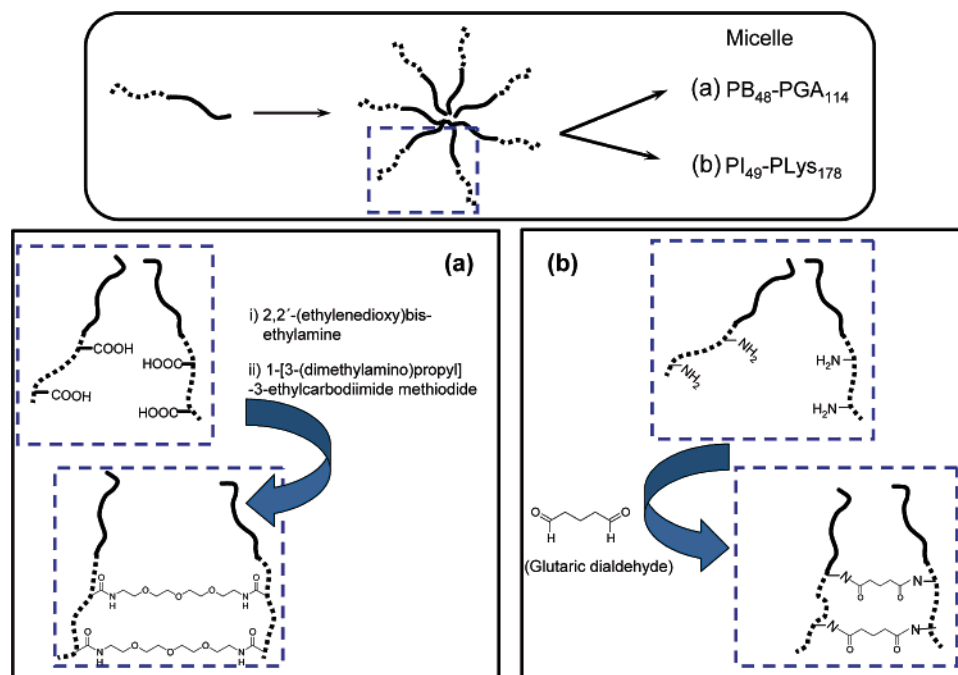


Figure 3. Schematic representation of the cross-linking strategies followed for PB₄₈-*b*-PGA₁₁₄ and PL₄₉-*b*-PLys₁₇₈.

As extensively used in peptide chemistry, reactions between amine and carboxylic acid groups required activation of the carboxylic function. The activating agent used was a water-soluble carbodiimide: 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (CDI). However, because carbodiimides have limited lifetimes in the presence of water and consequently decompose slowly,³¹ EDEA was added first to the micellar solution. The amount of EDEA was calculated with respect to the amount of carboxyl functionalities and was set to be 0.5 and 1 equiv. (50% and 100% of cross-linking were therefore expected). After 10–15 min of vigorous stirring, the carbodiimide activator was added to the solution. The amount of CDI used was 1 equiv. relative to the carboxylic acid groups in both cases. The reaction was then allowed to proceed overnight to guarantee the reaction to be completed.

The PL₄₉-*b*-PLys₁₇₈ copolymer at neutral pH contains primary amine groups on the coronal block. These amine groups were cross-linked in a single reaction step using glutaric dialdehyde (GDA) as a cross-linking agent. In this case, the amount of GDA added to fix the micellar structure was targeted to be 0.25 and 0.5 equivalent per amine function, i.e., 25% and 50% targeted cross-linking. Similar to the previous example the reaction was allowed to proceed overnight to ensure the total conversion of the aldehyde groups.

Characterization. ¹H NMR and IR spectroscopies were used to examine the formation of amide bonds during cross-linking. Unfortunately, both techniques were inadequate and no information could be extracted properly. All ¹H NMR spectra recorded in deuterated water afforded broad signals, and any characteristic peak before and after cross-linking could be distinguished. However, an increased broadness of the signals indicating a considerable reduction in the proton mobility was observed after cross-linking. Likewise, poor resolution in IR spectroscopy experiments prevented any distinction between the two types of amide bonds, i.e., those

newly formed and those contained in the original polypeptide backbone. However, valuable information was obtained by taking advantage of the charged shell of the micelles. Conductivity measurements were performed in order to evaluate the consumption of carboxyl and amine groups during the reaction. These groups are initially negatively (–COO[–]) and positively charged (–NH₃⁺). The ion density and therefore the conductivity should stay constant if they remain fully unreacted and be modified progressively along the reaction if the peptide groups are fruitfully attached to the cross-linking agent. As an illustrative example, Figure 4a shows the conductivity titration curve of 0.5% PL₄₉-*b*-PLys₁₇₈ with NaOH. The block copolymer was dissolved in neutral water and aliquots of 25 μL of NaOH (0.1 M) were successively added to the solution. Two transitions (5 and 15 mmol in the present example) have been systematically observed: the titration curves can then be divided into three distinct regions, based on the significant changes in their slope. The first one represents the neutralization reaction between the excess HBr present in the solution and NaOH: the decrease in conductivity is caused by the decrease of the H⁺ concentration since the mobility of H⁺ is larger than that of Na⁺. The second part corresponds to the reaction between the poly-L-lysine and NaOH, and the slight increase in conductivity is mostly due to the increase in free Na⁺ ions in solution. Finally, region 3 represents the excess of NaOH, where the Na⁺ and OH[–] ions contribute to the large increase in the conductivity.³² As a result, region 2 is related to the neutralization of the charged part of the PLys block and thus the number of residual amine groups can be determined. In Figure 4b, the ratio of reacted amine groups is represented as a function of the targeted cross-linking. As expected, the percent of amine groups that reacted effectively increases linearly with the targeted degree of cross-linking. However, the degree of cross-linking obtained is always lower than the targeted one due to several side

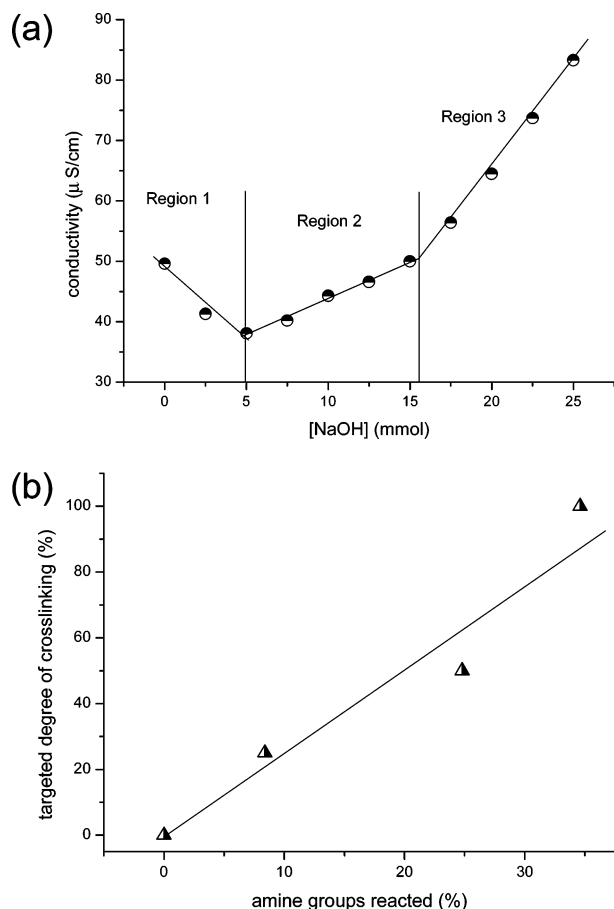


Figure 4. (a) Conductivity titration of 0.5% $\text{PI}_{49}\text{-}b\text{-PLys}_{178}$ with 0.1M NaOH at 25 °C. (b) Targeted cross-linking vs percent of amine groups reacted.

reactions that could interfere. First, a fraction of the cross-linking agent could react twice with the same chain to form an intramolecular loop. In other cases, the cross-linker can react only at one end with the polymer chain. The third side reaction concerns the carbodiimide used as activator of the carboxylic acid groups and the dialdehyde that decompose slowly in aqueous solution with a consequent decrease of the final degree of cross-linking. Some of these side reactions can statistically occur and modify the real degree of cross-linking. However, the last side reaction, that is the decomposition of carbodiimide and dialdehyde that respectively lead to a urea derivative and a dicarboxylic acid that will be neutralized in region 1, do not interfere in the analysis of the region 2.

In addition to the previous experiments, DLS analyses were performed, giving valuable information about the success of the cross-linking reactions. First, size distributions obtained from the CONTIN analysis of the autocorrelation functions at 90° were similar before and after the reaction, meaning that no changes in size occurred. However, in contrast to core cross-linking reactions, the stabilization of the shell can eventually be accompanied by undesirable intermicellar reactions. We thus used DLS analysis to examine the extent of these potential side reactions. For that purpose, the angular dependence of the micelles was measured before and after cross-linking at different polymer concentrations. The measurements were performed between 40° and 140°. The dependence of the decay frequency, Γ ,

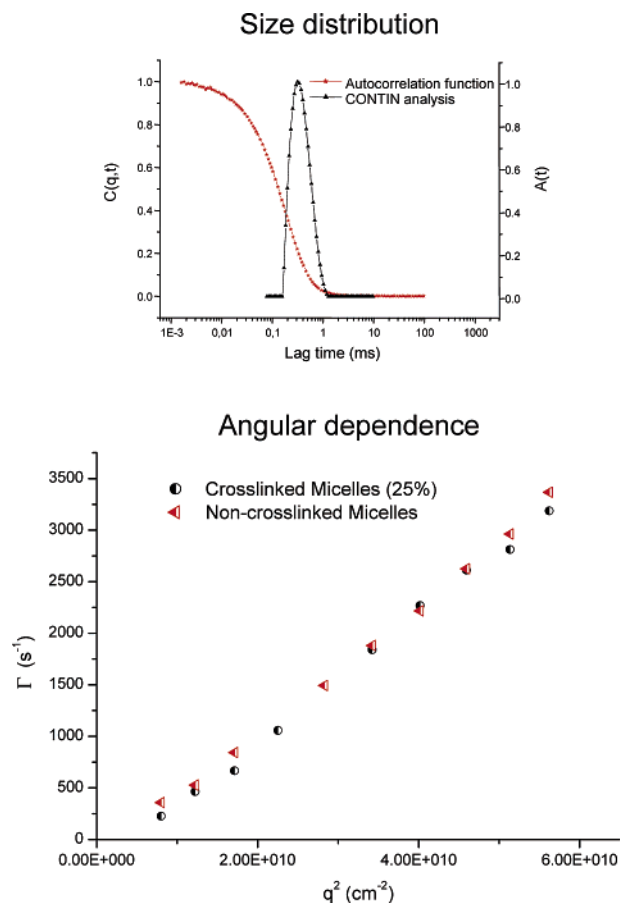


Figure 5. Plot of Γ versus q^2 for $\text{PI}_{49}\text{-}b\text{-PLys}_{178}$ micelles in aqueous solution with a concentration of 0.5 mg/mL at angles between 40° and 140°.

as a function of the square of the scattering vector q^2 (\mathbf{q} is defined as $\mathbf{q} = 4\pi n \sin(\theta/2)/\lambda$, where n is the refractive index of the solution, θ is the scattering angle, and $\lambda = 632.8$ nm is the wavelength of the incident laser light) can be plotted and is shown in Figure 5. After cross-linking of the micelles at a concentration of 0.5 mg/mL, the evolution of Γ exhibits a similar linear relationship with q^2 which confirms that the spherical structure of the micelle is maintained.²⁸ In addition, the slopes of these curves, which are related to the diffusion coefficient and thus to the hydrodynamic radii from the Stokes–Einstein relationship, were very similar, confirming that the micelles retained their integrity and that no observable intermicellar fusion can be detected.

Once it was verified that cross-linking occurred exclusively in each micelle separately, the swelling behavior of both cross-linked and non-cross-linked micelles was analyzed. For that purpose a cosolvent, which is a good solvent for the core-forming block (in this case tetrahydrofuran), was added in different amounts between 0 and 12% v/v (volume of THF to total volume of solution). The autocorrelation functions and the respective size distributions of the micelles are presented in Figure 6 for the $\text{PB}_{48}\text{-}b\text{-PGA}_{114}$ diblock copolymer micelles. For the non-cross-linked micelles (see Figure 6a) the addition of THF leads to a shift in the autocorrelation function to higher relaxation times, indicating a high increase in the aggregates size. In the meantime, the autocorrelation functions of the cross-linked micelles (Figure 6b) are not significantly modified on addition of THF to the solution,

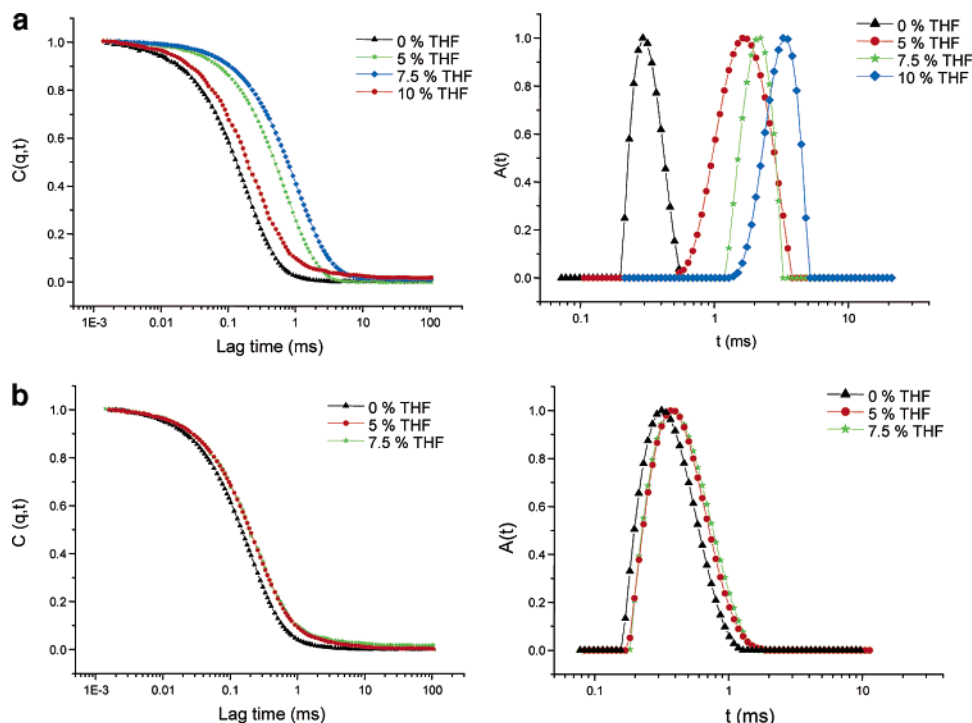


Figure 6. Autocorrelation functions and their CONTIN analysis for PB_{48} - b - PGA_{114} at different amounts of added THF: 0%, 5%, 7.5%, and 10% for (a) non-cross-linked micelles and (b) 50% cross-linked micelles.

indicating little change in their average size. These differences in the swelling behavior of these micelles before and after cross-linking can thus be directly related to a limited mobility of the PGA chains in the shell. Moreover, the evolution of the mean hydrodynamic radius R_H measured as a function of the volume of THF added to the solution is represented in Figure 7 for both systems. For targeted degree of cross-linking of 50% and 100% the expansion of the PB_{48} - b - PGA_{114} copolymer micelles was strongly limited and sizes remained almost constant even after addition of 7.5% THF. The PI_{49} - b - $PLys_{178}$ copolymer micelles were also cross-linked at a targeted degree of 25%. In this case, an increase in R_H from 44 to ~ 75 nm after addition of 12% v/v THF was observed. The reduction in the cross-linking density allows the micelles to expand to a greater extent compared to 50 or 100% cross-linking. One can thus anticipate that the degree of swelling can be controlled by the cross-linking density of the micelles.

Further support on the covalent stabilization was obtained from atomic force microscopy (AFM) experiments described in Figure 8 illustrating the PI_{49} - b - $PLys_{178}$ copolymer micelles. In acid conditions (pH ~ 3), the amine groups of the poly-(L-lysine) block are protonated and hence absorb on the mica substrate, which is negatively charged even at this low pH. After absorption of the non-cross-linked micelles (left image), spherical features randomly distributed on the surface were observed. These peaks have a mean size of 20–25 nm in diameter that is larger than the theoretical diameter of the polyisoprene (~ 7 –10 nm) calculated considering chain length, density, and the aggregation number, but that is also smaller than the D_H values of the micelles obtained from DLS experiments (~ 88 nm): this indicates that the core is surrounded by the polypeptide chains that are strongly absorbed on the mica surface. This fact was supported by

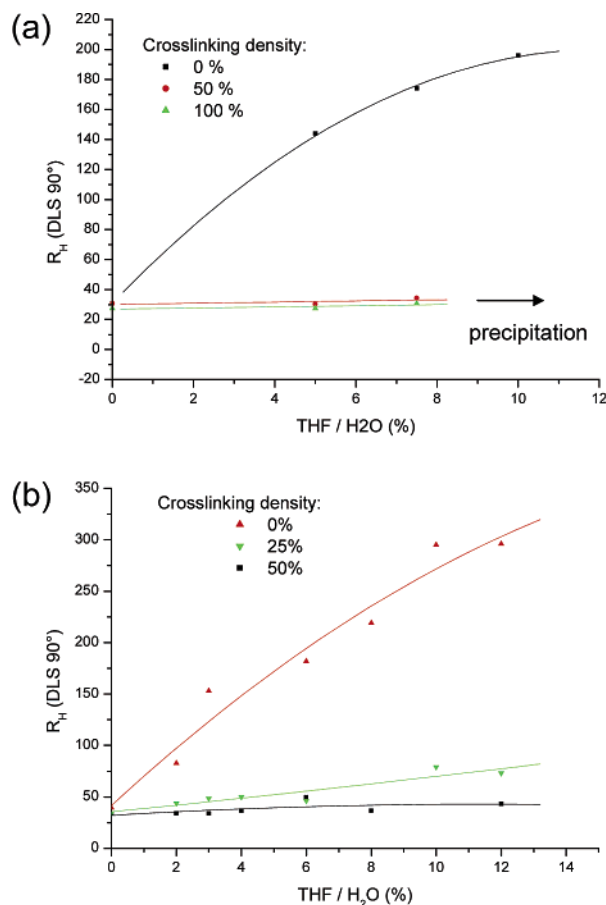


Figure 7. Hydrodynamic radius (R_H) of (a) PB_{48} - b - PGA_{114} and (b) PI_{49} - b - $PLys_{178}$ measured by DLS (90°) as a function of the volume fraction of THF added and for different targeted densities of cross-linking.

the smooth surface around the peaks that does not correspond to the mica substrate because repulsive interactions were

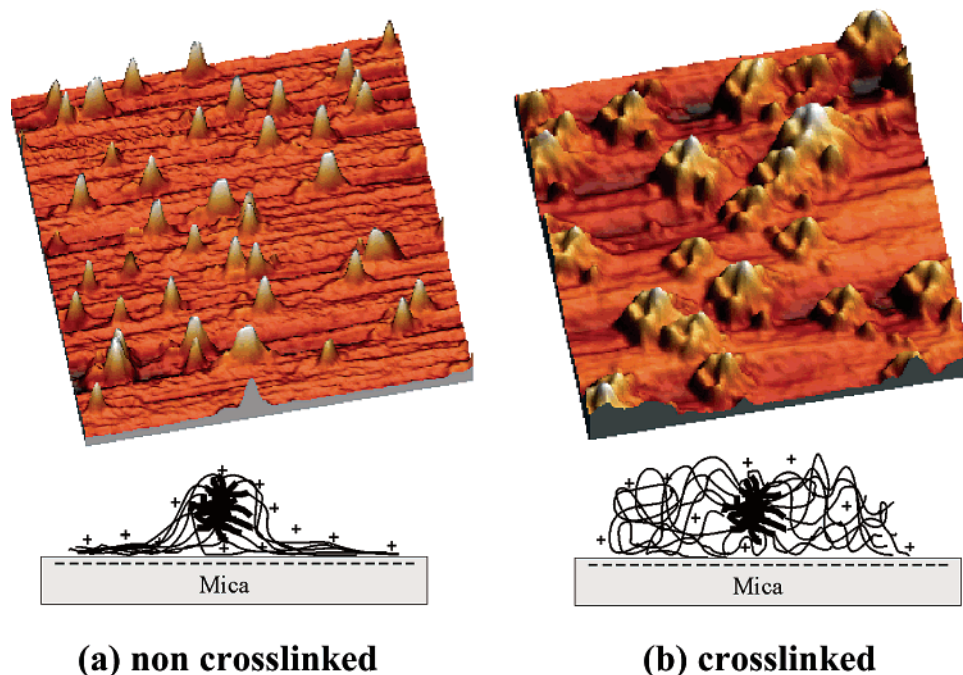
PI₄₈-*b*-PLys₁₇₈ micelles

Figure 8. AFM topography image of PI₄₈-*b*-PLys₁₇₈ micelles adsorbed on mica surfaces and preformed in an acid polymer solution. (a) Non-cross-linked micelles. (b) Cross-linked micelles.

observed when the AFM tip was approached. Indeed, electrostatic interactions between the poly(L-lysine) and the substrate create a flattened corona that cannot be distinguished in the images. In the meantime, the relatively high T_g of the polyisoprene core ($T_g = 35\text{ }^\circ\text{C}$) rendered it rigid enough to prevent flattening upon absorption at room temperature³³ (observation of peaks). Based on these observations, we propose a schematic representation of the micellar structure at the surface in Figure 8a. The image of the cross-linked micelles (right image) shows the core forming a cavity inside of a nonuniform environment. At least two main reasons explain this change in the morphology at the surface: first, after cross-linking the charge density of the poly-L-lysine block decreases, so the interaction between PLys and mica also decreases. In addition, the network formed in the shell increases its rigidity and then limit the deformation of the poly(L-lysine) block at the surface (Figure 8b).

Conclusion

In this contribution, we have reported the stabilization of micelles formed by self-assembly in aqueous solutions of two amphiphilic diblock copolymers, PB₄₈-*b*-PGA₁₁₄ and PI₄₉-*b*-PLys₁₇₈. Covalent capture of the morphology was accomplished via cross-linking of the water-soluble block at the periphery, i.e., PGA or PLys. Both strategies are based on the chemical reaction of two complementary functional groups; in the case of PGA, the presence of an activating agent is required. Conductivity, DLS measurements and AFM imaging confirmed the formation of a compact structure after cross-linking. First, a progressive decrease of the number of charged amine groups (not reacted) when the targeted percent of cross-linking increased could be observed.

Furthermore, the addition of a good solvent for the core (THF) limited gradually the swelling response from the cross-linked micelles as a function of the degree of cross-linking compared to the non-cross-linked ones. The stark contrast between the AFM images recorded before and after cross-linking confirmed a morphological change in the surface packing of the micellar shell.

Further studies will focus on the precise control of the amount of cross-linking in order to determine the porosity and the permeability of the created shell. Finally, the persistence of the “nano-objects” obtained, associated with the control of the porosity and the stimuli-responsive behavior reported previously, makes these systems very interesting potential candidates for controlled encapsulation and drug delivery purposes.

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