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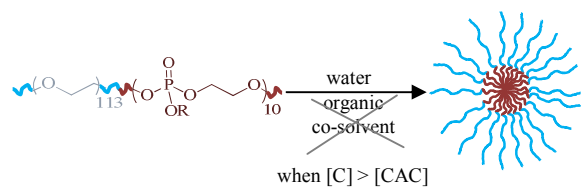
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Table of content :



Investigation of the micellization of degradable polyphosphoester based surfactants following a solvent-free process: the role of the pendent chain.

Synthesis and tensioactive properties of PEO-*b*-polyphosphate copolymers

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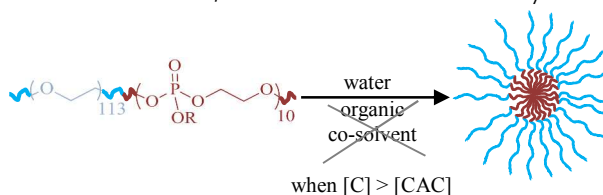
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Poly(ethylene oxide) (PEO)-*b*-polyphosphate copolymers made of hydrophilic PEO and hydrophobic polyphosphates are amphiphilic copolymers prone to self-assemble in water into nanoparticles. In this work, nanoparticles are obtained by the self-assembly of PEO-*b*-polyphosphate copolymers in water in absence of any organic co-solvent whatever the length of the pendant alkyl chain (between 4 and 7 carbon atoms) of the polyphosphate block. Remarkably, this solvent-free process remains efficient even for the most hydrophobic polyphosphate blocks. The critical aggregation concentration (CAC) of the block copolymers were determined by pyrene probe fluorescence. Finally, the efficiency of these copolymer surfactants to decrease the air/water interface was measured by air-bubble tensiometry.



Introduction

Amphiphilic block copolymers hold a special place in macromolecular chemistry due to their dual nature with one block exhibiting hydrophilicity and the other one lipophilicity. They are widely used in diverse applications^{1,2}, including detergency³, dispersion and foam stabilization^{4,5} and drug delivery systems⁶ in which they demonstrated some advantages such as a lower critical micellar concentration as compared to small size surfactants. These copolymers are capable to self-assemble in water, driven primarily by the hydrophobic effect but also by electrostatic interactions, inclusion complexes and hydrogen bonding.

The self-assembly of few non-ionic amphiphilic copolymers was investigated compared to the cationic, anionic, or amphoteric ones. Among the non-ionic copolymers displaying micellization ability, the majority of the research was performed on poly(ethylene oxide) (PEO) as the hydrophilic block with poly(propylene oxide) (commercially called “Pluronics”)^{7,8} or degradable aliphatic polyesters, i.e. poly(ϵ -caprolactone)^{9,10} (PCL) and poly(lactide)¹¹ (PLA) as the hydrophobic block.¹² Indeed, such PEO-based non-ionic surfactants being biocompatible and less toxic than ionic ones were extensively investigated for diverse oral and parenteral pharmaceutical formulations.^{13,14} A well-known example is

Tween-80 which is less toxic, less haemolytic and maintains nearly physiological pH in solution.¹⁵

At the end of the 1970s, Penczek and co-workers achieved a pioneering work establishing that polyphosphates can be synthesized by ring-opening polymerization (ROP) of cyclic phosphates.^{16,17} Organometallic compounds such as aluminum alkoxides¹⁸ and tin octanoate (Sn(Oct)₂)¹⁹ are commonly used to promote the ROP of cyclic phosphates. Nowadays these metal-based promoters are replaced by metal-free organocatalysts.^{20,21} Similarly to aliphatic polyesters (PCL, PLA, ...), polyphosphates are degraded by reaction with water. Indeed, Baran and Penczek showed that the kinetics of the polyphosphates hydrolytic degradation is affected by a number of parameters, such as temperature, pH and chemical structure of the pendant chains.²² Narendran and Kishore reported on a series of polyphosphates that exhibit a faster hydrolytic degradation at basic pH, leading to a weight loss of 3.9% in six hours (for the most stable polymer).²³ Wang *et al.* described a slower hydrolytic degradation for a PEO-*b*-polyphosphate amphiphilic block copolymers at 37°C and pH 7.4 in a phosphate buffer solution (a weight loss of 7% for the polyphosphate block in two months).²⁴ Consequently, polyphosphates turned out to be a valuable alternative to aliphatic polyesters as a degradable hydrophobic polymers for the elaboration of diblock amphiphilic polymers. In terms of applications, these polyphosphates appear particularly

appealing for the fabrication of surfactants or nanocarriers in the field of Drug Delivery systems.^{25–28}

In contrast to aliphatic polyesters, the chemical structure of polyphosphates is easily changed by simple modification of lateral groups. This task is achieved by the implementation of the ROP of cyclic phosphates prepared by the esterification reaction of 2-chloro-1,3,2-dioxaphospholane 2-oxide (COP) with the appropriate alcohol (scheme 1).^{29,30} This strategy was applied by Yang *et al.* in order to modify the hydrophobicity of the polyphosphate block of amphiphilic copolymers.³¹ It turned out that the longer the lateral chains are, the more hydrophobic the polyphosphate block is and the lower the Critical Aggregation Concentration (CAC) is. Nevertheless, in their work, the nanoparticles were prepared by dropwise addition of water into a solution of the amphiphilic diblock copolymer in an organic solvent (DMSO). The higher hydrophilicity of polyphosphates compared to aliphatic polyesters opens up the possibility to exploit them to prepare nanoparticles in the absence of any potentially toxic organic solvent³², which is an important advantage for several applications and especially for food, biomedical and detergency applications.

Our work aims at investigating the self-assembly without addition of organic co-solvent of PEO-*b*-polyphosphate copolymers in water and at the air-water interface. For this purpose, PEO-*b*-polyphosphate copolymers of increasing hydrophobicity by increasing the number of carbon atoms of pendant group were synthesized according to a similar strategy used by Yang *et al.*³¹ Besides the length, the effect of the alkyl group architecture was investigated on the CAC of the block copolymers determined by pyrene probe fluorescence. Finally, the efficiency of these copolymer surfactants to decrease the air/water interface was measured by air-bubble tensiometry.

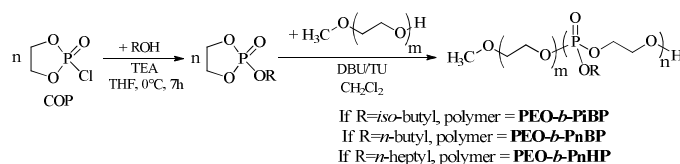
Results and discussion

The first step of our work is dedicated to the synthesis of PEO-*b*-polyphosphate copolymers. It was decided to use polyphosphates substituted by pendant *n*-butyl, *iso*-butyl and *n*-heptyl groups. The use of the ethyl group was discarded because of the known hydrosolubility of the corresponding polyphosphate³³, which is not suitable as the hydrophobic block for our study. On the one side, polymers substituted by the *n*-butyl and *iso*-butyl groups were selected in order to investigate the influence of the branching on the self-assembly of the corresponding amphiphilic copolymers in water. The tert-butyl group was discarded because such tertiary carbon could affect the stability of the resulting phosphate towards hydrolysis. On the other side, the comparison of the self-assembly of copolymers bearing pendant *n*-butyl and *n*-heptyl groups enabled the investigation of the effect of the length of the substituent in the absence of any organic co-solvent.

Monomer synthesis

The synthesis of cyclic phosphates is described in many works.^{21,31} Briefly, the cyclic phosphates substituted by a *n*-butyl group (nBP), an *iso*-butyl group (iBP) or a *n*-heptyl group

(nHP) were synthesized by the esterification reaction of the commercially available COP and the corresponding alcohol (1-butanol, *iso*-butanol or 1-heptanol) in the presence of a stoichiometric amount of TEA at 0°C for 7 hours (Scheme 1).



Scheme 1: Followed strategy for the synthesis of the PEO-*b*-polyphosphate copolymers

After filtration of the released triethylammonium hydrochloride salt, the monomers were purified by fractionated distillation under vacuum with an average final yield of 60% for nBP and iBP monomers and 50% for nHP. The structure of the different monomers was assessed by ¹H NMR, by ¹³C NMR and by ¹H-decoupled ³¹P NMR analyses (see in the supplementary information).

Synthesis of PEO-*b*-polyphosphate amphiphilic copolymers

Generally, hydrophilic PEO is used to synthesize non-ionic amphiphilic copolymers. Among the degradable amphiphilic copolymers, PEO-*b*-PCL copolymers are one of the most described in the scientific literature. Previous researches reported a molar mass of 5000 g mol⁻¹ as typical interesting length for the PEO block of such surfactant copolymer^{31,34,35}, notable because of the possible renal extraction when used in pharmaceutical fields³⁶. For sake of comparison, this length was selected for the PEO macro-initiator. Besides, a polymerization degree of 10 was targeted for each polyphosphate block for the sake of comparison with more commonly used PEO₁₁₄-*b*-PCL₈ copolymer surfactants.

Accordingly, the synthesis of PEO₁₁₄-*b*-PiBP₁₀, PEO₁₁₄-*b*-PnBP₁₀ and PEO₁₁₄-*b*-PnHP₁₀ copolymers was investigated by organocatalyzed ROP of cyclic phosphates in living conditions, by using monomethoxypoly(ethylene oxide) (MeO-PEO-OH) (5000 g mol⁻¹) as macro-initiator. From literature data²¹, a mixture of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 1-1-[3,5-bis(trifluoromethyl)phenyl]-3-cyclohexyl-2-thiourea (TU) appears as one of the most efficient catalytic system for polymerizing related cyclic phosphates. Based on these conditions (CH₂Cl₂, 0°C and 60 min for iBP and nBP monomers or 70 min for nHP monomer), the monomer to macro-initiator ratio was set to 10 and polymerization led to full conversion. Accordingly, the three targeted amphiphilic copolymers were successfully obtained in a controlled fashion as depicted by the good agreement between the theoretical and experimental molar masses for each copolymer (Table 1) and the narrow molecular weight distribution. The structures of the different PEO-*b*-polyphosphate copolymers and the molar masses of the corresponding polyphosphate block were determined by ¹H NMR analysis. The typical ¹H NMR

spectrum of PEO-*b*-PnHP is shown Figure 1 and $M_{nHP} = 222 \text{ g mol}^{-1}$ ($M_{iBP} = M_{nBP} = 180 \text{ g mol}^{-1}$).

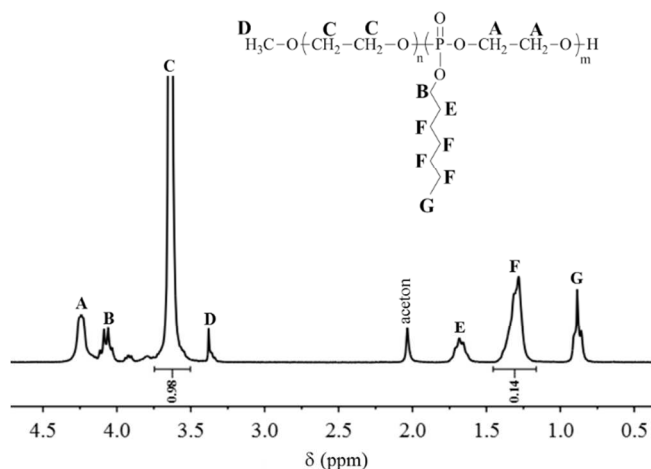


Figure 1: ^1H NMR spectrum of PEO-*b*-PnHP copolymer in CDCl_3

The molar mass of the PnHP block was measured by the comparison of the intensity of the signal of the PEO block at 3.6 ppm (Figure 2, peak C) and the typical signal of polyphosphate block at 1.3 ppm (Figure 2, peak F) according to equation 1, where I_C and I_F stand for the integral of the C and F peaks in Figure 2.

$$M_{n,NMR}(\text{PnHP block}) = DP_{\text{PEO}} \times \frac{I_F}{2 I_C} \times M_{\text{monomer}} \quad (1)$$

The ^1H -decoupled ^{31}P NMR spectrum (see supplementary material) of the PEO-*b*-PnHP copolymer confirmed the complete conversion of nHP monomer into the corresponding polymer by the shift of the signal from 18 ppm to -1 ppm.

SEC analysis confirmed also the success of the block copolymerization. Indeed, after polymerization, a shift of the PEO macro-initiator traces to higher apparent molar mass is observed in each case (Figure 2). Moreover, no trace of residual PEO is observed, which is in good agreement with a quantitative initiation by the PEO macro-initiator.

However, although the SEC trace of MeO-PEO-OH is monomodal, the SEC traces of the three different PEO-*b*-polyphosphate copolymers are bimodal. Indeed, a second population is observed at higher molar masses, which was attributed to the presence of a triblock copolymer, polyphosphate-*b*-PEO-*b*-polyphosphate coming from the unavoidable initiation of the cyclic phosphate polymerization by HO-PEO-OH, present in small amount in the commercial MeO-PEO-OH. The separation of these copolymer mixtures being tedious, they have been used without further purification for the following step, knowing that it represents about 10% of the sample.

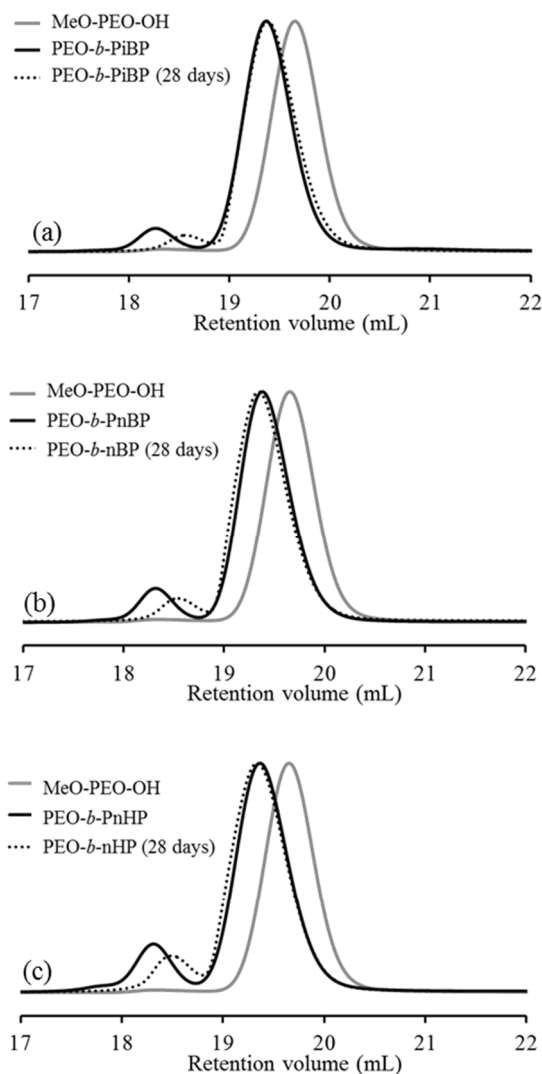


Figure 2: SEC traces in THF of (a) PEO-*b*-PiBP and MeO-PEO-OH, (b) PEO-*b*-PnBP and MeO-PEO-OH and (c) PEO-*b*-PnHP and MeO-PEO-OH. The curved dotted line represents the SEC chromatograms of the different copolymers after degradation in milli-Q water.

Aqueous behaviour of PEO-*b*-polyphosphate diblock copolymers

The main step of our work is to investigate the self-assembly of the diblock copolymers in water without the use of organic co-solvent. Indeed, the water contact angles of films of PnBP and PnHP homopolymers ($\sim 10.000 \text{ g mol}^{-1}$) were measured to be 7.6° and 14.8° , respectively (experimental details provided in supplementary information), showing the high hydrophilic character of these polyphosphates, even if both homopolymers are insoluble in water at room temperature. This fact in combination with the low T_g ($< -50^\circ\text{C}$) and the absence of crystallinity observed for the polyphosphate block of the copolymers, in contrast to the semi-crystalline PCL, would favour such solvent-free process (DSC traces of the amphiphilic copolymers are provided as supplementary information).

monomer	catalyst DBU/TU ratio <i>mmol</i>	Polym. time <i>min</i>	$M_{n,PEO}$ Macroinitiator <i>g mol⁻¹</i>	DP_{theo} PPhos	$M_{n,theo}^a$ PPhos <i>g mol⁻¹</i>	DP_{exp}^b PPhos	$M_{n,NMR}^b$ PPhos <i>g mol⁻¹</i>	$M_{n,SEC}^c$ total <i>g mol⁻¹</i>	M_w/M_n^c	HLB ^d
iBP	2.7/1.2	60	5000	10	1800	8	1500	5200	1.10	15.4 (18.6)
nBP	2.7/1.2	60	5000	10	1800	9	1600	5300	1.10	15.2 (18.4)
nHP	2.7/1.2	70	5000	10	2200	8	1800	5700	1.10	14.7 (17.6)

Table 1: Macromolecular characteristics of the different PEO-*b*-polyphosphate amphiphilic copolymers. ^aTheoretical value for the molar mass of the polyphosphate block determined by the initial monomer/initiator ratio. ^bPolymerization degree and molar mass of the polyphosphate block determined by ¹H NMR according equation 1. ^cMolar mass (calculated for the main peak) and molar mass distribution (calculated for the entire elugram) of the diblock copolymers determined by SEC in THF (PEO standards). ^dHLB calculated by the Griffin equation $20x[1-M_n(\text{hydrophobic block})/M_n(\text{total})]$ considering the phosphate block as hydrophobic, in bracket, HLB calculated by considering only the alkyl group as hydrophobic.

The self-assembly of amphiphilic copolymers depends on the hydrophilic-lipophilic balance (HLB), usually calculated by the Griffin equation. HLB calculated by considering as the hydrophobic segments the only alkyl side-chain (10 butyl or 10 heptyl), and keeping the polyphosphate backbone (-CH₂-CH₂-O-PO₃-) as part of the hydrophilic component are reported in Table 1. Based on this consideration, the HLB_{alk} obtained by the Griffin equation reaches 18.6, 18.4 and 17.6, while they were 15.4, 15.2 and 14.7 for the same equation but considering the full polyphosphate block as hydrophobic (Table 1). Due this strong hydrophilic character, it is questionable to see whether these copolymers are able to self-assemble into nanoparticles in aqueous media. It turned out that all the three copolymers synthesized in our work can be directly dissolved in aqueous media (water or phosphate buffer) at room temperature without the help of any organic co-solvent.

Hydrolytic stability. Before studying the surfactant properties of the copolymers, the hydrolytic stability of these polyphosphate-based copolymers was investigated in water. As shown in Figure 2, only the small peak of the triblock copolymers is slightly shifted to lower molecular weight. No shift of the main peak of the SEC traces is observed after 28 days evidencing no significant decrease of molar masses and thus no cleavage of the polyphosphate backbone by hydrolysis under these conditions. So, the diblock copolymers appear stable in milli-Q water at least for 28 days at room temperature, i.e. in the conditions used for the following studies.

Self-assembly in water by dynamic light scattering. The amphiphilic behaviour of the block copolymers was first investigated by measuring the intensity of scattered light of a concentrated solution for the different copolymers (3.8×10^{-4} mol/L) in milli-Q water. The size and size distribution (PDI) of the diffusers are reported in Table 2 as determined by Dynamic Light Scattering (DLS).

These DLS results show that all the three copolymers directly self-assemble in milli-Q water since diffusers have a size above 16 nm but remaining below 200 nm. Vangeyte *et al.*³⁴ described the self-assembly of a PEO₁₁₄-*b*-PCL₈

copolymer with a comparable DP into micelles with a diameter of about 20 nm. By comparison, the larger diameters observed for the PEO-*b*-PiBP and PEO-*b*-PnBP copolymers indicate the formation of loose aggregates rather than well-defined compact core-shell micelles. In contrast, very small and well-defined micelles are observed for the PEO-*b*-PnHP copolymer. The spherical morphology was confirmed by Transmission Electron Microscopy (TEM) for PEO-*b*-polyphosphate self-assembled nanoparticles (TEM images in supplementary information). As expected, the size of the nanoparticles measured by TEM, i.e. under vacuum, appears smaller than the diameter measured by DLS in aqueous medium where the PEO shell is swollen.

Self-assembly in water by fluorescence spectroscopy. In order to confirm the formation of hydrophobic pockets above the CAC, pyrene was added to the copolymer solutions of increasing concentration and the pyrene fluorescence spectrum was recorded for each copolymer concentration. Indeed, the fluorescence spectrum of pyrene depends on its environment, especially the intensities of the emission peak at 373 nm (denoted I₁), which corresponds to pyrene in aqueous environment, and at 383 nm (denoted I₃), corresponding to pyrene in hydrophobic environment, which is the case in the hydrophobic core of the nanoparticles. The measurement of the I₁/I₃ ratio as a function of the logarithm of copolymer concentration is shown in the supplementary information. From the slope break of these curves, the CAC could be obtained for the three copolymers (table 2). This pyrene experiment confirms the presence of self-assembled nanoparticles of the three PEO-*b*-polyphosphate copolymers above a given concentration depending on the length of the polyphosphate pendant groups.

In line with the recent report of Yang *et al.*³¹, we can conclude that the CAC decreases when the length of the pendant groups increases and thus when the hydrophobicity of the polyphosphate block increases. In addition, varying the architecture of the alkyl side-chain from iso-butyl to n-butyl appears to decrease slightly the nanoparticles size and increase the CAC of the copolymer of identical HLB. Remarkably, the used copolymers are able to self-assemble in water without the

help of organic co-solvent in contrast to the report of

Copolymers	D_h^a nm	PDI ^a	D_h^b nm	PDI ^b	CAC ^c mol/L	SCC ^d mol/L
PEO- <i>b</i> -PiBP	153 ± 2	0.114	169 ± 3	0.098	3.1 10 ⁻⁵	4.1 10 ⁻⁶
PEO- <i>b</i> -PnBP	145 ± 2	0.189	126 ± 3	0.168	4.6 10 ⁻⁵	2.2 10 ⁻⁶
PEO- <i>b</i> -PnHP	21 ± 2	0.224	16 ± 4	0.098	2.8 10 ⁻⁶	2.8 10 ⁻⁷

Table 2: Hydrodynamic diameter (D_h) and size distribution (PDI) of the PEO-*b*-polyphosphate assemblies in milli-Q water, determined by DLS measured ^afor a copolymer concentration of 1.9 10⁻⁴ mol/L and ^bfor a copolymer concentration of 3.8 10⁻⁴ mol/L, ^cCAC values determined by pyrene probe fluorescence spectroscopy and ^dthe SCC values by tensiometry.

Yang *et al.*³¹ on PEO-*b*-polyphosphate and to PEO-*b*-PCL copolymers as reported by Cajot *et al.*¹⁴.

It is noteworthy that above the CAC, the size of the assemblies varies slightly with the concentration. The size generally decreases when increasing the concentration (Table 2, compares columns 2 and 4) except for the iBP based copolymer. However, no dramatic changes of the nanoparticles size are observed with the concentration by diluting or increasing it above the CAC.

Behaviour of the PEO-*b*-polyphosphate at the air-water interface

The self-association in water of the diblock copolymers being demonstrated, the comparative ability to reduce the air/water interfacial tension was measured in function of the copolymer concentration. For all the three copolymers, a clear decrease of the interfacial tension is observed upon increasing the copolymer concentration (Figure 3). A clear break in the slope is observed in each case which occurs for quite similar concentration for both butyl polyphosphate (~3x10⁻⁶ mol/L) and corresponds to a levelling of the interfacial tension down to 30 mN/m.

In the case of PEO-*b*-PnHP copolymer, this break appears at a concentration of one order of magnitude lower (~ 3x10⁻⁷ mol/L) as compared for PEO-*b*-PiBP and PEO-*b*-PnBP in line with the lower CAC measured by pyrene fluorescence analysis. It is noteworthy that for PEO-*b*-PnHP copolymer, the interfacial tension does not stabilize after the slope break. It rather keeps decreasing slowly when we further increase the concentration, the interfacial tension remaining above 35 mN/m.

These slope change concentration (SCC) values were compared with PEO-*b*-PCL amphiphilic block copolymer presenting a similar DP for each block. Vangeyte *et al.*³⁵ determined by tensiometry the SCC value for PEO₁₁₄-*b*-PCL₈ copolymers which is approximately 8x10⁻⁶ mol/L. This value is similar than the SCC values for PEO₁₁₄-*b*-PiBP₉ and PEO₁₁₄-*b*-PnBP₉ copolymers whereas the SCC value for the most hydrophobic nHP system is much lower. Comparing the surface tension values at the slope break with those for the PEO₁₁₄-*b*-PCL₈ copolymers (~51 mN/m)³⁵, we observe that the surface tension values for the PEO-*b*-polyphosphate at the slope break are much lower, approximately 35 mN/m for PEO-*b*-PiBP and

PEO-*b*-PnBP copolymers and 47 mN/m for the most hydrophobic nHP system, showing the higher efficiency of PEO-*b*-polyphosphate copolymers to stabilize the air-water interface as compared to the PEO-*b*-PCL of the same DP.

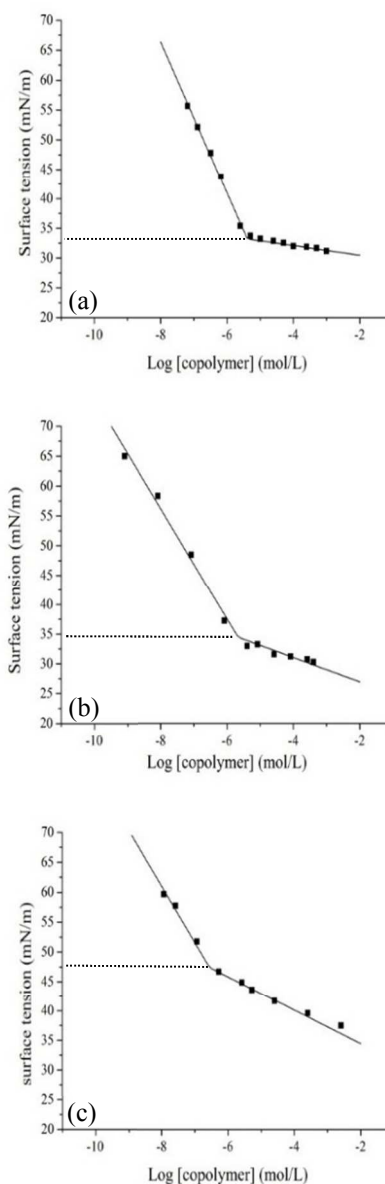


Figure 3: Plots of surface tension versus log[copolymer] for the PEO-*b*-polyphosphate copolymers: (a) PEO-*b*-PiBP, (b) PEO-*b*-PnBP and (c) PEO-*b*-PnHP

Conclusion

This study demonstrated that the amphiphilic block copolymers (PEO₁₁₄-*b*-PiBP₈, PEO₁₁₄-*b*-PnBP₉ and PEO₁₁₄-*b*-PnHP₈) are able to self-assemble in water into nanoparticles without the use of any organic co-solvent whatever the length of the pendant group along the polyphosphate block and thus whatever the hydrophobicity of the polyphosphate block. The surfactant properties of these copolymers including aqueous micellization and air/water interfacial tension investigations were systematically studied. The CAC decreases when the length of the pendant group increases and thus when the polyphosphate block is more hydrophobic. Accordingly, a low CAC can be achieved with a heptyl chain. In addition, we evidenced that the architecture of the alkyl chain while keeping constant the HLB has an effect on the size and CAC of the self-assemblies even if the particles size remains below 200nm in any case. The copolymers were synthesized by metal-free ring-opening polymerization of the corresponding cyclic phosphates initiated by a PEO macro-initiator. Such copolymers appear thus as a promising class of environmental-friendly neutral surfactant copolymers of interest in various fields of applications such as food, cosmetic, biomedical and detergency applications.

Experimental

Materials

2-chloro-1,3,2-dioxaphospholane 2-oxide (COP, Acros), Methanol (MeOH, Acros), diethyl ether (Chem-lab) and calcium hydride (CaH₂, Aldrich) were used as received without further purification. 2-methylpropan-1-ol (Aldrich), butan-1-ol (Aldrich), heptan-1-ol (Alfa Aesar), *N,N*-diethylethanamine (triethylamine, TEA, Aldrich) and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU, Aldrich) were dried over CaH₂ at room temperature and purified by distillation under reduced pressure just before use. Toluene (Chem-lab), tetrahydrofuran (THF, Chem-lab) and dichloromethane (CH₂Cl₂, Chem-lab) were dried on molecular sieves. Monomethoxy-PEO (MeO-PEO-OH, M_n=5000 g/mol, Aldrich) was dried by three azeotropic distillations with anhydrous toluene before use. Milli-Q water was used for the micelle's preparation. 1-1-[3,5-bis(trifluoromethyl)phenyl]-3-cyclohexyl-2-thiourea (TU), 2-(2-methylpropoxy)-1,3,2-dioxaphospholane 2-oxide (iBP) and 2-butoxy-1,3,2-dioxaphospholane 2-oxide (nBP) monomers were synthesized as described in the literature elsewhere.^{21,31,37}

Structural characterization techniques

¹H and ³¹P NMR analyses were performed on a Bruker Advance 250 MHz spectrometer in CDCl₃ at 25°C in the FT mode. The ³¹P NMR measurement was carried out with the ¹H powergate decoupling method using a 30° flip angle. ¹H NMR

chemical shifts are reported in ppm relative to Me₄Si. ³¹P NMR chemical shifts are reported in ppm relative to H₃PO₄. Size exclusion chromatography (SEC) was carried out in THF at 45°C at a flow rate of 0.7 mL/min with Viscotek 305 TDA liquid chromatograph. The PL gel 5 μm (10⁴ Å, 10³ Å and 100 Å) columns were calibrated with PEO standards.

Synthetic procedures

Monomer synthesis. 2-heptyloxy-1,3,2-dioxaphospholane 2-oxide (nHP) was synthesized by the esterification reaction of COP with 1-heptanol in the presence of TEA. Typically, COP (100g, 0.702 mol) in a THF solution (200 mL) was added dropwise, under stirring at 0°C, to a solution of the anhydrous alcohol and TEA in dry THF (200 mL) (COP/TEA/Alcohol = 1/1/1). After the complete addition, the solution was stirred at 0°C for 4 hours and the resulting mixture was filtrated to remove the alkyl ammonium salt before concentrating the filtrate under vacuum. Finally, the monomer was purified by fractional distillation under vacuum (10⁻⁴ Torr, T_b=105°C, yield=53%).

¹H NMR (250MHz, CDCl₃, 25°C, TMS): 0.9 ppm (t, 3H, -CH₂-CH₃), 1.3 ppm (t, 8H, -O-CH₂-CH₂-(CH₂)₄-CH₃), 1.7 ppm (m, 2H, -O-CH₂-CH₂-(CH₂)₄-CH₃), 4.1 ppm (dt, ²J_{HH} = 6.6 Hz and ³J_{PH} = 8.6 Hz, 2H, -O-CH₂-CH₂-(CH₂)₄-CH₃), 4.4 ppm (m, 4H, -O-CH₂-CH₂-O-). ¹H-decoupled ³¹P NMR (CDCl₃, 101 MHz): 18.1 ppm.

Polymerization from a PEO macro-initiator. The polymerization of nBP, iBP or nHP was performed in CH₂Cl₂ at 0°C with MeO-PEO-OH as a macro-initiator, DBU as a catalyst and TU as a co-catalyst with a ratio [PEO-OH]₀/[Monomer]₀/[DBU]₀/[TU]₀ = 1/10/1.3/0.5. In a glass reactor, TU (386 mg, 1.04 mmol) and the cyclic phosphate monomer (20.8 mmol) were dried by three successive azeotropic distillations with toluene and dissolved in CH₂Cl₂ ([Monomer]₀ = 1 mol/L) before being transferred under inert atmosphere into a second glass reactor containing 10 g (2.1 mmol) of MeO-PEO-OH, freshly dried by three azeotropic distillations. After complete solubilisation of PEO, 0.4 mL of DBU (2.7 mmol) was added under nitrogen atmosphere with a freshly flamed stainless steel capillary. After 60 minutes of polymerization (after 70 minutes for nHP), the solution was concentrated under vacuum and the polymer was precipitated in cold diethyl ether and filtrated. Residual DBU was eliminated overnight by dialysis against MeOH (Spectrum, membrane porosity = 1000 g mol⁻¹). After elimination of MeOH under vacuum, the residue was dissolved in THF and the amphiphilic copolymer was recovered by precipitation in cold diethyl ether before being dried under vacuum.

*For PEO-*b*-PnBP:* the ¹H NMR spectrum is in good agreement with the reported spectrum by Yang *et al.*³¹ ¹H-decoupled ³¹P NMR (101 mHz, CDCl₃, 25°C): -0.60 ppm. M_w/M_n (SEC) = 1.10

*For PEO-*b*-PiBP:* ¹H NMR (250MHz, CDCl₃, 25°C, TMS): 0.95 ppm (d, 6H, -CH-(CH₃)₂), 1.95 ppm (m, 1H, -CH-(CH₃)₂), 3.65 ppm (s, 4H, -C-O-CH₂-CH₂-O-C-), 3.85 ppm (m, 2H, -O-CH₂-CH-(CH₃)₂), 4.3 ppm (m, 4H,

–P–O–CH₂–CH₂–O–P–). ¹H-decoupled ³¹P NMR (101 MHz, CDCl₃, 25°C): -0.60 ppm. M_w/M_n (SEC) = 1.10

For PEO-*b*-PnHP: ¹H NMR (250MHz, CDCl₃, 25°C, TMS): 0.9 ppm (t, 3H, –CH₂–CH₃), 1.3 ppm (m, 8H, –O–CH₂–CH₂– (CH₂)₄–CH₃), 1.7 ppm (m, 2H, –O–CH₂–CH₂–(CH₂)₄–CH₃), 3.65 ppm (s, 4H, –C–O–CH₂–CH₂–O–C–), 4.1 ppm (m, 2H, –O–CH₂–CH₂–(CH₂)₄–CH₃), 4.25 ppm (m, 4H, –P–O–CH₂–CH₂–O–P–). ¹H-decoupled ³¹P NMR (101 MHz, CDCl₃, 25°C): -0.60 ppm. M_w/M_n (SEC) = 1.10

Characterization of the copolymer in water

Hydrolytic stability. The hydrolytic stability of the copolymers was monitored in Milli-Q water at room temperature over a period of 1, 7, 14 and 28 days. 100 mg of copolymer were dissolved in 35 mL of Milli-Q water. The concentration of the polymer was set at 3 mg mL⁻¹. At the predetermined times, 3 mL of the solution were collected, dried by lyophilisation before being analysed by SEC in THF.

Dynamic Light Scattering (DLS). Typically, 25 mg or 50 mg of the PEO-*b*-polyphosphate copolymer were directly dissolved in 20 mL deionized water and stirred overnight. Particle size and size distribution were acquired from freshly prepared copolymer solutions by dynamic light scattering (DLS). DLS measurements were performed using a Beckman Coulter Delsa Nano C Particle Analyzer and the data were treated by the Delsa Nano UI 2.21 software. The average size distribution of aqueous micellar solutions was determined based on the CONTIN method. All the measurements were carried out in a glass cell at 25°C at a measuring angle of 165°. Aqueous micellar solutions were filtered with a microfilter having an average pore size of 1.2 μm. All DLS measurements were repeated five times in order to check their reproducibility.

Transmission Electron Microscopy (TEM). The samples for TEM were prepared by slow evaporation of the copolymer aqueous solutions after DLS analysis on a formvar-coated grid. The excess of solutions was removed with a filter paper. The samples were analysed with a Philips CM100 microscope equipped with an Olympus camera and transferred to a computer equipped with a Megaview system.

Fluorescence spectroscopy measurements. CAC values in milli-Q water for the different amphiphilic block copolymers were determined by the pyrene probe fluorescence spectroscopy using a LS50B luminescence spectrometer (Perkin Elmer). Typically, solid pyrene (2.6 10⁻⁸ mol) was dissolved in aqueous solutions of the amphiphilic copolymer of increasing concentration from 1x10⁻¹⁰ to 1x10⁻³ mol/L. Milli-Q water was used to dissolve first the copolymer before adding it to vials containing the pyrene. Since the amount of needed pyrene is quite low (5.2 μg), a solution of pyrene (1.3 10⁻³ mol/L) in acetone was first prepared. 20 μL of the pyrene solution was transferred into glass vials and acetone was evaporated at room temperature before adding copolymer solution. After one night of stirring at room temperature, the fluorescence spectra of pyrene were recorded from 360 to 460 nm after an excitation at 339 nm. The emission and excitation slit widths were set at 2.5 and 3.0, respectively. The ratio of the peak intensities at 373 nm

and 383 nm (I₃₇₃/I₃₈₃) of the emission spectra were calculated for each copolymers solution and recorded as a function of the copolymer concentration.

Characterization of the air-water interface by tensiometry

The rising air-bubble method was used to study the efficiency of the PEO-*b*-polyphosphates copolymers to stabilize the air/water interface. Aqueous solutions of different copolymer concentrations ranging from 10⁻¹⁰ to 10⁻³ mol/L were prepared before being tested by a Tracker tensiometer (TECLIS, France). The method based on the analysis of a 5 μL air bubble deformation affords to measure the air/water interfacial tension at the equilibrium of adsorption for each copolymer.

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