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# Tuning the Thermo-Sensitivity of Micellar Systems through a Blending Approach

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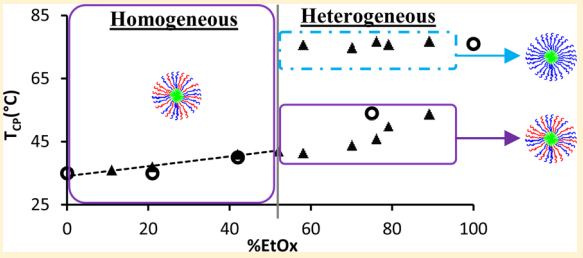
Supporting Information

**ABSTRACT:** This paper reports an original and easy route toward thermosensitive micelles based on a lipidic core and with adjustable cloud point temperatures ($T_{CP}$) through a simple blending approach between two copolymers of different $T_{CP}$. The cationic ring-opening polymerization (CROP) of various 2-alkyl-2-oxazoline monomers was first investigated from both mesylated and tosylated lipoinitiators and different lipid-*b*-poly(2-methyl-2-oxazoline), lipid-*b*-poly(2-ethyl-2-oxazoline), lipid-*b*-poly(2-isopropyl-2-oxazoline) and lipid-*b*-poly-((2-ethyl-2-oxazoline)-*co*-(2-isopropyl-2-oxazoline)) copolymers were synthesized. Blending of lipid-*b*-p(EtOx) and lipid-*b*-p(*i*PrOx) copolymers in various proportions were then prepared and their $T_{CP}$ investigated through UV−visible spectroscopy. Very interesting results were obtained as the blends exhibit a single $T_{CP}$ ranging from 35 to 45 °C. Furthermore, the blends $T_{CP}$ correspond to those of the statistical lipid-*b*-p((EtOx)-*co*-(*i*PrOx)) for the same p(EtOx)/p(*i*PrOx) content up to 52 wt % of p(EtOx). The blending approach is then an attractive strategy to control the $T_{CP}$ of micellar systems through a simple and easy formulation approach rather than fastidious syntheses.




## INTRODUCTION

Surfactants are widely present in industrial applications ranging from emulsion stabilization (painting formulation, cosmetics, ...) to viscosity regulation and catalyst support to biomedical applications. Advances in synthesis techniques now allow the preparation of environmental sensitive surfactants and the subsequent development of colloids with controlled stability vs the environment. However, the common approach is to design one specific molecule for one given application, with a sometimes complex composition and/or architecture. Despite the existing controlled polymerization techniques, it is usually quite a challenge to obtain strictly similar compositions and distribution between the initial comonomers ratio and the final copolymer. Therefore, a really fine-tuning of the self-assemblies properties is still challenging. Another limit of this usual approach is the necessity to step back to a new synthesis scheme when the required stability conditions are modified. Besides the numerous synthetic steps generated through this time-consuming approach, the drawback is even bigger when the FDA approval is required for each new (macro)molecule prior to any application.

By contrast with the design and the synthesis of new copolymers with novel microstructures, the cooperative self-assembly of two copolymers to manipulate the physicochemical behavior of the mixed system seems an efficient approach. Despite the possible fascinating properties of such micelles, there are only a few reports on their formation and behavior. The possibility to obtain mixed micelles through a blending approach was first demonstrated with copolymers presenting identical chemical nature but different block lengths.[1−4] This approach was extended to the combination of copolymers of different natures directed mainly to the formation of "oignon-like" structure (core−shell-corona).[5−7] However, Li et al. reported the formation of mixed micelles with double responsive channels by self-assembling poly(*tert*-butyl acrylate)-*b*-poly(*N*-isopropylacrylamide) and poly(*tert*-butyl acrylate)-*b*-poly(4-vinylpyridine),[8,9] and we have demonstrated that the combination of lipid-*b*-poly(acrylic acid) and lipid-*b*-poly(isopropyl-2-oxazoline) leads to the formation of mixed micelles presenting both pH and thermosensitive properties.[10,11] The blending approach is then an interesting pathway to combine polymer properties and stimuli-sensitivities. Really recently, Wright et al. investigated the mixing of two block copolymers, differing only in the ratio of comonomers in the hydrophobic block, and demonstrated that the blended micelles were structurally identical to those formed by a single block copolymer matching to the overall composition of the blends.[12,13] Therefore, the overall core content governs the micelles structure. Does the same statement apply to the hydrophilic corona and is it consequently possible to finely tune

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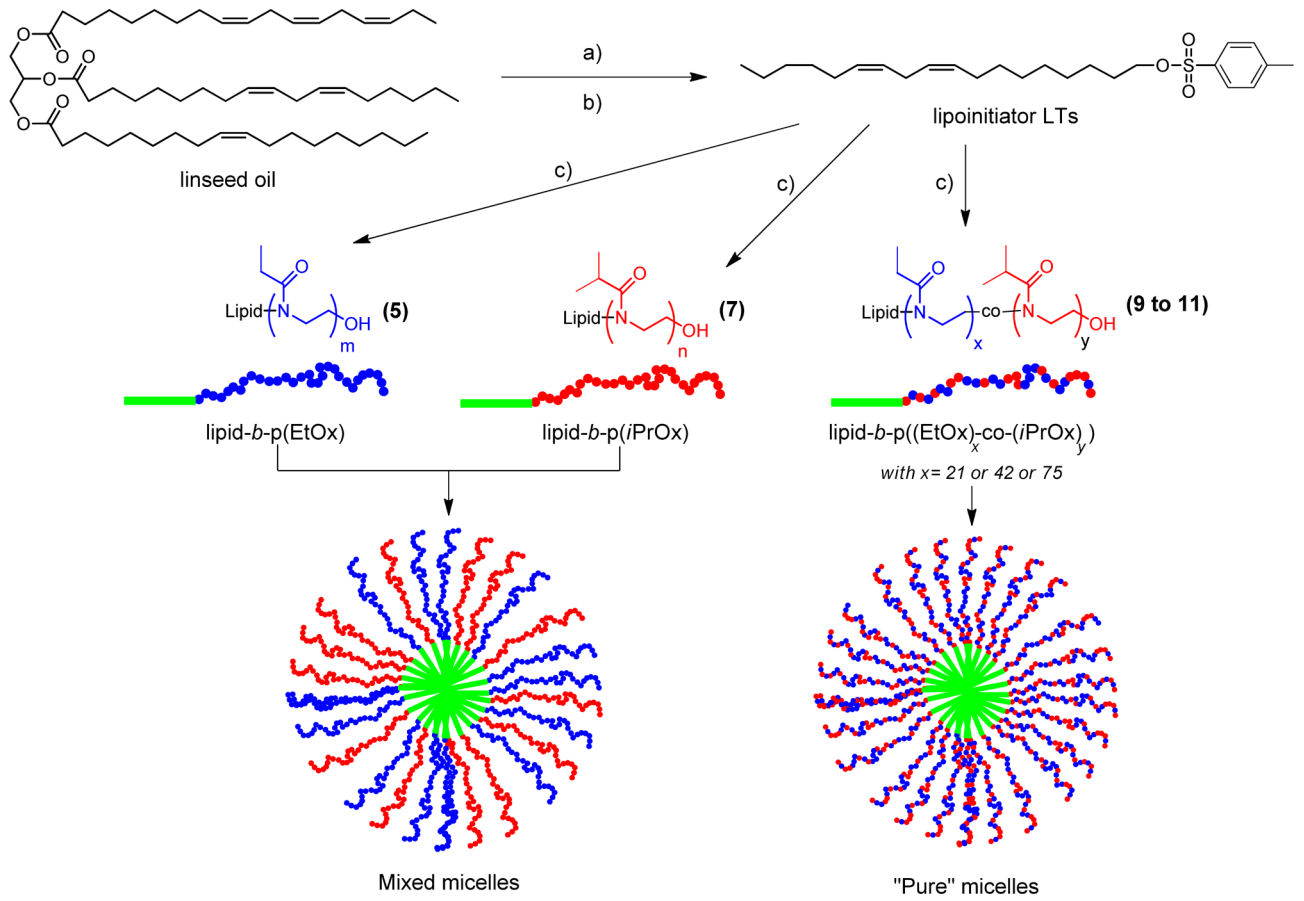
**Scheme 1. General Approach toward Thermosensitive Micelles with Tuned $T_{CP}$**[a]




[a]Key: (a) $LiAlH_4$, THF, RT, overnight; (b) DCC/DMAP, TsCl, $Et_3N$, $CHCl_3$, room temperature, overnight; (c) CROP 2-alkyl-2-oxazoline, $CH_3CN$, microwave, 140 °C, 30 min (for clarity purpose only the major aliphatic chain is represented on the lipoinitiator structure but one has to keep in mind that it is a statistical mixture).

the micelles stimuli-sensitivity through this simple blending approach? To answer this question, we investigate in the present paper the blending of two copolymers presenting different cloud point temperatures and studied the thermosensitivity of the final mixed micelles.

As the system's ability to equilibrate during or after the assembly is crucial to successfully form mixed micelles,[12,13] lipid chains were selected as the hydrophobic block (low $T_g$) to ensure the micelles' dynamic nature.[4,11] Poly(2-alkyl-2-oxazoline)s were chosen as the hydrophilic block knowing that the POx LCST varied with the pendant alkyl chain length (C2−C4),[14,15] the polymerization degree and the end-group polarity.[16,17] Park et al. reported the accurate tuning of the LCST of poly(2-isopropyl-2-oxazoline) through a gradient copolymerization with 2-ethyl-2-oxazoline.[18] Furthermore, the chemical structure of poly(oxazoline) can be easily modulated through the polymerization initiation or termination steps.[19] A few papers report lipopolyoxazolines based on poly(2-methyl-2-oxazoline) and synthesized from tosylated or halogenated lipoinitiators.[20−22] For instance, Woodle et al. studied the synthesis of distearoylphosphaditylethanolamine (DSPE)-*b*-poly(2-ethyl-2-oxazoline) by termination of the polymerization process with DSPE.[23] We also reported recently the preparation of lipid-*b*-poly(2-isopropyl-2-oxazoline) from a mesylated lipoinitiator and under microwave radiation.[11]

In the present paper, the thermal CROP of 2-methyl-2-oxazoline was initiated by mesylated or tosylated lipoinitiator to compare both leaving groups efficiency. The microwave-assisted CROP of 2-methyl-, 2-ethyl- and 2-isopropyl-2-oxazoline is then described to prepare a range of block copolymers using the more efficient lipoinitiator. The thermosensitivities of lipid-*b*-poly(2-isopropyl-2-oxazoline) (lipid-*b*-p(*i*PrOx)), lipid-*b*-poly(2-ethyl-2-oxazoline) (lipid-*b*-p(EtOx)) and statistic lipid-*b*-poly((2-isopropyl-2-oxazoline)-*co*-(2-ethyl-2-oxazoline)) (lipid-*b*-poly((*i*PrOx)-*co*-(EtOx))) copolymers are successively investigated. Finally, mixed micelles containing lipid-*b*-p(*i*PrOx) and lipid-*b*-p(EtOx) in different proportions are prepared and their cloud point temperatures are compared to statistical lipid-*b*-poly((*i*PrOx)-*co*-(EtOx)) copolymers presenting the same *i*PrOx/EtOx global content (Scheme 1).

## EXPERIMENTAL PART

**Materials.** Linseed oil was kindly provided by Novance. *p*-Methyl toluene sulfonate, *N,N′*-dicyclohexylcarbodiimide (DCC), 2-methyl-2-oxazoline, and 2-ethyl-2-oxazoline were purchased from Sigma-Aldrich. 2-Isopropyl-2-oxazoline was purchased from TCI. Lithium aluminohydride, triethylamine (TEA), 4-(dimethylamino)pyridine (DMAP), anhydrous THF, anhydrous acetonitrile and anhydrous chloroform were purchased from Acros. Silica Gel (60−200), cyclohexane and ethyl acetate were purchased from VWR.

**Instrumentation.** $^1H$ and $^{13}C$ NMR spectra were recorded on a Bruker AC-P 300 MHz spectrometer. Chemical shifts are reported in ppm relative to the deuterated solvent resonance. Molecular weights and molecular weight distributions were measured using size exclusion chromatography (SEC) on a Spectra-Physics apparatus equipped with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an RI Shodex refractive index detector with two mixed packed columns (Polar Gel L 300*7.5 mm). The eluent used is dimethylacetamide (DMAc) at a flow rate of 0.8 mL/min at 50 °C and poly(methyl methacrylate) standards were used for calibration. Fourier transform infrared (FTIR) spectra were recorded on a PerkinElmer spectrum 2000 FTIR, equipped with a diamond ATR (Attenuated Total Reflection) device (16 scans were performed). DLS measurements were recorded on a Malvern Zetasizer. MALDI−TOF-MS analyses were performed on a MALDI−TOF/TOF Bruker Ultraflex III mass spectrometer using a nitrogen laser for MALDI ($\lambda$ 337 nm) operating at an acceleration voltage of 25KV and reflectron lens potentials at 26.3 kV. Mixture of peptides was used for external calibration. The polymer sample was dissolved at 10 mg/mL in a mixture of acetonitrile and water (50/50 v/v). The matrixes used were HCCA ($\alpha$-cyano-4-hydroxycinnamic acid) and DHB (2,5-dihydroxybenzoic acid). Each matrix was dissolved at 10 mg/mL in a trifluoroacetic acid/acetonitrile solution (0.1% TFA/$CH_3CN$: 70/30). Solutions of matrix and polymer were mixed in a volume ratio of 10:4 respectively. The mixed solution was hand-spotted on a MALDI target and left to dry. The theoretical mass was expressed as followed:

$$M_{th} = n \times M_{EtOx,th} + m \times M_{iPrOx,th} + RM_{th}$$

Here $M_{th}$ is the calculated mass of the (co)polymer, $M_{EtOx,th}$ and $M_{iPrOx,th}$ are the mass of the ethyl and isopropyl repetitive units (99 and 113 g/mol, respectively), and $RM_{th}$ represents the calculated residual mass ($C_{18}H_{33}$− and −OH terminal groups).

**Fatty Alcohols Synthesis.** The linseed oil reduction into fatty alcohols was realized following a previously reported method.[10]

**Lipoinitiator (LTs) Synthesis.** A mixture of *p*-TsCl (2 equiv, 7.05 g) in anhydrous chloroform (40 mL) was added dropwise to a mixture of fatty alcohol (1 equiv, 5 g), TEA (3 equiv, 5.63 g) and DMAP (0.1 equiv, 0.22 g) in anhydrous chloroform (35 mL) under inert atmosphere. The reaction media was stirred overnight at 30 °C, then extracted with AcOEt (50 mL) and washed with $NaHCO_3$ and brine. The resulting solution was dried over anhydrous magnesium sulfate, filtered and concentrated under vacuum. The crude product was purified through a column chromatography over silica gel (cyclohexane/ethyl acetate =91:9) leading to a yellowish liquid with 57% yield.

$^1$H NMR ($CDCl_3$, 300 MHz), $\delta$ (ppm): 7.80 and 7.35 (d, 4H benzyl), 5.34 (m, 2H, −HC═CH−), 4.01 (t, 2H, $-CH_2-OS$), 2.80 (m, 2H, −HC═CH−**$CH_2$**−HC═CH−), 2.44 (s, 1H, $CH_3$), 2.05 (m, 2H, −HC═CH−**$CH_2$**−$CH_2$), 1.63 (m, **$CH_2$**−$CH_2$−OS), 1.25 (m, 2H, alkyl $CH_2$), 0.97 (t, 3H, $CH_3$ linolenic acid), 0.88 (m, 3H, $CH_3$ other acid). $^{13}$C NMR ($CDCl_3$, 75 MHz), $\delta$ (ppm): 144.8 (benzyl), 139−127.3 (−HC═CH−), 71 ($-CH_2-OS$), 32.1−20.9 (alkyl $CH_2$), 14.6 (alkyl chain). MS [M+ $Na^+$]: *m*/*z* 441 (linolenic), *m*/*z* 443 (linoleic), *m*/*z* 445 (olenic). IR: $\nu_{C=C}$ = 3009 $cm^{-1}$, $\nu_{CH_2,CH_3}$ = 2924 and 2853 $cm^{-1}$, $\nu_{S=O}$ = 1361 $cm^{-1}$, $\nu_{S=O}$ = 1180 $cm^{-1}$.

**CROP Polymerization.** The protocol for the thermal CROP is identical to the previously reported method.[19]

For all microwave-assisted polymerizations, the initiator (MeOTs or LTs) (1 equiv) and dried acetonitrile are first introduced in a microwave vial flushed with nitrogen. Then monomer(s) (4 M) is added via a syringe. The polymerization mixture is stirred at 140 °C during the appropriate reaction time and terminated with methanolic KOH (1 M) to introduce a hydroxyl group at the chain end. The polymer obtained is purified by dialysis (500 Da cutoff) against distilled water for 2 days changing the water every 6 h. The final polymer is recovered by lyophilization.

*Synthesis of Hydrophilic (Co)polymers Using MeOTs.* p(EtOx), **4**, $[EtOx]_0$:$[MeOTs]_0$ = 18:1; p(*i*PrOx), **6**, $[iPrOx]_0$:$[MeOTs]_0$ = 26:1; p((EtOx)-*co*-(*i*PrOx)), **8**, $[EtOx]_0$:$[iPrOx]_0$:$[MeOTs]_0$ = 2:17:1. The polymerizations run for 30 min and the purified polymers are white powders.

$^1$H NMR ($CDCl_3$, 300 MHz), $\delta$ (ppm): 3.7−3.1 (m, 4H, $N-CH_2-CH_2$), 3 (s, 1H, $-CH_3$), 3−2.5 (m, 1H, **−CH−**$(CH_3)_2$), 2.4−2.1 (m, 2H, **$-CH_2-CH_3$**), 1.15 (m, 9H, $CH_3$ isopropyl and ethyl group).

*Synthesis of Amphiphilic Copolymer Using LTs.* Lipid-*b*-p(MeOx), **3**, $[MeOx]_0$:$[MeOTs]_0$ = 26:1, 20 min of polymerization; lipid-*b*-p(EtOx), **5**, $[EtOx]_0$:$[LTs]_0$ = 26:1, 30 min of polymerization; lipid-*b*-p(*i*PrOx), **7**, $[iPrOx]_0$:$[LTs]_0$ = 26:1, 30 min of polymerization; lipid-*b*-p((EtOx)-*co*-(*i*PrOx)), **9**−**11**: $iPrOx_{79\%}$ = 17 equiv, $iPrOx_{58\%}$ = 13 equiv, $iPrOx_{25\%}$ = 5 equiv and $EtOx_{21\%}$ = 2 equiv, $EtOx_{42\%}$ = 6 equiv, and $EtOx_{75\%}$ = 12 equiv.

All the purified polymers are beige powders.

$^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ (ppm): 5.34 (m, 2H, − HC═CH−), 3.7−3.1 (m, 4H, $N-CH_2-CH_2$), 2.80 (m, 2H, − HC═CH−**$CH_2$**−HC═CH−), 3−2.5 (m, 1H, **-CH-**$(CH_3)_2$), 2.4−2.1 (m, 2H, **$-CH_2-CH_3$**), 1.85 (m, 2H, − HC═CH−**$CH_2$**−$CH_2$), 1.25 (m, 2H, alkyl $CH_2$), 1.15 (m, 9H, $CH_3$ isopropyl and ethyl group), 0.97 (t, 3H, $CH_3$ linolenic chain), 0.88 (m, 3H, $CH_3$ other alkyl chain).

**Preparation of Micelles.** Lipid-*b*-poly(2-alkyl-2-oxazoline) was first solubilized in methanol (2 mL) and then dropped into deionized water containing 0.1 M of NaCl. The solution was stirred overnight to evaporate the methanol. The solution was completed by deionized water (containing 0.1 M of NaCl) to achieve a copolymer concentration of 2 g/L and stirred a minimum of 3 h before analyses.

Lipid-*b*-p(EtOx) and lipid-*b*-p(*i*PrOx) solution prepared as described above were mixed together in order to form mixed micelles. Different solutions were prepared in order to obtain a range in EtOx concentration. All solutions were stirred a minimum of 3 h before analyses.

**Cloud Point Temperature Measurements.** Cloud points were determined by spectrophotometric measurements of the absorbance of micellar solutions at $\lambda$ = 500 nm and heated at a constant rate of 0.5 °C/min. The cloud points temperatures were reached when the absorbance increased by 10%.

**DLS Measurements.** Copolymer solutions at 2 g/L were prepared in deionized water containing 0.1 M of NaCl and filtered on a 0.45 $\mu$m cellulose filter prior to introduction in the Zetasizer device. Temperature is controlled with a Peltier. Eight measurements were performed by analysis.

**Fluorescence Measurements.** Sample solutions for fluorescence measurements were prepared as described previously.[24] The sample solutions were prepared by first adding known amounts of pyrene predissolved in acetone to a series of 10 mL volumetric flasks (to obtain a final pyrene concentration of $4.10^{-7}$ mol $L^{-1}$ after dilution). Acetone was allowed to evaporate and accurate amounts of a copolymer stock solution (2 g/L, 0.1 M NaCl) were added to each of the volumetric flask to reach various concentrations ranging from 1 mg/L to 2 g/L after dilution with a 0.1 M NaCl solution. The solutions were stirred at least 2 h prior the analysis. Excitation spectra of pyrene were recorded between 330 and 360 nm (bandwidth = 1.5 nm) at a fixed emission wavelength ($\lambda_{Em}$ = 374 nm, bandwidth = 1.5 nm).

## RESULTS AND DISCUSSION

**Polymerization of 2-Methyl-2-oxazoline (MeOx) Using Tosylated and Mesylated Initiators.** Two methods can be envisaged toward the preparation of lipid-*b*-poly(oxazoline) copolymers by (1) the "initiator route", where the lipidic block (lipoinitiator) is introduced from the beginning of the polymerization or (2) the "termination route", adding the fatty alcohol during the termination step of the CROP polymerization. The first method permitting a quantitative incorporation of the lipidic chain to the polyoxazoline block was preferred.[11]

In our previous study, the CROP polymerization of 2-isopropyl-2-oxazoline was initiated from a mesylated lipoinitiator (LMs), family of initiators very little studied. The polymerization control was not entirely satisfying. Herein, the study of the oxazoline polymerization was extended to more conventional lipidic initiator: tosylated lipoinitiator (LTs) and its efficiency was compared to that of LMs. Both initiators were


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synthesized in two steps from linseed oil: (1) the oil reduction with lithium aluminohydride leading to a statistical mixture of fatty alcohols (55.5% linolenic, 16.9% linoleic, 18% oleic and 9.6% of saturated chains as determined by $^1$H NMR); (2) the reaction of the hydroxyl end group with either mesyl or tosyl chloride. As previously reported, the mesylation reaction proceed in 77% yield and the final lipoinitiator can be easily purified through simple washing with water.[11] The purified tosylated lipoinitiator LTs (Scheme 1) is obtained with a yield of 57% after a column purification. The $^1$H NMR spectrum (Figure 2A) shows the appearance of a signal corresponding to the aromatic group ($\delta$ = 7.80 and 7.35 ppm) and the shift of the characteristic signal of the protons in $\alpha$ position (from $\delta$ = 3.63 to $\delta$ = 4.01 ppm) and in $\beta$ position (from $\delta$ = 1.5 to $\delta$ = 1.63 ppm) of the tosylate function. The FTIR spectrum (Figure S 3) shows the disappearance of the hydroxyl vibration band ($\upsilon$ = 3335 cm$^{-1}$) and the appearance of vibration bands corresponding to the introduced sulfate group ($\upsilon$ = 1360 and $\upsilon$ = 1180 cm$^{-1}$).

Both initiators were engaged in the CROP of MeOx in deuterated acetonitrile at 80 °C and the monomer conversion vs time was monitored by $^1$H NMR (Figure 1). The ln([M]$_0$/[M]) vs time shows some differences at the beginning of the polymerization with a longer latency time for LMs initiator (4 h vs 1 h). The latency has already been reported for other initiators.[25] This phenomenon may be explained by the low reactivity of the initiator coming from the low probability of encounter between the hydrophobic end fatty chain and a hydrophilic monomer MeOx. A first order kinetics can be observed in both cases showing a constant concentration of active species compatible with a living polymerization.

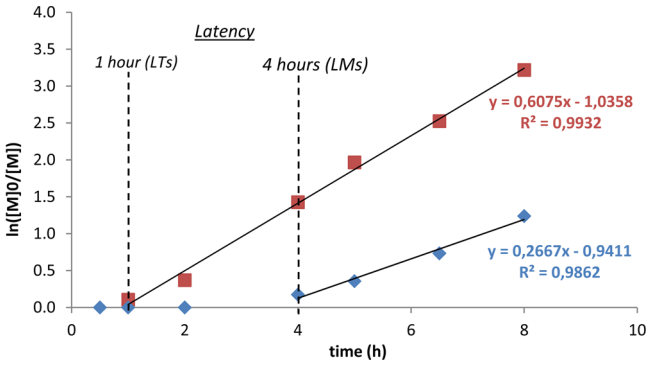


**Figure 1.** Kinetic plots for the thermal CROP of MeOx initiated by LTs (red ■) and LMs (blue ◆).

The polymerization rate constant ($k_p$) with both initiators were deduced from the slopes. The polymerization initiated from the tosylated lipoinitiator ($k_p$ (LTs) = 1.06.10$^{-3}$ L·mol$^{-1}$·s$^{-1}$) proceeds twice faster than from the mesylated one ($k_p$ (LMs) = 4.87.10$^{-4}$ L·mol$^{-1}$·s$^{-1}$). These values are consistent with those previously reported for propargyl toluene-4-sulfonate[26] or MeOTs.[27] In the literature, few studies described the use of mesylated initiators for the CROP of oxazoline even if it is well-known that the polymerization rate increases with the nucleophilicity of the initiator counterion: MeOTf > MeOTs > MeI.[28]

The average number molecular weight and the dispersity of p(MeOx) after purification were monitored by SEC analyses as summarized in Table 1 (runs 1 and 2). Using LTs and LMs, well-defined copolymers were obtained with a dispersity of 1.33 and 1.49, respectively. The presence of the lipidic chain in the final copolymers is confirmed by $^1$H NMR spectroscopy (Figure S 1 and Figure S 2) and allows one to determine the p(MeOx) polymerization degree through the relative integration of the lipidic CH$_2$ signal ($\delta$ = 1.30 ppm) vs the p(MeOx) CH$_2$ signal ($\delta$ = 3.40 ppm) (detailed formula in Supporting Information). The NMR analysis also permits the calculation of the efficiency of the initiator using the characteristic signals of initiator before and after reaction. For LTs, the calculation concerns the signals located at 7.60 and 7.76 ppm whereas for LMs, they appear at 2.50 and 3 ppm. A better efficiency was observed for LTs with 78 and 61%, respectively (Table 1 runs 1 and 2). Consequently LTs was chosen to pursue the study. To our best knowledge, there is only one previous paper reporting the use of a mesylated (lipo)initiator.[11] However, the results obtained show a real potential for this family of lipoinitiators (easier purification) and further studies are in progress.

The CROP under thermal condition allowed comparing the mesylated and tosylated initiators activity unlike microwave-assisted CROP, which occurred too fast. Wiesbrock et al. demonstrated that microwave radiations considerably reduce the polymerization time from days to minutes keeping the controlled character.[27,29] Thus, through microwave-assisted CROP in acetonitrile at 140 °C, lipid-*b*-p(MeOx) with a degree of polymerization of 49 was obtained only after 20 min. Due to the fast reaction, the kinetic could not be monitored by H$^1$

**Table 1. Polymerization Results and Cloud Point Temperatures of (Co)polymers in Solution at 2 g/L in 0.1 M NaCl**

| run | monomer | Initiator | %mol$_{EtOx}$/%mol$_{iPrOx}$ ($x$/$y$)[b] | $M_n$[c] (g/mol) | $Đ$[c] | eff.[b] (%) | $T_{CP}$[d] (°C) |
|---|---|---|---|---|---|---|---|
| 1 | MeOx[a] | LMs | – | 4500 | 1.49 | 61 | *f* |
| 2 | MeOx[a] | LTs | – | 3700 | 1.33 | 78 | *f* |
| 3 | MeOx | LTs | – | 2900 | 1.41 | 74 | *f* |
| 4 | EtOx | MeOTs | 100/0 | 2600 | 1.22 | *e* | *f* |
| 5 | EtOx | LTs | 100/0 | 3500 | 1.50 | 72 | 76 |
| 6 | *i*PrOx | MeOTs | 0/100 | 4200 | 1.27 | *e* | 58 |
| 7 | *i*PrOx | LTs | 0/100 | 5400 | 1.33 | 68 | 35 |
| 8 | EtOx/*i*PrOx | MeOTs | 20/80 | 3200 | 1.18 | *e* | 69 |
| 9 | EtOx/*i*PrOx | LTs | 21/79 | 4900 | 1.29 | 61 | 35 |
| 10 | EtOx/*i*PrOx | LTs | 42/58 | 4500 | 1.35 | 62 | 40 |
| 11 | EtOx/*i*PrOx | LTs | 75/25 | 3100 | 1.32 | 67 | 54 |

[a]Reaction conditions: 80 °C, acetonitrile. [b]Initiator efficiency determined by $^1$H NMR spectroscopy in CDCl$_3$. [c]$M_n$ and $Đ$ values calculated from SEC analysis in DMAc using PMMA standrards. [d]Cloud point temperature ($T_{CP}$) from spectrophotometric measurements. [e]Integration of CH$_3$ end group signal not possible by NMR characterization. [f]No $T_{CP}$ observed up to 85 °C.



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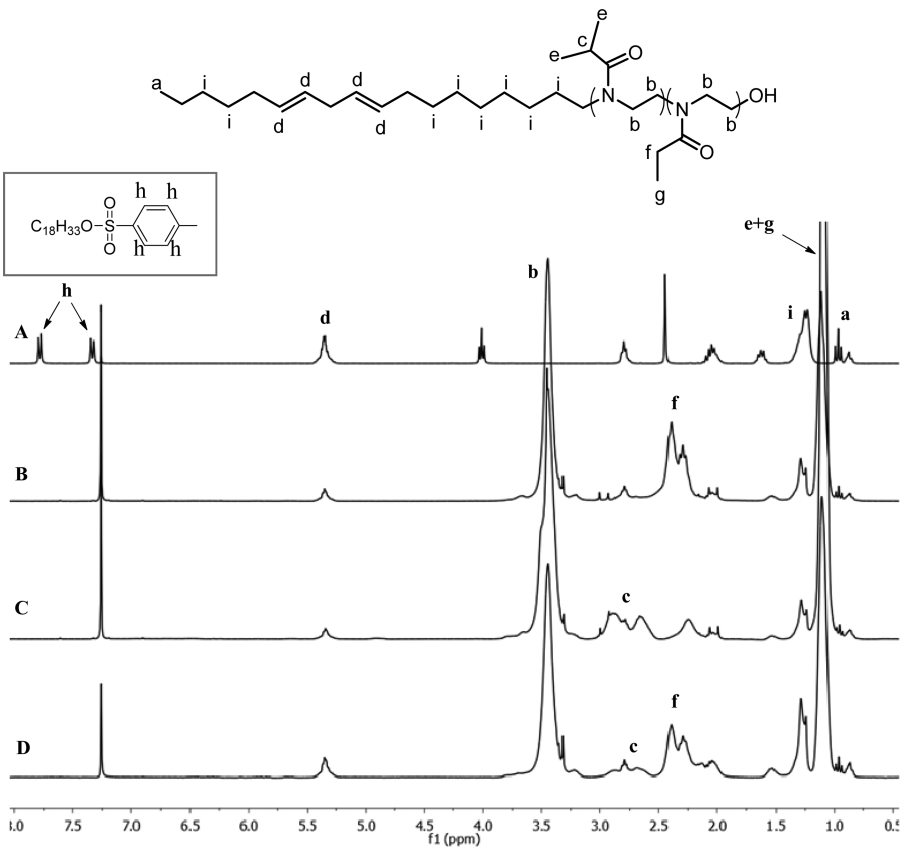


**Figure 2.** $^{1}$H NMR spectra of (A) LTs, (B) lipid-*b*-p(EtOx) **5**, (C) lipid-*b*-p(*i*PrOx) **7**, and (D) lipid-*b*-p((EtOx)-*co*-(*i*PrOx)) **11**. (for clarity purposes, only the major aliphatic chain is represented on the lipoinitiator structure but the statistical mixture contents an average value of 15 aliphatic CH).

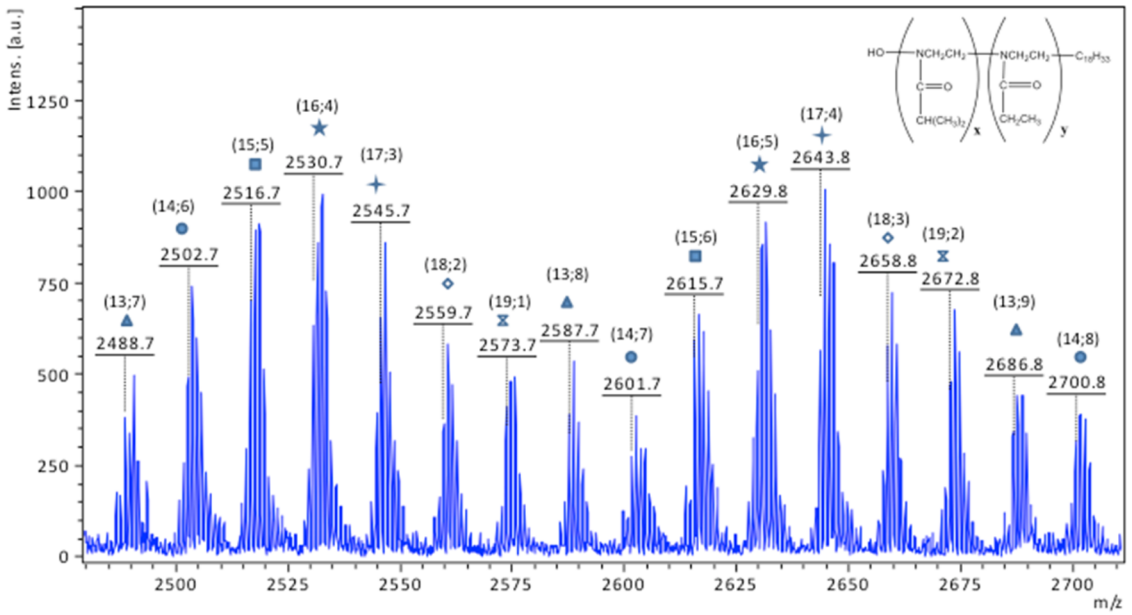


**Figure 3.** MALDI−TOF spectrum of **8** using HCCA matrix (zoom in the region 2400−2700 g/mol).

NMR spectroscopy. Nevertheless the efficiency of the lipoinitiator LTs was calculated (74%) and is comparable to thermal conditions (78%) (Table 1, runs 2 and 3).

**Microwave-Assisted Polymerization of EtOx and *i*PrOx from LTs and MeOTs.** Using the conditions of microwave-assisted CROP of MeOx (acetonitrile, 140 °C), amphiphilic thermoresponsive polyoxazolines were prepared by polymerization of EtOx and *i*PrOx. As expected, a slightly higher dispersity in molecular mass (1.50) is obtained when the CROP is initiated from our hydrophobic liponitiator compared to a classical initiator (1.22) (Table 1, runs 4 and 5). $^{1}$H NMR analysis of the final product shows the presence of characteristic


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signals of the lipidic chain at $\delta$ = 1.3 ppm (i) and of the $CH_2$ of the poly(EtOx) chain at $\delta$ = 3.4 ppm (b) (Figure 2B). The integration of both signals give the polymerization degree of the copolymer ($\overline{X_n}$) using the following formula:

$$\overline{X_n} = \frac{I_b/4}{I_i/15}$$

Similar results were obtained for *i*PrOx polymerization using MeOTs and LTs initiators in terms of polydispersity indexes (1.27 vs 1.33) and lipoinitiator efficiency (Table 1, runs 6 and 7). In both cases, the results confirmed that well-defined amphiphilic lipid-*b*-p(EtOx) and lipid-*b*-p(*i*PrOx) copolymers can be obtained from the original tosylated lipoinitiator LTs.

**Statistical Copolymerization of EtOx and *i*PrOx.** To investigate the influence of EtOx incorporation on the cloud point of the final amphiphilic copolymer, a series of lipid-*b*-p((EtOx$_x$)-*co*-(*i*PrOx$_y$)) copolymers with various EtOx/*i*PrOx ratios were synthesized by CROP polymerization of *i*PrOx and EtOx as summarized in Table 1 (runs 9−11). The composition of the final copolymer is calculated using the $^1$H NMR integrations corresponding to the two methyl groups of *i*Pr and the methyl group of Et at $\delta$ = 1.1 ppm (e + g) vs the integration of the backbone $-CH_2-CH_2$ protons (b) at 3.45 ppm (Figure 2D)

$$\begin{cases} I_b = 4n_e + 4n_i \\ I_{e+g} = 3n_e + 6n_i \end{cases}$$

with $n_e$ = number of EtOx units and $n_i$ = number of *i*PrOx units.

With our original lipoinitiator, the ratio of incorporated comonomers in the copolymer chain deviates from the feeding monomers. The phenomenon could be explained by the favored incorporation of EtOx related to *i*PrOx due to the slight difference of nucleophily/reactivity of these two monomers (EtOx > *i*PrOx). Lambermont-Thijs et al.[30] already reported a faster incorporation of MeOx compared to 4-ethyl-2-butyl-2-oxazoline during a statistical copolymerization revealing a decrease of polymerization rate with a steric hindrance increase. Well-defined lipid-*b*-p(EtOx$_x$ *i*PrOx$_y$) copolymers were synthesized with low, medium and high EtOx contents (lipid-*b*-p(EtOx$_{21\%}$-*co*-*i*PrOx$_{79\%}$), lipid-*b*-p(EtOx$_{42\%}$-*co*-*i*PrOx$_{58\%}$), lipid-*b*-p(EtOx$_{75\%}$ -*co*-*i*PrOx$_{25\%}$)) (Table 1, runs 9, 10, and 11).

Further characterizations of the copolymers structures were performed by MALDI−TOF spectrometry (Figure 3) in reflectron positive mode using $NH_4^+$ cation in the presence of acetonitrile and HCCA or DHB matrix. The latter matrix induced much more fragmentations than HCCA as shown in Figure S 5, parts a and b. As illustrated for lipid-*b*-p((EtOx)-*co*-p(*i*PrOx)), only one population of polymer was detected. The peaks were attributed to the family $(M + NH_4 + ACN)^+$ where M is the polymer chain constituted of EtOx ($x$) and *i*PrOx ($y$) repetitive units fully terminated by hydroxyl group. A good agreement between the experimental and the theoretical values were observed ($\Delta$m = 0.2). For instance, 2530.7 instead of 2530.9 g/mol was detected for $x$ = 16 and $y$ = 4. The massif was divided in successive waves, each one composed of seven peaks (marked by a specific symbol) with an increasing number of *i*PrOx units and a decreasing number of EtOx units in the polymer chain [$x$:7, ($x$ + 1):6, ($x$ + 2):5, ($x$ + 3):4, ($x$ + 4):3, ($x$ + 5):2 and ($x$ + 6):1]. Two consecutive peaks of the seven families were separated by $m/z$ of 113 corresponding to an *i*PrOx unit (Figure S 6). Interestingly, the top of each family corresponded to a *i*PrOx/EtOx ratio closed to 80% ($x$:$y$ = 16:4, 17:4, ...) in accordance with the NMR value.

**Thermosensitive Behavior in Aqueous Media.** Lipid-*b*-p(*i*PrOx), lipid-*b*-p(EtOx) and the corresponding statistical copolymers are all water-soluble at room temperature. Their cloud point temperatures were investigated by UV−visible spectrophotometry at a concentration of 2 g/L in distilled water containing 0.1 M NaCl (Figure 4). At this concentration,

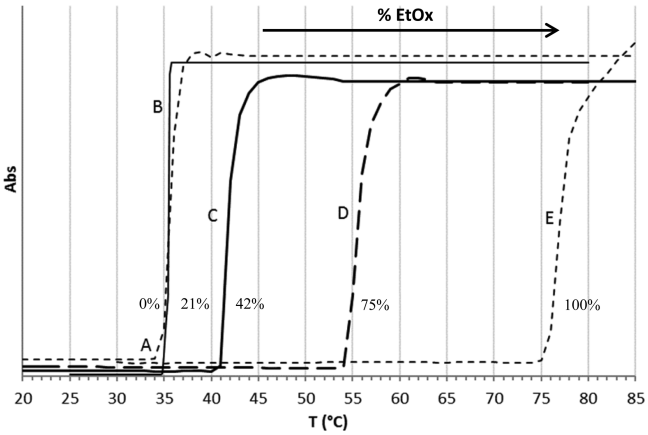


**Figure 4.** UV−visible light absorbance vs the temperature for (A) lipid-*b*-p(*i*PrOx) **7**, (B) lipid-*b*-p(EtOx$_{21\%}$-*co*- *i*PrOx$_{79\%}$) **9**, (C) lipid-*b*-p(EtOx$_{42\%}$-*co*-*i*PrOx$_{58\%}$) **10**, (D) lipid-*b*-p(EtOx$_{75\%}$-*co*-*i*PrOx$_{25\%}$) **11**, and (E) lipid-*b*-p(EtOx) **5**.

fluorescence measurements with pyrene confirmed the self-assembly of each amphiphilic copolymers (see Supporting Information). Indeed, the lipid-*b*-p(EtOx) **5** and lipid-*b*-p(*i*PrOx) **7** critical aggregation concentrations (CAC) were determined at 29 mg/L ($8.3 \times 10^{-6}$ mol/L) and 17 mg/L ($3.10 \times 10^{-6}$ mol/L) respectively. The copolymers present a CAC comparable to the lipid-*b*-p(*i*PrOx) **7** as illustrated for lipid-*b*-p(EtOx$_{42\%}$ -*co*-*i*PrOx$_{58\%}$) **10** with a CAC around 14 mg/L ($3.10 \times 10^{-6}$ mol/L). These values are consistent with previously reported values for amphiphilic lipid-*b*-poly(MeOx)[31] and lipid-*b*-poly(*i*PrOx).[11]

Park and Kataoka,[32] reported that no $T_{CP}$ is observed up to 90 °C for p(EtOx) with a molecular weight around 8000 g/mol. The same phenomenon occurs in our case where no turbidity appeared up to 85 °C. As expected, the introduction of an hydrophobic block in the copolymer structure decreases the $T_{CP}$ value to 76 °C for lipid-*b*-p(EtOx) (Table 1, run 5). Similarly lipid-*b*-p(*i*PrOx) present a $T_{CP}$ of 35 °C significantly lower than the corresponding p(*i*PrOx) homopolymer ($T_{CP}$ = 58 °C) (Table 1 runs 6 and 7). These results clearly confirmed the influence of a hydrophobic chain containing 18 carbons on the cloud point temperature of the copolymer as previously reported by Obeid et *al.*.[33] One of our goal was the tuning of the copolymer transition temperature through the incorporation of EtOx units in the hydrophilic block. The $T_{CP}$ of the statistical copolymers were thus investigated (Figure 4).

As expected, the progressive incorporation of EtOx units allows increasing the $T_{CP}$ of the lipid-based copolymers from 35 to 76 °C. Although, this evolution is not linear and at low EtOx content (21%), no influence on the $T_{CP}$ is observed. Above this critical threshold, the $T_{CP}$ progressively increases to 40 °C for 42% EtOx and to 54 °C for 75% EtOx (Figure 4 and Figure 6). This nonlinear evolution, in contradiction with the previous


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work of Kataoka et al.[18] could be due to the preorganization of the amphiphilic copolymer into micelles favorizing the polymer−polymer interactions. Below a certain pEtOx content, the p*i*PrOx collapse could then pull the whole hydrophilic chain away from the aqueous phase more easily.

The evolution of the size of the copolymer hydrodynamic diameters were also monitored by DLS. At 20 °C, the micelles' hydrodynamic diameters were about 10 nm for the pure lipid-*b*-p(*i*PrOx) **7** and lipid-*b*-p(EtOx) **5** copolymers and for the statistical copolymer lipid-*b*-p($EtOx_{42\%}$ $iPrOx_{58\%}$). Moreover, when their respective cloud point temperatures are reached, aggregates appear (Figure S 7). These results are in good agreement with the spectroscopic ones.

**Mixed Micelles Behavior.** Although the cloud point temperatures can be efficiently tuned by varing the copolymer composition, this approach requires the synthesis of different polymers, and therefore a new synthetic batch, for each desired properties. In the objective of finely tuning the transition temperature of micellar systems through a simple blending approach, lipid-p(*i*PrOx) **7** and lipid-p(EtOx) **5** were physically mixed in different proportions (21, 42, and 75% in EtOx) (from preprepared aqueous solution to obtain a final concentration of 2 $g{\cdot}L^{-1}$) and the final cloud point temperatures (Figure 5) were compared to the corresponding statistical copolymers with an identical *i*PrOx/EtOx ratio (Figure 6).

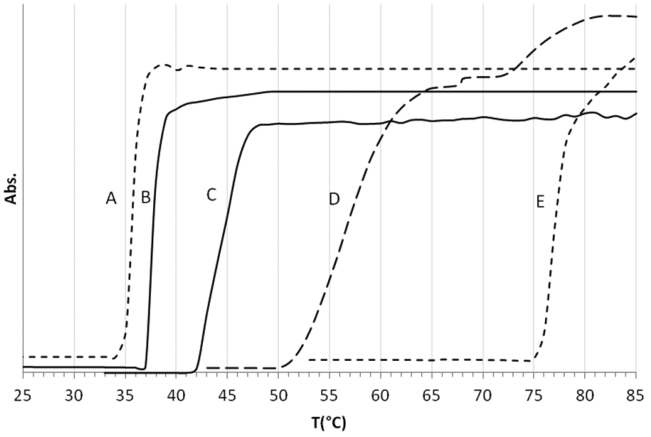


**Figure 5.** UV−visible light absorbance vs the temperature for (A) lipid-*b*-p(*i*PrOx) **7**, and blending of **7** with **5** at different ratios in EtOx (B) 21%, (C) 52%, (D) 79%, and (E) lipid-*b*-p(EtOx) **5**.

Figure 6 reveals a switch of behavior in water for the blending of **5** and **7** at 52% in EtOx. Up to this value, a single $T_{CP}$ is observed, corresponding to mixed micelles with a homogeneous composition in **5** and **7**. It is noticeable that the $T_{CP}$ is really close to the corresponding statistical copolymer indicating that the mixed micelles composition is consistent with the initial copolymers proportions. By contrast, above 52% in EtOx, two $T_{CP}$ are observed. The highest transition corresponds to the $T_{CP}$ of pure lipid-*b*-p(EtOx) **5**. Therefore, the system complete aggregation occurs in two steps, the mixed micelles aggregates first followed by pure lipid-*b*-p(EtOx) at a higher temperature. This behavior could result either from the initial coexistence of mixed micelles and pure lipid-*b*-p(EtOx) micelles or from a demixing of the two copolymers during heating as the lipid-based micelles are in dynamic equilibrium. DLS analyses of the mixed system containing 79 wt % in EtOx show micelles below 50 °C and only large aggregates above. As

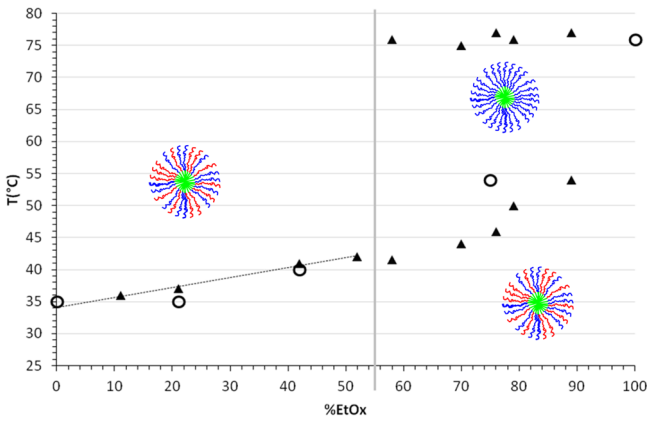


**Figure 6.** Cloud point temperature vs wt % in EtOx for (○) lipid-*b*-p($EtOx_x$ $iPrOx_y$) and (▲) the blending of lipid-*b*-p(*i*PrOx) **7** and lipid-*b*-p(EtOx) **5**.

large objects can easily hide smaller ones in DLS, it is therefore impossible to prove or dismiss the concomitant presence of unimers and/or micelles at 60 °C through this approach. A deviation appears between the cloud point temperatures of the statistical copolymer lipid-*b*-p($EtOx_{75\%}$ $iPrOx_{25\%}$) **11** and the corresponding mixed system as its composition differs from the targeted one due to the nonincorporation (or escape) of part of the lipid-*b*-p(EtOx) **5**. However, it is noticeable that $T_{CP}$ of the mixed micelles still increases almost linearly.

Following this result, the influence of the process for the mixed micelles preparation was investigated. Instead of mixing two preprepared micelles solutions, the solid copolymers were mixed together prior to solubilization into the aqueous phase. For the system containing 79% EtOx, the results obtained by spectrophotometry were strictly similar to the previous method. This confirms that the preparation process has no noticeable impact on the final properties due to the dynamic character of our micelles.

## CONCLUSION

We investigated the efficiency of two lipoinitiators for the thermal CROP of MeOx and the tosylated one showed a better efficiency than the mesylated one. The LTs was then engaged in the polymerization of different oxazoline monomers. Using microwave-assisted polymerization, the reaction time was considerably reduced and copolymers with controlled composition were successfully obtained according to NMR data. More specifically, lipid-*b*-p(EtOx), lipid-*b*-p(*i*PrOx) and lipid-*b*-p((EtOx)-*co*-(*i*PrOx)) were investigated in terms of thermoresponsiveness and results showed that the cloud point temperature can be tuned by varying the EtOx content into the copolymer. On a parallel approach, the blending of two copolymers with different $T_{CP}$, lipid-p(*i*PrOx) and lipid-p(EtOx) in different proportions was studied. Really interestingly, this blending approach allows tuning the transition temperature of the mixed system from 35 to 42 °C with results comparable to the corresponding statistical copolymer (with an identical EtOx/*i*PrOx content). However, above 52% EtOx, two transitions were observed showing a heterogeneous mixture containing both mixed micelles and pure lipid-*b*-p(EtOx) micelles. The phenomena causing this organization switch as well as an extension of the blending approach to different thermosensitive chains are currently under investigation.



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## ■ ASSOCIATED CONTENT

### Supporting Information

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$^1$H NMR spectra of LMs, LTs, lipid-*b*-p(MeOx) copolymers, LTs FTIR, CAC measurements by pyrene fluorescence, MALDI−ToF spectra of lipid-*b*-p($EtOx_{20\%}$-*co*-*i*$PrOx_{80\%}$) using (a) DHB and (b) HCCA matrixes, and DLS measurements (PDF)

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### Author Contributions

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## ■ ABBREVIATIONS

MeOx:2-methyl-2-oxazoline
EtOx:2-ethyl-2-oxazoline
*i*PrOx:2-isopropyl-2-oxazoline
LTs:tosylated lipoinitiator
LMs:mesylated lipoinitiator
HCCA:α-cyano-4-hydroxycinnamic acid
DHB:2,5-dihydroxybenzoic acid

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