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New amphiphilic graft copolymer based on poly(β-malic acid): synthesis and characterization

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Introduction

Micelle-like aggregates formed with amphiphilic copolymers have been recently receiving much attention as carriers for hydrophobic drugs [1-4]. Such amphiphilic block or graft copolymers have been found to form self-assemblies, nanosized micelle-like aggregates of various morphologies in aqueous solution. The hydrophobic part forms the core of the micelle as a preferential incorporation site for lipophilic drugs, while the hydrophilic corona or outer shell limits their uptake by reticuloendothelial systems and thus extents the *in vivo* life time of the drug carrier. Furthermore, these nanosized aggregates have the advantages of displaying a rather narrow size distribution, a low critical aggregation concentration compared to low molecular weight surfactants, a slow rate of dissociation and a high drug-loading capacity in biotechnological and pharmaceutical applications. Necessary compatibility between polymeric materials and living organisms, and strict control of degradation and bioassimilation by living systems have led to the demand for new biocompatible and biodegradable materials based on synthetic polymers bearing functional pendant groups [5]. Among these, the synthesis and controlled polymerization of functional cyclic (di)esters such as β-malolactonates is a straightforward way towards functional and biodegradable macromolecular architectures. Guérin et al. have reported on the synthesis and anionic polymerization of a large range of protected malolactonate (including optically active derivatives) yielding high molecular weight (co)poly(β-malic acid) based structures [6,7]. Quite recently, we reported on the tensioactive properties as well as micellization behavior in aqueous solution of amphiphilic poly([R,S]-β-malic acid-b-ε-caprolactone) diblock copolymers as obtained by a totally controlled synthetic pathway combining the anionic ring-opening polymerisation (ROP) of benzyl β-malolactonate (MLABz) with the coordination-insertion ROP of ε-caprolactone, followed by the selective removal of benzyloxy protective groups [8].

This paper aims at reporting on the synthesis of amphiphilic poly([R,S] hexyl β-malolactonate)-*graft*-poly([R,S] β-malic acid) copolymers (PMLAHex-*g*-PMLA) according to a four-step strategy. It involves first the anionic ring-opening copolymerization of [R,S] benzyloxypropyl β-malolactonate (MLABP) and [R,S] hexyl β-malolactonate (MLAHex) initiated by tetraethyl ammonium benzoate,

followed by the selective removal of the benzyloxy functions which generate free pendant hydroxyl functions suitable to initiate the ROP of MLABz after activation by tin(II) bis(2-ethylhexanoate) ($Sn(Oct)_2$). The final step relies upon the selective removal of benzyloxy protective groups of MLABz repeating units constituting the grafts. In order to demonstrate the amphiphilic character of the resulting PMLAHex-*g*-PMLA graft copolymers, their tensioactive properties have been investigated in a water/chloroform system by interfacial tension measurements using the pendant drop method.

Experimental

Materials

[R,S] benzyl β-malolactonate (MLABz), [R,S] benzyloxypropyl β-malolactonate (MLABP) and the [R,S] hexyl β-malolactonate (MLAHex) were synthesized and purified starting from aspartic acid as published elsewhere [9,10]. They were stored at – 18°C, MLABz was distilled under reduced pressure and both lactones were dried by three successive azeotropic distillations of toluene just before use. Tetraethyl ammonium benzoate (from Aldrich) was dried under vacuum during several hours. Trimethylsilyldiazomethane (2N in hexane from Aldrich), tin (II) bis(2-ethylhexanoate) ($Sn(Oct)_2$) (95%, from Aldrich) and hydrogen (Air Liquide, > 99.999%) were used without further purification. Toluene (Labscan, 99%) and tetrahydrofuran (Labscan, 99%) were dried by refluxing over CaH_2 and Na/benzophenone complex, respectively. Just before use, THF was further dried over low molecular weight ω-lithium styryl poly(styrene) and then distilled under reduced pressure.

Synthesis of the α-benzoate, ω-carboxylic acid poly(hexyl β-malolactonate-co-benzyloxypropyl β-malolactonate):

In a previously flamed and nitrogen purged round bottom flask, a mixture of hexyl β-malolactonate (90 mol %) and benzyloxypropyl β-malolactonate (10 mol %) was polymerized in bulk at 37°C for 15 days using tetraethyl ammonium benzoate (TEAB) ($[M]_0/[TEAB]_0 = 10^3$) as initiator [10]. After dissolution in THF, the copolymer was recovered by selective precipitation in an eight-fold excess of cyclohexane, filtration and drying under reduced pressure (conversion > 99%). Yield = 80%. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 0.8-0.9 (s, 3H), 1.2-1.4 (m, 2H), 1.65 (m, 2H), 1.9 (m, 2H), 2.9-3.1 (t, 2H), 3.65 (t, 2H), 4.1-4.3 (t, 4H), 4.5 (s, 2H), 5.5 (m, H), 7.3 (s, 5H). As determined by SEC (Figure 1) : M_n = 4,600, M_w/M_n = 1.50.

Methylation of α-benzoate, ω-carboxylic acid poly(hexyl β-malolactonate-co-benzyloxypropyl β-malolactonate):

In a previously flamed and nitrogen purged round bottom flask equipped with a three-way stopcock and a septum, 0.53 g of α-benzoate, ω-carboxylic acid poly(hexyl β-malolactonate-*co*-benzyloxypropyl β-malolactonate) (1.15×10^{-4} mol, M_n = 4,600) were dried by three successive azeotropic distillations of toluene (3 x 10 ml). Then, the dried polymer was dissolved in a mixture of 5 mL of toluene and 0.6 mL of anhydrous methanol. Trimethylsilyldiazomethane (1.04×10^{-3} mol, 0.5 ml) was then added allowing nitrogen gas evolution through a connected oil valve. After 1 h, the

reaction was stopped by the addition of a few drops of acetic acid (0.1 mol.L^{-1}) and the volatiles were removed out under reduced pressure. The polymer was recovered after drying under reduced pressure at 40 °C until constant weight. Yield>99%. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 0.8-0.9 (s, 3H), 1.2-1.4 (m, 2H), 1.65 (m, 2H), 1.9 (m, 2H), 2.9-3.1 (t, 2H), 3.5 (s, 3H), 3.65 (t, 2H), 4.1-4.3 (t, 4H), 4.5 (s, 2H), 5.5 (m, H), 7.3 (s, 5H). The ^{1}H-NMR spectrum is shown in Figure 2. As determined by SEC : M_n = 4,000, M_w/M_n = 1.50.

Synthesis of the poly(hexyl β-malolactonate-co-hydroxypropyl β-malolactonate):

In a round bottom flask, 0.53 g of the α-benzoate, ω-methyl ester poly(hexyl β-malolactonate-co-benzyloxypropyl β-malolactonate) was dissolved into 200 mL of acetone at r.t. and then added with 0.1 g of Pd/C 10wt%. A continuous flow of hydrogen was bubbled into the solution for 7 h. After filtration through Celite, a clear copolymer solution was obtained. The solvent was evaporated under reduced pressure (10 mmHg) before recovering the polymer by extensive drying at 40 °C under reduced pressure. Yield >99%. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 0.8-0.9 (s, 3H), 1.2-1.4 (m, 2H), 1.65 (m, 2H), 1.9 (m, 2H), 2.9-3.1 (t, 2H), 3.4 (s, 3H), 3.5 (t, 2H), 4.1-4.3 (t, 4H), 5.5 (m, H). As determined by SEC (Figure 1) : M_n = 4,000, M_w/M_n = 1.50.

Synthesis of the poly(hexyl β-malolactonate)-g-poly(benzyl β-malolactonate):

In a previously flamed and nitrogen purged round bottom flask equipped with a three-way stopcock and a septum, 0.475 g of the poly(hexyl β-malolactonate-co-hydroxypropyl β-malolactonate) (1.18×10^{-4} mol) were dried by three successive azeotropic distillations of toluene (3 x 5 ml). Then, the dried polymer was reacted with 1/400 equivalent of $Sn(Oct)_2$ compared to the content of hydroxyl functions (5.9×10^{-7} mol, 0.1 ml) in THF at 80 °C. After 15 minutes, β-benzyl malolactonate (MLABz) (2.34×10^{-3} mol, 0.48 g) was added and polymerized for 90 h at 80°C. After addition of a few drops of a HCl aqueous solution (0.1 mol.L^{-1}), the polymer was recovered by precipitation into 8 volumes of cold heptane, filtrated and dried under reduced pressure at 40 °C until constant weight. Yield =67%. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 0.8-0.9 (s, 3H), 1.2-1.4 (m, 2H), 1.65 (m, 2H), 1.9 (m, 2H), 2.9-3.1 (t, 2H), 3.4 (t, 2H), 3.7 (d, 2H and/or 1H), 4.1 (t, 4H), 5.2 (s, 2H), 5.5 (t, 1H), 7.5 (s, 5H). As determined by SEC (Figure 1) : M_n = 5,300, M_w/M_n = 1.44.

Synthesis of the poly(hexyl β-malolactonate)-g-poly(β-malic acid):

In a round bottom flask, 0.30 g of the poly(hexyl β-malolactonate)-g-poly(benzyl β-malolactonate) was dissolved into 200 mL of acetone at r.t. and then added with 0.06 g of Pd/C 10wt%. A continuous flow of hydrogen was bubbled into the solution for 4 h. After filtration through Celite, a clear copolymer solution was obtained. The solvent was evaporated (10 mmHg) before recovering the polymer by extensive drying at 40 °C under reduced pressure. Yield > 94%. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 0.8-0.9 (s, 3H), 1.2-1.4 (m, 2H), 1.65 (m, 2H), 1.9 (m, 2H), 2.2 (dd, 2H), 2.9-3.1 (t, 2H), 3.4 (s, 3H), 3.6 (t, 2H), 3.9 (t, 1H), 4.1-4.3 (t, 4H), 5.3 (m, H), 7.5 (s, 5H).

Characterization

^{1}H-NMR spectra were recorded using a Bruker AMX-300 apparatus at r.t. in $CDCl_3$ or CD_3COCD_3 (30 mg/0.6 ml). Size exclusion chromatography (SEC) was performed in THF at 35°C using a Polymer Laboratories liquid chromatograph equipped with a PL-DG802 degasser, an isocratic HPLC pump LC 1120 (flow rate = 1mL/min), a Rheodin manual injection (loop volume = 200 µL, solution conc. = 2 mg/mL), a PL-DRI refractive index detector and four columns : a PL gel 10 µm guard column and three PL gel Mixed-B 10 µm columns (linear columns for separation of MW_{PS} ranging from 500 to 10^6 daltons). Molar masses and molar masses distribution were calculed with reference to poly(styrene) standards. The interfacial tensions were determined using a DROP SHAPE ANALYSIS SYSTEM DSA 10 Mk2 equipped with a thermostated chamber and a Circulator Thermo HAAKE DC 10.

Results and discussion

A new amphiphilic graft copolymer including a hydrophobic poly([R,S] hexyl β-malolactonate) (PMLAHex) backbone and hydrophilic poly([R,S]-β-malic acid) (PMLA) branches has been synthesized according to a four-step strategy (Scheme 1). The first step consists in copolymerizing a mixture of [R,S] hexyl β-malolactonate (MLAHex, 90 mol %) and [R,S] benzyloxypropyl β-malolactonate (MLABP, 10 mol %) initiated by tetraethyl ammonium benzoate ($1^0/_{00}$ in mol) in bulk at 37°C for 15 days. As already demonstrated by some of us [11], the anionic (co)polymerization proceeds through the O-alkyl cleavage of the endocyclic ester bond leading to α-benzoate, ω-carboxylic acid P(MLAHex-co-MLABP) copolymer with complete comonomer conversion. The ^{1}H-NMR spectrum of the copolymer allows to determine a comonomer composition equal to the initial comonomer feed, as evidenced from the relative intensity of methyl protons of MLAHex repeating units at 0.9 ppm and benzylic protons of MLABP repeating units at 4.2 ppm. Size exclusion chromatography (SEC) shows a monomodal distribution characterized by a polydispersity index of 1.50 (Figure 1).

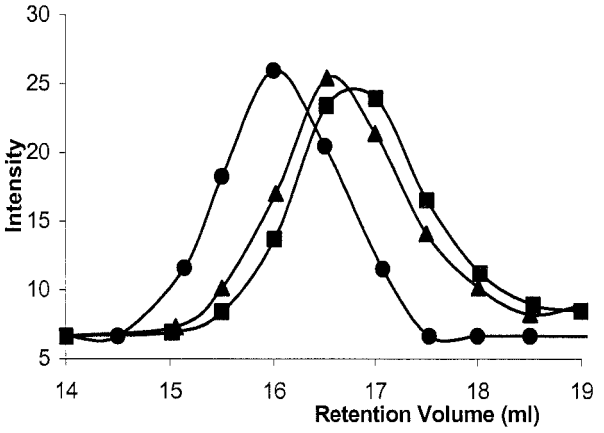
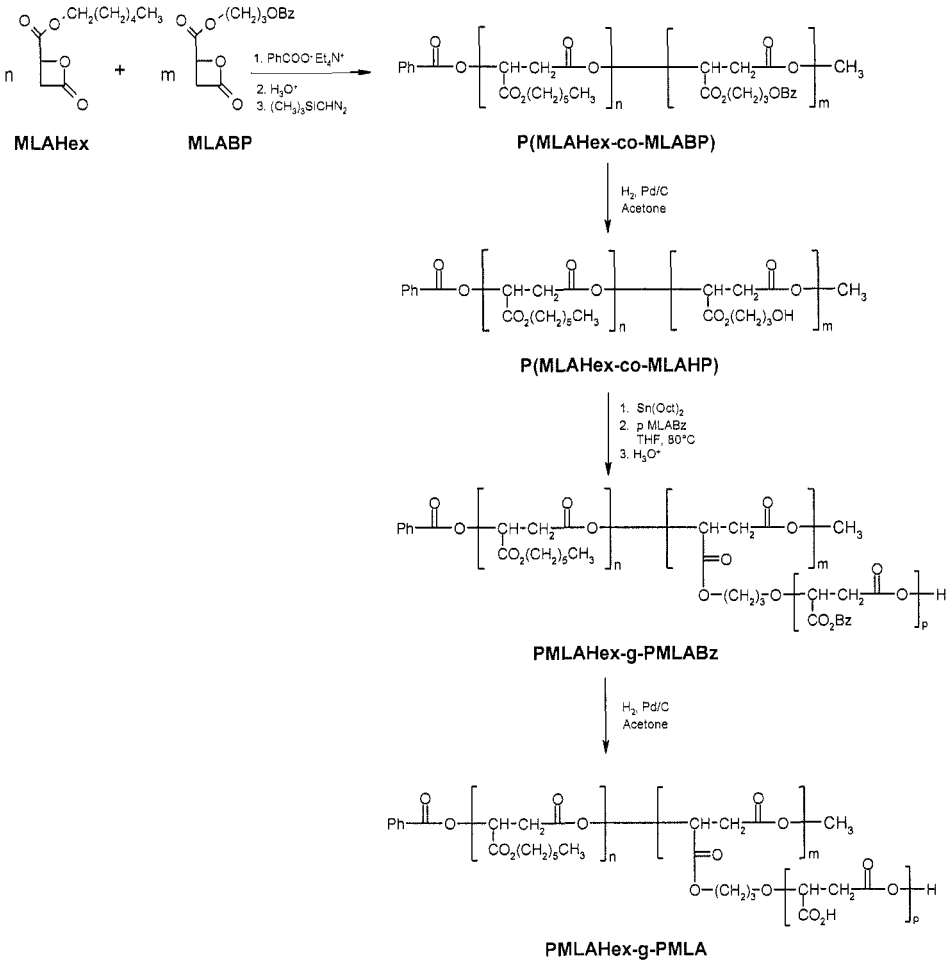


Figure 1 : SEC traces of α-benzoate, ω-carboxylic acid P(MLAHex-co-MLABP) (▲), α-benzoate, ω-methyl P(MLAHex-co-MLAHP) (■) and α-benzoate, ω-methyl PMLAHex-*g*-PMLABz (●).



Scheme 1 : Synthetic pathway to poly([R,S] hexyl β-malolactonate)-*g*-poly([R,S] β-malic acid) (P(MLAHex-*g*-PMLA)) graft copolymers.

In order to prevent any undesirable side-reactions with $Sn(Oct)_2$ that will be used as the catalyst for the graft synthesis, the carboxylic acid end-group of P(MLAHex-*co*-MLABP) has been reacted with an excess of trimethylsilyldiazomethane for 1 h at r.t. in toluene/methanol (9:1). The ^{1}H-NMR spectrum attests for the efficiency of the methylation to form α-benzoate, ω-methyl ester P(MLAHex-co-MLABP) with the appearance of the resonance signal at 3.4 ppm attributed to the ω-methyl ester end-group.

Figure 2 also shows that the MLAHex-to-MLABP comonomer molar ratio in the copolymer is kept at 9 and allows determining the actual molar mass from the relative intensity of methyloxycarbonyl protons (I_k) and the methylene protons (I_c)of the repeating units:

$$\overline{M_n} = \left(\frac{I_c x3}{I_k x2} x(0.9xMW_{MLAHex} + 0.1xMW_{MLABP}) \right) + 121 = 9{,}200.$$

As far as SEC is concerned, it is worth pointing out that both the polydispersity index and the apparent molar mass of P(MLAHex-*co*-MLABP) chains remain unchanged, M_{nSEC} = 4,000 and M_w/M_n = 1.50 (relative to a PS calibration).

In order to convert the benzyloxypropyl groups into hydroxypropyl functions, the second step consists in the catalytic hydrogenolysis of the protective groups statistically anchored along the poly(malate) backbone. The completion of the deprotection reaction has been attested by ^{1}H-NMR spectroscopy which displays the disappearance of the benzyloxy protons at 7.3 ppm. Actually, only the aromatic protons from the α-benzoate end-group still show up at ca. 7.3 ppm. Indeed, this is fully confirmed by the relative intensity ratio between these α-benzoate protons and the ω-methyl ester protons issued from the second extremity (at 3.4 ppm) which strictly equals the expected 3-to-5 ratio.

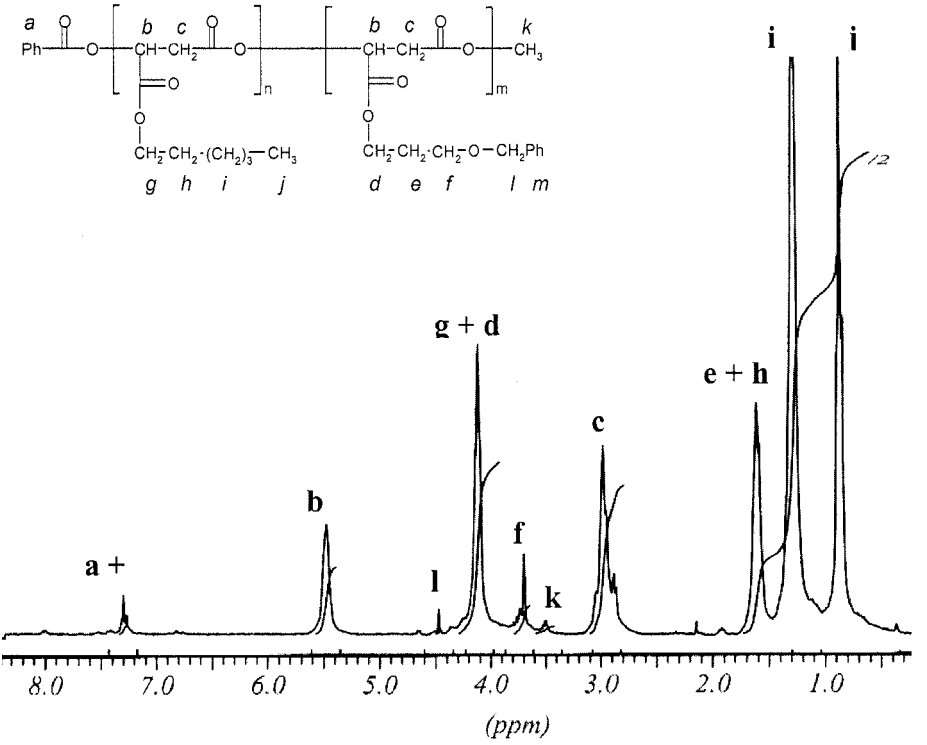


Figure 2 : ^{1}H-NMR spectrum of the α-benzoate, ω-methyl ester P(MLAHex-*co*-MLABP) (solvent = $CDCl_3$).

The third step of the copolymer synthesis involves the ring-opening polymerization (ROP) of [R,S] benzyl β-malolactonate (MLABz) from the pendant hydroxyl functions of P(MLAHex-co-MLAHP) previously activated by tin (II) bis(ethyl hexanoate) ($Sn(Oct)_2$). The hydroxyl groups have thus been treated with $Sn(Oct)_2$ in THF at 80°C with an initial hydroxyl-to-$Sn(Oct)_2$ molar ratio of 400. Such a low content in tin catalyst has actually been reported to enhance the control over the ROP of lactones like ε-caprolactone [12]. $Sn(Oct)_2$ has been proposed to react with hydroxyl functions with the fast and reversible formation of tin (II) alkoxyde initiating species [13,14]. The polymerization of MLABz has been carried out in THF at 80°C for an initial monomer concentration of 1.27 $mol.L^{-1}$ and an initial monomer-to-hydroxyl molar ratio of 10. Under such experimental conditions, the kinetics of polymerization was slow so that after 90h the monomer conversion reached 33 %, i.e. with a theoretical degree of polymerization ($DP_{theor.}$) of 3 (per graft) assuming a controlled process. In quite good agreement with $DP_{theor.}$, an experimental degree of polymerization of 4 can be estimated by comparing the relative intensity of the benzylic protons of MLABz repeating units along the grafts (at 5.15 ppm; $-CH_2-C_6H_5$) and the methylene protons from the main backbone (at 3.16 ppm; $-CH_2-CO_2-$). At this stage of this study, it appears that the ROP of MLABz likely takes place through O-alkyl bond scission even though one can not totally exclude the occurrence of O-acyl bond cleavage. Clearly, the study of the ROP mechanism is beyond the scope of this paper and will be the topic of a forthcoming publication. Interestingly, as shown in Figure 1, the SEC trace of the poly(hexyl β-malolactonate)-*g*-poly(benzyl β-malolactonate) graft copolymer (PMLAHex-*g*-PMLABz) is monomodal with a polydispersity index of 1.44, while the apparent molar mass expectedly increases (M_{nSEC} = 5,300) attesting for the efficiency of the grafting reaction.

The last step in the synthesis of the amphiphilic poly([R,S] hexyl β-malolactonate)-*g*-poly([R,S] β-malic acid) graft copolymer (PMLAHex-*g*-PMLA) consists in the catalytic hydrogenolysis of the benzyl ester functions along the PMLABz grafts. ^1H-NMR spectroscopy of the as-obtained copolymer shows the complete disappearance of benzylic protons attesting for the quantitative deprotection reactions. Interestingly, the amphiphilic character of the PMLAHex-g-PMLA graft copolymer (with a PMLA weight fraction of 0.11), has been evidenced by interfacial tension measurements. Purposely, solutions of PMLAHex-*g*-PMLA in chloroform with increasing concentrations have been dipped into Millipore Milli-Ro® water. Figure 3 shows the semi-logarithmic plot of interfacial tension (IFT) of the graft copolymer solution *vs.* concentration (expressed in $g.L^{-1}$). At 25 °C, the interfacial tension goes down from 29 mN/m to 12 mN/m, demonstrating the tensioactive properties of this new type of amphiphilic graft copolymers. The critical micelle concentration (CMC) has been determined from the intersection between the tangents drawn from higher concentration portion of the sigmoïdal plot [15] and calculated as 0.26 $g.L^{-1}$. For sake of comparison, it is worth noting that this value is much lower than CMC of some commercially available copolymers such as for example Pluronic® L43, characterized by a CMC around 4.07 $g.L^{-1}$ [16]

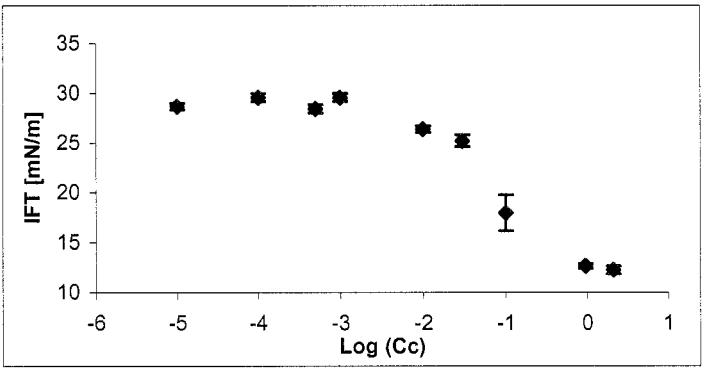


Figure 3 : Concentration dependence of the interfacial tension (IFT) of a P(MLAHex-*g*-PMLA) chloroform solution in water at 25°C.

In conclusion, we described a versatile route for the controlled synthesis of new amphiphilic graft copolymers based on poly(β-malic acid) combining anionic and coordination-insertion ring-opening polymerization (ROP) of different β-substituted β-lactones. The so-recovered PMLAHex-*g*-PMLA copolymers display amphiphilic properties and form micelles as evidenced by IFT measurements. We are currently investigating the tensioactivity of these graft copolyesters, and their ability to form micelles in function of their molecular composition. The result of this study will be reported in a forthcoming paper.

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