ARTICLE TYPE

Synthesis and self-assembly of amphiphilic polymers based on polyoxazoline and vegetable oil derivatives
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The synthesis and self-assembly of amphiphilic polymers based on unsaturated vegetable oils and poly(2-methyl-2-oxazoline)(POx) are reported. Two architectures of lipopolymers were explored starting from fatty methyl esters and raw vegetable oils. These latter were converted into macroinitiators for cationic ring-opening polymerization (CROP) of 2-methyl-2-oxazoline. Firstly, a thiol-ene coupling reaction in the presence of mercaptoethanol under UV irradiation was performed to introduce hydroxyl groups that were further transformed into initiating species used for cationic polymerization. According to this strategy, various lipopoly(2-methyl-2-oxazoline)s (LipoPOx)s with different lipidic/POx ratios were successfully obtained and characterized by ¹H NMR, SEC and MALDI-Tof analyses. Finally, the self-organization of the LipoPOx was studied using dynamic light scattering (DLS). Well-shaped nanoparticles were obtained with characteristic radii of 4.3 and 10.2 nm for the fatty ester- and triglyceride-based lipopolymers, respectively. The latter value is about twice the former one due to the higher lipidic fraction of the triglyceride-based polymer that promotes hydrophobic interactions. The relaxation time distributions of both systems were found to be monomodal indicating monodisperse colloidal suspensions.

INTRODUCTION

As a consequence of the depleting of fossil reserves and environmental issues, the use of renewable raw materials is important for sustainable development¹. The main bio-based raw materials are vegetable oils, polysaccharides, sugars and woods² that are chemically modified for the development of chemicals, polymers², amphiphilic copolymers, composites, blends³, plasticizers⁴, and coatings⁵.

Amphiphilic block copolymers are most often composed of hydrophilic blocks covalently linked to hydrophobic ones. They have the ability to self-assemble in bulk or in solution, leading to different soft materials⁶ such as micelles, vesicles⁷, polymersomes⁸, and gels. The peculiar behavior of these copolymers in solution allows their use as surfactants, emulsifiers⁹, drug carriers¹⁰, etc

Bio-based amphiphilic polymers can be either totally natural and made of renewable species for both the hydrophilic and hydrophobic parts (e.g. alkyl polyglycosides¹¹, nucleolipids¹², polysaccharide-block-polypeptide¹³ and oligosaccharide linked to hydrophobized oligosaccharide¹⁴), or partially bio-sourced where one block is from renewable resources. Nowadays, this latter class is being widely developed with the synthesis of diblock copolymers based on oligosaccharides¹⁵, peptides¹⁶ or lipids¹⁷.

Most vegetable oils are mainly composed of triglycerides, which are also known as triacylglycerols (TAG)s, with long alkyl chains (between 12 and 20 carbons). Vegetable oils and their derivatives (fatty alcohols, esters and acids) have been extensively investigated for the synthesis of oleochemical surfactants. These surfactants can be made ionic with the introduction of a negatively charged head, typically a sulfonate¹⁷ or carboxylate group¹⁸ (sulphonate of a glycerol ester or ester of a sulphated methyl glucoside). Positively charged heads, typically quaternary ammonium or amphoteric species such as betains, can also be encountered. Moreover non-ionic surfactants can be produced by associating lipids with hydrophilic molecules like diethanolamine or with hydrophilic polymers such as poly(ethylene oxide)(PEO)^{9,17}, poly(glycerol)¹⁹ or some poly(oxazoline)s: poly(2-ethyl-2-oxazoline) or poly(2-methyl-2-oxazoline)). Furthermore, we note that a study of block-copolymers based on poly(2-methyl-2-oxazoline) or poly(2-ethyl-

2-oxazoline) as the hydrophilic block and hydrophobic poly(2-oxazoline) synthesized from fatty acids has been recently reported²⁰.

In this work we focus on the synthesis of amphiphilic polymers composed of a natural lipidic hydrophobic part (a vegetable oil or its derivatives) and a hydrophilic chain based on poly(2-methyl-2-oxazoline), so called LipoPOx. Poly(2-methyl-2-oxazoline) is a more hydrophilic polymer than PEO²¹ and is used as a building block for various macromolecular architectures²². POx exhibits a low toxicity²³ whereas some recent studies suspected toxicity for PEO resulting in bioaccumulation²⁴. Thus poly(2-methyl-2-oxazoline) appears to be a good alternative to PEO-based polymers²⁵. Few synthetic routes in obtaining LipoPOx with various structures have been described in the literature so far. First linear lipopolymers were synthesized using lipidic initiators such as iodoalkyl²⁶, alkyl trifluoromethanesulfonate or alkyl tosylate²⁷ initiators for the polymerization of MOx (2-methyl-2-oxazoline). Furthermore, multi-step strategies were developed to obtain "pseudo-bicatenar" lipopolymers based either on diacylglycerol or glyceroldiethers. In this case, the synthesis and the use of 1,2-didodecanoylpropyl-*p*-toluene sulfonate²⁸ or 1,2-O-di-octadecyl-*sn*-glycerol tosylate or triflate²⁹ as the macroinitiator for the polymerization of MOx can be one approach. The other pathway consists in the preparation of poly(2-methyl-2-oxazoline) and then coupling a lipidic block such as distearylphosphatidylethanolamine^{25c} or 1,2-didodecanoylpropyl²⁸.

It should also be mentioned that a complex structure of lipopolymer was previously reported using a modified vegetable oil as the starting initiator for the synthesis of LipoPOx³⁰. In this case castor oil was modified with *p*-toluenesulfonyl chloride to initiate the cationic ring-opening polymerization (CROP) of MOx. As opposed to previous reported work in the literature, our strategy was based on the chemical modification of double bonds of the triglycerides without modification of the triacylglycerol structure. The main objective and the novelty of this contribution is the extension of the synthesis of LipoPOx to any unsaturated vegetable oils and to all fatty esters. Our strategy is described in Scheme 1. Following to this route, unsaturated lipids were converted into lipidic alcohols thanks to the addition of 2-mercaptoethanol (ME)

onto double bonds using the thiol-ene coupling (TEC) reaction. The resulting modified lipids were transformed into initiators for the polymerization of MOx. The strategy was first assessed using methyl oleate and afterwards applied to crude oil. We report here the ability of LipoPOx derived from fatty esters and triglycerides to self-organize in aqueous solution. It is important to point out that literature reported the self-organization of linear LipoPOx in water³¹ has been previously reported in the literature. In the present study, we were interested in the self-organization of branched LipoPOx based on crude oil and in the formation of nanoparticles.

R = Me
R = DAG = Diac

Scheme 1 General synthetic route towards amphiphilic polymers LipoPOx, MO = Methyl oleate, GSO = Grapeseed oil, HMO = Hydroxylated methyl oleate, HGSO = Hydroxylated grapeseed oil, LipoPOx = lipopolyoxazoline (amphiphilic polymer).

Experimental section

Materials

2-Methyl-2-oxazoline (MOx) was dried and distilled from CaH₂ and stored under a dry nitrogen atmosphere. Ether from VWR, tetrahydrofuran (THF), acetonitrile (ACN) and ethyl acetate (EtOAc) from Carlo Erba, anhydrous dichloromethane and chloroform (CHCl₃) from Sigma were distilled before use and stored under dry nitrogen. Grapeseed oil (GSO) (an usual commercially available oil), methyl oleate (MO) (purity 70%), 2,2-dimethoxy-2-phenylacetophenone (DMPA) (purity 99%), triethylamine (Et₃N), *N,N*-benzyl dimethylamine, pyridine, trifluoromethanesulfonic anhydride (Tf₂O), potassium carbonate (K₂CO₃) and piperidine from Aldrich, 2-mercaptoethanol (ME) and sodium iodide (NaI) from Sigma, MgSO₄ from VWR and *p*-toluenesulfonyl chloride (TsCl) and chloroacetyl chloride (CAC) from Fluka were used without further purification. Dialysis

membranes Spectra/Por[®] - MWCO 500-1000 were purchased from Spectrum Laboratories, Inc.

Characterization

Nuclear magnetic resonance (NMR) spectra were recorded on a RMN Bruker Avance I 300 MHz or on a Bruker Avance III 600 MHz spectrometers. Chemical shifts (¹H NMR) were referenced to the peak of residual CHCl₃ at 7.26 ppm. Chemical shifts (¹³C NMR) were referenced to CDCl₃ at 77 ppm. Fourier transform infrared (FTIR) spectra were recorded with a Perkin Elmer Spectrum 100 spectrometer equipped with an attenuated total reflectance (ATR) using a ZnSe crystal. The composition of crude oils was determined by gas chromatography (GC) analysis after conversion of the triglycerides into fatty acids (FA)s and methyl esters. The mixture was analyzed on an Agilent 6890 series using a Supelcowax 10 capillary column (SGE, Courtaboeuf, France): length, 30 m; internal diameter, 0.32 mm; film thickness, 0.25 μm and carrier gas: helium at a flow rate of 20 ml/min, splitting ratio: 100, injector temperature: 250°C, FID detector temperature: 270°C. The temperature profile was: 185–240°C at 4°C/min, 225°C for 10 min. The HPLC/ESI system consisted of a capillary HPLC system (Alliance 2790, Waters) and an ESI source of time-of flight (TOF) mass spectrometer (Q-TOF, Waters). The system was controlled by Masslynx software (Waters). The separations were performed on a BDS Hypersil C18 (Thermo) analytical column (3 μm particle size, 2.1 mm, 50 mm). In typical conditions, the elution was started with 100% of mobile phase A (ACN with 10 mmol of ammonium acetate) then solvent B (isopropanol) was increased from 0 to 100% in 15 min with a 15-min hold at 100% B. The flow rate was set at 0.2 mL/min, and the injection volume was 50.0 μL. LC/ESI-MS chromatograms were recorded in the positive ion mode with the capillary voltage set at 3000 V and cone voltage at 30 V while the dry temperature was set at 150°C and dry gas flow was maintained at 13 L/min. Size exclusion chromatography (SEC) was performed on a Spectra-Physics apparatus equipped with an RI Shodex refractive index detector. Two PL-gel mix C columns were used at 70 °C with a 0.8 ml/min flow rate of DMF (0.1% LiBr), calibrated using poly(methylmethacrylate) standards. Mass spectrometry analyses were conducted with a Bruker Ultra-Flex MALDI-ToF mass spectrometer, equipped with a nitrogen laser (LSI, 337 nm, 10 ns pulse length) and one detector. Mixture of peptides was used for external calibration. The ions were accelerated by a potential of 25 kV and reflected with a 26.3 kV potential. All measurements were recorded in the positive reflectron mode using α-cyano-4-hydroxycinnamic acid (HCCA) as matrix with NaI. For each spectrum 700 transients were accumulated. The resolution at *m/z* = 1263.8 was 6863. Dynamic light scattering (DLS) measurements were carried out at different scattering angles going from 20 to 155° using an ALV/CGS-8F S/N 069 apparatus (ALV, Langen, Germany) equipped with a 35mW red HeNe linearly polarized laser operating at a wavelength of 632.8 nm (JDSU, Milpitas, USA) and an ALV/LSE-5004 multiple τ digital correlator with a 125 ns initial sampling time. The copolymer solutions were loaded in 10 mm diameter cylindrical cells and maintained at a constant temperature of 25.0°C. The wave vector modulus (*q*) is equal to $(4\pi n/\lambda) \sin(\theta/2)$ where *n* represents the refractive index of the pure solvent (water in this case), *λ* is the laser wavelength, and *θ* designates the scattering angle. Data were collected typically for 300 s using the ALV correlator control software. The relaxation time distribution was obtained using the CONTIN analysis of the autocorrelation function^{(2)-1³²}.

Characterization of raw lipids

Methyl Oleate (MO). ¹H NMR (300 MHz, CDCl₃), δ = 0.79–0.88 (t, J = 7.0 Hz, 3H, CH₂-CH₃), 1.16–1.30 (m, 19H, CH₂), 1.6 (t, J = 7.0 Hz, 2H, CH₂-CH₂-C=O), 1.92–2.07 (m, 3.5H, CH₂-CH=CH-), 2.24–

2.37 (t, $J = 7.5$ Hz, 2 H, $\text{CH}_2\text{-CH}_2\text{-C=O}$), 2.74-2.78 (t, 0.26H, negligible), 3.66 (s, 3H $\text{CH}_3\text{-O-C=O}$), 5.21-5.42 (m, 2H, CH=CH).
 ^{13}C NMR (75.47 MHz, CDCl_3), $\delta = 13.04$ ($\text{CH}_2\text{-CH}_3$), 22.66-31.8 (CH_2), 34.9 ($\text{CH}_2\text{-C=O}$), 51.4 ($\text{CH}_3\text{-O-C=O}$), 127.9 (negligible),
 129.7 (CH=CH), 174.3 (C=O).
 FTIR (cm^{-1}): 3003 (C=C-H (*cis*), asymmetrical elongation), 2929 (C-H , asymmetrical elongation), 2853 (C-H , symmetrical elongation), 1745 ($\text{C=O}_{\text{ester}}$, elongation), 1459-1436 (CH_2 scissoring), 1355 (CH_3 symmetrical deformation), 1167 ($\text{C-O}_{\text{ester}}$ elongation), 725 (CH_2 rocking).

GrapeSeed Oil (GSO). ^1H NMR (300 MHz, CDCl_3), $\delta = 0.79$ -0.88 (m, 9 H, $\text{CH}_2\text{-CH}_3$), 1.16-1.30 (m, 51.5H, CH_2), 1.6 (m, 6.5 H, $\text{CH}_2\text{-CH}_2\text{-C=O}$), 1.92-2.07 (m, 10.8 H, $\text{CH}_2\text{-CH=CH-}$), 2.24-2.37 (t, $J = 7.5$ Hz, 6.1H, $\text{CH}_2\text{-C=O}$), 2.74-2.78 (t, $J = 5.9$ Hz, 4 H, $-\text{CH=CH-CH}_2\text{-CH=CH-}$), 4.11-4.17 (dd, 2 H, $^2J = 11.7$ Hz, $^3J = 5.7$ Hz, $^3J = 6$ Hz, $\text{CH}_2\text{-O}$), 4.26-4.32 (dd, 2 H, $^2J = 12$ Hz, $^3J = 4.2$ Hz, $\text{CH}_2\text{-O}$), 5.21-5.42 (m, 10.49 H, CH-O and CH=CH).
 ^{13}C NMR (75.47 MHz, CDCl_3), $\delta = 13.98$ ($\text{CH}_2\text{-CH}_3$), 22.49-31.8 (CH_2), 33.9-34.07 ($\text{CH}_2\text{-C=O}$), 61.9 ($\text{CH}_2\text{-O}$), 68.8 ($\text{CH}_2\text{-CH-O}$), 127.8 ($\text{CH}_2\text{-CH=CH-CH}_2\text{-CH=CH-}$), 130.05 ($\text{CH}_2\text{-CH=CH-CH}_2\text{-CH=CH-}$), 172.64-173.04 (C=O).
 FTIR (cm^{-1}): 3003 (C=C-H (*cis*), asymmetrical elongation), 2920 (C-H , asymmetrical elongation), 2850 (C-H , symmetrical elongation), 1742 ($\text{C=O}_{\text{ester}}$, elongation), 1459 (CH_2 scissoring), 1377 (CH_3 symmetrical deformation), 1157 ($\text{C-O}_{\text{ester}}$ elongation), 725 (CH_2 rocking).
 GC: 65.9% C18 :2, 0.1% C16 :1, 2.7% C18 :1, 0.5% C18 :3, 6.9% C16 :0 and 3.9% C18 :0.

Modification of lipids

Synthesis of hydroxylated lipid. Similar procedure was followed for the modification of MO or GSO. MO or GSO, ME (3 eq with respect to MO or 3 eq per double bond of GSO triglyceride), and 0, 0.02 or 0.1eq of DMPA with respect to MO or GSO were dissolved in a minimal amount of THF required to obtain a homogeneous medium.

In both cases, reactions were performed in a Pyrex photoreactor where a UV lamp was inserted in a water temperature-controlled (at about 20°C) double jacket tube immersed in a larger tube containing raw materials. The UV lamp was an HPK 125W high-pressure mercury vapor lamp of HeraeusNoblelight (maximum energy at a wavelength of 365 nm, with substantial radiations at 435, 404, 313 and 253 nm). After reaction, THF was removed and non-reacted ME was extracted by washing the organic phase several times (EtOAc/brine). The organic phase was then dried over MgSO_4 and solvent was evaporated by vacuum distillation. The resulting products were analyzed by NMR and IR spectroscopies.

Hydroxylated Methyl Oleate (HMO). ^1H NMR (300 MHz, CDCl_3), $\delta = 0.79$ -0.88 (t, $J = 7.0$ Hz, 3H, $\text{CH}_2\text{-CH}_3$), 1.16-1.7 (m, 27H, CH_2), 2.24-2.37 (t, $J = 7.5$ Hz, 2 H, $-\text{CH}_2\text{-(C=O)-}$), 2.56 (q, 1H, $J = 12.4$, 6.3 Hz, CH-S), 2.71 (t, 2H, $J = 6.0$ Hz, S-CH_2), 3.66 (s, 3H, $\text{CH}_3\text{-O-(C=O)-}$), 3.66-3.76 (t, 2H, $\text{CH}_2\text{-OH}$).
 ^{13}C NMR (75.47 MHz, CDCl_3), $\delta = 14.2$ ($\text{CH}_2\text{-CH}_3$), 22.7-31.9 (CH_2 , $\text{S-CH}_2\text{-CH}_2\text{-OH}$), 34.1 ($\text{CH}_2\text{-(C=O)-}$), 35.0 ($\text{CH}_2\text{-CH-S}$), 45.1 (CH-S-CH_2), 51.6 ($\text{CH}_3\text{-O-(C=O)-}$), 60.9 ($\text{CH}_2\text{-OH}$), 127.9-129.7 (negligible, residual double bonds), 174.4 (C=O).
 FTIR (cm^{-1}): 3440 (OH, stretching), 2923 (C-H , asymmetrical elongation), 2854 (C-H , symmetrical elongation), 1739 ($\text{C=O}_{\text{ester}}$, elongation), 1456-1433 (CH_2 scissoring), 1362 (CH_3 symmetrical deformation), 1170 ($\text{C-O}_{\text{ester}}$, elongation), 1043-1012 (C-C-OH mercaptoethanol), 725 (CH_2 rocking).

Hydroxylated GrapeSeed Oil (HGSO). ^1H NMR (300 MHz, CDCl_3), $\delta = 0.79$ -0.88 (9H, $\text{CH}_2\text{-CH}_3$), 1.16-1.50 (49H, CH_2), 1.5-1.7 (9.5H, $\text{CH}_2\text{-CH-S}$ and $\text{CH}_2\text{-CH}_2\text{-(C=O)-}$), 1.92-2.07 (8.35H,

$\text{CH}_2\text{-CH=CH-}$), 2.24-2.37 (t, 7H, $^3J = 7.5$ Hz, $\text{CH}_2\text{-(C=O)-}$), 2.5-2.8 (m, $\text{CH=CH-CH}_2\text{-CH=CH}$, CH-S and S-CH_2), 3.6-3.8 (dd, $\text{CH}_2\text{-OH}$), 4.11-4.17 (dd, 2H, $^2J = 11.7$ Hz, $^3J = 5.7$ Hz, $^3J = 6$ Hz, $\text{CH}_2\text{-O}$), 4.26-4.32 (dd, 2H, $^2J = 12$ Hz, $^3J = 4.2$ Hz, $\text{CH}_2\text{-O}$), 5.21-5.42 (m, 7.57H, CH-O and CH=CH).

^{13}C NMR (75.47 MHz, CDCl_3), $\delta = 14.1$ ($\text{CH}_2\text{-CH}_3$), 22.49-35 (CH_2), 45.1 (CH-S-CH_2), 61.9 ($\text{CH}_2\text{-O}$ and $\text{CH}_2\text{-OH}$), 68.8 ($\text{CH}_2\text{-CH-O}$), 127.8-130.05 ($\text{CH}_2\text{-CH=CH-CH}_2\text{-CH=CH}$), 172.9-173.3 (C=O).

FTIR (cm^{-1}): 3457 (OH, stretching), 3009 (C=C-H (*cis*), asymmetrical elongation), 2923 (C-H , asymmetrical elongation), 2850 (C-H , symmetrical elongation), 1738 ($\text{C=O}_{\text{ester}}$, elongation), 1462 (CH_2 scissoring), 1374 (CH_3 symmetrical deformation), 1167 ($\text{C-O}_{\text{ester}}$ elongation), 1043-964 ($\text{C-C-OH}_{\text{mercaptoethanol}}$), 725 (CH_2 rocking).

Synthesis of initiators for cationic ring-opening polymerization.

The reaction between hydroxylated lipids and *p*-toluenesulfonyl chloride (TsCl) was achieved as previously reported³³. Hydroxylated lipids were dissolved in distilled chloroform (0.5 M) stored under dried nitrogen atmosphere. 2 eq of triethylamine was introduced and the mixture was left under dry nitrogen stream for half an hour and cooled down in an ice bath. TsCl dissolved in distilled chloroform (0.5 M) was added dropwise at 0°C and left at room temperature during 24 hrs. The resulting product was purified by washing the organic part with brine (EtOAc/brine) several times, analyzed by NMR and LC-MS.

The reaction between hydroxylated lipids and trifluoromethanesulfonic anhydride^{27,29,34} was performed in a double jacket reactor. Anhydrous potassium carbonate (10 eq) and hydroxylated lipid (1eq, 1 M) were dissolved in distilled chloroform and stirred under dry nitrogen before cooling at -20°C. Then a solution of trifluoromethanesulfonic anhydride (2 eq) in distilled chloroform (4 M) was added dropwise. The mixture was stirred at -20°C for 2 hrs then 2 hrs at 0°C, and finally, let at RT till the end of reaction (20 hrs). The mixture was filtered, the solvent and the residues were removed under vacuum.

The reaction between hydroxylated lipids and chloroacetylchloride was achieved in a tricol equipped with a condenser. Hydroxylated lipid (1eq), triethylamine (1.1 eq) and anhydrous dichloromethane were mixed and cooled down in an ice bath and left under dry nitrogen stream. A solution of chloroacetyl chloride (1.5 eq) in dichloromethane was added dropwise. Reaction was completed after stirring at 0°C during 30 min and the product was purified by washing the organic part with brine (EtOAc/brine) several times.

Chloro-Methyl Oleate (Cl-MO). ^1H NMR (300 MHz, CDCl_3), $\delta = 0.79$ -0.88 (t, $J = 7.0$ Hz, 3H, $\text{CH}_2\text{-CH}_3$), 1.16-1.7 (m, 27H, CH_2), 2.24-2.33 (t, $J = 7.5$ Hz, 2 H, $-\text{CH}_2\text{-(C=O)-}$), 2.6 (q, 1H, $J = 12.4$, 6.3 Hz, CH-S), 2.73 (t, $J = 7.2$ Hz, 2H, S-CH_2), 3.65 (s, 3H, $\text{CH}_3\text{-O-(C=O)-}$), 4.06 (s, 2H, $\text{CH}_2\text{-Cl}$), 4.27-4.34 (t, $J = 7.1$ Hz, 2H, $\text{CH}_2\text{-O-(C=O)-CH}_2\text{-Cl}$).

^{13}C NMR (75.47 MHz, CDCl_3), $\delta = 14.2$ ($\text{CH}_2\text{-CH}_3$), 22.7-31.9 (CH_2), 34.2 ($\text{CH}_2\text{-(C=O)-}$), 35.0 ($\text{CH}_2\text{-CH-S}$), 40.9 ($\text{CH}_2\text{-Cl}$), 46.6 (CH-S-CH_2), 51.5 ($\text{CH}_3\text{-O-(C=O)-}$), 65.6 ($\text{CH}_2\text{-O-(C=O)-CH}_2\text{-Cl}$), 127.9-129.7 (negligible residual double bonds), 167.2 ($\text{O-(C=O)-CH}_2\text{-Cl}$, C-23), 174.4 (C=O).

FTIR (cm^{-1}): 2920 (C-H , asymmetrical elongation), 2850 (C-H , symmetrical elongation), 1736 ($\text{C=O}_{\text{ester}}$, elongation), 1459-1432 (CH_2 scissoring), 1377 (CH_3 symmetrical deformation), 1159 ($\text{C-O}_{\text{ester}}$, elongation), 776 (C-Cl , stretching), 725 (CH_2 rocking).

Chloro-GrapeSeed Oil (Cl-GSO). ^1H NMR (300 MHz, CDCl_3), $\delta = 0.79$ -0.88 (9H, $\text{CH}_2\text{-CH}_3$), 1.16-1.70 (63H, $\text{CH}_2\text{-CH}_2\text{-CH-S}$ and $\text{CH}_2\text{-CH}_2\text{-(C=O)-}$), 1.92-2.07 (9H, $\text{CH}_2\text{-CH=CH-}$), 2.24-2.37 (t, 7H, $^3J = 7.5$ Hz, $\text{CH}_2\text{-(C=O)-}$, H-4), 2.5-2.8 (m, 5H, $\text{CH=CH-CH}_2\text{-}$

CH=CH, CH-S and S-CH₂), 4.11-4.32 (m, 8H, CH₂-O, CH₂-O-(C=O)-CH₂-Cl, CH₂-Cl), 5.21-5.42 (m, 7.7H, CH-O and CH=CH). ¹³C NMR (75.47 MHz, CDCl₃), δ = 14.1 (CH₂-CH₃), 22.49-35.7 (CH₂ and S-CH₂), 40.8 (CH₂-Cl), 46.3 (CH-S), 62.1 (CH₂-O), 65.3 (CH₂-O-(C=O)-CH₂-Cl), 69.1 (CH-O), 127.8-130.05 (CH₂-CH=CH-CH₂-CH=CH), 167.2 (-(C=O)-CH₂-Cl), 172.9-173.3 (C=O). FTIR (cm⁻¹): 3009 (C=C-H (*cis*), asymmetrical elongation), 2920 (C-H, asymmetrical elongation), 2850 (C-H, symmetrical elongation), 1742 (C=O_{ester}, elongation), 1459 (CH₂ scissoring), 1377 (CH₃, symmetrical deformation), 1159 (C-O_{ester}, elongation), 772 (C-Cl, stretching), 725 (CH₂, rocking).

CROP of MOx using halogeno-lipid macroinitiators. All reactions were carried out under dry nitrogen atmosphere. NaI (1 eq) in dry acetonitrile was added dropwise to a 0.5 M solution of chloro-lipids in dry acetonitrile. The mixture was vigorously stirred 5 min at 78°C and MOx was added. The concentration of monomer in the final mixture was 4 M since transfer reactions can be avoided in the range from 4 to 7 M³⁵. The solution was stirred at 78°C during 24 hrs and samples were withdrawn at different times of polymerization. Each aliquot was quenched by addition of an adequate amount of piperidine (5 eq). After cooling, the polymer was isolated by 3 successive precipitations from cold diethyl ether (where the non-functionalized GSO, residual MOx and piperidine remained soluble). Dialysis of the final product was performed in water during 3 days. The desired chain length was reached by varying the ratio [M]/[I] ([MOx]/[macroinitiator]) or the polymerization duration.

Polymers based on MO (MO-POx). ¹H NMR (300 MHz, CDCl₃), δ = 0.79-0.88 (t, CH₂-CH₃lipid), 1.16-1.65 (m, CH₂ lipid), 1.67 (CH₂-CH₂-CH₂-N piperidine), 1.88 (CH₂-CH₂-N piperidine), 1.9-2.2 (CH₃-(C=O)-N POx), 2.27-2.31 (t, 2 H, CH₂-(C=O)-lipid), 2.6 (m, CH-S lipid), 2.73 (m, S-CH₂ lipid), 3.1-3.15 (t, CH₂-N piperidine), 3.3-3.6 (m, CH₂-CH₂-N-(C=O)-POx), 3.65 (s, CH₃-O-(C=O)-lipid), 4.1 (m, -(C=O)-CH₂-N-(C=O)-lipid), 4.1-4.3 (m, S-CH₂-CH₂-O lipid). ¹³C NMR (75.47 MHz, CDCl₃), δ = 14.2 (CH₂-CH₃ lipid), 22.7-31.9 (CH₂ lipid and piperidine), 34.1 (CH₂-(C=O)-lipid), 34.9 (CH₂-CH-S lipid), 43-50 (CH-S lipid, CH₂-CH₂-N-C=O POx, CH₂-N piperidine), 49.6 (-(C=O)-CH₂-N-(C=O)-lipid), 51.5 (CH₃-O-(C=O)-lipid), 64.0 (S-CH₂-CH₂ lipid), 169.2 (-(C=O)-CH₂-N-lipid), 170.9-171.6 (N-(C=O)-CH₃ POx), 174.4 (CH₃-(C=O)-lipid).

Polymer based on GSO (GSO-POx). ¹H NMR (300 MHz, CDCl₃), δ = 0.79-0.88 (t, CH₂-CH₃ lipid), 1.16-1.65 (m, CH₂ lipid), 1.67 (CH₂-CH₂-CH₂-N, piperidine), 1.88-2.2 (CH₂-CH=CH lipid, CH₃-(C=O)-N POx, CH₂-CH₂-N, piperidine), 2.27-2.31 (t, CH₂-(C=O)-lipid), 2.6 (m, CH-S lipid), 2.73 (m, S-CH₂ lipid), 2.77 (CH=CH-CH₂-CH=CH lipid), 3.1-3.15 (t, CH₂-N piperidine), 3.3-3.6 (m, CH₂-CH₂-N-(C=O)-POx), 4.1 (m, -(C=O)-CH₂-N-(C=O)-lipid), 4.1-4.3 (m, S-CH₂-CH₂ lipid), 4.11-4.32 (dd, CH₂-O glycerol), 5.25 (CH-O, glycerol), 5.3-5.42 (m, CH=CH, lipid).

Results and discussion

Grapeseed oil and its fatty esters were firstly converted into lipidic alcohols thanks to the addition of 2-mercaptoethanol (ME) onto double bonds using thiol-ene coupling (TEC) reaction. The resulting modified lipids were further transformed into macroinitiators for the polymerization of MOx. Then, the self-organization in aqueous solution of the obtained amphiphilic molecules was investigated.

I. Functionalization of vegetable oils and derivatives by thiol-ene coupling

The low reactivity of natural lipids requires a preliminary chemical modification of the backbone in order to enhance their reactivity. A wide range of chemical modifications has been explored so far. The most usual methodologies involved the modification of the double bonds into epoxy functions (epoxydation³⁶), (meth)acrylate functions (acrylation of alcohol³⁷ or epoxy groups³⁸), acid functions (oxidation³⁹), succinic anhydride group (maleinisation⁴⁰) and others. The introduction of alcohol groups can be achieved by hydroxylation⁴¹, ozonolysis and reduction⁴², phenolation⁴³, hydroformylation⁴⁴ and thiol-ene addition of hydroxylated mercaptan⁴⁵. This latter route named thiol-ene coupling (TEC) is the well-known addition of mercaptans onto unsaturations⁴⁶. The mechanism of this reaction can be either a nucleophilic type reaction when the base-catalyzed thio-Michael reaction is involved, or a radical one⁴⁷. Radicals can be produced by heating or exposure to UV radiation with or without any initiator. The mechanism of addition has been extensively studied^{48,49} and the influence of both structures of the thiols⁵⁰ and unsaturated alkyl chains⁵¹ were investigated. The effect of the initiator type⁵² was reported as well as the influence of the wavelength⁵³ and the intensity⁵⁴ of the light in the case of photochemical activation. This type of chemistry has been widely used on vegetable oils and their derivatives^{45,55}. Various parameters were studied such as reaction mechanism, kinetics^{55d}, configuration of the unsaturations and isomerization phenomena^{55b,c,56}, influence of conjugated and non-conjugated structure of lipids^{55j}, impact of the number of double bonds of the alkyl chain⁵⁵ⁱ and side reactions⁴⁵. The TEC reaction exhibits many advantages in functionalizing lipids. For instance, in the case of hydroxylated lipids, the alkyl chains can be modified with primary hydroxyl functions which are more reactive than secondary ones, generally obtained by ring-opening of epoxidized oils⁵⁷. Experiments can be performed under air since oxygen does not inhibit the reaction as is reported by several authors^{58,47,59}. In recent work on the TEC reaction^{45,60}, we showed that the thermal method may promote oxidation and oligomerization of vegetable oils^{55k}. In the present study, photochemical induced TEC was chosen instead of the thermal route to reduce side reactions occurring on lipids.

The thiol-ene reaction can be initiated at both 254 and 365 nm as previously reported⁵³. However, wavelength of 254 nm induces a direct cleavage of thiol groups in RS[•] and H[•], and in the presence of olefin, the H[•] radical is very quickly added onto double bonds of lipidic chains leading to a carbo-centered radical. This latter reacts with another thiol^{55c} by abstracting one proton. Consequently, and according to this mechanism, hydrogenated alkyl chains are produced as side products. Even if the mechanism operating at 365 nm is not well understood, this wavelength seems to be more appropriate for TEC and has been largely used. Under those conditions, the addition of thiol moiety onto double bonds is favored. Moreover, it has been highlighted that type I photoinitiators such as DMPA lead to the highest efficiencies at this wavelength⁵². For our systems, the TEC reaction was performed onto MO and AGSO at 365 nm with and without DMPA. MO was chosen as a model of vegetable oil because it is the simpler and the close species of vegetable oil exhibiting one double bond on the alkyl chain. This modeling aimed to validate each step of the general strategy to modify MO before applying it in a second part to crude oil.

First, the TEC reaction with MO was performed according to a protocol previously developed in our laboratory⁴⁵ (step 1, Scheme 1). An excess of thiol (3 eq of thiol per double bond) and 0.1 eq of DMPA are required for a total consumption of unsaturations and a short reaction time. The TEC reaction was monitored by ¹H NMR spectroscopy observing the disappearance of double bond peaks between 5.21 and 5.42 ppm. The yield reached a maximum after 4 hrs under UV exposure and all the double bonds were consumed as illustrated in Figure 1a. The addition of ME was confirmed by the quasi-total disappearance of double bond peaks (at 5.21-5.42 ppm)

and the appearance of new peaks at 2.56 ppm CH-S, 2.71 ppm S-CH₂ and 3.66-3.7 ppm CH₂-OH (H-2, H-3 and H-4, respectively). ¹³C NMR analysis confirmed this result with the quasi-disappearance of double bonds at 127.8-130.05 ppm (CH=CH) and the appearance of peaks at 45.1 (CH-S-CH₂) and 60.9 ppm (CH₂-OH). FTIR analysis showed the appearance of a large peak at 3430 cm⁻¹ corresponding to the alcohol function of the added mercaptoethanol, peaks at 1045-1012 cm⁻¹ characteristic of C-C-OH of ME and the disappearance of double bonds at 3003 cm⁻¹. This functionalized fatty ester, called hydroxylated methyloleate (HMO), was obtained with good yield (p_m = 94%). Furthermore, the FTIR spectra did not reveal any peak at 2560 cm⁻¹ (S-H vibration) confirming the total elimination of residual ME and the efficiency of the purification step.

Fig 1 ¹H NMR spectra of hydroxylated lipids: a. HMO and b. HGSO.

These experimental conditions were applied to grapeseed oil (GSO). As described in the experimental part, GSO exhibits around 4.75 double bonds per triglyceride. One of the objectives of our investigation was to obtain a triglyceride bearing only one hydroxyl group per molecule. Following our conditions, an excess of 3 eq of thiol reagent per double bond was reacted and the initial mixture was diluted with a minimum of solvent (THF) since starting reagents were immiscible. As in the case of methyloleate, similar new peaks were observed in ¹H NMR, ¹³C NMR and FTIR spectra. The influence of the use of photoinitiator and the UV time exposure was now investigated and the results are listed in Table 1. The conversion rate was expressed by the decrease of double bonds using ¹H NMR data according to equation 1:

$$\% \text{ of consumed C} = \text{C} = \frac{\int 5.2 \text{ ppm} - 5.5 \text{ ppm} (t) - 1}{\int 5.2 \text{ ppm} - 5.5 \text{ ppm} (t=0) - 1} \quad (1)$$

The use of DMPA as the photoinitiator increased drastically the conversion rate (Entries 1 and 3) 11% instead of 50% without DMPA after 4 hrs of UV exposure. Increasing the time to 8 hrs led to 63% of consumption of double bonds (Entry 2). One has to keep in mind that our goal was to insert only one hydroxyl function per triglyceride. As the photoinitiator enhanced drastically the reactivity, we decided to perform the reaction without DMPA where the conversion rate of the reaction depended only on the duration of UV exposure⁴⁵. Furthermore, we mention that the total elimination of residual DMPA according to usual purification procedure was difficult to achieve and that residual quantities of DMPA could generate early ageing of the final product. In our conditions, we observed that the conversion rate ranged from 11% (4 hrs of reaction) to 39% (48 hrs) (Entries 3 to 6). The theoretical conversion rate fitting with the desired mono-adduct compound is 27% (Entry 5). In order to be more accurate in the calculation, one notes that 27% of consumption of double bonds corresponds to 22% of added thiols. The percentage of added thiol was determined by ¹H NMR (equation 2):

$$\% \text{ thiol}_{\text{added}} = \frac{\int 2.7 \text{ ppm}}{2} \quad (2)$$

The low gap between the two percentages was attributed to side reactions as previously reported⁴⁵.

Table 1 Results of UV initiated TEC between ME and GSO using DMPA as photoinitiator.

Entry	Eq DMPA*	Irradiation duration (hrs)	% of consumed C=C
1	0.02	4	50
2	0.02	8	63
3	-	4	11
4	-	8	24
5	-	24	27
6	-	48	39

* Molar equivalent quantity of DMPA with respect to double bonds.

In our conditions, the analyses showed that TEC reaction did not lead to any degradation of the triglyceride as observed on ¹H NMR spectra (Figure 1b) where the characteristic peaks of the glycerol esters between 4.2 and 4.4 ppm for CH₂-O-C=O and at 5.2 ppm for CH-O-C=O remained unchanged. Hydroxylated grape seed oil (HGSO) was obtained in good yield (92%). Moreover, the presence of some di-hydroxylated triglycerides was observed in LC-MS and the various detected compounds are summarized in Table 2. Each species exhibited secondary alcohol appearing during the TEC reaction (mentioned OH in m/z data of Table 2).

Table 2 LC-MS data of HGSO (sample with 22% of addition).

Detected compounds		m/z
C=C	ME grafted	
2	2	1056.9 (M) ⁺ and 1078.9 (M) ^{Na+} TAG bearing 2 C=C + OH + 2 grafted ME
1	2	1058.9 (M) ⁺ and 1080.9 (M) ^{Na+} TAG bearing 1 C=C + OH + 2 grafted ME
0	2	1060.9 (M) ⁺ (TAG + OH + 2 grafted ME)
3	1	978.8 (M) ⁺ TAG bearing 3 C=C + OH + grafted ME
2	1	980.8 (M) ⁺ TAG bearing 2 C=C + OH + grafted ME
1	1	982.8 (M) ⁺ TAG bearing 1 C=C + OH + grafted ME
4	0	900.4 (M) ⁺ (TAG bearing 4 C=C + OH)
3	0	898.8 (M) ⁺ (TAG bearing 3 C=C + OH)

II Synthesis of lipidic macroinitiators

The alcohol groups of HMO or HGSO were used for the synthesis of a lipidic macroinitiator for the polymerization of MOx by cationic ring-opening polymerization (CROP). Generally, efficient initiators must have good leaving groups with appropriate nucleophilicity⁶¹ such as Lewis acids, strong protic acids and their corresponding esters, alkylhalides⁶¹, molecular iodine⁶² and rarely metallocenes⁶³. The chemical structure of the initiator has an influence on the initiation and the propagation steps during the CROP. If the counter-anion coming from the initiator is more nucleophilic than the monomer, a covalent mechanism occurs while in other cases, the mechanism proceeds as a ionic one^{61,64}. In the case of

polymerization of MOx, the nucleophilicity of this monomer is higher than that of counter-anion of the different possible types of initiator (I_2^{62} , R-I, ROTs and ROTf). Consequently, only the ionic mechanism occurred during the CROP of MOx. The type of initiator has also an influence on the conversion rate and it has been already shown that the covalent mechanism induced very low monomer conversion⁶⁵ unlike the ionic polymerization process. The macroinitiators were classified depending on the reaction rate⁶⁶: $MeOTf > MeOTs > MeI > benzyl\ bromide$.

In this work, the synthesis of three kinds of lipidic initiators based on sulfonate esters coming from *p*-toluenesulfonyl chloride (TsCl) or trifluoromethanesulfonic anhydride (Tf_2O) and chloroacetyl chloride was investigated (Scheme 2).

Fig. 2 1H NMR spectra of chloro-lipids (a. Cl-MO and b. Cl-GSO).

In summary, whatever the explored pathways, we assume that the addition of the reactants easily occurred on HMO and HGSO. But in the case of triflate and tosylate routes, the high reactivity of OTs and OTf species grafted onto the lipids rapidly induced side reactions leading to the degradation of the lipidic-based reagent. This result is not surprising considering the strong electrophilicity of esters of trifluoromethanesulfonic and *p*-toluenesulfonic acids. On the contrary, the reactivity of R-Cl reactant is low enough to ensure the stability of the macroinitiator. Thus, the strategy using CAC was successfully extended to GSO with 93% yield. No degradation of the glycerol part was observed in the 1H NMR spectrum with no change of the characteristic peaks between 4.2 and 4.4 and 5.2 ppm (Figure 2b).

III Cationic ring-opening polymerization of MOx using lipidic macroinitiators

As previously mentioned, chlorinated compounds initiated the CROP of oxazoline monomer but induced very low monomer conversion. To improve the reactivity of chlorinated initiators, an *in situ* conversion into iodide initiator was considered. In this case, the initiation step occurred in two stages: first, the *trans*-halogenation to convert CH_2-Cl into CH_2-I and then the initiation of polymerization. CH_2-I exhibits a weak bondable to initiate the polymerization by an ionic mechanism *via* an oxazolinium species with I^- as counter-anion (Scheme 3). The living nature of the CROP implies the use of terminating agents such as amines to stop the growth of the polyoxazoline chains. Piperidine is an appropriate terminating agent since it is a good nucleophile and a mild basic compound which should not damage the glycerol part. In our case, it has been previously checked that piperidine could not react with ester group by *trans*-amination despite its pK_a of 11.2.

Scheme 3 CROP mechanism of MOx using Cl-MO or Cl-GSO.

Study of the *trans*-halogenation

The use of chlorinated macroinitiator combined with a iodide salt (NaI or KI) has been already described in several studies^{65a,67}. Uyama *et al.*⁶⁸ showed that the kinetics of polymerization initiated

by a chlorinated initiator in the presence of NaI was quite similar to a polymerization directly initiated by iodinated compounds. A short study of the *trans*-halogenation for equimolar ratio of NaI and Cl-lipids was achieved. First the reaction was performed at room temperature and monitored by ^1H NMR spectroscopy during 2 hrs. The lowering of the characteristic peak of $\text{CH}_2\text{-Cl}$ at 4.06 ppm (H-5) and the appearance of the characteristic peak of $\text{CH}_2\text{-I}$ at 3.7 ppm (H-5') were followed and the conversion rate was evaluated by equation 3:

$$\% \text{ iodine initiator} = \frac{f_{3.7 \text{ ppm}}}{f_{3.7 \text{ ppm}} + 4.1 \text{ ppm}} \quad (3)$$

No degradation of the lipidic structure was observed and the characteristic peak of $\text{CH}_3\text{-O-C=O}$ at 3.6 ppm, (H-1, Figure 3) of the ester function remained unchanged.

Fig.3 Study of the *trans*-halogenation on the fatty ester macroinitiator Cl-MO.

After 15 min, 42% of chloride was exchanged with iodide and 2 hrs was required to reach a maximum of conversion with a percentage of *trans*-halogenation close to 80%. At room temperature, the *trans*-halogenation reaction is slow. Therefore the same experiment was performed at 80°C and complete reaction was observed after 5 minutes. As a consequence, the polymerization step was performed with a chlorinated macroinitiator in the presence of NaI at 80°C after waiting 5 min to insure complete *trans*-halogenation.

Study of the CROP of MOx

The CROP of MOx was performed in the presence of two lipidic macroinitiators: a fatty ester (Cl-MO) and the modified triglyceride (Cl-GSO). The resulting amphiphilic polymers based on polyoxazoline (MO-POx_n and GSO-POx_n) were synthesized with theoretical degrees of polymerization ranging from 8 to 50. The yields ranged from 15 to 86% depending on the length of polyoxazoline chains. The apparent degree of polymerization of polyoxazoline were calculated using equation 4:

$$\text{DP}_{n, \text{app}} = \frac{M_{n(\text{SEC})} - M_{\text{Macroinitiator}} - M_{\text{Termination agent}}}{M_{\text{monomer}}} \quad (4)$$

with $M_{n(\text{SEC})}$ determined by SEC (Figure 4, left). For both macroinitiators (Cl-GSO and Cl-MO), the experimental apparent degrees of polymerization ranged from 11 to 56. The polydispersity indexes ($\mathcal{D}$) ranging from 1.1 to 1.3 for MO-POx_n and from 1.2 to 1.5 for GSO-POx_n are usual values in the case of initiation involving a halogenated species²⁸. The evolution of M_n versus $([\text{M}]_0/[\text{I}]_{\text{trans}})$ in the case of MO-POx_n is represented in Figure 4, with $[\text{M}]_0$ the monomer concentration at $t=0$ and $[\text{I}]_{\text{trans}}$ the initiator concentration after *trans*-halogenation. The linear evolution indicated the absence of substantial transfer reactions during the polymerization. This

result is generally observed with common initiators but was never reported with initiators exhibiting relatively high molecular weights such as bio-based compounds derived from natural oils. In the case of triglyceride-based macroinitiators, polydispersity indexes ($\mathcal{D}$) were quite good considering that commercial crude oil was directly used. The gap between theoretical and experimental M_n may correspond to a partial efficiency of the macroinitiator.

Fig. 4 Molecular weights and polydispersity indexes for the polymerization of 2-methyl-2-oxazoline initiated by fatty ester- or triglyceride-based initiators (left). Evolution of M_n and polydispersity indexes (SEC) in the case of MO-POx_n (right). (* experimental value measured by SEC in DMF).

The copolymers systems were also characterized by ^1H NMR spectroscopy and the superposition of NMR spectra of Cl-MO and MO-POx_n is given in Figure 5. In the MO-POx_n spectrum, the typical peaks of the lipidic part were observed at 3.67 ppm for $\text{CH}_3\text{-O-C=O}$ (methyl ester group (H-1)), 2.3 ppm for $\text{CH}_2\text{-C=O}$ (H-2) and 0.88 ppm for terminal CH_3 (H-3). Moreover, the disappearance of the peak at 4.06 ppm characteristic of $\text{CH}_2\text{-Cl}$ group, the appearance of new peaks at 4.1 ppm corresponding to $(\text{C=O})\text{-CH}_2\text{-N}$ (H-7) and peaks belonging to the POx chains at 1.9-2.1 ppm ($\text{CH}_3(\text{C=O})$, H-10) and 3.4-3.6 ppm ($\text{CH}_2\text{-CH}_2$, H-8 and H-9) confirmed the simultaneous presence of POx and the fatty chain. The termination with piperidine was found to be effective with the characteristic peaks at 3.15 (N-CH₂, H-11), 1.88 (N-CH₂-CH₂, H-12) and 1.67 ppm (N-CH₂-CH₂-CH₂, H-13). The degree of polymerization of LipoPOx based on fatty ester was calculated using the integration of the characteristic signals of terminal CH_3 of fatty chain at 0.8 ppm related to that of CH_3 group of polyoxazoline unit at 2.2 ppm. The $\text{DP}_{n, \text{NMR}}$ values in DMF were close to theoretical ones. For instance, $\text{DP}_{n, \text{NMR}}$ of 10, 17 and 43 were calculated instead of 9.3, 14.1 and 49.2 theoretical values respectively. We noted that in Figure 5, CDCl_3 was preferred to DMF-d_6 as NMR solvent for a better attribution of each signal avoiding overlaps. Moreover in the same solvent (DMF), a good correlation between $\text{DP}_{n, \text{NMR}}$ and $\text{DP}_{n, \text{SEC}}$ was observed with a slightly higher values by SEC 13, 19 and 55 instead of 10, 17 and 43 by NMR spectroscopy.

Fig. 5 Comparison of ^1H NMR spectra of Cl-MO and MO-PO x_{13} .

Otherwise, MO-PO x samples were characterized by MALDI-ToF spectroscopy as illustrated in Figure 6 in the case of MO-PO x_{55} . Three series of peaks were observed, each one showing a difference of mass Δm between two successive peaks of 85.05 g/mol characteristic of the repetitive unit of PO x . These series of peaks were ascribed to MO-PO x_n -piperidine/ Na^+ species, MO-PO x_n -NH-CH $_2$ -CH $_2$ -OH/ Na^+ hydroxylamine⁶⁹ ones and MO-PO x_n -OH/ Na^+ hydroxyl terminated polymers.

Fig. 6 MALDI-ToF spectrum of MO-PO x_{55} .

All of the previous characterizations demonstrated the well-defined structure of MO-PO x_n amphiphilic polymers with lipidic and polyoxazoline parts associated in different ratios. Similar investigations were done in the case of polymers based on GSO-PO x_n . ^1H NMR spectra confirmed the efficacious polymerization of MO x starting from the triglyceride-based Cl-GSO macroinitiator (supporting information). The Maldi-ToF spectrum revealed the $\Delta m = 85.05$ g/mol confirming the presence of the polyoxazoline chains even if the chain ends were too complicated to be ascribed. Size exclusion chromatography (SEC) analysis was performed and Figure 7 represents the trace in the case of the GSO-PO x_{56} sample. The full black line allowed determining a molecular weight M_n of 5840 g/mol and M_w of 7760 g/mol. Moreover a slight shoulder was observed at lower elution time. Deconvolution of the trace using two Gaussian functions revealed two populations of copolymers. Using the value of the elution time for the maximum of each peak, M_w were calculated at 9700 g/mol for the first peak and 6510 g/mol for

the second one. Considering the gap between both M_w values, lipopolymers with the highest M_w could be ascribed to triglycerides bearing two PO x chains. This result is not surprising since previous LC-MS analysis revealed the presence of some di-hydroxylated triglycerides in minor quantity.

Fig. 7 Deconvolution of SEC trace for GSO-PO x_{56} using two Gaussian functions.

At last, the lipidic mass fraction f (molecular mass of the lipid part divided by the M_n of LipoPO x polymer) indicating the lipophilic character was evaluated (Figure 4). f ranging from 0.8 to 0.26 for MO-PO x_n and from 0.17 to 0.49 for GSO-PO x_n were obtained. This wide range shows the possibility to produce various amphiphilic polymers with tunable lipophilic / hydrophilic balance.

IV Self-assembly of LipoPO x copolymers in water

Volet *et al.*³¹ already showed that strictly linear LipoPO x were able to self-assemble in water. In the present systems, polymers based on lipids and polyoxazoline led to different architectures where grafted PO x chains were inserted in the middle of the lipidic chains. Thus the ability of self-organization in aqueous medium was investigated by the dynamic light scattering (DLS) technique for the two structures of lipopolymers: MO-PO x_n and GSO-PO x_n with a PO x length of respectively 13 (MO-PO x_{13}) and 11 (GSO-PO x_{11}) units as determined by SEC. From the DLS experiments, the autocorrelation functions ($g^{(2)}-1$) and the relaxation time distributions measured in both systems at 40, 90 and 140° are shown in Figure 8a and 8b. Whatever the scattering angle, the measurements exhibit single narrow relaxation time distributions. At a given scattering angle, the relaxation time ($1/\Gamma$) is given by the position of the maximum value of the autocorrelation function along the x-axis. The linear dependences of the relaxation frequency (Γ) on the square of the wave vector modulus (q^2) clearly indicate the Brownian diffusive motion of particles in both cases (Figures 8c and 8d). The value of the slope is equal to the diffusion coefficient (D) of the particles from which the hydrodynamic radius (R_H) is calculated for each sample using the Stokes-Einstein relation $R_H = \frac{k_B T}{6\pi\eta D}$ where k_B is the Boltzmann constant, T represents the absolute temperature of the sample and η is the viscosity of the pure solvent (water in this case)⁷⁰. The calculated values of R_H are 4.3 and 10.2 nm for MO-PO x_{13} and GSO-PO x_{11} , respectively⁷¹. Indeed, whatever the scattering angle, the relaxation time for GSO-PO x_{11} is higher than that for MO-PO x_{13} , reflecting the lower diffusion coefficient calculated for GSO-PO x_{11} . The two values of R_H differ by a factor of more than two. This behavior can be reasonably attributed to the fact that GSO-PO x_{11} (based on triglyceride) presents a more massive lipidic part than MO-PO x_{13} (based on fatty ester). The lipidic fractions represent 49.0% versus 26.0% in weight which promotes

hydrophobic interactions and drives the self-assembly process in water towards the formation of bigger nanoparticles so that the stabilization by the hydrophilic block (*i.e.* POx) can be readily achieved. No supra-aggregates or uncontrolled self-assemblies could be detected. Moreover, although SEC analysis revealed the presence of two size distributions, as shown in Figure 7 with the example of GSO-POx₅₆, the formed nanoparticles of GSO-POx₁₁, presenting one peak at $M_w = 4160$ g/mol and another at $M_w = 2510$ g/mol, remain surprisingly monomodal and quite monodisperse in size, as observed for MO-POx₁₃. No other peak is present in the relaxation time distribution. The self-assembly is thus not affected by the presence of the minor product. As a consequence, the fatty ester- and triglyceride-based LipoPOx self-assemble in monomodal quite monodisperse nanoparticles, forming nano-objects. The self-assembly of these lipopolymers is still under investigation and will be reported in a forthcoming article.

Fig. 8 DLS results of MO-POx₁₃ (C=2 g/L) (a: autocorrelation function ($g^{(2)}-1$) and relaxation time distribution curve, c: dependence of the relaxation frequency (Γ) on the square of the wave vector modulus (q^2)) and GSO-POx₁₁ (b: autocorrelation function ($g^{(2)}-1$) and relaxation time distribution curve, d: dependence of the relaxation frequency (Γ) on the square of the wave vector modulus (q^2)).

Conclusions

We report here an original synthesis of lipopolymers based on lipids and poly(2-methyl-2-oxazoline). Common vegetable crude oil was modified to make it reactive and used as the macroinitiator for the cationic ring-opening polymerization of the oxazoline monomer. The modification of the double bonds of lipids *via* soft thiol-ene chemistry allowed the synthesis of a halogenated macroinitiator. This macroinitiator leads to very good control of the polymerization of the 2-methyl-2-oxazoline with polydispersity index inferior to 1.5. Despite the complexity of the composition of crude oil and well-known drastic conditions required by a cationic type polymerization, we succeeded in the obtaining of amphiphilic polymers with a good composition control. The lipopolymers based on fatty esters and vegetable oils were shown to self-organize into nano-objects in aqueous medium without formation of any supra-aggregates or uncontrolled self-assembly. Well-shaped nanoparticles were obtained with characteristic radii of about 4.3 and 10.2 nm for the fatty ester- and triglyceride-based LipoPOx, respectively. Their relaxation time distributions were monomodal and highly monodisperse.

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