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L. Viora, T. Tichané, A. Taguet, X. Garric, J. Coudane, et al.. Syntheses of biodegradable graft copolymers from sodium caseinate and poly ϵ -caprolactone or poly lactic acid. Applications to the compatibilization of sodium caseinate/polyester blends. *Materials Today Chemistry*, 2023, 27, pp.101345. 10.1016/j.mtchem.2022.101345 . hal-03989285

HAL Id: hal-03989285

<https://imt-mines-ales.hal.science/hal-03989285v1>

Submitted on 14 Feb 2023

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Syntheses of biodegradable graft copolymers from sodium caseinate and poly ϵ -caprolactone or poly lactic acid. Applications to the compatibilization of sodium caseinate/polyester blends

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A B S T R A C T

Casein (and its sodium salt, sodium caseinate, SC) is an inexpensive natural milk protein that is used as a biodegradable biomaterial, especially to produce packaging films. However, to enhance some of its properties, it needs to be blended with other polymers, which should preferably be biodegradable such as poly lactic acid (PLA) and poly ϵ -caprolactone (PCL). New SC-g-PLA and SC-g-PCL graft copolymers have been prepared and unambiguously characterized, in particular by ¹H and DOSY NMR. The grafting degrees are high (between 24 and 35% by weight) and result in variations of properties, such as hydrophobicity and thermal properties. The microstructures of SC/PLA and SC/PCL blends were studied and compared, with and without the addition of the SC-g-PLA and SC-g-PCL copolymers to test the compatibilization capacity of these new biodegradable copolymers.

Keywords:

Casein
Chemical modification
Aliphatic polyesters
Grafting
Blends

1. Introduction

Renewable resources have gained interest in many domains, for example in chemical and polymer applications, especially in packaging and specifically for food packaging [1,2]. For some applications a water soluble material is desired and recent works describe the main materials that can be used [3]. In this area polysaccharides, such as chitosan [4,5], or alginate [6], proteins such as zein [7], or sodium caseinate [8,9] are used. Among the very large resources from nature, special attention can be paid to agricultural or animal products and by-products such as casein. Casein is extracted from milk of which it is one of the main constituents. Casein, often under a sodium caseinate form, is frequently used for the preparation of edible films for many food packaging applications [10–14]. Casein is readily soluble in water [15] at neutral or basic pH in concentrations up to 20% w/w, provided that the solutions are prepared at high temperature (80 °C). However, while casein can readily form films, these films are generally brittle and stiff due to strong casein-casein and casein-water interactions.

Plasticizers, particularly glycerol, must be added to increase the flexibility and elongation of the caseinate-based films [10,16,17]. These films are attractive for food applications because they adequately protect food products from their surrounding environment. All films made from these natural macromolecules are considered inherently biodegradable [1,2,18].

In other words, it should be convenient to increase the mechanical properties, such as Young modulus and tensile strength, and increase the hydrophobicity of the caseinate while maintaining its biocompatibility, biodegradability and partial water solubility. Tensile strength is a crucial factor in packaging applications since it determines the film thickness, which is related to the cost of the packaging. Some attempts were realized by adding starch or cellulose [10,17,19–22]. In other trials, aliphatic polyesters such as PLA or PCL were used as additives. These aliphatic polyesters, widely used in environmental and biomedical fields, are well known for their biocompatibility and biodegradability [23]. Moreover, PLA is derived from natural resources (corn and starch) and it is fully degraded into CO₂ and H₂O by naturally occurring microorganisms [24]. Tensile strength and elongation at break of casein samples are higher in the presence of PLA and depend on the proportion of PLA [25]. However, due to the large structural differences of PLA or PCL and caseinate, their blends are immiscible. To avoid or to limit this

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phenomenon, it is possible to graft PLA or PCL onto caseinate. Some grafting reactions on casein are described via Maillard reactions, especially with saccharides, polysaccharides [26–32] or fatty acids [25,33,34]. However, so far, no description of grafting biodegradable aliphatic polyesters onto casein has been found in the literature.

In this paper, the main part is devoted to the covalent grafting of PLA and PCL oligomers onto sodium caseinate, according to a Phase Transfer Catalysis (PTC) reaction in a biphasic medium, a mild method that respects the stability of the reagents. SC-g-PCL and SC-g-PLA Copolymers are synthesized and characterized by IR, ^1H , ^{13}C and DOSY NMR, DSC and TGA. SEM analysis of the microstructures of SC/PLA and SC/PCL blends, in the presence or absence of the copolymers, gives an idea of the compatibilizing ability of these two copolymers.

2. Materials and methods

2.1. Instrumentation and materials

2.1.1. Instrumentation

Infrared spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrophotometer using the attenuated total reflectance (ATR). Size exclusion chromatography (SEC) in DMSO was performed on Waters 515 (HPLC pump) equipment fitted with 2×300 mm long $8 \mu\text{m}$ Polargel M columns coupled with a Waters 410 refractometric detector at 60°C and a Waters 2996 PDA detector. The mobile phase was DMSO. Molar masses of PLA and PCL oligomers were measured by SEC using a Shimadzu Prominence system (Shimadzu Corp, Kyoto, Japan) equipped with a PLgel MIXED-C guard column (Agilent, $5 \mu\text{m}$, 50×7.5 mm), two mixed medium columns PLgel MIXED-C ($5 \mu\text{m}$, 300×7.8 mm), a Shimadzu RI detector 20 A and a Shimadzu UV-Vis detector SPD-20A. The mobile phase was THF with a flow of 1 mL/min at 35°C . Polystyrene standards were used for calibration. ^1H NMR spectra were recorded at room temperature using an AMX300 Bruker spectrophotometer operating at 300 MHz. Deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$) was used as solvent, chemical shifts were expressed in ppm. Diffusion ordered spectroscopy (DOSY) experiments were performed on a Bruker Advance III spectrometer using $\text{DMSO}-d_6$ as solvent. TGA thermograms were recorded on a TGA 2 Star System (METTLER TOLEDO) with AL-crucibles ($100 \mu\text{L}$) under N_2 with a heating rate of 10°C/min . DSC thermograms were recorded on a DSC3 Star System (Mettler Toledo) with AL-crucibles ($100 \mu\text{L}$) under N_2 with a heating rate of 10°C/min .

2.1.2. Materials

PLA (ref 3251D; $M_w = 55,000$) was obtained from NatureWorks LLC®. PCL (ref. 704105; $M_n = 45,000$), Lactic acid (85%), p-toluene sulfonic acid monohydrate, ϵ -caprolactone, thionyl chloride (97%), tetrabutyl ammonium bromide (98%) were purchased from Sigma Aldrich and used as received. Lotader® AX8900 (ethylene-methyl acrylate-glycidyl methacrylate terpolymer, 7–9% GMA, $M_w = 63\text{--}67^\circ\text{C}$) was obtained from Arkema. Sodium caseinate was obtained from Lactips (Saint-Paul-en-Jarez, Loire, France).

2.2. Experimental section

2.2.1. Synthesis of PLA oligomers

Lactic acid (356 g, 3.95 mol) is poured in a 500 mL round bottomed flask. p-toluene sulfonic acid (1 g) is then added, and the mixture is heated at 85°C under vacuum for 48 h to remove the water formed. The PLA oligomers are recovered without further purification. Molecular weight of PLA oligomers ($M_n \cong 750$) is measured by SEC in THF with polystyrene standards.

2.2.2. Synthesis of PCL oligomers

ϵ -Caprolactone (100 g, 0.88 mol) is poured into a round-bottomed flask. Lactic acid (3.75 g, 0.41 mol) and water (3 g, 0.166 mol) are then added. The mixture is heated at 120°C for 24 h. The molecular weight of PCL oligomers ($M_n \cong 1100$) is measured by SEC in THF with polystyrene standards.

2.2.3. Activation of PLA and PCL oligomers

2.2.3.1. Activation of PLA. Poly lactic acid oligomers (PLA, 20 g, $M_n = 750$, $2.67 \cdot 10^{-2}$ mol) were poured into a round bottomed flask equipped with a condenser, a NaOH trap at the top of the condenser and dry Argon bubbling. A large excess of thionyl chloride (50 mL) is added gently under mechanical stirring. Temperature is raised to 80°C for about 14 h. The thionyl chloride is then evaporated under reduced pressure. PLA chloride (PLA-COCl) is a viscous liquid that is used without further purification.

2.2.3.2. Activation of PCL. Poly(ϵ -caprolactone) oligomers (PCL, 40.4 g, $M_n = 1100$ g, $3.7 \cdot 10^{-2}$ mol) were poured in a round bottomed flask equipped with a condenser, NaOH trap at the top of the condenser and dry Argon bubbling. A large excess of thionyl chloride (35 mL) is added gently under mechanical stirring. The mixture is then heated to 80°C for about 14 h. The thionyl chloride is then evaporated under reduced pressure. PCL chloride (PCL-COCl) is a viscous liquid that is used without further purification.

2.2.4. Grafting of polyesters on sodium caseinate

2.2.4.1. Grafting of PLA on sodium caseinate. Sodium caseinate (10 g, $4.2 \cdot 10^{-4}$ mol) is dissolved in distilled water (250 mL) in a 2 L beaker under stirring at 8000RPM with an Ultra Turrax. Tetrabutyl ammonium bromide (4 g, $1.2 \cdot 10^{-2}$ mol) is added. A solution of activated PLA oligomers (24 g) in 250 mL of chloroform is then added slowly under Ultra Turrax stirring at 16,000 RPM. The pH of the solution is maintained at 8–9 by occasionally adding a solution (2 M/L) of NaOH. The reaction is continued for $\frac{1}{2}$ hour at room temperature. A solution of 60 mL HCl 2 M is added up to pH = 3. An emulsion is formed that is broken by adding ethanol (150 mL) before being centrifuged at 5000 RPM for 5 min. A gel is formed at the water/chloroform interface, which is recovered and washed twice with THF and finally dried under reduced pressure.

2.2.4.2. Grafting of PCL on sodium caseinate. Sodium caseinate (5 g, $2.2 \cdot 10^{-4}$ mol) is dissolved in distilled water (250 mL) in a 2 L beaker under stirring at 8000RPM with an Ultra Turrax. Tetrabutyl ammonium bromide (2 g, $0.6 \cdot 10^{-2}$ mol) is added. A solution of activated PCL oligomers (10 g, $8.9 \cdot 10^{-2}$ mol) in 250 mL of chloroform is then added slowly under Ultra Turrax stirring at 13,500 RPM. The pH of the solution is maintained at 8–9 by occasionally adding a solution (2 M/L) of NaOH. The reaction is continued for $\frac{1}{2}$ hour at room temperature. A solution of 30 mL HCl 2 M is added up to pH = 3. An emulsion is formed that is broken by adding ethanol (150 mL) before centrifuged at 5000 RPM for 5 min. A gel is formed at the water/chloroform interface, which is recovered, washed with methyl alcohol then twice with THF, and finally dried under reduced pressure.

2.2.5. Preparation of blends

Blends ($\cong 15$ g) were prepared by simple melt-mixing of the various components (SC, Polyester, eventually compatibilizing agent) for 5 min, using a DSM micro compounder. The melt mixing temperature was fixed at 180°C and 150°C for PLA and PCL, respectively and shear rate $>100 \text{ s}^{-1}$. The ratios SC/polyester are close to 20%/80% w/w.

3. Results and discussion

3.1. Syntheses of grafted caseinates, SC-g-PCL and SC-g-PLA

To obtain graft copolymers two methods are classically used, the grafting “from” method where some reactive groups of the caseinate, typically amine and alcohol functions, are used to initiate the polymerization of lactide or ϵ -caprolactone and the grafting “onto” method where activated preformed oligomers of PLA or PCL react on the reactive groups of the caseinate chain. In this paper, the grafting “onto” method is used, whose main advantage is that the molar masses of the grafted polyesters are well defined. However, the grafting degree is often lower than in the “grafting from” method, where the steric hindrance during the copolymer formation is lower. Therefore, only low molar mass polyesters are prepared and grafted, to maintain moderate steric hindrance. The reaction scheme is shown in Fig. 1.

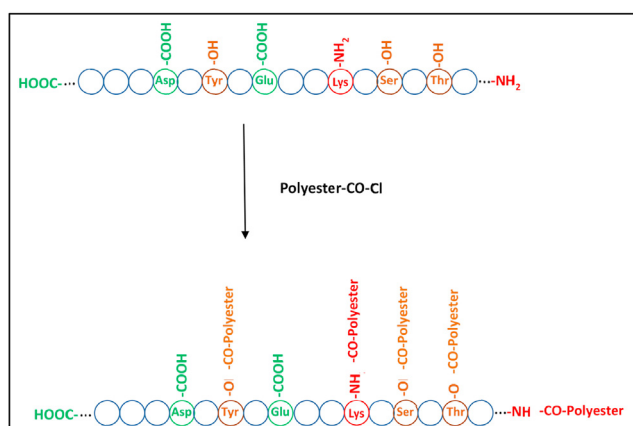


Fig. 1. Synthesis scheme of SC-g-PLA and SC-g-PCL preparation.

3.1.1. Syntheses of PLA and PCL oligomers

PLA and PCL oligomers are prepared by two different methods. PLA oligomers were synthesized by step polymerization of lactic acid. The molar mass of the oligomers is monitored during the polymerization by SEC in THF. The polymerization is stopped when the molar mass reaches 750, which corresponds to a degree of polymerization $\cong 10$. PCL oligomers were prepared by Ring Opening Polymerization of caprolactone in aqueous media using the Stanley method [35]. The reaction was stopped when the molar mass, measured by SEC in THF, reached 1100, which corresponds to a degree of polymerization $\cong 10$, similar to that of PLA. Both methods are assumed to give oligomers with an alcohol chain end and a carboxylic acid chain end.

3.1.2. Activation of acid chain-end of polyesters

Acyl chlorides are classical reagents for the activation of carboxylic acids to prepare esters or amides by reaction with alcohols or amines. Acyl chlorides are highly reactive compared to anhydrides but are rarely used to activate carboxylic acid chain end of polyesters [36,37]. For PLA and PCL oligomers we adapted the protocol of Ustariz-Peyret et al., described for PLA oligomers, in which thionyl chloride is used as solvent [37]. Activated polyesters are recovered after evaporation of thionyl chloride under reduced pressure. They are used without further purification. Activation is followed by the appearance of the acyl chloride FTIR band at 1795 cm^{-1} in activated PCL (PCL-COCl) and 1820 cm^{-1} in activated PLA (PLA-COCl) (Fig. 1SD).

3.1.3. Grafting of activated oligomers on sodium caseinate

To our knowledge, grafting of aliphatic polyesters onto caseinate is not described in the literature. However, grafting of polyesters onto cellulose [36] or starch [38] are mentioned. A casein-g-dextran copolymer in which the two homopolymers are water soluble at pH7 is described, via the Amadori rearrangement of the Maillard reaction [30]. One of the main problems in the synthesis of SC-g-Polyester is the difficulty in finding a common

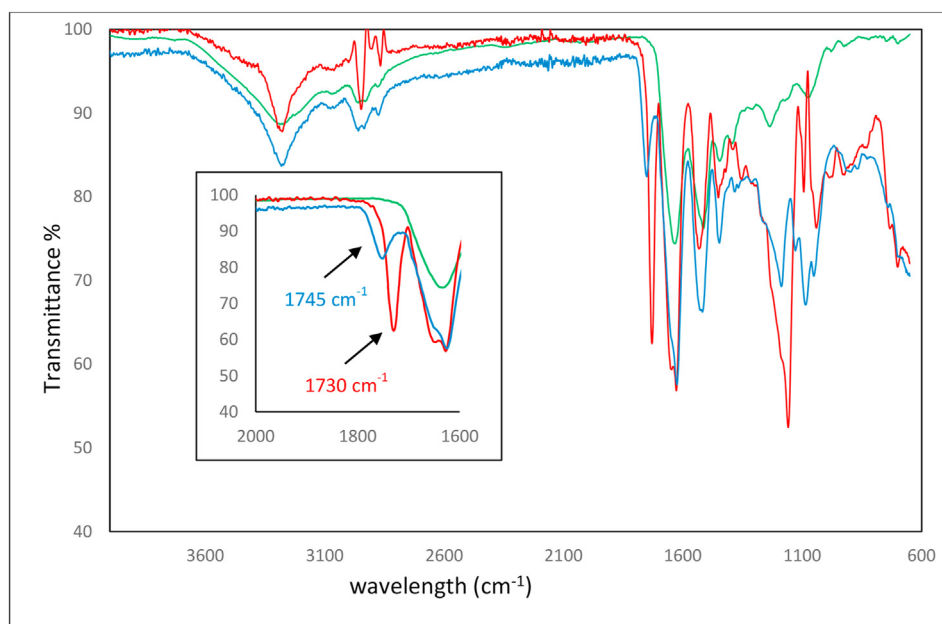


Fig. 2. FTIR spectra of caseinate (green), SC-g-PCL (red) and SC-g-PLA (blue) (Insert: enlargement $2000\text{--}1600\text{ cm}^{-1}$).

solvent for the hydrophilic caseinate and the hydrophobic polyesters. For this reason, we carried out the reaction in a biphasic medium consisting of a solution of caseinate in water and a solution of activated polyester in an organic solvent, dichloromethane or chloroform. A phase transfer catalysis reaction is performed in the presence of a surfactant, tetrabutyl ammonium bromide. A drastic stirring at about 15,000 RPM, is necessary to obtain a large aqueous phase/organic phase interface. The pH of the aqueous solution is maintained in the range 8–9 to leave the caseinate amine groups under a non-protonated form. To avoid remaining unreacted polyesters, the copolymers are purified by successive washes with THF. All reactions are carried out with an excess of acid chloride functions over the amine + alcohol functions (ratio (acyl chloride/amine + alcohol) \cong 2).

The amounts of activated polyesters were determined by considering the presence of approximately 30 free alcohol/phenol groups (from threonine, tyrosine and serine residues) and 14 free amine groups (from lysine residues) on a caseinate molecule, and

assuming that activated polyesters can react on both amine and alcohol groups.

3.2. Copolymers characterizations

3.2.1. FTIR

PCL grafting is highlighted on the FTIR spectrum by a peak at 1730 cm^{-1} on SC-g-PCL, characteristic of the PCL ester band, which is not present in caseinate. A similar band is observed at 1745 cm^{-1} on the FTIR spectrum of SC-g-PLA, characteristic of the PLA ester band, which is not present in caseinate (Fig. 2).

3.2.2. ^1H , ^{13}C and DOSY NMR

The ^1H NMR spectra were recorded in D_6 DMSO. The presence of characteristic PCL peaks on the SC-g-PCL (1.25 ppm Hd, 1.5 ppm Hc, 2.25 ppm Hb and 3.95 ppm Ha) can be clearly shown in the spectra of Fig. 3a. In the same way, Fig. 3b clearly shows the presence of PLA

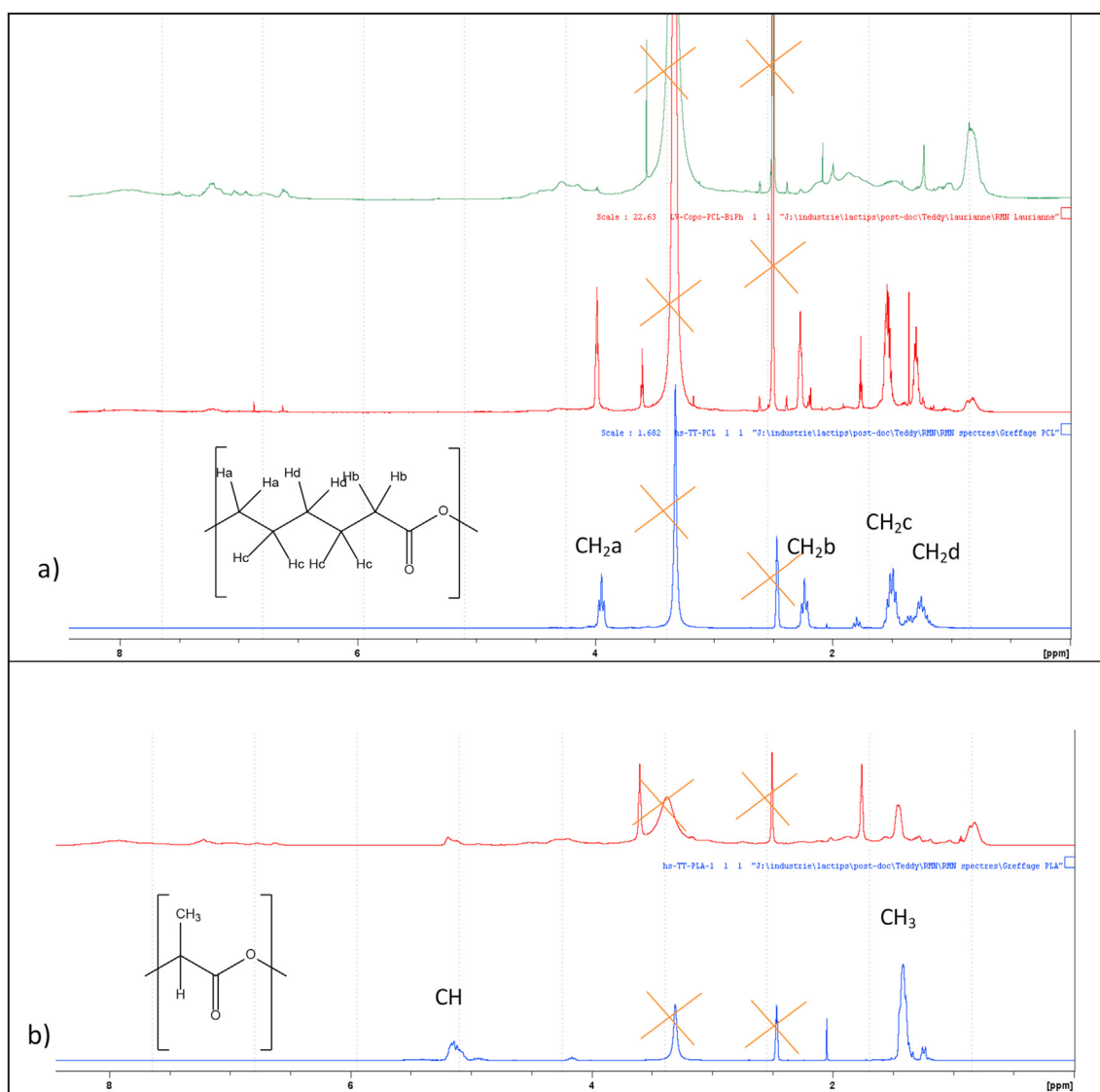


Fig. 3. ^1H NMR (D_6 DMSO) of a) PCL (blue), Copolymer SC-g-PCL (red) and SC (green) and b) PLA (blue), copolymer SC-g-PLA (red).

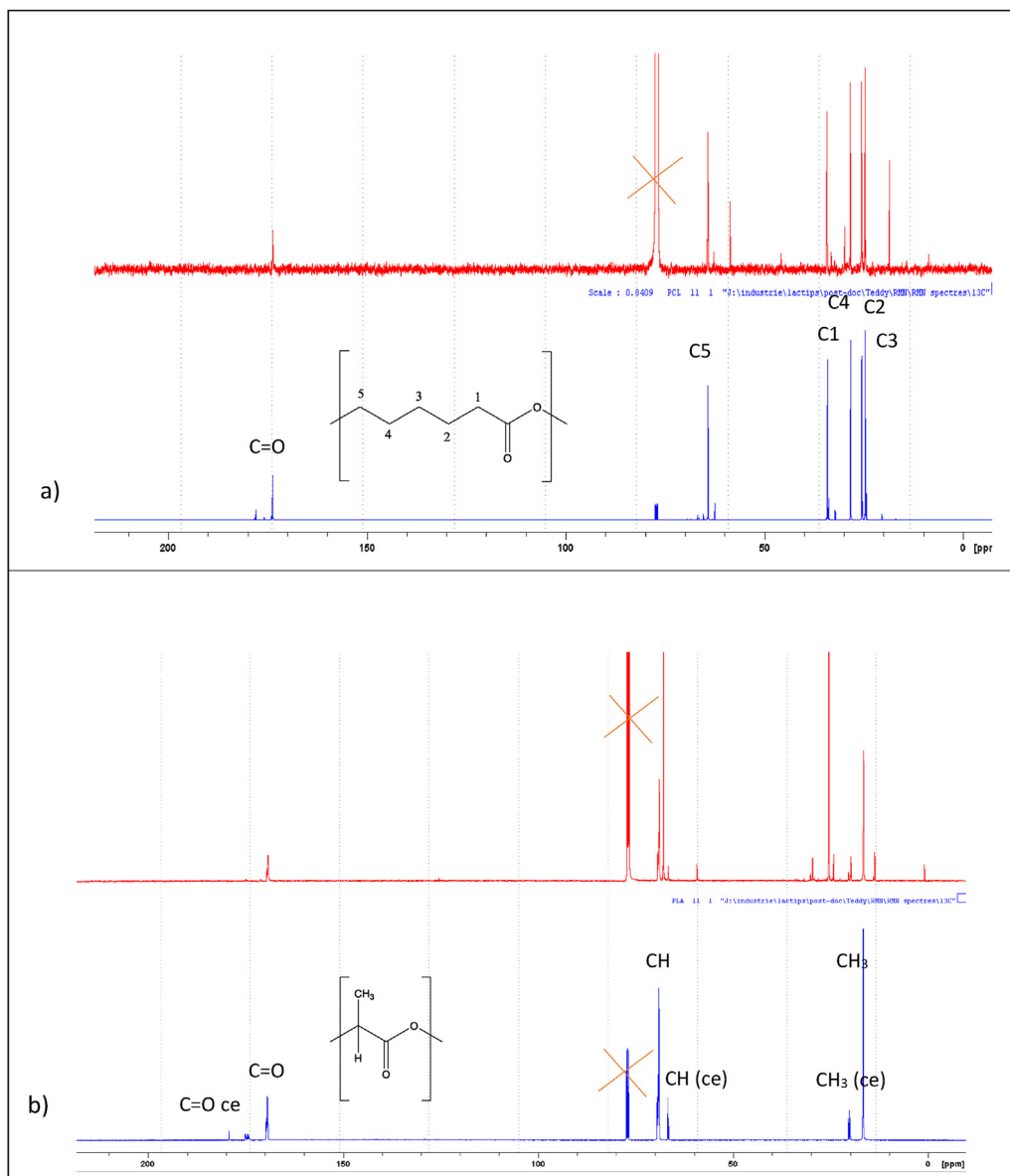


Fig. 4. ^{13}C NMR spectra of a) PCL (blue) and copolymer SC-g-PCL (red) and b) PLA (blue) and copolymer SC-g-PLA (red) (ce = chain end).

moiety in the copolymer SC-g-PLA (at 1.45 ppm (C–H) and 5.15 ppm (CH_3)).

^{13}C NMR spectra were recorded in CDCl_3 where the copolymers are quite soluble, thanks to the polyester moiety that is very soluble in CDCl_3 , while the SC is not soluble in CDCl_3 . The spectra of SC-g-PCL and SC-g-PLA clearly shows the presence of PCL and PLA chains in the copolymers (Fig. 4).

However, the ^1H and ^{13}C NMR spectra do not clearly show the possible presence of unreacted polyesters in the graft copolymers, which may bias the results. Therefore, DOSY NMR was used to distinguish between caseinate, copolymers and possible unreacted polyesters based on their different viscosities.

Fig. 5 shows the DOSY spectra of SC (blue) and purified SC-g-PCL (red) and the ^1H NMR of PCL (purple). The viscosity diffusion coefficient (DC) of caseinate ($F1 = -10.75 \log(\text{m}^2/\text{s})$) is higher than that of copolymer ($F1 = -11.2 \log(\text{m}^2/\text{s})$), due to the higher molar mass of copolymer. In addition, the spots at 1.25 ppm, 1.5 ppm, 2.25 ppm, and 3.95 ppm in the SC-g-PLA spectrum (red ovals) are characteristic of the PCL moiety, showing the efficient grafting of

PCL onto the caseinate chain. There is no evidence of free PCL oligomers at $\text{DC} = -10.1 \log \text{m}^2/\text{s}$ in the copolymer.

Similarly, Fig. 6 shows the DOSY spectra of PLA, SC and purified SC-g-PLA. It is shown that DC of SC-g-PLA ($-11.2 \log \text{m}^2/\text{s}$) is lower than that of SC ($-10.75 \log \text{m}^2/\text{s}$). In addition, spots appear at 5.1 ppm and 1.45 ppm in the spectrum of SC-g-PLA, characteristic of the methyne and methyl hydrogens of PLA, showing the effective grafting of PLA onto the caseinate chain. It is also shown that there are no free PLA ($\text{DC} = -10.1 \log \text{m}^2/\text{s}$) oligomers in this purified SC-g-PLA.

3.2.3. SEC analyses

SEC analyses were performed in DMSO where sodium caseinate, PLA and PCL oligomers and copolymers are sufficiently soluble, at least in low concentrations. Fig. 7a and Fig. 7b shows the chromatograms of unpurified SC-g-PCL and SC-g-PLA with UV detection at 280 nm (refractive index gives no trace in DMSO). Retention time of SC-g-PCL and SC-g-PLA are about 9.4 min and 9.7 min respectively and that of oligo PCL and oligo PLA are 14.35 min and 15.37 min respectively. The spectra of the two compounds, taken at

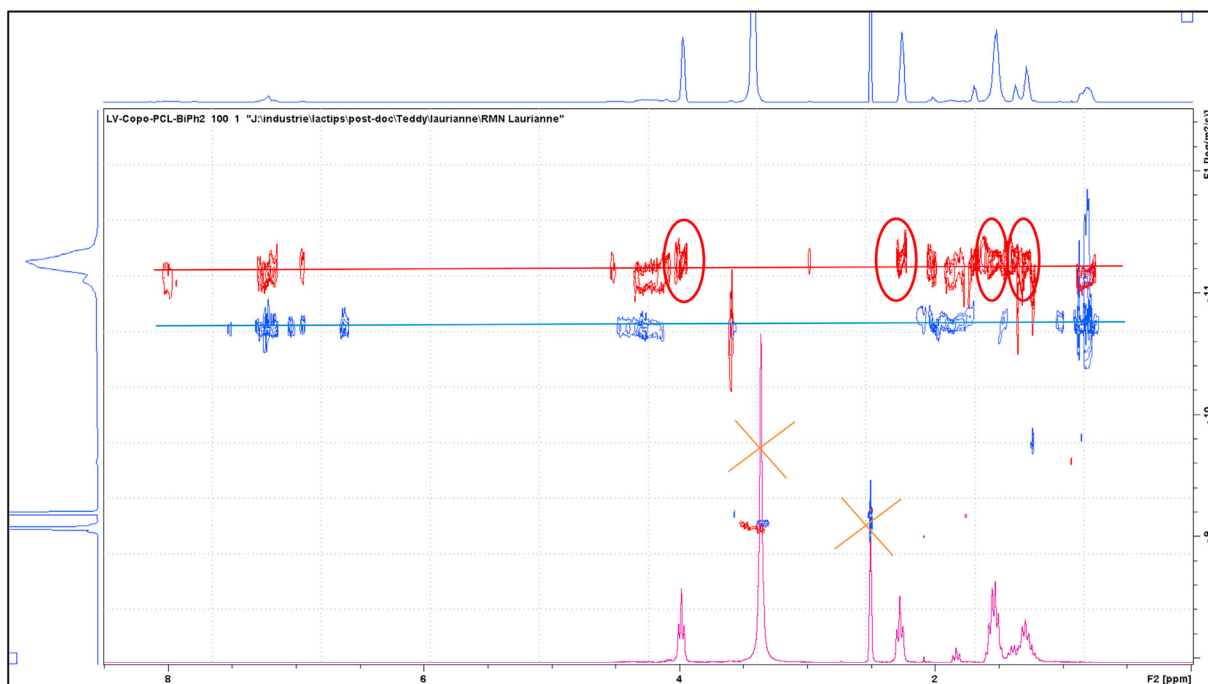


Fig. 5. DOSY NMR spectra of caseinate (blue), purified SC-g-PCL (red) and ^1H NMR of PCL (purple).

the peak top of the chromatograms, are shown in Fig. 7c and d with that of genuine caseinate.

3.2.4. Evaluation of the grafting degree

The copolymers considered free of homopolymers according to the DOSY, a grafting degree (GD) can be evaluated on the ^1H NMR spectra by the ratio of the intensities of peaks characteristic of PCL (I_{PCL} : CH_2 at 4 ppm), PLA (I_{PLA} : CH at 5.2 ppm) and caseinate (I_{cas} : CH_3 at 0.8 ppm).

Natural milk casein is composed of α -caseinate s1 (34–40%), α -caseinate s2 (12–16%), β -caseinate (37–42%) and κ -caseinate (8–15%). Among all the amino acids of caseinate, alanine, valine, leucine, isoleucine and threonine have methyl side groups giving an NMR signal around 0.8 ppm. Therefore, there are approximately 276 methyl protons from $\cong 92$ methyl groups in a caseinate chain. Similarly, there are 30 alcohol/phenol groups (from tyrosine, serine and threonine) and 14 free amine groups (from lysine) in a caseinate chain. The number of possible grafting points on a caseinate chain is thus 44.

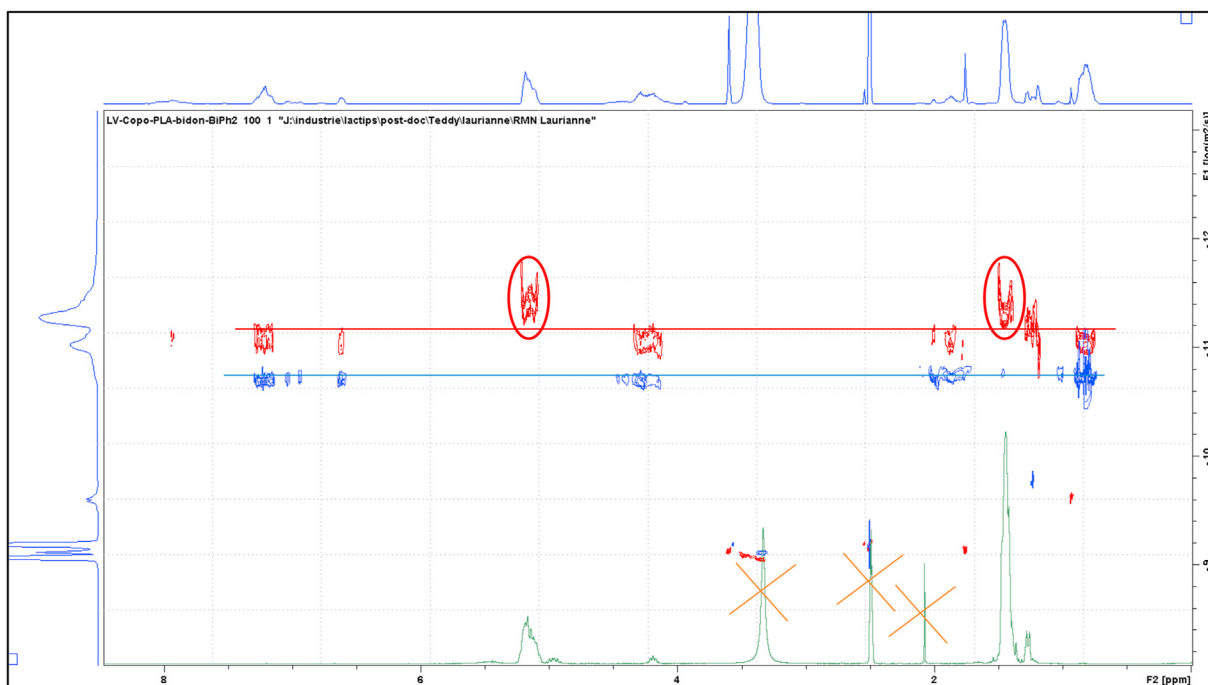


Fig. 6. DOSY spectra of SC (blue), SC-g-PLA (red) and ^1H NMR of PLA (green).

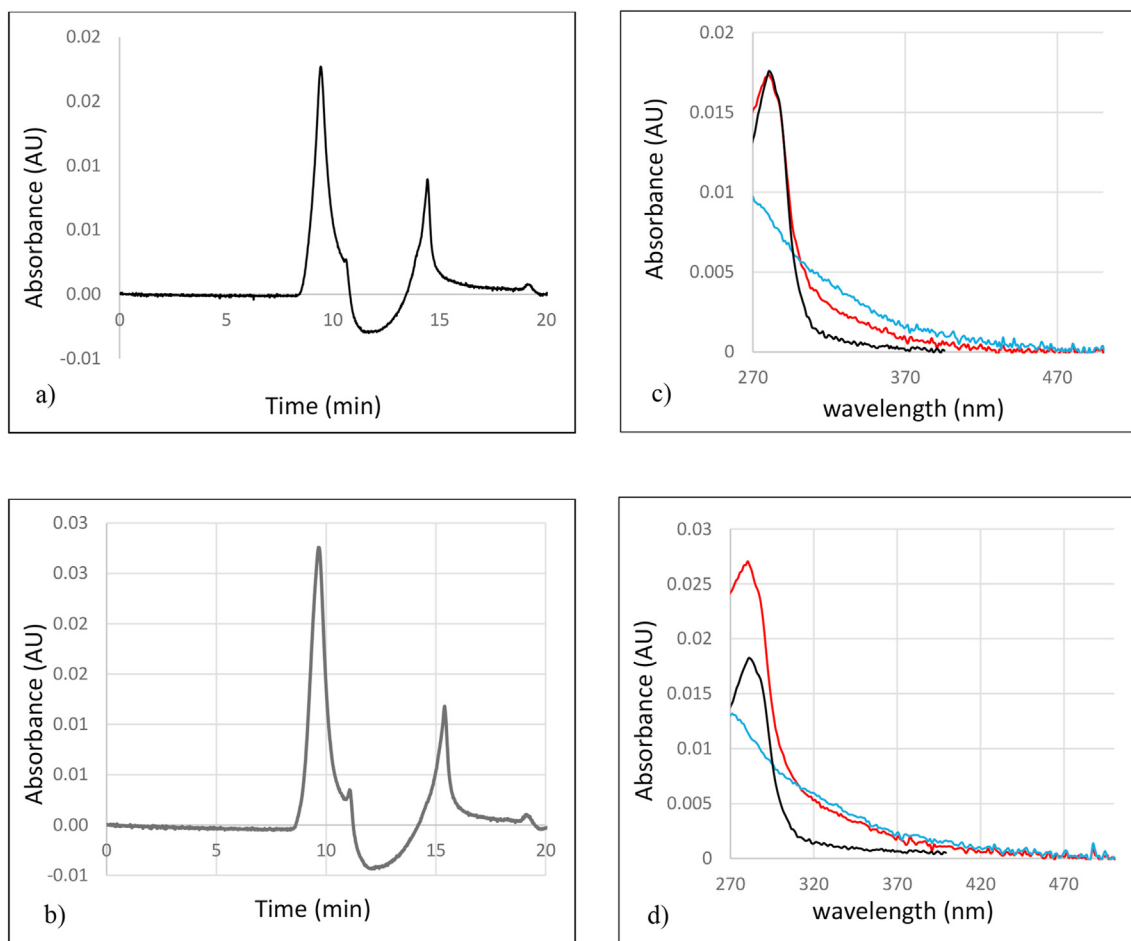


Fig. 7. Chromatograms of a) SC-g-PCL, b) Sc-g-PLA and spectra of c) SC-g-PCL (red), SC (black) Oligo-PCL (blue) and d) SC-g-PLA (red), SC (black), oligo-PLA (blue).

If PD is the degree of polymerization of PCL or PLA homopolymers, the effective number of grafting points (GN) on caseinate, i.e. the number of polyester chains grafted on a caseinate chain, as determined by NMR, is:

$$GN_{PCL} = I_{PCL} / I_{cas} * \frac{276}{2 * PD(PCL)} \text{ for PCL grafting}$$

and

$$GN_{PLA} = I_{PLA} / I_{cas} * \frac{276}{PD(PLA)} \text{ for PLA grafting}$$

The grafting degree (GD) is then: $GD = GN/44$. All results are summarized in [Table 1](#).

Given that molar mass of SC is about 22,000, the weight % (w%) of polyester is:

$$w\%_{PCL} = GN_{PCL} * M_{nPCL} / (22000 + GN_{PCL} * M_{nPCL})$$

$$w\%_{PLA} = GN_{PLA} * M_{nPLA} / (22000 + GN_{PLA} * M_{nPLA})$$

Table 1

Proportions of polyesters in copolymers and grafting degrees.

	Ratio: peak at 4 ppm (PCL) or 5.2 ppm (PLA)/peak 0.8 ppm	GN = number of grafted polyester chains on a SC chain	Grafting degree of polyester (%)	weight % of polyester
PCL	0.77	10.6	24	35
PLA	0.33	9.1	21	24

Grafting degrees are lower than those obtained with oleic acid under the same conditions (56%) (*Tichané et al., unpublished results*), showing the effect of the steric hindrance in this “polymer on polymer” reaction. The conformational constraints of caseinate molecules mean that not all the amine and alcohol groups are accessible, especially those located inside the protein molecules [39].

3.2.5. Thermal analyses

TGA thermograms were recorded from 20 °C to 550 °C under air at a heating rate of 10 °C/min. In the first part of the thermograms ([Figs. 8 and 9](#), inserts), from 20 °C to 150 °C, a water loss is observed that is depends of the hydrophobicity: the higher the hydrophobicity, the lower the water loss. The hydrophilic SC loses 8% of water between 20 and 150 °C while the hydrophobic polyesters lose 1% (PCL) and 2.5% (PLA) of water. It may be noted that PCL is more hydrophobic than PLA, which is a well-known property. The weight losses of the copolymers and blends of SC and polyester are between 2.5 and 8%. The 2nd part of the thermograms up to 550 °C describes the thermal stability of the materials. We will

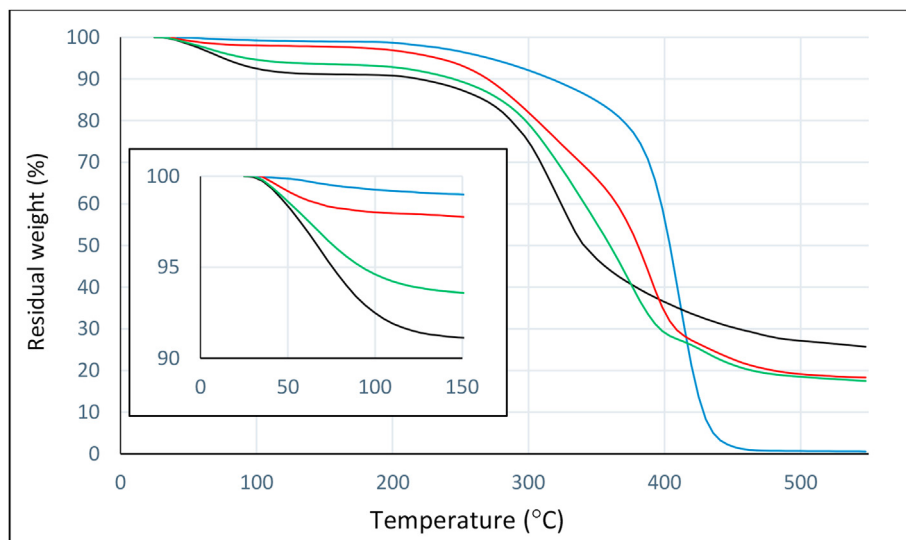


Fig. 8. TGA thermograms of SC (black), PCL (blue), SC-g-PCL (red), mix of SC and PCL (30%) (green) (Insert: curves enlargements).

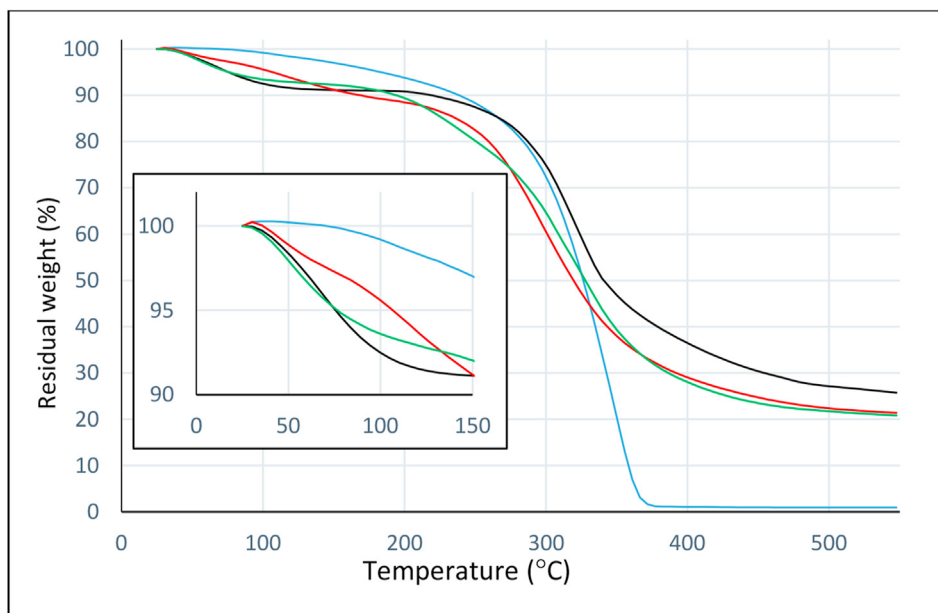


Fig. 9. TGA thermograms of SC (black), PLA (blue), SC-g-PLA (red), mix of SC and PLA (20%) (green) (Insert: curves enlargements).

focus on the T_{30} that corresponds to a 30% degradation of the material.

In the case of PCL-based compounds, a 30% degradation of PCL occurred at 390 °C and the degradation is total at 460 °C. The 30% degradation of SC occurred at 310 °C but at 500 °C only 72% weight loss is observed. T_{30} of SC-g-PCL (340 °C) and mix of PCL and SC (325 °C), are between the two values. At 500 °C the percentage degradation of SC-g-PCL (which contains around 35% of PCL) is close to that of the mixture containing 30% PCL, which confirms the calculations of the weight proportions of PCL in the copolymer, calculated by NMR.

In the case of PLA based compounds, results are different because PLA degrades more rapidly than PCL: $T_{30} = 305$ °C and a total degradation is observed at 370 °C. At 500 °C SC-g-PLA and a mixture of PLA and SC are between the two degradation values 72% for SC and 100% for PLA. It can be noted that at 500 °C the

percentage of degradation of SC-g-PLA (which contains around 24% of PLA) is close to the one of the mixture containing 20% PLA, which confirms also the calculations of the weight proportions of PLA in the copolymer, calculated by NMR.

-DSC thermograms of PCL-containing materials were recorded from 10 °C to 130 °C under N_2 at a 10 °C/min heating rate to observe the melting temperature of PCL moiety (Fig. 10). Melting temperature (T_{mPCL}) and ΔH fusion (ΔH_{fPCL}) of PCL are 42 °C and $-62.4 \text{ J} \cdot \text{g}^{-1}$ respectively. No melting temperature is observed in SC-g-PCL that contains 35% w/w PCL, showing that no crystalline form of PCL is present in SC-g-PCL, which confirms the formation of a conjugate. A "Normalized ΔH_{fPCL} " was defined in SC/PCL blends, considering the proportion of PCL in the blends: for a proportion of 20% PCL, the "normalized ΔH_{fPCL} " is $\Delta H_{fPCL} \cdot 5$. It is shown that the normalized ΔH_f hardly varies with the proportion of PCL in the mixture SC + PCL ($-62.4 \text{ J} \cdot \text{g}^{-1}$ in PCL, $-59.2 \text{ J} \cdot \text{g}^{-1}$ in SC + PCL

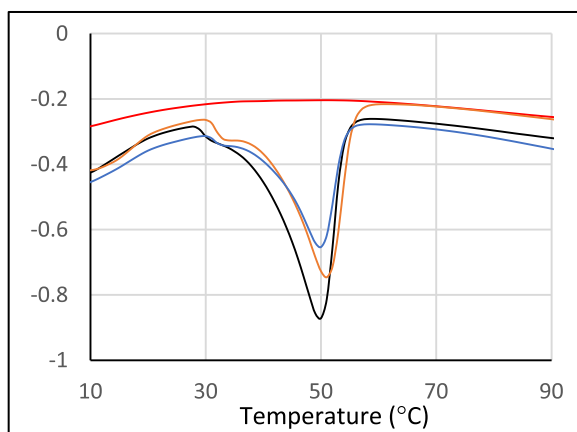


Fig. 10. DSC thermograms of SC/PCL blends 20% (blue), 30% (orange), 40% (black) and SC-g-PCL (red).

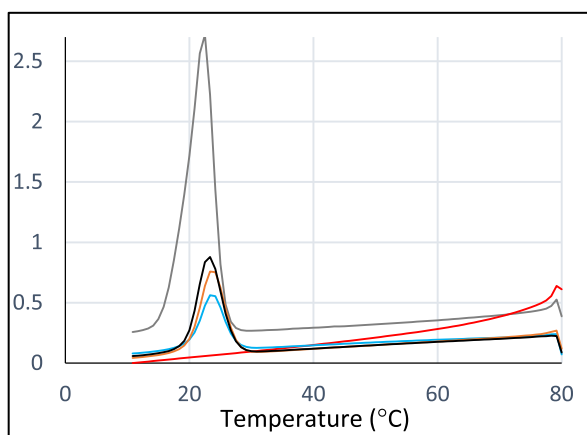


Fig. 11. DSC crystallization thermograms of pure PCL (grey) SC-g-PCL (red) and SC/PCL blends at 20% (blue), 30% (orange) and 40% (black).

20%, $-59.1 \text{ J}^* \text{g}^{-1}$ in SC + PCL 30%, $-52.0 \text{ J}^* \text{g}^{-1}$ in SC + PCL 40%). This result shows a significant lack of compatibility of the mixtures as PCL remains in a crystalline form.

Similarly, DSC thermograms of PCL-containing materials were recorded from 80 °C to 20 °C under N_2 to observe the potential

Table 2

Proportion of water-insoluble residues in casein and copolymers.

	residual w%		
	SC-g-PLA	SC-g-PCL	Casein
pH = 6	88	83	17
pH = 12	42	76	5

Table 3

Formulations of the blends.

Polyester (wt.%)	SC wt.%	Compatibilizer (wt.%)
PLA (80)	20	–
PLA (76)	19	Lotader® (5)
PLA (76)	19	SC-g-PLA (5)
PCL (80)	20	–
PCL (76)	19	Lotader® (5)
PCL (76)	19	SC-g-PCL (5)
PCL (78)	19.5	SC-g-PCL (2.5)
PCL (72)	18	SC-g-PCL (10)

crystallization of the PCL moiety (Fig. 11). The crystallization temperature of pure PCL occurred at 23 °C and did not vary in the SC + PCL blends at 20, 30 or 40% of PCL (ΔH_{cryst} remains between 58.5 and 63 $\text{J}^* \text{g}^{-1}$ for all these blends). The SC-g-PCL copolymer containing 35% of PCL fraction has a different behavior; it did not crystallize, showing once again that there is no crystalline form of PCL in the copolymer.

In the case of PLA-containing materials, there is no crystallinity because SC is not crystalline and the rate of crystallization of PLA is low and therefore its crystallinity is very low.

3.3. Copolymers properties

The grafting of hydrophobic polyesters should lead to an increase in the hydrophobicity of the material and consequently to a decrease in the water solubility.

3.3.1. Water solubility

To test water solubility of the copolymers, the same amount of sodium caseinate and copolymer was placed in two beakers containing the same amount of water, at pH = 6 and pH = 12. After rapid stirring for 24 h at room temperature, the resulting suspensions were centrifuged at 5000 rpm for 5 min and the non-soluble residue was separated, dried and weighed. The recovered weight/initial weight ratio was measured. The higher the latter, the more hydrophobic the compound. The values obtained are shown in Table 2. About 80% of the copolymers are recovered as insoluble compounds at pH6 instead of only 17% for casein. This shows a dramatic increase in hydrophobicity after grafting, due in part to the grafting of the hydrophobic polyester and in part to the decrease in the number of hydrophilic amine and alcohol functions after grafting. At pH12, 95% of the casein is soluble (as caseinate) in contrast to SC-g-PCL where 76% is insoluble. The decrease of the insoluble part in the copolymers is likely due to a partial degradation of the polyester chains during dissolution. This decrease is more important in the PLA chain (42% insoluble), which is known to be more readily degraded than the PCL chain. The ^1H and DOSY NMR spectra showed no significant difference between the soluble and insoluble fractions of the grafted caseinates.

3.3.2. Compatibilization of SC/PCL and SC/PLA blends

It is known that block or graft copolymers containing two different structures are good compatibilizing agents for mixtures of homopolymers having each of the two structures. Therefore, we investigate the compatibilizing ability of SC-g-PCL and SC-g-PLA for non-compatible blends of SC and PCL or PLA. Various formulations are shown in Table 3: Casein being an additive to modify the properties of polyester, the proportions of polyester and casein in the blends are close to 80% and 20% respectively. The proportions of compatibilizer are between 2.5% and 10% by weight, which are common values for an additive. As previously mentioned in the DSC studies, SC/PCL mixtures are only very partially compatible. The

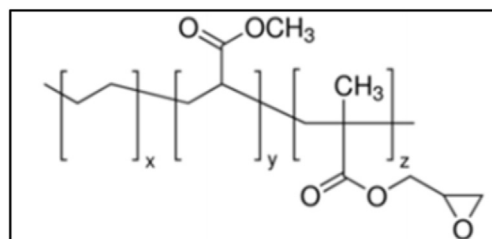


Fig. 12. Structure of LOTADER® AX8900.

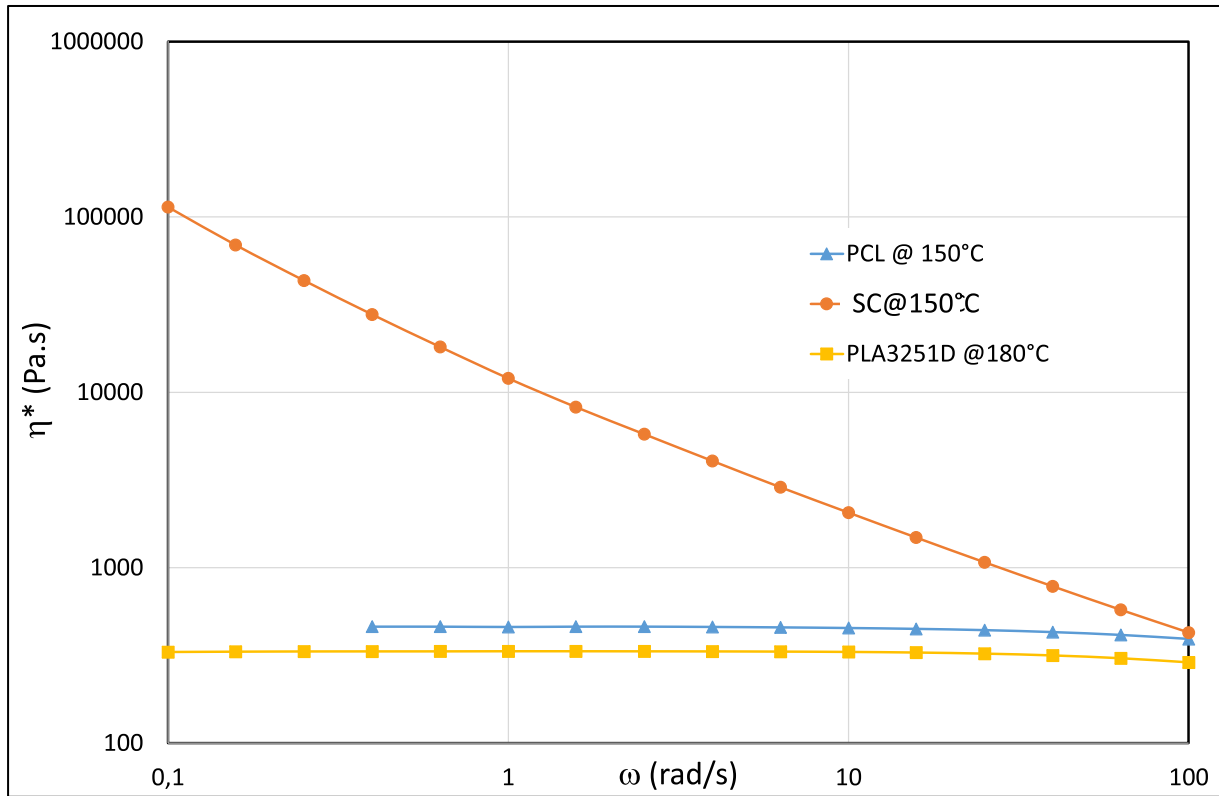


Fig. 13. Viscosities of PCL, PLA, and SC as function of ω (rad/s), experimental curves.

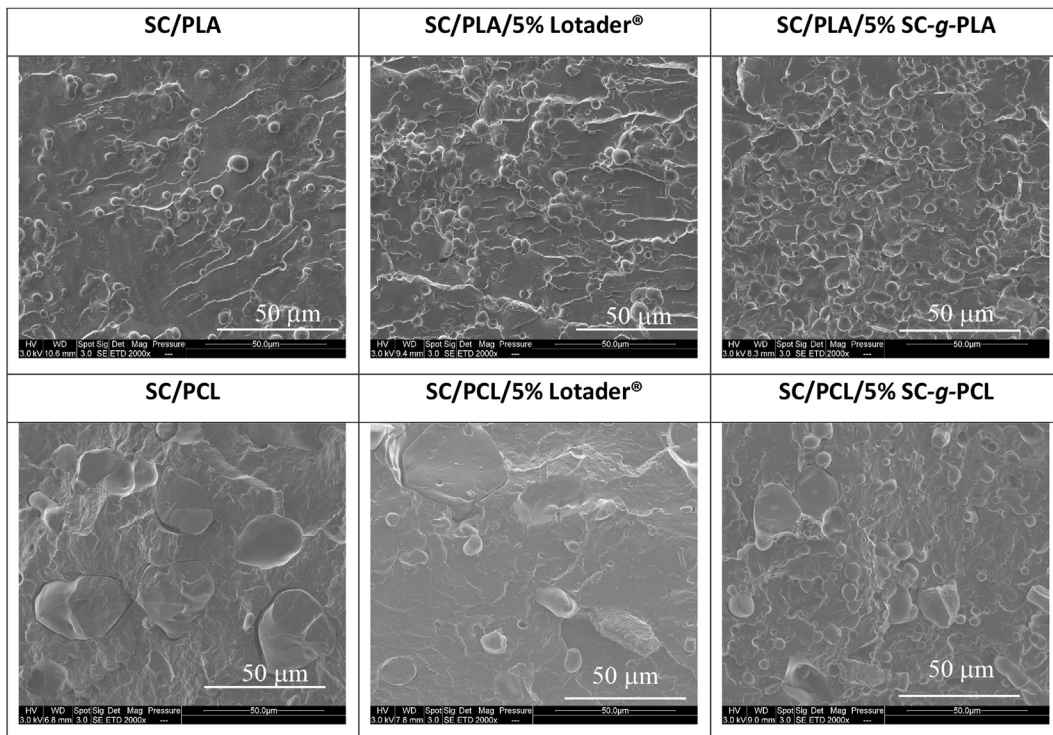


Fig. 14. SEM pictures of the microstructures of PLA/caseinate and PCL/caseinate with or without compatibilizers.

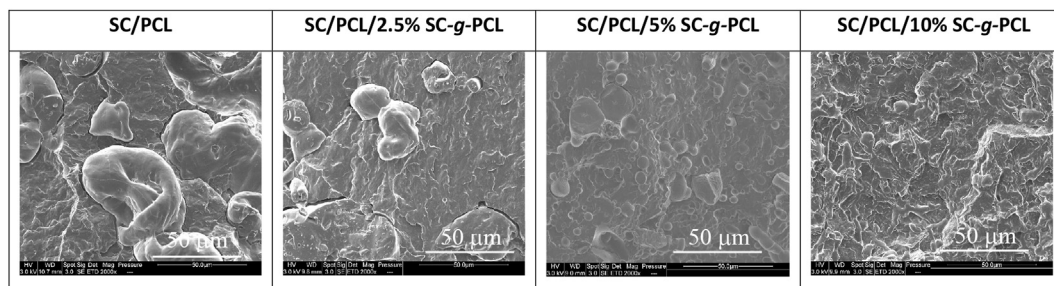


Fig. 15. Micrographs of SC/PCL blends containing various amounts of SC-g-PCL copolymer.

effect of compatibilization effect is studied by analyzing the microstructures of the various samples by SEM.

SC-g-PLA, SC-g-PCL and Lotader® AX8900 are added to PCL/SC and PLA/SC blends. Lotader® AX8900 is an industrially available ethylene-methyl acrylate-glycidyl methacrylate terpolymer, a compatibilizer currently used to increase the mechanical properties of polyester blends (PBT, PET ...) (Fig. 12). During the compounding at high temperature (>100 °C) the epoxy function is intended to react with the C–H of the polyesters chains [40,41].

To understand the final microstructure of a polymer blend, it is important to know their viscosity behavior. Indeed, several parameters dictate the final microstructure of an immiscible polymer blend [42], especially, viscosity ratio [43], elastic modulus of each phase, interfacial tension [44] and also proportions. All things being equal, the less viscous phase tends to encapsulate the most viscous one. An 80% polyester/20% SC blend leads to a polyester matrix with a caseinate dispersed phase. Fig. 13 shows the measured complex viscosities of the three components: at 150 °C for SC and PCL and 180 °C for PLA (Melting point of PLA is about 175 °C and measurements should be performed at higher temperature). It was not possible to measure the viscosity of SC at 180 °C due to thermal degradation. However, it can be assumed that the curve of SC at 180 °C should be much lower than that at 150 °C. The large caseinate zones ($\approx 25 \mu\text{m}$) for SC/PCL and finer caseinate nodules ($<10 \mu\text{m}$) for SC/PLA blends (Fig. 14) can be explained by the different viscosity ratio at the processing conditions. Indeed, for the SC/polyester blends, at the processing temperature (150 °C or 180 °C) and shear rate (higher than 100 s^{-1}), we have $\eta_{\text{PCL}} < \eta_{\text{SC}}$ (at 150 °C) and $\eta_{\text{SC}} < \eta_{\text{PLA}}$. Thus, it can be assumed that since the viscosity of the matrix is higher than that of the dispersed phase (not measured) in the case of the SC/PLA blend, this may explain the finer SC nodules, in contrast to the SC/PCL blend. In this latter case, at 150 °C, as the viscosity of the dispersed phase is close or higher than the one of the PCL matrix (under the processing conditions), this results in coarser microstructures.

Regarding the micrographs of the blends containing 5 wt% of compatibilizer (either Lotader® or casein-based copolymers), it seems that both compatibilizers have a poor influence on the caseinate/PLA blend, as the dispersed phase size is not changed when compatibilizers are added. This is not too surprising, as casein has been shown to have a certain affinity for PLA. In particular, it allows for better dispersion of cellulose/PLA mixtures [45]. On the contrary, SC-g-PCL tends to strongly decrease the size of the dispersed caseinate phase, from $\approx 25 \mu\text{m}$ to $\approx 5\text{--}10 \mu\text{m}$, unlike Lotader® that is almost ineffective at compatibilizing any of the blends. This decrease in the size of the dispersed phase is a classic emulsification effect, with the PCL portion of the copolymer having a strong affinity for the PCL matrix phase while the caseinate portion of the copolymer has a strong affinity for the caseinate dispersed phase of the blend. Then, the copolymer tends to be localized at the interface between the two polymer phases.

Further studies on the role of SC-g-PCL as a compatibilizer for PCL/SC blends have been performed. Increasing amounts of SC-g-PCL were added to a PCL/SC blend. Micrographs of the blends unambiguously show that increasing the amount of copolymer to 10 wt% results in a decrease in the size of the dispersed caseinate phase (Fig. 15).

4. Conclusion

The syntheses of two new graft copolymers based on a casein backbone, SC-g-PCL and SC-g-PLA are described. These copolymers contain fully biodegradable moieties and, for SC-g-PLA from renewable materials. The synthesis method uses a phase transfer catalysis reaction, which occurs in a biphasic medium, allowing the reaction of a hydrophilic compound and a hydrophobic compound. This mild synthesis method can be potentially extended to other hydrophobic polymers activated as acid chloride. The are high and yield grafted polyesters weight% of 24 and 35%. The copolymers are characterized in an unequivocal way by ^1H , ^{13}C and DOSY NMR. The thermal properties of these copolymers, analyzed by TGA and DSC, highlight the different structural characteristics of the copolymers compared to homopolymers blends. Finally, the compatibilizing capacity of these copolymers on casein/PCL and casein/PLA blends demonstrated by the study of the microstructures of the various blends.

Funding

This work was partly financed by the “Maison de la Chimie” for Teddy Tichane’s salary.

Authors contributions

Laurianne VIORA, carrying out the reactions.
 Teddy TICHANE, carrying out the reactions.
 Aurélie TAGUET, Methodology of compatibilization, realization of SEM tests.
 Xavier GARRIC, Validation.
 Jean COUDANE, Conceptualization, Writing - original draft.
 H el ene Van Den BERGHE, Writing - review.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mtchem.2022.101345>.

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