

# Bacterial polyesters grafted with poly(ethylene glycol): Behaviour in aqueous media

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## Abstract

An easy method for grafting of poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate) (PHOU) was developed. Oxidation of the pendant double bonds of PHOU into carboxyl groups to yield poly(3-hydroxyoctanoate-*co*-3-hydroxy-9-carboxydecanoate) (PHOD) and the esterification of the carboxyl side groups with poly(ethylene glycol) (PEG) were carried out in a single reaction solution. The grafting yield is dependent on the molar mass of the PEG graft. The maximum carboxyl group conversion (52%) was obtained with PEG  $M_n = 350$  and decreased with increasing molar mass of PEG (19% for PEG  $M_n = 2000$ ). Yields were determined by  $^1\text{H}$  and  $^{13}\text{C}$  NMR. Short PEG grafts lowered the glass transition temperature (PHOD-*g*-PEG 350  $-57^\circ\text{C}$ ) compared to PHOD ( $-19^\circ\text{C}$ ) and PHOU ( $-39^\circ\text{C}$ ). This effect depends on the COOH conversion and PEG chain length. Grafting enhanced the hydrophilic character of the modified polymers making them soluble in polar solvents, such as alcohols and water/acetone mixtures. PHOD-*g*-PEG films were more stable towards hydrolytic degradation as PHOD films. No obvious modification of films was observed after more than 200 days at pH 7.2 and  $37^\circ\text{C}$ . The molar mass of the grafted polymers decreased only slightly during this period, while PHOD films were hydrolyzed into soluble fragments.

**Keywords:** PHAs; Carboxyl PHAs; PHA-graft-PEG; Chemical modification; Stability; Hydrolysis

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## 1. Introduction

Poly(3-hydroxyalkanoate)s (PHAs) are biodegradable polyesters produced by a wide variety of bacteria [1]. They are non-toxic, biodegradable and biocompatible. Among bacteria able to accumulate PHAs, *Pseudomonas* sp. GP01 has been widely investigated because of its capacity to synthesize polymers with medium length side chains, from a large spectrum of carbon substrates [2,3]. Prominent examples are poly(3-hydroxyoctanoate) (PHO) and medium chain length PHAs with functional end groups in the side chains [4].

The unsaturated random copolymer poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate) (PHOU) can be produced by *Pseudomonas* sp. GP01 growing on a mixture of sodium

octanoate and undecenoic acid. The fraction of unsaturated repeat units in the polymer is closely related to the ratio of both carbon sources in the culture medium [5]. These polymers are particularly interesting because the double bonds hold the possibility of chemical modification. The unsaturated groups can be transformed into modifiable or hydrophilic functions like carboxyl or hydroxyl groups [6–9] which cannot be introduced directly by biosynthesis.

In medical and pharmaceutical applications the hydrolytic degradation of PHAs is required as the main degradation mechanism, while in environmental applications enzymatic degradation mechanisms (biodegradation) are predominant. PHO and PHOUs are semi-crystalline polymers with a low melting point ( $\leq 55^\circ\text{C}$ ) and low crystallinity due to the long pendant alkyl groups. These polymers ( $M_n = 50\,000\text{ g mol}^{-1}$ ) are highly hydrophobic and very stable with regards to hydrolysis. Renard et al. [10] blended the bacterial polymers physically with poly(lactic acid) (PLA) and PEG in order to improve the water

penetration into the hydrophobic polymer and thus to increase the hydrolysis rate. The more hydrophilic polymers PLA and PEG were, however, rapidly washed out before degradation in alkaline solution (pH 10) occurred [10].

Another strategy to increase the hydrolytic degradation of these polyesters is the introduction of carboxyl groups in the side chains by oxidation of the double bonds of PHOU [10]. Poly(3-hydroxyoctanoate-co-3-hydroxy-9-carboxydecanoate) (PHOD) was prepared by complete oxidation of PHOU containing 25% of unsaturated side groups. The weight of cast films in alkaline solution (pH 10) decreased very fast due to hydrolysis, reaching 100% after about 2.5 h. Renard et al. [10] supposed that the introduction of polar groups improves the water penetration into the polymer and thus promotes its hydrolysis.

However, the mechanical properties of PHOD are not satisfactory, because it is an amorphous and very sticky polymer ( $T_g = -19^\circ\text{C}$ ). Renard et al. [11] prepared nanoparticles of PHOD by a solvent displacement without any stabilizer. They observed that the nanoparticle solutions were unstable in salt solutions and that resuspension of the precipitates was impossible. The carboxyl groups can be grafted by direct esterification with PEG or PLA [12]. This offers the advantage that the hydrophilic/hydrophobic domains of the grafted polymers are controlled by the nature of grafted oligomers and the esterification yield. Furthermore, the grafting improves the mechanical properties of the polymers. Nanoparticles of PHOD-g-PEG, PHOD-g-PLA and unmodified PHO were prepared by the same solvent displacement as PHOD nanoparticles [11]. The authors found that the graft PEG units contributed significantly to the prevention of particle coalescence, because they acted probably as a surfactant. Such polymers are very promising for the encapsulation of agents for controlled drug delivery.

The aim of the present work was to set up an easier oxidation/grafting method for PEG onto oxidized PHOU and to study the influence of PEG graft on the hydrolytic stability. For that, we synthesized polymers grafted with PEG of different chain lengths. The obtained samples were subjected to hydrolytic degradation simulating physiological conditions (pH 7.2,  $37^\circ\text{C}$ ).

## 2. Experimental

All chemicals were purchased from Sigma–Aldrich (France) unless specified differently.

### 2.1. Synthesis of poly(3-hydroxyoctanoate-co-3-hydroxy-10-undecenoate) (PHOU)

The production process of PHOU by biosynthesis was previously reported [13]. Bacterial polyesters were extracted from lyophilized cells with  $\text{CH}_2\text{Cl}_2$  in a Soxhlet extractor, and subsequently purified by precipitation into ethanol. The molar composition of PHOU was 25% of unsaturated repeat units and 75% of 3-hydroxyoctanoate, as determined by  $^1\text{H}$  and  $^{13}\text{C}$  NMR.

### 2.2. Oxidation of PHOU

The oxidation procedure was described previously [6,9]. Typically, the reaction was carried out at a concentration of 20 g/L PHOU in a biphasic mixture of  $\text{CH}_2\text{Cl}_2$  and acetic acid (17 v/v%). The oxidizing agent  $\text{KMnO}_4$  (4 equivalents per double bond) was added in presence of 18-crown-6-ether (3 equivalents). The reaction was stirred at room temperature for 4.5 h. Then water and sodium bisulfite were added to reduce any excess oxidant. The organic phase was subsequently washed 4 times with twice its volume of water and dried over  $\text{MgSO}_4$ .

### 2.3. Grafting procedure of poly(3-hydroxyoctanoate-co-3-hydroxy-9-carboxy-decenoate) (PHOD)

After oxidation and phase separation, the organic layer was concentrated to 20 g/L PHOD. It was assumed that no material was lost during the previous washing steps and that therefore the yield of PHOD was 100%. Approximately 0.5 g of molecular sieve type 3 Å (Fluka, France) was introduced to yield anhydrous conditions. The molecular sieve was conditioned overnight at  $90^\circ\text{C}$  under vacuum. *N,N'*-Dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were added and, after their dissolution, monomethoxy poly(ethylene glycol) (PEG) was introduced. The reaction was carried out at room temperature.

The purification of the grafted polymer PHOD-g-PEG was carried out as follows. The organic solvent was gently evaporated under vacuum and the product was dissolved in 80 mL acetone (tech. quality). An equal volume of water (80 mL) was added without stirring which resulted in a white/bluish suspension. The suspension was dialyzed against water in order to remove unreacted products such as PEG, crown ether, salts, acetic acid (Spectra/Por membrane, molecular weight cut-off 6000–8000, Spectrum, France). Then the dialyzed suspension was evaporated under vacuum and the product was dissolved in  $\text{CH}_2\text{Cl}_2$ . Insoluble products such as dicyclohexylurea, being the hydrolyzed product of DCC, were removed by filtration at  $4^\circ\text{C}$  over a regenerated cellulose membrane (pore size  $0.47\text{ }\mu\text{m}$ , Interchim, France). The filtration was repeated two times.

### 2.4. Polymer characterization

The molar mass was determined by size exclusion chromatography (SEC) (Shimadzu LC-10AD). The fractionation was performed with a PL mixed C ( $5\text{ }\mu\text{m}$ ) column (Polymer Laboratories) preceded by a precolumn of the same type (Polymer Laboratories) using tetrahydrofuran (SDS, p.a.) as elution solvent. The fractions were detected by a refractive index detector (Shodex RI-71). The sample concentration was 10 mg/mL and injection volume  $50\text{ }\mu\text{L}$ . The calibration was carried out with polystyrene standards of low polydispersity purchased from Polysciences.

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{CDCl}_3$  on a Bruker Avance 300 apparatus Quantitative  $^{13}\text{C}$  NMR

measurements were carried out using the “INVGate” sequence with a pulse width of 30°, an acquisition time of 0.7 s, and a delay of 3 s between pulses [14].

Differential scanning calorimetry (DSC) measurements were carried out on a DSC Q100 apparatus (TA Instruments, Paris, France) in a temperature range of −100–100 °C at a heating rate of 20 °C/min. Melting points ( $T_m$ ) were determined after the first run from the maximum of the endothermic peak. The melting enthalpy ( $\Delta H_m$ ) was calculated from the area of the endothermic peak. Then, the sample was cooled to −100 °C and heated to 30 °C at a rate of 20 °C/min. The glass transition temperature ( $T_g$ ) was taken at the midpoint of the transition. The values are the average of two measurements.

## 2.5. Hydrolytic degradation of PHOD-g-PEG

PHOD-g-PEG films (30–40 mg) were cast out of CH<sub>2</sub>Cl<sub>2</sub> solution onto Teflon sheets. The film was dried to remove residual solvent to constant weight. The degradation studies were carried out in a phosphate buffer at pH 7.2 containing 7.54 g/L NaCl, 1.826 g/L Na<sub>2</sub>HPO<sub>4</sub>, and 0.2416 g/L Na<sub>2</sub>HPO<sub>4</sub>. To prevent microbial growth 0.1 g/L NaN<sub>3</sub> was added. The film samples were submersed with the Teflon support in 50 mL phosphate buffer. The bottles were maintained at 37 °C in a water bath. The buffer solution was renewed one time per two weeks in the first 3 months and then one time per month. For measuring, samples were removed from the buffer solution, washed abundantly

with water and dried under the extractor hood until constant weight (at least 7 days) was attained. The sample molar mass loss and polymer chain length (by SEC) were determined.

## 3. Results and discussion

### 3.1. NMR characterization of poly(3-hydroxyoctanoate-co-3-hydroxyundecenoate), PHOU

In order to better characterize PHOU and PHOD, a fine analysis of the PHOU <sup>13</sup>C NMR spectrum was carried out. Actually, PHOU is a copolymer of several repeat units. *Pseudomonas* sp. GPO1 metabolizes the carbon sources, sodium octanoate and undecenoic acid, by the β-oxidation pathway where the initial alkyl chain is shortened successively by two carbons. The metabolites of the β-oxidation can be directed towards the PHA-synthase system, where they are integrated in the growing polymer chain. Shorter monomer units, such as 3-hydroxyhexanoate, 3-hydroxynonenoate and 3-hydroxyheptenoate, are thus incorporated in the bacterial polymer. Fig. 1 displays the <sup>13</sup>C NMR spectrum. We observe the signals at 140 and 114 ppm assigned, respectively, to the CH and CH<sub>2</sub> carbons of the double bond [15]. Preusting et al. [14] produced PHAs using *n*-decane, 1-octene, or 1-decene as carbon source. They compared 75-MHz <sup>13</sup>C NMR spectra of PHA isolated from cells grown on *n*-decane and PHA isolated from cells

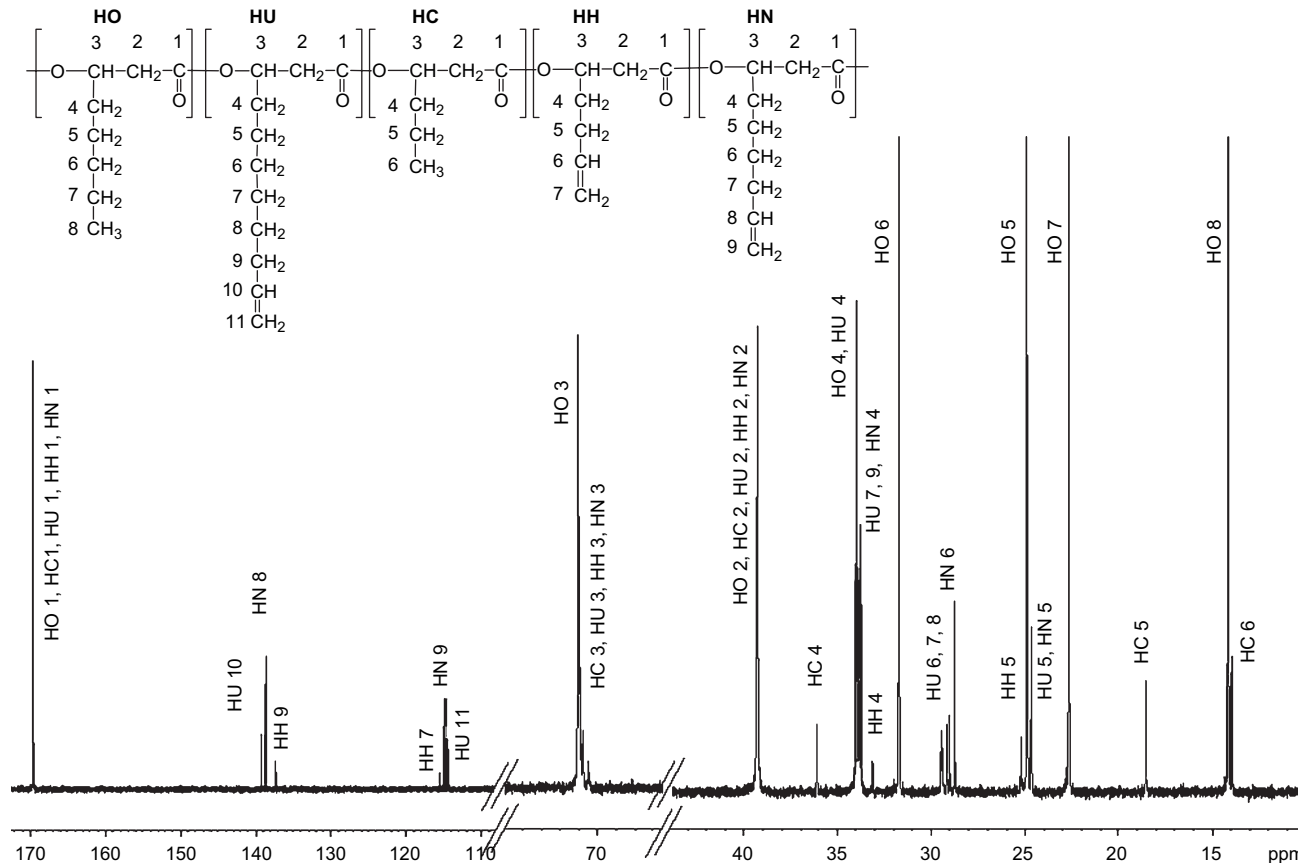


Fig. 1. <sup>13</sup>C NMR spectrum of PHOU. Abbreviations: HO, 3-hydroxyoctanoate; HU, 3-hydroxy-10-undecenoate; HC, 3-hydroxyhexanoate; HH, 3-hydroxy-6-heptenoate; HN, 3-hydroxy-8-nonenoate.

grown on 1-decene. They observed a cluster of resonances due to the different methylene carbon atoms between 14 and 40 ppm. Additional resonances were found between 114 and 119 ppm due to the terminal methylene carbon in the pendant group, and between 132 and 139 ppm assigned to the methine carbon of the terminal double bond. As shown in Fig. 1, the signals corresponding to the carbon atoms of saturated and unsaturated monomer units are present in the PHOU spectrum and can be assigned as described by Hany et al. and Langveen et al. [15,16]. Three well-separated peaks are observed around 137 and around 114 ppm. The complete assignment for carbon atoms was achieved with the help of the software CS Chem-Draw Pro 4.5 and the integration of the relative intensities of the signals corresponding to the five different monomer units present in the polymer.

The analysis of the relative signal intensities allowed the calculation of the molar composition of PHOU. It amounted to: 5% 3-hydroxyheptenoate (HH), 16% 3-hydroxy-8-nonenoate (HN), 7% 3-hydroxy-10-undecenoate (HU), 9% 3-hydroxyhexanoate (HC), and 62% 3-hydroxyoctanoate (HO). This result is consistent with the monomer composition determined by methanolysis of a PHOU containing 20 mol% of unsaturated subunits which yields 27% HN, 10% HU, and 1% HH [15,16]. The predominant unsaturated monomer unit in both cases is 3-hydroxy-8-nonenoate (HN). A similar result was also found by Preusting et al. [14]. When the bacteria were grown on 1-decene the quantity of 3-hydroxyoctanoate (33.5%) was higher than the one of 3-hydroxydecanoate. The

presence of smaller unsaturated monomers can be explained by the capability of alkane hydroxylase to oxidize the saturated end of alkenes to  $\omega$ -hydroxyl-1-alkenes which can be further metabolized to the corresponding fatty acids and degraded by the  $\beta$ -oxidation pathway.

### 3.2. Optimization of the grafting procedure with PEG 350

The synthesis of the grafted polymer PHOD-g-PEG is shown in Fig. 2. The grafting procedure previously described consists of two steps: (i) oxidation of PHOU and (ii) direct condensation of the carboxylic side groups of PHOD and the terminal hydroxyl groups of PEG [12]. After the first step, a procedure is necessary to purify the product which causes product loss by polymer aggregation and is time consuming. Additionally, long dialysis periods can lead to chain scission due to hydrolysis of ester bonds. More important, however, the oxidized polymer PHOD forms gels when dried under vacuum or by freeze drying due to intra- and intermolecular hydrogen bonds between carboxyl groups. Therefore an important quantity of soluble material and of carboxyl groups available for the grafting reaction is lost. A similar problem is observed for PHOU containing terminal alcohol functions in the side chains [8]. The manipulation of the sticky and easily crosslinking polymer PHOD is thus quite difficult.

In order to overcome this problem, a simplified process was developed to carry out oxidation and grafting reaction in one

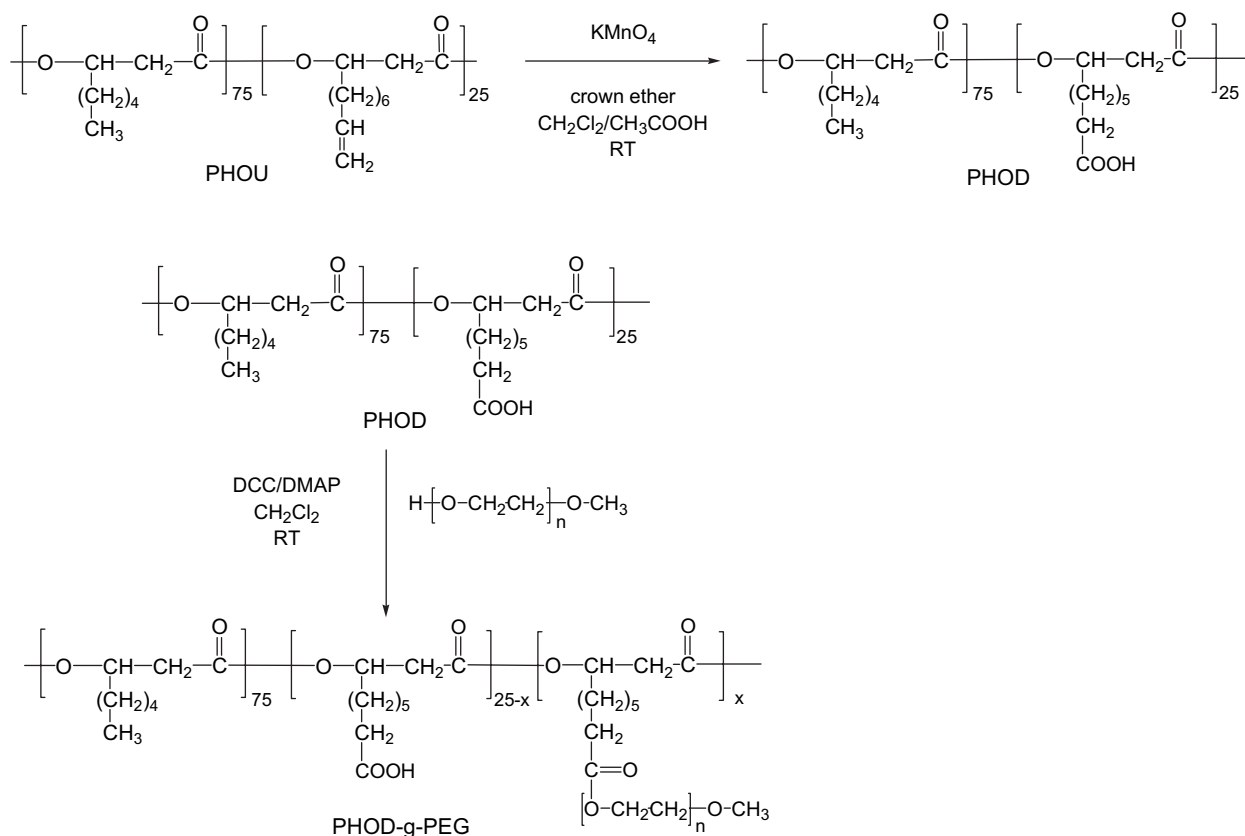


Fig. 2. Synthetic route of PHOU oxidation to PHOD and grafting of PEG. Abbreviations: DCC, dicyclohexylcarbodiimide; DMAP, dimethylaminopyridine.

reaction mixture. The main problems of doing the reaction in one pot are (i) increased hydrolysis of DCC because of traces of water in the reaction solution, and (ii) activation of traces of acetic acid by DCC which leads to the side reaction of grafting PEG with acetic acid. The optimization of the PHOU grafting in one reaction mixture was carried out with a monomethylated PEG having an  $M_n$  of 350.

The grafting was carried out as follows. At first, PHOU was oxidized by  $\text{KMnO}_4$  in  $\text{CH}_2\text{Cl}_2/\text{acetic acid}$  [9]. After this the grafting of PHOD was done with the following method. The reaction time was fixed to 24 h. PEG, DCC and DMAP were added simultaneously. The results of this procedure are shown in Fig. 3. It is important to note that 2 equivalents of DCC are necessary for observing a significant grafting of PEG 350 (12.5 mol% of repeat units with graft PEG). This result indicates partial hydrolysis of DCC, despite the drying of the reaction solution with  $\text{MgSO}_4$ . More drastic anhydrous conditions might thus be needed. To improve the grafting reaction conditions, a molecular sieve was added to the solution. We observe an improvement of the conversion in Fig. 3 (21%, filled bars). Furthermore, we can see that further addition of DCC (3 equivalents) had no effect on the conversion yield in the presence of the molecular sieve.

This result could be due to the assumed side reaction of grafting PEG with residual acetic acid. In order to address this issue, more PEG 350 equivalents were used in the same reaction mixture containing the molecular sieve. The results are shown in Fig. 4. We observe that the addition of a second equivalent of PEG 350 results in a doubling of the percentage of grafted PEG 350. Further increase of PEG 350 has only a small effect on the grafting rate. No significant differences between the addition of 3 and 4 equivalents were found. Moreover, it is seen in Fig. 4 that the maximum percentage of grafting is about 52% of PEG 350, meaning that only about 50% of the carboxylic functions are available for esterification. Similar results have been observed during the two-step process, where a maximum percentage of grafting of 56% was observed [12].

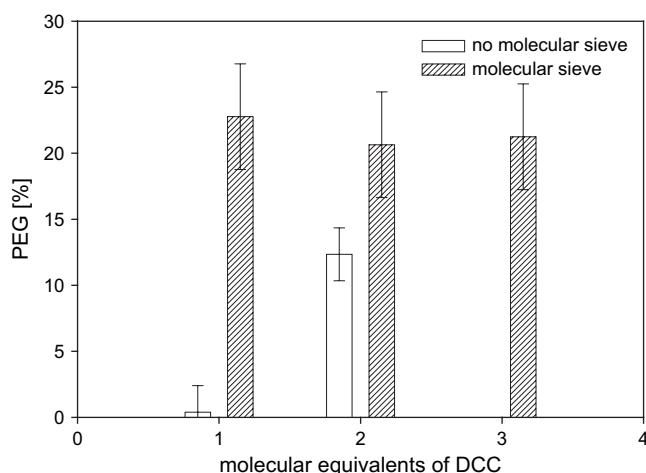


Fig. 3. Influence of the DCC quantity and the use of a molecular sieve (type 3 Å) on the conversion of repeat units. The DCC quantity is expressed in molar equivalents per oxidized repeat unit and the conversion in mole percent of repeat units grafted with PEG ( $M_n$  350).

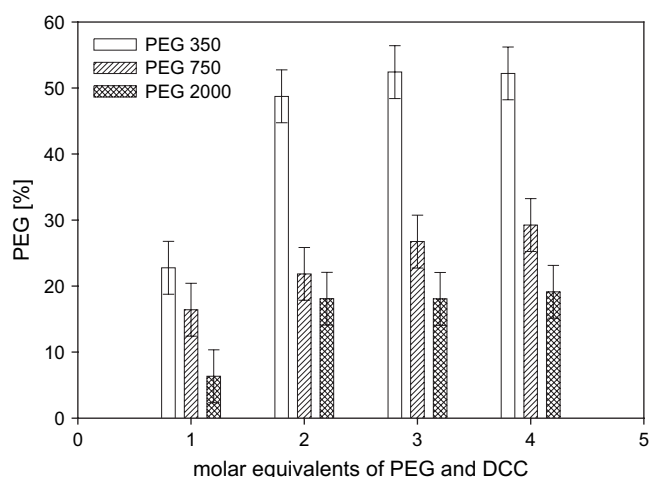


Fig. 4. Influence of the DCC and PEG quantity on the conversion of repeat units for different types of PEG ( $M_n$  350, 750 and 2000). The DCC and PEG quantity introduced are expressed in molar equivalents per oxidized repeat unit. The conversion is expressed in mole percent of grafted repeat units. The equivalents of DCC and PEG were introduced successively into the reaction mixture (first equivalent of DCC and PEG at the beginning, second after 6 h, third after 24 h, and fourth after 48 h). The conversion was measured before the introduction of the next equivalent (after 6, 24, 48, and 72 h).

### 3.3. PHOD-g-PEG NMR analysis

The reaction products were analyzed by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopy. Fig. 5 shows a typical  $^1\text{H}$  NMR spectrum of PHOD grafted with PEG having an  $M_n$  of 350  $\text{g mol}^{-1}$  (PHOD-g-PEG 350). No signals corresponding to the double bond of 3-hydroxy-10-undecenoate (4.9 and 5.7 ppm) were observed which confirms the quantitative oxidation. The percentage of conversion of the carboxy groups can be calculated by the ratio of the integration of the peak corresponding to the

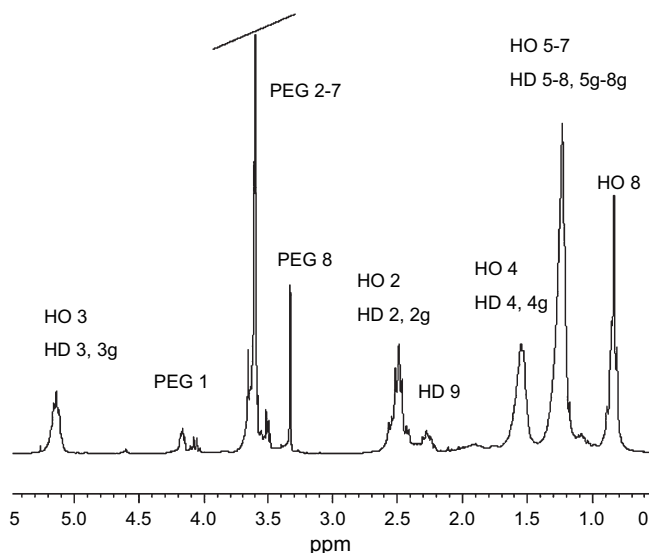


Fig. 5.  $^1\text{H}$  NMR spectrum of PHOD-g-PEG 350. (The labels correspond to the molecule structure given in Fig. 6.) Abbreviations: HO, 3-hydroxyoctanoate; HU, 3-hydroxy-10-undecenoate; HD, 3-hydroxy-9-carboxy-decanoate. The monomer units grafted with PEG are labeled "g".



CH<sub>2</sub> (PEG1) at 4.15 ppm in  $\alpha$  position of the ester group and the CH (HO3 and HD3) of the main polymer chain at 5.15 ppm:

$$\text{PEG} [\%] = \frac{I(\text{CH}_{2,\text{PEG1}})/2}{I(\text{CH}_{\text{HO3,HD3}})} \times 100 \quad (1)$$

The ratio of the integral of the CH<sub>3</sub> (PEG8) at 3.4 ppm and the CH (HO3 and HD3) allows to confirm the conversion rate and to verify the absence of free PEG in which case both values correspond. The calculation procedure is given in Eq. (2):

$$\text{PEG} [\%] = \frac{I(\text{CH}_{3,\text{PEG8}})/3}{I(\text{CH}_{\text{HO3,HD3}})} \times 100 \quad (2)$$

Fig. 6 shows the <sup>13</sup>C NMR spectrum of the graft polymer PHOD-g-PEG 350. The appearance of the signals corresponding to PEG (55–75 ppm) and to the oxidized terminal groups (173 ppm) are clearly observed, if compared to the <sup>13</sup>C NMR spectrum of PHOU (Fig. 1). The peak of the CH<sub>2</sub> of PEG (PEG1, 67 ppm) in  $\alpha$  position of the ester bond is shifted further than the signal of free PEG (60 ppm). The percentage of

grafting calculated with the help of the respective intensities of PEG 1 and the CH<sub>2</sub> of the main polymer chain (HO2, HU2, etc.) is slightly higher (60%) than the value calculated on <sup>1</sup>H NMR spectrum (52%).

### 3.4. Grafting procedure with PEG 750 and 2000

In order to synthesize grafted copolymers with different ratios of hydrophilic and hydrophobic groups and thus to modify the solubility and degradation characteristics, the grafting procedure was applied to PEG with different chain lengths (PEG 750 and PEG 2000). The percentage of grafting against the equivalents of PEG and DCC is shown in Fig. 4. As already observed for PEG 350, 2 equivalents of PEG and DCC are necessary for significant grafting. Furthermore, we see that the maximum conversion rate diminishes with the increase of the PEG chain length. For PEG 750 a conversion of 29% and for PEG 2000 a conversion of 19% is achieved. These results are explained by the increase of steric hindrance and the decrease of terminal hydroxyl group reactivity with increasing chain length.

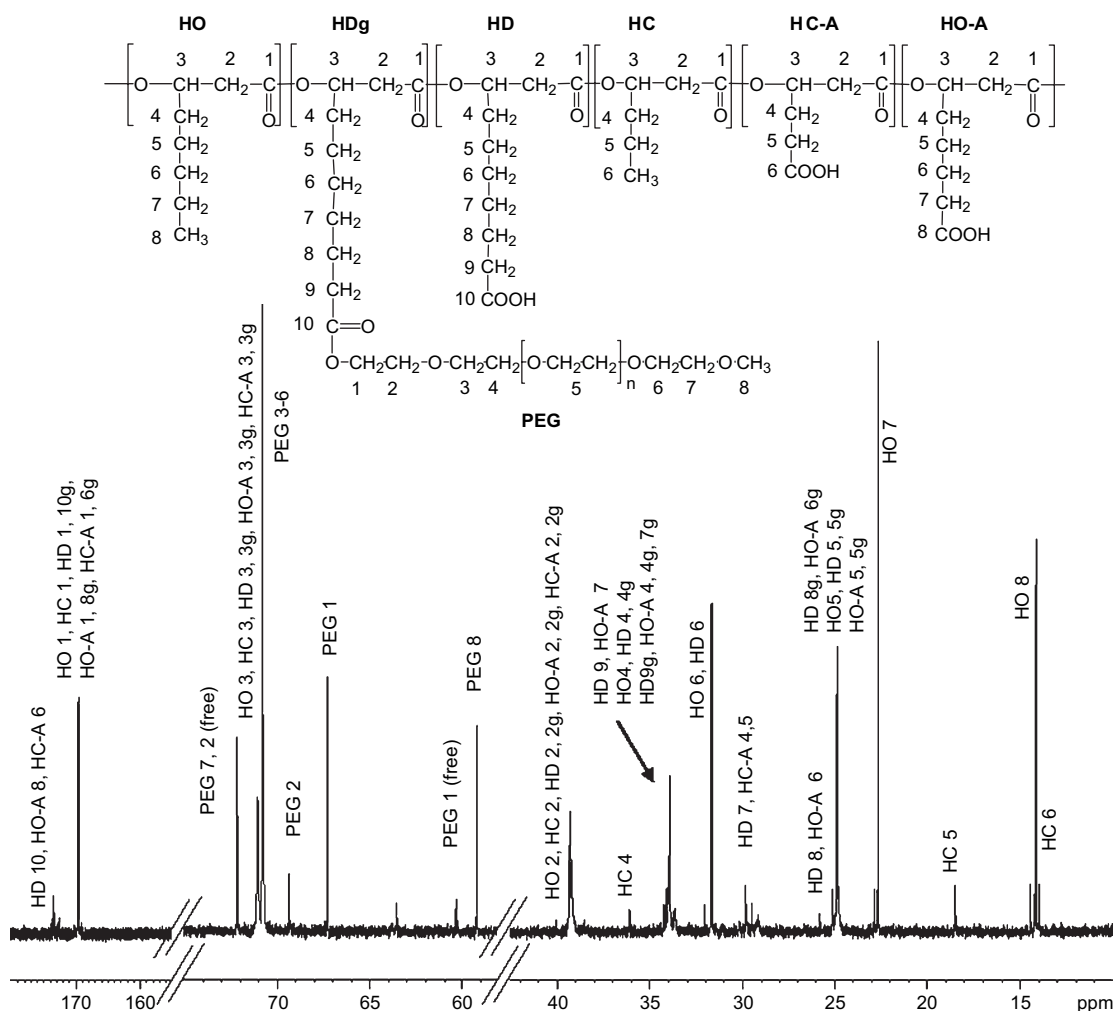


Fig. 6. <sup>13</sup>C NMR spectrum of PHOD-g-PEG 350. Abbreviations: HO, 3-hydroxyoctanoate; HU, 3-hydroxy-10-undecenoate; HC, 3-hydroxyhexanoate; HH, 3-hydroxy-6-heptenoate; HN, 3-hydroxy-8-nonenate; HD, 3-hydroxy-9-carboxy-decanoate; HC-A, 3-hydroxy-5-carboxy-hexanoate; HO-A, 3-hydroxy-7-carboxy-octanoate. The monomer units grafted with PEG are labeled “g”.

### 3.5. Characterization of PHOD-g-PEG

The physical characteristics of the graft PHOD are given in Table 1. PHOU oxidation results in a decrease of the average molar mass of the polymer. The decrease of the molar mass was also observed under milder reaction conditions, i.e. at 0 °C [6,9]. Nevertheless, molar mass remain high. A long dialysis period (7 days) is needed especially for the removal of PEG 2000, because the polymer grafts tend to solvate free PEG in solution. Because the pH of the dialysis solution is not controlled, a little decrease in  $M_n$  of PHOD-g-PEG 2000 was observed before the sample was satisfactorily purified. Fig. 7a shows the molecular size distribution of the different film samples after dialysis. The  $M_n$  of PHOD-g-PEG 2000 is slightly smaller than the  $M_n$  of the other polymers and followed by the peak corresponding to free PEG 2000.

Table 1 shows the thermal analysis of the polymer samples. The glass transition temperature ( $T_g$ ) of PHOD is significantly higher than that of PHOU. This may be linked to the formation of intermolecular hydrogen bonds stiffening the polymer. PHOU is semi-crystalline as shown by the small fusion peak at 40 °C, in contrast to PHOD which is an amorphous polymer. The grafting of PEG 350 results in a marked decrease of the  $T_g$ . We suppose that the grafted PEG increases the free volume in the polymer and gives a higher mobility of the chains. This hypothesis is confirmed by the decrease of  $T_g$  which is more pronounced when the conversion yield is lower. A relatively small quantity of grafts seems to increase strongly the mobility of the polymer chains and decrease the quantity of interactions present in PHOD. Increase of the percentage of grafting results in diminishing the chain mobility, which might be expected for sterically hindered polymers. The grafting of longer PEG chains results in further hindering the chain mobility which means a further increase of the  $T_g$ . An additional stiffening effect might be the crystallization of the larger PEG molecules forming intermolecular aggregates. The copolymers (PHOD-g-PEG 750 and PHOD-g-PEG 2000) show a melting peak corresponding to the crystallinity of the PEG. The melting enthalpy increases with the PEG length due to an increase of the crystallinity of the PEG block.

The introduction of hydrophilic side groups modifies the solubility parameters of the polymer. The results are summarized in Table 2. PHOU and PHOD are soluble in non-polar

Table 1  
Physical characteristics of graft PHOD determined by SEC and DSC

Sample	$M_n$	$M_w$	$T_g$ [°C]	$T_m$ [°C]	$\Delta H_m$ [J/g]
PHOU	80 000	158 000	−39	40	6
PHOD	34 000	49 000	−19	—	—
PHOD-g-PEG 350 (28%) <sup>a</sup>	31 000	66 000	−57	—	—
PHOD-g-PEG 350 (52%) <sup>b</sup>	30 000	64 000	−46	—	—
PHOD-g-PEG 750	26 000	46 000	−45	51	1.5
PHOD-g-PEG 2000	22 000	28 000	−31	55	52.0

<sup>a</sup> Polymer contains 7% of grafted repeat units.

<sup>b</sup> Polymer contains 14% of grafted repeat units;  $M_w$  weight average of molecular weight,  $M_n$  number average of molecular weight,  $T_g$  glass transition temperature,  $T_f$  fusion temperature. The values of enthalpy of fusion ( $\Delta H_m$ ) for copolymers are normalized by composition.

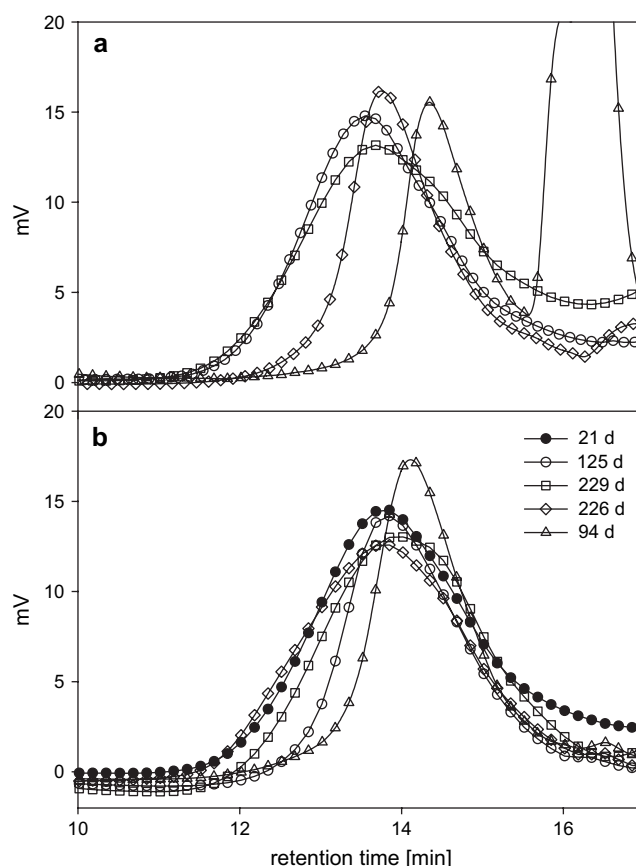


Fig. 7. Change molar mass of PHOD-g-PEG during hydrolytic degradation in phosphate buffer (pH 7.24, 37 °C). (a) SEC chromatograms of the different PHOD-g-PEG films before degradation. (b) Film samples after degradation. The degradation time in days is given in the legend. PHOD-g-PEG 350 (grafting 52%) ○, PHOD-g-PEG 350 (grafting 28%) □, PHOD-g-PEG 750 (grafting 29%) ◇, PHOD-g-PEG 2000 (grafting 19%) △.

solvents such as dichloromethane, chloroform, tetrahydrofuran. PHOU is insoluble in polar solvents like ethanol, methanol or water. The oxidation to PHOD results already in a better solubility of the polymers in polar solvents. Grafting does not change the solubility in non-polar solvents, but increases the polymer's hydrophilic character. Table 2 shows that the solubility of the graft polymer in acetone/water mixtures is gradually increased with the PEG chain length. All solutions are, however, slightly turbid, which indicates the existence of aggregates. The solubility of PHOD-g-PEG 2000 in acetone/water mixtures was not marked by a sharp transition between soluble and insoluble. The solution of PHOD-g-PEG 2000 in acetone/water 30/70% v/v was already turbid. Some small flocs were observed upon further increase of the water percentage. Even larger flocs were found in pure water, some of them were still observed after stirring the polymer suspension overnight.

### 3.6. Hydrolytic degradation of PHOD-g-PEG

The hydrolytic degradation of graft PHOD films at 37 °C and pH 7.24 was followed during approximately 7 months. The weight loss was recorded and compared to the weight loss of the non-grafted polymer PHOD under identical conditions.

Table 2  
Solubility of PHOD-g-PEG

Sample	PHOD	PHOD-g-PEG 350	PHOD-g-PEG 750	PHOD-g-PEG 2000
Chloroform	+	+	+	+
Dichloromethane	+	+	+	+
THF	+	+	+	+
Acetone	+	+	+	+
Methanol	+	+	+	+
Ethanol	+	+	+	+
Acetone/H <sub>2</sub> O 80/15	–	+	+	+
Acetone/H <sub>2</sub> O 80/20	–	±	+	+
Acetone/H <sub>2</sub> O 70/30	–	–	+	+
Acetone/H <sub>2</sub> O 40/60	–	–	+	+
Acetone/H <sub>2</sub> O 35/65	–	–	±	+
Acetone/H <sub>2</sub> O 30/70	–	–	–	+
Acetone/H <sub>2</sub> O 20/80	–	–	–	×
H <sub>2</sub> O	–	–	–	××

+ Soluble, – insoluble, ± turbid, × very turbid solution, ×× very turbid solution and appearance of flocs.

The PHOD sample was degraded into soluble fragments after 3 months while the graft films remained stable during the same period of time. The results are shown in Fig. 8. We observe a rapid weight loss of the polymer films during the initial two weeks, which is correlated to the length of PEG and with the unreacted carboxylate groups. After this first period, the film weight remains approximately constant during 200 days. The SEC analysis of the samples shows only an insignificant shift of the molar mass (Fig. 7b). The graft polymer is much more resistant to hydrolysis, at physiological pH, than PHOD. The graft groups of PEG, although hydrophilic, do not favor the penetration of water towards the main chain and thus are capable to stabilize the functionalized polymer. The unreacted carboxylate groups should be altered by the PEG grafts against hydrolysis. Water contact angles of PHOD (95°) and P(HOD-PEG) (95) are very close to water contact angle of PHO (104°) as determined otherwise [17]. This hypothesis agrees with the results obtained in the preparation of particles with PHOD-g-PEG. This modified PHOD-g-PEG could thus form particles with a hydrophobic

core shell, which is functionalized with carboxyl groups, and has an outer layer constituted of PEG arms, which could make inaccessible the remaining carboxyl groups. This layer is able to prevent the aggregation of the particles and stabilizes therefore their colloidal state.

The graft groups of PEG, although hydrophilic, do not favor the penetration of water towards the main chain and thus are capable of stabilizing the functionalized polymer.

#### 4. Conclusion

PHOU has been oxidized into PHOD and different PEG chains have been grafted by esterification. The new synthesis method makes the implementation of the reaction easier with only one dialysis step. The preparation is faster, the yield is increased and the crosslinking of the oxidized intermediate is limited. The resulting materials are more hydrophilic than PHOU and surprisingly stable towards hydrolysis. The family of PHO derivatives has been extended. It has been shown that with the help of chemical modification it is possible to prepare a wide range of polymers, from hydrophobic to hydrophilic with high stability by adjusting the nature, structure and percentage of grafted side groups. PHODs are promising promoters of the hydrolytic degradation. A better knowledge of these materials is important with regards to their potential use in the therapeutic domain. The tailor making of smart particles for drug release is thus possible by the combination of the tool's chemical functionalization, grafting, and synthesis of block copolymers.

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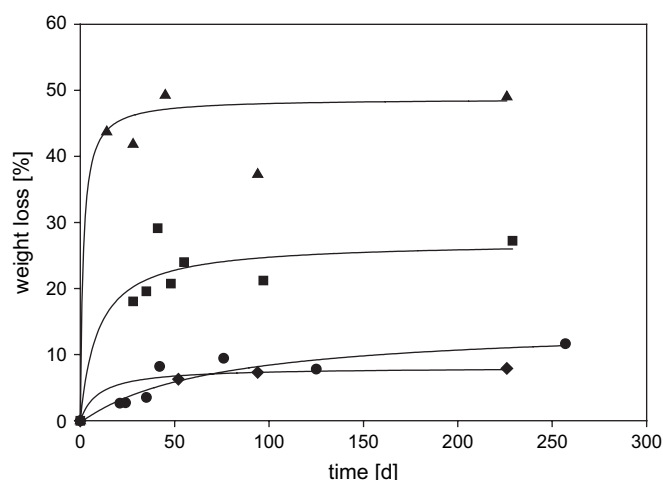


Fig. 8. Weight loss of different PHOD-g-PEG films in phosphate buffer (pH 7.24, 37 °C) during time: PHOD-g-PEG 350 (grafting 52%) ●, PHOD-g-PEG 350 (grafting 28%) ■, PHOD-g-PEG 750 (grafting 29%) ◆, PHOD-g-PEG 2000 (grafting 19%) ▲.



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