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Polymer 46 (2005) 639–651
polymer
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Different ways for grafting ester derivatives of poly(ethylene glycol) onto chitosan: related characteristics and potential properties

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Received 14 April 2004; received in revised form 24 November 2004; accepted 25 November 2004
Available online 19 December 2004

Abstract

The grafting of poly(ethylene glycol) functionalized by ester groups (MeO-PEG-ester) onto chitosan was studied and optimized using different reaction conditions. In a first procedure, the grafting was made from 6-*O*-triphenylmethyl-chitosan after protection of primary hydroxyl groups and in a second one, it was made directly onto chitosan. NMR spectroscopy was an important tool to study these reactions and the grafting is unequivocally showed up. Moreover, for each procedure, the solubility and surface properties of the obtained copolymers were evaluated and compared.

Keywords: Chitosan; Poly(ethylene glycol); Surface properties

1. Introduction

Chitosan is a cationic natural biopolymer produced by alkaline *N*-deacetylation of chitin, the most abundant natural polymer after cellulose. It ideally consists of 2-amino-2-deoxy-(1–4)-β-D-glucopyranose residues (D-glucosamine units) and has no or small amount of *N*-acetyl-D-glucosamine units. Chitosan and derivatives are used in various fields [1,2]: treating water [3–5], biomedical [6–12], cosmetic [13], agricultural [14], food industrial [15,16]. It shows some biological activities such as immunological [17], antibacterial [18], and wound healing activity [19]. Moreover, chitosan is non-toxic and biodegradable [20,21].

Chemical modification of chitosan to generate new biofunctional materials is of prime interest because the modification would not change the fundamental skeleton of chitosan, would keep the original physicochemical and biochemical properties and finally would bring new properties depending on the nature of the group introduced.

Chitosan has been already modified by acetylation reactions, or by hydroxypropyl, carboxymethyl, phthalimido, trityl, tosyl, sulfofurfuryl or saccharide groups or by chains degradation [22–30].

The water-soluble and non-toxic poly(ethylene glycol) (PEG) is frequently used for chemical modification of natural and artificial macromolecules for biomedical applications. Grafting PEG onto chitosan (PEG-*g*-chitosan) is considered to be a convenient way to synthesize for instance drug carriers [31].

PEG and derivatives have been used for the main following modifications of chitosan: by PEG [32–35], by PEG-sulfonate [36], by PEG-acid [37], by PEG-aldehyde [38–41], by PEG-ester [42,43], by PEG-acrylate [44], …

The first step to prepare PEG-*g*-chitosan consists in the functionalization of PEG with a suitable group. The most convenient method is the chemical modification of commercial PEG, available as a wide set of fractions characterized by given functionality and narrow molar mass distribution [45]. End group modification of α-monomethoxy, ω-hydroxy-PEG is the most interesting pathway to obtain reactive PEG intermediates for grafting

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0032-3861/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2004.11.104

on different compounds such as proteins, chitosan,…. Provided that the PEG is monofunctional, no cross-linking will occur upon reaction. Several α-monomethoxy, ω-hydroxy-PEG products with different molar masses are commercially available. However, this approach suffers from several difficulties as side-products and lack of monofunctionality of commercially available α-monomethoxy, ω-hydroxy-PEG, or difficulties in isolating the derivatives.

In some case, reactions to get PEG-*g*-chitosan are laborious and low yielding that prevent from routine or large scale synthesis. Moreover, concerning the preparation of functionalized PEG and chitosan derivatives, ^{1}H and ^{13}C NMR characterizations were not generally sufficiently clarified.

Herein, we report the preparation of PEG-*g*-chitosan by the reaction of a PEG-ester derivative with NH_2 groups of chitosan. A large part of this work deals with the NMR study of the modified chitosan and the intermediates. The first part consists in the modification of α-monomethoxy, ω-hydroxy-PEG functionalized by ester groups (MeO-PEG-ester) by reaction of the poly(ethylene glycol) sodium salt with methyl-chloroacetate to give a carboxymethyl-terminated poly(ethylene glycol). In the second part, we describe two procedures of grafting of the modified PEG with the amino groups of chtosan by ester aminolysis reaction (PEG-*g*-chitosan). According to the first way, the grafting reaction was carried on onto 6-*O*-triphenylmethyl-chitosan after protection of primary hydroxyl groups of chitosan. In the second way, the original chitosan was directly used. The two methods were compared, PEG-*g*-chitosans were fully characterized, their molar masses determined, and the grafting extent calculated. Moreover solubility and interfacial properties were evaluated.

2. Experimental section

2.1. Materials

Chitosan obtained from Fluka was purified by dissolution in aqueous hydrochloric acid (0.2%) to get a solution with a polymer concentration of 1% (w/v) and was precipitated in aqueous NaOH solution (pH > 7). The residue was washed several times with deionized water to attain the water conductivity and finally freeze-dried. The viscosity–average molar mass ($\bar{M}_v$) is 330,000 g mol^{-1} (determined by viscosimetry) [46] and the degree of deacetylation determined by ^{1}H NMR is 80% [47].

α-Monomethoxy, ω-hydroxy-PEG—poly(ethylene glycol) (MeO-PEG (**1**); $\bar{M}_w = 120$, 350, 550, 750 and 2000 g mol^{-1}), was purchased from Acros (called MeO-PEG 120, 350, 550, 750) and Sigma (MeO-PEG 2000) and used without further purification.

Organic solvents were purified by usual distillation before use. Other reagents were all chemical grade and used without further purification.

2.2. Equipment

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ for MeO-PEG and modified PEG; in D_2O/CD_3COOD at 75 °C for chitosan and grafted chitosan using a Bruker DRX400. Infrared spectra of PEG and modified PEG were obtained between NaCl windows, and IR spectra of chitosan and modified chitosan were obtained as KBr pellets using Perkin–Elmer 16 PC spectrometer.

Molar masses of MeO-PEG and MeO-PEG-ester were obtained by means of size exclusion chromatography (SEC) on a Water apparatus (Saint Quentin en Yvelines, France) equipped with Waters columns HR0.5 and HR4E using THF as eluant. The calibration curve was established using poly(ethylene glycol) standards.

The absolute determination of weight and number average molar masses (respectively, $\bar{M}_w$ and $\bar{M}_n$) of chitosan and PEG-*g*-chitosans together with molar masses distributions were performed by coupling on-line a size exclusion chromatography (SEC), a multi-angle laser light scattering (MALLS) and a differential refractive index detector (DRI). Acetic/acetate buffer (pH 4.7) and $LiNO_3$ 0.1 M used as carrier, was filtered through 0.1 μm filter unit (Millipore), carefully degassed (ERC-413), eluted at 0.5 mL min^{-1} flow rate (Flom HPLC pump 301), and clarified through a 0.45 μm filter upstream columns. The sample was injected through a 100 μl full loop. The SEC line consisted of two Syncropak CatSEC 1000 and 100 columns (Synchrom, Inc. USA) in series. The column packing is a polymerized polyamine gel. The MALLS photometer, a DAWN-F from Wyatt Technology inc. (Santa Barbara, USA) is fitted with a K5 cell and a He–Ne laser ($\lambda = 633$ nm). The collected data were analyzed using the Astra V-4.50 software package. The SEC-MALLS technique has been described elsewhere [48]. The concentration of each eluted fraction have been determined with the DRI (Shimadzu RID-6A, Japan) according to the known value of d*n*/d*C* (0.19 mL g^{-1}).

The interfacial properties were measured using the drop tensiometer (Tracker) from I.T. Concept (Longessaigne, France). It allowed the determination of the interfacial tension by analyzing the axial symmetric shape (Laplacian profile) of the rising air drop in polymer solution [49–51]. The solvent was 0.3 M acetic acid (AcOH)/0.05 M sodium acetate (AcONa) and the polymer concentration was 3 mg mL^{-1}. The drop area was taken equal to 15 mm^2, its volume being 5.7 mm^3. The drop area can be made to oscillate sinusoïdally at chosen amplitude and frequency. This was achieved by regulating the motor driving the piston in the syringe feeding the drop as to produce oscillations of the drop volume. The control unit records the area oscillations (dA) and the resulting interfacial tension oscillations (dσ). The complex elasticity ε was given as: $\varepsilon = d\sigma/dA$. The oscillating amplitude was 1.5 mm^2 as dA/A was taken equal to 10%. The oscillation period was 15 s.

2.3. Synthesis of MeO-PEG-ester **2**

The procedure of synthesis was described in Scheme 1. A solution of MeO-PEG-ONa was prepared by reaction of MeO-PEG **1** (20 g of MeO-PEG 120, 350, 550, 750 or 2000, 1 hydroxy equiv) with NaH (1.2 equiv) in THF (70 mL). The mixture was heated to 70 °C for 20 h, then cooled at room temperature and finally the solution was filtrated under N_2 to eliminate the excess of NaH. Then the solution of methyl-chloroacetate (1 equiv) in THF (20 mL) was added dropwise to the former solution of MeO-PEG-ONa and the mixture was heated at 70 °C for 24 h. THF was removed under reduced pressure. The residue was dissolved in dichloromethane (60 mL) extracted with water (60 mL). The dichloromethane solution was washed with saturated NaCl solution and dried over anhydrous magnesium sulfate. After removal of the solvent under reduced pressure, the PEG functionalized by ester groups was obtained (yield: 50% for **2a** and **2b** synthesized from MeO-PEG 120 and 350 g mol^{-1}, respectively, and 80% for **2c**, **2d**, **2e** synthesized from MeO-PEG 550, 750 and 2000 g mol^{-1}, respectively).

2.4. Synthesis of PEG-g-chitosan **9** from 6-O-triphenylmethyl-chitosan

The procedure of synthesis was described in Scheme 3. 6-*O*-Triphenylmethyl-chitosan was prepared from chitosan by the method reported by Nishimura et al. [52]. PEG-*g*-chitosan was prepared according to the following method. A solution of MeO-PEG-ester **2** (2 equiv) in THF was added to a solution of 6-*O*-triphenylmethyl-chitosan (0.5 g, 1 NH_2 equiv) in THF. The mixture was heated at 70 °C for three days. The polymer was purified by precipitation into acetone, washed three times with acetone and dried under vacuum. To deprotect its triphenyl methyl groups, the obtained solid in suspension in a mixture of chloroform with methanol (3.2% v/v) was treated for 5 h with a solution of acetyl chloride in methanol (8.8% v/v) with a volume corresponding to 10% of the volume of the mixture of chloroform with methanol [53].

The polymer was purified by precipitation into acetone, washed three times with acetone and dried under vacuum (yield: 50% for **9a** and **9b** synthesized from MeO-PEG-ester 120 and 350 g mol^{-1}, respectively, and 65% for **9c**, **9d**, **9e** synthesized from MeO-PEG-ester 550, 750 and 2000 g mol^{-1}, respectively).

Degree of MeO-PEG grafting was estimated by a colloidal titration method [54], where a negative colloid solution of PEG-*g*-chitosan was titrated with 1/400 N polyanionic solution of potassium poly(vinyl sulfate) by the conventional toluidine blue indicator method.

2.5. Synthesis of PEG-g-chitosan **10** from original chitosan

The procedure of synthesis was described in Scheme 4. A solution of MeO-PEG-ester (3 equiv) in acetic acid (0.2 N) was added to a solution of purified chitosan **4** in 0.2 N acetic acid (1 NH_2 equiv, 1% (w/v)). The mixture was heated at 70 °C for three days. The polymer was purified by precipitation into acetone and washed three times in acetone. The PEG-*g*-chitosan was dried under vacuum (yield: 90% for **10a** and **10b** synthesized from MeO-PEG-ester 120 and 350 g mol^{-1}, respectively, and 65% for **10c**, **10d**, **10e** synthesized from MeO-PEG-ester 550, 750 and 2000 g mol^{-1}, respectively).

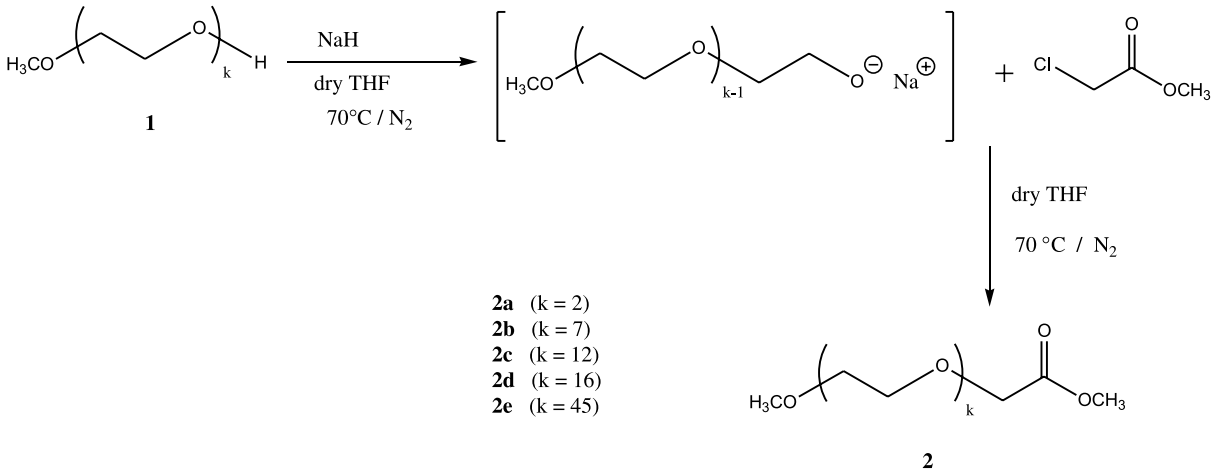
Degree of MeO-PEG grafting was estimated by the colloidal titration method [54].

3. Results and discussion

3.1. Preparation of MeO-PEG-ester

The reaction sequence used for the preparation of ester functionalized PEG is given in Scheme 1.

As representative of the great variety of chain lengths in the case of MeO-PEG, average molar masses from 120 to



Scheme 1. Synthesis of MeO-PEG-ester **2**.

2000 g mol^{-1} were investigated for the procedure described here. The modified PEG **2** was fully characterized by different spectroscopic and physico-chemical techniques such as SEC.

Synthesis of **2** is confirmed by the presence in the ^{1}H NMR spectrum of peaks at 3.7 and 4.1 ppm attributed to the methoxy and methylene groups, respectively, after the action of methyl chloroacetate (Fig. 1).

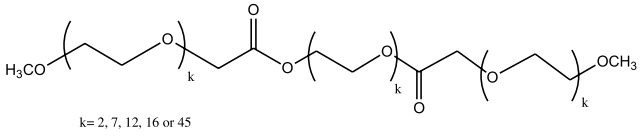
The presence of ester groups is unequivocally identified by ^{13}C NMR. Ester groups are characterized by singlets at $\delta = 51.4$ ppm and at $\delta = 170.5$ ppm corresponding to methoxy and carbonyl groups, respectively (Fig. 2).

These different results associated with the decrease in IR spectrum of the original band corresponding to the OH stretching vibration and with the appearance of the signal at 1750 cm^{-1} attributed to the carbonyl stretching vibrations, indicate that the reaction occurred.

All spectroscopic data were summarized in Table 1.

Nevertheless, the presence in ^{13}C NMR spectrum of peaks at $\delta = 61.4$ and 72.3 ppm corresponding to the $-CH_2-CH_2$-OH groups shows that even if the synthesis was carried out with an excess of methylchloroacetate, MeO-PEG did not react totally. The difference of structure between MeO-PEG-ester and MeO-PEG is too small to consider a good separation.

Furthermore, a peak in ^{13}C NMR spectrum at 170.10 ppm is observed (Fig. 2), which could correspond to a carbonyl group relative to a side product resulting from a transesterification reaction of **2** (compound **3**) as indicated below:



compound **3**

This was confirmed by SEC analysis chromatograms (Fig. 3, Table 2), that reveals for each MeO-PEG-ester analysis two peaks: the first one with a molar mass close to the original PEG and the second one attributed to the transesterified product **3**.

These side products could be eliminated by different ways:

- chromatography on silica using ethylacetate/acetone/dichloromethane (70/20/10) as eluant. This technic allows the purification of the product by elimination of **3** and of the starting MeO-PEG. Nevertheless the yield obtained is low,
- working in anhydrous conditions by distillating all reactive compounds and solvents, prevents the formation of **3**,
- carrying the reaction at 40 °C avoids the formation of **3**, but the quantity of starting PEG becomes then more important.

Moreover, **2** could be obtained from **3** according to the procedure described by Dejardin [55] where **3**, methanol and sulfuric acid have been refluxed for 15 h to get **2**.

3.2. Protection and deprotection reactions of chitosan functions

The protection of the primary hydroxyl function and of the amino function of chitosan was reported by Nishimura et al. [51]. The general procedure is described in Scheme 2.

The *N*-phthaloylation of chitosan is conducted at 130 °C using an excess phthalic anhydride in DMF. The characteristic absorptions due to phthalimido groups at 1778, 1716 and 722 cm^{-1} are observed in the IR spectrum (Fig. 4).

Nevertheless, compound **5** was ninhydrin positive

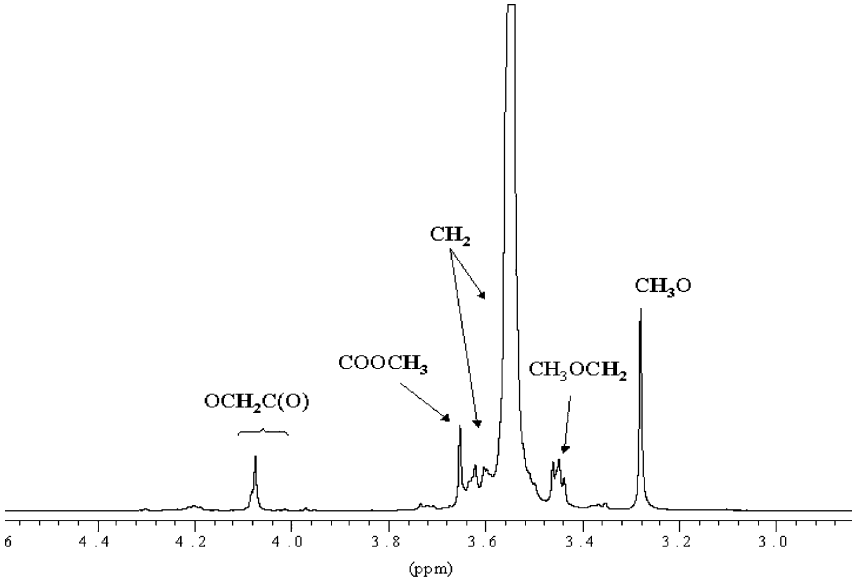


Fig. 1. ^{1}H NMR spectra of MeO-PEG **1** and MeO-PEG-ester **2**.

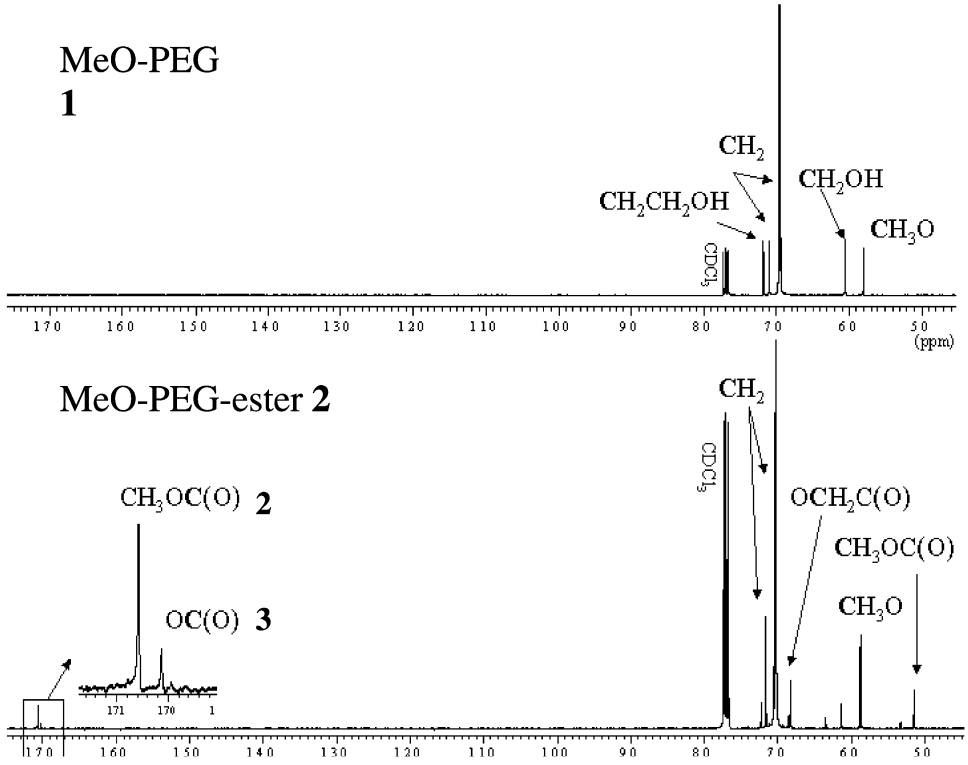


Fig. 2. ^{13}C NMR spectra of MeO-PEG **1** and MeO-PEG-ester **2**.

Table 1
Table1 NMR and IR data of MeO-PEG (**1**), MeO-PEG-ester (**2**), chitosan (**4**) and PEG-*g*-chitosan (**9** and **10**)

Compound	δ (ppm) ^{1}H NMR	^{13}C NMR	IR (cm^{-1})
1	3.3 (s, 3H, –OCH_3), 3.5 (m, 2H, –CH_2), 3.6 (m, *x*H[a], –OCH_2), 3.7 (m, 2H, –OCH_2)	58.8 (s, OCH_3), 61.3 (s, –CH_2-OH), 70.1 (m, –OCH_2), 71.7 (m, –CH_2), 72.3 (s, –CH_2)	3470 (νOH), 2870 (νCH), 1110 (νCO)
2	3.3 (s, 3H, –OCH_3), 3.4 (m, 2H, –CH_2), 3.6 (m, *x*H[a], –OCH_2), 3.7 (s, 3H, –C(O)OCH_3), 4.1 (m, 2H, –OCH_2C(O))	51.4 (s, –OCH_3, ester), 58.6 (s, –OCH_3), 68.2 (s, –OCH_2C(O)), 70.2 (s, –CH_2), 70.5 (s, –CH_2), 71.5 (s, –CH_2), 170.5 (s, –C(O))	1750 (νC=O, ester)
4	2.57 (s, 3H, –CH_3), 3.59 (s, 1H, H_2), 3.96–4.12 (m, 4H, H_2', H_6', H_5, H_4'), 4.12–4.50 (m, 5H, H_6, H_3, H_3', H_5', H_4), 5.13 (s, 1H, H_1'), 5.29 (s, 1H, H_1)	23.23 (s, –CH_3), 56.83 (s, C_2'), 57.10 (s, C_2), 61.46 (m, C_6), 61.73 (m, C_6'), 71.62 (m, C_3, C_3'), 75.90 (m, C_5, C_5'), 78.40 (m, C_4, C_4'), 99.21 (s, C_1), 102.25 (s, C_1'), 175.39 (s, –C(O)CH_3)	3470 (νOH, νNH), 1654 (νC=O, δN–H, amide), 1646 (δNH)
9	1.87 (s, –CH_3), 3.04 (m, H_2), 3.19 (s, –OCH_3), 3.35–3.76 (m, –CH, –CH_2, chitosan and PEG), 4.38–4.59 (m, H_1', H_1'', H_1'''), 4.72 (d, $^3J_{HH}$ = 7.7 Hz, H_1), 7.72–7.92 (m, CH phthalimide)	23.15 (s, –CH_3), 56.67 (m, C_2, C_2', C_2'', C_2'''), 56.89 (s, C_2), 58.97 (s, –OCH_3), 61.29 (s, C_6), 61.31–61.33 (m, C_6, C_6', C_6''), 70.33 (m, –CH_2), 70.54 (m, –CH_2), 71.04 (m, C_3, C_3', C_3'', C_3'''), 71.98 (s, –CH_2OCH_3), 75.32–76.06 (m, C_5, C_5', C_5'', C_5'''), 78.01–78.04 (m, C_4, C_4', C_4'', C_4'''), 98.49 (s, C_1), 102.14–102.17 (m, C_1', C_1'', C_1'''), 131.72–137.08 (C, CH phthalimide), 170.62 (s, CO phthalimide), 175.37 (s, –C(O)-PEG), 175.43 (s, –C(O)CH_3)	3446 (νOH, νNH), 2931 (νCH), 1778 (νC=O, phthalimide), 1716 (νC=O, phthalimide), 1650 (νC=O, δN–H, amide), 1558 (δNH)
10	1.83 (s, –CH_3), 2.96 (m, H_2), 3.14 (s, –OCH_3), 3.30–3.78 (m, –CH, –CH_2, chitosan and PEG), 4.27–4.45 (m, H_1', H_1''), 4.65 (d, $^3J_{HH}$ = 7.9 Hz, H_1)	23.11 (s, –CH_3), 56.90 (m, C_2, C_2', C_2''), 58.95 (s, –OCH_3), 61.26 (m, C_6, C_6', C_6''), 70.53 (m, –CH_2–), 71.22 (m, C_3, C_3', C_3''), 75.75 (m, C_5, C_5', C_5''), 78.08 (m, C_4, C_4', C_4''), 98.75 (s, C_1), 102.25 (s, C_1', C_1''), 175.36 (s, –C(O)-PEG), 175.42 (s, –C(O)CH_3)	3448 (νOH, νNH), 2924 (νCH), 1656 (νC=O, δN–H, amide)

[a] Depending on the molar mass of the starting MeO-PEG.

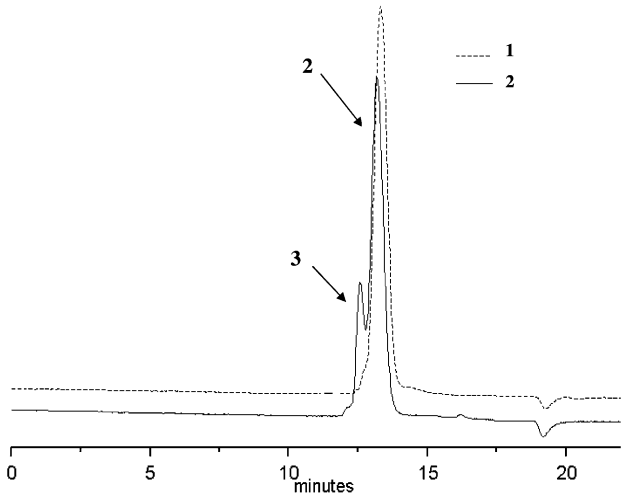


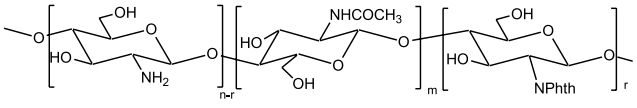
Fig. 3. SEC analysis of MeO-PEG **1** and MeO-PEG-ester **2** (**3** is the transesterified product).

showing that the protection of NH_2 functions is not complete.

The *O*-tritylation of **5** is carried on at 90 °C using an excess of chlorotriphenylmethane. The characteristic absorptions due to monosubstituted phenyl groups at 766, 748 and 702 cm^{-1} are unequivocally observed in the IR spectrum (Fig. 4).

Removal of the phthaloyl group from **6** is carried out by treatment with hydrazine hydrate at 100 °C. The deprotection of NH_2 function is confirmed in IR spectrum (Fig. 4) by the decrease of the phthalimido groups absorption bands. Nevertheless, the deprotection reaction is not complete even if the reaction is repeated. Indeed, IR spectrum of **7** shows residual absorption bands attributed to the phthalimido groups at 1716 and 1776 cm^{-1}.

Polymer **7** will be used for the grafting of PEG-ester onto chitosan. To evaluate the extent of NH_2 functions on the chitosan after the protection–deprotection reactions, the trityl groups were removed to obtained the polymer **8** [51].



Phth : phthalimido group

8

In the IR spectrum of **8**, no absorption bands attributed to the trityl groups are observed indicated that the deprotection of the primary hydroxyl group of chitosan is complete.

The extent of NH_2 functions was estimated by colloidal titration method [53,54] that shows that 20% of NH_2 functions remained protected by phthalimido groups.

Moreover, SEC-MALLS analysis of both chitosan and **8** are conducted to compare their weight average molar masses ($\bar{M}_w$). If the chitosan shows a $\bar{M}_w$ of 370,000 g mol^{-1} (in accordance with the viscosity–average molar mass ($\bar{M}_v$) equal to 330,000 g mol^{-1}), the $\bar{M}_w$ of sample of **8** has been found largely lower (i.e. only 25,000 g mol^{-1}). This result clearly evidences that the different protection–deprotection reactions degrades the original chitosan.

It is not so surprising regarding to the hard reaction conditions.

3.3. Preparation of PEG-g-chitosan

3.3.1. From 6-O-triphenylmethyl-chitosan

The grafting of MeO-PEG onto 6-*O*-triphenylmethyl-chitosan **7** was carried out with an excess of **2** that is eliminated by the precipitation of the polymer in acetone. The obtained polymer is treated by a solution of acetyl chloride in methanol [52] to deprotect its triphenylmethyl groups and the PEG-*g*-chitosan is precipitated in acetone and washed by soxhlet in THF (Scheme 3).

Deprotection of triphenylmethyl groups is confirmed by the disappearance in IR spectrum of their characteristic absorption bands. The spectroscopic characterization data are reported in Table 1.

^1H NMR spectrum of **9** shows in addition to peaks attributed to the chitosan, a peak at 3.2 ppm attributed to the methoxy groups of grafted MeO-PEG, peaks attributed to the methylene groups of PEG at 3.4 ppm and peaks corresponding to the residual phthalimido groups between 7.4 and 7.8 ppm (Fig. 5).

^{13}C NMR spectrum of **9** shows peaks attributed to chitosan and MeO-PEG (Fig. 6). The grafting is confirmed by the presence of the peak at 175.3 ppm attributed to the carbonyl groups resulting of the grafting reaction.

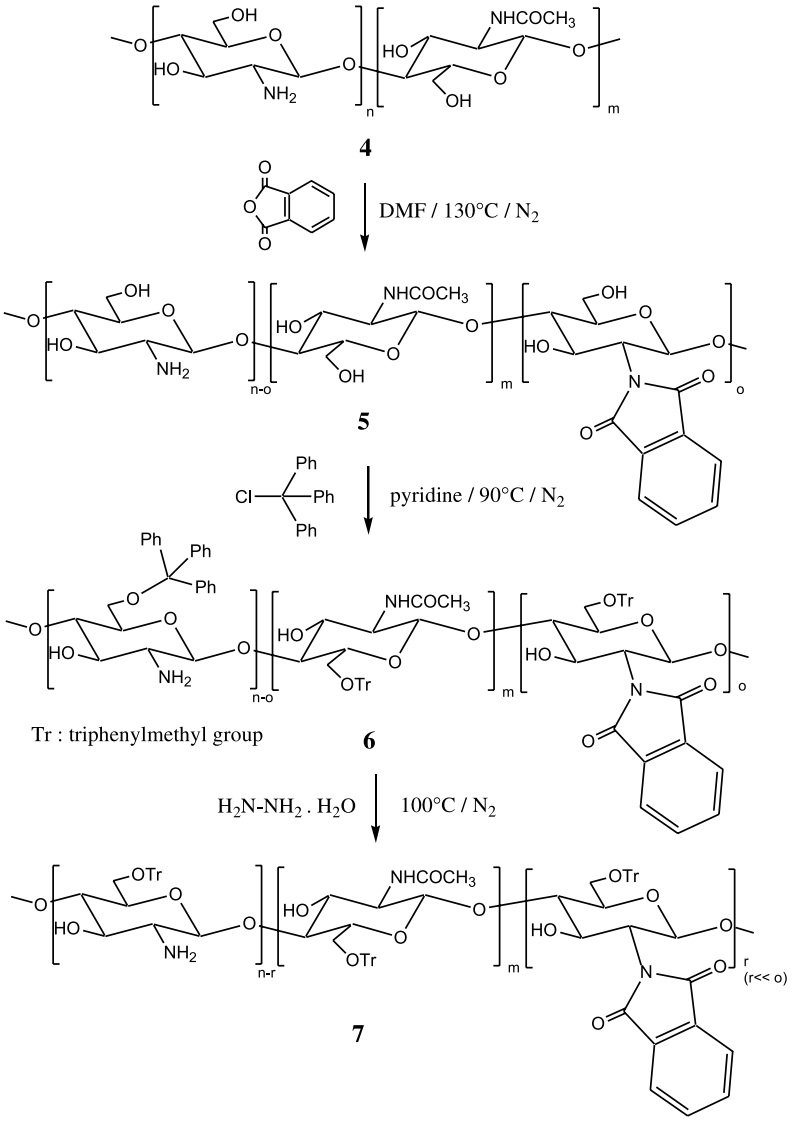
The extent of grafting (or the degree of substitution) was evaluated by colloidal titration and NMR ^1H spectroscopy. The obtained results were summarized in Table 3.

The grafting extents of MeO-PEG are very low. The

Table 2
SEC analysis of MeO-PEG-ester **2**

MeO-PEG-ester **2**	$\bar{M}_n$ (g mol^{-1})	$\bar{M}_w$ (g mol^{-1})	$I = \bar{M}_w/\bar{M}_n$	%
From[a] MeO-PEG 550	515	560	1.08	77.5
	1165	1205	1.03	22.5
From MeO-PEG 750	710	770	1.08	69.0
	1780	1920	1.08	31.0
From MeO-PEG 2000	2015	2105	1.04	81.5
	4015	4085	1.02	18.5

[a] Starting PEG.



Scheme 2. Protected–deprotected chitosan derivatives.

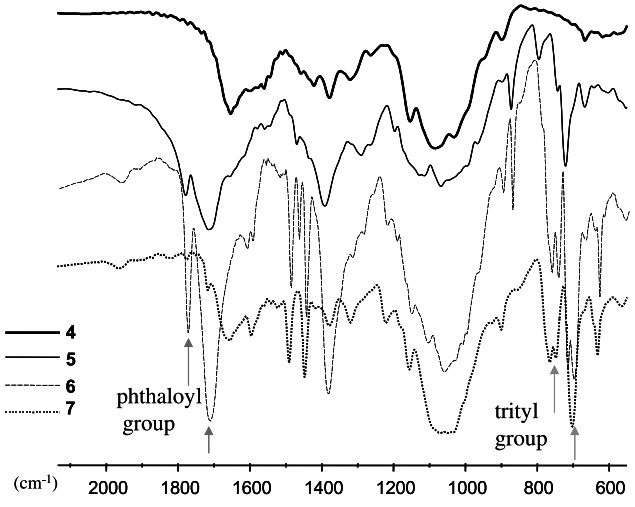


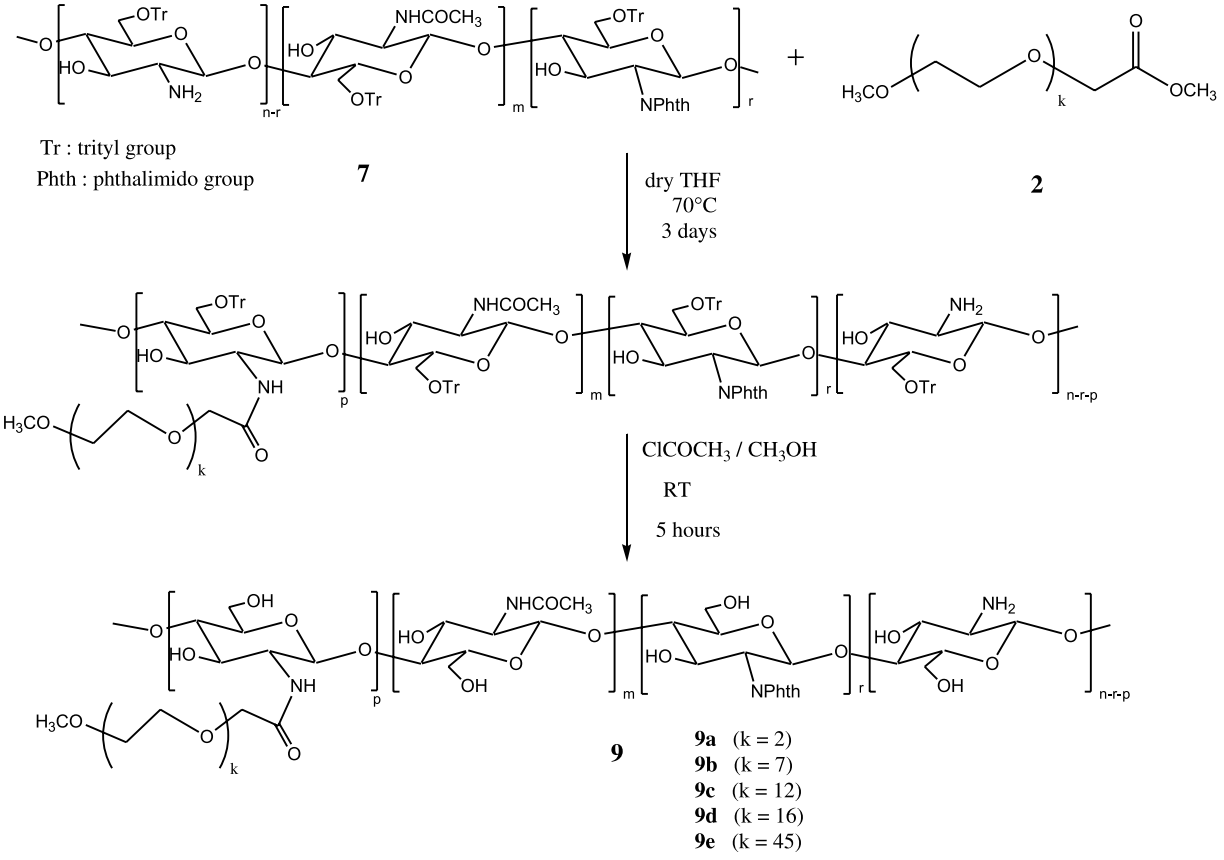
Fig. 4. Infrared spectra of protected–deprotected chitosan derivatives.

values do not seem to depend on the molar mass of the starting MeO-PEG. This low grafting extent could be due to the residual phthalimido groups (20%) leading to a steric hindrance that could limit the degree of substitution (DS) value.

The series of sample **9** was analyzed by SEC-MALLS, and all the $\bar{M}_w$ are around 30,000 g mol^{-1} and are not function of the molar mass of the starting MeO-PEG. This result is expected as polymers **9** are made according to the same procedure of polymer **8**, those molar masses are lower than the molar mass of chitosan showing the $\bar{M}_w$ for degradation of chitosan because of the reaction conditions.

3.3.2. From original chitosan

To limit the degradation of chitosan, the grafting of MeO-PEG was performed on the original chitosan (Scheme 4).



Scheme 3. Synthesis of PEG-*g*-chitosan **9**.

The reaction was carried out with an excess of **2** to increase the grafting extent. The excess of **2** was eliminated by soxhlet in THF. The spectroscopic characterization data were reported in Table 1.

Nevertheless, polymer **10** was ninhydrin positive showing that the grafting is not complete.

All peaks are attributed in ^{13}C NMR spectrum of **10** (Fig. 7). The peak corresponding to the carbonyl function of **2** is

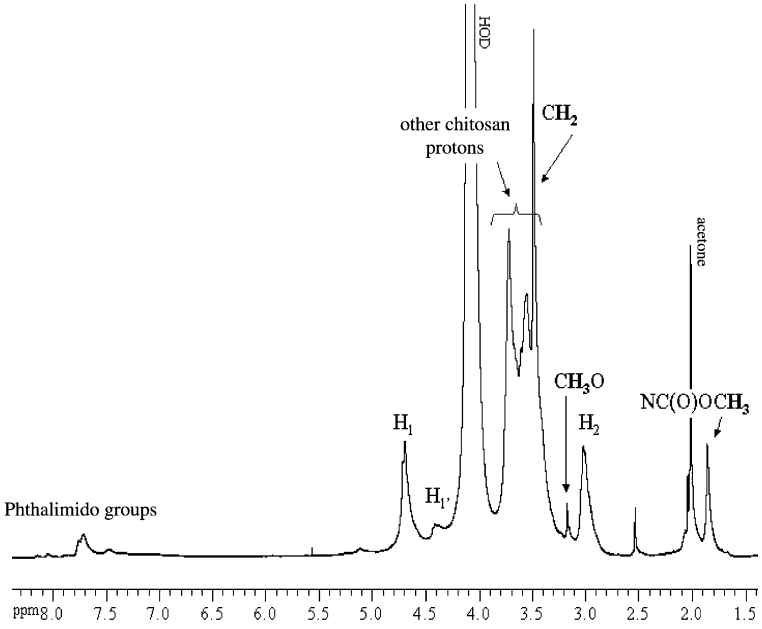


Fig. 5. ^{1}H NMR spectrum of **9**.

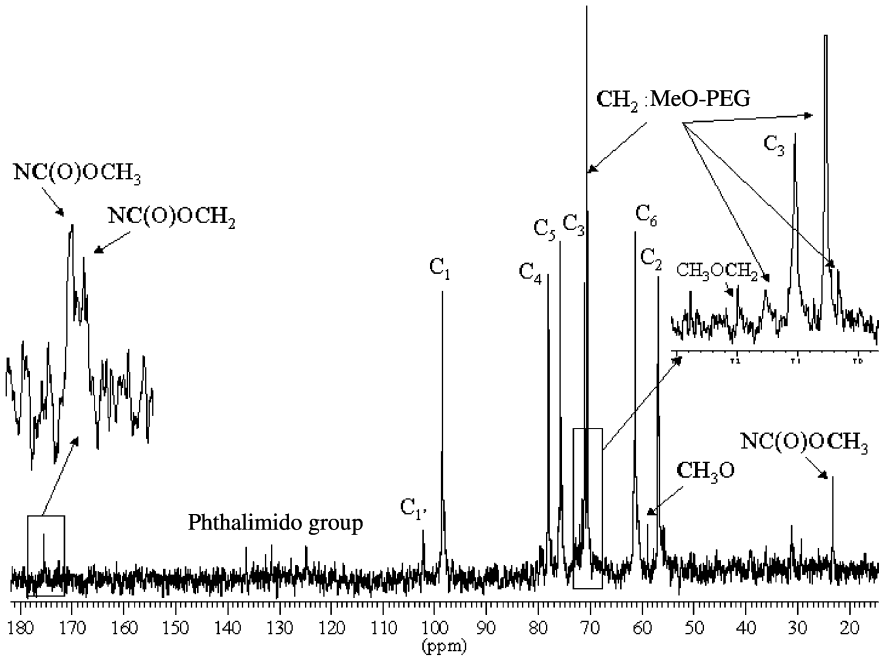


Fig. 6. ^{13}C NMR spectrum of **9**.

not visible indicating that the grafting reaction occurred. This is confirmed by the peak at 175.36 ppm corresponding to the amide linkage resulting from ester aminolysis reaction. The enlargement of this area shows clearly two different very closed peaks at 175.36 and 175.42 ppm corresponding, respectively, to the carbonyl signal of PEG-*g*-chitosan and to the carbonyl signal of the *N*-acetyl-D-glucosamine units of chitosan.

The grafting extents were estimated by colloid titration method [53,54] (Table 4).

This way of grafting allows higher DS of PEG on chitosan than in the case of the procedure using **7**. It do not seem to depend on the molar mass of the starting MeO-PEG. The maximum grafting extent of MeO-PEG is 20% for MeO-PEG 2000. The grafting extent could be increased by changing the experimental reaction conditions. When the reaction is carried on during six days instead of three, the degree of substitution rises to 22% and when the phenol is used as catalyst for the ester aminolysis reaction [56], it rises to 26%.

The series **10**, according to the different amounts of grafted PEG, was analyzed by SEC-MALLS. For the whole series, the $\bar{M}_w$ have been found around 250,000 g mol^{-1}. This results evidence that the $\bar{M}_w$ is not depending on the molar mass of the starting MeO-PEG. This weight average molar mass is lower than the initial chitosan $\bar{M}_w$, but the degradation of chitosan is largely less important by used this way of grafting compared to the previous way (i.e. samples **9**).

Nevertheless, more details concerning the composition of the **10a** could be obtained. In the Fig. 8, were reported both DRI and LS responses together with the molar masses distributions for the original chitosan and for the sample **10a**, which is largely representative of the series **10**.

It appeared that the **10a** presented two populations. The first population (around 3.5 mL) is consistent with the original chitosan. The maxima of LS signals for chitosan and first population of **10a** are observed at the same volume. As SEC is based on steric fractionation, both chitosan and **10a** eluted fractions observed at this maxima (3.5 mL) evidenced similar hydrodynamic volumes but the molar masses of **10a** were higher that for the chitosan. This means that the first population of **10a** was more compact according to the grafting of PEG or to aggregated structures. The second population of **10a** (about 4.5 mL) is largely lower in mass (i.e. about 80,000 g mol^{-1}) than chitosan evidencing that this reaction leads also to a degradation.

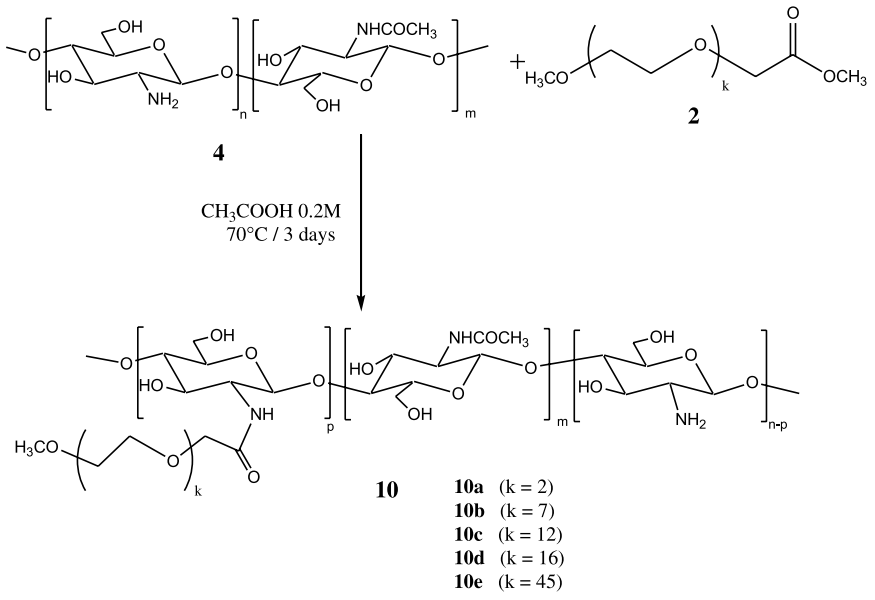
3.4. Properties

3.4.1. Solubility

According to the way of grafting of PEG onto chitosan, the solubility properties are different. Concerning the grafting from **7**, the PEG-*g*-chitosan **9** is soluble in acidic

Table 3
Grafting extent of PEG onto chitosan for derivatives **9**

	9a	**9b**	**9c**	**9d**	**9e**
DS	5.5%	3.5%	7.0%	7.2%	7.5%



Scheme 4. Synthesis of PEG-*g*-chitosan **10**.

Table 4
Grafting extent of PEG onto chitosan for derivatives **10**

	10a	**10b**	**10c**	**10d**	**10e**
DS	19.5%	16%	18.5%	13%	20%

water, water, DMSO, DMF. It should be reminded that this way of grafting led to a small grafting extent (between 4 and 7%) and caused an important degradation of the chitosan. This degradation should lead to an higher solubility of the modified polymer **9** in different solvents, particularly in water; indeed, chitosan **8** obtained after protection and deprotection process has a small molar mass and became soluble in water unlike **4**. Concerning the grafting from original chitosan, the PEG-*g*-chitosan **10** is only soluble in acidic water. Even if the DS is higher (between 13 and 20%), the PEG ratio is not enough important to allow the solubilization of PEG-*g*-chitosan **10** in water.

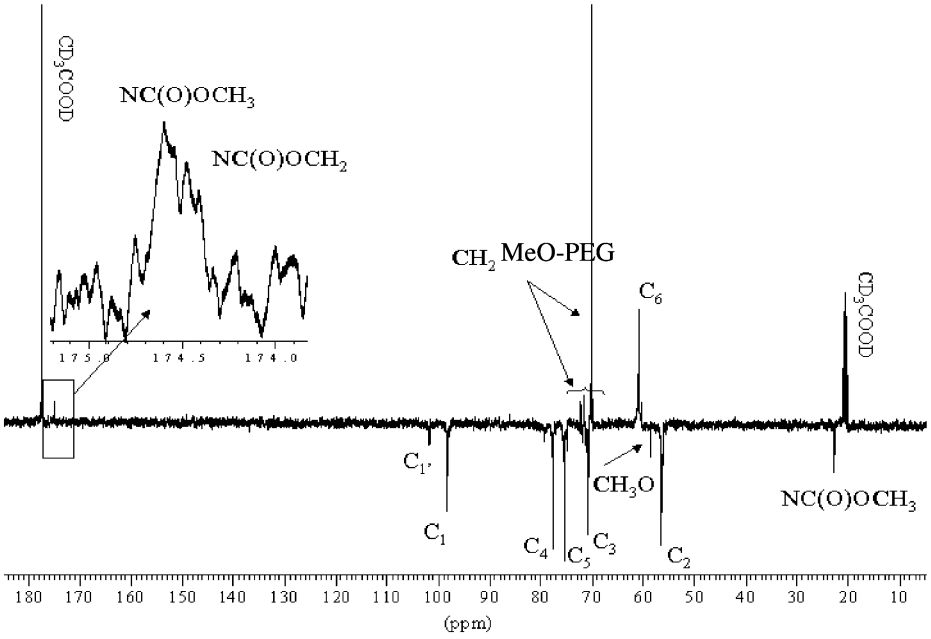


Fig. 7. ^{13}C NMR spectrum of **10**.

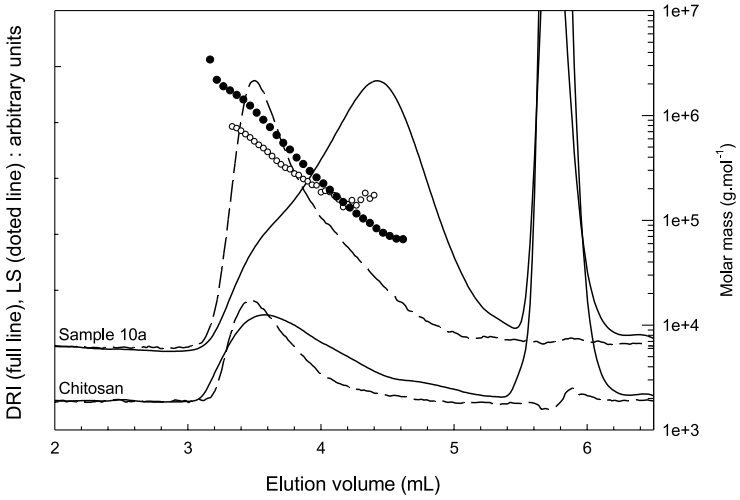


Fig. 8. SEC/MALLS analysis (full line: DRI, dotted line: LS) and molar masses distributions of chitosan (open circle) and sample **10a** (full circle). Dead volume at 5.8 mL.

3.4.2. Interfacial properties

Ouchi [57] has demonstrated the ability to include *N*-phenyl-1-naphthylamine, hydrophobic chemical, within chitosan-*g*-PEG aggregates, as the existence of hydrophobic cavities within these aggregates. Hence, it may be expected to observe tensioactive properties beyond the critical aggregation concentration determined to be around 0.7 g L^{-1} with the chitosan chain grafted with PEG which molar mass was 5000 g mol^{-1}.

Influence of the PEG chain length was evaluated for grafting reactions carried out with or without protection of primary hydroxyl groups of chitosan backbone (Figs. 9 and 10). In any cases modified polymers showed tensioactive properties.

The influence of the length of the PEG chain on surface tension was small. A slight decrease of surface tension and equilibrium times was observed for PEG 550-*g*-chitosan (**9c**, Fig. 9) compared to chitosan chains grafted with smaller molecular weight PEG chains (**9b**, Fig. 8), as an increase of the viscoelastic modulus of interfacial film which may allow to use these derivatives as emulsifier stabilizers.

By contrast chitosan grafted with PEG 2000 (**9e** and **10e**, Figs. 8 and 9) chains showed higher values of surface tension and viscoelastic modulus demonstrating less developed tensioactive properties. This may be explained by the larger length of grafted chain which modifies the rigidity of backbone and the ability of hydrophobic zones to structure near the surface, leading to a decrease of the diffusion of macromolecular chains towards air–liquid interface and of the organization of amphiphilic chains, increasing the equilibrium time.

The influence of the primary hydroxyl groups protection reactions on interfacial properties was demonstrated (Fig. 11).

The surface tension kinetics was quicker using protected chitosan sample, moreover the interfacial film viscoelastic modulus was the largest. This was due to the decrease of the

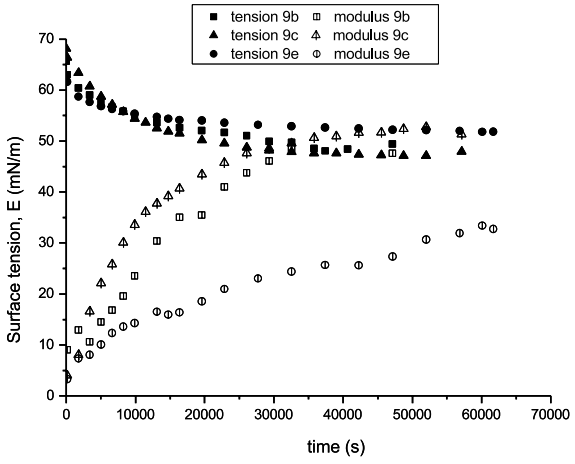


Fig. 9. Surface tension analysis for **9b**, **9c** and **9e** chitosan samples (protected primary hydroxyl groups): role of alkyl chain length.

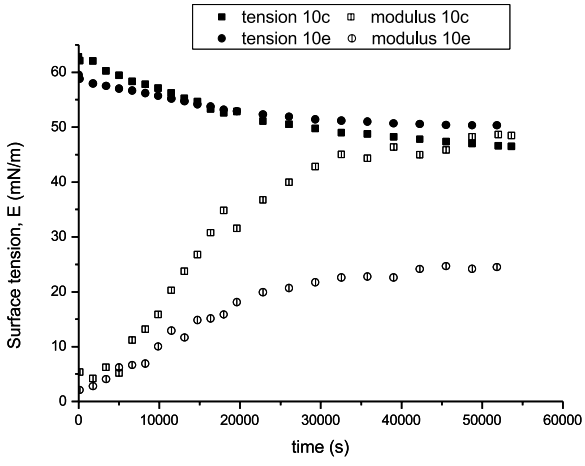


Fig. 10. Surface tension analysis for **10c** and **10e** chitosan samples (without protection of primary hydroxyl groups): role of alkyl chain length.

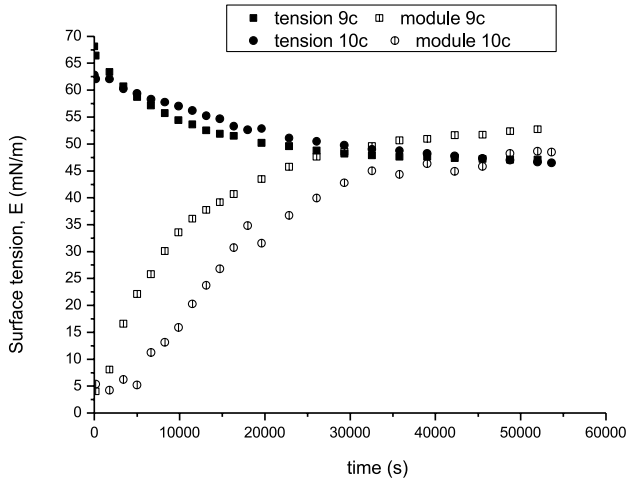


Fig. 11. Comparison of surface tension analysis between **9c** and **10c**: influence of protection and deprotection reactions.

molar mass during the protection reaction. This allowed a better diffusion of macromolecular chains towards the interface and their quickest reorganization.

4. Conclusion

We described in this work the synthesis of a PEG modified by ester functions; and two ways of grafting PEG onto chitosan. According to the way used, characteristics of PEG-*g*-chitosan are different. In the first way, the grafting occurred on 6-*O*-triphenylmethyl-chitosan **7** after protection of primary hydroxyl groups. The different protection and deprotection reactions lead to a degradation of the chitosan, and moreover the protection–deprotection reactions are not complete as demonstrated by the presence of residual phthalimido groups. These led to a solubilization of compound **9** in water despite of a small DS.

The second way allowed a fast and efficient PEG-grafting onto chitosan. In this case, the decrease of the chitosan molecular weight is less important and the DS higher than using the former way.

Surface properties of chitosan derivatives were evaluated and the different analyses showed the tensioactive properties of these compounds.

Acknowledgements

This work has been performed within the 'Réseau Matériaux Polymères-Plasturgie' interregional network (Pôle Universitaire Normand). We gratefully acknowledge financial support from the 'Ministère de la Recherche et des Nouvelles Technologies', the CNRS (Centre National de la Recherche Scientifique), the 'Région Basse-Normandie' and the European Union (FEDER funding) enlever for financial support.

References

[1] Desbrières J. L'actualité chimique. In: Société française de chimie ed. p. 39–44.
[2] Goosen MFA, editor. Applications of chitin and chitosan. Technomic Publishing Company; 1997.
[3] Yang TC, Zall RR. Ind Eng Chem Res 1984;23:168–72.
[4] Hirano S. In: Skjåk-Braek G, Anthonsen T, Sandford P, editors. Chitin and chitosan. Amsterdam: Elsevier; 1989. p. 37–44.
[5] Sandford PA. In: Skjåk-Braek G, Anthonsen T, Sandford P, editors. Chitin and chitosan. Amsterdam: Elsevier; 1989. p. 51–70.
[6] Hirano S, Noishiki Y, Kinugawa J, Higashijima H, Hayashi T. In: Gebelien GC, editor. Advances in biomedical polymers. New York: Plenum Press; 1987. p. 285–97.
[7] Sabnis S, Block LH. Int J Biol Macromol 2000;27:181–6.
[8] Genta I, Costantini M, Asti A, Conti B, Montanari L. Carbohydr Polym 1998;36:81–8.
[9] Okamoto Y, Kawakami K, Miyatake K, Morimoto M, Shigemasa Y, Minami S. Carbohydr Polym 2002;49:249–52.
[10] Bernkop-Schnürch A. Int J Pharm 2000;194:1–13.
[11] Knapczyk L, Krowczynski L, Marchut E, Brzozowski T, Marcinkiewicz J, Guminska M, Konturek SJ, Ptak W. In: Skjåk-Braek G, Anthonsen T, Sandford P, editors. Chitin and chitosan. Amsterdam: Elsevier; 1989. p. 543–730.
[12] Muzzarelli RAA. Chitin. Oxford: Pergamon Press; 1977. p. 255–65.
[13] Peter MG. Pure Appl Chem 1995;A32:629–40.
[14] Muzzarelli RAA. In: Skjåk-Braek G, Anthonsen T, Sandford P, editors. Chitin and chitosan. Amsterdam: Elsevier; 1989. p. 87–99.
[15] Muzzarelli RAA. Carbohydr Polym 1996;29:309–16.
[16] Ravi Kumar MNV. React Funct Polym 2000;46:1–27.
[17] Nishimura K, Nishimura S, Seo H, Nishi N, Tokura S, Azuma IJ. Biomed Mater Res 1986;20:1359–72.
[18] Tanigawa T, Tanaka Y, Sashiwa H, Saimoto H, Shigemasa Y. In: Brine CJ, Stanford PA, Zikakis JP, editors. Advances in chitin and chitosan. London: Elsevier; 1992. p. 206–15.
[19] Minami S, Okamoto Y, Tanioka S, Sashiwa H, Saimoto H, Matsuhachi A, Shigemasa Y. In: Yalpani M, editor. Carbohydrates, carbohydrate polymers. Illinois: ATL Press; 1993. p. 141–52.
[20] Sashiwa H, Saimoto H, Shigemasa Y, Ogawa R, Tokura S. Int J Biol Macromol 1990;12:295–6.
[21] Shigemasa Y, Saito K, Sashiwa H, Saimoto H. Int J Biol Macromol 1994;16:43–6.
[22] Hall LD, Yalpani M. J Chem Soc Chem Commun 1980;1153–4.
[23] Nishimura SI, Kohgo O, Kurita K, Vittavatvong C, Kuzuhara H. Chem Lett 1990;2:243–6.
[24] Nishimura SI, Miura Y, Ren L, Sato M, Yamagishi A, Nishi N, Tokura S, Kurita K, Ishii S. Chem Lett 1993;9:1623–6.
[25] Sashiwa H, Kawasaki N, Nakayama A, Muraki E, Yamamoto N, Aiba SI. Biomacromolecules 2002;3:1126–8.
[26] Xie W, Xu P, Liu Q. Bioorg Med Chem Lett 2001;11:1699–701.
[27] Sashiwa H, Shigemasa Y. Carbohydr Polym 1999;39:127–38.
[28] Tanida F, Tojima T, Han SM, Nishi N, Tokura S, Sakairi N, Seino H, Hamada K. Polymer 1998;39:5261–3.
[29] Amiji MM. Colloids Surf B 1998;10:263–71.
[30] le Dung P, Milas M, Rinaudo M, Desbrières J. Carbohydr Polym 1994;24:209–14.
[31] Ohya Y, Cai R, Nishizawa H, Hara K, Ouchi T. STP Pharma Sci 2000; 10(1):77–82.
[32] Wrzyszczynski A, Xia Q, Szosland L, Adamczak E, Linden LA, Rabek JF. Polym Bull 1995;34:493–500.
[33] Jiang WH, Han SJ. J Polym Sci Part A: Polym Chem 1998;36: 1275–81.
[34] Jiang WH, Han SJ. Eur Polym J 1999;35:2079–85.
[35] Angelova N, Manolova N, Rashkov I, Maximova V, Bogdanova S, Domard A. J Bioact Compat Polym 1995;10:285.
[36] Amiji MM. Carbohydr Polym 1997;32:193–9.

[37] Ouchi T, Nishizawa H, Ohya Y. Polymer 1998;39:5171–5.
[38] Muslim T, Morimoto M, Saimoto H, Okamoto Y, Minami S, Shigemasa Y. Carbohydr Polym 2001;46:323–30.
[39] Sugimoto M, Morimoto M, Sashiwa H, Saimoto H, Shigemasa Y. Carbohydr Polym 1998;36:49–59.
[40] Dal Pozzo A, Vanini L, Fagnoni M, Guerrini M, de Benedittis A, Muzzarelli RAA. Carbohydr Polym 2000;42:201–6.
[41] Bentley MD, Roberts MJ, Harris JM. J Pharm Sci 1998;87:1446–9.
[42] Saito H, Wu X, Harris JM, Hoffman AS. Macromol Rapid Commun 1997;18:547–50.
[43] Ohya Y, Cai R, Nishizawa H, Hara K, Ouchi T. STP Pharma Sci 2000; 10:77–82.
[44] Lee SJ, Kim SS, Lee YM. Carbohydr Polym 2000;41:197–205.
[45] Herman S, Hooftman G, Schacht E. J Bioact Compat Polym 1995;10: 145–87.
[46] Rinaudo M, Milas M, le Dung P. Int J Biol Macromol 1993;15:281–5.
[47] Domard A, Gey C, Rinaudo M, Terrassin C. Int J Biol Macromol 1987;9:233–7.
[48] Picton L, Mocanu G, Mihaï D, Carpov A, Muller G. Carbohydr Polym 1995;28:131–6.
[49] Cagna A, Esposito G, Riviere C, Housset S, Verger R. Thirty third international conference on biochemistry of lipids, Lyon, France; 1992.
[50] Benjamins J, Lucassen-Reynders EH. Proteins at liquid interfaces. Amsterdam: Elsevier; 1998 p. 341–83.
[51] Saulnier P, Boury F, Malzert A, Heurtault B, Ivanova T, Cagna A, Panaïotov I, Proust JE. Langmuir 2001;17:8104–11.
[52] Nishimura SI, Kohgo O, Kurita K, Kuzuhara H. Macromolecules 1991;24:4745–8.
[53] Hirrien M. Thesis, Grenoble University, France; 1996.
[54] Tôei K, Kohara T. Anal Chim Acta 1976;83:59–65.
[55] Dejardin JV. Bull Soc Chim Fr 1976;3–4:530–2.
[56] Jouffret F, Madec PJ. J Polym Sci, Part A: Polym Chem 1996;34: 2363–70.
[57] Ouchi T, Nishizawa H, Ohia Y. Polymer 1998;39:5171–5.