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Protein C-loaded monomethoxypoly (ethylene oxide)–poly(lactic acid) nanoparticles

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Abstract

This paper deals with the preparation and characterization of monomethoxypoly(ethylene oxide)–poly(lactic acid) (MPEO–PLA) nanoparticles containing protein C, a plasma inhibitor which regulates the mechanism of blood coagulation. Protein C was entrapped in MPEO–PLA nanoparticles using the double emulsion method. The influence of MPEO–PLA copolymers on the different parameters was evaluated: characteristics of protein C-loaded nanoparticles, in vitro release of the protein, evolution of the particle size with incubation time and MPEO release. The nanoparticle size does not depend on copolymer characteristics (MPEO and/or PLA block molecular weight). On the other hand, the efficiency of protein C entrapment is affected by the copolymer characteristics. The burst effect during the protein C release is increased with the hydrophilic character of the copolymer. Moreover, protein C adsorption on the particle surface during its release may be related to MPEO release. Only 25% of the released protein C is active, which clearly illustrates that it is altered during its encapsulation. The optimization of the experimental parameters which disturbed entrapped protein C activity, i.e. sonication time and organic solvent was investigated and has led to a preservation of protein C activity. Then, to optimize its entrapment efficiency, a blend PLA/MPEO–PLA (25/75) was used to prepare nanoparticles. This blend limited burst effect of protein C and its adsorption. However, protein C is only partially released which implicates further investigation for a potential therapeutic use.
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Keywords: Protein C; Monomethoxypoly(ethylene oxide)–poly(lactic acid); Biodegradable nanoparticles; Protein activity; Protein release

1. Introduction

Protein C is one of the plasma inhibitors which regulates the coagulation cascade in blood by modulating the anticoagulant response (Guillin,

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1987). Its deficiency in the general population is 1/300 (Gaussem et al., 1998). However, the clinical expression of the disease changes with the molecular anomaly. This vitamin K-dependent plasma glycoprotein has a molecular weight of 62 000 g/mol (Horellou et al., 1985). Protein C is the serine protease zymogen of activated protein C which possesses an anticoagulant activity, a profibrinolytic activity and probably an anti-inflammatory effect (Taylor et al., 1987). The increase in its plasmatic half-life (about 6 h) Marlar et al., 1993) would be suitable for the treatment of some disorders caused by its deficiency or by that of other plasma coagulation inhibitors. Thus, its encapsulation into biodegradable injectable colloidal carriers should protect it from degradation, ensure its transport and its delivery in the bloodstream (Couvreur, 1985). However, following intravenous injection, these colloidal carriers must be stealthy, otherwise they would be rapidly cleared from blood by the mononuclear phagocyte system (MPS; Verrechia et al., 1995; Fernandez-Urrusuno et al., 1996). The use of an amphiphilic diblock copolymer monomethoxypoly(ethylene oxide)–poly(lactic acid) (MPEO–PLA) to prepare nanoparticles can allow to delay phagocytosis (Bazile et al., 1991). In fact, the presence of MPEO chains on the nanoparticle surface limits the reduction of complement consumption owing to its steric repulsion effect (Jeon et al., 1991; Vittaz et al., 1996; Zambaux et al., 1999a). The protein nanoencapsulation and its delivery have been the goal of a lot of investigations for the last 10 years. However, most of the published works until now deal with the nanoencapsulation of model proteins such as albumin (Crotts and Park, 1997; Quellec et al., 1998) and only few authors have recently attempted the encapsulation of therapeutic proteins (Gaspar et al., 1998; Lemoine and Préat, 1998; Tobio et al., 1998). In fact, the major limitation in the formulation of therapeutic proteins is their release with a satisfactory biological activity. The feasibility to encapsulate protein C into biodegradable PLA nanoparticles prepared by the double emulsion method was reported in a previous paper (Zambaux et al., 1999b).

In this article, we report the preparation and characterization of protein C-loaded MPEO–PLA nanoparticles. Firstly, three diblock copolymers MPEO–PLA (10 000–45 000, 20 000–45 000 and 20 000–20 000 g/mol) were synthesized and characterized (Bouillot et al., 1998). Then, with the final aim to entrap active protein C in 200 nm MPEO–PLA nanoparticles, and to deliver it progressively, the influence of the MPEO–PLA copolymer on the characteristics of the protein C-loaded nanoparticles was studied in order to define the most convenient copolymer. Finally, the preservation of protein C activity as well as the optimization of its entrapment were investigated.

2. Materials and methods

2.1. Materials

D,L-Cyclic lactide (97%) was obtained from Lancaster, England. MPEO 10 000 and 20 000 g/mol were obtained from Shearwater Polymers, Inc. Poly(vinyl alcohol) (PVA) of molecular weight 13 000–23 000 g/mol (87–89% hydrolysed) was supplied by Sigma (Paris, France). Racemic D,L-PLA50 was obtained from Phusis (Le Versoud, France). Protein C was provided from Laboratoire Français du Fractionnement et des Biotechnologies (LFB; Les Ullis, France).

2.2. Synthesis and characterization of copolymers

The copolymers were synthesized by ring opening polymerization in solvent (Bouillot et al., 1998). Stannous octanoate was used as a catalyst. ^{1}H-nuclear magnetic resonance (NMR) was used to check that PLA chains were synthesized. The number average molecular weight ($\overline{M_n}$), the weight average molecular weight ($\overline{M_w}$) and polydispersity index (I) of the copolymers were evaluated by means of size exclusion chromatography (SEC) connected with a refractometer and a multiangle light laser scattering (MALLS; Wyatt Dawn, Milton Roy, Wyatt Technology Corporation). Their thermal properties were determined by differential scanning calorimetry (DSC; DSC 92, Setaram) and the measurements were conducted under helium flow at a rate of 10°C/min.

2.3. Preparation of protein C-loaded nanoparticles

Nanoparticles were prepared by a double emulsion technique as previously described (Zambaux et al., 1998). Briefly, 0.2 ml of an aqueous solution of protein C (flask of about 500 IU reconstituted into 0.35 ml of distilled water) was emulsified in 4 ml of methylene chloride containing 100 mg of copolymer or blend of PLA and copolymer, by sonication during 30 s in an ice bath (Scientific Vibra Cell at an output of 5 (15 W); Bioblock, France). Then, 8 ml of an aqueous solution containing a 3% (w/v) PVA solution was added to this first emulsion and sonicated during 30 s (pulsed) under the above conditions to obtain a double emulsion. This emulsion was then diluted into 160 ml of a 0.1% (w/v) PVA solution and the system was maintained under magnetic stirring for 5 min. Afterwards, the organic solvent was evaporated under vacuum using a rotating evaporator. Nanoparticles were recovered by centrifugation at 30 000 ***g*** for 20 min at 10°C, washed with distilled water (two washings) and freeze-dried. The amount of entrapped protein C into nanoparticles was indirectly determined, i.e. by dosing the amount of protein C in the supernatant of encapsulation. The entrapment efficiency (EE) of the process determined by enzyme linked immunosorbent assay (ELISA) indicates the percentage of protein C encapsulated with respect to the total amount used for the nanoparticle preparation. The protein C loading of the nanoparticles (L) indicates the encapsulated amount (IU) per 100 mg of nanoparticles.

Mean values of three batches are presented in tables and figures.

2.4. Particle size analysis

Particle size distribution (mean diameter and polydispersity index) was determined by photon correlation spectroscopy (PCS) using a Malvern spectrometer 4700 (Malvern Instruments, UK). Each nanoparticle preparation was analyzed in duplicate with 30 readings per nanoparticle sample suspended in distilled water. Mean particle size and polydispersity index (allows to determinate the dispersity of the suspension) were calculated for each sample. For a monodisperse system, polydispersity index should be between 0.03 and 0.06 (Müller, 1991). Mean values of three batches are presented in tables and figures.

2.5. Protein C antigenicity

The antigenicity of protein C was determined with the immunoenzymatic ELISA method according to the following procedure: microtitration plates (EIA/RIA, 96 wells; Costar) were coated for 1.5 h at 37°C with 100 μl of anti-human protein C polyclonal antibodies (Assera protéine C, Diagnostica Stago, Asnières, France) diluted 120 × in 50 mM sodium carbonate (coating buffer), pH 9.6. All washes (200 μl) and dilutions were operated with 130 mM NaCl, 5 mM Na_2HPO_4 and 1 mM KH_2PO_4, pH 7.2 containing 0.05% Tween 20 (v/v; PBS-Tween). The plates were blocked with 100 μl of 0.225% gelatin (v/v) for 1.5 h at 37°C. A 100 μl aliquot of sample or standard solution of protein C was added to the wells and incubated for 1.5 h at 37°C. A concentrate of protein C (46 IU/ml; LFB) was used as a standard; the successive dilutions of this standard were from 3200 to 25 600 × . A 100 μl aliquot of biotinylated anti-protein C monoclonal antibody (HC-2, Sigma, France) diluted 100 × was then added and incubated for 1.5 h at 37°C. The streptavidin–peroxidase complex (Sigma, France) diluted 40 × was incubated for 15 min at 37°C. After washing with PBS-Tween and 140 mM sodium acetate-citrate, pH 6, color was developed with 100 μl of 0.1 mg/ml TMB (3,3′,5,5′-tetramethylbenzidine; Fluka, France) and 0.05% (v/v) H_2O_2 in acetate-citrate. After 5 min of incubation at room temperature, the enzymatic reaction was stopped with 25 μl of 2 M H_2SO_4 and the absorbance was measured at 450 nm. Each determination was done in duplicate.

2.6. Protein C anticoagulant activity

Protein C anticoagulant activity was determined by measuring the activated partial thromboplastin time using a chronometric assay, Staclot protein C (Diagnostica Stago, Asnières, France). After mixing, 100 μl of deficient plasma in protein

C, 100 μl of protein C activator and 100 μl of sample to assay (diluted in Owren–Koller buffer) were incubated for 3 min at 37°C. After 3 min of incubation, 100 μl of 0.025 M $CaCl_2$ incubated were added and the chronometer was simultaneously set off (Option 4, BioMérieux, France). The chronometer was stopped with the formation of the clot. The time of coagulation is proportional to the percentage of protein C in the sample. The standard is a reference plasma (Protein C Calibrator, Diagnostica Stago, Asnières, France).

2.7. In vitro release studies

In vitro release studies were operated just after the preparation of protein C-loaded nanoparticles. In vitro release studies of a formulation were operated in triplicate and were performed by incubating 25 mg of nanoparticles in 15 ml of 0.05 M phosphate buffer, pH 7.4, stabilized with 0.2% NaN_3 (w/v). The nanoparticle suspensions were continuously stirred in a thermoshake (60 rpm) at 37°C. At preselected times, samples were collected and 2 ml were centrifuged at 35 000 ***g*** for 20 min. The removed supernatant was not replaced; it was checked that sink conditions were still satisfied at the end of the release.

2.8. MPEO assay

MPEO was assayed with barium chloride and iodine solution (Sims and Snape, 1980). The MPEO amount in the nanoparticles was determined after their destruction as described for PVA in a previous paper (Zambaux et al., 1998). At preselected times, to determine the released MPEO from copolymer nanoparticles, 1.5 ml of supernatant was collected and was centrifuged at 35 000 ***g*** for 20 min. Afterwards, the sample was treated with 1 N NaOH to hydrolyze PLA blocks in order to only assay MPEO block and then neutralized by 1 N HCl. Then, 0.5 ml of a 5% barium chloride (w/v) in 1 N HCl and 25 μl of an iodine solution (1.66% potassium iodide and 1.27% iodine in distilled water; w/v) were added to 1 ml of sample. After 5 min of incubation at room temperature in dark, the absorbance was measured at 535 nm against a blank. Solutions containing known amounts of MPEO (from 5 to 20 μl/ml) were used as a reference.

3. Results

3.1. Physico-chemical characterization of the MPEO–PLA copolymers

P-chemical characterization of the three synthesized MPEO–PLA copolymers, i.e. determination of their $\overline{M_n}$, $\overline{M_w}$, polydispersity index and thermal properties gave results reported in Table 1. First time, the polymerization of lactide was checked by ^{1}H-NMR by determining the number of PLA blocks which consequently led to the value of $\overline{M_n}$. In fact, the knowledge of the number of ethylene oxide units (provided by the supplier) and of the ratio of the integrated surface area of the different peaks relative to MPEO and PLA chains (obtained from ^{1}H-NMR) allowed to calculate the $\overline{M_n}$ of PLA in the copolymer. The obtained values were close to the expected ones (Table 1) and were confirmed by SEC–MALLS (size exclusion chromatography–multiple angle laser light scattering) experiments. Whatever the copolymer, the polydispersity index was about 1.4 and was close to those of commercial PLA50.

The thermal properties of the copolymers, i.e. glass transition temperature (T_g) and fusion temperature (T_f) are shown in Table 1. Because the use of these copolymers is an in vivo administration, the knowledge of their behavior at 37°C is interesting. Thus, a T_g smaller than 37°C will allow a good mobility and flexibility of the copolymer chains in materials at body temperature. An important decrease in T_g of copolymers was observed with the increase in MPEO molecular weight (Table 1). In the same way, T_g decreased with PLA molecular weight. The value of T_g of PLA50 and MPEO10–PLA45 were below 37°C and of the same order of magnitude, respectively 31 and 22°C. On the other hand, MPEO20–PLA45 and MPEO20–PLA20 copolymers have a T_g value of -9 and -37°C, respectively and a $T_f$of 58.6°C. However, whatever the synthesized copolymers, their glass transition temperature is below 37°C which means that they would be plastic materials at body temperature.

3.2. Influence of the MPEO–PLA copolymer on protein C nanoparticle characteristics

Table 2 depicts the influence of the polymer properties on the characteristics of MPEO–PLA nanoparticles. The particle size is close to 200 nm and does not depend on the copolymer characteristics (molecular weight of MPEO block or PLA block). It is likely that these amphiphilic copolymers in addition to the PVA effect act as stabilizers for the double emulsion. The entrapment efficiency of protein C decreases with the increase in MPEO molecular weight (from 10 000 to 20 000 g/mol) and increases with the PLA block length.

3.3. Influence of the MPEO–PLA copolymer on in vitro release of protein C

In vitro protein C release from copolymer nanoparticles is illustrated by Fig. 1. For each formulation, a more or less pronounced burst effect of protein C during the first 3 h is observed. In literature, this burst effect is often mentioned (Huang et al., 1999; Rojas et al., 1999). Afterwards, a decrease in the percentage of protein C in the release medium is obtained. Whatever the copolymer, about only 25% of the protein C molecules in the release medium are active. Of course, it was verified that free protein C in the supernatant did not get inactivated during the in vitro release conditions. In fact, the preparation conditions of the nanoparticles alter the protein C activity as it was observed in a previous paper in the case of PLA nanoparticles (Zambaux et al., 1999b).

Evolution of the copolymer particle size (mean diameter and polydispersity index) during the study of encapsulated protein C release is shown in Table 2. The mean diameter and the polydispersity index of MPEO10–PLA45 and MPEO20–PLA45 nanoparticles slowly decreased between $t = 0$ and 3 days. On the other hand, for MPEO20–PLA20 nanoparticles, a significant increase in their mean diameter and polydispersity index is observed. The results of the MPEO release study carried out during the release of encapsulated protein C are seen in Fig. 2. The MPEO release is slow (about 7% after 3 h to about 25% at $t = 3$ h) and is the greatest for MPEO10-PLA45 nanoparticles.

3.4. Optimization of the encapsulated protein C activity in MPEO20–PLA45 nanoparticles

In order to identify the experimental parameters which disturbed protein C activity during its encapsulation into PLA nanoparticles, the influ-

Table 1
Physico-chemical characteristics of the three synthesized MPEO – PLA copolymers

Polymer	Theoretical PLA $\overline{M_n}$ (g/mol)	PLA $\overline{M_n}$ (g/mol)[a]	Polymer $\overline{M_n}$ (g/mol)[b]	Polymer $\overline{M_W}$ (g/mol)[b]	I[c]	T_g (°C)[d]	T_f (°C)[e]
Commercial PLA50	–	–	37 800	49 500	1.3	31	–
MPEO10 –PLA45	45 000	38 000	50 700	71 500	1.4	22	–
MPEO20 –PLA45	45 000	41 000	59 800	86 100	1.4	−9	58.6
MPEO20 –PLA20	20 000	19 000	38 500	52 400	1.3	−37	58.6

[a] Calculated by ^{1}H-NMR.
[b] Determined by SEC–MALLS.
[c] Polydispersity index = $\overline{M_W}/\overline{M_n}$.
[d] Glass transition temperature.
[e] Fusion temperature.

Table 2
Influence of MPEO–PLA copolymer on the characteristics of protein C-loaded nanoparticles and evolution of their diameter and polydispersity index during the protein C release study[a]

Copolymer (EE[b] ± S.D.)	t (days)	Particle size (nm ± S.D)	Polydispersity index
MPEO20-PLA45 (57 ± 3)	$t=0$	195 ± 16	0.23 ± 0.02
	$t=1$	188 ± 9	0.25 ± 0.02
	$t=2$	178 ± 2	0.2 ± 0.03
	$t=3$	175 ± 7	0.18 ± 0.04
MPEO10-PLA45 (36 ± 3)	$t=0$	243 ± 6	0.24 ± 0.02
	$t=1$	226 ± 7	0.3 ± 0.016
	$t=2$	203 ± 6	0.23 ± 0.007
	$t=3$	194 ± 4	0.2 ± 0.008
MPEO20-PLA20 (70 ± 3)	$t=0$	204 ± 23	0.4 ± 0.04
	$t=1$	316 ± 33	0.7 ± 0.02
	$t=2$	262 ± 37	0.7 ± 0.04
	$t=3$	230 ± 29	0.6 ± 0.04

[a] See Section 2 for the preparation conditions.
[b] Entrapment efficiency.

ence of sonication time and of used organic solvent were investigated (Zambaux et al., 1999b). We showed that the decrease in sonication time from 30 s + 30 s (pulsed) to 10 s + 10 s (pulsed) increased the residual activity of protein C. On the other hand, the use of acetone with methylene chloride in a 1/1 volume ratio as an organic phase to prepare nanoparticles led to a twofold increase in residual activity of protein C compared with methylene chloride alone. Below 10 s + 10 s (pulsed) sonication, the double emulsion gave rise to nanoparticle diameter close to 400 nm with a very high polydispersity index (about 1). Concerning the organic solvent choice, acetone is often employed for plasmatic fractionation because it does not disturb the protein structure and activity. The protein C-loaded MPEO20–PLA45 nanoparticles were prepared under these conditions, i.e. an organic phase composed of a mixture of acetone and methylene chloride in a 1/1 volume ratio and a sonication time of 10 s + 10 s (pulsed). In vitro release of encapsulated protein C is illustrated by Fig. 3. Firstly, it was found that the presence of acetone in the organic phase did not influence protein C entrapment efficiency but decreased nanoparticle size below 200 nm. Protein C release was studied for 9 h because this period is consistent with the in vivo half-life of blank MPEO–PLA nanoparticles (Verrechia et al., 1995). The measurement of protein C antigenicity showed an important burst effect during the first hour (70% of the entrapped amount). The release continued slowly during the second hour and reached a plateau; the measurement of protein C activity also showed an important burst effect followed by a plateau. Furthermore, nearly all protein C molecules in the release medium were active.

3.5. Optimization of the protein C entrapment efficiency in MPEO20–PLA45 nanoparticles

We have just shown that it was possible to encapsulate active protein C in MPEO20–PLA45 nanoparticles. However, with these preselected conditions, protein C entrapment efficiency was only 33% and the protein C release was too fast. In order to overcome these drawbacks, a blend of PLA and MPEO20–PLA45 in 25/75 proportion was used. As shown in a previous paper, this ratio hampers the in vitro complement consumption by nanoparticles (Zambaux et al., 1999a). The presence of PLA in nanoparticle matrix did not mod-ify their size but increased protein C entrapment efficiency (47%) and the efficiency of nanoparticles recovery compared with MPEO20–PLA45 ones (respectively 49 and 35% (weight of nanoparticles/weight of initial polymer)). The release kinetics of protein C entrapped in these nanoparticles is illus-

trated by Fig. 3. The release profile related to protein C antigenicity showed a burst effect which was less pronounced (20% of the encapsulated amount) than for pure copolymer nanoparticles. In the same way, the released amount at $t = 2$ h (25% of the entrapped amount) was less important than for copolymer nanoparticles (80%). The release profile related to protein C activity showed a burst effect followed by a plateau. As expected, the protein C molecules in the release medium were active.

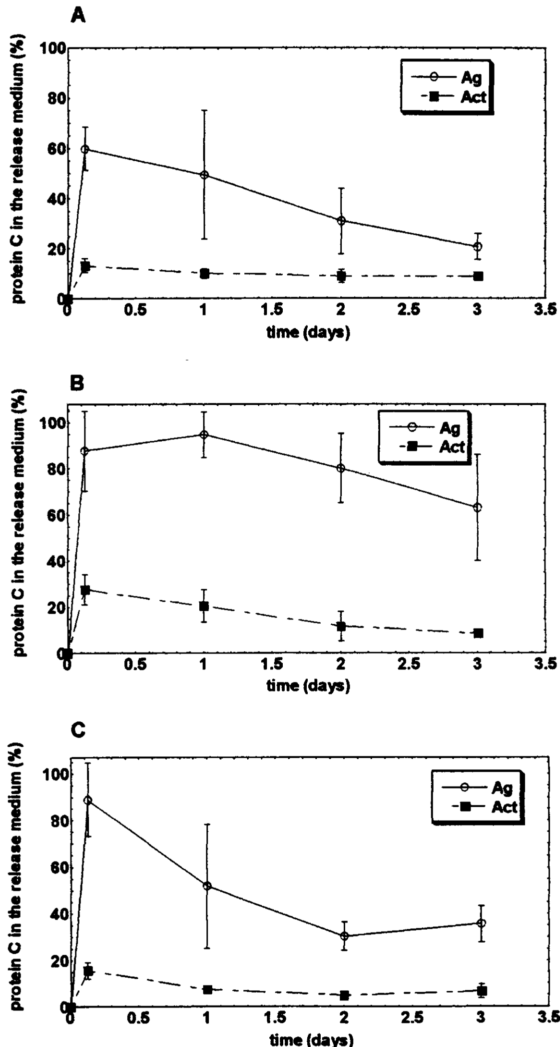

Fig. 1. Influence of MPEO–PLA copolymer composition on the kinetics of entrapped protein C release (Ag: antigenic activity, Act: anticoagulant activity). (A) MPEO10-PLA45; (B): MPEO20–PLA45; (C): MPEO20–PLA20. See Table 2 for the nanoparticle characteristics and Section 2 for the preparation conditions.

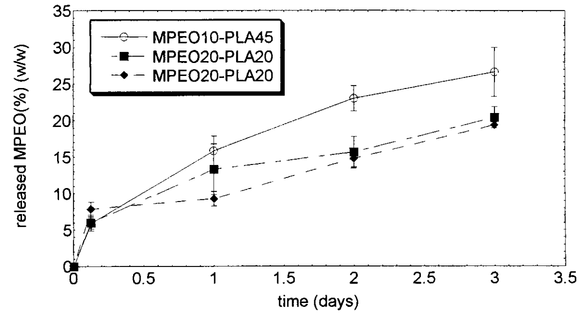

Fig. 2. MPEO release (%) during the study of protein C release from MPEO–PLA nanoparticles. %: weight of released MPEO /initial weight in the nanoparticles. See Table 2 for the nanoparticle characteristics and Section 2 for their preparation conditions.

Owing to the different results of the entrapment efficiency of protein C and in order to compare the amount of protein C in the release medium between PLA/MPEO–PLA blend and copolymer alone, the total amount of protein C was expressed in IU per 100 mg of nanoparticles (Fig. 4). The obtained release profiles showed that the blend allowed to decrease by twofold the burst effect of protein C whereas the amount in the

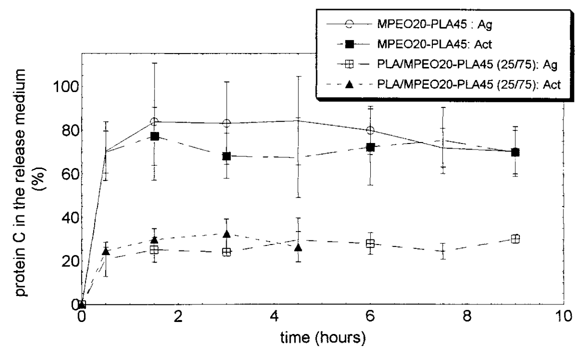

Fig. 3. Kinetics of protein C release (Ag and Act) from MPEO20–PLA45 and from PLA/MPEO20–PLA45 (25/75) nanoparticles. Preparation conditions of the nanoparticles: acetone/methylene chloride (1/1) and 10 s + 10 s (pulsed). The characteristics of MPEO20–PLA45 nanoparticles are: 185 ± 5 nm, polydispersity index = 0.3 and EE = 33 ± 2%. The characteristics of MPEO20–PLA45 (25/75) nanoparticles are: 193 ± 4 nm, polydispersity index = 0.25, EE = 47 ± 9% and L = 102 ± 12 IU/100 mg of nanoparticles.

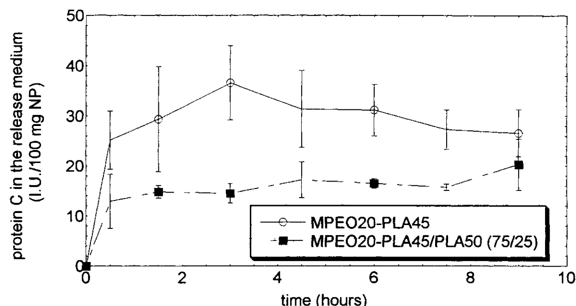

Fig. 4. Amount of protein C (Ag) in the release medium (per 100 mg of nanoparticles) as a function of the composition of nanoparticle matrix. See Fig. 3 for the nanoparticle characteristics.

supernatant was similar at $t = 9$ hours for both formulations (respectively 20 and 26 IU per 100 mg of nanoparticles for the blend and for the copolymer alone).

4. Discussion

The decrease in the MPEO10–PLA45 copolymer T_g in comparison with PLA alone indicated that MPEO plays the role of a plasticizer which was confirmed by the absence of a fusion peak; in this case, MPEO and PLA blocks were entangled and the presence of MPEO may lead to a better mobility and flexibility in the copolymer chains compared with pure PLA. On the other hand, for MPEO20–PLA45 and MPEO20–PLA20 copolymers, a T_g value of -9 and -37°C, respectively and a T_f of 58.6°C indicated a change in the chain distribution. One can notice that MPEO alone possesses crystalline domains, has a T_f value between 55 and 60°C and a T_g value of -60°C. So, in the MPEO20–PLA45 and MPEO20–PLA20 materials, crystalline zones of MPEO exist simultaneously with amorphous PLA zones. The two blocks were thus not entangled and it is likely that a phase separation occurred between the two blocks during the nanoparticle preparation which affected their morphology and so their characteristics (entrapment efficiency and release kinetic). Moreover, one can suppose that the decrease in the protein C percentage in the release medium was probably due to a protein C adsorption on the nanoparticle surface due to the release of MPEO. In fact, as MPEO was greatly hydrophilic and protrudes to the nanoparticle surface, water can facilitate the hydrolysis of the ester function between MPEO and PLA. Indeed, Quellec et al. (1998) showed that the hydrophilic character of the MPEO chains improved the nanoparticle water absorption. Consequently, the PLA chain hydrolysis and the nanoparticle erosion were facilitated as well as the diffusion of the soluble degradation products towards the supernatant. Thus, this phenomenon facilitated the adsorption of protein C from the release medium on nanoparticle surface. This adsorption phenomenon may be related to hydrophobic interactions between protein C and PLA chains that were evidenced in a previous work (Zambaux et al., 1999b). It was shown in this paper that when there was no residual surfactant on the nanoparticle surface of blank PLA nanoparticles, protein C could adsorb on their surface whereas it could not when there was residual surfactant.

The preservation of protein C activity during its nanoencapsulation was related to the presence of acetone in the organic phase in blend with methylene chloride that probably limited the contact between methylene chloride and protein C due to the reduction of the surface tension between the organic phase and the water one. Moreover, the presence of acetone allowed nanoparticles to solidify as a result of its solubility into water. Ghaderi and Carlfors (1997) reported that the addition of acetone to methylene chloride for microparticles preparation made it possible to improve the encapsulated lysozyme biological activity. In fact, for these authors, the more the lysozyme molecule was exposed to methylene chloride the more its activity was disturbed.

It is likely that the more hydrophobic character of PLA/MPEO20–PLA45 (25/75) nanoparticles compared to that of MPEO–PLA ones facilitated their sedimentation and the entrapment efficiency of protein C probably owing to hydrophobic interactions that exists between it and PLA (Zambaux et al., 1998). Moreover, PLA in the matrix of MPEO20–PLA45 nanoparticles decreased the water diffusion and lowered the protein C diffusion and consequently the burst effect. Thus, the

blend of PLA/MPEO20–PLA45 (25/75) was more convenient to prepare protein C-loaded nanoparticles than the copolymer alone because it improved the recovery efficiency of the nanoparticles, increased the entrapment efficiency of protein C and limited the release burst effect. However, the total released protein C was only of about 20% of the total encapsulated protein. Further experiments are underway in order to improve protein C entrapment efficiency, its loading and its progressive release.

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