Novel Bioresorbable and Bioeliminable Surfactants for Microsphere Preparation

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Purpose. The objective of this work is to prepare microspheres by the emulsion-solvent evaporation process using MPOE-PLA copolymers as the matrix material and/or the surfactant. This preparation was studied in order to avoid the use of PVA as the surfactant in the process.
Methods. Two series of MPOE-PLA copolymers were synthesised. The first, with a long and constant length PLA chain (45,000 $g.mol^{-1}$), was used as the matrix material, the second, with short PLA chains ($\leq$ 2,200 $g.mol^{-1}$), and different HLB as the surfactant. Microspheres were prepared by the "simple" and "double" emulsion methods. The steric stabilization effect of the copolymers was investigated using confocal microscopy and X-ray photoelectron spectroscopy (XPS).
Results. Confocal microscopy and XPS analysis showed that the microspheres prepared using MPOE5K-PLA0.5K as the surfactant and MPOE-PLA45K copolymers as the matrix material had an MPOE coating present at the microsphere surface. This hydrophilic layer ensures steric stabilization of the particles.
Conclusions. MPOE-PLA copolymers can be used for the preparation of particles instead of PVA and their use can be extended whenever a biocompatible and bioeliminable surfactant is required for biological or medical applications.

KEY WORDS: bioeliminable surfactants; amphiphilic properties; MPOE coating; microspheres.

INTRODUCTION

Encapsulation is used in the pharmaceutical field, mainly to improve the stability, the sustained release and the targeting of drugs. Numerous synthetic polymers have been used and are now available for use in controlled release systems; such polymers include poly(lactide) PLA, poly(lactide-co-glycolide) PLGA, poly(caprolactone), poly(anhydride), poly(orthoesthers), poly(cyanoacrylate). Most of the research on microencapsulation has involved the use of poly(lactide) and poly(lactide-co-glycolide), because of their biocompatibility, biodegradability and bioelimination, which were first established by Cutrigh et al (1), and Craig et al (2) using sutures. However, these two polymers have shown some limitations in achieving long-term release (3). Serious problems have been encountered, related to both the adsorption and denaturation of proteins or peptides (trypsin, insulin, bovine serum albumin) (4,5) in contact with PLA and PLGA hydrophobic matrices and to a low blood circulation half-life because of interactions with plasma proteins and phagocytic cells. Therefore, MPOE-PLA and PLA-POE-PLA copolymers consisting of 1 or 2 hydrophobic segments associated with a hydrophilic block (monomethoxypolyoxyethylene MPOE or polyoxyethylene POE) have also been investigated for use in drug delivery systems (6,7). Indeed, a POE or MPOE coating on the microsphere surface can be obtained by taking advantage of the amphiphilic properties of the copolymer (8). These hydrophilic segments can prevent protein adsorption (9) and increase the blood circulation half-lives of carriers (10,11).

Peptides and proteins are usually highly water soluble. Their encapsulation within a hydrophobic polymer can be carried out using a double water-oil-water emulsion process. Poly(vinyl alcohol) PVA, a non-biodegradable polymer, is usually required to stabilize large O/W droplets. However, high amounts of residual PVA adsorbed onto PLA and PLGA microspheres have been detected on the surface, despite the cleaning-procedure used (12). This presence of PVA is suspected to modify the surface properties of the delivery systems (13) and thus to change their behavior *in situ*. Moreover, Yamaoka et al (14,15) showed, in the case intravenous injections of PVA in mice, that small amounts of PVA are accumulated in the organs. Even if the health of mice was not affected in the short-term, Yamaoka et al (15) believed that it might become toxic long-term, particularly when multiple and regular injections are required to achieve the desirable treatment. The potential carcinogenic effect of PVA was shown in 1959 by Hueper (16).

The objective of this work was to find a new family of bioresorbable and biocompatible surfactant to use instead of PVA to prepare microspheres by an emulsion-solvent evaporation process. To this end, two series of MPOE-PLA copolymers were synthesised. The first series consists of short PLA chains ($<$ 2200 $g.mol^{-1}$) and is water-soluble. The second one possesses longer PLA chains ($\approx$45,000 $g.mol^{-1}$) and is water insoluble. The MPOE-PLA diblock copolymers, widely used to form the matrices of microspheres, were employed as emulsifying and stabilizing agents to prepare microspheres by taking advantage of their amphiphilic properties.

This paper describes the influence of the MPOE-PLA copolymer composition (e.g., the HLB) on the emulsion stability and the microsphere diameters. X-ray photoelectron spectroscopy analysis and a confocal analysis were carried out on the microspheres to investigate the stabilizing effect of the MPOE-PLA copolymers.

MATERIAL AND METHODS

Polymer and Copolymers Used to Form the Matrices of the Microspheres

D, L PLA was purchased from Phusis (France). A series of MPOE-D, L PLA was synthesised by ring-opening polymerization of D, L lactide on the hydroxyl end group of commercial MPOE, with stannous octoate as the catalyst, in solvent (xylene). The synthesized PLA block length was kept constant at 45,000 $g.mol^{-1}$ and the MPOE molar mass increased from 2,000 to 5,000, 10,000, 15,000, 20,000 $g.mol^{-1}$. The corresponding copolymers were named respectively MPOE2K-PLA45K, MPOE5K-PLA45K, MPOE10K-PLA45K, MPOE15K-PLA45K, and MPOE20K-PLA45K. 2 K, 5 K, 10

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K, 15 K, 20 K is the molecular weight indicated by the supplier (Shearwater Polymers Inc, USA) for MPOE; 45K is the molecular weight of the synthesized PLA block. The synthesis and the characterization of the diblock MPOE-PLA copolymers were fully described in a previous work (17).

We also synthesized a diblock copolymer MPOE5K-PLA45K, labeled with 1-pyrenemethanol, to study the location of the MPOE segments inside the microspheres. This copolymer was synthesised by condensation (T = 60°C) of 1-pyrenemethanol, after deprotonation with BuLi, on MPOE epoxide 5,000 $g.mol^{-1}$ (Shearwater Polymers Inc, USA) in THF. The amount of unreacted 1-pyrenemethanol was determined by high performance size exclusion chromatography (HPSEC), using two columns Lichrogel PS20 and PS400 (Merck, Germany) connected in series, with THF as eluent. 20 mole % of the final copolymer was labeled with 1-pyrenmethanol. The PLA chain was then synthesized as previously indicated by polymerization of lactide.

Polymer and Copolymers Used as Surfactants

Poly (vinyl alcohol) PVA (88% hydrolyzed, 13–23000 $g.mol^{-1}$) was purchased from Sigma (Germany). Four MPOE-PLA copolymers with different HLB were synthesized as described previously and named respectively MPOE5K-PLA0.5K, MPOE5K-PLA1.1K, MPOE5K-PLA2.2K, and MPOE10K-PLA2.2K.

Characterization of the MPOE-PLA Synthesized Surfactant Copolymers

^1H nuclear magnetic resonance (NMR) spectra of the copolymers in $CDCl_3$ were recorded using a Bruker AM 200 spectrometer, using tetramethylsilane as an internal standard. A vapor pressure osmometer (Knauer, Germany) was used to determine the molecular weight of the synthesized copolymers used as surfactants. The measurements were performed in THF at 45°C.

A tensiometer K-8 (Krüss, Germany) was used to determine the surface tension as a function of the aqueous polymer solution concentration and to determine at the same time the critical aggregation concentration of each synthesized copolymer.

Microsphere Preparation

By the Single Emulsion Method

A water-in-oil emulsion was obtained by mixing using an homogenizer (1 min) 2 mL dichloromethane or ethyl acetate containing 200 mg of polymer (PLA45K or MPOE2K-PLA45K) with 25 mL of distilled water containing 4 wt % of one of the synthesized copolymers as the surfactant (MPOE5K-PLA0.5K, MPOE5K-PLA1.1K, MPOE5K-PLA2.2K or MPOE10K-PLA2.2K). The mixture was then stirred for 15 min with a magnetic stirrer. During this period, the dichloromethane starts to evaporate and a thin layer of polymer is formed. The remaining solvent was removed using a rotary evaporator. The microspheres were collected by centrifugation (10,000 g for 9 min) and redispersed in distilled water. This cleaning procedure was repeated three times to remove the free surfactant. Finally, the microspheres were freeze-dried.

By the Double Emulsion Method

A water-in-oil emulsion was obtained by sonication (60s, 40W) of a mixture of 2 mL ethyl acetate containing 400 mg of polymer and 100 μL of distilled water. 3 mL of aqueous 4% MPOE5K-PLA0.5K copolymer was added and the solution was stirred with a homogenizer (30s) to make a (W/O)/W emulsion. The mixture was poured into 100 mL of distilled water and stirred for 15 min. The remaining solvent was removed using a rotary evaporator. The microspheres were collected by centrifugation (10,000 g for 9 min) and redispersed in distilled water. This cleaning procedure was repeated three times to remove the free surfactant. Finally, the microspheres were freeze-dried.

This preparation was then repeated without using any surfactant; 3 mL of distilled water was added to form the second emulsion.

It was checked using HPESC that the degradation of MPOE-PLA does not start during the 30 minutes required for the particle preparation.

Microsphere Characterization

A Coulter Multisizer II (Coultronics, USA) was used to measure the average diameter of the particles. The microspheres were observed first with an optical microscope then with a Scanning Electron Microscope (SEM) (Jeol JMS-T 330 A) after coating with a mixture of gold and palladium. Microspheres made with a blend PLA45K/MPOE5K-PLA45K labeled with 1-pyrenemethanol (70/30 wt%) were prepared and observed by means of a confocal microscope (MRC 1024 Bio-Rad, USA) to study the location of the MPOE chains inside the microspheres. X-ray Photoelectron Spectroscopy (XPS) (Hewlett Packard Hp 5950, USA) was used to show the stabilizing layer formed by the synthesized surfactant at the microsphere surface.

RESULTS AND DISCUSSION

All the copolymers used in this paper as the surfactants or the matrix material were synthesized by ring-opening polymerization of D,L lactide on the hydroxyl end group of MPOE with stannous octoate as the catalyst, in solvent (xylene). The polymerization of lactide in a solvent leads to a good control of the length of the PLA chains, and a low polymer polydispersity (17). Moreover, no PLA oligomers were obtained by this method because of the absence of transesterification reaction (17).

A series of surfactants (MPOE5K-PLA0.5K, MPOE5K-PLA1.1K, MPOE5K-PLA2.2K or MPOE10K-PLA2.2K) with different HLB values were synthesized according to this method. The molar ratio ethylene oxide/lactic acid (EO/LA) was calculated from the ^1H NMR spectra (18) and was found to be approximately equal to the theoretical value (Table I). Moreover, the comparison between the number average molecular weight of MPOE and that of the synthesized copolymers indicated that the PLA segments were obtained with the expected molecular weight. All these copolymers have a PLA chain $\leq$ 2,200 $g.mol^{-1}$ and are water-soluble.

The surface tension of the copolymer solutions was measured at the air-water interface at 25°C using a tensiometer. The surface tension was plotted as a function of the polymer concentration. The profile obtained was typical for surfactant

Table I. Characterisation of the MPOE-PLA Synthesized Surfactants

Surfactants	Yield (wt%)[a]	$\overline{M}_n$ of MPOE (g.mol^{-1})[b]	$\overline{M}_n$ of copolymers (g.mol^{-1})[b]	EO/LA theoretical[c]	EO/LA[d]	CAC $\times 10^6$ (mole.L^{-1})[e]
MPOE5K-PLA0.5K	95	4300	4700	13.6	13.6	18
MPOE5K-PLA1.1K	95	4300	5200	6.4	6.6	11
MPOE5K-PLA2.2K	93	4300	6200	3.2	3.4	6.5
MPOE10K-PLA2.2K	93	9100	11400	6.7	6.8	7.2

[a] Amount of copolymer after purification (mg) on total amount of MPOE + lactide (mg).
[b] Measured using a vapor pressure osmometer.
[c] Theoretical copolymer composition (molar ratio of units EO/LA).
[d] Molar ratio EO/LA calculated by ^{1}H NMR.
[e] Measured using a tensiometer.

solution (Fig. 1). At low concentration, the surface tension decreased linearly with log (polymer concentration), then, it stayed constant even at high concentration. The intersection of these two straight lines gives the Critical Aggregation Concentration CAC. The CAC values for all the copolymers are listed in Table I. The value of the CAC decreases with increasing the PLA chain length in the case of MPOE-PLA. Close values were obtained in the case of MPOE5K-PLA2.2K and MPOE10K-PLA2.2K copolymers. Indeed, the CAC is independent of the nature of the hydrophilic chains, but is related to the composition of the hydrophobic segment. The CAC decreases when the PLA chain length increases because the steric hindrance of the hydrophobic block leads to a saturation of the air-water interface at lower concentration; therefore the CAC is reached earlier. This phenomenon has been described in a previous study (19).

Preparation of Microspheres by the Single Emulsion Process Using the Synthesized Surfactants

We prepared microspheres made from PLA45K and MPOE2K-PLA45K by the single emulsion process. The various synthesized surfactants (MPOE5K-PLA0.5K, MPOE5K-PLA1.1K, MPOE5K-PLA2.2K or MPOE10K-PLA2.2K) were used as emulsifying and stabilizing agents to study their ability to form microspheres.

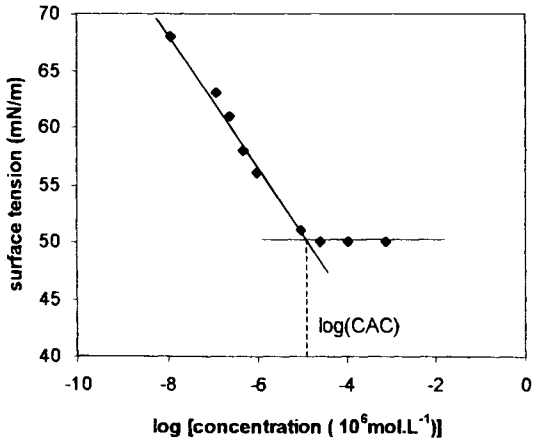

Fig. 1. Evolution of the surface tension at the interface water-air in the case of MPOE5K-PLA0.5K copolymer solutions at 25°C.

The preparation of PLA or MPOE-PLA microspheres by the emulsion-solvent evaporation process requires an oil phase, usually ethyl acetate or dichloromethane. However, none of the copolymer surfactants managed to stabilize the emulsion and to form microspheres, when dichloromethane was used to prepare the particles. Indeed, to be efficient the surfactant must exhibit a higher solubility in (and so affinity for) the water than in the organic phase. It will then adsorb at the oil-water interface, the hydrophobic segment in the oil phase, the hydrophilic block in the water phase and therefore form a stabilizing hydrophilic layer around the droplet.

MPOE homopolymers 5 K and 10 K are, however, both soluble in water and dichloromethane, unlike PLA homopolymers that are only soluble in dichloromethane. Therefore, the synthesized surfactants exhibited a higher affinity for the oil phase than the water phase, and their solubility in the dichloromethane increased with increasing the PLA chain length. Thus, most of the surfactant stayed solubilized in the organic phase and only a small amount was available to stabilize the droplet at the interface, which did not provide efficient steric stabilization.

Another solvent was thus required to form the oil phase in which the synthesized surfactants exhibit a lower solubility than in the water. Ethyl acetate was used because only PLA homopolymers are soluble in this solvent.

When PLA45K was used to form the matrix of the particles, spherical microspheres were obtained only with the MPOE5K-PLA0.5K synthesized surfactant. The mean diameter of the microspheres was measured using the Coulter Counter and was found to be 18 μm (Fig. 2). The oil-in-water emulsion was not stable and the droplets aggregated when MPOE5K-PLA2.2K copolymer was used as surfactant. In the case of the MPOE5K-PLA1.1K and MPOE10K-PLA2.2K synthesized surfactants, the emulsions were stable. However, non-spherical microspheres with irregular shapes were obtained.

When MPOE2K-PLA45K was used to form the matrix of the particles and MPOE5K-PLA0.5K was employed as the surfactant, spherical microspheres with a mean diameter of 29 μm were obtained. The other synthesized surfactants (MPOE5K-PLA1.1K, MPOE5K-PLA2.2K or MPOE10K-PLA2.2K) led to the formation of a mixture of microspheres and small "sticks" (Fig. 3). The yield of small sticks seemed to decrease with decreasing the PLA block length. We suppose that the formation of sticks is related to the coalescence of particles due to the lack of stabilizing efficiency of the surfactant used.

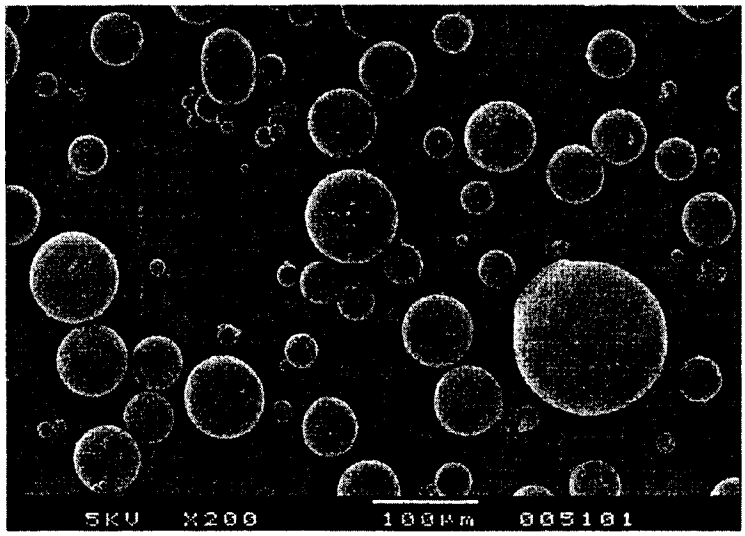

Fig. 2. SEM photograph of PLA45K microspheres prepared with MPOE5K-PLA0.5K copolymer as the surfactant.

Whichever polymer was used to form the matrix of the particles, spherical microspheres were obtained only when MPOE5K-PLA0.5K copolymer was used as the surfactant. The ability of a surfactant to stabilize an emulsion depends on its HLB (the composition and the length of both hydrophilic and hydrophobic segments). The composition corresponding to the MPOE5K-PLA0.5K copolymer seems to be the most efficient in the case of our preparation to stabilize an ethyl acetate in water emulsion.

The ability of the copolymer to stabilize the emulsion and to form microspheres increased with decreasing PLA chain length. These results are in good agreement with the stabilizing properties of these copolymers found in the case of microscopic decane-water-decane films and decane-in-water nanoemulsions (19). Moreover, on comparing the microspheres made of PLA and MPOE2K-PLA45K when using MPOE5K-PLA2.2K as surfactant, it was observed that the emulsion was better stabilized in the case of MPOE2K-PLA45K (spherical microspheres and small sticks were obtained) than in the case of PLA (the preparation lead to aggregate structure). This shows that MPOE2K-PLA45K copolymer also takes part in the stabilization of the emulsion because of its amphiphilic properties.

Fig. 3. Photograph of MPOE2K-PLA45K microspheres prepared with MPOE5K-PLA2.2K copolymer as the surfactant and obtained by means of an optical microscope.

Chemical Analysis of the Microsphere Surface

PLA microspheres prepared with MPOE5K-PLA0.5K copolymer as the surfactant were analysed using XPS.

According to the PLA formula, the chemical analysis should have theoretically shown a ratio O/C of 0.66 (20) and the presence of three different carbon types: CH_x, C-O, and COO in equal proportions 33% (21). However, the surface of PLA microspheres prepared with MPOE5K-PLA0.5K copolymer, as surfactant, exhibited a lower carbon content than expected; the calculated ratio was 0.84. Moreover, the three carbon types were not in equal proportions (21% CH_x, 53% C-O, 27% COO), a high amount of CO was detected. XPS analysis for MPOE homopolymer showed the presence of only one carbon type corresponding to ether functions (C-O). Therefore, this enrichment of O and of ether functions shows the presence of MPOE chains onto the microspheres and confirms that the MPOE5K-PLA0.5K copolymer migrates to the interface O/W to sterically stabilize the droplet and subsequently the particles. Despite the cleaning procedure, the MPOE5K-PLA0.5K copolymer remained strongly adsorbed onto the microspheres. Indeed, during the solidification of the particles, the PLA segment of the copolymer was trapped in the superficial layer of the PLA matrix. In fact, the use of MPOE5K-PLA0.5K copolymer as surfactant is also a new method to obtain a MPOE coating of PLA particles, which should be able to avoid protein adsorption and increase the blood circulation half-life of the carrier.

Table II. Mean Size of PLA45K and MPOE-PLA45K Microspheres Prepared Using MPOE5K-PLA0.5K Copolymer as the Surfactant and in Absence of Any Surfactant

Polymers	Mean particle size before freeze-drying (μm)	
	Surfactant: MPOE5K-PLA0.5K	Without surfactant
PLA45K	14 ± 1	[a]
MPOE2K-PLA45K	28 ± 1	46 ± 1
MPOE5K-PLA45K	47 ± 1	79 ± 2
MPOE10K-PLA45K	45 ± 1	101 ± 2
MPOE15K-PLA45K	40 ± 1	91 ± 2
MPOE20K-PLA45K	33 ± 1	79 ± 2

[a] No microsphere was obtained. Sizes measured by Coulter Multisizer II and expressed as the average of measurements with three batches.

Preparation of Microspheres by the Double Emulsion Method Using MPOE5K-PLA0.5K as the Surfactant

MPOE5K-PLA0.5K copolymer was used as the surfactant instead of PVA to prepare microspheres from PLA45K and the series of MPOE-PLA45K copolymers using the double emulsion process.

In all cases, spherical microspheres were obtained with a smooth surface. The mean diameter of the microspheres was measured using a Coulter Counter before freeze-drying. The results are listed in Table 2. The particle diameter increased from 14 to 47 μm as the molecular weight of the MPOE segment increased from 0 to 5 K. Then, it decreased again to 33 μm on further increasing the molecular weight of the MPOE chain.

This observation could be explained in terms of several effects, such as the hydrophilicity of the copolymer, their amphiphilic properties, the viscosity of the polymer solution, all of which have antagonist effects. Firstly, by increasing the MPOE chain length, the viscosity of the polymer solution increases and therefore the size of the resulting particles tends to increase. Secondly, the increase of the MPOE chain length also leads to high amount of water uptake, and thus to matrix expansion (22). Thirdly, the MPOE-PLA45K copolymers have amphiphilic properties, and thus they lower the interfacial tension of the O/W emulsion during the microsphere preparation. Moreover, the MPOE blocks are involved in steric stabilization of the droplets and then the microspheres.

Preparation of Microspheres in the Absence of Any Surfactant by the Double-Emulsion Method

As noticed previously, the MPOE-PLA45K, used to form the matrix of the microspheres also takes part in the stabilization of the emulsion. Therefore, we tried to prepare microspheres made from PLA45K and a series of MPOE-PLA45K copolymers by the double emulsion without adding any surfactant in the process. Except in the case of PLA45K, spherical microspheres were prepared (Fig. 4). Their mean diameters were measured using the Coulter Counter and are listed in Table 2. The mean diameter exhibited a similar evolution as described previously; it increased from 46 to 101 μm with increasing the molecular weight of the MPOE block from 2 to 10 K, then decreased to 79 μm whilst increasing the molecular weight of the hydrophilic segment further to 20 K.

This observation can be explained as before in terms of the hydrophilicity of the copolymer, their amphiphilic properties, and the viscosity of the polymer solution. Whichever the MPOE chain length, the MPOE-PLA45K amphiphilic copolymers stabilized the emulsion and led to the formation of microspheres. However, the MPOE chain length did influence the particle size.

The microspheres, obtained without any surfactant, possess a higher mean diameter than those prepared using MPOE5K-PLA0.5K as the surfactant. Indeed, the latter also decreases the ethyl acetate-water interfacial tension and allows the preparation of a smaller emulsion. It has to be noticed that the size distribution of the particles made with MPOE5K-PLA0.5K as the surfactant or without any surfactant appears broad according to the SEM pictures (Fig. 4).

We also prepared microspheres made from a blend of PLA45K/MPOE5K-PLA45K labeled with 1-pyrenemethanol (70/30wt%) by the single emulsion method.

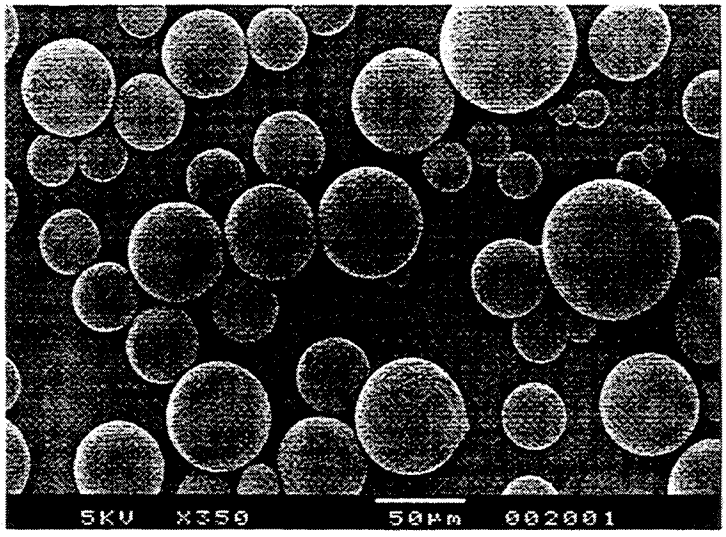

Fig. 4. SEM photograph of MPOE2K-PLA45K microspheres prepared without any surfactant in the process.

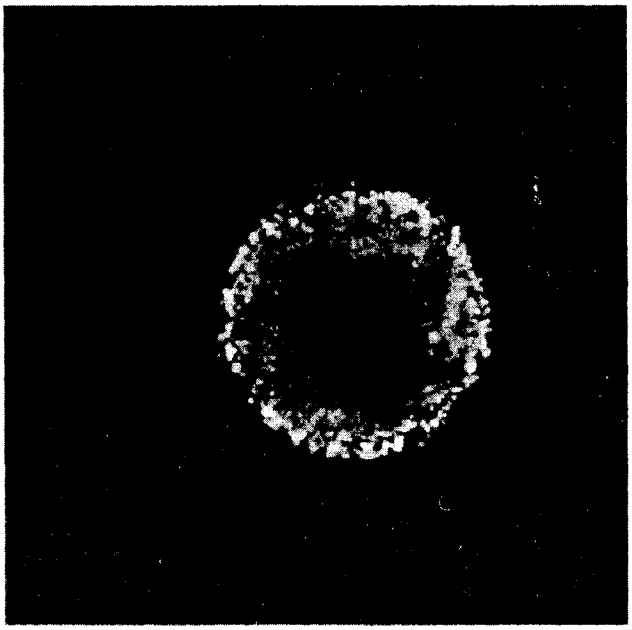

Fig. 5. Photograph realised with a confocal microscope of microspheres made of MPOE5K-PLA 45K labeled with 1-pyrenemethanol.

The confocal analysis showed that the fluorescent signal formed a ring around the microsphere surface (Fig. 5) and so proves that the MPOE chains have migrated towards the surface during the process. There is also a weak fluorescent signal in the rest of the particle corresponding to the MPOE chains that have not migrated and are distributed homogeneously inside the matrix. The preparation of microspheres made from MPOE-PLA45K amphiphilic copolymers by a emulsion-solvent evaporation process leads to a separation of the hydrophilic and hydrophobic chains and to a preferential location of the MPOE segments at the microsphere surface. The particles are coated by a MPOE layer that stabilizes the emulsion and subsequently the microspheres.

The use of a potential toxic surfactant such as PVA can be avoided by taking advantage of the amphiphilic properties of the MPOE-PLA45K copolymers. Moreover, Shakesheff *et al.* (21) showed the problem of competition between PVA and the MPOE chains for the occupation of the surface during the preparation of particles by the emulsion-solvent evaporation process. Therefore, the absence of surfactant in the process should lead to a more homogeneous MPOE coating of the particles. This coating should exhibit a greater efficiency in preventing the adsorption of protein or increasing the blood circulation half-lives of the carriers.

A surfactant is still required for the preparation of PLA microspheres. However, Carrio *et al.* (23) showed that surfactant-free PLAGA microparticles could be obtained in the case of short PLAGA polymers. Indeed, the latter exhibit amphiphilic property.

CONCLUSIONS

The use of PVA as surfactant for the preparation of microspheres by the emulsion-solvent evaporation process can be avoided in two different ways:

1. By using a MPOE5K-PLA0.5K surfactant. When ethyl acetate is used as the oil phase, this surfactant migrates at the O/W interface to form a stabilizing layer around the droplets and subsequently the particles. In accord with the model of the commercial "Pluronic" surfactants, the required behavior can be obtained by adjusting the HLB of the MPOE-PLA copolymers. The use of these copolymer is not restricted to the preparation of PLA and MPOE-PLA microparticles, but could be used whenever a biocompatible and bioeliminable surfactant is required for biological or medical applications.

The use of MPOE-PLA surfactant is also a new way to obtained a MPOE coating of PLA or PLGA hydrophobic particles, in order to prevent the protein adsorption or to increase the blood circulation half-lives of the carriers.

2. By taking advantage of the amphiphilic properties of MOE-PLA45K copolymers used to form the matrix of the microspheres. These amphiphilic properties are usually used to obtain a MPOE coating of the particles for the same reasons described previously. When particles made from MPOE-PLA45K copolymers are prepared by the emulsion-solvent evaporation process using ethyl acetate as the oil phase, MPOE chains migrate to the O/W interface to form a hydrophilic layer. This layer stabilizes the droplets and subsequently the particles, even in the case of short hydrophilic segment (2,000 $g.mol^{-1}$). The absence of surfactant in the process should lead to a homogeneous coating of the particles. Surface properties will not be affected by residual amounts of adsorbed surfactant. Thus, the MPOE coating should be more efficient in preventing the protein adsorption and increasing the blood circulation half-lives of the carriers. In the same way, the use of a potential toxic surfactant such as PVA is avoided.

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