Novel Bioresorbable and Bioeliminable Surfactants for Microsphere Preparation

P. Bouillot, V. Babak, and E. Delacherie

Received June 12, 1998; accepted September 15, 1998

**Purpose.** The objective of this work is to prepare microspheres by the emulation-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 (< 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 MPOESK-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) PLAs, poly(lactide-co-glycolide) PLGA, poly(caprolactone), poly(anhydride), poly(orthoesters), 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 (monomethoxy-polyoxyethylene 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 biodegradable and biocompatible surfactant to use instead of PVA to prepare microspheres by an emulation-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 long PLA chains (~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 PLAs 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
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 synthesized 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-pyrenemethanol. 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 CDCl₃ 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 HPSEC 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-T330 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 ≤ 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