Polylactide Microparticles Prepared by Double Emulsion/Evaporation Technique. I. Effect of Primary Emulsion Stability

Nicole Nihant,[1] Chantal Schugens,[1] Christian Grandfils,[1] Robert Jérôme,[1,2] and Philippe Teyssié[1]

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The process of microencapsulation of proteins by double emulsion/evaporation in a matrix of polylactide (PLA) can be divided into three successive steps: first, an aqueous solution of the active compound is emulsified into an organic solution of the hydrophobic coating polymer; second, this primary water-in-oil emulsion (w/o) is dispersed in water with formation of a double water-oil-water emulsion (w/o/w); third, the organic solvent is removed with formation of solid microparticles. This paper focuses on the effect of primary emulsion stability on the morphology and properties of polylactide microparticles loaded with bovine serum albumin (BSA) used as model drug. Depending on the stability of the primary emulsion, the internal structure of microparticles can be changed from a multivesicular to a matrix-like structure. Similarly, the average porosity can be controlled in a range from a few tenths of a micron to ca. 20 to 30 microns. This morphology control could find potential applications not only for the controlled drug delivery but also for the production of microporous particles intended for some specific applications, such as cell culture supports and chromatographic matrices. Although, the interplay of several processing parameters (polymer precipitation rate, polymer coprecipitation with interfacial compounds such as protein or surfactant, stirring rate, . . .) may not be disregarded, this study also indicated that a high loading of a hydrophilic drug can only be expected from a stable primary emulsion. When the stability of the primary emulsion is such as to prevent formation of macropores (>10 μm), the total pore volume is close to that of the originally dispersed aqueous drug solution.

KEY WORDS: microencapsulation; double emulsion–evaporation; emulsion stability; interfacial tension; protein encapsulation; polylactide; microparticles.

INTRODUCTION

Microencapsulation techniques have been developed to prepare microparticulate sustained drug delivery systems. Selection of the microencapsulation technique is primarily determined by the solubility of the drug in comparison with the coating polymer which is usually dissolved in an apolar organic solvent. For instance, evaporation of the organic solvent of an oil-in-water (o/w) emulsion is a method appropriate to the encapsulation of lipophilic drugs within a hydrophobic matrix (2–3). In contrast, water soluble drugs are easily immobilized in a hydrophobic polymer by either a non-aqueous phase separation technique (4–7) or the evaporation of the volatile solvent of the internal phase of an oil-in-oil (o/o) emulsion. In the latter case, drug is dispersed in the coating polymer solution, which is then emulsified in a continuous mineral oil phase (8).

More recently, a technique based on a water-oil-water (w/o/w) double emulsion has been proposed as an alternative method for the encapsulation of hydrophilic drugs. An aqueous solution of the active compound is emulsified into an organic solution of the hydrophobic coating polymer. This primary water-in-oil emulsion (w/o) is then dispersed in water with formation of a double water-oil-water emulsion (w/o/w). The organic solvent is finally removed with formation of solid microparticles. This microencapsulation process was patented in 1970 by Vrancken and Claeys (9) and de Jaeger and Tavernier in 1971 (10). Further, Kitajima and Kondo showed that the double emulsion/evaporation technique was suitable for immobilization of highly labile molecules, such as enzymes (11). Subsequently, enzymes and corrosion inhibitors were encapsulated within polystyrene microspheres (12). Ogawa *et al.* were the first to use the double emulsion technique to prepare an injectable poly(lactide-co-glycolide) microparticle dosage form for the sustained release of leuprolide acetate (13).

Until now, only one study by Alex and Bodmeier (14) has focused on the mechanism of microparticle formation from a double emulsion, showing that drug loading, porosity and surface morphology strongly depend on the way the coating polymer is precipitated. The purpose of this paper was to investigate the effect of the primary emulsion stability on the morphology and associated properties of polylactide microparticles loaded with bovine serum albumin (BSA) used as a model polymeric drug.

EXPERIMENTAL PART

Materials

Poly (D,L)-lactide [(D,L) PLA] was supplied by Boehringer Ingelheim, who reported an inherent viscosity of 0.9 dl/g (without disclosing the solvent and temperature used for the viscosity measurements). Mean number molecular weight (Mn) was estimated to be 51,000, with a polydispersity (Mw/Mn) of 1.95 by size exclusion chromatography (SEC) in THF at 30°C on the basis of a calibration with polystyrene standards, using a Hewlett Packard chromatograph 1090.

Methylene chloride (Merck, p.a.) and indigo carmine (Merck, p.a.) were used as received. Poloxamer 188, or Pluronic F68 (M.M = 8300) was used as a nonionic surfactant in the primary emulsion. Poly (vinyl alcohol) (Mowiol VP 3-83: supplied by Hoechst, Germany, with the following specifications: 17 mol % of vinyl acetate units and $\bar{M}w$ = 18,000) was chosen as a stabilizer for the second emulsion. Bovine serum albumin (BSA, Sigma A-7906) was the model drug encapsulated in the microparticles.

Methods

Microencapsulation Techniques

An Ultraturrax (IKA, T25) was used to emulsify 1 ml of an aqueous solution of BSA into 5 g of a polylactide solution

[1] Center for Education and Research on Macromolecules (CERM), University of Liège, Sart-Tilman B6, 4000 Liège Belgium.
[2] To whom correspondence should be addressed.

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in methylene chloride. The coating polymer solution concentration was 9 wt %. This w/o primary emulsion was stabilized by Pluronic F68. For the sake of comparison, the emulsion was also prepared without any surfactant. This primary emulsion was then dispersed under stirring into water (100 ml) containing 2.5 wt % polyvinyl alcohol (or PVA). A four-pitched blade impeller (rod diameter: 6 mm, blades 8 × 20 mm, pitched at 45°) was used at 800 rpm. The temperature was first maintained at 0°C for half an hour, then increased up to 20°C and kept constant for two hours in order to remove the solvent. Mircoparticles were finally collected by filtration, washed with water and lyophilized.

The effect of three parameters on the stability of the primary emulsion and the morphology of microparticles was investigated: (i) Concentration of BSA (0 to 3 wt %). (ii) Concentration of Pluronic F68 in the organic phase (0–10 wt %). The effect of this surfactant was analyzed in the presence of 0 and 0.5 wt % BSA in the aqueous phase. (iii) Concentration of poly(vinyl alcohol) in water: 0.1, 2.5 and 10 wt %.

Stability of the Primary Emulsion

Stability of the primary emulsion consisting of water dispersed within the polylactide solution in CH_2Cl_2 was estimated from:

(i) Demixing rate. The primary emulsion was prepared in an assay tube fitted with a rubber septum. The time required for initial macroscopic phase separation to occur was measured at room temperature.

(ii) Interfacial tension (IT). The Wilhelmy plate method (Prolabo-Tensiometer) was used at 25°C. Interfacial tension was measured between aqueous solutions of BSA (0.25, 0.5 and 1 wt % BSA) and CH_2Cl_2, on the one hand, and between water and polymer solutions (1 wt %) in CH_2Cl_2 containing various amounts of Pluronic F68 (0.5, 1 and 5 wt %), on the other hand. The interfacial tension between an aqueous solution of BSA (0.5 wt %) and organic solutions of Pluronic F68 (0.5, 1 and 5 wt %) was also measured.

Characterization of Microparticles

Dry microparticles were first metallized (Au-Pd sputtering; Balzers, SCP-20) and then examined by scanning electron microscopy (Jeol JSM-840 A Technics Co, Ltd, Tokyo). The pore volume and pore size distribution were measured by mercury intrusion porosimetry [Porosimeter 2000, Carlo Erba].

RESULTS AND DISCUSSION

Compared to the traditional w/o and o/w emulsions, preparation of stable w/o/w emulsions is a challenge. Stability of these emulsions is a critical requirement, even when the organic solvent is evaporated rapidly enough for the system to be frozen with formation of well-defined microparticles. Structure of the w/o/w emulsion when the polymer starts to precipitate from the organic phase is expected to dictate size and morphology of the final microparticles. In reference to the work by Florence and Whitehill (15), three main types of w/o/w emulsions have been identified, which are expected to give rise to microcapsules (Fig. 1A), multivesicular structures (Fig. 1B) and microspheres (Fig. 1C), respectively.

Several approaches have been proposed to improve the stability of w/o/w emulsions, such as gelation of the internal or external aqueous phase in the presence of a polymerizable surfactant (16). An alternative method is the noncovalent association of the surfactant with a polymeric compound dissolved in the internal aqueous phase (17).

Stability of the Primary Emulsion

Stability of the primary emulsion is a prerequisite for the successful stabilization of a multiple emulsion and the loading of a large amount of drug within the solid microparticles. Monitoring of the demixing process characterizes the emulsion stability, through measurements of the time required for creaming, sedimentation, flocculation or coalescence of the emulsion. This study focuses on the stability of a primary w/o emulsion stabilized by either BSA in the inner-aqueous phase, or Pluronic F68 in the organic phase, or a combination of the two. In the latter case, the Pluronic F68 concentration in the oil phase was increased while keeping constant the BSA concentration (0.5%) in water. BSA proved to be an efficient surfactant, and therefore, the emulsion stability was studied in relation to the BSA content. Pluronic F68 was first selected as a surfactant because of its known biocompatibility (18), although a high HLB (HLB = 29) is not very favourable to the formation of a primary water-in-oil emulsion. Thus, combining two surfactants might yield primary emulsions of a wide range of stability and features of the final microparticles.

Figure 2 shows how the phase separation time depends on the surfactant concentration. In the absence of any additive, phase separation is immediate, suggesting that the coating polymer has no interfacial activity. Stability of the water-in-oil emulsion is dramatically enhanced when small amounts of BSA are dissolved in the inner phase. Indeed, no phase separation is observed for three days when the BSA concentration is higher than 0.25 wt %. In spite of its hydrophilic character, Pluronic F68 decreases the phase separation rate, although it is much less efficient than BSA. In addition to an intrinsic interfacial activity, the beneficial effect of Pluronic F68 on the emulsion stability might also be due to the viscosity of the oil phase. This viscosity has been measured for solutions of PLA, Pluronic F68 and (PLA + Pluronic F68) solutions (10 wt % in each compound) in CH_2Cl_2 and found to be 47, 7 and 111 cP, respectively. The sharp increase in viscosity that occurs upon the addition of Pluronic F68 to the PLA solution is expected to contribute to

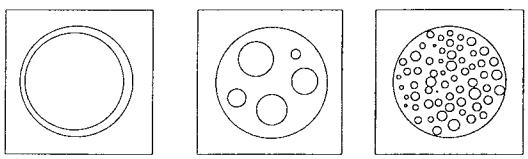

Figure 1: Sketch of the main types of internal morphology for microparticles prepared from w/o/w emulsions. A: microcapsule; B: multivesicular structure; C: microsphere (matrix-like structure).

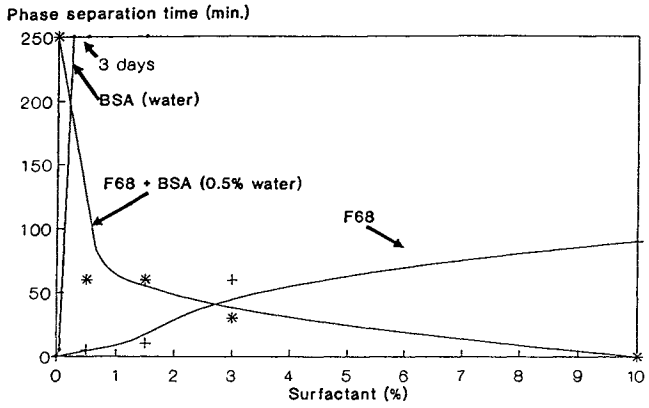

Figure 2: Time required for a phase separation to occur: effect of BSA content in the inner aqueous phase; effect of Pluronic F68 content in the organic phase; effect of Pluronic F68 content in the organic phase with 0.5 wt % of BSA in the inner aqueous phase.

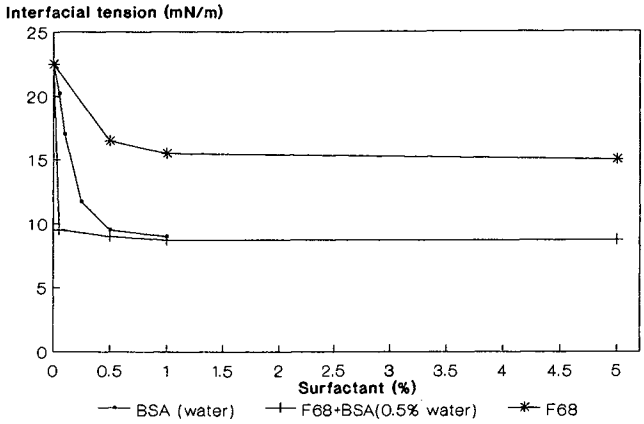

Figure 3: Water/CH_2Cl_2 interfacial tensions vs. concentration of: a. BSA in water (wt %); b. Pluronic F68 in CH_2Cl_2 (wt %); c. Pluronic F68 in CH_2Cl_2 (wt %), when 0.5 wt % BSA is dissolved in water.

the emulsion stability. This viscosity effect likely results from a favorable PLA-Pluronic F68 interaction, as it will be reported in a forthcoming paper (19).

When keeping the BSA concentration in water constant (0.5 wt %), an increasing concentration of Pluronic F68 in the oil phase has a detrimental effect on the emulsion stability (Fig 2). This observation results from an interfacial complexation of the two amphiphilic components (19). As a rule, these differences in the emulsion stability have been confirmed by the time dependence of the droplet size distribution of the primary emulsion (20).

Interfacial Tensions

Although the interfacial tension has a strong influence on the water-in-oil emulsification process, it is only one of the parameters that control the stability of the final emulsion. Nevertheless, measurement of the interfacial tension assesses the fate of a surfactant at an interface, e.g. complexation with, or displacement by, one of the compounds dissolved in one phase of the emulsion. It also allows one to compare the affinity of various surfactants for a specific interface.

A preliminary experiment focused on the possible activity of the coating polymer at the interface when dissolved in the oil phase. At a concentration smaller than 2 wt %, poly (D,L) lactide has no significant effect on the water/methylene chloride interfacial tension (22.5 mN/m, at 25°C). Addition of the protein into the aqueous phase was the next step to simulate formulation conditions. A semi-liquid continuous film forms immediately at the interface, which prevents any reliable measurement of the interfacial tension to be made. It is the reason why interfacial tensions have been measured in the absence of poly (D,L) lactide in the oil phase. According to figure 3, the interfacial activity of BSA is much higher than that of Pluronic F68. Indeed a very small concentration of BSA in water promotes a sharp decrease in the H_2O/CH_2Cl_2 interfacial tension. These experimental data agree qualitatively with the superiority of BSA over F68 in stabilizing H_2O/CH_2Cl_2 emulsions. However the interfacial tensions reported in figure 3 cannot account for the detrimental effect of F68 on emulsions stabilized by 0.5 wt % BSA, since the interfacial tension is essentially unaffected by the addition of Pluronic F68.

This apparent contradiction can be reconciled if the emulsion stability is assumed to be promoted by preferential interactions between BSA and poly (D,L)-lactide at the interface with formation of a semi-liquid film. Further, Law *et al.* (21) have reported that a series of surfactants of the poloxamer type, including Pluronic F68, are able to interact with BSA (i.e. a polyelectrolyte) through hydrophobic interactions and hydrogen bonding. Thus, an interfacial complexation of BSA and the coating polylactide occurs quickly and provides the water-in-oil emulsion with a high stability. When Pluronic F68 is added into the oil phase, it migrates to the interface and displaces polylactide chains which are then prevented from interacting with BSA and forming a semi-liquid phase at the interface. Competitive interactions between Pluronic F68 and polylactide for BSA will be thoroughly studied in the future.

Morphological Characteristics of Microparticles Stemming from w/o/w Emulsions, in Relation to the Surfactant Used in the Primary Emulsion

When microparticles stem from a w/o/w double emulsion, characteristics of the primary emulsion are critical, particularly when the organic solvent is highly volatile or partially miscible with the continuous phase. Indeed, the primary emulsion morphology foreshadows that one of the final particles, which are formed rapidly after the emulsification of the primary emulsion in water. The internal structure was probed by scanning electron microscopy of microparticle cross-sections. The internal porosity was also measured by mercury porosimetry.

When BSA is used as a surfactant in the primary emulsion, the internal morphology dramatically changes with the BSA concentration in the aqueous phase, i.e. with stability and phase size of the primary emulsion. At BSA contents smaller than 0.25 wt %, when demixing occurs within three days, the inner structure of the particules is very porous (figure 4). The mean size of the pores is then of the same magnitude as that of the dispersed phase of the primary emulsion. Figure 5 reports the pore size distribution in rela-

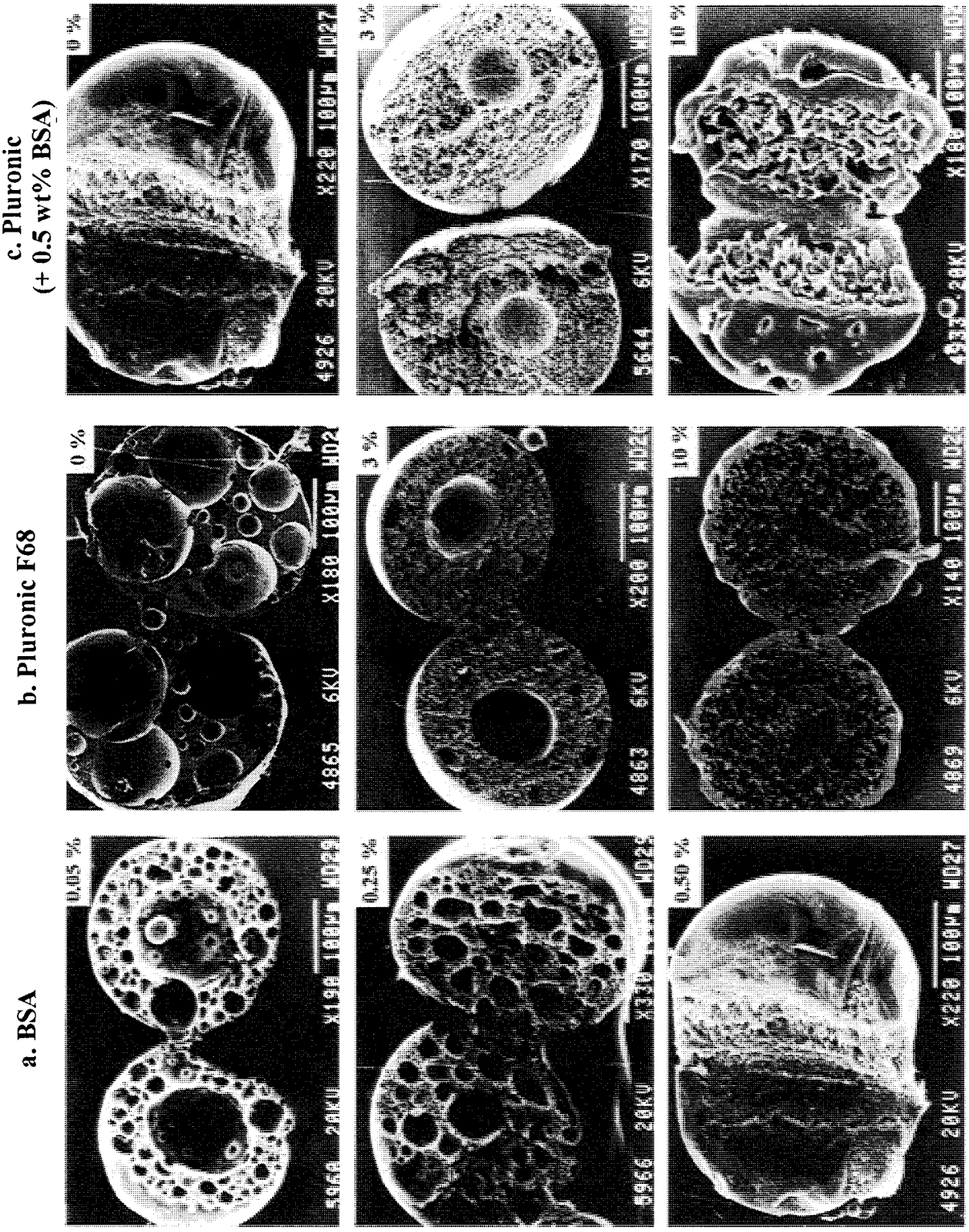

Figure 4: SEM of microparticles stemmed from a primary emulsion stabilized by: a. BSA in water (wt %); b. Pluronic F68 in CH_2Cl_2 (wt %); c. Pluronic F68 in CH_2Cl_2 (wt %), when 0.5 wt % BSA is dissolved in water.

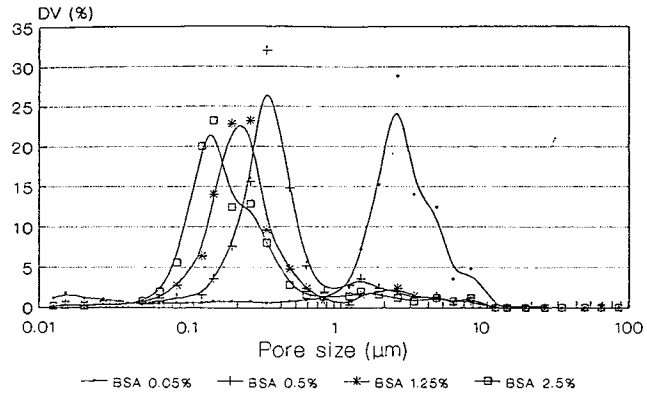

Figure 5: Pore size distribution of microparticles prepared from a double w/o/w emulsion. Effect of the BSA concentration used as a surfactant on the primary emulsion.

tion to BSA concentration in water, and supports the effectiveness of BSA as an emulsifier. The average pore size is shifted down from 10 microns to a tenth of micron when the BSA concentration is increased from 0.05 to 2.5 wt %. All the experimental observations are in agreement with an internal morphology dictated by the stability of the primary emulsion. The microparticle structure is indeed rapidly changing as the amount of BSA is increased from multivesicular spheres to microspheres, the porosity of which is of a few tenths of micron. SEM pictures also show that the microparticle surface is apparently homogeneous and smooth in all the cases, i.e. whatever the internal morphology. This contrast between the surface characteristics and the internal morphology suggests that the second emulsification step is not significantly dependent on the stability of the primary emulsion and the phase separation that occurs shortly after.

The nonionic surfactant, F68, is less effective than BSA in stabilizing the primary emulsion. Demixing of the emulsion is observed within 24 hours at the highest Pluronic F68 concentration. The internal morphology of the related microparticles as observed by SEM (Fig 4) is again closely related to emulsion stability. At the lowest F68 concentration, microparticles are highly porous and the macroporosity tends to decrease progressively as the concentration of the surfactant increases. However, even at a 10 wt % concentration of Pluronic F68 in CH_2Cl_2, the morphology is not yet that of a finely porous matrix as it is observed when the primary emulsion is stabilized by 0.5 wt % BSA in water.

Pore volume and average pore radius were calculated from porosimetry data, collected with an equipment that cannot detect pores larger than 10 μm. Table I shows that the pore volume is high and the average pore size is small for stable primary emulsions. Since the total pore volume is primarily dictated by the volume of the encapsulated aqueous phase of the primary emulsion, the encapsulation effiency is expected to change parallel to the final pore volume, all the other characteristic features being the same. Thus, if a drug is dissolved in the water droplets, the drug loading is expected to be as high as the primary emulsion is stable. On the basis of the double emulsion composition, a total pore volume of 2 cm^3/g can be calculated for a 100% encapsulation yield. Table I shows that this pore volume is

Table I. Pore Volume (cm^3/g) and Average Pore Radius (μm) of poly (D,L) Lactide Microparticles, Measured by Mercury Porosimetry. Effect of the Nature and Content of Surfactant Used in the Primary Emulsion

Content of F68 % w/w (CH_2Cl_2)	Pore volume (cm^3/g)	Average radius (μm)
0	0.88	1.49
0.5	1.50	1.01
3	1.56	0.33
10	1.50	0.21
Content of BSA % w/w (water)		
0	0.88	1.49
0.05	1.72	1.38
0.5	2.33	0.20
1.25	1.90	0.13
2.5	2.40	0.09

reached and sometimes exceeded when BSA is the emulsifier. A pore volume larger than the theoretical value might be due to a partial entrapment of the organic solvent in the microparticles. In the presence of F68, the pore volume does not exceed ca. 1.50 cm^3/g, which suggests an encapsulation efficiency of 75% at best. It will be reported elsewhere that the protein immobilization yield actually changes with the primary emulsion stability and the related microparticles porosity.

Finally, the addition of increasing amounts of Pluronic F68 to primary emulsions stabilized by 0.5 wt % BSA in water completely perturbs the microparticle morphology. According to figure 4, the originally spherical shape is deformed and the matrix-like internal morphology disappears in favor of a multivesicular structure. All these observations support a direct relationship between the stability of the primary emulsion and the final structure of the microparticles.

In the future, attention will be paid to the possible relationship between the internal morphology of microparticles, and drug content and kinetics of drug release. Furthermore, a detailed study will be performed to understand the interfacial events (e.g. polymer precipitation) that occur upon the emulsification process.

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