Microencapsulation by Coacervation of Poly(lactide-co-glycolide)—II: Encapsulation of a Dispersed Aqueous Phase

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Abstract: This paper is related to the phase separation of different copolyesters of lactides and glycolide solutions induced by the addition of silicone oil in order to promote microencapsulation of proteins. This coating process can be divided in three successive steps: phase separation of the coating polymer; adsorption of the coacervate droplets around the drug phase, and microcapsule solidification. This paper focuses on the physico-chemical analysis of the second step. The knowledge of the interfacial tensions between the three liquid phases allows understanding of the microscopic evolution of the phase separation medium.

Key words: microencapsulation, coacervation, surface tension, interfacial tension, polylactide, polylactide-co-glycolide, protein encapsulation.

INTRODUCTION

During the last decade, increasing attention has been paid to synthetic aliphatic polyesters for biomedical applications. Some of these polymers exhibit a set of valuable properties, i.e. absence of toxicity for living organisms, resorption after an appropriate period of implantation time and good mechanical properties.[1]

Polymers and copolymers of lactide and glycolide are known to be hydrolytically unstable and to release non-toxic by-products. Depending on their nature, composition and molecular weight, they offer a large range of biodegradability.[2] Because of this unique combination of biocompatibility and biodegradability, these polyesters have found applications in surgery (biodegradable sutures, artificial skins, resorbable prosthesis),[3–5] chemotherapy,[6,7] and galenic formulations.[8,9]

The ring-opening polymerisation of lactides (LA) and glycolide (GA) is a very convenient and well-controlled pathway to the related polyesters, in contrast to the traditional step polymerisation (polycondensation) method.[10,11]

Among the techniques used to encapsulate drugs in (co)polyesters of LA and GA, coacervation is well described in the patent and scientific literature.[12,13] It consists of a phase separation induced by the addition of the coating polymer solution with a constituent able to decrease the polymer solubility.[14–16] Two liquid phases thus appear: the polymer-rich coacervate and the supernatant, which is the major phase and is very poor in polymer. Accordingly, a drug initially dispersed in the polymer solution, can be encapsulated by the polymeric component.

Microencapsulation actually proceeds through three main steps: phase separation of the coating polymer; adsorption of those coacervate droplets around the drug phase; and the microcapsule solidification.

The first step has been investigated in detail in the authors' laboratory, as reported in a previous paper.[17] Composition and viscosity of the separate phases have been measured and discussed in relation to the nature and molecular characteristics of the coating polyester and the

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phase separation promoter (silicone oils). This paper focuses on the second step of the microencapsulation process, i.e. coating of a dispersed phase by coacervate droplets. In addition to microscopic observations,[18-20] measurements of the interfacial tension between coacervate, supernatant and a previously dispersed aqueous phase have been carried out. Although the knowledge of the interfacial tensions is of prime importance to control the encapsulation, the measurements have been either just suggested[21,22] or scarcely performed[23] in the literature.

Independently, Torza and Mason[24] have proposed a theory which allows some predictions to be made about phase morphology of a phase-separated ternary liquid system. Again, the basic assumption is that the interfacial tensions between the three coexisting phases play a major role. Nevertheless, the relative viscosity of the phases is also expected to control, at least kinetically, the phase situation. The volume fraction of the dispersed phase to be coated may also have an effect on the quality of the final microencapsulation.[25]

In order to check these parameters, desolvation of three copolyesters of LA and GA (PLGA 50/50, PLGA 75/25 and PLA) in methylene chloride has been analysed. Phase separation of polymer solutions has been induced by the addition of an incompatible polymer, i.e. silicone oils of various viscosities: 1000, 500 and 200 m.Pa.s. An aqueous solution of a proteolytic enzyme, α-thrombin, has been previously dispersed in the polymer solution in order to be coated. The encapsulation has been visualised by optical microscopy and related as much as possible to the experimental values of surface and interfacial tensions characteristic of the three-phase systems.

EXPERIMENTAL

Materials

Three (co)polyesters of (D,L) LA and GA were supplied by Boehringer Ingelheim, i.e. PLGA 50/50 Resomer" (RG505), PLGA 75/25 Resomer" (RG756) and (D,L)PLA

Resomer" (R206). PLGA were thus copolyesters, the LA/GA molar ratio of which was either 50:50 or 75:25.

Inherent viscosities were supplied by Boehringer as $0.07 \, m^3/kg$, $0.08 \, m^3/kg$ and $0.09 \, m^3/kg$, respectively, although solvent and temperature were undisclosed. Molecular weight ($\bar{M}_n$) was estimated by size exclusion chromatography. A Hewlett-Packard 1090 gel permeation chromatograph was used in tetrahydrofuran, at 30°C. It was calibrated with polystyrene standards. Experimental values of $\bar{M}_n$ were 20 000, 25 000 and 51 000, respectively. Silicone oils of various viscosity grades (200, 500, 1000 m.Pa.s) were purchased from Dow Corning (Cie. Com. Mat. Prem., Antwerp, Belgium).

Methylene chloride (Merck, p.a.) was used as solvent for the polyesters, and indigo carmine (Merck, p.a.), as a dye selectively soluble in the dispersed aqueous phase. α-Thrombin (T4648) was supplied by Sigma as a powdery lyophilisate.

Methods

Microscopic observations. This method has already been reported[14-17] for establishing the (co)polyester/silicone oil/methylene chloride (CH_2Cl_2) phase diagrams.

An Erlenmeyer flask fitted with a rubber septum was partially filled with a polymer solution (8% w/w) in CH_2Cl_2. A saturated aqueous solution of indigo carmine (150 μl) was then dispersed in the organic solution (25 g) under stirring. Silicone oil was added stepwise (1 ml aliquots) to the polymer solution in order to induce phase separation. After each addition, a sample was withdrawn from the Erlenmeyer and observed by optical microscopy (Leitz–Orthoplan) at 25°C. The encapsulation efficiency was estimated from the extent of the coating of the dispersed aqueous phase by the coacervate.

Measurements of surface and interfacial tensions. Surface tensions of polymer solutions (0.5%; 1%; 2% w/w) and silicone oil solutions (0.1%; 0.5%; 1% w/w) in CH_2Cl_2 and interfacial tensions of these solutions against water,

TABLE 1. Relative composition of the coacervate and supernatant phases as recovered after coacervation and dilution with methylene chloride

Systems		Polymer (%, w/w)		Silicone oil (%, w/w)		CH_2Cl_2 (%, w/w)	
		Coacervate	Supernatant	Coacervate	Supernatant	Coacervate	Supernatant
PLGA 50/50	—SO200	1	0.01	0.04	1	98.96	98.99
	SO500	1	0.01	0.11	1	98.89	98.99
	SO1000	1	0.01	0.15	1	98.85	98.99
PLGA 75/25	—SO200	1	0.01	0.04	1	98.96	98.99
	SO500	1	0.01	0.11	1	98.89	98.99
	SO1000	1	0.01	0.20	1	98.80	98.99
PLA	—SO200	1	0.01	0.09	1	98.91	98.99
	SO500	1	0.01	0.08	1	98.92	98.99
	SO1000	1	0.01	0.14	1	98.86	98.99

were measured at 25°C using the Wilhelmy plate method (Prolabo-tensiometer). Coacervate and supernatant phases, previously diluted with CH_2Cl_2, were characterised by the same method. When interfacial tensions were measured, pure water was used instead of an aqueous solution of the protein. Otherwise, a very thin film was systematically formed at the interface, which prevented the interfacial tension from being readily measured. This film more likely results from the interfacial coprecipitation of the coating polyester and the protein.

In order to avoid any deleterious effect of an exceedingly high viscosity on the measurements of surface and interfacial tensions, the separated coacervate and supernatant phases were diluted with methylene chloride until down to a 1% (w/w) polymer concentration.

Compositions of the coacervate and supernatant were analysed elsewhere[17] and are reported in Table 1.

Each surface and interfacial tension was measured at least three times: the standard deviation never exceeded 0·5 mN/m. Before each measurement the glass vessel was cleaned with a sulphochromic mixture and thoroughly rinsed with milli Q water (Millipore Corp., MA, USA).

RESULTS

Microscopic observations

As reported elsewhere,[14,17] the morphological evolution of the (co)polyester phase separation process depends on the (co)polymer composition and the coacervate viscosity. Four steps are clearly identified in the desolvation process of PLGA 50/50. More precisely, during the two first steps of the PLGA desolvation, coacervate droplets are of low viscosity, they coalesce and burst rapidly. The third step is referred to as the 'stability window' and is most appropriate for microencapsulation of a previously dispersed aqueous phase. Beyond the 'stability window' (step 4), the coacervate is highly viscous and an extensive aggregation is observed to occur, ultimately leading to polymer precipitation.[17] Transition from one step to the next one is rather diffuse for the more hydrophobic PLGA 75/25 compared to PLGA 50/50. When PLA itself is concerned, no characteristic step in the desolvation can be identified: actually, whatever the amount of silicone oil added to the PLA solution, the coacervate droplets are typically unstable and never get adsorbed onto the dispersed aqueous phase (Fig. 1(b)). In contrast, at any ternary composition in the 'stability window' of the phase diagram of PLGA 50/50 and PLGA 75/25, the coacervate droplets are very stable and the dispersed aqueous phase in uniformly coated (Fig. 1(a)).

Surface and interfacial tension measurements

Polymer solutions. Dissolution of each of the three (co)-polyesters under investigation does not significantly alter the surface of dichloromethane (33·1 mN/m). Indeed, in

(a)
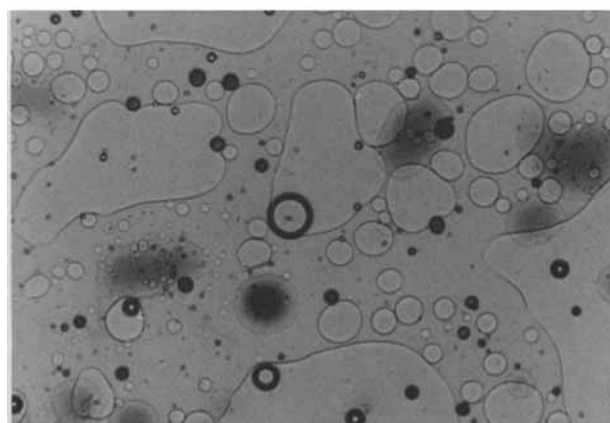
(b)

Fig. 1. Microscopic observations of (a) PLGA 50/50 'stability window' and (b) PLA.

the concentration range from 0·5 to 2% w/w, a surface tension of 32·5 mN/m is measured. Surface tension of dichloromethane is slightly decreased by the addition of a silicone oil, but in a manner which does not depend on the oil concentration, at least in the range of 0·1–1% (w/w) (~30 mN/m).

In contrast to surface tensions, interfacial tension of the (co)polyester solutions in CH_2Cl_2 against water significantly decreases as the hydrophobicity of the (co)polyester decreases (thus from PLA to PLGA 50/50) (Fig. 2(a)). Accordingly, the less hydrophobic PLGA 50/50 strongly interacts with water and the reverse is true for PLA. However, the interfacial tensions are observed to be independent of the polyester concentration, at least in the investigated range.

On the other hand, interfacial tension of silicone oil solution in CH_2Cl_2 against water is independent of molecular weight and concentration of the silicone oil (Fig. 2(b)). They are lower than the interfacial tensions of PLA solutions against water but higher than the values reported for the PLGA 50/50–water system. It is finally observed that the PLGA 75/25–water interface displays the same interfacial tension as that of the silicone oil–water interface.
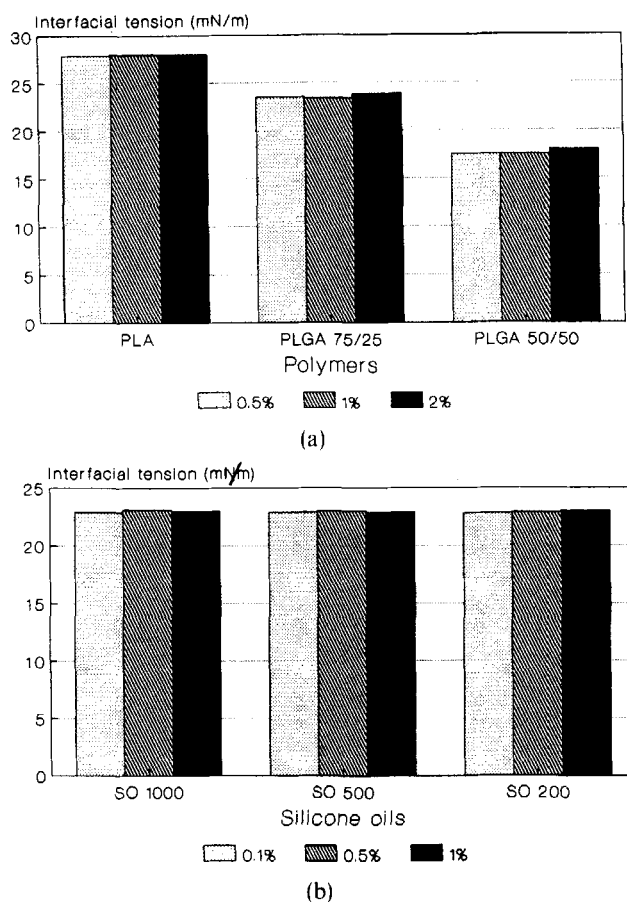
Fig. 2. Interfacial tensions of water against (a) (co)polyesters solutions in CH_2Cl_2, and (b) silicone oils solutions in CH_2Cl_2.

Fig. 3. Interfacial tensions of water against (a) coarcervate solutions in CH_2Cl_2 and (b) supernatant solutions in CH_2Cl_2.

Coacervate and supernatant phases. Surface tension of both the previously diluted coacervate and supernatant solutions (1%) (30 mN/m) is the same as the surface tension of silicone oil in CH_2Cl_2 (0·1–1% w/w). Although the weight concentration of the silicone oil is very small in the coacervate phase (<0·2% w/w), this compound has a marked effect on the CH_2Cl_2–air interface compared to the (co)polyester. Similarly, surface tension of the supernatant is dictated by the silicone oil and unaffected by small amounts of the polyester.

There is also a perfect analogy with the results of Fig. 2(a), when the interfacial tensions of the coacervate solutions against water are considered. They decrease from PLA to PLGA 50/50 (Fig. 3(a)), whereas the interfacial tension of the supernatant solutions against water does not depend on the (co)polyester used (Fig. 3(b)). It must be concluded that the presence of a silicone oil in the coacervate does not change the interfacial tensions of the (co)polyester solution against water (see Figs 1(a) and 2(a)). The same is true for the presence of the (co)polyester in the supernatant phase (Figs 2(b) and 3(b)).

DISCUSSION

Before attempting to interpret the interfacial tension data, it is worth reviewing briefly the theory developed by
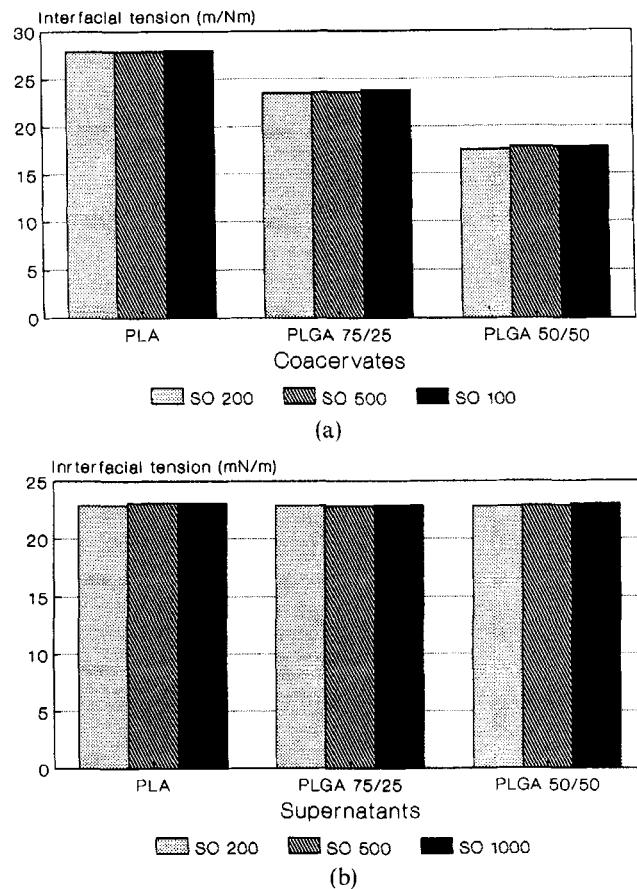
Torza and Mason.[24] The phase separation of the (co)polyester solutions in CH_2Cl_2 in the presence of a dispersed aqueous phase leads to the coexistence of three immiscible liquid phases, i.e. the aqueous phase (a), the coacervate (c) and supernatant (s).

The basic assumption of the theory is that the final equilibrium is dictated by the interplay of the interfacial tensions ($\sigma_{a,c}$; $\sigma_{s,c}$; $\sigma_{a,s}$) characteristic of each interface. Gravity, fluid motion and viscosity are supposed to have no effect on the equilibrium. According to Torza and Mason,[24] the relative values of the spreading coefficients (S) (eqns (1)–(3)) have a decisive effect on the phase morphology at the equilibrium.

$$S_a = \sigma_{s,c} - (\sigma_{a,s} + \sigma_{a,c}) \tag{1}$$

$$S_s = \sigma_{a,c} - (\sigma_{a,s} + \sigma_{s,c}) \tag{2}$$

$$S_c = \sigma_{a,s} - (\sigma_{s,c} + \sigma_{a,c}) \tag{3}$$

Figure 4 schematises the expected situation in relation to the spreading coefficients, i.e. the complete coating of a by C (Fig. 4(a)), the partial coating of a by C (Fig. 4(b)) and the lack of coating (Fig. 4(c)). The fourth phase configuration is mathematically impossible (Fig. 4(d)).

Obviously, configuration A is the ideal situation for the encapsulation of the dispersed aqueous phase by the coacervate. Equilibrium A actually requires a small
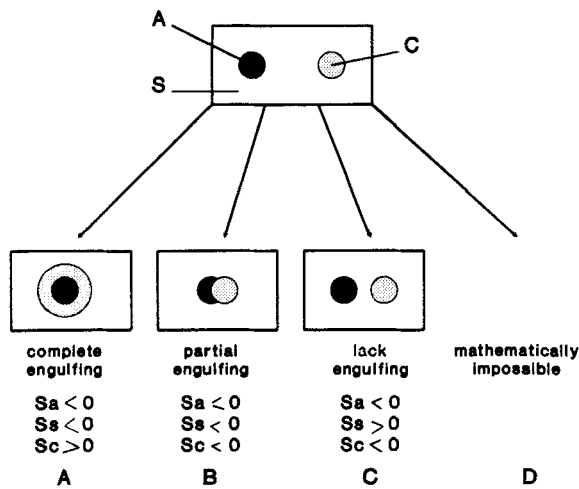
Fig. 4. Possible phase configurations at equilibrium.

enough coacervate/aqueous phase interfacial tension $(\sigma_{a.c})$, a high supernatant aqueous interfacial tension $(\sigma_{a.s})$ and $\sigma_{a.s} > \sigma_{s.c}$. The latter condition seems to be reasonable since the supernatant is partially miscible with the coacervate but not with water. In order to verify if such a model described by Torza and Mason[24] can be extended to the present coacervation procedure, the authors have measured interfacial tensions that exist in this ternary system.

Figure 5 shows the dependence of $\sigma_{a.s}$ and $\sigma_{a.c}$ on the nature of the coating (co)polyester for a common silicone oil (1000 m.Pa.s). Changing the viscosity of the silicone oil does not change the basic information provided by Fig. 5. Very clearly, when the hydrophobicity of the coating polyesters decreases, $\sigma_{a.c}$ diminishes in contrast to $\sigma_{a.s}$ which remains constant. As a result, $\sigma_{a.c}$ is higher than $\sigma_{a.s}$ when PLA is concerned, while $\sigma_{a.c} \simeq \sigma_{a.s}$ for the PLGA 75/25 copolyester. Finally, $\sigma_{a.c}$ is smaller than $\sigma_{a.s}$ when the lower hydrophobic PLGA 50/50 copolyester is the coating polymer.

By reference to Fig. 3, the coacervate comprising PLGA 50/50 and a silicone oil is thus expected to engulf
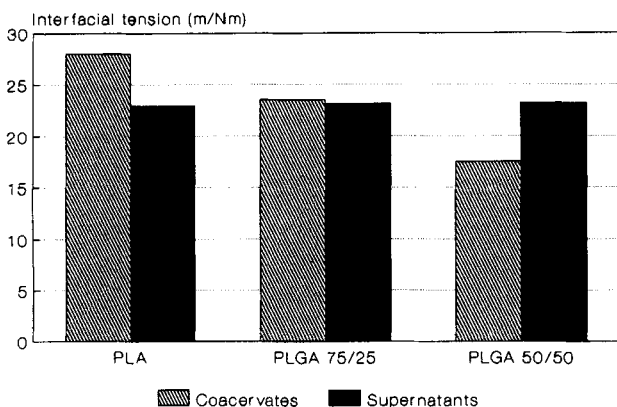

Fig. 5. Interfacial tensions of water against the 1% coacervate $(\sigma_{a.c})$ or supernatant $(\sigma_{a.s})$ phases, respectively (silicone oil of 1000 m.Pa.s).

the dispersed aqueous phase. Microscopic observations are in a complete agreement with this prediction. The aqueous dispersed phase is indeed regularly coated with coacervate droplets of PLGA 50/50.

When PLA is concerned, the relative values of the interfacial tensions allows the anticipation of a preferential coating of the aqueous phase by the supernatant rather than by the coacervate as actually observed by optical microscopy (Fig. 1). Finally, when PLGA 75/25 is the coating polymer, coacervate and supernatant are expected to compete for encapsulating the aqueous phase. It is the reason why the location of the stability window in the phase diagram is uncertain compared to the PLGA 50/50-silicone oil-CH_2Cl_2 system.

The experimental observations given in this paper give credit to the theoretical model proposed by Torza and Mason[24] which emphasises the key role of the interfacial tensions. It must be outlined that dissolution of a protein in the aqueous solution might deeply perturb the situation reported in this paper, because of the well-known surface activity of proteinic solutes.[26] As will be detailed in a forthcoming paper, the morphology of the phase separated ternary systems has a deep effect on the structural features (porosity etc.) of the final microcapsules, as well as on the fate of immobilised proteins.

CONCLUSIONS

Microencapsulation of drugs by coacervation ideally proceeds through three successive steps, i.e. polymer phase separation, adsorption of the coacervate on the dispersed aqueous phase and solidification of the microcapsules. The first step has been investigated, and characteristics of the phase-separated solutions of copolymers of lactide and glycolide in methylene chloride in the presence of a silicone oil have been analysed and reported elsewhere.[17]

As a follow-up to that preliminary study, encapsulation of a previously dispersed aqueous phase by the coacervate has been studied in this paper. The encapsulation efficiency was evaluated by optical microscopy, and these experimental observations supported by the relative values of the interfacial tensions characteristic of the three phase systems formed by coacervation. Clearly, this interfacial parameter is of the utmost importance for a successful microencapsulation process. It must, however, be pointed out that both coacervate and supernatant phase have been diluted to 1% solutions before surface and interfacial tensions are measured. As interfacial tensions between oil and water phases do not change when polymer concentration in the oil phase was ranged between 0·5 and 2%, it can be expected that this interface is saturated relatively to the coating polymer. The same remark is true for the silicone oil. Based on these considerations, increase in polymer concentration in the oil phases should only modify physico-chemical characteristic of the system other than its interfacial properties,

as such relative viscosity of the different phases. Within the limits of this restriction, experimental data support the theoretical predictions developed by Torza and Mason for the behaviour of three coexisting immiscible liquid phases.[24]

Accordingly, an effective encapsulation requires at least that the coacervate–aqueous phase interfacial tension be lower than that of the supernatant–aqueous phase interface. A higher interfacial tension of the supernatant against water compared to that of the supernatant–coacervate is also a favourable factor.

However, a more complete analysis should also take into account the possible influence of the relative viscosity of the phases on a non-equilibrium phase situation prevailing when microcapsules are prepared.

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