Microencapsulation by coacervation of poly(lactide-*co*-glycolide): 1. Physico-chemical characteristics of the phase separation process

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This paper describes the phase separation of different lactide and glycolide copolyester solutions, induced by the addition of silicone oil in order to promote protein microencapsulation. The phase diagrams of the ternary CH_2Cl_2–copolyester–silicone oil systems were established in relation to the composition of the copolyester and the viscosity, i.e. the molecular weight, of the silicone oil. The phase-separated systems were characterized in terms of weight, volume, composition and viscosity of the coacervate and supernatant. The effect of the nature of the coating polymer and the molecular weight of the coacervation agent (silicone oil) on the characteristics of the phase-separated system is discussed.

(Keywords: microencapsulation; coacervation; phase diagrams)

INTRODUCTION

The use of synthetic polymers in the medical field is rapidly and steadily increasing. The first application of these polymers was in the design of prostheses[1–3]. More recently, these materials have contributed to the reconstitution of some organs and to the formulation of drugs[4–6]. In this regard, major efforts have been made in order to control drug delivery. Critical requirements must, however, be fulfilled for a polymer to be used in biomedical applications, of which biocompatibility, in particular absence of toxicity, is the most important. Biodegradation is an additional advantage when the polymer must be removed after an appropriate period of implantation inside a living organism[7,8].

Polylactides and polyglycolide are accordingly of great interest since they are hydrolytically unstable and release only non-toxic byproducts. Polymers and copolymers of lactides and glycolide are thus widely used in medicine as biodegradable sutures, artificial skin, resorbable prostheses and as part of galenic formulations[9–11]. Moreover, ring-opening polymerization of lactides and glycolide provides a direct route to the related polyesters, in contrast to the traditional step-polymerization of lactic and glycolic acids[12,13].

Microencapsulation, i.e. coating of active compounds by a polymer, is currently used to produce galenic devices for controlled release of drugs[14]. This paper deals with coacervation as a microencapsulation method[15]. Coacervation is a phase-separation process which can occur when a polymer solution is added to a compound, decreasing the polymer solubility[16]. Two liquid phases accordingly appear: a polymer-rich phase, referred to as the coacervate, in equilibrium with a dilute polymer solution, i.e. the supernatant. When coacervation is promoted under well-controlled conditions, adsorption of the coacervate droplets onto the surface of a predispersed active drug can occur. Although coacervation is a well-known microencapsulation method, no basic relation has been clearly established as yet between the main physico-chemical characteristics of the phase-separating system and the final characteristics of the microspheres. In order to fill this gap, a model system has been considered which consists of methylene chloride as solvent, random copolymers of lactide and glycolide (poly(lactide-*co*-glycolide), PLGA) of various compositions (PLGA 50/50, PLGA 75/25, and polylactide, PLA) as coating material, and silicone oils of various viscosities as the coacervation promoter. Coacervate droplets are finally hardened by pouring the ternary mixture into a non-solvent of the coating, i.e. heptane[17,18].

In this paper, the phase diagrams of the ternary CH_2Cl_2–copolyester–silicone oil systems will be established in relation to the composition of the copolyester and the viscosity, i.e. the molecular weight, of the silicone oil. The phase-separated systems will be characterized in terms of weight, volume, composition and viscosity of the coacervate and supernatant. The effect of the nature of the coating polymer and the molecular weight of the coacervate agent (silicone oil) on the characteristics of the phase-separated system will be discussed.

EXPERIMENTAL

Materials

The coating polyesters were supplied by Boehringer (Ingelheim, Germany): PLGA 50/50 Resomer^R, PLGA

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75/25 Resomer^R, and (D,L)PLA Resomer^R (50/50 and 75/25 refer to the wt/wt% of D,L-lactide and glycolide in the copolyester). Inherent viscosities provided by the suppliers are 0.7 dl g^{-1} (PLGA 50/50), 0.8 dl g^{-1} (PLGA 75/25) and 0.9 dl g^{-1} ((D,L)PLA) (solvent and temperature not specified).

Molecular weights (M_n) were estimated by g.p.c. in tetrahydrofuran at 25°C (polystyrene calibration) and were found to be 20000, 25000 and 51000 for PLGA 50/50, PLGA 75/25 and (D,L)PLA, respectively. The polydispersity was 2.2 for the three polyesters.

Silicone oils (dimethylsiloxanes) with several viscosity grades (200, 500 and 1000 cSt), termed SO200, SO500 and SO1000, respectively, were obtained from Dow Corning and used as such.

Dichloromethane and indigo carmine, both of analytical grade, were supplied by Merck (Darmstadt, Germany).

Methods

Phase diagrams[19]. The coating polymer (2 g) was dissolved at 25°C in 25 ml of methylene chloride in an Erlenmeyer flask fitted with a rubber septum. A saturated solution (150 µl) of indigo carmine in water was then dispersed in the organic solution under magnetic stirring. Coacervation of the polyester was induced by stepwise addition of the silicone oil (1 ml aliquots). Progress of the phase separation was followed by observation of the ternary mixture by optical microscopy (Leitz-Orthoplan).

Weight and volume of the separated phases in equilibrium. A 2 wt% solution of the polyester in CH_2Cl_2 was added to a glass vessel covered by a lid with two holes (*Figure 1*). The central hole allowed a stirring screw to be connected to a driving motor (IKA type RW), and the four-pitched blade impeller was located a few millimetres above the bottom of the vessel.

The stirring rate was set at 200 turns min^{-1}. The silicone oil was added to the polymer solution through the second hole by using a polypropylene syringe. A tap was connected to the bottom of the glass vessel which was itself wrapped in a plastic bag and placed in a thermostated bath (20°C). When the appropriate amount of silicone oil had been added, the phase-separated system was quantitatively transferred under stirring into a calibrated centrifugation polypropylene tube* (Falcon) through the tap at the bottom of the vessel, avoiding, as much as possible, adsorption of the coacervate onto the glass walls. After centrifugation at 3000 rev min^{-1} for 10 min, the Falcon tube was weighed. The supernatant was then removed. The weight of the coacervate was determined by the difference between the weight of the tube plus coacervate phase and the weight of the empty tube. The phase volumes were measured from calibration lines marked on the polypropylene tube.

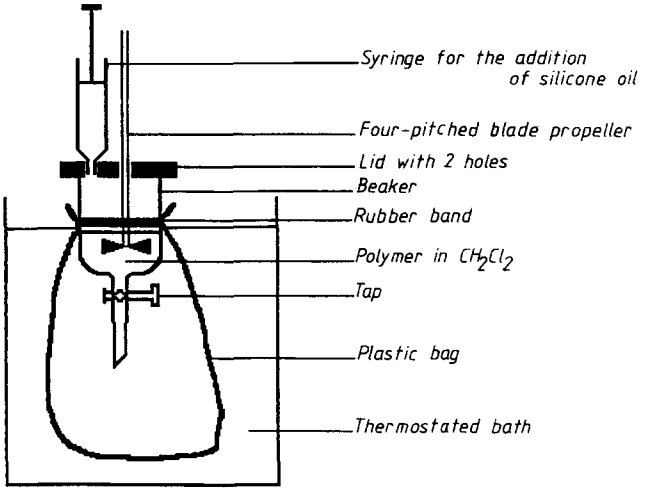

Figure 1 Experimental set-up

Chemical composition of the phases. After centrifugation, the supernatant phase and the coacervate were transferred into a previously weighed container.

To determine the weight of methylene chloride, the solvent was left to evaporate in a hood for 4 weeks at 25°C, and the weight loss was ascribed to CH_2Cl_2.

The polyester and silicone oil weight ratio was determined as follows. The ^{1}H n.m.r. spectra of the polyester and the silicone oil in $CDCl_3$ showed a well-resolved peak at $\delta = 1.53$ ppm, characteristic of the polyester (CH_3 of lactide units), and a peak at $\delta = 0.07$ ppm assigned to the CH_3 protons of the silicone oil. A 400 MHz Brucker apparatus was used (16 scans sample^{-1}) and various polyester/silicone oil mixtures of known composition were analysed in order to establish a calibration curve. The unknown samples (0.1 g) were then analysed by ^{1}H n.m.r. in $CDCl_3$ (1 ml). The relative intensity of the protons characteristic of the polymer and the silicone oil was reported on the calibration curve and the relative composition determined accordingly.

Viscosity of the coacervate. A cone/plate type Brookfield viscometer (HBTDV-IICP equipped with a no. CP-51 cone of 1.2 cm radius) was used to measure the viscosity of the coacervate (~1 ml on plate). Measurements were repeated at least three times for each sample.

RESULTS

Phase diagrams

A hydrophilic dye (indigo carmine) was selectively dissolved in the aqueous phase dispersed in the CH_2Cl_2 solution of the coating polyester in order to distinguish these two phases as clearly as possible. Upon addition of a silicone oil, phase separation occurs, the progress of which can be split into four successive steps, at least for favourable situations. (i) The addition of a relatively small amount of silicone oil gives the organic phase the aspect of a pseudo-emulsion. (ii) Increasing amounts of silicone oil induce clear phase separation. It must be stressed that these different situations do not correspond to thermodynamic equilibrium. Coacervate droplets can be observed, but are unstable and tend to coalesce into bigger entities which finally burst. (iii) When the amount of the added silicone oil is large enough, the dispersion becomes stable. The droplets adsorb onto the surface of the aqueous phase dispersion and surround it individually or tend to engulf many water particles. (iv) During the last step, the coacervate droplets extensively aggregate and precipitate.

* It was mandatory to use a polypropylene tube for the centrifugation of the phase-separated system since the coacervate droplets have a tendency to adsorb on glass walls, even after silanization[20]

The phase diagrams of the ternary CH_2Cl_2–polyester–silicone oil systems are reported in *Figures 2* and *3* for PLGA 50/50 and PLGA 75/25, respectively, in combination with three different silicone oils. The phase diagrams have been investigated in a limited composition range, since polymer solutions of a concentration smaller than 10 wt% have been reported to be most appropriate for the encapsulation process. Ruiz and Benoit[11] mentioned that well-defined microspheres are formed when the conditions for phase separation correspond to the third step (black zone in the phase diagrams), which they have referred to as the 'stability window'.

PLGA 50/50. Phase diagrams of PLGA 50/50 and silicone oils of various viscosities (200, 500 and 1000 cSt) are shown in *Figure 2*. It is obvious that phase separation occurs earlier when the viscosity, and thus the molecular weight, of the silicone oil increases. This is in complete agreement with the thermodynamic incompatibility of two polymers, which increases with the molecular weight of the associated partners.

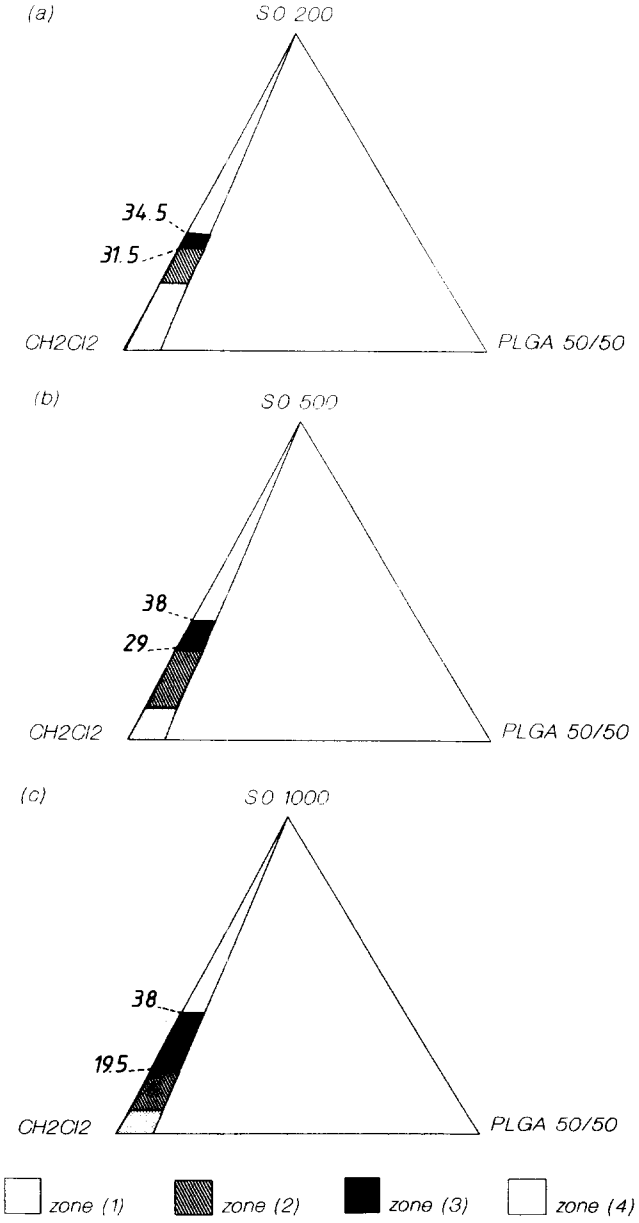

Figure 2 Phase diagrams of PLGA 50/50 using silicone oils with different viscosity as the phase inducer: (a) 200 cSt; (b) 500 cSt; (c) 1000 cSt

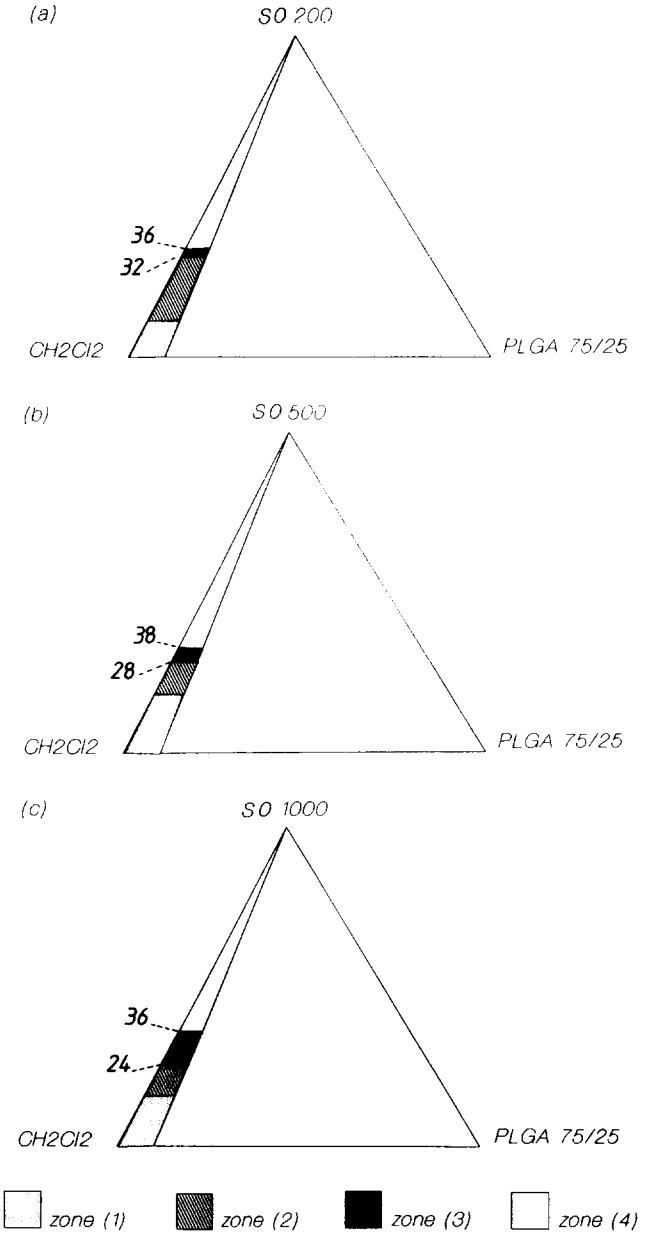

Figure 3 Phase diagrams of PLGA 75/25 using silicone oils with different viscosity as the phase inducer: (a) 200 cSt; (b) 500 cSt; (c) 1000 cSt

Table 1 Centre of the stability window for each silicone oil independent of the coating polyester

Viscosity of silicone oil (cSt)	Silicone oil (%)	Polymer (%)	CH_2Cl_2 (%)
200	33.1	1.3	65.6
500	31.4	1.35	67.25
1000	30	1.4	68.6

Interestingly, when the silicone oil is a more powerful desolvating agent, the stability window extends over a larger composition range. The composition of the central point of these windows is reported in *Table 1*.

PLGA 75/25. When the lactide content of the PLGA copolyester is increased from 50 to 75%, sharp transitions between the successive steps in the coacervation process are no longer observed. As a result, there is some

uncertainty as to the location of the four zones reported in the phase diagrams of *Figure 3*. Within the limits of accuracy, PLGA 75/25 appears to be more soluble in CH_2Cl_2 than the PLGA 50/50 counterpart; indeed the main desolvation steps of *Figure 3* are delayed compared to *Figure 2*. This could be related to an increase in the hydrophobicity of the copolyester when glycolide (–O–CH_2–CO–) units are replaced with lactide (–O–CH(CH_3)–CO–) units. The effect of the silicone oil viscosity is in qualitative agreement with that observed for PLGA 50/50.

PLA. The addition of increasing amounts of silicone oil to the PLA solution in CH_2Cl_2 does not allow well-defined steps to be distinguished in the desolvation process. It is thus clear that the coacervate has a decreasing tendency to form a continuous shell around the dispersed aqueous phase when the hydrophobicity of the coating polyester increases (from PLGA 50/50 to PLA). When PLA is used, the coacervate particles coalesce rapidly and form large entities containing numerous water droplets. In fact monolithic structures are formed rather than individual core–shell entities. As will be discussed in a subsequent paper[21] this modification is most likely related to the interfacial parameters between coacervate, water and supernatant.

Figures 2 and *3* also show that the composition at the centre of the stability window is nearly the same for PLGA 50/50 and PLGA 75/25 when the same silicone oil is used. It is worth pointing out that this particular composition of the ternary system has allowed non-aggregated microspheres to be produced not only for PLGA 50/50 and PLGA 75/25, but also for pure PLA. It should be kept in mind that the mode of agitation applied during phase-diagram establishment and microsphere preparation was different, so the contribution of the stirring rate during phase separation cannot be ruled out. On the basis of this observation, production of PLA microspheres is now feasible, although the stability window for that polyester has not been precisely identified. The experimental conditions most appropriate to microsphere formation for each silicone oil, whatever the coating polyester (centre of the stability window for the PLGA copolyesters), are reported in *Table 1*.

Figure 4 illustrates that well-shaped microspheres are only formed when the composition of the ternary system lies within the stability window.

Weights and volumes of the coacervate and supernatant

Tables 2 and *3* report the weight and volume, respectively, of the coacervate and supernatant which are formed at the ideal composition (*Table 1*) for microsphere formation. In agreement with *Tables 2* and *3*, *Figure 5* shows that volume and weight of the coacervate increase regularly from the PLGA 50/50–SO200 pair to the PLA–SO1000 combination.

Table 4 and *Figure 6* refer to the weight and volume of the coacervate and supernatant for the PLGA 50/50–SO1000 pair at various initial compositions of the

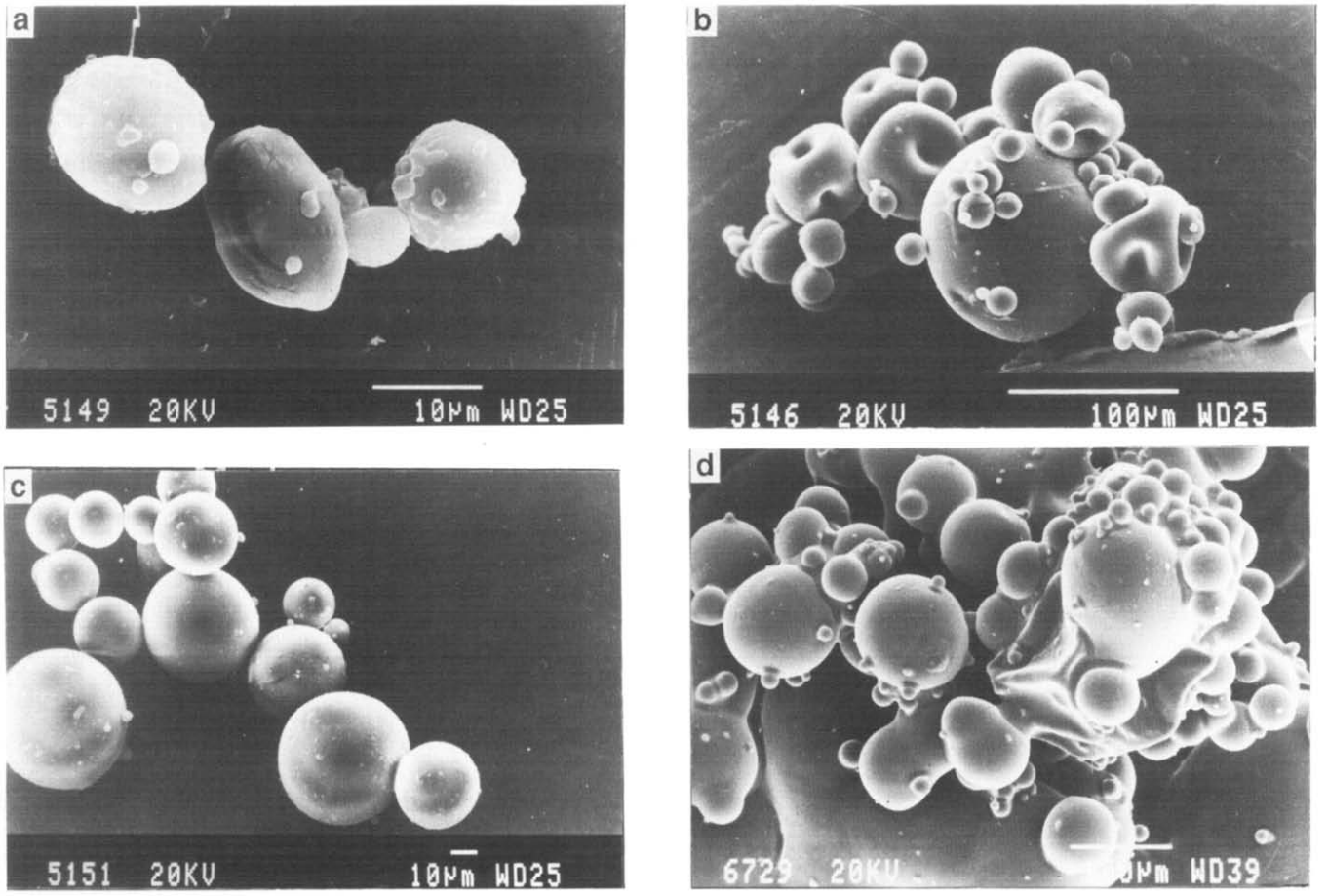

Figure 4 Morphology of microspheres according to the zone of the phase diagram: (a) beginning of step 2; (b) step 2; (c) stability window or step 3; (d) step 4

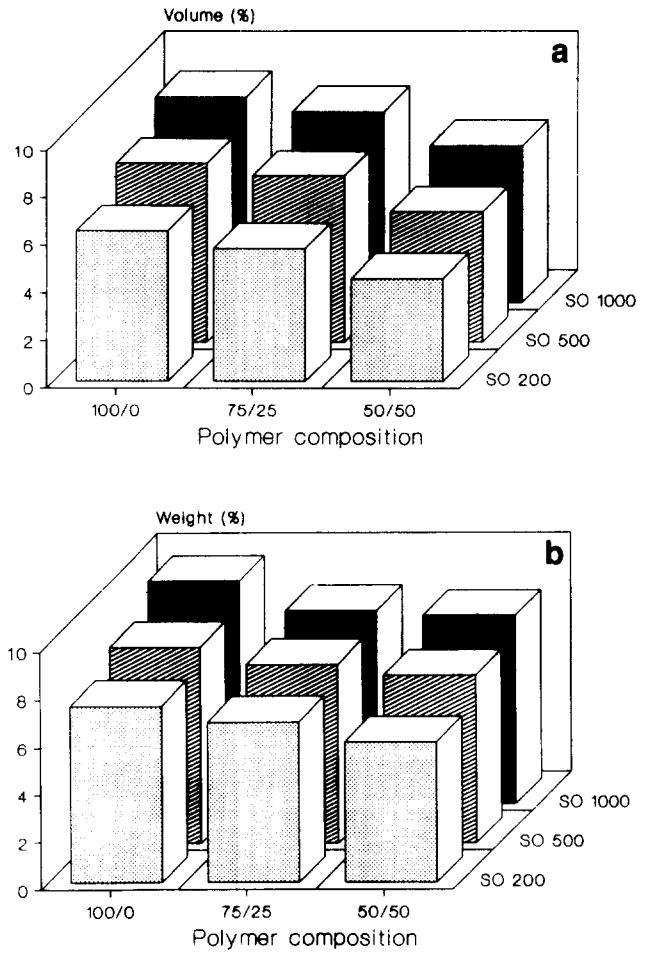

Figure 5 Volume (a) and weight (b) of the coacervate in the stability window according to the polymer–silicone oil system

Table 2 Coacervate weight (g)/supernatant weight (g) at the centre of the stability window

	PLGA 50/50	PLGA 75/25	PLA
SO200	3.3/51.7	3.7/51.3	4.1/51.0
SO500	3.8/49.8	4.0/49.5	4.4/49.1
SO1000	4.1/48.2	4.3/48.1	4.9/47.3

Table 3 Coacervate volume (ml)/supernatant volume (ml) at the centre of the stability window

	PLGA 50/50	PLGA 75/25	PLA
SO200	2.1/45.5	2.7/44.9	3.0/44.5
SO500	2.5/43.3	3.2/42.7	3.5/43.0
SO1000	3.0/41.6	3.6/41.3	3.9/40.9

Table 4 Weight and volume of the coacervate and supernatant for the PLGA 50/50–SO1000 pair at various initial compositions of the ternary system

	Beginning of step 2	End of step 2	Step 3	Step 4
Silicone oil volume (ml)	3.5	7.5	16.9	24
Coacervate weight (g)	9.4	6.2	4.1	3.4
Supernatant weight (g)	30.4	37.6	48.2	55.8
Coacervate volume (ml)	7.0	4.7	3.0	2.0
Supernatant volume (ml)	25	31	42	50

ternary system. The experimental data are consistent with a decrease in weight and volume of the coacervate when increasing amounts of SO1000 are added.

Chemical composition of the coacervate and supernatant

Although coacervate and supernatant have been separated from each other and recovered as quantitatively as possible, the total weight of the two phases is systematically smaller than the cumulative weight of the constituent compounds, although by less than 5%. It is also worth noting that the phase separation has been carried out in such a way that the coacervate might be somewhat contaminated by the supernatant, whereas residual supernatant might be left on the walls of the containers. It has also been observed that the polypropylene centrifugation tubes absorb a small amount of CH_2Cl_2. Since the weight of the coacervate is calculated from the weights of the empty tube and the tube filled with the coacervate (after centrifugation), an error in excess might be expected. As a result, the amount of the coating polyester in the coacervate is systematically in excess, since it is calculated from the relative percentage (determined by ^1H n.m.r.) of the polyester compared to the silicone oil in the previously dried coacervate. Due to these systematic errors in the chemical composition of the phases, the experimental data should be discussed only on a comparative basis.

Figures 7 and *8* illustrate the composition of the coacervate and supernatant, respectively, for the nine polyester–silicone oil pairs under consideration, as analysed in the stability window (see *Table 1* for the initial composition). As a rule, the quantity of methylene chloride in the coacervate increases when the silicone oil is more viscous and the coating polyester is more hydrophobic (from PLGA 50/50 to PLA). Only traces of silicone oil are reported whereas the quantity of the polyester is of the order of 20%, largely independent of the polyester–silicone oil pair. The amount of CH_2Cl_2 in the supernatant increases when the viscosity of the silicone oil decreases and when the polyester is less hydrophobic. This tendency is, of course, the opposite to that observed for the coacervate. The quantity of silicone oil in the supernatant also increases from SO1000 and SO200. Its dependence on the nature of the polyester is, however, unclear. The amount of polyester is negligible in that phase. Thus, the coacervate contains mainly solvent and coating polyester, although solvent and silicone oil are the main components of the supernatant.

Figure 9 shows that the amount of polyester increases and the relative amount of CH_2Cl_2 decreases in the coacervate as the phase separation proceeds. Simultaneously, the quantity of methylene chloride increases in the supernatant as well as the amount of silicone oil.

Coacervate viscosity

According to *Figure 10*, the viscosity of the coacervate recovered from the ternary systems, the initial composition of which is in the stability window (see *Table 1*), increases from the PLA–SO1000 pair to the PLGA 50/50–SO200 pair.

Table 5 supports the finding that the coacervate is more viscous as the amount of silicone oil in the CH_2Cl_2–PLGA 50/50–SO1000 ternary system increases.

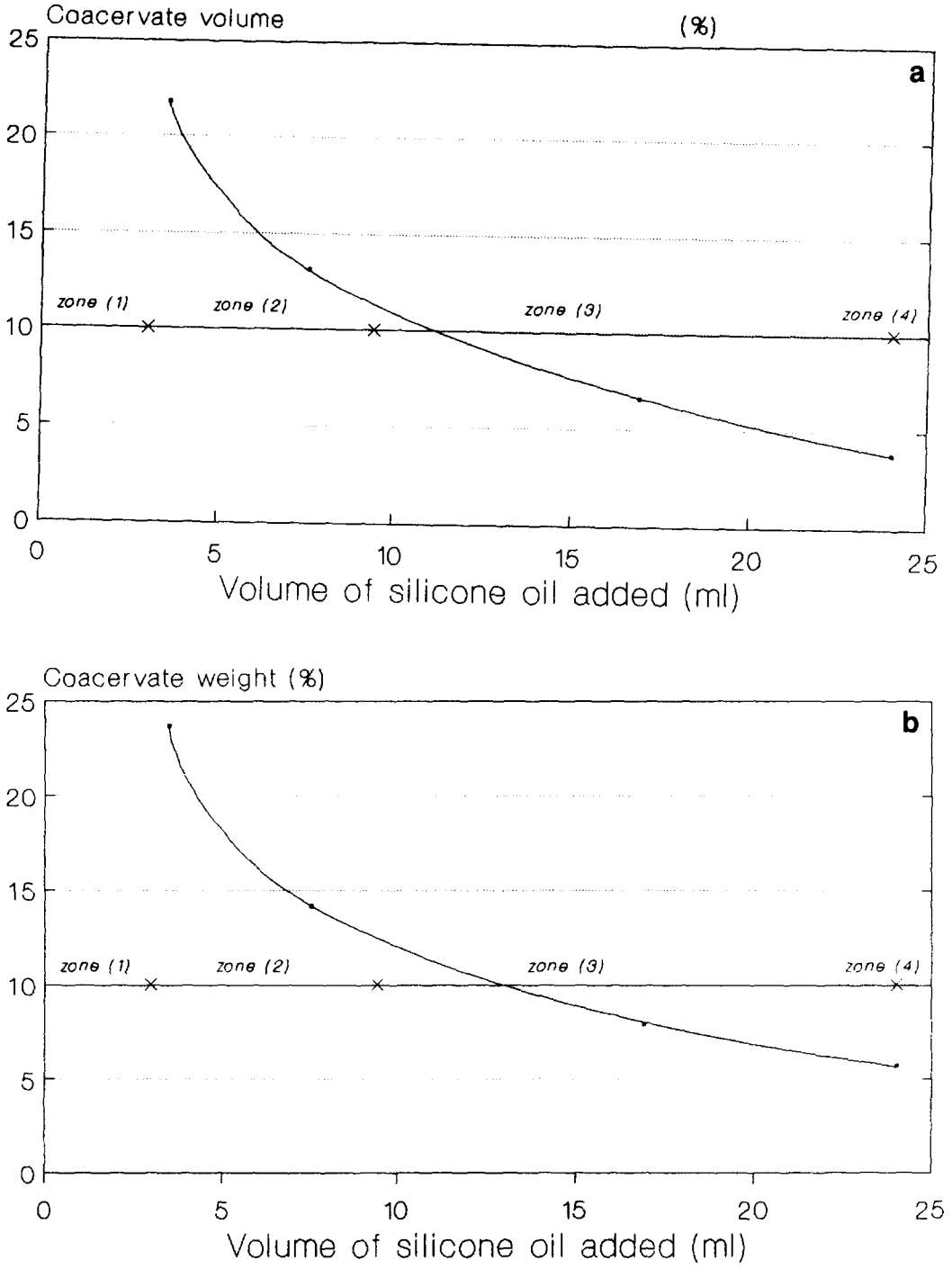

Figure 6 Volume (a) and weight (b) of the coacervate for the system PLGA 50/50–SO1000 according to the volume of silicone oil added

Table 5 Viscosity of the coacervate for the PLGA 50/50–SO1000 pair at various initial compositions of the ternary system

Phase diagram zone	Beginning of step 2	End of step 2	Step 3	Step 4
Volume of silicone oil (ml)	3.5	7.5	16.9	24
Viscosity of coacervate (cSt)	25	43	2070	4040

DISCUSSION

The phase diagrams of nine coating polyester–silicone oil–methylene chloride ternary systems have been prepared by observing the phase situation by optical microscopy when the polyester solution in CH_2Cl_2 (of up to 10% w/v) is progressively added with a silicone oil, i.e. a phase-separation inducer (*Figures 2* and *3*). The experimental observations are in complete agreement with those reported by Ruiz *et al.*[19] for poly(D,L-lactic acid-*co*-glycolic acid) copolymers with a composition close to 50/50 (lactic acid content from 56.4 to 50%), the phase inducer and the solvent being the same as in this study. Thus, a stability window has been identified which corresponds to a stable dispersion of coacervate droplets. This zone of the phase diagram has unique characteristics for preparing well-shaped and non-aggregated microspheres.

Three polyesters have been compared: they are polylactide chains containing 0, 25 and 50 mol% of

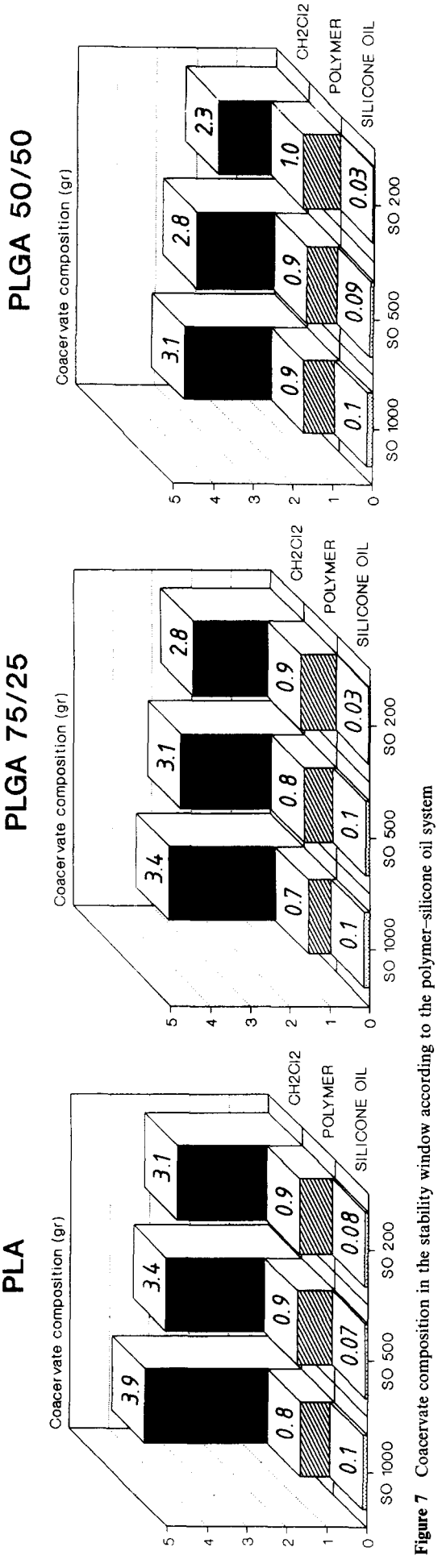

Figure 7 Coacervate composition in the stability window according to the polymer–silicone oil system

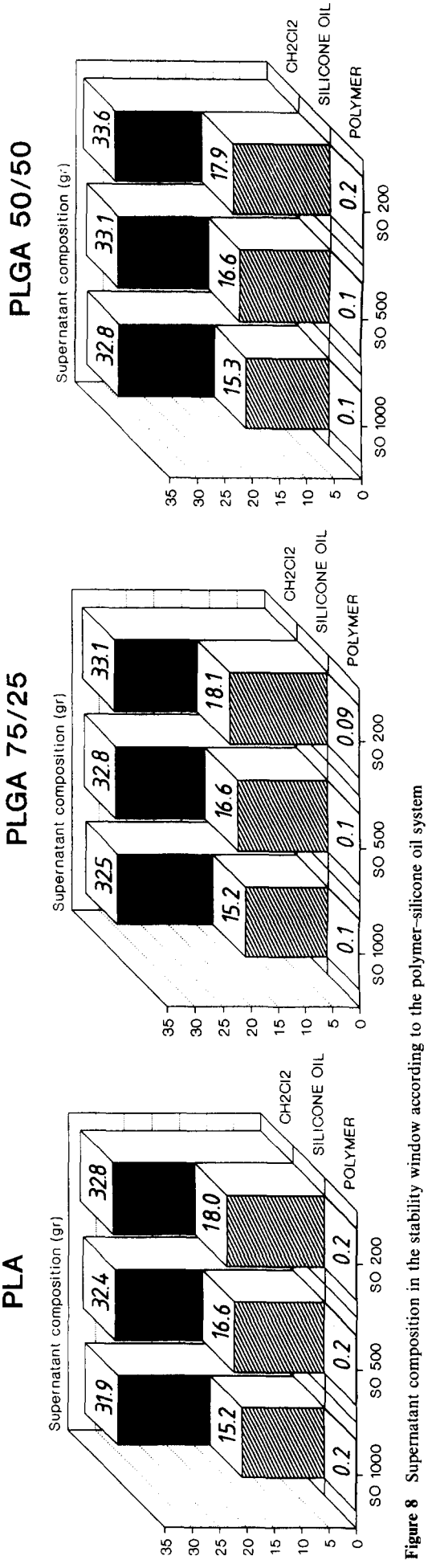

Figure 8 Supernatant composition in the stability window according to the polymer–silicone oil system

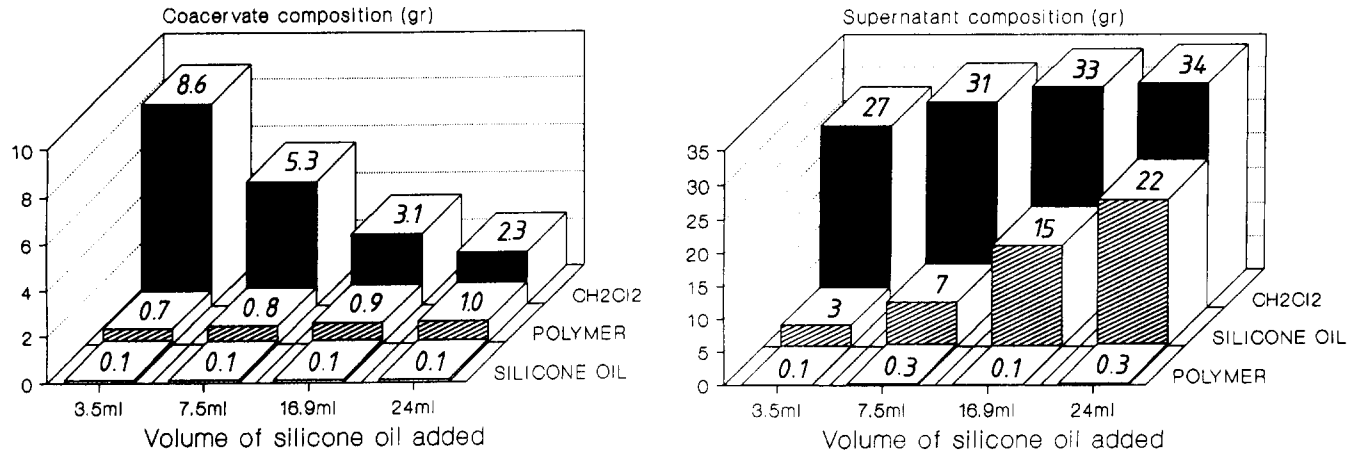

Figure 9 Composition of the coacervate and the supernatant for the system PLGA 50/50–SO1000 according to the volume of silicone oil added

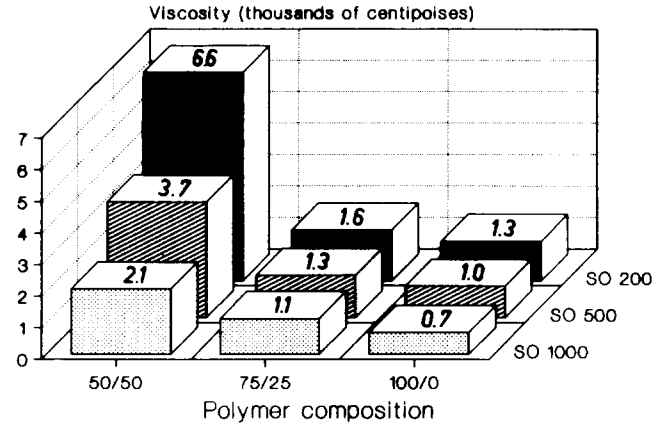

Figure 10 Viscosity of the coacervate

glycolic acid units distributed at random. With respect to the polyesters studied by Ruiz *et al.*, this series covers a much larger range of the hydrophobic side. The data published by Ruiz *et al.* suggested that the area of the stability window depended upon the polyester hydrophobicity, the largest surface being promoted by the polyester of the highest hydrophobic affinity. It should be pointed out, in contrast to the polymers used by Ruiz *et al.*[19], that the g.p.c. analysis profiles do not reveal the presence of oligomeric species in our coating materials. Therefore, their expected relative hydrophobicity can be mainly explored on their global chemical composition.

The experimental data concerning weight and volume of coacervate and supernatant phases confirm the decisive influence of the hydrophobicity of the coating polyester on the coacervation process induced by a silicone oil in CH_2Cl_2. When the polyester is more hydrophobic (from PLGA 50/50 to PLA), the weight and volume of the coacervate increase. The same evolution is noted when the silicone oil is more viscous (from SO200 to SO1000), i.e. when the molecular weight and the efficiency of the phase inducer increase. Thus, the highest weight and volume of coacervate correspond to the PLA–SO1000 pair, i.e. to the most solvated polyester and the silicone oil that has the weakest interaction with the solvent. As a consequence, the coacervate of the PLA–SO1000 pair is of lower viscosity.

In contrast, the ternary system based on the least solvated polyester (PLGA 50/50) and the oil that interacts most favourably with CH_2Cl_2 (SO200) gives rise to a coacervate of smaller weight and volume and of higher viscosity. When the PLGA 50/50 solution in CH_2Cl_2 is added with increasing amounts of a silicone oil, the coacervate is more desolvated, as it is supported by a decrease in weight and volume and an increase in viscosity (*Figure 6* and *Table 5*). The chemical composition of the coacervate shows that the percentage of the precipitated polyester increases with the amount of added silicone oil (*Figure 9*). This trend results from the amount of insolubilized polymer, which increases, and the solvation degree of the precipitated polymer, which decreases simultaneously. In agreement with these changes, the viscosity of the coacervate increases with the amount of phase-separation inducer. The seemingly monotonic changes in weight, volume and composition of the coacervate and supernatant phases cannot account, at least in a straightforward way, for the observation of distinct zones in the phase diagrams.

The experimental data of *Figures 2* and *3* clearly demonstrate that PLGA 50/50 leads to a larger stability window than PLGA 75/25, whatever the viscosity of the silicone oil. Finally, when the most hydrophobic PLA is concerned, no clear stability window is identified. Undoubtedly, and in contrast to the conclusions of Ruiz *et al.*[19], the less hydrophobic the lactide-based coating polymer, the larger the area of the stability window in CH_2Cl_2, at least when a silicone oil is the phase-separation inducer.

It is worth pointing out that the composition of the ternary system that corresponds to the middle of the stability window is practically independent of the lactide/glycolide molar ratio, at least in the 50/50 to 75/25 range. Interestingly, this 'ideal' composition for the production of microspheres of PLGA 50/50 and 75/25 has proved experimentally to be effective for PLA also, although no pertinent information could be derived from the phase diagram.

The width of the stability window is also observed to increase (*Figures 2* and *3*) with the viscosity of the silicone oil. This is in full agreement with results published by Ruiz *et al.*[19]. These authors argue that the viscosity of the continuous phase (i.e. the supernatant) should increase in parallel with the viscosity of the silicone oil, and contribute to the stabilization of the coacervate droplets. Although the viscosity of the supernatant was too low to be measured with a Brookfield viscometer,

Figure 8 shows that the quantity of CH_2Cl_2 and silicone oil in the supernatant does not change significantly with the grade of the oil. In agreement with the explanation by Ruiz *et al.*[19], the supernatant is expected to be more viscous when SO1000 is used instead of SO200. However, the viscosity of the supernatant phase does not appear to play a key role in the stability of the coacervate dispersion. Indeed, the composition, and thus the viscosity, of the supernatant is largely independent of the coating polyester, in contrast to the significant variation of the area of the stability window (*Figures 2* and *3*).

As a result, the viscosity of the separated phases should not be of determinant importance for the stability of the phase-separated system. That stability is more likely controlled by the coacervate/supernatant interfacial tension, which, as expected, depends on the nature of the coating polyester. The aqueous phase to be encapsulated has to be considered as well as the related coacervate/water and supernatant/water interfacial tensions. These characteristic features will be measured and related to the phase diagrams, in order to identify the experimental parameters that control the extent of the stability window. This still speculative view will be discussed in a forthcoming paper[21] and the influence of the data reported in this study correlated to the structural and morphological characteristics of the microspheres produced by coacervation in the stability window.

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