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Microencapsulation by coacervation of poly(lactide-co-glycolide) IV. Effect of the processing parameters on coacervation and encapsulation

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Abstract:

Attention has been paid to phase separation of poly(lactide-co-glycolide) solutions in CH_2Cl_2 induced by the addition of a silicone oil in order to promote protein microencapsulation. Since the process is very fast, the system is anytime out of equilibrium. The effect of the main processing parameters on the microencapsulation process has been analyzed and has highlighted that kinetics of the main encapsulation steps has a great effect on the characteristics of the final microspheres. These results have been discussed on the basis of a physico-chemical study of coacervation reported in previous papers of this series.

Keywords: Microencapsulation; Coacervation; Poly(lactide-co-glycolide); Microsphere; Protein encapsulation

1. Introduction

Poly (lactic acid) (PLA) and copolymers of lactic and glycolic acids (PLGA) are well-known biodegradable and histocompatible aliphatic polyesters. They are commonly used as biodegradable sutures [1-3] and they have more recently contributed to reconstruction of deficient or injured organs and to improved galenic formulations [4—6]. In this respect, they have been found suitable, in many instances, for the sustained release of drugs 'in vivo', for days or months.

Ring-opening polymerization of lactides and glycolide provides a direct access to the aforementioned polyesters [7,8]. In contrast to the traditional step-growth polymerization of lactic and glycolic acids, this polyaddition mechanism allows for the perfect control of the molecular characteristics of the final polymer, such as molecular weight, polydispersity and end-functionality.

During the last few years, several techniques for drug encapsulation have been developed, that currently use the aliphatic polyesters. A large variety of organic molecules have accordingly been encapsulated, that range from low molecular weight synthetic drugs to biological proteins [9]. Proteins are efficiently encapsulated by a modified solvent evaporation method based on double emulsions [10-12] and by a phase separation or coacervation process.

This paper focuses on the coacervation technique [13-15], which relies upon a decrease of the solubility of the coating polymer by addition of a third compound to the polymer solution in an organic solvent. The point has to be reached where two liquid phases are formed: the rich-in polymer coacervate and the supernatant liquid phase depleted in polymer. If a drug is initially dispersed in the polymer solution, it can be coated by the coacervate. As discussed in previous papers [16-18], microencapsulation by coacervation proceeds along three main steps: (1) phase separation of the coating polymer solution [16], (2) adsorption of the coacervate around the drug particles [17], (3) solidification of the microparticles [18]. The main characteristic features of these three steps have been analyzed elsewhere under standard conditions for the preparation of poly(lactide-co-glycolide) microspheres. A special attention has been paid to weight, volume, composition and viscosity of the coacervate and supernatant phases in connection with the size distribution, surface morphology and internal porosity of the final microparticles [18]. Since the phase equilibrium is never reached, the system is anytime out of equilibrium. Therefore, the processing parameters are expected to have a significant effect on the course of this three-step process and ultimately on the characteristics of the final microspheres.

The encapsulation technique has been implemented under the following conditions. Dissolution of a random equimolar lactide (LA)-glycolide (GA) copolyester (PLGA 50/50) in CH_2Cl_2. Addition of this coating polymer solution with an incompatible polymer: a silicone oil of several viscosities (1000,500 and 200 cSt). Aqueous solutions of α-thrombin or BSA, have been dispersed in the polymer solution prior to the addition of the silicone oil, in view of the encapsulation of these proteinic drugs. Compared to these conditions, the effect of four main processing parameters has been considered in this paper: (1) volume of the dispersed aqueous phase containing the drug to be encapsulated, (2) addition rate of the incompatible polymer to the coating polymer solution, (3) stirring rate of the dispersion, (4) drug to be encapsulated. Each of the experimental parameters has been modified independently, and its effect on the polymer coacervation and the characteristics of the final microspheres (size distribution, external morphology) has been analyzed.

2. Materials and methods

2.1. Materials

The coating polyester was supplied by Boehringer (Ingelheim, Germany), i.e. PLGA 50/50 Resomer® (RG 505). The 50 mol% composition was confirmed by NMR analysis. Inherent viscosity was provided by the supplier as 0.7 dl/g (PLGA 50/50), although solvent and temperature were undisclosed. Molecular weight (M_n) and polydispersity were measured by size exclusion chromatography (Hewlett-Packard 1090), in THF, at 30°C and found to be 20 000 and 2.2, respectively, by reference to a polystyrene calibration.

Silicone oils of several viscosity grades (200, 500, 1000 cSt) were purchased from Dow Corning (Seneffe, Belgium). Methylene chloride (p.a.) was used as solvent for PLGA 50/50, and indigo carmine (p.a.) as a dye selectively soluble in the dispersed aqueous phase. They were purchased from Merck (Darmstadt, Germany). Bovine Serum Albumin (A-7906), or BSA, and α-thrombin (T4648) were supplied by Sigma Chemie (Bornem, Belgium) as powdery lyophilisates.

2.2. Methods

Phase diagrams and microscopic observations [16]

2 g of PLGA 50/50 were dissolved in 25 g of methylene chloride at 25°C in a flask fitted with a rubber septum. Various volumes of a saturated solution of indigo carmine in water (150 µl, 300 µl, 600 µl, 1.5 ml and 3 ml) were dispersed in the organic solution under magnetic stirring. Phase separation of the polymer solution was induced by the stepwise addition of the 1000 cSt silicone oil (1 ml increments) and followed by observation by optical microscopy (Leitz-Orthoplan). The encapsulation efficiency was estimated from the extent of the coating of the dispersed aqueous phase by the coacervate.

Weight and volume of the separated phases

This method was reported elsewhere [16]. Briefly, 2 wt% solution of the PLGA 50/50 in CH_2C1_2 (1 g PLGA 50/50) was added into a glass vessel covered by a lid with two holes [12]. The central hole allowed a stirring screw to be connected to a driving motor (IKA type kw) (200 rpm) and the four-pitched blade impeller was located a few millimetres above the bottom of the vessel.

The 1000 cSt silicone oil was added to the polymer solution throughout the second hole at different rates (18; 5.7; 1.4; 0.65 ml/min). Coacervate and supernatant were separated by centrifugation at 3000 rpm in calibrated polypropylene (Falcon) tubes; weight and volume of each phase were measured [18].

Preparation of microspheres

This method was detailed elsewhere [18]. PLGA 50/50 (0.75 g) was dissolved in methylene chloride (37.5 g) in an Erlenmeyer flask fitted with a rubber septum. This solution was poured into a high beaker covered by a plastic sheet, thermostated at 20°C and stirred at 800 rpm. The aqueous solution containing BSA (7.5 mg) or α-thrombin (7.5 mg) (200 µl) was then added. After a few seconds, the stirring rate was decreased at 200 rpm and the (1000 cSt) silicone oil was added to the dispersion with a polypropylene syringe. Finally, the phase separated system was transferred into a high beaker containing 500 ml of heptane, thermostated at 20°C. The stirring rate was set at 300 rpm. After half an hour, the microparticles were washed with 500 ml of heptane and dried by

lyophilisation. These 'standard' conditions were systematically used in this study, excepted for the four aforementioned processing parameters that were changed independently.

Shape and surface of the microparticles were observed by scanning electron microscopy (Jeol JSM-840 A Technics Co, Ltd, Tokyo). Dry microcapsules were previously coated with a ca. 30 nm Au-Pd film under reduced pressure (Balzer, SCP-20).

Mean diameter and size distribution of the microparticles previously suspended in a saline buffer were analyzed with a Malvern Instrument (Mastersizer, S3:01; Worcestershire, U.K.).

3. Results and discussion

3.1. Effect of volume of the dispersed aqueous phase

The observation by optical microscopy of the microparticles prepared according to the standard coacervation technique [16,17] shows that a large part of them has failed to encapsulate the dispersed aqueous phase labelled with a dye. This observation may be the result of an excess of coacervate with respect to the dispersed aqueous phase or to a mismatching of the water-coacervate, water-supernatant and coacervate-supernatant interfacial tensions, as discussed by Torza and Mason [19].

***Table 1**: Stability window for the PLGA 50:50/silicone oil (1000 cSt)/CH_2C1_2 system in relation to the relative volume of the aqueous phase*

Aqueous phase/organic phase (wt%)	Stability window[a]
0.02	27.9-32.4
0.04	29.3-32.9
0.12	26.1-29.8

[a]wt% of silicone oil with respect to the organic phase(oil + polymer+ CH_2C1_2).

Since we have shown elsewhere that the interplay of the interfacial tensions in the standard system under investigation was favourable to the coating of the dispersed aqueous phase by the coacervate [18], it is worthwhile to consider the possible effect of an increasing volume of the aqueous phase at a constant weight of the coating polymer. In this respect, microspheres have been prepared in the presence of increasing volumes of dispersed aqueous phase. However, it has been ascertained in a preliminary step, that the phase diagram and particularly the 'stability window' of the ternary system is not modified by this parameter. It must be recalled that the stability window is a domain in the phase diagram where the dispersed aqueous phase is efficiently coated by the coacervate [13,16]. With respect to this window, when a defect or an excess of silicone oil is used, the coated aqueous droplets are unstable or aggregated, respectively.

The phase diagram for PLGA 50/50 (2 wt% in CH_2C1_2) and the 1000 cSt silicone oil has been established, as reported elsewhere [16], while changing the weight ratio of the dispersed aqueous phase and the organic phase (0.02; 0.04 and 0.12).

Table 1 shows that the desolvation process is not significantly affected by the relative volume of the aqueous phase, since the relative amount of the silicone oil (compared to the organic solution) required to observe the 'stability window' remains essentially unmodified. This merely indicates that the aqueous phase and the organic phase are immiscible, otherwise composition of the organic phase should be modified and the 'stability window' as well. Nevertheless, Table 1 shows that the 'stability window' gets narrower when the volume of the aqueous phase is increased, all the other conditions being kept constant.

Table 2: *Mean size (µm) of final microparticles in relation to stirring rate on dispersion of an aqueous solution of BSA in the PLGA solution in CH_2C1_2 prior to phase demixing*

Stirring rate (rpm)	Mean size (µm)
800	40.0±1.5
600	40.2 ±2.2
500	47.2±1.7
400	51.5±1.9
300	No microparticle

There is accordingly an increased instability of the coated aqueous droplets when their volume is increased. This experimental parameter thus affects the second phase of the microencapsulation, i.e. the coating process. This conclusion is confirmed by the morphology of the final microspheres as observed by scanning electron microscopy. Fig. 1 shows that spherical microparticles are observed at the lower aqueous phase content, whereas the particles tend to be deformed when the relative amount of the dispersed aqueous phase is increased. Independently of this detrimental effect, it is reported that the proportion of microparticles loaded with an aqueous solution of indigo carmine increases with the aqueous phase content. At a 0.12 aqueous phase/organic phase weight ratio, the microparticles are homogeneously stained. At water contents exceeding 0.12, microparticles become brittle and they spontaneously release the encapsulated dye solution during filtration. This observation is not surprising, since an increasing amount of aqueous phase with respect to the coacervate must result in a thinner polymer coating around the encapsulated aqueous droplets. As a rough piece of information, it may be calculated that the surface area of aqueous droplets of an average size of 20 µm changes from 9 to 90 dm^2 when the water volume is increased from 0.3 to 3.0 ml. From previous data about the size distribution of the coacervate droplets formed in our standard conditions [18], the surface area of these droplets is ca. 30 dm^2. Therefore, the coacervation phase which is in a large excess for 0.3 ml of aqueous phase to be coated is no longer enough to encapsulate 3 ml of aqueous droplets.

3.2. Effect of the stirring rate

The size distribution of microparticles is currently dependent on the stirring rate of the dispersion in the emulsion-evaporation technique [20,21]. Stirring should also affect the issue of the coacervation process, since the phase separated system consists of three immiscible liquid phases that tend to decrease rapidly the interfacial area by coalescence. Undoubtedly, the encapsulation efficiency and size of the final microspheres will change with the size distribution of the aqueous phase and the oil droplets, respectively. In order to discriminate the possible effect of these two populations of droplets on the encapsulation process, the stirring rate has been modified either just after the addition of the aqueous phase to the polymer solution (dispersion step) or during coacervation of the polymer solution (encapsulation step). In the first case, stirring rate should control the size distribution of the water-in-oil dispersion prior to the coating process. In the second case, average size of the coacervate droplets should also be controlled by the stirring rate.

Actually, when an aqueous solution of BSA (10 wt% with respect to PLGA) is dispersed in the polymer solution at decreasing stirring rates (800-300 rpm), the size of the aqueous droplets is qualitatively seen to increase, and Table 2 confirms that the size of the final microparticles changes in the same direction. Although this average size is not very sensitive to the stirring rate in the range from 800-600 rpm, it increases significantly when the rate falls down to 400 rpm. Surprisingly enough, at a still lower speed, i.e. 300 rpm, no microsphere can be collected and the coacervate droplets precipitate. This lower critical stirring speed might indicate that kinetics of coating of the aqueous droplets by the coacervate is too slow or that bigger coated droplets become unstable.

In a second series of experiments, the stirring rate has been kept constant during the aqueous phase dispersion (800 rpm), although it was changed during the addition of the silicone oil, i.e. the coacervation and coating steps. It is then observed that the stability of the coacervate droplets rapidly decreases, while their size increases as the stirring rate is decreased. At a stirring rate of 100 rpm or smaller, the coacervate tends to coalesce and to settle down. Table 3 shows that, in a parallel way, the mean size of the final microparticles changes in the same direction, which supports the assumption that the coacervate droplets are the main precursor for the microparticles and that one coacervate droplet tends to engulf one aqueous droplet [18].

As a result, the control of the average size of the microspheres in a range larger than 10-60 μm is not straightforward.

***Fig. 1**. Observation by scanning electron microscopy of microspheres prepared with an aqueous phase/organic phase wt ratio of 0.02 (a) and 0.12 (b).*

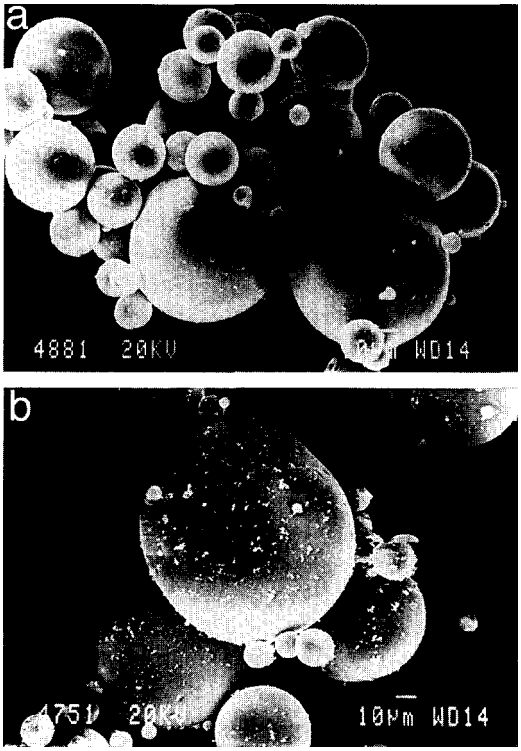

***Table 3:** Mean size (µm) of final microparticles in relation to stirring rate on addition of silicone oil (1000 cSt) to promote PLGA coacervation*

Stirring rate (rpm)	Mean size (µm)
200	40.0±1.5
180	43.1 ± 1.2
150	48.2 ± 2.1
130	58 ± 3.1
100	No microparticle

3.3. Effect of the addition rate of the silicone oil

Kinetics of the phase separation step is an additional parameter that might influence the whole microencapsulation process that occurs under non-equilibrium conditions. Indeed, the instantaneous concentration of the coating polymer in the supernatant and coacervate phases might be at variance, depending on time. Furthermore, the molecular weight distribution of the coating polymer in each phase should depend on the rate of polymer precipitation, as stated by Iso et al. [22], and analyzed later by Heinrich and Wolf [23]. At sufficiently high precipitation rates, polymer coils shrink so quickly that chains are not properly distributed between the two phases and a non-equilibrium distribution may persist until the microsphere solidification occurs. These potential effects have prompted us to check whether the addition rate of the silicone oil, i.e. the demixing agent, may have an effect on the demixing process.

Fig. 2 shows that the relative weight and volume of the coacervate with respect to the original polymer solution increase from 6.5 to 7.9% and from 5.35 to 6.6%, respectively, when the silicone oil, i.e. the phase separation promoter, is added at an increasing rate: 0.65-18 ml/min, respectively. These figures strongly support the conclusion by Heinrich and Wolf [23] that polymer coacervation is a non-equilibrated process whatever the way the phase separation is induced: cooling of the polymer solution in the study by Heinrich and Wolf [23] and addition of an immiscible polymer in this study.

In contrast to the two former processing parameters, the polymer precipitation rate has an effect on the size distribution of the coacervate droplets. Fig. 3 shows that this distribution is more uniform at a slower phase separation rate. This observation is actually in line with conclusions by Heinrich and Wolf [23] about the phase separation mechanism under non-equilibrium conditions. Under conditions close to the equilibrium, phase separation would occur by a nucleation and growth mechanism, prone to give dispersed phases of a homogeneous size. In contrast, a spinodal mechanism or a combination of these two mechanisms would prevail under fast coacervation conditions, accounting for a completely different size distribution of the dispersed phase.

Fig. 2. *Relative weight (%) and volume (%) of the coacervate in relation to the addition rate of the silicone oil (ml/min).*

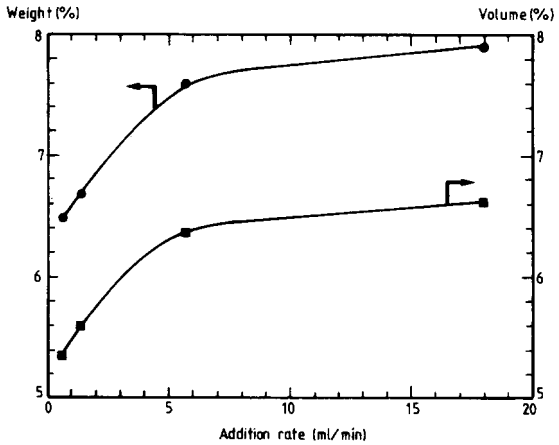

Fig. 3. *Size distribution of the coacervate droplets observed at two different addition rates of the silicone oil; (a) 18ml/min and (b) 5.7 ml/ min.*

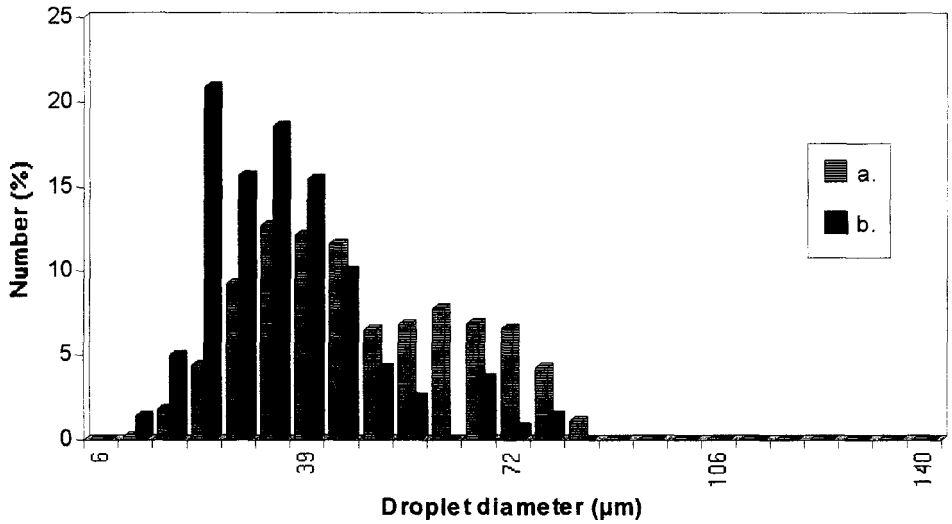

Table 4: *Mean size of PLGA50:50microparticles (µm) in relation to addition rate of silicone oil and encapsulated proteins*

Addition rate of silicone oil (ml/min)	BSA	Thrombin
18	40.0±1.5	38.3±1.1
5.70	39.1 ± 1.1	38.0 ± 2.0
1.40	50.9 + aggregates	No microparticles
0.65	53.1 + aggregates	No microparticles

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The critical addition rate of the silicone oil below which no microparticle is formed actually depends on the proteinic drug dissolved in the dispersed aqueous phase: 0.65 ml/min for a BSA solution and 5.7 ml/ min for the α-thrombin solution (Table 4). This observation highlights the effect of the interplay of the inter-facial tensions between the three phases: aqueous droplets, coacervate and supernatant phase on the encapsulation efficiency. Indeed BSA is known for a surface activity [24], so that the interfacial tension of a BSA aqueous solution against the coacervate and the organic supernatant phase, respectively, must be different compared to an α-thrombin aqueous solution, all the other conditions being the same. Unfortunately, as reported elsewhere [17], these interfacial tensions cannot be measured due to the systematic formation of a thin film at the interface as a result of the interfacial coprecipitation of the protein and the coating polyester.

The surface characteristic features of the microparticles also depend on the polymer precipitation rate. As shown in Fig. 4 and in agreement with previous data published by Donbrow et al. [25], the particle surface is smoother when the silicone oil is added more slowly. Consistently with Fig. 3 for the coacervate droplets, Fig. 5 confirms a much narrower size distribution of the final microparticles centered around 40 µm when the polymer precipitation is slow. Furthermore, when the addition of the silicone oil is fast, very small particles are observed in the range of 1-2 µm (Fig. 5a), the adsorption of which on the surface of the large particles is responsible for the micrographs reported in Fig. 4a. The parallel change in the size distribution of the coacervate droplets and the final microparticles when the rate of polymer coacervation is changed gives an additional credit to the origin of the microparticles that has to be found in the coacervate droplets.

From all these observations, it appears that microencapsulation by coacervation is a complex process that depends on the interplay of several kinetic parameters. If polymer precipitation is too fast compared to polymer deposition on the surface of the dispersed aqueous droplets, the encapsulation efficiency will inevitably be poor. Nevertheless, there is a lower critical coacervation rate below which the encapsulation does not occur at all. It must be pointed out that coating of the dispersed aqueous phase by the coacervate will be as difficult as the coacervate viscosity will be high. However, in addition to this kinetic effect, a high coacervate viscosity leads to a broad size distribution of the coacervate droplets and to microparticles covered with buds. The interfacial tension between the coexisting phases may not be ignored as a thermodynamic control of the encapsulation. It is worth noting that the silicone oil content of the final microparticles is far from being negligible (4.5 wt%) as measured by NMR.

Fig. 4. *Observation by SEM of BSA containing microparticles prepared at a silicone oil addition rate of (a) 18 ml/min, (b) 5.7 ml/ min.*

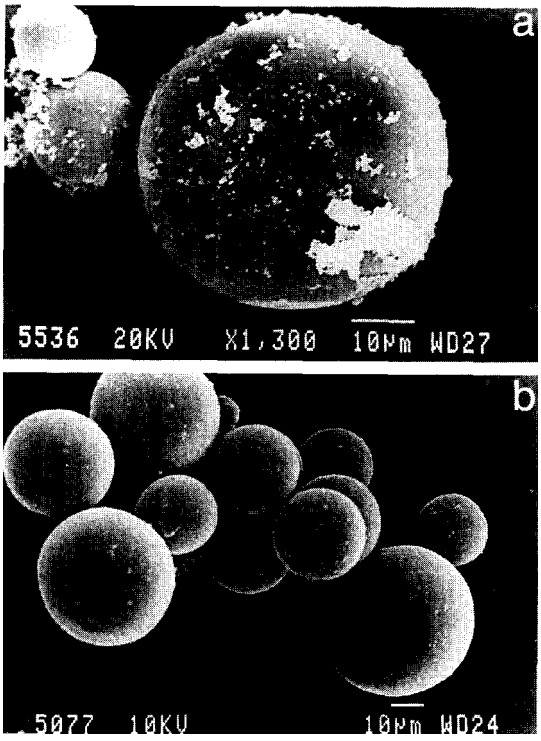

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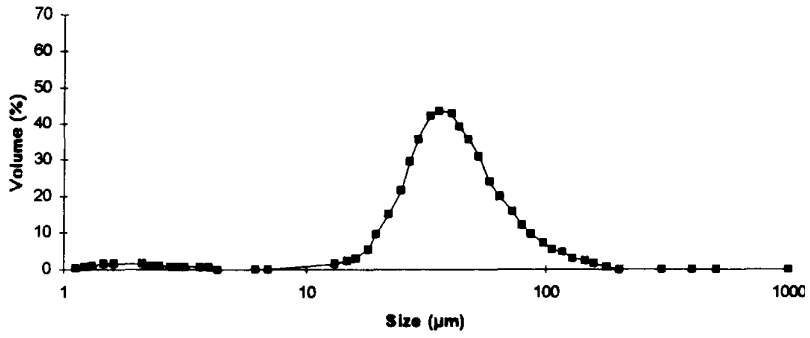
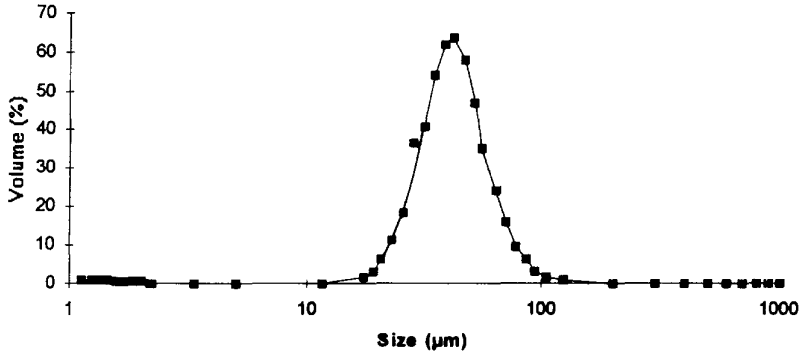
Fig. 5. *Size distribution of BSA containing microparticles prepared with a silicone oil added at (a) 18 ml/min and (b) 5.7 ml/min.*



a



b

4. Conclusions

This study has highlighted the effect that several formulation parameters can have on microencapsulation by coacervation of a coating polymer solution. This process proceeds along three steps, i.e. phase separation of the coating polymer, adsorption of the coacervate on the dispersed phase and hardening of the coating, that occur so quickly that they are far from equilibrium and thus sensitive to kinetic influences. So, the addition rate of silicone oil, i.e. the coacervation promoter, has a significant effect on the final microspheres and in the extreme, it can prevent encapsulation from occurring. The ratio of the dispersed aqueous phase to the oil phase and the stirring rate when dispersion is carried out also modify the fate of the coacervate droplets and thus the encapsulation efficiency. The protein drug to be encapsulated also affects the coating step, because it changes the interfacial tension of the dispersed solution against organic phases. Furthermore, it has been shown elsewhere that the protein can interact with the coating polymer and promote an interfacial complexation [10].

Several experimental observations point out that the coacervate droplets are the precursor for the final microspheres. Efficiency of microencapsulation by coacervation thus appears to be limited to rather critical conditions in terms of the system composition and kinetics of formulation and implementation. As a result, the main microparticle characteristics can only be modified in a limited range, compared to other available techniques, such as double emulsion (W/O/W) evaporation, which is a more versatile technique.

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