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Influence of various technological parameters on the preparation of spray-dried poly(ε-caprolactone) microparticles containing a model antigen

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This work evaluates the efficacy of the spray-drying technique to prepare poly(ε-caprolactone) (PCL) microparticles containing an entrapped model antigen (bovine albumin, BSA). The presence of a stabiliser was found to be an important parameter when preparing PCL microparticles containing a hydrophilic antigen. The effect of various technological parameters (concentration of the polymer and protein solutions, organic/aqueous phases ratio, nature of solvents and emulsion parameters such as duration and speed of agitation) on microparticle morphology and size, BSA entrapment and encapsulation efficiency was studied. Microparticles were characterized by a mean size from 9.56 ± 0.25 to $24.31 \pm 2.87\,\mu m$ and a BSA entrapment from 0.80 ± 0.02 to $24.21 \pm 0.23\%$ (w/w). SDS-PAGE electrophoresis and isoelectric focusing (IEF) confirmed the conservation of the physicochemical characteristics of the BSA entrapped within PCL microparticles produced by spray-drying. Together, these results showed that spray-drying is an efficient technique to overcome the key obstacle that represents the scaling-up of the manufacturing process to produce sufficient quantities of vaccine for clinical trials and, ultimately, commercialization.

Keywords: spray-drying, poly(ε-caprolactone), biodegradable microparticles, vaccine delivery systems.

Introduction

One of the most important current issues in vaccinology is the need for new adjuvants and delivery systems. Chang (1976) already suggested that an adjuvant effect could be achieved through the association of antigens with polymeric microparticles. In the late 1970s, it was discovered that solid particles of non-degradable polymers (silicone, polymethylmethacrylate), loaded with various antigens and implanted subcutaneously, generated a specific IgG immune response (Kreuter and Liehl 1978). The only disadvantage of these controlled release vaccines was that the matrix remained after the antigen had been released. Nowadays, many controlled release vaccine systems are polymers of polylactide (PLA) or polylactide-co-glycolide (PLG). These degrade to molecules normally

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found in the body as metabolic by-products and, hence, have no unwanted side effects. In addition to their proven safety record, these polyesters can be tailored to fit many delivery applications by altering some aspects of their manufacture, such as composition and particle size. However, the adjuvant effect achieved by the encapsulation of antigens within PLG microparticles has been demonstrated only relatively recently (Eldridge *et al.* 1991, O'Hagan *et al.* 1991a,b). In recent years, a number of studies have also described the induction of potent immune responses (systemic and/or mucosal) following mucosal (oral or nasal) administration with entrapped antigens (Eldridge *et al.* 1989, Almeida *et al.* 1993, O'Hagan 1994, Benoit *et al.* 1998, Baras *et al.* 1999). Nevertheless, the major drawback of these polymers is that their degradation generates an extreme acid environment (pH 2–3), in which many vaccines are found to lose their antigenicity (Gander *et al.* 1993, Park *et al.* 1995).

Poly(ε-caprolactone) (PCL) is also a biocompatible and biodegradable polyester polymer which has been under clinical evaluation world-wide for sustained delivery of levonorgestrel (Capronor^{TM}—a 1 year contraceptive). PCL degrades slowly and, therefore, does not generate an acid environment, unlike the PLA/PLG polymers. Moreover, its high hydrophobicity would allow a better targeting of the gut-associated lymphoid tissue (Eldridge *et al.* 1990, Baras *et al.* 1999) and, in doing so, make this polymer a prime carrier for oral vaccines.

The production technology mainly employed in the manufacture of all of these polyester microparticles has been solvent evaporation/solvent extraction from emulsion systems. Problems encountered with this method of production include the use of high shear forces and a long exposure time in organic solvents which might result in degradation of sensitive antigens.

Preparation of microparticles by spray-drying is an attractive alternative to conventional microencapsulation techniques. The mild processing conditions employed during spray-drying seem to be particularly suited to the formulation of protein- and peptide-entrapped microparticles. From a manufacturing viewpoint, this simple and rapid technique offers the advantage of being a single-step process which can readily be scaled up (Bodmeier and Chen 1988, Pavanetto *et al.* 1992). Spray-drying is already widely used in the pharmaceutical industry for a range of applications and has recently been investigated for the production of drug-entrapped PLA or PLG microparticles (Pavanetto *et al.* 1993, Conte *et al.* 1994, Baras *et al.* 1997a,b, Clarke *et al.* 1998). However, Giunchedi *et al.* (1994) claimed that lipophilic drug-entrapped microparticles which contained only PCL as polymer were not formed during the spray-drying process and the final product obtained was constituted by flakes or rods of irregular shape and remarkable dimensions ($>100\,\mu m$).

In this paper, factors influencing the preparation by spray-drying technique of poly(ε-caprolactone) microparticles entrapping a hydrophilic model antigen is reported. The influence of technological parameters, including concentration and viscosity of sprayed polymer solution, concentration of antigen solution, organic/aqueous phase ratio and nature of organic solvents on microparticle characteristics, are investigated. They will be selected to achieve a high degree of entrapment of the model antigen and to obtain particles suitable for vaccine administration, especially for oral immunization by targeting the gut-associated lymphoid tissue. The conservation of physicochemical characteristics

(molecular weight and isoelectric point) of the entrapped model antigen are also investigated.

Materials and methods

Materials

The following chemicals were purchased from commercial suppliers. Poly(ε-caprolactone) (molecular weight, 44 000) and polyvinyl alcohol (molecular weight, 12 000–23 000; 87–89% hydrolyzed) (PVA) were from Aldrich Chemical Co. (Bornem, Belgium). Albumin (from bovine serum, fraction V) (BSA) and Folin-Ciolcateu's phenol reagent were supplied by Merck (Darmstadt, Germany) and sodium dodecyl sulphate (SDS) was supplied by Sigma Chemical Co. (St. Louis, MO, USA). Dichloromethane (DCM) and chloroform (CFM) were purchased from UCB (Braine L'Alleud, Belgium). Low molecular weight standards for SDS-PAGE (range 14–94 kDa) and IEF standards (range pH 3.0–9.0) were supplied by Pharmacia Biotech (Uppsala, Sweden). Other materials were reagent grade.

Preparation of PCL microparticles with an entrapped water soluble antigen

An aqueous solution of BSA (50, 100, 250 or 500 mg/ml) containing (or not) PVA (10 mg/ml) in ultra-pure water (1 or 5 ml) was emulsified with an organic solution (50 or 100 ml) of PCL (0.5, 1 and 3%, w/w) in various solvents (DCM, CFM or a mixture DCM/CFM) using an Ultraturrax model T25 (IKA Laboratory Technology, Staufen, Germany) at high speed (8000–20 500 rpm for 5 or 10 min) and room temperature.

Microparticles were obtained by spraying this emulsion through the nozzle (0.5 mm) of a Büchi mini Spray Dryer Model 190 (Büchi Laboratoriums-Technik AG, Flawil, Switzerland). The process parameters were set as follows: inlet temperature (44–46 °C); pump setting (7.5 ml.min^{-1}); aspirator (setting 15); spray flow (5 bars and 400 litres/h). The solid microparticles that had precipitated into the bottom collector (34–35 °C outlet temperature) were harvested and kept at room temperature.

In these studies, the effects of formulation variables on microparticles characteristics such as surface morphology, particle size and BSA entrapment were investigated:

(a) Presence of an emulsion stabiliser (PVA, 10 mg/ml) in the aqueous phase.
(b) Concentration of the polymer solution: this was studied by variation in the concentration of polymer (0.5, 1 and 3%, w/v) dissolved in 100 ml of a DCM/CFM (1:1) mixture.
(c) Concentration of the BSA solution: this was studied by variation in the concentration of protein (50, 125 and 250 mg) in 1 ml of ultra-pure water containing 10 mg/ml PVA.
(d) Ratio of organic/aqueous phase volumes: the ratios investigated were 100/0.5, 100/1,100/5 and 50/1.
(e) Nature of organic solvents: the solvents studied were DCM, CFM and a DCM/CFM mixture in a ratio 1:1, 3:1 and 1:3.
(f) Emulsion parameters: stirring rates of 8000, 13 500 and 20 500 rpm and duration of agitation during emulsification of 5 or 10 min were investigated.

Microparticle characteristics

A scanning electron microscope Hitachi S-570 was used to assess the shape and the surface morphology. A small amount of microparticles were suspended in ultra-pure water, sonicated for 30 s at 50–70 Watts (Sonifier B-12, Branson Sonic Power Company). A drop of this suspension was placed on the sample holder, dried and observed after coating with gold-palladium under an argon atmosphere.

For particle size analyses, the microparticles sonicated and dispersed in filtered (0.1 μm) saline solution of 0.9% (w/v) NaCl were sized by employing a Coulter Multisizer (Coulter Electronics Ltd., Luton, UK) equipped with a 100 μm orifice and under continuous stirring. The particle size was expressed as volume mean diameter (VMD) in micrometres ± SEM ($n = 3$).

For evaluation of the BSA entrapment in microparticles, 30–50 mg of spray-dried microparticles, accurately weighted, were dissolved in 3.0 ml of 1 M NaOH containing 5% (w/v) SDS during 24 h under gentle agitation at room temperature (Hora *et al.* 1990). After centrifugation (4000 g for 10 min at room temperature), the supernatant was assayed following the method of Lowry *et al.* (1951) to determine the BSA concentration. The percentage of BSA entrapped per dry weight of spray-dried microparticles (w/w) was determined. The percentage of entrapment efficiency was expressed by relating the actual BSA entrapment to the theoretical BSA entrapment, as previously described (Jeffery *et al.* 1993, Conte *et al.* 1994). Each sample was assayed in triplicate. A problem related to the spray-drying process is the possibility to spray-dry unentrapped proteins. Before the study of protein loading, microparticles were washed to eliminate the unentrapped protein and to prevent an overestimation of the protein entrapment. However, during the washing process, a relatively important quantity of protein could be released from microparticles and induce an underestimation of the protein entrapment.

Conservation of the physicochemical characteristics of the entrapped antigen

The conservation of the molecular weight and the isoelectric point of the BSA entrapped within microparticles was assessed on released protein by using respectively polyacrylamide gel electrophoresis (SDS-PAGE) and IEF techniques. For SDS-PAGE analysis, protein samples were analysed on a 10% gel with the Mini-Protean system (Bio-Rad, Nazareth, Belgium), according to the method described by Laemmli (1970).

PhastGel IEF (Pharmacia Biotech, Uppsala, Sweden) was performed on precast homogeneous (5%T, 3%C) polyacrylamide gel that generates an isoelectric points (pI) gradient from 3.0–9.0 following the method of O'Farrel *et al.* (1977). All gels were fixed and stained with Coomassie brilliant blue R-250.

Statistical analyses

Statistical evaluation of the data used the Mann-Whitney's test for comparisons of the effect of various technological parameters on microparticle characteristics. p-values of 0.05 or less were considered significant.

Results and discussion

Shape of microparticles

In contrast to the double emulsion-solvent evaporation method, spray-drying, generally resulted in microparticle aggregation. The formation of insoluble aggregates was probably due to the high atomization air rate (approximately 400–500 l/h). This particular condition generates smaller droplets in the spray and, thus, increases the air-liquid interface for surface denaturation (Mumenthaler *et al.* 1994). The formation of aggregates will be minimized if spray-drying is conducted at low atomization rates (between 200–300 l/h). However, in this condition, the microdroplets were projected on the wall of the cylinder and did not reach the cyclone. Thus, an atomization air rate of 400 litres/h was used and a subsequent step of sonication was performed to disintegrate the aggregates.

Figures 1(*a–c*) showed microparticles obtained after sonication from 0.5–1% (w/v) PCL concentration at protein concentration of 50–250 mg/ml in ultra-pure

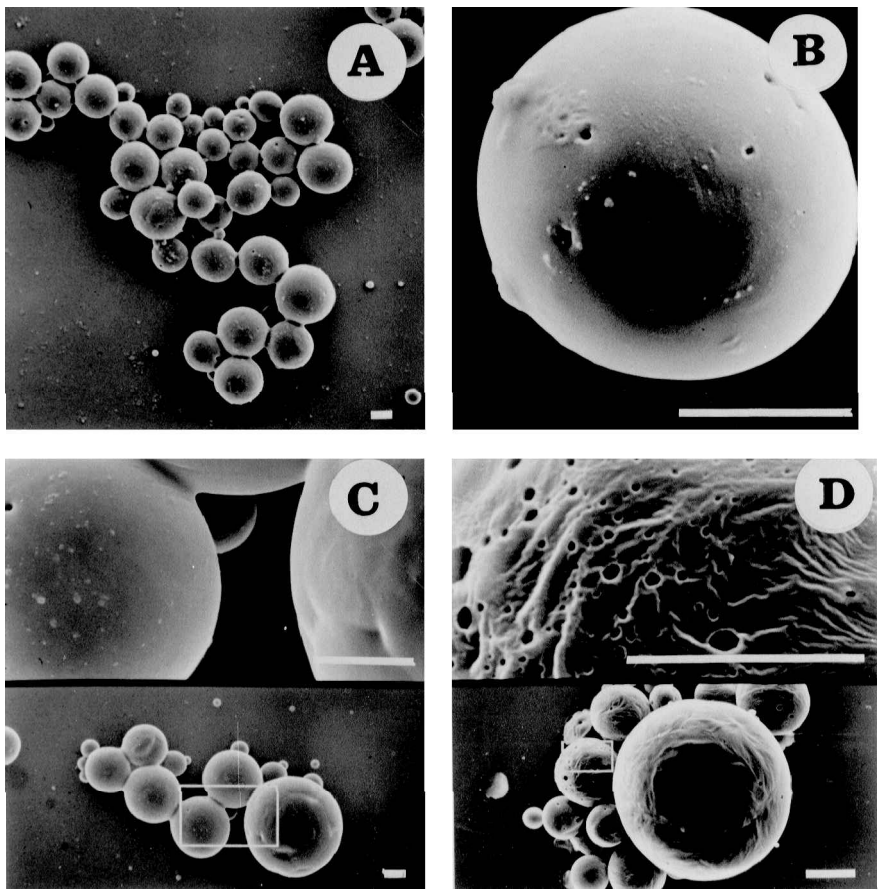

Figure 1. Scanning electron photomicrographs of microparticles produced from PCL in 100 ml of a DCM/CFM (1:1) mixture emulsified with 1 ml of ultra-pure water containing BSA and 10 mg/ml PVA. (A) 0.5% (w/w) PCL and 250 mg/ml BSA, (B) 1% (w/w) PCL and 250 mg/ml BSA, (C) 0. 5% (w/w) PCL and 50 mg/ml BSA and (D) 0. 5% (w/w) PCL without PVA. Horizontal bar = 10 μm.

water. These microparticles resulted in a spherical shape, whatever the PCL and protein concentrations.

The outer surface of particles appeared to be smooth with pits (figures 1(*a–c*). The number and size of pits increased according to the absence of the emulsion stabiliser (PVA) during the microparticles preparation (figure 1(*d*)).

Presence of an emulsion stabiliser in the aqueous phase

The addition of insoluble BSA in organic solvents (DCM or CFM) could give rise to a non-uniform distribution of large protein islands in the polymer matrix, presumably close to the microparticle surface (Cohen *et al.* 1991). So, an emulsion method that combines water and organic phases seemed suitable for encapsulation.

The stability of this emulsion is a critical requirement, especially when the organic solvent is evaporated rapidly of the system by spray-drying to created well-defined microparticles. Thus, the presence of an emulsion stabiliser (PVA, 10 mg/ml) seemed to be necessary.

Even if it was possible to produce microparticles in the absence of stabiliser (PVA), the process was always characterized by a low efficiency of encapsulation and the obtained microparticles showed a low protein loading (table 1). This result could be explained by the low stabilization of the microdroplets formed during the emulsification process in absence of PVA. The stabilization of the emulsion by the addition of PVA is a prerequisite for the loading of a large amount of BSA within the microparticles. Moreover, PVA allowed to produce weakly aggregated microparticles when compared to microparticles produced in absence of PVA (figures 1(*a–c*) and (*d*), respectively). The stabiliser acted as a protective polymer by being adsorbed at the oil/water interface of the droplets to produce a steric barrier which decreased the coalescence of the microparticles.

Concentration of the polymeric solution

An increase in the initial polymer concentration (from 0.5–3%, w/v) resulted in a significant decrease ($p < 0.05$) in BSA entrapment and also in the entrapment efficiency associated to an increase of the particle size (table 2). The highest BSA entrapment (24.21 ± 0.23%, w/w) correlated to the highest entrapment efficiency (73.60 ± 0.70%) was achieved with 0.5% (w/v) PCL concentration. A 1% (w/w)

Table 1. Effect of the presence of an emulsion stabiliser in the aqueous phase.

Initial BSA concentration (mg/ml)	Presence (+) or absence (−) of PVA (10 mg/ml)	Particle size (VMD ± SEM) (μm)	BSA entrapment (%, w/w)	Entrapment efficiency (%)
50	−	22.63 ± 2.02	2.05 ± 0.21	43.05 ± 4.41
50	+	19.12 ± 1.02	3.00 ± 0.47	63.60 ± 9.96
100	−	21.27 ± 2.17	2.46 ± 0.25	27.06 ± 2.75
100	+	18.92 ± 0.91	5.59 ± 0.71	62.05 ± 7.88
250	−	22.32 ± 1.02	4.05 ± 0.45	20.25 ± 2.25
250	+	19.84 ± 1.72	9.95 ± 0.41	50.15 ± 2.07
500	−	24.31 ± 2.87	0.80 ± 0.02	2.40 ± 0.06
500	+	20.21 ± 1.69	1.39 ± 0.12	4.20 ± 0.36

The PCL concentration was fixed at 1% (w/v) in 100 ml of a DCM/CFM mixture (1:1) and the aqueous phase was maintained at 1 ml of ultra-pure water ($n = 3$).

Table 2. Effect of the PCL concentration.

PCL concentration (%, w/v)	Particle size (VMD ± SEM)(μm)	BSA entrapment (%, w/w)	Entrapment efficiency (%)
0.5	15.88 ± 0.76	24.21 ± 0.23	73.60 ± 0.70
1	19.84 ± 1.72	9.95 ± 0.41	50.15 ± 2.07
3	23.33 ± 1.72	3.24 ± 0.53	42.25 ± 6.91

The BSA concentration was fixed at 250 mg in 1 ml of ultra-pure water (containing 10 mg/ml PVA) and the organic phase was maintained at 100 ml of a DCM/CFM (1:1) mixture ($n = 3$).

PCL concentration allowed one to obtain 9.95 ± 0.41% (w/w) of BSA entrapment (table 2).

At a 3% (w/w) concentration of PCL, which is the highest concentration that can be used without inducing plugging of the nozzle of the spray-dryer, the microparticles showed both low BSA entrapment (3.24 ± 0.53%, w/w) and entrapment efficiency (42.25 ± 6.91%) and were characterized by a mean size of 23.33 ± 1.72 μm (table 2).

These results could be related to the viscosity of the emulsion, which increased significantly with the polymer concentration and which could have reduced the efficiency of solution stirring (Jeffery *et al.* 1991) and increased the frequency of semi-formed droplets, so inducing an overall increase in the mean size of microparticles.

Moreover, a considerable loss of materials occurred during spray-drying. This loss depended on the technical features of the apparatus: much of the powder adhering to the cyclone walls was lost during spray-drying and was also related to the polymer concentration.

Concentration of the protein solution

There was a significant relationship ($p < 0.05$) between the initial concentration of BSA (50–250 mg/ml) and BSA entrapment (from 7.35 ± 0.58 to 24.21 ± 0.23%, ww) without effect on the mean size of microparticles ($p > 0.05$) (table 3). Nevertheless, a slight decrease of the entrapment efficiency (from 82.32 ± 6.50 to 73.60 ± 0.70%, $p < 0.05$) was also observed when the initial BSA concentration was increased from 50–250 mg/ml. It was postulated that, at a high protein/polymer ratio, the quantity of polymer present could be insufficient to cover the

Table 3. Effect of the BSA concentration.

Initial BSA concentration (mg/ml)	Particle size (VMD ± SEM)(μm)	BSA entrapment (%, w/w)	Entrapment efficiency (%)
50	16.12 ± 1.36	7.35 ± 0.58	82.32 ± 6.50
125	16.35 ± 1.12	15.40 ± 0.74	78.23 ± 3.76
250	15.88 ± 0.76	24.21 ± 0.23	73.60 ± 0.70

The PCL concentration was fixed at 0.5% (w/v) in 100 ml of a DCM/CFM mixture (1:1) and the aqueous phase was maintained at 1 ml of ultra-pure water (containing 10 mg/ml PVA) ($n = 3$).

protein completely. Due to the low quantity of antigen often available, 50 mg of BSA were used in further investigations.

Organic/aqueous phase ratio

As shown in table 4, increasing the volume of the aqueous phase (from 0.5–5.0 ml) while maintaining a constant volume of the organic phase (100 ml) resulted in a significant increase of the BSA entrapment (from 3.50 ± 0.37 to $10.34 \pm 0.05\%$, w/w, $p < 0.05$) without effect on the mean size of microparticles ($p > 0.05$). A high volume of aqueous phase (5.0 ml) and, thus, a higher quantity of protein in this one (250 mg) was concomitant to a lower entrapment efficiency, probably due to an insufficient quantity of polymer to encapsulate the protein completely.

The use of a reduced organic phase volume (50 ml) with the same volume of the aqueous phase (1 ml) induced a significant increase in the protein entrapment (from 7.35 ± 0.58 to $13.42 \pm 0.69\%$, w/w, $p < 0.05$) without significant modification of the mean size of microparticles and entrapment efficiency ($p > 0.05$) (table 4). The increased size was attributed to the increased viscosity of the organic phase, which might have caused a reduction in the efficiency of disruption of the oil phase (Jeffery *et al.* 1991).

Nature of solvents

The nature and the ratio between the two constituents of the organic solvent mixture seemed to modify both the BSA entrapment and the entrapment efficiency (table 5). Moreover, the use of dichloromethane or chloroform alone allowed one

Table 4. Effect of the organic/aqueous phase ratio.

Phase ratio (organic/aqueous) (ml)	Particle size (VMD ± SEM)(μm)	BSA entrapment (%, w/w)	Entrapment efficiency (%)
100/0.5	16.89 ± 2.10	3.50 ± 0.37	74.20 ± 7.84
100/1	16.12 ± 1.36	7.35 ± 0.58	82.32 ± 6.50
100/5	13.83 ± 0.53	10.34 ± 0.05	33.09 ± 0.16
50/1	18.78 ± 0.80	13.42 ± 0.69	83.20 ± 4.28

The PCL concentration was fixed at 0.5% (w/v) in a DCM/CFM mixture (1:1) and the concentration of BSA in ultra-pure water (containing 10 mg/ml PVA) was maintained at 50 mg/ml ($n = 3$).

Table 5. Effect of various solvents.

Solvent	Particle size (VMD ± SEM)(μm)	BSA entrapment (%, w/w)	Entrapment efficiency (%)
DCM/CFM (1:1)	16.12 ± 1.36	7.35 ± 0.58	82.32 ± 6.50
DCM/CFM (3:1)	12.67 ± 0.57	7.24 ± 0.60	81.09 ± 6.72
DCM/CFM (1:3)	17.53 ± 1.21	5.99 ± 0.37	67.09 ± 4.14
CFM	18.16 ± 0.77	8.51 ± 0.51	95.31 ± 5.71
DCM	9.56 ± 0.25	8.25 ± 0.32	92.40 ± 3.58

The PCL concentration was fixed at 0.5% (w/v) in 100 ml of the organic solvent and the concentration of BSA in 1 ml of ultra-pure water (containing 10 mg/ml PVA) was maintained at 50 mg/ml ($n = 3$).

to reach a high entrapment of the model protein. When DCM or CFM were used alone, more than 8% of the microparticle weight corresponded to the protein, with a 90% entrapment efficiency. Nevertheless, CFM in high proportion or alone always showed a high microparticle mean size (18.16 ± 0.77 μm). In contrast, both DCM and a DCM/CFM mixture in a ratio 3:1 were without effect on the microparticle size ($p > 0.05$) (table 5).

Emulsion parameters

An increase in the speed of agitation (from 8000–20 500 rpm) induced a significant decrease ($p < 0.05$) in BSA entrapment (from 7.35 ± 0.58 to 4.75 ± 0.53%, w/w), entrapment efficiency (from 82.32 ± 6.50 to 53.20 ± 5.94%) and mean size of microparticles (from 16.12 ± 1.36 to 12.63 ± 1.94 μm) (table 6). This phenomenon could be the result of the microdroplets destroying inside the emulsion due to the dramatic increase in the shearing strengths induced by the Ultraturrax during the stirring.

The modification of the duration of agitation during emulsification was without effect on BSA entrapment, entrapment efficiency and microparticle mean size ($p > 0.05$) (table 6).

Conservation of the physicochemical characteristics of the entrapped BSA

During the microencapsulation process, proteins were subjected to potentially damaging conditions, including exposure to organic solvents, high shear during the emulsification and, with the spray-drying technique, relatively high inlet temperature or spray-rate of feed. Although it is believed that the protein solution is uniformly dispersed throughout the organic phase and, consequently, that the protein is not actually in contact with the organic solvent, the possibility of degradation at the interface surrounding the aqueous phase cannot be discounted. These relatively adverse chemical and physical stresses could degrade the antigen entrapped within microparticles (Gander *et al.* 1995).

In addition to the potentially damaging conditions listed above, a major problem in the spray-drying of proteins is the risk of heat-induced degradation caused by exposure of the molecule to high temperatures. Indeed, the inlet temperature must be sufficient to evaporate the solvent without degradation of the entrapped protein. However, when spray-dried in a hot air stream that flows in the same direction as the descending spray droplets, the product can reach a

Table 6. Effect of emulsion parameters (stirring rate and duration of the agitation).

Stirring rate (rpm)	Time (min)	Particle size (VMD ± SEM) (μm)	BSA entrapment (%, w/w)	Entrapment efficiency (%)
8000	5	16.12 ± 1.36	7.35 ± 0.58	82.32 ± 6.50
8000	10	18.75 ± 2.02	7.01 ± 0.10	78.51 ± 1.12
13 500	5	13.89 ± 0.77	4.67 ± 0.45	52.30 ± 5.04
20 500	5	12.63 ± 1.94	4.75 ± 0.53	53.20 ± 5.94

The PCL concentration was fixed at 0.5% (w/v) in 100 ml of a DCM/CFM mixture (1:1) and the concentration of BSA in 1 ml of ultra-pure water (containing 10 mg/ml PVA) was maintained at 50 mg/ml ($n = 3$).

theoretical maximum temperature no higher than the temperature of the air stream at the dryer's outlet. In practice, it can generally be assumed that the actual maximum temperature is approximately 15–25 °C below the outlet air temperature (Mumenthaler *et al.* 1994). Moreover, typically during spray-drying, the period of exposure of the drying droplets to elevated temperature is approximately 5–30 s (Mumenthaler *et al.* 1994). Theoretically, these conditions would fail to degrade the protein, but the confirmation was important for the use of this technique for the antigen encapsulation by spray-drying.

The effect of the preparative processes on the conservation of the physicochemical characteristics of the entrapped BSA has been investigated by using respectively SDS-PAGE (figure 2) and IEF (figure 3) to detect potential protein degradation. SDS-PAGE analysis of the BSA released from microparticles (figure 2, lanes 2 and 3) showed that no larger or smaller molecular weight fragments of BSA were present, when compared with unentrapped BSA (figure 2, lane 4). IEF indicated identical bands for the native BSA (figure 3, lane 4) and BSA released from microparticles (figure 3, lanes 2 and 3). The isoelectric point of BSA was pI 4.8, as compared with the isoelectric point markers (figure 3, lanes 1 and 5).

SDS-PAGE and IEF analyses showed that the physicochemical characteristics of the entrapped BSA were not significantly affected by the entrapment procedure. According to Cohen *et al.* (1991), the data suggested that the model antigen (BSA) did not chemically interact with the polymer matrix and that it was physically held by the dense polymer matrix. Moreover, they confirmed that the method of BSA entrapped within PCL microparticles using a spray-drying technique, which involved an organic solvent such as dichloromethane and chloroform, did not lead to a significant irreversible aggregation or degradation of the entrapped antigen.

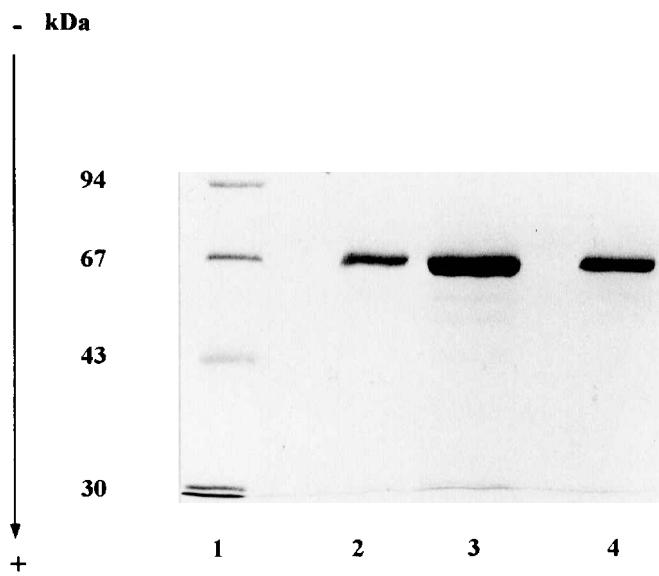

Figure 2. Coomassie R 250-stained SDS-PAGE electrophoretic gel of BSA released from spray-dried microparticles of PCL (lane 2 : 0.8 µg, lane 3 : 5 µg. Lane 1: molecular weight markers. Lane 4: unentrapped purified BSA (0.8 µg).

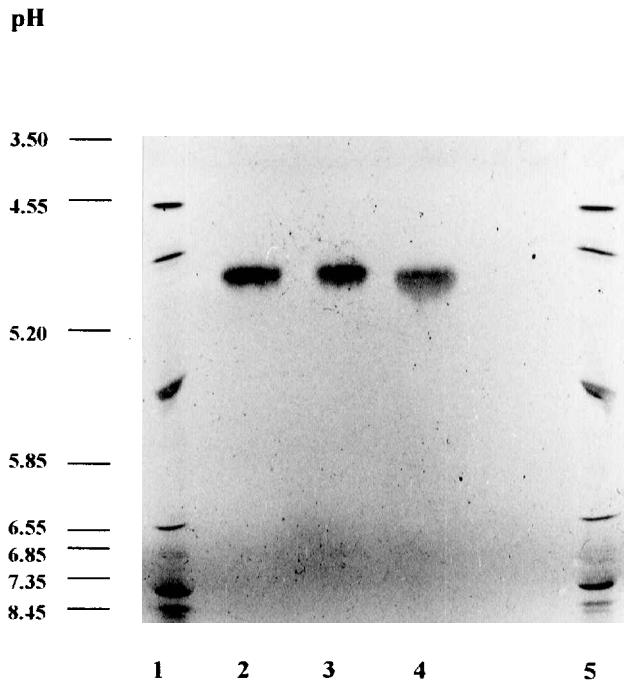

Figure 3. Coomassie R 250-stained isoelectric focusing gel of BSA (0.6 µg) released from spray-dried microparticles of PCL (lanes 2 and 3). Lanes 1 and 5: isoelectric point markers. Lane 4: unentrapped purified BSA.

Conclusion

This work confirmed that spray-drying is a process capable of achieving a high encapsulation efficiency of a model antigen in microparticles produced from PCL alone, while maintaining its integrity. The modification of various parameters and the presence of an emulsion stabiliser, which was essential to entrap a hydrophilic drug, allowed one to obtain these well-formed PCL microparticles. The use of PCL for vaccine delivery systems seems to be important for oral administration. Indeed, its hydrophobicity would favour the uptake of microparticles by the gut-associated lymphoid tissue. The *in vitro* stability of PCL, its low cost and lack of toxicity are other characteristics strengthening the interest to produce PCL microparticles.

This investigation has also provided an understanding of the effects of some process parameters on microparticle size and BSA entrapment into microparticles, without significant modification of the physicochemical characteristics of the entrapped model antigen. This last result confirmed the potential of the spray-drying technique for the encapsulation of antigens. The selection of appropriate conditions has enabled the preparation of smooth, spherical PCL microparticles, 10–20 µm in mean size with a high protein encapsulation (up to 24%, w/w) and entrapment efficiency (up to 95%).

Recently, the laboratory has produced PCL microparticles by the double emulsion-solvent-evaporation method (Benoit *et al.* 1999, Youan *et al.* 1999). These microparticles allowed one to obtain a long-lasting humoral immune

response following a single oral or nasal administration (Benoit *et al*. 1998, Baras *et al*. 1999). Spray-drying is a well-adapted technique to produce microparticles as vaccine carriers.

Indeed, it has been shown that a single administration of polylactide and polylactide-co-glycolide microparticles produced by spray-drying technique also resulted in a long-lasting humoral immune response (Baras *et al*. 1997a,b). This technique is easy to scale-up, simpler and more rapid than the double emulsion-solvent evaporation technique, the most common technique used to encapsulate antigens. Finally, this work is the first approach to overcome the key obstacles that represent the scaling-up of the manufacturing process to produce sufficient quantities of vaccine for clinical trials and, ultimately, commercialization.

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