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Influences of process parameters on nanoparticle preparation performed by a double emulsion pressure homogenization technique

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Abstract

The preparation of nanoparticles (NP) as an improved colloidal carrier system for proteins was investigated. Bovine serum albumin (BSA) was used as model drug. Owing to the high solubility of the protein in water, the double emulsion technique has been chosen as one of the most appropriate method. In order to both reaching submicron size as well as increasing the grade of monodispersity compared to previous preparation techniques, a microfluidizer as homogenization device was used. All experiments were performed using two biodegradable polymers, poly[D,L-lactic-co-glycolic acid] 50/50 (PLGA) and poly[ε-caprolactone] (PCL). The homogenization procedure has been optimized with regard to particle size and monodispersity by studying the influence of the homogenization time as well as the amount of polymer and surfactant in the external aqueous phase. The drug loading has been improved by varying the concentration of the protein in the inner aqueous phase. By increasing the protein concentration in the inner aqueous phase the polydispersity was slightly higher, while the particle size was not influenced significantly. The BSA encapsulation efficiency decreased with higher protein concentration in the inner aqueous phase. All release profiles were characterized by a initial burst effect, a higher release rate was obtained after 4 weeks for PLGA NP (60%) compared with PCL NP (47%).

Keywords: Nanoparticles; Encapsulation; Double emulsion technique; Poly[lactic-co-glycolic acid]; Poly[ε-caprolactone]; BSA

1. Introduction

For many reasons it is important to protect drugs against degradation. Especially for proteins it is one of the most important problems to avoid their denaturation during the transport to their site of action. Colloidal drug carriers are interest-

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ing in the field of drug delivery systems because of their small size allowing them to permeate through biological barriers (Nakada et al., 1996). Among various colloidal drug delivery systems, nanoparticles (NP) potentially represent one of the most promising approaches to this aim. In previous works the protein loading of NP was carried out by adsorbing the protein on the particle surface (Armstrong et al., 1997) involving a limited loading capacity and uncontrolled desorption of the drug. For this reason it seems to be advantageous to incorporate the drug inside the NP. Several polymerization techniques have been applied for the incorporation of hydrophilic compounds into NP. However, residual monomers could interact with the protein leading to its denaturation. In addition, residues of the polymerization medium can be more or less toxic. Therefore new encapsulation techniques were developed by using preformed polymers. Many authors (Alex and Bodmeier, 1989; Cohen et al., 1991) have previously shown that the double emulsion technique was the most appropriate method to encapsulate hydrophilic drugs and proteins within microparticles. This technique has been later applied to NP (Blanco and Alonso, 1997; Lemoine and Préat, 1998; Tobío et al., 1998), by using sonication or Ultraturrax. However, in each case the polydispersity remained relatively high. In order to prepare NP with a lower size polydispersity we investigated their preparation by the double emulsion technique by using a pressure homogenization device. The optimization of this preparation process was carried out with the model protein bovine serum albumin (BSA, MW 65 000 Da) by changing the most influencing process parameters during the preparation step.

2. Methods

For all NP preparations two biodegradable polymers, poly[D,L-lactic-co-glycolic acid] 50/50 (PLGA) (MW 40 000 Da) and poly[ε-caprolactone] (PCL) (MW 42 000 Da) were used. NP were prepared by using the multiple emulsion (w/o/w) technique, previously applied to the preparation of both micro- and nanoparticles. The adjustment was based on the use of a homogenizer in the two-step emulsification process, thus reducing considerably the size of the dispersed droplets. Briefly, BSA previously dissolved in water was emulsified by magnetic stirring in methylene chloride containing the polymer (PLGA and PCL). This w/o emulsion was thereafter poured into the external aqueous phase containing polyvinylalcohol (PVA) (MW 30 000 Da, 88% hydrolyzed) and homogenized in a homogenizer (AML 2, Guérin, Mauze, France) in an ice bath. After evaporation of the methylene chloride under reduced pressure, the polymer precipitated and the NP were isolated by centrifugation and washed twice before lyophilization. NP were thereafter analyzed for their size distribution and their surface potential by using a Zetasizer II® (Malvern Instruments, UK). The encapsulation efficiency was determined by measuring the non-encapsulated amount of BSA with the Lowry–Peterson protein assay (Peterson, 1977) in the supernatant recovered after ultracentrifugation and washing of the NP. This method was also applied to all samples analyzed during the in vitro release experiments.

3. Results and discussion

The choice of a particular method of encapsulation is usually determined by the solubility characteristics of the drug. In our approach, the double emulsion-evaporation process was adopted since it is known to be superior to other incorporation methods in terms of stability of proteins (Tabata et al., 1993). The particle size, polydispersity and BSA encapsulation efficiency have been optimized by changing the homogenization procedure and varying the amount of BSA. The characterization of NP by imaging was effected by two different imaging procedures, atomic force microscopy (AFM) and scanning electron microscopy (SEM). As observed in Fig. 1 NP appear spherical with a relatively monodispersed size.

The pressure homogenization time decreased the NP polydispersity up to an interval of 3 min without changing the NP size significantly. After 3 min the longer homogenization time the higher the NP size and polydispersity (Fig. 2A).

Before 3 min the shear stress of the homogenization device leads probably to a relative homogeneous droplet size with a low polydispersity index. After 3 min the stability of the double emulsion seems to be decreased involving the uncontrolled coalescence of the droplets leading to an increase of both size and polydispersity.

By increasing the polymer concentration in the organic phase an increase of the size as well as the polydispersity of NP was observed. These results

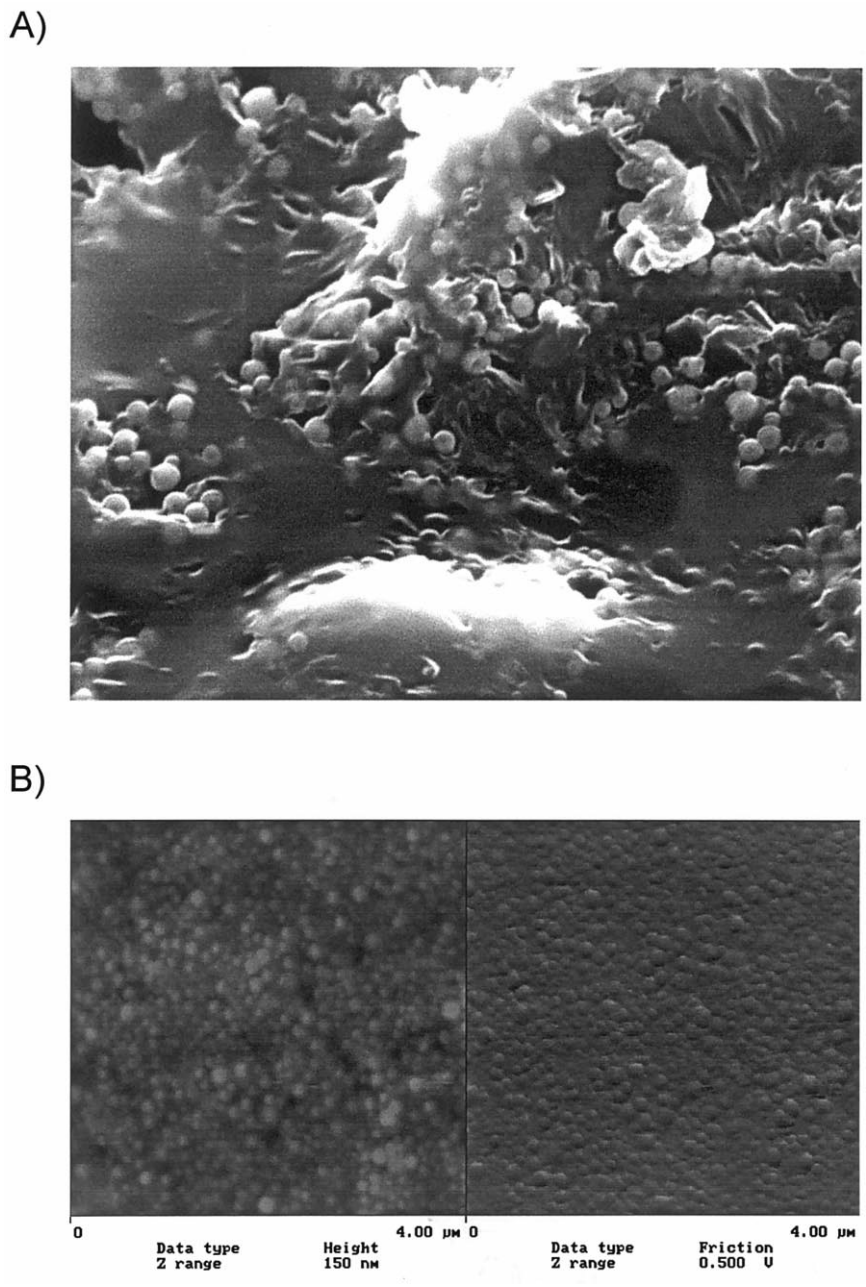

Fig. 1. Images of NP taken by SEM (A) and AFM (B) (non contact mode).

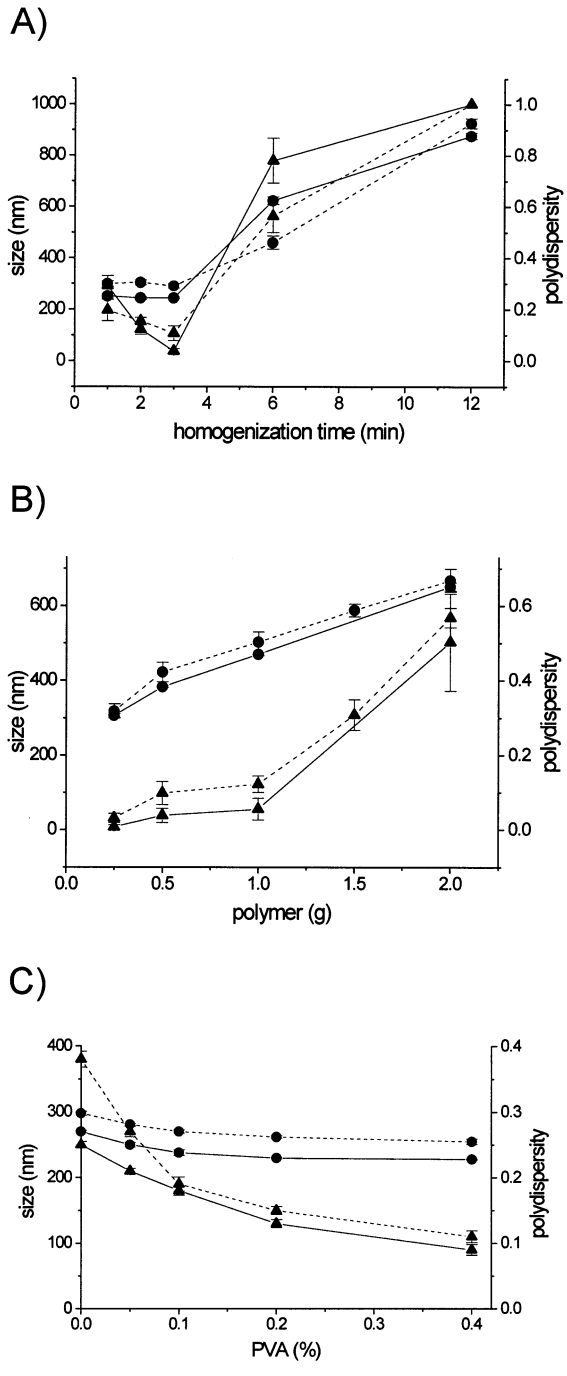

Fig. 2.

correlated with those previously observed by Ogawa et al. (1988). Indeed, they stated that an increase in polymer concentration led to an increase of the viscosity of the first emulsion and consequently the aggravated dispersion of the inner aqueous phase in the organic phase. This first emulsion subsequently led to less efficient reduction of the emulsion droplet size during the second emulsification step.

The more the PVA concentration was increased, the more the PVA molecules covered the interface between organic phase and external aqueous phase. An improved protection of the droplets from coalescence was obtained, leading consequently to smaller emulsion droplets than at lower PVA concentrations. Since NP were formed from the emulsion droplets after the solvent evaporation, their size is dependent upon the size and the stability of the emulsion droplets.

As shown in Fig. 3A, blank NP were slightly smaller than protein loaded NP. However, by measuring the amount of BSA in the inner aqueous phase the size of the resulting NP did not change.

On the contrary the polydispersity was slightly increased, but in all cases it was much lower than in other published work (Tobío et al., 1998) especially for PLGA NP (Fig. 3B). The concentration of BSA in the internal aqueous phase had a non-negligible influence on the encapsulation efficiency (Fig. 3C). The optimal encapsulation efficiency reached 85% and 84% for both PLGA and PCL NP when the lowest BSA amount was used. Moreover, by increasing BSA concentrations it diminished down to a plateau. The difference in osmotic pressure between the internal and external aqueous phases could be responsible for the decrease in entrapment efficiency. The osmotic

Fig. 2. (A) Effect of the homogenization time on particle size (PLGA, —●—; PCL ---●---) and the polydispersity of respectively PLGA (—▲—) and PCL (---▲---) NP. Data are shown as mean ± SE. (B) Effect of the polymer mass in the organic phase on the size (PLGA, —●—; PCL, ---●---) and the polydispersity of respectively PLGA (—▲—) and PCL (---▲---) NP. Data are shown as mean ± SE. (C) The effect of the PVA concentration in the external aqueous phase on the size (PLGA, —●—; PCL ---●---) and the polydispersity of respectively PLGA (—▲—) and PCL (---▲---) NP. Data are shown as mean ± SE.

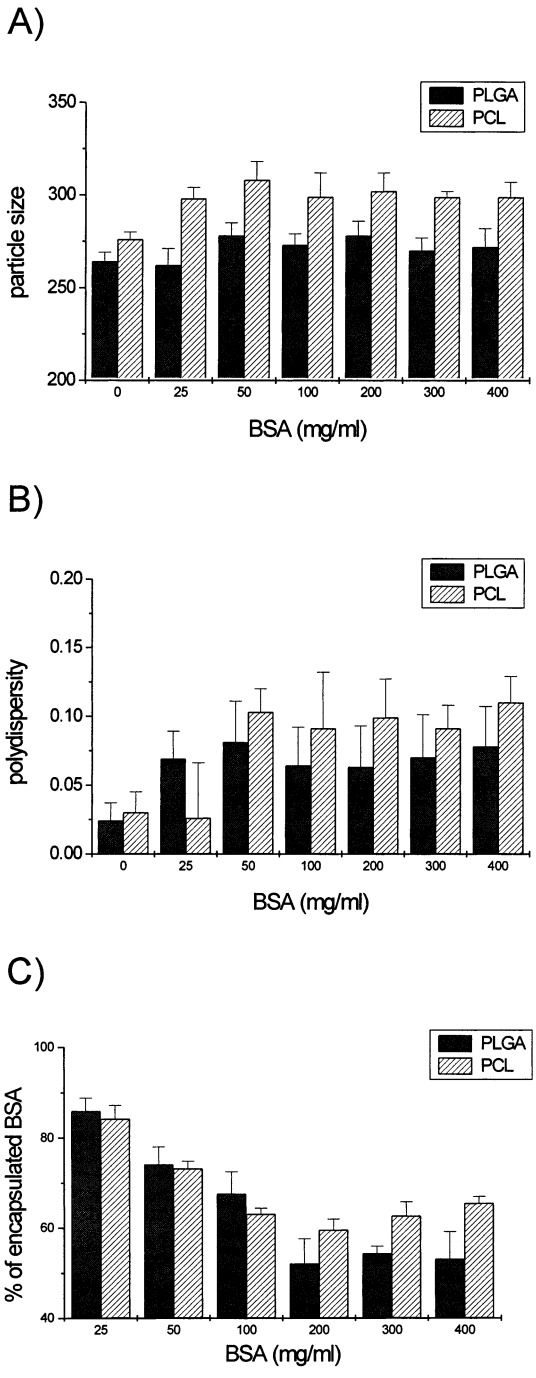

Fig. 3.

pressure difference did in fact increase with increased BSA loading, leading to a rupture of the lipophilic droplets, and an exchange between the internal and external aqueous phases, with a consequent loss of BSA.

NP were investigated in order to characterize their properties as a controlled protein delivery system. The in vitro protein release profiles obtained for each formulation showed a three phase composition (Fig. 4):

1. a first initial burst release due to the drug desorption from the particle surface;
2. a plateau for an certain period, resulting from the only diffusion of the drug dispersed into the polymer matrix; and
3. a constant sustained release of the drug over four weeks resulting from the diffusion of the protein through the polymer wall as well as its erosion.

In conclusion, the present NP can be expected to be used as carriers of hydrophilic compounds

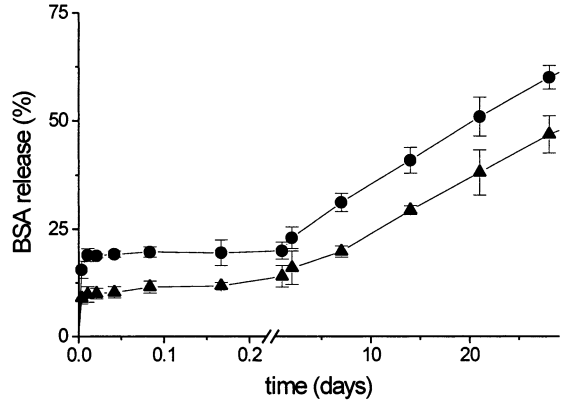

Fig. 4. Release profiles of BSA from PLGA (●) and PCL (▲) NP in phosphate buffer at 37°C and pH 7.4 after 2 h (a) and 30 days (b). Data are shown as mean ± SE.

Fig. 3. (A) Influence of the BSA concentration in the inner aqueous phase on the polydispersity. Data are shown as mean ± SE obtained from three formulations. (B) Influence of the BSA concentration in the inner aqueous phase on the polydispersity. Data are shown as mean ± SE obtained from three formulations. (C) Entrapment efficiency of BSA loaded NP prepared by the double emulsion technique versus the amount of the polymer in the organic phase (PLGA (●) and PCL (▲) NP). Data are shown as mean ± SE.

prepared by a w/o/w extraction method. Furthermore, the present NP are attractive for parenteral or transmucosal application because of their monodispersed submicron-sized structure and their biodegradability. Moreover, the preliminary protein release test from the PLGA and PCL NP in vitro proved that the present NP had the properties of a sustained release form.

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