DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 20(16), 2535-2545 (1994)

PREPARATION METHODS OF BIODEGRADABLE MICROSPHERES ON BOVINE SERUM ALBUMIN LOADING EFFICIENCY AND RELEASE PROFILES

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ABSTRACT

The solvent evaporation and multiple phase methods for preparing poly-(*d*, *l*) lactide microspheres of bovine serum albumin (BSA) were compared. The effects of poly (vinyl alcohol) concentration and external aqueous phase temperature on the loading efficient of BSA microspheres prepared by multiple phase emulsion method were evaluated as well. The BSA loading efficient of microspheres by multiple phase emulsion method was much higher than that by solvent evaporation method. The high aqueous solubility of BSA contributes to the low loading efficieny in the solvent evaporation method, suggesting that this method is inappropriate for proteins with high water solubility. The loading efficieny of microspheres, whcih were prepared by multiple phase emulsion method, increased with PVA concentration but decreased with external aqueous phase temperature. The burst phenomenon of release profiles of microspheres was influenced by poly (vinyl alcohol) concentrations and the external aqueous phase temperature. Considering the duration sustained release, 0.5% w/v of poly (vinyl alcohol) is most appropriate among the concentrations tested for preparing BSA microspheres by multiple phase emulsion method.

INTRODUCTION

Recently, protein and polypeptides have been used to form microspheres or microparticles, since biodegradable microspheres have promise for the prolonged delivery of proteins and polypeptides via the injectable route. Candidate biodegrable materials include albumin, gelatin, poly(*d, l* lactide-glycolide) copolymers, etc, (1-6). Poly(*d, l* lactide-glycolide) copolymers have become popular over the last few years (7-9), because of their biodegradability and the potential for administration by parenteral routes.

Deciding a method for preparing the microspheres is essentially based on the properties of chosen drug and polymer, the morphology of microspheres, and the release profiles of drug from the microspheres will based upon the preparation method (10-11).

In this study, bovine serum albumin (BSA) and poly-(*d, l*) lactide (PLA) microspheres were prepared by using solvent evaporation and multiple phase emulsion methods. The effects of surfactant concentration and microencapsulation temperature on the loading efficiency of BSA in the microspheres and the release profile of BSA from the microspheres were studied as well.

MATERIALS AND METHODS

Solvent Evaporation Method (O/W method):

150 mg bovine serum albumin (BSA) (< 75 μ) (H_2O content 3.1%, 96-99% (GE), Sigma Chemical Company, St. Louis, MO) was dispersed into the poly-(*d, l*) lactide (PLA) (Medisorb 100DL, Medisorb Technologies Intern. L.P., Cincinnati, OH) solvent solution [PLA, 350 mg; methylene chloride (HPLC grade, Baxter Healthcare Corporation, McGaw Park, IL), 3ml]. The whole dispersion was emulsified into an external aqueous phase [deionized water, 50 ml; poly(vinyl alcohol) (PVA) (88% mole hydrolyzed, PolyScience, Inc., Warrington, PA), 0.25% w/v] at 25 °C. The microspheres were collected by

filtration, washed with deionized water, and dried in a vacuum oven at 25 °C overnight.

Multiple Phase Emulsion Method (W/O/W method):

150 mg BSA was dissolved in 0.5 ml deionized water, and homogenized in 3 ml PLA solution (PLA, 450 mg; methylene chloride, 3 ml) for 1 min to form water-in-oil emulsion. The w/o emulsion was then poured into an external aqueous phase (deionized water, 50 ml; PVA, 0.125%~0.75% w/v) at 5, 25 or 50 °C water bath. The method for collecting the microspheres was identical to that of the solvent evaporation method. Also, for both solvent evaporation and multiple phase emulsion methods, the information about microsphere size and morphological characteristics were obtained using scanning electron microscopy.

Microspheres Evaluation:

BSA-loaded microspheres (20 mg) were dissolved in 7 ml methylene chloride, and the suspension was centrifuged at 3000 rpm for 10 min. The clear supernatant was discarded, and the same procedure was repeated twice. Finally, the residue was dissolved in pH 7.4 buffer solution [containing 0.1% w/v sodium dodecyl sulfate (Aldrich Chemical Company, Inc., Milwaukee, WI]. The loading efficiency for BSA in the microspheres was given by

$$\text{loading efficiency} = \frac{\text{real loading}}{\text{experimental loading}}$$

Dissolution of microspheres:

The in-vitro BSA release profiles were carried out in a horizontal shaker (37 °C, 75 rpm). The BSA-loaded microspheres (20 mg) were placed into a 10-ml glass vial filled with pH 7.4 buffer solution. Periodically, the vial was removed form the shaker, and centrifuged at 3000 rpm for 10 min. The whole sample solution was taken out by using a syringe. Followed by replacement with fresh

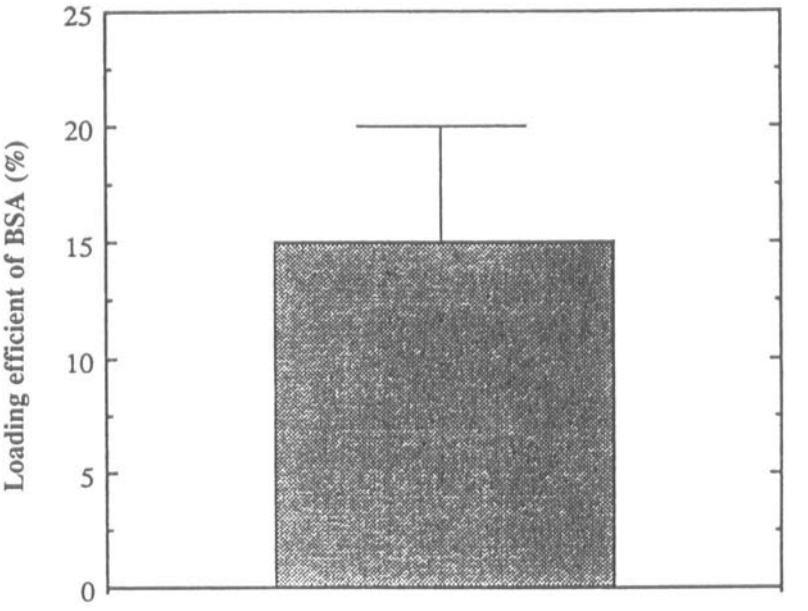


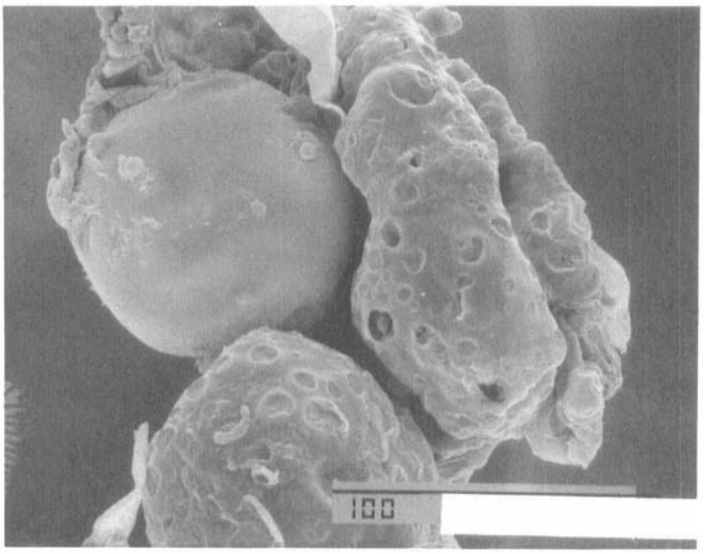
Figure 1
The loading efficiency of BSA-microspheres prepared by solvent evaporation method.

buffer solution. The amount of BSA released in the sample solution was monitored using a the UV Spectrophotometer at 278 nm.

RESULTS AND DISCUSSION

The loading efficiency of BSA in the microspheres prepared from solvent evaporation is presented in Figure 1, with supporting scanning electron micrograph of the microspheres in Figure 2(a). The low loading efficiency of BSA in the microspheres could be due to the high solubility of BSA in water, since most of BSA would dissolve in the external aqueous phase during microencapsulating. Therefore, simple solvent evaporation method is not adequate for preparing BSA microspheres.

(a)



(b)

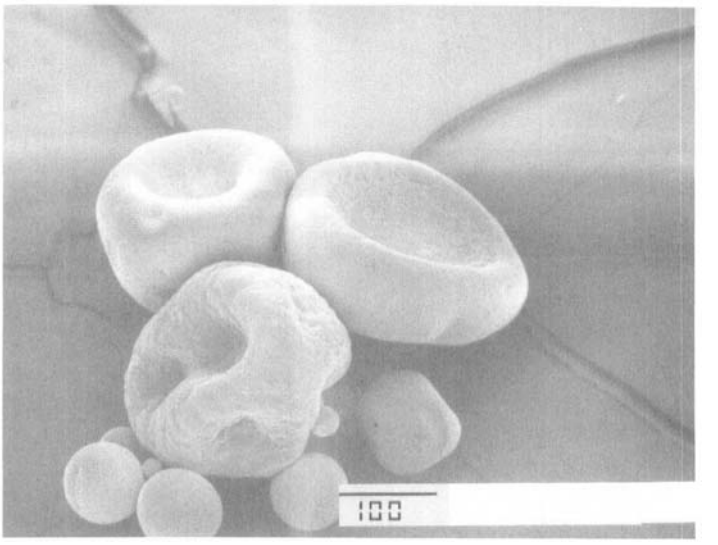
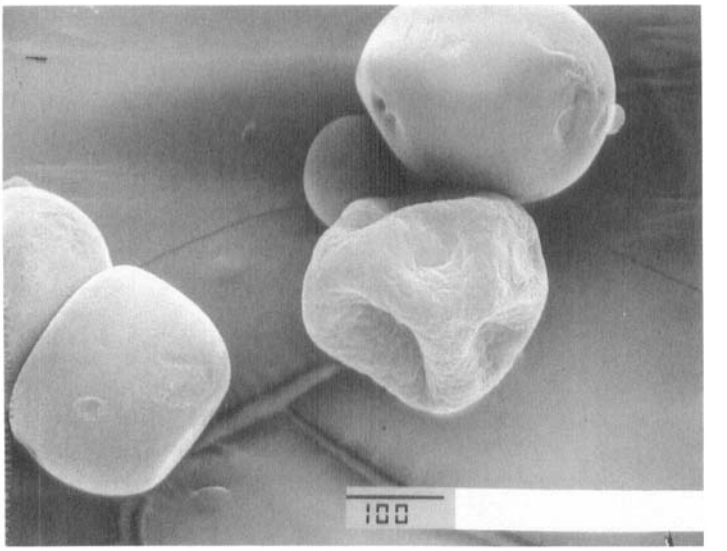


Figure 2

Scanning electron micrographs of BSA microspheres. (a) microsphere prepared by solvent evaporation method, (b) microspheres prepared by multiple phase emulsion method at 25 °C, 0.125% PVA, (c) microspheres prepared by multiple phase emulsion method at 25 °C, 0.5% PVA, (d) microspheres prepared by multiple phase emulsion method at 25 °C, 0.75% PVA, (e) microspheres prepared by multiple phase emulsion method at 5 °C, 0.75% PVA, (f) microspheres prepared by multiple phase emulsion method at 50 °C, 0.75% PVA. Bar at bottom represents 100 μ.

(continued)

(c)



(d)

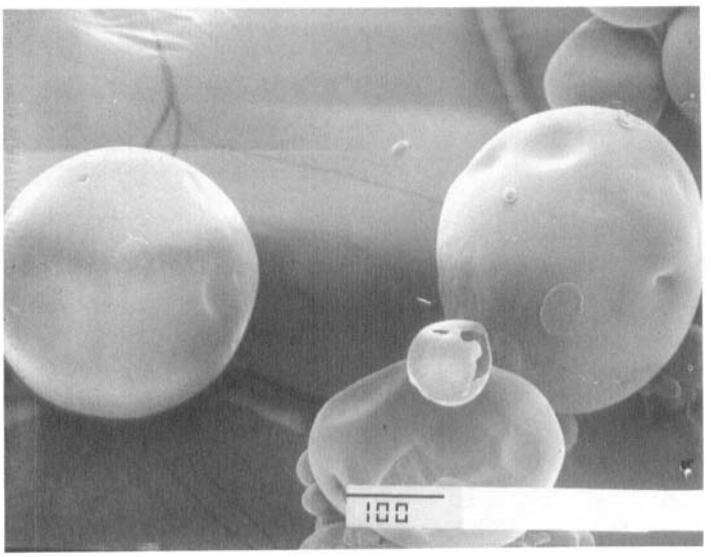
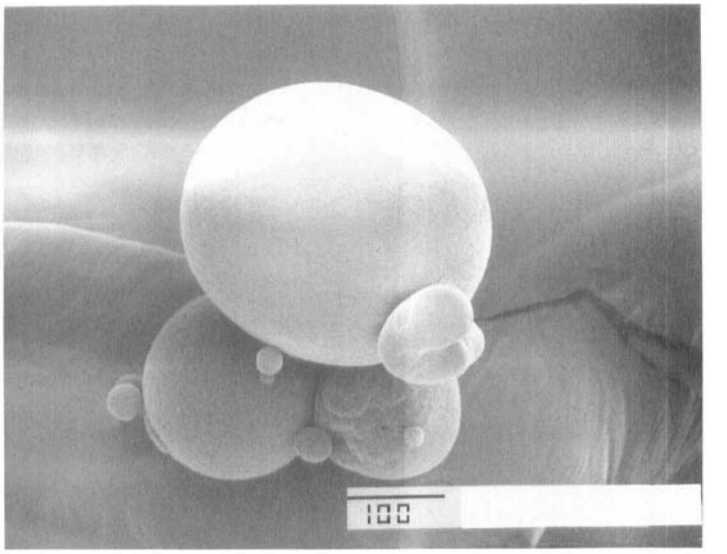
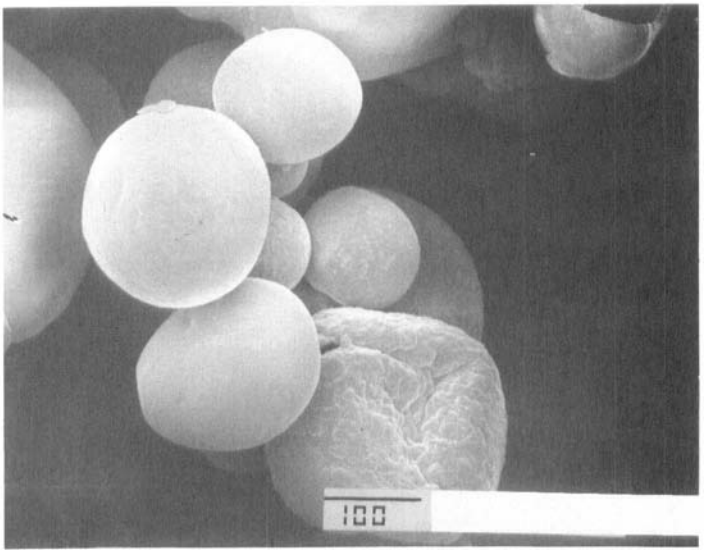


Figure 2 Continued.

(e)



(f)



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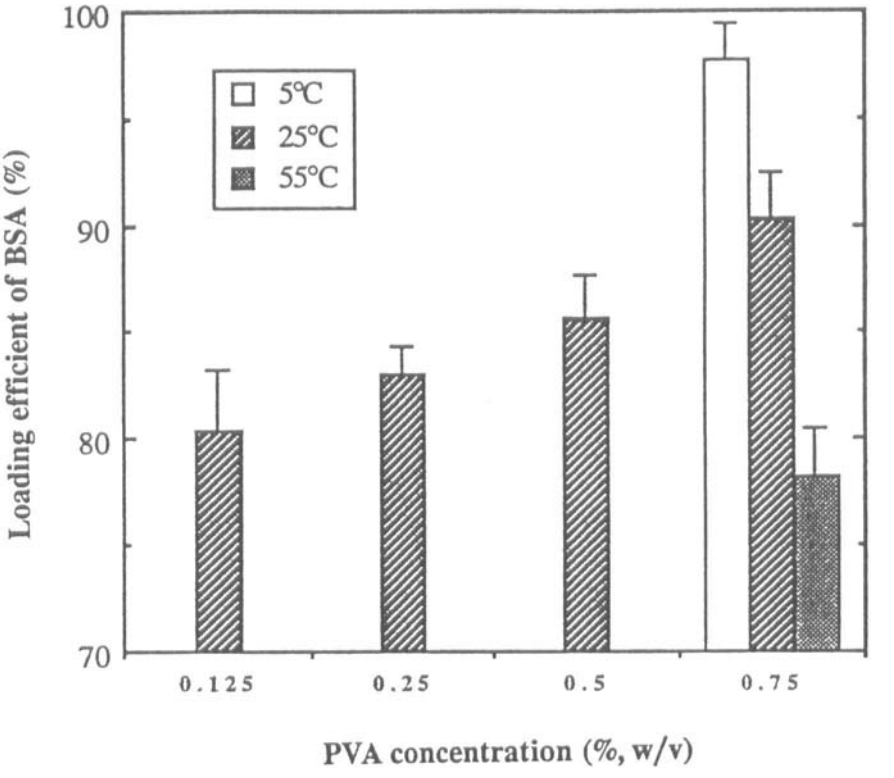

Figure 3
The loading efficiency of BSA-microspheres prepared by multiple phase emulsion method.

The effects of PVA concentration and external aqueous phase temperature on the loading efficiency of BSA microspheres which were prepared from multiple phase emulsion method are shown in Figure 3, and the scanning electron micrographs are shown in Figures 2(b) to 2(f). The loading efficiency of BSA microspheres by this method was higher than that of the solvent evaporation method, and the loading efficiency of BSA in the microspheres was found to increase with PVA concentration in the external aqueous phase and decrease when increasing the external aqueous phase temperature. Since increasing the surfactant concentration would decrease the interface tension between the internal w/o emulsion and the external aqueous phase, the higher amount of w/o emulsion

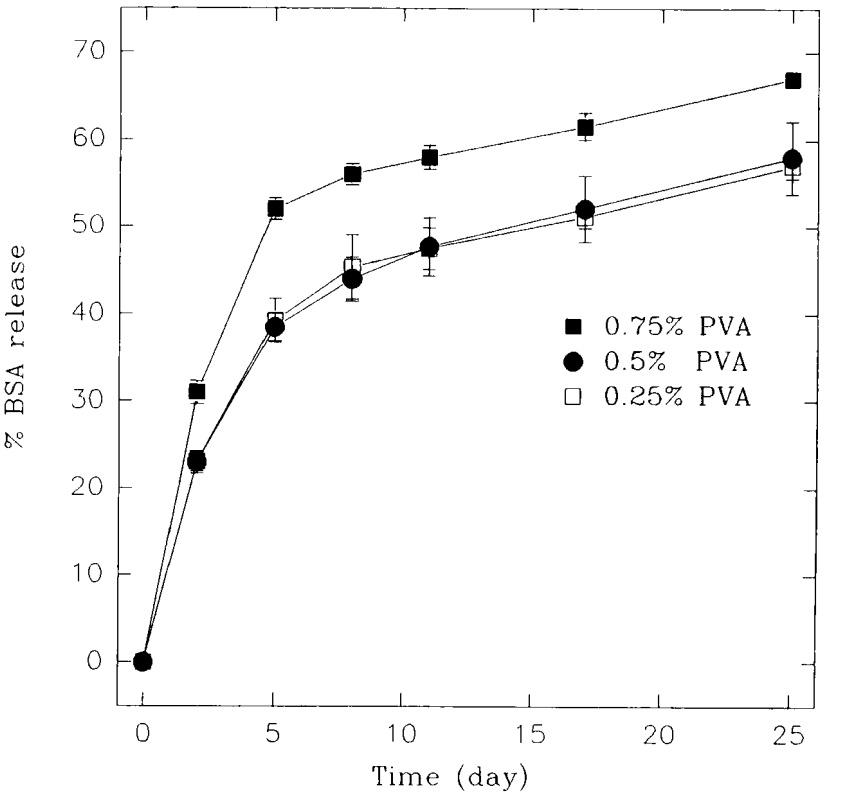

Figure 4
Dissolution profiles of BSA-microspheres which contained different PVA concentrations and were made at 25°C.

would be entrapped by the external aqueous phase and higher loading efficiency of BSA in the microspheres was expected. Because a higher solvent evaporation rate existed in the higher external aqueous phase as a function of increasing temperature, the less amount of w/o emulsion would be encapsulated in the external aqueous phase and the loading efficiency of BSA in the microspheres would be expected to decrease.

The dissolution profiles of BSA-loaded microspheres are shown in Figures 4 and 5. Except for the burst effect during initial release step, BSA release from the microspheres was similar for all the microspheres which were prepared from multiple phase emulsion method at different PVA concentrations and

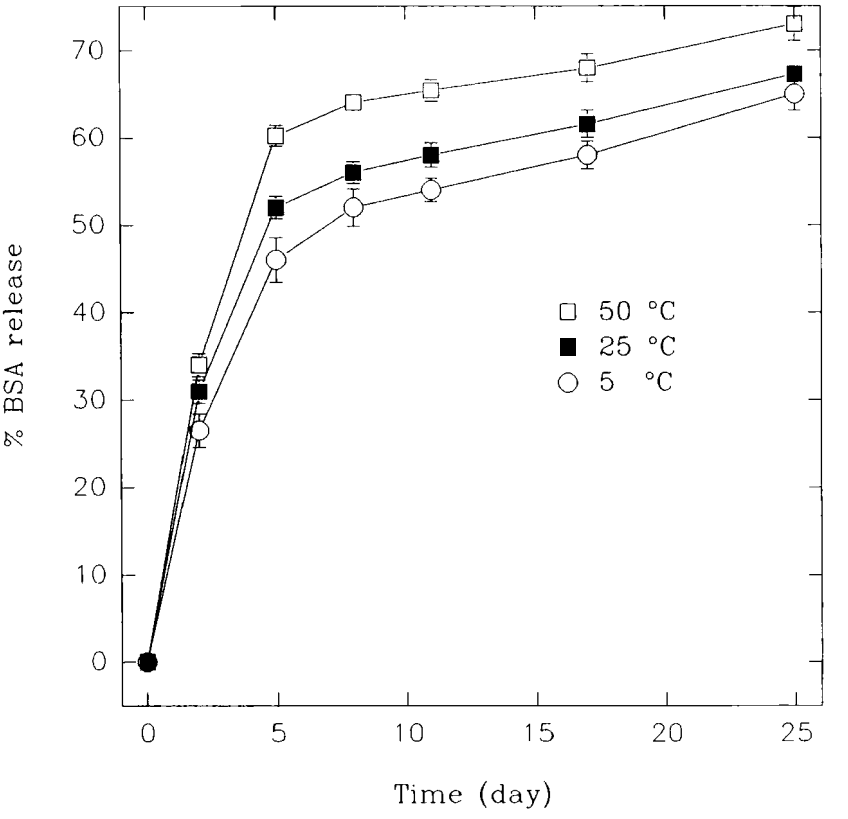

Figure 5
Dissolution profiles of BSA-microspheres which were prepared at different temperatures with 0.75% PVA.

temperatures. The burst phenomenon of release from the microspheres was influenced by PVA concentrations and the external aqueous phase temperature. The burst release of BSA was lower in the microspheres prepared at lower PVA concentration and lower temperature. Considering the duration of sustained release, 0.5% w/v of PVA was most appropriate of the concentrations tested for preparing BSA microspheres by the multiple phase emulsion method.

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