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Note

Biodegradation and drug release studies of BSA loaded gelatin microspheres

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Abstract

Certain variations in the process parameters (emulsification time, surfactant concentration) were performed in order to prepare BSA-loaded gelatin microspheres with high loading efficacy and particle size ranging from 1 to 10 μm using a procedure originally employed by Tabata and Ikada. The mathematical modelling of drug release in the presence of collagenase showed a biphasic release pattern, where the rate constant for the initial time release confirmed the influence of the particle size and/or enzymatic degradation rate on drug release rate.

Keywords: Gelatin microspheres; BSA; Collagenase; Biodegradation; Drug release

Polymer based microparticulated delivery systems intended for oral antigen delivery should maintain the integrity and activity of the antigen, augment the immunopotentiating effect and modulate the type of antibody response by acting as regulated and sustained release systems (Alpar et al., 2000). Gelatin microspheres proved to evoke immune response at the mucosal effector tissues, suggesting the possibility to develop the oral vaccine (Nakamura et al., 1998). Variations in particle size can control particle uptake into mucosa subepithelia regions, while the control of mechanical characteristics as well degradation kinetics via glutaraldehyde crosslinking could be achieved (Friess, 1998). Parameters strictly linked to the drug release kinetics from the gelatin matrix are the rate of water uptake, drug dissolution/diffusion rate and the polymer glass-rubbery transition including matrix erosion/degradation rate (Brazel and Peppas, 2000). Gelatin degradation by all cell types can occur either after phagocytosis or by extracellular protease acting at either neutral or acid pH. At neutral pH, only specific collagenases simply attack the Gly-X-Y sequence. Thus, the adjuvant effect is derived not only from the function of gelatin itself as an accelerator of Mϕ phagocytosis, but also from the function of cross-

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linked gelatin microspheres as carriers in the GIT to protect the antigen from enzymatic degradation and to deliver it to the immune-inductive sites (Peyer's patches) in a controlled manner.

Variations in the process parameters were performed in order to prepare microspheres with high loading efficiency and an optimal size for uptake into mucosa subepithelia: 1–10 μm (Alpar et al., 1998). "Model" protein, BSA, had facilitated the understanding of the release kinetics. A key parameter appears to be particle size, so the correlation between the biodegradation and drug release rates in a presence of collagenase, and drug and polymer particle size was established.

The microspheres were prepared by emulsification of aqueous solution of gelatin (10% w/V) and radiolabelled [^{131}I]BSA (2.5% w/V) into the particles in an oil (Kreuter, 1994) and stabilized by crosslinking with glutaraldehyde-saturated toluene (0.05 mg/mg gelatin) at 0 °C for 4 h. By variations in the process parameters, time of emulsification and sorbitan monooleate concentration, particles in a range of 7.03 ± 1.23 to 1.19 ± 1.96 μm were prepared (laser diffractometry; Fritsh particle size analysette D LAB/22, Germany). Two factorial analysis of variance ($\alpha = 0.05$) confirmed the differences in particle size due to the extended time of emulsification and interaction between process variables. High percentage of encapsulation, calculated as a percent of total radioactivity present in the supernatant and in the filtrate after microsphere isolation ("well" counter Scaler Type N 529 D, EKCO Electronics, UK) was observed at all series of microspheres (80–95%, respectively). The biodegradation (of blank microspheres) and release studies (of BSA-loaded gelatin microspheres) were carried out in calcium containing phosphate buffered dispersion of microspheres (0.5 mg/ml) in the presence of collagenase (0.1 mg/ml) by simulating in vivo conditions (37 °C, 75 Str/min). At regular time intervals, the dispersion of blank microspheres was centrifuged (8000 rpm for 15 min), and the amount of gelatin in the sediments was determined according to a ninhydrin method (Sawicki et al., 1961). The amount of BSA released was determined by measuring the radioactivity present in the supernatants at regular time

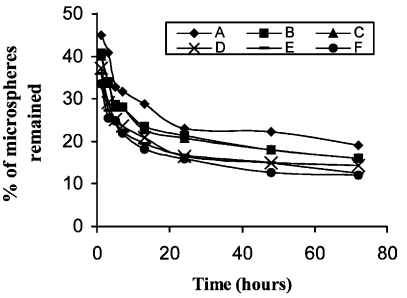

Fig. 1. Degradation profiles of gelatin microspheres with different mean diameters; (A) 7.03 μm; (B) 5.89 μm; (C) 4.63 μm; (D) 3.20 μm; (E) 2.29 μm; (F) 1.99 μm.

intervals. In vitro release profiles were described using biexponential function (Kreuter, 1994).

Degradation data (Fig. 1) showed that the preparation procedure provides prolonged enzymatic degradation at all series of gelatin microspheres, suggesting complete in vivo degradation. During 72 h at 37 °C, between 84 and 90% of gelatin microspheres were degraded.

After 24 h at 37 °C the release of BSA from glutaraldehyde treated gelatin microspheres in the presence of collagenase was between 80 and 93% of the total amount of the encapsulated drug (Fig. 2). In all series the drug release profiles showed a biphasic modulation characterized by an initial relatively rapid release period, followed by a slower release phase. Namely, at the series of gelatin microspheres with mean diameter smaller than 4 μm, in the initial time release (3 h) between

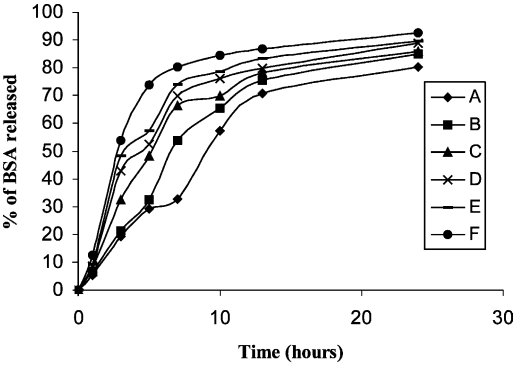

Fig. 2. BSA release (%) from gelatin microspheres with different mean diameters; (A) 7.03 μm; (B) 5.89 μm; (C) 4.63 μm; (D) 3.20 μm; (E) 2.29 μm; (F) 1.99 μm.

30 and 55% of total drug was released. The initial time release for the same percent of total drug ($\approx 37\%$) at the series of gelatin microspheres with mean diameter higher than 5 μm was ≈ 6 h.

These data are in correlation with biodegradation rate, so the higher percent released at the series of gelatin microspheres with mean diameter smaller than 4 μm in the initial time release is probably a result of the complete water penetration into the spheres supported by the faster enzymatic degradation and thus, more free drug liberation. Thus, high correlation coefficients between the particle size and degradation rate ($r = 0.970$) and, also, the particle size and drug release rate were noticed ($r = 0.945$), confirming the statistically significant difference among the series with different particle size.

The drug release constants obtained by the semilogarithmic plots confirmed that the drug release rate in the initial time ($0.058 < \alpha < 3.21\ h^{-1}$) is faster at series with lower values for mean diameter, suggesting the influence of degradation and diffusive processes, while the drug liberation in the late release time ($0.066 < \beta < 0.078\ h^{-1}$) is influenced by the diffusive processes, only.

In conclusion, emulsification of aqueous solution of gelatine and BSA into the particles in oil and proposed variations in the process parameters, as well, enable preparation of microspheres with high drug-loading efficacy and an optimal size for an uptake into the gut-associated lymphoid tissues and systemic immunoresponsive organs. The biodegradation and drug release rates data support the potential adjuvant efficacy of gelatin microspheres.

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