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Preparation of gelatin microspheres of bleomycin

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Summary

A new method for preparing hydrophilic, cross-linked, solid gelatin microspheres of 15–28 μm size has been developed. Two main advantages of this method are the addition of the cross-linking agent in an organic medium to the aqueous gelatin medium and the use of concentrated high polymer solutions as the organic phase for polymeric dispersions. Gelatin microspheres containing bleomycin were prepared by this novel method using concentrated solutions of polymethylmethacrylate.

Microspheres have been used as sustained release targeting agents for anticancer drugs by many investigators (Zolle et al., 1970; Kramer, 1974; Sezaki et al., 1982; Hashida et al., 1979). The use of albumin microspheres as drug carriers has been studied to an increasing extent. However, current methods of albumin microsphere preparation involve either thermal denaturation at elevated temperatures (110–165°C) or chemical cross-linking in vegetable oil or isooctane emulsions (Ishizaka et al., 1981; Senyei et al., 1981). Because small amounts of surfactants are needed to disperse such microspheres in water, they appear to be somewhat hydrophobic. Surface hydrophilicity is important, because the hydrophilic albumin surface may enhance surface physical and chemical behaviour in vivo and the surfactants presently used to disperse the human serum albumin microspheres may influence tissue interactions, drug activity and its release. Recently, a new method of preparation of hydrophilic albumin microspheres without any surfactants has been reported (Longo et al., 1982).

The present report is concerned with the preparation of gelatin microspheres by a new procedure which yields hydrophilic, solid microspheres. Gelatin was selected as a model macromolecule because of its ready availability, its relatively low antigenicity and its previous use in parenteral formulations.

Gelatin microspheres containing the anti-cancer agent bleomycin (BLM) were prepared by using concentrated polymethylmethacrylate (PMMA) solution. PMMA was prepared in our laboratory by using potassium persulphate ($K_2S_2O_8$) and sodium bisulphite ($NaHSO_3$) redox system in the aqueous medium. The polymer solution was cooled and precipitated with isopropanol. The isolated PMMA was dried at room temperature initially and stored over calcium chloride in a vacuum

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desiccator. Both distilled and undistilled monomers were used in the above preparation. The experimental conditions for the preparation and characterization of PMMA using Gel Permeation Chromatography for molecular weight and their distribution is given below. Preparation: monomer 20 ml; initiator $K_2S_2O_8$ 0.8 g, $NaHSO_3$ 0.4 g; time 3 h; temperature 65–80°C; water = 200 ml. Characterization: PMMA prepared from undistilled MMA — $\overline{M}_n$ 7.698×10^4, $\overline{M}_w$ 7.818×10^4, $\overline{M}_z$ 7.940×10^4, $\overline{M}_v$ 7.817×10^4, $\overline{M}_w/\overline{M}_n$ 1.016; PMMA prepared from distilled MMA — $\overline{M}_n$ 9.107×10^4, $\overline{M}_w$ 9.489×10^4, $\overline{M}_z$ 9.947×10^4, $\overline{M}_v$ 9.488×10^4, $\overline{M}_w/\overline{M}_n$ 1.042.

Microspheres were prepared by dissolving 300 mg of gelatin in 1 ml of warm water (40°C); 1.5 g PMMA was dissolved in 6 ml of a chloroform: toluene (1 : 1) mixture and added to the gelatin solution; the mixture was shaken well. Glutaraldehyde in toluene was used for cross-linking. The cross-linking solution was prepared by mixing 2 ml of glutaraldehyde with 2 ml of toluene in a test tube and shaken intermittently for 2 h. After phase separation, the resulting toluene solution of glutaraldehyde was added to the gelatin dispersion. The dispersion was then mixed and left for the completion of cross-linking of gelatin (2 h). The microspheres were allowed to settle and the excess PMMA was removed by treating with toluene followed by acetone. Finally, the microspheres were given a rapid wash of distilled water and dried at room temperature. A free-flowing powder was obtained. Gelatin microspheres containing the drug were prepared exactly in the same fashion as reported above, except that the drug was added to the gelatin solution before mixing with PMMA.

The molecular weight characterization of PMMA by GPC clearly indicated that $\overline{M}_z > \overline{M}_w > \overline{M}_n$ and the number average molecular weight was in the order of 10^4–10^5, both in the case of undistilled and distilled monomers. However, the molecular weight of PMMA from distilled monomer is slightly higher than that of the polymer prepared from undistilled monomer. The polydispersity is found to be close to 1, which indicated that the PMMA had very narrow molecular weight distribution. The concentration of the polymer dispersant solutions was important in the preparation of gelatin microspheres. It was found by Longo et al. (1982) that in the preparation of albumin microspheres, less than 25% (w/w) polymer concentrations were not sufficient to prevent coagulation when small microspheres were desired and concentrations greater than 30% retarded complete removal of the polymer. It was also indicated that low molecular weight PMMA was unsatisfactory at concentrations of 25–30% (w/w) since coagulation occurred. Hence, in our studies, we have always used 25% PMMA solution of molecular weight 10^4–10^5 for the preparation of microspheres.

The size of the microspheres was determined using an optical microscope with the help of a micrometer. The shape of the coated gelatin particles was invariably spherical. At least 150 microspheres were randomly selected and their size was measured by the optical microscope. The size distribution of naked and bleomycin-incorporated microspheres was obtained as illustrated in Fig. 1. The majority of naked microspheres indicated that they were in the range of 15–20 μm diameter, whereas the BLM-loaded microspheres showed that the majority of the spheres lie in the range of 20–30 μm diameter.

The morphological characteristics of the microspheres using the optical microscope have been carried out for the control and the drug-loaded microspheres. It is shown from the optical microphotograph (Fig. 2) that BLM is encapsulated in the gelatin matrix surrounded by a polymer coating. The polymer coating on microspheres apparently affords dispersion stability and prevents coagulation before and during glutaraldehyde cross-linking. However, when the microspheres, both naked and drug-loaded, were left in water for 12 days, complete rupture of the polymer coating was observed. Scanning electron microscopic (SEM) studies of the microspheres and the in vitro release studies of bleomycin in phosphate buffer pH 7.4 at 37°C are currently in progress.

Two main advantages of this method for gelatin microsphere preparation as compared to other methods are the addition of the cross-linking agent in an organic medium to the aqueous gelatin dispersion and the use of concentrated high poly-

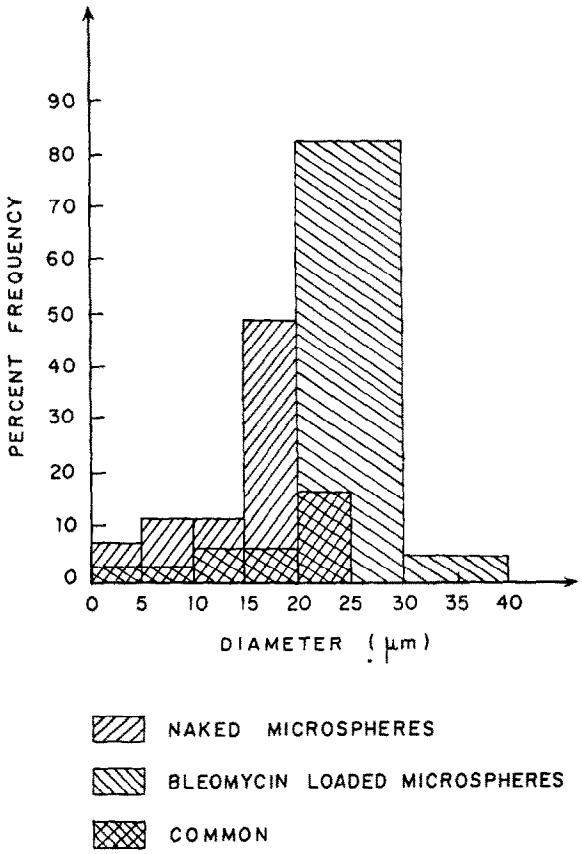

Fig. 1. Particle size distribution of microspheres.

mer solutions as the organic phase for polymeric dispersions. Concentrated polymeric solutions as the organic dispersion phase afford excellent dispersion and control, wide particle size, and smooth uniform spheres without the need for surfactants. The availability of a relatively high surface concentration of aldehyde groups on the microspheres facilitates a variety of chemical modifications.

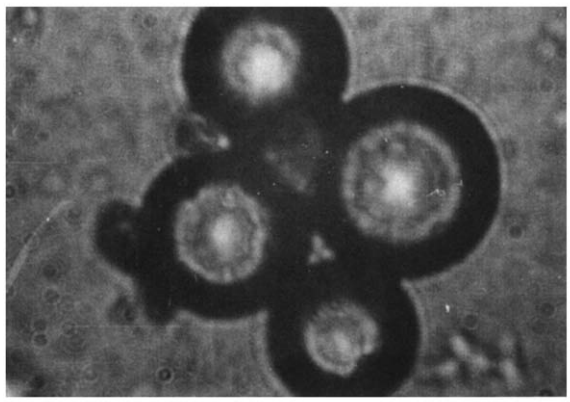

Fig. 2. Optical microphotograph of gelatin microspheres containing bleomycin. Average particle size 28 μm.

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