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Potential use of albumin microspheres as a drug delivery system. I. Preparation and in vitro release of steroids

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Summary

Albumin microspheres were prepared by two different stabilization processes: chemical denaturation and heat denaturation. The extent of stabilization was characterized by the solubility and the swelling properties of the microspheres. In vitro drug (prednisolone) release rates were determined for the different microsphere preparations and the results were correlated to the stability of the microspheres. Heat denaturation had a significant effect on the in vitro release rates; the more denatured the albumin, the slower the drug release rate. Chemical denaturation, using glutaraldehyde, did not have a marked effect on drug release from the microspheres. Two of the major limitations of albumin microsphere systems, i.e. poor drug entrapment and premature release ("burst effect") have been overcome using prednisolone loaded heat denatured microspheres.

Introduction

Biodegradable albumin microspheres appear to be an exploitable delivery system for sustained and controlled release of drugs (Morimoto and Fujimoto, 1985). Albumin microspheres have been proposed as drug delivery systems for targeting to various organs and tissues, including tumors and the cells of the reticuloendothelial system (Kramer, 1976; Illum and Davis, 1982; Widder et al., 1979; Tomlinson and McVie, 1983). The aim of this study is to evaluate the potential use of albumin microspheres to deliver steroidal anti-inflammatory drugs, for example, by direct injection into joints for the treatment of rheumatoid arthritis.

Corticosteroidal drugs show a high incidence of adverse side-effects. To reduce these untoward effects intra-articular therapy has been investigated as a means of localizing the activity of various drugs including, hydrocortisone (Hollander et al., 1951), prednisolone (Rothermich and Phillips, 1957; Murdoch and Will, 1962), and triamcinolone (Dixon et al., 1972). Multiple dosing is necessary as steroids are rapidly cleared from the joints. However, this is deleterious to the articular cartilage (Chandler, 1959; Salter et al., 1967).

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Liposomes have been investigated as a means of increasing retention of drugs in the synovium and thereby reducing the number of doses required. Shaw et al. (1976) reported that the anti-inflammatory effect of cortisol palmitate was sustained for 3–4 days when incorporated into liposomes.

Microspheres, prepared from rabbit serum albumin cause only slight hyperplasia of the synovial membrane when injected into rabbit knee joints, compared to a severe inflammatory response provoked by other biodegradable polymers; polylactic acid, poly-butylcyanoacrylate and gelatin (Ratcliffe et al., 1984). Due to this relative biocompatibility, albumin microspheres may provide the best means of sustaining the release and reducing the clearance of steroidal anti-inflammatory drugs from joints. Drug release from microspheres as reviewed by Tomlinson et al. (1984) is characterized by an initial rapid release of drug ("burst" release), followed by slower release of the remaining drug. For highly water-soluble drugs, release may be as high as 90% in the first 10 min (Tomlinson et al., 1984). Yapel (1979) also reported biphasic drug release from albumin microspheres, however, by careful manipulation of various parameters he was able to achieve a monophasic release profile for microspheres containing epinephrine. Morimoto et al. (1980) and Sugibayashi et al. (1979) have obtained sustained release of 5-fluorouracil in vivo, over a period of 1 week by intra-peritoneal injection of heat-stabilized microspheres into mice with Ehrlich ascite carcinoma.

The purpose of the present study was to prepare microspheres which would have an acceptable in vivo drug release profile, over a period of weeks or months. Two methods of stabilization of the albumin microspheres are investigated; chemical denaturation using glutaraldehyde, and heat denaturation. The extent of stabilization of the microspheres is measured by the effect on albumin solubility, and on the swelling properties of the microspheres. In an attempt to determine whether drug release is affected by the method of stabilization, these parameters are related to the in vitro drug release profiles obtained for the two microsphere systems.

Microspheres of large particle diameter (23 μm) were selected for this study for the following reasons: significant quantities of water-insoluble drug may be incorporated; drugs can be incorporated in the form of a micronized powder suspension; there will be a smaller surface area available for drug release compared to smaller microspheres; and these microspheres may be retained within the joint cavity longer than smaller microspheres, since they may not be phagocytosed. It is anticipated that biodegradation of the albumin would be more rapid within the phagocytic cell. Ratcliffe et al. (1984) reported that particles smaller than or equal to 6 μm are phagocytosed when injected into the knee joint. All these factors may contribute to achieving the desired effect of sustained drug release.

Materials and Methods

Rabbit serum albumin (RSA) fraction V, highly refined olive oil, prednisolone, and Tween 80 were obtained from Sigma U.K. The prednisolone was micronized at 3M Health Care, Loughborough, U.K. (particle size 2.4 μm, geometric weight-mean diameter, and 0.62 μm, geometric standard deviation). Polycarbonate membrane filters, 0.2 and 0.8 μm pore diameter were obtained from Nucleopore. Diethylether, potassium phosphate, and sodium hydroxide were of Analar grade.

Preparation of RSA microspheres

The method of Tomlinson et al. (1984) was used with slight modification. 0.4 ml of a 25% w/v solution of RSA in distilled water was added to 125 ml of olive oil in a baffled flat-bottomed glass beaker, 60 mm in diameter by 110 mm in height, with 4 baffles (93 × 6 × 6 mm). A heildolph mixer, with a 4-bladed axial flow impeller was used to emulsify the two phases. The oil was prestirred for 30 min at the desired speed. The water-in-oil emulsion was then stirred for a period of time, dependent on the method of stabilization, and the extent of stabilization required. Drug-loaded microspheres are prepared by suspending micronized drug particles with a mean diameter of 2.4 μm in the aqueous RSA solution.

In initial studies, it was shown that the drug-to-RSA ratio affected microsphere formation. The particle size range of the microspheres increased and drug content became less uniform as the weight ratio of drug-to-microsphere was increased. Microspheres would not form at drug-to-microsphere weight ratios of 1 : 2 or higher amounts of drug. A drug-to-microsphere weight ratio of 1 : 5 was selected as optimum to produce microspheres with a high drug loading, yet maintain a narrow particle size range. The aqueous solubility of prednisolone is 5 mM, therefore at most only 2.5% of the added drug will go into solution in a preparation containing a drug-to-microsphere weight ratio of 1 : 5. The drug is therefore contained within the microspheres as solid particles around which the RSA fuses upon denaturation.

Unstabilized microspheres

These were prepared at room temperature as described above. The emulsion was stirred for 60 min to allow equilibration, following which 60 ml of diethylether were added and the resultant mixture was stirred for a further 10 min. Diethylether causes reversible denaturation of the albumin molecules at the surface of the microemulsion droplets, thereby preventing agglomeration. The microspheres were collected on a 0.8 μm polycarbonate filter, and residual oil was removed by washing with diethylether. The microspheres were lyophilized for 18 h.

Chemically stabilized microspheres

These were prepared as for unstabilized microspheres, with the addition of 0.1 ml of the desired concentration of glutaraldehyde solution following the initial emulsification period.

Heat-stabilized microspheres

These were prepared as for unstabilized microspheres, but stirring was continued while the beaker containing the emulsion was heated in an oil bath at the desired temperature for the desired length of time.

Particle size analysis of microspheres

Particle size analysis was carried out by two methods: Coulter analysis (stabilized microspheres only) using 0.9% NaCl as the electrolyte and Tween 80 to wet the microspheres; and by light microscopy using a microscope fitted with a calibrated graticule. Microsphere samples were analyzed by microscopy either dry (freeze-dried) or following wetting in a 0.1% v/v Tween 80 solution in phosphate buffer. A minimum of 200 microspheres were counted and size distributions were plotted as cumulative percentage undersize from which number average mean diameters were obtained.

Determination of the water solubility of the microspheres

The percentage of the total RSA which dissolved, when a known weight of non-loaded, 23 μm diameter microspheres was added to a known volume (15 ml) of pH 7.0 phosphate buffer at 37 °C, was determined by UV analysis at 278 nm. Phosphate buffer was added to the microspheres in sample vials and the resultant suspensions were sonicated periodically in a bath sonicator for 2 h. The suspensions were then filtered through polycarbonate filters and diluted, if necessary, prior to determination of the UV absorbance of the solution.

In vitro drug release

The release rate of drug from the 23 μm diameter, prednisolone microspheres into phosphate buffer (pH 7.0) at 37 °C, was determined under sink conditions by UV analysis correcting for RSA absorbance. Approximately 20 mg of microspheres were added with stirring to 350 ml of phosphate buffer (pH 7.0) in a water-jacketed beaker at 37 °C. The dissolution medium was analyzed for prednisolone content by UV spectroscopy at 248 nm, using a flow-through cell method. A filter was used to prevent any microspheres travelling into the cell.

Microsphere preparations with half-life ($t_{1/2}$) values for drug release longer than 2 h were not analyzed as described above; instead 2 ml samples were withdrawn at given time intervals and analyzed. The samples were filtered through 0.8 μm nucleopore filters. Any microspheres collected on the filters were flushed back into the dissolution apparatus using replacement buffer.

Results and Discussion

The effect of heat and chemical denaturation on the solubility and swelling properties of RSA microspheres

To investigate the effect of crosslinking on the stability of RSA microspheres (23 μm diameter) the percentage of soluble RSA was determined, on suspending unstabilized and stabilized microspheres in pH 7.0 phosphate buffer at 37 °C. Chemically stabilized microspheres were prepared by incorporating different amounts of glutaraldehyde over the concentration range 0–5% w/v glutaraldehyde in the microspheres. Heat-stabilized microspheres were prepared by heating at 130 °C for 30 min to 24 h. 130 °C was selected for these studies since albumin denatures very rapidly at higher temperatures, and even after very short stabilization times the microspheres are highly crosslinked and swelling effects are insignificant.

The relative stabilities of the microspheres in phosphate buffer are illustrated in Fig. 1A for chemically stabilized microspheres, and Fig. 1B for heat-stabilized microspheres. The percentage of soluble RSA is shown to decrease rapidly as the glutaraldehyde content of the microspheres is increased from 0 to 0.5% w/w. Further increase in glutaraldehyde concentration did not significantly affect the RSA solubility. At glutaraldehyde concentrations below 0.1% w/w, stabilized microspheres were not formed. The time employed for heat stabilization also had a marked effect on the stability of the microspheres (Fig. 1B). Periods of 0–1.5 h did not significantly alter the aqueous solubility of the microspheres. On heating for longer periods, the microspheres become progressively more insoluble. After a period of 6 h, further heating did not affect RSA solubility. From this study, it would appear that albumin microspheres heated for 6 h at 130 °C have a similar extent of stabilization as those treated with glutaraldehyde to a final glutaraldehyde content of 0.5% w/w in the microspheres.

Microscopic examination of microspheres

Freeze-dried microspheres were seen to be spherical in most cases. However, in samples of lightly heat-stabilized microspheres, or chemically

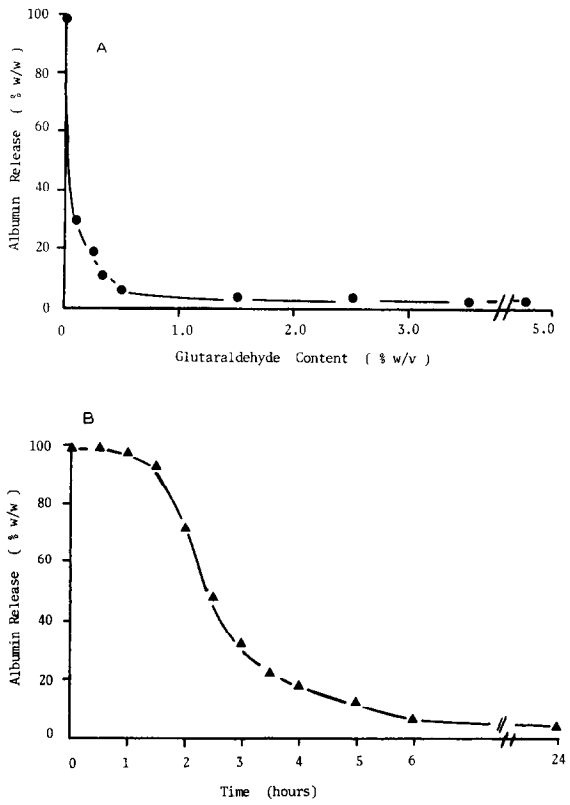

Fig. 1. The effect of the extent of stabilization on the albumin solubility of RSA microspheres, in pH 7 phosphate buffer at 37 °C. A: chemically stabilized. B: heat stabilized.

stabilized microspheres containing less than 0.5% w/w glutaraldehyde, small proportions of "split" microspheres and of crystalline albumin were also present. Fig. 2 is a photomicrograph of "split" microspheres containing prednisolone drug powder. When dispersed in phosphate buffer, stabilized (intact and spherical) freeze-dried, non-loaded microspheres underwent a degree of swelling according to the extent of stabilization. The microspheres retained their spherical shape on swelling, unless their degree of stabilization was very low (less than or equal to 0.1% w/w glutaraldehyde, or less than or equal to 1 h heat stabilization at 130 °C) when the microspheres either dissolved, or so much water was incorporated that their shapes became distorted.

The effect of stabilization on swelling of

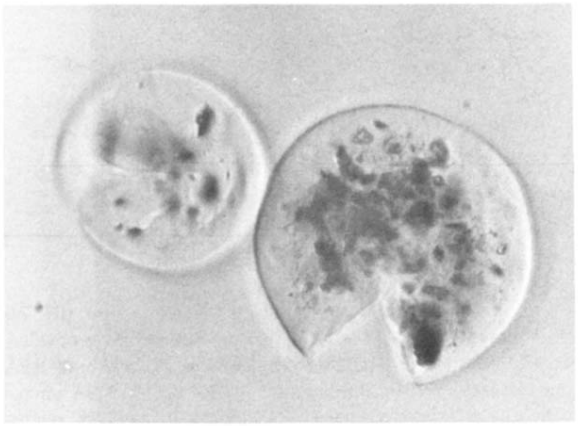

Fig. 2. Photomicrograph of 'split' microspheres containing prednisolone. The total magnification is ×2000.

freeze-dried microspheres in pH 7.0 phosphate buffer at 25 °C was investigated (Table 1a and b). The average diameter of the microspheres prior to swelling was 23 μm. It is seen that the degree of swelling was dependent on the extent of stabilization for both heat and chemically stabilized microspheres, decreasing as the extent of stabilization increased. An increase in the glutaraldehyde content of the microspheres from 0.1 to 2.0% w/w, dramatically reduced swelling; however, further increase in the glutaraldehyde content did not significantly effect swelling. The percentage solubility of the microspheres may be used to give an indication of the degree of swelling of microspheres. These two effects follow the same trend for both heat and chemically stabilized microspheres.

TABLE 1

The effect of the extent of stabilization on swelling of freeze-dried RSA microspheres, in pH 7 phosphate buffer, at 25 °C

(a) Chemically stabilized		(b) Heat stabilized	
Glutaraldehyde content (% w/w)	% Increase in size on wetting	Time of heating at 130 °C (h)	% Increase in size on wetting
0.2	140.7	0.5	125.8
0.5	124.1	1.0	95.2
1.0	53.1	1.5	85.9
2.0	8.6	2.0	72.4
2.5	6.7	2.5	54.2
4.0	7.4	3.0	49.5
5.0	8.5	6.0	32.9

In vitro drug release from chemical and heat stabilized microspheres

Chemical stabilization

The heat denaturation process is limited by the heat stability of the drug. There is no such limitation to the extent of stabilization possible using the chemical denaturation process, assuming that the drug does not react with glutaraldehyde. Thus chemical crosslinking may be the most effective method of stabilizing the microspheres. Glutaraldehyde reacts with the amino residues of albumin, crosslinking and hence denaturing the albumin and rendering it insoluble. Sokoloski and Royer (1984) describe the various proposed mechanisms of interaction of glutaraldehyde with albumin. It was considered that slower drug release rates might be achieved when the extent of denaturation was increased.

The effect of both glutaraldehyde concentration and the time of exposure of the microspheres to glutaraldehyde, upon drug release from the microspheres was studied. The glutaraldehyde content ranged from 0.2 to 5% w/w of microspheres. The in vitro release of prednisolone into pH 7.0 phosphate buffer at 37 °C is shown in Table 2 in terms of the time for 50% release ($t_{1/2}$) and the time for total drug release from the microspheres ($t_{100\%}$). Drug release was very rapid for microspheres with a glutaraldehyde content of 1.0–5% w/w. Release

TABLE II

The effect of glutaraldehyde content on the rate of drug release from RSA microspheres; into pH 7 phosphate buffer at 37 °C

Glutaraldehyde content (% w/w)	$t_{1/2}$ drug release (min)	$t_{100\%}$ drug release (min)
0.2	5.5	30
0.5	6.0	22
1.0	3.8	8
2.0	2.0	7
2.5	1.5	8
4.0	1.75	10
5.0	2.5	8

was complete within 10 min and the $t_{1/2}$ values were almost identical, except for the 1.0% w/w preparation. Interestingly, microspheres with low glutaraldehyde content, 0.3 and 0.5% w/w, had slower drug release rates, release being complete within 30 and 22 min, respectively. This may be related to the considerable degree of swelling which these microspheres underwent in the presence of water (both these preparations more than doubled their size on contact with water; Table 1). Diffusion of drug through the swelling albumin coating must be the rate-controlling step for drug release from these microspheres. Freeze-dried microspheres with glutaraldehyde content equal to or greater than 2.0% w/w increase in size only slightly (approximately 10%) on contact with water. Thus although the microspheres become more rigid with increasing amounts of glutaraldehyde this does not reduce the permeability of prednisolone. Therefore crosslinking with glutaraldehyde is not an effective mechanism of reducing the diffusion of this low molecular weight material. The effect of the time of exposure of the microspheres to glutaraldehyde was also studied. The time of exposure was increased from 1 hour up to 24 h. However, this did not have any significant effect on the drug release profile.

Heat stabilization

Heat denaturation of albumin causes intra- and inter-molecular crosslinking of the albumin through the formation of lysinoalanine, *N*-(DL-2-amino-2-carboxyethyl)-L-lysine (Sokoloski and Royer, 1984). Heat denaturation at high temperature promotes disulfide bond rupture and disulfide interchange. The more crosslinks formed, the less permeable the albumin microsphere should be and hence drug release should be slower. The maximum temperature which prednisolone would withstand without a significant level of degradation was 160 °C. 4% of prednisolone decomposed under these conditions. 155 °C was selected as a suitable stabilization temperature.

Microspheres containing 20% w/w prednisolone were heat stabilized at 155 °C for periods of 1–24 h. As shown in Fig. 3, the drug release rate from the microspheres into pH 7.0 phosphate buffer (37 °C) decreased significantly as the heating time was increased. Microspheres stabilized for 3 h have a $t_{1/2}$ value for prednisolone release of 0.25 h, whereas those stabilized for 24 h have a $t_{1/2}$ value of 25 h. These profiles are of a hyperbolic shape, suggesting that the formulations may conform to Higuchi's matrix dissolution model (Huguchi, 1963). However, plots of square-root of time ($t_{1/2}$) against cumulative percentage drug release are not linear, implying that drug release from the microspheres does not fit Higuchi's model (Fig. 4). Baker and Lonsdale (1974) proposed a different equation for drug release from spheres. Fig. 5 shows the data plotted according to Baker and Lonsdale's formulation, where F is the fraction of drug released up to time t. The micro-

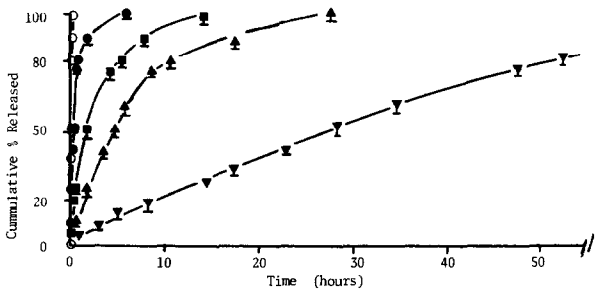

Fig. 3. In vitro release rates of prednisolone into pH 7.0 phosphate buffer at 37 °C. Key: ○, micronised drug powder; ●, RSA microspheres heat stabilized at 155 °C for 3 h; ■, RSA microspheres heat stabilized at 155 °C for 6 h; ▲, RSA microspheres heat stabilized at 155 °C for 12 h; ▼, RSA microspheres heat stabilized at 155 °C for 24 h.

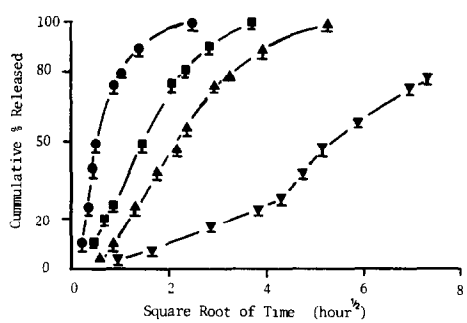

Fig. 4. In vitro release rates of prednisolone as a function of the square-root of time, from heat-stabilized (155 °C) RSA microspheres. Key: ●, 3 h stabilization; ■, 6 h stabilization; ▲, 12 h stabilization; ▼, 24 h stabilization.

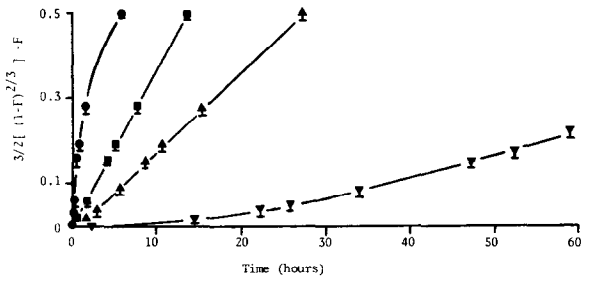

Fig. 5. Relationship between calculated value of $3/2[(1-F)^{2/3}]-F$ and time for heat stabilized (155 °C) RSA microspheres, where F is the fraction of drug released up to time t. Key: ●, 3 h stabilization; ■, 6 h stabilization; ▲, 12 h stabilization; ▼, 24 h stabilization.

sphere preparations stabilized for 6 and 12 h give linear plots and hence appear to fit the Baker and Lonsdale model. Those stabilized for only 3 h show a positive deviation from linearity while those stabilized for 24 h show a slight negative deviation from linearity. Jun and Lai (1983) found a linear relationship for nitrofurantoin release from albumin microspheres (average diameter 400 μm) using this model. The Baker and Lonsdale model appears to be an appropriate model for drug release from albumin microspheres in the size range 23–400 μm.

Unlike the chemical stabilization method studied, heat stabilization does significantly sustain drug release from the microspheres and eliminates one of the major limitations of albumin microspheres, i.e. premature release. This is possibly due to the differing effects of the chemical and heat methods of protein denaturation on the structure of proteins (Lapanje, 1978). Heat denaturation of albumin at high temperatures results in breakage and reformation of disulfide bonds and hence considerable rearrangement of the molecular conformation. This must result in a tightly woven structure with consequently reduced permeability to prednisolone as shown by the slow drug release rates. Chemical denaturation results in the formation of crosslinks by chemical reaction of glutaraldehyde with the albumin molecules, and would not be expected to have the same effect on the structure of the microspheres as heat denaturation.

Conclusions

These studies have shown that the extent of stabilization of lightly stabilized RSA microspheres can be correlated to the percentage of water-soluble RSA present in the microspheres and that drug release rates from these microspheres can be related to the degree of swelling on contact with water. However, microspheres with similar extents of stabilization (as characterized above) do not have similar drug (prednisolone) release rates, if different methods of denaturation are used.

The desired sustained release of prednisolone was achieved in vitro by incorporating the drug into RSA microspheres which were highly stabilized by heat denaturation. There was no evidence of premature ("burst") release of drug from these microspheres. The microspheres were rigid and resistant to swelling in aqueous environments. Crosslinking with glutaraldehyde also provided RSA microspheres which were rigid and resistant to swelling. However, a corresponding change in the release characteristics of these microspheres was not observed. In fact, the release rate of prednisolone increased slightly with increase in the amount of glutaraldehyde added in the crosslinking process.

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