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PREPARATION, CHARACTERIZATION AND IN-VITRO RELEASE KINETICS OF SALBUTAMOL SULPHATE LOADED ALBUMIN MICROSPHERES

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ABSTRACT

Salbutamol sulphate loaded Bovine serum albumin microspheres were prepared by heat denaturation method. The effects of such preparation conditions as denaturation temperature, denaturation time, protein concentration and phase volume ratio on the extent of drug loading, size and size distribution and drug release were studied. An increase in protein concentration from 5% w/v to 15% w/v increased the mean particle size from 8.5 μm to 16.6 μm and decreased the drug loading from

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46% w/w to 18% w/w. A decrease in the phase volume ratio substantially lowered mean particle size and size distribution. An increase in the severity of denaturation conditions lowered both the drug incorporated and drug released. The kinetics of drug release from microspheres were compared to the theoretical models of Higuchi diffusional release and first order release. Both the models gave an adequate fit to the data. Scanning electron microscopy revealed that the dummy microspheres are spherical with smooth surfaces. As the drug-protein ratio increased, the microspheres exhibited rough surfaces showing the presence of drug crystals.

INTRODUCTION

Targeting of drugs to specific sites or organs in the body is currently gaining much interest (1). Targeting increases the specific distribution of a drug and hence combined with lower dosing increases the therapeutic efficacy of a drug substance. In recent years there has been a considerable interest in the use of colloidal carriers, to include microspheres, as a means of delivering drugs (2-4). Microspheres have often been used for the purpose of drug targetting following parenteral administration. One simple application of microspheres for delivery to the vascular compartment is

their direction to the lungs. Passive targeting of microspheres to lung can be achieved by exploiting the fact that microspheres reasonably large in size (more than 7 μm) will be retained after intravenous administration by a simple process of mechanical entrapment in the capillary beds of the lung (5-6). This process has been employed for many years in diagnostic imaging (7). The same process has been used for drug delivery and examples of possible clinical applications have included the treatment of respiratory diseases (emphysema) and for cancer chemotherapy (8). Biodegradable microspheres have been shown to lodge within the capillary networks of the lungs and then, by a process of biodegration and diffusion, to release the incorporated agent (9).

Salbutamol sulphate β_2 agonist is widely used as a branchodilator in asthmatic conditions. But the conventional oral delivery in the form of tablet warrants multiple dosing regimen (3 to 4 times daily) as this drug is rapidly absorbed coupled with fast elimination with a plasma half life of 1.5 to 2 hrs (10). Thus salbutamol is a potential candidate for lung targeting as microspheres.

Microsphere characteristics like size, size distribution, surface topography, payload and release

kinetics are greatly affected by various processing and formulation variables such as drug-albumin ratio, denaturation temperature and time, phase volume ratio, type of oil, stirring speed, homogenisation speed and time, etc. The present investigation aims at optimizing the processing and formulation variables to get microspheres of optimum physicochemical characteristics suitable for lung targeting.

MATERIALS AND METHODS

Materials

Solbutamol sulphate I.P. (obtained from T.P.S. Laboratories, Bombay), Bovine serum albumin fraction V (CDH, Delhi), paraffin liquid light (S.D. Fine Chemical, Bombay), solvent ether (Jaywanthi Chemicals). All other chemicals used were of the best available quality.

Methods

Preparation of Microspheres - The microspheres were prepared by emulsion polymerization technique (11,12). Aqueous solutions of Bovine serum albumin of various concentrations (5, 10, 12.5, 15% w/v) were prepared by dissolving specified amounts of albumin in water.

100 mg of salbutamol sulphate was dissolved in 2 ml each of the above Bovine serum albumin (BSA) solutions and mixed well. To this specified amounts of light liquid paraffin oil containing 0.1% span 80 was added, ice cooled to 4°C, and homogenized at 6000 rpm for 3 min by Remi high speed homogenizer (Model RQ/127A, Remi Lab instruments, Bombay).

The resultant w/o emulsion was transferred to the burette of the microsphere preparattion apparatus (12). The emulsion was added dropwise at a rate of 100±10 drops per minute to 100 ml of light liquid paraffin preheated to specified temperature and stirred at a constant speed. The stirring was continued for half an hour to allow the formation of microspheres. The microsphere oil suspension was allowed to cool to 24°C. The microspheres were washed with 60 ml of anhydrous solvent ether by centrifuging at 3000 rpm for 15 minutes (C.M.B. Centrifuge, Bombay). The washing was repeated three more times with similar amounts of ether to remove any traces of oil associated with the microspheres. The microspheres were vaccum dried overnight and transferred to an airtight container and stored as a free flowing powder at 4°C in dark. The batch specifications are given in the Table 1.

Table 1. Batch specifications of salbutamol sulphate loaded microspheres.

	Batches									
	1	2	3	4	5	6	7	8	9	10
Variable Parameters										
Albumin content (mg)	100	200	200	250	250	250	300	300	300	300
Light liquid paraffin (ml)	15	15	15	15	30	15	15	30	15	15
Denaturation temp.(^{o}C)	125	125	150	125	125	150	125	125	150	150
Denaturation time (hrs)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	3
Constant Parameters										
Drug content	100 mg									
Aqueous phase volume	2 ml									
Span 80	0.1% w/v									
Homogenization speed	6000 rpm									
Homogenization time	3 min									
Stirring speed	800 rpm									
Centrifugation speed	3000 rpm									

Determination of Salbutamol Sulphate Content in Microspheres - 10 mg of drug loaded microspheres were homogenized in 0.5 M acetic acid and the homogenate was centrifuged at 3000 rpm for 10 min and the solution was allowed to remain overnight at 4°C to allow the digestion of microspheres. Specified volume of the supernatant solution was withdrawn and analysed after suitable dilution on a Beckman - 21 UV Spectrophotometer at 276 nm. Plain microspheres prepared without drug were treated similarly and used as blank.

Optical Microscopy - The microspheres were sized using light optical microscope fitted with a caliberated micrometer. Three hundred microspheres were counted from each batch.

Scanning Electron Microscopy - Scanning electron microscopy (SEM) was used to study the surface topography of microspheres. The samples were prepared by mounting the microspheres on a brass stubb with gold coating for 5 min on a JEOL sputter coater. The samples were examined and photographed on a scanning electron microscope (JEOL JSM 840A).

In-Vitro Drug Release - A modified method of Miyazaki et al. (13) was employed to study the drug release from

microspheres. The donor compartment consisted of a hollow glass tube of 2.4 cm internal diameter. The cellophane membrane was soaked for 12 hrs in 5% glycerine water and thoroughly washed with distilled water. The membrane was tied to one end of the tube. The tube was immersed to a depth of 5 mm in 50 ml of phosphate buffer saline pH 7.4 as receiver fluid kept in 100 ml beaker on a magnetic stirrer. Twenty five milligram sample of microspheres was taken into the donor compartment and 2 ml phosphate buffer saline was added to it. The receiver fluid was continuously stirred at 100 rpm with a magnetic bead. Aliquots (5 ml) from the receiver fluid were withdrawn at regular intervals for a specified period of time and the same amount was replaced with a fresh buffer. The absorption was measured on Beckman-21 UV Spectrophotometer at 276 nm. Dummy microspheres prepared without drug were also subjected to release study and used as a control. Similarly, 2.5 and 5 mg of the pure drug powder was taken in the donor compartment with 2 ml phosphate buffer saline and subjected to in-vitro release study as above.

RESULTS AND DISCUSSION

Table 2 shows the size and drug loading of microspheres. It is found that the increase in albumin concentration

Table 2. Size of Microspheres and Drug Loading

Batch No.	Mean particle size(um)(±S.D.)	Percentage drug entrapped	Payload % w/w
1	8.5 (± 3)	96	46
2	10.8 (± 4.09)	70.57	23.5
3	10.7 (± 5.58)	67.5	22.4
4	11.2 (± 5.37)	76	21.7
5	9.44 (± 1.35	55	15.7
6	9.01 (± 1.11)	51.7	14.7
7	16.61 (± 7.59)	64.4	18
8	10.46 (± 6.147)	49.3	12.3
9	11.61 (± 4.13)	53.66	13.4
10	11.95 (± 5.38)	41.1	10.4

increases both mean size and size distribution of microspheres. The mean particle size increased from 8.5 to 16.61 μm with an increase in the protein concentration from 5% w/v to 15% w/v. More than 80% of the microspheres were above 7 μm in batches 7, 9 and 10. It is known that albumin microspheres increase in size with an increase in albumin concentration (14,15). This effect can probably be attributed to a higher relative viscosity of the protein solution (16).

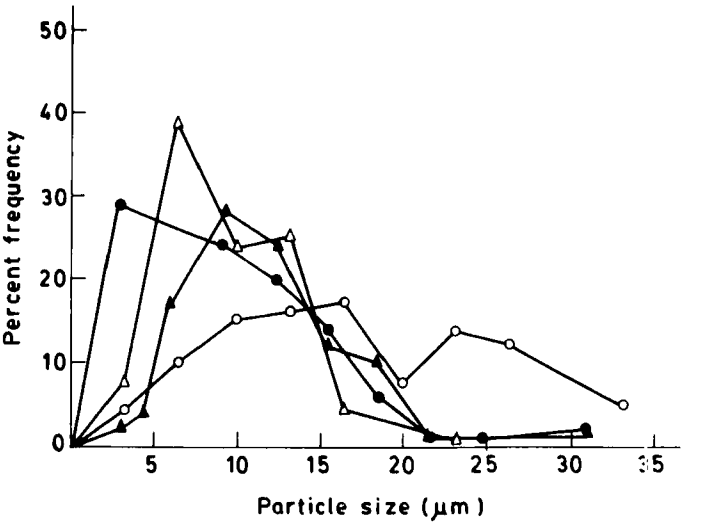


FIGURE 1

Effect of protein concentration and phase volume ratio on the particle size and size distribution.

Key: (▲) Batch 4, (△) Batch 5, (o) Batch 7, (●) Batch 8

A decrease in the aqueous to oil phase ratio narrowed down the particle size considerably with more than 30 to 35% particles below 7 μm in batches 5 and 8. It is observed that the doubling of oil phase decreased the mean particle size from 11.2 to 9.44 μm in batches 4 to 5 and 16.61 to 10.46 μm in batches 7 to 8, respectively. Figure I shows the effect of phase volume ratio and protein concentration on the size and dize distribution in these batches. Reddy et al. (17) reported that the low aqueous to oil phase ratios produced small and uniformly distributed microspheres.

The scanning electron microscopy revealed that the plain microspheres are spherical in shape with smooth surfaces (Fig. 2a, 2b). The drug loaded microspheres are also spherical but with rough surface due to the presence of drug crystals on the surface (Fig. 2c,d,e and f). Increasing the drug albumin ratio increased the drug crystals on the microsphere surface.

It is evident from the data in Table 2 that the drug loading is affected by the protein-drug ratio, denaturation temperature and time and phase volume ratio. An increase in these parameters decreased the drug loading. For example, an increase in the protein concentration from 5% w/v to 15% w/v lowered the pay load from 46% w/w to 18% w/w in the batches 1 to 7, respectively. Tomlinson and Burger have made similar observations (18). Similarly, an increase in the denaturation temperature from 125^{o}C to 150^{o}C reduced the drug loading substantially in all the batches. An increase in the denaturation time from 30 min to 3 hrs in batches 9 and 10 decreased the pay load from 13.4% to 10.4% w/w, respectively. Similar results have been reported elsewhere (19,20). This may be attributed to the fact that the degree and extent of carrier denaturation may affect the partitioning of drug into the oil and washing media, thus affecting its entrapment (21,22).

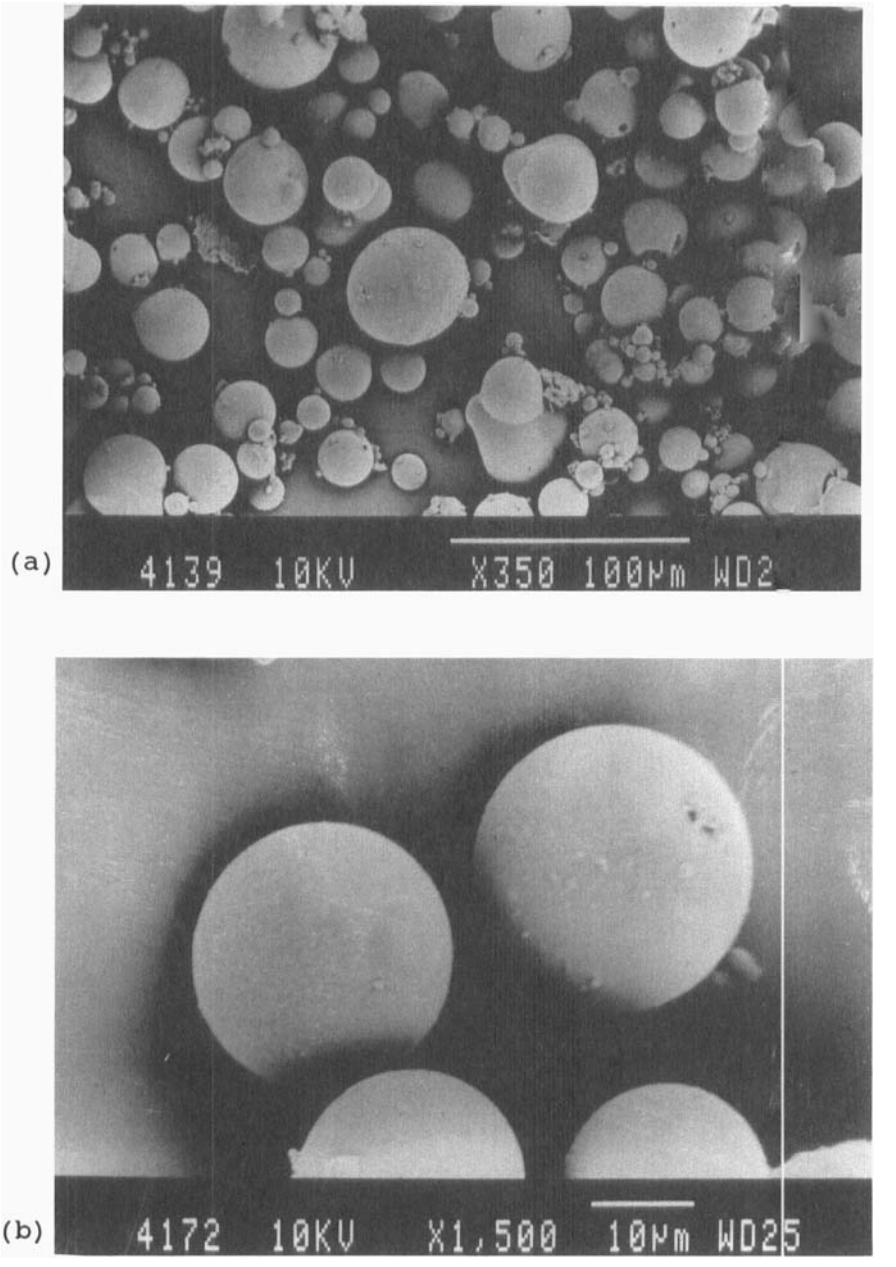
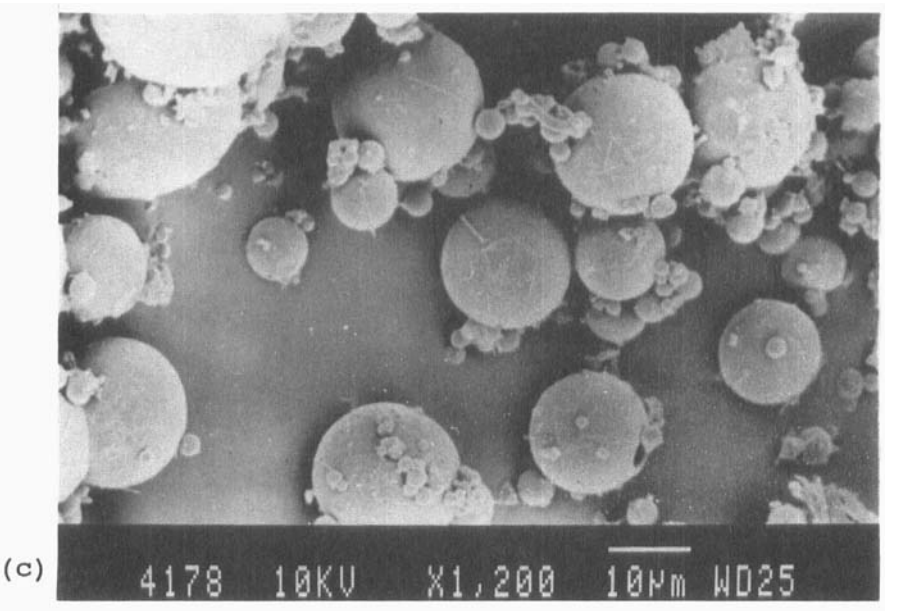


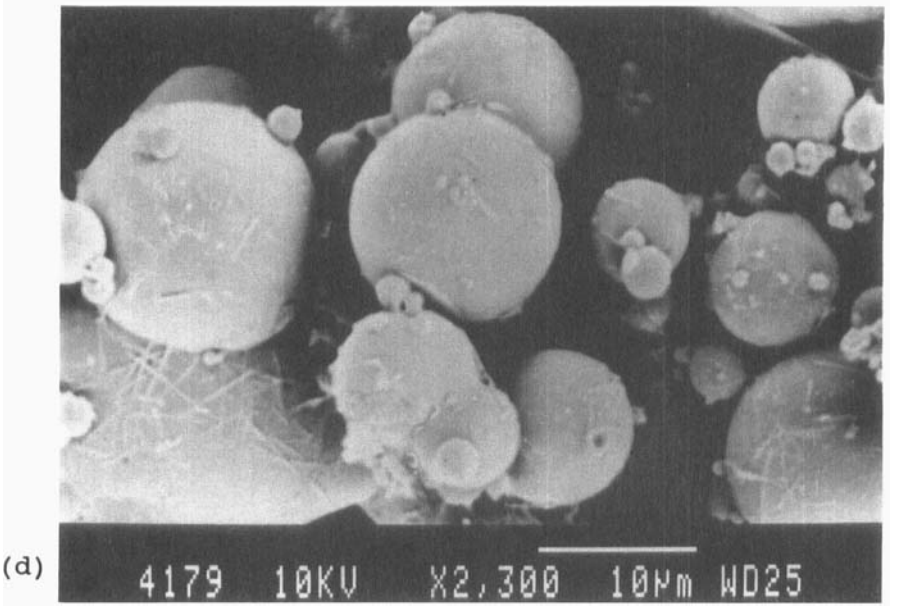
FIGURE 2

Scanning electron micrographs of microspheres.

Key: (a) Plain microspheres (X350)

(b) Plain microspheres (X1500),(c) Batch 4 (X1200), (d) Batch 4 (X2300), (e) Batch 9 (X1000), (f) Batch 9 (X2500)





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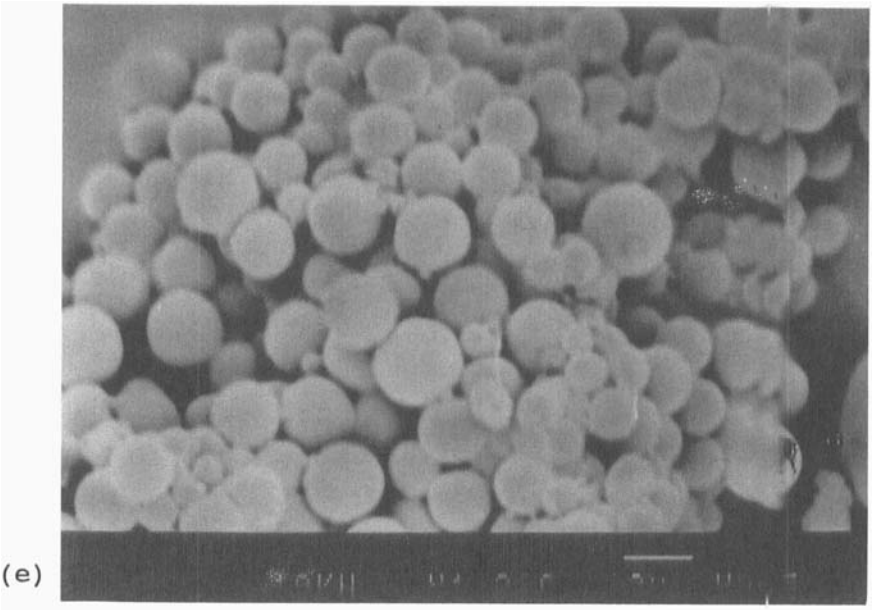

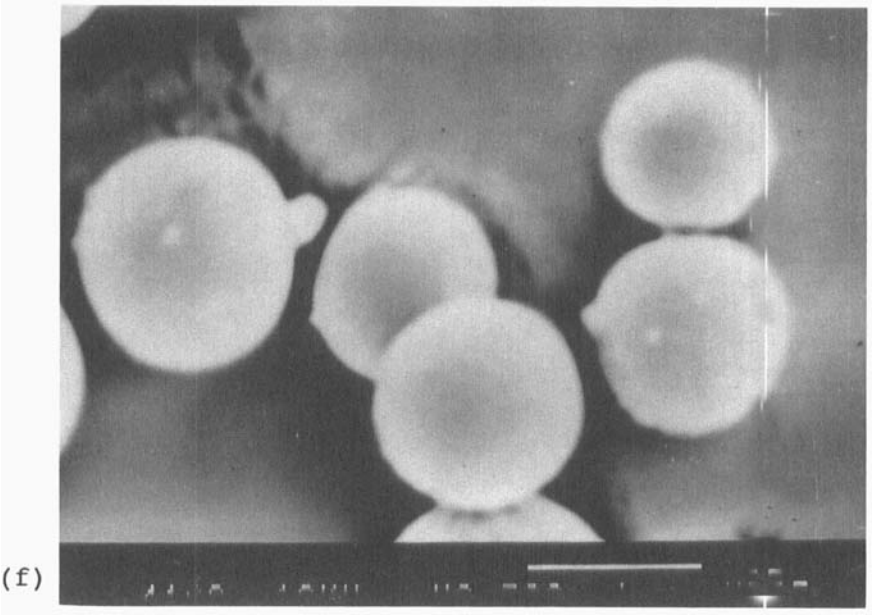

FIGURE 2 Continued

As the particle size decreased with an increase in oil phase ratio the pay load decreased proportionately. This is in accordance with the fact that for a given volume of colloidal carrier a decrease in particle size increases its exposed surface area resulting in loss of more drug due to its partitioning in the oil/and or the carrier washing media (23).

The release of plain drug from the donor compartment is characterized by a very fast release (about 30%) in the first five minutes followed by an exponential decrease ($r = 0.9994$). The drug release pattern is the same with 2.5 and 5 mg of plain drug. The drug release from microspheres is remarkably slow compared to the plain drug. Figures 3, 4 and 5 show the in-vitro release profile of salbutamol sulphate from microspheres. Generally all the batches exhibited a high initial release (burst effect) upto 3 hrs releasing about 40% of the drug, followed by a more gradual terminal release. Such release pattern has been reported previously also (24). In all the cases, increasing the particle size and severity of denaturation conditions decreased the rate of drug release. Figure 3 shows the effect of denaturation temperature and time on the release profile of drug from batches 7, 9 and 10. The fastest and slowest drug release were obtained from the microspheres of batches 1

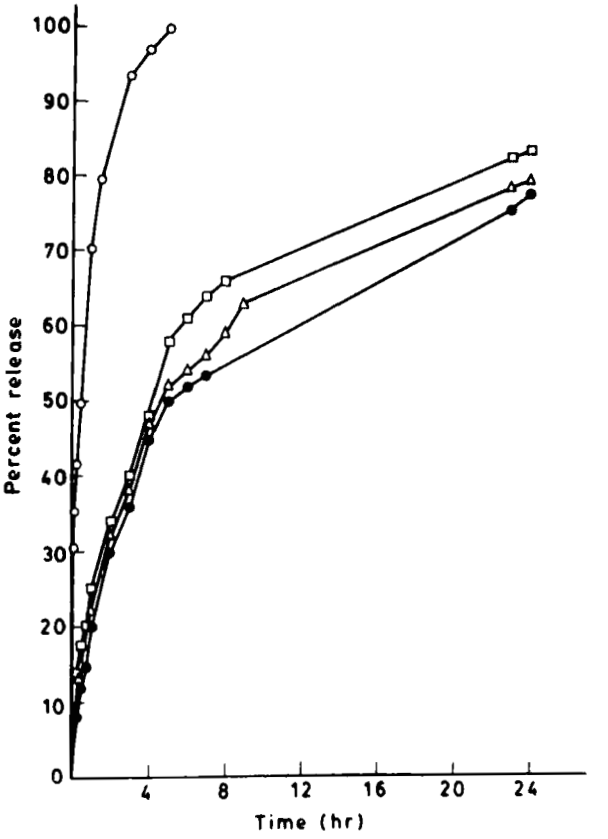

FIGURE 3

Effect of denaturation temperature and time on in-vitro release profile of salbutamol sulphate from microspheres.
Key: (o) Plain drug, (□) Batch 7, (△) Batch 9
(●) Batch 10

and 10, respectively. The drug release in batch 10 is more gradual and prolonged compared to all other batches. Drug release from microspheres can take place via various paths such as total microsphere disintegration, microsphere hydration, surface erosion, particle

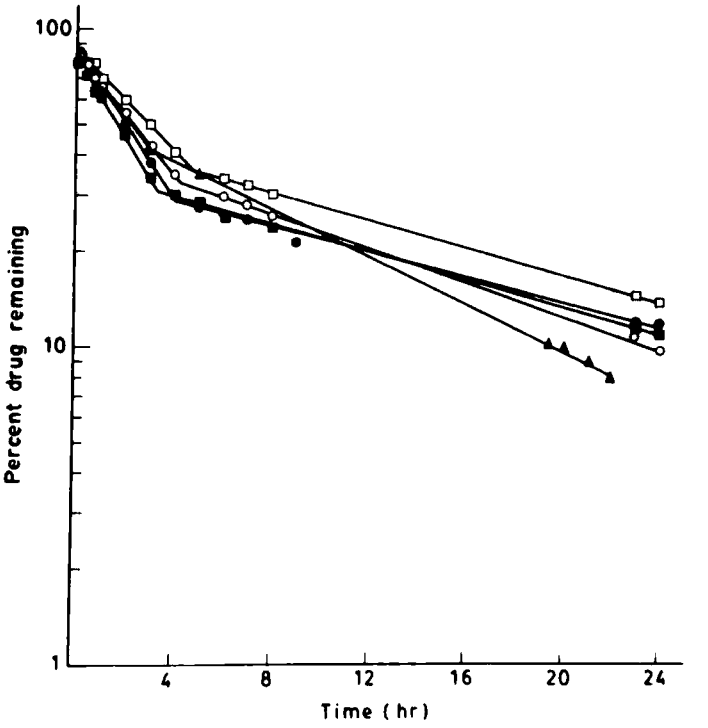

FIGURE 4

Log% drug remaining vs. time profile of in-vitro release of salbutamol sulphate from microspheres.

Key: (▲) Batch 1, (o) Batch 2, (●) Batch 3, (□) Batch 4, (■) Batch 5.

diffusion and leaching (18). The release rate of drug is highly dependent on the drug loading, particle size and denaturation conditions.

Typically the release of drug from microspheres increased with decrease in the particle size of microspheres. This may be due to the fact that the

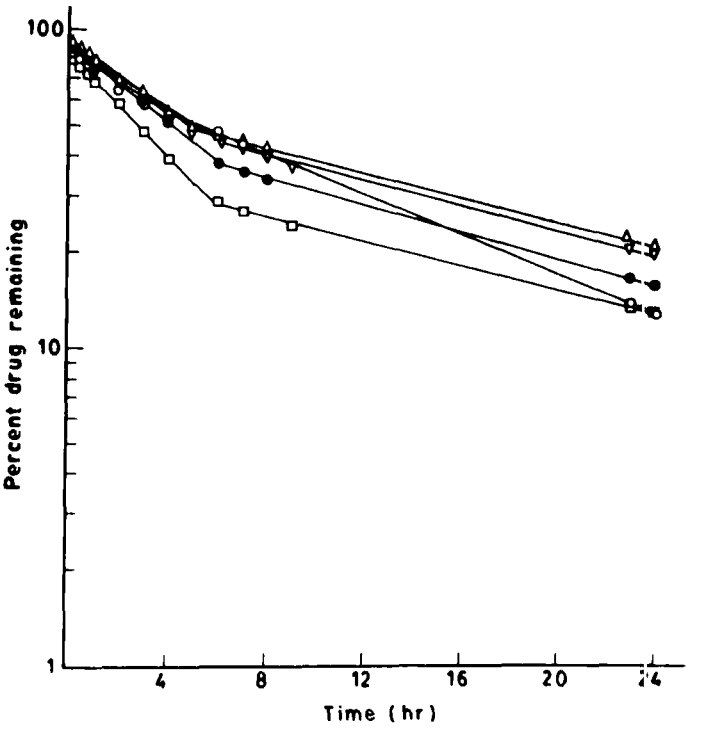

FIGURE 5

Log% drug remaining vs. time profile of in-vitro release of salbutamol sulphate from microspheres.

Key: (□) Batch 6, (●) Batch 7, (o) Batch 8
(▽) Batch 9, (△) Batch 10.

smaller particles offered more surface area to release the drug. Thus, it is possible to modulate drug release rates by varying the size of the microspheres.

The decrease in the rate of drug release from microspheres synthesized by increasing the severity of denaturation conditions might be attributed to the lower

Table 3. In-vitro release kinetics of salbutamol sulphate from microspheres.

Batch No.	Initial Phase			Terminal Phase		
	Time (hrs)	Higuchi Model	First order Model	Time (hrs)	Higuchi Model	First order Model
1	0.25-3	r= 0.9935 m=29.1606	r= -0.9833 m= -0.0937	3-22	r= 0.9994 m=11.5866	r= -0.9996 m= -0.0372
2	0.25-4	r= 0.9993 m=31.1015	r= -0.9983 m= -0.0966	6-24	r= 0.9993 m= 7.9668	r= -0.9995 m= -0.0258
3	0.25-3	r= 0.9992 m=38.5509	r= -0.9969 m= -0.1240	5-24	r= 0.9917 m= 5.8773	r= -0.9939 m= -0.0187
4	0.25-4	r= 0.9918 m=28.7137	r= -0.9948 m= -0.0812	6-24	r= 0.9997 m= 8.0998	r= -0.9929 m= -0.0225
5	0.25-3	r= 0.9974 m=36.7696	r= -0.9959 m= -0.1284	4-24	r= 0.9963 m= 6.4667	r= -0.9984 m= -0.0210
6	0.25-4	r= 0.9956 m=30.4952	r= -0.9984 m= -0.0823	3-24	r= 0.9734 m=11.9366	r= -0.9965 m= -0.0525
7	0.25-4	r= 0.9980 m=21.8744	r= -0.9932 m= -0.0525	6-24	r= 0.9985 m= 9.0432	r= -0.9993 m= -0.0208
8	0.25-3	r= 0.9968 m=22.0852	r= -0.9945 m= -0.0597	4-24	r= 0.9985 m=14.4556	r= -0.9999 m= -0.0314
9	0.25-6	r= 0.9961 m=22.7420	r= -0.9919 m= -0.0512	7-24	r= 0.9940 m=10.0952	r= -0.9967 m= -0.0192
10	0.25-5	r= 0.9982 m=24.4575	r= -0.9957 m= -0.0553	6-24	r= 0.9987 m=10.3936	r= -0.9991 m= -0.0189

r = Correlation coefficient; m = Rate constant.

amounts of drug incorporated in the microspheres and/or to increase in the density and hardness of the albumin matrix (25,26).

Table 3 shows the in-vitro release kinetic data. The release profile obtained from various batches is compared with Higuchi $\sqrt{t}$ and first order kinetic models. It can be observed from the data in Table 3 that both the models provide an adequate fit to the experimentally derived data. Similar observations were made by Gupta et al. (27) also.

In conclusion, synthetic conditions of the batch 9 produced more than 80% microspheres above 7 μm with 13.4% pay load and 63% drug release over 9 hrs. Thus, the microspheres of the above batch are suitable for targeting to lungs.

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