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ALBUMIN MICROSPHERES : EFFECT OF PROCESS VARIABLES ON SIZE DISTRIBUTION AND IN VITRO RELEASE

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ABSTRACT

Albumin microspheres used as target drug delivery systems were prepared from egg albumin by polymerization technique using glutaraldehyde as the cross linking agent. The present study was designed to evaluate the effect of process variables on the microsphere size distribution and **in vitro** drug release. Phase volume ratio and speed of agitation exerted greater influence on the microsphere size distribution whereas the albumin concentration and cross linking time effected only the yield and surface characteristics of the microspheres respectively. Lower phase volume ratios resulted in small and uniform microspheres with smooth surfaces in narrow size range. Speed of agitation exhibited an inverse relationship with size. **In vitro** release pattern of drug from the microspheres showed a biphasic profile and the release rates were prolonged upon increase in the concentration of cross linking agent and cross linking time.

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INTRODUCTION

It is desirable to deliver the drug to its specific target organ and control release to obtain higher therapeutic index and lower adverse effects ex: cancer chemotherapy). Microspheres are colloidal drug delivery systems intended for parenteral administration to achieve target specificity. Microspheres can be prepared from a variety of carrier materials (ex: albumin). Albumin microspheres are chemically and physically stable, non-immunogenic [1] and biodegradable [2] amenable to preparation in large batches and capable of accomodating a wide variety of drug molecules in a relatively non-specific fashion[3]. They are rapidly removed from the vascular system by phagocytosis[4], potentially localized in the reticulo-endothelial system and found within the cytoplasm of tumor cells[5]. Several research groups investigated albumin microspheres as the prominent drug carriers in the chemotherapy of microbial infections[5-8]. Radiologists utilize the phagocytic activity of the reticuloendothelial system to achieve target specificity in the delivery of radiolabelled albumin and sulfur colloids[9-11].

Preparation of albumin microspheres involves either thermal denaturation at elevated temperatures (110-165°C) or chemical cross-linking in vegetable oil or iso-octane emulsions[12,13]. Yapel[14] reviewed the general methods of preparation.

The characteristics of albumin microspheres depend upon numerous process variables due to the fact that both emulsion and suspension technology are involved. Relative tissue and or organ distribution of albumin microspheres in human body when given intravenously, is a function of microsphere size[14]. Thus by simply altering the size, it is possible to achieve localization of microspheres in a particular organ and or tissue.

The present study was designed to identify the process variables which may be important to control the size distribution of microspheres in a reproducible manner and to evaluate their influence on **in vitro** release characteristics.

EXPERIMENTAL

Materials

Egg albumin (Fresh hen's eggs), castor oil I.P., glutaraldehyde. (Riedel-De Haenag Seelze Hannover), toluene, acetone and sulfadi azine.

Preparation of Albumin Microspheres

Albumin microspheres were prepared by emulsion polymerization method. 3 ml of egg albumin was added to a miture of 15 ml castor oil and 10 ml toluene held in 4" x 1.5" glass tube at appropriate stirring speed (rpm) using a mixer fitted with 3 blade glass stirrer. The particle size was checked by observing a drop of miture under optical microscope.

Glutaraldehyde saturated toluene solution was prepared by mixing equal volumes of glutaraldehyde and toluene in a test tube and after shaking for 10 minutes, the mixture was allowed to separate. The upper toluene layer saturated with glutaraldehyde was pipetted and added drop wise to the albumin dispersion. In the present study glutaraldehyde concentration of approximately 0.7% v v was employed.

The dispersion was then mixed at an appropriate speed until cross linking reaction was completed (approx: 5 h). At the end the suspension of microspheres was washed free of oil with 4 ml volumes of toluene for 4 times at 1500 rpm for 2 minutes in high speed centrifuge fitted with variable speed adjusting system. Then the microspheres were washed 3 times with 5 ml volumes of acetone at 2500 rpm. Between each washing microspheres were centrifuged the supernate discarded and resuspended. At the end of final washing with acetone microspheres were suspended in 10 ml of acetone and transferred into a paper dish for air drying at room temperature($25^{o}C$). Upon drying yellow to yellowish orange coloured free flowing fine powder was obtained.

Drug entrapped Microspheres

Albumin microspheres containing sulfadiazine were prepared under the conditions phase volume ratio = 3:30, albumin concentration

TABLE 1

	Process variable	No. of batches made	Constant conditions
1.	Phase volume ratio 2:30, 3:30, 4:30, 5:30, 6:30	5	Albumin conc. 12.32% w v Agitation = 5500 rpm, Cross-linking time = 5 h
2.	Speed of Agitation 1100, 4100, 5500, 7200 rpm	4	Phase volume ratio = 3:30; Albumin conc.= 12.32% w v; Cross-linking time = 5 h
3.	Albumin conc. 12.32, 8.21, 6.16% w v,	3	Phase volume ratio = 3:30 Agitation = 5500 rpm; Cross-linking time = 5 h
4.	Cross linking time 0.5, 2, 3, 4, 5 h	5	Phase volume ratio=3:30, Agitation = 5500 rpm· Albumin conc.= 12.32% w v.

12.32% w v, speed of agitation = 5500 rpm. and drug to albumin ratio= 0.2 (75 mg drug and 370 mg albumin). Since sulfadiazine is water insoluble. it was finely dispersed in fresh egg albumin drug-albumin mixture was kept for equilibration for 15 minutes before its addition to the castor oil toluene mixture.

SIZE DISTRIBUTION

Size distribution analysis was done by optical microscopy by spreading a minute quantity of microspheres on a clean glass slide. Diameters of 4000-500 microspheres were measured on an average. To investigate the effect of different process variables on size distribution of microspheres, each time one variable was varied keeping the others constant (Table 1). From the results obtained, optimum level of that variable was kept constant in the subsequent evaluation.

IN VITRO DRUG RELEASE

Method by Kim **et al**[15] was adopted for **in vitro** drug release studies. At periodic time intervals for 24 h 5 ml of medium were

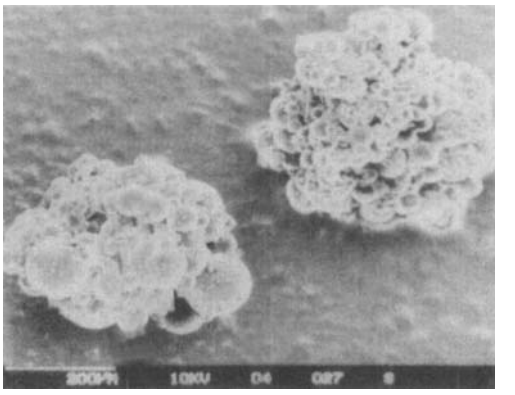

Plate-I: Aggregates of Albumin microspheres.

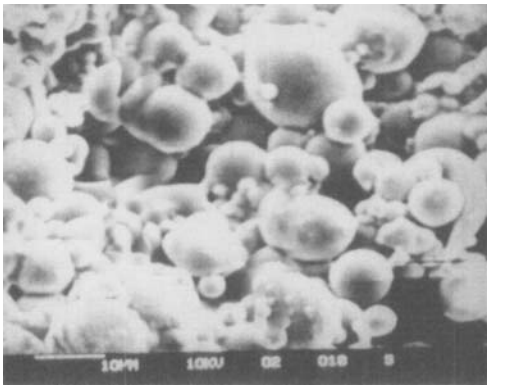

Plate-II: Albumin microspheres seen together with non spherical beads.

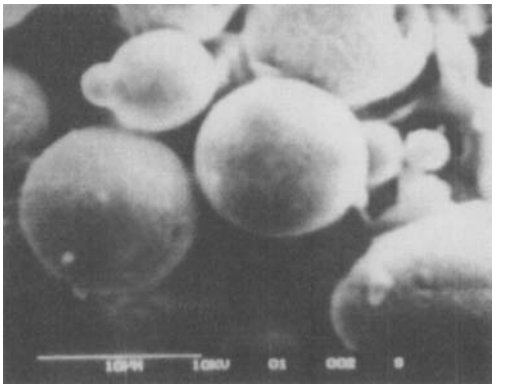

Plate-III: Albumin microspheres showing smooth surfaces.

removed and filtered through a membrane filter (0.45 um). The amount of drug released was estimated by UV spectrophotometry at 535 nm [16].

The influence of the variables - amount of glutaraldehyde and cross-linking time on drug release were investigated.

(a) Amount of Glutaraldehyde

Three batches of microspheres containing sulfadiazine were prepared corresponding to 0.5, 0.71 and 1.41% v v glutaraldehyde concentrations respectively calculated with reference to the external phase volume. Cross-linking time was 5 h for all the three batches.

(b) Time of Cross-linking

Two batches of microspheres containing sulfadiazine were prepared at 3 h and 5 h cross linking times respectively using 0.7% of glutaraldehyde.

RESULTS AND DISCUSSION

It is observed from Figure 1 that both average microsphere size range increased with an increase in phase volume ratio. With 2:30, the mean (± SD) diameter of microspheres was 2.51 ± 1.07 With 3:30, the mean (± SD) diameter of microspheres increased slightly to 3.33 ± 1.37 um. With both phase volume ratios, microspheres with uniform and smooth surfaces were obtained without any visible sign of aggregation. With 4:30 and 5:30 there was a marked increase in the microsphere size with 4.12 ± 2.5 um and 6.04±3.04 um respectively together with signs of aggregation (Plate I). With 5:30 non-spherical beads were also observed which were difficult tobe removed (Plate II). With 6:30, not microspheres were formed and the material was converted into huge lumps.

Since microspheres are stabilized while they are in globule form, the factors affecting the size distribution of globules will affect the size distribution of microspheres. Increased volume

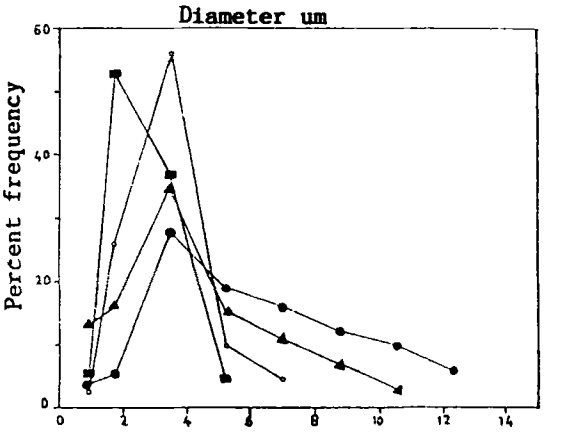

Fig.1. Effect of phase volume ratio on size distribution (■)2:30 (○)3:30 (▲)4:30 (●) 5:30.

of internal phase leads to diminished shearing efficiency of the mixer owing to the increased internal phase volume, resulting in larger globule size and increased size range. When the internal phase volume is large the mean distance between the globules is smaller leading to flocculation of globules which may increase the chances of coalescence which can also result in inter microsphere cross linking leading to aggregation of the microspheres prepared with 4:30 and 5:30 phase volume ratios.

It is observed from Figure 2 that the average microsphere size decreased with increasing speed of agitation (rpm). At 7200 rpm, microspheres were smooth with mean (± SD) diameter of 2.81 ± 1.17 um with a narrow size range. At 5500 rpm, microspheres were smooth showing a slight increase in the mean (± SD) diameter (3.02 ± 1.10 um) (Plate III). At 4100 rpm, there was a marked increase in the mean (± SD) microsphere diameter (9.96 ± 4.61 um) and the smoothness and shape of the microspheres were affected considerably. At 1100 rpm, mean (± SD) diameter was even larger (16.63 ± 7.6 um) and contained non-spherical beads.

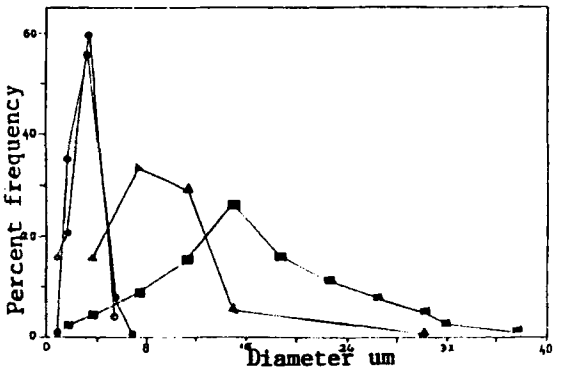

Fig.2 Effect of rpm on size distribution (○)7200 rpm (●)5500 rpm (▲)4100 rpm (■) 1100 rpm.

Increase in stirring speed results in more efficient shearing of albumin solution into fine globules. From Figure 2 it can be observed that the size distribution curves of microlspheres prepared at 7200 and 5500 rpm are almost superimposible which implies that the efficiency of mixer is no longer enhanced under the given conditions with an increase in rpm.

It can be observed from Figure 3 that the mean diameter of microspheres decreased with decreasing concentrations of albumin solution, though the differences were not significant. The mean (± SD) microsphere diameter at 12.32, 8.21 and 6.16% w v was 3.51 ± 1.2, 3.15 ± 1.07 and 2.98 ± 1.08 um respectively. An increase in concentration may reduce the efficiency of the mixer due to increased viscosity.

It is observed from Figure 4 that the mean diameter of microspheres decreased to a slight extent with increased cross-linking time. Microspheres prepared with a cross linking time of 0.5 h could not be recovered as swelling and subsequent aggregation of the microspheres resulted. The Mean (± SD) diameter of microspheres

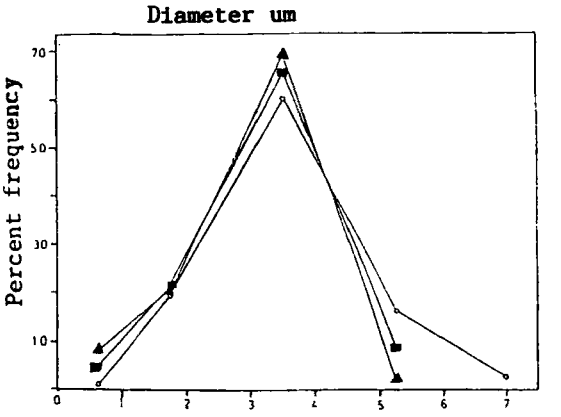

Fig.3.Effect of albumin concentration on size distribution (●)12.32w/v%,(■)8.21w/v%, (▲)6.16 w/v%.

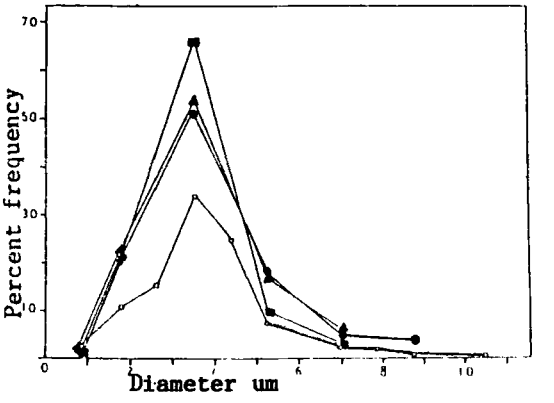

Fig.4 Effect of cross-linking time size distribution.(●) 2 hr, (●) 3 hr, (▲) 4 hr, (■) 5 hr.

prepared with cross linking times of 2, 3, 4 and 5 h was 3.68 ± 1.41. 3.75 ± 1.68, 3.59 ± 1.44 and 3.39 ± 1.16 um respectively. At higher cross-linking times, uniform microspheres with smooth surfaces were obtained. Swelling of the microspheres prepared with cross-linking time of 0.5 h indicates that the cross-linking reaction is slow and the matrix stability has not been achieved within that time. The uniform and smooth surfaces of the microspheres prepared with longer cross-linking times can be attributed to better and prolonged shearing action.

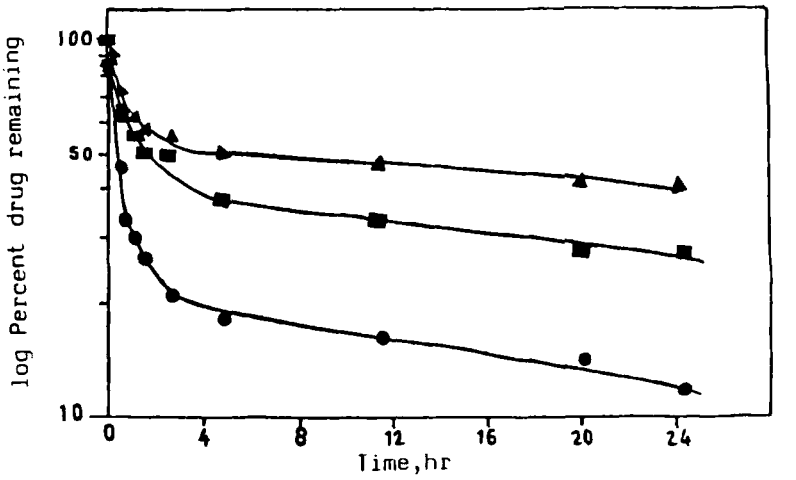

Fig.5: Effect of glutaraldehyde concentration on in vitro drug release (●)0.5%v/v, (■)0.71%v/v, (▲) 1.4% v/v

In the release studies, a plot of log percent drug remaining in the microspheres, prepared using different glutaraldehyde concentrations versus time, presented in Figure 5 reveals that drug release has taken place in two distinct phases. An initial fast releasing phase of 1-2 h during which 40-70% of the entrapped drug was released followed by a slow releasing phase which lasted for several hours. At the end of 24 h some drug was still retained in the microspheres and this amount was proportional to the glutaraldehyde concentration used. From the Figure, rate constants for drug release and half-life values were computed for both fast and slow release phases and given in **Table 2.**

It is clear from the Table that the rate of drug release was decreased with increased glutaraldehyde concentration for both fast and slow release phases.

Biphasic drug release pattern was also observed for the microspheres prepared with different cross linking times. The amount

TABLE 2

	Release phase		k (h^{-1})	$t_{\frac{1}{2}}$ (h)
Glutaraldehyde concentration (% v/v)				
0.5	a.	fast	0.886	0.78
	b.	slow	0.022	31.94
0.71	a.	fast	0.460	1.50
	b.	slow	0.017	39.92
1.41	a.	fast	0.307	2.26
	b.	slow	0.013	52.70
Cross-linking time				
3 h	a.	fast	1.096	0.63
	b.	slow	0.029	23.58
5 h	a.	fast	0.460	1.50
	b.	slow	0.017	39.92

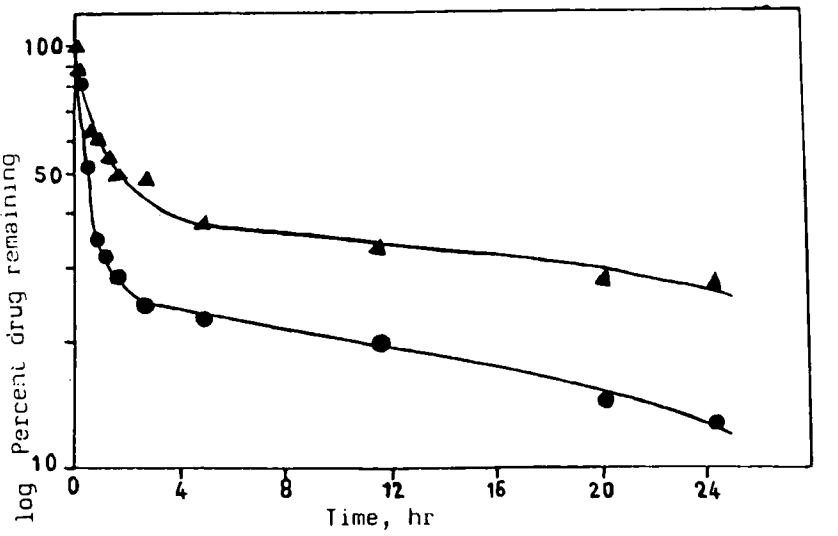

Fig.6.: Effect of cross-linking time on in vitro drug release (●) 3 hr, (▲) 5 hr.

of drug retained within the microspheres, at the end of 24 h **in vitro** study, was more for microspheres prepared with 5 h cross-linking time. The rate constants and half-life values were computed for both fast and slow release phases see also Table 2 .

The initial rapid drug release ("burst effect") may be due to loosely held drug on the surface or just beneath the surface of the microspheres. The slow release phase may be due to slow diffusion of drug that has been deeply entrapped in the albumin microsphere matrix. Microspheres maintained their spherical shape even after 24 h release period. Since glutaraldehyde is responsible for matrix formation. increased glutaraldehyde concentration will increase the matrix density resulting in the formation of more stable and rigid spheres which show less tendency to swell. Swelling of microspheres noticed after 24 h of drug release might have resulted in the relaxation of the albumin microsphere matrix facilitating rapid release of drug by diffusion through the pores. Hence, at higher glutaraldehyde concentrations, drug release rates were lower presumably owing to lesser degree of swelling.

CONCLUSIONS

(1) Since albumin microspheres were stabilized in globule form, factors affecting globule size distribution also affected the size distribution of microspheres. Since both speed of agitation and phase volume ratio had a strong influence on the size distribution of microspheres, desired size range can be obtained by controlling them. However, albumin concentration and time of cross-linking did not influence the size distribution much. These two parameters can be adjusted to improve the yield and smoothness of the product respectively.

(2) **In vitro** drug release showing distinct biphasic release pattern. Biphasic drug release may be desirable for injectable drug delivery systems, since a therapeutic 'loading dose' can be provided initially in the fast release phase followed by a subsequent slower and sustained release of drug necessary to maintain therapeutic blood levels.

(3) The possible mechanism of drug release **in vitro** is by diffusion through matrix pores.

(4) Amount of cross-linking agent and time of cross-linking exerted a strong influence on **in vitro** drug release. Hence desired drug release rates may be achieved by controlling these two variables.

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