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Albumin microspheres as a means of drug delivery to the lung: analysis of the effects of process variables on particle sizes using factorial design methodology

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Abstract

Albumin microspheres (MS) in the size range of 7–11 μm in diameter were prepared using high-speed homogenisation and subsequent heat denaturation. The effects of several process variables on the particle size were evaluated by both empirical optimisation and factorial design. Using analysis of variance, the pH of the protein solution and the stirring rate during heat denaturation were shown to have significant effects on the particle size of the resultant MS ($p < 0.01$). MS prepared at low pH values and/or slow stirring rates had larger particle sizes. A small amount of butan-1-ol (< 10% v/v) in the oil phase improved the appearance of MS but had no significant effect upon size. An inter-relationship was shown to exist between pH, stirring rate and butan-1-ol content ($p < 0.01$). Albumin concentration and phase volume ratio had slight but insignificant effects ($p > 0.1$) on the particle size.

Key words: Albumin; Microsphere; Process variables; Factorial design; Particle size

1. Introduction

Albumin microspheres (MS) are biodegradable particles that can be produced in a size range of 1–200 μm in diameter, being either physical or chemical solidification of an albumin emulsion in an organic phase (Gupta and Hung, 1989). Since they possess many desirable characteristics such as biocompatibility (Ratcliffe et al., 1984), biodegradability (Lee et al., 1981), ease of preparation on a large scale and ability to entrap a wide variety of drugs (Kramer, 1974), albumin MS have become one of the most extensively investigated carriers for targeting drugs to various organs and tissues (Davis et al., 1985). The in vivo disposition of albumin MS depends largely on their particle size and route of administration (Gupta and Hung, 1989). Particles larger than 7 μm, when given intravenously, can be trapped in the capillary bed of the lungs and based upon this characteristic, some workers have used albumin MS to

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target drugs to the lungs (Willmott et al., 1985; Pande et al., 1991; Tripathi and Singh, 1992).

Different methods have been reported for the preparation of albumin MS (Arshady, 1990). The routine synthesis involves emulsification of albumin solution in an oil phase and solidification by heat denaturation or chemical cross linking. It has been demonstrated that many process variables can influence the size of albumin MS. These factors include protein concentration, phase volume ratio, stirring rate, etc. (Gallo et al., 1984; Reddy et al., 1990). However, most studies involving the preparation of albumin MS have been conducted under empirically optimised conditions (Gupta and Hung, 1989), i.e., investigating one variable whilst keeping the other conditions constant. Furthermore, none of the previous studies has employed sound statistical design when examining the influence of process variables on the particle size of albumin MS. Thus, in the present work, factorial design has been used to investigate the effects of several process variables on the particle size and the data compared statistically using analysis of variance (ANOVA).

2. Materials and methods

2.1. Materials

Bovine serum albumin fraction V (96–99% albumin) was purchased from Sigma Chemical Co. (Poole, U.K.). Butan-1-ol (general grade) and oleic acid (specified lab. reagent) were obtained from BDH Laboratory Suppliers (Poole, U.K.) and Fisons Scientific Equipment (Loughborough, U.K.), respectively. Citric acid, disodium phosphate and all other chemicals were general laboratory reagents.

2.2. Preparation of albumin solutions

Albumin was dissolved in 0.1 M citric acid buffer solution to obtain the required concentration. The solution was adjusted to the desired pH with dilute hydrochloric acid and then centrifuged at $1900 \times g$ for 15 min to remove insoluble material before storing in a refrigerator at 8°C until required.

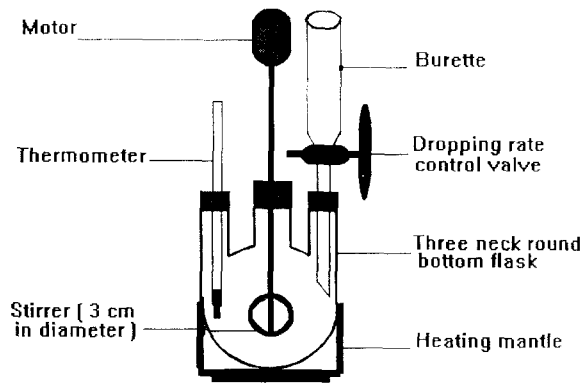

Fig. 1. Apparatus employed for the heat denaturation of albumin MS.

2.3. Preparation of albumin MS

A modified version of the method described by Shafi et al. (1990) was adopted. A prescribed amount of butan-1-ol was mixed with 50 ml oleic acid, contained in a 100 ml round bottom flask. Albumin solution (3 ml) was added dropwise and emulsified using high-speed homogenisation for 5 min. The speed of the homogeniser (Kika Werk Ultra-Terrax) was monitored with a stroboscope (Xenon Stroboflash Model 60). The w/o emulsion so prepared was added dropwise at a rate of about 40 drops/min into 150 ml of preheated oleic acid with constant stirring (Fig. 1). The mixture was heated at 115 ± 2°C for 15 min, after which it was allowed to cool to room temperature.

The suspension was centrifuged at $1900 \times g$ for 10 min, the supernatant was decanted and microspheres were resuspended in 50 ml of butan-1-ol. After sonication in a bath for 5 min, the suspension was centrifuged under similar conditions. The microspheres were washed three times with butanol as described previously. Finally, they were transferred to a petri dish, allowed to dry at room temperature and were then stored as a dry powder in the dark.

2.4. Particle size measurement

Dry microspheres were suspended in liquid paraffin and sonicated in a water bath for 5 min to aid dispersion. The suspension was mounted on a microscope slide and particle size was measured using a microscope (Nikon Labophot) connected to a video camera and analysed using image analysis software. 300 particles were measured for each batch and mean weight diameters were recorded.

2.5. Investigation of the single effect of each variable by empirical optimisation

Parameters examined in this study include albumin concentration, phase volume ratio, homogenisation speed, stirring rate, butan-1-ol content in the oil phase and pH of the albumin solution. One factor was changed while the other five factors were kept constant (Table 3). The constant level of each factor was: albumin concentration, 300 mg/ml; phase volume ratio, 1.5:30 (aqueous phase to oil phase); homogenisation speed, 6000 rpm; butan-1-ol content, 10% (v/v); pH of albumin solution, 6; and stirring rate, 1000 rpm.

2.6. Preparation of albumin MS according to factorial design

Empirical optimisation is acceptable only when the factors are independent of one another, but factorial design can reveal not only the single effect of each variable but also the interactions between them. Thus, the design enables the investigators to move beyond a single-dimensional view of behaviour to a richer and more revealing multidimensional view. The most commonly used factorial design involves the use of two levels of each variable. The variables investigated in this study were: butan-1-ol concentration; pH of albumin solution; stirring rate during the heat denaturation; phase volume ratio and albumin concentration. Each factor has a low and a high level (see Table 1). Thus, 32 combinations (2^5) should be run, but in this study only 16 combinations (half replicate) were investigated according to the protocol listed in Table 2 (Hinchen, 1969).

Table 1
Five factors at two levels and three constant parameters used to prepare albumin MS

Factors		Values	Abbreviation
Variables			
(1)	Butan-1-ol concentration (ml/100 ml)	10	A_2
		0	A_1
(2)	pH of albumin solution	6	B_2
		3	B_1
(3)	Stirring rate (rpm)	1000	C_2
		500	C_1
(4)	Phase volume ratio	3:30	D_2
		1.5:30	D_1
(5)	Albumin concentration (mg/ml)	300	E_2
		100	E_1
Constant			
(1)	Homogenisation	6000 rpm for 10 min	
(2)	Heating process	115 ± 2°C for 30 min	
(3)	Centrifugation	1900 × g for 10 min	

3. Results and discussion

Table 3 lists the single effects of each variable on the particle size. It is apparent that all six of

Table 2
Combinations of variables of $2^5/2$ factorial design

Expt no.	[Butan-1-ol] (ml/100 ml) A	pH B	Stirring rate (rpm) C	Phase volume ratio D	[Albumin] (mg/ml) E
1	0	3	500	1.5:30	100
2	0	3	500	3:30	300
3	0	3	1000	1.5:30	300
4	0	3	1000	3:30	100
5	0	6	500	1.5:30	300
6	0	6	500	3:30	100
7	0	6	1000	1.5:30	100
8	0	6	1000	3:30	300
9	10	3	500	1.5:30	300
10	10	3	500	3:30	100
11	10	3	1000	1.5:30	100
12	10	3	1000	3:30	300
13	10	6	500	1.5:30	100
14	10	6	500	3:30	300
15	10	6	1000	1.5:30	300
16	10	6	1000	3:30	100

the examined factors had an influence upon the resultant particle size to a greater a lesser extent. Increase in the homogenisation speed from 4000 to 8000 rpm resulted in a general decrease in the particle size, whereas increasing the homogenisation speed up to 10000 rpm increased the mean diameter. Increasing the butan-1-ol concentration caused an initial decrease in particle size, however at high concentrations of butan-1-ol (13% v/v) the particle size was found to increase. At low concentrations, butan-1-ol is likely to reduce the interfacial tension between the aqueous and oil phases, thereby decreasing the particle size. At high concentrations, butan-1-ol may decrease the viscosity of the oil phase and the latter has been reported to be inversely proportional to particle size (Arshady, 1990). The pH of the albumin solution was found to have a marked effect on particle size. The particles were observed to be largest at the extremes of the pH range employed (Table 3) while lower pH exerted greater influence. At pH about 5 the lowest particle size was obtained. This phenomenon might be attributed to the overall change of electric charge of albumin molecules at pH values above or below the isoelectric point. Decreasing albumin concentration and/or the phase volume ratio tended to decrease the particle size, but the influence of these factors was minor compared with that of pH. Stirring rate also produced large differences in particle size, the slower the stirring rate, the larger the particle size (Table 3). These results are in agreement with those reported previously (Gallo et al., 1984; Reddy et al., 1990).

Table 3
Effects of various factors on the mean diameter of albumin microspheres

Entry	Variables	Values	Mean diameter ± 1SD
(1)	Homogenisation speed (rpm)	4000	8.58 ± 1.59
		6000	7.84 ± 1.32
		8000	7.65 ± 1.04
		10000	8.34 ± 1.21
(2)	Butan-1-ol concentration (ml/100 ml)	1	7.85 ± 1.02
		5	7.83 ± 1.13
		9	7.21 ± 1.23
		13	8.65 ± 1.12
(3)	pH of albumin solution	3	11.59 ± 2.35
		5	7.45 ± 1.25
		7	7.87 ± 1.15
		9	8.39 ± 1.96
(4)	Albumin concentration (mg/ml)	150	7.56 ± 1.57
		250	7.48 ± 1.14
		350	8.15 ± 1.08
		450	8.63 ± 1.33
(5)	Phase volume ratio	1.5:30	7.01 ± 1.05
		2.0:30	7.33 ± 1.13
		2.5:30	7.95 ± 1.26
		3.0:30	8.08 ± 1.19
(6)	Stirring rate (rpm)	400	11.34 ± 2.57
		600	10.56 ± 1.78
		800	8.44 ± 1.24
		1000	7.45 ± 1.12

Table 4
Particle size of albumin microspheres for each combination

Expt no.	Preparation conditions [a]	Mean diameter (μm)	
(1)	$A_1 B_1 C_1 D_1 E_1$	8.38	8.62
	$A_1 B_1 C_1 D_2 E_1$	8.75	8.13
(2)	$A_1 B_1 C_2 D_1 E_2$	11.21	11.32
	$A_1 B_1 C_2 D_2 E_1$	11.51	11.86
(3)	$A_1 B_2 C_1 D_1 E_2$	8.04	7.28
	$A_1 B_2 C_1 D_2 E_1$	8.31	8.64
(4)	$A_1 B_2 C_2 D_1 E_1$	6.59	6.74
	$A_2 B_2 C_2 D_2 E_2$	5.89	6.48
(5)	$A_2 B_1 C_1 D_1 E_2$	11.32	11.77
	$A_2 B_1 C_1 D_2 E_1$	11.28	10.88
(6)	$A_2 B_1 C_2 D_1 E_1$	7.82	8.05
	$A_2 B_1 C_2 D_2 E_2$		
(7)	$A_2 B_2 C_1 D_1 E_1$	8.78	7.93
	$A_2 B_2 C_1 D_2 E_2$		
(8)	$A_2 B_2 C_2 D_1 E_2$	7.28	7.43
	$A_2 B_2 C_2 D_2 E_1$	7.36	6.54

[a] See Table 1.
[b] No MS formed.

Table 4 summarises the observed values of the mean diameter measured according to factorial design. The resultant particle size varied between 7 and ~11 μm. The combinations containing both the high level of phase volume ratio (D_2) and albumin concentration (E_2) did not produce any microspheres as the excess albumin in the oil phase aggregated into a large non-dispersible mass. Therefore, these combinations were not considered in the ANOVA model. Any two methods of preparation, having the same A, B and C levels, produced particles which were not significantly different in size ($p > 0.1$). This indicates

Table 5
Summary of the analysis of variance based on the particle sizes observed

Source	Basic ratio	Sum of the squares	df	MS	F
Butanol concentration [A]	[A] = 2367.6	[A] − [T] = 0	1	0	0
pH [B]	[B] = 2407.4	[B] − [T] = 39.8	1	39.8	258 [a]
Stirring rate [C]	[C] = 2373.6	[C] − [T] = 6	1	6	39 [a]
$A \times B$	[AB] = 2409.3	[AB] − [A] − [B] + [T] = 1.9	1	1.9	12 [a]
$A \times C$	[AC] = 2392.6	[AC] − [A] − [C] + [T] = 19	1	19	123 [a]
$B \times C$	[BC] = 2415.6	[BC] − [B] − [C] + [T] = 2.2	1	2.2	14 [a]
$A \times B \times C$	[ABC] = 2462.1	[ABC] − [AB] − [AC] − [BC] + [A] + [B] + [C] − [T] = 25.6	1	25.6	166 [a]
S/ABC	[ABCs] = 2466	[ABCs] − [ABC] = 3.7	24	0.154	

[a] $p < 0.01$.

From the data above, it can be seen that butan-1-ol had no significant effect on the particle size. However, microspheres prepared in the presence of butan-1-ol exhibited a more regular shape than those prepared in its absence, as shown in Fig. 2.

that albumin concentration and phase volume ratio combined had no significant effect on particle size over the investigated range. Consequently, any two combinations having the same A, B and C level were treated as one combination, with four samples in each group (Table 4). As four combinations were deleted, batches of their correspondent combinations were prepared and analysed in duplicate so that every group contained four samples. As a result, the five-factor design could be simplified to a three-factor design. The results of the analysis of variance are (Keepel, 1982) listed in Table 5.

The pH of albumin solutions exerted a significant effect on particle size ($p < 0.01$), lower pH values tending to induce the formation of larger

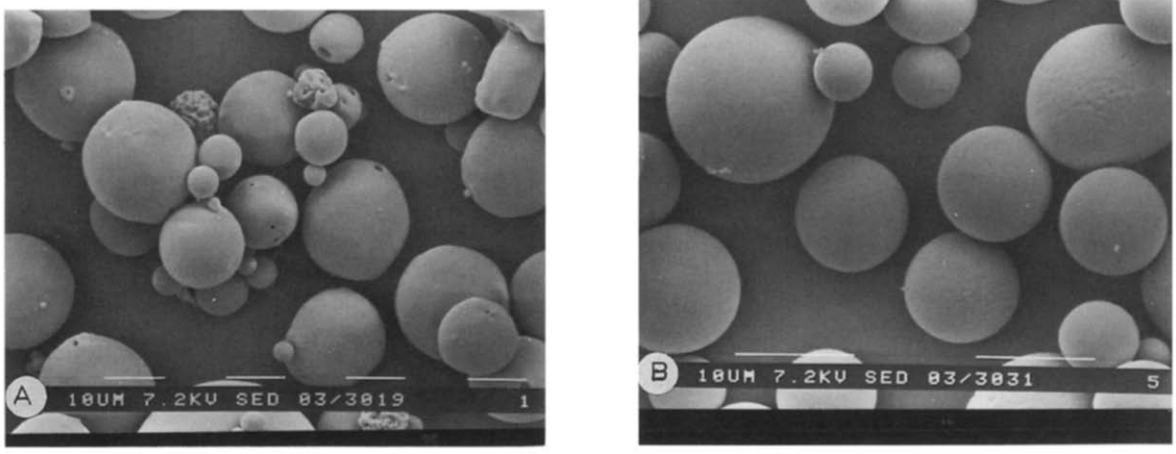

Fig. 2. Scanning electron micrographs of albumin MS: (A) without butan-1-ol; (B) with 9% (v/v) butan-1-ol.

microspheres. As the pH of the albumin solutions may play an important role in drug entrapment, especially for lipophilic acidic and basic drugs, this factor should be carefully controlled during the preparation of drug-entrapped microspheres. Stirring rate also exhibited statistically significant effects on particle size ($p < 0.01$), a higher stirring rate producing smaller particles. Table 5 also shows that there is evident interdependence ($p < 0.01$) among the three factors. Empirically optimised design cannot reveal the real effects of these factors. Hence, it can be demonstrated that statistical techniques of the type described should be employed when optimising process variables.

In conclusion, this study demonstrates the use of factorial design in the analysis of the effects of several process variables on the particle size of albumin MS. All of the investigated variables can influence the particle size, but only pH of albumin solution and stirring rate during heat denaturation exhibited statistically significant effects. Higher stirring rate and neutral pH value have separate or joint effects in reducing the particle size. In order to prepare albumin MS with an appropriate particle size these two factors should be carefully investigated and controlled. Butan-1-ol in the oil phase exhibits the ability to improve the appearance of albumin MS, causing the production of regularly shaped particles, but it had no significant effect upon particle size. Over the investigated range the amount of albumin is shown to have no significant effect upon particle size, however, excess albumin should be avoided as it tends to aggregate, preventing the formation of MS. The albumin MS prepared in the study have mean diameters from 7 to 11 μm, indicating they may be of an appropriate size to be used as carriers for targeting to the lung after parenteral administration.

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