ELSEVIER

International Journal of Pharmaceutics 151 (1997) 47–55

international journal of pharmaceutics

Preparation of hydrophobic and hydrophilic albumin microspheres and determination of surface carboxylic acid and amino residues

A.B. MacAdam [a], Z.B. Shafi [1,a], S.L. James [a], C. Marriott [b], G.P. Martin [b,*]

[a] *Department of Pharmacy, University of Brighton, Moulescoomb, Brighton, UK*
[b] *Department of Pharmacy, Kings College, University of London, Manresa Road, Chelsea, London, UK*

Received 2 October 1996; revised 15 January 1997; accepted 21 January 1997

Abstract

Albumin microspheres (MS) have been studied extensively as delivery systems for targeting drugs since they are biodegradable, non-toxic, relatively easy to prepare and their size range can be controlled. A method for albumin MS production was developed which was faster, processed larger quantities of starting material than previous methods, and had chemically reactive groupings on the MS surface to which ligands could be attached. Relatively hydrophobic, hydrophilic and also carboxymethylated MS were manufactured. The number of carboxylic acid residues was determined on the surface of these MS using ^{14}C-glycine ethyl ester hydrochloride as a probe, and the number of amino groups was determined using ^{14}C-sodium acetate as a probe. The number of carboxylic acid residues per unit surface area for the hydrophobic, hydrophilic and carboxymethylated MS was 2.1×10^4, 4.1×10^4 and 8.4×10^4, respectively, and the number of amino acid residues was 2.2×10^3, 5.0×10^2 and 5.0×10^2, respectively. © 1997 Elsevier Science B.V.

Keywords: Albumin microspheres; Hydrophobic; Hydrophilic; Carboxymethylated; Crosslinking; Glutaraldehyde; Surface carboxylic acid residues; Surface amino residues; ^{14}C-Glycine ethyl ester hydrochloride; ^{14}C-Sodium acetate

1. Introduction

Microparticulate drug carrier and delivery systems have been produced from a wide range of different materials. Albumin microspheres (MS) have been extensively studied in previous work as suitable for drug delivery (Kramer, 1974; Widder

* Corresponding author.
[1] Present address: Pharmaceutical Sciences, Wyeth-Ayerst Research, Fareham Road, Gosport, Hants., UK.

PII S0378-5173(97)04886-2

et al., 1980; Lee et al., 1981; Tomlinson et al., 1984; Longo and Goldberg, 1985; McArdle et al., 1988; Ghassabian et al., 1996) since they are biodegradable (Morimoto and Fujimoto, 1985; Nakagawa et al., 1987), relatively easy to prepare and by varying the preparation conditions, their size range can be controlled (Tomlinson and Burger, 1995). They are reported to be relatively non-toxic (Edman et al., 1983; Nakagawa et al., 1987) and produce no short term adverse immunological responses on serial injection, although skin sensitivity has been reported (Rhodes et al., 1969).

The principle of preparation of albumin MS is to suspend an aqueous solution of albumin in an external non-polar phase, thus producing an emulsion of albumin droplets. Covalent stabilisation—often by the use of a fixative such as glutaraldehyde (Oner and Groves, 1993; Bachtsi and Kiparissides, 1996)—of the albumin results in the production of stable MS. Since this method of production employs a lipophilic external phase, it is claimed that this method produces MS with a hydrophobic surface due to preferential orientation of albumin amino acid residues at the aqueous/lipophilic interface during synthesis, such that, hydrophobic regions of the albumin molecule are exposed externally to the lipophilic phase, in contrast to the situation predicted for native albumin in an aqueous environment (Longo and Goldberg, 1985). Such alignment of the albumin molecule may promote a hydrophobic interaction between the lipophilic phase (usually an oil) and the albumin, resulting in some adsorption of the oil during synthesis, which adheres to the MS even after washing, increasing their surface hydrophobicity (Longo and Goldberg, 1985).

A technique has been described, however, which it is claimed produces albumin MS with an increased hydrophilic surface (Longo et al., 1982). Increasing the hydrophilicity of the MS is considered advantageous because such particles may exhibit more desirable surface physical and chemical properties for use as delivery systems in vivo. In addition such MS would not require surfactants to aid their aqueous dispersion and, moreover, a hydrophilic surface may facilitate aqueous chemical modification of the surface groups (Longo and Goldberg, 1985). These modified MS, termed 'hydrophilic MS', were produced by altering the external phase from a hydrophobic oil to poly(methylmethacrylate) (PMMA) dissolved in equal amounts of toluene and chloroform, a solvent/polymer combination, which is more hydrophilic than an oil. It is claimed that this process introduces a relatively high surface concentration of aldehyde groups which facilitates a variety of chemical modifications including aminoethanol capping of the aldehyde groups to enhance hydrophilicity and the binding of enzymes, antibodies or other protein ligands as well as the covalent attachment of amino-functional drugs (Longo et al., 1982).

The methods so far described for both 'hydrophobic' and 'hydrophilic' MS have a common disadvantage of allowing the processing of only small quantities of albumin, in the order of 100 mg (Tomlinson and Burger, 1995) and 150 mg (Longo et al., 1982). The time required for the stabilisation of the MS by crosslinking with glutaraldehyde varies according to the method employed, for the hydrophobic MS described by Tomlinson and Burger (1995), crosslinking took between 1–4 h whereas hydrophilic MS required a longer time of between 5–6 h (Longo et al., 1982).

It was the purpose of this investigation to develop a method of albumin MS production which was faster, processed larger quantities of starting materials than previous methods, and produced MS which have chemically reactive groupings on the surface to which ligands could be attached, whilst ensuring a final particle size of less than 50 μm. A technique for the modification of the MS surface to increase available reactive surface carboxylic acid residues was also developed and the number of carboxylic acid residues on the MS surface was determined using a radioactive molecule as a probe, glycine ethyl ester hydrochloride, which reacts via an amino group with carboxylic acid groups on the MS surface. The number of amino groups was also determined using radiolabelled sodium acetate which reacts via the carboxylic acid group with amino groups on the MS surface.

2. Materials and methods

2.1. Materials

Oleic acid, butan-1-ol, glutaraldehyde, sodium hydroxide (pellets) and Cocktail T-'Scintran' were supplied by BDH (Poole, Dorset, UK).

Bovine serum albumin (fraction V powder, 96–99% albumin) and iodoacetic acid (free acid) were supplied by Sigma (Poole, Dorset, UK).

Polymethylmethacrylate (DA100) (intrinsic viscosity 1.2; average molecular weight 500 000 Da) was kindly donated by ICI Chemicals and Polymers Ltd. (Wilton, Middlesborough, Cleveland, UK).

^{14}C-Sodium acetate and 1-^{14}C-glycine ethyl ester HCl was supplied by Du Pont (UK) Ltd., NEN Products (Stevenage, Herts., UK).

2.2. Methods

2.2.1. Production of hydrophobic MS

Oleic acid (50 ml) was poured into a 100 ml glass measuring cylinder (internal diameter 27 mm). Butan-1-ol (7 ml) and 1 ml glutaraldehyde (16.7% v/v) were added and the liquids mixed for 15 s using an homogeniser (Standard laboratory mixer emulsifier Silverson, Bucks, U.K.). The paddles were set to rotate at 6100 rpm, measured by stroboscopic timing and during the mixing process the cylinder was raised and lowered through a vertical plane once per second. Bovine serum albumin (BSA) solution (3 ml, 33.3% w/v) was added slowly over a 1 min period of continued mixing to the measuring cylinder using a 5-ml syringe fitted with a 40 gauge needle. After a further 5 min of mixing, the hydrophobic MS produced were harvested by centrifugation at $1000 \times g$ for 3 min. The albumin MS were resuspended in butan-1-ol and centrifuged under the same conditions to obtain a pellet. Following a further two repetitions of this washing procedure, the MS were transferred to uncovered petri dishes and left for 24 h to air-dry at 20°C.

2.2.2. Production of hydrophilic MS

PMMA (12.5 g), toluene (35 ml) and chloroform (35 ml) were mixed using a magnetic stirrer and follower bar for 10 min. The mixture was transferred to a 100 ml measuring cylinder, 1 ml glutaraldehyde (25% v/v) added and the liquids mixed for 30 s using the homogeniser. BSA solution (3 ml, 33.3% w/v) was added slowly over a 30 s period of continued mixing to the measuring cylinder using a 5 ml syringe fitted with a 40 gauge needle. The mixture was left to stand for 30 min then, a solution of glycine (10 ml, 10% w/v) was added and after remixing, left to stand for a further hour. The hydrophilic MS formed were washed 3 times using the method described above, except acetone was substituted for butan-1-ol as the washing solvent. Drying was carried out by freezing the MS at −20°C followed by freeze drying for 24 h.

2.2.3. Production of carboxymethylated MS

Hydrophobic MS (200 mg) were added to 20 ml sodium hydroxide (1M) and mixed using a magnetic stirrer and follower bar for 5 min. Iodoacetic acid (50 mg) was added to the MS suspension and mixing continued for 2 h. Hydrochloric acid (20 ml, 1 M) was added to the mixture and the MS were washed using the method described for the hydrophobic MS, except distilled water was substituted for the butan-1-ol as the washing solvent. The washing procedure was carried out until after centrifugation the decanted supernatant had the same pH as the original distilled water. The carboxymethylated MS were then freeze dried.

2.2.4. Particle characterisation

2.2.4.1. Scanning electron microscopy. Small samples of MS (1–5 mg) were stuck to carbon black stubs before gold sputter coating. The stubs were examined under a scanning electron microscope (ISI 100A, Akashi Beam Technology, Japan) using a tungsten source, a working distance of 15 mm and an accelerating voltage of 3–5 kV to avoid penetration.

2.2.4.2. Particle size analysis by laser light diffraction. Particle size analysis (PSA) was carried out by a laser light scattering instrument (Malvern multisizer, Malvern instruments Ltd., Worcestershire, UK), the suspending solvent being Tween 80 (1% w/v) in normal saline.

2.2.4.3. Density determination. Density measurements were performed by using a multivolume pycnometer (Pycnometer 1305, Micromeritics, Nercross, GA, USA). Accurately weighed samples were placed in a 5 cm^3 cup and inserted into the multivolume pycnometer. Helium was introduced into the sample chamber, the pressure recorded and the gas was expanded into a precisely measured volume, which resulted in a pressure drop. The sample volume and hence density were calculated from the two pressure readings.

2.2.4.4. Isoelectric point. The isoelectric point of the MS was determined by measuring the electrophoretic mobility using a particle microelectrophoresis apparatus (Rank Mark 1, Rank Bros., Cambridge, UK). The mean velocity at pH values ranging from 3 to 10 (suspending buffer 1 mM NaCl), was calculated by timing particle migration over a distance of 1 mm.

2.2.5. Estimation of the surface carboxylic acid residues on MS

Reactive amino groups on a blocked radioactive glycine derivative were linked to carboxylic acid residues on the surface of the microsphere using the water soluble peptide condensing agent 1-ethyl-3(3-dimethyl aminopropyl)-carbodiimide (EDAC). MS (5 mg) were suspended in 1.8 ml sodium chloride solution (0.1 M, pH 7.0). The MS were dispersed by sonication for 10 min using an ultrasonic bath. EDAC was dissolved in 0.1 ml sodium chloride solution (0.1 M, pH 7.0), added to the MS suspension and mixed at 4°C, using a magnetic stirrer and follower bar for 10 min. The amount of EDAC to be added was determined by a series of experiments in which increasing concentration of the carbodiimide were employed in an effort to determine optimal condensation conditions. For the actual amounts used for each type of MS (see Section 3). ^{14}C-Glycine ethyl ester hydrochloride (2×10^3 MBq/mM, 11×10^3 Bq in 0.1 ml) was added to the MS suspension and mixed at 4°C for 2 h. The MS were recovered from the mixture by centrifugation at $1000 \times g$ for 3 min, after which a washing procedure was carried out employing either 5 ml urea (8 M) twice and 5 ml saturated sodium chloride solution once, or 5 ml distilled water three times. The MS were resuspended in 5 ml cocktail T scintillation fluid and counted in a liquid scintillation counter (1209 Rack-beta, Pharmacia, U.K.) for 1 min. The surface carboxylic acid residues of the three different types of MS (hydrophobic, hydrophilic and carboxymethylated) were thus determined.

2.2.6. Estimation of the surface amino residues on MS

Amino groups on the MS surface were probed using EDAC to condense the ^{14}C-acetic acid carboxylic acid residue. MS (5 mg) were suspended in 1.8 ml sodium chloride solution (0.1 M. pH 7.0). The MS were dispersed by sonication for 10 min, using an ultrasonic bath. EDAC was dissolved in 0.1 ml sodium chloride solution (0.1 M, pH 7.0) and 0.1 ml ^{14}C-sodium acetate (2.1×10^3 MBq/mM, 11×10^3 Bq in 0.1 ml) added. Following 10 min incubation at 4°C, the mixture was added to the MS and mixed using a magnetic stirrer and follower bar for 2 h. The MS were recovered from the mixture by centrifugation at $1000 \times g$ for 3 min, and the washing procedure was carried out employing either 5 ml urea (8 M) twice and 5 ml saturated sodium chloride solution once or 5 ml distilled water three times. The MS were transferred to 5 ml scintillation fluid and counted as described above. The surface amino residues of the three different types of MS (hydrophobic, hydrophilic, carboxymethylated) were thus determined.

3. Results

3.1. Scanning electron microscopy

Results from examining the MS using the SEM revealed the hydrophobic and carboxymethylated MS to be discrete and spherical, whereas the hydrophilic MS were discrete but irregular (Figs. 1–3). Whilst too few MS can be captured to yield a reliable estimate of particle diameter, it was clear that the hydrophilic MS were larger than the other two types—a result supported by PSA.

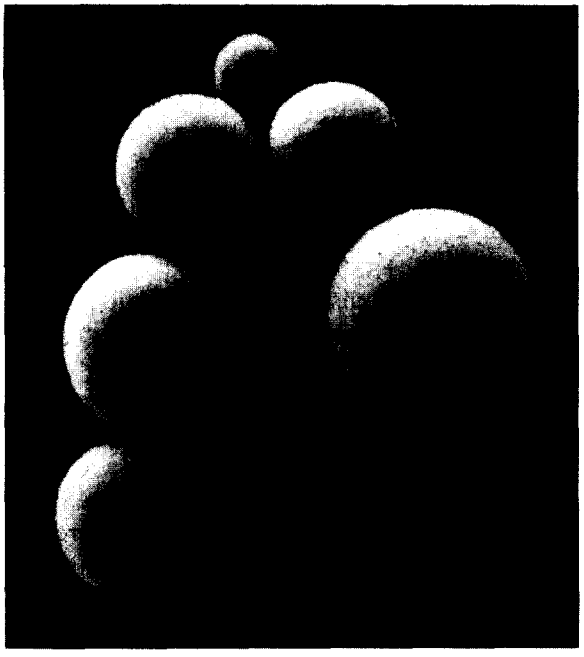

Fig. 1. Scanning electron micrograph of hydrophobic MS (× 12 000).

3.2. Physical characteristics

Table 1 shows that the mean volume diameters of the hydrophobic MS were the smallest and the hydrophilic MS the largest; however, the carboxymethylated MS had a mean diameter closer in value to the hydrophobic. The specific surface area followed the expected pattern related to the mean diameters being lowest for the hydrophilic MS and similar for the hydrophobic and carboxymethylated MS. The density of the three different types of MS was the same, although the isoelectric point of the hydrophilic MS was found to be lower than the value obtained for both the hydrophilic and carboxymethylated MS (Table 1).

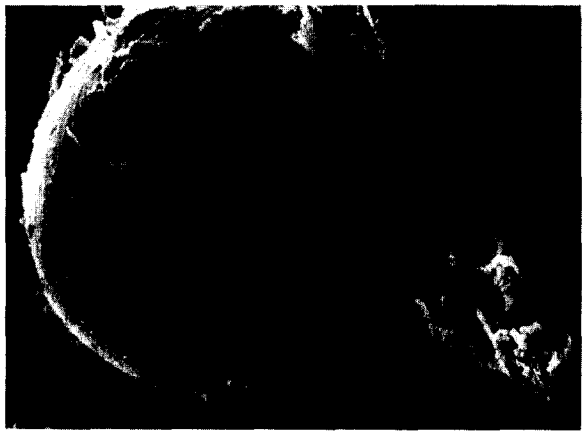

Fig. 2. Scanning electron micrograph of hydrophilic MS (× 5000).

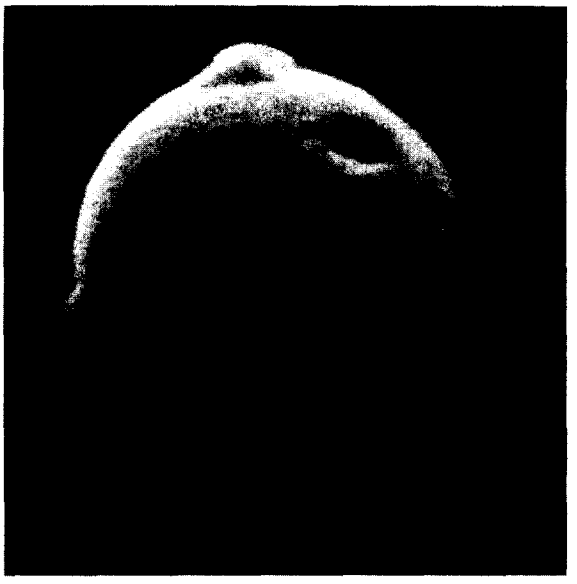

Fig. 3. Scanning electron micrograph of carboxymethylated MS (× 24 000).

3.3. Estimation of surface carboxylic acid and amino residues on the MS

The quantity of EDAC required for the maximum binding of ^{14}C-glycine ethyl ester hydrochloride to 5 mg of hydrophobic, hydrophilic and carboxymethylated MS was 6 mg ml^{-1}, 5 mg ml^{-1} and 4 mg ml^{-1}, respectively. The amount required for maximum binding of ^{14}C-sodium acetate was 5 mg ml^{-1}, 5 mg ml^{-1} and 4 mg ml^{-1}, respectively. A representative curve showing the measure of radioactive sodium acetate and glycine ethyl ester hydrochloride bound with increasing concentration of EDAC to carboxymethylated MS is shown in Fig. 4. The DPMs obtained at maximum binding was reduced after washing with 8 M urea and saturated sodium chloride solution compared with the counts obtained at maximal binding following washing with distilled water

Table 1
Physical characteristics of the albumin MS

	Hydrophobic MS	Hydrophilic MS	Carboxymethylated MS
Density (kg m^{-3}) $\times 10^3$	1.48	1.49	1.48
Isoelectric point (pH)	4.3	3.7	4.3
Mean volume diameter (μm $\pm$ S.D.)	9.68 $\pm$ 3.0	26.22 $\pm$ 2.71	10.19 $\pm$ 3.1

(Table 2). There was a reduction in the binding of ^{14}C-glycine ethyl ester of 40.6%, 14.8% and 19.7% for the hydrophobic, hydrophilic and carboxymethylated, respectively, and a reduction in the binding of ^{14}C-sodium acetate of 68.9%, 84.8% and 91.7% for the hydrophobic, hydrophilic and carboxymethylated MS (Table 2).

From the DPM for the isotope glycine ethyl ester hydrochloride and the specific activity of the isotope, the total number of carboxylic acid residues on the MS surface was calculated to be 2.5×10^{14} and this corresponded to an approximate number of carboxylic acid residues on each hydrophobic MS of 6.3×10^6. The comparable values for the other types of MS are given in Table 3. As the mean volume diameters for the three types of MS are different, the results may be better represented in terms of number of carboxylic acid residues per unit surface area as also shown in Table 3. The results expressed as the number of chemical groups per unit surface area indicate that there are double the number of carboxylic acid groups available per unit area of carboxymethylated MS compared with the hydrophilic MS, and there are approximately double the number of carboxylic acid groups available per unit area of hydrophilic MS compared with the hydrophobic MS. There are fewer amino groups than carboxylic acid groups on all three types of MS, the hydrophobic MS having the greatest number of amino groups whilst the hydrophilic and carboxymethylated MS have approximately the same number.

4. Discussion

The approach of using an homogeniser, whose specific function is to produce emulsions by high shear conditions, enabled larger quantities of albumin to be processed in comparison to previous studies.

As the cause of the hydrophobicity of the MS is believed to be due to the influence of oily external phase, if the external phase was rendered more hydrophilic, the resultant surface of the MS may have more hydrophilic groups exposed. Therefore, a less hydrophobic external phase was employed which included the polymer PMMA. The hydrophilic MS produced in this manner were not however as smooth or spherical as the hydrophobic MS. It is suggested that a polymer coat remained on the MS surface even after washing. MS prepared by Longo et al. (1982) also had a similar appearance. An indication of the presence of a polymer coat was derived from the fact that hydrophilic MS sediment quickly following dispersion in an aqueous solvent, in the manner of a flocculated system; this could be the result of polymer bridging between MS. A disadvantage of any coat which is not covalently bound to the MS surface is the potential lack of any long-term stability in an aqueous environment.

An alternative approach to increasing the hydrophilicity of the MS was to attempt to modify the preformed MS with a reagent capable of producing hydrophilic residues on the surface. Iodoacetic acid has been employed previously to introduce carboxymethyl groups into various amino acids, the reaction being pH-dependent (Gurd, 1967). Reaction of iodoacetic acid with histidine, lysine and aspartate residues occurs at low pH, whereas reaction with phenolic amino acids tends to occur at high pHs. Since the hydrophobic MS surface is likely to be rich in phenolic amino acids, the reaction was carried out in 1 M sodium hydroxide. This strategy appears successful since the resultant carboxymethylated MS pro-

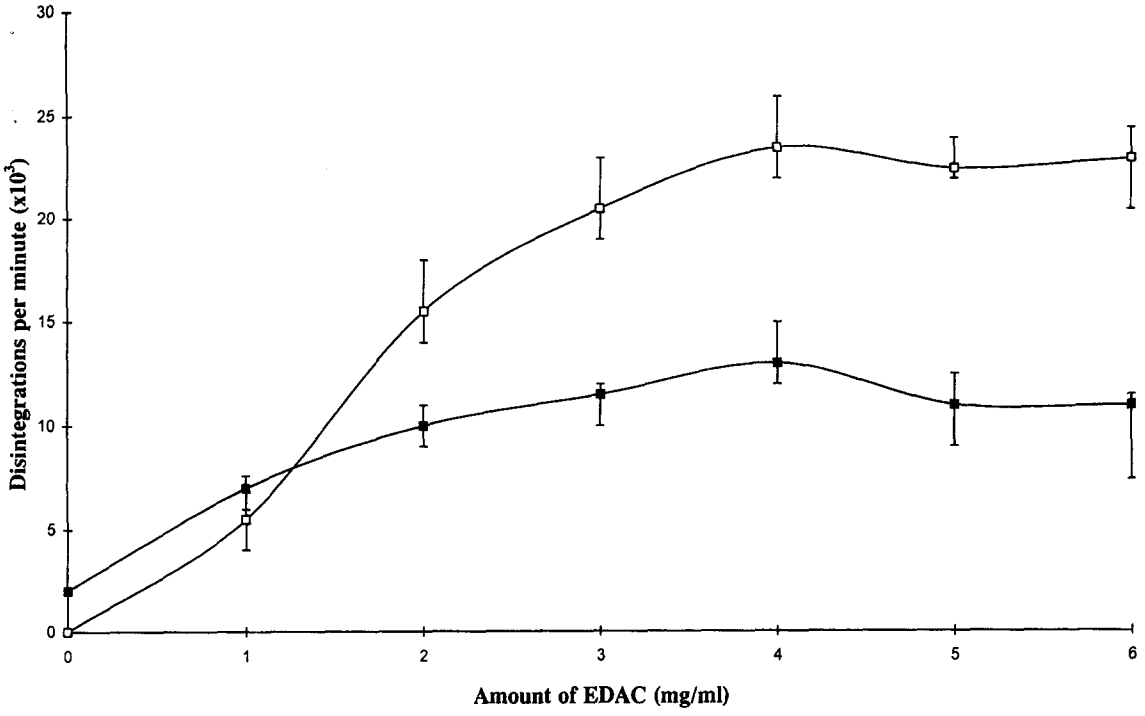

Fig. 4. Measure (mean ± S.D.) of radioactive sodium acetate (■) and glycine ethyl ester hydrochloride (□) bound with increasing concentration of EDAC to carboxymethylated MS (5 mg).

duced in this study dispersed well in an aqueous environment, sedimenting slowly, similar to a deflocculated system, and showed a marked increase in carboxylic acid groups per unit MS surface area.

SEM of the hydrophobic and carboxymethylated MS revealed they were smooth and spherical, whereas, although the hydrophilic MS were spherical, the surfaces had an irregular appearance.

The MS were found to be polydisperse in size, ranging from under 1 μm to 100 μm. However, the majority of the hydrophobic and carboxymethylated MS were in the size range 2–15 μm, whereas for the hydrophilic MS, the corresponding size range was 5–40 μm. The larger particle size is likely to be due to the altered shear conditions as a result of the presence of PMMA. In addition, since PMMA was adsorbed to the MS, increased swelling of the particles is likely in the aqueous suspending solvent. Zhang et al. (1995) found a similar polydisperse system with particle size range of 58.8–256 μm, for cisplatin albumin MS using an emulsion-heat stabilisation method.

The results of the density measurements showed all the MS had a similar density. This may have been expected since the MS were composed of the same material, in fact the material for all MS production was from the same batch of BSA. This does suggest, however, that any PMMA coating on the hydrophilic MS is likely to be thin, as a substantial coat would alter the particle density. It also suggests that excessive swelling of the MS is not present.

The isoelectric points obtained from measurement of electrophoretic mobility were the same for the hydrophobic and carboxymethylated MS, at pH 4.3, whereas for the hydrophilic MS it was at pH 3.7. The isoelectric point of BSA is 4.9 and a possible explanation for the fall in the isoelectric point of the MS compared to albumin is that MS are stabilised by the cross-linking agent, glutaraldehyde, which reacts with the primary amino

Table 2
DPMs associated with MS after employing different washing solvents

Microspheres	Mean DPM ($\times 10^{-4} \pm$ S.D., n = 5,)	
	^{14}C-Glycine ethyl ester	^{14}C-Sodium acetate
Hydrophobic		
Washed with water	8.08 ± 0.60	1.77 ± 0.13
Washed with urea and saturated sodium chloride	4.82 ± 0.81	0.55 ± 0.05
Hydrophilic		
Washed with water	19.71 ± 1.19	1.58 ± 0.12
Washed with urea and saturated sodium chloride	16.79 ± 2.14	0.24 ± 0.05
Carboxymethylated		
Washed with water	23.06 ± 2.02	1.58 ± 0.16
Washed with urea and saturated sodium chloride	18.51 ± 1.09	0.13 ± 0.02

groups of the albumin. The difference in the number of carboxylic acid groups could be observed in the electrophoretic mobility of the MS in the pH range 7 to 10, where the carboxymethylated MS had a greater mobility than the hydrophobic MS, indicating a larger overall negative charge and hence more ionised carboxylic acid groups.

From the estimation of the surface carboxylic acid and amino residues, it was determined that more carboxylic acid residues than amino residues were found on the surface of each type of MS. This may have been anticipated since the primary mechanism involved in the production process of all types of MS is the cross-linking of all primary amino groups with glutaraldehyde. Such a cross-linking procedure would decrease the number of amino groups available for linking to the radioactive sodium acetate.

To ensure that only one radioactive probe molecule interacted with a chemical group on the surface of the MS, those with either one amino group or one carboxylic acid group were employed. ^{14}C-glycine ethyl ester has one reactive amino group and ^{14}C-sodium acetate has one reactive carboxylic acid group. Linkage of ^{14}C-glycine ethyl ester and ^{14}C-sodium acetate to the three types of MS showed a typical saturation curve where, as the amount of EDAC increased, so did the number of the radioactive probe molecules. A point was eventually reached after which an increase in EDAC produced a slight decrease in the number of molecules of both isotopes that bound. This could be because at high concentrations of EDAC, crosslinking between MS occurs and this decreases the number of available groups on the surface of the MS for the radioactive probe molecules to bind. The amount of EDAC required for maximal binding of both probes was established. The amount of isotope covalently bound to each type of MS was determined by employing urea (8 M) and saturated sodium chloride solutions as the washing solvents. The former removes probe molecules bound by hydrogen bonding and the latter by

Table 3
An estimate of the number of carboxylic acid and amino residues present on the surface of hydrophobic, hydrophilic and carboxymethylated MS in terms of number of groups per MS and number per unit area of MS

	Microspheres		
	Hydrophobic	Hydrophilic	Carboxymethylated
DPM at maximum binding glycine ethyl ester	4.8×10^4	1.7×10^5	1.8×10^5
No. COOH groups/MS	6.3×10^6	5.1×10^7	2.7×10^7
No. COOH groups/mm^2	2.1×10^4	4.1×10^4	8.4×10^4
DPM at maximum binding sodium acetate	5.5×10^3	2.4×10^3	1.3×10^3
No. NH_2 groups/MS	6.6×10^5	6.8×10^5	1.8×10^5
No. NH_2 groups/μm^2	2.2×10^3	5.0×10^3	5.0×10^2

ionic interaction. Washing in water allows such binding to remain intact. Less of the radioactive glycine ethyl ester hydrochloride was bound by hydrogen and/or ionic bonding to the carboxymethylated or the hydrophilic MS in comparison with the hydrophobic MS. However, most of the binding of the radioactive sodium acetate to the carboxymethylated MS was due to hydrogen and/or ionic bonds. This was also the case for hydrophilic and hydrophobic MS. This is surprising in the context of the increasing probe binding with increasing EDAC, which implies covalent interactions. The urea washings may be so vigorous that it removes part of the MS surface.

Thus, in conclusion a method is described for the preparation of hydrophobic albumin MS. These can be rendered hydrophilic by either coating with PMMA or carboxymethylating the MS resulting in surface carboxylic acid residues suitable for attachment of ligands as shown by Shafi et al. (1995). Moreover, the number of surface carboxylic acid residues and amino residues can be determined using the radioactive probes ^{14}C-glycine ethyl ester and ^{14}C-sodium acetate.

References

Bachtsi, A.R. and Kiparissides, C., Synthesis and release of oil containing poly(vinyl alcohol) microcapsules prepared by coacervation. *J. Control. Release*, 38 (1996) 49–58.

Edman, P., Sjoholm, I. and Brunk, V., Acrylic MS in vivo. VII. Morphological studies in mice and cultured macrophages. *J. Pharm. Sci.*, 72 (1983) 658–665.

Ghassabian, S., Ehtezazi, T., Forutan, S.M. and Mortazavi, A.S., Dexamethasone-loaded magnetic albumin microspheres: preparation and in vitro release. *Int. J. Pharm.*, 130 (1996) 49–55.

Gurd, F.R.N., Carboxymethylation. *Methods Enzymol.*, 11 (1967) 532–541.

Kramer, P., Albumin MS as vehicles for achieving specificity in drug delivery. *J. Pharm. Sci.*, 63 (1974) 1646–1647.

Lee, T., Sokoloski, T. and Royer, G., Serum albumin beads: an injectable biodegradable system for the sustained release of drugs. *Science*, 213 (1981) 233–235.

Longo, W.E. and Goldberg, E.P., Hydrophilic albumin MS. In Widder, K. and Green, R., (Eds), *Drug and Enzyme Targeting. Methods in Enzymology.*, Vol. 112, Academic Press, London, 1985, pp 18–26.

Longo, W.E., Iwata, H., Lindheimer, T.A. and Goldberg, E.P., Preparation of hydrophilic MS using polymeric dispersing agents. *J. Pharm. Sci.*, 71 (1982) 1323–1328.

McArdle, C.S., Lewis, H., Hansell, D., Kerr, D.J., McKillop, J. and Wilmott, N., Cytotoxic-loaded albumin MS: a novel approach to regional chemotherapy. *Br. J. Surg.*, 75 (1988) 132–134.

Morimoto, Y. and Fujimoto, S., Albumin MS as drug carriers. *CRC Crit. Rev. Ther. Drug Carrier Syst.*, 2 (1985) 19–63.

Nakagawa, Y., Takayama, K., Ueda, H., Machida, Y. and Nagai, T., Preparation of bovine serum albumin nanospheres as drug targeting carriers. *Drug Design Deliv.*, 2 (1987) 99–107.

Oner, L. and Groves, M.J., Properties of human albumin microparticles prepared by chilled cross-linking technique. *J. Pharm. Pharmacol.*, 45 (1993) 866–870.

Rhodes, B.A., Zolle, I., Buchanan, J.W. and Wagner, H.N., Jr., Radioactive albumin MS for studies of the pulmonary circulation. *Radiology*, 92 (1969) 1453–1459.

Shafi, Z.B., Martin, G.P., Olliff, C.J. and James, S.L., Surface modification of albumin microspheres. *J. Drug Target.*, 3 (1995) 53–56.

Tomlinson, E. and Burger, J.J., Incorporation of water soluble drugs in albumin. In MS. Widder K. and Green, R. (Eds), *Drug and Enzyme Targeting. Methods in Enzymology*, Vol. 112, Academic Press, London, 1995, pp. 27–43.

Tomlinson, E., Burger, J.J., Schoonderwoerd, E.M.A. and McVie, J.G., Human serum albumin MS for intra-arterial drug targeting of cytostatic compounds. In Davis, S.S., Illum, L., McVie, J.G. and Tomlinson, E. (Eds), *Pharmaceutical Aspects and Release Characteristics. Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects.* Elsevier, Amsterdam, 1984, pp. 75–89.

Widder, K.J., Senyei, A.E. and Ranney, D.F., In vitro release of biologically active adriamycin by magnetically responsive albumin MS. *Cancer Res.*, 40 (1980) 3512–3517.

Zhang, Y.Q., Jiang, X.T., Sun, Q.R., Zhang, G.Q. and Wang, Y., Studies on CDDP-albumin MS for hepatic arterial chemoembolisation. *Yao Hsueh Hsueh Pao* (China), 30(7) (1995) 543–8.