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Albumin microspheres. III. Synthesis and characterization of microspheres containing adriamycin and magnetite

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Summary

Adriamycin-associated bovine serum albumin microspheres (Adr-BSA), plain magnetic BSA microspheres (Fe-BSA) and adriamycin-associated magnetic BSA microspheres (Adr-Fe-BSA) were prepared by heat stabilization at different temperatures and evaluated for their size, hydration characteristics, drug and/or magnetite entrapment, Fe_3O_4 distribution within particles and drug release properties. It was demonstrated that microspheres with a mean diameter of less than 1 μm can be prepared at temperatures between 105 and 150 °C, in the presence of adriamycin and/or magnetite. Equilibrium hydration with these particles was attained in about 2 h after soaking in normal saline at 37 °C. The degree to which the particles increase in size was dependent on their stabilization temperature. The entrapment of adriamycin was influenced by the presence of magnetite as well as the temperature employed during the carrier stabilization. Maximum entrapment of adriamycin in Adr-Fe-BSA microspheres, after 4 washings, was obtained at 120 °C. The presence of adriamycin significantly affected the entrapment and distribution of Fe_3O_4 in albumin microspheres. The release rate of adriamycin entrapped within Adr-BSA and Adr-Fe-BSA microspheres was dependent on the presence of magnetite as well as the stabilization temperature of the carrier.

Introduction

Albumin microspheres have been used extensively in the diagnosis of abnormalities of reticuloendothelial system and in the measurements of blood flow (Blanchard et al., 1965; Del Maestro et al., 1979; Rhodes et al., 1969; Wagner et al., 1969; Zolle et al., 1970a). Recently, this carrier has been investigated for active as well as passive drug targeting (Kramer and Burnstein, 1976; Morimoto et al., 1980; Tomlinson et al., 1982; Widder et al., 1978; Willmott et al., 1985). An understanding of the physicochemical properties of albumin microspheres would be of value in gaining a better appreciation of their use in vivo. The optimum dosage regimen for a drug administered via microspheres is dependent on the size, drug content, hydration and drug release characteristics of these particles (Oppenheim, 1981). In the case of drug targeting using magnetic microspheres, the magnetite content of the carrier must also be considered (Bartlett et al., 1984; Elliot et al., 1984; Gupta et al., 1986a; Morimoto et al., 1981; Morris et al., 1984; Ranney, 1986; Sugibayashi et al., 1982; Widder et al., 1979a).

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The particle size of a drug carrier can affect the degree of drug entrapment (Kojima et al., 1984; Tomlinson, 1983; Wakiyama et al., 1981; Yapel, 1979), drug release profile (Cavalier et al., 1986; Duc-Mauger et al., 1986; Tomlinson et al., 1984) and the hydration characteristics of the carrier (Burger et al., 1985). It has also been postulated that an increase in size of albumin microspheres due to hydration can alter its body distribution (Sugibayashi et al., 1979a). Use of submicron size microspheres minimises the incidence of pulmonary embolism often encountered with particles greater than 7 μm or particles which aggregate upon their in vivo administration (Hampton, 1958; Illum and Davis, 1982; Mattsson et al., 1986; Schroeder et al., 1978; Sjoholm and Edman, 1984; Wagner et al., 1964; Zolle et al., 1979b).

The retention of magnetic microspheres at the target site is dependent on the magnetite content of the carrier and the magnitude of the applied magnetic field (Driscoll et al., 1984; Ishii et al., 1984; Kato, 1982; Senyei et al., 1978). Although high magnetite content allows the use of smaller magnetic fields, it reduces the effective space available within the carrier for drug entrapment (Zimmermann, 1983). Hence, the incorporation of drug and magnetite needs to be delicately balanced.

It has been suggested that peripheral distribution of Fe_3O_4 incorporated in albumin microspheres exhibits high response towards the applied magnetic field (Senyei et al., 1978; Widder et al., 1979b). This property therefore remains a desirable characteristic of Fe-BSA and Adr-Fe-BSA microspheres.

This investigation has been undertaken to examine Adr-BSA, Fe-BSA and Adr-Fe-BSA microspheres stabilized at different temperatures, with respect to their particle size, hydration characteristics, and drug and/or magnetite entrapment. The distribution pattern of Fe_3O_4 in Fe-BSA and Adr-Fe-BSA microspheres has been examined using transmission electron microscopy (TEM). Dynamic dialysis has been used to illustrate the effect of magnetite and stabilization temperature on the release rate of adriamycin from Adr-BSA and Adr-Fe-BSA microspheres.

Materials and Methods

Materials

The materials and the apparatus used in the synthesis and analysis of Adr-BSA microspheres have been discussed earlier (Gallo et al., 1984; Gupta et al., 1986b). Variable speed Heidolph stirrers, model RZRI, and Dawe Soniprobe type 7530 A, were used for the synthesis of microspheres. Adriamycin hydrochloride was donated by Farmitalia Carlo Erba, Milan (Italy). Magnetite was obtained as a 25–35% w/v aqueous suspension of Fe_3O_4 from Ferrofluidics (Nashua, NH). A Shimadzu Atomic Absorption AA-640-12 (P/N 204-29900) was used to analyse the iron content of magnetic microspheres.

Synthesis of microspheres

(a) Fe-BSA microspheres. To 250 μl of aqueous BSA solution (400 mg/ml), about 100 mg of magnetite was added while vortexing. 30 ml of cotton seed oil (4 °C) was added to it and the mixture ultrasonicated for 2 min at 125 W. The magnetic emulsion was then added (100 $\pm$ 10 drops/min) to 100 ml of preheated cottonseed oil (105–150 °C), stirred at 1500 rpm. Heating and stirring of the oil were continued for 10 min after the addition of emulsion. The resulting suspension was ice-cooled to 20 °C and washed 4 times with 60 ml of anhydrous ether, each time centrifuging at 3000 *g* for 15 min. The washed microspheres were suspended in 10 ml of ether and the unincorporated Fe_3O_4 removed by transferring the suspension into a tared tube, in presence of a 300 Gauss bar magnet placed at the rim of the decanting tube. The weight of the microspheres was determined after evaporating the ether under a gentle stream of oxygen-free nitrogen, and stored as an ether suspension (25 mg/ml) or as a free flowing powder at -15 °C until used.

(b) Adr-BSA microspheres. The method of preparing these microspheres has been described earlier (Gupta et al., 1986b, 1987a). Stabilization temperatures of either 105, 120, 135 or 150 °C were used.

(c) Adr-Fe-BSA microspheres. These were prepared in the same manner as the Fe-BSA micro-

spheres except that 200 μl of aqueous adriamycin hydrochloride solution (50 mg/ml) was incorporated into the protein solution before emulsification.

Size analysis of microspheres

Scanning electron microscopy (SEM) was used for the size analysis of microspheres prepared in the presence of drug and/or magnetite at different stabilization temperatures. At least 200 particles were measured per batch.

Hydration study

The three types of microspheres (Fe-BSA, Adr-BSA and Adr-Fe-BSA), stabilized at 4 different temperatures (105, 120, 135 and 150 °C), were subjected to 4 washings before carrying out the hydration study (Gupta et al., 1986b and c). Each washing was performed by sonicating about 50 mg of microspheres in 5 ml of normal saline for 5 min, and then centrifuging at $5000 \times g$ for 5 min. The 4 times washed microspheres were suspended in 5 ml of normal saline and maintained on a rotator (40 rpm) at 37 °C. Five-hundred microlitre samples were removed at predetermined time intervals over an 8 h period and immediately centrifuged at $5000 \times g$ for 5 min. The aqueous supernatant was discarded and the microsphere pellet washed twice with 10 ml of absolute alcohol (Gupta et al., 1986b). The alcohol washed microspheres were dispersed in a small volume of ether, a drop of which was placed on a SEM stub. The ether was allowed to evaporate and the microsphere layer on the stub was coated with 30 nm gold layer. These stubs were maintained in vacuum until observed on SEM for the particle size and shape of microspheres. The internal matrix of these microspheres was examined using TEM (Gupta et al., 1986b).

Analysis of free adriamycin

Free adriamycin was analysed using a reversed-phase ion-pairing HPLC method (Gallo et al., 1986).

Analysis of adriamycin content of microspheres

Adriamycin content of Adr-BSA microspheres at different stabilization temperature and washing levels was determined as described earlier (Gupta et al., 1986b). The same procedure was adopted for analysing the adriamycin content of Adr-Fe-BSA microspheres.

Analysis of iron content of magnetic microsphere

The following method was adopted for analysing magnetite in Fe-BSA and Adr-Fe-BSA microspheres using atomic absorption at 248.3 nm: To about 5 mg of microspheres, 2 ml of concentrated hydrochloric acid was added and the contents heated at 60 °C for 2 h. To 0.5 ml of the hydrolysate, 0.5 ml of 20% v/v trichloroacetic acid was added to precipitate the proteins. The tube was centrifuged at $5000 \times g$ for 5 min and the supernatant diluted suitably before subjecting to atomic absorption analysis.

Analysis of distribution of Fe_3O_4 in magnetic microspheres

Details of examining the internal matrix of microspheres using TEM has been discussed earlier (Gupta et al., 1986b). The same procedure was used to investigate the distribution of Fe_3O_4 in Fe-BSA and Adr-Fe-BSA microspheres.

Release study with Adr-BSA and Adr-Fe-BSA microspheres

Dynamic dialysis technique was used to study the drug release characteristics of Adr-BSA and Adr-FE-BSA microspheres stabilized at different temperatures. A detailed description of this method, and the mathematical model used therein to account for the zero-order release of adriamycin from Adr-BSA microspheres in aqueous media, has been made earlier (Gupta et al., 1987b). About 50 mg of 4 times washed microspheres were suspended in 7 ml of Tris buffer (pH 4.0) (Janseen et al., 1985) and dialysed against 128 ml of buffer at 25 °C. One-hundred microlitre samples of the medium outside the dialysis bag were removed over a 72 h period and assayed for adriamycin concentration using HPLC. Dissolution studies were carried out in triplicate for each type of microspheres.

Data analysis

Analysis of variance (ANOVA) with factorial design (Box et al., 1978) was used to analyse the

difference in particle size, drug and magnetite content of different types of microspheres, stabilized at different temperatures.

Heat-stabilized Adr-BSA microspheres have been shown to exhibit zero-order release of adriamycin (Gupta et al., 1986b) and a mathematical model to determine the release rate has been suggested (Gupta et al., 1987b). In the present study, the release study data were analysed using the same mathematical model.

All statistical analyses were performed using SAS computer package (SAS, 1985).

Results and Discussion

The mean diameters of all of 3 types of microspheres prepared at different temperatures are listed in Table 1. These data indicate that particles with mean diameter less than 1 μm can be readily prepared in the presence of adriamycin and/or magnetite. However, wide coefficients of variation (50–80%) in size were observed for all types of microspheres regardless of their stabilization temperature. Table 1 also lists the mean diameter of different microspheres after 2 h of hydration in normal saline at 37 °C. It was found that particles tend to aggregate upon hydration with simultaneous formation of pores and cavities. No detectable change in particle diameter was observed when the microspheres were hydrated for more than 2 h (see Fig. 1). Hence, 2 h appears to be a good approximation of the equilibrium hydration time for these particles. In addition, it was found that irrespective of the stabilization temperature and presence of drug and/or magnetite, the diameter of microspheres after hydration for 2 h is significantly greater than those of the unhydrated particles.

In order to investigate the effect of stabilization temperature and the presence of adriamycin and/or magnetite on the size of microspheres, before and after equilibrium hydration, the data listed in Table 1 was divided into two groups: (i) diameter of particles before hydration; and (ii) diameter of particles after hydration. ANOVA for a 3×4 factorial design, without interaction, was then used to analyse each of the two groups. It was found that the presence of drug and/or magnetite have no effect on the size of unhydrated or hydrated microspheres. Interestingly, the stabilization temperature, which has no effect on the particle size of unhydrated microspheres, show significant influence on the size of the hydrated microspheres ($P = 0.0027$). After hydration, the size of microsphere stabilized at 105 or 120 °C, is significantly greater than the size of microspheres stabilized at 135 or 150 °C. However, no difference could be detected between the size of microspheres stabilized at 105 or 120 °C, or 135 and 150 °C.

Table 2 presents the drug entrapment data for Adr-Fe-BSA microspheres, prepared at different temperatures. Drug-associated microspheres are invariably washed before their in vivo administration (Gupta et al., 1986b and c; 1987a; Ovadia et al., 1982; Ranney, 1985; Widder et al., 1979a and b). Hence, the effect of washing on the entrapment of adriamycin was also included as a part of this study. Since less than 3% w/w of the total

TABLE 1

Mean diameter (μm ± S.D.) of microspheres before and after 2 h of hydration [a]

Stabilization temperature [b] (°C)	Adr-BSA microspheres		Fe-BSA microspheres		Adr-Fe-BSA microspheres	
	Before	After	Before	After	Before	After
105	0.69 ± 0.41	1.29 ± 0.46	0.70 ± 0.39	1.15 ± 0.41	0.68 ± 0.39	1.26 ± 0.41
120	0.73 ± 0.54	1.25 ± 0.51	0.69 ± 0.43	1.13 ± 0.37	0.75 ± 0.44	1.23 ± 0.45
135	0.69 ± 0.36	1.06 ± 0.43	0.72 ± 0.35	1.07 ± 0.39	0.70 ± 0.41	1.02 ± 0.37
150	0.71 ± 0.44	1.08 ± 0.57	0.74 ± 0.45	1.02 ± 0.40	0.74 ± 0.38	1.07 ± 0.40

[a] Means of 200 particles.

[b] Maintained at ±5 °C level.

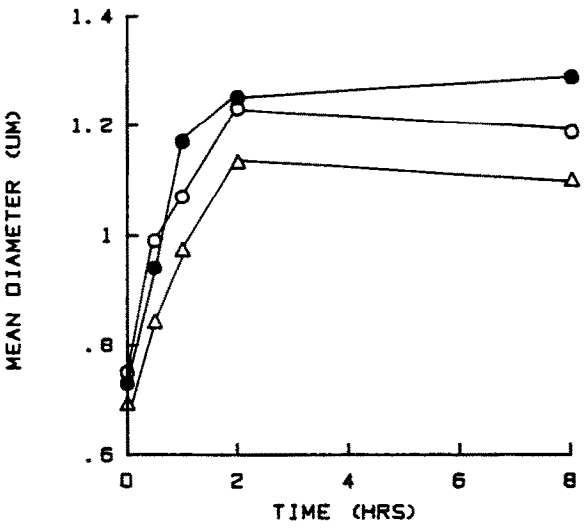

Fig. 1. Plot showing the change in size of different types of microspheres, heat-stabilized at 120 ± 5 °C, as a function of their hydration time in normal saline at 37 °C. Symbol representation: ●, Adr-BSA microspheres; (○), Adr-Fe-BSA microspheres; and △, Fe-BSA microspheres.

associated drug (i.e. surface adsorbed plus entrapped drug) was released during the fifth washing, a total of 4 washings represents an optimal washing condition for Adr-Fe-BSA microspheres. Similar washing level has been reported for Adr-BSA microspheres (Gupta et al., 1986b and c; 1987a).

As shown in Table 2, the stabilization temperature markedly affects the incorporation of adriamycin in magnetic particles. Less drug incorporation at high temperatures is mainly due to drug decomposition at temperatures above 120 °C (Gupta et al., 1987a, Widder et al., 1980). These results are at variance to those reported by other workers (Miyazaki et al., 1986a, b and c) where stabilization temperature has been shown to have no effect on the incorporation of adriamycin in fibrinogen microspheres. One probable explanation to this anomaly is the use of a non-specific method for drug analysis by these workers. At low temperatures, such as 105 °C, the majority of associated drug exists as surface-adsorbed fraction and is readily removed during the washing steps (Gupta et al., 1987a). Hence use of high as well as low stabilization temperatures result in microspheres with a low degree of drug entrapment.

After 4 washings of Adr-Fe-BSA microspheres, the stabilization temperature of 120 °C provides maximum drug entrapment (9.1 ± 1.8 μg adriamycin-HCl/mg of magnetic carrier). This value is considerably less than that reported by Widder et al. (1978, 1979a, 1980, 1981) (between 23 and 56 μg/mg of magnetic carrier). This difference is probably due to low drug : protein ratios employed, altered techniques of carrier synthesis and drug analysis, and to the washing procedure.

Table 2 also lists the drug entrapment values of unwashed and 4 times washed Adr-BSA microspheres. Separate ANOVA for 2 × 4 factorial design were used to determine the effect of stabilization temperature and presence of Fe_3O_4 on the

TABLE 2

Effect of stabilization temperature and washing level on the entrapment of adriamycin in Adr-BSA and Adr-Fe-BSA microspheres

Stabilization Temperature [a] (°C)	μg adriamycin/mg microsphere (mean ± S.D.) [b]							
	Adr-Fe-BSA microspheres						Adr-BSA microspheres [c]	
	0 [d]	1	2	3	4	5	0	4
105	68.9 ± 8.7	26.1 ± 3.9	16.4 ± 1.9	9.2 ± 2.2 [e]	6.9 ± 1.1	5.2 ± 0.9	100.2 ± 9.4	10.8 ± 1.3
120	38.6 ± 6.2 [e]	20.9 ± 4.3	14.8 ± 3.2	11.0 ± 1.9	9.1 ± 1.8	8.3 ± 1.6	40.7 ± 3.1	14.0 ± 0.7
135	19.4 ± 6.7 [e]	13.3 ± 4.8	10.2 ± 3.9	8.8 ± 4.1	8.0 ± 2.6	7.4 ± 2.2	20.9 ± 1.1	12.9 ± 1.0
150	10.3 ± 4.3	7.9 ± 3.8	7.0 ± 3.1 [e]	6.2 ± 2.7	5.6 ± 3.0	5.3 ± 2.3	24.5 ± 2.3	15.4 ± 1.0 [e]

[a] Maintained at ± 5 °C level.
[b] Means of four batches of microspheres.
[c] Adapted from Gupta et al. (1987a).
[d] Washing level.
[e] Means of 3 batches of microspheres.

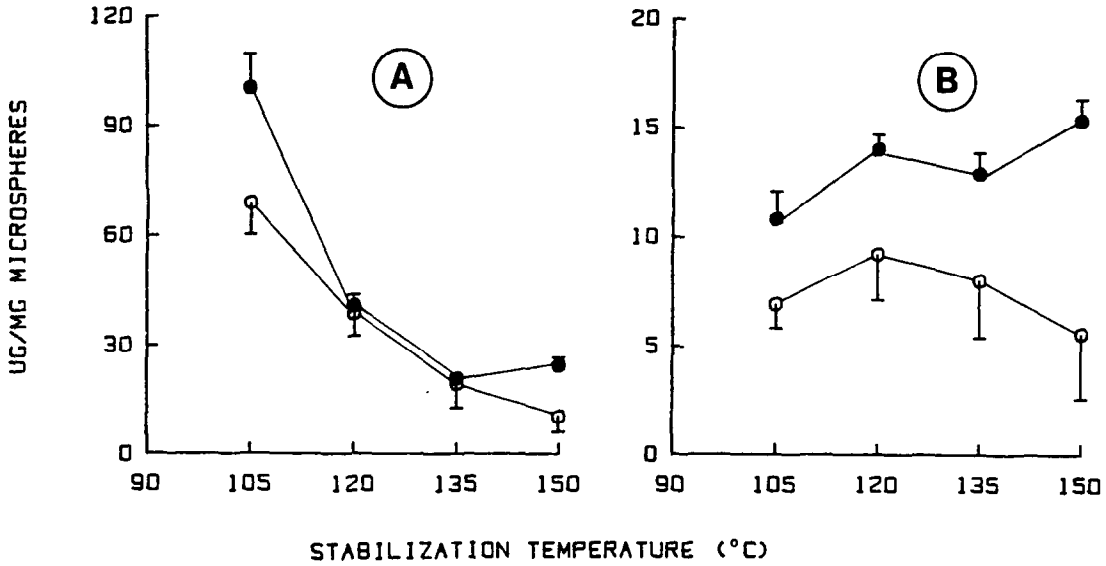

Fig. 2. Plots of mean adriamycin content associated per mg of (A) unwashed and (B) four times washed microspheres. Symbol representation: ●, Adr-BSA microspheres; and ○, Adr-Fe-BSA microspheres.

drug content of unwashed and 4 times washed albumin microspheres. The interaction between the stabilization temperature and microsphere type (i.e. Adr-Fe-BSA and Adr-BSA microspheres) was found to be significant for unwashed ($P = 0.0002$) as well as the 4 times washed ($P = 0.0186$) particles. This leads to the conclusion that regardless of the washing condition, the drug content of the two types of microspheres does not change uniformly over the range of stabilization temperature studied. The unwashed Adr-BSA microspheres stabilized at 105 and 150 °C, contain significantly higher amounts of adriamycin than the Adr-Fe-BSA microspheres. At 120 and 135 °C, the difference in the associated drug content is minimal (see Fig. 2a). When 4 times washed microspheres are compared, the drug entrapment in Adr-BSA microspheres, at all stabilization temperatures, is significantly greater than that in the Adr-Fe-BSA microspheres (see Fig. 2b). These results indicate that presence of magnetite probably reduces the space available within the particulate carriers for effective entrapment of a chemotherapeutic agent.

The magnetite content associated with the 4 times washed Fe-BSA and Adr-Fe-BSA microspheres, is listed in Table 3. Washing procedure was found to have no effect on the entrapment of magnetite in both Fe-BSA and Adr-Fe-BSA microspheres. ANOVA for a 2×4 factorial design was used to illustrate the effect of stabilization temperature and presence of adriamycin on the entrapment of Fe_3O_4 in albumin microspheres. It was found that stabilization temperature of microspheres has no effect on the inclusion of magnetite. However, the presence of adriamycin significantly increases its incorporation ($P = 0.0014$). In general, 18–23% w/w Fe_3O_4 can be readily

TABLE 3

Effect of presence of adriamycin and stabilization temperature on the incorporation of Fe_3O_4 in Fe-BSA and Adr-Fe-BSA microspheres [a]

Stabilization temperature [b] (°C)	% w/w Fe_3O_4 [c]	
	Fe-BSA	Adr-Fe-BSA
105	17.3 ± 3.8	20.1 ± 4.2
120	18.5 ± 2.9	21.2 ± 2.4
135	17.9 ± 2.6	22.6 ± 1.6
150	18.1 ± 3.2	23.7 ± 3.3

[a] Using 4 times washed microspheres.
[b] Maintained at ± 5 °C level.
[c] Means of 4 batches of microspheres.

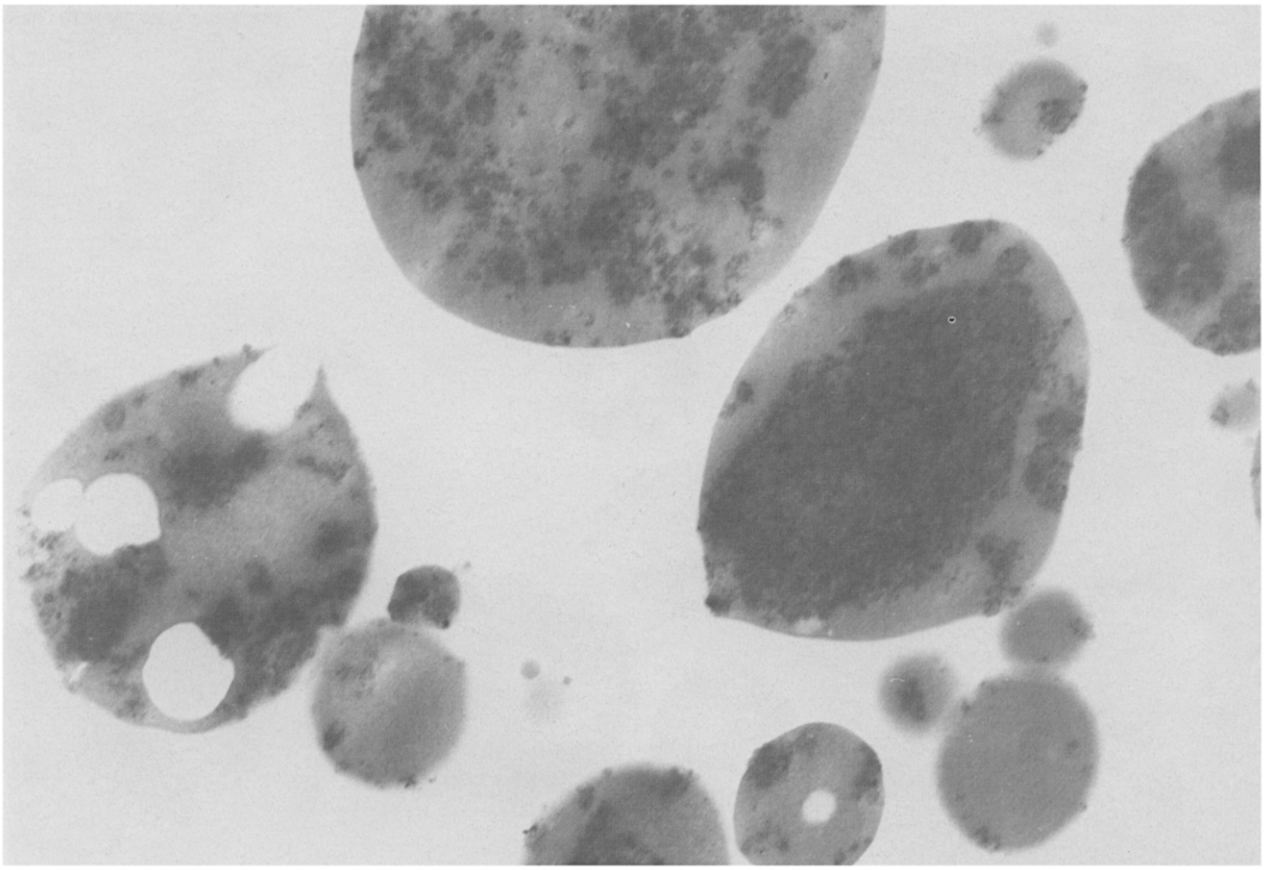

Fig. 3. A transmission electron microscope photograph of Fe-BSA microspheres prepared by heat-stabilization at 120 ± 5 °C. (Magnification: 15,000 ×).

incorporated in these particles, and this figure closely resembles the values reported by Widder et al. (1979a and b). Figs. 3 and 4 display the TEM photomicrographs of Fe-BSA and Adr-Fe-BSA microspheres prepared at 120 ± 5 °C. Figs. 3 and 4 demonstrate that in Fe-BSA microspheres, the majority of Fe_3O_4 is localized in the centre of the carrier matrix. However, in Adr-Fe-BSA microspheres, the magnetite is distributed throughout the peripheral region. No explanation can be offered for these observed differences in the magnetite entrapment and its distribution in albumin microspheres, in the presence and absence of adriamycin. Based on the suggestion that peripheral distribution of incorporated Fe_3O_4 promotes magnetic response of the carrier (Senyei et al., 1978; Widder et al., 1979b), the Adr-Fe-BSA microspheres formulated in the present study appear suitable for drug targeting using an external magnetic field.

Biphasic zero-order release profiles were obtained for the 4 times washed Adr-BSA and Adr-Fe-BSA microspheres using the dynamic dialysis technique, at all stabilization temperatures. Table 4 lists the initial and the terminal release rates of adriamycin from these particles. Regression equations were fitted through the terminal release rate constants of microspheres as a function of their stabilization temperatures. The relationships obtained for Adr-BSA and Adr-Fe-BSA micro-

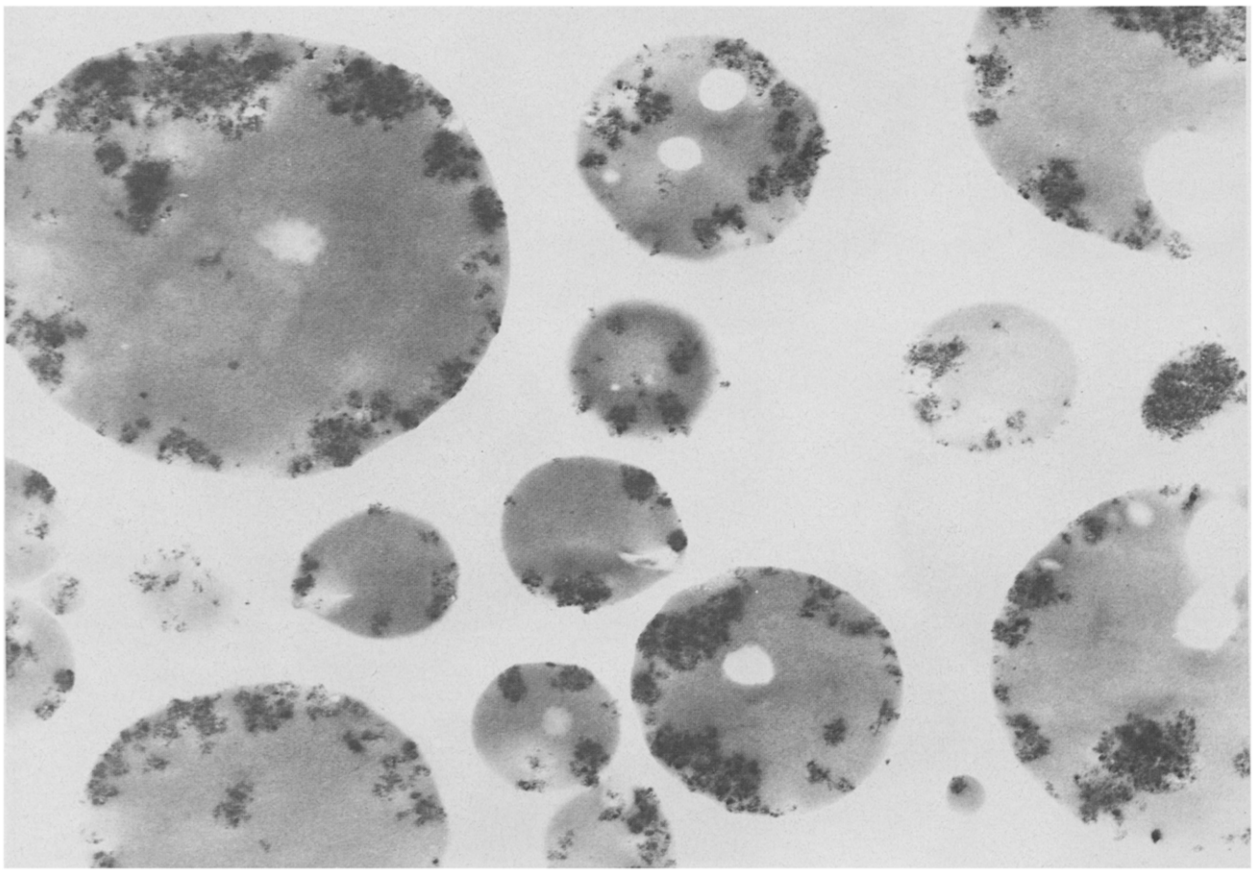

Fig. 4. A transmission electron microscope photograph of Adr-Fe-BSA microspheres prepared by heat-stabilization at 120 ± 5 °C. (Magnification: 15,000 ×).

TABLE 4

Effect of carrier stabilization temperature and presence of magnetite (Fe_3O_4) on the release rate constant of adriamycin from Adr-BSA and Adr-Fe-BSA microspheres [a]

Stabilization temperature [b] (°C)	Release rate constant (mean ± S.D.) [c]			
	Adr-BSA microspheres		Adr-Fe-BSA microspheres	
	Initial	Terminal	Initial	Terminal
105	$63.6 \times 10^{-2} \pm 29.3 \times 10^{-3}$	$8.2 \times 10^{-2} \pm 7.5 \times 10^{-3}$	$29.6 \times 10^{-2} \pm 59.4 \times 10^{-3}$	$8.1 \times 10^{-2} \pm 9.0 \times 10^{-3}$
120	$59.0 \times 10^{-2} \pm 36.2 \times 10^{-3}$	$5.0 \times 10^{-2} \pm 5.0 \times 10^{-3}$	$199.5 \times 10^{-2} \pm 41.1 \times 10^{-2}$	$3.9 \times 10^{-2} \pm 3.0 \times 10^{-3}$
135	$36.5 \times 10^{-2} \pm 4.0 \times 10^{-3}$	$2.4 \times 10^{-2} \pm 4.0 \times 10^{-4}$	$178.7 \times 10^{-2} \pm 67.8 \times 10^{-2}$	$1.7 \times 10^{-2} \pm 1.0 \times 10^{-3}$
150	$23.3 \times 10^{-2} \pm 7.9 \times 10^{-3}$	$1.3 \times 10^{-2} \pm 9.0 \times 10^{-4}$	$39.1 \times 10^{-2} \pm 11.8 \times 10^{-2}$	$1.0 \times 10^{-2} \pm 7.0 \times 10^{-4}$

[a] Based on the biphasic zero-order release model (Gupta et al., 1987b).
[b] Maintained at ±5 °C level.
[c] Means of 3 release studies. Units: μg/mg microspheres.

spheres, respectively, were:

ln(release rate constant)

$$= 1.90 - 0.042 \times (\text{stabilization temperature});$$

and ln(release rate constant)

$$= 2.41 - 0.047 \times (\text{stabilization temperature})$$

These results are similar to those obtained with Adr-BSA microspheres using the conventional dissolution method (Gupta et al., 1986c). Comparison of the two equations indicated that their intercepts are statistically non-significant. However, their slopes are significantly different ($P = 0.0234$). This therefore leads to the conclusion that stabilization temperature of microspheres, as well as the presence of Fe_3O_4 significantly affects the release rate of the entrapped adriamycin. At a constant stabilization temperature between 105 and 150 °C, the release rate of adriamycin from Adr-BSA microspheres is significantly greater than that observed from the Adr-Fe-BSA microspheres. These results indicate that the Adr-Fe-BSA microspheres possess greater tortuosity and/or lower porosity than that of the Adr-BSA microspheres.

Conclusions

The results of this investigation suggest that irrespective of the presence of drug and/or magnetite, albumin microspheres with a mean diameter less than 1 μm can be synthesised at a temperature range of 105–150 °C. Use of submicron size particles will reduce the clearance of incorporated drug (DeLuca et al., 1980; Kanke et al., 1980; Scott et al., 1967). This, in turn, would increase the duration of drug action. In vitro hydration studies carried out with these microspheres indicate significant increase in particle diameter in 2 h. However, the increase in size was not to the extent of 5–10-fold as postulated by Sugibayashi et al. (1979a). This difference in the characteristics of microsphere hydration may in part be due to wide variation in their size distribution and differences in their synthesis.

The amount of drug entrapped in this carrier, as analysed after 4 washings, is significantly influenced by the presence of magnetite as well as the stabilization temperature. The 4 times washed microspheres containing 18–23% w/w Fe_3O_4, show a maximum entrapment of adriamycin (about 1% w/w) at 120 °C.

Although peripheral distribution of incorporation Fe_3O_4 is attained in Adr-Fe-BSA microspheres, their magnetic response, in comparison to Fe-BSA microspheres, still remains to be determined. Dialysis results indicate that the rate of release of adriamycin from Adr-BSA and Adr-Fe-BSA microspheres may be controlled by altering their stabilization temperature. Based on the results of this study it is evident that albumin microspheres are promising candidates for drug delivery and targeting.

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References

Bartlett, J.M., Richardson, R.C., Elliot, G.S. and Blevins, W.E., Localization of magnetic microspheres in 36 canine osteogenic sarcomas. In S.S. Davis, L. Illum, J.G. McVie and E. Tomlinson (Eds.), *Microspheres and Drug Therapy, Pharmaceutical Immunological and Medical Aspects*, Elsevier, Amsterdam, 1984, pp. 413–426.

Blanchard, R.J.W., Grotenhuis, I., LaFave, J.W. and Perry, J.F., Jr., Blood supply to hepatic V2 carcinoma implants as measured by radioactive microspheres. *Proc. Soc. Exp. Biol. Med.*, 118 (1965) 465–468.

Box, G.E., Hunter, W.E. and Hunter, J.S., *Statistics for Experimenters*, Wiley, New York, 1978.

Burger, J.J., Tomlinson, E., Mulder, E.M.A. and McVie, J.G., Albumin microspheres for intra-arterial tumour targeting I. Pharmaceutical aspects. *Int. J. Pharm.*, 23 (1985) 333–344.

Cavalier, M., Benoit, J.P. and Thies, C., The formation and characterisation of hydrocortisone loaded poly[(±)-lactide] microspheres. *J. Pharm. Pharmacol.*, 38 (1986) 249–253.

Del Maestro, R.F., Schlosser, R. and Agerup, B., Multiple cerebral and spinal cord blood flow measurements using radioactive microsphere method. In D.H. Lewis (Ed.), *Biobliotheca Anatomica, No. 18, Current Advances in Basic and Clinical Microcirculatory Research*, Karger, Basel, 1979, pp. 201–263.

DeLuca, P., Kanke, M., Bivins, B., Slack, J., Simmons, G., Yokel, R., Shroeder, H. and Sniecinski, I., The fate and effects of Ce labelled microspheres following IV or IA administration in beagle dogs, *2e Cong. Int. de Technol. Pharmaceutique. Paris, Tome III*, 1980, pp. 27–36.

Driscoll, C.F., Morris, R.M., Senyei, A.E., Widder, K.J. and Heller, G.S., Magnetic targeting of microspheres in blood flow. *Microvasc. Res.*, 27 (1984) 353–369.

Duc-Mauger, A., Benoit, J.P. and Puisieux, F., Preparation and characterization of cross-linked human serum albumin microspheres containing 5-fluorouracil. *Pharm. Acta Helv.*, 61 (1986) 119–124.

Elliot, G.S., Blevins, W.E., Richardson, R.C., Janas, W., Bartlett, J.M., Hale, J.R. and Silver, R.L., Methods of vascular access for delivery of microspheres to distal extremity tumours in dogs – a large animal model. In S.S. Davis, L. Illum, J.G. McVie and E. Tomlinson (Eds.), *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects*. Elsevier, Amsterdam, 1984, p. 437.

Gallo, J.M., Hung, C.T. and Perrier, D.G. Analysis of albumin microsphere preparation. *Int. J. Pharm.*, 22 (1984) 63–74.

Gallo, J.M., Hung, C.T. and Perrier, D.G., Reversed-phase ion-pair HPLC of adriamycin and adriamycinol in rat serum and tissues. *J. Pharm. Biomed. Anal.*, 4 (1986) 483–490.

Gupta, P.K., Gallo, J.M., Hung, C.T. and Perrier, D.G., Influence of stabilization temperature on the entrapment of adriamycin in albumin microspheres. *Drug Dev. Ind. Pharm.*, 13 (1987a) 1471–1482.

Gupta, P.K., Hung, C.T. and Perrier, D.G., Albumin microspheres I. release characteristics of adriamycin. *Int. J. Pharm.*, 33 (1986b) 137–146.

Gupta, P.K., Hung, C.T. and Perrier, D.G., Albumin microspheres II. Effect of stabilization temperature on the release of adriamycin. *Int. J. Pharm.*, 33 (1986c) 147–153.

Gupta, P.K., Hung, C.T. and Perrier, D.G., Quantitation of release of doxorubicin from colloidal dosage forms using dynamic dialysis. *J. Pharm. Sci.*, 76 (1987b) 141–145.

Gupta, P.K., Morris, C. and Hung, C.T., Evaluation of magnetic albumin microspheres in site-specific delivery of adriamycin in rats. *Proc. Univ. Otago Med. Sch.*, 64 (1986a) 63–64.

Hampton, J.C., An electron microscope study of the hepatic uptake and excretion of submicroscopic particles injected into the blood stream and into the bile duct. *Acta Anat.*, 32 (1958) 262–291.

Illum, L. and Davis, S.S. The targeting of drugs parenterally by use of microspheres, *J. Parent. Sci. Technol.*, 36 (1982) 242–248.

Ishii, F., Takamura, A. and Noro, S., Magnetic microcapsules for in vitro testing as carrier for intravascular administration of anticancer drugs: preparation and physiochemical properties. *Chem. Pharm. Bull.*, 32 (1984) 678–684.

Janseen, M.J.H., Crommelin, D.J.A., Storm, G. and Hulshoff, A., Doxorubicin decomposition on storage. Effect of pH, type of buffer and liposome encapsulation. *Int. J. Pharm.*, 23 (1985) 1–11.

Kanke, M., Simmons, G.H., Weiss, D.L., Bivins, B.A. and DeLuca, P.P., Clearance of ^{141}Ce-labelled microspheres in specific organs following intravenous and intra-arterial administration in beagle dogs. *J. Pharm. Sci.*, 69 (1980) 755–762.

Kato, T., Encapsulated drugs in targeted cancer therapy. In S.D. Bruck (Ed.), *Controlled Drug Delivery, Vol. II*, CRC Press, Boca Raton, FL, 1982, p. 189.

Kojima, T., Nakano, M., Juni, K. Inoue, S. and Yoshida, Y., Preparation and evaluation in vitro of polycarbonate microcapsules containing local anesthetics. *Chem. Pharm. Bull.*, 32 (1984) 2795–2802.

Kramer, P.K. and Burnstein, T., Phagocytosis of microsphere containing anticancer agent by tumor cells in vitro. *Life Sci.*, 19 (1976) 515–520.

Mattsson, J., Naredi, P., Hafstrom, L. and Peterson, H.-I., Intratumoral distribution of microspheres. *Anticancer Res.*, 6 (1986) 563–566.

Miyazaki, S., Hashiguchi, N., Hou, W.-M., Yokouchi, C. and Takada, M., Antitumour effect of fibrinogen microparticles containing adriamycin on Ehrlich ascites carcinoma in mice. *Chem. Pharm. Bull.*, 34 (1986a) 2632–2636.

Miyazaki, S., Hashiguchi, N., Hou, W.-M., Yokouchi, C. and Takada, M., Preparation and evaluation in vitro and in vivo of fibrinogen microspheres containing adriamycin. *Chem. Pharm. Bull.*, 34 (1986b) 3384–3393.

Miyazaki, S., Hashiguchi, N., Yokouchi, C., Takada, M. and Hou, W.-M., Antitumour effect of fibrinogen microspheres containing doxorubicin on Ehrlich ascites carcinoma. *J. Pharm. Pharmacol.*, 38 (1986c) 618–620.

Morimoto, Y., Akimoto, M., Sugibayashi, K., Nadai, T. and Kato, Y., Drug carrier property of albumin microspheres in chemotherapy. IV. Antitumour effect of single shot or multiple shot administration of microspheres entrapped 5-FU on Ehrlich ascites or solid tumour in mice. *Chem. Pharm. Bull.*, 28 (1980) 3087–3092.

Morimoto, Y., Okumura, M., Sugibayashi, K. and Kato, Y., Biomedical applications of magnetic fluids. II. Preparation and magnetic guidance of magnetic albumin microspheres for site-specific delivery in vivo. *J. Pharmacol. Dyn.*, 4 (1981) 624–631.

Morris, R.M., Poore, G.A., Howard, D.P. and Sefranka, J.A., Selective targeting of magnetic albumin microspheres containing vindesine sulphate: total remission in Yoshida sarcoma-bearing rats. In S.S. Davis, L. Illum, J.G. McVie and E. Tomlinson (Eds.), *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects*, Elsevier, Amsterdam, 1984, pp. 439–440.

Oppenheim, R.C. Solid colloidal drug delivery systems: nanoparticles. *Int. J. Pharm.*, 8 (1981) 217–234.

Ovadia, H., Carbone, A.M. and Paterson, P.Y., Albumin mag-

netic microspheres: a novel carrier for myelin basic protein. *J. Immunol. Meth.*, 53 (1982) 109–122.

Ranney, D.F., Targeted modulation of acute inflammation. *Science*, 227 (1985) 182–184.

Ranney, D.F., Drug targeting to the lungs. *Biochem. Pharmacol.*, 35 (1986) 1063–1069.

Rhodes, B.A., Zolle, I., Buchanan, J.W. and Wagner, H.N., Jr., Radioactive albumin microspheres for studies of the pulmonary circulation. *Radiology*, 92 (1969) 1453–1460.

SAS User's Guide: *Statistics*, 5 edn., North Carolina, 1985.

Schroeder, H.G., Simmons, G.H. and DeLuca, P.P., Distribution of radiolabelled subvisible microspheres after intravenous administration to beagle dogs. *J. Pharm. Sci.*, 67 (1978) 504–507.

Scott, G.B.D., Williams, M.S. and Marriott, P.M. The phagocytosis of colloidal particles of different sizes. *Br. J. Exp. Pathol.*, 48 (1967) 411–416.

Senyei, A., Widder, K. and Czerlinski, G., Magnetic guidance of drug carrying microspheres. *J. Appl. Phys.*, 49 (1978) 3578–3583.

Sjoholm, I. and Edman, P., The use of biocompatible microparticulates as carriers of enzymes and drugs in vivo. In S.S. Davis, L. Illum, J.G. McVie and E. Tomlinson (Eds.), *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects*, Elsevier, Amsterdam, 1984, pp. 245–262.

Sugibayashi, K., Akimoto, M., Morimoto, Y., Nadai, T., and Kato, Y. Drug-carrier property of albumin microspheres in chemotherapy. III. Effect of microsphere-entrapped 5-fluorouracil on ehrlich ascites carcinoma in mice. *J. Pharmaco.-Dyn.*, 2 (1979b) 350–355.

Sugibayashi, K., Morimoto, Y., Nadai, T., Kato, Y., Hasegawaw, A. and Arita, T., Drug carrier property of albumin microsphere entrapped 5-fluorouracil. *Chem. Pharm. Bull.*, 27 (1979a) 204–209.

Sugibayashi, K., Okumura, M. and Morimoto, Y., Biochemical applications of magnetic fluids III. Antitumor effect of magnetic albumin microsphere-entrapped adriamycin on lung metastasis of AH 7974 in rats. *Biomaterials*, 3 (1982) 181–186.

Tomlinson, E. Microsphere delivery systems for drug targeting and controlled release. *Int. J. Pharm. Technol Prod. Mfr.*, 4 (1983) 49–57.

Tomlinson, E., Burger, J.J., Schoonderwoerd, E.M.A., Kuik, J., Schlotz, F.C., McVie, J.G. and Mills, S., Preparation and characterization of albumin microspheres for intra-arterial tumour targeting of cytotoxic compounds. *J. Pharm. Pharmacol.*, (1982) 88P.

Tomlinson, E., Burger, J.J., Schoonderwoerd, E.M.A. and McVie, J.G., Human serum albumin microspheres for intra-arterial drug targeting of cytostatic compounds. Pharmaceutical aspects and release characteristics. In S.S. Davis, L. Illum, J.G. McVie and E. Tomlinson (Eds.), *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects*, Elsevier, Amsterdam, 1984, pp. 75–90.

Wagner, H.N., Jr., Rhodes, B.A., Sasaki, Y. and Ryan, J.P. Studies of the circulation with radioactive microspheres. Invest. Radiol., 4 (1969) 374–386.

Wagner, H.N., Jr., Sabiston, D.C., Jr., McAfee, J.G., Tow, D.E. and Stern, H.S., Diagnosis of massive pulmonary embolism in man by radioisotope scanning. *N. Engl. J. Med.*, 271 (1964) 377–384.

Wakiyama, N., Juni, K. and Nakano, M., Preparation and evaluation in vitro of polylactic acid microspheres containing local anesthetics. *Chem. Pharm. Bull.*, 29 (1981) 3363–3368.

Widder, K., Flouret, G. and Senyei, A., Magnetic microspheres: synthesis of a novel parenteral drug carrier. *J. Pharm. Sci.*, 68 (1979a) 79–82.

Widder, K.J., Morris, R.M., Poore, G., Howard, D.P., Jr. and Senyei, A.E., Tumor remission in Yoshida sarcoma-bearing rats by selective targeting of magnetic albumin microspheres containing doxorubicin. *Proc. Natl. Acad. Sci. U.S.A.*, 78 (1981) 579–581.

Widder, K.J., Senyei, A.E. and Ranney, D.F., Magnetically responsive microspheres and other carriers for the biophysical targeting of antitumor agents. In S. Garattini, A. Goldin, F. Howking, I.J. Kopin and R.J. Schnitzer (Eds.), *Advances in Pharmacology and Chemotherapy*, Academic, New York, 16 (1979b) p. 213.

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

Widder, K.J., Senyei, A.E. and Scarpelli, D.G., Magnetic microspheres: a model system for site specific drug delivery in vivo. *Proc. Soc. Exp. Biol. Med.*, 58 (1978) 141–146.

Willmott, N., Cummings, J., Stuart, J.F.B. and Florence, A.T., Adriamycin-loaded albumin microspheres: preparation, in vivo distribution and release in the rat. *Biopharm. Drug Disp.*, 6 (1985) 91–104.

Yapel, A.F., Jr., Albumin medicament carrier system. *U.S. Patent*, (1979) 4,147,767.

Zimmermann, U., Cellular drug-carrier systems and their possible targeting. In E.P. Goldberg (Ed.), *Targeted Drugs*. Wiley, New York, 1983, p. 153.

Zolle, I., Hosain, F., Rhodes, B.A. and Wagner, H.N., Jr., Human serum albumin microspheres for studies of the reticuloendothelial system. *J. Nucl. Med.*, 11 (1970a) 379.

Zolle, I., Rhodes, B.A. and Wagner, H.N., Jr., Preparation of metabolized radioactive human serum albumin microspheres for studies of the circulation. *Int. J. Appl. Radiat. Isotopes*, 21 (1970b) 155–167.