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Albumin microspheres. I. Release characteristics of adriamycin

Pramod K. Gupta, C.T. Hung and D.G. Perrier *

Department of Pharmacy, University of Otago, Dunedin (New Zealand)

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Summary

Several models have been investigated for their appropriateness in describing the release of adriamycin from heat-stabilized bovine serum albumin microspheres. Drug release from the microspheres can be adequately described by bi-exponential first-order, bi-phasic zero-order and Higuchi's square-root of time equations. Theoretical possibilities for accepting bi-phasic zero-order release characteristics are discussed.

Introduction

The application of magnetic and non-magnetic albumin microspheres (0.2–100 μm) to the site-specific delivery of antineoplastic drugs has been well documented (Kramer, 1974; Sugibayashi et al., 1979; Widder et al., 1979a). Passive drug targeting using colloidal carriers has been reported by Blanchard et al. (1965), while active drug targeting using magnetic albumin microspheres has been described by Widder et al. (1979a and b). The future prospects of this colloidal drug delivery system have been reviewed by several investigators (Widder et al., 1979b; Illum and Davis, 1982; Tomlinson, 1983; Gardner, 1985). However, little is known about the release mechanism of drug from this delivery system. Therefore, this investigation was undertaken to evaluate the release characteristics of adriamycin from heat-stabilized bovine serum albumin microspheres.

Theoretical considerations

It has been demonstrated by Higuchi (1961, 1963) that the release of dispersed solid drug under sink conditions from one surface of an inert porous matrix can be described by the following relationships:

$$Q = \sqrt{\frac{D_s \cdot \epsilon \cdot S}{\tau}(2A - \epsilon \cdot C_s)C_s \cdot t}$$

$$= \sqrt{D_{eff} \cdot S(2A - \epsilon \cdot C_s)C_s \cdot t} \quad (1)$$

or

$$(Q_0 - Q) = Q_0 - \sqrt{\frac{D_s \cdot \epsilon \cdot S}{\tau}(2A - \epsilon \cdot C_s)C_s \cdot t}$$

$$= Q_0 - \sqrt{D_{eff} \cdot S(2A - \epsilon \cdot C_s)C_s \cdot t} \quad (2)$$

* *Present address:* Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 1A1.
Correspondence: C.T. Hung, Department of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand.

where D_s is the diffusion coefficient of the drug in the permeating fluid, ϵ is the porosity of the matrix, τ is the tortuosity of the matrix, S is the exposed surface area, A is the total amount of drug present in the matrix per unit volume, C_s is the solubility of the drug in the dissolution medium, Q is the amount of drug released, Q_0 is the original amount of drug present in the microspheres, D_{eff} is drug diffusivity in the matrix, and t is the time during which release of drug occurs. Although drug release from granular spherical pellets by diffusion is very complex, it has been shown by Higuchi (1963) that such a process can be approximated by Eqn. 1, provided more than 50% of the entrapped drug is within the carrier.

Release of triamcinolone diacetate from human serum albumin microspheres has been reported by El-Samaligy and Rohdewald (1982) to follow first-order behaviour, i.e.

$$\ln(Q_0 - Q) = \ln Q_0 - K \cdot t \qquad (3)$$

where Q_0, Q and t are as defined above, and K is the first-order release rate constant.

More recently, a bi-exponential first-order model has been employed to describe the drug release characteristic from albumin microspheres (Tomlinson et al., 1984). The relationship used was

$$(Q_0 - Q) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \qquad (4)$$

where Q_0, Q and t are again as described above, A and B are the zero time intercepts for the initial and terminal release phases, and α and β are the apparent first-order initial and terminal release rate constants, respectively.

The mechanism of drug release from albumin microspheres is dependent on the location of the drug in the carrier as well as on the properties of the microsphere matrix. Drugs can be associated with albumin microspheres by either adsorption onto the particle surface or inclusion in the microsphere matrix (Widder et al., 1979b; Tomlinson et al., 1984). The possible sites of drug association with the microspheres are depicted in Fig. 1. It is obvious that the drug release rate from one site will differ widely from the others. For example, the release of drug from site A can be achieved by drug dissolution or by drug desorption from the particle surface. Depending on drug solubility in the dissolution medium, the physical state of drug included in the carrier, and the affinity of drug for the carrier, the drug release profile from the site A of the microsphere can follow either a zero-order or a first-order model. Provided the albumin matrix of the microsphere is stable during in vitro dissolution, the release of drug from sites B and C will be diffusion controlled and can be described by Eqn 1. Drug entrapped within the microsphere, i.e. at site D may also be available during in vitro dissolution studies by diffusion through the albumin matrix. If a homogeneous matrix is assumed, Eqns. 1 or 2 can be used to describe drug release from site D of the carrier. In deriving Eqns. 1 and 2, it has been assumed that the properties of the carrier matrix remain constant throughout the release. Such an assumption may not be valid in the case of albumin microspheres. It has been reported that albumin microspheres swell in aqueous media (Zolle et al., 1970), and consequently, the thickness of the diffusion layer and the exposed surface area increase.

According to Fick's first law:

$$d(Q_0 - Q)/dt = -D_{eff} \cdot S(C_m - C)/h \qquad (5)$$

where Q_0, Q, D_{eff} and S are as described previously, C is the concentration of drug in the dis-

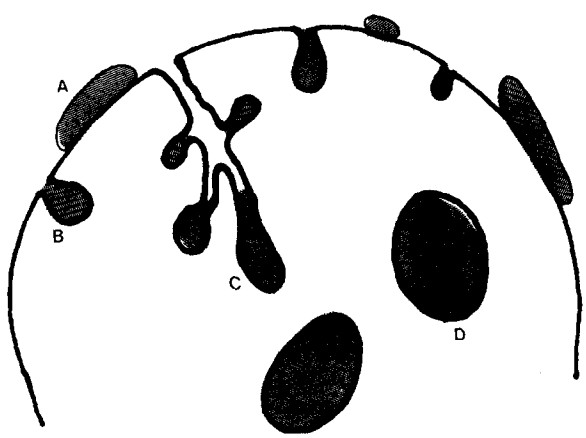


Fig. 1. A hypothetical picture of an albumin microsphere showing possible sites of location of drug.

solution medium at any time t, C_m is the drug concentration inside the microspheres and h is the diffusion layer thickness. Here, an increase in diffusion layer thickness will reduce the release of drug from the carrier. On the other hand, as the drug dissolves, the matrix structure is weakened and causes particle erosion. This effect will tend to increase the porosity and the surface area of the particles. In addition, the tortuosity of the microsphere matrix will be decreased. Ultimately, this will result in an increase in D_{eff} and S, and hence, the quantity of the drug released from the microsphere. However, if the diffusion layer thickness were to increase to the same degree as that of drug diffusivity and the particle surface area, and C_m was much greater than C, release would appear as a zero-order process, i.e.

$$d(Q_0 - Q)/dt = -K_0 \quad (6)$$

where K_0 is a constant equal to $D_{eff} \cdot S \cdot C_m/h$, which occurs only when C_m is constant.

Therefore, the release of drug from albumin microspheres appears complex. The purpose of this investigation was to identify the model which best describes the relationship of drug release from the microsphere as a function of in vitro dissolution time. The model identified can then be employed in the evaluation of the drug release characteristics from albumin microspheres prepared under different conditions. Results obtained should also provide some insight on the mechanism of drug entrapment in this colloidal drug delivery device.

Experimental

Apparatus and materials

The apparatus used for the formulation and size analysis of albumin microspheres has been described previously (Gallo et al., 1984). The chromatographic system for quantitation of adriamycin consisted of a Waters Associate 6000A pump, a Rheodyne 7125 injector with a 100 μl sample loop and a Schoeffel FS 970 fluorometer. The chromatographic column was stainless steel, 100 $\times$ 4.6 mm i.d., and packed with ODS-Hypersil (Shandon Southern Products). Transmission electron microscopy (TEM) for particle structure analysis was performed using a Siemens ELMISKOP 102. The dissolution apparatus for the microspheres was a 100 $\times$ 50 mm i.d. double-walled beaker, externally coated with black epiglass lacquer. A Heidolph stirrer fitted with a 20 mm long $\times$ 8 mm wide teflon-coated rectangular stirring blade was used in the dissolution studies.

Adriamycin hydrochloride was kindly donated by Farmitalia Carlo Erba (Milan, Italy). Bovine serum albumin (BSA) was obtained from Sigma Chemicals (No. 7030). Cottonseed oil was purchased from Real Foods, Dunedin, New Zealand. Hydrochloric acid, orthophosphoric acid, potassium dihydrogen phosphate, sodium chloride, sodium dihydrogen phosphate, sodium lauryl sulphate and tris-(hydroxy methyl) methylamine were supplied by The British Drug House. Absolute alcohol was obtained from May & Baker. Acetonitrile (HPLC grade) and anhydrous ether was obtained from J.T. Baker. Glassware was silanized with Aquasil from Pierce Chemicals. Water was double glass-distilled and MilliQ filtered.

Methods

Preparation of adriamycin-associated BSA microspheres

Aqueous solutions of BSA (400 mg/ml) and adriamycin hydrochloride (50 mg/ml) were prepared. Two-hundred μl of the drug solution was mixed with 250 μl of the BSA solution in a 50 ml double-walled beaker with the aid of an ultrasonic bath. Thirty ml of ice-cooled cottonseed oil was then added to the beaker and the mixture emulsified with an ultrasonic probe for 2 min at a 125 W setting. The resulting emulsion was added dropwise (100 $\pm$ 10 drops/min) to 100 ml of preheated (135 $\pm$ 5°C) and constantly stirred (1500 rpm) cottonseed oil. After the emulsion was added, heating and stirring were maintained for another 10 min. The suspension was then ice-cooled to 20°C. Sixty ml of anhydrous ether was added to the microsphere-in-oil suspension and the mixture centrifuged at 3000 $\times g$ for 15 min. The ether phase was discarded and the washing with ether

repeated thrice. The finally washed microspheres were transferred to a tared test-tube and the ether evaporated under a gentle stream of oxygen-free nitrogen. The microspheres obtained were then weighed and resuspended in ether (25 mg/ml). The suspension was stored in an air-tight container at −15°C until used.

Analysis of free adriamycin

A reversed-phase ion-pair HPLC method (Gallo et al., 1986) was employed in the analysis of adriamycin in all studies. The mobile phase consisted of 50% v/v acetonitrile in water with 80 mM sodium lauryl sulphate and 30 mM potassium dihydrogen phosphate with the final pH adjusted to 2 with orthophosphoric acid. A flow rate of 2 ml/min was employed. The excitation and emission wavelengths were set at 470 and 580 nm, respectively. The injection volumes were between 40 and 100 μl.

Analysis of drug content in microspheres

The total adriamycin associated with microspheres was analyzed for the surface drug and entrapped drug.

(a) Surface drug. To an aliquot of the ether suspension of adriamycin microspheres, representing 5 mg of microspheres, 2 drops of Tween 80 was added and the suspension briefly vortexed. Ether was then evaporated under oxygen-free nitrogen. To the microsphere residue, 1 ml of normal saline was added (Widder et al., 1979a) and the suspension was placed in an ultrasonic bath for 5 min. The suspension was then centrifuged at 5000 × *g* for 5 min. This supernatant, the first washing, was analyzed for drug content. The microspheres were washed in the same manner three more times. This resulted in the second, third and the fourth washings. The adriamycin content in all supernatants were analyzed by the described HPLC method.

(b) Entrapped drug. Microspheres obtained after four washings were digested overnight in 5 ml of 0.5 M acetic acid. The digested homogenate was centrifuged at 5000 × *g* for 5 min and the adriamycin content in the supernatant assayed by HPLC. The digested microsphere residue was redigested to ensure total recovery of the entrapped drug.

Drug content in the microspheres after 100 h of dissolution was carried out by recovering and washing the microspheres with absolute alcohol (to remove all residual water and prevent flocculation of the particles). These microspheres were then digested and the adriamycin content in the homogenate determined as described above.

Dissolution studies of adriamycin associated BSA microspheres

About 40 mg of the four times washed microspheres was suspended in 135 ml of Tris-buffer (pH 4.0) (Janssen et al., 1985). The suspension was then transferred into the double-walled beaker of the dissolution apparatus. To prevent drug loss during the dissolution study due to photodegradation, the dissolution assembly was protected from light. The medium was maintained at 25°C and stirred at 200 rpm with the teflon-coated stirrer. Two hundrerd μl of the dissolution medium samples were collected for 100 h at scheduled intervals via a microsyringe, and immediately analyzed for their adriamycin content by HPLC. Each measurement was made in triplicate and if an individual measurement differed from the other by more than 5%, further replicate measurements were performed.

Hydration studies with adriamycin-associated BSA microspheres

After one hour of dissolution, the microspheres from the dissolution apparatus were isolated. To the hydrated microspheres, 10 ml of absolute alcohol was added and the tube briefly sonicated before subjecting to centrifugation for 5 min at 5000 × *g*. Washing of microspheres with absolute alcohol was repeated once more in the same manner to ensure the complete removal of aqueous media. Size-analysis, surface-analysis and the matrix examination of these particles was then carried out using scanning electron microscopy (SEM) and TEM.

Analysis of the adriamycin-associated BSA microsphere matrix by TEM

A few drops of microsphere ether suspension

was placed in a beam capsule and the ether allowed to evaporate at room temperature. The capsule was filled with Agar 100 and then polymerized by heating overnight at 70°C. The solid agar matrix with microspheres was then cut into semi-thin sections using a glass knife. The sections were stained with 2 drops each of 1% w/v toluidine blue in 1% w/v borax and 1% w/v methylene blue in 1% w/v borax. The staining was fixed by heating and the excess dye washed out with water. The desired sections were prepared for ultra-thin sectioning and placed on a copper grid. These were again stained with saturated aqueous solution of uranyl acetate for 20 min at 60°C and excess chemical removed by washing with double-distilled water. This was followed by further staining with Reynolds lead acetate solution for 10 min and dried after washing off the excess stain with water. The grid with ultra-thin sections of microspheres was then observed under the TEM.

Analysis of data

For all sets of data, $Q_0 - Q$ was plotted as a function of time according to the three relationships being considered, i.e. $Q_0 - Q$ versus $\sqrt{t}$ (Eqn. 2), $\ln(Q_0 - Q)$ versus t (Eqns. 3 and 4), and $Q_0 - Q$ versus t (Eqn. 6). Since all curves were multiphasic, the method of residuals (Gibaldi and Perrier, 1982) was applied. The terminal amount–time data and the residual amount–time data were fitted using linear regression to generate release rate constants.

Results and Discussion

It has been reported that in drug-associated albumin microspheres, about 40% of the total drug is released rapidly and the remaining 60% released slowly (Widder et al., 1979a). The so-called rapidly released drug is probably due to the desorption of the surface drug, as represented by sites A in Fig. 1. This adsorbed drug has little value in drug targeting as it will be rapidly removed from the particle surface upon intra-arterial administration. Therefore, the use of unwashed microspheres in the dissolution studies will not provide the actual drug release characteristics from this carrier at the target site. Four times washed adriamycin-associated BSA microspheres were used in the present investigation since most of the surface adriamycin was removed by such a washing procedure. The amount of drug recoverd after each washing and after the digestion of the particles is presented in Table 1. As can be seen from these data approximately 38% of the adriamycin associated with the unwashed microspheres was readily removed surface drug. This agrees well with the observation of Widder et al. (1979a).

TABLE 1

ANALYSIS OF ADRIAMYCIN IN THE WASHING MEDIA AND THE DIGESTED HOMOGENATE OF THE ADRIAMYCIN-ASSOCIATED BSA MICROSPHERES

Variables studied	n	Adriamycin (μg/mg microsphere ± S.D.)
Surface drug		
first washing	5	4.71 ± 1.09
second washing	4	1.77 ± 0.38
third washing	5	0.92 ± 0.18
fourth washing	5	0.52 ± 0.10
		total 7.92
Entrapped drug		
first digestion	5	12.52 ± 1.10
second digestion	4	0.39 ± 0.06
		total 12.91

Protection of the dissolution assembly from light is essential to prevent the photodegradation of adriamycin in the dissolution media (Tavloni et al., 1980). A teflon-coated stirrer was employed to avoid interaction between adriamycin and iron (Bachur et al., 1984). Several aqueous solutions such as isotonic phosphate buffer (pH 7.4), normal saline, Tris-buffer with and without sodium chloride (pH 4.0) (Janssen et al. 1985) and normal saline with 0.1% w/v of Tween 80 (Widder et al., 1979a) were tested for their suitability as the dissolution medium for adriamycin. It was found that adriamycin was only stable in the Tris-buffer with 0.9% w/v sodium chloride (pH 4.0). No sign of adriamycin degradation was observed in this solution for up to 100 h of incubation at 25°C in the absence of light. Stability studies of adriamy-

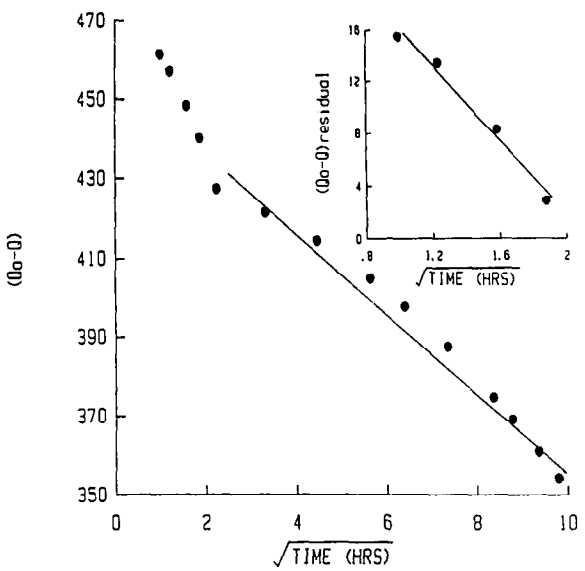


Fig. 2. Plot of $(Q_0 - Q)$ versus $\sqrt{t}$ for adriamycin released from 40 mg of BSA microspheres (see Eqn. 2). The inset is a plot of the residual values verus $\sqrt{t}$.

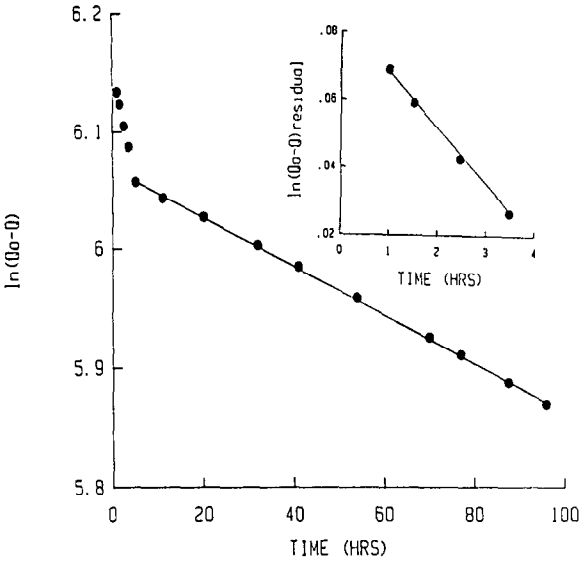


Fig. 3. Plot of $\ln(Q_0 - Q)$ versus t for adriamycin released from 40 mg of BSA microspheres (see Eqn. 3). The inset is a plot of the residual values versus t.

cin in the Tris-buffer at 37°C, however, indicated that substantial drug decomposition occured, with a decomposition half-life around 140 h. Therefore, the dissolution studies were carried out using washed microspheres in the Tris-buffer with sodium chloride (pH 4.0) maintained at 25°C.

The Higuchi square-root of time plot for the release of adriamycin from the BSA microspheres is shown in Fig. 2. The first-order and zero-order plots of the same set of data are shown in Figs. 3 and 4, respectively. Application of the method of residuals to the data indicated that it was biphasic regardless of the plotting method (i.e. Higuchi square-root of time, first-order or zero-order). Of the two phases, the initial fast release phase lasts for less than 6 h and appears to be a combination of the release of surface and possibly entrapped adriamycin as well as particle hydration. The terminal slow release phase is probably primarily due to the release of entrapped drug.

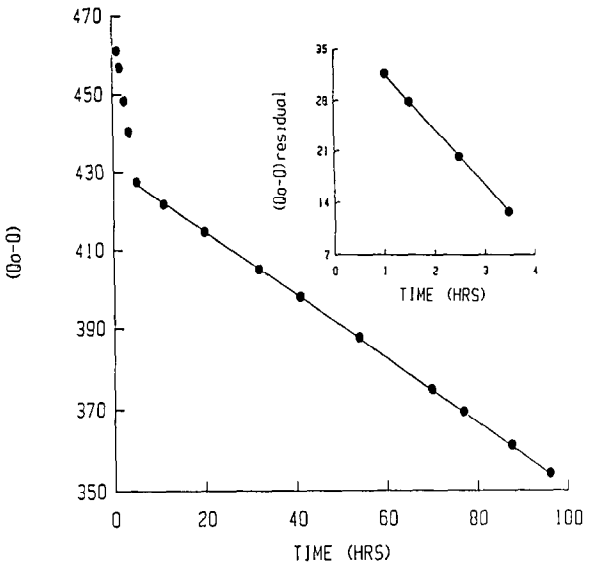
The initial and the terminal release rate constants for each plot are listed in Table 2. It can be

TABLE 2

RELEASE RATE CONSTANTS [a] OF ADRIAMYCIN per mg OF BSA MICROSPHERES USING DIFFERENT MODELS

Model employed	Initial release rate constant ± S.D. (r^2)	Terminal release rate constant ± S.D. (r^2)
Higuchi square-root of time ($\mu g \cdot h^{1/2}$)	$361 \times 10^{-3} \pm 201 \times 10^{-4}$ (0.993)	$263 \times 10^{-3} \pm 850 \times 10^{-4}$ (0.991)
First order (h^{-1})	$0.4 \times 10^{-3} \pm 1.5 \times 10^{-4}$ (0.999)	$0.1 \times 10^{-3} \pm 0.25 \times 10^{-4}$ (0.999)
Zero-order ($\mu g \cdot h^{-1}$)	$190 \times 10^{-3} \pm 0.34 \times 10^{-4}$ (0.999)	$19.7 \times 10^{-3} \pm 17 \times 10^{-4}$ (0.999)

[a] Means of three sets of data analyzed by the method of residuals. Refer to text for details.



observed that all three models provide an adequate fit to the experimentally derived data set. Therefore, there is no unique model to describe the release of drug from the BSA microspheres. One possible explanation is that the duration of sample collection relative to the half-time of drug release in the terminal phase is comparatively short.

In view of the relatively inert properties of the BSA microspheres, it is reasonable to assume that the release of entrapped drug from this carrier is diffusion controlled as described by Eqn. 5. First-

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Fig. 4. Plot of $(Q_0 - Q)$ versus t for adriamycin released from 40 mg of BSA microspheres (see Eqn. 6). The inset is a plot of the residual values versus t.

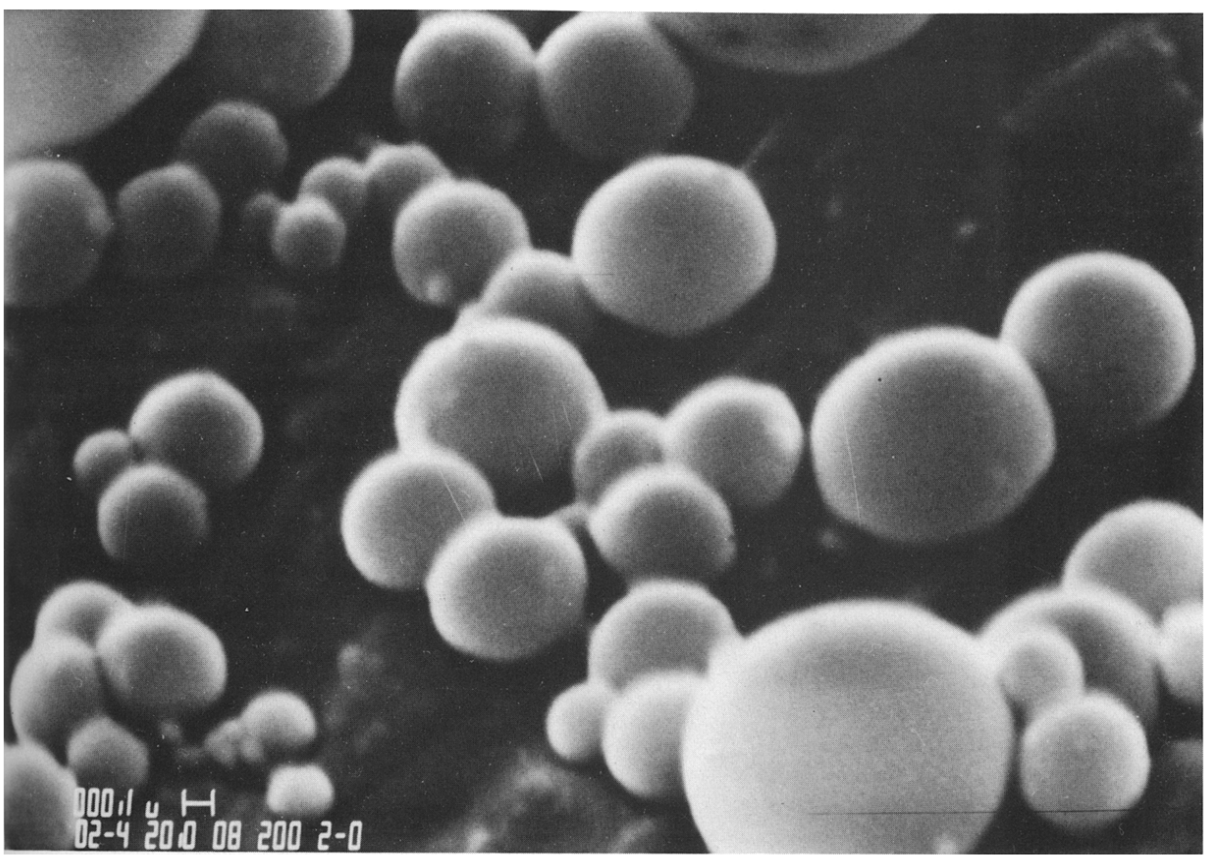

Fig. 5. Scanning electron microscope photograph of adriamycin-associated BSA microspheres prepared by heat-stabilization at 135 ± 5°C (magnification: 20,000 ×; note 0.1 μm line).

order release will occur only under non-sink conditions, i.e. $(C_m - C) \neq C_m$. In this study, it was found that more than 8 μg of the adriamycin (60% of the entrapped drug in the four times washed microspheres) still remained in 1 mg of carrier after 100 h of dissolution. In addition, the adriamycin concentration in the dissolution medium at any given time during the release study was much less than the solubility of the drug (6 g/100 ml) (personal communication, Farmitalia Carlo Erbra). Consequently, sink conditions applied throughout the release study, i.e. $(C_m - C) \sim C_m$ and C_m can be regarded as a constant. Therefore, it is unlikely that the release of adriamycin from BSA microspheres follows first-order pattern.

In the Higuchi model, Eqn. 2, the diffusivity (D_{eff}) is assumed to be constant during the dissolution process. However, in this study the D_{eff} and the exposed surface area (S) of Eqn. 2 are not constant as they will increase as a result of particle hydration. Representative adriamycin-associated BSA microspheres prepared by heat-stabilization at $135 \pm 5°C$ are shown in Fig. 5. The average diameter of these particles was 0.69 ± 0.36 μm. The external and the internal structure of the adriamycin associated BSA microspheres, after subjecting them to dissolution for 1 h at 25°C, are shown in Figs. 6 and 7, respectively. The average diameter of these hydrated microspheres was found to be 0.93 ± 0.48 μm. It can be observed that cavities are formed on the surface as well as within the microspheres upon hydration, and are probably caused by the dissolution of entrapped adriamycin. These observations not only cast doubt upon the validity of using the Higuchi equation to describe the release of adriamycin

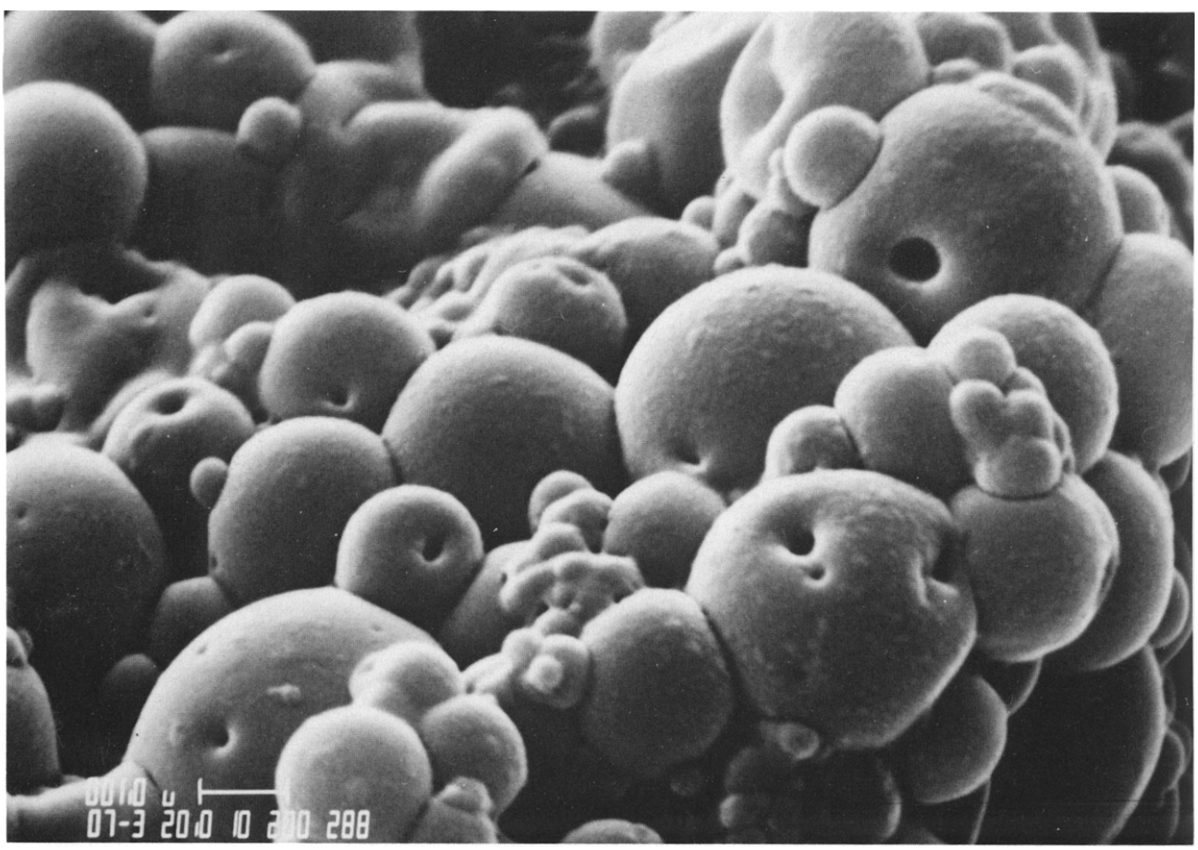

Fig. 6. Scanning electron microscope photograph of adriamycin-associated BSA microspheres prepared by heat-stabilization at $135 \pm 5°C$ and subjected to 1 h of dissolution at 25°C (magnification: 7000×; note 1 μm line).

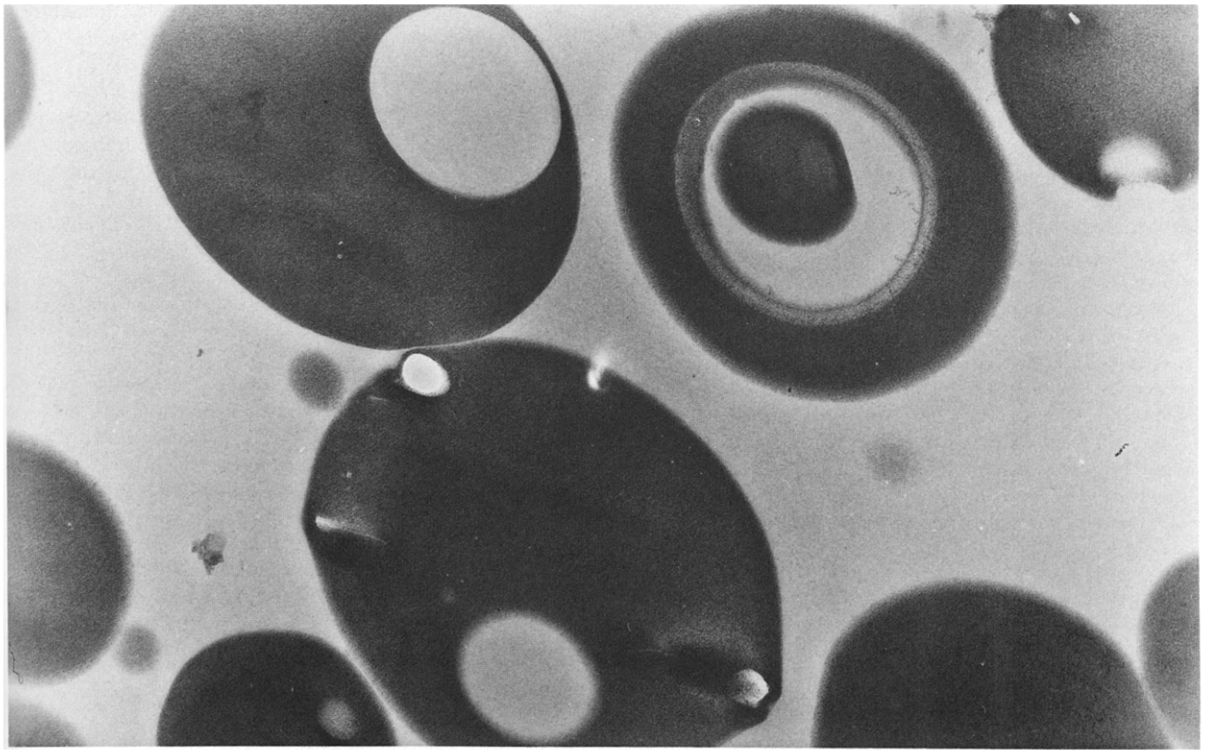

Fig. 7. Transmission electron microscope photograph of adriamycin-associated BSA microspheres under same conditions as for Fig. 6 (magnification: 30,000 ×).

from the BSA microspheres, but also support the contention that drugs can be associated with this carrier in a number of ways, as illustrated in Fig. 1.

Figs. 6 and 7 also indicate that the average size and the surface area of the BSA microspheres increase during the dissolution study. This would lead to an increase in the drug diffusion layer thickness, and hence decrease in drug release from the carrier. As discussed previously, the effect of increase in drug diffusivity and particle surface area due to particle hydration may be compensated by an increase in the diffusion layer thickness. Under these circumstances, the coefficient, $(D_{eff} \cdot S/h)$, of Eqn. 5 can be regarded as a constant. In addition, it has been demonstrated in this study that $(C_m - C)$ is approximately equal to C_m. Under these situations, Eqn. 5 represents a zero-order process. Therefore, this suggests that the release of adriamycin or any other water-soluble drug from BSA microspheres should be adequately described by a zero-order process. The bi-phasic release profile observed in this study is probably due to the release of drug from sites B and C, as well as increased drug release due to particle hydration at the initial stage and from site D at the terminal stage.

Since a substantial amount of drug associated with the albumin microspheres is present in the slowly released form, this delivery device can be very well exploited for the purpose of a sustained or controlled release of other water-soluble drugs.

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