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# Albumin microspheres. II. Effect of stabilization temperature on the release of adriamycin

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## Summary

The effect of stabilization temperature on the rate of release of adriamycin from bovine serum albumin (BSA) microspheres has been evaluated. Microspheres were prepared at 105, 120, 135 and 150 °C and washed 1–4 times to remove the surface drug before the release study. Release profiles depend on the number of washing steps employed. Microspheres subjected to two or less washings exhibit a tri-phasic zero-order release of adriamycin, whereas four washings of the carrier shows a bi-phasic zero order release pattern. The in vitro dissolution results indicate that the rate of release of adriamycin from the microspheres decreases with an increase in stabilization temperature of the carrier and the release of entrapped drug can be controlled.

## Introduction

Microparticulate drug delivery devices have recently gained attention in targeting chemotherapeutic agents. Various biodegradable materials such as polyalkylcyanoacrylate (Couvreur et al., 1980; El-Samaligy and Rohdewald, 1982; Lowe et al., 1985), polycarbonate (Kawaguchi et al., 1982; Kojima et al., 1984), polylactic acid (Juni et al., 1985; Krause et al., 1985; Wakiyama et al., 1982), gelatin (El-Samaligy and Rhodewald, 1982; Marty et al., 1978; Yoshioka et al., 1981) and starch (Artursson et al., 1984; Schroder and Mosbach, 1982) have been used as carriers for drug targeting. However, albumin is the most favoured carrier for studying passive as well as active drug targeting (Kramer, 1974; Scheffel et al., 1972; Sugibayashi et al., 1979; Widder et al., 1979). Passive drug targeting to the reticuloendothelial system and lungs using albumin microspheres (1.4–45 $\mu$m in size) has been demonstrated by several investigators (Burger and McVie, 1985; Illum and Davis, 1982; Morimoto et al., 1985; Willmott et al., 1985). Targeting of drugs outside the reticuloendothelial system has been achieved by Senyei et al. (1981), Sugibayashi et al. (1982) and Widder et al. (1979; 1981) with submicron magnetic albumin microspheres.

In a recent review on drug targeting, Florence and Halbert (1985) have emphasized that drug release from its carrier should be controlled to allow negligible drug levels in the systemic circulation, but maintaining a high level at the target site.

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When albumin microspheres are used for drug targeting, the systemic drug release from the carrier is minimized by washing the microspheres before in vivo administration. It has been reported by several investigators that albumin microspheres with minimal surface drug may be obtained by sonicating the carrier briefly in a suitable dispersion medium (Senyei et al., 1981; Sugibayashi et al., 1979; Tomlinson et al., 1984); however, as many as four washings of microspheres have been employed by Ovadia et al. (1982) to remove the non-incorporated myelin basic protein. Conversely, the drug level at the target site is governed by the drug release characteristics of the carrier (Illum and Davis, 1982). Widder et al. (1979) and Sugibayashi et al. (1979) have reported that the drug release profile from albumin microspheres is affected by the stabilization temperature of the carrier. Sokoloski and Royer (1984) have further suggested that albumin microspheres may be employed for controlled release of drug. However, the effect of carrier stabilization temperature on the release of drug from albumin microspheres has not been evaluated quantitatively.

This investigation has been undertaken to elucidate the effect of stabilization temperature and the extent of washing of BSA microspheres on the in vitro release characteristics of adriamycin from the carrier.

## Experimental

The materials and methods for preparing adriamycin-associated BSA microspheres, quantitation of adriamycin, and the procedure for carrying out the in vitro dissolution study have been discussed previously (Gupta et al., 1986). In this investigation, the albumin microspheres were subjected to 5 min sonication in 1 ml of normal saline in each washing step, because sonication of microsphere suspensions for less than 5 min resulted in non-homogeneous dispersions. In addition, such a redispersion technique did not cause microsphere disintegration.

Adriamycin associated BSA microspheres were prepared by heat stabilization at four temperatures (105, 120, 135 and 150°C). The microspheres were subjected to in vitro release study after washing for 1–4 times. It has been shown earlier that release of adriamycin from BSA microspheres follows a zero-order model (Gupta et al., 1986). In this study, therefore, zero-order release rate constants of adriamycin from the microspheres were determined from the plot of the amount remaining with the carrier, i.e. $(Q_0 - Q)$ per mg of microspheres, as a function of the release time. Since a multiphasic release pattern was obtained in all cases, the data were analyzed by the method of residuals (Gibaldi and Perrier, 1982).

## Results and Discussion

The particle size of the microspheres used in this investigation ranged between 0.69 and 0.73 $\mu$m in diameter. Depending upon the stabilization temperature and the extent of washing of the microspheres, the adriamycin hydrochloride content associated with the carrier was between 10.77 and 45.69 $\mu$g/mg of BSA microspheres. However, the stabilization temperature has no effect on the hydration characteristics of the microspheres. The average diameter of the microspheres, regardless of the stabilization temperature, after 1 h of hydration was $1 \pm 0.5$ $\mu$m.

Fig. 1 displays the release profile of adriamycin from the microspheres stabilized at 105°C. The plot demonstrates the effect of four washing levels on the release pattern of adriamycin. Application of the method of residuals to the data indicated a tri-phasic zero-order release of adriamycin from the one, two and three times washed microspheres with the initial and intermediate release phases lasting for 6 and 24 h, respectively. A bi-phasic release pattern was obtained from the four times washed microspheres. Fig. 2 shows the release pattern of adriamycin from the BSA microspheres stabilized at 150°C. Comparison of Figs. 1 and 2 shows that similar drug release profiles were obtained except that a bi-phasic zero-order drug release pattern is observed after three washings of the microspheres stabilized at 150°C. Such observations further support our previous proposal that drugs may be mechanically associated with

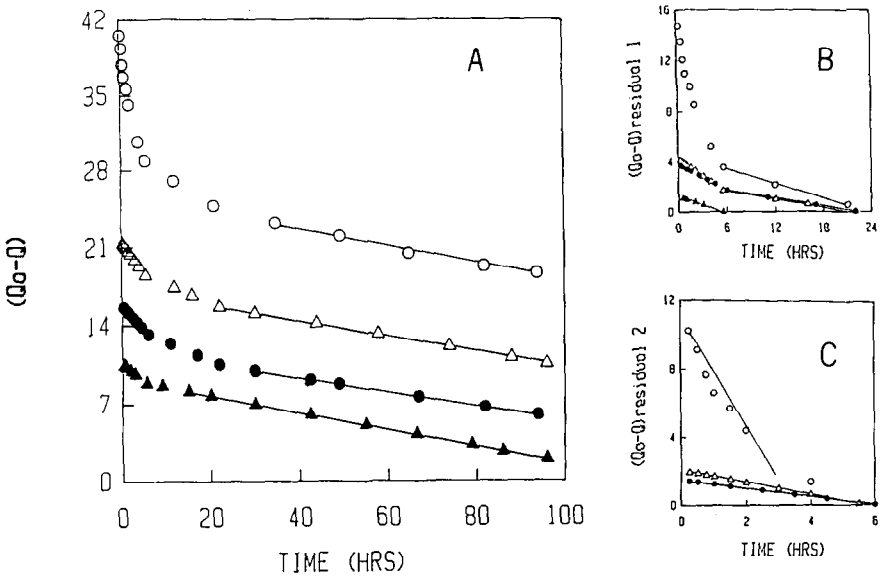


Fig. 1. A: plot of $(Q_0 - Q)$ versus t for adriamycin released per mg of BSA microspheres prepared by heat stabilization at 105°C. B: plot of first residual $(Q_0 - Q)$ versus t. C: plot of second residual $(Q_0 - Q)$ versus t. ○——○, first washing; △——△, second washing; ●——●, third washing; ▲——▲, fourth washing.

BSA microspheres by different ways (Gupta et al., 1986). Repeated washing of this drug carrier will eliminate the loosely entrapped surface drug and consequently, reduces the complexity of its drug release characteristics.

The release rate constants of adriamycin at different phases of the microspheres stabilized at different temperatures and washing levels are presented in Tables 1–4. The release rate constants of adriamycin at the initial and intermediate phases

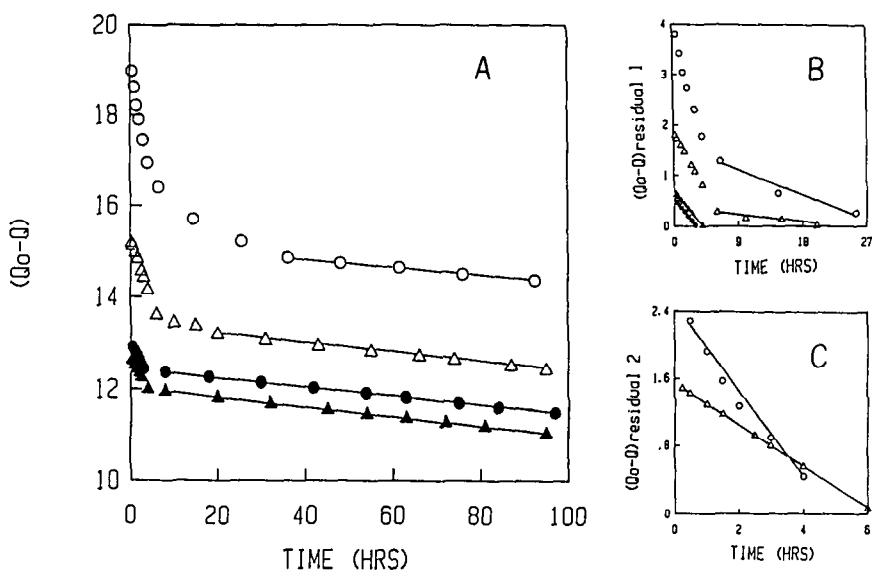


Fig. 2. A: plot of $(Q_0 - Q)$ versus t for adriamycin released per mg of BSA microspheres prepared by heat stabilization at 150°C. B: plot of first residual $(Q_0 - Q)$ versus t. C: plot of second residual $(Q_0 - Q)$ versus t. Symbols representation same as in Fig. 1.

are substantially higher than those at the terminal phase. The release of drug at these two phases is probably due to the drug dissolution from the peripheral binding sites and hydration of the microspheres. The slow release rate at the terminal phase is due to the release of the entrapped drug from the core of the microspheres (Gupta et al., 1986). Tables 1–4 also demonstrate that the release rate constants of adriamycin at the initial and intermediate phases are affected by both sta-

TABLE 1

RELEASE RATE CONSTANT [a] OF ADRIAMYCIN per mg OF BSA MICROSPHERE STABILIZED AT 105°C [b]

| Washing number | A [c] | Initial release rate constant ± S.D. | B | Intermediate release rate constant ± S.D. | C | Terminal release rate constant ± S.D. |
|---|---|---|---|---|---|---|
| One | 9.7 | $224.0 \times 10^{-2} \pm 278.0 \times 10^{-3}$ | 4.5 | $19.0 \times 10^{-2} \pm 10.9 \times 10^{-3}$ | 25.8 | $7.8 \times 10^{-2} \pm 4.7 \times 10^{-3}$ |
| Two | 2.0 | $36.1 \times 10^{-2} \pm 2.4 \times 10^{-3}$ | 2.2 | $9.8 \times 10^{-2} \pm 2.3 \times 10^{-3}$ | 17.2 | $6.9 \times 10^{-2} \pm 0.4 \times 10^{-3}$ |
| Three | 1.5 | $24.3 \times 10^{-2} \pm 2.2 \times 10^{-3}$ | 2.3 | $10.4 \times 10^{-2} \pm 0.9 \times 10^{-3}$ | 11.9 | $6.2 \times 10^{-2} \pm 0.2 \times 10^{-3}$ |
| Four | 1.2 | $22.1 \times 10^{-2} \pm 1.8 \times 10^{-3}$ | | | 9.3 | $7.6 \times 10^{-2} \pm 0.2 \times 10^{-3}$ |

[a] Mean of three sets of data analyzed by the method of residuals. Units: μg/mg carrier/h.
[b] Maintained at ±5°C level.
[c] A, B and C refer to extrapolated intercepts at zero time.

TABLE 2

RELEASE RATE CONSTANTS [a] OF ADRIAMYCIN per mg OF BSA MICROSPHERES STABILIZED AT 120°C [b]

| Washing number | A | Initial release rate constant ± S.D. | B | Intermediate release rate constant ± S.D. | C | Terminal release rate constant ± S.D. |
|---|---|---|---|---|---|---|
| One | 2.6 | $78.3 \times 10^{-2} \pm 84.1 \times 10^{-3}$ | 2.7 | $12.5 \times 10^{-2} \pm 7.3 \times 10^{-3}$ | 19.9 | $3.6 \times 10^{-2} \pm 0.9 \times 10^{-3}$ |
| Two | 2.4 | $49.0 \times 10^{-2} \pm 1.1 \times 10^{-3}$ | 1.9 | $9.3 \times 10^{-2} \pm 0.8 \times 10^{-3}$ | 15.6 | $3.7 \times 10^{-2} \pm 3.1 \times 10^{-3}$ |
| Three | 1.4 | $23.8 \times 10^{-2} \pm 1.0 \times 10^{-3}$ | 1.3 | $5.2 \times 10^{-2} \pm 0.8 \times 10^{-3}$ | 12.9 | $4.0 \times 10^{-2} \pm 5.3 \times 10^{-3}$ |
| Four | 0.8 | $20.1 \times 10^{-2} \pm 2.2 \times 10^{-3}$ | | | 11.3 | $4.2 \times 10^{-2} \pm 1.8 \times 10^{-3}$ |

Meaning of superscripts as Table 1.

TABLE 3

RELEASE RATE CONSTANTS [a] OF ADRIAMYCIN per mg OF BSA MICROSPHERES STABILIZED AT 135°C [b]

| Washing number | A | Initial release rate constant ± S.D. | B | Intermediate release rate constant ± S.D. | C | Terminal release rate constant ± S.D. |
|---|---|---|---|---|---|---|
| One | 1.8 | $52.4 \times 10^{-2} \pm 37.9 \times 10^{-3}$ | 1.4 | $5.4 \times 10^{-2} \pm 1.9 \times 10^{-3}$ | 12.3 | $1.8 \times 10^{-2} \pm 0.6 \times 10^{-3}$ |
| Two | 1.7 | $28.2 \times 10^{-2} \pm 1.7 \times 10^{-3}$ | 0.7 | $3.1 \times 10^{-2} \pm 1.0 \times 10^{-3}$ | 11.4 | $1.9 \times 10^{-2} \pm 1.5 \times 10^{-3}$ |
| Three | 0.9 | $20.5 \times 10^{-2} \pm 1.7 \times 10^{-3}$ | 0.4 | $2.3 \times 10^{-2} \pm 0.2 \times 10^{-3}$ | 11.1 | $1.8 \times 10^{-2} \pm 0.6 \times 10^{-3}$ |
| Four | 1.0 | $19.1 \times 10^{-2} \pm 3.4 \times 10^{-3}$ | | | 10.8 | $2.0 \times 10^{-2} \pm 1.7 \times 10^{-3}$ |

Meaning of superscripts as Table 1.

TABLE 4

RELEASE RATE CONSTANTS [a] OF ADRIAMYCIN per mg OF BSA MICROSPHERES STABILIZED AT 150°C [b]

| Washing number | A | Initial release rate constant ± S.D | B | Intermediate release rate constant ± S.D. | C | Terminal release rate constant ± S.D. |
|---|---|---|---|---|---|---|
| One | 2.4 | $51.2 \times 10^{-2} \pm 34.0 \times 10^{-3}$ | 1.5 | $5.2 \times 10^{-2} \pm 11.7 \times 10^{-3}$ | 15.2 | $0.9 \times 10^{-2} \pm 0.2 \times 10^{-3}$ |
| Two | 1.5 | $24.7 \times 10^{-2} \pm 0.9 \times 10^{-3}$ | 0.3 | $1.4 \times 10^{-2} \pm 5.8 \times 10^{-3}$ | 13.4 | $1.0 \times 10^{-2} \pm 3.2 \times 10^{-3}$ |
| Three | 0.5 | $17.7 \times 10^{-2} \pm 1.3 \times 10^{-3}$ | | | 12.4 | $0.9 \times 10^{-2} \pm 2.8 \times 10^{-3}$ |
| Four | 0.6 | $16.2 \times 10^{-2} \pm 1.4 \times 10^{-3}$ | | | 12.0 | $1.0 \times 10^{-2} \pm 1.8 \times 10^{-3}$ |

Meaning of superscripts as Table 1.

bilization temperature and the extent of washing of the carrier. The decrease in drug release rate with the increase in stabilization temperature has been interpreted as a result of increase in matrix tortuosity at higher temperatures (Sugibayashi et al., 1979; Widder et al., 1979). The decrease in drug release rate constants at these two phases upon washing is due to the removal of the loosely deposited drug near the surface of the microspheres. The release of adriamycin from different drug binding sites of the BSA microspheres can be described by the following equation (Gupta et al., 1986)

$$d(Q_0 - Q)/dt = D_{eff} \cdot S \cdot (C_m - C)/h \qquad (1)$$

where $Q_0$ is the initial amount of drug per mg of microspheres, Q is the amount of drug released per mg of microspheres in time t, $D_{eff}$ is the effective diffusivity of the drug from BSA microspheres, S is the surface area of the microspheres, $C_m$ is the drug concentration inside the microspheres, C is the concentration of drug in the dissolution medium, h is the thickness of the diffusion layer and t is the time during which release of drug occurs. Since the coefficient $D_{eff} \cdot S/h$ can be regarded as a constant, and $C_m$ is much greater than C, Eq. 1 can be simplified to the following zero-order relationship:

$$d(Q_0 - Q)/dt = -K \cdot C_m \qquad (2)$$

where $K = D_{eff} \cdot S/h$ (Gupta et al., 1986). Reduction of the drug concentration of the microspheres, i.e. $C_m$, at the peripheral drug binding sites upon washing will, therefore, decrease the observed release rate constant ($K \cdot C_m$) at the initial and intermediate release phases.

The release rate constants at the terminal phase are affected only by the stabilization temperature of microspheres. As with the other two phases, the release of drug at this phase decreases with the increase in the stabilization temperature. However, the release rate constants are relatively insensitive to the extent of microsphere washing. This further supports the concept that the release phase is governed by the entrapped drug inside the microsphere which cannot be removed readily by the washing procedure.

In this work it has been revealed that the terminal drug release rate constants of adriamycin from the BSA microspheres exhibit a log-linear relationship with the stabilization temperature of the carrier (shown in Fig. 3). Such findings indicate that the terminal drug release rate can be controlled by adjusting the stabilization temperature of the BSA microspheres.

The intercepts A, B and C of Tables 1–4 represent the quantity of adriamycin ($\mu$g/mg of microspheres) available for release at the initial, intermediate and terminal phases respectively. As seen from Tables 1 and 2, substantial reduction in A and B upon washing is obtained from microspheres stabilized at 105 and 120°C. These results demonstrate that a large portion of drug associated with the BSA microspheres prepared at comparatively low stabilization temperatures is loosely bound to the carrier. Four washings appear necessary for the removal of the undesirable surface drug from these microspheres. However, for the microspheres stabilized at higher temperatures, i.e.

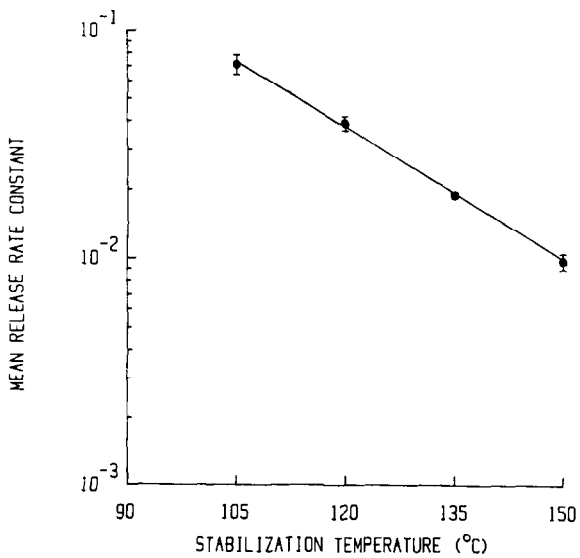


Fig. 3. Log-linear plot of the mean release rate constant of adriamycin at the terminal phase as a function of the stabilization temperature of BSA microspheres. Bar represents the S.D. of four release rate constants at different washing levels for each stabilization temperature.

135 and 150°C, three washings of the carrier are adequate (see values of A and B in Tables 3 and 4).

Alternatively, the quantity of drug entrapped within the microspheres, as reflected by the C terms of Tables 1–4, is not affected to the same extent as the surface drug by the washing procedure. After four washings of the carrier, 88–95% of the total associated drug remains inside the microspheres. This fraction of drug can therefore be made available to the target site with minimal drug release in the systemic circulation.

In conclusion, this study demonstrates that release of a water-soluble drug from BSA microspheres can be controlled by adjusting the stabilization temperature of the carrier. In addition, several washings of the microspheres, prior to their in vivo use, are required to minimize the systemic release of drug from this drug targeting device.

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