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Albumin microspheres. IV. Effect of protein concentration and stabilization time on the release rate of adriamycin

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Summary

The effects of protein concentration and heat-stabilization time at 120 °C on the release rate of adriamycin from microspheres have been investigated. The albumin concentration and the stabilization time of the microspheres were varied from 20 to 40% w/v and from 2.5 to 10 min, respectively. The release studies carried out using dynamic dialysis demonstrated that irrespective of the protein concentration and stabilization time, the initial release rate was significantly higher than the terminal release rate of adriamycin from these particles. Whereas variation in the albumin concentration was shown to cause comparable changes in the initial and the terminal release rate constants of adriamycin, the variation in the stabilization time predominantly affected the terminal release rate constants. The results indicate that protein concentration as well as the heat-stabilization time of albumin microspheres may be used to control the release of entrapped drug.

Albumin microspheres have been extensively investigated for drug delivery by several workers both in vitro (Gupta et al., 1986a and b, 1987a and b, 1988a and b; Willmott et al., 1985a) and in vivo (Fujimoto et al., 1985; Illum et al., 1987; Lee et al., 1981; Morimoto et al., 1980; Ratcliffe et al., 1987; Sugibayashi et al., 1979; Willmott and Cummings, 1987; Willmott et al., 1985b). Tissue histological studies have proved that albumin microspheres possess better bio-acceptability than other polymeric carriers (Ratcliffe et al., 1984; Waser et al., 1987). Physicochemical characterization of a drug delivery device allows identification of the factors which may affect their efficacy in vivo. In addition, such studies provide information on the formulation of these particles with the desired release characteristics.

The therapeutic efficacy of a drug delivery system is dependent upon its release characteristics at the target site (Illum and Davis, 1982). Prolonged release of incorporated drug in a controlled manner minimises its frequent dosing, often warranted in conventional chemotherapy. In addition it reduces the chances of systemic toxicities associated with the drug.

Several factors are known to govern the release pattern of drug(s) from albumin microspheres. These include drug and protein concentration of

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the carrier (Sokoloski and Royer, 1984; Tomlinson et al., 1984), particle size (Dau-Mauger et al., 1986; She and Sokoloski, 1986) and the degree of carrier stabilization (Gupta et al., 1986b, 1988a; Lee et al., 1981). When heat is used for the denaturation of protein, the release behaviour of included drug(s) is affected by the stabilization temperature as well as the length of heat denaturation. Most published reports have considered the effect of stabilization temperature on the drug release characteristics from albumin microspheres (Gupta et al., 1986b, 1988a; Sugibayashi et al., 1979). However, the effect of stabilization time on the release of drug from these particles has not been considered.

This investigation was undertaken to investigate the effect of protein concentration and stabilization time of albumin microspheres, at 120 °C, on the release rate of the incorporated adriamycin hydrochloride. Results obtained from this study are envisaged to assist the design and development of albumin microspheres with predictable drug release characteristics.

The materials and the apparatus used in the synthesis and analysis of adriamycin-associated albumin microspheres have been discussed earlier (Gallo et al., 1984; Gupta et al., 1986a). Adriamycin hydrochloride was donated by Farmitalia Carlo Erba, Milan (Italy).

The adriamycin-associated albumin microspheres were prepared as follows. To 250 μl of aqueous albumin solution (20, 30 or 40% w/v), 200 μl of aqueous adriamycin hydrochloride solution (50 mg/ml) was incorporated while vortexing. 30 ml of cotton seed oil (4 °C) was then added and the mixture was ultrasonicated at 125 W for 1.5 min using a Dawe Soniprobe (type 7530 A). The primary emulsion was added (250 ± 25 drops/min) to 100 ml of preheated cottonseed oil (120 °C), stirred at 1500 rpm (Variable speed Heidolph stirrer, model RZRI). Heating and stirring of the oil were continued for 2.5, 5.0, 7.5 or 10 min after the addition of emulsion. The resulting suspension was ice-cooled to 20 °C in about 10 ± 2 min, centrifuged (Sorvall RC2-B; 3000 *g*; 15 min) and the microspheres were subsequently washed with 60 ml of anhydrous ether. This procedure was repeated for a total of 4 washings. Depending upon the albumin concentration, 50–100 mg microspheres were obtained per batch which were stored as an ether suspension (25 mg/ml) or as a free flowing powder at −15 °C till used.

The different types of microspheres were examined under scanning electron microscope (SEM) before and after their release studies in Tris buffer (pH 4.0) at 25 °C. An aliquot of suspension of fresh microspheres in ether was allowed to evaporate on a SEM stub at room temperature and then observed under the microscope after gold coating (20 nm) (Gupta et al., 1986a). The microspheres subjected to release studies were processed for SEM examination by adding absolute alcohol to a pellet of microspheres and vortexing the tube, followed by its centrifugation at 3000 *g* for 15 min. The supernatant was discarded and microspheres were resuspended in fresh alcohol. SEM stubs containing these microspheres were prepared in a manner similar to that discussed above.

The adriamycin content of the microspheres was determined by digesting them in 0.5 M acetic acid and analysing the supernatant by HPLC (Gallo et al., 1986; Gupta et al., 1986a). Dynamic dialysis technique was used to study the drug release characteristics of adriamycin associated albumin microspheres (Gupta et al., 1987b). About 50 mg microspheres were washed 4 times in normal saline to remove the surface-associated drug, then suspended in 7 ml of Tris buffer (pH 4.0) and dialysed against 128 ml of buffer at 25 °C. 100 μl samples of the medium outside the dialysis bag were removed over a 72 h period and assayed for adriamycin concentration using HPLC. Dialysis studies were carried out in triplicate for each type of microspheres. Regression analysis (Draper and Smith, 1981) was applied to the initial and the terminal release rate constants, obtained using the previously suggested mathematical model (Gupta et al., 1987b). All statistical analyses were performed using SAS computer package (SAS, 1985).

Adriamycin hydrochloride is heat-decomposed at temperatures > 120 °C (Widder et al., 1980). It has been shown in our laboratory that use of temperatures > 120 °C, for the stabilization of adriamycin associated albumin microspheres, reduces the total as well as the entrapped drug

TABLE 1

Effect of albumin concentration and stabilization time on the adriamycin content of albumin microspheres

Stabilization time (min)	Adriamycin content (% w/v albumin)		
	20	30	40
2.5	4.91 ± 1.23	3.62 ± 0.88	2.38 ± 0.22
5	4.14 ± 0.98	2.84 ± 1.17	1.83 ± 0.39
7.5	3.54 ± 1.10	2.33 ± 0.57	1.52 ± 0.20
10	3.02 ± 0.14	1.91 ± 0.06	1.24 ± 0.14

[a] Stabilization temperature of 120 °C was used and the microspheres were washed four times with normal saline before the analysis of drug content. Values are % w/w, means of 3 studies ± S.D.

content of these particles (Gupta et al., 1987a, 1988a and b). Although the use of temperatures < 120 °C increases the total amount of adriamycin associated with albumin microspheres, it fails to allow a high degree of drug entrapment (Gupta et al., 1987a). In addition we found that the use of 20 to 40% w/v of protein along with a stabilization time of 2.5 to 10 min, at 120 °C, allows entrapment of 1–5% w/w of adriamycin in albumin microspheres (Table 1). In view of these reasons, a temperature of 120 °C, albumin concentration of 20–40% w/v and stabilization time of 2.5–10 min were considered in this study.

Scanning electron microscopic observations of different batches of microspheres revealed that the particles had a mean diameter < 1 μm. In all cases the particles were spherical in shape. Changes in the albumin concentration, in general, did not affect the surface characteristics of the microspheres. However when lower stabilization times were used (e.g., 2.5 or 5 min), the microspheres often demonstrated presence of rough surface. Examination of the microspheres after their dissolution in Tris buffer (pH 4.0) at 25 °C revealed the presence of pores and cavities in the matrix. These observations are similar to those reported previously (Gupta et al., 1986a) and suggest that variation in the protein concentration and/or stabilization time of albumin microspheres does not affect the release mechanism of the entrapped adriamycin.

Following dynamic dialysis studies, all batches of microspheres demonstrated bi-phasic zero-order release profile of adriamycin, similar to those reported previously (Gupta et al., 1986a). Regression equations were fitted to relate the initial as well as the terminal release rate constants as a function of their protein concentration and stabilization time. The relationships obtained for the initial and the terminal release phases, respectively, were:

$$\ln(\text{release rate constant}) = 1.405 - 2.969 \times (\text{albumin concentration}) - 0.0575 \times (\text{stabilization time}) \quad (1)$$

$$\ln(\text{release rate constant}) = 0.215 - 2.969 \times (\text{albumin concentration}) - 0.126 \times (\text{stabilization time}) \quad (2)$$

Comparison of the two equations indicated that their intercepts are significantly different ($P = 0.0001$). In addition, the coefficients for the stabilization time were also found to be significantly different ($P = 0.0367$). However the coefficients for the albumin concentration were found to be statistically non-significant. No interaction was detected between the albumin concentration and the stabilization time at 120 °C. These results indicate that for a constant stabilization time between 2.5 to 10 min, at 120 °C, variation in the concentration of albumin alters the drug release rate constant of both initial and terminal phases to the same extent (see Fig. 1). However, for a constant level of albumin concentration between 20 and 40% w/v, the changes in the stabilization time of the carrier at 120 °C does not affect the release rate constants of the two phases to the same degree.

Fig. 2 shows the effect of stabilization time, at 120 °C, on the release rate of adriamycin during the initial and terminal phases, from the microspheres prepared using 20 and 40% w/v of albumin. The values plotted in this figure were determined using Eqns. 1 and 2. Comparison of the slopes for the initial and terminal release rate constants, as a function of stabilization time, indi-

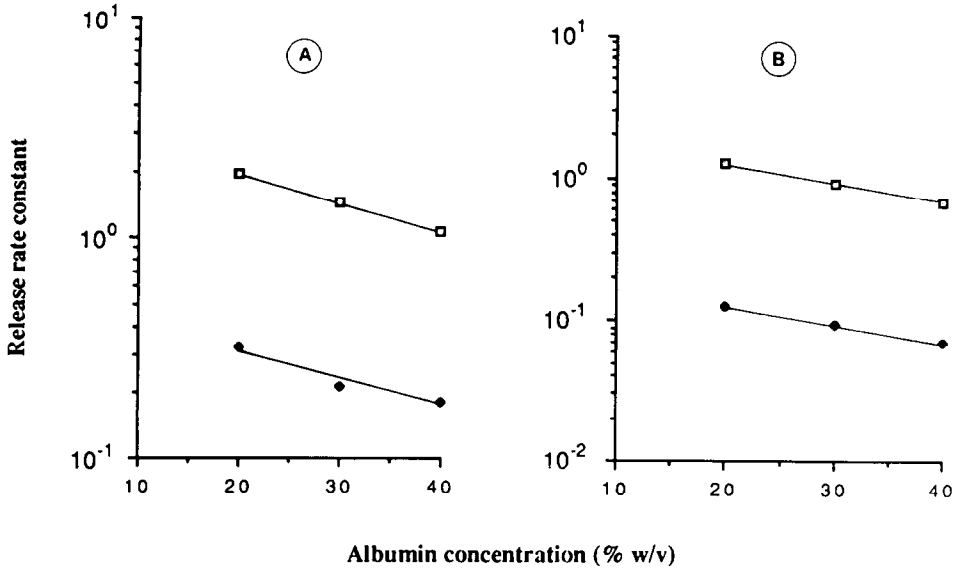

Fig. 1. Plots showing the effect of protein concentration on the release rate constant (μg/mg) of adriamycin from albumin microspheres, heat-stabilized at 120 ± 5 °C for (A) 2.5 min and (B) 10 min. (□), Initial release rate constant; (◆), terminal release rate constant. Each point represents a mean of 3 release studies.

cates that irrespective of the albumin concentration, stabilization time affects the terminal release rate constants at least 2 times more than the initial release rate constant.

In conclusion, the results of this study suggest that either protein concentration and/or stabilization time may be used to control the release of drug from albumin microspheres. Hence these

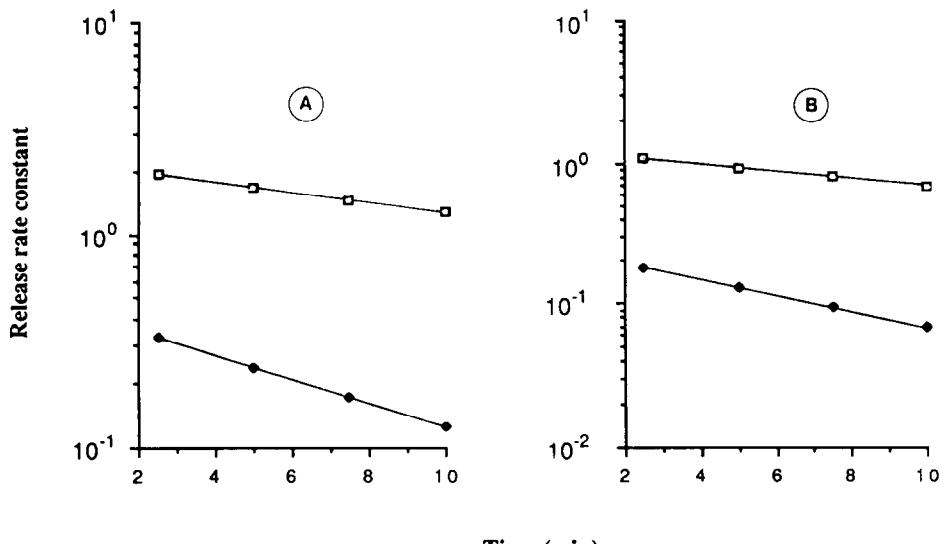

Fig. 2. Plots showing the effect of stabilization time of albumin microspheres, at 120 ± 5 °C, on the release rate constant (μg/mg) of adriamycin from microspheres prepared using (A) 20% w/v of albumin, and (B) 40% w/v of albumin. Symbols same as for Fig. 1. Each point represents a value predicted by Eqns. 1 or 2. See text for discussion.

parameters may be employed to develop biodegradable microspheres with desired drug release characteristics in the bio-phase.

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