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# INFLUENCE OF STABILIZATION TEMPERATURE ON THE ENTRAPMENT OF ADRIAMYCIN IN ALBUMIN MICROSPHERES

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## ABSTRACT

Adriamycin associated bovine serum albumin (BSA) microspheres have been prepared by the method involving emulsion and suspension technology. Stabilization of the carrier matrix was achieved by heat treatment at 105, 120, 135 and 150°C.

Following zero to four washings, each of these four batches of microspheres have been evaluated for the

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amount of associated adriamycin using HPLC. At high stabilization temperatures, migration of adriamycin to the microsphere surface is reduced leading to increased drug entrapment. Results demonstrate that the proportion of entrapped to total drug increases with increase in stabilization temperature of the carrier.



## INTRODUCTION

In the preparation of albumin microspheres as drug targeting systems, two methods are well established for achieving the matrix stabilization of carrier: heat treatment at temperatures between 100 and 165°C,[1-5] and chemical crosslinking using aldehydes.[4-6]

Several workers have indicated that the total drug associated with the carrier exists in two forms: a loosely bound fraction of drug which is adsorbed onto the surface of the particles, also called the surface drug; and the slowly releasable fraction, called the entrapped drug.[3-7] The percentage surface drug has been estimated as about 40% irrespective of the method of matrix stabilization.[4] However, using the chemical crosslinking approach, it has recently been estimated that the percentage of surface drug can vary between 40 and 93%.[6] Since the surface drug is of little value in drug targeting, washing steps have invariably been employed by all workers before the in-vivo use of this delivery device.[3,4,6,8] Little quantitative information however has been published on the effectiveness of such procedures in removing unwanted rapidly released surface bound drug.

The amount of drug incorporated into microspheres is said to affect its release characteristics.[5,6,9]

In addition, it is known that variation in temperature of the heat stabilization method can influence the release pattern of the entrapped drug.[3,4] Hence, it is possible that the temperature employed during stabilization of carrier may affect the amount of drug entrapped within the particle. No quantitative information on how the stabilization temperature can alter the percentage of total drug which is entrapped is available.

Chemotherapeutic agents are intrinsically thermally unstable and it is obvious that such delivery systems prepared by high temperature stabilization will contain decomposed products. Adriamycin has been shown to form up to 22% of degradation products at temperatures of 135°C using microbiological techniques for quantitation of drug.[10]

In the present study the influence of stabilization temperature of albumin microspheres on the total and entrapped drug content is examined. This, in turn, is used to identify the effect of stabilization temperature on the dependence of entrapped to total associated drug ratio. As part of this work, the effect of repeated washing of the carrier is also investigated. Adriamycin, an anticancer agent, already extensively studied by other workers[4,5,10] is employed as the test drug and its quantitation, in presence of its concomitant degradation products, is carried out using HPLC.

## EXPERIMENTAL

### Materials and Method of Preparing Adriamycin Associated B.S.A. Mirospheres

The materials and apparatus used for the synthesis of BSA microspheres have been described previously.[1,7]

Adriamycin Hydrochloride was kindly donated by Farmitalia Carlo Erba, Milan (Italy). All reagents used in the study were analytical grade and glassware was silanised with Aquasil® before use.

An aqueous phase consisting of 250µl of the BSA solution (400mg/ml) and 200µl of adriamycin HCl (50mg/ml) was mixed with 30ml of cottonseed oil (4°C). The mixture was ultrasonicated for 2 min at 125W setting and the resultant emulsion added (100 ± 10 drops/min) to 100ml of oil maintained at a fixed temperature (105, 120, 135 or 150°C) and stirred at 1500 rpm. Heating and stirring were maintained under the same conditions for another 10 min after the addition of emulsion. The resulting microsphere in oil suspension was ice-cooled to 20°C and then washed with 60ml of anhydrous ether. The suspension was then centrifuged at 3000xg for 15 min and the supernatant discarded. After three more washings, the microspheres were suspended in a small volume of ether. This suspension was transferred to a tared test tube and the ether evaporated in a gentle stream of oxygen free nitrogen gas. This yielded the mass of microspheres recovered. The final product was dispersed in ether to give a suspension of 25mg/ml and stored at -15°C until used.

## Analysis of Free Adriamycin

A reverse-phase ion-pairing HPLC technique[11] was adopted for analysing adriamycin.

## Analysis of Adriamycin Content Associated with BSA Microspheres

To an aliquot of the ether suspension of adriamycin associated BSA microspheres (representing 10mg of

microspheres) two drops of Tween 80 were added and the ether evaporated. The microspheres were then washed for zero to four times. Each washing was carried out by sonicating the microspheres in 1ml of normal saline for 5 min. This was found to be the minimum time in order to achieve a homogeneous suspension of the microspheres. The microspheres were isolated by centrifuging the suspension at 5000xg for 5 min. The particles were then digested for 12 hrs in 5ml of 0.5M acetic acid.[12] The homogenate was centrifuged at 5000xg for 5 min and the supernatant analysed for adriamycin concentration by HPLC. To ensure complete recovery of the drug a second digestion of the residue was carried out. The total drug entrapment reported was the combined adriamycin content obtained after two digestions of the microspheres. This procedure was found to recover more than 99% of the total adriamycin content.

## RESULTS AND DISCUSSION

Figure 1 is a representative photomicrograph of the albumin microspheres prepared by heat stabilization. It was found that irrespective of the temperature of heat stabilization, these had a mean particle diameter of 0.7 ± 0.5μm.

The total percentage of undecomposed adriamycin associated with the microspheres stabilized at various temperatures is shown in Figure 2. These values represent the drug contents determined in the absence of any washing procedure. As anticipated, the concentration of adriamycin decreases with the increase in

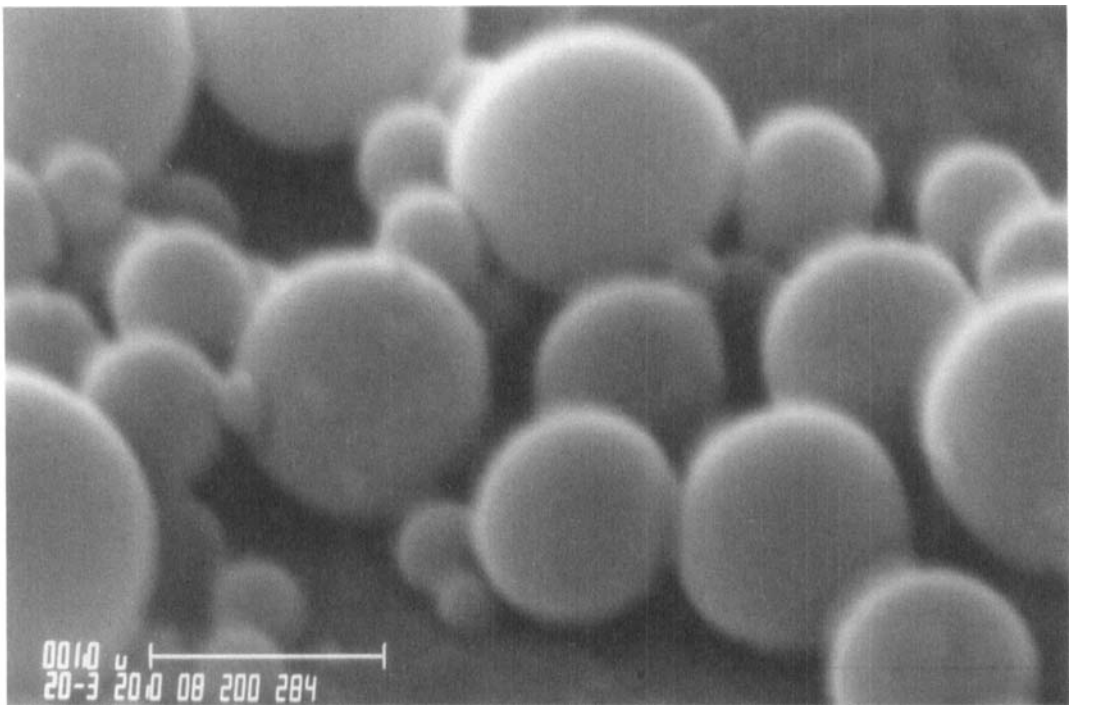


FIGURE 1

Scanning Electron Microscopy photomicrograph of adriamycin associated BSA microspheres prepared by heat stabilization at 120°C.

temperature due to the unavoidable decomposition of the chemotherapeutic agent. The specificity of the method of assay is shown in Figure 3 which shows chromatograms of an undecomposed adriamycin solution and a solution obtained by digestion of the microspheres stabilized at 150°C.

Table 1 shows the amount of adriamycin associated with the microspheres stabilized at different temperatures and subjected to various number of washings. Inspection of Table 1 indicates that as surface drug

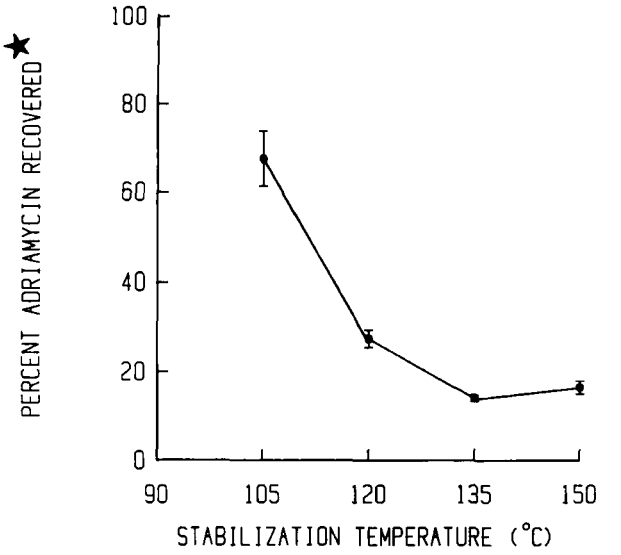


FIGURE 2

Plot of percentage of adriamycin recovered with BSA microspheres versus stabilization temperature. Bar represents S.D. of four sets of data.
*The total (surface plus entrapped) adriamycin associated with the BSA microspheres.



is removed by repeated washings, the proportion of entrapped drug increases at higher stabilization temperatures of the carrier. To our knowledge this clear differentiation between the proportions of entrapped and surface drug has not been made before. It appears therefore that the content of entrapped adriamycin is increased at higher stabilization temperatures when adequate removal of the surface drug is carried out and that the initial total drug content shown in Figure 2 is of limited value when considering drug targeting.

Figure 4 shows the percentage of the total drug considered to be entrapped within the microspheres

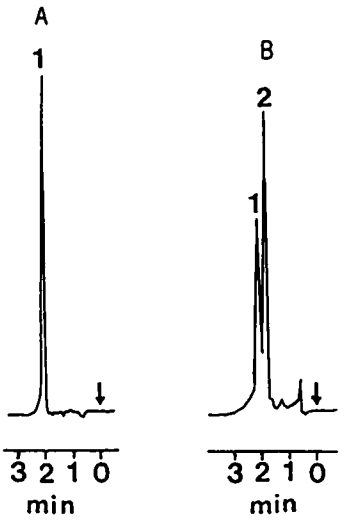


FIGURE 3

Chromatograms of stable and heat decomposed adriamycin. A. Peak obtained from stable adriamycin solution (injection volume 80µl; detector sensitivity 0.2). B. Peak due to the solution obtained after digesting the microspheres heat stabilized at 150°C (injection volume 30µl; detector sensitivity 0.2). Peak identification: 1 - adriamycin peak; 2-heat decomposed product(s).

calculated as the ratio of the adriamycin content found after four washings to that obtained originally. It can be observed that in contrast to the data in Figure 2 these values increase as temperature of stabilization is increased. These quantitative results are not inconsistent with the wide estimates suggested earlier.[4,6] It is appreciated that the choice of four washings to remove surface drug is to some extent arbitrary. It is noted, however, that in all cases the decrease after four washings was in the region of 1 ± 0.7%. Since continued washing will remove

TABLE 1

Effect of Stabilization Temperature and Extent of Washing on the Entrapment of Adriamycin in BSA Microspheres

| Stabilization[a] temperature (°C) | µg adriamycin/mg microsphere[b] ± S.D. | | | | |
|---|---|---|---|---|---|
| | Number of Washes | | | | |
| | 0[c] | 1 | 2 | 3 | 4 |
| 105 | 100.2 ± 9.4 | 45.7 ± 2.3 | 20.4 ± 1.4 | 13.7 ± 1.3 | 10.8 ± 1.3 |
| 120 | 40.7 ± 3.1 | 26.1 ± 1.0[c] | 19.3 ± 0.9 | 15.8 ± 0.8 | 14.0 ± 0.7 |
| 135 | 20.9 ± 1.1 | 16.1 ± 1.0 | 14.3 ± 0.9 | 13.4 ± 0.9[c] | 12.9 ± 1.0 |
| 150 | 24.5 ± 2.3 | 19.6 ± 1.3[c] | 17.7 ± 1.2 | 16.1 ± 1.1 | 15.4 ± 1.0[c] |

[a] Maintained at ±5°C level.

[b] Mean of four batches analysed.

[c] Mean of three bathces analysed.

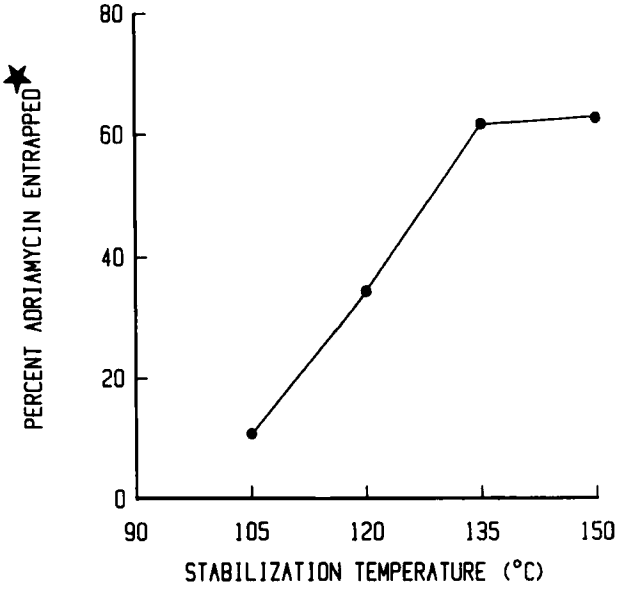


FIGURE 4

Plot of percent adriamycin entrapped versus stabilization temperature.
*Data obtained as a ratio of entrapped drug, after four washings, to the total amount associated originally.



entrapped drug also, it appears that the column 6 of Table 1 represents a good approximation to the entrapped drug values.

These results are at variance with opinions expressed by other workers[4,10] and a possible explanation can be suggested from observations in the unrelated field of drug granulation. It has been demonstrated that the entrapment of a water soluble drug in lactose granules is dependent on the drying temperature.[13] At low drying temperatures, the water within the granules evaporates slowly enhancing the migration of the entrapped drug to the surface of the granules. However

at high temperatures, instantaneous evaporation of the water inside the granule reduces the migration of the drug increasing the drug entrapment. It is possible that the same phenomenon governs the movement of a water soluble drug, such as adriamycin, in albumin microspheres.

In conclusion the present results indicate unequivocally that over the temperature range studied, more drug is entrapped within the microspheres the higher the temperature of stabilization. The apparent higher values at the lower temperatures are the result of decreased thermal degradation but are in point of fact irrelevant for drug targeting since a higher proportion of drug is associated with the surface regions.



## ACKNOWLEDGEMENTS

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