Effect of Heating Temperature and Time on Pharmaceutical Characteristics of Albumin Microspheres Containing 5-Fluorouracil

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INTRODUCTION

Albumin microspheres have been a subject of great interest in targeting drugs, especially anticancer drugs, to targets such as the lung, the intestine, and the liver.[1,2] Though a number of methodologies have been proposed and used to prepare albumin microspheres,[3] the 2 methods that are routinely used to prepare albumin microspheres are the heat denaturation method and the chemical crosslinking method. Both methods involve preparation of a water/oil emulsion where the inner phase contains droplets of aqueous albumin solution while the external phase is mostly oil. Subsequently, this emulsion is subjected to either heat treatment, where the emulsion is heated for some time at a temperature that may range from 90°C to 180°C, or the chemical crosslinking method, where usually glutaraldehyde is added in a small amount to the emulsion. The final result of both treatments is denaturation/crosslinking and consequent solidification of albumin. At the end of the process, the emulsion is transferred into suspension, where solid and spherical albumin particles are suspended in an external oily phase. These microspheres are separated by centrifugation and subsequent washing with organic solvents.

Though a number of parameters that may affect characteristics of albumin microspheres have been identified and studied,[4-6] not much attempt has been made to study the effect of heating temperature and time on desirable attributes of albumin microspheres. In the present case, an attempt has been made to determine and explain the effect of change in heating temperature and time on characteristics of albumin microspheres prepared by a heat denaturation method.

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5-Fluorouracil (5-FU) is an anticancer drug that is mainly used to treat breast cancer and gastrointestinal tract cancer. It has a very short biological half-life (10-20 minutes) that necessitates frequent administration of the drug. However, frequent administration of the drug may lead to severe side effects such as mucosal ulceration in the gastrointestinal tract, anorexia, diarrhea, and even shock.[7] Attempts have been made to control these complications by encapsulation of 5-FU using biodegradable polymers.[8,9] In the present case, 5-FU was selected to prepare a delivery system that can be used in the future for targeting anticancer drugs by chemoembolization.

KEYWORDS: albumin microspheres, particle size, 5-fluorouracil (5-FU), drug entrapment efficiency

MATERIALS AND METHODS

Materials

Materials used in the study included egg albumin flakes (Laboratory Rasayan grade, SD Fine Chem Ltd, Boisar, India), diethyl ether extrapure (LR grade, Suvidhinath Laboratories, Baroda, India), cottonseed oil (NK Proteins Ltd, Mehasana, India), and 5-fluorouracil (Biochem Pharmaceutical Limited, Ahmedabad, India).

Methods

Preparation of Abumin Microspheres

Albumin microspheres were prepared by the method suggested by Gupta et al,[10] with minor modification. In brief, 0.5 mL of 10% aqueous albumin solution containing 0.02% wt/vol of drug (5-FU) was added dropwise using a 22-gauge hypodermic needle to 20 mL of cottonseed oil in a beaker of 100-mL capacity

at room temperature. After the addition was made, a magnetic stirrer (DBK 5048, Shital Scientific Industries, Baroda, India) was stirred at the highest possible speed for 10 minutes. The water/oil emulsion thus obtained was added dropwise to hot cottonseed oil (20 mL) maintained at one of the 3 temperatures (90°C, 120°C, 150°C) with simultaneous stirring for one of the 3 times (5 minutes, 15 minutes, 25 minutes) on a magnetic stirrer, keeping stirring speed at an intermediate level. Later, the suspension containing microspheres and oil was left for 24 hours at room temperature. Supernatant oil was removed by decantation, and microspheres were washed with diethyl ether 3 times. After the third wash, microspheres were suspended in 5 mL of diethyl ether and were stored at 4°C. Nine batches were prepared, with each batch prepared in triplicate.

Determination of Drug Entrapment Efficiency

Efficiency of drug entrapment for each batch was calculated in terms of percentage entrapment as per the following formula:

$$\text{Percentage Entrapment} = (\text{Practical Yield}/\text{Theoretical Yield}) \times 100 \quad (1)$$

Theoretical yield—that is, the amount of drug that should be present in microspheres—was determined by calculation assuming that all the drug present in the albumin solution used gets entrapped in microspheres and no loss occurs because of partitioning of drug into oil phase, washing by diethyl ether, or squeezing due to heat-induced shrinking.

Practical yield—that is, the amount of drug that is actually present in microspheres—was determined by the following method[11]: 10 mL of ethereal suspension of albumin microspheres was taken in a previously weighed 10-mL volumetric flask. It was kept at 40°C for 1 hour to remove diethyl ether completely. The volumetric flask was again weighed, and the weight of the microspheres was obtained. Enough 5% vol/vol HCl in 95% ethanol was added to make the volume 10 mL. The solution was left for 24 hours at room temperature. The supernatant was collected by centrifugation, and the drug content in the supernatant was determined by UV spectrophotometry at 264 nm using a UV visible spectrophotometer (Spectrascan-2200 (Chemito, Nashik, India).

Determination of Mean Particle Size and Size Distribution

Particle size analysis was performed by optical microscopy[12] using a compound microscope (Model 1669, Getner, Ambala cantt, India). A small volume of ethereal suspension of microspheres was taken on a clean slide and was allowed to air-dry. The slide containing the dry film of albumin microspheres was mounted on the stage of the microscope and a size of at least 300 particles was measured using a calibrated ocular micrometer. This process was repeated for each batch prepared. Particle size distribution was determined by plotting a percentage frequency polygon using the data obtained during particle size determination

Data Analysis

Values of mean particle size, size distribution, and drug entrapment efficiency were subjected to 2-tailed paired *t* test for means to determine the significance of the effect of change on the heating temperature and time.

RESULTS AND DISCUSSION

The result of determination of mean particle size and drug entrapment efficiency for each batch is given in **Table 1**. The effect of each parameter will be discussed separately.

Effect of Heating Temperature on Mean Particle Size and Size Distribution

Increase in heating temperature from 90°C to 120°C led to a decrease in mean particle size (**Table 1**) and size distribution (**Figures 1**, **2**, and **3**) in all the batches irrespective of the time of heating. The effect was found to be highly significant ($P < .01$) in all the batches. However, further increase in temperature from 120°C to 150°C did not produce any significant change ($P > .4$) in mean particle size and size distribution except in batches prepared by heating for 5 minutes, where mean particle size and size distribution decreased significantly ($P < .01$). Our observation is in agreement with the observation made by Gupta et al.[10] This observation can be explained in terms of various processes that occur during heating and that are sensitive to process parameters. The processes that occur during addition of emulsion to hot oil and subsequent heat-induced stabilization are congealing of albumin, evaporation of water present in droplets added, and coalescence and aggregation of droplets and particles. The time of initiation and extent of this process will differ with the variation in the process parameters (heating temperature and time in the present case). Increase in the heating temperature from

Table 1. Effect of Heating Temperature and Time on MPS and PE*

Heating Temperature (^{o}C)	Heating Time					
	5 Minutes		15 Minutes		25 Minutes	
	MPS, µm	PE, %	MPS, µm	PE, %	MPS, µm	PE, %
90	12.86 ± 0.05	69.36 ± 0.82	13.50 ± 0.22	31.35 ± 0.97	12.22 ± 0.06	12.24 ± 1.72
120	10.33 ± 0.16	68.23 ± 1.63	10.43 ± 0.06	18.26 ± 0.89	10.30 ± 0.05	20.05 ± 2.95
150	9.40 ± 0.1	66.73 ± 1.31	10.67 ± 0.039	8.43 ± 1.26	10.24 ± 0.11	6.78 ± 0.82

*MPS indicates mean particle size; PE, percentage entrapment.

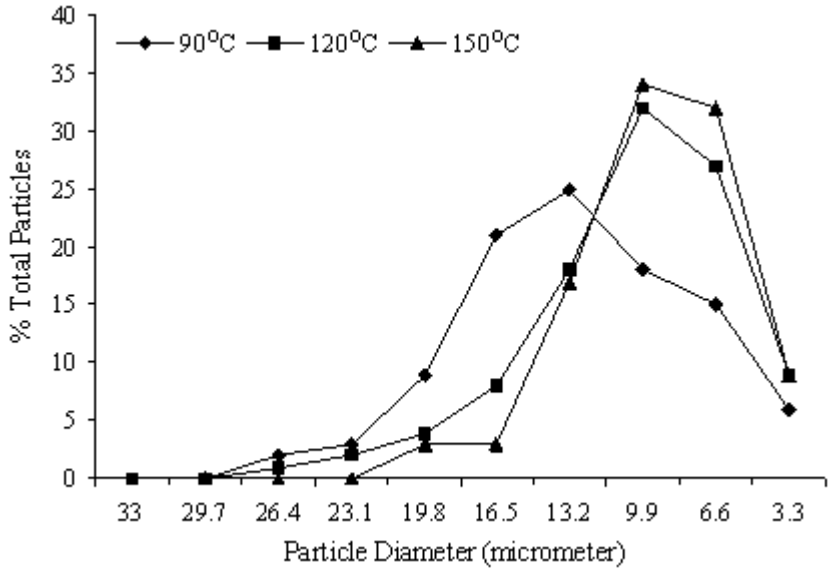

Figure 1. Effect of heating temperature at 5 min on particle size distribution.

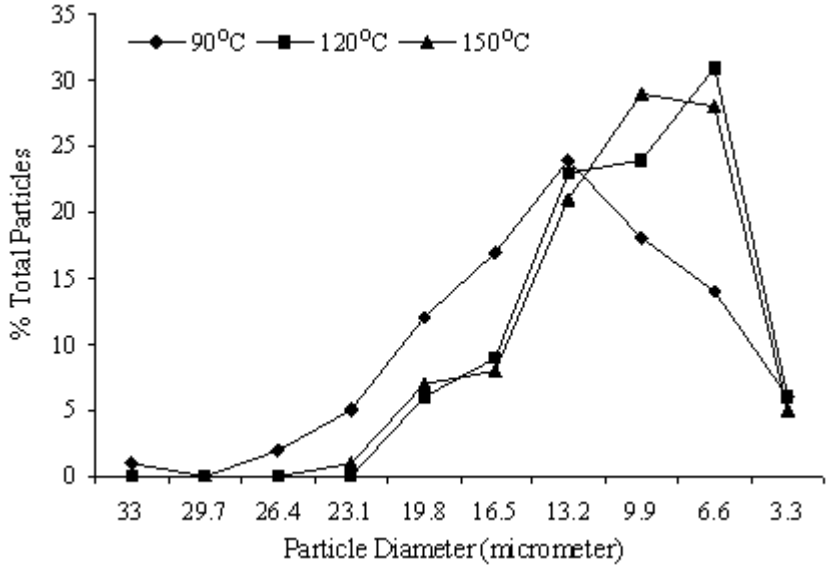

Figure 2. Effect of heating temperature at 15 min on particle size distribution.

90°C to 120°C increases the degree of congealing or rigidization of albumin,[13] which ultimately results in shrinking of the particles. Furthermore, 90°C may be too low a temperature for conversion of water in liquid state into vapor and hence complete removal of water present in the inner phase. This process starts essentially when the temperature of the hot oil is 110°C or higher.[14] However, when the heating temperature is increased to 120°C, almost all the water is evaporated, and this decreases the size of the microspheres. The decrease in particle size with increase in temperature has also been reported by Gallo et al.[13] When heating temperature is increased to 150°C, almost the same effect as that produced at 120°C occurs and hence no further change is observed.

Effect of Heating Time on Mean Particle Size and Size Distribution

Irrespective of heating temperature, batches prepared at different heating times did not show any significant difference in mean particle size (**Table 1**). A similar result was obtained for particle size distribution of the microspheres, except that in batches prepared by heating at 90°C, particle size distribution became more skewed (because of an increase in the number of particles at a lower size range) with increase in heating time. The pattern of change in mean particle size, though insignificant, was common at all the temperatures, as shown in **Table 1**. The increase in heating time from 5 minutes to 15 minutes increased the mean particle size. However, further increase in the heating time from 15 minutes to 25 minutes decreased the mean particle size. A similar change was observed in the size distribution in batches prepared at the lower temperature (90°C). The latter effect, especially at the lower temperature, was similar to that found in an earlier report, where increase in duration of chemical crosslinking reduced the mean particle size and size distribution measured in terms of SD.[15] This may be due to initial swelling of the particles due to evaporation of water during rigidization, leading to an increase in mean particle size; mean particle size then decreases as the water vapor is lost because of prolonged heating. The change may be very swift at higher temperatures (120°C and 150°C). However, at a lower temperature (90°C) the process becomes more dependent on time of heating, as temperature may not be high enough for conversion of water into vapor. This may be the reason that change in heating time at the lower temperature (90°C) has a significant effect on mean particle size and size distribution.

The observed effect of heating temperature and time on mean particle size and size distribution implies that the process responsible for change in particle size during heating was influenced more by temperature, while time of heating produced a negligible effect.

Effect of Heating Temperature on Drug Entrapment Efficiency

The effect of heating temperature on drug entrapment efficiency was found to depend on heating time (**Figure 4A**). While in the case of albumin microspheres prepared by heating for 5 minutes, percentage drug entrapment efficiency remained almost constant, it changed significantly ($P < .05$) in batches prepared by heating for 15 and 25 minutes. While drug entrapment efficiency decreased with increase in heating temperature from 90°C to 120°C in batches prepared by heating for 15 minutes, it increased in batches prepared by heating for 25 minutes, as shown in **Figure 4A**. Further increase in heating temperature from 120°C to 150°C decreased drug entrapment efficiency irrespective of time of heating. The overall result of the increase in temperature was a decrease in drug entrapment efficiency. The observed change in drug entrapment efficiency with heating temperature for different heating times was difficult to explain and may be further studied.

Effect of Heating Time on Drug Entrapment Efficiency

As is evident from **Table 1** and **Figure 4B**, there was a steep fall in drug entrapment efficiency as the heating time was increased from 5 minutes to 15 minutes. This was in line with the observation made for adriamycin-loaded magnetic albumin microspheres by Gupta et al.[16] In drug-loaded albumin microspheres, the drug remains present on the surface as well as in the matrix. It is the surface-bound drug that is loosely held and is usually lost to the external oily phase during preparation or to solvents during the washing stage.[17] In the present case too, the sharp decrease in drug entrapment efficiency may be due to the loss of the drug that is present on the surface of the microspheres to the external phase. As per Bertucci et al,[18] serum albumin contains a binding site for 5-FU. Though it needs to be established whether the same holds true for egg albumin, the change in drug entrapment efficiency may be due to some interaction between 5-FU and albumin. Further experiments may be carried out to establish whether leaching during

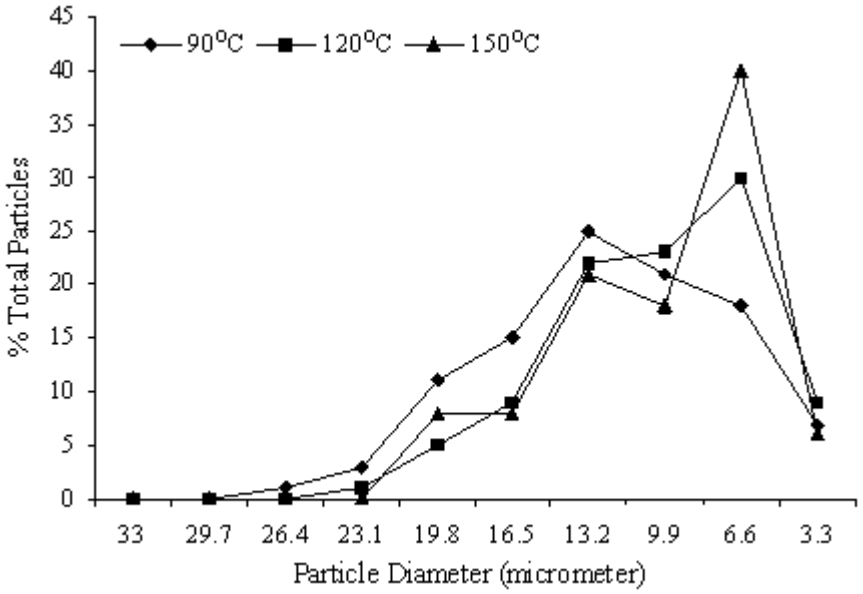

Figure 3. Effect of heating temperature at 25 min on particle distribution.

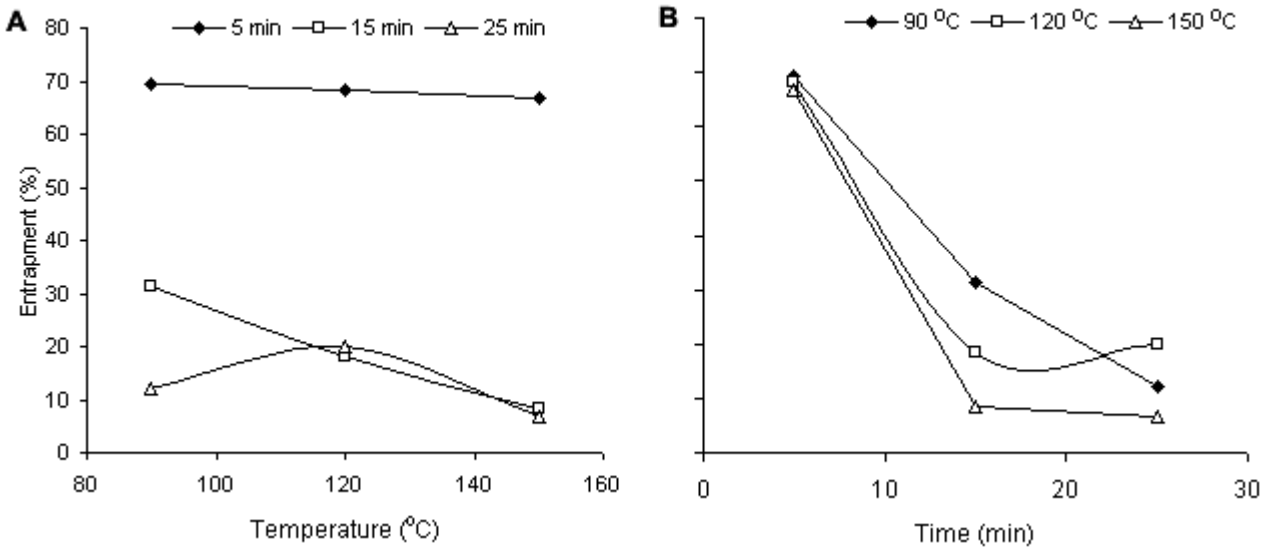

Figure 4. Effect of (A) heating temperature and (B) heating time on albumin entrapment in microspheres.

washing, leaching during binding, or both types of leaching are responsible for the observed decrease in drug entrapment efficiency. Further increase in heating time from 15 minutes to 25 minutes did not produce any significant change ($P > .05$), as the bulk of the drug present on the surface may have already been lost to the external phase.

CONCLUSION

In this study, we found that both heating temperature and heating time affect mean particle size, particle size distribution, and drug entrapment efficiency of albumin microspheres. The change in heating temperature may affect the particle size of the product, especially when heating is carried out at a lower temperature (90°C-120°C). Hence the temperature should be selected on the basis of desired size range. Given

that it is desirable for a maximum amount of the drug used in the preparation to become entrapped in microspheres, heating temperature and heating time for denaturation of albumin should be selected cautiously, as both have a significant effect on drug entrapment efficiency. In the present case, the highest entrapment was found in batches prepared by heating at 90°C for 5 minutes. However, the extent of stabilization at the selected temperature and the time of heating should also be taken into consideration, as they may affect the release of drugs to target tissue.[17]

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