RESEARCH ARTICLE

Preparation and evaluation of biodegradable microspheres of methotrexate

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The objective of the present study is to prepare sustained-release methotrexate microspheres of bovine serum albumin in different ratios by the emulsion cross-linking method. The prepared microspheres were subjected to various physicochemical evaluation and *in vitro* release studies. The drug release from microspheres of 1:6 ratio is the most constant and prolonged drug release is diffusion followed by erosion. The characteristics of the prepared microspheres are conducive to the formulation of the sustained release drug delivery system.

Key words: *Bovine serum albumin, methotrexate, scanning electron micrographs*

INTRODUCTION

Microspheres are the colloidal drug delivery system. Microspheres are characteristically free-flowing powders consisting of proteins/synthetic polymers that are biodegradable in nature and ideally having a particle size less than 200 μm. Biodegradable microspheres can be utilized to direct drugs to certain organs through capillary blockade. Its success depends on the size of the microspheres used and on the mode of administration (intravenous/intra-arterial).[1] The microspheres can also be used for targeting anticancer drugs to the tumor.

Methotrexate (MTX) is an antineoplastic antibiotic whose mechanism is similar to alkylating agents. It is a highly toxic drug with a very low therapeutic index.[2] It causes toxicities like stomatitis, gingivitis, glossitis, ulceration, and bleeding of the mucous membrane when given orally and hematological effects like leucopenia, thrombocytopenia, anemia, hemorrhage from various sites in single-dose intravenous administrations, and also some hepatic toxicities by administering as conventional dosage forms. Sustained and targeted delivery of MTX will reduce these toxicities considerably by maintaining a low and constant level of drug in the blood.[3,4]

Microspheres are reported to possess high specificity toward tumor sites and good controlled release properties were exhibited by using biodegradable polymers.[5,6] Hence, it was envisaged to prepare microspheres of MTX using biodegradable carrier albumin and evaluate them for sustained release.

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MATERIALS AND METHODS

MTX was obtained from Dabur Research Foundation, India. Bovine serum albumin (BSA), Span80, and glutaraldehyde were gift samples from S.D. Fine Chem. Ltd., India. Trypsin was received as a gift sample from Loba Chemie, Bombay, India.

Preparation of microspheres of MTX

BSA microspheres containing MTX were prepared by the emulsion technique.[7,8] An aqueous solution of BSA (12.3–36.6% W/V) was adjusted to pH 10. Take 1 ml of BSA solution and to this add 0.061 g of MTX and dissolve. This solution was dispersed in a 10-ml solution of Span80 in toluene (13%) by vortexing. This dispersion is stirred at 300 rpm and gradually added to 0.5–1 ml of glutaraldehyde in distilled water (10%), which was maintained at pH 10. After initial cross-linking (i.e. 20 s), add 20 ml of acetone with continuous stirring for 5 h. The microspheres formed were washed with acetone five times and with water three times and then dried in a vacuum desiccator to get free-flowing microspheres in a powder form. By varying the drug:polymer ratio, six batches of microspheres were prepared[9-11] [Table 1].

Physicochemical evaluation of the microspheres

Morphology

The surface morphology of the microspheres was observed by scanning electron microscopy (SEM) (model JSM-5310; JEOL, Tokyo, Japan). The microspheres were mounted in metal stubs using a double-sided adhesive tape. After being vacuum coated with a thin layer

Table 1: Composition of MTX microspheres of bovine serum albumin

Batch code	Drug:Polymer ratio
BSA-1	1:1
BSA-2	1:2
BSA-3	1:3
BSA-4	1:4
BSA-5	1:5
BSA-6	1:6

(100–150 A°) of gold, the microspheres were examined by SEM at different magnification.[12,13]

Size analysis

It is carried out by using a compound microscope at ×45. Dried microspheres were first redispersed in distilled water and placed in a glass slide and the number of divisions of the calibrated eye piece was counted by a micrometer using a stage micrometer. The average size of the particles was determined.[14]

Melting point

A small amount of the microspheres was taken and they were ground to remove the coating material and then subjected to melting point determination.

Infrared spectroscopy

Triturate about 1 mg of the microspheres with approximately 300 mg of dry, finely powdered potassium bromide Infrared (IR). Grind the mixture thoroughly, spread it uniformly in the die, and compress under vacuum at a pressure of about 800 Mpa. Mount the resultant disc in a holder in the IR spectrophotometer and record the spectra in the IR region of 4000–625/cm^{-1}. Compare the positions and the relative intensities of the absorption bands of the microspheres obtained with that of the pure MTX.

Thin layer chromatography

A pure sample of MTX 5 mg was dissolved in 0.5% W/V solution of ammonium carbonate to produce 4.17 ml. Similarly, 5 mg-equivalent MTX microspheres and 5 mg of BSA were separately dissolved in 0.5% W/V solution of ammonium carbonate. These solutions were spotted in a thin layer chromatography (TLC) plate and marked. Then, the plate was placed in the closed vessel containing 15.6% W/V solution of sodium dihydrogen phosphate adjusted to pH 4.8 with sodium hydroxide solution as the mobile phase. The developed spots were noted and the Rf values were measured. The drug stability was studied by means of comparison of the Rf values.

Drug content determination

Microspheres of 5 mg were accurately weighed and dispersed in 15 ml of KCl–HCl buffer solution of pH 2 by continuous stirring for 15 min in a magnetic stirrer. Then, 10 ml of pH 2 buffer solution containing trypsin 5 mg/ml was added to the above solution and then shaken for 48 h in 37°C. Complete dissolution of the matrix components was confirmed by light microscopy. MTX concentration of the resulting solutions was determined.

Determination of % of drug encapsulated in the microspheres

$$\% \text{ of drug encapsulated} = \frac{\text{Drug content in microspheres}}{\text{Drug included in the formulation}} \times 100$$

In-vitro drug release studies[15-18]

The drug release studies of the microspheres were carried out for up to 24 h using the dissolution test apparatus (paddle method). Five hundred milliliters of phosphate buffer pH 7.2 was taken as the dissolution medium. Microspheres containing 50 mg-equivalent of MTX were accurately weighed and tied in the muslin cloth. The paddle was rotated at 50 rpm. The dissolution medium was thermally controlled at 37°C. Samples of 1 ml were withdrawn from the dissolution media at suitable time intervals. The sample volume was replaced by an equal volume of fresh medium. The drawn 1 ml samples were made up to 10 ml and analyzed using a UV spectrophotometer at 303 nm.[19]

RESULTS AND DISCUSSION

In the present study, BSA microspheres loaded with MTX were prepared by the emulsion cross-linking method described by Helmut Vienstein *et al.*, with modification in solvent, surfactant, energy used for emulsification, and pH. The inner aqueous phase constitutes BSA (12.3–36.6% W/V) and drug dissolved in alkaline medium. This solution is dispersed in the continuous phase of toluene emulsified with 13% V/V Span80. The microspheres formed were stabilized with 10% glutaraldehyde solution in water. Microspheres of drug polymer ratio 1:1 could not be obtained, indicating the insufficiency of the proportion of the polymer for the formation of the microspheres. Microspheres of all the other five batches were discrete and free flowing.

Morphology

The microspheres were found to be spherical with quite smooth surfaces when viewed microscopically (×45 and ×100).

SEM of the microsphere shows that microspheres have a spherical shape with porous outer skins [Figures 1-3].

Size distribution

Batch (BSA-II) was prepared by using a mechanical stirrer at a speed of 200 rpm. As the size range of the microspheres obtained was above 20 μ, the emulsification was subsequently carried out in a magnetic stirrer at a speed of 300 rpm. The microspheres of the other batches fall in the narrow range of 5–25, with the average being 6–8 μ [Tables 2-4].

Melting point

The melting points of the free drug and the drug in the microspheres were found to be the same (192°C), indicating

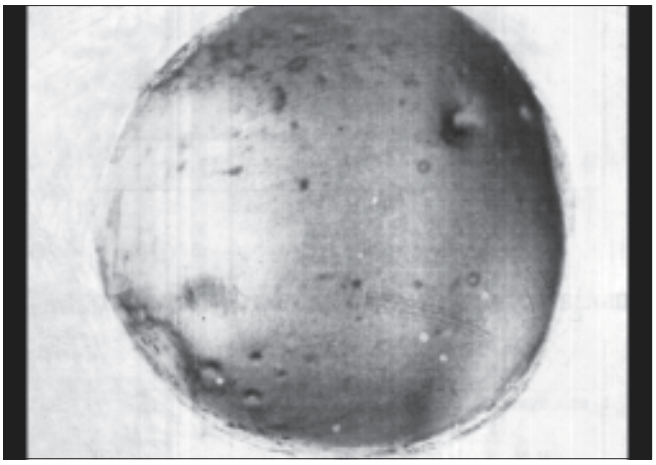

Figure 1: Microsphere 3

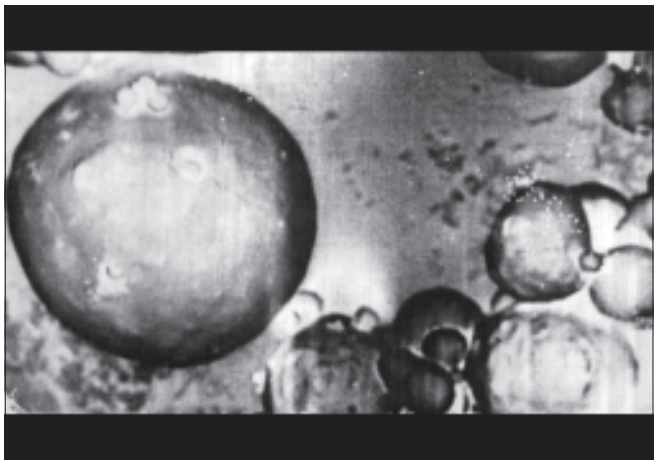

Figure 2: Microsphere 1

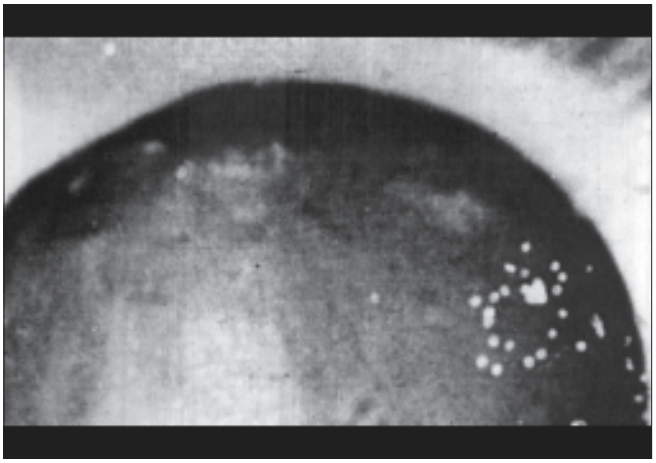

Figure 3: Microsphere 2

Table 2: Size distribution analysis data

Batch code	Average size in μm
BSA-2	20.989
BSA-3	6.14
BSA-4	8.058
BSA-5	7.33
BSA-6	7.96

Table 3: Drug content and percentage of drug entrapped

Batch code	Amount of MTX in 5 mg of the microsphere	Entrapment efficiency in %	Drug remaining un-encapsulated in %
BSA-2	0.5	30.12	69.88
BSA-3	0.9	72	28
BSA-4	0.85	85	15
BSA-5	0.75	90.04	9.96
BSA-6	0.7	98.04	1.96

that there is no change in the nature of the entrapped drug due to the process of formulation of the microsphere.

IR spectroscopic studies

The IR spectra of the free drug and the microspheres were recorded. The identical peaks corresponding to the functional groups and BSA features confirm that neither the polymer nor the method of preparation has affected the drug stability.

Table 4: Data of the biphasic release pattern of MTX from the microspheres

Batch code	Cumulative % release of MTX in the first phase		Cumulative % release of MTX at the 24th h
	Time (h)	Cumulative % release	
BSA-2	17	28	95
BSA-3	18	24.04	64.15
BSA-4	20	24	53
BSA-5	21	24.7	51.03
BSA-6	21	25.29	48.65

Thin layer chromatographic studies

The drug stability in the prepared microspheres was also tested by the TLC method. The Rf values of the prepared microspheres (0.38) were compared with the Rf value of the pure MTX (0.39). It also confirms that neither the polymer nor the method of preparation has affected the drug stability.

Drug content and percentage of drug entrapped

The microspheres were analyzed for the drug content uniformity and the encapsulation efficiency. MTX was found to be encapsulated 30–72% in two batches (BSA-2 and 3), 85% in one batch (BSA-4), and more than 90% in two batches (BSA-5 and 6), which shows that if there is an increase in the concentration of the polymer, the encapsulation efficiency also increases. From the encapsulation efficiency data, we can state that there is no wastage of the drug and hence this method is economical.

***In vitro* drug release studies**

The dissolution of the pure drug was complete within 50 min, indicating that the solubility of the drug in the dissolution medium is not a limiting factor or a constraint to drug release.

The drug release from all the batches (BSA-2 to 6) is sustained over 24 h. A biphasic release pattern was observed with all the batches, which is a slow first phase followed by a rapid release. This is in concurrence with the observations made with the release from the biodegradable microspheres. The second rapid release after 17–21 h may be attributed to the increased permeability of the microspheres and facilitated diffusion of the drug through the newly generated pores and surfaces and also the degradation of the polymer, which might have enhanced the release. As the polymer ratio increased, the time taken for the change in the release behavior was also prolonged, although relatively high microspheres were obtained in BSA-2 and its release was unusually high. This may be due to the lower polymer proportion. A perfect positive correlation was observed with the release behavior in all the batches, showing a constant controlled delivery except the slight 1st-h burst effect. This might have been due to the un-entrapped drug. The identical slopes calculated for the straight lines in the drug delivery plots indicated a similar rate of drug delivery in all the batches.

CONCLUSION

The obtained microspheres are fine and free flowing, the method followed is economical to get reproducible microspheres, and the drug:polmer ratio has an impact on the drug encapsulation efficiency and *in vitro* drug release. The drug release from microspheres of 1:6 ratio is the most constant and prolonged (24 h), the mechanism of dug release being diffusion followed by erosion and characteristics of the prepared microspheres being conducive to the formulation of a sustained-release drug delivery system.

In the present study, an extensive attempt has been made to incorporate the maximum amount of drug in the microspheres by applying a minimum economic implement.

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