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Research Article

DEVELOPMENT AND EVALUATION OF METHOTREXATE LOADED BSA MICROSPHERES

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Abstract: Methotrexate (MTX) loaded bovine serum albumin (BSA) were prepared by emulsion cross linking method. The mean diameter of the microspheres was affected by the type of emulsion stabilizer, polymer concentration, aqueous and organic phase volume and stirring speed. The prepared microspheres were subjected to various physicochemical evaluation and *in vitro* release studies. The results indicated that the microspheres system studied well be a promising tool for sustained release delivery of methotrexate.

Key words: Microspheres, methotrexate, bovine serum albumin

INTRODUCTION

Microparticulate delivery systems are reliable means of delivering the drug to the desired concentration at the site of interest without untoward effect. It has added advantages over the conventional delivery system which include increased bioavailability, subject variability and drug induced toxicity and side effect.[1-3]

Microspheres are the colloidal drug delivery system. Microspheres are characteristically free-flowing powders consisting of protein/synthetic polymers that are biodegradable in nature and ideally having a particle size less than 200µm. 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.[4]

Methotrexate **(**MTX) is among the most commonly used disease antirheumatic drugs (DMARDs) in the treatment of patients with rheumatoid arthritis (RA). It is taken once a week in doses commonly ranging between 7.5 and 20 mg. Whereas in some clinical trials the weekly dose was divided into 3 equal aliquots taken in 12-hour intervals,[5,6] patients with RA generally take the entire dose at once. The most common route of administration is oral, but parenteral (intramuscular, subcutaneous, and occasionally intravenous) routes are preferred for some patients. Although bioavailability of oral MTX is similar to that of parenteral MTX, [7-9] there are reports of patients who respond to intramuscular injections but not to oral MTX. A possible explanation for this observation is that the bioavailability of oral MTX relative to intramuscular MTX was 13.5% lower at the usual maintenance dose (mean, 17 mg) than at the 7.5-mg dose commonly used at the initiation of MTX therapy.[10]

MATERIALS AND METHODS

Methotrexate was obtained from Khandelwal Laboratory Pvt. Ltd. Mumbai as a gift sample. Bovine serum albumin, Acetone, Span 80, Toulene, Glutaraldehyde was purchased from CDH chemicals.

Preparation of BSA microspheres containing MTX: BSA microspheres were prepared by emulsion technique. About 5gm of BSA was dissolved in 25 ml of water pH was adjusted to 10. Take 5 ml of BSA solution and to this add 45 mg of methotrexate and dissolve. This solution was dispersed in a 10 ml solution of span 80 in toluene (0.4% w/v). This dispersion was dispersed is stirred at 200 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 add 20 ml of acetone with continuous stirring for 5 h. This microspheres formed were washed with acetone 5 times and water 3 times and then dried.[11]

Process variables: Various process variables, which could affect the preparation and properties of the microspheres shown in table 1, were identified and studied. The method of preparation was accordingly optimized. Concentration of emulsifying agent and stirring rate of microspheres preparation were selected for optimization of formulation.

Physicochemical evaluation of the microsphers:
Morphology: The surface morphology of the microspheres was observed by scanning electron microscopy (SEM) (FEI Quanta -200 MK2, Netherland)
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.
Percent yield of microspheres: The prepared microspheres were collected and weighed. The weighed microspheres were divided by the total weight of all the non-volatile components used for the preparation of the microspheres. Percent yield of microspheres of different formulation batches was reported in Table 4.2. Percent yield was calculated by using following formula,

% yield = (Weight of microspheres collected / Weight of all non volatile components) × 100

Percentage drug entrapment: Percent drug entrapment of all the batches prepared was determined ultra-violet spectrophotometrically to study the effect of various variables. An accurately weighed 100 mg microspheres containing MTX were washed with specific amount of methylene chloride. Then microspheres were dissolved in 20 ml of ethanol. The solution was filtered with a whatman filter paper (#40) and analyzed spectrophotometrically at 307 nm using UV-visible spectrophotometer. Percent drug entrapment of microspheres of different formulation batches was reported in Table 2.
% drug entrapment = (Calculated drug content / Theoretical drug content) × 100
In vitro drug release studies: Basket type dissolution apparatus (Dissolution rate test apparatus USP/ IP/ BP STD) has been employed for dissolution study of microspheres. Microspheres 100 mg was filled in gelatin capsule. The capsule was placed in basket. Basket was placed in 900 ml of dissolution medium (PBS pH 7.4). The basket was rotated at 100 rpm and the temperature of dissolution medium was thermostatically controlled at 37±0.5^{0}C. The pH of the dissolution medium was kept for 2 h. Sample were withdrawn from the dissolution medium at various time intervals using a pipette and replaced with fresh phosphate buffer solution pH 7.4, respectively and analyzed using UV-spectrophotometer at 307 nm.

RESULTS AND DISCUSSION

In the present study, BSA microspheres loaded MTX were prepared by the emulsion cross-linking method, with modification in solvent, surfactant, energy used for emulsification, and pH. The inner aqueous phases constitute 5 g BSA and to this add 45mg of drug. This solution is dispersed in the continuous phase of toluene emulsified with span80. The microspheres formed were stabilized with glutaldehyde solution in water. Microspheres of all the other batches were discrete and free flowing.

Morphology
SEM of the microsphere shown that microspheres have a spherical shape with porous outer skins (figures 1 and 2).

Size distributon
The average particle size of microspheres was found to be in the size range of 33.90 ± 3.97 to 30.32 ± 3.22 at 100 rpm stirring speed, 30.67 ± 3.74 to 28.01 ± 4.63 at 200 rpm stirring speed and 27.32 ± 3.28 to 24.57 ± 3.78 at 300 rpm stirring speed in different concentration ratio of emulsifying agent. The average particle size of BSA microspheres decreased as agitation speed increased from 100 to 300 rpm. This was expected because high turbulence caused frothing, results in decreased in mean particle size of microspheres. These results are in agreement with the result demonstrated that an increased in stirring rate shows a decreased in the mean particle size of microspheres because of high turbulence.

Melting Point:
The melting points of the free drug and the drug in the microspheres were found to be same (192°C), indicating that there is a no change in the nature of the entrapped drug due to the process of formulation of the microspheres.

Percent yield and drug entrapped:
The percent yield was found to be 77.06 ± 3.1 to 89.01 ± 1.3 %. It was found that a stirring speed higher than 300 rpm results in the formulation of needle shaped microspheres, where as a stirring speed below 200 rpm showed non uniform shape of microspheres with agglomeration. The average percent drug entrapment was found to be in range of 65.78 ± 1.6 to 75.60 ± 2.9 % and it was observed that the formulation developed in 0.4 % w/v of emulsifying agent (span 80) facilitates better entrapment. At 200 rpm speed and 0.4% w/v emulsifying agent showed highest drug entrapment in microspheres without any agglomeration.

In vitro drug release studies:
In vitro drug release profile of MTX from different formulation batches in drug released from formulation prepared at 100 rpm as compared to formulation prepared at 200 and 300 rpm stirring speed, which was due to increased average particle size which provide less surface area for dissolution, but the concentration of emulsifying agent was not significantly affect the in vitro drug profile of MTX. The microspheres of BSA also released the drug in pH 7.4, which may be due to the presence

of pores on the microspheres surface. Released of drug from microspheres in pH 7.4 may be due to diffusion process.

Table: 1 Formulation code and variables used in the preparation of microspheres.

S. No	Formulation code	Concentration of emulsifying agent (%)	Stirring rate (rpm)
1	MM 1a	0.2	100
2	MM 2a	0.4	100
3	MM 3a	0.6	100
4	MM 4a	0.8	100
5	MM 1b	0.2	100
6	MM 2b	0.4	200
7	MM 3b	0.6	200
8	MM 4b	0.8	200
9	MM 1c	0.2	200
10	MM 2c	0.4	300
11	MM 3c	0.6	300
12	MM 4c	0.8	300

Table: 2 Particle size, % Yield and % Drug entrapment of different formulation batches (based on emulsifying agent concentration and stirring rate).

S. No	Formulation code	Average particle size (µm)	Yield (%)	Drug entrapment (%)
1	MM 1a	33.90 ± 3.97	79.89 ± 1.2	68.76 ± 1.2
2	MM 2a	32.06 ± 3.67	83.48 ± 0.9	69.00 ± 2.9
3	MM 3a	30.32 ± 3.22	79.66 ± 1.8	67.08 ± 3.1
4	MM 4a	33.44 ± 3.09	78.00 ± 0.6	65.78 ± 1.6
5	MM 1b	29.03 ± 2.90	86.30 ± 0.4	72.98 ± 1.6
6	MM 2b	30.67 ± 3.74	89.01 ± 1.3	75.60 ± 2.9
7	MM 3b	29.87 ± 3.06	83.48 ± 1.6	71.71 ± 2.3
8	MM 4b	28.01 ± 4.63	87.45 ± 0.7	74.09 ± 2.6
9	MM 1c	27.32 ± 3.28	79.52 ± 2.5	69.89 ± 3.6
10	MM 2c	26.12 ± 2.67	77.06 ± 3.1	71.07 ± 4.1
11	MM 3c	26.98 ± 2.34	79.33 ± 0.7	65.88 ± 3.4
12	MM 4c	24.57 ± 3.78	81.19 ± 1.2	69.00 ± 2.3

Values are average of three reading ± standard deviation.

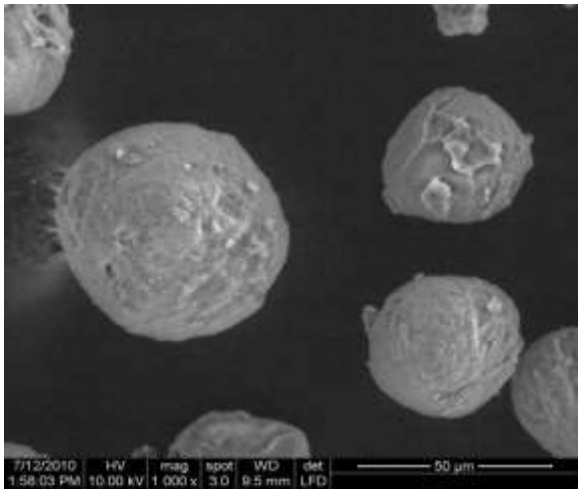

Fig. 1: Scanning electron photomicrograph of BSA microspheres (1000x).

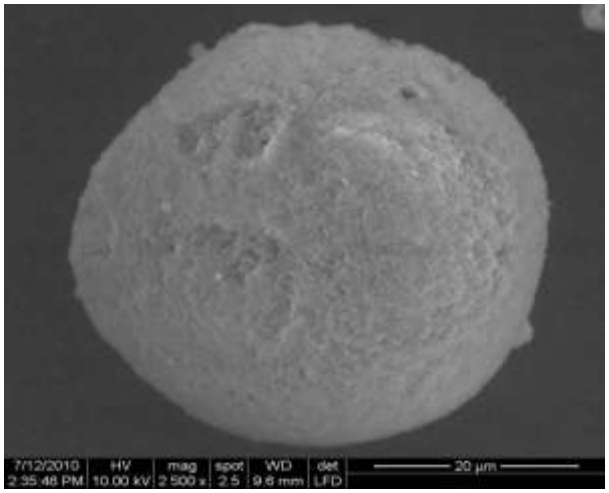

Fig. 2: Scanning electron photomicrograph of BSA microsphere (2500x).

CONCLUSION

The obtained microspheres are fine and free flowing, the method followed is economical to get reproducible microspheres, and the drug:polymer ratio has an impact on the drug encapsulation efficiency and *in vitro* drug release. The drug release from microspheres is the most constant and prolonged, the mechanism drug release being diffusion followed by erosion and characteristics of the prepared microspheres.

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