RESEARCH PAPER

Accepted 15 January 2001
Revised 21 December 2000
Received 19 June 2000
Indian J. Pharm. Sci., 2001, 63(2) 123-127

A Study on Albumin Microspheres Containing Metronidazole

V. SANKAR[1], V. RAVICHANDRAN[1], V. SIVANAND[1], S. RAGHURAMAN AND G. VELRAJAN*
Department of Pharmaceutics
*S. Chattanatha College of Pharmacy, Kodikurichi, Tenkasi-627 807, Tamilnadu
[1]Fathima College of Pharmacy, Kadayanallur-627 759, Tamilnadu

The particulate form of albumin has been regarded as a potential carrier of drugs for either site-specific localization or their local application into autonomically discrete sites. Various formulations of metronidazole-loaded albumin microspheres were prepared by heat stabilization process and chemical stabilization process. Effect of stirring rate on size distribution, effect of albumin concentration, effect of cross linking agent on microspheres were the parameters investigated Angle of repose, drug content, *in vitro* and *in vivo* release rate studies were also carried out. *In vitro* release profile for formulations containing metronidazole-loaded albumin microspheres with cross linking agent shows slow sustained release up to 24 h. It also obeys first order kinetics. The studies conducted in rabbits confirm sustained release. Hence albumin microspheres prepared by the heat stabilization process and chemical stabilization process could be used for the treatment of hepatic amoebiasis where the sustained action is needed.

Amoebiasis is one of the most prevalent diseases in tropical countries[1]. Metronidazole is the most effective agent available for the treatment of all forms of amoebiasis including hepatic amoebiasis[2]. Doses in the range of 1.5-2.5 g are administered daily for up to 7 days. The frequent dosing is associated with certain disadvantages like unpleasant taste, anorexia, epigastric distress and abdominal cramps[3]. It is official in IP[4] and BP[5]. The use of albumin microspheres in drug delivery was first suggested by Kramer[6]. Albumin microspheres can satisfy the criteria of ability to bind and release high concentration of drugs, stability after synthesis with a clinically acceptable shelf life[7]. Solid biodegradable microspheres with a drug dispersed throughout particle matrix have received much attention in recent years not only for prolonged release but also for targeting of drug to specific sites[8]. In the present investigation, an attempt was made to encapsulate the drug in albumin microspheres to overcome the above drawbacks and to sustain the release of drug.

*For Correspondence

MATERIALS AND METHODS

Metronidazole IP was procured from Aspamin Pharmaceuticals (P) Ltd., Madurai sunflower oil was purchased from L.T.C Agrotech Ltd, Secunderabad. Gluteraldehyde was obtained from S.D. Fine Chem. Ltd., Boisar. All other chemicals and buffers used were of analytical grade.

Heat stabilization process[9]:

Metronidazole (100 mg) is dissolved in 3 ml of water and then 5 ml of 2% egg albumin is added. The aqueous phase consisting of drug and albumin is added drop wise to 10 ml of sunflower oil and the resulting emulsion in added drop wise to 100 ml of preheated oil which is at 120° with constant stirring at 400 rpm. The stirring was continued for 30 min. Oil phase was decanted. The micospheres were kept washed with petroleum ether. The microspheres were kept in a desicator for 24 h. Dried microspheres were passed through Sieve No. 30 and stored in glass vials. The same procedure was repeated with 800, 1500 and 2000 rpm and with 3%, 4% and 5% albumin concentration.

Chemical stabilization process:

In this process, a cross linking agent or chemical stabilising agent such as glutaraldehyde (2 ml) was added after the addition of emulsion to pre heated oil at 120° with constant stirring at 400 rpm. Stirring was continued for 30 min. Oil phase was decanted. The microspheres were washed with petroleum ether. The microspheres were kept in a desicator for 24 h. Dried microspheres were passed through Sieve No. 30 and stored in glass vials. The same procedure was repeated at 800, 1500 and 2000 rpm and with 3%, 4% and 5% albumin concentration.

Particle size analysis:

Size analysis of all the batches of prepared microspheres were out using a set of standard sieves ranging from 10-120 mesh[10]. The sieves were arranged in such a way that the decreasing order of mesh size, 10 mesh on the top and 120 mesh in the bottom. The microspheres were passed through set of sieves and the amount retained on each sieve was weighed. The arithmetic average diameter (D_{av}) was determined by dividing the total weight size by 100.

Angle of repose:

A funnel was fixed in a stand in such a way that the tip of the funnel was at the height of 6 cm from the surface. The microspheres of 30/40 sieve size were poured through the funnel, so that they form a conical heap on the surface. The height and radius of heap was measured and the angle of repose was calculated.

Drug content determination:

One hundred milligrams equivalent metronidazole-loaded microshperes of 30/40 mesh size were accurately weighed and transferred to a 100 ml standard flask. To this, 50 ml of phosphate buffered saline (pH 7.4) and 50 ml of 0.1 N HCl was added and shaken vigorously for 15 min. Then it was filtered through a Whatman filter paper and the drug concentration was analysed spectrophotometrically at 277 nm[4] against a blank prepared in the same way, but without drug.

***In vitro* release:**

The USP XXII dissolution rate testing apparatus was employed to study the release profile of metronidazole using phosphate buffer (pH 7.4) as a dissolution medium[11]. One hundred milligrams equivalent of metronidazole containing albumin microspheres (30/40 mesh size) was filled in hard gelatin capsules and utilized for the study. The dissolution medium was stirred at a rate of 75 rpm maintaining a temperature at 37±0.5°. Five millilitre samples were withdrawn at 1 h time intervals for 24 h. The concentration was determined by analyzing spectrophotometrically at 277 nm. The same procedure was repeated with 100 mg equivalent of metronidazole tablet and the results were compared.

***In vivo* release:**

Male healthy white rabbits weighing 1.5 to 2 kg were taken for the studies. Rabbits were fasted overnight and were divided into 3 groups of 4 rabbits each. The rabbits were kept in a cage with husk bedding. To group I, a tablet containing 100 mg of metronidazole was given orally with the help of a plastic tube. To the group II, 100 mg equivalent of metronidazole containing albumin microspheres (30/40 mesh size) without the cross linking agent was filled in empty hard gelatin capsules and given orally. To the group III, 100 mg equivalent of metronidazole containing albumin microspheres with the cross linking agent was filled in hard gelatin capsule and given orally. Then 0.5 ml blood samples were withdrawn from the marginal ear vein of the rabbit at hourly intervals using a syringe containing 0.5 ml of 3.8% sodium citrate to prevent clotting. To the above sample 0.5 ml of 0.5 N NaOH and 0.5 ml of 10% $ZnSo_4$ was added to precipitate blood protein. Then it was filtered through a Whatman filter paper and the samples were analysed at 277 nm against a blank. This process was continued up to 8 h.

RESULTS AND DISCUSSION

The particles were mostly discrete, round or spherical and free flowing. The data showing the effect of stirring rate on microspheres has been presented in Table 1. It was found that on increasing the rate of stirring from 400 rpm to 2000 rpm the size of microspheres reduced from 90 μm to 5 μm in heat stabilization process and from 100 μm to 10 μm in chemical stabilization process. But on increasing the stirring rate the drug entrapment is reduced. It was found that in the formulation prepared by heat stabilization process (A_1) (2% protein concentration at 400 rpm) near by 41% of drug was entrapped where as in the case of formulation (A_4) (5% protein concentration at 2000 rpm) only 24% of drug was entrapped. The same effect was confirmed and reported in Table 1 for the chemical stabilization process.

The formulations which exhibit different mean particle sizes are reported in Table 1. It was found that when

TABLE 1: PARTICLE SIZE RANGE OF ALBUMIN MICROSPHERES PREPARED BY TWO DIFFERENT PROCESS

Product	Rpm	Size range (μm)	Protein concentration (% w/v)	Mean particle size (μm)
A_1	400	60-90	2	51.3
A_2	800	40-50	3	62.3
A_3	1500	20-30	4	70.8
A_4	2000	5-20	5	81.5
B_1	400	60-100	2	59.8
B_2	800	50-70	3	71.2
B_3	1500	25-40	4	84.5
B_4	2000	10-20	5	92.1

A_1 to A_4 and B_1 to B_4 represents microspheres prepared by heat stabilization process and chemical stabilization process respectively at different rpm and at different protein concentration.

the protein concentration increases, the particle size also increases under heat and chemical stabilization processes. Inclusion of a cross linking agent, slightly alters the particle size as shown in Table 1. Even though the particle size is increased the drug entrapped is not improved in heat and chemical stabilization processes. It may be due to the increased stirring of the medium by the mechanical stirrer.

The arithmetic average diameter (d_{av}) was about 351±1.26 microns for formulation A_1 (2% protein concentration at 400 rpm without cross linking agent) whereas it was around 391±0.91 microns for formulation B_1 (2% protein concentration at 400 rpm. with cross linking agent). This shows inclusion of cross linking agent increases the average diameter to a smaller extent and the particles were found to be free flowing in nature. The size distribution analysis for the various batches prepared with the cross linking agent were depicted in fig. 1.

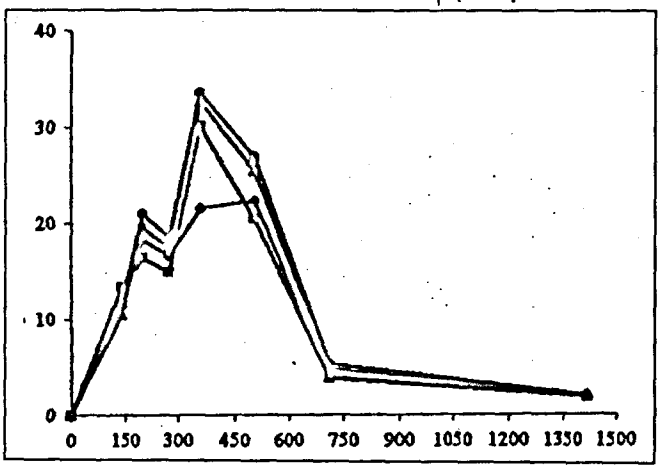

Fig 1: Size distribution analysis
Size analysis from metronidazole microspheres containing a cross linking agent at (-o-) 2% protein concentration at 400 rpm, (-▲-) 3% protein concentration at 800 rpm, (-□-) 4% protein concentration at 1500 rpm and (-◆-) 5% protein concentration at 2000 rpm.

It was found that all the prepared microspheres have a tanθ value in the range of 17-22° which indicates that all the formulations are free flowing in nature. The values

TABLE 2: ANGLE OF REPOSE AND DRUG CONTENT OF THE MICROSPHERES

Product code	Angle of repose θ	Per cent drug content*
A_1	21°.28'	40.34±0.62
A_2	20°.40'	32.38±0.49
A_3	19°.68'	28.62±0.56
A_4	17°.72'	24.82±0.38
B_1	22°.48'	46.42±0.39
B_2	20°.34'	42.62±0.26
B_3	19°.27'	36.49±0.49
B_4	18°.44'	31.42±0.89

*Data are average of three determinations

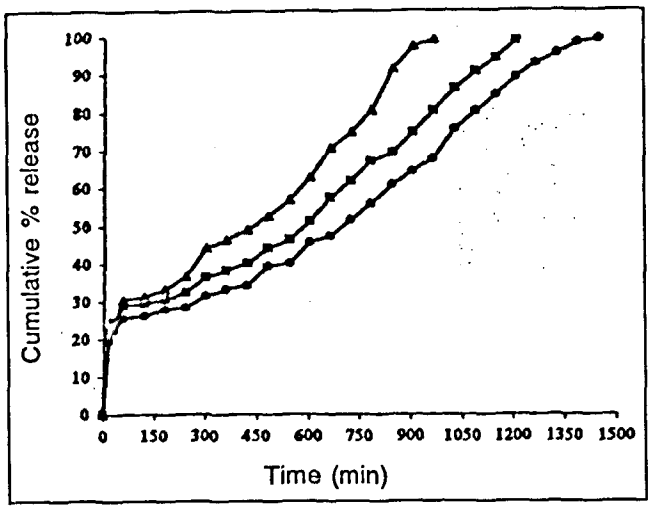

Fig. 2: Cumulative % release of metronidazole from tablet and microspheres
(-▲-) represents tablet containing 100 mg of metronidazole (-□-) represents albumin microsphere containing 100 mg equivalent of metronidazole prepared with 2 % Protein concentration at 400 rpm without the cross linking agent. (-○-) represents albumin microsphere containing 100 mg equivalent of metronidazole prepared with 2% Protein concentration at 400 rpm with the cross linking agent.

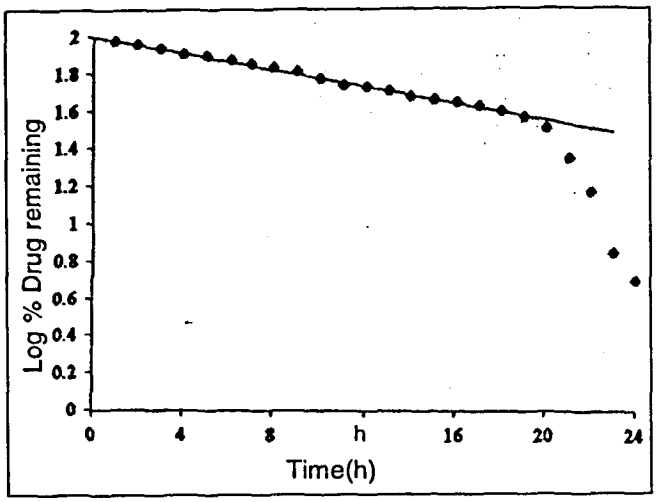

Fig. 3: Release pattern of metronidazole
(-◆-) First order release kinetics for metronidazole microspheres prepared with 2% protein concentration at 400 rpm with the cross linking agent.

are shown in Table 2. The amount of drug loaded in different formulations are reported in Table 2. It was found to be in the range of 24 to 40% in case of albumin microspheres prepared by heat stabilization process whereas in the case of albumin microspheres prepared by chemical stabilization process it was found to be in the range of 31 to 42%. Inclusion of cross-linking agent in the chemical stabilization process enhances the drug entrapment. The standard deviation among the three values were found to be small. This indicates that the drug is distributed almost uniformly throughout in a batch of microsphere. This technique was found to be reproducible. But however the entrapment decreases with respect to increase in rpm.

Cumulative percentage of drug release at different time intervals are depicted in fig. 2. The percentage release of metronidazole from the tablets at 15 min was 22% where as in the formulation A_1 (2% protein concentration at 400% rpm without cross linking agent) the percentage release was 21% and 19% for formulation B_1 (2% protein concentration at 400 rpm with cross linking agent). This data indicates that the burst effect was limited with the cross linking agent. Glutaraldehyde is widely used in protein chemistry as a cross linking agent and it reacts primarily with amino group of proteins in biological systems and forms efficient cross linking with albumin. Due to this drug is held securely in the matrix making the drug diffusion slower. The percentage release of drug at 1440 min confirms the sustained release of drug from the formulation B_1 (2% protein concentration at 400 rpm) with a cross linking agent.

Though other formulations prepared with cross linking agent had shown first order release[12], the amount of drug released was less when compared to formulation B_1 (2% protein concentration at 400 rpm). The release pattern was indicated in fig. 3.

In vivo drug release profile shown in Table 3 indicates *in vivo* release profile from a metronidazole tablet and from albumin microspheres loaded with metronidazole (n=2). The sustained release of drug from formulation B_1 (2% protein concentration at 400 rpm). with a cross linking agent for 8 h and the same release of drug can be expected to 24 h. Due to efficient cross linking with albumin the cumulative mean % release from the formulation B_1 (2% protein concentration at 400 rpm) at 8 h was 37% where as it was higher at 43% for the formulation. A_1 (2% protein concentration at 400 rpm) without cross linking agent and 55% for metronidazole tablets. This also confirms the sustained release of drug from the albumin microspheres loaded with metronidazole.

In conclusion, the microspheres prepared at optimum speed (400 rpm) with 2% protein concentration with a

TABLE 3: *IN VIVO* RELEASE PROFILE FROM A METRONIDAZOLE TABLET AND MICROSPHERES

Time in h	Cumulative mean % of release[x]	Cumulative mean % release[*]	Cumulative mean % release[+]
1	24.19±0.32	21.42±0.72	18.42±0.31
2	28.14±1.92	23.39±0.48	20.26±1.04
3	31.36±1.06	28.26±1.26	24.38±1.62
4	37.28±0.92	32.42±1.06	28.42±1.51
5	41.46±0.52	35.62±0.94	31.42±0.32
6	47.32±1.98	38.92±1.23	32.12±0.41
7	51.47±0.86	40.46±1.24	34.38±0.28
8	55.38±0.71	43.26±0.32	37.42±0.31

Data are mean of two determinations. x indicates release from 100 mg metronidazole tablets. *indicates release from formulation A_1 (2% protein concentration without cross linking agent. + indicates release from formulation B_1 (2% protein concentration) with cross linking agent.

cross linking agent shows good percentage drug entrapment and sustained release. Moreover the diffusion of drug from the cross linked structure depend on the degree of cross-linking agent. Hence while formulating microspheres rpm should be taken into account as an important criteria. In this study chemical stabilization process was found to be superior over heat stabilization process in the drug entrapment. Hence the methods employed for preparing microspheres and the parameters observed were reproducible albumin microspheres loaded with metronidazole can be used for the treatment of hepatic amoebiasis were sustained action is needed.

REFERENCES

1. Tracy, J.W. and Webster, L.T., In; Hardman, J.G., Limbard, L.E., Molinoff P.B. and Gilman, A.G., Eds., Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th Edn., McGraw-Hill, New York, 1996, 995.
2. Tirpathi, K.D., In; Medical Pharmacology, 3rd Edn., Medical Publishers, New Delhi, 1994, 742.
3. Reynolds, J.E.F., Eds., In; Martindale: The Extra Pharmacopoeia, 30th Edn., The Pharmaceutical Press, London, 1993, 516.
4. Pharmacopoeia of India, 3rd Edn., Vol. 1, Controller of Publications, Ministry of Health, Govt. of India, New Delhi, 1985, 319.
5. British Pharmacopoeia, Vol. 1, HMSO Publications Centre, London, 1993, 430.
6. Krammer, P.A., **J. Pharm. Sci.,** 1974, 63, 1646.
7. Illum, L. and Davis, S.S., In; Polymer in controlled drug delivery, Wright Bristol, New York, 1987, 25.
8. Davis, S.S. and Hansrani, P., **Int. J. Pharm.,** 1985 63, 69.
9. Desy, P.B., In; Microencapsulation and Related Drug Processes, Drugs and Pharmaceutical Sciences, Vol. 20, Marcel Dekker, Inc., New York, 1984, 225.
10. Coben, L.J. and Libermann, H.A. In; Lachman, L., Liberman, H.A., and Kanig, J.L., Eds., The Theory and Practice of Industrial Pharmacy, 3rd Edn., Varghese Publication, Bombay, 1987, 27.
11. The United states Pharmacopoeia XXIII, United States Pharmacopoeial Convention, Rockville, MD., 1995, 1019.
12. Brahmankar, D.M. and Jaiswal, B.S., In; Jain, M.K., Eds., Biopharmaceutics and Pharmacokinetics, a Treatise, 1st Edn., Vallabh Prakashan, New Delhi, 1995, 270.