Development of a Protein-Based Model Microparticle Drug Delivery System

A. BHATTACHARYA AND A. MUKHERJEE*
Division of Pharmaceutical Technology, Department of Chemical Technology,
University College of Science & Technology, Calcutta University
92, A.P.C. Road, Calcutta -700009

Accepted 19 September 1999
Received 11 May 1999

A new generation model drug mianserine is microencapsulated in protein. Lipid soluble pure mianserine was emulsified under optimized conditions in aqueous protein solution using isopropanol as co-emulsifier. Resultant clear microemulsion can readily be used through a micronized spray in denaturation solvent environment to produce fairly uniform protein coated microcapsules. Particle size distribution, particle character was analysed in SEM and *in vitro* drug release studied.

Microparticulation have come up as an efficient tool for drug delivery and sustained release. Several attempts were taken to exploit various advantages of microparticulation including some non-conventional approaches like oral peptide delivery[1] and systemic drug targeting[2]. Systemic microparticular drug delivery systems can utilise two main systems-phagocytable and non-phagocytable system. Drug distribution in non-phagocytable systems often followed by sheer particle size distribution while in case of phagocytable systems size distribution as well as opsonization character play an important role in drug distribution. Phagocytable microparticles containing drug candidate can readily concentrate in liver and other reticulocyte processing area differentiated mainly by it's particular character[3,4]. Some earlier approaches tried to take these advantages to deliver several chemotherapeutics[5].

This work is a successful attempt to microencapsulate a newer multiple serotonin receptor modulator, mianserine in a narrow particle size distribution. The method essentially involves a suitable microemulsion (o/w) formation with the drug in the internal phase and the protein (albumin) in external phase. Heat denaturation technique[6] was particularly avoided. This microemulsion can readily be converted into microcapsules by atomized spray in a denaturing-recoiling media. The resultant microparticles can then be harvested out, washed and stored for sufficient time period. Particle size distribution, drug release and SEM (Scanning Electron Microscopy) characterization of these uniform microcapsules were undertaken.

Mianserine hydrochloride was obtained as a gift from Torrent Pharmaceuticals, Ahmedabad. BSA (bovine serum albumin) was purchased from SRL, India. Solvents used were of HPLC grade, water was double distilled and used after filtration through millipore 0.45 μ. Hitachi U2000 spectrophotometer was used for analytical purposes. SEM was carried out in Hitachi-model No. 415 A. Scanning electron microscope.

BSA was chosen for initial studies as it is a well known protein material and several studies have already utilised BSA as polymer substance[7]. However, several loopholes were observed in BSA encapsulation studies so far, these include heat denaturation rendering the method nonviable for handling of many drugs which are heat sensitive. Many other method utilise water soluble drugs only leading to high protein binding therefore, in effect, producing protein-bound microparticulation and not microencapsulation. Systems using emulsion and cross linker addition do not produce stable emulsion and do not take care of ionic character of the medium resulting invariably in much wider particle size distribution which are often not much of practicable use.

***For correspondence**

One and half milligrams of mianserine hydrochloride was dissolved in 10 ml double distilled and filtered (millipore 0.4μ) water. The basic drug mianserine was extracted in 10 ml dichloromethane. This was emulsified in 30 ml of 0.5% aqueous solution of BSA using Tween 80 (0.4 ml) as emulsifier under magnetic stirring for half an hour. The protein : drug ratio was 100:1. The formed emulsion was transformed into microemulsion by adding coemulsifier isopropanol dropwise. About 32 ml isopropanol was required for this purpose. The formed microemulsion was sprayed into acetone with the help of a compressed air driven nebulizer. The microcapsules formed were then separated by cold centrifugation (0°) at 10000 rpm for 10 min., washed quickly with little cold water and dried at room temperature under vacuum. Lyophilisation of the supernatant by precooling in liquid N_2 also produced some yield (≅10% of total) of similar particle size distribution and nature. Two other sets having protein:drug ratio 50:1 and 10:1 were also performed in a similar manner.

The mean particle diameter obtained was at 12 μ following this technique and % occurrence at mean was 64%. Fig. 1 represents the scanning electron micrograph of drug loaded BSA microparticles.

For total release studies of drug load 10 mg of loaded microcapsules were taken in a 100 ml round bottommed flask 50 ml of 1 N HCl was added to it and refluxed for 10 h. It was cooled to room temperature, made alkaline and extracted with three successive 15 ml portions of dichloromethane. The drug content of this organic layer was measured by determining UV absorption at 278.5 nm. The drug release from the microcapsules having protein : drug ratio 100:1, 50:1 and 10:1 were 63%, 81% and 86% respectively.

For *in vitro* drug release studies, ten mg of loaded microcapsules were taken in a test tube and 10 ml of water was added to it. It was kept at 37° with continuous shaking. Two ml of sample was withdrawn at regular time intervals and was immediately replaced with 1 ml of water.

The withdrawn sample was made alkaline the drug was extracted in 4 ml of dichloromethane. The amount of drug present in the organic layer was measured as described above (Fig. 2).

The initial objective of this work was to produce a stable microemulsion. Several emulsifiers were tried for

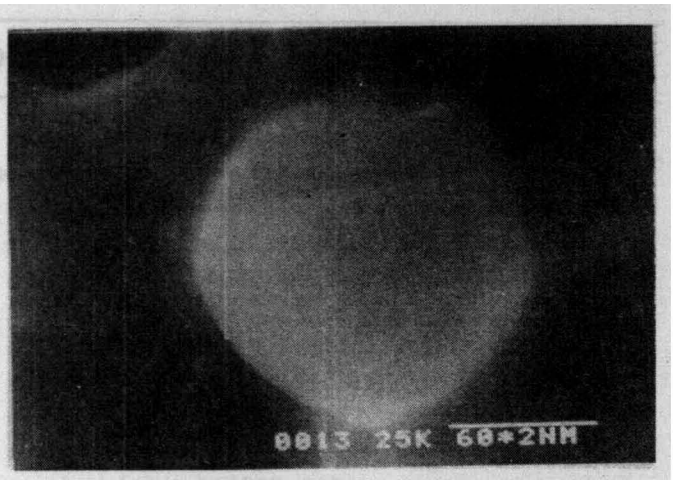

Fig. 1 : Scanning electron micrograph of BSA coated mianserine microparticle at x 5000 at 25 KV; BSA : mianserine ratio 10:1

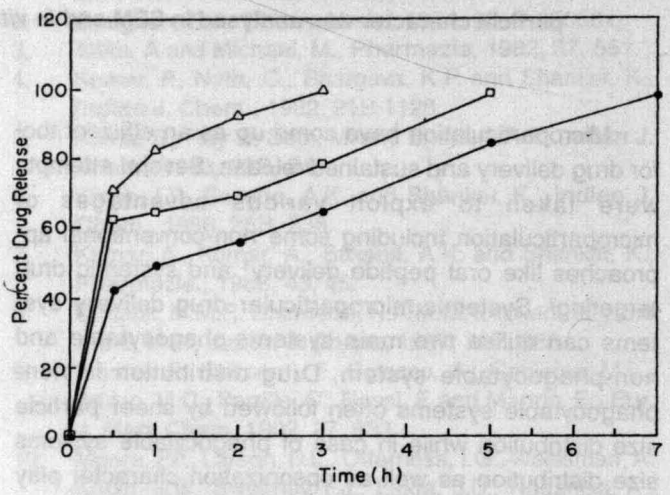

Fig. 2 : *In vitro* release of mianserine microcapsules from different protein: drug ratios (●-100:1, ❐ 50:1, Δ-10:1)

this purpose which included span 80, glyceryl monostearate, lecithin and tween 80 and the last provided excellent stable emulsion. Sonication (20 KHz) used during initial emulsification produced stable emulsion quickly. However, when this stable emulsion was converted to microemulsion by addition of a coemulsifier (isopropanol) no difference was observed in final microemulsion formation as compared to simple stirring method. The microemulsion processes was optimized by measurement of solution turbidity in a turbidity meter (100 nt) and about 32 ml of isopropanol was altogether necessary. Presence of ketone bodies and particulate matter seriously affect protein microemulsion at this stage. Other

coemulsifiers tried included glycerine and polyethylene glycol without much improvement.

The particle size distribution did not vary much in 10:1 and 50:1 formulations. But in 100:1 formulation several larger irregular particles were seen in photomicrography and SEM. In the SEM studies perfectly spherical particles were obtained with 10:1 and 50:1 formulations. They have a smooth external surface suggesting a recoiled protein coated structure. A biphasic nature of drug release curve was observed in all the drug: protein ratio. It has taken seven hours for 100% drug release from 100:1 formulation, five hours from 50:1 formulation and three hours from 10:1 formulations.

For the purpose of analysis a standard curve was first drawn by dissolving known amount of mianserine hydrochloride in water; the solution pH was adjusted to pH 9 with dil. NaOH and then extracted in dichloromethane. This provided an excellent fitting curve ($Y= 0.0156 + 0.1007 x$; $r^2 = 0.9932$); (Y= absorbance, x = concentration).

Total drug release from loaded microparticles were measured by digesting the loaded microparticles in 1 N HCl and the drug released was measured in UV spectrophotometer by extraction. Mianserine being largely protein bound and reactive[8] a prololonged acid hydrolysis apparently resulted in loss of drug material in acid hydrolysis.

In general, this method can be utilised for drug delivery of various lipid soluble substances like steroid hormones, peptides and antigens. HSA (human serum albumin) can readily replace BSA for this purpose, while regulated delay in drug release can be attained using controlled cross linking of albumin hydrogel coat. Work is in progress in this direction.

ACKNOWLEDGEMENTS

One of the author A.B. is thankful to CSIR for the grant of a Senior Research Fellowship.

REFERENCES

1. Nagareya, N., Uchida, T. and Matsuyama, K., **Chem. Pharm. Bull.**, 1998, 46, 1613.
2. Illum, L. and Davis, S.S. **Life Sci.**, 1987, 40, 1553.
3. Kramer, P.A. and Burnstein, T., **Life Sci.**, 1976, 19, 515.
4. Sugibayashi, K., Morimoto, Y., Nadai T., Kato, Y., Hasegawa, A. And Arita, T., **Chem. Pharm. Bull.**, 1979, 27, 204.
5. Poste, G. and Kirsh, R., Biotechnology, 1983, 1, 869.
6. Magdassi, S. and Vinetsky, Y., In; Benita, S.. Eds: Microencapsulation methods and Industrial applications, Vol 73, Marcel Dekker, Inc., New York 1996, 21.
7. Kramer, P.A., **J. Pharm. Sci.**, 1974, 63, 1646.
8. Rasmussen, J.K., **J. Pharm. Biomed Anal,** 1998, 16, 1159.