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Biodegradable microparticulate system of captopril

P.M. Dandagi *, V.S. Mastiholimath, M.B. Patil, M.K. Gupta

Department of Pharmaceutics, K.L.E.S's College of Pharmacy, JNMC Campus Nehru Nagar, Belgaum 590010, Karnataka, India

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Abstract

Albumin microparticles have found many applications in diagnosis and treatment in recent years and more than 100 diagnostic agents and drugs have been incorporated into albumin microparticles. In the present study, bovine serum albumin (BSA) based microparticles bearing captopril were prepared by an emulsification–heat stabilization technique. Four batches of microparticles with varying ratio of drug and polymer were prepared. The prepared microparticles were studied for drug loading, particle size distribution, in vitro release characteristics, in vivo tissue distribution study and stability studies. The microparticles had mean diameter between 2 and 11 μm of which more than 70% were below 5 μm and incorporation efficiency of 41–63% was obtained. In vitro release profile for formulations containing captopril-loaded albumin microparticles with heat stabilizing technique shows slow controlled release up to 24 h. The in vivo result of drug-loaded microparticles showed preferential drug targeting to liver followed by lungs, kidneys and spleen. Stability studies showed that maximum drug content and closest in vitro release to initial data were found in the formulation stored at 4 °C. In the present study, captopril-loaded BSA microparticles were prepared and targeted to various organs to a satisfactory level and were found to be stable at 4 °C.

Keywords: Microparticles; Captopril; Bovine serum albumin; Captopril-loaded microparticles; Emulsification–heat stabilization technique

1. Introduction

The main problems associated with systemic drug delivery include an uneven bio-distribution throughout the body, a lack of drug targeting specificity, the necessity of a large dose to achieve high local concentration and adverse side effects due to such high doses. There is now a growing realization that innovative delivery of drugs would not only increase safety and efficacy levels, but also improve the overall performance of the drug (Catherine et al., 2003). The therapeutic benefits of new systems include increased efficacy of the drug site-specific delivery, decreased toxicity/side effects, increased convenience, shorter hospitalizations, viable treatments for previously incurable diseases, potential for prophylactic applications and lower healthcare costs—both short and long term and better patient compliance (Lee and Robinson, 2000; Lalla, 1991).

Microparticles are small solid particulate carriers containing dispersed drug particles either in solution or crystalline form. The importance of microparticles has been growing because of their use as carriers for drugs or other therapeutic agents. Microparticles are made from natural and synthetic polymers. Different materials have been used for microparticles systems like albumin, gelatin, starch, ethyl cellulose and synthetic polymers such as poly lactic acid, poly cyanoacrylates and poly hydroxybutyrate. Routes of administration are by injection, i.e. intravenous, intramuscular and intraarticular or by the nasal route. Microparticles suffer from a number of disadvantages in their use as carrier systems. Some of these are: they are cleared and taken up from the circulation by the reticuloendothelial cells, burst effect, i.e. premature drug release is seen, target site specificity of microparticles could be improved and poor entrapment of drugs (payload characteristics) is seen (Dvane et al., 2003).

Currently captopril is routinely used in the management of hypertension. Captopril is marketed in the conventional dosage form of tablet in usual strength of 12.5–50 mg. When the drug is administered by oral route it undergoes first pass hepatic metabolism. The bioavailability of captopril is reduced to 55% in presence of food and its half-life is less than 2 h (Ooi and Colucci, 2001). These limitations of captopril in conventional dosage form can be overcome by administering captopril by other routes of administration. The present work is aimed at

* Corresponding author. Tel.: +91 831 2471834; fax: +91 831 2472387.
E-mail address: pmdandagi@yahoo.com (P.M. Dandagi).

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preparing biodegradable microparticles as possible drug carrier for targeting and controlled release.

2. Materials and methods

2.1. Materials

Captopril BP was supplied by Macleods Pharmaceutical Ltd. (Mumbai, India) and bovine serum albumin was purchased from (BSA Fraction V) LOBA Chemie Pvt. Ltd. (Mumbai, India). Vegetable oil (refined sunflower oil) was purchased from Parakh Foods Limited (Pune, India).

2.2. Formulation of microparticles of captopril

BSA microparticles containing captopril were prepared by emulsification–heat stabilization technique. Seventy-five milligrams of BSA was dissolved in 2 ml deionised water containing 0.1% Tween 80, to which 50 mg captopril was added and used as the aqueous phase. The oil phase comprised of 3 ml sunflower oil and 1 ml petroleum ether with 1% Span 80 (as emulsifier), which were mixed together and allowed to stir for 10 min at 800–1000 rpm on a magnetic stirrer. The aqueous phase was added drop wise to the oil phase and stirred at the same rpm for 30 min to form the primary emulsion. This emulsion was then added to 75 ml of sunflower oil preheated to 95–100 °C using 21 No. needle and stirred at 1000–1200 rpm for 15 min to allow the formation and solidification of microspheres. The suspension was then allowed to cool to room temperature with continuous stirring using a magnetic stirrer. On cooling, 150 ml of anhydrous ether was added. The suspension containing the microparticles was centrifuged at 3500 rpm for 30 min and the settled microparticles were washed for three times with ether to remove traces of oil on microparticles surfaces. The obtained microparticles were then vacuum dried in a desiccator overnight and stored at 4 °C in dark (Arshady, 1990; Tabassi and Rajavi, 2003). Four batches of microparticles were prepared by the above-mentioned method and labeled as CM-1, CM-2, CM-3 and CM-4 (Table 1) (Hakan et al., 2000).

2.3. Characterization of microparticles

2.3.1. Entrapment efficiency

2.3.1.1. Unentrapped drug. Ten milliliters of pH 7.4 phosphate buffer saline (PBS) was added to 10 mg of microparticles and this mixture was kept in an ultrasonicator. After centrifugation, the supernatant was filtered through a 0.45 μm filter and the filtrate was suitably diluted with buffer solution. The absorbance of the resulting solution was measured at 217 nm.

2.3.1.2. Entrapped drug. The residue obtained on centrifugation was mixed with 5 ml 0.1N acetic acid and stored at 4 °C for one night. In acetic acid the denaturated protein gets precipitated out, which is then later centrifuged at 5000 rpm. The absorbance of the filtrate was observed at 230 nm after filtration though a 0.45 μm filter and after suitable dilution.

$$\text{Loading efficiency} = \frac{M_{\text{actual}}}{M_{\text{theoretical}}} \times 100$$

where, M_{actual} is the actual amount of drug in formulations determined by above experiment and $M_{\text{theoretical}}$ is the theoretical amount of drug in formulations calculated from the quantity in the fabrication process (Shenoy et al., 1997; Li et al., 1997; Ramesh et al., 2002).

2.3.2. Particle size distribution

Particle size analysis was done by scanning electron microscopy using JEOL JSM-T330A scanning microscope (Japan). Dry microparticles were placed on an electron microscope brass stub and coated with gold in an ion sputter. Pictures of microparticles were taken by random scanning of the stub. The diameters of about 100 microparticles were measured from the optical microscope of each batch. Finally, average mean diameters were obtained (Pednekar and Singh, 2004; Chang and Gupta, 1996).

2.3.3. In vitro release studies

Drug-loaded microparticles equivalent to 25 mg of drug were weighed and transferred into a 100 ml conical flask. To this 50 ml of 7.4 pH PBS was added, then the flasks were kept in a metabolic shaker and the shaker was adjusted to 50 horizontal shakes/min at 37 ± 0.5 °C. One milliliter of the drug releasing media was withdrawn at various time intervals of 15 min, 1, 2, 4, 6, 8, 12, 16 and 24 h and replaced by the same volume of phosphate buffer saline. These samples were filtered though 0.45 μm membrane filter. The filtrate was diluted suitably. The drug was estimated in each batch by UV–vis spectrophotometer at 217 nm. For batch CM-1, the drug release study was carried by HPTLC method for comparing the results, which are coming from UV spectroscopy (Oner and Groves, 1993; Shenoy et al., 1997).

2.3.4. In vivo drug distribution studies

This study was carried out to compare the targeting efficiency of drug-loaded microparticles with that of the free drug in terms of percentage increase in targeting to various organs of reticuloendothelial system like liver, lungs, kidney and spleen.

Thirty healthy adult Albino mice weighing 35–40 g were selected, a constant day and night cycle was maintained and they were fasted for 12 h. The animals were divided into six groups each containing five mice. Groups I, II and III received microparticles equivalent to 494 mcg of captopril intravenously in the tail vein after redispersing in sterile phosphate buffer saline

Table 1
Formulation design, drug entrapment efficiency and size distribution of microparticles of different formulations

Batch	Formula (drug:polymer)	Drug entrapment efficiency (%)	Average particle size (μm)
CM-1	1:1.5	63.06	2.15
CM-2	1:2.0	53.63	2.58
CM-3	1:2.5	47.90	7.75
CM-4	1:3.0	41.20	10.55

solution. Microparticles from batch CM-2 were selected for the study. Groups IV and V received 494 mcg of pure captopril drug intravenously and Group VI was kept as solvent control and received only saline phosphate buffer.

After 1 h the Groups I, IV and VI mice were sacrificed and their liver, lungs, kidney and spleen were isolated. The Group V was sacrificed after 2 h and Groups II and III were sacrificed after 12 and 24 h, respectively. The individual organs of each mice were homogenized with adding small quantity of phosphate saline buffer pH 7.4 and 0.1N acetic acid, then kept in fridge for 12 h to precipitate protein and then centrifuged at 15,000 rpm to obtain the supernatant. The supernatant was filtered though an ultra filter membrane of pore size 0.2 μm and drug content was estimated using UV spectrophotometer at 230 nm (Dhanaraj et al., 2001; Sawant et al., 2001).

2.3.5. Stability studies

All the four batches of captopril microparticles were tested for stability. All the preparations were divided into three sets and were stored at 4 °C (refrigerator), ambient temperature/humidity and 40 °C (thermostatic oven). After 15, 30 and 60 days, drug content of all the formulations was determined by the method discussed previously in entrapment efficiency section. In vitro release study was also carried out of the best one formulation (Oliva et al., 1999; Kulkarni et al., 2004).

3. Results

3.1. Percentage practical yield

Percentage practical yield increased as the amount of polymer added to each formulation decreased. Maximum yield was found to be 63.81% in CM-1.

3.2. Particle size analysis

Scanning electron photomicrographs of all the four formulations are shown in Fig. 1. Average particle size of bovine serum albumin microparticles of captopril was found to be 2.15, 2.58, 7.75 and 10.55 μm for CM-1, CM-2, CM-3 and CM-4, respectively (Table 1) (Shishkova, 1999).

3.3. Drug entrapment efficiency

The amount of drug bound per 10 mg of microparticles was determined in the prepared four batches. Drug entrapment efficiency was calculated from drug content. The maximum entrapment was found in CM-1 (63.06%) (Table 1).

3.4. In vitro release studies

The cumulative percent drug release of pure drug was found to be 98.04% at 2 h. Cumulative percent of drug release after 24 h was 88.75, 81.37, 77.56 and 75.50% for CM-1, CM-2, CM-3 and CM-4, respectively, by UV spectroscopy. It was observed that the drug release from the formulations decreased with increase in the amount of polymer added in each formulation. It indicates that when compared with pure drug, the in vitro release of microparticles is prolonged over a period of 24 h (Table 2).

The in vitro release of all the four batches of microparticles showed a bi-phasic release with an initial burst effect. In the first hour, drug release was 23.79, 27.76, 28.82 and 29.19% for CM-1, CM-2, CM-3 and CM-4 formulations, respectively. Afterwards the drug release followed a steady pattern approximating zero-order release. The mechanism for the burst release in the first hour can be attributed to the drug loaded on the microparticles surface or imperfect albumin entrapment.

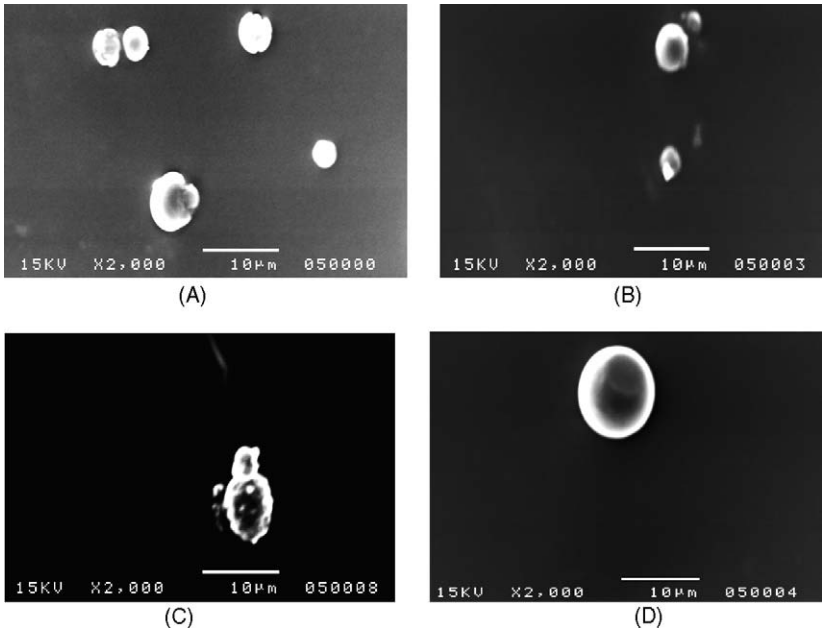

Fig. 1. Scanning electron microscopy of prepared microparticles: CM-1 (A), CM-2 (B), CM-3 (C) and CM-4 (D).

Table 2
In vitro release studies of different formulations

Time (T, h)	Cumulative percentage of drug released			
	CM-1	CM-2	CM-3	CM-4
0.25	19.36	21.92	22.80	27.32
1	23.79	27.76	28.82	29.19
2	37.18	35.18	34.96	32.96
4	48.98	40.83	37.88	34.45
6	58.49	44.77	41.88	38.23
8	64.41	50.42	46.00	42.55
12	71.30	59.76	53.88	50.69
16	76.11	70.47	66.44	65.59
24	88.75	81.37	77.56	75.50

An alternative method to estimate the drug release concentration of formulation CM-1 was carried out by HPTLC. The graph of HPTLC is shown in Fig. 2.

The regression coefficient for formulations CM-1 to CM-4 of zero-order plot were found to be 0.9238, 0.9796, 0.9873 and 0.9915, respectively. The regression coefficient for formulations CM-1 to CM-4 of first-order plot were found to be −0.9898, −0.9972, −0.9928 and −0.9850, respectively. Hixson Crowell plot regressions coefficient of formulations CM-1 to CM-4 were found to be −0.9765, −0.9961, −0.9941 and −0.9889, respectively. The regression coefficient for formulations CM-1 to CM-4 of Higuchi matrix plot was found to be 0.9858, 0.9963, 0.9875 and 0.9619, respectively. The 'n' values for CM-1 to CM-4 were 0.3592, 0.2883, 0.2585 and 0.2181, respectively, which is less than 0.45.

3.5. In vivo tissue distribution studies

The average distribution efficiency of drug-loaded microparticles was found to be 11.74% in liver, 7.69% in lungs, 5.67% in spleen and 8.50% in kidneys after 24 h, whereas accumulation of pure drug in liver was 7.69%, in lungs it was 4.86%, in spleen 2.02% and in kidneys 2.63% of the injected dose after 2 h (Table 3).

3.6. Stability studies

In vitro release studies revealed that the formulation (CM-2) stored at 4 °C showed 82.84% release, the one which was stored at ambient temperature/humidity showed 83.37% and 40 °C batch showed 83.68% release after 24 h.

4. Discussion

In the present study, an attempt was made to formulate captopril as microparticulate drug delivery system in order to localize drug at the absorption site, enhance its bioavailability, reduce dose, there by improving patient compliance. Microparticulate system of captopril was formulated using bovine serum albumin (natural polymer) as carrier by emulsification–heat stabilizing method. Prior to formulation, preformulation studies were car-

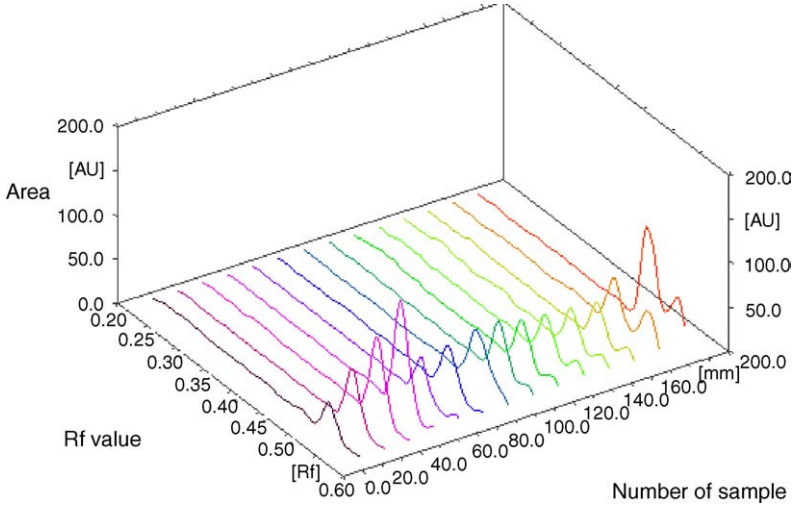

Fig. 2. Plots of cumulative percentage drug released vs. root time for different formulations of captopril microspheres (in vitro release studies) [Higuchi matrix]: CM-1 (♦), CM-2 (■), CM-3 (▲) and CM-4 (●).

Table 3
In vivo tissue distribution studies of bovine serum albumin microparticles of captopril and pure drug

Organs	Drug content (%)				
	Group I after 1 h	Group II after 12 h	Group III after 24 h	Group IV after 1 h	Group V after 2 h
Liver	34.01	25.10	11.74	22.67	7.69
Lungs	20.65	19.03	7.69	17.81	4.86
Kidney	17.41	15.79	8.50	10.53	2.63
Spleen	13.77	11.74	5.67	7.69	2.02

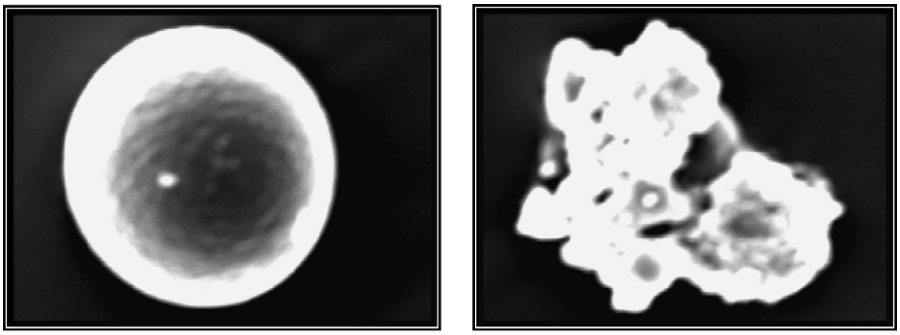

Fig. 3. Scanning electron microscopy of before and after in vitro release microparticles of CM-2: before erosion (A) and after erosion (B).

ried out in order to establish compatibility between drug and polymer by IR spectroscopy.

Percentage practical yield was found to be maximum in formulation CM-1. Percentage practical yield increased as the amount of polymer added to each formulation decreased, although it may not be dependent upon drug concentration in the formulation. Particle size of the drug-loaded microparticles revealed that the particles were in micron range. The particle size of the albumin microparticles is 2–11 μm. The microparticles described in this study were spherical, smooth surfaced and without porous. Drug entrapment efficiency was found to be maximum in CM-1. It was observed that drug entrapment efficiency increased with increase in concentration of drug added in consecutive formulations.

In vitro release study was analyzed using various mathematical models. Cumulative percent of drug release with respect to time was found to be highest for formulation CM-1 and lowest for formulation CM-4. Based on the regression coefficient values, the best-fit model for CM-1, CM-2 and CM-3 was first-order and CM-4 followed zero-order release. Results of Hixson Crowell plot indicated that CM-3 and CM-4 appear to fit this model. It was also observed that CM-2 followed Higuchi matrix suggesting drug release by diffusion (Fig. 3).

The '*n*' values of Peppas suggest Fickian release and Hixson Crowell regression data show that formulations also appear to release drug by erosion mechanism and the release is drug dissolution limited (Fig. 4).

Overall, the curve fitting into various mathematical models was found to be average and the in vitro release of formulations best fitted into the Peppas model followed by Higuchi's and Hixson Crowell model.

On the basis of drug content, particle size morphology, in vitro release and release kinetics, formulation CM-2 was selected as an optimum formulation for in vivo and stability studies. Present study shows that the targeting efficiency of drug-loaded microparticles over free drug was higher, which may provide increased therapeutic efficacy. Moreover, higher concentration of drug targeted to various organs may help in reduction of dose required for the therapy and thereby dose related side effects could be minimized. The in vivo drug targeting studies revealed the following order of targeting (tissue distribution): liver > lungs > kidneys > spleen.

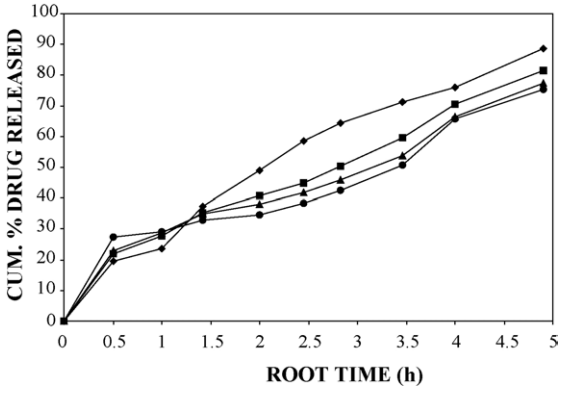

Fig. 4. 3D view of HPTLC of in vitro release profile of CM-1 formulation. First four tracks for standard calibration peak, tracks 5–13 are drug release samples and last track for maximum drug concentration in the formulation.

Drug content data of stability studies indicate that maximum drug was retained by formulation CM-2 when stored at 4 °C. Drug content of the formulation CM-2 stored at various temperature conditions reduced in the following order: 4 °C > RT > 40 °C. In vitro release data of stability studies indicate that very less variation in release was found at 4 °C followed by room temperature and 40 °C. From the above studies, it can be concluded that 4 °C is the most suitable temperature for storage of captopril microparticles.

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References

Arshady, R., 1990. Albumin microspheres and microcapsules: methodology of manufacturing techniques. J. Control. Release 14, 111–131.

Catherine, C.B., Stephen, W., Charles, S., Curtis, A.G., 2003. Potential drug-cell delivery routes using magnetic nanoparticles. Eur. Cells Mater. 6, 29–34.

Chang, W.C., Gupta, K.R., 1996. Stabilization of tetanus toxoid in poly(DL-lactic-coglycolic acid) microspheres for the controlled release of antigen. J. Pharm. Sci. 85, 129–132.

Dhanaraj, S.A., Gowthamarajan, K., Shanthi, K., Suresh, B., 2001. Albumin microspheres containing methotrexate: a lung specific delivery system. Indian J. Pharm. Sci. 63, 196–199.

Dvane, T., Birnbaum, B.L., 2003. Microparticle drug delivery systems. In: Brown, D.M. (Ed.), Drug Delivery Systems in Cancer Therapy. Humana Press Inc., Totawa, pp. 117–136.

Hakan, H., Suheyla, L., Mustfa, A.A., 2000. Preparation of bovine serum albumin microspheres containing Dexamethasone sodium phosphate and in vitro evaluation. Turk. J. Med. Sci. 30, 125–128.

Kulkarni, G.T., Gowthamarajan, K., Suresh, B., 2004. Stability testing of pharmaceutical products: an overview. Indian J. Pharm. Educ. 38, 194–202.

Lalla, J.K., 1991. Introduction to controlled release and oral controlled drug delivery systems. East. Pharm. 45, 25–28.

Li, L.K., Wang, N., Wu, S., 1997. A novel biodegradable system based on gelatin nanoparticles and poly(lactic-co-glycolic acid) microspheres for protein and peptide drug delivery. J. Pharm. Sci. 86, 891–895.

Lee, T.W., Robinson, J.R., 2000. Controlled release drug delivery systems. In: Gennaro, A.R. (Ed.), Ramington: The Science and Practice of Pharmacy, vol. 1, 20th ed. Lippincott Williams and Wilkinsi, Philadelphia, pp. 903–928.

Oliva, A., Santovena, A., Llabrews, M., Farina, J.B., 1999. Stability study of human serum albumin pharmaceutical preparations. J. Pharm. Pharmacol. 51, 385–392.

Oner, L., Groves, M.J., 1993. Properties of human albumin microparticles prepared by a chilled cross-linking technique. J. Pharm. Pharmacol. 45, 66–870.

Ooi, H., Colucci, W.S., 2001. Pharmacological treatment of heart failure. In: Hardman, J.G., Limbird, L.E., Gilman, A.G. (Eds.), Goodman and Gilman's. The Pharmacological Basis of Therapeutics, 10th ed. McGraw-Hill, New York, p. 910.

Pednekar, D.V., Singh, K.K., 2004. Particle size analysis—newer developments. Indian J. Pharm. Educ. 38, 130–133.

Ramesh, D.V., Medlicott, N., Razzak, M., Tucker, I.G., 2002. Microencapsulation of FITC-BSA into poly(ε-caprolactone) by a water-in-oil-in-oil solvent evaporation technique. Trends Biomater. Artif. Organs 15, 31–36.

Shenoy, B.D., Kini, D., Udupa, N., 1997. Formulation and in vitro evaluation of centchoman-loaded biodegradable microspheres. Indian J. Pharm. Sci. 60, 41–44.

Shishkova, V., 1999. Albumin microspheres as a drug delivery system for Dexamethasone: pharmaceutical and pharmacokinetics aspects. J. Pharm. Pharmacol. 51, 385–392.

Sawant, K.K., Tapar, K.K., Murthy, R.S.R., 2001. Novel complex coacervates of albumin and gelatin as carriers for 5-fluorouracil. Indian J. Pharm. Sci. 63, 295–298.

Tabassi, S.A.S., Rajavi, N., 2003. Preparation and characterization of albumin microspheres encapsulated with propranolol HCl. DARU 11, 137–141.