International Journal for Pharmaceutical Research Scholars (IJPRS)
ISSN No: 2277-7873
RESEARCH ARTICLE **V-1, I-4, 2012**

Design and Characterization of Cisplatin Magnetic Microspheres

***Vyas MB, Shah SK**

**Department of Pharmaceutics, Sardar Patel College of Pharmacy, Vidyanagar Vadtal Road, Bakrol, Anand - 388115 Gujarat, India*

Manuscript No: IJPRS/V1/I4/00179, Received On: 05/10/2012, Accepted On: 19/10/2012

ABSTRACT

The present study is aimed at the overall improvement in the efficacy, reduction in toxicity and enhancement of therapeutic index of cisplatin. Magnetically responsive biodegradable microparticulate delivery system of cisplatin has been developed by phase separation emulsion polymerization technique by using bovine serum albumin. The formulations were evaluated with respect to particle size analysis, entrapment efficiency, magnetite content, *in vitro* magnetic responsiveness, *in vitro* drug release studies, *in vivo* drug targeting studies and stability studies. The formulated magnetic microspheres were found to be spherical with average particle size of 3-12 µm in diameter and incorporation efficiency up to 56.37%. Result of X-ray diffractometry confirmed the presence of magnetite in prepared cisplatin magnetic microspheres. The total percentage of Fe_2O_3 in the microspheres was found to be 42.53% to 55.48%. *In vitro* drug release after 24 hours was 89.60%, 82.22%, 78.41% and 76.35% for formulation F1, F2, F3 and F4 respectively. Results of *in vitro* magnetic responsiveness and *in vivo* tissue targeting proved that the retention of microspheres in presence of magnetic field was significantly high than those in the absence of the magnetic field. Stability studies revealed that 4º is the most suitable temperature for storage of cisplatin loaded magnetic microspheres. Overall, this study shows that the magnetic albumin microspheres can be retained at their target site *in vivo*, following the application of magnetic field, and are capable of releasing their drug content for an extended period of time.

KEYWORDS

Cisplatin, magnetic microspheres, phase separation emulsion polymerization.

INTRODUCTION

Magnetic drug delivery by particulate carriers is a very efficient method of delivering a drug to a localized disease site. Very high concentrations of chemotherapeutic or radiological agents can be achieved near the target site, such as a tumor, without any toxic effects to normal surrounding tissue or to the whole body[1-3]. Magnetic carriers receive their magnetic responsiveness to a magnetic field from incorporated materials such as magnetite, iron, nickel, cobalt, neodymium–iron–boron or samarium–cobalt.

***Address for Correspondence:**
Mehul B. Vyas
Department of Pharmaceutics,
Sardar Patel College of Pharmacy,
Bakrol, Anand, Gujarat, India.
E-Mail Id: mehulvyas_85@yahoo.co.in

Magnetic carriers are normally grouped according to size. At the lower end, we have the ferrofluids, which are colloidal iron oxide solutions. Encapsulated magnetite particles in the range of 10–500 nm are usually called magnetic nanospheres and any magnetic particles of just below 1–100_m are magnetic microspheres[4]. In recent years, considerable interest has been shown in the use of albumin microspheres as platforms for active drug targeting as well as producing a sustained and controlled rate of drug release[5-7]. In the previous studies, a phase separation emulsion technique was developed for the preparation of the microspheres. Stabilization of the albumin microspheres matrix was accomplished by

either heat denaturation at various temperatures (110-190°) or crosslinking with carbonyl compounds in an ether phase reaction. The degree of stabilization controls the rate of drug diffusion out of the carrier as well as the extent of carrier degradation[8]. Magnetic albumin microspheres are capable of being retained in the capillaries by using extracorporeal magnets. Electron microscopic studies of rat tail skin perfused with microspheres in a 8000 G magnetic field of 30 m duration showed that microspheres were internalized by endothelial cells and trapped between the plasma membranes of two adjacent endothelial cells and hence were not cleared by the reticuloendothelial system[9]. The studies confirm second order drug targeting (targeting to specific organs or tissues)[10] in the target tissue of healthy animals[11]. Targeting by magnetically responsive albumin microspheres has a high efficiency. For example, doxorubicin hydrochloride, entrapped in magnetic albumin microspheres, has been shown to cause enhanced drug concentration in the target tissue compared with the administration of free drug[12]. In addition, the amount of drug reaching the heart and liver was reduced. Dexamethasone sodium phosphate is synthetic glucocorticoids, which could be used in the treatment of lymphocytic tumors and lymphomas. It also has anti-inflammatory effects as well as preventing the cell mediated immune reactions[13].

In this study, magnetic microspheres prepared from starch and serum albumin using a "phase separation emulsion polymerization" was employed for drug targeting and as drug carriers. An elaborate technology, which has now been commercialized, was introduced by Ugelstad et a[14]. using acrylates and polystyrene microspheres in which iron (III) oxide is incorporated using a swelling-oxidation process.

MATERIALS AND METHODS

Cisplatin was a kind gift sample from Sun Pharma Mumbai and Cipla Pvt. Ltd. Bangalore. Bovine serum albumin and tween 80 were received from Himedia, Mumbai. Potassium dihydrogen phosphate, dimethyl sulphoxide, ferrous sulphate, sodium thio sulphate were generously gifted by S. D. Fine Chemicals Ltd, Mumbai.

Preparation of Coated Magnetic Particles[15]

The magnetite was prepared by reacting 10% w/v ferrous sulphate (containing 5% of tween 80) with 20% w/v NaOH solution, followed by washing of the precipitate with dilute ammonia in order to get magnetite free of sulphate ions. This precipitate of magnetite was then dried at 100º and passed through sieve 300#. The magnetite particles thus formed tend to agglomerate due to their surface energy and under the influence of induced magnetic field. To overcome this disadvantage the magnetite particle were coated with a non magnetic material that is silicon oil to reduce mutual attractive forces between the particles. The coating was done by packing the magnetite particle in a funnel and percolating 1% w/v solution of silicon oil in ether through the magnetite. The oil coating also imparts hydrophobicity to the particles and facilitates their uniform dispersion in water.

Preparation of Magnetically Responsive Microspheres[16]

Magnetic microspheres of cisplatin were prepared by phase separation emulsion polymerization technique. 1 ml solution of bovine serum albumin (in freshly prepared PBS pH 7.4) containing dispersed magnetite (30 % w/w of albumin) and 0.5 ml of dimethyl sulfoxide containing 10 ml of dissolved cisplatin) was added into 10 ml of cottonseed oil (4º) containing 4 % w/v of surfactant span 20 and the above mixture was stirred for 10 m. The resultant emulsion was then added drop wise (50 ±10 drops per m) using glass syringe with 23-gauge needle into 150 ml of cottonseed oil (preheated to 130 ± 5º) along with stirring at 1400 rpm. Heating and stirring of the oil were continued for 10 m after the addition of emulsion. The resulting suspension was then allowed to cool to room temperature with continuous stirring and washed three times with 60 ml anhydrous diethyl ether, each time

centrifuging at (3000 xg, 15 m). The washed microspheres were suspended in 10 ml anhydrous ether and unincorporated Fe_2O_3 was removed by transferring the suspension into a tared tube, in presence of a 300 g bar magnet placed at rim of the decanting tube. The magnetic microspheres thus obtained were dried in desiccators and stored in airtight amber colored bottles at low temperature. Four batches of magnetic microspheres viz. F1, F2, F3 and F4 were prepared by employing the above method.

Evaluation of Magnetic Microspheres

Particle Size Analysis[17]

Particle size analysis was done by scanning electron microscopy (SEM). SEM has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface. Particle size analysis was done by SEM using JEOL JSM-T330A scanning microscope.

Drug Entrapment Efficiency[18]

Magnetic microspheres equivalent to 10 mg of cisplatin were weighed and suspended in 10 ml solution (0.5 ml 0.1N HCl + 9.5 ml PBS) for 5 m. The suspension was then filtered through 0.45 µ filter.

Table 1: Drug entrapment efficiency and percent magnetite entrapped in magnetic microspheres

Formulations	% Magnetite entrapped	% Drug entrapment efficiency
F1	42.53±0.221	39.82±0.168
F2	47.76±0.223	46.76±0.214
F3	54.34±0.268	53.19±0.257
F4	55.48±0.321	56.37±0.355

Each value represents mean ±SD for (n=3)

The residue obtained after filtration was then digested in 5 ml solution containing 2.5 ml of 50 % v/v trichloroacetic acid and 2.5 ml 0.1N HCl and kept for 24 h to precipitate the protein. The digested homogenate was centrifuged for 5 m and the supernatant was analyzed for drug content by measuring the absorbance at 210 nm by UV-Vis spectrophotometer (UV-1201 Shimadzu, Japan) after appropriate dilutions with PBS.

$$\text{Entrapment efficiency} = \frac{\text{experimental drug content} \times 100}{\text{Theoretical drug content}}$$

Determination of Percent Magnetite Content[19, 20]

Determination of Fe_2O_3 content in prepared magnetically responsive microspheres was conducted by employing a conventional titrimetric method using thiosulphate and potassium iodide for quantitative analysis. An accurately weighed amount of magnetic microspheres (after destruction by gentle heating) was dissolved in mixture of water (200 ml) and conc. HCl (200 ml) by heating it to the boiling point. The solution was boiled for 15 s and cooled rapidly. Then potassium iodide (3 g) was added and kept in dark for 15 m, the liberated iodine was then titrated with 0.1 N sodium thiosulphate using starch as indicator. A blank titration was carried out. The difference between titrations gave the amount of iodine liberated by ferric ion.

Each ml of 0.1 N sodium thiosulphate ≡ 0.005585g of ferric ion

***In vitro* Drug Release Studies**[21]

Magnetic microspheres equivalent to 10 mg of cisplatin were weighed and transferred into a conical flask containing 50 ml of PBS pH 7.4. Then the flask was kept in a metabolic shaker and the shaker was adjusted to 50 horizontal shakes per m at 37º ± 0.5º. 1 ml aliquot of release medium was withdrawn at time intervals of 15 m, 30 m, 1 h, 2 h, 4 h, 8 h, 16 h and 24 h and replaced by the same volume of PBS. These samples were filtered through 0.45 µ membrane filter. The filtrate was diluted suitably and estimated by UV-Visible spectrophotometer (UV-1201 Shimadzu, Japan) at 210 nm.

***In vivo* Drug Targeting Studies**[15, 16, 22]

This study was carried out after obtaining the due permission for conduction of experiments from relevant ethics committee (K.L.E.S's College of Pharmacy, Belgaum) which is registered for "Teaching and Research on Animals" by committee for the purpose of control and supervision of experiments on animal, Chennai (Registration number 221/CPCSEA).

This study was carried out to compare the targeting efficiency of cisplatin loaded magnetic microspheres with that of free drug in terms of percentage increase in targeting to various organs of reticuloendothelial system like liver, lungs, spleen and kidneys. Experiments were performed on rats of 225-235 g weight. All the experiments were carried out in accordance with the protocols approved by the Institutional animal ethics committee (K.L.E.S College, Belgaum, India).

Eight healthy adult **Sprague Dawley** rats weighing 225-235 g were divided into 2 groups, each containing 4 rats. The rats of first group were anaesthetized and a dose of drug loaded magnetic microspheres equivalent to 607 mcg of cisplatin in sterile PBS solution was introduced through polyethylene tubing, cannulated through caudal artery of the tail. The magnetic field was applied at preselected target site, with the help of electromagnet having field strength 7000 Oe. The magnet was removed from the site after 30 m of administration of the magnetic microspheres. Two rats were sacrificed after 1 h and the other two after 3 h. The organs such as tail, lung, liver, spleen and kidney were isolated. The individual organs of each rat were homogenized separately and digested with 2 ml solution containing 1 ml of 50 % v/v trichloroacetic acid and 1 ml 0.1 N HCl and kept for 24 h in refrigerator to precipitate the protein. Then the drug was extracted after multiple washings and centrifuged at (3000 xg, 15 m) to obtain the supernatant. The supernatant was filtered through an ultra filter membrane of pore size 0.22 µ and subjected to extraction procedure. The rats of second group, taken as control were administered with the same dose of magnetic microspheres of cisplatin in the absence of magnetic field and the same procedure was followed.

Table 2: In vivo Targeting Studies of Cisplatin Loaded Magnetic Microspheres

Organs	Group I(with magnet)		Group II (without magnet)	
	% Drug content after 1h	% Drug content after 3h	% Drug content after 1h	% Drug content after 3h
Tail section	57.61±0.361	41.97±0.220	3.62±0.090	2.31±0.061
Lungs	1.81±0.055	3.46±0.087	8.23±0.218	14.81±0.238
Liver	10.69±0.226	15.63±0.242	32.1±0.248	46.1±0.246
Spleen	2.79±0.069	3.95±0.096	9.22±0.223	18.11±0.244
Kidney	3.29±0.084	5.59±0.213	5.27±0.210	9.38±0.225

Each value represents mean ±SD for (n=3)

Stability Studies[23]

Information on the stability of drug substance is an integral part of systematic approach to

Table 3: Stability studies for percent drug content (after storage at 4º, ambient temperature and humidity and at 30º / 65% RH)

Formulations	Percent drug content at 4º			Percent drug content at ambient temperature and humidity			Percent drug content at 30º / 65% RH		
	After 15 d	After 30 d	After 60 d	After 15 d	After 30 d	After 60 d	After 15 d	After 30 d	After 60 d
F1	39.74 ± 0.322	39.62 ± 0.300	38.88 ± 0.315	39.36 ± 0.325	39.10 ± 0.291	38.54 ± 0.285	39.30 ± 0.301	38.95 ± 0.299	38.30 ± 0.283
F2	46.64 ± 0.385	46.38 ± 0.389	45.64 ± 0.379	46.29 ± 0.380	45.89 ± 0.382	45.12 ± 0.370	46.25 ± 0.387	45.76 ± 0.368	44.62 ± 0.360
F3	53.02 ± 0.522	52.88 ± 0.495	52.26 ± 0.530	52.96 ± 0.520	52.32 ± 0.548	51.96 ± 0.498	52.88 ± 0.495	52.15 ± 0.501	51.58 ± 0.488
F4	65.18 ± 0.551	56.04 ± 0.549	55.38 ± 0.541	56.14 ± 0.545	55.94 ± 0.540	54.78 ± 0.499	56.08 ± 0.501	55.70 ± 0.544	54.42 ± 0.498

stability evaluation. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under influence of the variety of environmental factors such as temperature, humidity and light, and to establish a shelf life for the drug product and recommended storage conditions. All 4 batches of cisplatin magnetic microspheres were tested for stability. All the preparations were divided into 3 sets and were stored at, 4º in refrigerator , 30º ± 2º / 65 % RH ± 5 % RH in humidity control oven (GINKYA IM 3500 series), ambient temperature and humidity. Drug content of all the formulations was determined after 15d, 30d and 60d. *In vitro* release study of a selected formulation was also carried out after storage for 1 mo.

RESULTS AND DISCUSSION

Different magnifications were used while taking these photomicrographs. Average particle size of magnetic microspheres of cisplatin was found to be 3.27 $\pm$ 0.0766, 5.39 $\pm$ 0.1932, 7.61 $\pm$ 0.846, 12 $\pm$ 0.1066 µ for F1 to F4 respectively. Particles of formulations F1, F2 and F4 were found to be smooth, oval and discrete whereas particles of formulation F3 were slightly rough surfaced but discrete. Scanning electron photomicrographs of formulations F3 and F4 are shown in Fig. 1a and Fig. 1b respectively.

Drug entrapment efficiency was calculated from the drug content. The drug content in four batches of cisplatin magnetic microspheres was studied. The amount of drug bound per 10 mg of magnetic microspheres was determined in each batch. Table no. 1 shows the results of the drug entrapment efficiency and percent magnetite content in each of these formulations. It was observed that the entrapment efficiency increased with the increase in concentration of polymer in the formulations. The maximum

entrapment efficiency was found to be 56.37% in formulation F4.

Determination of magnetite content in prepared magnetically responsive microspheres was conducted by employing a conventional titrimetric method using thiosulphate and potassium iodide for quantitative analysis. The amount of magnetite content per 10 mg of microspheres was determined in all formulations. It was observed that entrapment of magnetite increased with increase in concentration of polymer added in consecutive formulations.

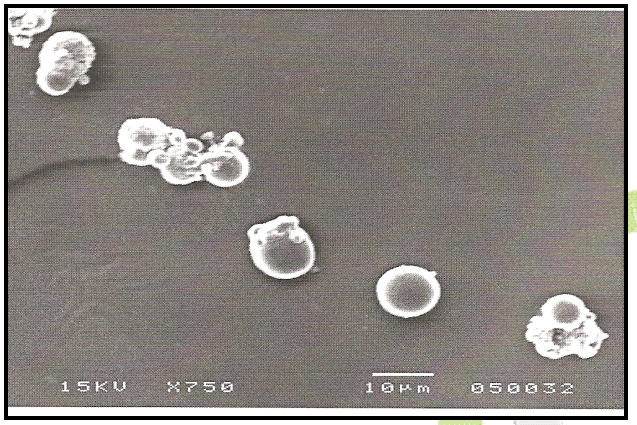

Figure1a: Scanning electron photomicrographs of formulations F3

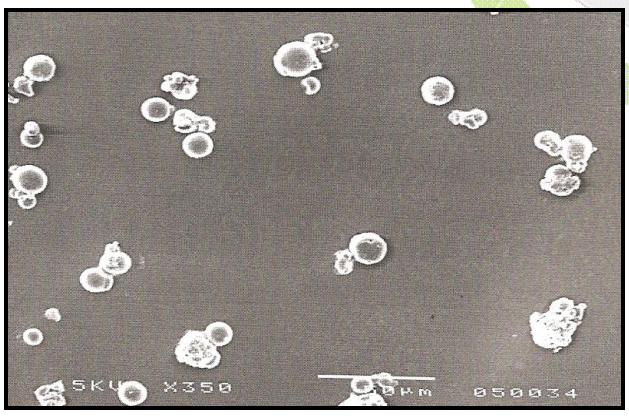

Figure 1b: Scanning electron photomicrographs of formulations F4

The maximum magnetite content was found to be 55.48% in formulation F4. Pure cisplatin and all the four formulations of cisplatin loaded magnetic microspheres were subjected to *in vitro* release studies. These studies were carried out using metabolic shaker in PBS pH 7.4. The cumulative percent drug release of pure drug was found to be 93.60% at 3 h. Cumulative percent drug release after 24 h was 89.60%, 82.22%, 78.41% and 76.35% for F1, F2, F3 and F4 respectively by UV spectroscopy.

The release data obtained for pure drug and formulations F1, F2, F3 and F4 are showed in Fig. 2 It shows plots of cumulative percent drug released as a function of time for pure drug and for different formulations of cisplatin loaded magnetic microspheres. It was observed that the drug release from the formulations decreased with increase in ratio of polymer added in each formulation. When compared with the pure drug, the *in vitro* release of microspheres is prolonged over a period of 24 h. The *in vitro* release of all the four batches of microspheres showed a bi-phasic release with an initial burst effect even after washing. In the first hour, drug release was 38.64%, 36.03%, 35.81% and 33.81% for F1, F2, F3 and F4 respectively. Afterwards the drug release followed a steady pattern approximating zero order release. The burst release in the first hour even after washing indicates need of increase in washing period in order to overcome burst effect.

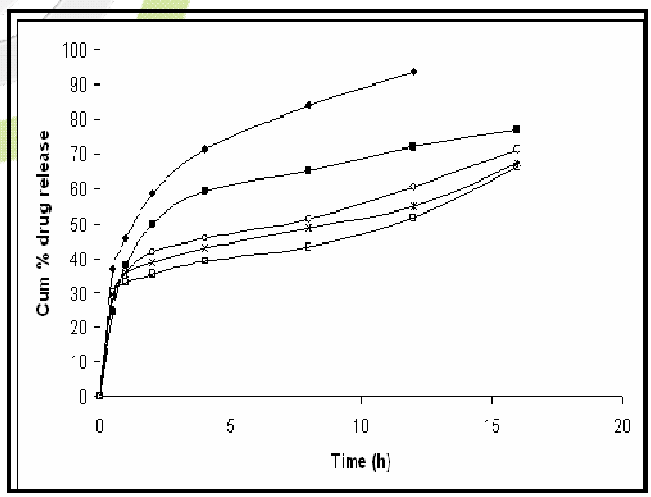

Figure 2: *In vitro* release profile of cisplatin from magnetic microspheres.

In vitro release profile for different formulations of cisplatin magnetic microspheres

Pure drug(-♦-),Formulation F1(-■-), Formulation F2(-o-),Formulation F3(-×-), Formulation F4(-□-)

In vivo drug targeting studies showed that after 1 h of administration of magnetic microspheres, 57.61% of drug were recovered from the rat tail under the influence of magnetic field. The drug concentration in other organ was found to be 10.69% in liver, 3.29% in kidney, 2.79% in spleen and 1.81% in lungs. Though the magnetic field was removed at 30 m after the administration of magnetic microspheres, 41.97% of the drug was recovered from the tail after 3 h of the administration Table no.2.This localization may have appeared due to the penetration of the microspheres by endocytosis in the tail section, lack of phagocytosis and slow rate of blood flow in the tail. These results were compared with those obtained from control rats (without magnetic field). In control group of rats only 3.62 % drugs was recovered after one hour and 2.31 % was recovered after 3 h from the tail. However the highest concentration was found in liver (46.1%) and spleen (18.11%).

Stability studies of the prepared microspheres were carried out, by storing all the formulations F1 to F4 at 4º in refrigerator, ambient temperature and humidity and 30º ± 2º / 65 %RH ± 5 % RH in humidity control oven for 60 d. Two parameters namely residual percent drug content and *in vitro* release studies were carried out. The results of drug content after 15 d, 30 d and 60 d are shown in Table no.3. These studies reveal that there is a reduction in drug content after storage for 60 d at 4º, ambient temperature and humidity and 30º ± 2º / 65 %RH ± 5 % RH. It was also revealed that out of the four formulated batches, the one stored at 4º showed maximum residual drug followed by that stored at ambient temperature and humidity and 30º ± 2º / 65 %RH ± 5 % RH. *In vitro* release studies, which were carried out after storing a selected formulation F3 at 4º, ambient temperature and humidity and30º ± 2º / 65 %RH ± 5 % RH for 60 d. *In vitro* release studies reveal that the formulation stored at 4º showed 80.56% release, the one which stored at ambient temperature and humidity showed 82.74% and formulation stored at 30º ± 2º / 65 %RH ± 5 % RH showed 84.48% release after 24 h. These results indicate that the drug release from the formulation stored at 30º ± 2º / 65 %RH ± 5 % RH was highest followed by formulation stored at ambient temperature and humidity and 4º. On comparing these data with the previous release data of F3, it was observed that there was an overall increase in the drug release. These results may be attributed to erosion of particles to some extent during storage.

ACKNOWLEDGEMENT

The authors sincerely thank the Analytical Research (HINDALCO) Belgaum, for providing the facility of scanning electron microscopy. We heartily thank Sun pharmaceuticals Mumbai and Cipla Pvt Ltd, Bangalore to procure gift samples of cisplatin.

REFERENCES

1. Widder KJ, Senyei AE, Ranney DF, “Magnetically responsive microspheres and other carriers for the biophysical targeting of antitumor agents”, Adv Pharmacol Chemother 1979, 16, 213–71.
2. Gupta PK, Hung CT, “Magnetically controlled targeted micro-carrier systems”, Life Sci, 1989, 44, 175-86.
3. Hafeli U, Schütt W, Teller J, Zborowski M, “Scientific and Clinical Applications of Magnetic Carriers”, 1st ed New York: Plenum Press, 1997.
4. Meyers PH, Cronic F, Nice CM, “Experimental approach in the use and magnetic control of metallic iron particles in the lymphatic and vascular system of dogs as a contrast and isotopic agent”. Am J Roentgenol Radium Ther Nucl Med, 1963, 90, 1068-77.
5. Morimoto Y, Okumura M, Sugibayashi K. Kato Y, “Biomedical application of magnetic fluids.ll. Preparation and magnetic guidance of magnetic albumin microspheres for site-specific drug delivery *in vivo*”. J Pharm Dvn, 1981, 4, 624-31.

6. Widder KJ, Seneyei AE, "Magnetic microspheres: a vehicle for selective targeting of drugs". Pharm Ther, 1983, 20, 377-95.

7. Gallo JM, Gupta PK, "Evaluation of drug delivery following the administration of magnetic albumin microspheres containing adriamycin to the rat" J Pharm Sci, 1989, 78, 190-94.

8. Widder KJ, Seneyei AE, Ranney DE, "*In vitro* release of biologically active adriamycin by magnetically responsive albumin microspheres", Cancer Res, 1980, 40, 3512-17.

9. Widder KJ, Seneyei AE, Scarpelli DG, "Magnetic microspheres: a model system for site specific drug delivery *in vivo*". Proc Soc Exp Biol Med, 1978, 58, 141-46.

10. Thies C, "Dispersed system for parenteral administration. In Rosoff, M. (Ed.), Controlled Release of Drug Polymers and Aggregate Systems". New York: Publishers Inc; 1989.

11. Gupta PK, Hung CT, Rao NS, "Ultra structural disposition of adriamycin-associated magnetic albumin microspheres in rats", J Pharm Sci, 1989, 78, 290-94.

12. Gupta PK, Hung CT, "Effect of carrier dose on the multiple tissue disposition of doxorubicin hydrochloride administration via magnetic albumin microspheres in rats" J Pharm Sci, 1989, 78, 745 -48.

13. Haynes RC, "Adrenocorticotropic hormone: Adrenocortical steroids and their synthetic analogs; Inhibitors of adrenocortical steroid biosynthesis", In Goodman Gillman, A., Rall. T.W., Nies A.S. and Taylor P. (Eds.). The pharmacological basis of therapeutics, 8th Edition, Vol.2. Maxwell Macmillan International Editions, New York, 1991.1457.

14. Ugelstad J, Ellingsen T, Berge A, Helgee OB, US Pat.1987; 4 654-267.

15. Bhadra S, Choubey D, Agrawal GP, "Target oriented microspheres of diclofenac sodium", Indian J Pharm Sci, 2003, 65, 503-509.

16. Gupta PK, Hung CT, "Albumin microspheres V. Evaluation of parameters controlling the efficacy of magnetic microspheres in the targeted delivery of adriamycin in rats". Int J Pharm., 1990, 59, 57-67.

17. Jacob JS, Characterization of delivery systems: Microscopy. In: Mathiowitz E, editor. Encyclopedia of Controlled Drug Delivery vol 1. John Wiley and Sons, Inc. New York 1999:242-3.

18. Ramesh DV, Medlicott N, Razzak M, Tucker IG, „Microencapsulation of FITC-BSA into poly (ε-caprolactone) by a water-in-oil-in-oil solvent evaporation technique" Trends Biomater Artif Organs, 2002, 15(2), 31-36.

19. Avivi S, Felner I, Novik I, Gedanken A, "The preparation of magnetic proteinaceous microspheres using the sonochemical method" Biochimica et Biophysica Acta, 2001, 1527, 123-9.

20. Qadry JS, Qadry SZ, "A textbook of inorganic pharmaceutical and medical chemistry. 5th ed. Ahmedabad: B S Shah prakashan; 1992.

21. Santhi K, Dhanraj S, Ponnusankar S, Suresh B, "Study on formulation and targeting efficiency of amphotericin B nanospheres", Indian J Pharm Sci, 2000, 6, 421-3.

22. Augey V, Cociglio M, Galtier M, Yearoo R, Pinsani V, Bressolle F, "High performance liquid chromatographic determination of cis-dichlorodiammineplatinum (II) in plasma ultra filtrate, J Pharma Biomed Ana, 1995, 13, 1173-8.

23. The European Agency for the evaluation of medicinal products. Stability Testing Guidelines: Stability testing of new drug substances and products. London:ICH-Technical Coordination, EMEA. [online]. 2003 [cited 2003 Feb 20];[21 screens]. Available from: URL:http://www.emea.eu.int

