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# Target Oriented Microspheres of Diclofenac Sodium

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**Bovine serum albumin microspheres of diclofenac sodium were prepared by emulsion polymerization technique and the gelatin microspheres were prepared by gelation and dehydration technique. These microspheres were prepared for targeting of diclofenac sodium to inflammation site. The silicone coated magnetite particles were prepared and used for the preparation of magnetic microspheres. Effect of different formulation and process variables was studied on particle size, drug entrapment, *in vitro* release rate, magnetic responsiveness, biodistribution and stability of the microspheres.**

Diclofenac has good bioavailability after oral administration, peak plasma levels occur in 2 to 3 h. Diclofenac also exhibits hepatic first pass effect in humans and only 50 percent is bioavailable after 2 h. Diclofenac has a short biological half-life and a narrow therapeutic index[1]. The drug has to be administered relatively frequently, and peak levels achieved after this has been reported to have side effects like gastrointestinal disturbances and headache[2]. Thus localization of the drug at the site of inflammation is desired.

The main problem associated with most of the carriers is that they are rapidly taken up by the reticuloendothelial systems, present in liver and spleen, thus first order targeting is not attained. Targeting of the drug can be done by incorporating it in magnetite bearing microspheres[3,4]. Thus localization of the drug at a particular site and at a specific rate can be achieved by the application of external magnetic field of appropriate strength.

Thus, it was aimed to prepare and characterize target-oriented microspheres of diclofenac sodium. To achieve this, magnetic microspheres were prepared using silicone coated magnetite particles. For comparative studies, two types of microspheres were prepared, i.e. bovine serum albumin (BSA) microspheres by emulsion polymerization technique[5,6] and gelatin microspheres by gelation dehydration technique[7].

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## MATERIALS AND METHODS

BSA was procured from Spectrochem Pvt. Ltd., Mumbai. Gelatin was purchased from Loba Chemie, Mumbai. Ferrous sulphate was obtained from E. Merck, Mumbai and was used for the present work. Each of these chemicals was of extra pure grade. The other chemicals were of reagent grade. Diclofenac sodium was procured as gift sample from Torrent Pharmaceuticals Ltd., Ahmedabad. Microspheres were prepared as per the following procedures using different concentrations of albumin 200, 250 and 300 mg/ml at various stirring rates 800, 1200 and 1600 rpm.

### Preparation of nonmagnetic BSA microspheres by heat stabilization technique[5]:

One millilitre of aqueous solution of 250 mg albumin and 100 mg diclofenac sodium was added into 50 ml of soyabean oil at about 4°. Four drops of Span 20 were also added to it and the above mixture was stirred for 5 min. The resultant emulsion was added dropwise to 100 ml of soyabean oil (preheated to 125°) and stirred at 1200 rpm for 10 min. After allowing the suspension to cool to room temperature with continuous stirring, the mixture was washed three times with 60 ml of anhydrous ether and

centrifuged. The sediment thus obtained was resuspended in 10 ml anhydrous ether and stored in air tight bottle at low temperature. These were prepared at various stabilization temperatures that included 100°, 125° and 150°.

**Preparation of nonmagnetic BSA microspheres by chemical stabilization technique[6]:**

The primary emulsion was prepared by the same process as used above. The resultant emulsion was added to 100 ml of soyabean oil stirred at 1200 rpm. To this mixture, 30 ml of 0.2 M formaldehyde solution was added slowly and stirring was continued for 1 h. The separation, purification and storage of the chemically stabilized microspheres were done in the same manner as for heat stabilized microspheres. The concentration of cross linking agent formaldehyde was varied in the range of 0.1, 0.2 and 0.3 M.

**Preparation of nonmagnetic gelatin microspheres[7]:**

Aqueous solution of gelatin (10% w/v) was prepared by heating at 55° and was slowly added with stirring to paraffin oil containing various concentrations of sorbitan monooleate (2%, 4% and 6%), maintained at 0-5°. Stirring was done at 2400 rpm for 1.5 h. To this dispersion, isopropanol was added at 5° with continuous stirring. Microdrops thus formed were resuspended in isopropanol containing 25 ml of 0.2 M formaldehyde used as crosslinking agent. Excess carbonyl reagent was removed by isopropanol, pelleting the microspheres by centrifugation and decanting the supernatant. The microspheres were washed two times in this manner and subsequently stored at low temperature.

**Preparation of coated magnetite particles[8]:**

The magnetite was prepared by reacting 10% w/v solution of ferrous sulphate (containing 0.5% of Tween 80) with 20% w/v solution of sodium hydroxide, followed by washing of the precipitate with dilute ammonia in order to get magnetite free of sulphate ions. This was then dried at 100° and passed through #300 sieve. The magnetite particles thus formed tend to agglomerate due to their surface free energy and under the influence of induced magnetic field (when magnetic field is applied). Therefore the magnetite particles were coated with a nonmagnetic material such as silicone oil reduces the mutual attractive forces between the particles. The coating was done by packaging the magnetite particles in a funnel and percolating a 1% w/v solution of silicone oil in ether through the magnetite. The oil coating also imparts hydrophobicity to the particles and facilitates their uniform dispersion in water.

**Preparation of magnetic microspheres:**

For the preparation of magnetic BSA microspheres, the coated magnetite particles (25% w/v of albumin) were dispersed in the aqueous solution of albumin and diclofenac sodium. This was followed by the same steps as for the preparation of nonmagnetic microspheres (given earlier). For the preparation of magnetic gelatin microspheres, the coated magnetite particles (25% w/v of albumin) were dispersed in the aqueous solution of gelatin at 55° and the oily phase containing 4% w/v of surfactant was added to it, while maintaining the temperature at 0-5°. The remaining procedure was same as for the preparation of nonmagnetic microspheres (described earlier in the text).

In an attempt to see the effect of different process variables on the properties of microspheres, few formulations were also prepared by varying only one parameter and keeping all other conditions the same. The dispersions of microspheres, prepared by various methods, were observed under optical microscope at 10x100 magnification, to determine their particle size.

**Drug entrapment efficiency[9]:**

Five milligrams of microspheres were digested with 1 ml of 50% v/v trichloroacetic acid and kept for 24 h to precipitate the protein. The digested homogenate was centrifuged for 5 min. and the supernatant was analyzed for drug content, by measuring absorbance at 276 nm after appropriate dilutions with phosphate buffer pH 7.8.

***In vitro* magnetic responsiveness:**

An apparatus was designed to study the magnetic responsiveness (fig. 1). A 250 ml separating funnel, used as reservoir (A) was fitted with a stopcock (B) to regulate the flow of fluid from the reservoir. A rubber tubing (C) of length 10 cm formed the link between the reservoir and a L-shaped glass tubing D (30 cm long vertically and 40 cm long horizontally). This tube had its end bent to a length of 6.5 cm in the upward direction and it was attached to another rubber tube of 2.5 cm. A screw cock (E) was fitted to this rubber tube to adjust the flow rate of the fluid. A trap (F) was made at the middle of the horizontal portion of the glass tube with an exit tube (making an angle with it). To this trap a rubber tubing 2.5 cm in length was joined and closed with the help of a pinchcock (G). A pair of horse shoe magnet (H) of strength 7000 Oe was kept perpendicularly over the trap.

The microspheres were initially dialyzed using cellulose

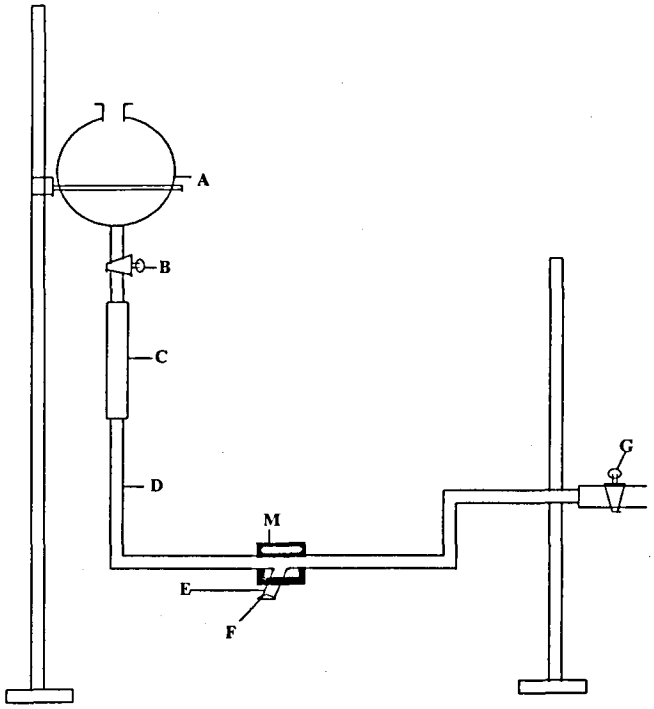


**Fig. 1: Apparatus for *in vitro* magnetic responsiveness study.**

**Apparatus for studying *in vitro* magnetic responsiveness of diclofenac loaded magnetic microspheres having components as: A-Reservoir; B-Stopcock for reservoir; C-Rubber tubing; D-Glass tubing; E-Trap for microspheres; F-Pinchcock over rubber tubing; G-Screwcock over rubber tubing and M-horse shoe magnet.**

tubing in order to remove the entire free drug. The flow rate was adjusted to 1 ml/sec with the help of stopcock (B). The suspension of microspheres in isotonic saline solution containing 1 drop of Tween 80 was injected slowly at the upper end of rubber tubing C. The magnets were so placed that the glass tube lay in the centre of the magnetic field. Ten minutes after the injection, the flow of fluid from A was stopped with the help of stopcock. The microspheres, retained by the magnets, were collected from the trap and analyzed for the drug content.

### *In vitro* release rate studies:

Ten millilitres suspension of microspheres was dialyzed against 50 ml phosphate buffer saline (pH 7.4), maintained at 37±1° on a hot plate magnetic stirrer. Two millilitres of the sample was withdrawn every 1 h and analyzed for drug content for 8 h.

### *In vivo* localization:

Eight Sprague Dawley rats, weighing 225±12 g, were divided into two groups of 4 rats. The tail of each rat was demarcated into four parts with picric acid solution. The rats of first group were anaesthetized and a dose of drug loaded microspheres equivalent to 300 µg of diclofenac sodium was introduced through polyethylene tubing, cannulated through caudal artery, at the exposed section 1 of the tail. Section 3, the preselected target site, was placed between the poles of the bipolar magnet having a magnetic field strength 7000 Oe. The magnet was removed from the site (section 3) after 30 min 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, liver, spleen and kidney were separated from the rat's body and were sliced. The sliced organs were homogenized and digested with 1 ml of 50% trichloroacetic acid for 12 h to precipitate the protein. Then the drug was extracted by multiple washings and centrifugation. The drug content of the supernatant was analyzed spectrophotometrically, at λ=276 nm. The rats of second group, taken as control were administered same dose of nonmagnetic microspheres of diclofenac sodium and the same procedure was followed for distribution of drug in various organs.

### Stability studies[10,11]:

Both the magnetic and nonmagnetic forms of heat stabilized BSA microspheres were tested for stability. All the preparations were stored in 3 different amber colored vials, closed with rubber closures. The vials were stored at 37±2°, 45±2° and 55±2°, respectively, in thermostatic ovens. After intervals of 7, 14 and 21 d, the samples were withdrawn and analyzed for the drug, spectrophotometrically at 276 nm.

## RESULTS AND DISCUSSION

On increasing the albumin concentration from 200 mg/ml to 250 mg/ml, the particle size of the microspheres decreased, while it again increased by further increasing the albumin concentration. The optimum concentration of albumin was thus found to be 250 mg/ml that produced microspheres of size 3.87 µm. Similarly, size of the microspheres decreased initially on increasing the stirring rate from 800 to 1200 rpm, but further increase in the stirring rate to 1600 rpm lead to agglomeration. Thus, 1200 rpm was found to be the optimum stirring rate. Temperature used for stabilization of microspheres showed an important effect on the size and shape of the microspheres. At 100°, the microspheres were found to be unstable and tended to

agglomerate and form irregular shaped microspheres, having average size 4.81 μm. The optimum temperature of 125° produced microspheres having average size 3.92 μm. On further increasing the temperature to 150°, the particle size again increased to 4.28 μm. In chemically stabilized microspheres, the concentration of formaldehyde used as crosslinking agent affected the particle size. At 0.1 M concentration of formaldehyde, the particles formed were larger (5.71 μm) and were of irregular shape. Formaldehyde concentration of 0.2 M was found to be optimum, producing particles of 4.7 μm. Further increase in concentration of formaldehyde yielded particles of larger size. This may be attributed to the development of a gelled structure over the microspheres at higher concentration of the cross-linking agent. For comparative study microspheres of gelatin were also prepared and were found to be of greater size as compared to albumin microspheres. On studying the effect of surfactant concentration it was found that 4% concentration was optimum, producing particles of 5.5 μm. At concentrations lower or higher than 4%, the particles obtained were of larger size.

The drug loading efficiency of nonmagnetic-heat stabilized microspheres was found to be 7.6% whereas, that of magnetic-heat stabilized microspheres was 6.9%. Similarly, nonmagnetic-chemically stabilized microspheres entrapped 7.2% and magnetic-chemically stabilized microspheres entrapped only 6.4% of the total drug taken. The drug loading efficiency of magnetic and nonmagnetic gelatin microspheres were found to be 6.5% and 5.7%, respectively (Table 1). Thus this study showed that drug loading efficiency of magnetic microspheres was marginally less than that of nonmagnetic microspheres. Also, the drug entrapment efficiency of gelatin microspheres was found to be less than that of BSA microspheres.

The magnetic responsiveness, after 10 min., was found to be higher for heat stabilized BSA microspheres (78%), at 25% magnetite concentration as compared to chemically stabilized microspheres (61%). The gelatin microspheres were found to be the least responsive (58%) (Table 2). It was also observed that magnetic responsiveness increased with increase in magnetite concentration from 20% to 30% (Table 3).

TABLE 1: PERCENT DRUG LOADING IN MICROSPHERES.

| Sample | Amount of microspheres taken (mg) | Drug content (%)* | |
|---|---|---|---|
| | | Non-magnetic | magnetic |
| A | 5 | 7.6±0.5 | 6.9±0.3 |
| B | 5 | 7.2±0.3 | 6.4±0.2 |
| C | 5 | 6.5±0.4 | 5.7±0.2 |

A: Heat stabilized BSA microspheres, B: Chemically stabilized BSA microspheres and C: Gelatin microspheres. *Mean±S.D. (n=5)

It was observed that after second or third hour sampling, the *in vitro* release from all the microsphere formulations, irrespective of their method of preparation, followed almost a linear relationship when the cumulative percent drug release was plotted against square root of time. This result can be correlated with Higuchi model for drug release. The rate of release was found to be effected by the process variables employed during the preparation of microspheres, such as albumin concentration, stirring rate, stabilization temperature, concentration of cross-linking agent, method of preparation etc. With the increase in albumin concentration, a decrease in release rate was observed. The effect of stirring rate showed that the release

TABLE 2: *IN VITRO* MAGNETIC RESPONSIVENESS OF DIFFERENT MAGNETIC MICROSPHERES SYSTEMS.

| Sample | Amount of drug incorporated in microspheres (mg) | Amount of drug in microspheres retained by magnets (mg) | Drug retained* (%) |
|---|---|---|---|
| A | 3.45±0.15 | 2.69±0.11 | 78±7.53 |
| B | 3.20±0.11 | 1.95±0.09 | 61±4.31 |
| C | 2.85±0.14 | 1.65±0.05 | 58±4.48 |

A: Heat stabilized BSA microspheres, B: Chemically stabilized BSA microspheres C: Gelatin microspheres. *Mean±S.D. (n=3)

TABLE 3: *IN VITRO* MAGNETIC RESPONSIVENESS AT DIFFERENT MAGNETITE CONCENTRATIONS.

| Magnetite concentration (%) | Percent magnetite retained by magnets* | | |
|---|---|---|---|
| | Heat stabilized BSA microspheres | Chemically stabilized BSA microspheres | Gelatin microspheres |
| 20 | 68±4.1 | 56±2.3 | 49±1.4 |
| 25 | 78±4.8 | 61±3.2 | 58±2.4 |
| 30 | 84±3.9 | 72±3.6 | 70±4.1 |

*Mean±S.D. (n=5)

rate was maximum at 1200 rpm. This indicates that at perfect sink conditions, rate of release is governed by the size of the particles. It was observed that increase in stabilization temperature also decreases rate of release. In chemically stabilized microspheres, the release rate was decreased with an increase in concentration of cross-linking agents. These results suggest that the higher degree of stabilization resulted in increased rigidity and reduced porosity, thus decreasing the release rate. Also the gelatin microspheres exhibited lower release rate than heat stabilized BSA microspheres and chemically stabilized BSA microspheres. After 8 h, the cumulative amount of drug released by gelatin microspheres was found to be only 18%, whereas that released by heat stabilized BSA microspheres was 23.5% and that by chemically stabilized BSA microspheres was 21.5% (fig. 2)

The *in vivo localization* studies showed that after 1 h of the administration of magnetic microspheres, 61% of the drug was recovered from the rat's tail section 3 under the influence of magnetic field. The drug concentration in other organs was found to be 2.5% in lungs, 10.5% in liver, 2.5% in spleen, 5.5% in kidney and 3% in blood (fig. 3). Though the magnetic field was removed 30 min. after the administration of microspheres, 49% of the drug was recovered from the rat's tail section 3 after 3 h of the administration (fig. 4). This localization may have appeared due to the penetration of the microspheres by endocytosis in the rat's tail section, lack of phagocytosis and slow rate of blood flow in the tail. These results were compared with that obtained from nonmagnetic microspheres (i.e. in the control rats). In control group of rats, only 3.5% drug was recovered after 1 h (fig. 3) and 2% was recovered after 3 h from the rat's tail section 3 (fig. 4). However the largest concentration of the drug was found in the liver (44%) and spleen (17%).

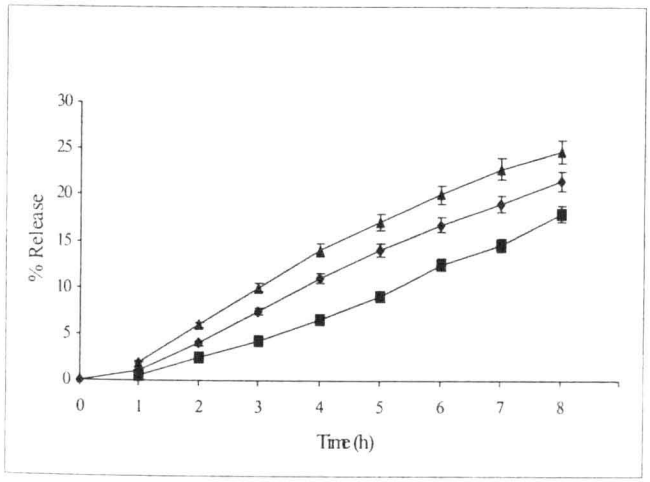


**Fig. 2: *In vitro* release rate profile of microspheres.**

**Release rate of diclofenac sodium from gelatin microspheres (-■-), heat stabilized BSA microspheres (-▲-) and chemically stabilized BSA microspheres (-♦-).**

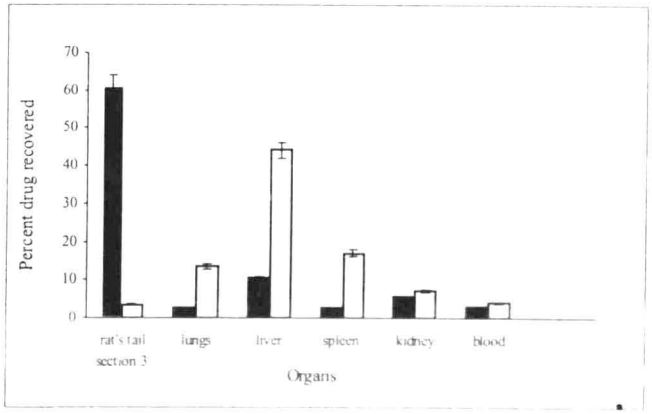


**Fig. 3: *In vivo* localization of microspheres after 1 h of administration.**

**Concentration of diclofenac sodium in various organs as compared to rat's tail section 3 after 1 h of administration of Magnetic microspheres (■) and non-Magnetic microspheres (□) .**

TABLE 4: DEGRADATION RATE CONSTANT AND SHELF LIFE OF THE MICROSPHERES AT DIFFERENT TEMPERATURES.

| Sample | Degradation rate constant (K) | | | $K_{25}°$ | $T_{10\%}$ (Days) |
|---|---|---|---|---|---|
| | 37±2° | 45±2° | 55±2 | | |
| Heat stabilized BSA nonmagnetic microspheres | $6.58x10^{-3}$ | $1.31x10^{-3}$ | $3.61x10^{-3}$ | $1.25x10^{-4}$ | 832 |
| Heat stabilized BSA magnetic microspheres | $9.87x10^{-3}$ | $1.64x10^{-3}$ | $3.94x10^{-3}$ | $1.58x10^{-4}$ | 658 |

$T_{10\%}$: Shelf life of the formulations at 25°

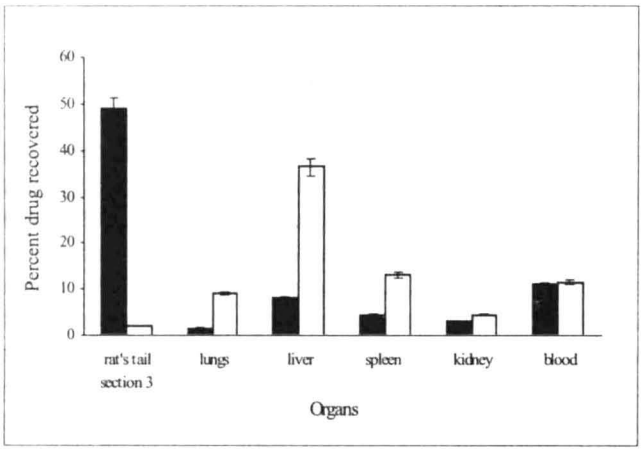


**Fig. 4: *In vivo* localization of microspheres after 3 h of administration**

**Concentration of diclofenac sodium in various organs as compared to rat's tail section 3 after 3h of administration of Magnetic microspheres (-■-) and non-Magnetic microspheres (-□-) .**

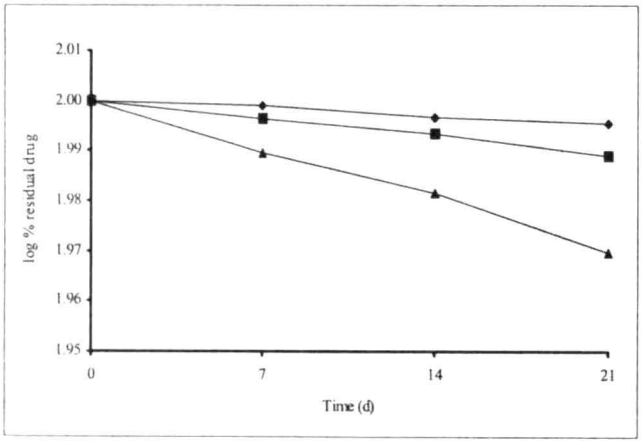


**Fig. 5: Stability of nonmagnetic microspheres at different temperature.**

**Stability studies of non-magnetic microspheres at 37° (-♦-), 45° (-■-), 55° (-▲-).**

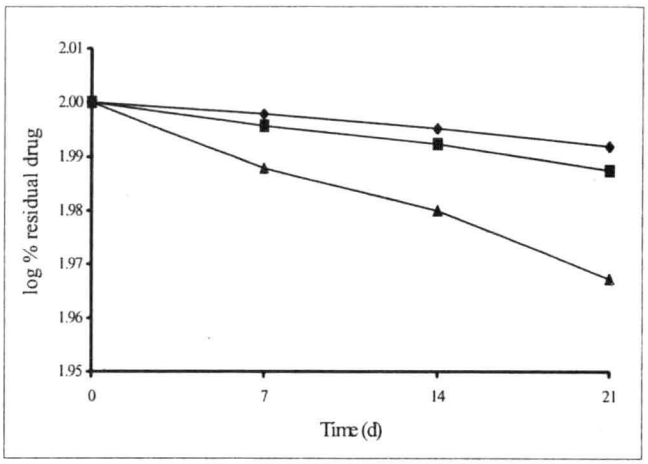


**Fig. 6: Stability of magnetic microspheres at different temperature.**

**Stability studies of magnetic microspheres at 37° (-♦-), 45° (-■-), 55° -(-▲-).**

During the stability studies, a first order rate of degradation was obtained both for nonmagnetic microspheres and magnetic microspheres (fig. 5, fig. 6 respectively). The shelf life for magnetic microspheres was found to be 658 d, which was less as compared to nonmagnetic microspheres (Table 4).


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