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Preparation and characterization of anastrozole loaded magnetic poly (epsilon-caprolactone) microspheres for anticancer activity

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ABSTRACT

The objective of the present study was to formulate and evaluate magnetic microspheres of anastrozole using polycaprolactone as the encapsulating material to achieve targeted drug delivery. Anastrozole loaded magnetic PCL microspheres were prepared by O/W emulsion solvent evaporation technique and characterized in terms of morphology, particle size, entrapment efficiency, drug loading, FTIR, DSC studies, magnetite content, magnetic properties, in vitro drug release and in vitro release mechanism. Microspheres were smooth and spherical in shape with an average size of 10.2-11.24µm. Encapsulation efficiency and drug loading were found to be good and the formulations exhibited superparamagnetic behaviour with saturation magnetization of 7.66 emu/g. FTIR studies showed the absence of chemical interaction between polymer and drug. DSC studies revealed amorphous state of drug in the magnetic microspheres. The average magnetite content was 18.22%w/w which could be sufficient to direct the microspheres to their target site. The in vitro release studies showed initial burst effect followed by sustained effect over a period of 21 days. Among all the batches formulated, formulation F3 containing drug to magnetite ratio 1:3 shows more controlled release behaviour. Results suggest that the prepared magnetic microspheres might be potentially used as carrier for targeted delivery.

Keywords: Anastrozole, magnetic microspheres, targeted drug delivery, PCL.

INTRODUCTION

Controlled release drug delivery systems are designed to deliver the drug at specific release rates within a predetermined time period. A major problem associated with these systems is that they do not exclusively deliver the drug to the target organ. This may be achieved by targeted drug delivery systems. The objective of drug targeting is to achieve desired pharmacological response at a selected site and minimize the toxic side effects on healthy cells and tissues **[1]**. Magnetic targeted drug delivery system by particulate carriers has emerged as a promising approach that can deliver the pharmaceutical drugs to target site using an external magnetic field **[2]**. Magnetic microspheres usually consist of a magnetic core and polymer shell. Magnetic core consists of magnetic materials such as magnetite (Fe_3O_4), nickel, cobalt, neodymium, iron, iron-boron or samarium-cobalt which are responsible for magnetic properties **[3].** Magnetite is a common iron oxide which exhibits superparamagnetic properties and is biodegradable, biocompatible, nontoxic and well tolerated by the body **[4,5].** The polymer shell around the magnetite core is made of biocompatible and biodegradable polymers. It might have the function of transporting and releasing the drug during its degradation process.

A wide variety of synthetic biodegradable polymers such as poly(alkylcyanoacrylates), polyanhydrides, polyorthoesters and polyesters such as poly(lactic acid), poly(glycolic acid), poly(ε-caprolactone) and their copolymers have been used **[6].** In this study, polycaprolactone is selected as the encapsulating material as it is biodegradable, nontoxic, biocompatible polymer with slow degradation and is most suitable for long term delivery

for a period of more than one year **[7,8].** It has high permeability to many therapeutic drugs and hence it is well suited for controlled drug delivery **[9].**

Anastrozole is a potent selective nonsteroidal aromatase inhibitor used for the treatment of breast cancer in postmenopausal women. Anastrozole inhibits the enzyme aromatase which is responsible for converting androgens to estrogens. For adjuvant treatment of early breast cancer in postmenopausal women, anastrozole is given as an oral dose of 1mg daily and therapy may be continued upto 5yrs **[10].** Inorder to get prolonged therapeutic effect and to target the drug at its site of action, in the present study, magnetic microspheres of anastrozole are formulated using polycaprolactone as an encapsulating material and the formulated microspheres were characterized by particle size analysis, Scanning electron microscopy (SEM), Entrapment efficiency, Drug loading, Fourier transform infrared spectroscopy (FT-IR), Differential scanning calorimetry (DSC) and evaluated for their magnetic properties, magnetite content and in-vitro drug release characteristics.

MATERIALS AND METHODS

2.1. Materials

Anastrozole was obtained as a gift sample from Sun Pharmaceutical Industries Ltd, Mumbai. Poly ε-caprolactone (M.wt.45,000) and iron(II/III)oxide nanopowder (magnetite) (size < 50nm) were purchased from Sigma-Aldrich, Switzerland. Polyvinyl alcohol (PVA) was obtained from Burgoyne burbidges Co. India, Mumbai. All other chemicals used were of analytical grade.

2.2. Preparation of magnetic microspheres of Anastrozole

The magnetic microspheres containing anastrozole were prepared by O/W emulsion- solvent evaporation technique. The microspheres were prepared by conception of drug and polymer at a ratio of 1:10 and varying the amount of magnetite in the formulations. Four such batches were prepared with drug and magnetite ratio of 1:1, 1:2, 1:3 and 1:4. Required quantity of PCL, magnetite and drug were dissolved in a mixture of 10 ml of dichloromethane: methanol and the mixture was sonicated for 10 min to make the organic phase. 1% w/v PVA aqueous solution (20ml) was prepared and the organic phase was slowly added to the aqueous phase. The mixture was emulsified with the help of a high speed homogenizer [IKA T 25 digital Ultra-Turrax] at 5000 rpm for 20 min and then probe sonicated for 10 min. The formed emulsion was further stirred for 3 hrs under a mechanical stirrer at 1000 rpm to evaporate the organic phase. The resulting magnetic microspheres were washed 3 times with water and collected by centrifugation at 9000 rpm for 10 min and dried in an oven at 50^0 C. The microspheres were then stored in a desiccator for further use. Different formulations were prepared by varying the concentration of magnetite as specified in table 1.

Table 1. Composition of anastrozole loaded polycaprolactone magnetic microspheres

Formulation Code	Drug (mg)	Polymer (mg)	PVA (%)	Magnetite (mg)
F 1	100	1000	1.0	100
F 2	100	1000	1.0	200
F 3	100	1000	1.0	300
F 4	100	1000	1.0	400

2.3. Morphology by SEM

The morphological characteristics of the samples were studied using scanning electron microscopy (SEM, JOEL-JFC 5300) (S-4800, Hitachi Co. Ltd. Japan). Magnetic microspheres were dispersed in distilled water, dripped in aluminium foil and evaporated. The dried magnetic microspheres were mounted on a copper stub**,** coated with gold palladium under vacuum using an ion sputter coater (JEOL JFC 1100E) and then observed under scanning electron microscope.

2.4. Particle size determination by optical microscopy method

Particle size and size distribution of the formulated magnetic microspheres was determined by optical microscopy. The eye piece micrometer was calibrated with the help of a stage micrometer. The microspheres were dispersed on a glass slide and the size of 300 particles was measured by using a micrometer attached with a microscope. The average particle size was determined using Edmundson's equation $D_{mean} = \varepsilon n d/\varepsilon n$. Where n= No. of microspheres counted, d = mean size range.

2.5. Determination of drug loading and encapsulation efficiency

50 mg of magnetic microspheres were accurately weighed and dissolved in 20 ml of dichloromethane: methanol mixture in a conical flask. The flasks were placed in an orbital shaking incubator and were shaken at 100 rpm for 24hrs. The resulting solution was filtered by using a whatmann filter paper, diluted with respective solvent system

and concentration was determined using the UV/ Vis spectrophotometer (Perkin Elmer- LAMBDA 25) at 214 nm. Drug loading and encapsulation efficiency were estimated by using the equations 1 and 2 respectively.

Drug loading = mc/mt x 100% ---------- 1

Encapsulation efficiency = mc/ mo x 100% ---------- 2

Where mc is mass of anastrozole in magnetic microspheres, mo is the total mass of the drug and mt is the total mass of magnetic microspheres.

2.6. Fourier Transform Infra Red Spectroscopy (FTIR) analysis

FTIR spectra of anastrozole, PCL, magnetite, and magnetic microsphere formulations were obtained using a FT-IR spectrophotometer (Perkin Elmer-Spectrum RX) using KBr pellet method. The samples were mixed with potassium bromide and pressed into pellets and were scanned in the IR range of 400- 5000cm^{-1}at 25^0 C.

2.7. Thermal analysis

DSC of pure drug, polycaprolactone, magnetite and magnetic microsphere formulations was performed by using Perkin Elmer DSC-7 to determine the state of anastrozole in the microspheres. Samples were heated in aluminium pans using dry nitrogen gas. The analysis was performed over a heating range of 30- 500°C at a rate of 10°C min-1.

2.8. Determination of magnetic property

A vibrating sample magnetometer (DMS 1600) was used to measure the magnetic properties of Fe_3O_4/PCL microparticles. The samples were placed in Teflon sample holder and the magnetic properties were determined by increasing magnetic field over the sample. The measurements were carried out at room temperature in the field range of ± 1 T.

2.9. Determination of magnetic content

The content of magnetite present in the formulations was estimated quantitatively by atomic absorption spectroscopy (AAS) [Elico SL 173]. 100 mg of magnetic microspheres were accurately weighed and digested with 5ml of HCl. The digested solution was made upto 500ml with de-ionized water and was thoroughly filtered using whatmann filter paper. The weight percentage of iron in the solution was assayed by AAS at 248 nm.

$$\text{Wt \%} = \frac{\text{ppm(mg/L) X Volume in mL X dilution factor X } 10^{-4}}{\text{Weight of Sample in grams}}$$

Where, Ppm (mg/L) - result obtained from the instrument, Volume in ml- volume required for digestion, weight of sample in gm- 0.100gm for all the samples.

2.10. In vitro drug release studies

The in vitro release studies for formulated magnetic microspheres were performed in phosphate buffer (pH 7.4) using dialysis bag at 37.5 ± 0-5^o C. Drug loaded magnetic microspheres equivalent to 10 mg of anastrozole were put into a dialysis bag immersed in 100 ml of phosphate buffer (pH 7.4) in a conical flask. The flask was placed in an orbital incubator shaker [Remi CIS 24] and rotated at 50 rpm. At predetermined time intervals, 5 ml of the sample was withdrawn and the same volume of fresh medium was replaced. After suitable dilution, amount of drug released in media was determined by measuring the absorbance using UV-Visible spectrophotometer at 214nm.

2.11. In vitro drug release kinetic studies

To study the drug release kinetics and mechanism, the cumulative release data obtained from in vitro release studies were fitted to various models: zero order (Q v/s t), first order (Log(Q_0-Q) v/s t), Higuchi (Q v/s $t^{1/2}$) and Korsemeyer Peppas model (log Q v/s log t). Where Q is the cumulative percentage of drug released in time t and (Q_0-Q) is the cumulative percentage of drug remaining after time t. Nature of release of the drug from the formulated magnetic microspheres was inferred based on the correlation coefficients obtained from the plots of the kinetic models. The model with highest correlation coefficient close to unity was taken as the appropriate model.

RESULTS AND DISCUSSION

3.1. Morphology by SEM Analysis

The morphology of PCL magnetic microspheres was investigated using scanning electron microscopy. The microspheres obtained from various batches were free flowing. Scanning electron micrographs showed that the

microspheres were spherical in shape and had relatively smooth surface without any pores as shown in Figure. 1 and Figure 2. The smooth surface reveals complete removal of the organic phase from the microspheres during the fabrication process. The microspheres were individually homogeneously distributed without the evidence of collapsed spheres.

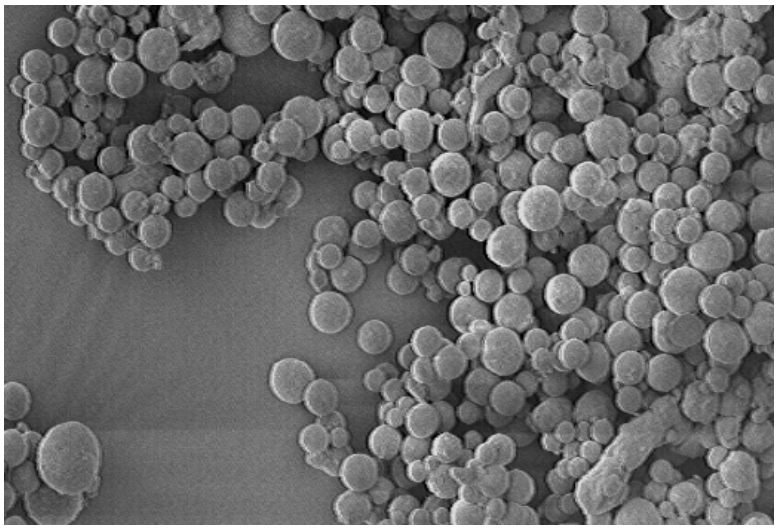

Figure 1. SEM image of anastrozole loaded polycaprolactone magnetic microspheres

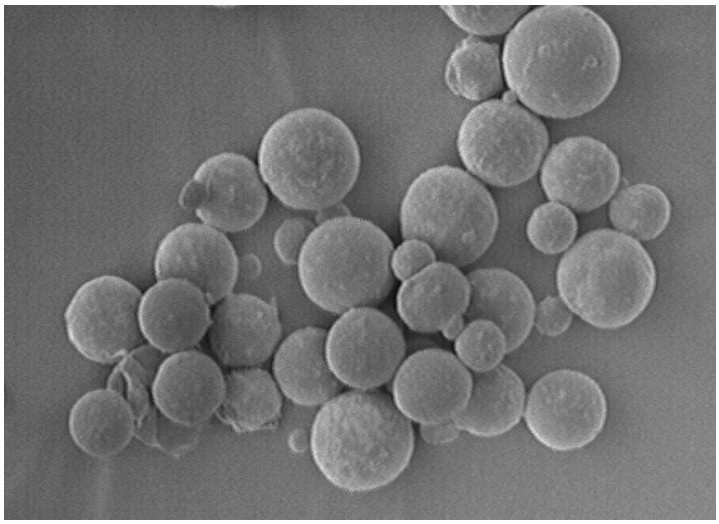

Figure 2. SEM image of anastrozole loaded polycaprolactone magnetic microspheres

3.2. Particle size determination by optical microscopy method

The PCL magnetic microspheres were prepared by solvent evaporation method using 1% polyvinyl alcohol as surfactant. The concentration of surfactant utilised in the formulation provides an optimum particle size. The average particle size of the drug loaded magnetic PCL microsphere formulations was found to be in the size range of 10.2µm to 11.2µm. The size of the particle was influenced with the addition of increasing concentration of magnetite. As there is an increase in the amount of magnetite incorporated a simultaneous increase in the particle size was observed. The size and size distribution of anastrazole loaded PCL magnetic microspheres are shown in figure 3.

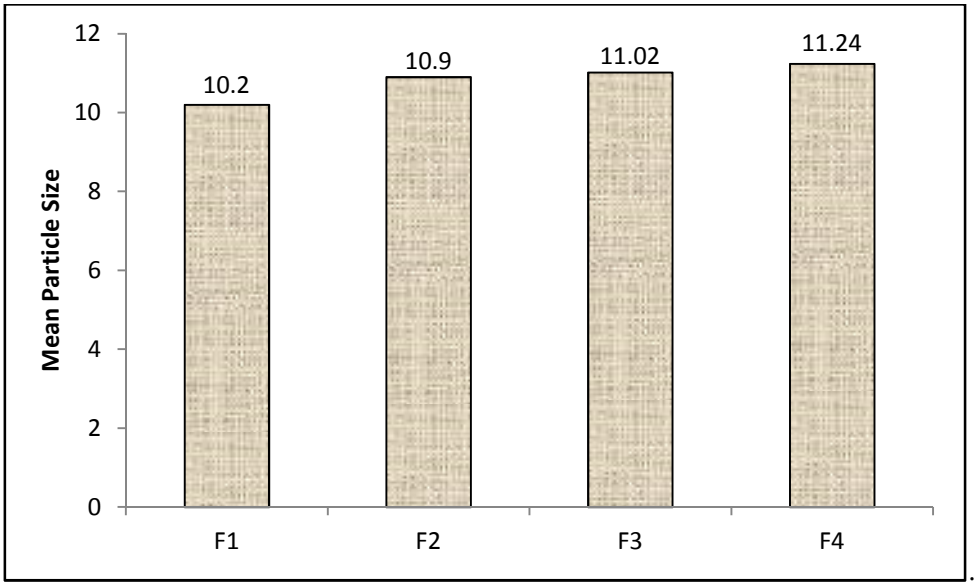

Figure 3. Average particle size of anastrozole loaded polycaprolactone Magnetic microspheres formulations F1-F4

3.3. Determination of drug loading and encapsulation efficiency

As shown in table 2, the entrapment efficiency and % drug loading of the magnetic PCL microsphere formulations (F1-F4) was found to be in the range of 81.32% to 84.03% and 7.65% to 13.10% respectively. Entrapment efficiency and drug loading decreased with increasing the concentration of magnetite due to the entrapment of the magnetite within the microsphere formulation. Entrapment efficiency was found to be good in all the formulations and a maximum of 84.3% was obtained in the formulation F1. This increased encapsulation efficiency is due to the greater proportion of polymer with respect to amount of drug. **[11]**

Table 2. Percentage drug loading and percentage encapsulation efficiency of formulation batches F1 – F4

Formulation code	Drug/polymer ratio	Drug/magnetite ratio	Average particle size (µm)	Encapsulation efficiency (%)	Drug loading (%)
F1	1:10	1:1	10.2	84.03	13.10
F2	1:10	1:2	10.9	83.62	11.33
F3	1:10	1:3	11.02	82.26	9.08
F4	1:10	1:4	11.24	81.32	7.65

3.4. Fourier Transform Infra Red Spectroscopy (FTIR) analysis

The FT-IR spectra of anastrozole, PCL, Fe_3O_4 nanoparticles magnetic microspheres were analyzed. The FTIR spectra of anastrozole (Figure 4) exhibit a characteristic band at 3045.9cm^{-1} which is characteristic of aromatic C-H stretch of benzene. Characteristic absorption band at 2234.7 cm^{-1} was observed which is assigned to aliphatic C≡N stretch of nitrile. The C=N hetero aromatic stretching was observed at 1605.3 cm^{-1} and 1273.6 cm^{-1}. Characteristic broad band at 3433.8 cm^{-1} due to stretching of aromatic nitrile was observed. FTIR spectrum of PCL shows a strong carbonyl stretching band at 1724 cm^{-1} revealed the ester carbonyl bond (Figure 5). The spectrum of Fe3O4 nanoparticles (Figure 6) exhibited a characteristic band at 588.3 cm^{-1} due to the iron oxide structure. The spectra of magnetic microsphere formulations (Figure 7) were identical to that of polymer and did not display the intense bands characteristic of drugs because they were of low intensity, less pronounced bands and were hidden or masked by the bands produced by the polymer. Hence this indicates the absence of chemical interaction between polymer and drug in magnetic microspheres and the presence of drug as a molecular dispersion in the polymer matrix.

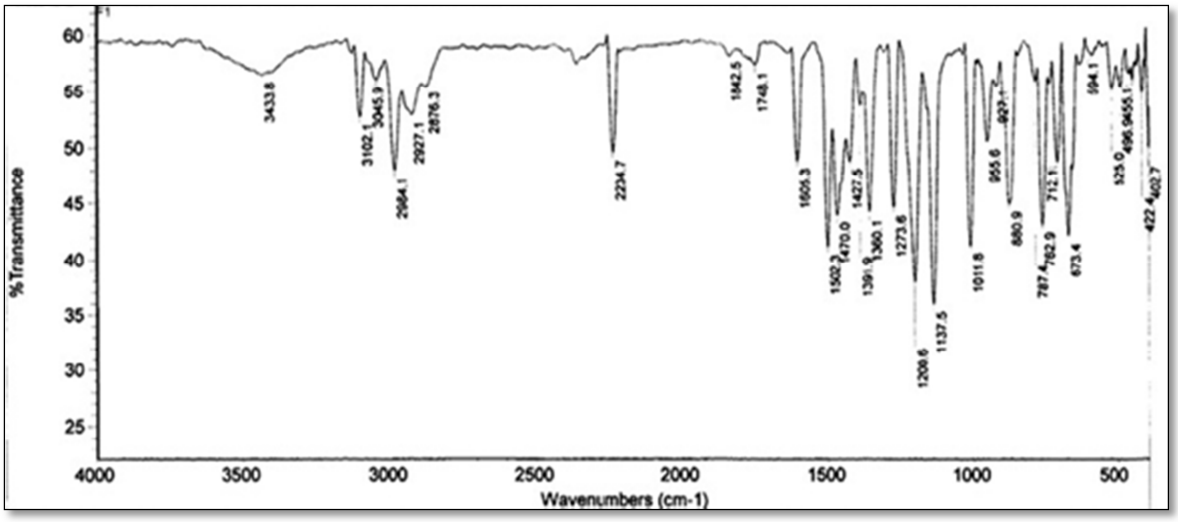

Figure 4. Infrared spectrum of Anastrozole

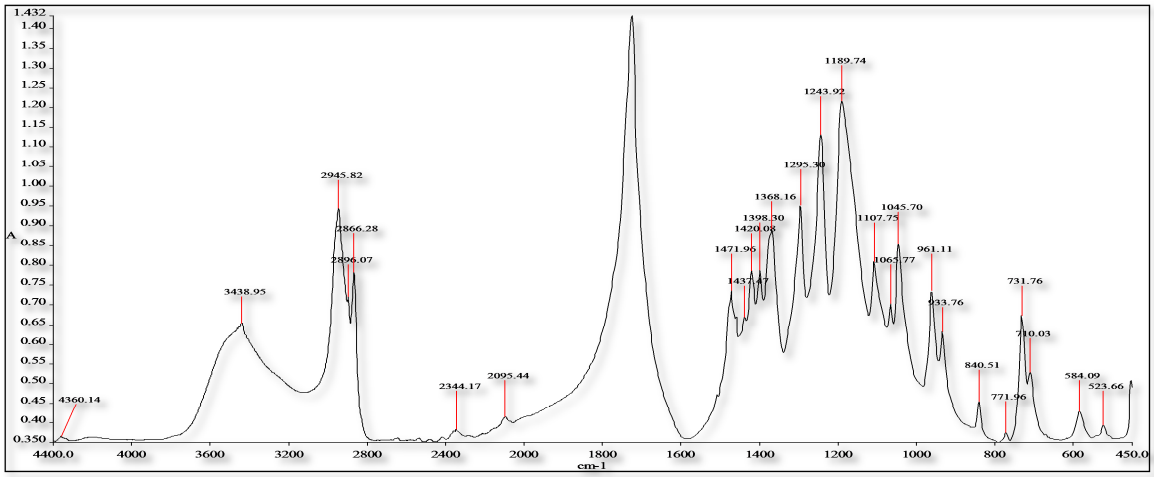

Figure 5. Infrared spectrum of polycaprolactone

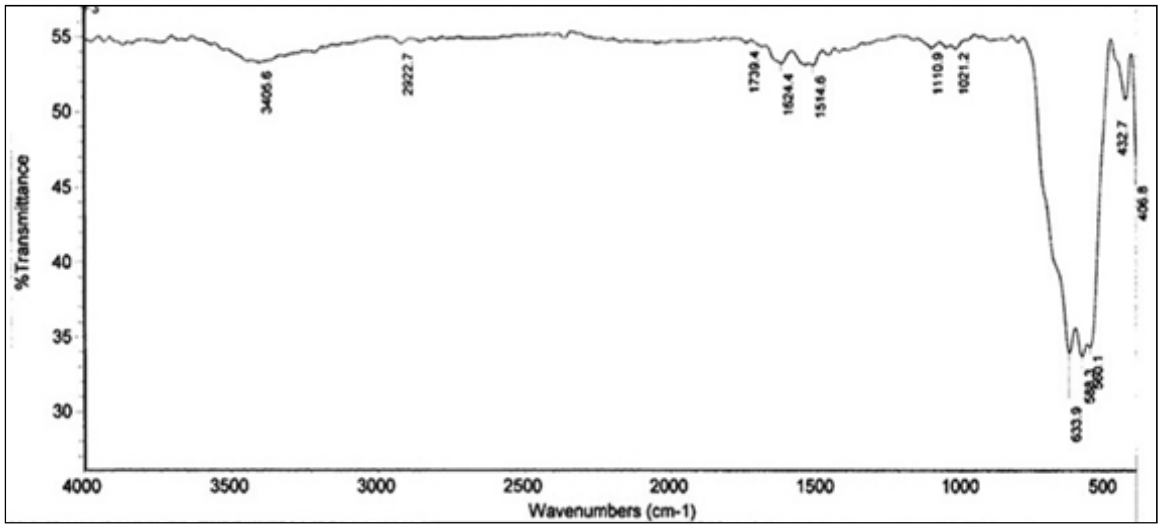

Figure 6. Infrared spectrum of Fe_3O_4 nanoparticles

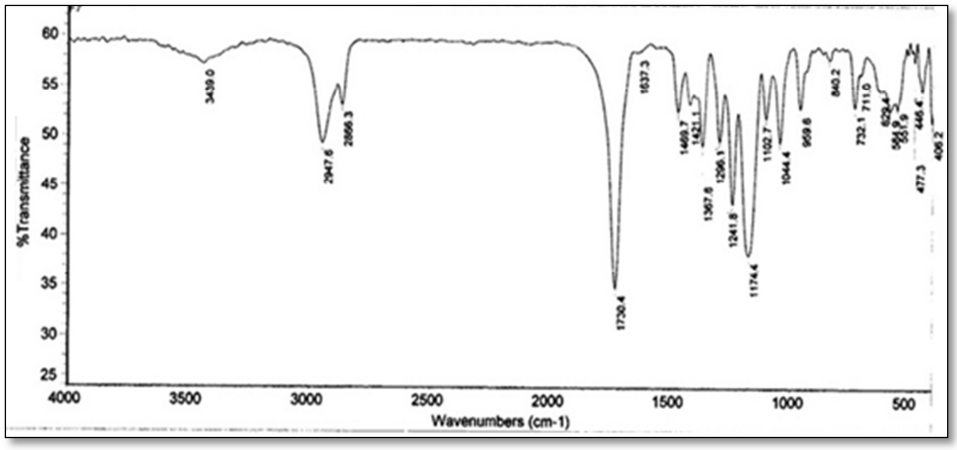

Figure 7. Infrared spectrum of anastrozole loaded polycaprolactone magnetic microspheres

3.5. Thermal analysis

The state of anastrozole in the magnetic microspheres was investigated by DSC. (Figures 8-11) shows DSC thermograms for pure drug, PCL, magnetite and drug loaded PCL magnetic microspheres. The glass transition temperature of pure drug was present at 88.1 ^{o}C and 178.8^{o}C and for polymer it was present at 68.1^{o}C and 365.2^{o} C. The thermogram of drug loaded magnetic microsphere exhibits similar shape and position to that of PCL polymer and the peak corresponding to the melting point of free drug was disappeared. Absence of detectable melting peak of drug in the formulation indicates amorphous or molecular dispersed state of entrapped drug. Thus the result suggests that the drugs were at molecular level at polymer melting temperature, and that the polymer maintained its characteristics in the microsphere formulations. The amorphous form of anastrozole in magnetic microsphere contributes to the strong intermolecular forces between anastrozole and polycaprolactone during the formulation of microspheres. This amorphous nature of the drug may have pronounced pharmaceutical significance as it could lead to increased solubility and finally to an improved biological activity.

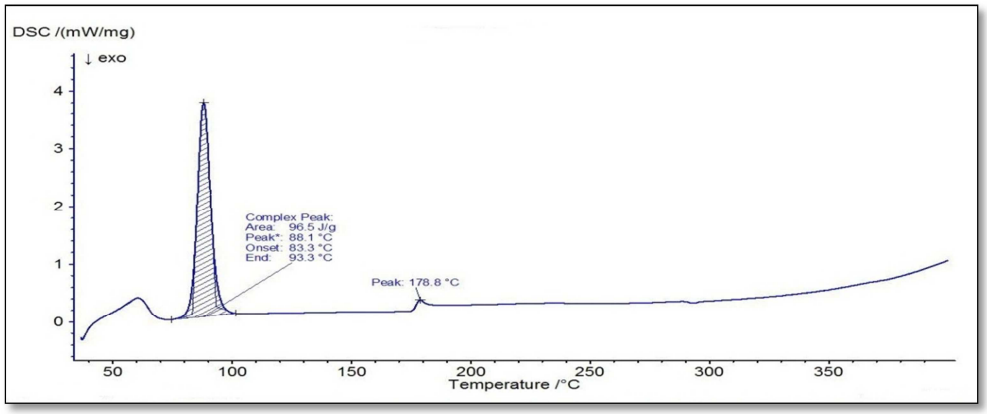

Figure 8. DSC thermogram of pure drug

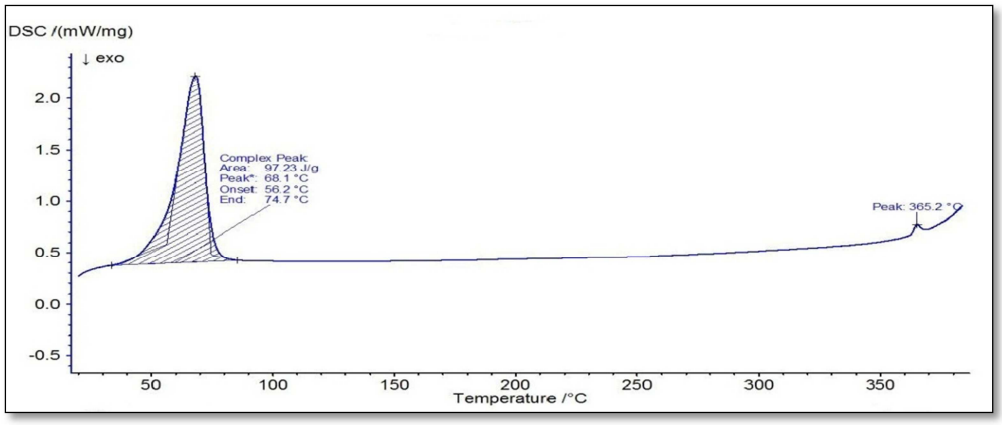

Figure 9. DSC thermogram of polycaprolactone

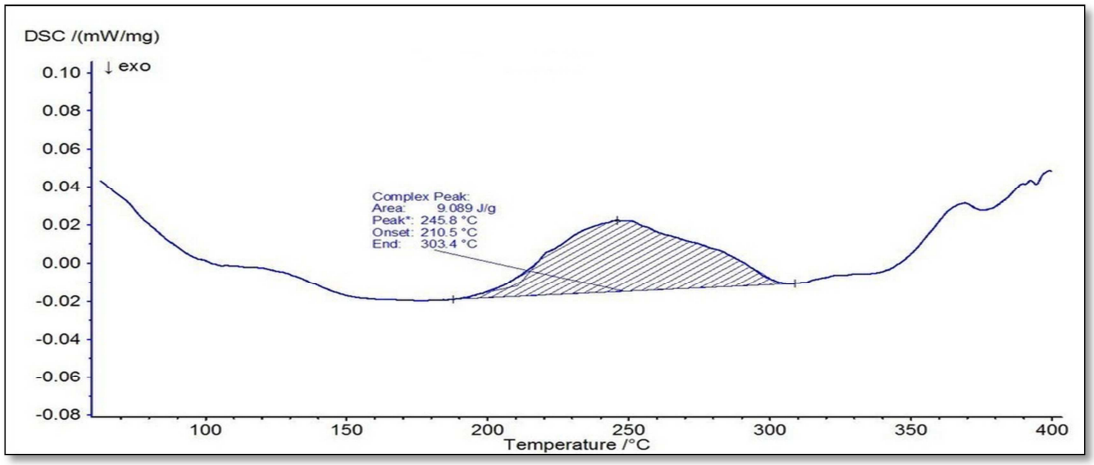

Figure 10. DSC thermogram of Magnetite

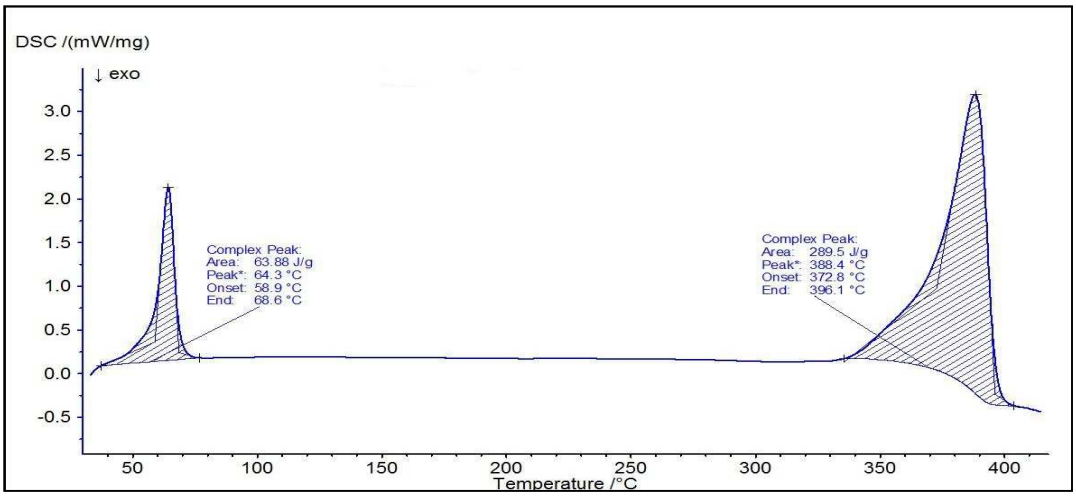

Figure 11. DSC thermogram of anastrozole loaded magnetic PCL microspheres

3.6. Determination of magnetic property

Magnetic properties of encapsulated magnetite in the PCL microspheres were investigated using Vibrating Sample Magnetometer (VSM). The superparamagnetic behaviour of polymer magnetic microsphere prevents magnetic microspheres from aggregation and enables them to redisperse rapidly when the magnetic field is removed **[3]**. The magnetization curve of the drug loaded magnetic microspheres and Fe_3O_4 nanoparticles at room temperature were shown in the (Figure 12). The saturated magnetization (σs) of magnetite and magnetic microspheres were found to be 22.19emu/g and 7.66emu/g respectively. It was observed that the saturation magnetization of magnetic PCL microspheres was smaller than that of bulk magnetite which is 84emu/g a typical value reported in the literature for

this material **[12].** The prepared magnetic microspheres exhibited superparamagnetic behaviour with no coercivity and remanence. This indicates single domain magnetic Fe_3O_4 nanoparticles remained in these magnetic microspheres.

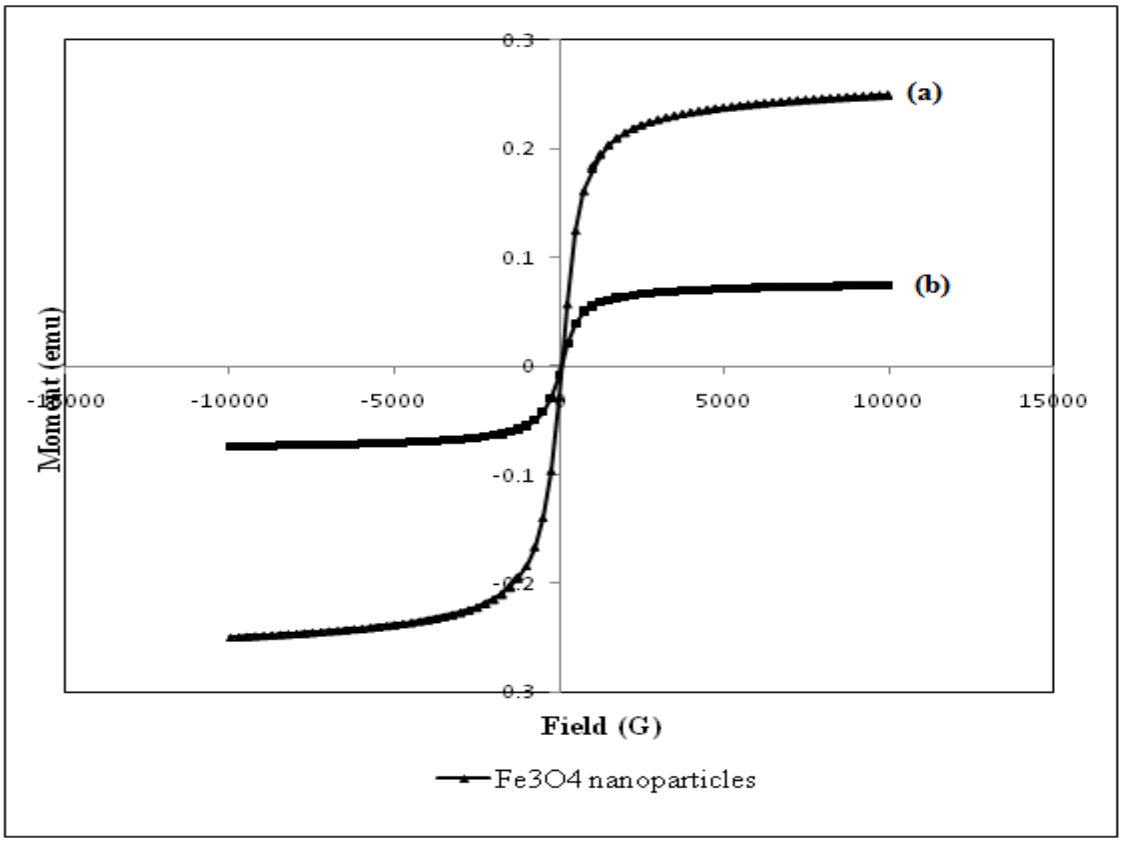

Figure 12. Magnetization curves obtained by vibrating sample magnetometer (VSM) at room temperature. (a) Fe_3O_4 nanoparticles (b) Fe_3O_4 / PCL magnetic microspheres

3.7. Determination of magnetic content

The amount of magnetite loaded in the magnetic PCL microspheres was determined by AAS and the results were shown in the table 3. The amount of magnetite among the formulations F1-F4 was found to be 22.17- 25.46%w/w. This concentration of magnetite within the magnetic microspheres could be sufficient to direct the microspheres to reach their target site. It was previously reported by Gupta and Hung that 15 – 20% w/w magnetite is sufficient to achieve 100% retention of the magnetic carrier using 8000G magnet in an arterio-capillary flow of 0.005 – 0.1 cm/s **[13].** It was also observed that a higher amount of magnetite (upto 30%) was considered sufficient to withstand arterial pressure under a magnetic field **[14].** Based on the above research findings, 25.46%w/w of magnetite included in PCL microspheres could be sufficient to achieve the expected degree of localization of the microspheres.

Table 3.Magnetite content of the magnetic PCL microspheres

Formulation code	% Magnetite content
F1	22.17
F2	24.52
F3	25.01
F4	25.46

3.8. In vitro drug release studies

Figure 13 shows the invitro release profile of anastrozole from the prepared magnetic microspheres. Formulations F1, F2, F3 and F4 having drug to polymer ratio 1:10 and drug to magnetite in the ratios 1:1, 1:2, 1:3 and 1:4 shows a biphasic pattern with initial burst release of 26.23%, 24.25%, 27.69%, and 25.38% respectively at the end of 24hrs and the remaining drug was slowly released over a period of 21 days due to higher concentration of the polymer. At the end of 21 days, formulations F1, F2, F3 and F4 exhibits 89.05%, 88.65%, 86.15% and 87.43% respectively. Initial burst release of the drug at the end of 24hrs is due to the presence of poorly encapsulated drug bound to the surface of the microspheres **[15,16].** The slow and controlled release of anastrazole from the microsphere formulation may be due to diffusion of drugs from polymer as well as due to erosion of polymer. The phenomenon

seems to be more accentuated by the high hydrophobicity of polycaprolactone **[17].** It was observed that there were no significant changes in the drug release of the formulations F1-F4 with the increase in the concentration of the magnetite. Among all the batches formulated, formulation F3 containing drug to magnetite ratio 1:3 shows more controlled release behaviour with 86.15% of release at the end of 21 days.

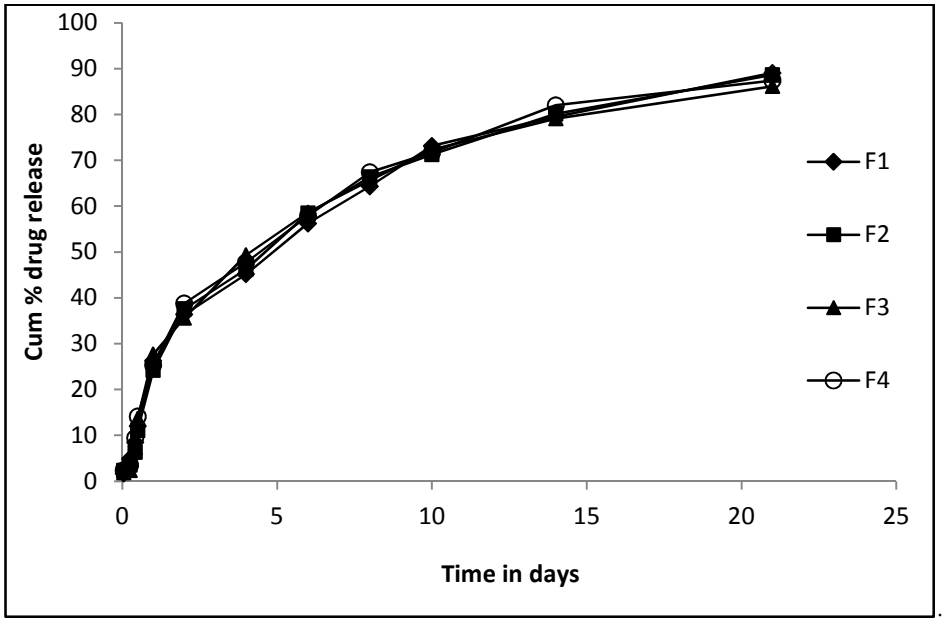

Figure 13. Comparative release profile of formulations F1 to F4

3.9. In vitro drug release kinetic studies

The release data obtained was fitted to various kinetic models such as zero order, first order, Higuchi, and Peppas models in order to conclude the mechanism of release of anastrozole from the magnetic PCL microspheres. The drug release pattern of the formulations F1 to F4 shows best fit with the highest correlation coefficients for by Higuchi. This indicates that the release of anastrozole from PCL magnetic microspheres is controlled by diffusion. Formulation F3 shows R^2 value 0.962 which is highest correlation factor with Higuchi. The results were shown in the table 4.

Table 4.Kinetic data of anastrozole loaded PCL magnetic microspheres

Batch	Zero	First	Higuchi	Peppas
	R^2	R^2	R^2	R^2
F1	0.839	0.980	0.976	0.716
F2	0.828	0.976	0.970	0.711
F3	0.807	0.955	0.962	0.710
F4	0.818	0.963	0.968	0.677

CONCLUSION

Based on our previous studies, magnetic microspheres of anastrozole were successfully prepared by O/W emulsion solvent evaporation technique and the microspheres were spherical in shape with smooth surface. The average particle size was found to be in the range of 10.2-11.24µm. High percentage of encapsulation efficiency and drug loading was achieved and the microspheres exhibits superparamagnetic behaviour with saturation magnetization of 7.66 emu/g. FTIR and DSC studies confirmed the absence of chemical interaction between drug and polymer and amorphous state of anastrozole in the magnetic microspheres. The in vitro release studies showed initial burst effect followed by sustained effect over a period of 21 days. Among all the formulations, F3 showed more sustained release behaviour. Hence it can be concluded that the magnetic PCL microspheres of anastrozole could deliver the drug for a prolonged period of time at the target site and might be potentially used as carrier for targeted delivery.

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