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Ultrasonically controlled release and targeted delivery of diclofenac sodium via gelatin magnetic microspheres

Muniyandy Saravanan[a,c,*], Kesavan Bhaskar[a], Gomathinayagam Maharajan[b], Kalathil Sadasivan Pillai[c]

[a] *Department of Pharmaceutics, Vel's College of Pharmacy, Old Pallavaram, Chennai, Tamilnadu 600 117, India*
[b] *Department of Physiology, Sri Jayendra Saraswathi Institute of Medical Sciences and Research, Perumbakkam, Tamilnadu 601 302, India*
[c] *Department of Toxicology, International Institute of Biotechnology and Toxicology, Paddapai, Kanchipuram District, Tamilnadu 601 301, India*

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Abstract

In the present work, an attempt was made to target diclofenac sodium to its site of action through magnetic gelatin microspheres. The gelatin magnetic microspheres loaded with 8.9% w/w of diclofenac sodium and 28.7% w/w of magnetite were formulated by emulsification/cross-linking with glutaraldehyde. The formulated microspheres were characterized by particle size distribution, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray diffraction and in vitro release studies. The in vivo distribution and targetability of gelatin magnetic microspheres after i.v. administration were studied in rabbits. The formulated microspheres were below 5 μm and spherical in nature as evidenced by the SEM photographs. DSC and X-ray diffraction studies revealed the absence of drug–polymer interaction. Encapsulated diclofenac sodium was released slowly more than 18 days. Application of sonication, as external stimuli to enhance drug release, during release study, has slightly increased the release rate. The formulated microspheres were injected intravenously after keeping a suitable magnet near the target area. The quantity of drug available at the target and non-target area was determined by HPLC. About 5.5% of injected dose localized near the target organ. Majority of injected dose was recovered from lungs, spleen and liver indicating localization of microspheres in these organs. Further studies are required to improve the targeting efficiency of gelatin microspheres by modifying surface properties to overcome phagocytosis and by selecting suitable particle size to avoid the entrapment of microspheres in non-target organs.

Keywords: Gelatin magnetic microspheres; Diclofenac sodium; In vivo distribution of magnetic microspheres; Controlled and targeted drug delivery

* Corresponding author.
E-mail address: msaravanan72@hotmail.com (M. Saravanan).

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1. Introduction

Targeting the drug with magnetic microspheres was first described by Widder et al. (1979), who used magnetically responsive biodegradable drug carrier with the capacity to localize both carrier and therapeutic agent, by magnetic means to a specified in vivo target site. These magnetic microspheres consist of magnetite (Fe_3O_4) particles, which are responsible for magnetic property, and a therapeutic agent entrapped in the biodegradable polymer matrix. Magnetite are biocompatible iron (II, III) oxide particles with no toxicity (Lübbe et al., 1996), hence many authors utilized this material in the preparation of magnetic microspheres, to target toxic drugs particularly for anti-cancer therapy (Kato, 1983; Gupta and Hung, 1989). Very few literatures are available in the development of magnetic microspheres with anti-inflammatory drugs. Lalla and Ahuja (1991) attempted to develop magnetic microspheres with anti-inflammatory drug in order to target it into inflammatory site.

Diclofenac sodium is one of the drugs of choice to treat arthritis because of its potential anti-inflammatory and analgesic activity and this is the only approved NSAID available for parenteral delivery. Because of shorter biological half-life, diclofenac sodium should be given frequently to maintain its therapeutic activity. It also has high percentage of protein binding and it undergoes pre-systemic metabolism. To overcome these problems, many authors developed sustained release formulations with an intention to maintain effective diclofenac concentration for prolonged period (Tuncay et al., 2000a,b). Gastric ulcers, gastrointestinal bleeding, blood dyscrasias and anaphylaxis are potential life threatening side effects of diclofenac sodium (Carson et al., 1989). To minimize the side effects, particularly to avoid gastric ulcers, diclofenac sodium is marketed as enteric coated and sustained release tablets. But even these formulations have shown GI toxicity in clinical studies (Davies, 1999).

To overcome the toxicity produced by the diclofenac sodium and to get prolonged therapeutic effect, in the present study, gelatin magnetic microspheres are formulated to target the drug at its site of action. Gelatin is well-known biocompatible and biodegradable polymer, widely used in various pharmaceutical applications and hence selected for the present study. The development of magnetic microspheres by using gelatin as carrier material is a new area and however a meager amount of research work has been carried out (Wu et al., 1993; Saravanan et al., 2003a) in this area. The gelatin magnetic microspheres were prepared by emulsification and cross-linking technique by using glutaraldehyde as cross-linking agent. The formulated microspheres were characterized by loading efficiency, entrapment/encapsulation efficiency, size distribution, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray diffraction and in vitro release studies. The in vivo distribution of formulated magnetic microspheres, in presence of a suitable magnet at target site, after intravenous administration was studied in rabbits to find out the targeting efficiency of the microspheres.

2. Materials and methods

2.1. Materials

Gelatin, type-B, 300 bloom strength was purchased from Sigma Chemicals, USA. Magnetite powder, less than 1 μm was supplied by Liquids Research Limited, UK. Diclofenac sodium gifted by MARAL, Chennai, India. Anhydrous ether, isopropyl alcohol, toluene, span 80 and glutaraldehyde were purchased from S.D. Fine Chemicals Ltd., Boisar, India and Sesame oil (Idhayam) was purchased from Food World, Chennai, India. Neodymium magnet, 8000 G field strength and 400 G/cm field gradient, 30 mm diameter and 4 mm thickness was purchased from ABY systems (PVT) Ltd., Red Hills, Chennai, India. Healthy white New Zealand rabbits weighing 1.5–2 kg were procured from animal house, Vel's College of Pharmacy, Chennai, India. All other reagents used were of analytical/HPLC grade.

2.2. Methods

2.2.1. Preparation of gelatin magnetic microspheres

Magnetite of lesser particle size (<1 μm) was employed in the preparation. Gelatin (750 mg) was dissolved in 3 ml of phosphate buffer (pH 7.4) heated to 60 °C. Diclofenac sodium (125 mg) was dissolved separately in 3 ml of phosphate buffer (pH 7.4) by heating and added to gelatin solution. Required quantity

(375 mg) of magnetite was wetted with 1 ml of 50% v/v ethanol, added to gelatin–diclofenac sodium solution and the mixture was homogenized (Remi, India, 5000 rpm). Then, the resulting mixture was added drop wise to 100 ml of oil phase (75 ml of sesame oil and 25 ml of anhydrous ether containing 1% v/v span 80) pre-heated to 45 °C and emulsified by stirring with help of hand blender (10,000 rpm/10 min). After getting the required globule size, the stabilized emulsion was stirred with help of a stirrer attached to a motor (Remi, India, approximately1000 rpm).

Ten millilitres of glutaraldehyde-saturated toluene solution was added slowly and stirring was continued for 6 h at room temperature. The cross-linked microspheres were allowed to sediment by placing 8000 G magnet at the bottom of the beaker. Then, the microspheres were washed with anhydrous ether to remove sesame oil. Then, it was washed thrice with 10 ml of 5% w/v sodium metabisulphite, twice with 10 ml of water and twice with 10 ml of isopropyl alcohol. During each washing, the microspheres were collected by placing a magnet of 8000 G strength at the bottom of the beaker. After washing, the microspheres were dried at 45 °C, transferred to glass vials and stored in a desiccator.

2.2.2. Determination of drug loading and encapsulation efficiency

Drug-loaded microspheres (100 mg) were digested with 10 ml of 1N sodium hydroxide (Saravanan et al., 2002) at room temperature for 12 h. The solution was filtered and analyzed (Rani et al., 1994) at 277 nm, to determine the amount of diclofenac sodium present in the microspheres. The drug loading in microspheres was estimated by using the formula:

$$L = \frac{Q_m}{W_m} \times 100$$

where L is the percentage loading of microspheres, Q_m the quantity of diclofenac sodium present in W_m g of microspheres. The amount of diclofenac sodium encapsulated in the microspheres was determined using the formula:

$$E = \frac{Q_p}{Q_t} \times 100$$

where E is the percentage encapsulation of microspheres, Q_p the quantity of drug encapsulated in microspheres (g) and Q_t is the quantity of drug added for encapsulation (g).

2.2.3. Determination of magnetite content

The magnetite content in microspheres was estimated quantitatively by hydrolyzing an aliquot of the microspheres in concentrated hydrochloric acid and assaying the resultant hydrolysate for iron by atomic absorption spectroscopy at 248 nm (Gupta et al., 1988) using AAS/127 instrument (electronic corporation, India).

2.2.4. Particle size analysis

Diclofenac sodium-loaded gelatin magnetic microspheres were analyzed for their size and size distribution. Microspheres were dispersed in water, vortexed for 3 min and ultrasonicated for 30 s before sampling. The particle size was measured by laser diffraction (SHIMADZU SALD 1100, Japan) and plotted for size distribution using the software supplied by the manufacturer.

2.2.5. Scanning electron microscopy

The sample for the SEM analysis was prepared by sprinkling the microspheres one side of double adhesive stub. The stub was then coated with gold using Jeol JFC 1100 sputter coater. The SEM analysis of the microspheres was carried out by using Jeol JSM 5300, Japan. The microspheres were viewed at an accelerating voltage of 15–20 kV.

2.2.6. Differential scanning calorimetry

DSC of diclofenac sodium, magnetite and microspheres were performed using Perkin-Elmer DSC-7 model. The instrument was calibrated with indium. All the samples (≈5 mg) were heated in aluminum pans using dry nitrogen as the effluent gas. The analysis was performed with a heating range of 50–200 °C and at a rate of 20 °C/min.

2.2.7. X-ray diffraction

Diclofenac sodium, magnetite and microspheres were subjected to X-ray diffraction study in an X-ray diffractometer (XD-D_1, Shimadzu, Japan), in the range 5–70° of 2θ. The working conditions were CuKα radiation, 30 kV, 20 mA and with a slit of 1.1–0.3 mm.

2.2.8. In vitro release studies

The in vitro release studies (Thanoo et al., 1993; Saravanan et al., 2003b) of drug-loaded microspheres were carried out at 37 °C in phosphate buffer (pH 7.4). Each batch of microspheres containing 20 mg of diclofenac sodium was individually added to 100 ml of phosphate buffer (pH 7.4) in flasks. The flasks were shaken (60 oscillations/min) in an incubator (Remi, India) at 37 °C. One millilitre of sample was withdrawn at regular time intervals and same volume of phosphate buffer was replaced. The diclofenac sodium present in the sample was estimated at 277 nm using UV visible spectrophotometer (Shimadzu 1601).

To find out the effect of ultrasonication on in vitro release, the flasks containing gelatin microspheres equivalent to 20 mg of drug in 100 ml of phosphate buffer (pH 7.4) were subjected to ultrasonification (Lifesonic, Lifeline systems, India, with following operative conditions: continuous mode, 2.5 W/cm^2, quartz crystal probe, 230 V, 50 Hz) 2 h before each sampling. One millilitre of sample was withdrawn at regular time intervals and same volume of phosphate buffer was replaced. The diclofenac sodium present in the sample was estimated at 277 nm using UV visible spectrophotometer (Shimadzu 1601).

2.2.9. Release kinetics

Data obtained from in vitro release studies were fitted to various kinetic (Costa and Lobo, 2001; Saravanan et al., 2003c) equations. The kinetic models used are zero order, first order and Higuchi equation. The following plots were made: Q_t versus t (zero order kinetic model); log ($Q_0 - Q_t$) versus t (first order kinetic model); Q_t versus square root of t (Higuchi model); $Q_t^{1/3}$ versus t (Hixson and Crowell model) and $Q_t^{2/3}$ versus t (modified root cube equation). Where Q_t is the amount of diclofenac sodium released at time t and Q_0 is the initial amount of diclofenac sodium present in microspheres. Further, to find out the mechanism of drug release, first 60% drug release was fitted in Korsmeyer–Peppas model.

$$\frac{M_t}{M_\alpha} = kt^n$$

where M_t/M_α is the fraction of drug released at time t, k the rate constant and n is the release exponent. The n value is used to characterize different release mechanisms.

2.2.10. In vivo study

The percentage of injected drug available at site of action was determined in normal rabbits (n = 5) as follows. Before injection, microspheres containing 10 mg of drug were dispersed in 0.5 ml of normal saline and sonicated for 2 min to get uniform dispersion. The contents were transferred to 1 ml syringe with 27-gauge needle. Then, gelatin magnetic microspheres was administered intravenously in ear marginal vein. A magnet of 8000 G strength was placed just above (median side of left thigh) the left knee (target) with help of adhesive tapes before injection. The institutional ethical committee for animal experimentation, Vel's College of Pharmacy, Chennai, India approved all experimental procedures.

After 1 day of post injection, animals were sacrificed (intra-peritoneal injection of phenobarbitone, 45–55 mg/kg) and the drug present in various organs was estimated by HPLC. The various organs such as liver, lung, kidney, heart, spleen, left and right femoral artery were homogenized in a tissue homogenizer (Remi, India) to get a uniform suspension. Magnetic microspheres present in the suspensions were separated by using 8000 G magnet and washed with normal saline. The magnetic microspheres thus obtained were kept in 50 ml of sodium hydroxide for 12 h at room temperature in order to digest the microspheres and release the drug in sodium hydroxide. In case of synovium, the synovial fluid was directly added to 50 ml of 1N sodium hydroxide.

After the digestion process, the contents were filtered to remove suspended matters. Five millilitres of filtrate was taken in a test tube and shaken well with 5 ml of methanol for 30 min. The solution was centrifuged (Remi, India, 3000 rpm) and 20 μl of clear supernatant liquid was injected into HPLC, Shimadzu, VP series, version consist of ODS C_{18}, Hycersil®, 250 mm × 4.6 mm, 5 μm particle size column. Water/methanol/glacial acetic acid (69:28:3) was used as mobile phase and pumped with help of two LC-10 VP pump (Shimadzu) at a rate of 2 ml/min. The quantity of diclofenac sodium present in the effluent was determined by using SPD-10 AVP UV/Visible detector at 275 nm. Typical chromatogram of diclofenac sodium present in the microspheres isolated from lungs are shown in Fig. 1. The percentage of administered dose available at the target and various organs determined by HPLC is given in Table 2.

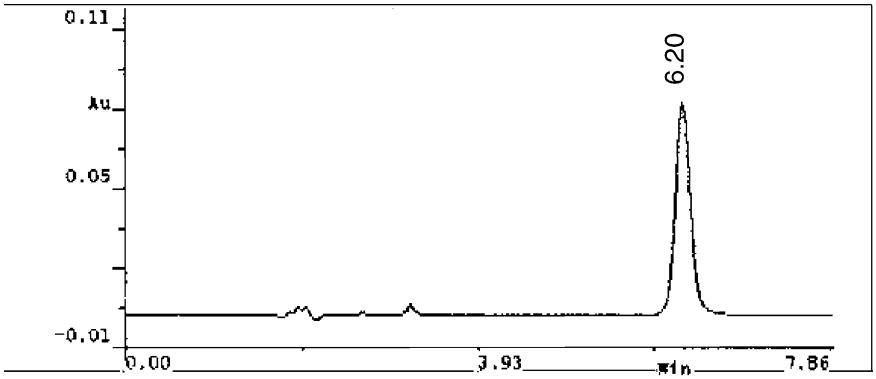

Fig. 1. A typical chromatogram showing the peak of diclofenac sodium at a retention time of 6.20 min obtained from the content of lungs after intravenous administration of gelatin magnetic microspheres.

3. Results and discussion

3.1. Preparation of gelatin magnetic microspheres

The procedure followed to prepare the magnetic microspheres produced good yield of microspheres. As shown in Table 1, the percentage of yield was 84, which indicated low loss of microspheres during preparation and recovery. The formulated magnetic microspheres were with free flowing nature. When the suspension of microspheres was placed near a magnet of 8000 G, their magnetic response was good and they were readily attracted by the magnet. This property was exploited in the recovery of the microspheres as described in the preparation procedure. The sesame oil with 1% v/v span 80 was found to produce spherical microsphere without aggregation. Glutaraldehyde-saturated toluene solution was used to cross-link and stabilize the magnetic microspheres. After stabilization, the unreacted glutaraldehyde was neutralized by using sodium metabisulphite.

Since gelatin magnetic microspheres was prepared for intravenous injection, a size range of less than 5 μm was prepared by optimizing the preparation conditions. Magnetite particles of less than 1 μm were used in this formulation. The stirring speed and gelatin/drug ratio was optimized by observing the particle size under microscope attached with micrometer. The process of drug localization by magnetic microspheres is based on the competition between forces exerted on the blood compartment, and magnetic forces exerted between the microspheres and applied magnetic field. When the magnetite forces exceed the linear blood flow rates in arteries (10 cm/s) or capillaries (0.05 cm/s), the microspheres are retained at the target site. It has been suggested that at the arterio–capillary blood flow rate of 0.005–0.1 cm/s, 20% w/w magnetite is sufficient to achieve 100% retention of the magnetic carrier using 8000 G magnet (Gupta and Hung, 1989). In an in vitro experiment it was demonstrated that 28% w/w of magnetite in nanoparticles is necessary for their effective targeting. Many authors formulated magnetic microspheres with magnetite content of 15–22% w/w and reported their targeting efficiency (Gupta and Hung, 1989) in various tissues. After reviewing all above factors available in the literature, in the present study, magnetic microspheres were prepared with higher amount of magnetite (30% w/w) to withstand arterial pressure under a magnetic field produced by 8000 G magnet.

3.2. Drug loading, entrapment and encapsulation efficiency

The drug present in the microspheres must be completely extracted by suitable method during the determination of drug content. Sodium hydroxide solution was used to digest the gelatin in order to extract the encapsulated diclofenac sodium. Since the drug is soluble in sodium hydroxide, it is possible to get complete extraction of drug from the microspheres (Saravanan

Table 1
Physicochemical parameters of gelatin magnetic microspheres loaded with diclofenac sodium

Gelatin (mg)	Diclofenac sodium (mg)	Magnetite (mg)	Yield		Percentage of drug loading		Percentage entrapped	Percentage encapsulated	Magnetite content	
			Milligram	Percentage	Theoretical	Actual			Theoretical	Actual
750	125	375	1053 ± 68	84 ± 5.44	10	8.9 ± 0.74	89 ± 7.4	74.9 ± 6.2	30.9	28.7 ± 1.9

Values are mean ± S.E. ($n = 3$).

et al., 2002). Gelatin magnetic microspheres showed good loading, entrapment and encapsulation efficiency as given in Table 1.

3.3. Determination of magnetite content

The targeting through magnetic microspheres is mainly influenced by the magnetic field applied at the target region and also by the magnetite content of the microspheres (Gupta and Hung, 1990). It has been demonstrated that the microspheres containing magnetite of 20–28% w/w could be effectively targeted at the site by using the magnet of 5000–8000 G (Gupta and Hung, 1989). The magnetite content of the formulated magnetic microspheres was estimated by the atomic absorption spectroscopy. The prepared gelatin magnetic microspheres showed good percentage of magnetite entrapment as given in Table 1. The content of magnetite present in the gelatin microspheres was 28.7% w/w, which is sufficient to retain the microspheres at the site of targeting by using a magnet of 8000 G.

3.4. Particle size analysis

Controlled release microspheres formulated for parenteral administration should be free flowing powder. The spherical particles should be less than 250 μm and ideally less than 125 μm in the diameter (Tice et al., 1990). The desired size of microspheres to be injected mainly depends upon the route of administration. In general, less than 5 μm size is used for intravenous route, less than 125 μm is used for intra-arterial and intra-articular route. Particles of this size can be administered easily by suspending them in a suitable vehicle and injecting them using a conventional syringe (Tice et al., 1990) with an 18- or 20-gauge needle.

The size distribution of gelatin microspheres loaded with diclofenac sodium for intravenous injection (Fig. 2) was between 0.4 and 5 μm. The average particle size of these microspheres was found to be 2.4 μm. The formulated microspheres were well below the size that can be injected through i.v.

3.5. Scanning electron microscopy

The size and shape of diclofenac sodium-loaded gelatin magnetic microspheres were further studied by

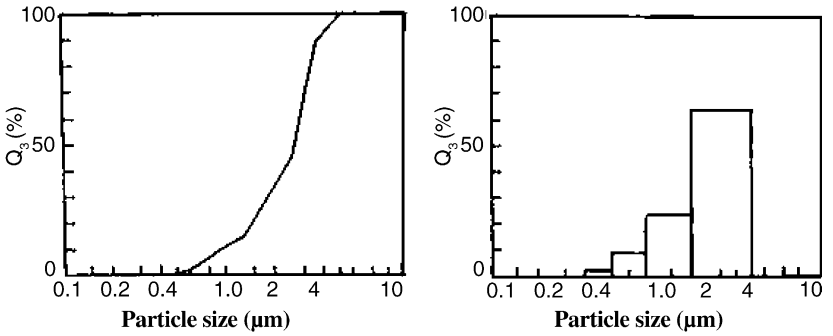

Fig. 2. Particle size distribution of gelatin magnetic microspheres loaded with diclofenac sodium.

SEM. As shown in Fig. 3, the formulated microspheres were spherical and compact in nature. The particle size of the formulated gelatin microspheres was less than 5 μm as evidenced by the SEM photograph. As shown in photograph, the particles were less aggregated and they were readily dispersed in water.

3.6. DSC

In our previous publication (Saravanan et al., 2003a), based on Fourier infrared spectroscopy, we have reported the compatibility of diclofenac sodium and gelatin. In the present study, the DSC analysis were performed to find out the physical nature of diclofenac sodium entrapped in the gelatin microsphere and also to confirm absence of drug–polymer interaction. The thermogram of diclofenac sodium showed (Fig. 4) a peak at about its melting point (297 °C). Because of its higher melting point, which is beyond the scanning temperature, thermogram of magnetite (Fig. 4) showed no peaks. The physical mixture of diclofenac sodium and gelatin microspheres (1:2) produced a peak about the melting point of the drug. The thermogram

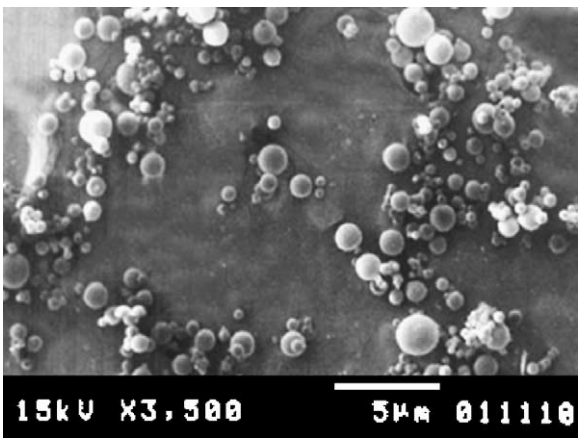

Fig. 3. SEM of gelatin magnetic microspheres loaded with diclofenac sodium.

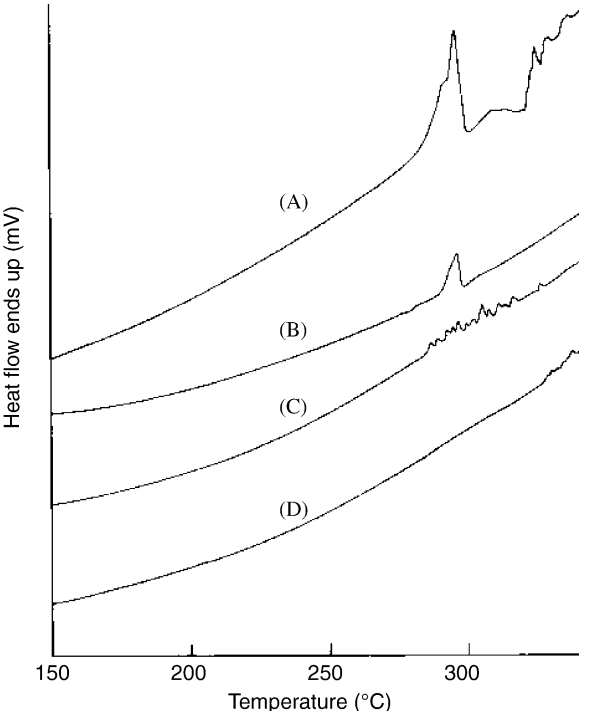

Fig. 4. Thermogram of diclofenac sodium (A), diclofenac sodium and unloaded gelatin magnetic microspheres (1:2) physical mixture (B), unloaded gelatin magnetic microspheres (C) and gelatin magnetic microspheres loaded with diclofenac sodium (D).

of unloaded gelatin magnetic microspheres showed no peak as given in Fig. 4 and indicated the amorphous nature of carrier particles. Diclofenac sodium peak was absent in the thermogram of drug-loaded gelatin magnetic microspheres, which revealed the amorphous nature of entrapped drug in the formulated microspheres.

3.7. X-ray diffraction

The physical nature of diclofenac sodium entrapped in gelatin microspheres was further confirmed by X-

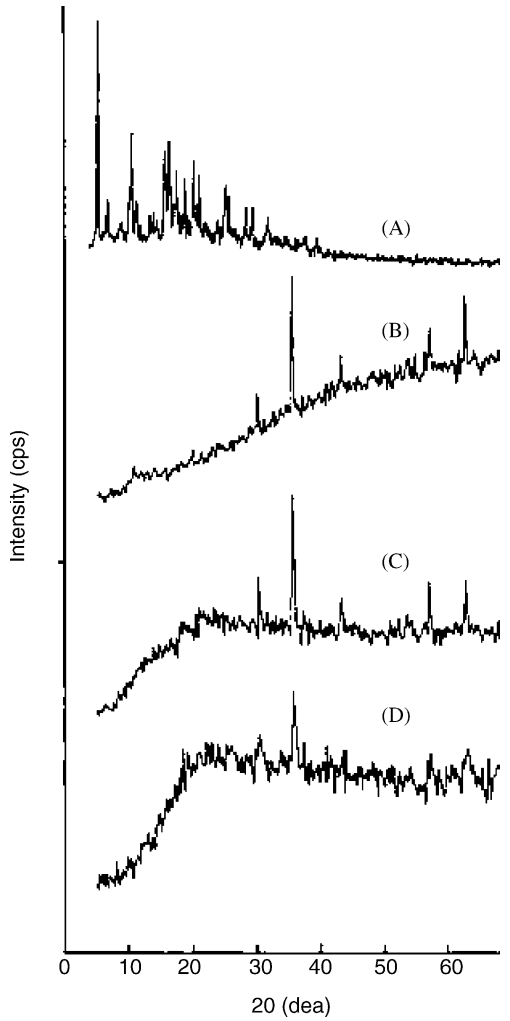

Fig. 5. X-ray diffraction patterns of diclofenac sodium (A), magnetite (B), gelatin magnetic microspheres without drug (C) and gelatin magnetic microspheres loaded with diclofenac sodium (D).

Table 2
Square parameters of the model equations applied to the release of diclofenac sodium from gelatin magnetic microspheres formulated for intravenous administration

Batch no.	Zero order equation		First order equation		Higuchi model		Korsmeyer–Peppas		Hixson–Crowell		Modified cube-root equation	
	r^2	k_0 (day^{-1})	r^2	k_1 (day^{-1})	r^2	k_H (day$^{-1/2}$)	r^2	n	r^2	k_{HC} (day$^{-1/3}$)	r^2	k_{MC} (day$^{-2/3}$)
A	0.9158	5.05	0.9890	0.1126	0.9963	22.01	0.9941	0.63	0.5652	0.1936	0.8045	1.0380
B	0.8779	6.06	0.9834	0.1333	0.9939	23.99	0.9997	0.52	0.5201	0.2410	0.7511	1.2540

A and B represent release kinetics in phosphate buffer (pH 7.4) without and with ultrasonification, respectively.

ray diffraction studies. The diffraction pattern of diclofenac sodium, magnetite, unloaded gelatin magnetic microspheres and diclofenac sodium-loaded gelatin magnetic microspheres were given in Fig. 5. The diffraction pattern of magnetite showed few characteristic peaks (Fig. 5), which were also presented in loaded and unloaded gelatin magnetic microspheres (Fig. 5). Characteristic peaks of magnetite in gelatin magnetic microspheres confirmed the presence of magnetite particles in the gelatin microspheres. It can be observed that the X-ray diffraction patterns of diclofenac sodium were showed sharp peaks due to crystalline nature of the drug. However, these drug peaks were disappeared in the X-ray diffraction patterns of diclofenac sodium-loaded gelatin microspheres (Fig. 5). It was thought that the diclofenac sodium showed its specific crystal peaks when existed in a crystalline form but after drug entrapped into the microspheres, the drug can be existed as a molecular dispersion in the microspheres. Aceves et al. (2000) and Ryu et al. (2000) observed similar patterns with other drug/carriers.

3.8. In vitro release studies

The drug release from microsphere was slow (Fig. 6) in phosphate buffer (pH 7.4) and extended up to 21 days. The release studies were also performed with application of ultrasonic waves, as an external stimulus to enhance/modify the drug release. The application of ultrasonification slightly enhanced the drug release from the magnetic microspheres (Fig. 6) and the drug was released within 15–18 days. This may be due to faster diffusion of dissolution medium or migration of drug molecules during the application of ultrasonic waves. The release rate was initially faster and then became slower as time progress.

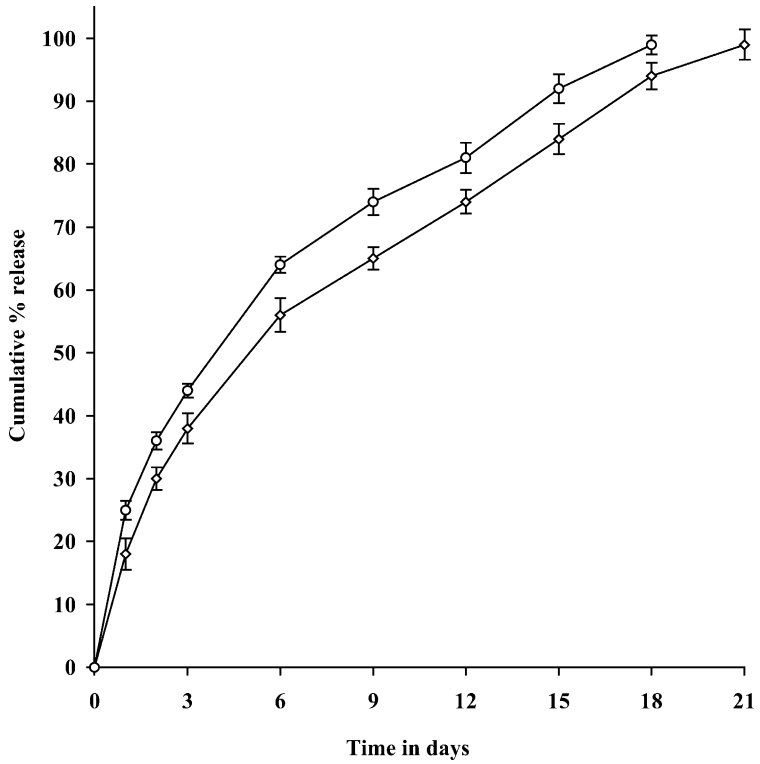

Fig. 6. The in vitro release of diclofenac sodium from gelatin magnetic microspheres prepared for intravenous administration. The figure illustrate the drug release in phosphate buffer (pH 7.4) without (–◇–) and with the application of ultrasonification (–◯–). Values represent mean of six determinations and bars represent ±S.E.

3.9. Release kinetics

The data obtained from the first 90% release were fitted to various kinetic equations to determine the mechanism of drug release and release rate. As indicated (Table 2) by higher correlation coefficient (r^2), the drug release from gelatin microspheres followed Higuchi model (diffusion controlled) than the first order and zero order equations. Hixson–Crowell and modified cube-root equations (dissolution controlled) showed poor correlation. These findings indicated that the drug release from the formulated gelatin microspheres were diffusion controlled. In our previous work, we reported the same kind of finding in gelatin magnetic microspheres, which was formulated with different gelatin/drug/magnetite ratio (Saravanan et al., 2003a). To confirm the release mechanism, the data of first 60% were applied to Korsmeyer–Peppas equation to find out the release exponent *n*, which indicates the mechanism of drug diffusion. The data were well fitted with the equation as indicated by high correlation (r^2) coefficient and the mechanism of diclofenac sodium release from formulated gelatin magnetic microspheres was found to be non-Fickian diffusion (anomalous transport, since the *n* value was between 0.5 and 1).

3.10. In vivo studies

The primary objective of this study was to target diclofenac sodium to its site of action for the effective treatment of inflammation. The magnetic microspheres were formulated with an intention to produce a depot near the target organ, by placing a suitable magnet near the target organ, particularly in the artery, which is supplying blood to the target organ. From the depot, drug will be released slowly and carried to the target organ through blood. By localizing the drug carrier near the target organ, unwanted distribution of drug to non-target organ can be avoided. This approach will localize the drug only at target site (since the drug is localized at the target area, lower dose of drug need to be administered) and minimize the drug-induced toxicity.

The formulated gelatin magnetic microspheres were tested for its targeting efficiency in rabbits. The microspheres were injected intravenously after keeping a suitable magnet at target site (above the left knee) and the targeting efficiency of microspheres was determined by measuring diclofenac sodium content in various organs. About 4.4 and 1.1% of injected dose were recovered from the left femoral artery and synovial content of left knee, respectively, after one day of post injection. The placement of magnet near the target joint after i.v. injection, able to localize the magnetic microspheres containing 5.5% of injected diclofenac sodium as depot in the artery and in the joint, which is adjacent to the magnet/target. The diclofenac sodium was below the detectable amount in non-targets such as right femoral artery and right knee synovium as given in Table 3. The targeting efficiency of microspheres and availability of drug-loaded magnetic microspheres at target area (a depot will be formed in left femoral artery due to presence of magnet) after intravenous administration was markedly low. This might be due to distribution/entrapment of microspheres in non-target organs (Table 3) and phagocytosis of administered microspheres by the macrophages/reticulo endothelial system (Kumar and Banker, 1996). Majority of injected dose was recovered from lungs, spleen and

Table 3
In vivo distribution (after i.v. injection) of gelatin magnetic microspheres in presence of magnet above the left knee (target site)

Organ	Dose recovered/organ (mg) mean ± S.E. (n = 5)	Percentage of drug distributed mean ± S.E. (n = 5)
Heart	0.27 ± 0.17	2.7 ± 1.7
Liver	1.45 ± 0.58	14.5 ± 5.8
Lungs	2.47 ± 0.78	24.7 ± 7.8
Spleen	1.89 ± 0.45	18.9 ± 4.5
Kidney	0.14 ± 0.1	1.4 ± 1
Synovial content of left knee (target site)	0.11 ± 0.12	1.1 ± 1.2
Synovial content of right knee	–	–
Left femoral artery (target site)	0.44 ± 0.23	4.4 ± 2.3
Right femoral artery	–	–

(–) Not detectable.

liver indicating localization of microspheres in these organs.

4. Conclusion

The formulated diclofenac sodium-loaded gelatin magnetic microspheres showed good entrapment and encapsulation efficiency with spherical geometry. DSC and X-ray diffraction studies confirmed the absence of drug–polymer interaction and amorphous nature of entrapped drug in the microspheres. The in vitro release profile revealed the ability of microspheres to prolong the drug release for more than 18 days. The application of ultrasonic waves slightly enhanced the drug release as seen in in vitro release study. This may be exploited in in vivo conditions to release the drug as and when required, by simply applying ultrasonic waves near the depot. The quantity of i.v. injected magnetic microspheres localized at the target site was only 4–5% and majority of microspheres was localized in liver, spleen and lungs. Further studies are required to improve the targeting efficiency of gelatin microspheres by modifying surface properties to overcome phagocytosis and by selecting suitable particle size to avoid the entrapment of microspheres in non-target organs.

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