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Preparation, evaluation and bioavailability studies of indomethacin-bees wax microspheres

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Abstract The present study envisages the preparation of microspheres containing indomethacin (IM) as model drug and bees wax as carrier, and to compare the in vitro release and pharmacokinetics of prepared IM formulation with commercially available oral formulation Microcid®SR. The microsphere formulations were prepared by meltable emulsified dispersion and cooling induced solidification. Surface morphology of microspheres has been evaluated using scanning electron microscopy (SEM). The SEM images revealed the spherical shape of microspheres and more than 98.0% of the isolated microspheres were in the size range 115–855 μm. Differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy studies indicated that the drug after encapsulation with bees wax was stable and compatible. A single dose randomized complete cross over study of IM (75 mg) microspheres was carried out on 8 healthy Albino sheeps. Plasma IM concentrations and other pharmacokinetic parameters obtained were statistically analyzed. The T_{max}, C_{max}, $AUC_{O\text{-}24}$ and $T_{1/2}$ values of Microcid®SR and optimized formulation were 3.0 h, 2038 ± 51.31 ng/ml, 9528 ± 129.65 ng/ml h^{-1}, and 2.59 ± 0.02 h^{-1}; and 3.2 h, 1940 ± 22.61 ng/ml, 8751 ± 41.32 ng/ml h^{-1}, and 2.68 ± 0.02 h^{-1}, respectively. Beeswax microspheres showed controlled release and it can be concluded that both the prepared formulation and Microcid®SR are bioequivalent.

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1 Introduction

The use of waxes and fats at Greek-Roman period was described by Gomelins celsis. The earliest use of waxes and fats was traced by Lipowasky in 1938. The coated particles approach to control the drug release was introduced by SKF in the early 1950. In recent years, various uses of wax and fat microspheres in the pharmaceutical field have come into fore front, involving the microspheres technology [1]. Over the past decades, treatment of illness has been accomplished by the administration of drugs to the human body through various conventional dosage forms. However, to achieve and maintain the drug concentrations within the therapeutic range, it is often obligatory to take the dosage form several times a day. This results in an undesirable see-saw pattern of drug levels in the body. As the cost involved in developing a new drug entity is very high, several advancements have been made to develop new techniques for drug delivery. The growing interest in controlled drug delivery release is because of its benefits like increased patient compliance, which is due to reduced frequency of administration and less undesirable side effects. Different waxes and fats have been used as barrier coatings due to their non toxic and biocompatible nature.

Oral controlled release dosage forms such as microparticles are becoming more popular than single unit dosage forms. The uniform distribution of these multiple unit dosage forms along the gastro intestinal tract could result in

more reproducible drug absorption and reduced risk of local irritation. The goal of any drug delivery system is to provide a therapeutic amount of drug (s) to the proper site in the body in order to promptly achieve and there by to maintain the desired drug concentrations during treatment. This idealized objective can be achieved by targeting the drugs to a specific organ or tissue with the help of controlling the release rate of the drug during the transit time in gastro intestinal tract. Poorly water-soluble drugs, which are lipophilic in nature easily mix with waxes/fat and show good absorption rate. The wax matrix material used in the current study has good pharmaceutical and biological properties. However, reported methods are not suitable for all drugs and it depends on the nature of drug and its end use. Among the reported conventional methods different strategies have been developed in recent years to design different types of wax and fat microspheres loaded with hydrophilic and lipophilic drugs using toxic solvents. The use of such solvents during formulation is of environmental concern and also faces challenge to human safety.

To overcome these problems, in the present investigation, water is used to prepare wax microspheres by meltable dispersed emulsified cooling induced solidification method. Furthermore, the process was optimized to produce microspheres to give better yield with spherical geometry and predictable dissolution pattern. Indomethacin (IM) is an important indole acetic acid non- steroidal anti-inflammatory drug, commonly used in the treatment of rheumatoid arthritis and other severe inflammatory diseases [2]. Now a days IM is widely used in the treatment of active stages of moderate to severe stages of rheumatoid arthritis. IM should be dosed at least 2–3 times per day. Due to its narrow therapeutic index, the frequency of adverse effects is dose related [3]. Considering the long therapeutic regimen of osteoarthritis therapy, the administration of IM may induce adverse side effects on gastro intestinal tract (GIT) as well as central nervous system (CNS), renal and cardiac systems [4]. The occurrence of these adverse effects can be reduced by the use of controlled release formulations [5]. Oral conventional dosage forms are administered 2–3 times a day to maintain adequate and effective therapeutic concentration in blood. However, it fails to protect the patients against morning stiffness [6]. Thus, the development of controlled release formulation of IM have several advantages over the other conventional dosage forms, such as reduction in occurrence of high initial peak plasma concentrations, protection against morning stiffness, prolonged duration of action, improved bioavailability, patient compliance and reduction in adverse effects [7]. The side effects could be lowered by controlling the drug release and by adjusting the absorption rate. This can be achieved by employing suitable modification in the manufacturing process [8].

Previous experimental results demonstrated that the waxes are biocompatible, non-immunogenic material used for the entrapment of drug and its controlled drug release in the intestinal tract [9]. Delivering the drug in the intestinal environment from wax microspheres could be manipulated by suitable coating techniques [10]. Desired plasma levels can be achieved without the risk of side effects using once a day dose of controlled release preparation [11]. These findings suggested that the kinetic control is an effective route for preventing the toxicity of IM. The goal of the present study are to formulate, characterize and study the in vitro drug release of IM microspheres and to compared with commercially available oral formulation Microcid®SR (75 mg capsule). Furthermore, the pharmacokinetics of IM optimized formulation and Microcid®SR 75 mg capsule were compared in healthy Albino sheeps.

2 Materials and methods

2.1 Materials

Indomethacins (IM), pure drug and mefanamic acid (MA), the internal standard were kindly donated by Micro Labs (Bangalore, India). Indomethacin is a white to pale yellow crystalline powder, which is insoluble in water and soluble in ethanol, ether, acetone, etc. It has a melting point between 155 and 161°C and has molecular weight of 357.8. Bees wax, Tween-80, all other chemicals and solvents used were of analytical grade and purchased from Ranbaxy Fine chemicals (New Delhi, India). Commercially available oral capsule formulation (Microcid®SR 75 mg, Micro Labs Ltd., India) is used for the present study.

2.2 Preparation of microspheres

Required quantity of bees wax was melted in a china dish kept on water bath. To the melted wax mixture, IM which was previously passed through sieve No. 100 was added and stirred to obtain a homogeneous mixture. The resultant mixture was poured into 150 ml of phthalate buffer solution (pH 4.5), previously heated to a temperature higher than melting point of carnauba wax. The surfactant, tween 80 (1.0–2.0% w/w) was added to the above mixture and stirred mechanically at 1200 rpm using a stirrer (RQ-127A). Spherical particles are produced due to dispersion of molten wax in the aqueous medium. The mixture was stirred continuously above the melting point of wax at 1200 rpm for 7 min. The temperature of the reaction mixture was cooled rapidly and brought down to 10°C by the addition of cold water. The resultant solid spheres were collected by filtration and washed with water to remove surfactant residue. Air-drying was carried out at room

Table 1 Micromeritic properties of the drug loaded IM-bees wax microspheres

Formulation code	Size[a] (μm)	Yield[a] (%)	Angle of repose[a] (θ)	Carr's index (% I)	Tapped density[a] (g/cm^3)
F1	347	81.82	25.56	11.00	0.3958
F2	355	86.25	26.47	11.32	0.3462
F3	342	82.29	26.92	10.22	0.3888
F4	339	84.54	28.24	12.48	0.3887

[a] Standard deviation $n = 3$, F1 = IM (3.0) + BW (8.8), F2 = IM (3.0) + BW (9.0), F3 = IM (3.0) + BW (8.9) and F4 = IM (3.0) + BW (9.1). IM is Indomethacin and BW is bees wax

temperature for 48 h to give discrete, solid, free flowing microspheres. A total of four formulations were prepared by varying the wax to drug ratios (Table 1).

2.3 Microsphere characterization

Tap density of the prepared wax microspheres was determined using tap density tester and percentage Carr's index (% I) was calculated using the formula;

$$\text{Carr's index } (\%\ \text{I}) = (\text{tapped density} - \text{bulkdensity})/\text{tapped density} \quad (1)$$

Angle of repose (θ) was assessed to know the flow ability of wax microspheres, by a fixed funnel method.

$$\text{Tan}(\theta) = \text{height}/\text{radius} \quad (2)$$

Scanning electron microscope (SEM) photomicrographs were recorded using Joel- LV-5600 SEM, USA. To determine the sphericity, the tracings of wax microspheres (magnification 45×) were taken on a black paper using Camera Lucida (model -Prism type, Rolex, India) and circulatory factor was calculated by the equation;

$$S = p^2/(12.56 \times A) \quad (3)$$

where, A is area (cm^2) and p is perimeter (cm).

In order to find the size distribution and size analysis of the wax microspheres sieve analysis technique and scanning electron microscopic (SEM) studies were used, respectively. The FTIR spectra of the samples were obtained using FT-infrared spectrophotometer (Shimadzu, 8033, USA) by KBr pellet method in the wave number range 600–4000 cm^{-1}. All dynamic DSC studies were carried out using DuPont thermal analyzer with 2010 DSC module. The instrument was calibrated using high purity indium metal as standard. The DSC scans of the samples were recorded in the temperature range ambient to 225°C in nitrogen atmosphere at a heating rate of 10°C/min.

In order to evaluate the drug loading, 100 mg microspheres were dissolved in 100 ml of % methanol. The resulting solution was analyzed using UV/Visible spectroscopy (Shimadzu-1601, Japan) at 319 nm after sufficient dilution with pH 7.2 phosphate buffer solution [12].

2.3.1 *In vitro drug release studies*

USP XXI dissolution apparatus, type II was employed to study the percentage of drug release from the prepared formulations. A quantity of drug loaded microspheres (IM equivalent to SR 75 mg capsule) were subjected for dissolution study in 900 ml dissolution medium for 2 h in pH 1.2 hydrochloric acid buffer and 6 h in pH 7.2 phosphate buffer at 100 rpm and a temperature of 37 ± 0.5°C using Tween 80 (0.1% w/v) to increase the wettability of microspheres. Drug concentrations were determined by withdrawing 10 ml of aliquots using guarded sample collectors at intervals of 30 min for first 4 h and at 60 min intervals for the next 4 h.

2.3.2 *Peppas model fitting*

Koresmeyer–Peppas model is used to evaluate the mechanism of drug delivery. The Koresmeyer–Peppas equation is as follows;

$$M_t/M_\infty = 1 - A\left(\exp^{-kt}\right) \quad (4)$$

$$\log(1 - M_t/M_\infty) = \log A - kt/2.303 \quad (5)$$

where, M_t/M_∞ is the fractional amount of drug released and t is the time in hours. In this study, the release constant, k and constant, A was calculated from the slopes and intercepts of the plot of ln $(1 - M_t/M_\infty)$ versus time (t).

The optimized formulation was subjected for stability studies, which were stored at in glass bottles at 25°C/60% RH (Relative humidity), 30°C/65% RH and 40°C/75% RH for a period of 90 days. A total of 100 mg of microspheres from each batch of formulations was taken at the end of 30, 60, and 90th days and were subjected for in vitro drug release studies.

2.3.3 *In vivo studies*

The in vivo release studies have been conducted on four male and four female healthy adult albino sheeps. The sheep's ages were in the range 6–8 years and their body weight ranged between 30 and 35 kg. A written approval was obtained from the Institutional ethical committee of

JSS Medical College Hospital and JSS College of Pharmacy, Mysore, India. Detailed verbal and written information on the study was provided to the Veterinary Surgeon, Central Animal Facility, JSS Medical College Hospital and permission was obtained. The study was conducted as an open, randomized complete cross over design in which a single 75 mg dose of IM (Microcid®SR 75 mg capsule and formulation F2) was administered to fasted, healthy adult males and females on two different occasions, separated by a wash out period of 2 weeks between dosing interval. The content uniformity of marketed product and optimized formulation have been estimated as per USP specification [13]. The contents of 5 units of Microcid®SR 75 mg capsule and formulation F2 were individually combined and weighed to the average weight of each unit. Drug was extracted from the respective dosage forms using methanol (80%). Methanolic extract was suitably diluted and drug content was determined.

All the animals have been shifted to the clinical trial laboratory from animal house at 7.00 AM after over night fast of 10 h. After shaving near the neck, an 18 gauge (1.3 × 45 mm, 96 ml/min) canula was inserted in to a jugular vein and kept with heparinised saline lock for ensuing 24 h blood sampling. Test medications (marketed product and optimized formulation) were administered to the sheeps, fed with banana and 200 ml water. Light food was provided at 3rd hour followed by two standard meals at 7 and 11th hour following drug administration. Blood samples (5 ml) were collected at 0 h (pre dose interval) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 16, 20 and 24 h post dose intervals. Blood samples were centrifuged (eltek-TC 4100 D centrifuge, Mnf. by Elektroshaft, Bombay, India) at 1500 rpm for 10 min. The separated plasma was stored at –20°C prior to analysis. Any other type of food was not permitted after 12 h administration of test medication. All subjects remained ambulatory and strenuous physical activity was prohibited during the first 12 h of blood sampling. Plasma concentration of drug from the collected samples was quantified by modified HPLC method.

2.3.4 Extraction procedure

Internal standard Mefanamic acid (MA) (100 µl) and citrate buffer (pH 3.0, 500 µl) were added to 10 ml screw capped glass tubes containing 500 µl of spiked plasma. The tubes were extracted gently with 7 ml of petroleum ether: dichloromethane (50:50) for 5 min on a rotary shaker and centrifuged at 800 rpm for 5 min. The organic phase was transferred to a watch glass and evaporated to dryness at 40°C. The residue was re-suspended in 100 µl of mobile phase and 25 µl was injected to the column. Quantification was achieved by the measurement of the peak area ratio of the indomethacin to the internal standard (mefanamic acid). The limit of detection of IM in plasma was 100 ng/ml (500 µl of plasma injected).

2.4 Chromatographic studies

Plasma concentrations of IM were quantified by high performance liquid chromatography (HPLC) method [12]. The HPLC system consisted of HPLC-Shimadzu (Tokyo, Japan) LC-6A model, fitted with a μ-Bondapack C_{18} (4.6 × 250 mm) column of particle size 5 µm (Supelco, Bellefonte, PA). The flow rate was maintained at 1 ml/min, and the drug concentration was detected using a UV/visible detector (SPD- 6Av). The mobile phase consisted of 80% methanol and 0.02 M sodium acetate buffer (60:40 v/v). The pH of the acetate buffer was 3.6. The column was heated to 40°C and wavelength of 320 nm was used.

2.4.1 Statistical data analysis

The pharmacokinetic parameters were calculated using the Quick calk, computer PK calculation programme. The peak plasma concentration (C_{max}) and time needed to reach peak plasma concentration (T_{max}) were computed directly from plasma level profiles as a measure of the rate of absorption of the drug from each product. The elimination rate constant (K_{el}) was calculated from the terminal elimination phase of logarithm of drug concentrations against time curve by the method of least square regression analysis. The biological half life ($T_{1/2}$) was determined by the relation;

$$T_{1/2} = 0.693/K \quad (6)$$

The extent of absorption for the drug (Microcid®SR 75 mg capsule and formulation F2) in different subjects from the area under the plasma concentration time curve from zero to 24 h (AUC_{0-24}) were calculated by the trapezoidal rule method. Area under the plasma concentration time curve from zero to infinity ($AUC_{0-\infty}$) was calculated using the formula;

$$AUC_{0-\infty} = AUC_{0-T} + C_{24}/K \quad (7)$$

where, C_{24} = drug concentrations in plasma at 24 h. The drug plasma concentration and pharmacokinetic parameters were analyzed by paired *t*- test and analysis of variance (ANOVA) at 95% confidence limits. Difference between two related means was considered statistically significant when their *P* values were equal to or less than 0.05.

3 Results and discussion

The effect of pH on drug entrapment, drug and wax ratio, amount of surfactant, stirring speed and time, and volume of aqueous phase used were optimized during the preparation of wax microspheres. It was found that, when the pH value of the external aqueous phase was acidic, the solubility of the drug was reduced and the encapsulated amount of the drug increased. The maximum drug load of 22.49% was obtained at pH 4.2 (phthalate buffer). As the pH increased from 4.2 to 7.0, the percent of IM loading was reduced from 22.49 to 5.21%.

In order to produce the spherical, discrete microspheres, an optimum drug to wax phase ratio of 1:3 w/w was used. It was found that the higher amount of drug to wax ratio (2:3) produces aggregate masses during the cooling process. It may be due to the increased amount of drug ratio, responsible for reduced the melting point of the wax, leads to aggregate mass. SEM photographs also indicated the presence of the crystals on the surface of the microspheres and resulted microspheres were unsuitable for pharmaceutical uses.

Incorporation of drug into wax microspheres requires the addition of a surfactant at an optimum concentration to reduce the interfacial tension between the hydrophobic material and the external aqueous phase. An attempt was made to incorporate the drug into the wax microspheres without the addition of a surfactant. But the process failed and resulted in formation of aggregate cake like mass during the solidification of wax. It may be due to the repulsion resulting from high interfacial tension between the hydrophobic waxy material and external aqueous phase. It was found that the surfactant having a hydrophilic lipophilic balance (HLB) value of 15 or more was suitable to increase substantial dispersion of wax and promotes drug incorporation in the microspheres. In order to obtain an optimal surfactant concentration, various concentrations ranging from 1.0 to 2.0% (w/w) of the total formulation were studied. Concentration of the surfactant (Tween-80) at 1.8% w/w of wax was used to obtain discrete microspheres with good flow properties after cooling.

The important factor that influences the size distribution of microspheres was the time and speed of stirring. A stirring speed of 900 rpm and stirring duration of 5 min was used to obtain reproducible microspheres. It was observed that with an increase in the stirring speed from 900 to 1100 rpm, there was a reduction in the average size and recovery yield of the microspheres. It was due to small sized wax microspheres, which were lost during successive washings. When the stirring speed was lower than 900 rpm, larger pellets were formed. It was also observed that an increase in stirring time from 5 to 7 min (at a stirring speed of 900 rpm) caused a reduction in the recovery yield of microspheres. When the stirring time was lower than 5 min, it was observed that some amount of melted material was adhered on to the sides of the beaker during the cooling process resulting in lower recovery of yield.

It was also noticed that 150 ml of aqueous phase was suitable for producing spherical microspheres and the resultant microspheres did not have any surface irregularities and are non-aggregated. As the volume of external phase increased, the yield was reduced and the resultant microspheres were also irregularly shaped. When the volume of the aqueous phase was less than 150 ml, the resultant microspheres were highly aggregated in nature and difficult to separate as an individual microsphere. Temperature of the aqueous phase was maintained 5°C higher than the melting point of the wax. It was also observed that when the temperature of the aqueous phase was less than the melting point of the wax (5°C), big flakes were produced.

The obtained micromeritic properties of the IM formulations were presented in Table 1. Sieve analysis data indicated that 53–60% of the prepared microspheres were in the size range of 115–855 μm. It was observed that an average size of the microspheres lies in the range 342–355 μm. The values of θ (angle of repose) were in the range 25.5–28.2, indicating reasonable good flow potential for the microspheres. The measured tapped density values lies in the range 0.3462–0.3958 g/cm^3. The percent Carr's index (% I) ranged from 10.22 to 12.48%, suggesting good flow property of the prepared microspheres.

SEM photomicrograph showed that the wax microspheres (F2) were spherical in nature and had a smooth surface with inward small pores on the wall of the microspheres (Fig. 1). This property may be due to removal of the solvent during in situ drying process. The rate of solvent removal from the microspheres exerts an influence on the morphology of the final microsphere. SEM photographs revealed the absence of crystals of the drug on the surface of microspheres indicating uniform distribution of the drug on the walls of the microspheres. The sphericity of the prepared microspheres was confirmed and the calculated values were nearer to one.

DSC studies were performed on pure drug; empty microspheres and drug loaded microspheres (Fig. 2). The pure drug exhibits a sharp endothermic peak at 161.3°C (Table 2). From the DSC thermograms, it was observed that the absence of the endothermic peak at 161.3°C in the drug loaded microspheres indicates that the drug is distributed at molecular level in microspheres [14].

The FTIR spectra for IM and formulation F2 is shown in Fig. 3. The characteristic IR absorption peaks of IM at 3410 (aromatic C–H stretching), 2624 (carboxylic acid stretching), 1695 (C=O stretching), 1600 (C=C stretching), 1450 (O$-CH_3$ deformation) and 1230 cm^{-1} (O–H deformation)

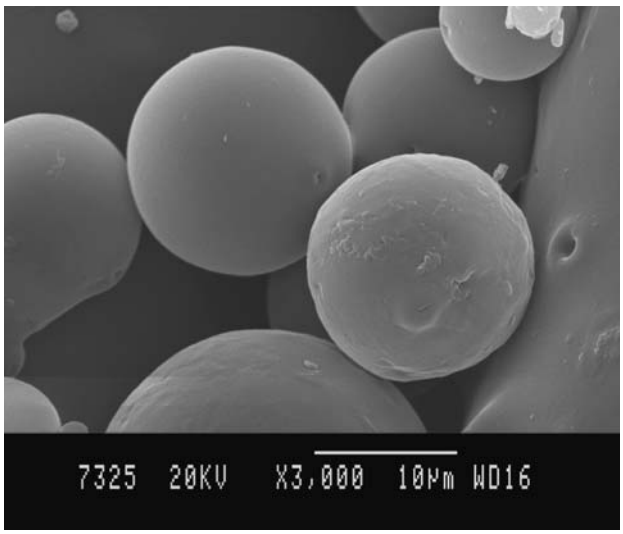

Fig. 1 SEM photomicrograph of formulation F2 showing spherical in nature

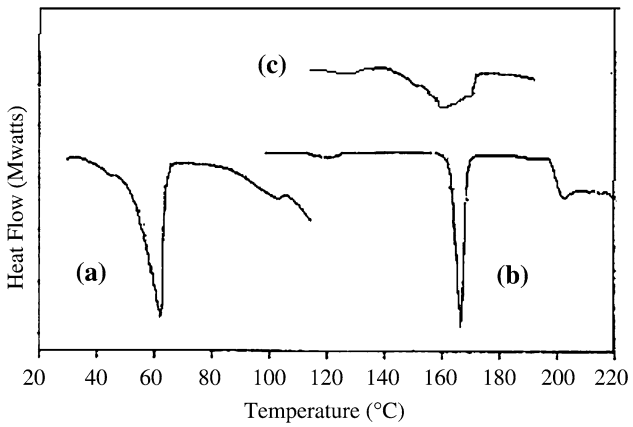

Fig. 2 DSC thermograms of, (a) Beeswax, (b) indomethacin drug, and (c) IM loaded wax microspheres (F2)

Table 2 DSC data for pure drug and optimized formulation

Formulation	Melting range (T_o–T_c) (°C)	Melting temperature (T_m) (°C)
Pure drug	158.35–163.4	161.3
F2	152.12–169.5	161.8

were not altered after successful encapsulation of drug, indicating no chemical interactions between drug and wax. The comparison and interpretation of this region in our spectra agrees with the reported result elsewhere [14].

The percent of drug loading in the formulations lies in the range 18.23–22.49%. The encapsulation efficiency (%) of the drug was found to be more for formulation F2 (90.03%) as compared to F1 (80.12%), F3 (84.58%) and F4 (83.21%) formulations (Table 3). From this result, it can be concluded that the formulation F2 had more encapsulation efficiency.

From the release studies, it was observed that, there is no significant release of drug from wax microspheres at gastric pH indicating that the used wax is gastro-resistant in nature [15, 16]. At the end of 8th hour, in vitro drug release from formulation F2 (94.8%), was faster than the other formulations {F1 (86.3%), F3 (78.8%), and F4 (86.8%)} in the intestinal environment as shown in Fig. 4. From the figure, it was clear that the drug was released in a biphasic manner consisting of initial fast release stage followed by a slow release at intestinal pH**.** After initial burst effect, the subsequent release of drug was slow and this release might be from the surface accumulated drug. The in vitro drug release of the optimized formulation (F2) was compared to marketed product.

The data obtained from in vitro drug release studies of marketed product and optimized formulation F2 was fit into Peppas model [17]. From the plot of log M_t/M_∞ versus t, the parameters such as release constant (k), constant (A) and the regression coefficient (R^2) were calculated and are given in Table 4. In both the cases the value of A were found to be less than 0.5. This result indicates that the release of drug from the microspheres was found to be by Fickian diffusion.

The measured average drug content uniformity of Microcid® SR 75 mg capsule (74.44 mg) and formulation F2 (74.46 mg) are presented in Table 5. The percent of drug content uniformity of Microcid®SR 75 mg capsule and formulation F2 are 99.23 and 99.28%, respectively. Hence, the percent of drug content uniformity in both the products were well within the limits as per United State Pharmacopoeia and National formulary specification [13].

Microcid®SR 75 mg capsule and formulation F2 were subjected for stability studies for 90 days. It was observed that in vitro drug release from Microcid®SR 75 mg capsule and formulation F2 at the end of 90 days (8th hour), were 94.73 and 98.13%, respectively. However, no significant change in in vitro drug release from both the products was noticed after the study period, indicating good stability for the prepared formulation.

Recovery of the IM from the plasma was calculated by comparison of peak height ratio after direct injection of IM or MA to the peak height of the same concentrations of the analytes extracted from plasma. In both the cases the absolute IM recovery from plasma was over 90%. The extraction solvent selected in this investigation gave higher recoveries and clean extracts than other solvents tested. Plasma spiked with 500 ng/ml of IM and 1000 ng/ml of MA, the retention time for IM and MA were 5.32 and 8.25 min, respectively. Sensitivity of HPLC assay qualitative confirmation of the purity of IM and MA peaks were

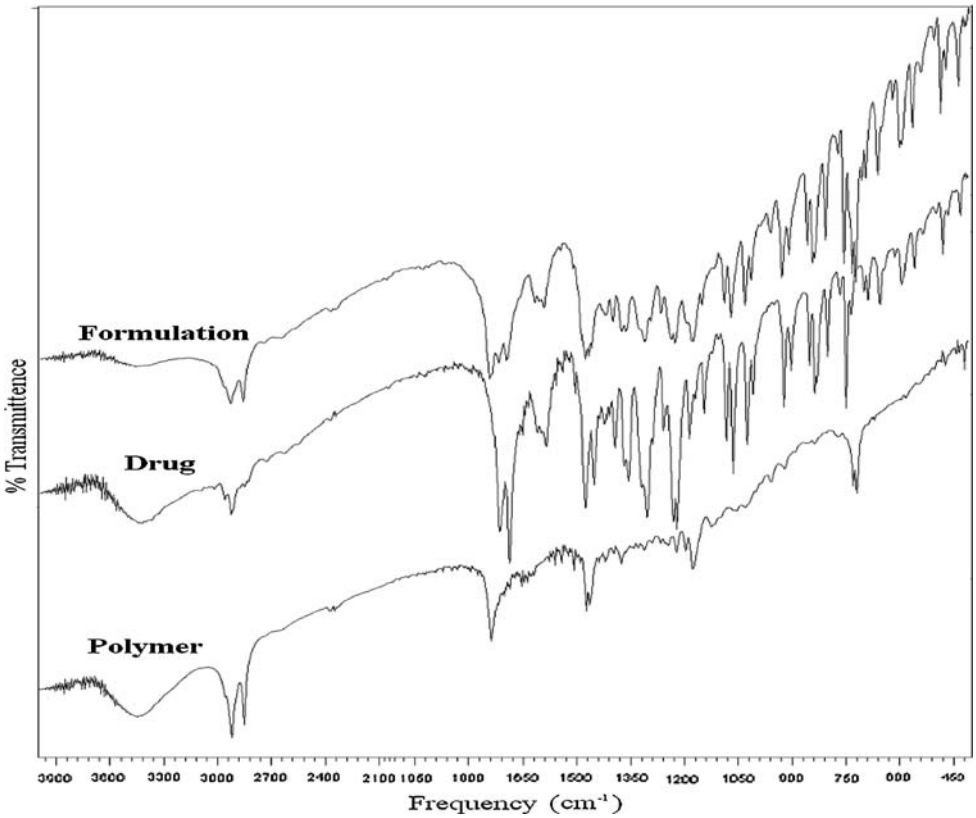

Fig. 3 FTIR spectra of indomethacin and IM loaded wax microspheres (F2)

Table 3 Drug loading properties of IM-bees wax microspheres

Formulation code	Drug loading[a] (%)	Encapsulation efficiency[a] (%)
F1	18.23 ± 0.11	80.12 ± 0.22
F2	22.49 ± 0.08	90.03 ± 0.34
F3	20.15 ± 0.15	84.58 ± 0.58
F4	19.50 ± 0.13	83.21 ± 0.49

[a] Standard deviation $n = 3$

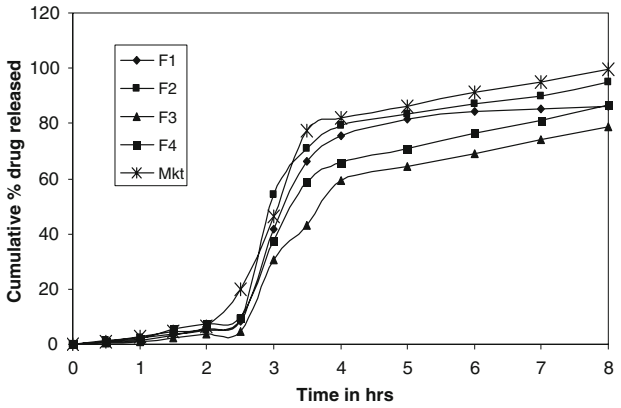

Fig. 4 Dissolution time profiles of indomethacin microspheres (F1–F4) and marketed product

Table 4 Data obtained from Peppa's model fitting for the microsphere formulations and commercially available product

Formulation	Constant A[a]	Release constant k[a] $(min^{-n})10^2$	Regression coefficient[a] (R^2)
F1	0.34	1.78	0.9656
F2	0.31	1.71	0.9682
F3	0.39	1.46	0.9664
F4	0.42	1.55	0.9639
Microcid®SR	0.38	1.68	0.9610

[a] Standard deviation $n = 3$

Table 5 Content uniformity for Microcid®SR and formulation F2

Product	Averaged drug content uniformity (mg)[a]	Percent (IM) labeled claim
Microcid®SR	74.44 ± 0.44	98.23–101.56
Formulation F2	74.46 ± 0.46	98.07–103.25

[a] Standard deviation $n = 3$

Percent (IM) labeled claim for the products Microcid®SR and formulation F2 is 75 mg. United State Pharmacopoeia 24/National formulary 19 specification is 92.5–107.5%

obtained (Table 6). The limit of quantitation was 50 ng/ml of IM in plasma when 0.5 ml plasma was placed. The obtained mean correlation coefficients for the standard

Table 6 Absolute recovery results obtained for indomethacin from plasma

Sampling time (h)	Drug present in ng/ml (A)	Drug added in ng/ml (B)	Drug recovered in ng/ml (C)	Drug conc. recorded in ng/ml (C−A)	% of drug recovered C−A × 100/B Mean ± SD[a]
0.5	10	50	56	46	92.0 ± 1.23
2.0	10	100	107	97	97.0 ± 1.46
4.0	10	200	205	195	97.5 ± 1.02
6.0	10	300	307	297	99.0 ± 0.71
8.0	10	400	408	398	99.5 ± 0.37

[a] Standard deviation $n = 3$

curves ($n = 7$) was 0.997. Assay was shown to be sensitive; capable of reliably detecting IM concentrations in plasma as low as 50 ng/ml. Interferences from endogenous compounds were overcome by using an acidic buffer (citrate buffer pH 3.0) to alter the pH of the aqueous phase before extraction. To prevent the substantial interferences from endogenous compounds, strong acid like HCl was employed. When the sample solvent was injected at a stronger concentration than mobile phase, column life gets shortened. This phenomenon, which has been described for the analysis of antibiotics, can occur in ion pair chromatography [18].

The mean plasma concentration as a function of time is shown in Fig. 5 and the calculated pharmacokinetic parameters of Microcid®SR and F2 formulations are given in Table 7. After oral administration of both the products, more mean C_{max} value was observed for Microcid®SR 75 mg capsule (2038 ± 51.31 ng/ml) than formulation F2 (1940 ± 22.61 ng/ml). However, the difference in the C_{max} values obtained for Microcid®SR 75 mg capsule and formulation F2 were statistically insignificant. The peak plasma levels for Microcid®SR 75 mg capsule and formulation F2 lies in the range 1978–2086 ng/ml and 1912–1955 ng/ml, respectively. Mean plasma concentrations of IM for both the products in all experimental conditions were within the therapeutic concentration range (300–3000 ng/ml) [5]. The C_{max} values for both the products do not exceed the above limit in all subjects. It was observed the plasma concentrations of IM fall below detection limit (50 ng/ml) after 24 h in all animals following administration of either product. On the basis of the therapeutic concentration range of IM, it could be concluded that the therapeutic effects of both formulations would be probably be maintained for about 12 h following a single dose administration. Thus it could be predicted that the two controlled release formulations included in this study are associated with a similar onset of therapeutic response following a single dose administration under fasting conditions. Further more, it could be predicted that both controlled release formulations in this study are associated with a similar onset of therapeutic response, following a single dose administration under fasting conditions.

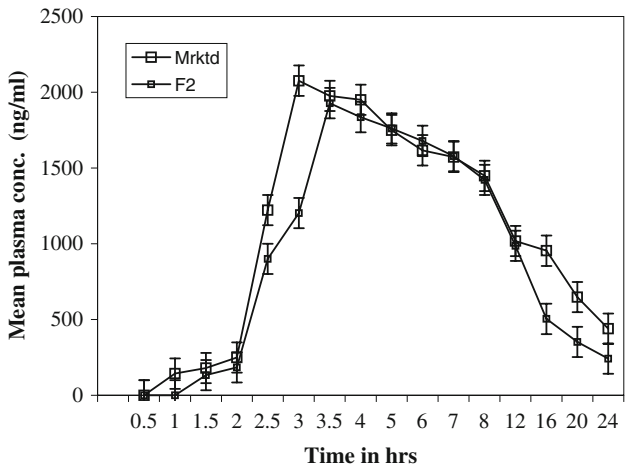

Fig. 5 The plot of mean plasma concentration as a function of time profiles of IM from Microcid®SR and formulation F2

The time taken to reach peak plasma concentration T_{max} of IM was little higher in case of formulation F2 compared to Microcid®SR, but no statistical significance differences between two products is observed (Table 7). The calculated mean $T_{1/2}$ values for Microcid®SR and formulation F2 were 2.59 ± 0.02 h^{-1} and 2.68 ± 0.02 h^{-1}, respectively. There was not much difference in the $T_{1/2}$ for IM, between both the formulations and no statistical significance differences were observed between both the products.

The systematic availability of IM can be determined by comparison of the area under the plasma concentration (AUC) versus time curves. The mean AUC_{0-24} values for Microcid®SR and formulation F2 were 9528 ± 129.65 ng/ml h^{-1} and 8751 ± 39.87 ng/ml h^{-1}, respectively. The slower in vitro release of IM from the products Microcid®SR and F2 formulations may be responsible for the decreased AUC values when compared to the reported conventional dosage forms [5]. The average value of the individual and mean AUC_{0-24} ratio at 95% confidence limit is within acceptable limits, indicating that the tested

Table 7 Comparison of mean values of pharmacokinetics obtained for products Microcid®SR and formulation F2 after oral administration

Parameters	Marketed formulation	Formulation F2	*P* value
T_{max} (h)[a]	3.0	3.2	<0.04
C_{max} (ng/ml)[a]	2038 ± 51.31	1940 ± 22.61	<0.04
$T_{1/2}$ (h^{-1})[a]	2.59 ± 0.02	2.68 ± 0.20	<0.04
AUC^{a}_{0-24} (ng/ml h^{-1})	9528 ± 129.65	8751 ± 39.87	<0.04
$AUC^{a}_{0-\infty}$ (ng/ml h^{-1})	9978 ± 132.92	8971 ± 41.32	<0.04
K_a (h^{-1})	0.3565 ± 0.003	0.3728 ± 0.003	<0.04
K_{el} (h^{-1})	0.2643 ± 0.003	0.2595 ± 0.002	<0.04
Mean residence time[a] (MRT)	4.69 ± 0.02	4.73 ± 0.01	<0.04

[a] Standard deviation $n = 3$

products are bioequivalent. The observed mean $AUC_{0-\infty}$ values for products Microcid®SR and formulation F2 were 9978 ± 132.92 ng/ml h^{-1} and 8971 ± 41.38 ng/ml h^{-1}, respectively and does not show any significant statistical difference between both these products.

K_a, K_{el}, and MRT values for Microcid®SR and formulation F2 are given in Table 7. The difference between the values obtained from the two formulations was not statistically significant. However, a small difference between both products related to C_{max}, K_a, K_{el}, T_{max}, $T_{1/2}$, AUC_{0-24}, $AUC_{0-\infty}$ and MRT, depends on IM release from the formulations. From the study, it was noticed that the reduced fluctuations and elevated mean plasma concentration of IM from both the products, responsible in protecting patient against morning stiffness [6].

The individual and mean AUC_{0-24} ratios for Microcid®SR and F2 were 1.12 ± 0.1 and 0.82 ± 0.1 ng/ml h^{-1}, respectively. The average values of the individual and mean AUC_{0-24} ratios at 95% confidence limit (0.87–1.14), was within acceptable limit for bioequivalent products [19]. In order to obtain in vitro-in vivo correlation, drug absorption profiles were compared for Microcid®SR and formulation F2 using the cumulative fraction of the drug absorbed in vivo against cumulative fraction of the drug dissolved in vitro up to 8 h. From the study it was noticed that both products showed an adequate correlation [20]. Currently accepted criteria in the US for bioequivalence for most dosage forms requires that, the mean pharmacokinetic parameters of the test dosage forms should be within 80–120% of the reference dosage form using 90% confidence interval. Pharmacokinetic parameters clearly indicate that the parameters of F2 are in good agreement with Microcid®SR.

On the basis of FDA recommendation [21], the two products, Microcid®SR and formulation F2 can be considered bioequivalent. No untoward effects were observed by any of the subjects after the administration of either product. Thus, the two formulations can be considered similar, because all the subjects very well tolerated. These observations clearly indicates the absence of high peak concentrations (>5000 ng/ml), which are very often associated with adverse effects due to drug accumulation [4], because of the accumulation effect. The products Microcid®SR and formulation F2 investigated in the present study were found to be bioequivalent.

4 Conclusions

The objective of the study was to prepare and evaluate wax microspheres loaded with IM by optimized meltable dispersion emulsified cooling induced solidification method for controlled release. The method employed was simple, rapid, and economical and does not imply the use of toxic organic solvents. The results of the drug entrapment and micromeritic properties, exhibited fairly good spherical nature as evidenced by SEM photomicrograph. The compatible state of the drug loaded wax microspheres were evaluated by FTIR and DSC. Both the formulations were found to be bioequivalent and both the formulations showed an adequate correlation between cumulative fractions dissolved in vitro and cumulative fractions absorbed in vivo. Optimized formulation F2 and marketed product Microcid®SR showed similarity in drug release profiles and in vivo bioequivalent behavior. From the present work, it can be concluded that the prepared wax microspheres demonstrate the potential use of wax for the development of controlled drug delivery systems for water insoluble or lipophilic drug.

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