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COMPARATIVE BIOAVAILABILITY STUDIES OF TWO INDOMETHACIN CONTROLLED RELEASE FORMULATIONS IN HEALTHY ALBINO SHEEP'S

Aravindram A. S.[1], D. V. Gowda†[2], Raghunandan V.[2] and Afrasim Moin[2]

ABSTRACT

The purpose of the present study was to compare the *in vitro* release and to find out whether the bioavailability of a 75 mg indomethacin capsule (Microcid®SR - reference - product A) produced by Micro labs, India was equivalent to optimized formulation (indomethacin loaded carnauba wax microspheres – test – product B). Indomethacin (IM) loaded carnauba wax microspheres were prepared by meltable emulsified dispersion cooling induced solidification method. 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 345 - 360 µm. Differential scanning colorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy and stability studies indicated that the drug after encapsulation with carnauba wax was stable and compatible. A single dose, randomized, complete cross over study of IM (75 mg) microspheres was carried out on 8 healthy male and female Albino sheep's under fasting conditions. The plasma was separated and the concentrations of IM were determined by HPLC-UV method. Plasma IM concentrations and other pharmacokinetic parameters obtained were statistically analyzed. The Cmax, Tmax, AUC_{0-24} and T1/2 values of Microcid®SR and optimized formulation were 2015 ± 31.31ng/ml, 3.0 h, 9699 ± 120.54 ng/ml h^{-1}, and 2.59 ± 0.03 h^{-1} and 1932 ± 24.43 ng/ml, 3.2 h, 7912 ± 43.43 ng/ml h^{-1}, and 2.62 ± 0.03 h^{-1}, respectively. Based on this study, spermaceti microspheres showed controlled release and it can be concluded that both the optimized formulation and Microcid®SR capsule are bioequivalent in term of the rate and extent of absorption.

KEYWORDS: Bioavailability, Bioequivalence, Carnauba wax, Indomethacin, Pharmacokinetics Release kinetics.

INTRODUCTION

Bioavailability and bioequivalence of drug products have emerged as critical issues in pharmacy and medicine during the last three decades. In recent years, various uses of wax and fat microspheres in the pharmaceutical field have come into forefront, involving the microspheres technology [1].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 thereby 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 and show good absorption rate. 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 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 fat 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.

Carnauba wax is used in the current study has good pharmaceutical and biological properties [2]. Carnauba, also called Brazil wax and palm wax, is a wax of the leaves of the palm, *Copernicia prunifera*. It is known as queen of waxes and usually comes in the form of hard yellow-brown flakes. It is obtained from the leaves of the carnauba palm by collecting them, beating them to loosen the wax, then refining and bleaching the wax. Carnauba wax is hard, but oily to the touch, and is devoid of taste or smell, making it very useful as an ingredient in cosmetics, as a pharmaceuticalexcipient, especially in cerates [3].

Indomethacin (IM) is a non-steroidal, anti-inflammatory agent with anti-pyretic, analgesic properties and it is a indole derivative designated chemically as 1-(p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid. Indomethacin has a molecular weight of 357.7 and a molecular formula of $C_{18}H_{16}ClNO_4$ [4]. 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 [5].

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 [6]. The occurrence of these adverse effects can be reduced by the use of controlled release formulations [7]. 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 [8].

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 [9]. 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 [10]. 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 [11]. Delivering the drug in the intestinal environment from wax microspheres could be manipulated by suitable coating techniques [12]. The chief characteristics of enteric coating are their impermeability to gastric juice, but susceptibility to intestinal juice [13,14]. Desired plasma levels can be achieved without the risk of side effects using once a day dose of controlled release preparation [15]. These findings suggested that the kinetic control is an effective route for preventing the toxicity of IM.

The aim of the present study are to formulate, characterize and study the *in vitro* release of IM from microspheres and to compared with commercially available oral formulation Microcid®SR (75 mg capsule). Furthermore, to investigate the pharmacokinetic and bioavailability of two different oral IM formulation (optimized microsphere formulation and Microcid®SR 75 mg capsule) following in single dosing in healthy Albino sheeps in order to prove the bioequivalence between the both preparation.

MATERIALS AND METHODS

Materials

Indomethacins (IM), pure drug and mefanamic acid (MA), the internal standard were kindly donated by Micro Labs (Bangalore, India). IM is an odorless, pale yellow to yellow tan crystalline substance. It is lipid-soluble, practically insoluble in water and sparingly soluble in alcohol. IM has a pKa of 4.5 and is stable in neutral or slightly acidic media and decomposes in strong alkali. The suspension has a pH of 4.0-5.0 and it has a melting point between 155°C and 161°C and has molecular weight of 357.8. Carnauba wax (Melting point 82 – 86 °C), Tween 80, all other chemicals and solvents used were of analytical grade and purchased from Ranbaxy Fine chemicals (New Delhi, India). Commercially available oral

[1]Department of Pharmaceutics, Farooquia College of Pharmacy, Mysore
[2]Department of Pharmaceutics, JSS College of Pharmacy, JSS University, Mysore
†Corresponding author: dvgowda4@gmail.com

capsule formulation (Microcid®SR 75 mg, Micro LabsLtd., India) is used for the present study.

Preparation of microspheres

9 gm of carnauba wax was melted in a china dish kept on water bath. To the melted wax mixture, IM (3 gm) previously passed through sieve No. 100 was dispersed in melted wax mass and stirred to obtain a homogeneous mixture. The resultant mixture was then poured into 150 ml of phthalate buffer solution (pH 4.5), previously heated to a temperature higher than melting point of carnauba wax (> 5°C). The surfactant, Tween 80 (1.4 % w/w) was added to the above mixture and stirred mechanically at 900 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 900 rpm for 5 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 temperature for 48 h to give discrete, solid, free flowing microspheres. A total of five formulations were prepared by varying the wax to drug ratios (Table 1).

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;

Carr's index (%I) = (tapped density – bulk density)/tapped density (1)

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

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

Scanning electron microscopic studies and sphericity determination

Scanning electron microscope (SEM) photomicrographs were recorded using Joel- LV-5600 SEM, USA. To determine the sphericity, the tracings of wax microspheres (magnification 459) were taken on a black paper using Camera Lucida (model -Prism type, Rolex, India) and Circulatory factor was calculated by the equation;

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

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

Differential scanning calorimetry (DSC)

All dynamic DSC studies were carried out on DuPont thermal analyzer with 2010 DSC module. Calorimetric measurements were made with the help of an empty cell (high purity alpha alumina discs of DuPont Company) as the reference. The instrument was calibrated using high purity indium metal as standard. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10°/min. The runs were made in triplicate.

Fourier transforms infrared spectroscopy (FTIR)

FTIR spectra of pure drug, empty microspheres and drug loaded microspheres were obtained using KBr pellet method (applying 6000 kg/cm ^{2}). Spectral measurements were obtained by powder diffuse reflectance on a FTIR spectrophotometer (Shimadzu, Model 8033, USA) in the wave number region 400 -4000cm^{-1} to drug excipient interactions if any.

Estimation of drug loading

Drug incorporated wax microspheres of each batch was selected and powdered in a mortar. Drug was extracted from wax microspheres using methanol, filtered and analyzed for drug content after suitable dilution. Estimation of IM was accomplished UV/Visible spectroscopy (Shimadzu – 1601, Japan) at 319 nm after sufficient dilution with pH 7.2 phosphate buffer.

***In Vitro* studies**

USP XX1 dissolution apparatus type II was employed to study percentage of drug release from various formulations prepared. Encapsulations of the drugs-loaded microspheres were avoided, as dissolution of shell will add one more parameter to the result. Accurately weighed quantities of drug (IM - equivalent to 75 mg) loaded microspheres of each batch were taken in 900 ml dissolution medium (IM - 2 h in pH 1.2 hydrochloric acid buffer and 6 h in pH 7.2 phosphate buffer and stirred at 100 rpm by maintaining at a temperature of 37±0.5°. The drug concentrations were determined by withdrawing the 10 ml of aliquots using guarded sample collectors periodically at an interval of 30 min for first 4 h and at 60 min interval for the next 4 h. Release studies were carried out in triplicate.

Stability studies

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. 100 mg of microspheres from each batch of formulations was taken at the end of 30th, 60th and 90th days and were subjected for *in vitro* release studies.

***In vivo* studies**

The in vivo release studies have been conducted on four male and four female healthy adult albino sheep's. The sheep's ages were in the range 7 – 9 years and their body weight ranged between 33 and 36 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 F3) 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 [16].

The contents of 5 units of Microcid®SR 75 mg capsule and formulation F3 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 6.00 AM after overnight fast of 10 hrs. After shaving near the neck, an 18 gauge (1.3 X 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 sheep's, fed with banana and 200 ml water. Light food was provided at 3rd hour followed by two standard meals at 7 and 11th h 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 [17].

Extraction procedure

Internal standard Mefanamic acid (MA) (100 µL) and cit rate 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 at900 rpm for 5 min. The organic phase was transferred to a watch glass and evaporated to dryness at40 °C. The residue was re-suspended in 100 µL of mobile of mobile phase and 25 µL was injected to the column. Quantification was achieved by the measurement of the peak area ratio of the IM to the internal standard (mefanamic acid). The limit of detection of IM in plasma was 100 ng/ ml (500 µL of plasma injected).

Chromatographic studies

The IM concentrations in plasma were assayed using a fully validated high performance liquid chromatography with ultra violet detection (HPLC-

UV) method [16], with respect to adequate sensitivity, specificity, linearity, recovery, accuracy and precision (both within and between days).The HPLC system consisted of HPLC-Shimadzu (Tokyo, Japan) LC-6A model, fitted with a μ -Bondapack C18 (4.6 X 250 mm) column of particle size 5μm (Supelco, Bellefonte, PA). The flow rate was maintained at 1 μL/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. Calibration standards, controls, and samples were processed in batches. The Stability of the samples under frozen conditions, at room temperature, and during freeze-thaw cycle was also determined.

Pharmacokinetic and Statistical data evaluation

The pharmacokinetic parameters were calculated using the Quick calk, computer PK calculation programme. The peak plasma concentration (Cmax) and time needed to reach peak plasma concentration (Tmax) 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 (Kel) 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;

$$T_{1/2} = 0.693/K \quad (8)$$

The extent of absorption for the drug (Microcid®SR 75 mg capsule and formulation F3) 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-inf}) was calculated using the formula;

$$AUC_{0-\infty} = AUC_{0-T} + C_{24}/K \quad (9)$$

where, C24 = drug concentrations in plasma at 24 hrs. The drug plasma concentration and pharmacokinetic parameters were analyzed by paired t- test and analysis of variance (ANOVA) at 95% confidence limit. Difference between two related means was considered statistically significant when their P values were equal to or less than 0.05.

RESULTS AND DISCUSSIONS

Evidence have [10-12] shown in the recent years that waxy materials have the physical properties and behavior suitable to prepare gastro resistant, biocompatible, biodegradable microspheres to release the entrapped drug in the intestinal lumen [14,15,18]. In the present study, a modified novel meltable dispersion emulsified cooling induced solidification method was employed using inert carnauba wax and non-toxic solvents to entrap the drug. In the present study, various parameters were studied such as drug and wax ratio, stirring speed and time, amount of surfactant added, volume of the aqueous phase used, effect of pH on drug entrapment, temperature of the aqueous phase and rapid cooling studies. Therefore the influence of the above parameters was highlighted. When the pH value of the external aqueous phase was highly alkaline, the solubility of the drug was reduced and the encapsulated amount of the drug increased. The maximum drug load 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 23.52 to 4.92 % .

Table1. **Drug and wax ratio for the prepared microspheres formulations**

Formulation	Drug (gm)	Carnauba wax (gm)
F_1	2.8	8.8
F_2	2.9	8.9
F_3	3.0	9.0
F_4	3.1	9.1
F_5	3.2	9.2

In the present study, it was found that 150 ml of aqueous phase suitable for producing the spherical microspheres. 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 irregularly shaped. When the volume of the aqueous phase was less than 150 ml, the resultant microspheres were highly aggregated in nature and highly impossible to distinguish as an individual microsphere. In order to avoid the formation of irregularly shaped larger particles, in the present method, 150 ml of aqueous phase was used.

Incorporation of drug into carnauba wax microspheres required the addition of tween 80 as a surfactant, at an optimum concentration to reduce the interfacial tension between the hydrophobic material and external aqueous phase. An attempt was made to incorporate drug in the wax microspheres without the addition of a surfactant. But the process was a failed, as it resulted in an aggregate cake like mass during the solidification of wax. This may be due to repulsion resulting from high interfacial tension between the hydrophobic waxy material and external aqueous phase. It was found that tween 80 having a HLB value of 15 was suitable to increase substantially dispersion of waxy material in external aqueous phase and promote drug incorporation in the wax microspheres. To obtain an optimal surfactant concentration, various concentrations ranging from 0.8 to 2.3 % (w/w) of the total formulation were tested. Discrete microspheres with good flow properties using an optimum concentration of surfactant 1.4 % w/w (tween 80) were used. Concentrations of tween 80 ranging from 0.8 to 2.3 % w/w failed to produce reproducible microspheres. The resultant wax microspheres were composed of irregular masses, which were not possible to distinguish as individual microspheres. A similar surfactant concentration was reported for carnauba wax and bees wax microspheres prepared by meltable dispersion method [10,11,12].

Temperature of the aqueous phase was maintained at 5 ^{0}C higher than the melting point of the carnauba wax in the corresponding formulations. From SEM studies it was observed that the resultant microspheres were free from surface irregularities, except some wrinkles. It was also observed that when the temperature of the aqueous phase was less than the 5°C than the melting point of the big flakes were produced.

In the present study, to produce the spherical discrete microspheres, an optimum drug to wax phase ratio of 1:3 w/w was used. It was found that higher the amount of drug to wax ratio (2:3) produces aggregate masses during the cooling process. It may be due to reduced melting point of the waxy materials. SEM photographs also indicated the presence of the crystals on the surface of the microspheres. The resultant microspheres were unsuitable for pharmaceutical uses. Hence an optimum 1:3 ratio was used to prepare microspheres (Table 1). Similar parameters were reported by Gowda et al for waxes/fat microspheres [10, 12, 14].

It was observed that the average size of the microspheres ranged between 345 to 360 mm presented in Table 2. The important factor that influences the size distribution of microspheres is the optimum stirring speed and stirring time. A stirring speed of 900 rpm and stirring time of 3 min was used to obtain reproducible microspheres. It was observed that with the increase in the stirring speed from 900 to 1100 rpm there was a decrease in the average size of the spheres and recovery yield of the microspheres. It is due to small sized wax microspheres, which were lost during successive washings. When the stirring speed was lower than 900 rpm, larger pellets were a formed. It was also found that an increase in stirring time, from 2 to 4 min (at a stirring speed of 900 rpm), there was a decrease in the recovery yield of microspheres. With the stirring time lower than 2 min, it was observed that some amount of melted material adhered to the sides of the beaker during the cooling process resulting in lower recovery of yield.

Micro particulate drug delivery systems are formulated as single unit dosage forms in the form of capsule or tablet. Such microparticulate systems should possess the better and adequate micromeritic properties. The obtained micromeritic properties are given in Table 2.The values of angle of repose were well within the range, indicating reasonable good flow potential for the microspheres. The tapped density values ranged between 0. 45 g/cm^3 to 0. 52 g/cm^3. The results of % compressibility index ranges from 9.31% to 15.02%, suggests good flow characteristics of the microspheres [Table 2]. The better flow property indicates reasonable and good flow potential of prepared microspheres.

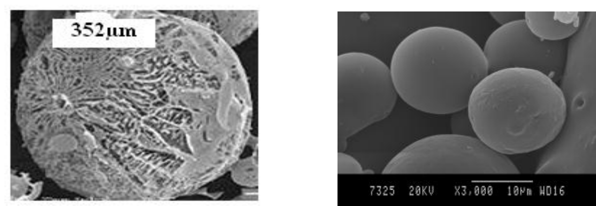

Figure 1. SEM microphotographs of wax microspheres loaded with IM formulation (F_3)

Table 2. Micromeritic properties of the drug loaded wax microspheres

Formulation	Average size (μm)	Yield (%)	Angle of repose (θ^0)	% Compressibility index	Tapped density (g/cm ^{3})
F_1	345	90.32	26.31	9.31	0.45
F_2	349	92.21	27.12	10.34	0.47
F_3	352	96.32	27.98	12.54	0.50
F_4	354	89.54	24.89	11.67	0.49
F_5	360	89.92	25.98	15.02	0.52

Values shown in the table mean percent of 3 batches (n = 3)

SEM photographs showed that the wax microspheres were spherical in nature, had a smooth surface with inward dents and shrinkage, which is due to the collapse of the wall of the microspheres Figure 1 photograph reveal the absence of crystals of the drug on the surface of microsphere, indicating uniform distribution of the drug within the microspheres. The rate of solvent removal from the microspheres exerts an influence on the morphology of the final product [15]. The sphericity factor obtained for the microspheres nearer to the value 1, confirming the sphericity of the microsphere.

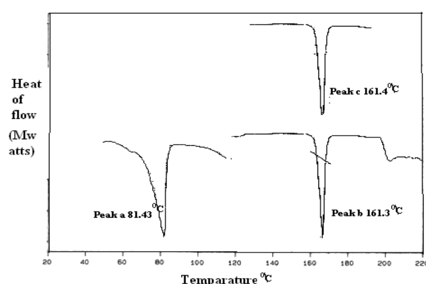

Figure 2. DSC thermograms of carnauba wax, pure indomethacin and indomethacin loaded wax microspheres, peak a= carnauba wax, peak b = indomethacin, peak c = indomethacin loaded wax microspheres (F_3)

DSC studies were performed on pure drug, empty and drug-loaded microspheres have shown sharp endothermic peaks. IM exhibits a sharp endothermic peak at 161.3 ^{0}C presented in fig. 2. It was observed that absence of the endothermic peak of the drug at 161.4 ^{0} C in the drug loaded wax microspheres indicates, that the drug is uniformly distributed at molecular level in the microspheres [14].

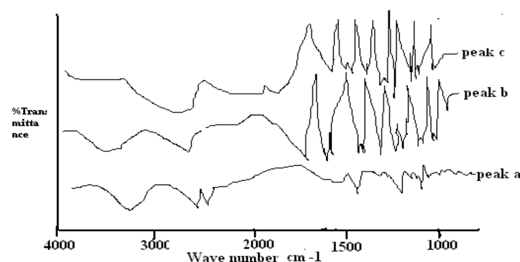

Figure 3. FTIR spectra of carnauba wax (peak a), indomethacin (peak b) and indomethacin loaded wax microspheres (peak c - F_3)

FTIR spectra for IM and formulation F_3 is shown in Fig.3. The characteristic IR absorption peaks of IM compared the IR spectra at 3417 (aromatic C-Hstretching), 2620 (carboxylic acid stretching), 1693 (C=O stretching), 1600 (C= C stretching), 1449 (O - CH_3 deformation) and 1235 cm $^{-1}$ (O-H deformation) were not alter after successful encapsulation of drug, indicating no chemical interactions between the drug and wax used. A comparison and interpretation of this region in our spectra agrees with their conclusions [14,18]. The percent of drug loading in the formulations was found to be in the range of 19.46 - 23.52 %. It was low in the formulation F_4 (19.46) and more in F_3 (23.52).. The encapsulation efficiency (%) was found to be more in formulation F_3 (93.24 %) as compared to F_1(89.12%), F_2(89.79%), F_5 (88.32%) F_4 (87.53%).From this result, it can be concluded that the formulation F_3 had more encapsulation efficiency.

From the release studies it was observed that, there is no significant release of drug at gastric pH from wax microspheres. At the end of 8th h, in vitro drug release from F_3 (94.78 %), was slower than Microcid®SR (99.35 %) in the intestinal environment. Drug was released in a biphasic manner consisting of initial fast release followed by a slow release in intestinal pH from the wax microspheres [2,10,12,18]. The decreased *in vitro* drug release from wax microspheres might be due to more hydrophobicity and influence of molecular weight of wax. The in vitro drug release was considerably retarded from the wax microspheres when compared Microcid®SR. The rate of drug release followed first order release kinetics and numerical data fitted into Peppa's model showed that, the mechanism of drug release from wax microspheres was non fickian diffusion presented in Table 3. After an initial burst effect, the subsequent release of drug from microspheres was slow.

Table3. *In vitro* release kinetic parameters for carnauba wax microspheres

Formulation	n	k	R^2
F_1	0,532	0.0148	0.9957
F_2	0.547	0.0147	0.9971
F_3	0.524	0.0152	0.9986
F_4	0.535	0.0145	0.9956
F_5	0.576	0.0139	0.9973
Microcid®SR	0.532	0.0156	0.9996

Values shown in the table mean percent of 3 batches (n=3)

Microcid®SR 75 mg capsule and formulation F_3 were subjected for stability studies for 90 days. It was observed that in vitro drug release from Microcid®SR 75 mg capsule and formulation F_3 at the end of 90 days (8th h), were 99.35 and 94.76 %, 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. The measured average drug content uniformity of Microcid®SR 75 mg capsule is 74.75 mg and formulation F_3 is 74.63mg. The percent of drug content uniformity of Microcid®SR 75 mg capsule and formulation F_3 are 99.66 and 99.51%, 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 [16].

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 5.52 and 8.23 min, respectively. Sensitivity of HPLC assay qualitative confirmation of the purity of IM and MA peaks were obtained (Table 4). The limit of quantification was 50 ng/ml of IM in plasma when 0.5 ml plasma was placed. The obtained mean correlation coefficients for the standard curves (n = 6) was 0.998. 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 employed.

The mean plasma concentration as a function of time is shown in Fig. 4 and the calculated pharmacokinetic parameters of Microcid®SR and F_3 formulations are given in Table 5. After oral administration of both the products, more mean C_{max} value was observed for Microcid®SR 75 mg capsule (2015 ± 31.3ng/ml) than formulation F_3 (1932 ± 24.43ng/ml). However, the difference in the C_{max} values obtained for Microcid®SR 75 mg capsule and formulation F_3 was statistically insignificant. The peak plasma levels for Microcid®SR 75 mg capsule and formulation F3 lies in the range 1982–2096 ng/ml and 1916 –1963 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) [9]. The Cmax values for both the products do not exceed the above limit in all animals. It was observed the plasma concentration 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. Furthermore, 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.

Table 4. Absolute recovery results obtained for indomathacin 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 x 100 / B Mean ± SD*
0.5	10	50	57	47	94.0 ± 1.12
2 .0	10	100	105	95	95.0 ± 1.35
4 .0	10	200	207	197	98.5 ± 1.42
6.0	10	300	309	299	99.66± 0.32
8.0	10	400	407	397	99.25 ± 0.28

*Standard deviation n = 3

Table 5. Comparison of mean values of pharmacokinetics obtained for products Microcid®SR & formulation F2 after oral administration

Parameters	Microcid®SR	Formulation F_3	P value
T_{max} (h) *	3.2	3.0	< 0.05
C_{max} (ng /ml) *	2015 ± 31.3	1932 ± 24.43	< 0.05
$T_{1/2}$(h^{-1}) *	2.59 ± 0.02	2.62 ± 0.20	< 0.05
AUC_{0-24}* (ng/ml h^{-1})	9699±120.54	7912 ± 43.43	< 0.05
$AUC_{0-\infty}$* (ng/ml h^{-1})	9894±115.22	8798 ± 32.77	< 0.05
K_a * (h^{-1})	0.3812±0.002	0.3728 ± 0.002	< 0.05
K_{el}* h^{-1})	0.2713±0.004	0.2422 ± 0.004	< 0.05
Mean residence Time* (MRT)	4.54 ± 0.03	4.65 ± 0.03	< 0.05

*Standard deviation n = 3

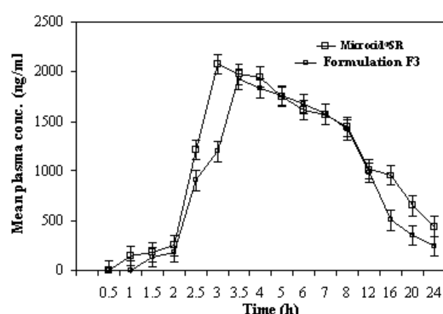

Figure 4. Mean plasma concentrations – time profiles of IM from Microcid®SR and formulation F_3

The time taken to reach peak plasma concentration T_{max} of IM was little higher in case of Microcid®SR compared to formulation F_3, but no statistical significance differences between two products is observed (Table 5). The calculated mean $T_{1/2}$ values for Microcid®SR and formulation F_3 were 2.59 ± 0.02 h^{-1} and 2.62 ± 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. Mean rate of absorption K_a for Microcid®SR was 0.3812 ± 0.002 h^{-1} and for formulation F_3 0.3648 ± 0.002 h^{-1} and mean elimination rate constant K_{el} for Microcid®SR and for formulation F3 was 0.2713 ± 0.004 h^{-1} and 0.2422 ± 0.004 h^{-1}, respectively.

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 F_3 were 9699 ± 120.54 ng/ml h $^{-1}$ and 7912 ± 43.43 ng/ml h^{-1} respectively. The Slower *in vitro* release of IM from the products Microcid®SR and F_3 formulations may be responsible for the decreased AUC values when compared to the reported conventional dosage forms [18]. The average value of the individual and mean AUC $_{0-24}$ ratio at 95% confidence limit (0.8 – 1.25) was within acceptable limits for bioequivalent products (19). In order to obtain *in vitro-in vivo* correlation, drug absorption profiles were compared for Microcid®SR and formulation F_3 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. Pharma co kinetic parameters clearly indicate that the parameters of F_3 are in good agreement with Microcid®SR. The observed mean AUC $_{0-\infty}$ values for Microcid®SR and formulation F_3 was 9894 ± 115.22 ng/ml.h^{-1} and 8798 ± 32.77 ng/ml.h^{-1} does not show any significant statistical difference between the products.

On the basis of FDA recommendation [21], the two products, Microcid®SR and formulation F_3 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 plasma concentrations (> 5000 ng/ml), which are very often associated with adverse effects due to drug accumulation [8], because of the accumulation effect. The products Microcid® SR and formulation F_3 investigated in the present study were found to be bioequivalent.

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 F_3 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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