Bioequivalence Study of Indomethacin from Loaded Spermaceti Wax Microspheres on Healthy Albino Sheep

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ABSTRACT

The present study aims at probing *in vitro* release and bioequivalence of Indomethacin (IM) loaded spermaceti wax microspheres with Microcid®SR. The microspheres of spermaceti wax have been prepared by meltable emulsified cooling solidification method and the conditions such as drug/wax ratio; pH and amount solvent; and rotational speed and time are optimized. The drug loaded spermaceti microspheres were characterized by SEM, DSC, FTIR and stability studies. The bioavailability of optimized formulation has been established by randomized cross over study on 10 fasting healthy albino sheep on two separate occasions. Plasma IM concentrations and pharmacokinetics parameters were statistically analyzed. Spermaceti microspheres have shown controlled release and it can be concluded that prepared formulation and Microcid®SR are bioequivalent in terms of rate and absorption.

Keywords: Indomethacin, Spermaceti, Release Kinetics, Pharmacokinetics, Bioequivalence.

INTRODUCTION

Bioavailability and bioequivalence of drug products have emerged as critical issues in pharmacy and medicine during the last three decades. Bioavailability is a pharmacokinetic term that indicates the rate and extent to which the drug ingredient is absorbed from a drug product and becomes available in the systemic circulation. 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, the 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 administer the dosage form several times a day. This results in an undesirable see-saw pattern of drug levels in the body. Due to cost and time involvement in developing a new drug entity, several modification routes 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 microspheres which are becoming more popular than single unit dosage forms. The uniform distribution of these multiple unit dosage forms along the gastro intestinal track 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 thereby to maintain the desired drug concentrations during treatment. This idealized objective can be achieved by

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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 the gastro intestinal tract. Poorly water-soluble drugs, which are lipophilic in nature, easily mix with waxes and show good absorption rate. The spermaceti used in the current study has good pharmaceutical and biological properties [2].

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 faces challenge to human safety. To overcome this problem, water is used as solvent in the present study to prepare wax microspheres. Furthermore, the process was optimized to produce microspheres to give better yield with spherical geometry and predictable dissolution pattern. Spermaceti is a wax present in the head cavities of the sperm whale (*Physeter macrocephalus*). Spermaceti is extracted from sperm oil by crystallization at 6°C, when treated by pressure and a chemical solution of caustic alkali. Spermaceti forms brilliant white crystals that are hard but oily to the touch, and are devoid of taste or smell, making it very useful as an ingredient in cosmetics, as a pharmaceutical excipient, especially in cerates and ointments [3]. Indomethacin (IM) is a non-steroidal, anti-inflammatory agent with anti pyretic, analgesic properties discovered and developed by the Merck Sharp and Dohme Research laboratories. IM is an 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 $C_{18}H_{16}ClN0_4$ [4]. Nowadays IM is widely used in the treatment of active stages of moderate to severe stages of rheumatoid arthritis. IM should be administered at least 2 to 3 times a day. 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 (RCS)[5] . The occurrence of these adverse effects can be reduced by the use of controlled release formulations [6]. Oral conventional dosage forms are administered 2 to 3 times a day to maintain adequate and effective therapeutic concentration in blood. However, conventional dosage forms fails to protect the patients against morning stiffness [7]. Thus, the development of controlled release formulation of IM has 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 [8]. 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 [9]. Previous experimental results demonstrated that the waxes are biocompatible, non-immunogenic material is used for the entrapment of drug and its controlled drug release in the intestinal tract [10]. Delivering the drug in the intestinal environment from wax microspheres could be manipulated by suitable coating techniques [11]. The aim of the present study is to formulate, characterize and study the *in vitro* release of IM from optimized spermaceti microspheres (F_3) and compare this with commercially available oral formulation Microcid®SR (75 mg capsule). Furthermore, to investigate the pharmacokinetics and bioavailability of two different oral IM formulations (optimized microsphere formulation F_3 and Microcid®SR 75 mg capsule) following single dosing in healthy Albino sheep in order to prove the bioequivalence between both products.

Materials and Methods

Materials

Indomethacin (IM), pure drug and Mefanamic Acid (MA), the internal standard was kindly donated by Micro Labs (Bangalore, India). IM is an odorless, pale yellow to tan yellow crystalline substance. It is lipid-soluble, practically insoluble in water and sparingly soluble in alcohol. IM has a pk_a 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. It has a melting point between 155 and 163^{0}C and has molecular weight of 357.8. Spermaceti, Span-20, other chemicals and solvents used were of analytical grade and obtained from Ranbaxy Fine chemicals (New Delhi, India). Commercially available oral capsule formulation (test formulation - Microcid®SR 75 mg, Micro Labs Ltd., India) is used for the present study.

Preparation of Microspheres

The required quantity of spermaceti was melted in a china dish kept in a 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 spermaceti (> 5^{0}C). The surfactant, span 20 (2.4% 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 the dispersion of molten wax in the aqueous medium. The mixture was stirred continuously above the melting point of wax at 1500 rpm for 5 min. The temperature of the reaction mixture was cooled rapidly and brought down to 10^0 C by the addition of cold water. The resultant solid spheres were collected by filtration and washed thoroughly 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 drug to wax ratios (Table 1).

Microsphere Characterization

The 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 (θ) was assessed to know the flowability of spermaceti microspheres, by a fixed funnel method.

Tan (θ) = Height/Radius (2)

Scanning Electron Microscope (SEM) photomicrograph was 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).

In order to know the size distribution and size analysis of the wax microspheres, sieve analysis technique and SEM studies were used, respectively. The FTIR spectra of the samples were obtained using FT-infrared spectrophotometer (Shimadzu, 191 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 DSC194 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 ^{0} C under nitrogen gas purge at a heating rate of 10^0 C/min. In order to evaluate the drug loading, 100 mg of microsphere was 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.

***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 ± 0.5^0 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 regular intervals of 30 min for first 4 h and 60 min intervals for the next 4 hours.

Peppa's Model Fitting

Koresmeyer–Peppa's model is used to evaluate the mechanism of drug delivery. Koresmeyer –Peppa's

equation is as follows:

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

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

where, M_t /M_∞ is the fractional amount of drug released and t is the time in h. In this study, the release constant k, and constant A, were calculated from the slope and intercept, respectively of the plot of ln (1- M_t/M_∞) versus time (t).

A differential factor (f_1) and similarity factor (f_2) were calculated from dissolution data according to the following equations:

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \times 100 \quad (6)$$

$$f_2 = 50 \log \left\{ \left[1 + \left(\frac{1}{n}\right) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (7)$$

where, f_1 is differential factor, f_2 is similarity factor, n is number of points, R_t is dissolution value of the reference at time, t and T_t is dissolution value of test formulation at time, t. The acceptable ranges for f_1 and f_2 were 0 to 15 and 50 to 100, respectively.

The optimized formulation F_3 (equivalent to 75 mg drug) and Microcid®SR were subjected for stability studies, which were stored 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. An equivalent amount of drug from F_3 and Microcid®SR were taken at the end of 15, 45 and 90th days and were subjected for *in vitro* drug release studies. The average drug content uniformity for F_3 and Microcid®SR has been estimated as per USP specification[12]. Drug was extracted from F_3 and Microcid®SR using methanol (80%) and methanolic extract was suitably diluted and average drug content uniformity was determined.

Subjects and Study Design

The study was performed at clinical trial laboratory and was conducted according to the Declaration of Helsinki and GLP guidelines. The study protocol was reviewed and approved by the Institutional Ethical Committee of JSS Medical College and Hospital, Mysore and JSS College of Pharmacy, Mysore, India. Reference formulation was F_3 (single 75 mg dose of IM) and test formulation was Microcid®SR 75 (single 75 mg dose of IM). Ten healthy sheep: 5 males and 5 females, aged between 7 to 9 years, body weight within normal range (BMI – 18.10 – 22 93 kg/m^2). Detailed written information on the study was provided to the Veterinary Surgeon, Central Animal Facility, JSS Medical College and Hospital, Mysore and permission was obtained.

Treatment Phase and Blood Sampling

The study was conducted as an open, single blind, two period, randomized cross over study, in which a single 75 mg dose of IM from F_3 and Microcid®SR was administered to fasting, healthy sheep, separated by a wash out period of two weeks between dosing intervals. Animals were brought to clinical trial laboratory at night before drug administration and they were fasting from any food except water from 21:00 PM. In the morning 06:00 AM of the dosing day, after an overnight fast, a pre-dose pharmacokinetic blood sample was taken. F_3 and Microcid®SR were administered to the sheep at 07:00 AM with banana and 200 ml water. Venous blood samples (heparinised saline lock 18 gauge canula inserted into a jugular vein) were withdrawn 10 ml immediately before administering F_3 and Microcid®SR (pre-dose interval) and 5 ml each blood sample was collected 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, Elektroshaft, Bombay, India) at 1500 rpm for 10 min. The separated plasma was stored at –20 °C prior to analysis. Diet type and timing were strictly followed. After two weeks of the first drug administration (wash out period), the same procedure was repeated. Food was provided 4th and 10th h after drug administration. The amount of food and water intake for each animal was standardized during the first 24 h of blood sampling. Plasma concentration of drug from the collected samples was quantified by HPLC method. One

veterinary surgeon and two nurses with sufficient qualification and training were present at dosing time and stayed at the site until the last animal left the study unit.

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 900 rpm for 5 min. The organic phase was transferred to a watch glass and evaporated to dryness at 40 ^{o}C. The residue was re-suspended in 100 µL of mobile phase. Calibration standards, controls, and samples were processed in batches.

HPLC Analysis

The IM concentrations in plasma were assayed using a fully validated High Performance Liquid Chromatography (HPLC) [13]. 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, UV/visible detector). The mobile phase was acetate buffer (pH 3.6) which consists of 80% methanol and 0.02 M sodium (60:40 v/v). The column was heated to 40 ^{o}C, the flow rate was 1.0 mL/min with the injection volume 25 µL. Detection was performed with UV detector at 320 nm. Quantification was achieved by the measurement of the peak area ratio of the IM to the internal standard MA. The limit of detection of IM in plasma was 50ng/ml. The stability of the samples under frozen conditions, at room temperature, and during freeze-thaw cycle was determined.

Pharmacokinetic and Statistical Data Evaluation

The pharmacokinetic parameters were determined using the Quick calk (computer pharmacokinetic calculation programme, India). C_{max} and t_{max} of the drug from each formulation, was obtained directly from the observed data. AUC_{0-t} is calculated by the trapezoidal rule method. The area under the plasma concentration time curve from zero to infinity ($AUC_{0-\infty}$) was calculated using the formula;

$$AUC_{0-\infty} = AUC_{0-t} + C_t/K_{el} \quad (8)$$

where, C_t is the last quantifiable concentration, K_{el} is the terminal elimination rate constant was determined by least square regression analysis during the terminal log linear phase of the concentration – time curve. The biological half life ($T_{1/2}$) was calculated as;

$$T_{1/2}=0.693/K_{el.} \quad (9)$$

Quick calk was used to perform the statistical analysis of $C_{max,}$ AUC_{0-t} and $AUC_{0-\infty}$ using analysis of variance (ANOVA) after transformation of the data to their logarithmic (ln) values. Using the error variance (S^2) obtained from the ANOVA, 90 % Confidence Intervals (CI) were calculated from the following equation;

$$90\ \%\ CI = (\bar{X}_{F3} - \bar{X}_{Microcid}) \pm t_{0.1(v)} \sqrt{(S^2 \times 2/_n)} \quad (10)$$

where, $\bar{X}_{F3}$ and $\bar{X}_{Microcid}$ are means of the ln transformed values for the formulation F3 and Microcid®SR, S^2 is the error variance obtained from the ANOVA, n is the number of subjects, $t_{0.1}$ is the t values for 90 % CI, v is the degree of freedom of the error variance from the ANOVA. The acceptance criteria for bioequivalence were that the 90 % CI of the geometric mean ratios 0.80 – 1.25 for the $C_{max,}$ AUC_{0-t} and $AUC_{0-\infty.}$ The t_{max} difference was analyzed using original data Wilcoxon matched – pairs test. The difference between two related means was considered statistically significant, when their P values were equal to or less than 0.05.

Results and Discussion

Preparation and Characterization of Microspheres

Recently, many scientists have reported wax and fat materials which show that the physical behaviors are suitable to prepare gastro resistant, biocompatible and biodegradable microspheres to release the entrapped drug in the intestinal lumen [11,1,15,14]. A modified novel meltable dispersion emulsified cooling induced solidification method was employed using spermaceti (FDA approved inert wax) and water as solvent to entrap the drug. In the present study, the effect of drug and wax ratio, stirring speed and time, amount of surfactant, volume of the aqueous phase, pH on drug entrapment, temperature of the aqueous phase and rapid cooling conditions were

optimized during the preparation of spermaceti microspheres. Therefore, the influences of the above parameters were interpreted. The maximum drug load of 23.34% was obtained at pH 4.5 (phthalate buffer). As the pH increased from 4.5 to 7.0, the percent of IM loading was reduced from 23.34 to 4.98 %.

In order to produce the spherical, discrete microspheres, an optimum drug to wax ratio was 1:3 w/w. It has been 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 the reduced melting point of the spermaceti, which 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 spermaceti 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 spermaceti microspheres without the addition of a surfactant. But the process failed and resulted in the formation of aggregate cake like mass during the solidification of spermaceti. It may be due to the repulsion resulting from high interfacial tension between the hydrophobic waxy spermaceti and external aqueous phase. It has been found that the surfactant having a Hydrophilic Lipophilic Balance (HLB) value of 8.6 was suitable to increase substantial dispersion of spermaceti and promotes drug incorporation in the microspheres. In order to obtain an optimal surfactant concentration, various concentrations ranging from 1.5 to 2.4 % (w/w) of the total formulation were studied. The concentration of the surfactant (span 20) at 2.4 % w/w of spermaceti were used to obtain discrete microspheres with good flow properties after cooling.

The key factor that influences the size distribution of microspheres was the time and speed of stirring. A stirring speed of 1200 rpm and stirring duration of 5 min were used to obtain reproducible microspheres. It was observed that with an increase in the stirring speed from 800 to 1400 rpm, there was a reduction in the average size and reduction in the recovery yield of the microspheres. It has been due to the small sized spermaceti microspheres, which were lost during successive washings. When the stirring speed was lower than 800 rpm, larger microspheres were formed. It has also been observed that an increase in stirring time from 5 to 8 min (at a stirring speed of 1200 rpm) caused a reduction in the recovery yield of microspheres. When the stirring time was lower than 5 min, it has been 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 has also been noticed that 150 ml of aqueous phase was ideal in obtaining spherical microsphere without 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 ^{0}C higher than the melting point of the spermaceti. It was also observed that when the temperature of the aqueous phase was less than the melting point of the spermaceti (5 ^{0}C), big flakes were produced.

The obtained micromeritic properties of the IM formulations were presented in Table (1). Sieve analysis data indicated that about 58 – 61 % of the prepared microspheres were in the size range 120 –800 µm. It has been observed that an average size of the microspheres lies in the range 332 – 351 µm. The measured tapped density values lies in the range 0.3231–0.3665 g/cm^3. The values of θ (angle of repose) and percent Carr's index (% I) were in the range 24.2 – 27.9 and 9.31 to 11.98 %, respectively, indicating reasonable good flow potential for the microspheres.

Table 1: Micromeritic properties of the drug loaded IM – spermaceti microspheres.

Formulation code (Drug : Wax ratio)	Size[a] (µm)	Angle of repose (Θ) [a]	Carr's index (%)[a]	Tapped density (g/cm^3)[a]
F_1 (1.2:3.0)	339	24.2	10.81	0.3315
F_2 (1.4:3.0)	346	25.5	9.38	0.3231
F_3 (1.0:3.0)	351	27.9	9.38	0.3562
F_4 (1.6:3.0)	338	26.3	9.98	0.3665
F_5 (1.8:3.0)	332	25.9	9.56	0.3433

[a] Standard deviation n = 3

SEM Studies

SEM photomicrograph showed that formulation F3 was spherical and had a smooth surface with inward dents on the wall of the microspheres (Figure 1). This property may be due to removal of the solvent during in situ drying process and the rate of solvent removal from microspheres exerts an influence on the morphology. SEM photomicrographs revealed the absence of crystals of the drug on the surface, indicating uniform distribution of the drug on the walls of microspheres. The sphericity of the prepared microspheres was confirmed and calculated values were nearer to one.

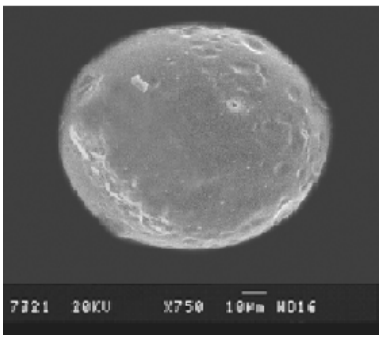

Figure 1. SEM photomicrograph of formulation F_3 showing spherical in nature.

DSC and FTIR Studies

The DSC thermogram for pure drug; microspheres and drug loaded microspheres are shown in Figure 2.

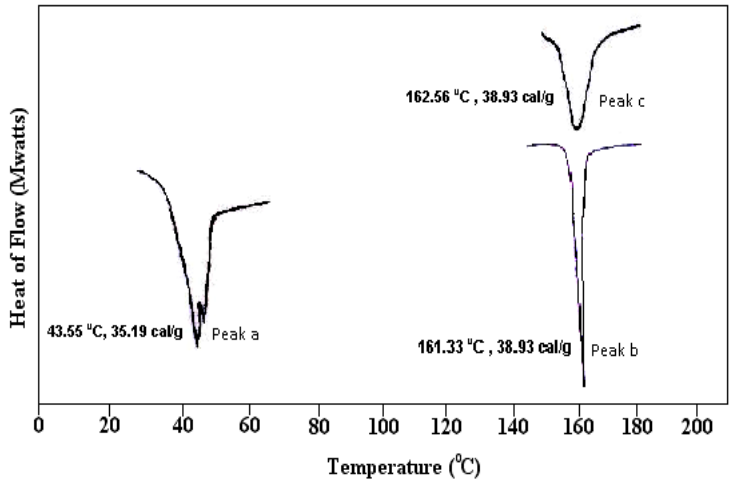

Figure 2. DSC thermograms of Spermaceti (peak a), Indomethacin drug (peak b) and IM loaded spermaceti microspheres (peak c).

The pure drug exhibits a sharp endothermic peak at 162.2°C. The DSC thermograms reveal the presence of an endothermic peak at 162.5^0 C in the drug loaded microspheres, drug is within microspheres [15]. The FTIR spectra for IM and F_3 are shown in Figure 3.

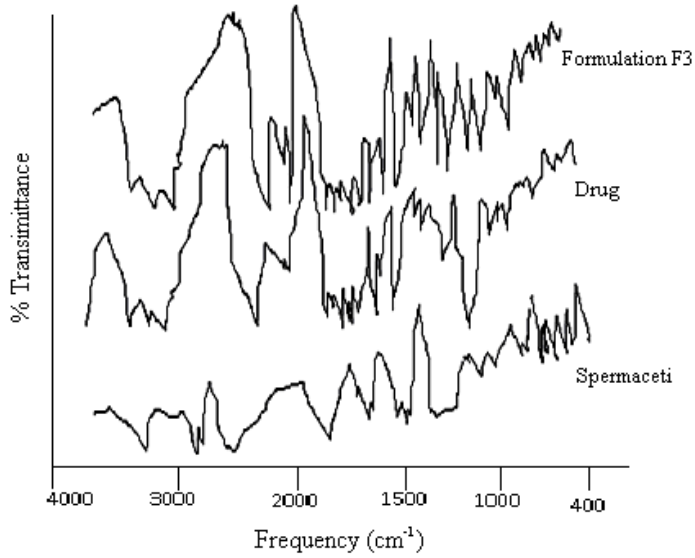

Figure 3. FTIR spectra of IM and IM loaded spermaceti microspheres.

The characteristic IR absorption peaks of IM at 3415 (aromatic C–H stretching), 2630 (carboxylic acid stretching), 1688 (C=O stretching), 1612 (C=C stretching), 1442 (O-CH_3 deformation) and 1234 cm^{-1} (O–H) were not altered even after the successful encapsulation of drug with wax, indicating no chemical interactions between drug and spermaceti. The comparison and interpretation of this region of spectra have good agreement with the reported result elsewhere [15].

Drug Loading and *in vitro* Release Studies

The percent of drug in the microspheres lies in the range 19.12–23.34%. The encapsulation efficiency (%) of the drug was found to be more for formulation F_3 (94.19 %) as compared to F_1, F_2, F_4 and F_5 formulations. From this result, it can be concluded that the formulation F_3 had more encapsulation efficiency. From the release studies, it has been observed that, there is no significant release of drug from spermaceti microspheres at gastric pH indicating that the used spermaceti is gastro-resistant in nature [7,1]. At the end of 8th h, *in vitro* drug release from formulation F_3 (94.80 %), was faster than the other formulations {F_1 (86.33 %), F_2 (78.82 %), F_4 (86.87%), F_5 (84.93 %) and Microcid®SR 75 mg capsule (99.41 %)} in the intestinal environment as shown in Figure 4.

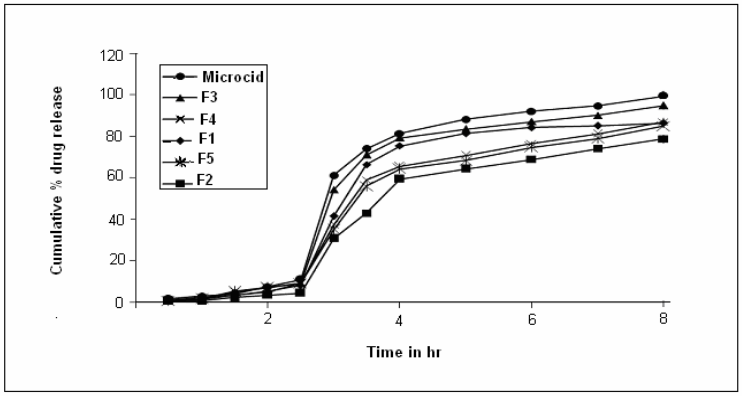

Figure 4. Dissolution time profiles of IM loaded microspheres (F_1–F_5) and Microcid®SR.

From the figure, it is clear that the drug was released in a biphasic manner from microspheres, consisting of initial burst release of drug; it may be due to surface accumulated drug, followed by a slow release at intestinal pH. *In vitro* drug release from the optimized formulation (F_3) was compared to marketed product (Microcid®SR). The *in vitro* drug release studies data obtained from F_3 and Microcid®SR was fit into Peppa's model (Gowda et al., 2005). The release constant k and regression coefficient (R^2) were obtained in the range between 1.48 to 1.72 (min^{-1} x $10^{2)}$ and 0.9911 to 0.9975, respectively. The obtained values of A are less than 0.5 this indicates that the drug release from F_3 and Microcid®SR by Fickian diffusion.

Differential factor (f_1) and similarity (f_2) factor were calculated from dissolution profile for F_3 and Microcid®SR and the results were compared. The obtained values f_1 and f_2 for F_3 and Microcid®SR are 10.76 and 12.24 and 79.23 and 81.56, respectively, which suggests that the drug release from the products F_3 and Microcid®SR are almost identical.

Drug Content Uniformity and Stability Studies

The estimated average drug content uniformity for F_3 (74.57 mg) and Microcid®SR (74.62 mg) are presented in Table (2).

Table 2: Content uniformity for Formulation F_3 and Microcid®SR.

Formulation	Average drug content Uniformity (mg)[a]	Percent (IM) labeled claim [a]
F_3	74.57 ± 0.35	98.27 -102.43
Microcid®SR	74.62 ± 0.39	98.12 -103.13

[a] Standard deviation n = 3

The percent of drug content uniformity in both products was well within the limits as per the United States Pharmacopoeia and National Formulary Specification [12]. *In vitro* drug release from F_3 and Microcid®SR at the end of 90 days (8th h), was found be to 94.75% and 99.35%, respectively. However, after stability study period (up to 90 days), no significant change was noticed for both systems, indicating that drug was in a stable state in F_3 and Microcid®SR.

***In vivo* Studies**

Plasma IM was recovered from F_3 and Microcid®SR, which reflects recovery, accuracy, precision, linearity, specificity and adequate sensitivity evaluated by HPLC method. Plasma spiked with 500 ng/ml of IM and 1000 ng/ml of MA, the retention time for IM and MA were 5.45 and 8.18 min, respectively. 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. Mean plasma concentrations versus time profiles of IM after administration of both formulations in healthy subjects (n = 10) are shown in Figure 5, it has been noticed that the mean plasma concentration time profiles of the product F_3 and Microcid®SR were almost identical. The calculated pharmacokinetic parameters such as C_{max}, t_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ for F_3 and Microcid®SR are presented in Table (3) and are used for bioequivalence evaluation.

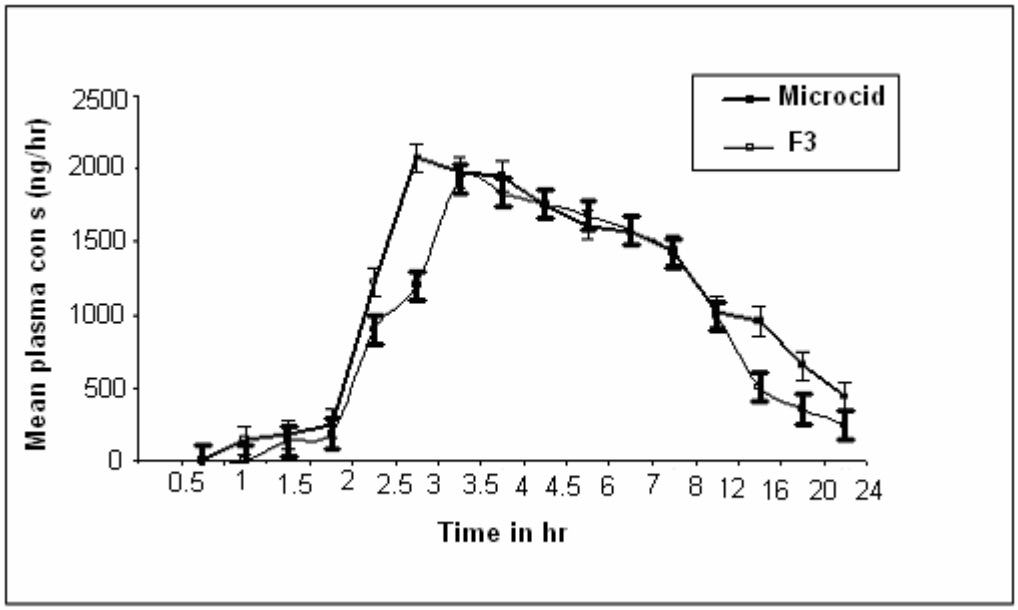

Figure 5. The plot of mean plasma concentration as a function of time profiles of IM from formulation F_3 and Microcid®SR.

In this study, mean C_{max}, t_{max}, AUC_{O-t}, $AUC_{O-\infty}$ and $T_{1/2}$ of IM from F_3 and Microcid®SR were 1959 ng/ml, 3.6 h, 8817 ng/ml h^{-1}, and 9012 ng/ml h^{-1} and 2064 ng/ml, 3.2 h, 9648 ng/ml h^{-1} and 9992 ng/ml h^{-1}, respectively. The 90% confidence intervals for geometric mean ratios of F_3 and Microcid®SR for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the acceptable limits (80 –125%) of bioequivalence which implies that the bioequivalence criteria were met. Based on bioequivalence guidelines, C_{max}, AUC_{0-t} and t_{max} were the main parameters in order to assess possible bioequivalence between both preparations. The C_{max} values for both products do not exceed the above limit in all animals (300–3000 ng/ml) [8] and no statistical significance differences between the two products. In each animal, the mean AUC_{0-t}, values of IM was higher than 80% as compared to the values of $AUC_{0-\infty}$ for both the formulations, indicating that the sampling time was sufficiently long to ensure an adequate description of the absorption phase [16]. Using Wilcoxon matched pairs test on the original data, the difference between t_{max} values of both formulations was not significantly different. Compared to the previous research performed by Gowda et al. (2009) in which the obtained pharmacokinetic parameters such as C_{max}, t_{max}, AUC_{0-t} and $AUC_{0-\infty}$, IM loaded bees wax microspheres and Microcid®SR 1940 ng/ml, 3.2 h, 8751 ng/ml h^{-1} and 8971 ng/ml h^{-1} and 2038 ng/ml, 3.0 h, 9528 ng/ml h^{-1} and 9978 ng/ml h^{-1}, respectively. The above pharmacokinetic parameter values are lesser as compared to the data obtained for spermaceti wax microspheres. This result clearly indicates that the spermaceti wax microspheres exhibits better bioavailability.

The calculated mean $T_{1/2}$, K_{el}, and K_a, values for F_3 and Microcid®SR were 2.89 h^{-1} and 2.62 h^{-1}, 0.2798 h^{-1} and 0.2832 h^{-1} and 0.3865 h^{-1} and 0.3964 h^{-1} respectively and no statistical significance differences were observed between both formulations. In the present study, the intra subject Coefficient of Variance (% CV) obtained from the ANOVA for IM was 3.35 %, it means that the study only required a sample size of less than 10 subjects. Therefore, this study had an adequate power to confirm a statistical conclusion. In order to obtain *in vitro - in vivo* correlation, absorption profiles were constructed for formulation F_3 and Microcid®SR using the fraction of IM

absorbed *in vivo* against fraction of IM dissolved *in vitro*. It has been observed that both products showed an adequate correlation [17] between Cumulative Fractions Dissolved (CFD) *in vitro,* Cumulative Relative Fraction Absorbed (CRFA) - *in vivo*. Correlation coefficient obtained for F_3 and Microcid®SR was 0.9811 and 0.9929, respectively. On the basis of FDA recommendation [18], formulation F_3 can be considered bioequivalent on for with Microcid®SR. There were no adverse events encountered and no deviation during the study.

Conclusions

The method employed in this experimental investigation to prepare microsphere was simple, rapid, economic and free from toxic organic solvent. The results of drug loaded microsphere exhibited better drug entrapment and micromeritic properties. SEM photomicrographs evidenced spherical nature of the microsphere. The compatible state of the drug loaded spermaceti microspheres were confirmed by FTIR and DSC studies. The present study demonstrates the potential use of spermaceti wax for the development of controlled drug delivery systems for water insoluble or lipophilic drug. Based on the pharmacokinetic and statistical results, it can be concluded that optimized formulation F_3 and Microcid®SR exhibited similarity in drug release profile and bioequivalent.

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دراسة التكافؤ الحيوي للاندوميثاسين من الشمع المحمل من ميكروسفير سبيرماسيتي في الأغنام البيضاء

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ملخـص

تهدف هذه الدراسة إلى التحقيق في التحرير والتكافؤ الحيوي لمادة اندوميثاسين المحمل مع ميكروسفير سبيرماسيتي Microcid للتحريرالثابت . وقد تم إعداد الميكروسفير من شمع سبير ماسيتي بواسطة طريقة مستحلب قابل للانصهار وظروف التبريد والتجميد؛ على سبيل المثال: نسبة الدواء / الشمع ، درجة الحموضة وكمية المذيب، سرعة الدوران ودراسة الثبات . وتم تكوين التوافر الحيوي الامثل من خلال الدراسة العشوائية على 10 عينات من الاغنام البيضاء في حالتين منفصلتين. تم التحليل الاحصائي لتركيزات البلازما والمؤشرات للحركات الدوائية . وقد أُظهر ميكروسفير سبير ماسيتي تحريراً ثابتاً. ويمكن القول إن التوليفة الجديدة تكافؤ ميكروسيد بما يتعلق بمعدل ومقدار الامتصاص .

الكلمات الدالة: اندوميثاسين ، سبيرماسيتي، تحرير حركي، حركية الدواء ، التكافؤ الحيوي.

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