

Research Article

Preparation and Characterization of Sugar Cane Wax Microspheres Containing Indomethacin

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Abstract

Purpose: To formulate and characterize indomethacin (IM) microspheres prepared with sugar cane wax microspheres.

Methods: Microspheres were prepared by melt-emulsified dispersion and cooling-induced solidification method. The microspheres were characterized by scanning electron microscopy (SEM) and differential scanning calorimetry (DSC) as well as for drug loading, and in vitro and in vivo release in albino sheeps.

Results: SEM images showed that the microspheres were spherical in shape and more than 98.0 % of the isolated microspheres were in the size range 345 - 360 μm . The C_{max} , T_{max} , AUC_{0-24} and $T_{1/2}$ values were 2123 ± 30 ng/ml, 3.1 h, 9734 ± 126 ng/ml h^{-1} , and 2.68 ± 0.03 h^{-1} for the reference product, Microcid[®] SR, and 1989 ± 26 ng/ml, 3.0 h, 8013 ± 79 ng/ml h^{-1} , and 2.79 ± 0.12 h^{-1} , respectively, for the test formulation.

Conclusion: Based on this study, it can be concluded that the developed indomethacin-loaded wax microspheres and Microcid[®] SR capsule are bioequivalent in terms of rate and extent of absorption.

Keywords: Indomethacin, Bioavailability, Controlled release Sugarcane wax, Release kinetics.

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INTRODUCTION

Bioavailability and bioequivalence of drug products have emerged as critical issues in pharmacy and medicine in the last three decades. Also, in recent years, wax and fat-based microspheres have found applications in drug delivery [1]. The goal of any drug delivery system is to provide therapeutic amount of drug(s) to the proper site in the body in order to promptly achieve and maintain the desired drug concentrations during treatment. 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 they depend on the nature of drug and its end use. Among the reported conventional methods, different strategies have been developed in recent years to design wax microspheres loaded with hydrophilic and lipophilic drugs using toxic solvents. The use of such solvents during formulation is of environmental concern and also detrimental to human safety.

Sugarcane wax has good pharmaceutical and biological properties. Nearly 60 - 70 % of octacosanol present in sugarcane wax has been shown to reduce cholesterol and triglyceride levels. It contains long-chain carbohydrates with an OH radical or CHO radical, often known as octacosanol and octocosanol [2]. The lipid content, i.e., "cane wax", is extracted from cane molasses. The wax is hard, but oily to touch, and is devoid of taste or smell, making it very useful as an ingredient in food and as a pharmaceutical excipient, especially in cerates [3].

Indomethacin (IM) is a non-steroidal, anti-inflammatory agent with antipyretic, analgesic properties and it is an indole derivative. It is widely used in the treatment of moderate to severe stages of rheumatoid arthritis and is given 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 regimen of osteoarthritis therapy, the administration of IM may induce

adverse side effects on the gastrointestinal tract (GIT), as well as the central nervous, 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 a controlled release formulation of IM would afford several advantages over the other conventional dosage forms, including reduction in 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 drug release and modifying absorption rate. The present study is designed to achieve this.

EXPERIMENTAL

Materials

Indomethacins (IM), pure drug and mefenamic acid (MA), the internal standard, were kindly donated by Micro Labs (Bangalore, India). Sugarcane wax (melting point, 45 – 48 °C) was procured from Mandya Sugar Factory, Karnataka, India. Tween 80, and all other chemicals and solvents used were of analytical grade and purchased from Ranbaxy Fine Chemicals (New Delhi, India). A commercially available oral capsule formulation (Microcid[®] SR, 75 mg, Micro Labs Ltd, India) was used as a reference standard in this study.

Preparation of microspheres

Sugarcane wax (9 g) was melted in a china dish over a hot water bath and IM (3 g), previously passed through a 120 μ aperture sieve, was dispersed in it and stirred to obtain a homogeneous mixture. The mixture was

poured into 150 ml of phthalate buffer solution (pH 5.0), previously heated to a temperature higher (by $> 5^{\circ}\text{C}$) than the melting point of sugarcane wax. A surfactant, Tween 80 (0.2 % w/w), was added and stirred mechanically at 900 rpm with a stirrer (model RQ 127A, Remi Mumbai, India). The resulting spherical particles were stirred continuously above the melting point of wax at 900 rpm for 5 min. The temperature of the mixture was cooled rapidly to 10°C by the addition of cold water. The solid spheres formed 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 ratio (Table 1).

Microsphere characterization

Wax microspheres were determined using a tap density tester and Carr's index (% I) was calculated using Eq 1.

$$\% I = (\text{tapped density} - \text{bulk density}) / \text{tapped density} \dots\dots\dots(1)$$

Angle of repose, Tan (θ), was assessed by a fixed funnel method to ascertain the flowability of the microspheres, and calculated as in Eq 2.

$$\text{Tan } (\theta) = h/r \dots\dots\dots (2)$$

where h is the height of the pile and r the radius of the base of the pile.

Scanning electron microscopy

Scanning electron microscope (SEM) photomicrographs were recorded using Joel-LV-5600 SEM, USA. To determine sphericity, the tracings of wax microspheres (magnification, x459) were taken on a black paper using Lucida camera (Prism type, Rolex, India), and the sphericity factor (S) was calculated using Eq 3.

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

where, A is the area (cm^2) and p the perimeter of the microsphere (cm)

Differential scanning calorimetry (DSC)

Dynamic DSC characterization was carried out on the microspheres using a DuPont thermal analyzer with a 2010 DSC module..

Determination of drug loading

The amount of drug incorporated in the microspheres was evaluated by first powdering the microspheres (100 mg) in a mortar. The drug was extracted from the microspheres using methanol (100 ml), filtered and analyzed for drug content after suitable dilution with phosphate buffer (pH 7.2), using a UV/visible spectrophotometer (Shimadzu – 1601, Japan) at a wavelength of 319 nm.

In vitro studies

USP XX1 dissolution apparatus type II was employed to study drug release from the microspheres. An accurately weighed quantity of the microspheres (equivalent to 75 mg of IM) was placed in 900 ml dissolution medium (hydrochloric acid buffer, pH 1.2), stirred at 100 rpm, for 2 h. in the dissolution medium was changed to (phosphate buffer, pH 7.2) and the dissolution process continued for another 6 h. The temperature was maintained at $37 \pm 0.5^{\circ}\text{C}$. The drug concentrations were determined by withdrawing the Aliquots (10 ml) of the dissolution medium were withdrawn at time intervals using guarded sample collectors, and analyzed spectrophotometrically at 319 nm.

In vivo studies

In vivo release studies were conducted in healthy adult albino sheep. Their age was in the range 6 - 8 years and body weight 30 to 32 kg. A written approval (no. 004/2001) was obtained from the institutional ethical committee of JSS Medical College Hospital

and JSS College of Pharmacy, Mysore, India. The study was an open, randomized complete cross-over design in which a single 75 mg dose of IM (Microcid® SR 75 mg capsule or test formulation F3) was administered to fasted, healthy adult males and females on two different occasions, separated by a washout period of 2 weeks between dosing. Blood samples (5 ml) were collected at 0 h (pre-dose) 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. The blood samples were centrifuged at 1500 rpm for 10 min and the separated plasma stored at -20 °C prior to analysis [18].

Chromatographic studies

IM concentrations in plasma were assayed using a fully validated high performance liquid chromatography (HPLC) [16]. The HPLC system used (model LC-6A, Shimadzu, Tokyo, Japan) was fitted with a μ -Bondapak C18 (4.6 x 250 mm) column of particle size (5 μ m, Supelco, Bellefonte, PA, USA). The flow rate was maintained at 1 μ L/min; drug concentration was detected using a UV/visible detector (SPD- 6Av). The mobile phase consisted of 80 % methanol and 0.02M sodium acetate buffer (60:40 v/v). The pH of the acetate buffer was 3.6. The column was heated to 40 °C and the wavelength was set at 320 nm.

Pharmacokinetic and data analysis

Pharmacokinetic parameters were calculated using Quick calc, computer PK calculation programme. The extent of absorption of Microcid® SR 75 mg

capsule and test formulation (F3) in sheep was derived from the area under the plasma concentration time curve from zero to 24 h (AUC_{0-24}) calculated by the trapezoidal rule method. Area under the plasma concentration time curve from zero to infinity (AUC_{0-inf}) was calculated as in Eq 4.

$$AUC_{0-\infty} = AUC_{0-t} + C_{24}/K \dots\dots\dots (4)$$

where, C_{24} = drug concentration in plasma at 24 h, K the constant, t the time. 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

Micromeritic properties

Mean size of the microsphere ranged from 345 to 360 μ m, as presented in Table 1. A wax:drug ratio of 1:3 was found to be optimum for the preparation of the microspheres (Table 1). A similar finding was previously reported by Gowda *et al* for wax/fat microspheres [10,12,14].

The micromeritic properties of the microspheres are given in Table 1. The angles of repose, tap density and car's index were well within limits, indicating good flow of the microspheres as shown in Table 1.

Table 1: Composition and physicochemical properties of indomethacin microspheres

Formulation	Drug (g)	Sugarcane wax (g)	Mean size (μ m)	Yield (%)	Angle of repose (θ^0)	Carr's index (%)	Tapped density (g/cm^3)
F ₁	2.8	8.8	245	92.41	27	10	0.46
F ₂	2.9	8.9	249	93.45	26	10	0.48
F ₃	3.0	9.0	252	96.02	27	12	0.51
F ₄	3.1	9.1	254	90.12	25	11	0.48
F ₅	3.2	9.2	260	90.49	26	15	0.51

SEM photographs showed that the microspheres were spherical, non-aggregated (Fig 1a), and had generally a smooth surface with some inward dents and shrinkage (Fig 1b), due probably to the collapse of the wall of the microspheres. The rate of solvent removal from the microspheres exerts an influence on the morphology of the final product [15].

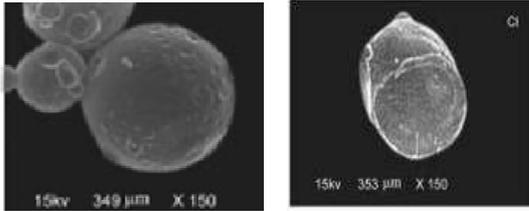


Fig 1: SEM photomicrograph of; (a) the exterior surface of indomethacin (IM)-loaded F₃ microsphere (b) interior of the same microsphere

Thermal characteristics

DSC studies on the pure drug, as well as on the blank and drug-loaded microspheres showed sharp endothermic peaks (Fig 2). IM exhibited a sharp endothermic peak at 161.3 °C while.

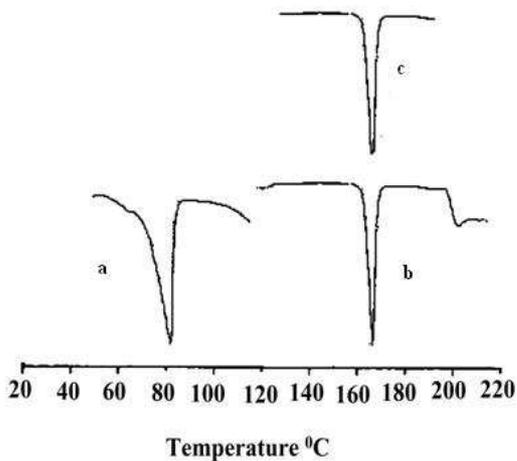


Fig 2: DSC thermogram of (a) pure indomethacin, (b) plain microspheres, and (c) indomethacin-loaded microspheres (F₃)

Drug-loading and encapsulation efficiency of the microspheres

Drug loading in the formulations was in the range of 19.1 - 22.1 % while encapsulation efficiency ranged from 86.1 - 91.2 %.

Drug content

The drug content of Microcid[®] SR capsule and formulation F₃ were 99.66 and 99.51 %, respectively. Thus, the drug content was well within the stipulated limits (98% w/v to 107% w/v) of the United State Pharmacopoeia /National Formulary [16] for the two formulations.

Drug release

There was no significant release of drug at gastric pH from the test microspheres. At the end of 8 h, *in vitro* drug release from F₃ (95.3 %), was lower than from Microcid[®] SR (99.1 %). Drug release was biphasic manner consisting of initial fast release followed by a slow release in intestinal pH from the wax microspheres [2,10,12,18].

Pharmacokinetics of indomethacin

The mean plasma drug concentration as a function of time is shown in Fig 3 while the determined pharmacokinetic data for Microcid[®] SR and F₃ formulations are given in Table 2. Mean plasma concentrations (C_{max}) of IM for both preparations were within therapeutic concentration range (300 – 3000 ng/ml) in humans. [9]. C_{max} fell below detection limit (50 ng/ml) after 24 h in all animals following administration of either product. On the basis of the data obtained, it can be predicted that the therapeutic activity of both formulations would be sustained for about 12 h following a single dose administration.

The time taken to reach peak plasma concentration (T_{max}) for both the reference product and the test formulation (F₃) were

Table 2: Pharmacokinetic parameters of Microcid[®] SR

Parameter	Microcid [®] SR	F3	Pure IM	p value
T _{max} (h)	3.0	2.9	3.0	< 0.05
C _{max} (ng/ml)	2142 ± 39	2089 ± 16	2189 ± 26	< 0.05
T _{1/2} (h ⁻¹)	2.48 ± 0.13	2.49 ± 0.11	2.65 ± 0.12	< 0.05
AUC ₀₋₂₄ (ng/ml h ⁻¹)	8634 ± 106	8523 ± 89	8713 ± 69	< 0.05
AUC _{0-∞} (ng/ml h ⁻¹)	9974 ± 78	9315 ± 103.11	9897 ± 81	< 0.05
K _a (h ⁻¹)	0.3921 ± 0.001	0.3882 ± 0.003	0.389 ± 0.003	< 0.05
K _{el} (h ⁻¹)	0.283±0.004	0.282±0.002	0.286±0.002	< 0.05
MRT*	4.68 ± 0.03	4.53 ± 0.02	4.61 ± 0.03	< 0.05

T_{1/2} values for Microcid[®] SR and formulation F₃ were similar: 2.48 ± 0.13 and 2.49 ± 0.11 h⁻¹, respectively. So also were the rate of absorption (K_a), 0.392 ± 0.001 and 0.388 ± 0.003 h⁻¹, respectively; elimination rate constant (K_{el}), 0.284 ± 0.012 h⁻¹ and 0.282 ± 0.001 h⁻¹, respectively; and AUC_{0-∞}, 997 ± 78 and 9315 ± 103 ng/ml.h⁻¹, respectively.

not statistically different ($p < 0.05$, as shown in Table 2).

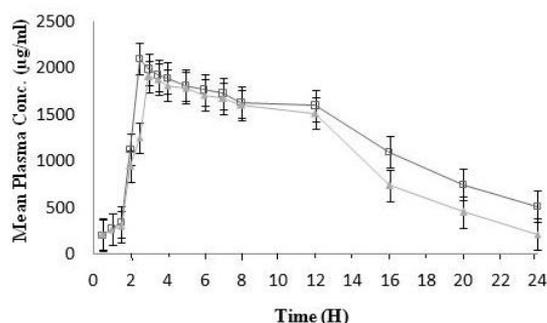


Fig 3: Mean plasma drug concentrations – time profiles of IM from Microcid[®] SR (□) and (▲) formulation F3

DISCUSSION

Waxy materials have the physical properties suitable for preparing gastro-resistant, biocompatible and biodegradable microspheres to release entrapped drug in intestinal lumen [13-15,18].

Using an aqueous medium that was alkaline, indomethacin (IM) solubility decreased while that of the encapsulated drug increased.

The decrease *in vitro* drug release from the microspheres might be due largely to the hydrophobicity and influence of the molecular weight of wax. *In vitro* drug release was considerably retarded from the wax microspheres when compared to Microcid[®] SR. The rate of drug release followed first order release kinetics and the numerical data fitted into Peppas model which means that, the mechanism of drug release from the wax microspheres was non-Fickian diffusion. On the basis of United States Food and Drug Administration (FDA) recommendation, the two products, Microcid[®] SR and formulation F₃, can be considered bioequivalent. Thus, the two formulations can be considered similar in terms of release characteristics,. Absence of high peak plasma drug concentrations (> 5000 ng/ml) are often associated with indomethacin adverse effects due to drug accumulation [8].

CONCLUSION

The test and reference formulations were bioequivalent based on both the *in vitro* and *in vivo* data obtained, Thus, indomethacin microspheres encapsulated in sugar wax microspheres by melt method showed good potential for controlled drug delivery systems.

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