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PREPARATION AND EVALUATION OF MICROSPHERES OF DICLOFENAC SODIUM-RICE BRAN WAX

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ABSTRACT

Objective: The objective behind the study was to develop Diclofenac sodium loaded biocompatible microspheres to reduce the side effects of Diclofenac sodium and sustain the release of drug for a prolonged action. **Methods:** The microspheres were prepared by emulsification technique; the lipophilic wax was poured into water dropwise using Tween-80 as surfactant. **Results:** The prepared microspheres were evaluated for particle size, drug entrapment efficiency (EE) and in vitro drug release study. The mean particle sizes were found to be 8.58µm, 9.387 µm and 9.98 µm respectively for the three formulations M_1, M_2 and M_3 representing drug polymer ratio 1:1, 1:2 and 1:3 respectively. The values of cumulative percentage of drug released during 7 hours for the three formulations M_1, M_2 and M_3 were found to be 68.02, 65.02 and 60.24 respectively. **Conclusions:** The result reveals that the particle size and EE increases as the polymer content in the formulation increases. The increase in polymer concentration prolonged the release of drug from the microspheres with a sustained release pattern. Hence, the microspheres of Diclofenac sodium with rice bran wax were satisfactorily prepared.

KEYWORDS: *Diclofenac sodium, Rice bran wax, Sustained release, Biocompatible microspheres.*

INTRODUCTION

Controlled release drug delivery employs drug-encapsulating devices from which therapeutic agents may be released at controlled rate for long periods of times, ranging from days to months. Such systems offer numerous advantages over traditional methods of drug delivery, including tailoring of drug release rates, protection of fragile drug and increased patient comfort compliance. Polymeric microspheres are ideal vehicles for many controlled delivery applications due to their ability to encapsulate a variety of drugs, biocompatibility, high bioavailability and sustained drug release characteristics. The term 'microsphere' describes a monolithic spherical structure with the drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersion of particles.[10]

Microsphere based drug delivery system have received considerable attention in recent years. Microspheres provide constant and prolonged therapeutic effects, which will reduce the dosing frequency and thereby improve the patient compliance. Microsphere morphology allows a controllable variability in degradation and drug release.[6]

Diclofenac sodium is the sodium salt form of diclofenac, a benzene acetic acid derivate and nonsteroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic and anti-inflammatory activity. Normally, 50 mg of diclofenac sodium is prescribed into 3 - 4 divided doses in a day.

The biological half - life of diclofenac sodium has been reported as 1 – 4 hr. The side effects of diclofenac sodium like gastric ulcers on frequent oral administration are well known. Hence microspheres of diclofenac sodium can be prepared for the purpose of sustaining its release & reducing the dosing frequency.

Rice bran wax is the vegetable wax extracted from the bran oil of rice (Oryza sativa). It is a major wax resource in East Asia, where rice is main food. Rice bran wax is edible and can serve as the substitute for carnauba wax in most applications due to its relatively high melting point. Crude rice bran wax is dark brown in color and has its own typical physical and chemical composition. This wax is purified in several steps and a purified, light weight and slightly non sticky wax is obtained.[2]

The utilization of rice bran wax in pharmaceuticals is meager or hardly there is any report in spite of large production. Therefore the rice bran wax utilization in

pharmaceuticals is worth investigating. The aim of the present work was to develop microspheres of diclofenac sodium and rice bran wax using spontaneous emulsification solvent diffusion technique.[8]

EXPERIMENTAL

Materials

The crude rice bran wax was obtained from Maheshwari Solvent Extraction Plant, Gondia (MH), which was dark brown in color and contained non waxy impurities and fixed oil. All other reagents were analytical or pharmaceutical grade and used as received.

Purification of crude wax

The crude wax (100 g) was soxhleted with ethyl acetate (300 ml) for 30 min at 85°C. The mixture in thimble was cooled up to 25°C and was subjected to decolorization with 2% hydrogen peroxide at 90°C for 1 hour and secondary decolorization with sodium chloride (15% *w/v*) at 100°C for 1 hour. The purified wax obtained was then used for further study.[9]

Characterization of purified wax

The rice bran wax obtained after purification was standardized to determine its physicochemical properties like solubility, melting range, acid value, peroxide value, saponification value, iodine value, unsaponifiable matter as per pharmacopoeial procedure.[1,4]

Preparation of microspheres of diclofenac sodium - rice bran wax

Weighed amount of RBW was taken in a china dish and melted. Weighed amount of drug diclofenac sodium was dispersed into the melted wax. The melted mixture was added to 100 ml distilled water at above 80 degrees. Tween 80 in the concentration of 1.8% w/w as surfactant was added into the mixture using a magnetic stirrer set at 800 rpm for not more than 15-20 minutes which was then cooled to room temperature, filtered and washed so as to get spherical shaped microspheres after air drying.[7]

Table no 1: Formulation Composition of Diclofenac Sodium Rice Bran Wax Microspheres

Sr. No.	Ingredients	M_1	M_2	M_3
1.	RBW (g)	1	2	3
2.	Diclofenac sodium(g)	1	1	1
3.	DS:RBW	1:1	1:2	1:3
4.	Tween 80 (%w/w)	1.8	1.8	1.8
5.	Distilled water (ml)	200	200	200

Evaluation and characterization of microspheres

Fourier transform infrared studies (FTIR studies)[12]

The FT-IR spectra of the samples were obtained to ascertain the compatibility between diclofenac sodium and rice bran wax by FT-IR method. The FTIR spectra for pure drug, empty microspheres and drug loaded microspheres were obtained using KBr powder method. Spectral measurements were obtained by powder diffuse reflectance on a FT-IR spectrophotometer in the wave number region of 400-4000 cm^{-1} to find out drug excipient interaction if any.

Particle size and size distribution[3]

The particle size of microspheres was determined using an optical microscopy method. Approximately 100 microspheres were counted using calibrated microscope.

Determination of percentage yield

Percentage yield was determined by calculating the initial weight of raw materials and the finally obtained weight of microspheres. Percentage yield was calculated by using the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Micromeritic properties[12]

Determination of bulk and tapped density

A fixed weight of the prepared microspheres was poured in a 100 ml graduated cylinder, the powder was allowed to settle with no outer force and the volume occupied was measured as V_b (initial bulk volume). After carrying out the procedure as given in the measurements of bulk density, the cylinder containing the sample was tapped using a mechanical tapped density tester. The cylinder was tapped for 100 times initially followed by an additional tap of 50 times for proper settlement and the tapped volume (V_t) was measured to the nearest graduated unit. The bulk and tapped density was calculated, in grams per ml, using the formula:

Bulk Density $\rho_b = M / V_b$

Tapped Density $\rho t = M / V_t$

Where (M) is the mass of the powder.

Determination of Carr's index

The percentage compressibility (Carr's index) was determined from the following equation:

$$\text{Carr's Index} = \frac{V_b - V_t}{V_b} \times 100$$

Where V_b and V_t are initial bulk volume and initial tapped volume respectively.

Determination of angle of repose

The fixed funnel method was employed to measure the repose angle. A funnel was secured with its tip at a given height (h) above a graph paper that was placed on a flat horizontal surface. The blend carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. The radius (r) of the base of the conical pile, was measured. The angle of repose, α, was calculated using the following formula.

$\alpha = \tan^{-1}(h/r)$

Determination of Hausner's ratio

The Hausner's ratio is a number that is correlated to the flowability of a powder or granular material. The Hausner ratio is calculated by the formula

$$H = \frac{\text{Initial Tapped Density } (\rho t)}{\text{Initial Bulk Density } (\rho_b)} \times 100$$

***Determination of drug loading efficiency*[5]**

One hundred mg of drug-loaded microspheres of each batch were randomly taken. The microspheres were added to a tarred volumetric flask. Sufficient phosphate buffer was added to the volumetric flask to extract the microspheres. The extracts were filtered and collected into 100 ml volumetric flask and made up to the volume with phosphate buffer (pH 7.4). The solutions were subsequently suitably diluted with pre warmed phosphate buffer (pH 7.4) and spectrophotometric absorbance was taken at 276 nm, against a blank prepared with microspheres containing no drug. The corresponding calculations were calculated from a standard curve prepared for diclofenac sodium. Drug loading efficiency (DL) was calculated according to the following equation,

$$DL\ (\%) = \frac{\text{Weight of drug in microspheres} \times 100}{\text{Weight of microspheres}}$$

The weight of drug in microspheres was calculated from the experiment for drug content determination.

***Determination of drug entrapment efficiency*[5]**

Entrapment efficiency is defined as the percentage of drug incorporated into microspheres relative to the total drug added. The EE was determined using the following equation.

$$EE\ (\%w/w) = \frac{\text{Weight of drug in microspheres} \times 100}{\text{Weight of drug used in the preparation of microspheres}}$$

***In-vitro drug release studies*[12]**

The microspheres were tested for the *in vitro* release of DS in simulated GI fluids. An accurately weighed amount of microspheres, were added to 500 ml of dissolution medium and the drug release from microspheres was processed using USP rotating paddle dissolution apparatus at 100rpm and at 37±0.5 °C. Perfect sink condition was maintained during the drug dissolution study period. The dissolution of the drug was conducted in two media pH 1 (0.1 N HCL) as well as pH 7.4 (Phosphate buffer) representing stomach and intestinal conditions respectively.

The simulation of GI pH variations was accomplished by modifying the pH of the dissolution at various time intervals. The pH of the dissolution medium was kept at 1.2 for 2 hour with 0.1N HCl. Then, the pH was changed to 7.4 using phosphate buffer (pH 7.4). The release rate analysis was run for another 5 hour. At predetermined time intervals, aliquots (5ml) were withdrawn, filtered through 0.45 membrane filter and replaced with equal volumes of pre warmed fresh medium to maintain constant volume and keep sink condition. After appropriate dilution, the sample solution was analyzed for diclofenac sodium by UV absorbance method at 276 nm. All release tests were performed in triplicate. The effects of drug–polymer ratio on *in vitro* drug release of DS loaded albumin microspheres were also evaluated.

RESULTS AND DISCUSSION

Purification and characterization of wax

The rice bran wax procured from the mill was not purified therefore purification of the wax was carried out as per reported method. The purified wax was characterized for solubility, melting range, acid value, peroxide value, saponification value, iodine value and unsaponifiable matter. Table 2 summarizes the characteristics of purified wax. The crude wax was dark brown in colour which became yellowish white on purification. No significant changes were observed in the taste and odour of the wax. Both, the crude and purified wax were found to be soluble in ether, benzene, chloroform and CCl_4 and insoluble in water, acetone and ethanol. The melting point showed a little decrease after purification. For purified wax, the acid value was low indicating lesser degree of unsaturation, lower iodine value showed less degree of unsaturation. The values obtained were in the range of optimum values as stated for purified rice bran wax.

Preparation of microspheres

The drug loaded microspheres of rice bran wax were prepared in different drug: polymer ratio. Incorporation of drug into wax microspheres and drug release from microspheres requires the addition of a Tween 80 at an optimum concentration to reduce the interfacial tension between the hydrophobic material and external aqueous phase. Also, an optimum stirring time and speed is required for a proper yield of microspheres. An increased stirring time and speed led to smaller sized microspheres which were lost during washing and filtration.

Evaluation and characterization of microspheres

Fourier transform infrared studies (FTIR studies)

The IR spectra are shown in Figure 1. The IR spectrum of pure drug (diclofenac sodium) (Fig.1a) shows a characteristic peak at 3386 cm^{-1} due to N-H stretching frequency of secondary amine. The absorption bands at 1305 and 1282 cm^{-1} resulted from C-N stretching and the peaks at 1556 and 1574 cm^{-1} due to C=C stretching and C=O stretching of carboxylate group, respectively. The C-Cl stretching characteristic peak was observed at 746 cm^{-1}. The IR spectrum of diclofenac sodium with rice bran wax as microspheres (Fig.1b) and plain rice bran wax microspheres (Fig.1c) shows all the principal characteristic peaks related to diclofenac sodium without any change in their position, indicating no possibility of chemical interaction between the drug and rice bran wax.

Particle size and size distribution

The size of the prepared microspheres was measured by the optical microscopy method using a calibrated stage micrometer. The obtained mean particle size is given in Table no 3. When varying the drug–polymer ratio from 1:1 to 1:3, the mean diameter of microspheres was in the range between 8.58 µm and 9.98 µm. The size increased with increasing polymer concentration. Optical microscopic analysis revealed the spherical shape of microspheres with more or less uniform size distribution.

Determination of percentage yield

The percentage yield of prepared microspheres was calculated. It was observed that as the drug-polymer ratio varies from 1:1 to 1:3, the percentage yield increases. The percentage yield of different concentrations of microspheres is given in Table no 3.

Micromeritic properties

Table 4 shows the results of the micromeritic properties of the prepared microspheres. It is clear that the rheological parameters like angle of repose and bulk density of all the microspheres confirms better flow and packing properties. All the prepared microspheres showed good flowability, as represented in terms of angle of repose. Hausner's ratio of all the preparations was less than 1.25 indicating good flow while the % compressibility was less than 20% again indicating good flow. The improvements of micromeritic properties suggest that the prepared microspheres can be easily handled.

Determination of drug loading efficiency

The drug loading efficiency of microspheres was calculated using a standard calibration curve of Diclofenac Sodium. The drug loading in different ratios of microspheres is shown in Table no 5. It was observed that the drug loading decreased as the concentration of RBW was increased.

Determination of drug entrapment efficiency

The EE increases as the polymer content in the formulation increases. The EE for formulation M_1, M_2 and M_3 were found to be 53.08%, 74.92% and 90% respectively. The increase in matrix content is expected to raise the EE by providing more space to incorporate the drug. Increment of the polymer content also reduces the escaping of drug into the external phase, which accounts for an increase in EE. The values of drug loading and dug entrapment efficiency are shown in Table no. 5.

In-vitro drug release studies

DS-loaded RBW microspheres were subjected to *in vitro* dug release studies in the presence of simulated GI fluids using USP dissolution test apparatus I. The studies were carried out in 500 ml of the dissolution medium, stirred at 100rpm at 37 degrees C. The dissolution profiles for both batches were studied using acid buffer solution of pH 1.2 for 2 h (simulated gastric fluid), pH 7.4 for remaining 5 h. The in vitro release profiles of DS from the microspheres in simulated GI fluids are depicted in Figure No.2. About 10 to 12% of drug was released from the formulations in the initial 15 minutes. This may be due to the drug desorption and release from the surface of microspheres. A sustained release of 68.02%, 65.02% and 60.24% respectively were found for formulations M_1, M_2 and M_3 over the entire period of study. The increase in polymer concentration prolonged the release of drug from the microspheres.

Table no 2: Physicochemical Characteristics of Crude And Purified Wax.

PARAMETERS	CRUDE WAX	PURIFIED WAX
STATE	Solid	Solid
COLOUR	Dark Brown	Yellowish White
ODOUR	Characteristic	Characteristic
TASTE	Bland	Bland
SOLUBILITY	Soluble in ether, benzene, chloroform and CCl_4 Insoluble in water, acetone and ethanol	Soluble in ether, benzene, chloroform and CCl_4 Insoluble in water, acetone and ethanol
MELTING RANGE	82^o-86^o C	78^o-82^o C
ACID VALUE	21.01 mg KOH/g	9.46 mg KOH/g
PEROXIDE VALUE	31.74 mEq/Kg	11.31 mEq/Kg
SAPONIFICATION VALUE	112.75 mg KOH/g	75.62 mg KOH/g
IODINE VALUE	10.51 g/100 g	7.13 g/100 g
UNSAPONIFIABLE MATTER	58 %w/w	42 %w/w

Table no 3: Mean Particle Size And Percentage Yield of Microspheres.

Sr.No	Formulation Code	Drug:Polymer Ratio	Mean Particle Size	% Yield
01.	M_1	1:1	8.58 $\pm$ 0.5	70%
02.	M_2	1:2	9.387 $\pm$ 0.5	76%
03.	M_3	1:3	9.98 $\pm$ 0.5	85%

Table no 4: Micromeritic Properties of Prepared Microspheres.

Sr. No.	Ratio	Bulk density(g/ml)	True density (g/ml)	Carr's index (%)	Hausner's ratio	Angle of repose
01.	1:1(M_1)	0.28	0.34	18	1.21	25.42
02.	1:2(M_2)	0.34	0.41	15	1.20	26.68
03.	1:3(M_3)	0.40	0.44	10.5	1.1	26.46

Table no 5: Drug Loading And Entrapment Efficiency Of Microspheres.

Sr No.	Formulation	Drug:Polymer Ratio	Drug Loading (%)	Entrapment Efficiency (%)
01.	M_1	1:1	26.54	53.08
02.	M_2	1:2	24.97	74.92
03.	M_3	1:3	22.5	90.00

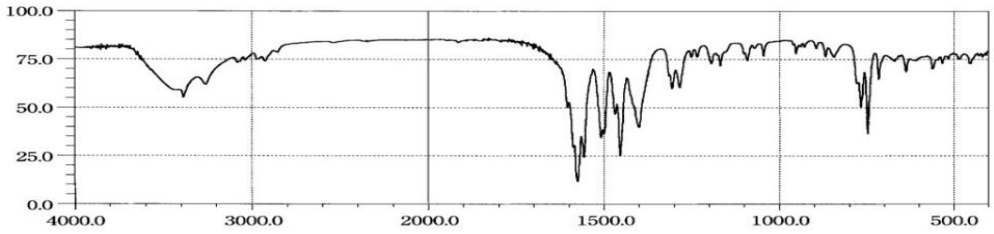

Figure 1a: IR Spectra of Pure Diclofenac Sodium.

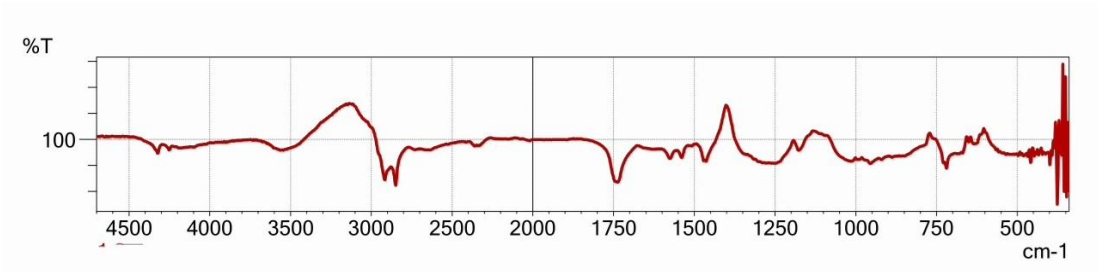

Figure 1b: IR Spectra of Diclofenac Sodium And Rice Bran Wax Microspheres.

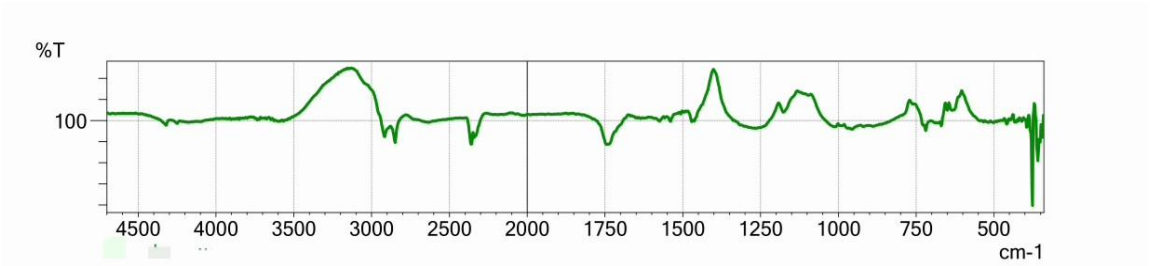

Figure 1c: IR Spectra of pure rice bran wax microspheres.

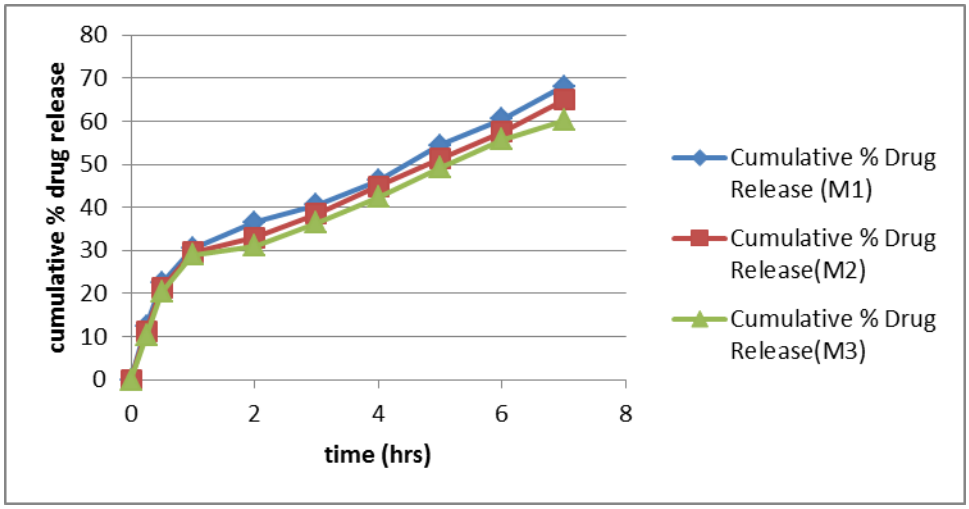

Figure No 2: Cumulative % Drug Release of Different Formulations of Microspheres.

CONCLUSIONS

Diclofenac sodium loaded rice bran wax microspheres were prepared by emulsification method. Three batches of formulations, of drug: polymer ratio i.e. 1:1, 1:2 and 1:3, were prepared and evaluated for particle size, drug loading capacity, drug entrapment efficiency and *in vitro* drug release. The microspheres were spherical with more or less uniform size distribution and an increase in particle size was observed as the polymer concentration was increased. The encapsulation efficiency increased as the polymer content in the formulation increased. *In vitro* release studies demonstrated a sustained release pattern with an initial burst release. In our study, it was observed that an increase in polymer content delayed the release of drug from the formulation. The results demonstrated a potential use of wax for the preparation of sustained release devices for water insoluble drugs. Hence, the basic objective of formulating a sustained release formulation of diclofenac sodium using a biodegradable polymer was satisfactorily attained.

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