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Preparation, evaluation and bioavailability studies of Indomethacin loaded PEA polymeric microspheres for controlled drug delivery

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ABSTRACT

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. The present study aims to the preparation of poly ester amide (PEA) microspheres containing indomethacin (IM) as a model drug, and to compare the In vitro release and pharmacokinetics of prepared IM formulations with commercially available Microcid®SR. In the present study, water is used to prepare PEA polymer microspheres by meltable dispersed emulsified cooling induced solidification method. Surface morphology of prepared microspheres has been evaluated using scanning electron microscopy (SEM). The SEM images revealed the spherical shape of microspheres with size ranges132 µm to 796 µm. Differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy studies indicated that the drug after encapsulation with PEA polymer was stable and compatible. A single dose randomized complete cross over study of IM (75mg) microspheres was carried out in healthy albino rabbits. Plasma IM concentrations and other pharmacokinetic parameters were statistically analyzed.

Keywords: Microspheres, PEA polymer, controlled release, Indomethacin.

INTRODUCTION

Oral route has been the most popular and successfully used for sustained delivery of drugs because of convenience and ease of administration, greater flexibility in dosage form design and ease of production and low cost of such a system. The growing interest in controlled drug delivery release is because of its benefits like increased patient compliance due to reduced frequency of administration and less undesirable side effects. Microencapsulation of drugs in a hydrophobic matrix such as polymer, control the release of drugs. The term "control" includes phenomena such as protection and masking, reduced dissolution rate, facilitation of handling and spatial targeting of the active ingredient. The characteristics of microspheres containing drug should be correlated with the required therapeutic action and are dictated by the materials and methods employed in the manufacture of the delivery systems. Different poly (ester) amides (PEA) polymers have been used as barrier coatings due to their hydrophobic nature. Oral controlled release dosage forms such as micro particles are becoming more popular than single unit dosage forms. The uniform distribution of these multiple unit dosage forms along the gastro intestinal tract could result in more reproducible drug absorption and reduced risk of local irritation.

Poorly water-soluble drugs, which are lipophilic in nature easily, mix with PEA polymer and show good absorption rate. The PEA polymeric materials used in the current study have good pharmaceutical and biological properties. Different strategies have been developed in recent years to design different types PEA polymer microspheres loaded with hydrophilic and lipophilic drugs using toxic solvents. The use of such solvents during formulation is of environmental concern and challenges to human safety. To overcome this problem, in the present study, water is used to prepare PEA polymer 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. Various drugs are loaded with PEA, which will delay the action, due to slow dissolution of the drug. Recently it has been reported that lipid soluble drugs gives better absorption if they are embedded in PEA polymer microspheres. Since dissolution is an important prerequisite for drug absorption in most of acidic or basic drugs, the used carriers influence the drug absorption to a great extent [1-3].
This write up will highlight a systematic study of the PEA microspheres to develop controlled drug delivery systems.

Factors affecting the release of drugs from PEA polymer microspheres are as follows, [4]

1. Molecular weight of the drug
2. Physicochemical properties of the drug
3. Type and amount of PEA polymer material used
4. Size and density of the microspheres
5. Presence of adjuvants
6. pH of the dissolution medium
7. Presence of enzymes

PEA polymer has been used as drug carriers to achieve controlled drug delivery for the past few decades. However PEA polymer microspheres have gained prominent interest owing to their versatile properties such as non immunogenic, biodegradable and capacity to encapsulate drugs. Indomethacin was chosen as a model drug. It is a drug of choice for maniac disease due to its narrow therapeutic index; a controlled release dosage form is highly desirable [5].

MATERIALS AND METHODS

Indomethacin (IM), pure drug was kindly donated by Micro Labs (Banglore, India). White to pale yellow, crystalline powder, odorless. Practically insoluble in water.
All other chemicals and reagents used were of analytical grade.

Preparation of PEA microspheres

Required quantity of PEA polymer was melted at a temperature at 265º C for 5 min separately in a water bath. Lithium carbonate (LC) which was already passed through sieve No.100 was dispersed in the melted PEA polymer mass and stirred for 30 min to obtain a homogeneous melt. These individual mixtures were poured into 150 ml of buffer solutions (pH 4.2 pthalate buffer, to minimize the solubility of drug) previously heated to a temperature (90ºC). Surfactant Tween 80 (1.8%w/w) was added to the mixture. The whole mixture was mechanically stirred using a stirrer (RQ-127A) fitted with a four blade impeller of approximately 53mm diameter. The molten mass produces spherical particles due to dispersion in the aqueous medium. The mixture was stirred continuously at 90ºC for 2 minutes. After 2 minutes the temperature of the mixture in the beakers was cooled rapidly and brought down to 10^{O}C by the addition of cool water. The resultant solid spheres collected by filtration were extensively washed with water to remove any drug and surfactant residues. Air-drying at room temperature for 48 hours gave discrete, solid free flowing microspheres.
A total of four formulations were prepared by varying polymer to drug ratio.

Characterization of Microspheres

Size distribution and size analysis: Size distribution of the PEA microspheres were studied by sieve analysis technique. The separations of the microspheres into various size fractions were carried out. Drug loaded microspheres (10g) were placed on the top of series of six standard bronze sieves in the range of 1000 - 106 μm (Test sieves, India), arranged in the order of decreasing aperture size. The sieves were mounted on mechanical sieve shaker (C.M equipments, India) and operated for a period of 30 min, which is adequate for complete separation.

Micromeritics properties:

Tap density of prepared PEA 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 done in order to know the flow ability of PEA microspheres, by a fixed funnel method.

Tan (θ) = height/radius. [2]

Scanning electron microscopic (SEM) study: SEM photographs were taken with a scanning electron microscope Model Joel- LV-5600, USA, at the required magnification at room temperature. The photographs were observed for morphological characteristics and to confirm spherical nature of the microspheres.

Determination of the sphericity: To determine the sphericity, the tracings of PEA microspheres (magnification 45x) were taken on a black paper using camera Lucida, (Model - Prism type, Rolex, India) and circulatory factor was calculated [6].

Drug content: Uniformity of drug content for the best formulation and their corresponding commercial formulation was determined. The content of 5 units were combined and weighed to the average weight of each unit. The amount equivalent to the content of each unit was determined. In brief, drug was extracted from the respective dosage forms using methanol (80 %). Methanolic extract was suitably diluted and drug content was determined. The results are expressed as percentage claim.

Estimation of drug loading: Drug incorporated (100 mg) PEA microspheres of each batch were selected and powdered in a mortar. Drug from PEA microspheres was extracted using methanol (80 %),were filtered and analyzed after necessary dilution. *In-vitro* studies were carried out for all the batches of the prepared formulations and their corresponding commercial formulations [7-9].

Differential scanning calorimetry (DSC): All dynamic DSC studies were carried out on Du Pont thermal analyzer with 2010 DSC module. Calorimetric measurements were made with empty cell (high purity alpha alumina discs were used for Indomethacin and Indomethacin and high purity gold discs was used for Indomethacin of Du Pont 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 10^0C/min. The runs were made in triplicate.

Fourier Transform Infrared Radiation Measurements (FT-IR): FT-IR analysis was carried out for pure drug and for microspheres with and without drug using KBr pellet method on FTIR spectrophotometer type Shimadzu model 8033, USA.

In Vitro 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 75 mg) were subjected for dissolution study in 900 ml of dissolution medium for 2 h in pH 1.2 hydrochloric acid and buffer pH 7.2 phosphate buffer. The dissolution media was maintained at 37^0C ± 0.5^0C and stirred at 100 rpm. Drug release from the formulations were determined by withdrawing 10 ml of samples using guarded pipette at 30 minutes interval for first four hours and one hour interval for the remaining four hours. Samples withdrawn were estimated after appropriate dilution. Release studies were carried out in triplicate.

Release studies of prepared microspheres were compared to commercially available Microcid SR®-75 in order to interpret the release pattern required.

***In vivo* studies**

Subjects: Four male and four female healthy adult rabbits were included in this study. Provisions were made for all observed signs and symptoms occurring during the study period to be recorded in full.

Ethical review: Written approval obtained from local ethical committee of Farooqia College of pharmacy, Mysore, India.

Study design and doses: Microcid SR®-75 capsules coded as product – A and indomethacin loaded in PEA polymer microspheres coded as product B. The study was an open, randomized complete cross over in which a single 75 mg dose of each product
(Product A and B) was administered to fasting, healthy adult males and females on two different occasions, separated by a wash out period of 2 weeks between dosing**.** The content uniformity of marketed product and optimized formulation have been estimated as per USP specifications (13). Contents of 5 units of Microcid SR®-75 capsule and formulation IM1 were individually combined and weighed to average weight of each unit.

Procedure: All the animals were reported to the clinical trial laboratory from animal house at 7.00 AM after overnight fast of 10 hours. After shaving near the neck, an 18 gauge (1.3 x 45 mm, 96ml / min) canula was inserted in to a jugular vein and kept with heparinised saline lock the ensuing 24 h blood sampling. Test medication products A and B were administered to the subjects with banana and 200ml water. A light food was provided at 3^{rd} h followed by two standard meals at 7^{th} and 11^{th} h following drug administration. Blood samples (5 ml) were collected at 0 h (pre dose), 0.5, 1,1.5, 2, 2.5, 3, 3.5, 4, 5,6, 7, 8, 12, 16, 20, 24 h post dose. Blood samples were centrifuged (eltek- TC 4100 D Centrifuge, Elektroshaft, Bombay, India) at 1500 rpm for 10 min. plasma separated and stored at – 20^0C prior to analysis. Any other types of food not permitted after 12 h after administration of the test medication. All subjects remained ambulatory and strenuous physical activity was prohibited during the first 12h of blood sampling.

Chromatographic condition: Serum concentrations of IM were quantified by a modifications of the HPLC method described for IM by Johnson et al [10].
The mobile phase consisted of 80 % methanol in 0.02M sodium acetate buffer (60: 40 v/v). The pH of the acetate buffer was adjusted to pH 3.6 with acetic acid and the mobile phase was filtered (0.45 µm pore size) and degassed by sonication under vacuum. The HPLC system was allowed to equilibrate at a flow rate of 1ml / min. The column was heated to 40^0C and the wavelength of the detector was set to 320 nm to optimize elution of both IM and MA. MA was used as internal standard. The retention time for IM was 5.32 minutes and that for MA (internal standard) was 8.25 minutes.

Extraction procedure: Internal standard (100 µl) and citrate buffer (pH 3.0, 500 µl) were added to 10ml screw capped glass tubes containing 500 µl of spiked plasma. The samples were

extracted gently with 7ml of petroleum ether: Dichloromethane (50: 50) v/v for 5 min on a rotary shaker and centrifuged at 600 rpm for 5 min. The organic phase was transferred to 10 ml conical test tubes and evaporated to dryness at 40^0C. The residue was resuspended in 100 µl of mobile phase and 25 µl was injected to the column. Quantification was achieved by the measurment of the peak area ratio of the indomethacin to the internal standard (mefanamic acid). The limit of detection of indomethacin in plasma was 100 ng/ml (500 µl of plasma injected) with a coefficient variation (%) ranging between 3.8 - 8.

Statistical Data Analysis: The pharmacokinetic parameters were calculated using the Quick calk, computer PK calculation programmer. The peak plasma concentration (C_{max}) and time needed to reach peak plasma concentration (T_{max}) were computed directly from plasma level profiles as a measure of the rate of absorption of the drug from each product. The elimination rate constant (K_{el}) was calculated from the terminal elimination phase of logarithm of drug concentrations against time curve by the method of least square regression analysis. The biological half life ($T_{1/2}$) was determined by the relation $T_{1/2} = 0.693/K$. The extent of absorption for the products (A&B) in different subjects from the area under the plasma concentration time curve from zero to 24 h (AUC_{0-24}) was calculated by the trapezoidal rule method. Area under the plasma concentration time curve from zero to infinity ($AUC_{0-\infty}$) was calculated using $AUC_{0-\infty} = AUC_{0-T} + C_{24} / K$. Where C_{24} = drug concentrations in the plasma at 24 h. The drug plasma concentration and pharmacokinetic parameters were analyzed by analysis of variance (ANOVA) at 95% confidence limits. Difference between two related means was considered statistically significant when their P values were equal to or less than 0.05.

RESULT AND DISCUSSION

In the present study, a novel dispersed Emulsified Cooling Induced Solidification Method was optimized by using inert PEA polymeric materials and nontoxic solvent to entrap the drug. Effect of pH on drug loading capacity, effect of surfactant concentration on drug incorporation, drug and polymer ratio, stirring sped, and volume of aqueous phase used were optimized during the preparation of polymeric microspheres.

Table 1. Effect of pH on the loading capacity of Indomethacin into PEA polymer

Formulations Code	pH	Drug loading (%)
IM1	4.2	22.36%
IM2	5.2	17.24%
IM3	6.2	13.72%
IM4	7.2	11.38%

*Standards are expressed as mean ± SD (n =3)

Loading of drug into PEA polymer was found to be pH dependent. Loading capacity of IM into PEA obtained at different pH ranges are given in Table 1. At pH 4.2 (pthalate buffer) a

maximum of 22.36 % of IM was loaded which was the maximum amount of drug loading encountered in respect to all the formulation. As the pH was increased from 4.2 to 7.2, percent of IM loaded was reduced from 22.36 to 11.38 %. It is because when pH value of external phase was acidic, solubility of drug was reduced and encapsulated amount of drug was increased.

Incorporation of drug into PEA polymeric microspheres required the addition of 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 liquid drug in the PEA polymer microspheres without the addition of a surfactant. But the process failed and resulted in an aggregate cake like mass during the solidification of PEA polymer. It may be to repulsion resulting from high interfacial tension between the hydrophobic PEA polymer material and external aqueous phase. An optimum concentration of a surfactant to obtain discrete microspheres with good flow properties, 1.8 % w/w (Tween-80) for PEA polymer was used. To obtain an optimal surfactant concentration, various concentrations ranging from 0.5 to 2.0 % (w/w) of the total formulation were tested. Concentrations of surfactant (Tween-80) ranging from 0.5 to 1.7 % w/w did not give reproducible microspheres. The resultant PEA polymer microspheres were composed of irregular masses and not possible to distinguish as individual microspheres. It was found that surfactant having a HLB value of 15 was more suitable to increase substantially dispersion of PEA polymer, promote drug incorporation in the microspheres. Solid, discrete, free flowing microspheres were produced, after cooling. A similar surfactant concentration was reported for beeswax microspheres prepared by a melt able dispersion method [11].

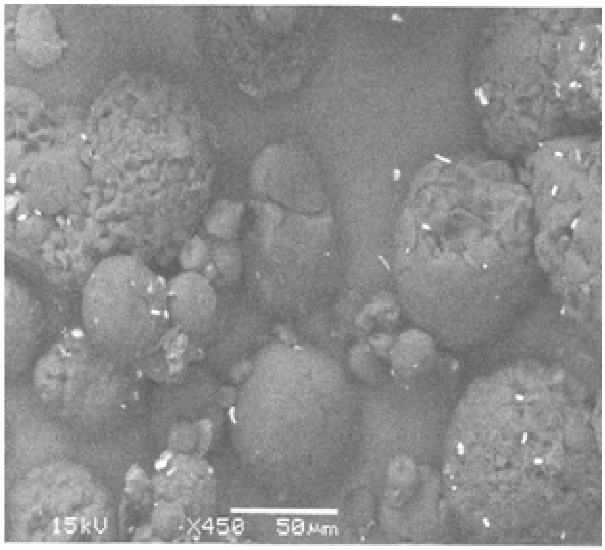

Figure 1. SEM photomicrograph of formulations showing irregular surface and presence of drug crystals on microspheres surface

In the present study, to produce spherical discrete microspheres, an optimum of drug to polymer ratios is essential. Keeping this in view drug to polymer ratio ranging from 1:4 to 1:1 w/w was used, having a formulation code of IM4 to IM1 respectively. A higher amount of drug to

polymer ratio is also under taken but it was noticed that when higher amount of drug to PEA polymer ratio (2:5) was used, microspheres found to get aggregated and form a mass during the cooling process. From the SEM photographs, Figure. 1 it was observed that the prepared microspheres were irregular in shape, with surface irregularities and presence of the drug crystals on the surface of the microspheres**.** The presence of drug particles on the surface of the microspheres may be due to the sudden diffusion of drug from PEA polymer spheres and deposited on the surface at the time of emulsification. The produced microspheres were unsuitable for pharmaceutical uses.

In the present study it was found that 150 ml of aqueous phase was suitable for producing spherical microspheres. Resultant microspheres obtained using this amount of aqueous phase was free from surface irregularities and can be recovered as discrete matter. However an increase in amount of aqueous phase and decrease in amount of aqueous phase was also taken into consideration to prepare spherical microspheres. It was found that as the volume of external phase increased, the yield was reduced and the resultant microspheres were irregular in shape. When the volume of the aqueous phase was less than 150 ml, the resultant microspheres were found to be aggregated like a mass which is highly impossible to distinguish as a individual and discrete microspheres. So in order to avoid the formation of irregularly shaped larger particles, and to produce spherical microspheres, in the present method 150 ml of aqueous phase was used.

Temperature of the aqueous phase was maintained at 90°C. The resultant microspheres were free from surface irregularities, except some wrinkles on surface. It was also observed that when the temperature of the aqueous phase was less than 90°C, big flakes were produced.

Another important factor taken into consideration was effect of stirring speed on size distribution of drug loaded microspheres. It was observed as the stirring speed was increased considerably, reduction with the average size of the microspheres was observed as shown in Table 2. A stirring speed of 700 rpm to 1000 rpm was taken into consideration. When the stirring speed was lower than 900 rpm, leads to the formation of pellets. Microspheres prepared at different stirring speed (700 – 1000 rpm) were prepared and their size was measured by SEM.

From the above results it can be concluded that the drug and PEA ratio, temperature of the aqueous phase, surfactant concentration, stirring speed were well controlled to produce better quality of microspheres.

Table 2. Effect of stirring speed on size for IM loaded PEA microspheres

Formulation Code	Stirring speed (rpm)	Average size (µm)*
IM1	700	363± 06
IM2	800	348± 04
IM3	900	335± 07
IM4	1000	323± 01

*Standards are expressed as mean ± SD (n =3)

Micromeritic properties

Prepared PEA microspheres were subjected to micromeritic studies and presented in Table 3 Sieve analysis data indicated that upto 70% of prepared microspheres 132 µm to 796 µm. It was observed that an average size of microspheres lies in range between 323 µm to 363 µm. The value of angle of repose (θ) were in range between 24.36 ± 2.03 to 28.19 ± 1.29, indicating good flowing behavior of prepared microspheres. Measured tapped density lies within range of 0.3541 ± 2.54 to 0.4896 ± 1.41 g/cm^3. The percent Carr's index (%I) was found to be in range from11.44 ± 1.51 % to 15.14 ± 3.19 %, suggesting good flow property of the prepared PEA microspheres.

Table 3. MIcromeritic properties of IM loaded PEA microspheres

Formulation Code	Size(µm)*	Yield (%)*	Angle of Repose (θ)*	Carr's index (%I)*	Tapped Density (g/cm^3)*
IM4	323± 01	80.12 ± 1.03	24.36 ± 2.03	13.53 ± 2.59	0.3854 ± 1.73
IM3	335± 07	88.33 ± 2.41	25.82 ± 1.13	11.52 ± 1.69	0.3541 ± 2.54
IM2	348± 04	91.75 ±1.06	27.56 ± 2.34	11.44 ± 1.51	0.4896 ± 1.41
IM1	363± 06	89.85 ± 1.05	28.19 ± 1.29	15.14 ± 3.19	0.4657 ± 1.10

*Standards are expressed as mean ± SD (n =3)

The scanning electron micrographic photographs (SEM) were obtained to identify the morphology of the prepared PEA microspheres are presented in **Figure 2**. SEM photographs showed that the PEA microspheres were spherical in nature, had a smooth surface with inward dents and shrinkage due to the collapse of the wall of the microspheres. Thus the removal of solvent from microspheres exerts an influence on the morphology of the end product. These characteristics are correlated with waxes microspheres reported earlier by Giannola et al.[11]SEM photographs reveal the absence of drug particles on the surface of microsphere showing uniform distribution of the drugs in the walls of the microspheres.

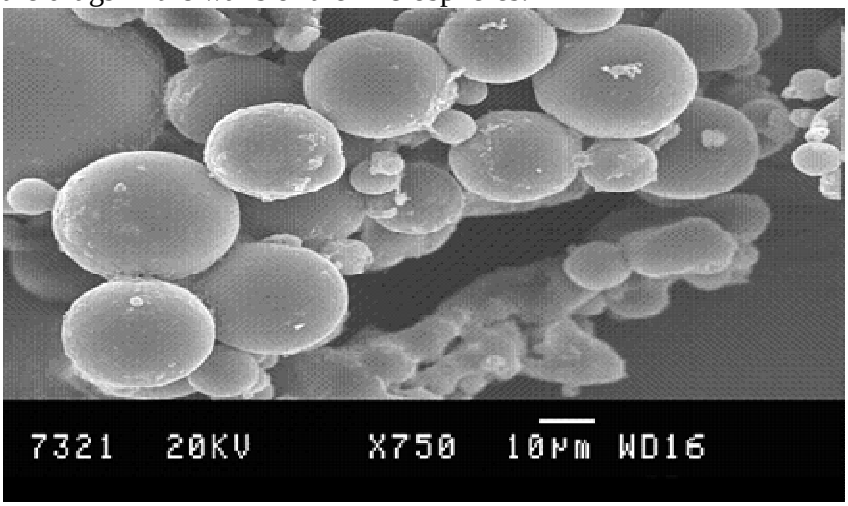

Figure 2: SEM photographs of IM loaded PEA microspheres showing spherical shape.

Differential Scanning Calorimetry (DSC) and FTIR studies

To understand the compatible state of the drug, DSC studies were carried out on pure drug, microspheres with drug loaded microspheres and without drug; the thermo grams obtained for Indomethacin are shown in Figure 3. The melting point of the PEA polymer was 265 ^{0} C and melting point of the drug is 152 to 169^{0}C**.** Pure drug exhibits a sharp endothermic peak at to 161^{0}C. It was observed that absence of the endothermic peak at to 161^{0}C in the drug loaded microspheres indicated, that the drug is molecularly distributed in the microspheres. A comparison and interpretation of these results in our study agrees with Tamilvanan et al [12].

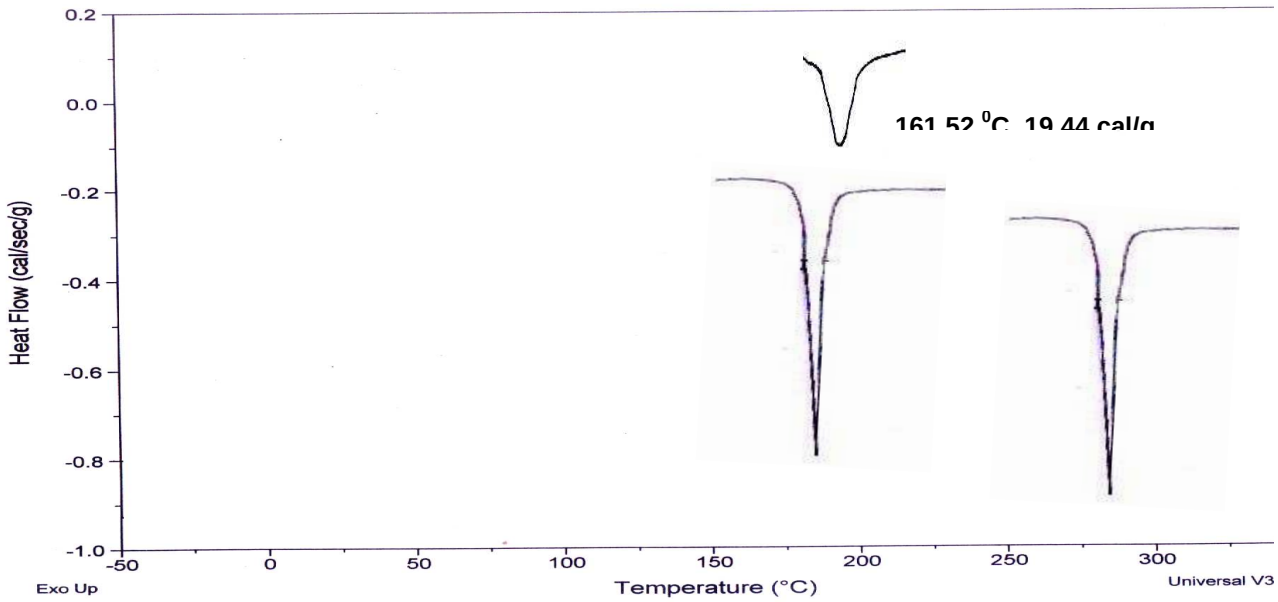

Figure 3. DSC thermograms of pure drug IM (Peak a), PEA Polymer (peak b), IM loaded in PEA polymer (peak c).

FTIR spectra showed in Figure 4 revealed that the characteristics peaks of drug occur after successful encapsulation, without any change in their position, indicating no chemical interactions between the drug and used PEA microspheres.

Encapsulation efficiency

Encapsulation efficiency (%) of prepared PEA IM microspheres was found to be highest in formulation IM1 (92.58%) as compared to IM2 (83.56%), IM3 (76.56%), IM4 (70.27%). This effect may be presence of more amount of polymer in IM1 formulation which in turns enhances its ability to entrap more amount of drug with in matrices.

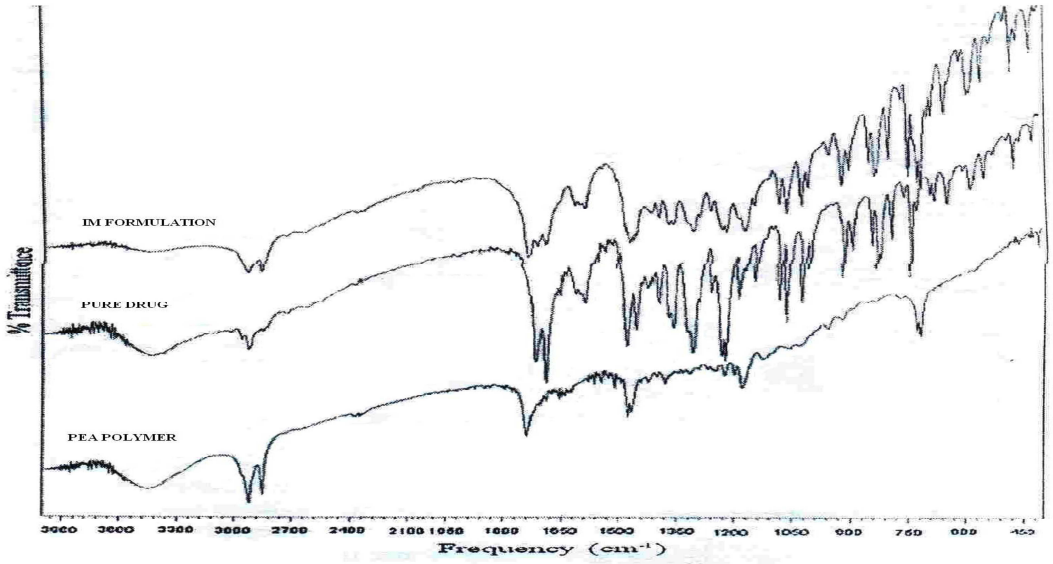

Figure 4. FTIR spectra of Indomethacin and IM loaded PEA microspheres.

In vitro drug release

From the release studies it was observed that, there is no significant release of drug at gastric pH from PEA microspheres and this indicates that the used PEA were gastro resistant in nature. Drug was released in a biphasic manner consisting of initial fast release stage followed by a slow release at intestinal pH from PEA microspheres. Numerical fits indicated that the first order release was most appropriate one for describing initial release behavior. The first order release gave consistently higher values for the correlation coefficient 0.9922, 0.9856, 0.9751, 0.9854 for the prepared formulations. At the end of 8th hour, drug release in the intestinal environment for the prepared formulations ranges from 77.81% for IM1 and MIcrosid SR® – 75, it was 97.59% as shown in Figure 5. The *In vitro* drug release was considerably retarded from the PEA microspheres when compared to marketed product MIcrosid SR® – 75.

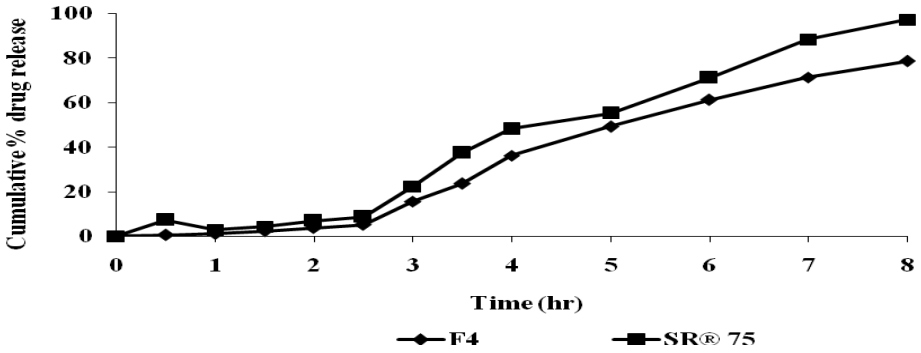

Figure 5. Drug release profile of Formulation IM4 and marketed product Microsid SR® 75.

Initial drug release from PEA microspheres at intestinal environment by biphasic manner and associated with an initial burst release of drug, microspheres was observed respectively. This release might be from the surface accumulated drug. After initial burst effect, the subsequent release of drug was slow and the same behavior of drug from wax was reported by Giannola *et al* [11].

In the present study, statistically estimated values of n at the 95 % confidence limit, for prepared microspheres n = 0.57 and 0.45 indicating fickian transport. This conclusion is agrees with the Kumbar *et al.* have also reported such low values of n for Diclofenac Sodium loaded microspheres[13].

Recovery of the IM from the plasma was calculated by comparison of peak height ratio after direct injection of the IM or MA to the peak height of the same concentrations of analytes extracted from plasma. In both products the recovery of IM was above 90%. Sensitivity of HPLC assay qualitative confirmation of the purity of IM and MA peaks were obtained in Table 4**.** The limit of quantitation was 50 ng/ml of IM in plasma when 0.5 ml plasma was placed. The obtained mean correlation coefficients for the standard curves (n=7) was 0.998. Assay was shown to be sensitive, capable of detecting IM concentrations in plasma as low as 50 ng/ml. Interferences from endogenous compounds were overcome by using as acidic buffer (citrate buffer pH 3.0) to alter the pH of the aqueous phase before extraction.

Table 4. Recovery results obtained for IM from plasma

Sampling time (h)	Drug present in ng/ml (a)	Drug added in ng/ml (b)	Drug recovered in ng/ml (c)	Drug conc. Recorded in ng/ml (c-a)	% of drug recovered c-a × 100/b. Mean ± SD[a]
0.5	10	50	54	44	90.0 ± 1.01
2.0	10	100	106	93	93.0 ± 1.21
4.0	10	200	209	191	95.0 ± 1.43
6.0	10	300	303	301	98.7 ± 1.32
8.0	10	400	405	393	97.4 ± 1.51

***In vivo* Studies**

In vivo studies were carried out for Microcid®SR capsule (product A) and indomethacin loaded PEA microspheres (product B), both containing 75 mg of indomethacin, on adult albino rabbits. Blood samples were withdrawn at different time intervals and plasma concentrations of indomethacin was estimated, results of which are presented in Table 5 and the profile is presented in Figure 6 for both the products in all the eight subjects.

From the data obtained, it may be observed that after oral administration, peak plasma concentration C_{max} of 2064 ± 55.78 ng/ml was observed after 3.0 hours for products A and 1939 ± 20.32 ng /ml for products B. From the comparison of the mean values of plasma concentrations of product A and B, it was observed that product B has significantly lower plasma concentrations. But the mean plasma concentrations of indomethacin for both the products in all subjects were within the therapeutic concentration range (300- 3000 ng/ml). It was observed that

the plasma concentrations of indomethacin in all animals after 24 hours of oral administration were below 50 ng/ml for both the products. It was also observed from the studies that the therapeutic concentration range of indomethacin maintained for about 12 hours following a single oral dose administration for both product A and Product B. From the data obtained, it may be observed that the time taken to reach peak plasma concentration T_{max} was 3.0 hours for product A and 3.5 hours for product B. Though T_{max} was little high for product B, statistical significance differences between the two products was not significant. Mean rate of absorption K_a for product A was 0.3822 ± 0.002 h^{-1} and for product B 0.3668 ± 0.029 h^{-1} and mean elimination rate constant K_{el} for product A 0.2723 ± 0.004 h^{-1} and for product B 0.2417 ± 0.01 h^{-1}. Similarly mean elimination half life $t_{1/2}$ for products A was 2.65 ± 0.03 h^{-1} and for product B 2.86 ± 0.01 h^{-1} . From the above data it may be observed that $C_{max,}$, and $t_{1/2}$ for both products differ marginally and statistical difference was not significantly.

Table 5.Mean plasma concentration of indomethacin from product A and B

Time (hr)	Product A Conc. ng/ml *	Product B Conc. ng/ml *
0.5	00.00	00.00
1.0	143.62 ± 4.59	00.00
1.5	179.62 ± 8.10	138.5 ± 5.09
2.0	248.5 ± 8.60	184.62 ± 6.23
2.5	1222.5 ± 19.01	900 ± 5.31
3.0	2088 ± 50.33	1202.5± 21.63
3.5	1976.37 ± 12.72	1928.12 ± 21.30
4.0	1400 ± 21.98	1311.25 ± 15.38
5.0	900 ± 20.59	806.12 ±1.59
6.0	516.87 ± 10.28	471.12 ± 8.85
7.0	473.25 ± 13.39	380.62 ± 11.2
8.0	342.37 ± 21.81	321.66 ± 6.31
12.0	311.62 ± 8.81	205.12 ± 2.32
16.0	218.12 ± 11.51	186.12 ± 5.64
20.0	154.5 ± 11.16	140.25 ± 9.23
24.0	63.87 ± 7.56	65.12 ± 5.24

*Standards are expressed as mean ± SD (n =3)

The mean AUC_{0-24} values for products A& B was 9687 ± 132.87 ng/ml. h^{-1} and 8353 ± 40.04 ng/ml.h^{-1}. The systemic availability of indomethacin, as determined by comparison of the area under the plasma concentration time curves (AUCs), is lower for both the formulations. The reported bioavailability of orally and rectally administered IM is 100% and 80 % relative to intravenous dose. As for as comparison of the two formulations are concerned, the statistical analysis indicated that the product B exhibited a smaller and non-significant reduction in the AUC values. The slower *In vitro* release of indomethacin from the products A & B may be responsible for the decreased AUC values. Statistical analysis indicated that the product B exhibited only a smaller and non significant reduction in the AUC values. The observed mean AUC $_{0-\infty}$values for product A & B was 9860 ± 129.22 ng/ml.h^{-1} and 8627 ± 46.88 ng/ml.h^{-1} does not show any

significant statistical difference between the products. Comparative profile of various pharmacokinetic parameters is shown in Table 6.

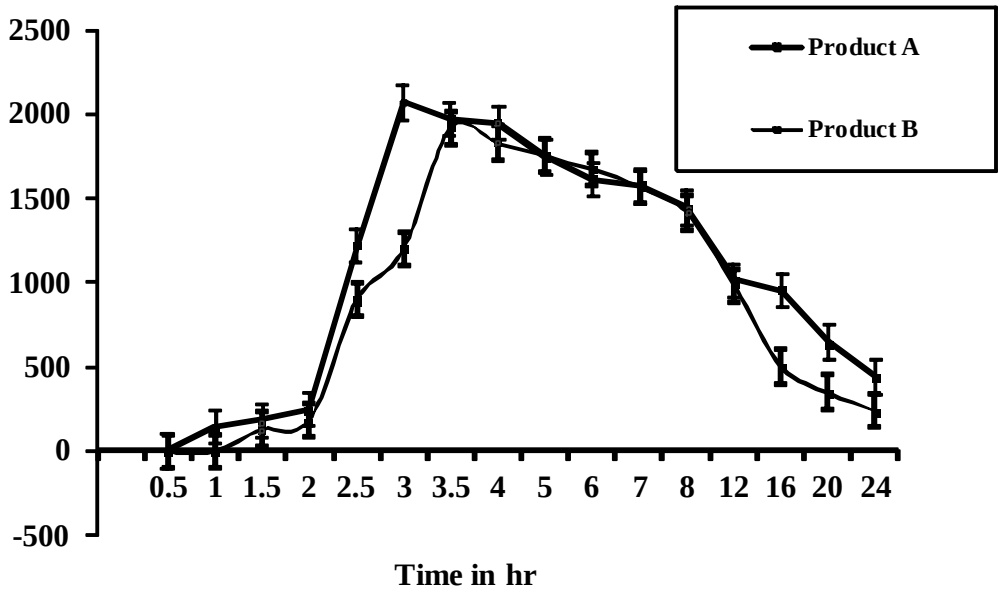

Figure 6. Mean plasma concentrations time profiles of product A and product B
Product A - Microcid®SR and Product B – IM loaded in PEA microspheres.

Table 6. Statistical comparison of the mean values of pharmacokinetic parameters product A & B

Parameters	Product A	Product B	P
C_{max}	2064 ± 55.78 ng /ml	1939 ± 20.32 ng /ml	< 0.05
T_{max}	3.0 h	3.5 h	< 0.05
K_a	0.3822 ± 0.002 h^{-1}	0.3668 ± 0.002 h^{-1}	< 0.05
K_{el}	0.2723 ± 0.004 h^{-1}	0.2417 ± 0.01 h^{-1}	< 0.05
$t_{1/2}$	2.65 ± 0.03 h^{-1}	2.86 ± 0.20 h^{-1}	< 0.05
AUC $_{0-24}$	9687 ± 132.87 ng/ml h^{-}	8353 ± 40.04 ng/ml h^{-}	< 0.05
AUC $_{0-\infty}$	9860 ± 129.22 ng/ml h^{-}	8627 ± 46.88 ng/ml h^{-}	< 0.05

On the basis of FDA recommendation, the two products, Microcid®SR and formulation found to be bioequivalent. No untoward effects were observed by any of the subjects after administration of either product.

CONCLUSION

Present work aimed to prepare and evaluate microspheres loaded with Indomethacin by novel dispersed Emulsified Cooling Induced Solidification Method for controlled release of drug. Method employed was simple and economical, without using any toxic solvents. Results of drug entrapment and micromeritic properties exhibited good results. It was found that microspheres prepared were spherical as indicated by SEM studies. Compatibility studies were done by FTIR and DSC studies. Results indicate that polymer and drug are compatible with each other. Optimized formulation and marketed formulation shows comparable drug release profile. Optimized formulation and marketed productMicrocid®SR showed similarity in drug release profiles and in vivo bioequivalent behavior. From the present data it is concluded that prepared formulation controlled the drug release in a satisfactory fashion, which in turn demonstrate the potential use of PEA polymer for the development of controlled drug delivery system.

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