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Preparation and characterization of naproxen loaded lipid microsphere by melt solidification technique

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Abstract
The present study envisages formulation of wax microparticulate drug delivery system. Naproxen, a well-known non-steroidal anti-inflammatory drug was encapsulated with waxy polymers to provide sustained action and to minimize gastro esophageal side effects by avoiding the Release of drug in the upper gastrointestinal tract. Congealable disperse encapsulation method using biodegradable waxes such as beeswax, ceresin wax microspheres using a wetting agent was the method of choice. The formulations were prepared by keeping the amount of drug fixed to 250mg and the total amount of lipids bees wax and ceresin wax were used in varying concentration. Solid, discrete, reproducible free flowing microspheres were obtained. These microspheres have free flowing and good packing properties and shows the characterization values well within the limit that are angle of repose, % Carr's index and tapped density. In-vitro drug release was studied in a paddle type dissolution Apparatus for hours in phosphate buffer having ph 7.4. After 24 hours, the release of drug was 89.86% for F6 which contains beeswax. The release mechanisms were explored and explained with zero order, first order, Higuchi and korsmeyer-peppas models. Microspheres surface Morphological study was done by scanning electron microscopy (SEM). Drug polymer incompatibility studies were Performed by differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR). The Absence of endothermic melting peak of naproxen in DSC thermogram revealed that the drug might be dispersed in the polymer as solid solution or in a metastable molecular dispersion. The drug loaded in microspheres was found to be stable and compatible with waxes as confirmed by FTIR studies.

Keywords: naproxen, microsphere, melt dispersion technique, lipids

1. Introduction
Controlled drug delivery systems, which are used to deliver drugs at predetermined rates at predefined time periods, have been used to overcome the shortcomings of various conventional drug formulations [1]. Naproxen 6-methoxy-α-methyl-2-naphthalene acetic acid is a new nonsteroidal anti-inflammatory drug (NSAID), most useful drug for showing effective anti-inflammatory and analgesic properties and mainly used in osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. [2, 3] The gastrointestinal (GI) disturbances, peptic ulceration, blurring of vision, tinnitus, depression and GI bleeding along with short half-life (2–4 h) has lead to the design of controlled release formulations of naproxene[4]. Naproxen is rapidly and efficiently absorbed after oral administration. Also For reduction of rate of administration and to better patient compliance naproxen is a suitable for making controlled release dosage form.[5,6]. Due to its low melting point and hydrophobic nature many attempts have been made to develop wax based controlled release formulations Melt dispersion technique has been reported for the development of naproxen microspheres[8,9]. The natural and synthetic polymers in microsphere preparation, acts as matrixing agents like they are biocompatible, biodegradable, nonimmunogenic, can entrap wide range of water insoluble compounds and are economic [10]. In these techniques naproxen-wax melt was emulsified and then cooled to obtain microspheres. The drug:wax ratios were significantly high from 1:3 to 1:5 (50–80% wax) with the drug loading in the range of 10–30% [11, 12]. This technique is especially suitable for drugs, which are insoluble in water. Taking into account the above considerations, lipid microspheres of naproxen using bees wax and ceresin wax as a lipid carrier were prepared by modified melt dispersion technique. The objective of the present study was to develop controlled release naproxen lipids employing the strength of melt solidified bond and to impart sphericity with minimum amount of excipient. The lipids microspheres were characterized using scanning electron microscope (SEM) and FT-IR. The effect of variables on the yield, micromeritic properties, crushing strength and various release parameters was evaluated [13-15]

2. Materials and methods
2.1 Materials
Naproxen was a gift sample from Taj Pharmaceutical Ltd, Mumbai India. All other chemicals used were of analytical grade.

2.2 Preparation of Wax Microspheres:
Naproxen Lipid microspheres were prepared by modified melt dispersion technique.[8] Weighed amount of ceresin wax were melted separately in china dish using water baths. Drug previously passed through sieve no.100 was dispersed in the melted wax mass and stirred to obtain a homogeneous melt. These individual mixtures were poured into 200 ml of mixture of dispersant medium containing 100ml of pH 7.4 Phosphate buffer solution (to minimize the solubility of drug) and 100ml of PVA (1%), which was previously heated to a temperature higher than melting point of wax (>+ 5°). Tween 80 (2% w/w) was added to the mixture

containing waxes The whole mixture was mechanically stirred at 900 rpm using a stirrer. Spherical particles are produced due to dispersion of molten wax in the aqueous medium. The mixture was stirred continuously at 900 rpm at a higher temperature (>+ 5°) of the melting point of lipids for 3 min to form an o/w emulsion. The temperature of the mixture in the beakers was cooled rapidly to 4°C by the addition of cold water. The resultant solid spheres collected by filtration were extensively washed with water to remove any drug and surfactant residues. Air drying was carried out at room temperature for 48hr produced discrete, free flowing solid microspheres. Similarly above process was carried out with Bees wax by melted in china dish at a temperature of 75°C. Total 6 formulations were prepared by varying concentration of both lipids as shown in table 1.

Table 1: Formulation of Naproxen Microspheres

Formulation Code	Quantity of Lipids		Drug (mg)
	Ceresin Wax (mg)	Bees Wax (mg)	
F1	750	-	250
F2	1000	-	250
F3	1250	-	250
F4	-	750	250
F5	-	1000	250
F6	-	1250	250

3. Evaluatory parameters

3.1 Particle Size Analysis of microspheres

The size distribution of the Microspheres was determined using the particle size analyzer (Beckman Coulter, Delsanano C, Brea, USA) equipped with a dry accessory system. Sample was diluted with water and temperature maintained at 25°C.

3.2 Determination of bulk density and tapped density

An accurately weighed quantity of the powder (W), was carefully poured into the graduated cylinder and the volume (V_o) was measured, then the graduated cylinder was closed with lid, set into the density determination apparatus. The density apparatus was set for 100 taps and after that, the volume (V_f) was measured and continued operation till the two consecutive readings were equal.

The bulk density, and tapped density were calculated using the following formulas:

Bulk density $= W / V_o$

Tapped density$= W / V_F$

Where, W = weight of the powder,, V_O = initial volume, V_F = final volume

3.3 Angle of Repose

Angle of repose was calculated by fixed funnel standing method. The angle of repose (θ) is calculated by the following formula, $\theta = \tan^{-1} (h/r)$

Where, h = pile height of microspheres, r = radius of the circular are formed by the microspheres on the ground.

3.4 FT-IR spectroscopy

FT-IR spectra were taken from dried samples. FTIR spectra of pure drug (2mg), empty microspheres and drug loaded microspheres were obtained using KBr pellet method (applying 600 kg/cm^2). Spectral measurements were obtained by powder diffuse reflectance on a FTIR spectrophotometer (Shimadzu, Model 8033,USA)in the wave number region 400 -4000CM^{-1} to drug excipient interactions.

3.5 Differential scanning calorimetric analysis

Differential Scanning Calorimetry (DSC) analysis was undertaken to characterize the changes, if any, during thermal exposure of the samples. The test was carried out using thermal analysis system (Shimadzu, DSC-60). Calibration with the standard (aluminium) were undertaken prior to subjecting the samples for study (between 30-300ºC), which were heated at 10ºC/min in an aluminium pan under a nitrogen atmosphere while using an empty pan as the reference in this instrument. The instrument automatically calculated onsets of melting point and enthalpy of fusion.

3.6 In vitro release studies[17]

In-vitro dissolution studies of Naproxen Microspheres were performed using USP type-II (Paddle) Type dissolution test apparatus. 900ml of buffer is used as a dissolution medium. The medium was maintained at 37±0.5ºC at a speed of 100rpm. The *in vitro* dissolution studies were performed at different pH in 0.01N HCL for 2hrs simulated gastric fluid with simulated intestinal fluid and dissolution was continued for 8 hours up to 24hrs. An accurately weighed sample equivalent to 250mg drug was responded in dissolution medium consisting 900ml of buffer and dissolution was done up to 24hrs.At prefixed time intervals 1ml of sample was withdrawn and filtered through 0.4 µm membrane filter. Then the withdrawn is diluted to 10ml. The volume of the dissolution medium was adjusted to 900ml at every sampling time by replace same 1ml of dissolution medium. Then the samples were analyzed Spectrophotometrically at 271nm. The amount of drug released was calculated using the standard calibration curve for naproxen.

3.7 Surface topography by SEM

Scanning electron microscopy (JEOL 5400, Tokyo, Japan) was used to determine the shape, surface topography and texture as well as to examine the morphology of fractured or sectioned surface. The shape and surface morphology of lipid loaded naproxen microspheres were investigated using SEM. SEM is a commonly used method for characterizing drug delivery systems, owing in large part to simplicity of sample preparation and ease of operation. Sample spreads on the small square plate and coated with a gold ion for 5-6 mins. The prepared sample was kept inside the chamber and images captured with different magnifications.

3.8 Entrapment efficiency [16]

Entrapment efficiency in microspheres is very important to study the efficiency of the process. Entrapment efficiency of all the batches prepared to study the effect of various variables was determined spectrophotometrically using UV 2300, Schimadzu. Entrapment efficiency was calculated by using following formula:

$$\text{Entrapment efficiency} = \frac{\text{Drug entrapped}}{\text{Theoretical drug content}} \times 100.$$

3.9 Drug Content

Naproxen drug incorporated wax microspheres of each batch was selected and powdered in a mortar. 100 mg of drug loaded wax microspheres was accurately weighed and added into 100mL volumetric flask. To this, 100mL DCM

was added and stirred for 60min, till the entire drug leached out. The solution was filtered and 1mL was withdrawn from this solution and added in to 10mL volumetric flask and volume was made to 10mL (10µg/mL) with phosphate buffer pH 6.8. Drug content was estimated UV spectrophotometrically at 271 nm using pH 6.8 phosphate buffer as a blank.

3.10 Drug release kinetics

To study the exact mechanism of drug release from the microsphere, drug release data were analyzed according to zero-order, first-order, Higuchi's, and Peppa's equation. The criteria for selecting the most appropriate model were chosen on the basis of goodness of fit test.[11]

4 Results and discussion

For the preparation of lipid microspheres of naproxen bees wax or ceresin wax is used in varying concentration. Drug is insoluble in water. The volume of pH 7.4 phosphate buffer and PVA 1% used is about 200ml if the reduced volume is not sufficient for the formation of microspheres. If the volume is reduced irregular shaped particles are found as well clumps are formed. Tween80 is used as emulsifier in 2% concentration. Bees wax or ceresin wax is used as lipids in varying concentration just to check effect on particle size and drug release[15]. Without emulsifier formulation is not possible. Speed is optimized at 900 rpm below that speed particle size is increased

4.1 Microsphere Size Analysis

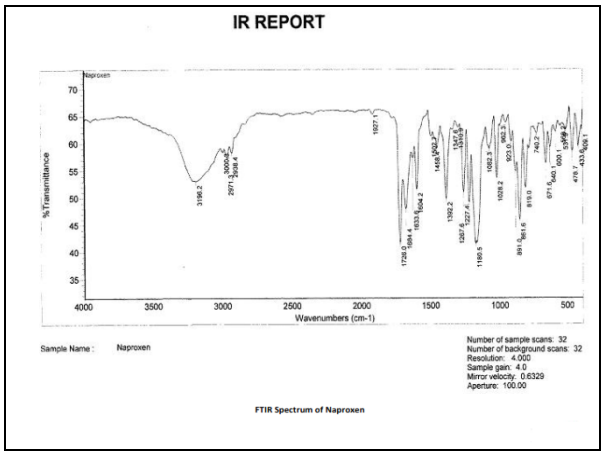
Particle Size distribution plays a very important role in determining the release characteristics of the microspheres The particle size of the prepared Microspheres was determined by particle size analyzer (Beckman Coulter). The Average particle size of the naproxen loaded Microspheres were found to be 1.94±3.8µm. Results are shown in Table 2.

4.2 Angle of Repose

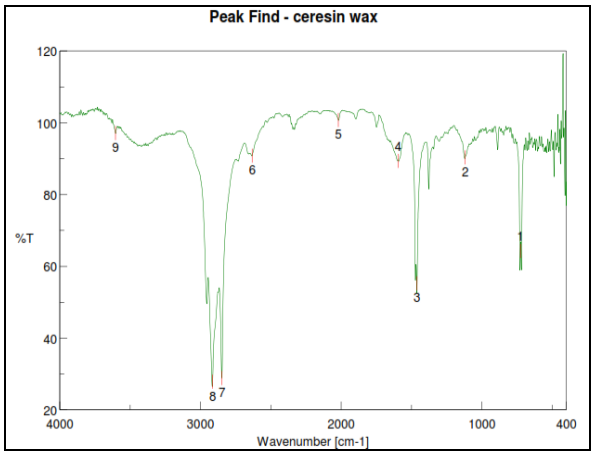
Angle of repose was assessed to know the flow ability of wax microspheres. Tap density of the prepared microspheres was determined using bulk density tester and % Carr's index was calculated and found to be satisfactory. All the formulations show good flow property. Results of all the formulations are shown in Table 2.

4.3 Fourier Transform Infrared Spectroscopy (FTIR)

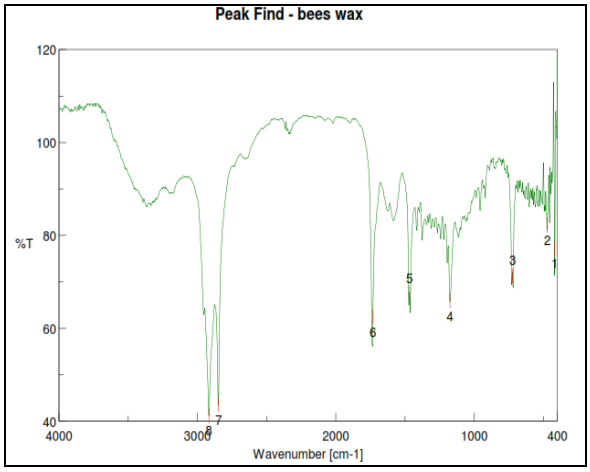
An FTIR spectrum shows that both the drug and polymer are compatible with each other. The physicochemical compatibility of the drugs and the polymer was obtained by FTIR studies Fig. 1 shows FTIR spectra of blank bees wax, ceresin wax microspheres, pure drug, formulation F3 and F6. IR spectra indicates that IR frequency bands of the -OH and C=O and groups having stretched at 3196cm^{-1} and 1227 cm^{-1} respectively are not affected in the presence of Lipids[18].



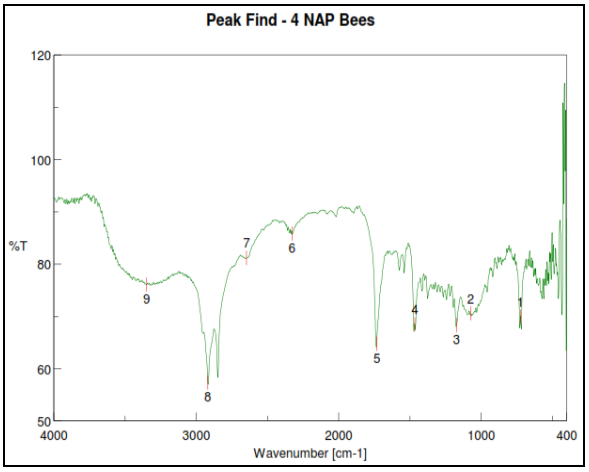
(a) Naproxen



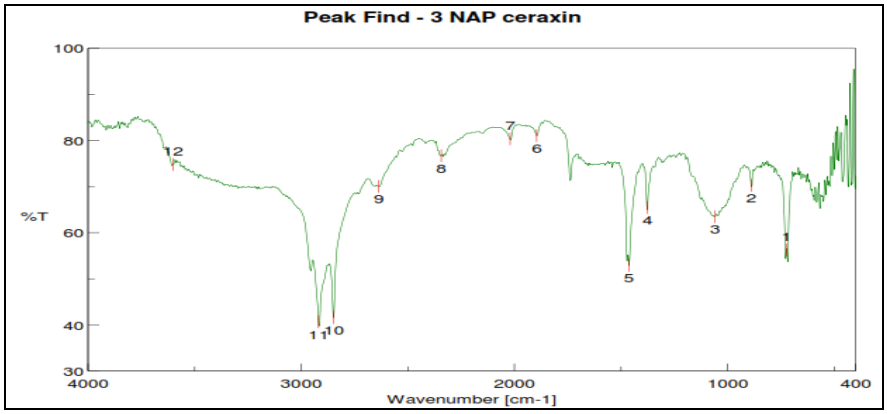
(b) Ceresin wax



(c)Bees Wax



(d) F6 formulation

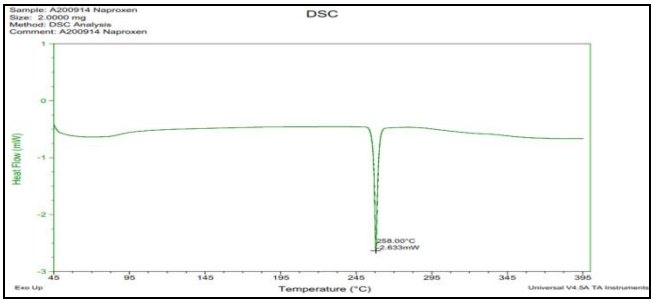


(e) F3 formulation

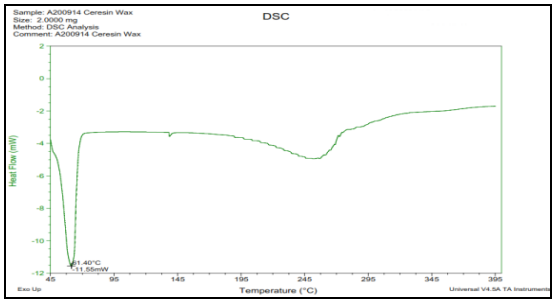
Fig 1: FTIR Spectra of (a) Naproxen, (b) Ceresin wax, (c) Bees wax (d) F6 Formulation prepared Microsphere (e) F3 Formulation prepared Microsphere

4.4 Differential Scanning Calorimetric studies.

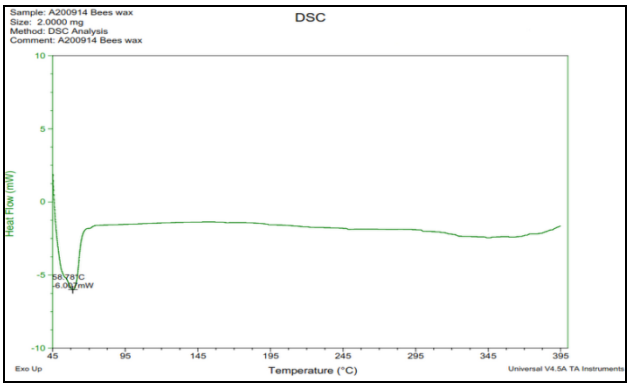
The Naproxen, physical mixture of Naproxen and polymers and the prepared microsphere were subjected to DSC study. The endothermic melting peak was found at 258^0C Fig.2. This although with slight shifting but remaining within a range. The peak was absent in the DSC thermogram of the prepared microsphere. The melting peak was also found in the DSC thermogram of the physical mixture of Naproxen and lipids disappearance of the endothermic peak corresponding to the encapsulated drug melting point, indicates the dispersion of drug in the lipids as solid solution or as a metastable molecular dispersion.[9]



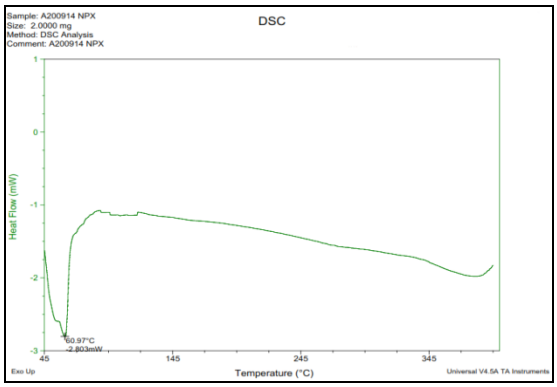
(a) Naproxen



(b) Ceresine Wax



(c) Bees Wax



(d) Physical Mixture

Fig 2: DSC Thermogram of (a)pure Naproxen, (b) Ceresinwax, (c) Bees wax (d) Physical Mixture prepared Microsphere

4.5 Scanning Electron Microscopy

SEM observed the shape and surface characterization of microspheres and only Optimized batch is selected for SEM analysis. SEM photographs were taken using scanning electron microscope JEOL 5400, Tokyo, Japan, at suitable magnification at room temperature. SEM showed that the lipid microspheres were spherical in nature, had a smooth surface. Result is shown in Fig. 3.

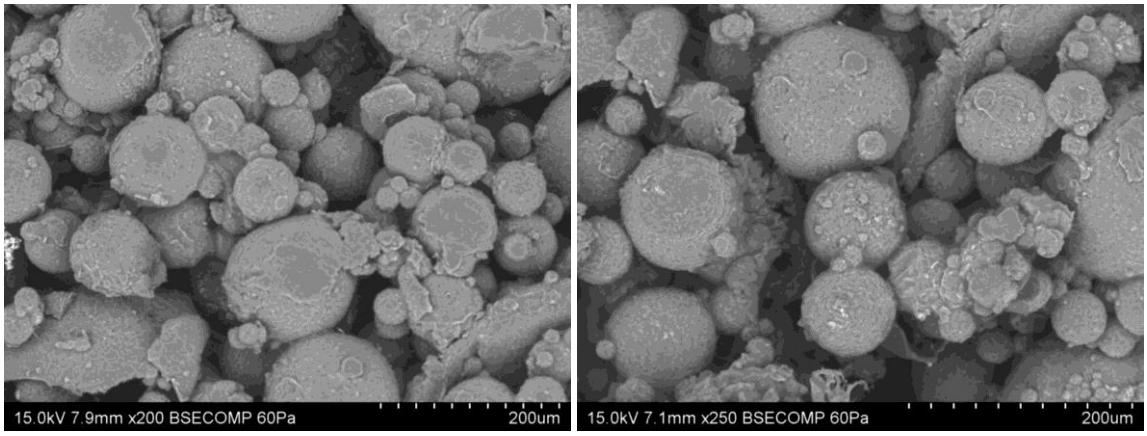


Fig 3: SEM Shows shape and size of microspheres.

4.6 Drug Content and Entrapment Efficiency

Entrapment Efficiency and Drug Content was found in the range of 40-85%. The drug is insoluble in water so the drug release during preparation is avoided. The mixing of drug with wax it shows uniform distribution and entrapment of drug. It was observed that the drug release from the formulations increased with increase in polymer concentration. The formulations F3 and F6 showed the longer duration of drug release for 24hrs in simulated intestinal fluid, in addition to completing retarding the drug release in gastric medium. This is due to the polymer Bees wax. The drug release from waxy microspheres was considerably retarded from the waxes. So that F6 was taken as a best formulation to achieve a prolonged maintenance of effective concentrations of drug. It was observed that the encapsulation efficiency increases with increase in polymer concentration; Formulation F6 shows maximum entrapment efficiency. Results of all the formulations are shown in Table 2.

Table 2: Micromeritic properties of the drug loaded lipid microsphere

Formulation	% Yield (%w/w)	Mean particle size (microns)	Angle of repose	Tap Density	Carr's Index	Drug entrapment (%)	Drug Content (mg)
F1	98.69	6.6 ±1.4	27.31	2.01±0.01	12.27±0.7	45±0.32	11.48
F2	95.47	5.13 ± 1.9	27.75	2.743±0.007	13.85±0.34	62±0.694	12.50
F3	91.44	3.22 ± 2.1	25.95	4.457±0.011	15.10±0.306	73±0.481	12.17
F4	97.12	8.22±1.3	28.06	2.11±0.01	11.53±0.67	47±0.32	11.99
F5	93.10	4.50±1.25	27.63	2.747±0.006	13.47±0.34	57±0.401	11.57
F6	89.86	11.94±3.8	21.82	4.557±0.005	14.85±0.244	78±0.481	13.00

4.7 In-Vitro Dissolution Studies

It was observed that the drug release from the formulations increased with increase in polymer concentration this is because more will be the wax concentration more time is taken to diffuse the drug molecule. From the release studies it was observed that, formulation F6 shows extended release up to 12 hrs. There is initial burst release followed by constant release. Fig 4.

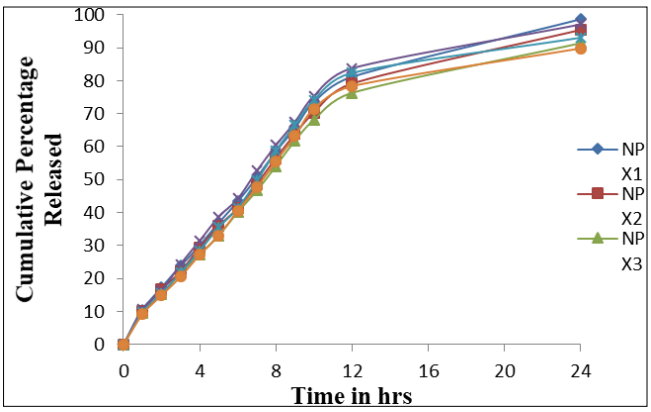


Fig 4: *In Vitro* Release data of Naproxen Microsphere

4.8 Drug release kinetics

The linear regression analysis of Naproxen Microspheres shown as R^2 values in (Table 3). When the data were plotted according to the zero-order equation, for all formulations (F1 to F6) showed a fairly linear, with regression (R^2) values between (0.806 to 0.855) clearly indicate that the drug wasn't released as per zero order mechanism. All the formulation expressed by Higuchis plots shows linearity with regression coefficient (R^2) value as (0.923 to 0.954) also not close to infinity indicate the drug release process is not as per Higuchis plot. The first-order plots of all formulations were found to be highly linear, and close to infinity as indicated by their high regression (R^2) values as (0.958 to 0.989). Therefore, it was ascertained that the drug permeation from these formulations could follow either near First-order or First order kinetics. Hence the release mechanism was shifted from the zero order to Higuchis, followed by first order release kinetics. The drug release mechanism, the data were fitted to Peppas equation. In the present study also it was observed (Table 3) that no value was obtained between (0.782 to 0.816) for all formulations. These values, suggesting that more than one mechanism may be involved in release kinetics. In the case of formulation F6 with Bees wax shows non Fickian Diffusion with n value as (0.816).

Table 3: In-Vitro Release Kinetics of Naproxen Prepared Microspheres

Formulation code	Mathematical models (release kinetics)				
	Zero order kinetics	First order kinetics	Higuchi's	Peppa's	
	r^2	r^2	r^2	r^2	n
F1	0.854715	0.963628	0.954373	0.976251	0.782229
F2	0.853652	0.983606	0.953792	0.975839	0.78593
F3	0.847283	0.98961	0.949033	0.973677	0.799856
F4	0.823566	0.985245	0.939341	0.968373	0.78314
F5	0.806285	0.964546	0.92324	0.963712	0.810971
F6	0.813129	0.958002	0.927569	0.965244	0.816725

5 Conclusions

Naproxen may be an excellent candidate for consideration in drug delivery system as sustained
release dosage form. Microsphere of the mentioned polymers can be used as a vehicle to modulate the drug release for a sustained activity for 24 hours. Naproxen loaded microspheres were formed by melt dispersion technique which was found to be reproducible and also may be ideal method to prepare the microsphere in large scale. In this research, other factors such as types of solvent, rpm, stirring time and temperature were kept constant. The Naproxen lipid microspheres were spherical with smooth surface and good micromeritics properties. It can be concluded that there is no vigorous treatment to the formulation so the yield of the product is best as well as particle size can also be optimized. The formulations F3 and F6 showed the longer duration of drug release for 24hrs in simulated intestinal fluid, in addition to completing retarding the drug release in gastric medium. This is due to the polymer Bees wax. The drug release from waxy microspheres was considerably retarded from the waxes. So that F6 was taken as a best formulation to achieve a prolonged maintenance of effective concentrations of drug. The drug release from the formulations increased with increase in polymer concentration. All the particles are having spherical shape. It releases the drug 90% upto 24hrs.so it can be assumed that it can be controlled release form. The release kinetics for all the batches were best fitted to Korsmeyer-Peppas model, and Higuchi's diffusion was prominent. The SEM study was performed to characterize the surface morphology of the prepared microsphere. The SEM reports depicted that the particles' surface morphology was drastically changes as the ratio between the polymer changes. FTIR showed a successful formulation technique of preparing microsphere as showing the presence of the drug within the microsphere.

Thermal analysis by DSC was also performed for pure drug (Naproxen), lipids, and the prepared microspheres. In the prepared microspheres the endothermic melting peak of Naproxen was found with slight shifting but remained within an acceptable range but the endothermic melting peak of Naproxen disappeared in the thermogram for all the prepared batches. Particle size analysis was done by particle size analyzer clearly showed that the size of the particles was within in the range (1-1000µm). The mean particle size increased with the increase of lipid concentration. If the formulation and process variables are optimized successfully, the method of microsphere formation used in this study may be an ideal means of drug delivery device.

6. Acknowledgement

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