

Evaluation of Gum Katira as a Model Sustained Release Adjuvant in the Preparation of Etodolac Loaded Microsphere

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ABSTRACT

Aims: The aim of present study was to evaluate Gum Katira obtained from the plant *Cochlospermum religiosum* (Family Cochlospermaceae) as a matrix forming pharmaceutical excipient for a novel drug delivery system. **Materials and Methods:** Gum Katira was used for the drug release retarding material in microsphere formulation using Etodolac as a model drug. Etodolac was found to be compatible with the matrix material Gum Katira by conducting the various physicochemical and instrumental analysis. **Result:** Etodolac loaded Gum Katira microsphere (ELGKM) was compared with Etodolac loaded sodium alginate microsphere (ELSAM) and blank microsphere (without gum matrix), which subsequently revealed that the drug release rate of ELGKM was better for sustained and controlled release. **Conclusion:** In our experiment, it was found that the drug release mechanism best fitted in Korsmeyers Peppas model on comparing the correlation coefficient values of different mathematical models. The result of this study indicates that ELGKM (1% w/v Gum Katira) would be desired formulations in delivering the drug with controlled and sustained release pattern.

Key words: Etodolac, Gum Katira, Gum Matrix, Rheogram, Sustained release.

INTRODUCTION

Novel drug delivery system has reached its pinnacle in last few years. Sustained drug delivery system is advantageous with its prolonged drug release mechanism which is desirable from the therapeutic point of view, for the treatment of chronic pain syndromes like nocturnal asthma, angina pectoris and rheumatoid arthritis.¹ Sustained drug delivery systems maintain the drug level at the therapeutic optimum which is needed in the blood in a numbers of pathological conditions. Therefore, the preparation of controlled and sustained drug delivery system is one of the most important tasks of pharmaceutical technologist.² Research has been performed on prodrug, pH, time dependent and polysaccharide based drug delivery system leading to unsatisfactory results, so further investigation on natural gum based microsphere was initiated.³⁻⁵ Multiunit sustained release formulation like microspheres

pass through the gut as if a solution avoiding the vagaries of gastric emptying and different transient rates and thereby, release drugs more uniformly, and spread over a large area of absorbing mucosa decreasing dose dumping and preventing exposure to high drug concentration.^{6,7}

Drug release retarding polymers are the key performers in designing a dosage form for oral sustained release formulation.⁸ The natural polysaccharides (excipients of natural origin) are the attractive alternative product because of their reliability, biocompatibility, biodegradability, sustainability, low toxicity and low cost compared to synthetic product.⁹ Natural Polysaccharide such as guar gum, xanthan gum, and locast bean gum has been investigated for their use in Novel drug delivery system.¹⁰ They remain undigested in stomach but get degraded by different anaerobic microflora in the intes-

Submission Date :02-06-2015
Revision Date :24-07-2015
Accepted Date :19-07-2015

DOI: 10.5530/ijper.50.1.19
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tine.¹¹ Gum Katira was selected as the matrix forming material because of its well-established biocompatibility, non toxic and safe material for the use in food and pharmaceutical industries.¹² Gum Katira is a novel heteropolysaccharide isolated from *Cochlospermum religiosum* and consists of D-galactose, D-galactouronic acid and L-rhamnose in a molecular ratio 2:1:3 respectively together with traces of a ketohexose.¹³ This gum is pale, semi transparent and swells to form a pasty transparent mass with water.¹⁴ Different physiochemical properties of Gum Katira such as solubility, percentage of swelling, and rheogram etc have been studied. Increment in shear stress on increasing shear rate confirmed the pseudoplastic nature that may be a suitable characteristic for the controlled and sustained release drug delivery formulations.¹⁵ The instrumental techniques like SEM, DSC, X ray diffraction, FT-IR, etc were performed to establish that Gum Katira has no interactions with the ingredients used in the microsphere formulation containing a polymeric substance as a carrier and a coating material.

Etodolac, a non steroidal anti-inflammatory drug, used in the treatment of various acute and chronic pain syndromes like osteoarthritis, rheumatoid arthritis and other types of joint pain.¹⁶ Etodolac is taken as a model drug because of its short half life, low water solubility and ready absorbability throughout the gastrointestinal tract.^{17,18} However, its immediate release formulations shows severe side effects like gastric irritation, bleeding, ulceration and eventually wall perforation especially in chronic dosing led to the formulation of microsphere, a sustained release dosage form.¹⁹

Eudragit which is one of the most thoroughly investigated synthetic polymers for sustained drug delivery system is being used as a coating material in this formulation.^{20,21} Eudragit RS 100 and RL 100 are taken due to their biocompatibility, good stability, easy fabrication and insolubility in gastric juice.²²⁻²⁵

In contrary to conventional dosage forms like tablets and capsules, formulations which are present in the market with better effectiveness also have higher side effects on gastrointestinal system²⁶ and cardiovascular system²⁷ on administration of more than 400mg dose. The use of microsphere for drug delivery is not limited to any specific illness, rather they can be widely applied in many situations where continuous or controlled drug administration is essential.

MATERIALS AND METHODS

Materials

Crude Gum Katira was collected from the Seoni district of Madhya Pradesh. Etodolac was obtained as a gift sam-

ple from Fleming Laboratories Limited Medak district, Andhra Pradesh, India. Eudragit RS100 and Eudragit RL100 were purchased from the Yarro Chem Products Mumbai (India), Dichloromethane, Tween 80 and Span 20 (Merck, India), and all other reagents were of analytical grade obtained commercially and used as received.

Methods

Collection and Purification of Gum Katira

Crude gum was collected as dried exudates from the branches of the fibrous exudates of plant *Cochlospermum religiosum* (Hindi: Galgal) in the month of December from Seoni district of Madhya Pradesh, India. For purification larger dust particles were removed manually and air dried well. Dried gum exudates was then kept overnight in water. Gum was allowed to swell fully to form ribbon shaped transparent mass. Again larger visible foreign particles were removed carefully. The transparent mass was stirred vigorously using mechanical stirrer for 3 hrs. The remaining minute dust particles were then removed manually. The finally obtained white material was then oven dried at 70°C for 2 days. Particle size of oven dried material was reduced as much as possible by using mortar and pestle and finally kept in air dried container for optimum storage.

Solubility

The solubility of Gum Katira was evaluated randomly in water, acetone, chloroform, ethanol and most of the other organic solvents. As a result, the gummy exudates were found to be slightly soluble in water and insoluble in most of organic solvents used like Ethanol, chloroform and acetone.

Particle size and Melting Point determination

The particle size of the Gum Katira was determined by the help of Zetasizer (ASU), Model No. Nano ZS 90, Malvern, UK. The melting point of the Gum Katira was determined by digital melting point apparatus (model VMP-D, Veego, Mumbai). For this the gum powder was taken in the capillary tube of the apparatus. As the temperature of the apparatus rises, solid starts transforming into liquid but in case of natural polysaccharides the transition from solid to liquid is followed by charring during the melting process. Gum Katira powder starts charring at 80-90°C.

Determination of Moisture content

Moisture content of Gum Katira was determined by Carl Fischer titration method. The gum powder was dispersed in methanol, stirred well for 5 minutes to remove the water and titrated with standardized Karl Fischer

reagent till the end point. Moisture content was calculated using the following formula.

$$\text{Moisture content (\%)} = V_1 \times W_c / S_w \times 100$$

Where V_1 is the volume of Karl Fischer reagent used, W_c is the water equivalent and S_w is the weight of sample. Water equivalent is 5.2; 15 ml of reagent is equal to 75 mg of water.

Microbial Load Study

Microbial load of the Gum Katira was observed on fresh sample and 10 months older sample. The sample was analyzed for total aerobic microbial count and fungal count by pour plate method. 1 $\mu\text{m}/\text{ml}$ of gum dispersion was incubated in soybean casein digest sugar medium, poured in petridish and allowed to solidify. The plate was left for 1 hr at 10°C and incubated at 37°C for a day. The total no of colonies formed were counted. Total fungal count was done using potato dextrose agar medium. The plates are incubated at room temperature for 72 hrs.

Swelling characteristic of Gum Katira

Rate of swelling/ hydration of ELGKM and ELSAM in same concentration were evaluated to study the mechanism of drug release from the hydrated gum and extent of water uptake by the gum. Swelling capacity was evaluated in terms of weight gain by the microsphere. Accurately weighed microspheres were placed on petridishes having the dissolution media (pH 1.2 HCl media for 2 hrs and pH 6.8 phosphate buffers for 10 hrs). Swollen Microspheres were withdrawn periodically from the medium and weighed in an electronic balance (Model No. D455001482, Shimadzu corp, Japan) after removing the excess surface water using tissue paper. The same process was repeated for 12 hrs and gain in weighed was recorded. Percentage swelling was calculated according to the below mentioned formula:

$$\% \text{ swelling of Gum} = [(S_t - S_0) / S_0] \times 100$$

Where S_0 is the initial weight of microspheres and S_t is the weight of hydrated Microspheres.

The rate of swelling of ELGKM and ELSAM in pH-1.2 HCl media and pH-6.8 phosphate media were determined using the following relationship.

$$\text{Rate of Swelling} = \text{Slope of } [\% \text{ Swelling of Gum vs. } \sqrt{\text{Time}}] \text{ curve.}$$

Rheological Study

The Rheological properties were determined by using Rheometer (Anton Paar, and Model No. MRC 102, Austria). For this Gum Katira (1% ω/ν , 2% ω/ν) dispersion and sodium alginate (1% ω/ν) solution were prepared with distilled water and allowed to stand for 24 hrs. Then the gum solution was analyzed at different shear stress vs shear rate and shear rate vs viscosity at different time intervals at 12th hr and 24th hr respectively.

Toxicity Study

The acute toxicity study was carried out in adult Swiss albino mice (20-25 g) by “fixed dose” method of OECD (Organization for Economic Cooperation and development) guideline no.423 and 407 respectively. The subacute toxicity study was conducted on male wistar rat (150-200 g). The solution of gum katira at dose of 100, 200 and 400 mg/kg body weight was administered orally to three groups of six rats respectively in every 24 hrs for 30 days and a control received vehicle of same volume. The toxic manifestation i.e. body weight, mortality and behavioral changes were regularly monitored. After thirty days all the surviving animals were fastened overnight and anaesthetized with ether and the heparinized blood samples were collected for determination of haematological parameters followed by sacrificing the animals for the collection of internal organs kidney and liver. The collected organs were then preserved in 10% formaldehyde solution for histopathological examination. The research was conducted in accordance with the ethical rules on animal experimentation approved by Ethical committee, Department of Pharmaceutical Technology, Jadavpur University (Approval No: 147/1999/CPCSEA).

Determination of λ_{max} of Drug

A solution of Etodolac having a conc. 1 $\mu\text{g}/\text{ml}$ was prepared in acid (pH-1.2) and phosphate buffer (pH-6.8) separately. UV spectrum was taken using double beam spectrophotometer (MULTISKAN GO, Thermo Scientific). The solution was scanned in the range of 200-400 nm. The wavelength of maximum absorption of Etodolac was found to be 224 nm in both the cases.

Determination of Melting Point of Drug

The melting point of Etodolac was determined by open capillary method using melting point apparatus VEEGO, (Model No.VMP-DS). The melting point of Etodolac was found to be 150.5-151.5°C.

Preparation of Microsphere

Etodolac microspheres were prepared by double-emulsion solvent evaporation technique. Matrix material Gum Katira (50 mg) was mixed with distilled water to form a homogeneous mixture in magnetic stirrer for 30 minutes with a constant temperature of 40-45°C. Etodolac (50 mg) was added to the homogeneous mixture and stirring was continued for another 30 minutes. This mixture was then dispersed in a solution of Eudragit RS and RL 100, Dichloromethane, Acryflow and span 20 through a 20 gauge syringe. The above mixture was homogenized well for 5 minutes using homogenizer to form W1/O emulsion. Another solution

of distilled (100 ml) water containing Tween 80 was subjected to mechanical stirring (700-800 rpm) and to it the previously prepared W₁/O emulsion was added drop wise using a 16 guage syringe to form W₁/O/W₂ emulsion with a continues stirring for 1-1.5 hrs. The resultant microspheres formed were washed with distilled water followed by air drying for 24 hrs and final storage in desiccators for further use.

Percentage of Yield and Encapsulation efficiency of the Microspheres

The percentage of yield is calculated to know the maximum practical yield of the products. Etodolac loaded microspheres were prepared having production yield near about 80-95%. The percentage yield of prepared formulation was determined by using the formula:

$$\text{Percentage Yield} = \left[\frac{\text{Practical Yield}}{\text{Theoretical Yield}} \right] \times 100$$

Crushed and Powder 30 mg of Etodolac loaded Microspheres were dissolved in 5 ml of Dichloromethane (DCM). This solution was stirred for 1hr using magnetic stirrer for the complete dissolution of Polymer in DCM. 45 ml of phosphate buffer pH-6.8 was added to this solution. This solution was magnetically stirred for 1 hr at 40-45°C and then filtered. About 4 ml of filtered solution was taken and added to 21 ml of fresh phosphate buffer to make 25 ml of aliquot. The absorbance of the final solution was measured at 224 nm using double beam UV-Visible spectrophotometer against phosphate buffer pH-6.8 as blank and calculation was done for the percentage of drug loading in sample. Drug loading efficiency was found to range between 45-60%. Efficiency of drug loading for each batch was calculated in terms of percentage of drug loading (PDL) as per the following formula:

$$\text{PDL} = \left[\frac{\text{Practical drug content}}{\text{Theoretical drug content}} \right] \times 100$$

Scanning Electron Microscope Analysis

Scanning Electron Microscope (SEM) studies were carried out by using JEOL MAKE (UK), (MODEL-JSM6360). Microspheres were mounted on conducting stubs using double sided adhesive tape and vacuum coated with gold palladium film using a sputter coater (Edward S-150, UK). Images were taken using 17 kV electron beam intensity in a scanning electron microscope to examine the surface morphology of the samples.

FTIR Spectroscopic Study

Fourier Transform Infra Red (FTIR) spectroscopy was performed on IR- Prestige-21, Shimadzu make, Japan. FTIR study was carried on Etodolac, Gum Katira and Microsphere shows their characteristic peaks in the region from 400-4000 cm⁻¹.

Differential Scanning Calorimetric Analysis

Differential Scanning Calorimetric (DSC) was performed to study the changing thermal behavior of the drug, polymer and gum during formulation. Thermograms are evaluated to detect the melting point and change in enthalpy of melting of the samples. Thermograms of Etodolac, Gum Katira and formulation were prepared by Model No. Pyris Diamond TG/DTA, Perkin Elmer (SINGAPORE), Nitrogen Atmosphere (150 ml/min). Platinum crucible was used with alpha alumina powder as reference to study the changing thermal behavior of the drug, polymer and the gum.

XRD Study

X-ray diffractometry of Gum Katira, Etodolac and Etodolac loaded Microsphere were performed by a X-Ray Diffractometer using Model no. Ultima-III, Regaku make (Japan), Cu target slide 10 mm. to observe the physical state of Etodolac in the Microsphere.

In vitro Drug Release Study

In vitro drug release studies were performed by using USP II dissolution test apparatus (Model TDP-06P, Electro Lab, Mumbai, India). Dissolution studies of all microspheres were carried out in 0.1 N HCl, pH-1.2 acidic media for an initial 2 hrs followed by USP phosphate buffer, pH 6.8 for the rest of the period at 75 rpm maintained at a temperature of 37°C ± 0.5°C. Aliquots were withdrawn at specific time interval and replenished immediately with the same volume of fresh solution. The drug release was analyzed by UV-Visible Spectrophotometer (MULTISKAN GO, Thermo Scientific) at 224 nm. All release studies were triplicated. The drug release behavior of ELGKM was compared with ELSAM and blank Microsphere.

Statistical analysis

The data obtained were analyzed statistically using one-way ANOVA and t-test by using Graph Pad prism 5.

RESULTS AND DISCUSSIONS

Physiochemical Evaluation of Gum Katira

Solubility

Gum Katira is slightly soluble in water and form reddish brown color dispersion. It is practically insoluble in ethanol, acetone, chloroform and most of the organic solvent. The gum forms a viscous colloidal dispersion with warm water. This type of matrix forming solu-

Table 1: Haematological Parameters of rat after 30 days oral Administration of Gum Katira

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
Haemoglobin (gm%)	14.41 ± 0.043	14.40 ± 0.034	14.45 ± 0.036	14.38 ± 0.021
RBC (10 ⁶ /μl)	8.83 ± 0.067	8.92 ± 0.084	9.01 ± 0.101	8.95 ± 0.076
WBC (10 ³ /μl)	13.97 ± 0.068	14.05 ± 0.065	13.93 ± 0.093	14.16 ± 0.073
Neutrophil (10 ³ /μl)	21.83 ± 0.792	22.83 ± 0.703	21.83 ± 0.703	22.50 ± 0.428
Monocyte (10 ³ /μl)	2.26 ± 0.071	2.21 ± 0.060	2.30 ± 0.044	2.35 ± 0.042
Lymphocyte (10 ³ /μl)	73.67 ± 0.494	73.50 ± 0.428	73.83 ± 0.477	72.50 ± 0.619
Eosinophil (10 ³ /μl)	2.20 ± 0.077	2.11 ± 0.079	2.35 ± 0.076	2.41 ± 0.047
Platelets (10 ³ /μl)	1232.00 ± 1.065	1241.00 ± 1.801	1243.00 ± 0.763	1239.00 ± 1.054
SGOT (U/L)	88.96 ± 0.424	89.10 ± 0.427	89.40 ± 0.503	89.65 ± 0.356
SGPT (U/L)	32.53 ± 0.630	32.24 ± 0.660	31.32 ± 0.550	33.13 ± 0.294

Values are expressed as mean ± SEM of 6 rats in each group. P<0.05 was considered significant. No significant difference was observed in any parameter.

tion is suitable for the development of prolonged drug release formulation.²⁸

Particle size and Melting point determination

The particle size of gum was in the range of 100-200 μm. The calculated average diameter of the Gum Katira powder is found to be about 123 μm, which is within the desired range of 75-125 μm for most of the gum powder particles used in various sustained release formulation. The natural products have a range of melting point rather than a definite melting point of pure products. During the melting process the transition of solid phase to liquid phase of gums is always accompanied by charring. Similar, charring characteristic of Gum Katira at 80-90°C confirms it as a novel excipient for sustained drug delivery system.²⁹

Moisture content determination

Moisture content of gummy excipients used in formulations can influence the chemical and physical stability of active pharmaceutical ingredients and also the formulation. The moisture content of natural polysaccharide used as drug release excipients should be ≤ 15% in according to the pharmacopoeia and WHO guidelines.³⁰ Moisture content of Gum Katira powder was found to be 13.21 ± 0.0% which is within the mentioned limits. So, in terms of moisture content Gum Katira can be successfully used in a sustained release formulation.

Microbial Load Study

The sample was analyzed for total aerobic microbial count and fungal count by pour plate method. Total aerobic count should be less than 1000 CFU/g and total fungal count should be less than 100 CFU/g to pass the microbial limit test.³¹ Microbial count study shows that total number of colonies for aerobic count is 210 CFU/g and total fungal count 93 CFU/g which is

within the mentioned limits. Therefore the Gum Katira can be used for formulation development.

Toxicity Study of Gum Katira

Acute toxicity study was conducted on mice with the gum katira which revealed no behavioral changes and also no mortality was observed even at a dose level of 2.0 g/kg body weight, after 24 hours of per-oral administration. Subacute toxicity study was conducted on rat whose haematological analysis (Table 1) after thirty days of oral administration showed no significant variance in the level of hemoglobin, RBC, WBC, neutrophil, eosinophil, monocyte, lymphocyte, SGOT, SGPT and platelets in treated animals as compared to that of the control group animals. Also, the histopathological studies (Figure 1) of treated animals revealed no significant tissue damage of the kidney and liver and were comparable to that of the control group. So, no abnormalities in histopathological studies were found. On the basis of the toxicity study conducted it can be concluded that the gum katira is safe to be used in the formulation.

Percentage swelling of Gum

Hydrophilic polymers undergo swelling at a rate proportional to the amount of polymer irrespective of the nature of the drug. Due to stress induced by ingresses water the polysaccharide matrix initially shows rapid swelling, with progressive increment in size along with entanglement of polymeric chain which forms a periphery gelatinous layer around the core through which diffusion of drug molecules takes place.³² Thickness of gelatinous layer determines the drug release from matrix. Drug diffusion also depends on the viscosity of the swollen region which in turn depends on the amount of polymer in the matrix. During the swelling characteristic study it was observed that initially both the formulations showed rapid swelling and at around 8-9th

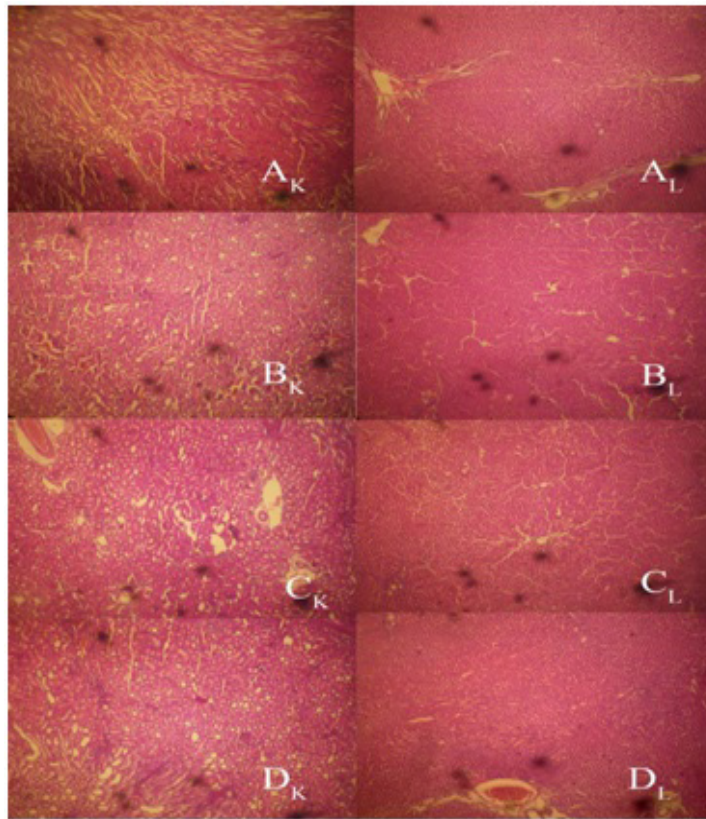


Figure:1 Histopathology of Kidney and Liver of rat after 30 days oral administration of Gum Katira

(AK-Control Kidney, AL-Control Liver; BK-Treated 100 mg/kg Kidney, BL-Treated 100 mg/kg Liver; CK-treated 200 mg/kg Kidney, CL-Treated 200 mg/kg Liver and DK-treated 400 mg/kg Kidney, DL-treated 400 mg/kg Liver)

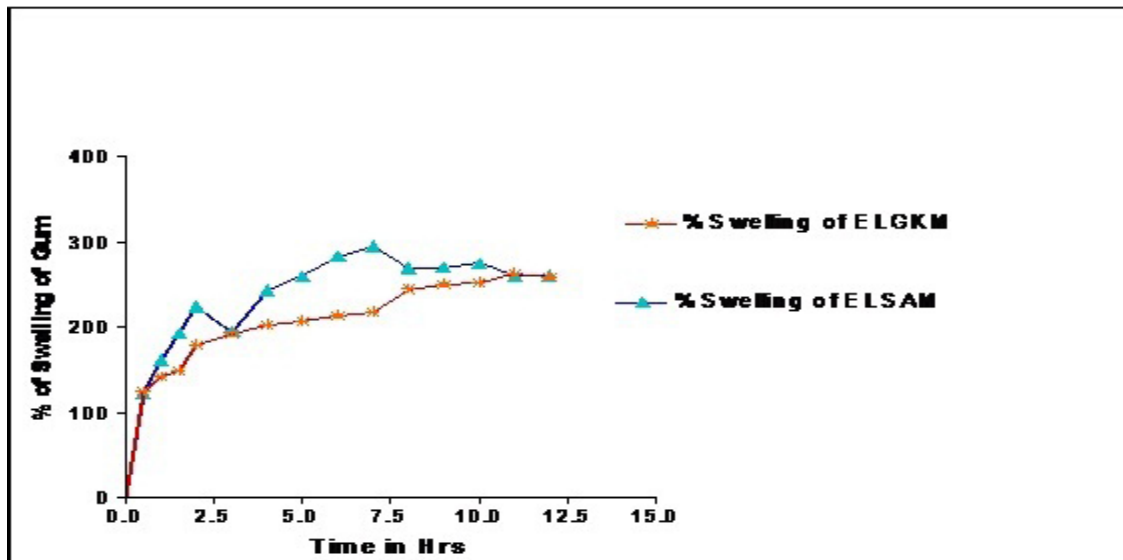


Figure 2: Comparison of % of Swelling between ELGKM and ELSAM in SGF (pH-1.2) and SIF (pH-6.8)

hrs they attained maximum swelling after which there was a percentage decrease in swelling due to erosion of the axial layer, which promotes water penetration in the preceding layers.³³ The swelling performance of 1% w/v (gummy) of ELGKM and ELSAM are shown in the (Figure 2). The maximum percentage of swelling of

Gum Katira was achieved at 11th hrs but Sodium alginate at 7th hr after which they start losing their integrity. ELGKM showed slow and continuous swelling up to 11 hrs followed by decrease in swelling percentage which may be due to polymeric relaxation, whereas ELSAM showed faster swelling followed by immedi-

Table 2: Swelling rate of ELGKM and ELSAM in acidic (pH-1.2) and buffer (pH-6.8) solution		
Formulation	Rate of swelling	Correlation Coefficient(R ²)
ELGKM (1% Gum Katira)	49.874	0.9728
ELSAM (1% Sodium alginate)	48.246	0.7379

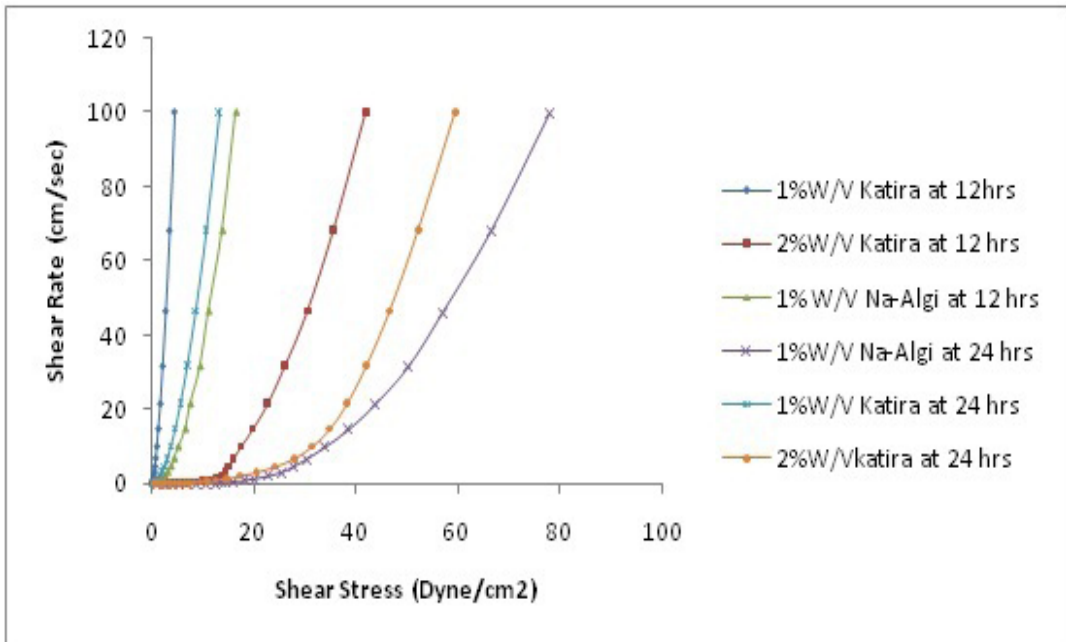


Figure 3: Rheogram of Gum Katira and Sodium Alginate solution of different concentration on different times

Table 3: Composition of Drug loaded Microsphere							
Formulation Code	Drug (mg)	Gum (% w/v)	Eudragit RS 100 (% w/v)	Eudragit RL 100 (% w/v)	Acryflow (% w/v)	Span 20 (µl)	Tween 80 (µl)
F _A (Katira)	100	0.50	3.75	1.25	0.30	100	20
F _B (Katira)	100	1.00	3.75	1.25	0.30	100	20
F _C (Katira)	100	2.00	3.75	1.25	0.30	100	20
F _D (Katira)	100	3.00	3.75	1.25	0.30	100	20
F _E (Alginate)	100	1.00	3.75	1.25	0.30	100	20
F _F (Blank)	100	0.00	3.75	1.25	0.30	100	20

% w/v = Amt. of ingredients (mg) present in 100 ml solvent

ately decrease in swelling as compared to ELGKM. It is also observed that percentage of swelling increases with increase in gum concentration up to a certain limit after which further increase in gum concentration decreases swelling due to inhibition of water penetration into the matrix. In the (Table 2), the slope of percentage swelling vs. time indicates that rate of swelling of ELGKM in the dissolution media (pH-1.2 acidic media and pH-6.8 phosphate buffer) is greater than ELSAM. So, it is expected that the drug release from ELGKM should be more prolonged than that of ELSAM.

Rheological Study

In pseudoplastic flow the consistency curve begins at the origin. As the shear stress increases progressively, shear rate also increases, but the curve is not linear. These types of curves are generally exhibited by Non-Newtonian system such as polymeric solution of sodium alginate.³⁴ The rheogram of Gum Katira(1% w/v, 2% w/v) and sodium alginate (1%w/v) solution at different time interval (12th and 24th hrs) are graphically presented in (Figure 3). The rheograms of aqueous dispersion of both gums indicate pseudoplastic flow. But it

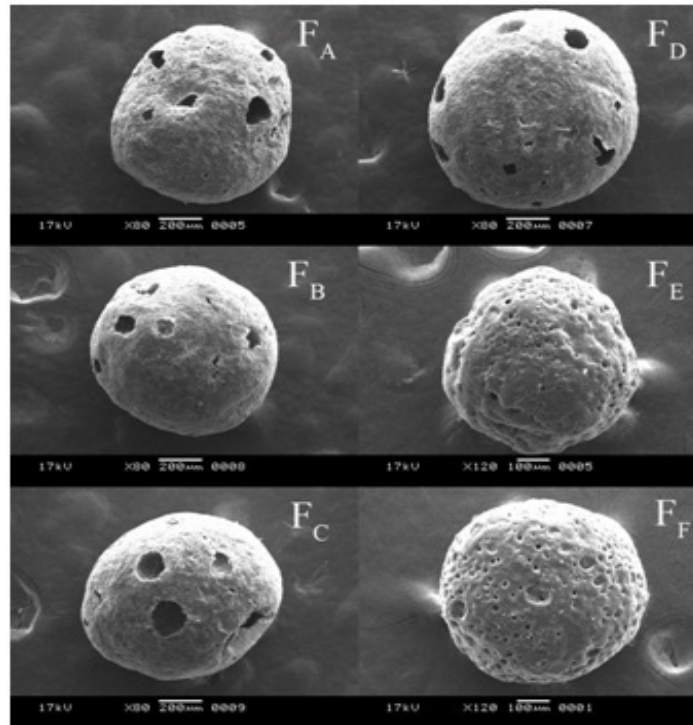


Figure 4: SEM of Etodolac loaded gum katira and sodium alginate Microsphere and Blank Microspheres.

F_A = [0.5 %w/v of Gum Katira], F_B = [1.0 % w/v of Gum Katira], F_C = [2.0 % w/v of Gum Katira], F_D = [3.0 % w/v of Gum Katira], F_E = [1.0 % w/v of sodium alginate], F_F = [without gum, Blank]

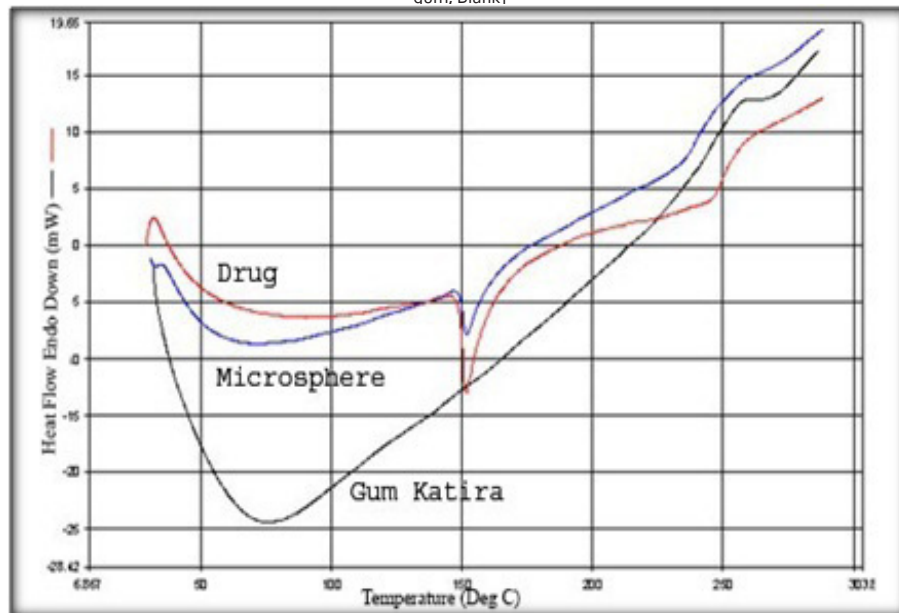


Figure 5: DSC of Gum Katira, Etodolac and Microsphere

was also seen that at lower concentration of Gum Katira the curve does not follow proper pseudoplastic flow which is due to very weak molecular interaction among the polymer molecules. In the study, the viscosity of gum solution decreases with increase in rate of shear and so no single value can be used to express its viscosity. The entire system is most satisfactory representation of Gum Katira as a pseudoplastic material.

Characterization/ Evaluation of Microsphere Percentage of Yield and Encapsulation Efficiency

The percentage yield and encapsulation efficiency of Etodolac were optimum in all formulations. Yet, due to variation of polymer type, stirring speed and polymer drug ratio some formulation were affected. Like with the increase in rate and duration of stirring the drug loading was seen decreasing. Also, with increase in amount

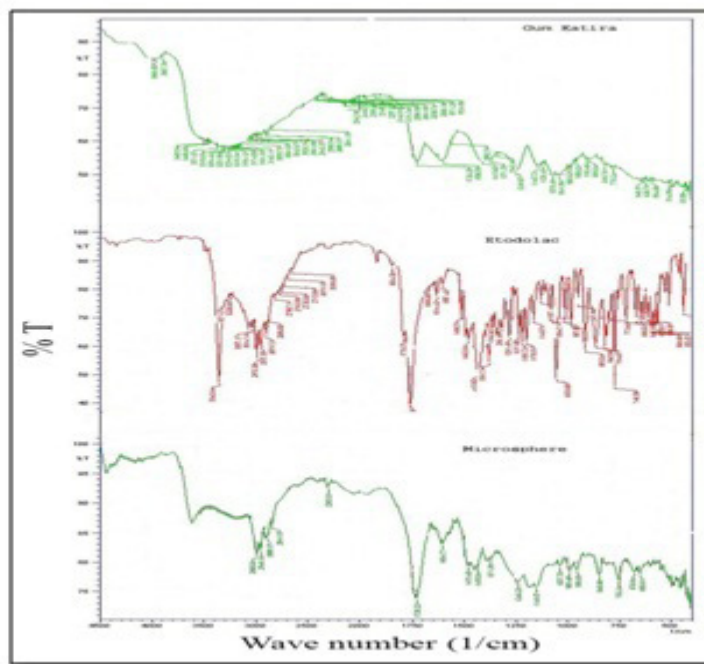


Figure 6: FTIR spectrum of Gum Katira, Drug Etodolac and Microsphere

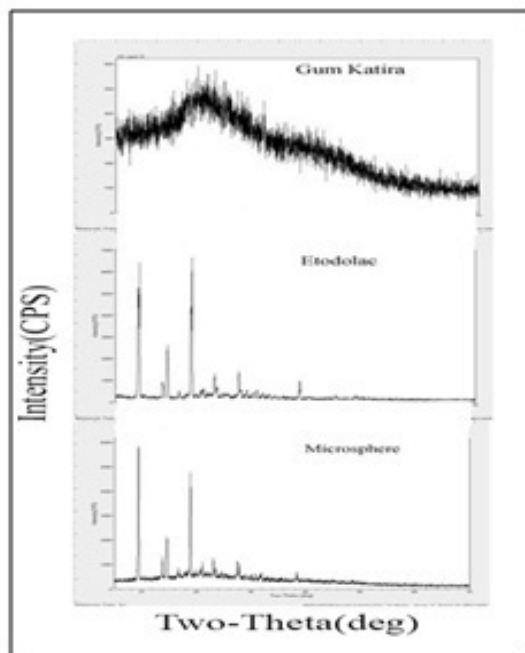


Figure 7: XRD study of Gum Katira, Drug Etodolac and Microsphere

of coating polymer, Eudragit the drug loading increases to some extent. But, the drug loading increases upto an optimum ratio (gum katira: Etodolac 1:2) of Gum Katira and Etodolac after which any increase in the ratio decreases the drug loading (Table 3).

Scanning Electron Microscope

The SEM is used to determine the particle size distribution, surface morphology, texture and also to examine the morphology of fracture or sectioned surface of

the prepared microsphere. The absence of pore in the formulation is of major importance for the underlying drug release mechanisms because drug release through water filled cavities is much faster than through dense polymeric matrix network. SEM of ELGKM (F_A , F_B , F_C & F_D), ELSAM (F_E) and Blank microsphere (F_F) are shown in the (Figure 4). All microspheres were spherical in shape; smooth surfaced and had minute pores on the surface. The surface of ELGKM was found to be

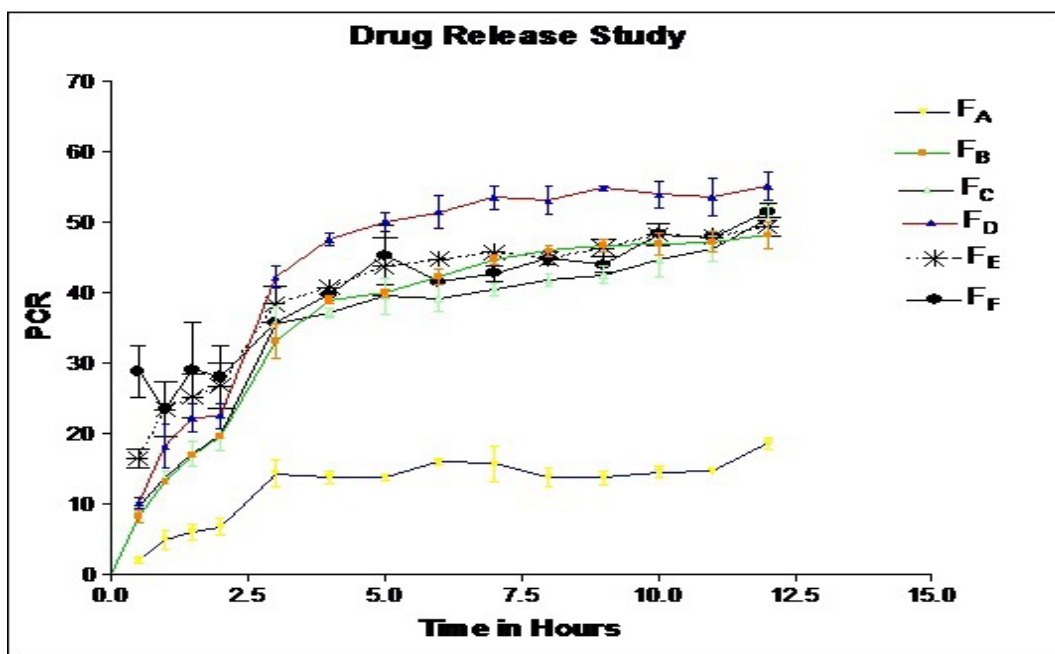


Figure 8: Drug release from ELGKM with different % of Gum Katira, ELSAM and Blank Microsphere in SGF (pH-1.2) and SIF (6.8) in a continuous process

smooth bearing small pores whereas in case of ELSAM and Blank microsphere the surface was rough bearing larger pores. In the study, the formulations with Gum Katira bearing smooth surface showed slower rate and prolonged drug release as compared to the formulation without Gum Katira.³⁵

Differential Scanning Calorimetry

DSC was performed to study the changing thermal behavior of the drug, polymer and gum during formulation. A sharp symmetric endothermic peak represents the melting point of the pure sample whereas a broad asymmetric curve in the DSC thermogram conveys about the presence of impurities in the sample. Generally, large endothermic and exothermic peak indicates the thermal decomposition of the sample. Also, the broad and large endothermic peak usually indicates the loss of water present in the compound.³⁶ Amorphous compound gives a broad peak due to the gradual decomposition of exudates structure but a crystalline solid require a fixed amount of energy for decomposition and gives a sharp peak. In (Figure 5), a sharp endothermic peak at 15°C and broad endothermic peak at 50-90°C suggests the melting point of Etodolac and melting range of Gum Katira respectively. The thermogram of the microsphere showed little endothermic peak shift from 151.90°C to 152.12°C which is negligible. From the DSC conducted, it is clear that Gum Katira does not produce any thermal changes with the drugs and other ingredients used in microsphere formulations. So, both gum and Etodolac

are compatible with each other as they are essentials for sustained drug delivery system.

FTIR Study

FTIR was conducted to study chemical interaction between the active ingredient, Etodolac and all the other excipients used in the formulation. The entire observed spectrum is given in (Figure 6) where the characteristic peaks of ELGKM was compared with that of the standard spectrum of Etodolac and no chemical interaction was identifiable in IR profile of ELGKM. Etodolac having ether form shows the C-O stretching vibration at 1033.85 cm⁻¹. The other characteristic bands are shown at 1728.22 cm⁻¹ for C=O stretching vibration of the COOH group, at 2970.38 cm⁻¹ for N-H stretching vibration of secondary amine group and at 748.38 cm⁻¹ for C-H stretching vibration of aromatic group.³⁷ So, FTIR study suggests that there is no appearance of new peak and disappearance of existing peak which denotes an absence of interaction between the drug and excipients used in microsphere formulations.

XRD study

According to the powder crystalline theory the crystalline substance have a well defined structure. Atoms, ions and molecules are arranged in a three dimensional lattice which results in tight packing of components so well defined edges and faces diffract X-ray in a regular pattern. Amorphous solids have curved and irregular surface so does not give well resolved dif-

Table 4: Kinetic parameters of drug release from ELGKM, ELSAM and Blank Microsphere

Formulation Code	Zero Order		First Order		Higuchi		Korsmeyers Peppas			Hixon Crowell	
	K_0	R^2_0	K_1	R^2_1	K_h	R^2_h	n	K_{kp}	R^2_{kp}	K_{hc}	R^2_{hc}
F _A	1.029	0.6513	-0.008	0.6761	4.814	0.7688	1.181	0.661	0.8557	0.080	0.6004
F _B	3.311	0.8032	-0.006	0.8015	15.17	0.9116	3.012	0.988	0.9939	0.120	0.7326
F _C	3.078	0.8168	-0.019	0.8157	13.98	0.91	1.035	1.171	0.9403	0.111	0.7457
F _D	3.570	0.7319	-0.022	0.6697	16.61	0.8566	1.057	1.253	0.9151	0.1162	0.6756
F _E	2.501	0.7896	-0.020	0.9183	11.50	0.9013	0.684	1.357	0.9486	0.0798	0.7387
F _F	2.111	0.8598	-0.015	0.8415	9.306	0.9021	0.463	1.441	0.8564	0.063	0.8287

K' value (Rate of release), n value (release exponent) and Correlation coefficient ' R^2 ' according to different kinetic equation for ELGKM, ELSAM and Blank microsphere respectively. All the data (K) were found to be significant at the level of $P < 0.0001$ assessed by One-way ANOVA using graph pad prism 5

Table 5: Interpretation of Diffusional Release Mechanism from Polymeric films

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.45 < n < 0.89	Non-Fickian Transport	t^{n-1}
0.89	Case II transport	Zero order release
> 0.89	Super case II transport	t^{n-1}

fractrogram.³⁸ The powdered X ray diffraction of Gum Katira, Etodolac and formulation are shown in the (Figure 7) where, the diffraction pattern of pure Etodolac and Etodolac loaded Gum Katira microsphere shows crystalline peak but Gum Katira alone gives amorphous peak. The result demonstrates that the crystalline peak of drug and formulation are almost similar but peak intensity diminishes in the formulation diffractrogram due to dilution of the drug with gum and polymer. So, the conducted XRD experiment also suggests that Etodolac is compatible with Gum Katira. Therefore the Gum Katira can be used as a novel matrix forming excipient for the sustained drug delivery system.

Dissolution Profile and Release rate kinetics

The mechanism of drug release from the microspheric formulation is biphasic in nature. Dissolution was carried out and it was observed that the drug release from the microspheres in acidic media pH-1.2 was too low in comparison to USP phosphate buffer media pH-6.8. Where, in buffer media at pH-6.8 the drug release gradually increased and followed a steady state (Figure 8). Such release profile of the drug is observed mainly due to variation in solubility of drug and percentage swelling of gum at different pH. The drug is less soluble at pH-1.2 and highly soluble at pH-6.8 which accounts for higher rate of drug release in phosphate buffer at pH-6.8 than in acidic media at pH-1.2. Also initial slow drug release reflects the time consuming processes of diffusion through the polymer wall, as well as the formation of pores and channels within the microspheres. Therefore, at pH-6.8 where the percentage of swelling of gum

is high as observed in (Figure 2) there is an increase in pore size which accounts for better and increased drug release. The collected drug release data from the dissolution test were evaluated using number of kinetic models like Zero order³⁹ release kinetics Eq. 1, First Order⁴⁰ Eq. 2, Higuchi's square root of time equation⁴¹ Eq. 3, Korsmeyer and Peppas equation⁴² Eq. 4, and Hixon-Crowell's cube root of time equation⁴³ Eq. 5.

$$C = K_0 t \quad (1)$$

Where, K_0 is zero order rate constant expressed as concentration/ time and t is the time.

$$\log C = \log C_0 - K_1 t / 2.303 \quad (2)$$

Where, C_0 is the initial concentration of drug and K_1 is first order constant.

$$Q = K_h t \quad (3)$$

Where, K_h is the constant reflecting the design variables of the system.

$$M_t / M_\infty = K_{kp} t^n \quad (4)$$

Where, M_t / M_∞ is the fraction of drug release, K_{kp} is the release rate constant, n is the diffusional release exponent indicative of the drug release mechanism and t is the dissolution time.

$$Q_0^{1/3} - Q_t^{1/3} = K_{hc} t \quad (5)$$

Where, Q_t is the amount of the drug released in time t , Q_0 is the initial amount of drug in the formulation and K_{hc} is the rate constant for Hixon-Crowell rate equation. The best fitted model was analyzed by comparing the correlation coefficient value of different mathematical models in (Table 4). From the recorded data the correlation coefficient value of ELGKM ($r^2 = 0.9939$) is found to be higher than that of ELSAM ($r^2 = 0.9486$) in equal percentage of gum. Thus, Korsmeyers Peppas model was the

best satisfactory mathematical model for ELGKM (1% w/v Gum Katira). Based on Korsmeyers Peppas model, the magnitude of release exponent “n” correspond to the release mechanism supercase II transport or anomalous transport, which can be concluded on comparing the value of $n=3.012$ which is greater than the value 0.89 (Table 5). Similarly, for ELSAM the “n” value i.e. $n=0.684$ is within the range of 0.45 to 0.89 thus it follows non-fickian transport release mechanism. In this regard, the drug release from microspheric formulation of Gum Katira followed the supercase II transport mechanism whereas formulation with sodium alginate and Blank microsphere followed non fickian transport mechanisms.

CONCLUSION

The evaluation of the different physiochemical parameters of Gum Katira reveals its potential to be a superior matrix for sustained release. The stability in terms of rheology and safety profile showed the acceptability of formulations composing of Gum Katira. The microsphere prepared by double emulsion solvent evaporation method delivers the drug in a regular fashion for an extended period of time. Consequently, it can be concluded that the gum exudates of *Cochlospermum religiosum*, when incorpo-

rated with etodolac may be potential pharmaceutical formulation for the safe management of pain therapy.

ACKNOWLEDGEMENT

The authors would like to thank University Grants Commission (UGC), New Delhi, India for the financial purport. The authors also greatly acknowledge Mr. Soumen Mukhopadhyay, Deputy Drug Controller of India, East Zone for extending his support in this research.

ABBREVIATION

ANOVA	: Analysis of Variance
CFU	: Colony forming unit
ELGKM	: Etodolac loaded gum katira micro sphere
ELSAM	: Etodolac loaded sodium alginate microsphere
OECD	: Organization for economic cooperation and development
SGOT	: Serum glutamic oxaloacetic transaminase
SGPT	: Serum glutamic pyruvic transaminase

SUMMARY

- Gum Katira is a natural gum obtained from the plant *Cochlospermum religiosum* (Family Cochlospermaceae).
- On the basis of its physiochemical characters, Gum Katira is a good drug release retarding agent. and thus it acts as an excipient in the novel drug delivery system.

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Dr. T.K. Chatterjee: An Ex-UGC (Govt. of India) Research Scientist and presently a Faculty member of the Department of Pharmaceutical Technology of Jadavpur University, Kolkata, has been actively engaged in Pharmacology & Toxicology research and teaching for the last 3 decades. He has been resourceful contributor of more than 108 research papers in journals of National and International repute, and he has to his trust worthiness as a distinguished author of ten books on pharmacology that attracted widespread recognition from various organizations of Pharmaceutical, Medical and allied sciences. International conferences in different countries like Sweden, Belgium, Germany and U.K. have also been enriched by his resourceful papers, a number of times. He was facilitated by different organization awarding him with prestigious awards like Ram Mohan Puraskar, Aadi Samman, NIMA Award, Nagarjun Puraskar for his contribution in Pharmaceutical Sciences. With the discovery of a new antibiotic MT-81 (Patent No. 156916), Dr. Chatterjee’s research work stands in high esteem embracing indepth study on the subject, and thus adding a new plume into his ever increasing contribution in the fields of Medical healthcare.

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