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The impact of processing variables on the physical properties of etodolac loaded gum Katira microsphere

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ABSTRACT

The study's objectives were to formulate a sustained release microsphere formulation for a poor water-soluble drug and determine the influence of processing parameters in the preparation of etodolac-loaded gum katira microspheres (ELGKM) following Wp/O/Wm emulsion solvent evaporation technique. The amount of gum katira, mechanical stirrer's stirring speed, the ratio of coating material, volume, and pH variation of the inner phase of the Wp/O/Wm emulsion system was varied to examine their effect on drug entrapment efficiency (DEE), particle size, surface morphology and *in vitro* drug release characteristics in dissolution media (pH-1.2 & pH-6.8). Scanning Electron Microscopic (SEM), Differential Scanning Calorimetric **(**DSC), and Fourier Transform Infrared (FTIR) studies were performed to find out the physicochemical as well as biopharmaceutical characterization of drug-loaded microsphere formulations. Based on experimental results, it was found that some processing variables significantly affect drug entrapment efficiency, particle size, and drug release kinetic in dissolution media. ELGKMs prepared with 50 mg Gum Katira, the ratio of Eudragit®RS100: Eudragit®RL100 at 7:1, at a stirring speed of 900 rpm showed the best formulation subjected to DEE, average particle size, and drug release profile. So, the study suggested that under the proper control of processing parameters, the Wp/O/Wm emulsion solvent evaporation technique would be a suitable method for preparing ELGKM.

Keywords: Gum Katira, Wp/O/Wm emulsion, Processing Variable, Microspheres, Pain management.

Running Title: Influence of processing parameters in etodolac loaded gum katira microsphere.

INTRODUCTION

Nowadays, pain-related pathological conditions like arthritis, osteoporosis, spondylitis, and gout are increasing widely. Elderly patients are affected mainly by these acute and chronic uncontrolled pain symptoms. To get rid of intolerable pain, patients take medicines of different kinds of conventional dosage forms like tablets, capsules of NSAIDs, steroids, and opium derivatives. They face extensive side effects like gastric irritation, peptic ulceration, gastric bleeding, chronic nephritis, and even gastrointestinal tract perforation (GIT).

So, it is now a matter of concern to design a novel pharmaceutical dosage form that will provide a sustained drug release kinetic and prolonged therapeutic drug action with the least side effects. The sustained drug delivery system in the form of drug-loaded microspheres is a suitable candidate to achieve this goal easily [1]. The drug-loaded microsphere formulations have been accepted greatly for their uniqueness and numerous clinical advantages. Drug release from microspheres is delayed and prolonged, with a low risk of dose dumping. Microspheres spread uniformly in GIT and provide more reproducible drug absorption with the least gastric mucosa. On chronic dosing, undesirable effects and dosage frequency can be reduced compared to single-unit dosage forms[2]. Etodolac, USFDA approved COX enzyme inhibitor, is developed to treat arthritis, acute and chronic pain, and inflammation [3]. Due to the suitable physiochemical, biopharmaceutical, and pharmacological properties, etodolac has been chosen as a model drug for the sustained release microsphere formulation [4]. Etodolac-

loaded gum katira microspheres (ELGKMs) were prepared by the Wp/O/Wm emulsion solvent evaporation technique. It is a simple, versatile, and practical reproducible method for the entrapment of poorly water-soluble drugs in microspheres formulation. Widely used Eudragit polymer was applied as coating polymer whereas Gummy exudate, gum katira was used as a model sustained release adjuvant in the drug-loaded microspheres. Gum katira is well established biocompatible, non-toxic and safe matrix-forming material used in the food and pharmaceutical industry [5].

The different physicochemical and biopharmaceutical characteristics of drug-loaded microspheres were analyzed by various instrumental techniques like SEM, DSC, FTIR, etc. The solid-state characteristics, drug release profile, and drug entrapment efficiency in dissolution media (pH-1.2 and pH-6.8) of etodolac-loaded gum katira microspheres depended primarily on the various applied processing parameters. The experimental research work aimed to find out the optimized ELGKMs and how the applied processing parameters influenced the particle size, drug entrapment efficacy, and drug release profile of drug-loaded microspheres.

Based on research findings, it can be concluded that by properly controlling processing parameters, ELKGMs can be formulated with satisfied particle size, drug entrapment efficacy, prolonged drug release profile, and maximum therapeutic efficacy with the least side effects.

MATERIALS AND METHODS

Materials

M/S Fleming Laboratory Limited, Dist gifted Etodolac. Medak, Andhra Pradesh, India. Crude Gum Katira was collected as dried exudates from the branches of the fibrous exudates of the plant *Chochlospermum religious* in December from the Seoni District of Madhya Pradesh. After purification, physiochemical and toxicological evaluations finally obtained gum katira was kept for future research work [5]. Eudragit®RS100 and Eudragit®RL100 polymer granules were obtained as a gift sample from Evonik Rohm, Pharma Polymers, Germany. Span 80, Acryflow and Tri-Sodium Orthophosphate (Loba Chemie Pvt. Ltd. India), Hydrochloric Acid 35%, Tween 80, Dichloromethane, Potassium dihydrogen phosphate (Merck Specialties Pvt. Ltd, India), and all others analytical grade chemicals were purchased and used as received.

Methods

Preparation of ELGKM [9]

Wp/O/Wm emulsion solvent evaporation technique was used to prepare ELGKM. 50 mg Gum Katira with 4ml of phosphate buffer (pH-6.8) was stirred to make a homogenous mixture in a magnetic stirrer, and then Etodolac was given to prepare a homogenous mixture and stirred for another 30 minutes. This 4 ml mixture was dispersed drop by drop using a 20-gauge syringe into another 50 ml prepared organic Dichloromethane solution of Eudragit®RL100 (250g) and Eudragit®RS100 (1750g) with 100mg of Acryflow (lubricating agent) and Span 80 (30µl).

The above mixture was homogenized well for 5-10 minutes using a mechanical stirrer (1000rpm) to produce W1/O emulsion. The prepared W1/O emulsion was added drop-wise by a 16-gauge syringe into a 100 ml of acidic aqueous solution (pH-4.0) containing Tween 80 (50µl) and then was stirred on a magnetic stirrer continuously for 2-2.5 hours to form Wp/O/Wm emulsion. The formed microspheres were washed three to four times with distilled water, followed by air drying for 24 hours, and the final product was stored in desiccators for experimental purposes.

Estimation of Drug contents in microsphere

Crushed and powdered of ELGKMs (30mg) were dissolved in 5ml of Dichloromethane (DCM). The solution was agitated for 10 minutes with a magnetic stirrer to dissolve the polymer in DCM. 10 ml of methanol was added to this solution. At 40-45oC, this solution was magnetically stirred for 2 minutes before filtration of the solution. 1 ml of filtered solution and 9ml of fresh methanol were added to make 10ml of the aliquot. The absorbance of the final filtered solution was measured at 224 nm using a double beam UV-Visible spectrophotometer (MULTISCAN GO, Thermo Scientific) against methanol as blank. The mathematic calculations were done

to measure etodolac loading in the sample microspheres. Drug Entrapment Efficiency (DEE) was found to range between 45-75%. Drug Entrapment Efficiency (DEE) and Drug Loading (DL) for each batch were calculated in terms of percentage as per the following equations 2 and 3, respectively [6]. The percentage yield of the prepared microsphere formulation was determined by the following equation [7]:

Percentage Yield= [Practical Yield/Theoretical Yield] *100 (Eq.1)

Percentage of Drug encapsulation:

Percentage of Drug Encapsulation= [Encapsulated Drug Mass/Introduced Drug Mass] *100 (Eq.2)

Percentage of Drug Loading= [Encapsulated Drug Mass/Microsphere Mass] *100 (Eq.3)

Particle size analysis

The particle size analysis of ELGKM was determined by a simple sieving method. A 5g of microsphere formulations were taken on the top of a nest of British Standard Sieves (Geological India). They were arranged with the coarsest sieve on the top ranging from Mesh No. 30 to 140. The arranged sieve set was shaken for 10 minutes on a mechanical sieve shaker (EGG 80432, Geologist' Syndicate Pvt. Ltd., Kolkata, India). The Microspheres retained on each sieve were collected and weighed to determine the percentage of microspheres passing. The distribution of particle size of maximum retained microspheres was determined, and the average diameter of maximum retained was calculated [36].

Morphological analysis of microsphere

Scanning Electron Microscope (SEM) was used to determine surface morphology and texture and to examine the morphology of the cross-sectioned surface of the prepared microsphere. SEM studies were carried out using JEOL MAKE (UK) (Model-JSM6360). ELGKMs 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). In a scanning electron microscope, images were captured at a voltage of 17 kV [29].

Differential Scanning Calorimetric Analysis

Differential Scanning Calorimetric analysis was performed to study the changing thermal behavior of the drug, polymer, and gum in microsphere formulation. Thermograms were evaluated to detect purity and change in enthalpy of melting of the samples. Model No prepared Thermograms of Etodolac, Gum Katira, and formulation. Pyris Diamond TG/DTA, Perkin Elmer (SINGAPORE), Nitrogen Atmosphere (150ml/min). The platinum crucible was used with alpha alumina powder as a reference for the study [30].

FTIR Spectroscopic Study

Fourier Transform Infrared (FTIR) spectroscopy was performed on IR- Prestige-21, Shimatzu makes, Japan. FTIR spectra of finely powdered pure etodolac and ELGKMs were measured in the region from 400-4000cm^{-1}. The individual IR spectrum is presented in (Figure 1)[28].

In vitro drug release kinetic study

In vitro drug release kinetic studies were performed using the USP II dissolution test apparatus (Model TDP-06P, Electro Lab, Mumbai, India). Dissolution studies of all microsphere formulations were carried out in simulated gastric fluid (SGF) (0.1 N HCl, pH-1.2) for an initial 2hrs and followed by in simulated intestinal fluid (SIF) (USP phosphate buffer, pH 6.8) for the next 10hours at 75rpm maintained at a temperature of 37ºC± 0.5º C. Aliquots were withdrawn at a specific time interval and immediately replaced with the same amount of fresh solution. The drug release into dissolution media was analyzed by UV-Visible Spectrophotometer (MULTISKAN GO, Thermo Scientific) at 224 nm. All release studies were triplicated [31]. The collected drug release data from the dissolution test were analyzed using several kinetic models like Zero order release kinetics (Eq. 1), First Order (Eq. 2), Higuchi's square root of time equation (Eq. 3), Korsemeyer and Peppas equation (Eq. 4), and Hixon–Crowells cube root of time equation (Eq. 5).

$C=K_0t$ (1)[32]

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

LogC=LogC0–K1t/2.303 (2)[33]

Where C0is the initial concentration of the drug and K1is the first order constant.

Q=Kht (3)[34]

Where Khis is the constant that indicates the design variables of the system.

Mt/M∞= Kkptn (4) [31]

Where Mt/M∞is the fraction of drug release, Kkpis the release rate constant, n is the diffusion release exponent indicative of the drug release mechanism, and t is the dissolution time.

Q01/3–Qt1/3= Khct (5) [35]

Where Q is the amount of the drug released in time t, Q0is the initial amount of drug in the formulation, and Kh cis is the rate constant for Hixson- Crowell rate equation.

The best-fitted model was evaluated by comparing the correlation coefficient value of different kinetic models.

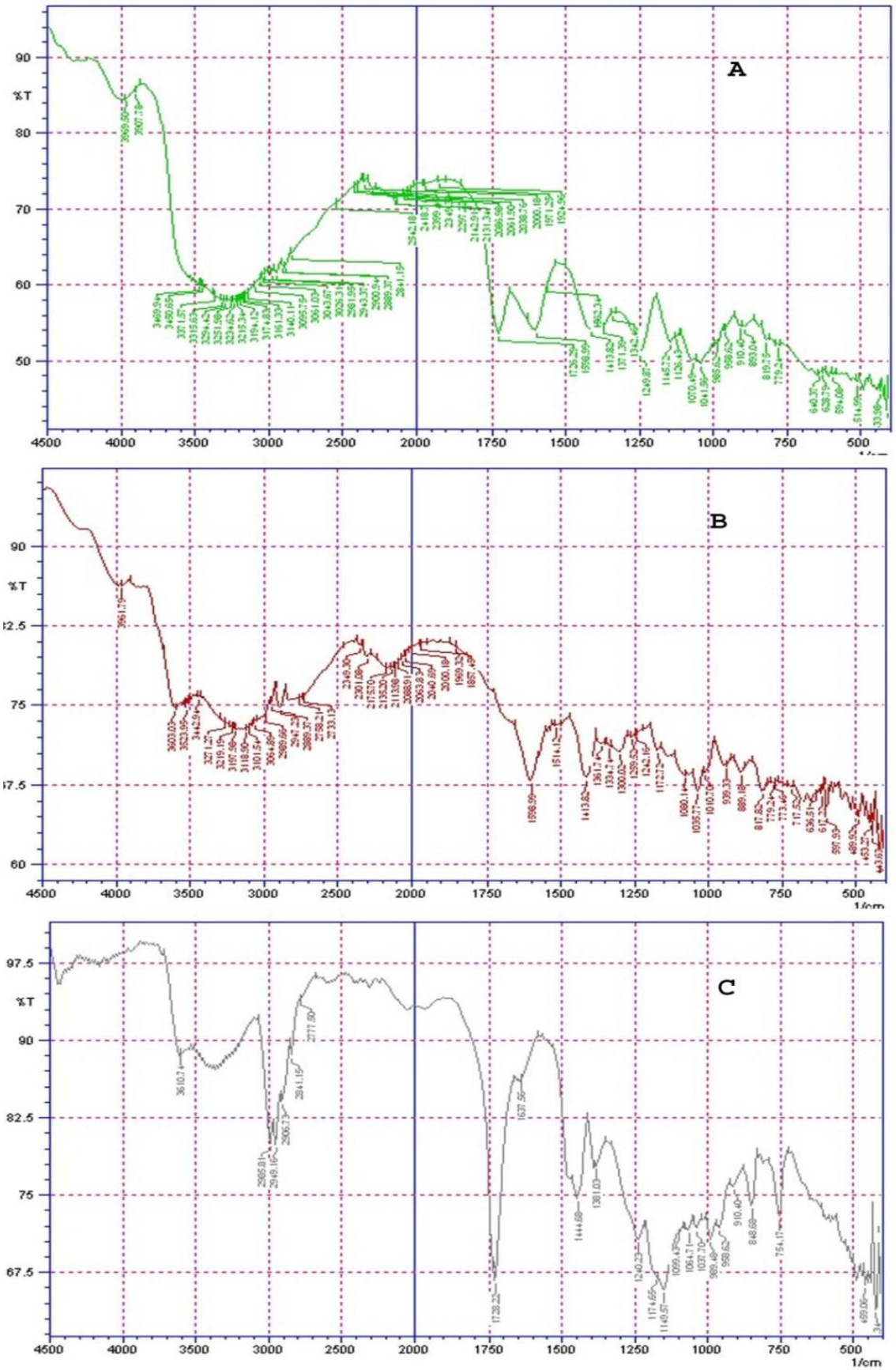
Statistical Analysis

The provided data of drug content, average particle size, and *in vitro* drug release study were analyzed statistically with one-way ANOVA and t-test using Graph Pad Prism 3.

RESULTS AND DISCUSSIONS

FTIR Study

Gum katira is a well-established biocompatible, nontoxic, and safe matrix-forming material. The individual and combination of Eudragit®RS100 and Eudragit®RL100 were used as a coating material for sustained drug release microspheres formulation. The FTIR study was conducted to find any chemical interaction between the active ingredient, etodolac, and other excipients (Gum Katira, Sodium Alginate, EudragitRL 100) used in the microsphere formulation. The characteristic peak of etodolac in the formulation was compared with other peaks of excipients used in the formulation. The entire observed spectrum is presented in (Figure 1).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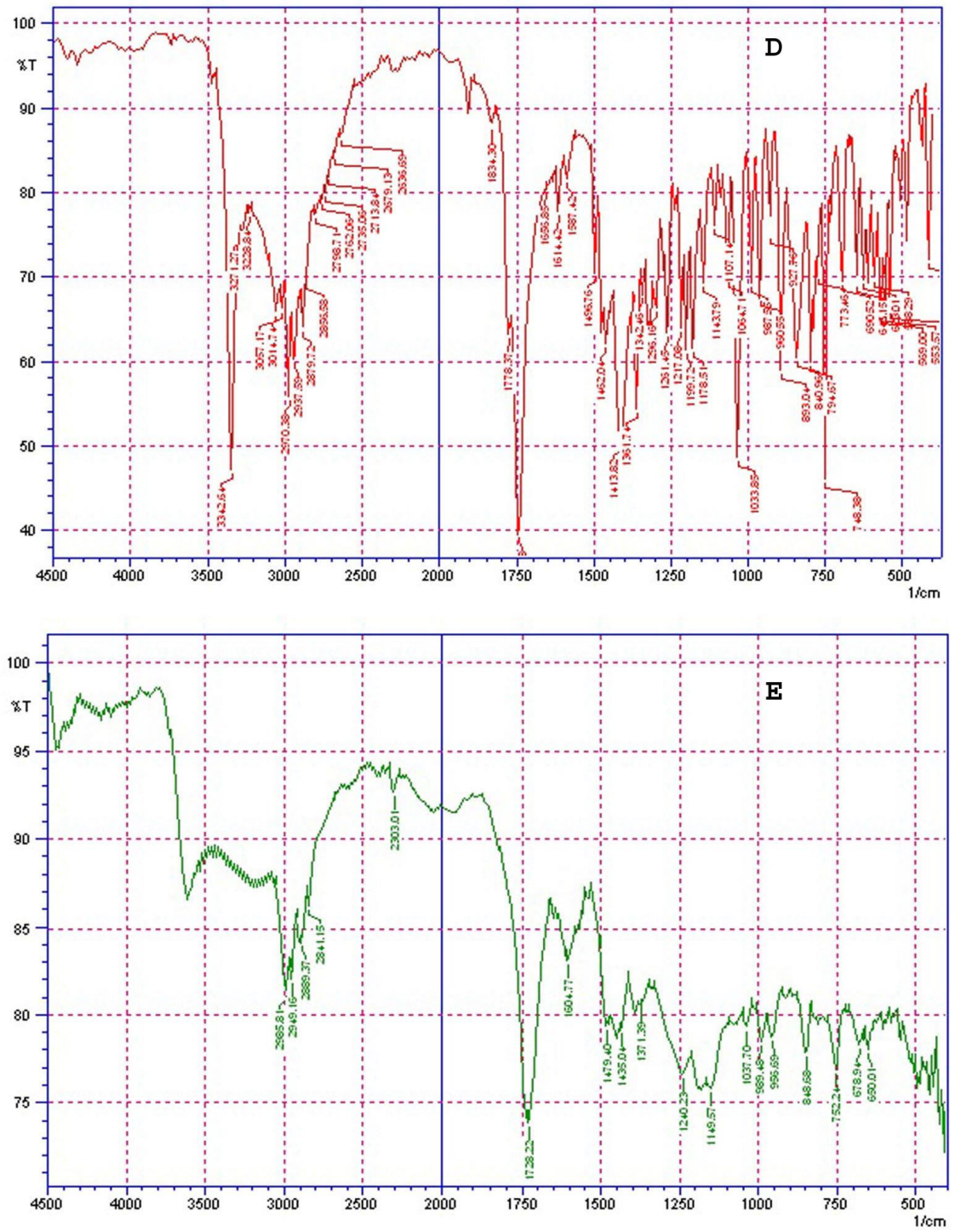

Figure (1): FTIR spectrum of Gum Katira (A), Sodium Alginate (B), EudragitRL100 (C), Etodolac (D), and Formulated Microsphere (E).

Any chemical interaction was not found in the IR profile of formulated microsphere. The ether group in Etodolac shows the C-O stretching vibration at 1033.85 cm-1and the C=O stretching vibration of the COOH group at 1728.22 cm-1. At the same time, the N-H stretching vibration of the secondary amine group in Etodolac shows at 2970.38 cm-1, and the C-H stretching vibration of the aromatic group at 748.38 cm-1[28]. So, FTIR studies reveal that there is no appearance of a new peak and disappearance of the existing peak, indicating no chemical interaction between the etodolac and other excipients used in microsphere formulations.

Effect of amount of gum katira in microspheres preparation

The drug entrapment efficiency of microspheres without gum katira was found to be low in microspheres, but it was significantly increased with gum katira, which was used as drug release retarding material in the microsphere formulations. However, the amount of gum katira in the inner phase of DEE was increased up to a limiting value (50mg), beyond which it was decreased. It was assumed that up to a certain amount of gum katira, the low viscosity of the inner phase has less chance to leach etodolac to the external aqueous phase due to less osmotic pressure difference. Similarly, the higher viscosity of the W1 phase of primary emulsion aggravated the formation of numerous pores, which enhanced the leakage of etodolac to the external aqueous phase [8].

The particle size of the microsphere was changed with the volume of the inner phase (Table 1). Because of the high viscosity of the W1 phase, it was difficult to break it into tinny droplets, leading to larger microspheres [9].

Table (1): Effect of processing variables on the properties of ELGKMs.

Processing Variables	Prepared Batches	%DEE	Average Particle Size(µm)	% Drug Release at 12^{th}hr	Best fitting drug release model
Amount of Gum Katira (mg)					
25	FA1	62.25	194.67±8.5	74.58±0.46	Korsemeyer andPeppas model, n =0.679, K_{kp}=1.202, R^2= 0.955
50	FA2	71.81	236.66±3.5	71.59±0.76	Korsemeyer and Peppas model n =0.924, K_{kp}= 0.915, R^2=0.990
75	FA3	70.45	277.00±5.3	69.24±1.05	Korsemeyer and Peppas model n =0.673, K_{kp}=1.171, R^2= 0.978
100	FA4	66.034	294.00±10.3	62.88±1.47	Higuchi model K_h= 32.35 R^2=0.981
Stirring speed (rpm)					
500	FB1	68.67	289.67±6.8	71.85±2.04	Korsemeyer and Peppas model n =0.682, K_{kp}= 1.082, R^2=0.971
700	FB2	72.12	273.67±4.5	75.35±0.72	Higuchi Model R^2=0.99, K_h= 23.99
900	FB3	71.89	242.67±6.4	80.22±0.85	Korsemeyer and Peppas model n =0.659, K_{kp}=1.185, R^2= 0.993
1100	FB4	65.63	172.00±7.0	74.54±1.53	Korsemeyer and Peppas model n =0.518, K_{kp}=1.345, R^2= 0.971
Eudragit® RS100 :Eudragit®RL 100					
1:0	FC1	71.48	285.67±5.1	66.72±1.09	Korsemeyer and Peppas model n = 0.615, K_{kp}= 1.139, R^2= 0.978
7:1	FC2	70.45	253.67±6.0	80.81±1.40	Korsemeyer and Peppas model n = 0.657, K_{kp}= 1.186, R^2= 0.977
1:7	FC3	58.86	238.33±3.0	83.99±0.65	Higuchi Model R^2= 0.978, K_h= 30.09
0:1	FC4	51.78	211.67±3.0	91.80±1.00	First Order Model R^2= 0.955, K_F= -0.1052

In the W1 phase, the drug release profile of microspheres was significantly changed with varying amounts of gum katira. In the W1 phase, an increased amount of gum katira reduced the drug release rate in the dissolving media (Fig.2.A). The viscous solution within

a certain limit may provide drug diffusion resistance from the inner core to the exterior dissolving media [10].

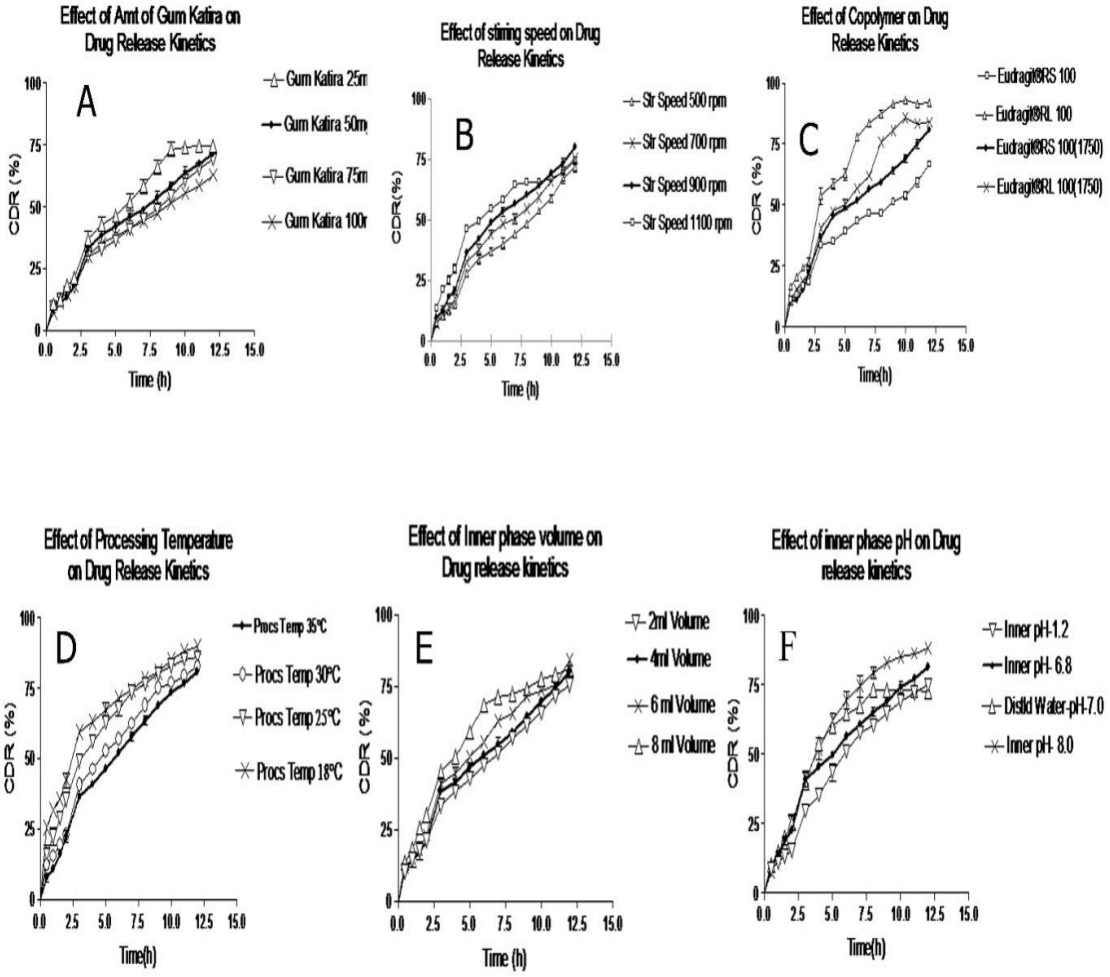

Figure (2): Drug release profile of microsphere)

Effect of stirring speed during the secondary emulsification process

The stirring speed of the mechanical stirrer was found to significantly influence drug content, average particle size, surface morphology, and drug release kinetics, which were being investigated. The stirring speed was maintained at 500, 700, 900, and 1100 rpm, respectively. An increasing stirring speed from 500rpm to 1100rpm caused a marked reduction in average particle size and DEE of the microsphere's formulation (Table 1). Higher stirring speed provides a higher shearing force to break down the emulsion droplets into smaller droplets, prevents the agglomeration of immature microspheres, and finally forms the smaller microspheres [11]. The formations of smaller emulsion droplets have a larger surface area, and the drug diffuses out from the microspheres faster before they harden. So, the maximum amount of drug was lost, leading to microspheres with low drug content [12]. It was observed that particles were smooth and spherical at 900 rpm, whereas below and above this speed, the particles were not in proper shape and size (Fig 3. A and Fig 3. C). At the time of the secondary emulsification process, variation in the stirring speed of the mechanical stirrer also affected the drug release behavior in dissolution

media (Fig 2. B). Small particles had a faster drug release characteristic compared to larger particles. Microsphere particles' increased surface area and porous surface morphology are possible causes of this phenomenon [13].

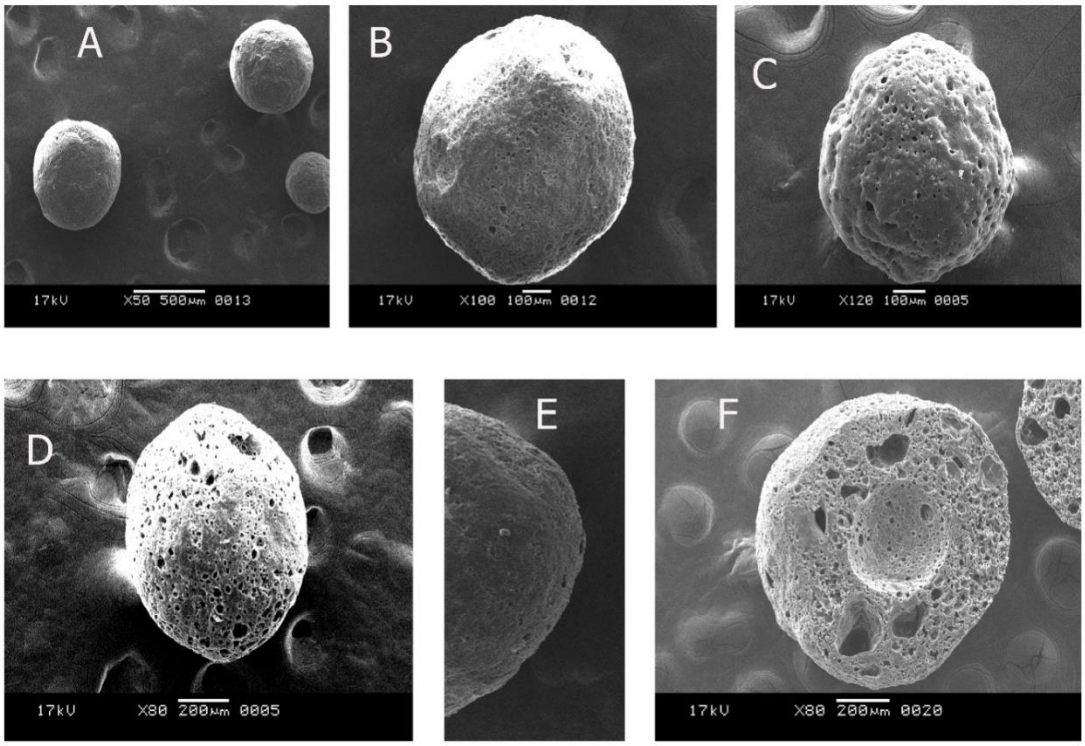

Figure (3): Scanning electron microscopy (SEM) analysis of ELGKMs (Fig 3. **A**: Formulated ELGKM, **B**: Optimized ELGKM, **C**: ELGKM with a rough and porous surface, **D**: ELGKM with a smooth and porous surface, **E**: Surface morphology of optimized ELGKM, **F**: Cross-sectional surface of ELGKM).

Effect of co-polymer ratio in the preparation of microspheres

To examine the drug content, particle size, and drug release behavior, microsphere formulations were made using varied ratios of Eudragit®RS100 and Eudragit®RL100 (1:0, 7:1, 1:7, 0:1). Combination of Eudragit®RS100 and Eudragit®RL100 were used in the preparation of microsphere to achieve an appropriate coating matrix structure and a superior drug release profile. When the amount of Eudragit®RS100 was increased, the drug content also increased (Table1). The increased concentration of Eudragit®RS100 in dichloromethane may increase the viscosity of the organic phase. The viscous organic phase can prevent drug migration from the inner to the outer aqueous phase [14].

SEM studies revealed that microspheres become denser with the amount of Eudragit®RS100 (Fig3.F). A large amount of Eudragit®RS100 in an organic solvent increased the frequency of collisions, resulting in the fusion of semi-formed particles and an increase in the number of microspheres [15]. The different ratios of co-polymer influenced the drug release kinetics significantly. It was observed that drug release rates from Eudragit®RS100 coated microspheres were very slow and incomplete, whereas Eudragit®RL100 coated microspheres showed a relatively higher drug release rate and complete (Fig 2. C). The relative increase in drug release rate could have happened because of a greater number of quaternary ammonium groups in Eudragit®RL100[16].

Effect of processing temperature in microspheres preparation

The DEE of microspheres prepared at 35ºC was higher than those prepared at 18ºC. The faster evaporation rate of the organic solvent (DCM) at elevated temperature causes the eudragit polymer to solidify quickly, which may be a possible reason for increasing drug content [17]. It was found that as the processing temperature increased, the size of the particles of the microspheres also increased. The microspheres were hardened rapidly, and the surface became rougher at a high temperature (35ºC), whereas microsphere formulations fabricated at18ºC have a smooth surface. Because of the evaporation and quick phase separation of DCM during the hardening and shrinking stage, the surface of the prepared microspheres becomes rougher (Fig. 3E) [18]. The rough and uneven surface of the microsphere is beneficial for the attachment with the payer's patch in the intestine, providing a suitable environment for prolonged drug release [19]. Differential Scanning Calorimetric **(**DSC) was also performed to study the changing thermal behavior of the drug, polymer, and gum katira used in microsphere formulation, but a significant change was not found.

The drug release characteristic of microsphere formulation prepared at different processing temperatures has been represented in (Fig2D). The drug release profile revealed that microspheres formed at higher temperatures had a slower drug release rate than microspheres fabricated at lower temperatures (Table 2). The microspheres made at low temperatures were highly porous with rough surfaces (Fig 3. C). A similar observation was reported elsewhere [20].

Table (2): Effect of processing variables on the properties of ELGKMs.

Processing Variables	Prepared Batches	%DEE	Average Particle Size(µm)	% Drug Release at 12thhr	Best fitting drug release model
Processing Temperature					
18ºC	FD1	52.72	173.00±8.0	89.77±1.63	Korsemeyer and Peppas model n = 0.764, K_{kp}= 1.131, R^2= 0.985
25ºC	FD2	61.23	190.00±9.6	85.89±1.23	Higuchi model R^2=0.980, K_h= 17.36
35ºC	FD3	69.38	230.33±4.2	82.71±0.94	Higuchi model R^2= 0.989, K_h= 26.82
45ºC	FD4	71.45	251.33±3.5	80.67±1.10	Korsemeyer and Peppas model n = 0.891, K_{kp}= 0.936, R^2= 0.983
Inner Phase Volume					
2ml	FE1	62.56	203.67±5.0	75.53±2.00	Higuchi model R^2= 0.975, K_h=23.92
4ml	FE2	73.25	243.00±8.0	80.24±1.91	Korsemeyer and Peppas model n = 0.637,K_{kp}= 1.196, R^2= 0.976
6ml	FE3	71.34	261.33±7.0	84.47±1.53	Korsemeyer and Peppas model n = 55.33,K_{kp}= 22.75, R^2= 0.961
8ml	FE4	70.23	292.00±4.0	81.09±1.07	Higuchi model R^2= 0.973, K_h= 26.60
pH of Inner Phase					
pH-1.2	FF1	49.065	218.00±5.6	75.02±0.98	Korsemeyer and Peppas model n= 0.589, K_{kp}= 1.258, R^2=0.994
pH-6.8	FF2	74.11	242.00±7.0	81.44±0.87	Korsemeyer and Peppas model n= 0.674, K_{kp}= 1.224, R^2= 0.987
pH-7.0	FF3	63.123	231.00±6.6	72.48±1.73	Korsemeyer and Peppas model n= 0.838, K_{kp}= 1.167, R^2=0.958
pH-8.0	FF4	55.64	228.00±3.0	88.18±1.21	Korsemeyer and Peppas model

Processing Variables	Prepared Batches	%DEE	Average Particle Size(µm)	% Drug Release at 12thhr	Best fitting drug release model
					n =0.499, K_{kp}= 1.382, R^2= 0.911

Impact of inner phase volume in microspheres preparation

To maximize drug content, particle size, and drug release profile, microsphere formulations were made by applying a double emulsion solvent evaporation technique with varying the inner phase volume. DEE was seen to be higher in 4ml of the inner phase compared to 8ml of the inner phase of the double emulsion system. It can be explained that the lower volume of the inner phase(W1) may form a relatively thick layer of the organic phase (O) which can function as a barrier to the diffusion of drugs to the external acidic aqueous phase (pH-4.0) [21].

SEM analysis of the prepared microsphere formulation suggested that variation in the volume of the inner phase of Wp/O/Wm emulsion did not have an impact on the spherical shape of the microsphere but influenced the surface morphology of microspheres. Microspheres became porous with an increase in inner phase volume of Wp/O/Wm emulsion (Fig 3.D). An increase in inner phase volume tended to produce larger microspheres (Table 2). An increase in the volume of the W1 phase increased the number of droplets dispersed in a given volume of the organic phase (DCM). It may cause coalescence between the dispersed droplets, slightly increasing the microsphere's diameters [22].

The microsphere formulation with higher inner phase volume released its maximum drug content earlier than the microsphere prepared with lower inner phase volume in both dissolution media (Fig 2. E). During the microsphere's preparation, surface pores in microspheres may be formed due to water leakage through the organic phase. That was probable for faster drug release than the microspheres with higher inner phase volume [23].

Effect of pH variation of inner phase in microspheres preparation

It was found that pH variation in the inner phase of Wp/O/Wm emulsion significantly affects the DEE of ELGKM formulations. The solubility of etodolac in the inner phase of the Wp/O/Wm multi-emulsion determined its drug entrapment efficacy in the core matrix of ELGKMs. The Shake Flask Method performed a solubility study of etodolac (Table 3) [24]. Due to the lower pKa value of etodolac (pKa=4.65), it is more soluble in a solvent having a higher pH value [25]. The study observed that DEE was higher in the formulation with phosphate buffer (Inner phase, pH-6.8) in comparison to pH-8, pH-7.0, or pH-1.2. Low DEE at pH-8 or 7 can be attributed to the high permeability of polymer Eudragit to water and the leaching of the drug into the external aqueous phase [26].

Additionally, DEE was also increased with a decrease in pH of the external aqueous phase (Wm) (pH<4.65), which should be lower than the pKa of etodolac. The research study explained that a drug's intrinsic dissolution rate would decrease if the solvent's pH were lower than the pKa [25]. From the experiment, it can be said that DEE was higher at pH-6.8 compared to other pH values. The pH variation of the inner phase (Wp) did not significantly affect the particle size of microspheres (Table-2). From *in vitro* drug release profile of ELGKM formulation (Fig2.F), it was found that an increase in pH of the inner phase showed a faster drug release profile. Eudragit®RL100 is highly permeable to a higher alkaline media and would cause the formation of rough and porous microspheres shown in (Fig. 3. C) [27]. Microspheres prepared with higher pH values followed a faster drug release characteristic in both dissolution media.

Table (3): Solubility study of etodolac in different solvent.

Test Sample	Phosphate Buffer (pH-6.8)		Acid Buffer (pH-1.2)		Methanol		Distilled Water	
	Conc. (mg/ml)	Mean (mg/ml)	Conc. (mg/ml)	Mean (mg/ml)	Conc. (mg/ml)	Mean (mg/ml)	Conc. (mg/ml)	Mean (mg/ml)
Test1	1.929	2.044 ±0.10	0.174	0.1063 ±0.05	119.532	119.0 ±0.97	1.585	1.180 ±0.47
Test2	2.083		0.076		117.897		0.657	
Test3	2.119		0.069		119.626		1.298	

CONCLUSION

ELGKMs release etodolac, which is significantly more dependent on the volume and pH of the inner phase of multi-emulsion than the other processing variables. Out of the many formulations tried, the microspheres made with 50 mg Gum Katira, the ratio of Eudragit®RS100: Eudragit®RL100 at 7:1, at a stirring speed of 900 rpm done at a processing temperature of 35°C and with 4ml phosphate buffer (pH-6.8) as inner phase showed the best formulation with DEE (> 70%), average particle size (about 250µm), drug release more than 80% of their drug content during 12 hours and release profile followed the Korsemeyer and Peppas kinetic model. It can be assumed that microsphere formulations with applied processing variables may regularly release etodolac for an extended period in GIT to minimize gastric complications and subsequently provide a better drug therapy for NSAID drug molecules in managing acute and chronic pain.

Ethics approval and consent to participate

Ethical approval

Not applicable.

Consent for publication

All the authors have read and approved the manuscript for publication.

Availability of data and materials

Not applicable.

Author's contribution

Biswajit Das: Involved in conceptualization, writing-original draft, data curation, formal analysis, methodology development, supervision, validation, visualization, and writing review & editing of the manuscript. **Tathagata Roy:** Involved in formal analysis, validation, visualization, and writing review & editing of the manuscript. **Dr. Tapan Kumar Chatterjee:** Involved in conceptualization, formal analysis, and supervision of the research work.

Competing interest

The authors declare that there is no conflict of interest.

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