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Formulation and Characterization of Modified Release Microspheres of Lornoxicam Using Okra Gum as Natural Polymer and Ethyl Cellulose as Synthetic Polymer

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Authors' contributions

This work was carried out in collaboration between both authors. Author AKS designed, conducted the study and wrote the article. Author NV supervised the whole study. Both authors read and approved the final manuscript.

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ABSTRACT

Objective: The nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications in the world because of their demonstrated efficacy in reducing pain and inflammation. The arthritis, pain and inflammation are effectively treated with Lornoxicam, an effective NSAIDs. Because the drug is weakly acidic, it is absorbed easily in the GI tract, and has a short biological half-life of 3 to 5 hours. To meet the objectives of this investigation, we developed a modified release dosage form to provide the delivery of lornoxicam at sustained rate which was designed to prolong its efficacy, reduce dosage frequency, and enhance patient compliance. The present research work was focused on the development of lornoxicam microspheres using natural polymer like okra gum extracted from the pods of *Abelmoschus esculentus Linn.* and synthetic polymer like ethyl cellulose along with sodium alginate prepared by Ca^{2+} induced ionic-gelation cross-linking in a complete aqueous environment were successfully formulated.
Materials and Method: The microspheres were prepared by using sodium alginate with natural polymer (okra gum) and synthetic polymer (ethyl cellulose) in different ratios by Ca^{2+} induced ionic-

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gelation cross-linking. The formulations were optimized on the basis of drug release up to 12 hrs. The physicochemical characteristics of Lornoxicam microspheres such as drug polymer interaction study by Fourier Transform Infrared (FTIR) and further confirmation by Differential Scanning Calorimetry (DSC) and X-ray Diffraction (XRD). The formulated microspheres were characterized for particle size, percentage drug entrapment efficiency, micromeritic properties, surface morphology, percentage swelling index, *in-vitro* drug release study and mechanism of drug release.

Results and Discussion: The FTIR Spectra revealed that there was no interaction between polymer and Lornoxicam which was further confirmed by DSC and XRD. All the formulated Lornoxicam microspheres were spherical in shape confirmed by SEM. The microspheres exhibited good flow properties and also showed high percentage drug entrapment efficiency. All the batches have excellent flow properties with angle of repose in the range of 25.38° ± 0.04 to 30.41° ± 0.07, carr's index and hausner's ratios in the range of 10.40% ± 0.018 to 16.66% ± 0.012 and 1.128 ± 0.09 to 2.225 ± 0.01, respectively. The optical microscopic studies revealed that the mean particle size of all the formulations were found in the range of 819.46 ± 0.07 to 959.88 ± 0.02 µm and percentage of drug entrapment were found to be between 72.35 ± 0.02 to 90.00 ± 0.05. Swelling index of prepared microspheres revealed that with increasing the polymer ratios, there were increase in the swelling of prepared microspheres, showing in the range of 600.76 ± 0.42 to 690.11 ± 0.03% for okra gum microspheres at the end of 9 hr in comparison with ethyl cellulose microspheres which ranges between 179.71 ± 0.07 to 227.73 ± 0.05% at the end of 7 hr. *In-vitro* drug release of prepared microspheres formulation code LSO4 and LSE4 were found to be 88.654 ± 0.25% and 93.971 ± 0.20% respectively at the end of 12 hr. It was suggested that increase in polymer concentration, the drug release from the prepared microspheres got retarded producing sustained release of lornoxicam. *In-vitro* drug release data obtained were fitted to various release kinetic models to access the suitable mechanism of drug release. Drug release from lornoxicam-loaded alginate-okra gum microspheres followed a pattern that resembled sustained release (Korsemeyer-Peppas model) (R^2 = 0.9925 to 0.9951), and n ≤ 1 indicated anomalous diffusion (non-Fickian), supercase-II transport mechanism LSO4 (n = 1.039) over a period of 12 hour underlying *in-vitro* drug release. Moreover, zero order model (R^2 = 0.9720 to 0.9949) were found closer to the best-fit Korsemeyer - Peppas model.

In addition, the drug release from lornoxicam-loaded alginate-ethyl cellulose microspheres also follow Korsemeyer-Peppas model (R^2 = 0.9741 to 0.9973) with near to Hixson-Crowell model (R^2 = 0.9953 to 0.9985) and n < 1 indicated non-Fickian diffusion or anomalous transport mechanism. Moreover, first order model with non-Fickian diffusion mechanism (R^2 = 0.9788 to 0.9918) were found closer to the best-fit Korsemeyer-Peppas model/ Hixson-Crowell model.

Conclusion: The present study conclusively demonstrates the feasibility of effectively encapsulating Lornoxicam into natural polymer (okra gum) and synthetic polymer (ethyl cellulose) to form potential sustained drug delivery system. In conclusion, drug release over a period of 12 hrs, could be achieved from these prepared microspheres. A pH-dependent swelling and degradation of the optimized microspheres were also observed, which indicates that these microspheres could potentially be used for intestinal drug delivery.

Keywords: Microspheres; sustained release; lornoxicam; Okra pods mucilage/gum; ethyl cellulose; sodium alginate; ionotropic gelation.

1. INTRODUCTION

Conventional immediate-release (IR) dosage forms are not able to maintain stable plasma levels for an extended period of time; they tend to have a short duration of action, which means multiple daily doses need to be taken. In contrast, multi-dose therapy may be more appropriate for shorter-term health conditions such as colds and flus, migraines, and neuralgia, in which the treatment may last a couple of days. Multiple daily dosing is, however, undesirable and inconvenient for patients with long-term chronic conditions, which may require treatment for several months or even years. It may result in missed and made-up times as well as compromised quality of life [1]. Additionally, modified-release (MR) dosage forms are increasingly becoming more popular, as they are an effective alternative to IR solid dosage forms for oral administration. It is the goal of these dosage forms to release a drug slowly over an extended period of time, which is why they are also known as delayed-release

(DR) and sustained-release (SR) medications [2].

There has been a broad range of research exploring the application of microspheres to the development of formulations and as potential carriers in the novel drug delivery segment. Microspheres are based on polymer-based frameworks ranging from 1-1000 µm in size [3, 4]. In addition to providing a reduced risk of side effects, decreased dosing frequency, and improved patient compliance, microspheres can be used for drug delivery applications [5]. Recent research has focused on natural biodegradable polymers for controlling drug release. Sodium alginate, okra mucilage, guar gum, linseed mucilage, and tamarind gum are among the most prevalent biodegradable polymers used for the advancement and design of new drug delivery systems [6].

Alginates have been utilized broadly to control the pattern of drug release for a longer time frame due to its hydrogel properties and fewer handling necessities. Many researchers are reported that alginates have many pharmaceutical applications in formulation of various drug delivery system [7]. Alginates undergo ionotropic gelation in the presence of divalent cations (e.g., Ca^{2+}) and trivalent cations (e.g., Al^{3+}), where ionic cross-linking interactions occur between groups of alginate carboxylic acids ($-COO^{-}$) and the respective cations [8-10].

Based on the mechanism for ionotropic-gelation technique, distinct calcium alginate microspheres were designed and tested for the release of different drugs. Such alginate microspheres of calcium showed different patterns of drug release [11-13].

From the last few decades, the exercise of natural polymers for the development and improvement of a variety of sustainable drug delivery systems has been of huge interest and these polymers remain attractive primarily due to their easy availability, cost-effectiveness, biodegradability and biocompatibility, when compare with synthetic polymers. Importantly, natural polymers can also undergo chemical changes [14-15]. A number of researcher's reports demonstrated that several natural polysaccharides such as xanthan gum, sodium alginate, cellulose ethers, locust bean gum, okra gum, guar gum and tamarind seed gum have been tested for drug delivery systems in the hydrophilic matrix [16-17]. These polymers have hydrogel forming properties but sodium alginate has been widely used as matrix among these polymers in various drug delivery applications [18]. Much attention has been paid over the past few years to the manufacturing of biopolymeric microspheres prepared using ionotropic gelation technique based on sodium alginate [19].

Natural polymers have recently been recognized as a major interest in controlled drug delivery systems since biodegradable, stable and non-toxic biopolymers [5]. Among natural polymers, okra mucilage from *Abelmoschus esculentus Linn.* pods is one of the advantageous polysaccharides currently being studied in the field of pharmaceuticals [20-21]. It is a polysaccharide consisting of D-galactose, L-rhamnose and L-glactonoric acid, and it has been found that the repeating units of the gum are (1-2)-rhamnose and (1-4)-galacturonic acid residues with side chains of disaccharide and a degree of acetylation i.e. DA=58. These polysaccharides produce extremely viscous solution with a slimy appearance when extracted with water and it is one of the beneficial polysaccharides currently being studied in the pharmaceutical industry [22]. However, for the study of mixing alginate with okra mucilage, few studies have been carried out. In the present research, we were used different ratios of okra mucilage in blended form with fixed ratio of pure drug lornoxicam to sustain the release of drug for 12 hrs and further we compared the drug release from microspheres prepared using ethyl cellulose and sodium alginate.

Ethylcellulose (EC) is a noncaloric, noncorrosive, tasteless, odorless, biodegradable, biocompatible, hydrophobic polymer widely used in controlled drug delivery systems. Ethylcellulose release is highly dependent on the porosity of the hydrophobic compact. Despite its water-absorption abilities, ethyl cellulose requires the addition of channeling agents to change kinetics of drug release [23].

Lornoxicam is a congener of tenoxicam and a new NSAID belongs to the oxicamcategory.It has potent analgesic and anti-inflammatory properties that belong to class II (low solubility and high permeability) of biopharmaceutical classification system. Lornoxicam is 90 to 100% bioavailable. The gastrointestinal tract is responsible for rapidly and almost completely absorption of Lornoxicam. Owing to its relatively short plasma half-life (3-5 hrs), it is recommended to take lornoxicam twice or thrice

a day in divided daily doses to control the therapeutic plasma concentration. Such characteristics make lornoxicam a suitable drug candidate to produce into sustained release microspheres [24].

The goal of this study was to design microspheres using alginate with okra gum and ethyl cellulose for sustained drug delivery of lornoxicam using inotropic gelation technique. Then, prepared microspheres were characterized for drug loading, drug entrapment efficiency, particle size, micromeritics properties, SEM (Scanning electron microscopy), FTIR (Fourier transform infrared spectroscopy), DSC (Differential Scanning Calorimetry) and XRD (X-ray Diffraction). The swelling kinetics of the formulated lornoxicam microspheres in 0.1 N acidic buffer pH 1.2 and alkaline phosphate buffer pH 6.8 were tested to confirm the functionality of *Abelmoschus esculentus Linn.* mucilage and ethyl cellulose for sustained drug release effect. The drug release were investigated in the physiological media of the gastrointestinal tract, i.e., simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Drug release mechanism were evaluated using different release kinetic models such as Zero order model, First order model, Higuchi model, Korsmeyer-Peppas model and Hixson-Crowell model.

2. MATERIALS AND METHOD

2.1 Materials

Lornoicam was obtained from Tirupati Medicare Ltd. Nahan Road; Paonta Sahib, District Sirmour, Himachal Pradesh-173025, India. Sodium alginate (having a viscosity of 5.5 cps in a 1% w/v aqueous solution at 25^{o}C), Ethyl cellulose and calcium chloride were procured from SD Fine Chemicals Pvt. Ltd., Mumbai, India. The fruit of *Abelmoschus esculentus Linn.* were procured from local market of Moradabad (Uttar Pradesh) and was confirmed by local peoples. All other chemicals were of analytical grades.

2.2 Extraction & Isolation of Okra gum

The method for extracting okra gum was based on procedure provided by Tavakoli et al. [25]. 1 kg of fresh, unripe and delicate okra fruits (pods) were taken from nearby vegetable market. All the fruits were cleaned and thinly sliced using a sharp knife. Due to absence of mucilage in okra seeds, the seeds were removed from okra fruits. To isolate and extract the mucilage from okra fruits, the sliced mass of okra fruits were steeped in distilled water overnight.

After soaking, the sticky gum (mucilage) was extracted; and filtered by using a white muslin cloth. Acetone was used to precipitate the gum by adding 3 part of acetone in 1 part of gum extract. In addition, the precipitated gum was then dried for about 2 weeks in desiccator containing anhydrous calcium chloride. Particle size reduction of prepared okra gum was done by using stainless steel grinder followed by passing through sieve no. 120 to obtain uniform particles of dried okra gum powder. The dried okra gum powder were stored in airtight glass containers.

2.3 Preparation of Lornoxicam Loaded Alginate-okra Gum/ Ethyl Cellulose Microspheres

Formulation batches of lornoxicam loaded alginate-okra gum/ ethyl cellulose microspheres were prepared by ionic-gelation technique using $CaCl_2$ as ionic cross-linking agent. Polymeric aqueous dispersion mixtures of Okra gum and Ethyl cellulose in different concentrations (0.25%, 0.5%, 0.75% and 1.0% w/v) with Sodium Alginate at constant ratio (1.0% w/v) were prepared in distilled water at 1000 rpm using magnetic stirrer (Remi Motors, India) for 15 min. to produce homogenous dispersion of all ingredients. Subsequently, lornoxicam was added to the above polymeric dispersion. All the formulations were prepared with an equal proportion of pure drug (50 mg) to polymer dispersion mixture (sodium alginate in constant ratio 1.0 % w/v) with different ratios of okra gum and ethyl cellulose (0.25%, 0.5%, 0.75% and 1.0% w/v) were used. Final drug-polymer mixture solutions were agitated at 1000 rpm for 25 min. using a homogenizer (Remi Motors, India) for producing uniform dispersion. Lornoxicam containing polymeric dispersion mixture were sonicated for 5 min. until they were free from bubbles. The prepared homogenized bubble-free lornoxicam loaded alginate-okra gum/ ethyl cellulose mixture were extruded drop wise into aqueous solution of $CaCl_2$ (fixed concentration i.e. 7% w/v at room temperature) through a 21 G needle (0.51 mm inner diameter) using a 10 ml hypodermic syringe. The distance of falling of the drops were 5 cm. The added droplets were retained into $CaCl_2$ solution for 20 min. for complete the curing reaction and to produce spherical rigid microspheres. The prepared

Table 1. Formulation composition of lornoxicam microspheres using natural polymer (okra gum)

Formulation Code	Pure Drug (Lornoxicam) (mg)	Sodium Alginate (% w/v)	Okra Gum (% w/v)	Calcium Chloride (% w/v)	Stirring Speed(rpm)
LSO1	50	1.0	0.25	7	800
LSO2	50	1.0	0.50	7	800
LSO3	50	1.0	0.75	7	800
LSO4	50	1.0	1.0	7	800

Table 2. Formulation composition of lornoxicam microspheres using synthetic polymer (ethyl cellulose)

Formulation Batches	Pure Drug (Lornoxicam) (mg)	Sodium Alginate (% w/v)	Ehyl Cellulose (% w/v)	Calcium Chloride (% w/v)	Stirring Speed(rpm)
LSE1	50	1.0	0.25	7	800
LSE2	50	1.0	0.50	7	800
LSE3	50	1.0	0.75	7	800
LSE4	50	1.0	1.0	7	800

microspheres were then collected by decantation, subsequently washed with distilled water atleast two times in order to remove Cl^- ions and separated out by filtration. The prepared microspheres were air dried at 37ºC for 24 hrs and the dried microspheres were stored in a desiccator at room temperature until further used [26]. Formulation variables of different lornoxicam loaded alginate-okra gum microsphere batches were given in Table 1; and lornoxicam loaded alginate-ethyl cellulose microsphere batches were given in Table 2.

3. CHARACTERIZATION AND EVALUATION OF PREPARED MICROSPHERES

3.1 Determination of Percentage Yield

After the microspheres were dried, the weight of each microsphere batches were calculated by considering the total weight of the drug and polymer used during preparation [27].

The percentage yield was calculated using the following formula:

$$\text{Percentage Yield} = \frac{\text{weight of microspheres recovered}}{\text{weight of polymers} + \text{weight of drug}} \times 100$$

3.2 Estimation of Drug Entrapment Efficiency (DEE)

Approximately 100 mg of microspheres were taken from each batch and grinded with pestle and mortar. Then dried powder was added to a 250 ml phosphate buffer, pH 6.8, and was left at 37°C ± 0.5°C for 24 hours with occasional shaking. After removing the polymer with 0.45 mm filter paper, the absorbance of lornoxicam were measured at 376 nm using a UV spectrophotometer (Shimadzu, Japan) [28]. The entrapment efficiency was calculated using the following formula:

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Actual drug content in microspheres}}{\text{Theoretical drug content in microspheres}} \times 100$$

3.3 Particle Size Analysis by Optical Microscopy

A stage micrometer was used to determine the particle size, and the average particle size was calculated by measuring approximately 200 particles with the help of a calibrated stage micrometer. A small drop of suspension (dried microspheres in 10 ml liquid paraffin) was placed on a glass slide and the diameter of atleast 100 particles were measured by using calibrated optical microscope [29].

3.4 Micromeritics Properties of Prepared Microspheres

The microspheres were characterized for micromeritic properties such as particle size, bulk density, tapped density, compressibility index, Hausner's ratio and angle of repose to investigate the flow properties of prepared microspheres [30].

(i) Bulk density

$$\text{Bulk density} = \frac{\text{Mass of Prepared Microspheres}}{\text{Bulk Volume}}$$

(ii) Tapped density

$$\text{Tapped density} = \frac{\text{Mass of Prepared Microspheres}}{\text{Volume of the Microspheres after Tapping}}$$

(iii) Compressibility index

$$\text{Carr's compressibilityindex (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

(iv) Hausner's ratio

$$\text{Hausner's Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

(v) Angle of repose

The maximum angle conceivable between the surface of a powder pile and the horizontal plane is defined as the angle of repose. The funnel method was used to determine the angle of repose of each formulation batch of prepared microspheres. The microspheres were allowed to pass through the funnel orifice onto a flat piece of paper that was placed on a horizontal surface to form a pile of microspheres.

The angle of repose wascalculated by the following equation.

$$\theta = \tan^{-1} h / r$$

Here, θ - Angle of repose; h - Height of microspheres above the flat surface; r - Radius of the circle formed by the microspheres heap.

3.5 Evaluation of Swelling Behavior of Microspheres

Swelling index was determined by measuring the extent of swelling of microspheres firstly in acidic buffer having pH 1.2 and then in phosphate buffer having pH 6.8 withthe use of empty tea bags. 100mg of microspheres were accurately weighed and placed in 500ml of appropriate buffer and left at room temperature for 24 hours. At certain intervals, these sample bags of swollen microspheres were removed, and the surface were dried with tissue paper to absorb excess water and then weighed [5].

The swelling index of prepared microspheres were calculated by using following formula:

$$\text{Swelling index (\%)} = \frac{\text{Weight of } microspheres \text{ after swelling} - \text{Dry weight of } microspheres}{Dry\ weight\ of\ microspheres} \times 100$$

3.6 Compatibility Study by Fourier Transform-infrared (FTIR) Spectroscopy Analyses

Powdered samples were analyzed as potassium bromide pellets using Fourier transform-infrared (FTIR) spectroscopy (Perkin Elmer Spectrum RX I, USA). Samples were mixed with potassium bromide at a ratio of 1:9 in the sample holder and pelletized at 100 kg pressure, then scanned between 3800-400 cm^{-1} at a resolution of 4 cm^{-1} with a scan speed of 1 cm/sec [31].

3.7 Surface Morphology Analysis

Scanning electron microscopy (SEM) was used to examine the morphology of prepared microspheres. The microspheres were carbon-glued to the support and then coated with gold in the high vacuum evaporator using a gold sputter module. After that, samples were examined at 15 kV using a scanning electron microscope (JEOL JSM- 6490 LV scanning microscope, Tokyo, Japan) [32].

3.8 Differential Scanning Colorimetry

The drug's compatibility with polymers were investigated using DSC technique. The melting point of the blends, as well as the absence of a significant shift in the melting point or the formation of a new exothermic/endothermic peak in the blend, indicated that the drug and polymer were compatible. Although the slight changes related to peak shape, peak height and their width; might be due to the variation in geometry of the mixtures [33].

3.9 Powder X-ray Diffraction (P-XRD) Analysis

XRD studies were carried out to determine the physical state of lornoxicam, polymers, and lornoxicam microspheres by using X-ray diffractometer (D8 Advance Eco, Bruker, Germany) at 40 kV and 30 mA of current. Scanning was done at a rate of 10 per minute, with a range of 50-500 [34].

3.10 *In-vitro* Drug Release Study

The *in-vitro* drug release studies of each formulation batches of prepared microspheres containing lornoxicam were carried out using USP dissolution test apparatus type-II [Electrolab (TDT-08L)]. Precisely weighed quantities of microspheres equivalent to 16 mg of drug lornoxicam from the total weight of prepared microsphere formulations were taken subjected to dissolution studies, they were packed in tea bags and placed in the basket of dissolution apparatus. The drug release from each formulation batches of prepared microspheres were subjected to dissolution medium consisted of 900 ml of 0.1N HCL (pH 1.2) for first 2 hours, followed by pH 6.8 phosphate buffer for the remaining time period up to 12 hours. The temperature of the dissolution medium was maintained at 37 ± 0.5^0C. A rotational speed of 50 rpm of dissolution basket were maintained through out the experiment. Samples (10 ml) were withdrawn at regular time intervals for a total of 12 hours and replaced with the same volume of test medium to maintain sink conditions. The withdrawn samples were suitably diluted, filtered through a 0.45µ membrane filter and analyzed spectrophotometrically at 376 nm for the presence of lornoxicam. The tests were conducted in triplicate [35].

3.11 Analysis of *in-vitro* Drug Release Kinetics and Mechanism

A variety of mathematical models were used to evaluate the *in-vitro* drug release kinetics, including the zero order, first order, higuchi, hixson-crowell cube-root model and Korsmeyer-Peppas model [5, 36].

Zero-order model: $Q = kt + Q_0$; Where Q_0 represents the starting value of Q, and Q represents the drug release time in time t; k is the rate constant.

First-order model: $Q = Q_0e^{kt}$; Where Q_0 represents the starting value of Q, and Q represents the drug release time in time t; k is the rate constant.

Higuchi model: $Q = kt^{0.5}$; Where Q refers to the amount of drug released over time t, and k refers to the rate constant.

Hixson-Crowell cube-root Model: $Q^{1/3} = kt + Q_0^{1/3}$; Where, Q is the amount of drug released in time t, Q_0 is the initial amount of the drug and kt is the rate constant.

Korsmeyer-Peppas model: $Q = kt^n$; where Q indicates as the amount of drug released per unit of time t, k as the rate constant and n as the diffusional exponent, which indicates the release mechanism for the drug.

A squared correlation coefficient (R^2) was calculated to determine the accuracy and predictive ability of the models. Again, the Korsmeyer-Peppas model was employed to distinguish between competing release mechanisms: Fickian release (diffusion-controlled release), non-Fickian release (anomalous release), and case II release (relaxation-controlled release). Fickian release occurs when n is equal to or higher than 0.43. N values between 0.43 and 0.85 are considered non-Fickian release. Whenever, n is equal to or greater than 0.85, it is a case-II transport [37].

4. RESULTS AND DISCUSSION

4.1 Preparation of Lornoxicam Loaded-Alginate Okra Gum/ethyl Cellulose Microspheres

We prepared lornoxicam loaded-alginate okra gum/ethyl cellulose microspheres using CaCl2 as cross-linking agent by means of ionic gelation. As a result of adding Sodium Alginate-Okra gum/Ethyl Cellulose polymeric dispersion mixtures containing lornoxicam to aqueous solutions containing CaCl2, rigid wet microspheres were formed. A negative charge – COO^- group of Sodium Alginate might form an electrostatic ionic interaction when exposed to $CaCl_2$ containing aqueous solutions in the current study as a cross-linking medium. Calcium ions exert an electrostatic ionic interaction with sodium alginate to form calcium alginate in an "egg box" model so that the calcium ions fit in electronegative cavities of the sodium alginate [38-40]. Cross-linking occurs at the junction zones, which are polyvalent cations that cause interfacial adhesion between polysaccharides [39]. Two polymer chains join together when Ca^{2+} ions and Na^+ ions compete for the same space within Sodium Alginate. There is a close ion-pair interaction between Ca^{2+} and $–COO^-$ of sodium alginate in the interstices between two polyuronate chains, and other electronegative oxygen atoms can coordinate [41].

Table 3. Percentage yield of prepared microsphere formulations using okra gum [LSO (1-4)] & ethyl cellulose [LSE (1-4)]

S. No.	Formulation code	Percentage yield (%)
1.	**LSO1**	81.43±0.39
2.	**LSO2**	83.53±0.02
3.	**LSO3**	85.79±0.17
4.	**LSO4**	89.26±0.15
5.	**LSE1**	77.26±0.05
6.	**LSE2**	79.65±0.04
7.	**LSE3**	82.25±0.08
8.	**LSE4**	85.44±0.04

The values are expressed as mean ± SD, n =3.

4.2 Percentage Yield

The Percentage yield of lornoxicam microspheres prepared with different concentration of polymers were presented in Table 3. The Percentage yield were found to be increased with increasing polymer concentration, progressively increased the percentage yield, possibly due to the presence of more calcium binding sites in polymer chains and hence more cross linking [41]. In the present study, we found (see in Table 3) that the percentage yield of lornoxicam-loaded Alginate-Okra gum microspheres having more percentage yield as compare to lornoxicam-loaded Alginate-Ethyl cellulose microspheres.

4.3 Drug Entrapment Efficiency

The entrapment efficiency can be defined as the % amount of drug entrapped inside the polymer. There are various factors which affect the entrapment efficiency of drug in microspheres. Some are nature of drug, polymer concentration, drug polymer ratio, stirring speed, concentration of cross-linking agents [22]. The entrapment efficiency of the drug in prepared microspheres (Okra gum & Sodium Alginate) was found to be between 76.14±0.03 to 90.00±0.05% and entrapment efficiency of the drug in prepared microspheres (Ethyl Cellulose & Sodium Alginate) was found to be between 72.35±0.02 to 79.77±0.04% presented in Table-4. We observed that by increasing the amount of Okra gum in polymer blends, drug entrapment was increased. This could be caused by the fact that the polymer-blend solutions became more viscous with increasing ratio of okra gum addition. In that way, any drug leakage into the cross-linking solution could have been prevented. Typically, the most significant entrapment efficiency of microspheres was obtained when mucilage was utilized as a matrix network with alginate; this might be due to the expansion in consistency of the polymeric arrangement featuring alginate, including other factors that prevent drug leaching [5]. Previous studies on the development of natural polysaccharide blends containing Sodium Alginate and other plant polysaccharides came up with similar results [18-24].

In the same pattern as above, drug encapsulation efficiency was also observed with ethyl cellulose blends. Thus, the crosslinking of the polymer was also increased along with the compactness of the insoluble matrix formed. Consequently, more drugs would be encapsulated within the microspheres. In this regard, the results are in agreement with the findings of Racovita et al. [19] and Ramteke et al. [26]. Furthermore, increasing the concentration of crosslinking agent failed to enhance the drug penetration because of potential saturating of $–COO^-$ binding sites in guluronic acid chain of the alginate polymer, preventing more calcium ion penetration. The drug entrapment efficiency of prepared microspheres were good, because the drug was poorly soluble in aqueous medium.

Table 4. Percentage drug entrapment of prepared microsphere formulations using okra gum [LSO (1-4)] & ethyl cellulose [LSE (1-4)]

S. No.	Formulation code	Drug entrapment efficiency (%)
1.	**LSO1**	76.14±0.03
2	**LSO2**	83.43±0.02
3	**LSO3**	87.53±0.06
4.	**LSO4**	90.10±0.05
5.	**LSE1**	72.35±0.02
6.	**LSE2**	74.37±0.04
7.	**LSE3**	76.61±0.06
8.	**LSE4**	79.77±0.04

The values are expressed as mean ± SD, n =3

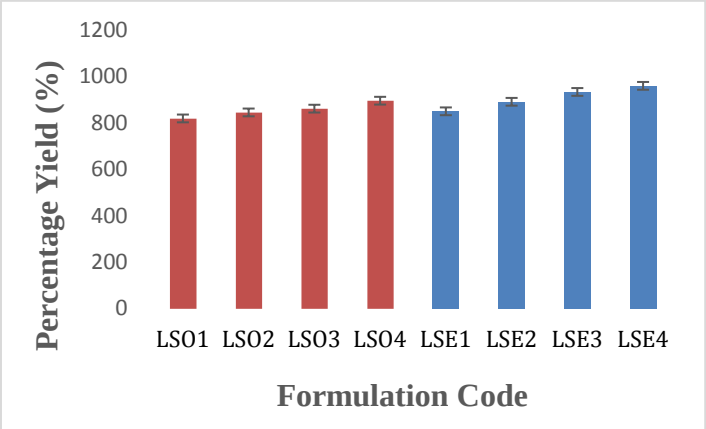

Fig. 1. Percentage yield of prepared microsphere formulations of lornoxicam
Where, LSO stands for lornoxicam loaded alginate-okra gum microspheres
LSE stands for lornoxicam loaded alginate-ethyl cellulose microspheres

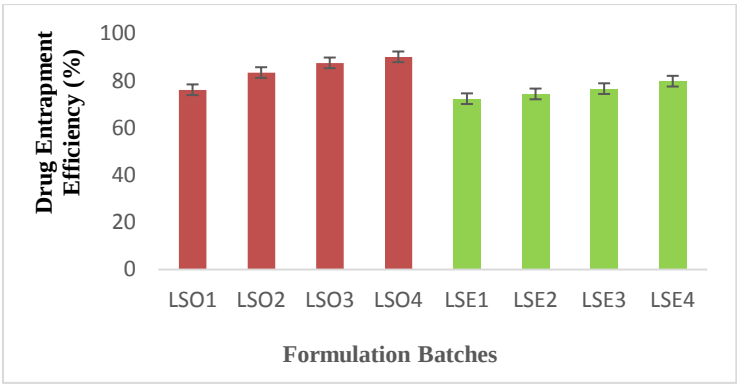

Fig. 2. Drug Entrapment Efficiency of prepared microsphere formulations of lornoxicam

4.4 Particle Size Analysis

Particle size of lornoxicam loaded alginate-Okra gum/Ethyl cellulose microspheres were prepared sucessfully with the help of ionic gelation method. Optical microscopy was used to analyse the mean particle size of microspheres. The mean particle size of lornoxicam loaded alginate-Okra gum/Ethyl cellulose microspheres were found to be in the range of 819.46 ± 0.07 µm to 895.91 ± 0.05 µm (shown in Table 5), i.e. the increase in concentration of sodium alginate to okra gum polymer (1.0:0.25) affected the particle size of microspheres and the mean particle size was found to be 819.46 ± 0.07 µm, where as at polymer concentration (1.0:1.0), it was 895.91 ± 0.05 µm. This increase in particle size may be due to increase in the viscosity of droplets with increase in polymer concentration, which results in larger droplets of emulsion.

In contrast, the mean particle size of lornoxicam-loaded ethyl cellulose-Alginate microspheres were found to be in the range of 850.18 ± 0.10 µm to 959.88 ± 0.02 µm (see in Table 5), i.e. the increase in concentration of sodium alginate to ethyl cellulose polymer (1.0:0.25) affected the particle size of microspheres and the mean particle size was found to be 850.18 ± 0.10 µm, where as at polymer concentration (1.0:1.0), it was 959.88 ± 0.02 µm. This increase in particle size may be due to increase in the viscosity of the medium at a higher ethyl cellulose concentration resulting in enhanced interfacial tension. Shearing efficiency is also diminished at higher viscosities. This results in the formation of larger particles.

4.5 Micromeritic Parameters of Prepared Microspheres

Micromeritic parameters like angle of repose, bulk density, tapped density, carr's index, hausner's ratio of all formulations are presented in Table 6. The rheological parameters like angle of repose, bulk density and tapped density of

microspheres confirms better flow and packaging properties. All the formulations showed excellent flow ability represent in terms of angle of repose (<30^{o}). Micromeritic studies were conducted to all the formulations in which LSO4 formulation has given a good bulk density.

Table 5. Particle Size of prepared microsphere formulations of lornoxicam using okra gum [LSO (1-4)] & ethyl cellulose [LSE (1-4)]

S. No.	Formulation Code	Mean particle size (µm)
1.	**LSO1**	819.46±0.07
2	**LSO2**	845.37±0.09
3	**LSO3**	861.74±0.08
4.	**LSO4**	895.91±0.05
5.	**LSE1**	850.18±0.10
6.	**LSE2**	891.22±0.04
7.	**LSE3**	933.65±0.03
8.	**LSE4**	959.88±0.02

The values are expressed as mean ± SD, n =3

As compared to all the formulations, LSO4 formulation has shown the good bulk density and tapped density i.e. 1.354±0.005 and 1.199±0.013gm/ml respectively. All the formulations have excellent flow properties but LSO4 has given the best flow property as compared to other formulations.

4.6 Surface Morphology of Microspheres

The surface morphology of sodium alginate-okra gum/ethyl cellulose microspheres loaded with lornoxicam was examined with the help of scanning electron microscope. It was observed that the microspheres prepared with sodium alginate-okra mucilage almost spherical and regular in shape [33]. Thus, the drug was entrapped strongly in polymeric network, also the surface smoothness was found to be increased with increasing in okra gum concentration. The formulation containing Sodium alginate-okra mucilage in the ratio of (1.0:0.25) exhibited microsphere with smooth and wrinkled at surface (Fig. 4A), as the concentration of okra mucilage was increased from (1.0:0.50, 1.0:0.75, 1.0:1.0), the surface smoothness was also increased (Fig. 4B).

On the other hand, microspheres prepared with different concentrations of ethyl cellulose indicating the discrete, uniform, and rough surface morphology due to higher concentration of drug uniformly dispersed at the molecular level in the sodium alginate matrices. There are no crystals on surface which states that the drug is uniformly distributed (Fig. 5A & 5B).

4.7 FTIR Spectroscopy

The FTIR spectra of pure drug lornoxicam, sodium alginate, isolated okra gum, ethyl cellulose, lornoxicam-loaded okra gum-alginate microspheres (LSO4), lornoxicam-loaded ethyl cellulose-alginate microspheres (LSE4), all of them are indicated in figures. In the FTIR spectrum of pure lornoxicam, principal absorption peaks were appearing at 3423 cm^{-1} due to the -NH stretching, 3066.5 cm^{-1}due to aromatic/heterocyclic C-H stretching. The other prominent absorption bands appeared at 1637.62 cm^{-1} due to –C=O stretching. The peaks at 1425.3 cm^{-1} correspond to O=S=O group, 828.6 cm^{-1}due to –C-Cl stretching vibrations (Fig. 6a).

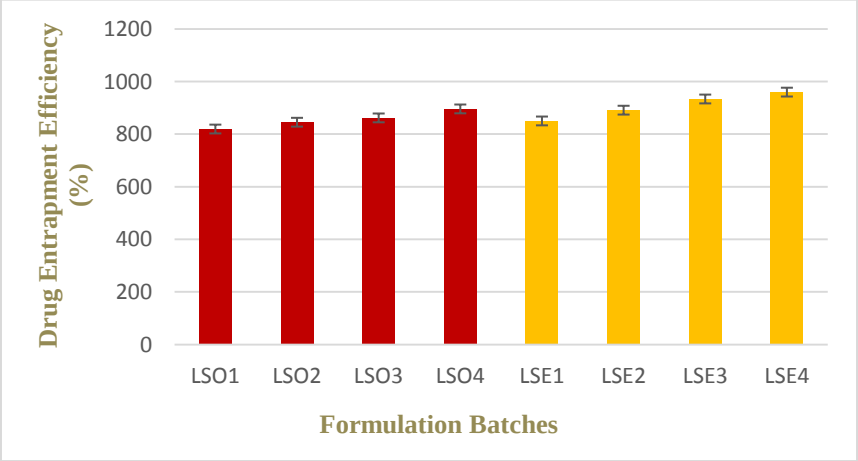

Fig. 3. Particle size of different formulations of prepared lornoxicam microspheres

Table 6. Flow Properties of prepared microsphere formulations using okra gum [LSO (1-4)] & ethyl cellulose [LSE (1-4)]

Formulations Code	Bulk Density (g/mL)	Tapped Density (g/mL)	Carr's Index (%)	Hausner's Ratio	Angle of Repose (θ)
LSO1	1.241±0.002	1.212±0.001	16.66±0.012	1.138±0.07	26.35±0.03
LSO2	1.077±0.003	1.256±0.001	11.73±0.015	1.217±0.08	26.33±0.02
LSO3	1.178±0.011	1.337±0.012	15.32±0.011	1.219±0.04	27.49±0.03
LSO4	1.354±0.005	1.199±0.013	10.40±0.018	1.128±0.09	25.38±0.04
LSE1	1.038±0.002	1.126±0.001	12.76±0.015	1.130±0.02	28.34±0.01
LSE2	1.036±0.003	1.428±0.002	14.63±0.014	2.055±0.04	30.41±0.07
LSE3	1.138±0.002	1.228±0.002	15.31±0.014	1.121±0.02	27.31±0.03
LSE4	1.148±0.002	1.148±0.002	13.31±0.016	2.225±0.01	29.50±0.03

The values are expressed as mean ± SD, n =3.

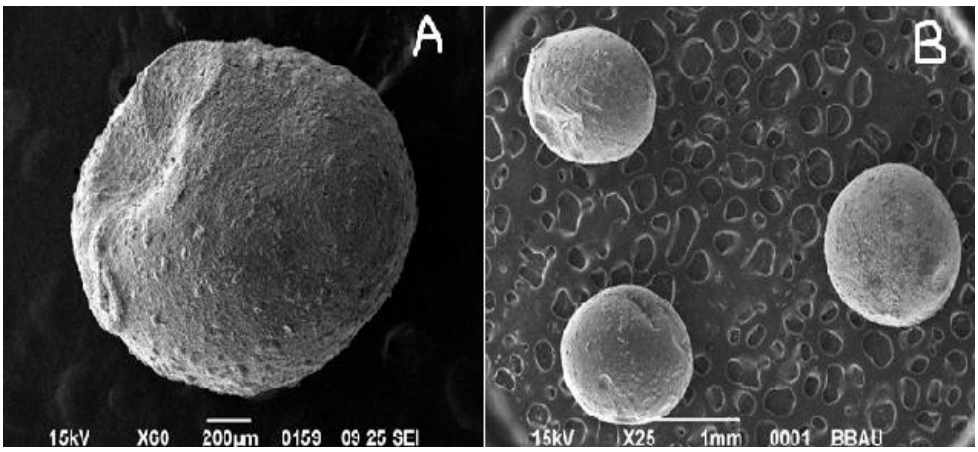

Fig. 4. The surface morphology of lornoxicam-loaded okra gum-alginate microspheres (LSO4) visualized by SEM, (A) at 200 µm (B) 1 mm

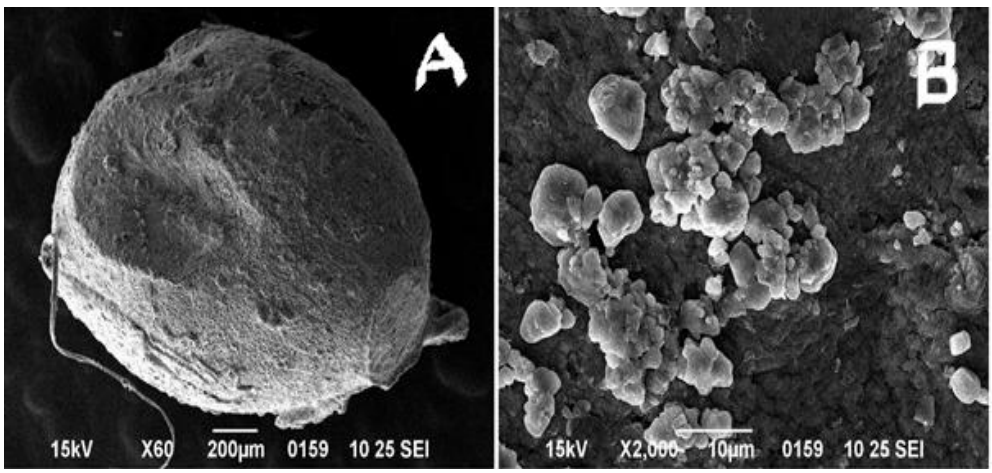

Fig. 5. The surface morphology of lornoxicam-loaded ethyl cellulose-alginate microspheres (LSE4) visualized by SEM (A) at 200 µm (B) 10µm

In the FTIR spectrum of Sodium Alginate, the characteristic peaks were appeared 1415.7 cm^{-1} and 1611.1cm^{-1}, for symmetric and asymmetric -C=O stretching vibrations of $–COO^-$ anions, respectively. In addition, a wide band at 3434.6cm^{-1} were appeared due to the –OH stretching vibrations (Fig. 6b).

The spectrum of isolated Okra gum showed an identical small peak at 1422.7cm^{-1}due to -C–H bend, a small peak at 1727.8 cm^{-1} due to -C=O stretch, peak at 2928.0 cm^{-1} due to C–H stretch and a broad band at 3372.3 cm^{-1} due to –OH stretching vibrations (Fig. 6c).

In Fig. 7, the FTIR spectra of Ethyl Cellulose, shows absorption bands for –C–O–C– stretching vibrations (1052 cm^{-1}), –CH stretching bands (2969 cm^{-1} and 2875 cm^{-1}), –CH bending (1370 cm^{-1}) and –OH stretching (3473 cm^{-1}) (Fig. 6d).

In the FTIR spectrum of lornoxicam-loaded okra gum-alginate microspheres (LSO4) and lornoxicam-loaded Ethyl cellulose-alginate microspheres (LSE4), various characteristic peaks of pure lornoxicam, Sodium Alginate, isolated Okra gum and Ethyl cellulose were appeared without any significant shifting or deviation of these above mentioned characteristic peaks, which were evidenced in their individual spectrum (see in Fig. 6e & 6f). Although there were slight shift in the peaks of IR spectra but considered to be not significant. Therefore, it was clear that lornoxicam maintained its identity after formulation of lornoxicam-loaded okra gum-alginate microspheres (LSO4) and lornoxicam-loaded Ethyl cellulose-alginate microspheres (LSE4) through ionic-gelation technique and there was no drug-excipient interactions.

4.8 Differential Scanning Calorimetry (DSC) Analysis

The DSC curve of pure drug lornoxicam (Fig. 7a), Okra gum (Fig. 7b), Sodium Alginate (Fig. 7c), Ethyl cellulose (Fig. 7d), lornoxicam-loaded alginate-Okra gum microspheres (Fig. 7e), and lornoxicam-loaded alginate-Ethyl cellulose microspheres (Fig. 7f) are shown in figures. It was evident from the DSC curves that pure drug lornoxicam was typical of a crystalline substance, exhibiting a sharp exothermic peak (221.84^{o}C and 278.59^{o}C) at temperature corresponding to its melting point (Fig. 7a). DSC curve of okra gum/mucilage showed a broad endothermic peak at 123.17ºC as can be seen in Fig. 7b. Sodium alginate peaks occurred at temperatures > 85^{o}C (Fig. 7c). DSC curve of Ethyl Cellulose has an endothermic peak at temperatures around 120ºC and also a small peak at 226ºC (Fig. 7d).

The thermograms of lornoxicam-loaded alginate-Okra gum/Ethyl cellulose microspheres showed the existence of the drug exothermic peak but the intensity of the peak was slightly diminished in the formulation, which can be due to the dilution factor (see in Fig. 7e & 7f), which could indicate the absence of interactions between pure drug lornoxicam and polymers used to prepare microspheres. The DSC profile of the drug appeared at the temperature corresponding to its melting point in the lornoxicam-loaded alginate-Okra gum/Ethyl cellulose microspheres but with loss of its sharp appearance. It appears that there is a significant reduction of drug crystallinity in the microspheres. The DSC study apparently revealed that the drug was compatible with the polymer and neither drug decomposition nor drug-polymer interactions occurred in the freshly prepared microspheres.

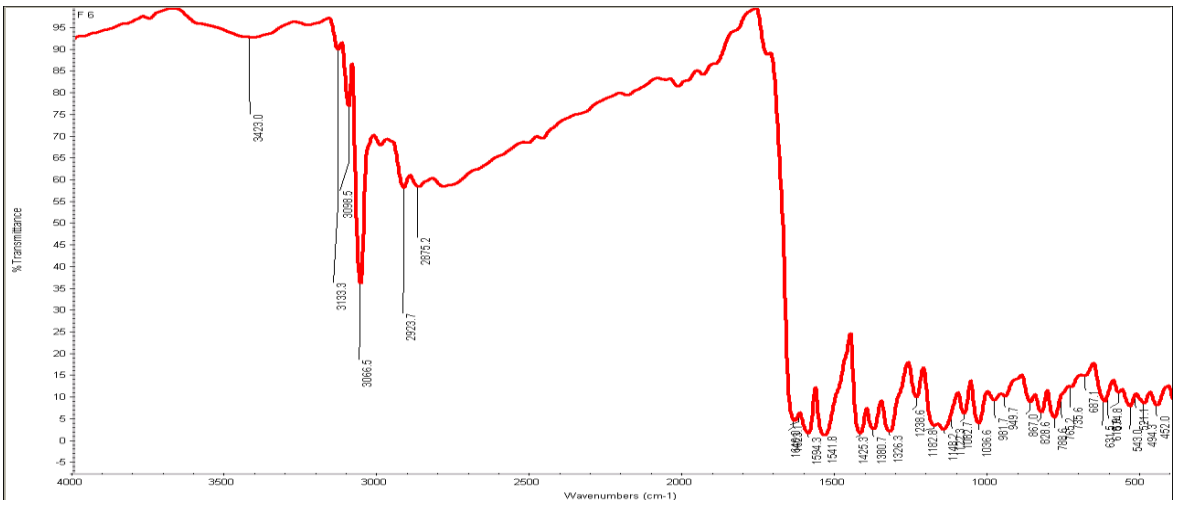

Fig. 6 (A). FTIR Spectra of Pure drug Lornoxicam

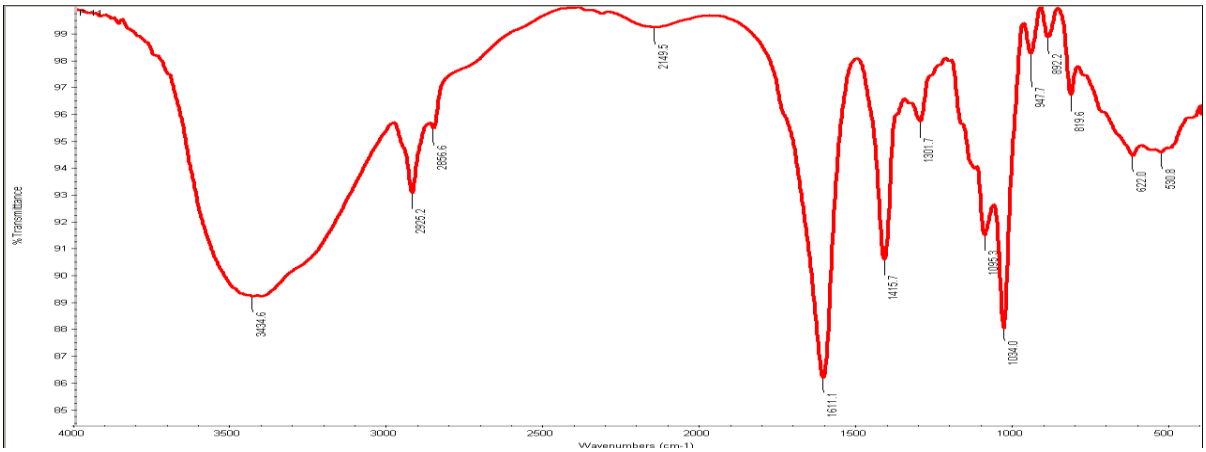

Fig. 6 (B). FTIR Spectra of Sodium Alginate

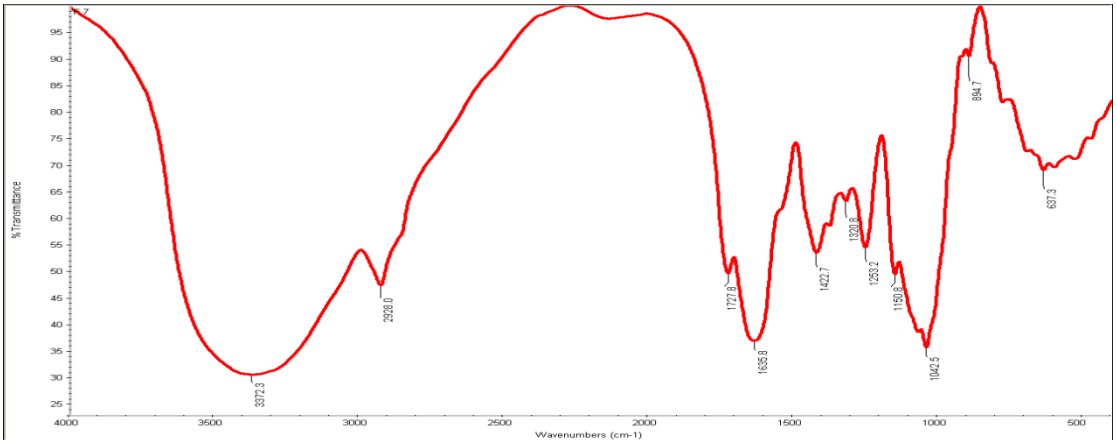

Fig. 6 (C). FTIR Spectra of isolated Okra Gum

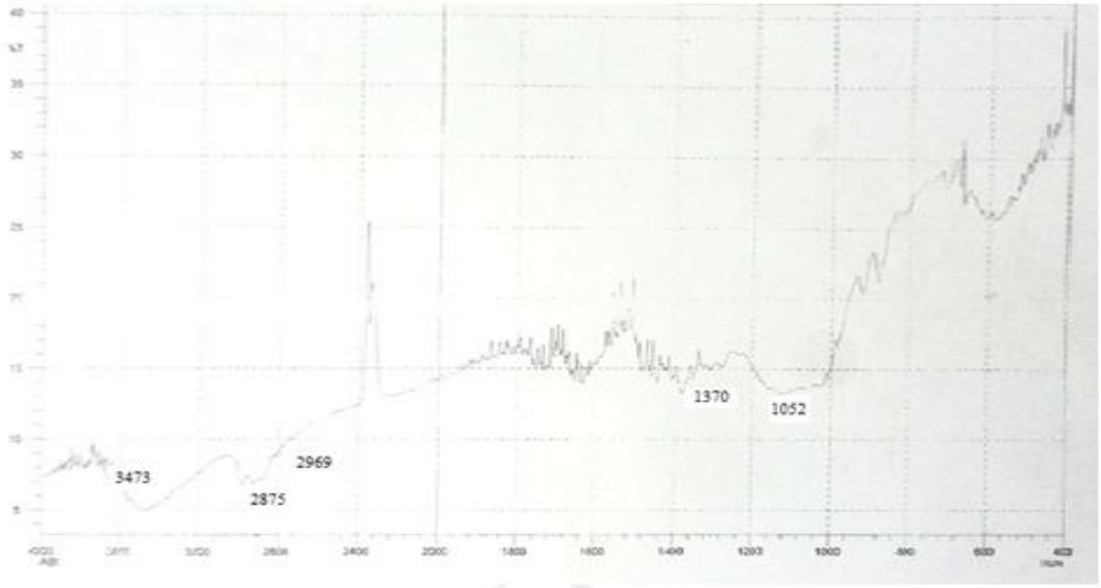

Fig. 6 (D). FTIR Spectra of Ethyl Cellulose

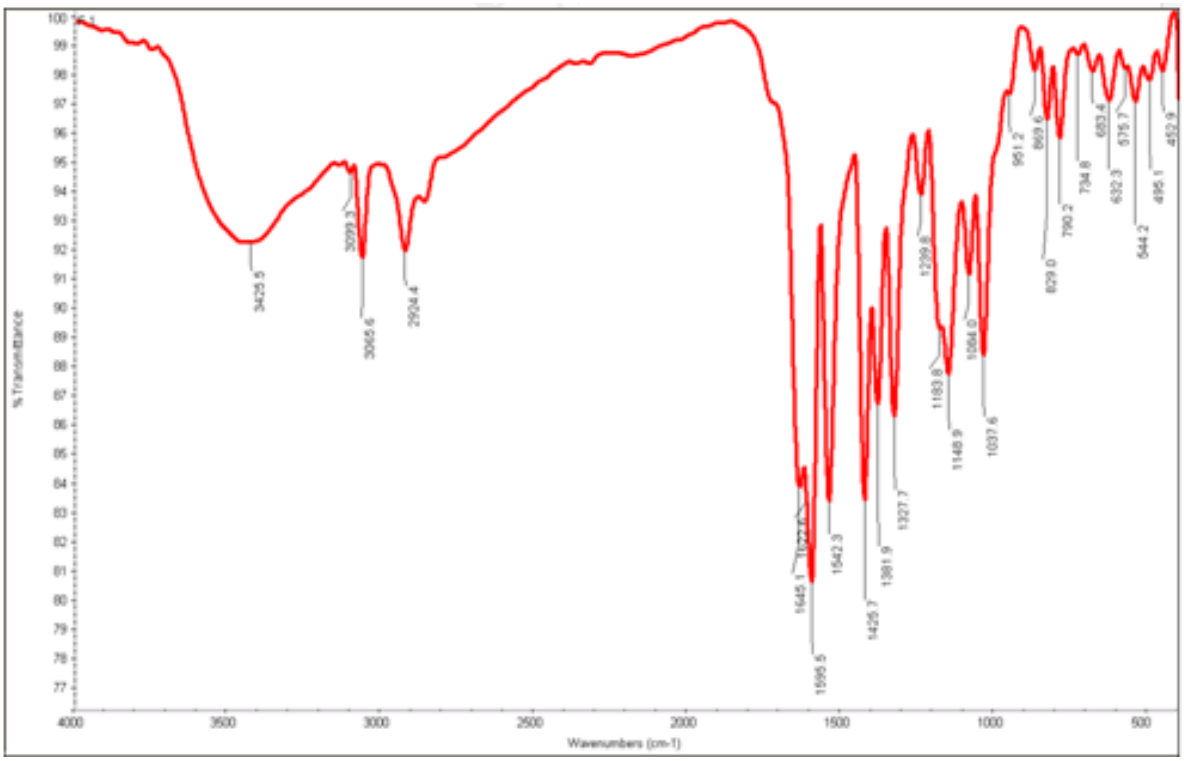

Fig. 6 (E). FTIR Spectra of lornoxicam-loaded okra gum-alginate microspheres

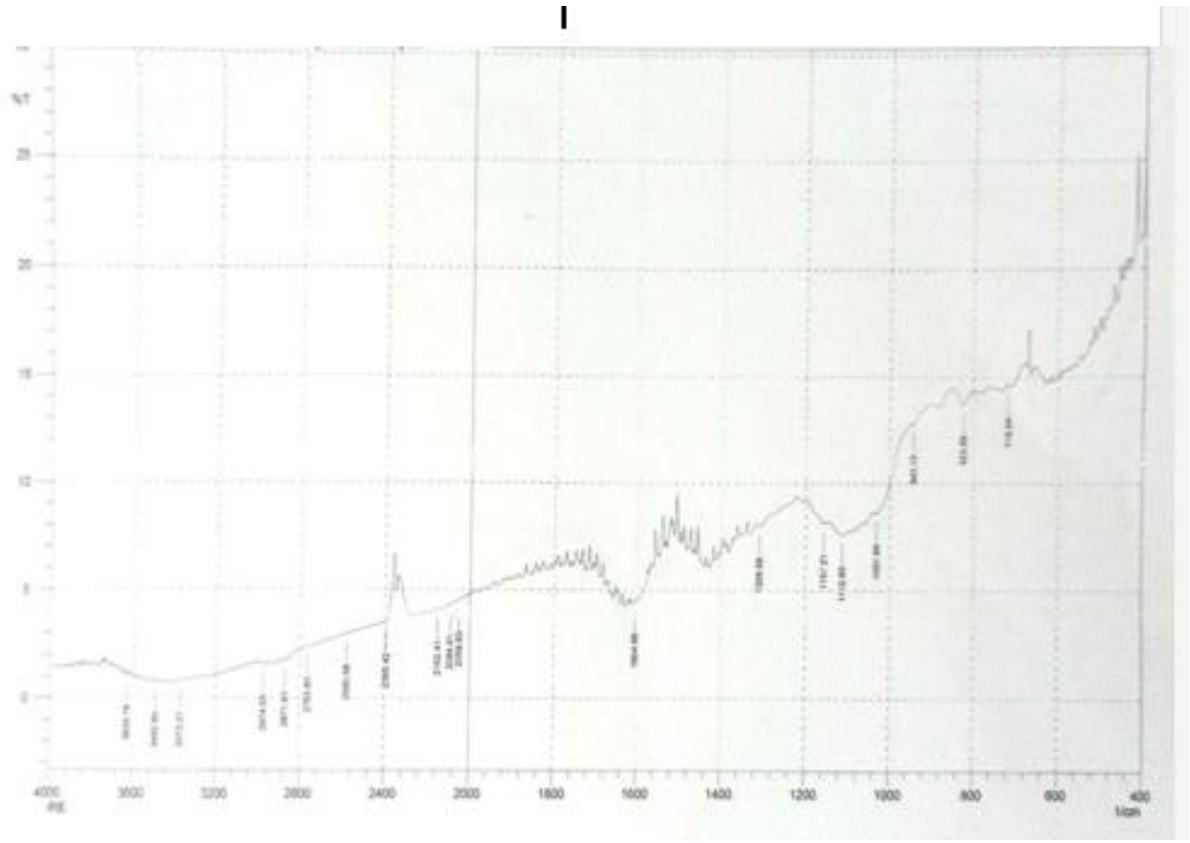

Fig. 6 (F). FTIR Spectra of lornoxicam-loaded ethyl cellulose-alginate microspheres

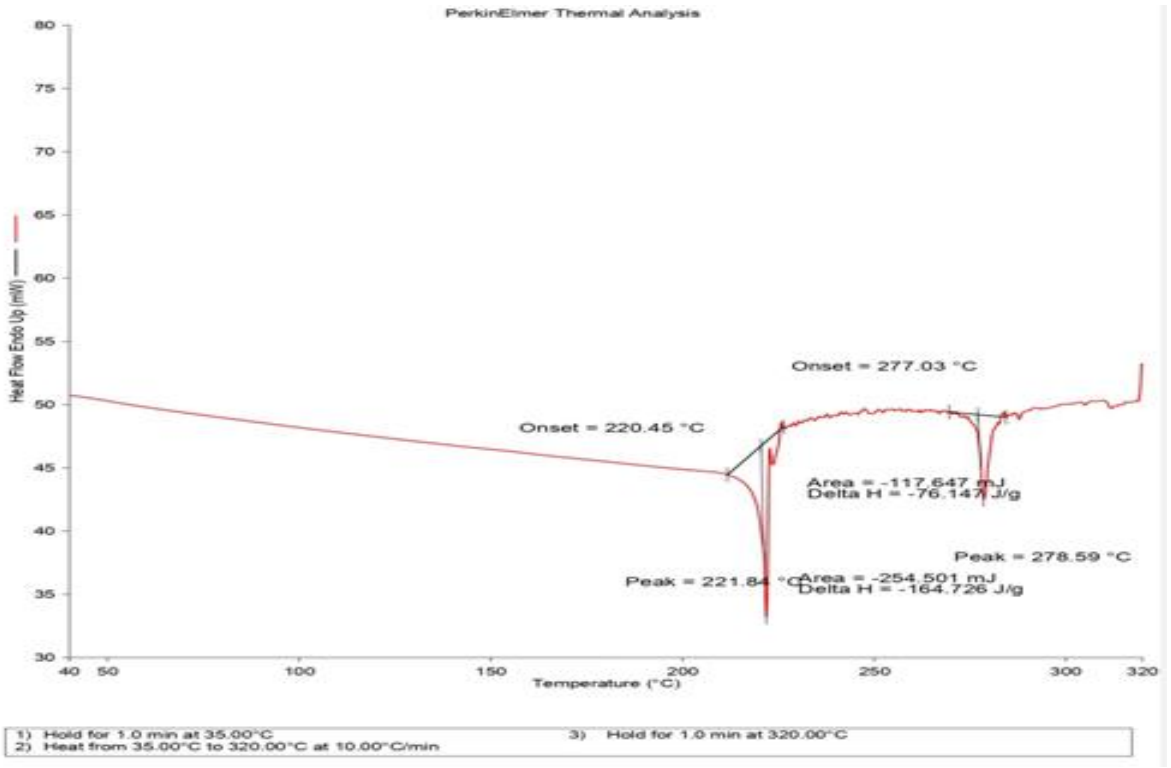

Fig. 7 (A). DSC thermogram of Pure drug Lornoxicam

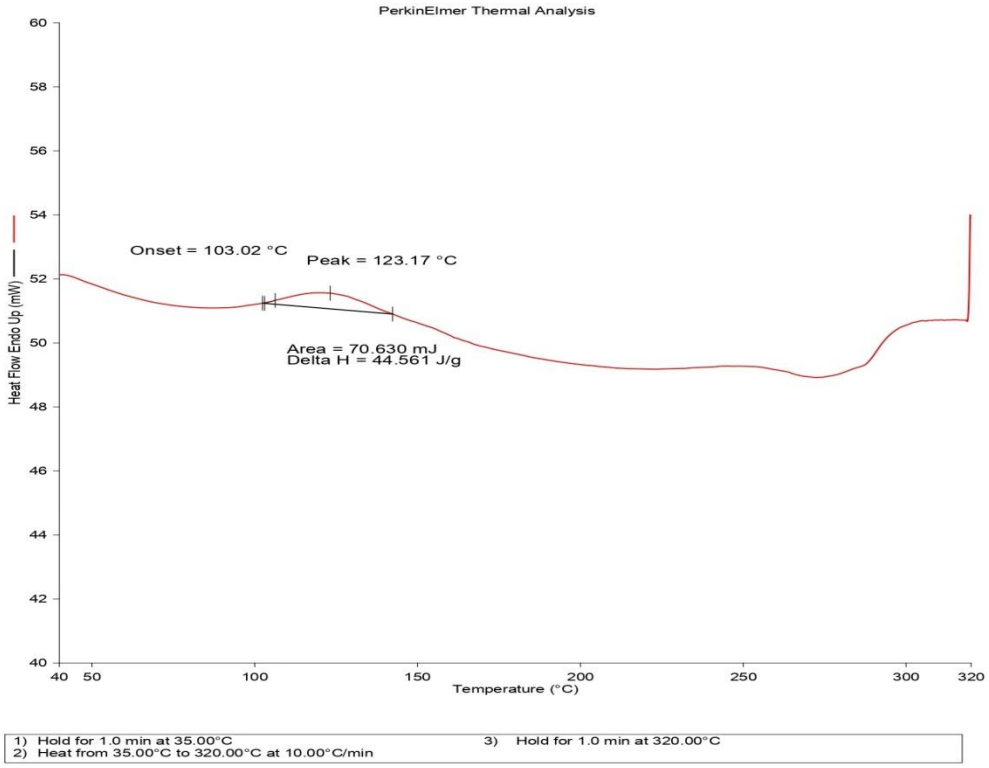

Fig. 7 (B). DSC thermogram of isolated Okra mucilage

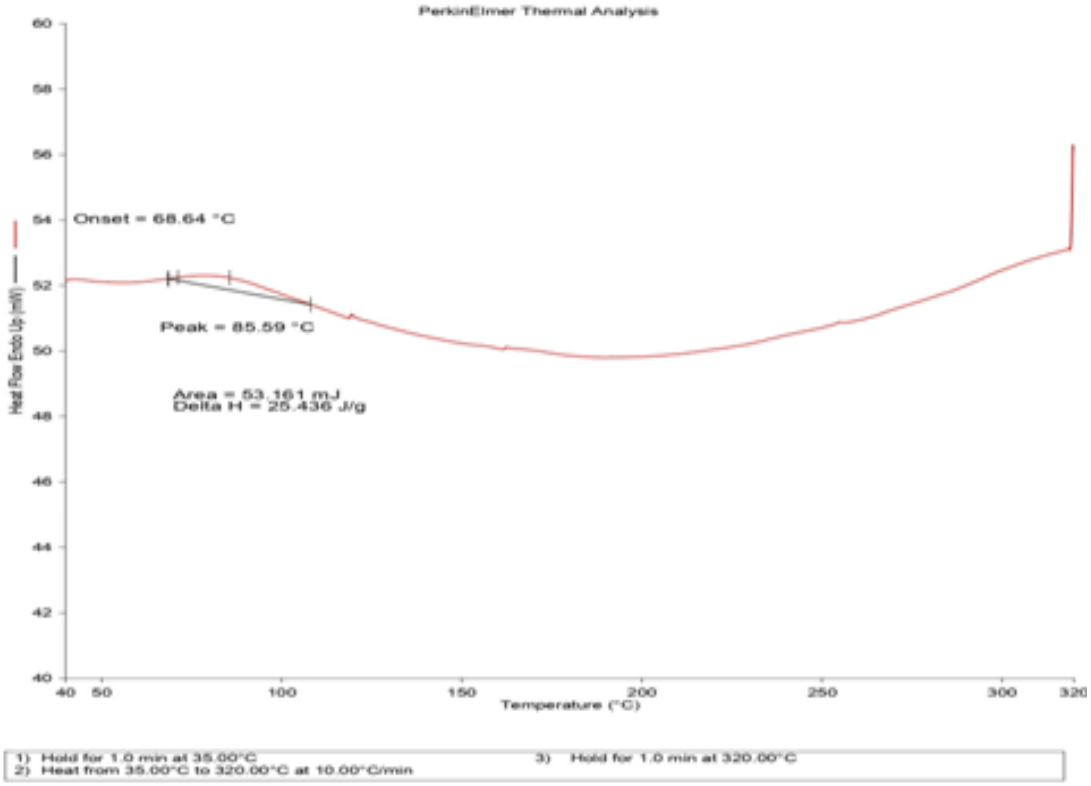

Fig. 7 (C). DSC thermogram of Sodium Alginate

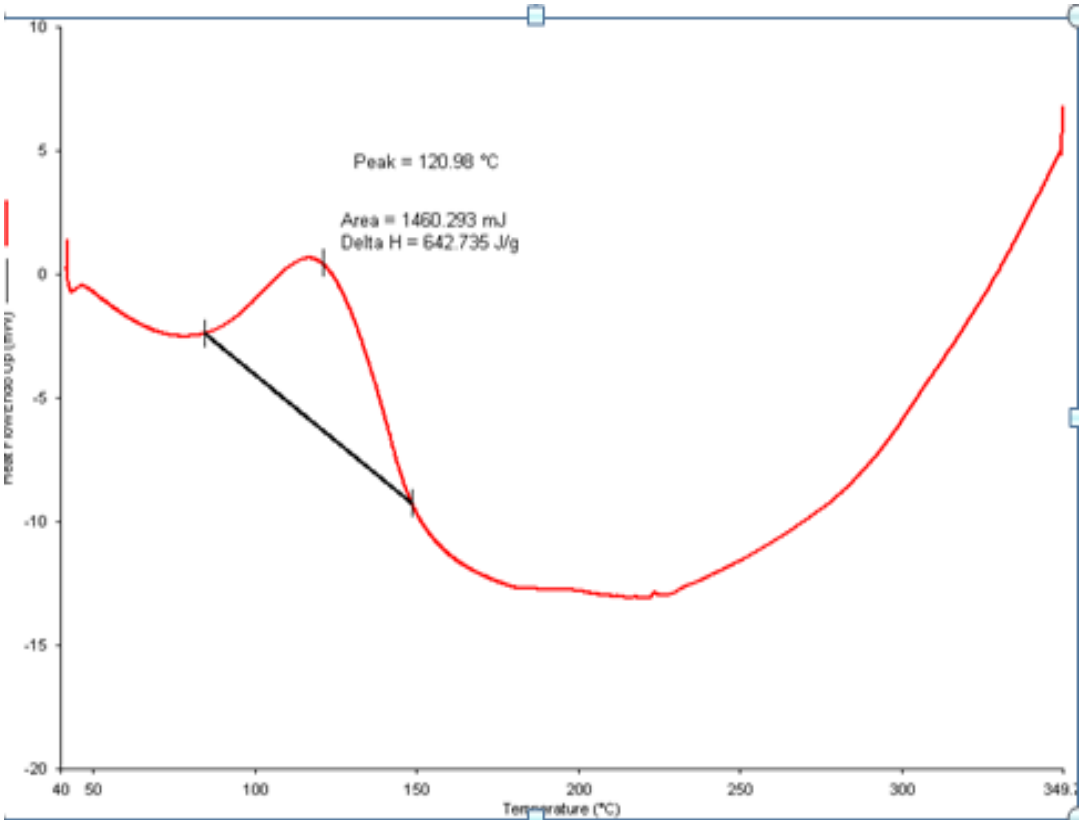

Fig. 7 (D). DSC thermogram of Ethyl Cellulose

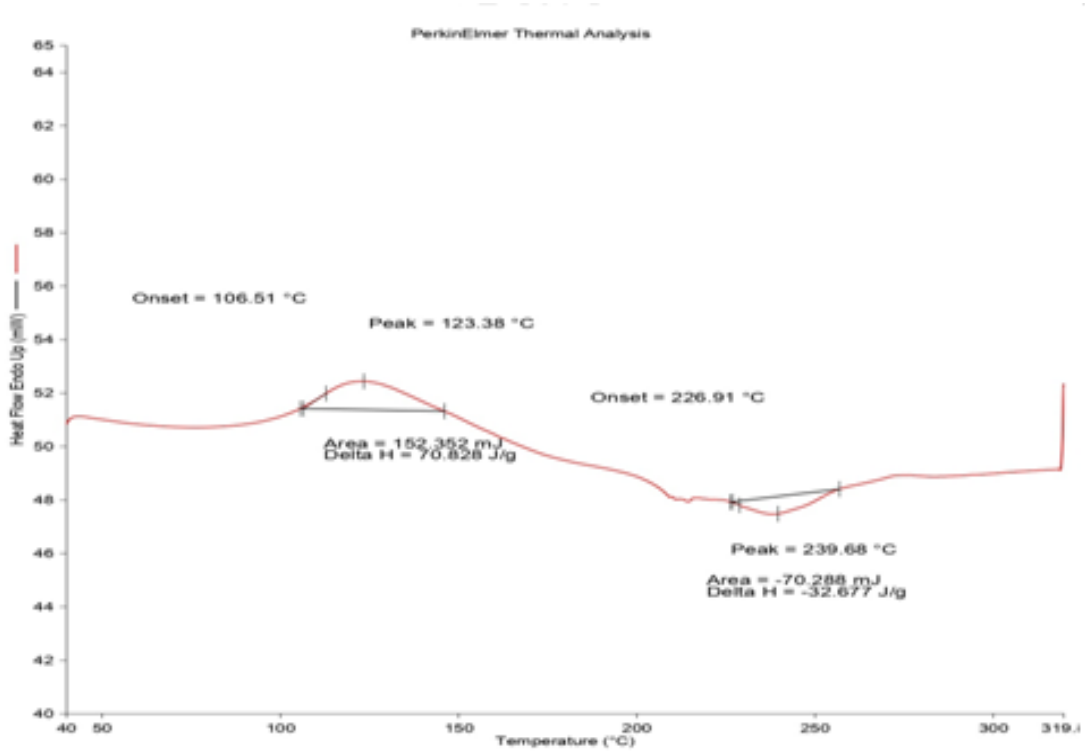

Fig. 7 (E). DSC thermogram of lornoxicam-loaded Okra gum-alginate microspheres

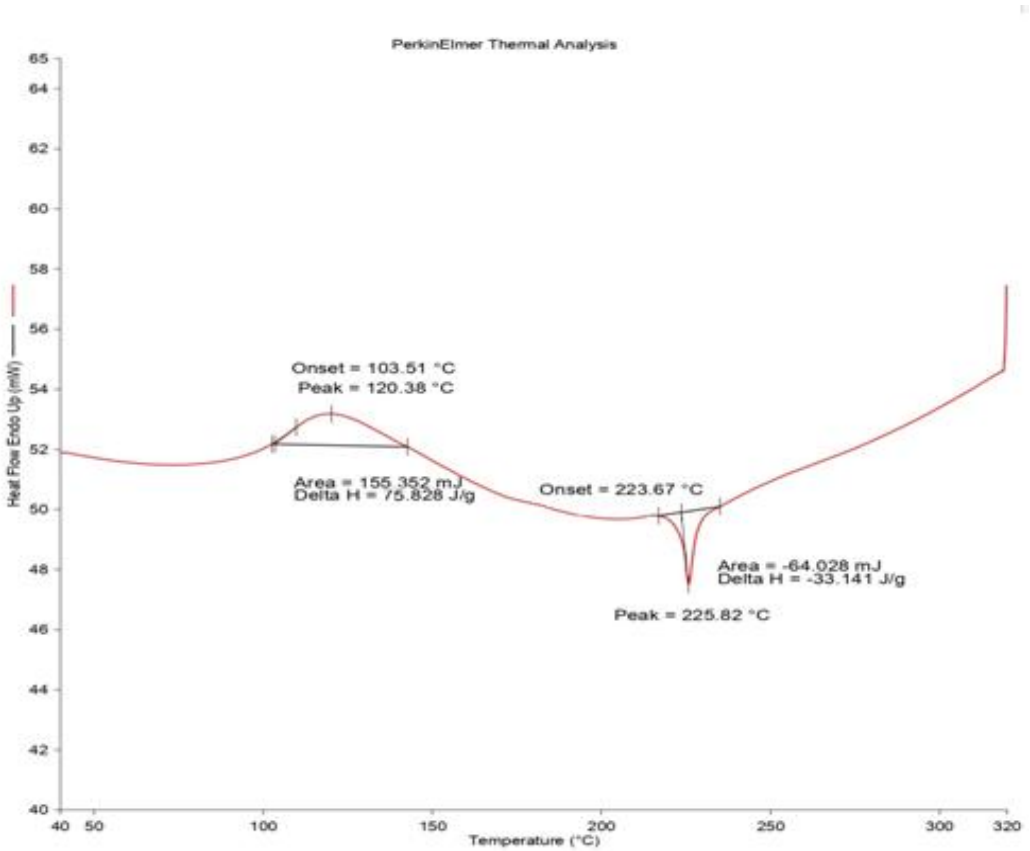

Fig. 7 (F). DSC thermogram of lornoxicam-loaded ethyl cellulose-alginate microspheres

4.9 Powder X-ray Diffraction (P-XRD) Analysis

The XRD patterns of pure drug Lornoxicam, optimized lornoxicam-loaded alginate-okra gum microspheres (LSO4) and lornoxicam-loaded alginate-ethyl cellulose microspheres (LSE4) at different scattering angles from 0^o to 100^o are presented in Fig. 8b and 8c, respectively. The diffractogram of lornoxicam showed important characteristic peaks at different scattering angles ranges from 3^o to 40^o with different signal intensities, which refers to the crystalline nature of lornoxicam (see in Fig. 8a). It was observed that the broad peaks in a diffractogram were at around 13.6° and 25.8° for lornoxicam. The intensity of these characteristic peaks of lornoxicam was found decreased significantly in the diffractogram of optimized lornoxicam-loaded alginate-okra gum microspheres (LSO4) and lornoxicam-loaded alginate-ethyl cellulose microspheres (LSE4) indicating the reduction of crystallinity of lornoxicam and existence of most of the lornoxicam in amorphous state within the optimized lornoxicam microspheres. However, decreases in the peak intensity and the baseline shift of the diffractogram were also observed due to presence of polymers in microspheres when compared to the pure lornoxicam. The changes of lornoxicam crystals within optimized formulations of lornoxicam microspheres could be due to progressive amorphization and/or dissolution of the drug (Lornoxicam) inside the polymeric-blend matrix during formulation.

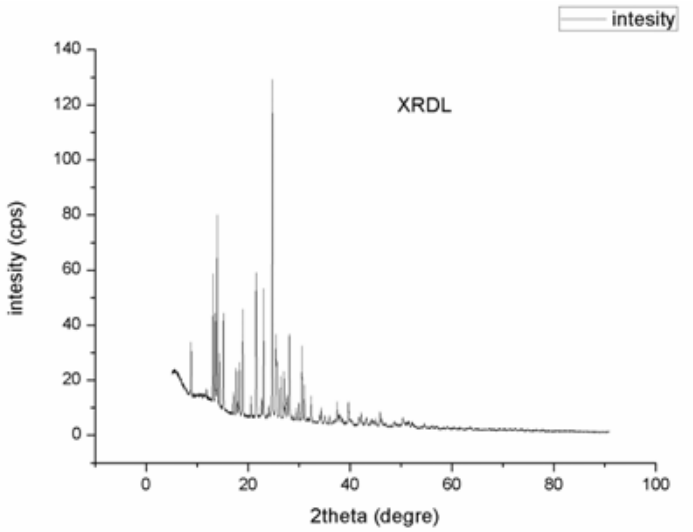

Fig. 8 (A). XRD Pattern of Pure Drug Lornoxicam

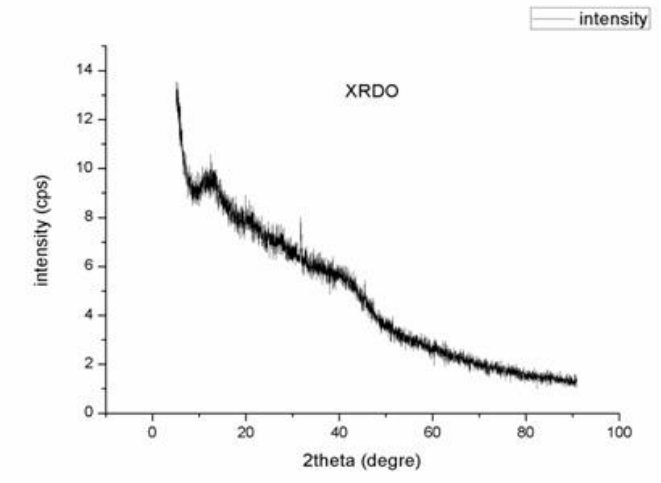

Fig. 8 (B). XRD Pattern of Lornoxicam-loaded alginate-okra gum microspheres (LSO4)

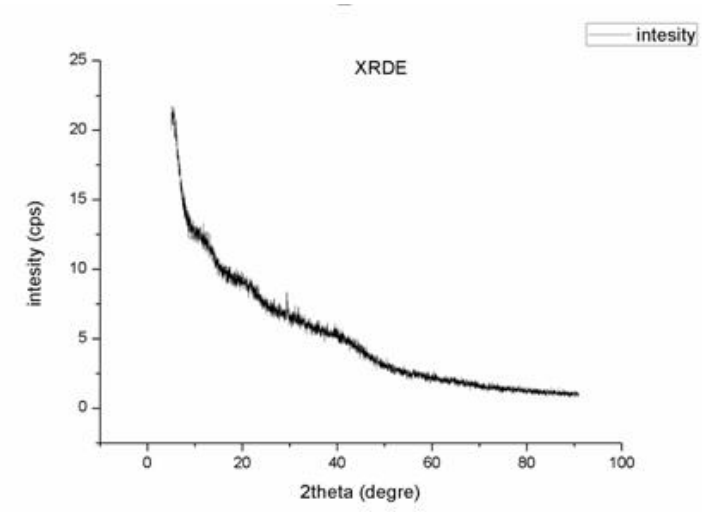

Fig. 8 (C). XRD Pattern of Lornoxicam-loaded alginate-ethyl cellulose microspheres (LSE4)

4.10 Swelling Behavior of Microspheres

The swelling behaviour of Lornoxicam-loaded alginate-okra gum/ethyl cellulose microspheres were evaluated in both gastric acidic pH (0.1 N HCl, pH 1.2) and intestinal alkaline pH (phosphate buffer, pH 6.8). The results of swelling of microspheres were shown in Fig. 9A & 9B. The concentration of polymers has a positive effect on the swelling index as it increases with increasing the concentration of polymers especially with the amount of okra gum because it is a macromolecular polysaccharide having good swelling capability. Lornoxicam-loaded alginate-okra gum/ethyl cellulose microspheres initially had a lower swelling index in acidic pH compared to alkaline pH. When calcium alginate-based microspheres are exposed to acidic pH, swelling is minimal. A minimum swelling in acidic pH may result from proton-Ca^{2+} ion exchange, form insoluble alginic acid regions and then solvent penetration into the gel-network. We observed maximum swelling at pH 6.8 in phosphate buffer after 9 hours, followed by erosion and dissolution of microspheres. According to previous work, the swelling of calcium alginate in presence of Ca^{2+} capturing agents like phosphates is caused by progressive displacement of Ca^{2+} ions within the calcium alginate microspheres [42]. As a consequence, phosphate ions (in phosphate buffer) can induce swelling of alginate-based microspheres by virtue of their calcium sequestrant properties [43]. The swelling of okra gum-alginate microspheres in phosphate buffer, pH 6.8, could therefore be explained by the ion exchange between Ca^{2+} ions present in okra gum-alginate microspheres and the Na^{+} ions present in phosphate buffer under the influence of calcium-sequestrant phosphate ions. As a result, matrix erosion and dissolution of microspheres caused by disaggregation of okra gum and alginate matrix were observed. It has been suggested that these microspheres slowly erode as a result of degradation of the polymeric backbone into smaller molecular weight components [44]. Based on the results, it appears that the dried microspheres swelled slightly in an acidic pH condition. Hence, the microspheres will subsequently swell to an appreciable size when they are transferred to alkaline pH of the stomach, thereby forming a thick layer surrounding the surface of the microspheres, largely sustaining the release of incorporated drug (lornoxicam). Despite the higher pH in the intestine, these microspheres were found to get rapidly dissolved in the higher pH of okra gum-alginate, making them suitable for the intestinal delivery of lornoxicam. Moreover, the obtained results indicated that okra gum has a higher swelling proportion than ethyl cellulose, which may be due to its higher hydrophilicity than cellulose [45]. A significant increase in polymer concentration was also found to increase swelling percentage [46]. In a study, Yadav and Jain, reported that Metformin microballons swelling increased in correlation with a higher concentration of sodium alginate [47].

Table 7. Swelling Index of prepared microspheres of okra gum in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8)

Time in Hrs	Swelling Index (%)			
	LSO1	LSO2	LSO3	LSO4
0	0	0	0	0
1	31.43±0.01	51.43±0.59	55.35±0.92	69.17±0.04
2	88.12±0.09	110.06±0.28	118.83±0.31	131.34±0.22
3	150.63±0.51	169.78±0.72	180.37±0.23	205.09±0.91
4	220.29±0.32	244.16±0.05	257.71±0.41	284.16±0.72
5	285.31±0.21	305.24±0.32	325.05±0.02	359.48±0.29
6	350.28±0.17	379.74±0.82	405.17±0.21	439.14±0.09
7	430.67±0.19	463.41±0.08	490.23±0.52	529.06±0.31
8	520.44±0.66	561.58±0.56	589.58±0.81	625.81±0.12
9	600.76±0.42	630.94±0.42	655.89±0.06	690.11±0.03
10	536.99±0.99	582.16±0.05	607.64±0.12	647.26±0.99
11	450.15±0.75	501.03±0.23	547.18±0.09	585.91±0.81
12	375.33±0.02	430.58±0.12	480.04±0.31	525.36±0.29

The values are expressed as mean ± SD, n =3.

Table 8. Swelling Index of prepared microspheres of ethyl cellulose in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8)

Time in Hrs	Swelling Index (%)			
	LSE1	LSE2	LSE3	LSE4
0	0	0	0	0
1	15.11±0.21	20.11±0.99	28.11±0.17	39.11±0.33
2	35.23±0.78	43.23±0.41	53.23±0.31	65.23±0.03
3	63.28±0.09	68.28±0.84	76.28±0.01	90.28±0.91
4	91.62±0.72	101.62±0.49	112.62±0.73	126.62±0.02
5	127.65±0.26	139.65±0.11	150.65±0.28	163.65±0.93
6	158.09±0.32	169.09±0.53	184.09±0.07	207.07±0.46
7	179.71±0.07	194.73±0.12	212.73±0.19	227.73±0.05
8	175.69±0.33	190.70±0.99	208.77±0.77	223.75±0.96
9	171.70±0.41	187.31±0.81	199.71±0.49	216.7±0.33
10	168.31±0.88	182.31±0.06	194.31±0.21	213.31±0.48
11	166.48±0.46	175.48±0.97	190.48±0.99	211.48±0.07
12	164.92±0.08	172.92±0.34	187.92±0.52	208.92±0.55

The values are expressed as mean ± SD, n =3.

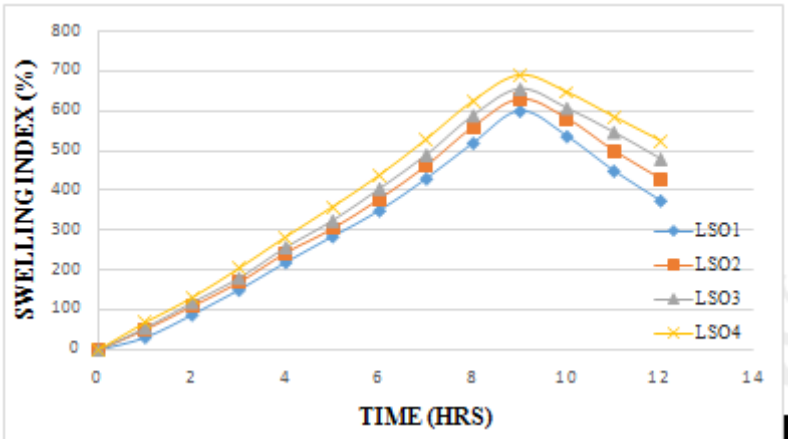

Fig. 9 (A). Swelling behavior of different formulations of okra gum microspheres in 0.1 N HCl, pH 1.2 and phosphate buffer, pH 6.8 (Mean ± S.D., n = 3)

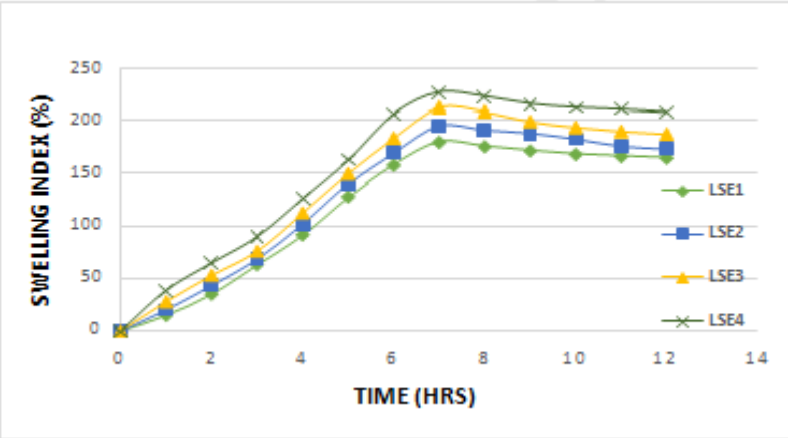

Fig. 9 (B). Swelling behavior of various formulations of ethyl cellulose microspheres in 0.1 N HCl, pH 1.2 and phosphate buffer, pH 6.8 (Mean ± S.D., n = 3)

4.11 *In-vitro* Drug Release Studies

For the *in-vitro* drug release studies, all the lornoxicam-loaded okra gum-alginate microspheres (LSO1 to LSO4) were placed in 0.1 N HCl for 2 h, then in phosphate buffer for 10 h at pH 6.8. The *in-vitro* drug release was prolonged over 12 hours with these microspheres (Fig. 10). It was found that all formulations were found to exhibit negligible drug release in acidic media pH 1.2 due to shrinkage of the gel network created by alginate microspheres [20]. At the initial stage, a small amount of drug might be released from these microspheres due to drug adhering to their surfaces. Afterward, we observed faster drug release from microspheres in phosphate buffer (pH, 6.8), which could have resulted from their higher swelling rate at alkaline pH. Furthermore, we found that by increasing the ratio between okra gum to sodium alginate in the polymer-blends, the drug release was slowed down. A higher okra gum content in alginate-okra gum microspheres (i.e., sodium alginate: okra gum 1.0: 1.0) would be required to bind well with water and possibly form a viscous gel structure. This could block the pores on the surfaces of the microspheres and ensure sustained release of the drug from these microspheres. The results of the *in-vitro* study of drug release also demonstrated that with a 7% w/v concentration of cross-linker ($CaCl_2$) in cross-linking solutions, the drug release was reduced significantly. Alginate and Ca^{2+} ions form a tight junction between the residues of alginate with the Ca^{2+} ions during ionic gelation of β-D-mannuronic acid and ∞-L-guluronic acid residues. Using a high concentration of cross-linkers in the ionic-gelation of alginate based microspheres leads to a rigid polymer structure caused by the contraction of microvoids. In this case, the higher degree of cross-linking could facilitate poor entry of the liquid dissolution medium into the polymer matrix, potentially delaying the release of the drug. A similar result was obtained in previous investigation of ionically-gelled plant polysaccharide-alginate microspheres [20-26]. For instance, *in-vitro* studies were carried out on lornoxicam-loaded ethyl cellulose-alginate microspheres (LSE1 to LSE4), when the sample was placed in 0.1 N HCl (pH, 1.2) for the first two hours and then in phosphate buffer up to 10 hours (pH, 6.8). Interestingly, these microspheres released drugs *in-vitro* for up to 12 hours (Fig. 11). Calcium alginate, which shrinks at acidic pH (as calcium alginate is pH sensitive), and poor solubility of calcium alginate at lower pH, probably contribute to the reduced amount of drug released from ethyl cellulose-alginate microspheres in acidic medium. Ethyl cellulose-alginate microspheres release the drug depending on several factors, including penetration of the dissolution medium into the microspheres, eventual swelling and diffusion of the swollen matrix, dissolution of the alginate matrix, and following dissolution of the leached out drug. Microspheres released 23.227±0.34% to 34.599±0.34% of the drug in the first 2 h in SGF (0.1 N HCl pH, 1.2), and after that the remaining drug was released in the SIF (pH, 6.8 phosphate buffer), which lasted up to 12 h, where releasing 93.971±0.20% to 98.717±0.23% of the drug, as shown in Table 9. Despite the fact that the drug is insoluble in water, we observed that the initial burst release and per cent cumulative drug release decreased as the ethyl

cellulose concentration was increased. In microspheres with increase ethyl cellulose concentration, polymer matrix density increases, which results in increased diffusional path length and consequently more retardation of drug release.

4.11.1 Influence of change in pH of dissolution medium

The *in-vitro* release of the model drug lornoxicam from different formulations was conducted first in 0.1 N HCL (pH 1.2). The % drug release at pH 1.2 was much lower as compared to pH 6.8, which exhibit pH sensitivity of prepared microspheres. The Okra gum-sodium alginate and Ethyl cellulose-sodium alginate microspheres were swelled at pH 1.2 but were intact and don't dissolved, and almost all the formulations exhibit minimum (approx. 34.599±0.34%) drug release in the gastric environment which could be attributed because of high degree of crosslinking between the oppositively charged polyelectrolyte, and the same has been supported in literature. The prepared polyelectrolyte exhibited drug release in a controlled manner at pH 6.8, and it was also found that the drug release was decreased as the concentration of polymer was increased. The drug release rate could be slower because of the diffusion of lornoxicam from the internal microsphere environment to the external environment. This indicate that crosslinked okra gum-sodium alginate and ethyl cellulose-sodium alginate microspheres should be produced stronger electrostatic interaction with negatively charged chloride ions.

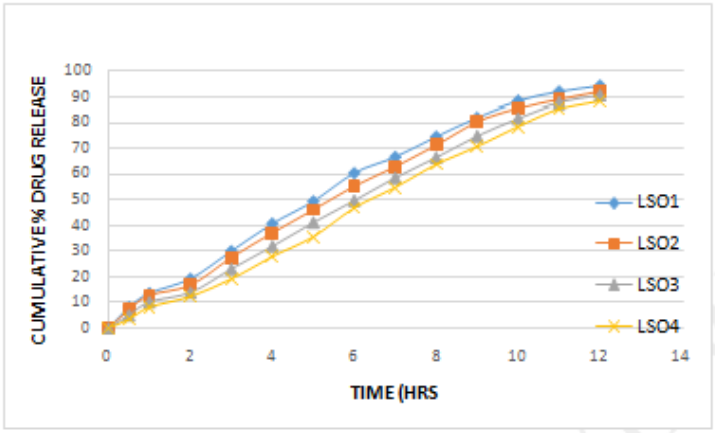

Fig. 10. *In-vitro* release profile of microspheres (LSO1 to LSO4 formulations) at different pH conditions (1-2 hours at pH 1.2; and 3-12 hours at pH 6.8) (Mean ± S.D., n = 3)

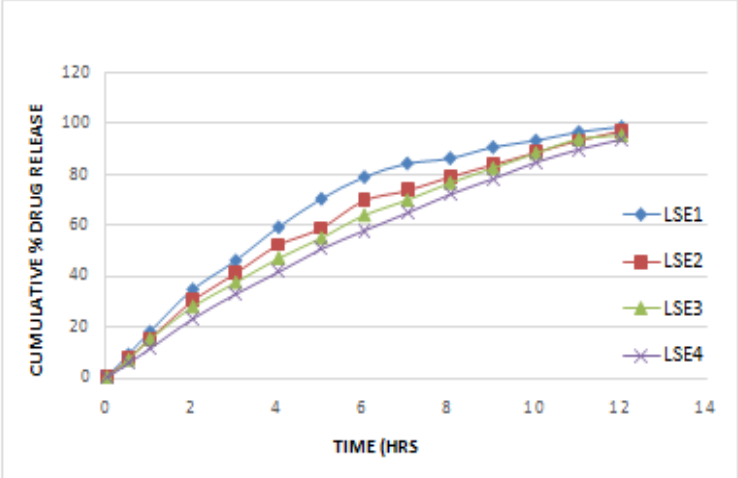

Fig. 11. *In-vitro* release profile of microspheres (LSE1 to LSE4 formulations) at different pH conditions (1-2 hours at pH 1.2; and 3-12 hours at pH 6.8) (Mean ± S.D., n = 3)

Table 9. % Cumulative Drug Release of prepared microspheres using okra gum and ethyl cellulose

Time (in hrs)	Cumulative percent drug release							
	LSO1	**LSO2**	**LSO3**	**LSO4**	**LSE1**	**LSE2**	**LSE3**	**LSE4**
0	0	0	0	0	0	0	0	0
0.5	8.263±0.05	7.324±0.26	5.152±0.2	3.556±0.55	8.886±0.64	7.353±0.34	6.905±0.23	5.751±0.34
1	13.751±0.03	12.886±0.41	10.353±0.34	8.245±0.72	17.832±0.17	15.045±0.87	14.993±0.34	11.396±0.45
2	19.396±0.01	16.832±0.30	14.045±0.30	12.396±0.17	34.599±0.34	30.266±0.40	27.871±0.28	23.227±0.34
3	30.227±0.04	27.599±0.35	23.266±0.35	19.227±0.80	45.996±0.40	41.148±0.36	37.395±0.40	32.979±0.30
4	40.979±0.05	37.162±0.25	32.124±0.32	27.979±0.28	59.181±0.86	52.241±0.04	46.921±0.86	41.691±0.39
5	49.691±0.01	46.181±0.28	41.421±0.28	35.691±0.05	70.355±0.28	59.035±0.35	54.918±0.23	50.765±0.42
6	60.765±0.07	55.355±0.31	50.035±0.30	46.921±0.11	78.964±0.36	69.971±0.38	63.862±0.36	58.054±0.13
7	67.054±0.13	62.964±0.15	58.971±0.34	54.918±0.57	84.302±0.34	73.909±0.39	69.796±0.60	64.968±0.36
8	74.968±0.07	71.302±0.30	66.909±0.36	63.862±0.51	86.364±0.28	79.281±0.24	76.594±0.32	72.332±0.16
9	82.332±0.06	80.364±0.35	75.281±0.26	70.796±0.34	90.717±0.17	84.089±0.17	82.399±0.17	78.425±0.15
10	88.425±0.07	85.717±0.45	82.089±0.35	78.594±0.52	93.367±0.60	88.909±0.04	88.281±0.46	84.971±0.30
11	91.971±0.04	89.367±0.09	87.909±0.28	85.399±0.56	96.749±0.40	93.678±0.43	93.737±0.34	89.989±0.02
12	94.213±0.09	92.331±0.28	90.612±0.23	88.654±0.25	98.717±0.23	97.399±0.45	95.281±0.28	93.971±0.20

The values are expressed as mean ± SD, n =3.

4.11.2 Influence of Polyelectrolyte complexation on drug release

The % cumulative drug release data (tabulated in above Table 9 and Fig. 11), the effect of increase in concentration of okra gum have profound effect over drug release, the formulation having okra gum-sodium alginate (1.0:1.0) presented slower release for longer time, this could be attributed because of lower ability of negatively charged moieties to interact with those of the positively charged Na-alginate molecules. As the concentration of okra gum was increased, the drug release decrease to a considerable extent, this decrease in the drug release could be attributed to the fact that at higher concentration of okra gum:sodium alginate (0.75:1.0 and 1.0:1.0) might be produced relatively stronger network as compare to those formulation having okra gum:sodium alginate at lower concentration (0.25:1.0 and 0.5:1.0), the drug release decreased with increase in the concentration of negatively charged polyelectrolyte i.e. okra gum:sodium alginate. The results (mentioned in Table 9 and shown in Fig. 11) indicates that okra gum:sodium alginate concentration have significant impact over the drug release.

4.12 *In-vitro* Drug Release Kinetics and Mechanism

The release kinetics of lornoxicam-loaded okra gum-alginate microspheres LSO (1-4) were analyzed using various mathematical models like zero order, first order, Higuchi, Korsmeyers-Peppas and Hixson Crowell models. The R^2 and RMSC (Regression Model selection criteria) values of the models were calculated by DD Solver 1.0 software for the assessment of precision of these models. The results of the release kinetic studies of the *in-vitro* drug release data of microspheres is shown in Table 10. It was found that release from microspheres followed the Korsmeyer-Peppas model (R^2 = 0.9925 to 0.9951) and finally the best fitting of Korsmeyer-Peppas model was verified by comparing MSC values and it was found (4.484-4.955). Moreover, zero order model (R^2 = 0.9720 to 0.9949) was found closer to the best-fit Korsemeyer – Peppas model. The estimation of diffusional type decided from Korsmeyer-Peppas model was n ≤ 1 and shown the release design from these microspheres followed anomalous diffusion (non-Fickian), supercase-II transport mechanism LSO4 (n = 1.039) over a period of 12 hour underlying *in-vitro* release, controlled by swelling and unwinding of polymeric gel, this could be ascribed because of polymer disintegration and polymeric chain relaxation [28].

In addition, the drug release from lornoxicam-loaded alginate-ethyl cellulose microspheres also follow Korsemeyer-Peppas model (R^2 = 0.9741 to 0.9973) with the addition of Hixson-Crowell model (R^2 = 0.9953 to 0.9985) and n < 1 indicated non-Fickian diffusion or anomalous transport mechanism (shown in Table 11). Moreover, first order model with non-Fickian diffusion mechanism (R^2 = 0.9788 to 0.9918) was found closer to the best-fit Korsemeyer-Peppas model/ Hixson-Crowell model. The polymer ratio (sodium alginate: ethyl cellulose) was found to influence the release of drug from the formulations. As the polymer level is increased, the drug release rates were found to be decreased. Drug release was found to follow

Table 10. Results of curve fitting of *in-vitro* lornoxicam release data from different lornoxicam loaded okra gum-alginate microspheres using different kinetic models

Model Parameters		Formulation Code			
		LSO1	LSO2	LSO3	LSO4
Zero-order	R^2	0.9720	0.9836	0.9943	0.9949
	MSC	3.2246	3.7719	4.8592	4.9881
First-order	R^2	0.9666	0.9603	0.9480	0.9355
	MSC	3.0490	2.8903	2.6447	2.4471
Higuchi model	R^2	0.9259	0.9088	0.8787	0.8499
	MSC	2.2516	2.0591	1.7969	1.6023
Korsmeyer-Peppas model	R^2	0.9925	0.9932	0.9950	0.9951
	MSC	4.4848	4.5893	4.9149	4.9556
	n	0.795	0.851	0.949	1.039
Hixson-Crowell	R^2	0.9885	0.9835	0.9738	0.9625
	MSC	4.1157	3.7704	3.3287	2.9886

R^2: squared correlation coefficient; MSC: Model selection criteria; n: diffusional exponent.

Table 11. Results of curve fitting *in-vitro* lornoxicam release data from different lornoxicam loaded ethyl cellulose-alginate microspheres using different kinetic models

Model parameters		Formulation code			
		LSE1	LSE2	LSE3	LSE4
Zero-order	R^2	0.8318	0.9062	0.9410	0.9705
	MSC	1.3548	1.9667	2.4469	3.1694
First-order	R^2	0.9918	0.9908	0.9850	0.9788
	MSC	4.3717	4.2913	3.8155	3.5002
Higuchi model	R^2	0.9685	0.9678	0.9587	0.9372
	MSC	3.0309	3.0369	2.8053	2.4128
Korsmeyer-Peppas model	R^2	0.9741	0.9903	0.9957	0.9973
	MSC	3.1630	4.1698	5.0121	5.4952
	n	0.577	0.647	0.698	0.773
Hixson-Crowell	R^2	0.9985	0.9984	0.9976	0.9953
	MSC	6.0506	6.0355	5.6355	5.0022

R^2: squared correlation coefficient; MSC: Model selection criteria; n: diffusional exponent.

near first order kinetics and mechanism of drug release was observed to be following Korsmeyer-Peppas model (Anomalous transport) and Hixson-Crowell model.

5. CONCLUSION & SUMMARY

In conclusion, the present study illustrates the suitability of microspheres loaded with lornoxicam for oral administration and can be considered as more effective alternative to conventional dosage forms. Lornoxicam-loaded alginate-okra gum and ethyl cellulose microspheres were prepared using isolated natural and ecofriendly okra gum: sodium alginate and ethyl cellulose: sodium alginate polymer blends using $CaCl_2$ as cross-linking agent by ionic-gelation method. It was observed that modified release microspheres of lornoxicam can be prepared successfully using both the polymers individually. Formulation code LSO (1-4) indicates data obtained for lornoxicam loaded microspheres containing natural polymer, the observed data reveals that these prepared microspheres have good sustained release behaviour with enhanced flow properties as compared to lornoxicam loaded alginate-ethyl cellulose microspheres, the drug release was sustained up to 12 h for prepared formulations by ionic-gelation method using natural polymer. These microspheres also exhibited pH dependent swelling. As we know that alginate is a naturally occurring biocompatible, biodegradable, biopolymer and is capable of rate and/or time controlled drug release, so dosing frequency can be reduced and as ionic-gelation method is water based technique, so it involves total aqueous system avoiding any residual solvent in microspheres. The percentage yield of these microspheres were higher, the FTIR, XRD and DSC studies indicates that there was no chemical interaction between the drug and polymers.

The formulation code LSE (1-4) represents the data for formulated lornoxicam loaded microspheres containing synthetic polymer by ionic-gelation method. It was observed that prepared microspheres were lower in flow properties as compared to lornoxicam loaded alginate-okra gum microspheres, their drug entrapment efficiency were lower as compared to prepared lornoxicam microspheres using natural (okra gum) polymer. Formulations comprising of synthetic (ethyl cellulose) polymer were able to provide controlled drug delivery by controlling the release of drug from microspheres up to 12 h, as concluded from dissolution data. The viscosity and increasing polymer ratio was the parameters controlling the release of drug from formulations, FTIR data revealed no polymer-drug interaction, XRD results indicated low-crystalline nature of microspheres.

Finally, it can be concluded that among all the eight formulations, the best designed formulation of lornoxicam loaded microspheres was LSO4 comprising of natural polymer, as it fulfilled entire requirements for sustained delivery of lornoxicam. Therefore, one can assume that okra gum are natural biopolymer used in pharmaceutical dosage forms by providing sustained release drug delivery systems and avoiding the side effects. The entire process is feasible in an industrial scale and demands pilot study. The results of drug release indicates that rate and extent of drug release decreased significantly with increase in the concentration of

polymers. Thus, the utility of isolated okra gum was proved as a potential sustained drug release polymer-blends with sodium alginate in the development of controlled lornoxicam release ionically-gelled microspheres for oral use.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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