

DEVELOPMENT OF GASTRO RETENTIVE MICROBEADS FOR SUSTAINED RELEASE OF FEXOFENADINE AND MONTELUKAST

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ABSTRACT

In allergic rhinitis, montelukast (leukotriene receptor antagonist) in combination with fexofenadine (antihistamine) provide improved and complimentary effects and reduce the allergic symptoms effectively. Montelukast has less bioavailability due to hepatic first pass metabolism and fexofenadine have low permeability. So sustained release delivery is crucial for these drugs. One of the techniques to overcome this challenge is the development of polymeric microspheres or microbeads for enhanced bioavailability and prolong the action of drug in body.

The objective of the study is to produce a potential microencapsulated formulation having the combination of fexofenadine hydrochloride and montelukast sodium. Microbeads were formulated by using polymer, eudragit RS100. Single emulsion solvent evaporation method was used for the preparation of formulation. The developed drug loaded polymeric microbeads showed that percentage floating ranged from 82.1 - 90.4%. Entrapment efficiency of microbeads were found between 68.8- 80.9%. FTIR results revealed absence of drug-polymer interaction. *In-vitro* release studies shown that from all the prepared formulations of both drugs the optimum formulation was released up to 24hrs and percentage cumulative drug release was 87.40% and 89.78% respectively. The acute toxicity study showed the safety of the developed system.

Formulated microbeads were significantly efficient to achieve a sustained release of fexofenadine and montelukast with prolonged therapeutics release up to 24 hours. The developed gastro retentive floating drug delivery systems showed excellent physicochemical properties and sustained drug release pattern, thereby improving the bioavailability of the drugs.

Keywords: Floating microbeads, Gastroretentive, Fexofenadine HCl, Montelukast, Emulsification-solvent evaporation.

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INTRODUCTION

All drug delivery systems aimed to provide a therapeutic amount of drug to the proper site in the body and then maintain desired drug concentration [1]. Oral, Intravenous (IV), intramuscular (IM), intranasal (IN) and intradermal (ID)/transdermal administration are the major drug delivery routes. Other routes, such as ocular or rectal, have also been developed for localized, site-specific drug administration without unwanted systemic side effects.

Among all the routes that are available for administration of the drug, most predominant was

oral pathway and has attracted the most attention due to its unique advantages, including noninvasive, ease of administration, safe, economical, patient compliance and feasibility. Additionally, a large surface area (>300 m²) lined with a viscous mucosal layer paves the way for drug attachment and subsequent absorption [2]. In particulate drug delivery, the distinction is often gained by micro-particles. Increased surface area by reduction in particle size results faster dissolution, commonly by a small manner of magnitude. Increasing or enhancing

bioavailability in some drug cases it may be sufficient to achieve the goal.

Microparticles can be described in terms of solid, size ranges from one to thousand micrometer in diameter. Microbeads or microspheres are spherical, in nature, polymeric microparticulate system ranges in size from 0.5 to 500 micrometer [3, 4]. They are micrometric matrix systems and essentially spherical in shape. Structurally they are made up of dispersion phase by using miscible polymers either one or more in which drug particles can be dispersed, in either solution or microcrystalline form, at the molecular or macroscopic level containing dispersed drug.

Drugs, either hydrophilic or lipophilic, with relatively high efficiency, can be incorporated into polymeric microparticles. These types of drug carrier system have been proved physicochemically stable, regarding both *in vivo* and during storage aspects, more as compared to liposomes [5]. Natural polymers such as Chitosan, Xanthan gum, Sodium alginate Eudragit, HPMC, Ethyl cellulose and synthetic polymers Eudragit, HPMC, Ethyl cellulose are used in floating system to target the drug delivery at specific region in the upper GI tract. Eudragit RS 100 was used as drug carrier/polymer for the development of microbeads.

This polymer has been firstly introduced in 1968 [6]. Structurally defined as copolymer of Ethyl acrylate, methyl methacrylate along with low content of methacrylic acid ester with quaternary ammonium groups. In the form of salts ammonium groups are present, that ultimately enhance its permeability [7, 8]. Over single-drug therapy DCT has various advantages. It can reduce the required concentration of each drug that are used individually. It can completely or partially inhibit more than one target and also delay the drug resistance [9]. Oral H1-antihistamine with leukotriene receptor antagonists is one of the strong pillars in management of allergic rhinitis. Enhanced and complimentary effects can be provided by the combination therapy of montelukast with antihistaminic, also effective in reduction of the symptoms and potentially reduced cost as compared to monotherapy. Due to low permeability of fexofenadine and hepatic first pass metabolism of montelukast leading to masked the bioavailability that ultimately increases the dosing frequency. One of the techniques to make them more bioavailable is to incorporate in polymeric microparticles.

Montelukast is referred as a leukotriene receptor antagonist includes that it is orally active and inhibits the cysteinyl leukotriene CysLT1 receptor selectively [10, 11]. Fexofenadine belongs to the class called diphenylmethanes that is of organic in nature. Referred to those compounds that contain a diphenylmethane moiety, which means they have a methane and two hydrogen atoms that are replaced

by two phenyl groups. Fexofenadine hydrochloride is an antihistaminic drug that is used in the treatment of hay fever and other allergic symptoms. It was developed as alternative therapy to terfenadine. As compare to other antihistamines fexofenadine is non sedative as it not crosses the blood-brain barrier so produce less sleepiness as compare to the first-generation histamine-receptor antagonists [12].

The objective of the present study was to develop microbeads having combination of fexofenadine and montelukast one of the strong pillars in management of allergic rhinitis and this combination is not available in market. To investigate effects on morphology and drug release of different concentrations of polymer. To determine *in vitro* effective and sustain release pattern of the developed system. Characterization of the developed microbeads for physicochemical characteristics using zeta sizer, FTIR, DSC and SEM and to evaluate drug entrapment efficiency. To reduce the dosing frequency and dose dumping in patients suffering from allergic rhinitis and determine toxicity or safety profile of formulation in rats.

MATERIAL AND METHODS

Fexofenadine HCl, as a gift, was obtained by SURGE Laboratories (Bhikhi, Pakistan). Montelukast sodium was obtained by Saffron Pharmaceuticals Pvt Ltd, Faisalabad, Pakistan. Eudragit RS 100 which served as drug carrier/ polymer was purchased from SURGE Laboratories (Bhikhi, Pakistan). Magnesium stearate (Antisticking agent) gifted by Saffron Pharmaceuticals Pvt Ltd, Faisalabad, Pakistan. Dichloromethane was purchased from Sigma-Aldrich Corporation. Ethanol was got from Sigma-Aldrich Corporation. Methanol was purchased from Sigma-Aldrich Corporation. Distilled water was purchased from Scientific World, Faisalabad, Pakistan. Sodium lauryl sulphate (SLS), act as surfactant, gifted by Saffron Pharmaceuticals Pvt Ltd, Faisalabad, Pakistan. N-hexane was purchased from Sigma-Aldrich Corporation. Hydrochloric acid was purchased from Sigma-Aldrich Corporation. Tween 80 was purchased from Sigma-Aldrich Corporation. All chemicals that utilized in this study were used as received and of analytical grade.

Preparation of Fexofenadine HCl Microbeads

In 10ml of a mixture of dichloromethane and ethanol required amount of the polymer and magnesium stearate were added (1,0.2 v/v) with continuous magnetic stirring at 300 rpm at 22°C. Purposive amount of fexofenadine powder under same condition of stirring was dissolved in the polymeric solution. The prepared dispersion was poured dropwise (1.5-2 ml/min) by using syringe into 100 ml of 0.2% w/v SLS aqueous solution and emulsified by strong stirring (800 rpm) at room temperature using a

three-blade overhead stirrer. Stirring was continued for 1-1.5 hour until all solvent evaporated. Prepared microparticles were permitted to settle and solidified by adding n-hexane. Filtration was done and collected microbeads placed in the hot air oven at 35°C for 1hour for proper solvent evaporation. After drying microbeads stored in air tight glass vials and stored in desiccator. Different formulations will be prepared by altering the concentrations of drug and polymer.

Preparation of Montelukast Sodium Microbeads

Calculated polymer and magnesium stearate were dissolved in proposed amount of dichloromethane with non-stop magnetic stirring at 300 rpm at 22°C. Predetermined amount of drug was dissolved in the polymer solution with continuous stirring. The prepared dispersion poured drop-wise (1.5-2 ml/min) by using syringe into 100 ml of 0.2% w/v SLS aqueous solution and was emulsified by strong stirring (830 rpm) at room temperature using a three-blade overhead stirrer. Stirring was continued for 1hr and 20 minutes till all solvent evaporated.

Prepared microbeads were permitted to settle and solidified by adding n-hexane. Filtration was done and collected microbeads placed in the hot air oven at 35°C for 1hour for proper solvent evaporation. After the drying process microbeads were then be stored in air tight glass vials and stored in desiccator. By changing the ratio of drug and polymers different types of formulation were prepared.

EVALUATION OF MICROBEADS

Percentage Yield

Prior to calculate the percentage yield, prepared microbeads were completely dried in hot air oven maintained at 37°C for 24 h and then weighed. Percentage yield calculated as.

$$\text{Yield (\%)} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Scanning Electron Microscopy

Prepared microbeads morphology e.g. surface and shape were analyzed SEM (Jeol JSM-5200). Scanning Electron photomicrographs of microbeads were taken. Prior to consideration, the small amount of microbeads was spread on adhesive tape after that adhesive tape implied on gold coated aluminum stub. A Scanning electron photomicrograph was taken at an acceleration voltage of 20KV [13, 14].

Entrapment Efficiency

Amount of microbeads were taken that contained 10 mg of drug then suspended in 10 ml of methanol. Vortexed and sonicate it. For determination of drug entrapment efficiency solution was filtered and then

checked absorbance by using a UV/visible spectrophotometer [15]. It was calculated by

$$\text{EE (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Determination of Particle Size

Photon Correlation Spectroscopy (PCS) with Zetasizer 3000 was used to analyzed Particle size (Malvern Instrument, Malvern, UK). Double distilled water that was filtered through a 0.22 µm filter (Millipore®) was used to dilute suspension. After that the filter aqueous suspension was placed in clear disposable zetacell. The computer can provide the mean size and the distribution width of the microbeads. At least three times for each batch of sample under identical condition analysis was carried out and mean value were calculated. Zeta potential of drug loaded microbeads was carried out by using same colloidal suspension with the help of same instruments [16].

Fourier Transform Infrared Spectroscopy

For drug excipient interaction studies Infra-Red spectroscopy can be done by Fourier transformed infrared spectrophotometer. To confirm interactions between the selected drug and polymer Perkin Elmer (model Impact 410, Wisconsin, MI, and USA) spectrophotometer was used. Size of the microparticles were reduce into fine particles in pestle and mortar then 1 mg of grounded microparticles mixed with 100 mg of KBr (Merk IR Spectroscopy grade) then compressed under a hydraulic pressure of 20 psi for 10 minutes to produce the pellets. The spectra of prepared microparticles were analyze in the wave number range of 4000-650 cm⁻¹. FTIR study was performed on pure drug, polymer and drug loaded microparticles [10, 17].

Differential Scanning Calorimetric Analysis

To find the changes during thermal exposure of samples differential scanning calorimetric (DSC) were performed on a Modulated DSC V1.1A TA instrument 2000 (USA). Instrument was standardized by Tin (232°C), Zinc (419.5°C) and indium (156°C) as internal standards. Placed the Samples (2–10 mg) in aluminum pans and sealed the pan. Aluminum pan under a nitrogen atmosphere were used to heat the prob from 25 to 400 °C at rate of 10°C /min, in this instrument an empty pan used as a reference. Enthalpy of fusion and melting point was calculated by instrument [10].

Flow Properties of Microbeads

The microbeads were categorized on the basis of their angle of repose, tapped density, Carr's index flow properties, bulk density, and Hausner's ratio.

Table 1: Design of formulation of fexofenadine HCl microbeads.

Code	Polymer	Drug polymer ratio	Organic solvent ratio (DCM,ethanol)	Mg.stearate (mg)	SLS (Surfactant conc)	External phase Distilled water (mL)
FX1	Eudragit RS100	1:1	1:0.1	40	0.2% w/v	100
FX2	Eudragit RS100	1:2	1:0.2	40	0.2% w/v	100
FX3	Eudragit RS100	1:3	1:0.3	40	0.2% w/v	100
FX4	Eudragit RS100	1:4	1:0.4	40	0.2% w/v	100

Table 2: Design of formulation of montelukast sodium microbeads.

Code	Polymer	Drug polymer ratio	Organic solvent ratio (DCM,ethanol)	Mg.stearate (mg)	SLS (Surfactant conc)	External phase Distilled water (mL)
MN1	Eudragit RS100	1:1	1:0.1	40	0.2% w/v	100
MN2	Eudragit RS100	1:2	1:0.1	40	0.2% w/v	100
MN3	Eudragit RS100	1:3	1:0.1	40	0.2% w/v	100
MN4	Eudragit RS100	1:4	1:0.1	40	0.2% w/v	100

Bulk Density

It is the ratio of untapped material mass to volume including interparticulate void spaces. It was determined by accurately weighed amount of the beads and transferred into 10 ml measuring cylinder. The volume occupied by the beads was noted. Its unit is g/ml. Formula for the determining of bulk density

$$\text{Bulk density (d}_b\text{)} = \frac{\text{Weight of microbeads}}{\text{Bulk Volume of microbeads}}$$

Tapped Density

Tapped density is the ratio of mass to volume of tapped material. Tapped density calculated by accurately weighed amount of the beads and transferred into 10 ml measuring cylinder. It was subjected to tapping for 100 times and the volume occupied by the beads was calculated [18]. Its unit is g/ml. It was determined by following formula

$$\text{Tapped Density (d}_t\text{)} = \frac{\text{Weight of microbeads}}{\text{Tapped Volume of microbeads}}$$

Hausner's ratio

It is the ratio of tapped density to bulk density [19]. Hausner's ratio was calculated as

$$\text{Hausner's ratio} = \text{Tapped density} / \text{Bulk density}$$

Carr's compressibility index

Carr's compressibility index is the sign of compressibility of a powder [20]. It was determined by following formula

$$\text{Compressibility index (C.I) \%} = \frac{d_t - d_b}{d_t} \times 100$$

where d_t is tapped density and d_b is bulk density.

Angle of Repose

Angle of repose is a method used for describing the flow properties of a powder. Funnel angle of repose can be found by using funnel method. In this technique powder move downward with the help of gravitational force without using external force and on a plane surface form a conical heap. After the

formation of conical heap, the circumference of the heap is drawn and then the height of heap is calculated. Lower would be the height if greater the flow of the powder and as a result longer will be the diameter of the circumference of the heap [21]. Angle of repose was calculated as

$$\text{Angle of Repose } \theta = \tan^{-1} \frac{h}{r}$$

In vitro Buoyancy Study

To calculated in vitro buoyancy study 0.15 g/150mg of microspheres were spread on the surface of USP dissolution apparatus (type II) which was filled with 900 ml of simulated gastric fluid SGF (pH 1.2) or 0.1N HCl containing 0.02% tween 80. Paddle rotating at 100 rpm which agitated the medium and maintained at 37°C for 12 hours. Separation of layer of buoyant microbeads was done by filtration. Both types floated and settled portions were dried in a desiccator and then weighed [22]. It was calculated by

$$\text{Percentage Buoyancy} = \frac{\text{Weight of floating microbeads}}{\text{Initial weight of floating microbeads}} \times 100$$

In vitro Release Study

From floating microbeads, the drug release rate was carried out using the USP type II dissolution paddle assembly. 120 mg of fexofenadine HCl and 10 mg montelukast sodium floating microbeads were added in 900 ml of 0.1 N HCl (pH 1.2) maintained at 37 ± 0.5°C and stirred at 100 rpm withdrawn sample at predetermined intervals then filtered through a 0.45 µm Millipore filter up to 18 hrs. After each withdrawal equal volume of dissolution medium was added in the vessel to maintain sink volume. Samples that were taken diluted with 0.1 N HCl and analyzed spectrophotometrically at 259 nm and 367 nm to find the percentage drug release was plotted against time to calculate the release pattern [23].

***In vivo* Toxicity Studies**

Ten male albino rats of about weight of 120-130 gm kept in animal house in department of Government College University Faisalabad for six days before the start of study. They were delivered chow in diet and water. Animals were placed in two different cages, grouped wise in a well-ventilated room, at room temperature of $27 \pm 1^\circ\text{C}$ and humidity up to 60-65% with 12 hours light-dark cycle. The study was conducted after the approval from Animal Ethics Committee of department of pharmacy Government College University Faisalabad and study was performed according to the rules for laboratory animals use and care. Rats were divided into two groups of rats (n=5) each. Group 1, was served as normal control and received only normal saline. Group 2, the treatment group, received combination of both drugs microbeads FXMN1 through oral route in one single dose for seven days [24]. Group I, (Normal Control) received normal saline 2ml/kg. Group II, (Treatment Control) treated combination of FXMN1 microbeads through oral route (the best microbead formulation that exhibited sustained release rate weighed equivalent to 120mg and 10mg respectively). Rats were fasted before dosing. Food was withheld for 3–4 h before the administration of test substance. Animals had free access to water. Following the fasting period, body weights of the animals were recorded. After 2 hours of the administration of test substance animals were provided with food. After dosing, rats were observed individually, once during the first 30 min and periodically during the first 24 h. Special attention was given to the first 4 h. Animals were observed for 7 days for any signs of toxicity. Observations included physical or behavioral changes such as skin and fur, eyes and mucus membranes, respiration, circulation, autonomic nervous system (ANS) and behavior pattern. Observations were also recorded for

the presence of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Individual records of the animals were maintained. All animals were sacrificed on the 8th day following an overnight fasting. Animals were exposed to chloroform. Incisions were quickly made in the neck region of the animals and blood samples collected from the heart into gel activated tubes for biochemical analysis. Serum was separated and serum marker enzymes of liver function were analyzed, i.e. Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), Aspartate Transaminase (AST), Total Bilirubin (TB) [25].

RESULTS AND DISCUSSION

Formulation of Fexofenadine HCl and Montelukast Microbeads

Using Eudragit RS 100 drug loaded microbeads formulated by single emulsion solvent evaporation technique by different amount of drug and prepare different polymer formulation.

Percentage Yield

FX1-FX4 and MN1-MN4 all formulations have percentage yield in satisfactory range of 84.6-92.04%. The values are given in **Table 3**. The percentage yield of different formulations of microbeads varied from 84.6-92.04%. Results shown that all the batches of fexofenadine and montelukast formulation have percentage yield greater than 50% exhibit that formulation methods of microbeads were suitable. B. Raja Narender et al., (2016), percentage yield of the formulation increases as we change the polymer ratio from 1:1 to 1:4. In this work, when the drug, polymer ratio was changed from 1:1 to 1:4 respectively the percentage yield of microbeads may be decrease due to the loss of small particles during the process of washing and filtration. Another aspect for decreased percentage yield of the microbeads may be due to sticking of polymer to the stirrer and agglomeration during microbead production

Table 3: Percentage yield, encapsulation efficiency and floating properties of FX and MN- loaded ERS100 microbeads.

Formulation	Percentage yield (%)	Encapsulation efficiency (EE%)	Floation (%)
FX1	86.4	71.1	85.6
FX2	84.2	76.6	88.5
FX3	90.4	78.3	90.4
FX4	85.7	80.9	86.2
MN1	87.8	68.8	89.1
MN2	92.04	72.1	82.8
MN3	91.3	75.4	88.3
MN4	89.1	79.9	86.7

Morphological Studies of Microbeads

Morphological studies of microbeads were observed with scanning electron microscopy at 15kV by means of magnification power 500x, 1x, 2.5x. Results were shown in **Fig. 1** of formulation FX1, FX2 and MN1, MN2 respectively. SEM was performed to access surface and morphological characteristics of prepared microbeads. The obtained results depict that shape of

prepared microbeads were almost spherical. Kajale Archana D et al., 2016 observed similar results in their study.

Flow Properties

Hausner's ratio

Formulations FX1- FX4 and MN1-MN4 ratio ranged from 1.12-1.19. Result showed that all the formulations have good flow properties **Fig. 2**.

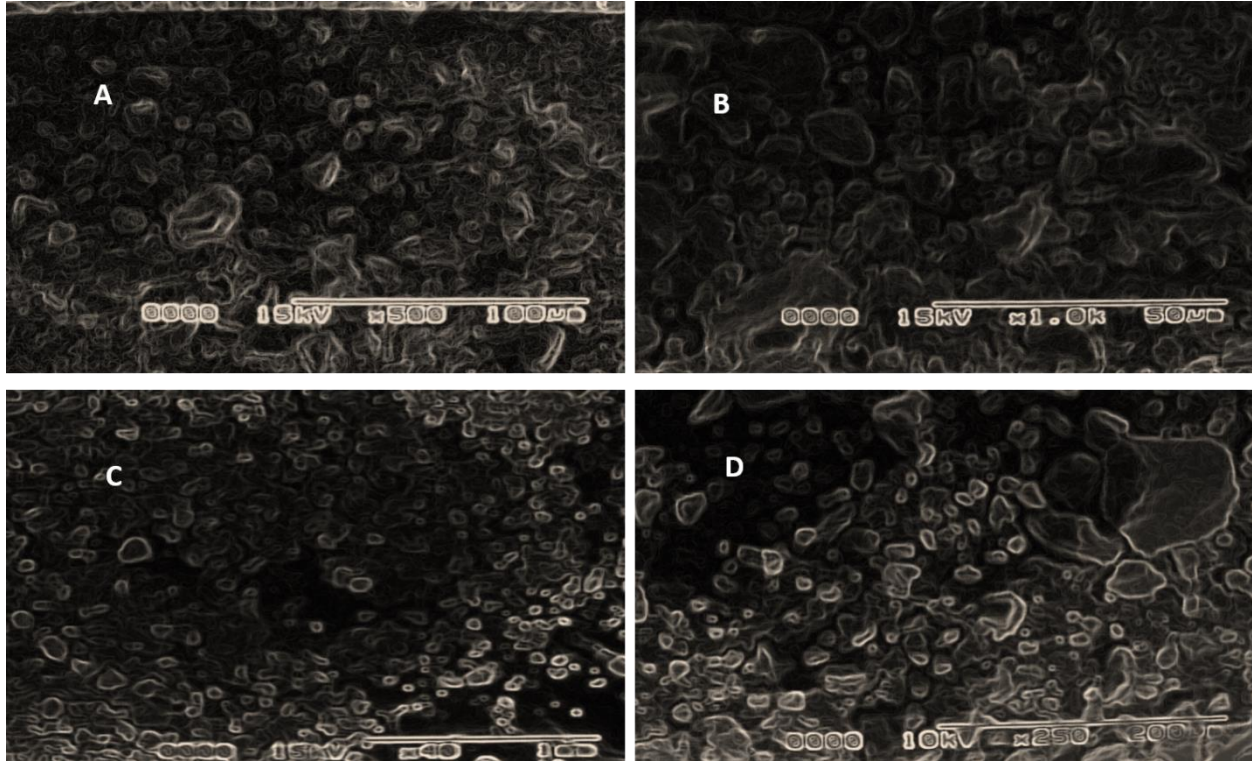


Figure 1: SEM images of (A (FX1) B(FX2), C(MN1), D(MN2)).

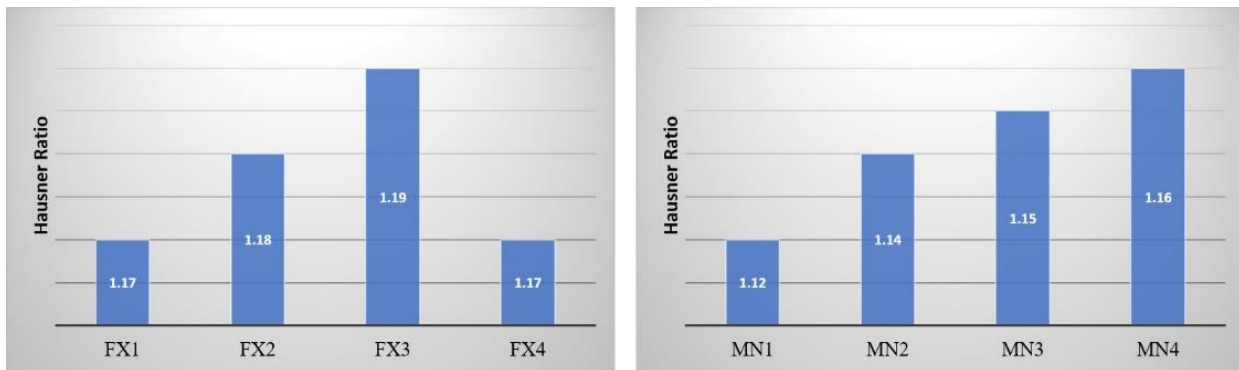


Figure 2: Hausner's ratio of formulation FX1-FX4 and MN1- MN4.

Carr's Compressibility Index

Values regarding compressibility index was calculated by using the values of bulk and tapped density. Tapped and bulk density provided information about flowability of powders. Carr's index of formulation ranged from 11-16%. Results showed that all formulations have low index values and showed good flowability **Fig. 3**.

Angle of Repose

If we see angle of repose and flowability have an opposite relation. If angle of repose has less value it means material have good flow property. If angle of repose has ranged between 25-30 then it means all formulations have excellent flow properties. Results showed in **Fig. 4**.

Entrapment Efficiency % (EE)

Encapsulation efficiency of all formulations prepared by solvent evaporation method were found between 68.8- 80.9% are display in **Table 3**. Encapsulation efficiency results ranged from 68.8- 80.9% and highest value is for FX4 and MN4 i.e. 80.9 and 79.9% respectively. The encapsulation efficiency was good for preparation. The entrapment efficiency increased progressively with increasing polymer concentration. The factors that influence the entrapment efficiency of the drug in microbeads e.g. drug-polymer ratio drug nature and speed at which stirring done etc. Normally polymer shows low encapsulation efficiency in low concentration. Girish

Kumar Tripathi et al., (2011) detected that when increases the polymer concentration the encapsulation efficiency increases. This is because when increases the polymer concentration as a result the availability of excess of the polymer as a result formation of larger size of beads capturing more amount of the drug meanwhile it slows the release rate.

Particle Size Analysis

Size of the particles was found by zeta sizer and optimum formulations FX1 and MN1 have size 2.4 μ m and 0.7 μ m respectively. Two important micromeritic properties, size distribution particle size that affect physical stability, drug dissolution rate and in-vivo behavior. Polydispersity Index (PDI) is the indicative of closeness of size distribution and stability of systems. Results of the particle size analysis of optimum formulation FX1 and MN 1 was obtained from Zetasizer 3000 (Malvern Instrument, Malvern, UK). The sharp peak in size distribution plots (indicate narrow size range and uniformity in particle size in formulations. The prepared formulations were in the optimum range and the size distributions were relatively monodisperse in both formulations FX1 and FX2 with the PDI values 0.232 and 0.385 respectively. PDI value less than 0.5 is indicative of very narrow size distribution range with very good control over particle size [16, 26].

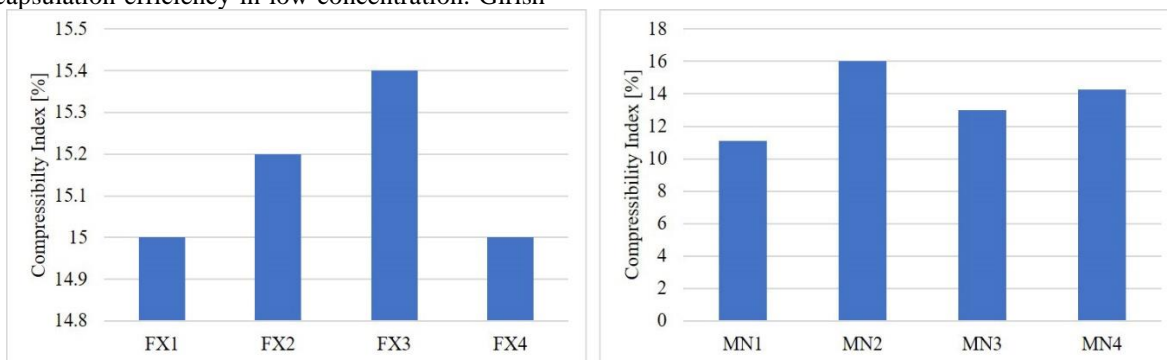


Figure 3: Carr's index of formulations FX1-FX4 and MN1-MN4.

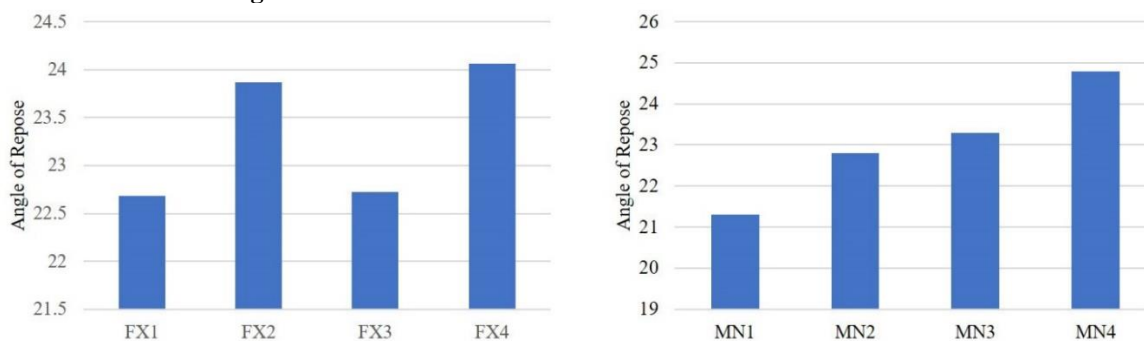


Figure 4: Angle of repose of formulations FX1-FX4 and MN1-MN4.

Fourier Transform Infrared Spectroscopic

Physical state of drugs and polymer individually and prepared microbeads by the combination of polymer and drugs were studied by FTIR for assessing the drug-polymer interaction. The results of FTIR of the drugs and the polymer was shown in **Fig. 5**. FTIR spectroscopic study was used in compatibility studies to evaluate formulation composition and interaction between carrier molecules. FTIR graph is given as wavenumber (cm^{-1}) verses percentage transmittance. The FTIR spectrum of pure fexofenadine hydrochloride showed absorption peaks at 3269.9 cm^{-1} , 1636 cm^{-1} , 1494 cm^{-1} and 1241.2 cm^{-1} represents O-H stretching vibrations, carbonyl (C=O) stretching of Carboxylic acid, aromatic C=C stretching and C-O stretching vibration of tertiary alcohol functional groups individually. All peaks confirmed the structure of pure drug [27].

Fig. 5 represents the FTIR spectra of pure montelukast sodium. The very broad peak at 3369.5 cm^{-1} showed stretching vibration of tertiary hydroxyl group. Absorption peak at 2929.7 cm^{-1} showed the stretching nodes of aromatic CH group. A sharp and intense peak at 1722 cm^{-1} represent carboxylic acid in form of salt. Absorption peak at 1550 cm^{-1} represents stretching of amide group. FTIR spectra of the pure drug (montelukast Na) was found similar to the standard spectrum of drugs [28].

Spectra of eudragit RS100 shown in **Fig. 5**. The mild intense absorption peak at 2985.6 cm^{-1} represents the stretching vibration of C-H group. At 1382.8 cm^{-1} a sharp peak is due to C-N groups vibration. An intense peak at 1140.6 cm^{-1} characterizes the stretching vibration of C-O group. C=O stretching vibrations representative spectral peak at 1722 cm^{-1} . All the characteristics peaks confirmed the structure of eudragit RS100 [29].

In case of fabricated microbeads of fexofenadine hydrochloride the absorption at 3269.9 cm^{-1} was shifted to 3291.2 cm^{-1} . The absorption peak at 1636 cm^{-1} shifted to 1632.8 cm^{-1} due to complex bonding. The absorption peak at 1494 cm^{-1} shifted to 1490.9 cm^{-1} and absorption peak at 1241.2 cm^{-1} was shifted to 1278.5 cm^{-1} in case of fabricated microparticles. Results shown that there is no polymer drug interaction since the significant absorption peaks of polymer and drug were retain in prepared microbeads.

In case of developed microbeads of montelukast sodium, the absorption peak at 3369.5 cm^{-1} was shifted to 3300 cm^{-1} due to complex bonding between drug and polymer. The absorption peak at 2929.7 cm^{-1} was shifted to around $2900-300 \text{ cm}^{-1}$. Absorption peak at 1722 cm^{-1} was shifted to 1700 cm^{-1} and meanwhile absorption peak at 1550 cm^{-1} shifted to 1661 cm^{-1} in case of microparticles. So, it was showed

that there is no polymer drug interaction as peaks were retained in fabricated microbeads.

Differential Scanning Calorimetric Analysis

The DSC thermogram of pure drugs, Eudragit RS 100 and selected batch of microbeads of were shown in **Fig. 6**. Differential scanning calorimeter was used to characterize the physical state of the drug. The DSC thermograms of the drug, polymer and the formulation, exhibited peaks corresponding to their melting point, transition temperature. The DSC curve of pure fexofenadine HCl indicated endothermic response corresponding to a melting point of 223°C showed crystalline anhydrous nature which was found similar to the standard peak of pure drug [30]. In case of developed microbeads, it gives rise to wider degree of onset of melting process and showed deviation from the pure sample peak and shifted to 278°C as a broad peak which could be due molecularly dispersion/dissolution of fexofenadine HCl [31]. The DSC thermogram of eudragit RS 100 showed exothermic peak at 422°C was found similar to the standard peak of polymer [32]. It was much beyond the melting point of the drugs alone or in formulations so the polymers did not affect the melting point of the drugs [33]. Montelukast sodium, in pure form, when subjected to DSC studies, it gave sharp peak at 141°C which was found similar to the peak in the previous studies [34]. In case of developed microbeads, the crystalline peak of montelukast was shifted to 172°C which was probably due to amorphization of the drug, due to the strong physical entrapment in the polymer matrix or drug polymer heat prompted interaction at molecular level. Similar results were reported which showed crystalline to amorphous state transformation of drug, pioglitazone- eudragit RS 100 electro sprayed nanobeads [35].

In vitro Release Studies of Microbeads Incorporated with Fexofenadine and Montelukast

In vitro release studies of microbeads were done by comparing the release profile ratio of drug and polymer. In vitro release studies of microbeads were achieved in 0.1 N HCl for 24 hrs. Plotted a graph between percentage collective drug release and time and presented in **Fig. 7**.

The percentage cumulative drug release from gastro retentive floating microbeads was decreased with increase in polymer concentration. Fexofenadine HCl and montelukast sodium released at acidic pH 1.2 up to 24 hours. All the prepared formulations of FX, the optimum formulation was FX1 as initial burst of drug was 16.85% and after 24 hours percentage cumulative drug release was 87.40% and in case of montelukast sodium microbeads, MN1 was considered as optimum formulation, the initial burst release was 15.1% and 89.78% drug was released

after 24hrs. Eudragit RS 100 form colloidal gel barrier and controlled the water intake in the microbeads and the release of drugs from

microbeads. The release of drug from eudragit RS 100 was slow as it is less permeable and for extended period of time [1, 15].

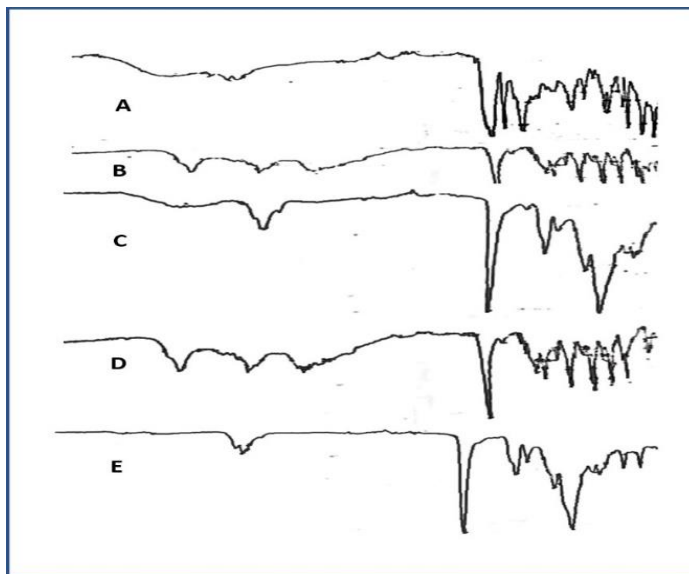


Figure 5: FTIR spectra of fexofenadine HCl pure drug (A), optimum formulation FX1 (B), montelukast sodium (C), optimum formulation MN1 (D) and eudragit RS100 (E).

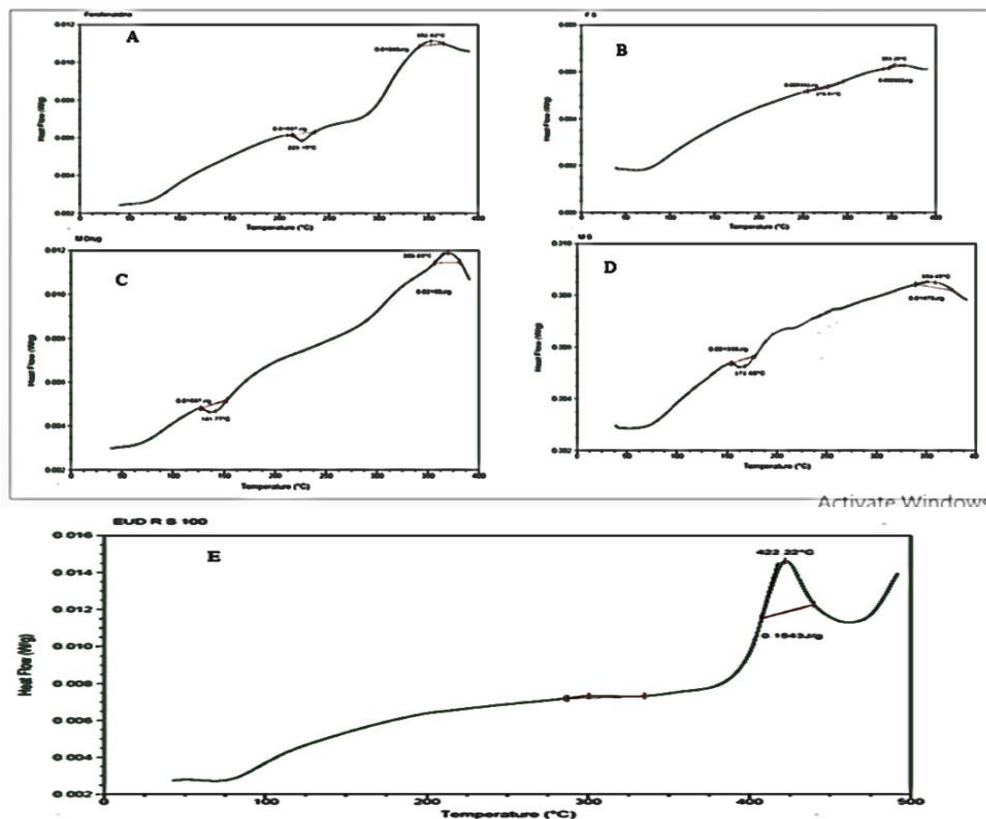


Figure 6: DSC thermograms of fexofenadine (A), FX1 (B), montelukast (C), MN1 (D) and Eudragit RS100 (E).

Release Kinetics

Results of in vitro release studies of microbeads of all the formulation of FX and MN were put in equations i-e zero order, first order, Higuchi and Korsmeyer-Peppas. The best fitted model was found by compare the R^2 values. Results were display in **Table 4 and 5**. By comparing the correlation coefficients of respective FX formulations, it was found that drug followed Higuchi model (R^2 0.9834-0.9871) showed that mechanism for release adopted by the microbeads was diffusion controlled. Meanwhile comparison of correlation coefficients of MN formulation it was observed that drug followed Korsmeyer-Peppas Model (R^2 0.9955-0.9971), n

value of MN formulation was higher than 0.5 which showed cell transport [36].

In vivo Acute Toxicity Studies

In the present study, animals were administered as a single dose of microbeads daily and monitored for seven days. No mortality or symptoms of toxicity was observed in any of the rats. No drastic change was observed in body weight of the rats between the control group and the treatment group during seven days. Meanwhile no any physical and behavioral change was observed in rats and the food and water consumption remained unaffected.

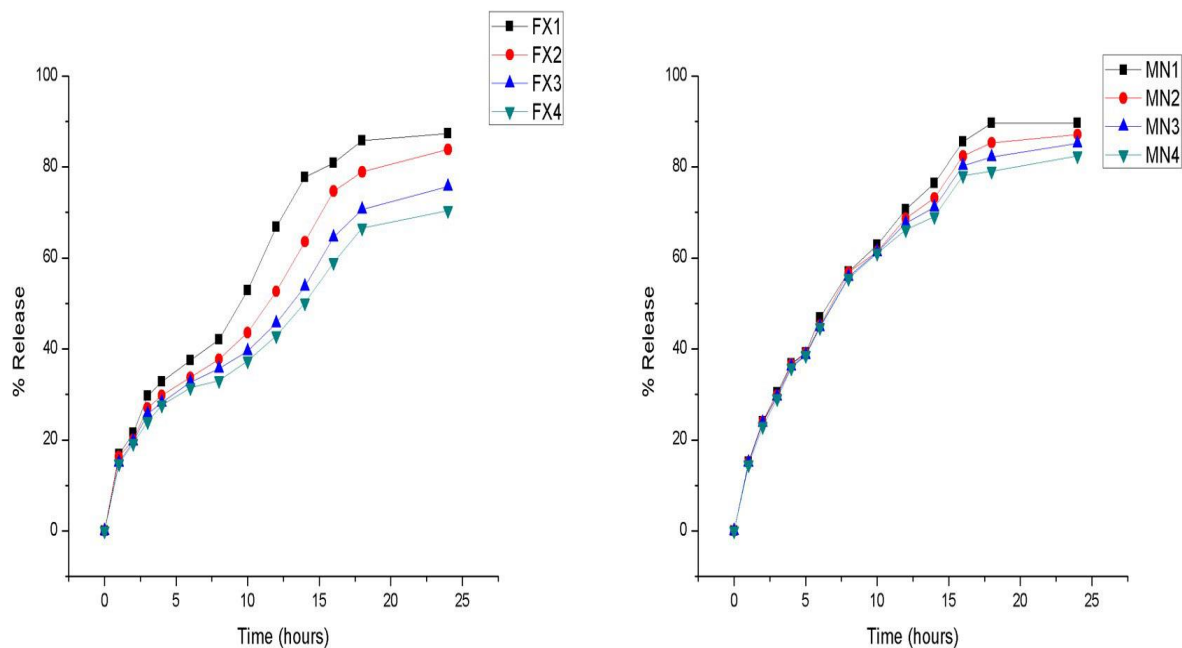


Figure 7: In vitro release rate of fexofenadine HCl and montelukast sodium loaded Eudragit RS100 microbeads.

Table 4: Release kinetics of fexofenadine HCl formulations.

Code	Polymer-Drug ratio	Zero order	First order	Higuchi	Korsmeyer-Peppas	
		R2	R2	R2	R2	N
FX1	1:1	0.7299	0.8663	0.9871*	0.9851	0.491
FX2	1:2	0.7043	0.8378	0.9842*	0.9828	0.477
FX3	1:3	0.6994	0.8311	0.9834*	0.9825	0.475
FX4	1:4	0.7629	0.8601	0.9661*	0.9629	0.519

*showed highest values

Table 5: Release kinetics of montelukast sodium formulations.

Code	Polymer-Drug ratio	Zero order	First order	Higuchi	Korsmayer-Peppas	
		R2	R2	R2	R2	N
MN1	1:1	0.9022	0.9738	0.9782	0.9958*	0.636
MN2	1:2	0.9021	0.9733	0.9789	0.9963*	0.635
MN3	1:3	0.8912	0.9677	0.9814	0.9955*	0.621
MN4	1:4	0.9061	0.9751	0.9782	0.9971*	0.641

*showed highest values

Table 6: LFTs of control and treatment group.

	ALT	AST	ASP	TB
Control	35.6 ± 3.9	38 ± 3.8	192 ± 6.8	0.33 ± 0.034
Treated	36.2 ± 5.16	38.6 ± 6.3	173 ± 26.37	0.34 ± 0.030

Values expressed as Mean ±SD

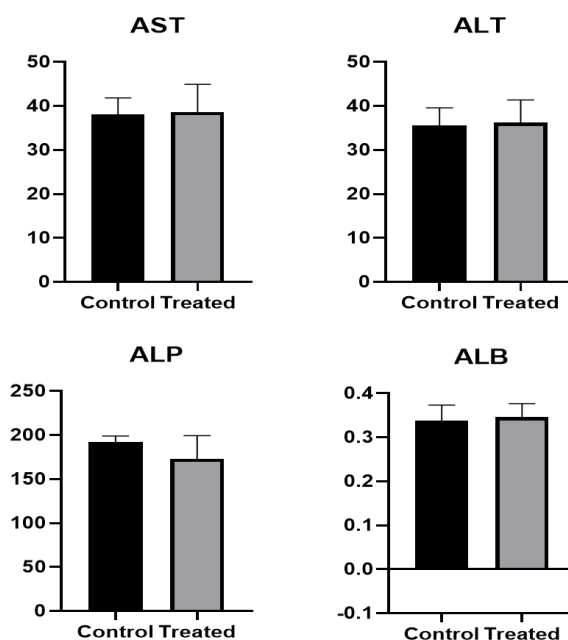


Figure 8: Graphs of AST, ALT, ALP, ALB. Data were presented as Mean ±SD.

CONCLUSION

In a nut shell, allergic rhinitis is the most prevailing disease of our community. It was concluded from this study that formulated sustained polymeric microbeads have excellent flow properties, good entrapment efficiency up to 80.9% and buoyancy was retained up to 12 hr. Fabricated microbeads showed sustained release up to 24 hours. Results of FTIR, DSC showed that there was no any interaction

between polymer and selected drugs. In vivo toxicity study indicated that developed formulation is safe and did not cause liver toxicity. So, it has been demonstrated that, eudragit RS 100 loaded fexofenadine hydrochloride and montelukast sodium microbeads can be an excellent choice in drug delivery system.

Declaration of Interest

The authors declare no competing financial interest.

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