

## DEVELOPMENT AND *IN VIVO* EVALUATION OF MUCOADHESIVE MICROSPHERES USING PIRENZEPINE

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### ABSTRACT

**Objective:** The current investigation objective was to fabricate gastroretentive mucoadhesive microspheres of pirenzepine and to investigate the pharmacokinetic parameters of optimized formulation in comparison with a marketed product.

**Methods:** Pirenzepine mucoadhesive microspheres prepared using ionotropic gelation technique. Evaluation parameters and characterization such as Fourier transform infrared (FTIR) and scanning electron microscopy were performed. *In vivo* bioavailability studies were conducted in rabbits. The technique used was found to be handling easy, inexpensive, and reproducible process.

**Results:** Among the total 14 formulations, M13 formulation was optimized and showed free flowing with good packability. FTIR studies investigated incompatibility were not observed between drug and excipients. The optimized formulation (M13) showed best cumulative percentage drug release of pirenzepine up to 99.07±0.17% within 12 h whereas marketed product displayed the drug release of 95.23±0.21% within 1 h. The release mechanism from microspheres followed the zero-order and Korsmeyer-Peppas model ( $R^2=0.951$  and  $0.994$ ), respectively. Optimized formulation (M13) was stable at 40°C±2°C/75% RH±5% RH for 6 months. Form *in vivo* studies, the optimized formulation bioavailability was much higher than the marketed product.

**Conclusion:** Microspheres would be a promising drug delivery system could play a potentially significant role in pharmaceutical drug delivery in a controlled manner for an extended period of time for effective management of gastritis.

**Keywords:** Pirenzepine, Mucoadhesive microspheres, Gastritis, *In vivo* Bioavailability studies.

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### INTRODUCTION

Gastritis is a chronic inflammatory disease of the stomach mucosa. Nowadays, the occurrence and frequency of gastritis are increasing, especially in developed countries [1]. Conventional drug delivery systems (DDS) cannot achieve prolongation of plasma drug concentration and effective bioavailability this is because of gastric emptying, pH of the stomach, etc., which can be overcome by developing gastric retention and long-acting release drug products [2]. There is need to develop new drugs and novel formulations as an alternative to existing formulations. Oral controlled DDS continues to be the most accepted and popular one among all the delivery systems [3]. To achieve this goal a variety of system has been developed including mucoadhesive and hydrogels among these the mucoadhesive DDS offers several advantages for those drugs associated with poor bioavailability and narrow absorption window in the gastrointestinal tract (GIT) upper part [4]. Mucoadhesive system had selected in the present research work. From the scientific and patent literature and due to advancements in controlled DDS, it is marked that if gastroretentive dosage forms retain in GIT for a time, then the drug is released slowly over a long period of time [5]. Mucoadhesive microspheres have advantages such as efficient absorption, enhanced bioavailability of drugs, maximum utilization of drug, and a much more intimate contact with mucous membrane [6]. It clearly indicated that these dosage forms can control the drug release at gastric region without getting cleared from the GIT; hence, it avoids the fluctuations and reducing the requirement of several administrations [7].

Pirenzepine, a selective antimuscarinic agent, is being investigated for clinical efficacy in the treatment of gastritis and ulcer. In contrast to the traditional antimuscarinic agents, pirenzepine shows selectivity for muscarinic receptors. Pirenzepine associated with low bioavailability (25%), hence, is rapidly metabolized into its inactive metabolite within

liver and colonic environment so the efficacy would be reduced and requires multiple dosing for maintaining therapeutic effect throughout the day. One approach to avoid this problem would be control the drug release, hence, increases the bioavailability at *in situ* level [8].

In oral controlled drug delivery, one of the finest microparticulate systems is microspheres. Microspheres can be defined as solid, spherical particles size ranging from 1 to 1000  $\mu\text{m}$ . The drug may be dispersed either in solution/microcrystalline form [9]. Microspheres offer advantages such as reducing dosing frequency and fluctuations and maintaining drug within the therapeutic range. Several varieties of biodegradable polymers were used for the preparation of microspheres. Polymeric DDS displays several advantages over the conventional dosage forms, and it includes enhanced efficacy, patient compliance, reduced toxicity, and to control the encapsulated drug release. Sodium alginate is an anionic natural polysaccharide, prepared by a mixture of D-mannuronic acid and L-glucuronic acid [10]. Sodium alginate is extensively used as a carrier for drug delivery due to its biocompatibility and low toxicity. The widely used method for pirenzepine microspheres preparation is an ionotropic gelation method [11]. This technique offers several advantages such as simple method of preparation no need to use organic solvent, and, also easier to control. Sodium alginate could form a gel in the presence of multivalent cations such as  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ba}^{2+}$ , and  $\text{Al}^{3+}$  by ionic cross-linking to form microspheres; it has been widely used in sustained drug release. Hence, in this study calcium chloride is selected as a cross-linking agent and because of its nontoxic and biocompatibility. Chitosan and sodium carboxymethylcellulose (CMC) are used as mucoadhesive agents [12].

In our present research work, the main aim of the study was to develop and evaluate gastroretentive mucoadhesive microspheres

of pirenzepine and to investigate the pharmacokinetic parameters of optimized formulation in comparison with a marketed product.

## MATERIALS AND METHODS

### Materials

Pirenzepine was procured as a sample of a gift from Splendid laboratories, Pune, India. Sodium alginate was used as polymer obtained from Pruthvi Chemicals, Mumbai. Calcium chloride was purchased from SD fine chemicals Mumbai, India. Chitosan and sodium CMC were purchased from Rubicon Labs, Mumbai, India. All other chemicals used were of analytical grade.

### Formulation of pirenzepine mucoadhesive microspheres

Chitosan and sodium CMC as a mucoadhesive agent, sodium alginate as a microsphere core forming agent, and calcium chloride as cross-linking agent were used for the formulation and preparation of pirenzepine microsphere.

### Preparation of mucoadhesive microspheres

Pirenzepine mucoadhesive microspheres were prepared by ionotropic gelation method using sodium alginate in combination with mucoadhesive polymers, namely chitosan and sodium CMC as mentioned in Table 1. Initially, sodium alginate solution was prepared by solubilizing the weighed quantity of it, chitosan and sodium CMC in deionized water using gentle heat, being stirred magnetically. In between the stirring time, 100 mg of pirenzepine was added to 100 ml of each percentage solution to form homogeneous dispersions at 500 rpm, maintained room temperature. The mixtures were sonicated for 30 min to eliminate air bubbles that may have been formed during the stirring process. The above dispersion (100 ml) was extruded dropwise through 20G needle fitted with a 10ml syringe into 100 ml of 7% w/v and 10% w/v of calcium chloride solution, being stirred at 500 rpm for 10 min. Later, the microspheres were collected by decantation and washed repeatedly with deionized water. The pirenzepine microspheres could dry at 60°C for 2 h in a hot-air oven [13].

### Evaluation parameters of pirenzepine mucoadhesive microspheres

#### Micromeritic properties

Micromeritic properties were used for the assessment of flowability and characterization of microspheres such as angle of repose, bulk density, tapped density, Carr's index, and particle size.

#### Swelling index

The swelling index was determined by suspending the weighed quantities of microspheres in 0.1N HCl with pH 1.2 and allowed to swell for the specified period. The excess surface adhered liquid drops of swollen microspheres were removed using blotting paper and then weighed it with the help of a microbalance. The swollen microspheres

were dried in an oven at 60°C for 5 h or until showed the constant weight [14]. The swelling index was determined using the initial weight of microspheres with respect to the weight of microspheres after drying (final weight) as per the formula below mentioned.

Swelling index =  $(\text{Mass of swollen microspheres} - \text{Mass of dry microspheres}) / \text{mass of dried microspheres} \times 100$ .

#### Percentage yield

The prepared microspheres were collected, dried and weighed. The percentage yield was calculated by taking the weight of dried microspheres divided by the total weight of drug and all excipients used in the microspheres preparation. It was determined using the following formula:

percentage yield =  $[\text{Total weight of microspheres} / \text{Total weight of drug and polymer}] \times 100$

#### Entrapment efficiency

The weighed quantity of prepared pirenzepine microspheres was transferred in a mortar and crushed. The crushed microspheres were dissolved in 50 ml of methanol then transferred into 100 ml conical flask and made the volume up to the mark using methanol. The above solution was agitated to dissolve the drug, all excipients and to extract the drug. The solution was filtered through a membrane filter (0.45 μm) to separate shell fragments. The solution was diluted suitably, and the absorbance was estimated at the  $\lambda_{\text{max}}$  of 280 nm by using a double beam spectrophotometer (Shimadzu, UV-1800) [15]. The amount of drug entrapped was determined using the following equation.

Percentage drug entrapment =  $(\text{Calculated drug concentration} / \text{Theoretical drug concentration}) \times 100$

#### Ex vivo mucoadhesion study

The microspheres mucoadhesive property was assessed by *ex vivo* mucoadhesion method using a chicken small intestinal tissue. The mucosal membrane was excised and washed with saline. 5 cm of jejunum portion was separated and averted with a glass rod. About 100 microspheres were spread uniformly on the tissue specimen. Then, both ends of the segment were tied using a thread. The tissue specimen was suspended in a 50 ml tube containing 40 ml of saline at 37°C and stirred horizontally. The tissue specimen was removed from the medium at specified time periods such as 1, 2, 3, 4, 5, 6, 7, and 8 h, then immediately immersed into a tube containing 40 ml of fresh saline and unbound microspheres were counted [16]. The adhering percent was calculated using the formula shown below.

Mucoadhesion =  $(\text{No. of microspheres adhered} / \text{No. of microspheres applied}) \times 100$

Table 1: Formulation trials for pirenzepine mucoadhesive microspheres

Formulation code	Pirenzepine (mg)	Sodium alginate (%)	Sodium CMC (mg)	Calcium chloride (%)
M1	50	1	100	7
M2	50	1.2	150	7
M3	50	1.4	200	7
M4	50	1.6	250	7
M5	50	1.8	300	7
M6	50	2	350	7
M7	50	2.2	400	7
Formulation code	Pirenzepine (g)	Sodium alginate (%)	Chitosan (mg)	Calcium chloride (%)
M8	50	1	10	10
M9	50	1.2	15	10
M10	50	1.4	20	10
M11	50	1.6	25	10
M12	50	1.8	30	10
M13	50	2	35	10
M14	50	2.2	40	10

CMC: Carboxymethyl cellulose

*In vitro drug release studies*

The *in vitro* drug release from prepared mucoadhesive microspheres was studied using USP dissolution apparatus II. Accurately, weighed the quantity of microspheres equivalent to 100 mg of drug was transferred into 900 ml of 0.1N HCl (pH1.2) medium maintained at  $37\pm 0.5^\circ\text{C}$  and stirring at 100 rpm. Aliquots of samples were withdrawn at specified time intervals, filtered and diluted with similar medium finally assayed at 280 nm using a UV-Visible spectrophotometer. The samples were withdrawn and replaced with same dissolution medium at predetermined time intervals [17]. All the samples were analyzed in triplicate.

*Analysis of in vitro drug release kinetics and mechanism*

The *in vitro* release data from optimized microspheres formulation containing pirenzepine were determined kinetically using different mathematical models such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas model.

*Drug-excipient compatibility studies**Fourier transform infrared spectroscopy (FTIR)*

The spectral analysis can be used to identify the functional groups in the pure drug and drug-excipient compatibility. Pure pirenzepine FTIR spectra and optimized formulation were recorded using FTIR (SHIMADZU). A weighed quantity of KBr and drug excipients was taken in the ratio 100:1 and mixed by mortar. The samples were made into a pellet by the application of pressure [18]. Then, the FTIR spectra were recorded in the wavelength region between 4000 and 400/cm.

*Scanning electron microscopy (SEM) studies*

Microsphere surface character includes size and shape was examined with the help of SEM. The microspheres were dried completely before analysis and SEM carried out at different magnifications of 15.0kv $\times$ 7.0 mm, 15 kv $\times$ 7.3 mm, and 15 Kv $\times$ 6.4 mm [19].

**Table 2: Micromeretic properties pirenzepine mucoadhesive microspheres**

Formulation code	Particle size ( $\mu\text{m}$ )	Bulk density ( $\text{g}/\text{cm}^3$ )	Tapped density ( $\text{g}/\text{cm}^3$ )	Angle of repose	Carr's index (%)
M1	65.29 $\pm$ 0.13	0.63 $\pm$ 0.05	0.62 $\pm$ 0.17	29 $^\circ$ .67 $\pm$ 0.11	09.34 $\pm$ 0.16
M2	73.43 $\pm$ 0.04	0.65 $\pm$ 0.23	0.69 $\pm$ 0.22	30 $^\circ$ .54 $\pm$ 0.23	11.12 $\pm$ 0.23
M3	78.67 $\pm$ 0.09	0.67 $\pm$ 0.18	0.73 $\pm$ 0.30	28 $^\circ$ .15 $\pm$ 0.31	14.23 $\pm$ 0.30
M4	79.45 $\pm$ 0.21	0.69 $\pm$ 0.33	0.75 $\pm$ 0.15	22 $^\circ$ .91 $\pm$ 0.17	10.32 $\pm$ 0.19
M5	83.42 $\pm$ 0.12	0.72 $\pm$ 0.14	0.79 $\pm$ 0.29	27 $^\circ$ .93 $\pm$ 0.27	11.87 $\pm$ 0.22
M6	85.34 $\pm$ 0.09	0.75 $\pm$ 0.26	0.82 $\pm$ 0.33	25 $^\circ$ .54 $\pm$ 0.32	13.95 $\pm$ 0.35
M7	87.12 $\pm$ 0.13	0.76 $\pm$ 0.12	0.91 $\pm$ 0.26	23 $^\circ$ .91 $\pm$ 0.18	10.20 $\pm$ 0.17
M8	69.43 $\pm$ 0.09	0.66 $\pm$ 0.06	0.61 $\pm$ 0.12	30 $^\circ$ .91 $\pm$ 0.20	09.34 $\pm$ 0.21
M9	72.46 $\pm$ 0.09	0.68 $\pm$ 0.24	0.63 $\pm$ 0.22	27 $^\circ$ .91 $\pm$ 0.15	09.11 $\pm$ 0.37
M10	76.89 $\pm$ 0.10	0.72 $\pm$ 0.37	0.68 $\pm$ 0.32	30 $^\circ$ .24 $\pm$ 0.26	12.12 $\pm$ 0.11
M11	85.94 $\pm$ 0.11	0.74 $\pm$ 0.21	0.72 $\pm$ 0.17	27 $^\circ$ .93 $\pm$ 0.11	10.23 $\pm$ 0.28
M12	88.94 $\pm$ 0.11	0.79 $\pm$ 0.38	0.75 $\pm$ 0.21	22 $^\circ$ .34 $\pm$ 0.37	11.34 $\pm$ 0.30
M13	89.04 $\pm$ 0.21	0.58 $\pm$ 0.25	0.59 $\pm$ 0.13	19 $^\circ$ .54 $\pm$ 0.26	8.41 $\pm$ 0.12
M14	91.45 $\pm$ 0.21	0.83 $\pm$ 0.10	0.83 $\pm$ 0.05	24 $^\circ$ .91 $\pm$ 0.10	13.45 $\pm$ 0.22

**Table 3: Percentage yield, entrapment efficiency, swelling index, and mucoadhesiveness of pirenzepine mucoadhesive microspheres formulations**

Formulation code	Percentage yield (%)	Entrapment efficiency (%)	Swelling index (%)	Mucoadhesiveness (%)
M1	75.45 $\pm$ 0.24	76.02 $\pm$ 0.13	72.11 $\pm$ 0.31	69.14 $\pm$ 0.11
M2	81.38 $\pm$ 0.16	82.03 $\pm$ 0.28	78.34 $\pm$ 0.12	78.22 $\pm$ 0.25
M3	82.97 $\pm$ 0.32	84.04 $\pm$ 0.21	82.89 $\pm$ 0.25	71.29 $\pm$ 0.31
M4	85.00 $\pm$ 0.08	86.00 $\pm$ 0.11	84.56 $\pm$ 0.37	78.73 $\pm$ 0.16
M5	87.02 $\pm$ 0.14	88.72 $\pm$ 0.22	85.23 $\pm$ 0.18	80.54 $\pm$ 0.29
M6	96.03 $\pm$ 0.22	95.03 $\pm$ 0.16	94.12 $\pm$ 0.22	95.26 $\pm$ 0.33
M7	92.01 $\pm$ 0.31	90.01 $\pm$ 0.28	84.23 $\pm$ 0.10	85.10 $\pm$ 0.26
M8	81.08 $\pm$ 0.15	80.02 $\pm$ 0.39	69.12 $\pm$ 0.21	83.37 $\pm$ 0.32
M9	83.00 $\pm$ 0.20	82.05 $\pm$ 0.17	70.12 $\pm$ 0.37	82.16 $\pm$ 0.16
M10	84.00 $\pm$ 0.33	85.00 $\pm$ 0.22	75.22 $\pm$ 0.23	85.24 $\pm$ 0.08
M11	89.00 $\pm$ 0.17	88.25 $\pm$ 0.31	84.34 $\pm$ 0.24	87.18 $\pm$ 0.27
M12	92.35 $\pm$ 0.13	91.26 $\pm$ 0.11	91.09 $\pm$ 0.31	92.50 $\pm$ 0.19
M13	98.90 $\pm$ 0.20	97.07 $\pm$ 0.23	96.08 $\pm$ 0.10	95.50 $\pm$ 0.16
M14	90.72 $\pm$ 0.11	89.67 $\pm$ 0.25	90.03 $\pm$ 0.12	88.12 $\pm$ 0.25

**Table 4: *In vitro* cumulative percentage drug release of pirenzepine mucoadhesive microspheres formulations M1–M7 and marketed product**

Time (h)	M1	M2	M3	M4	M5	M6	M7	Marketed product
0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
1	15.89 $\pm$ 0.15	15.11 $\pm$ 0.15	14.07 $\pm$ 0.16	14.04 $\pm$ 0.18	13.23 $\pm$ 0.16	11.32 $\pm$ 0.14	12.12 $\pm$ 0.21	95.23 $\pm$ 0.21
2	25.40 $\pm$ 0.16	26.23 $\pm$ 0.11	24.80 $\pm$ 0.16	24.40 $\pm$ 0.16	22.34 $\pm$ 0.13	21.90 $\pm$ 0.22	22.23 $\pm$ 0.21	-
4	38.20 $\pm$ 0.19	38.90 $\pm$ 0.14	37.40 $\pm$ 0.13	37.20 $\pm$ 0.12	39.30 $\pm$ 0.15	36.84 $\pm$ 0.16	36.90 $\pm$ 0.23	-
6	51.30 $\pm$ 0.16	49.90 $\pm$ 0.11	51.70 $\pm$ 0.11	50.30 $\pm$ 0.19	50.39 $\pm$ 0.16	51.08 $\pm$ 0.19	44.90 $\pm$ 0.11	-
8	63.30 $\pm$ 0.16	61.20 $\pm$ 0.13	60.30 $\pm$ 0.14	61.30 $\pm$ 0.18	66.23 $\pm$ 0.11	66.03 $\pm$ 0.18	64.20 $\pm$ 0.15	-
10	69.90 $\pm$ 0.11	70.10 $\pm$ 0.12	70.40 $\pm$ 0.12	69.90 $\pm$ 0.18	70.12 $\pm$ 0.14	81.07 $\pm$ 0.14	70.10 $\pm$ 0.16	-
12	76.30 $\pm$ 0.13	80.20 $\pm$ 0.16	83.50 $\pm$ 0.13	86.30 $\pm$ 0.16	88.34 $\pm$ 0.16	94.21 $\pm$ 0.15	86.24 $\pm$ 0.14	-

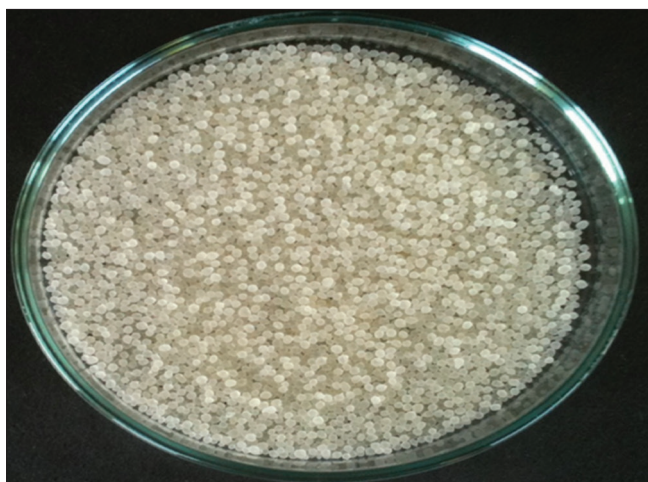


Fig. 1: Pirenzepine mucoadhesive microspheres

AQI



Fig. 2: Pictorial diagram showing mucoadhesive property of mucoadhesive microspheres in Chic Intestine

#### Stability studies

Stability testing was carried out at  $40^{\circ}\text{C}\pm 2^{\circ}\text{C}/75\% \text{RH}\pm 5\% \text{RH}$  for 6 months using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at specified intervals 0, 30, 60, 120, and 180 days period according to ICH guidelines. Various *in vitro* parameters such as percentage yield, encapsulation efficiency, and cumulative percentage drug release studies were conducted [20].

#### *In vivo* bioavailability studies

##### Pharmacokinetic study

The optimized mucoadhesive formulation M13 was used for *in vivo* studies. The animals used for *in vivo* experiments were of either sex New Zealand white rabbits weighing, 2–3 kg. The study included 12 animals, and six rabbits were kept in each group. Rabbits were kept on fasting 12 h before administration of the drug and until 24 h post dosing. Standard diet and water *ad libitum* were given for rabbits

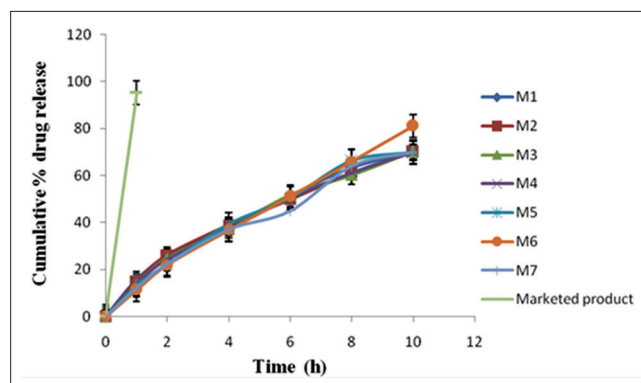


Fig. 3: *In vitro* cumulative percentage drug release of pirenzepine mucoadhesive microspheres formulations M1–M7 and marketed product

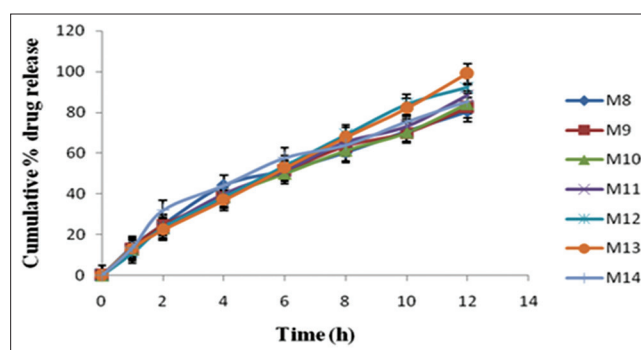


Fig. 4: *In vitro* cumulative percentage drug release of pirenzepine mucoadhesive microspheres M8–M14

throughout the study. The protocol of animal study was approved by the Institutional Animal Ethics Committee with no: P31/VCP/IAEC/2015/9/DBP/AE15/Rabbits.

The Group A received optimized formulation through oral route administration of drug solution. The Group B received oral administration of marketed formulation (tablet) was suspended in 1.0 ml saline and administered orally using a rubber tube under non-anesthetic condition. Blood samples (0.5 ml) at different time intervals 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 20, and 24 h were withdrawn by marginal ear vein. Heparin was added to collected blood samples to prevent blood clotting. The samples were centrifuged at 5000 rpm for 5 min at  $-20^{\circ}\text{C}$  until analysis. The several pharmacokinetic parameters such as  $C_{\text{max}}$ ,  $T_{\text{max}}$ ,  $\text{AUC}_{0-t}$ ,  $\text{AUC}_{0-\infty}$ , elimination half-life ( $t_{1/2}$ ), and elimination rate constant (Kel) were calculated. The kinetic parameters were calculated by compartmental method using WinNonlin 3.3<sup>®</sup> software (Pharsight Mountain View, CA USA). All values are expressed as the mean  $\pm$  standard deviation (SD) [21].

#### Statistical analysis

All the data were expressed as the mean  $\pm$  SD. Statistical analysis was carried out employing ANOVA using the GraphPad Instat software followed by Tukey–Kramer multiple comparison tests. A value of  $p < 0.05$  was considered statistically significant [22].

## RESULTS AND DISCUSSION

### Mucoadhesive microspheres

The results of micromeritic properties studies are given in Table 2. The particle size of M1–M14 varied from  $65.29\pm 0.13 \mu\text{m}$  to  $91.45\pm 0.21 \mu\text{m}$ . The bulk density and tapped density of prepared microspheres were found to be  $0.58\text{--}0.83 \text{g/cm}^3$  and  $0.59\text{--}0.91 \text{g/cm}^3$ , respectively. The angle of repose value was found to be  $19^{\circ}.54$  to  $30^{\circ}.91$ , indicating that the prepared microsphere flow properties fall in the category of

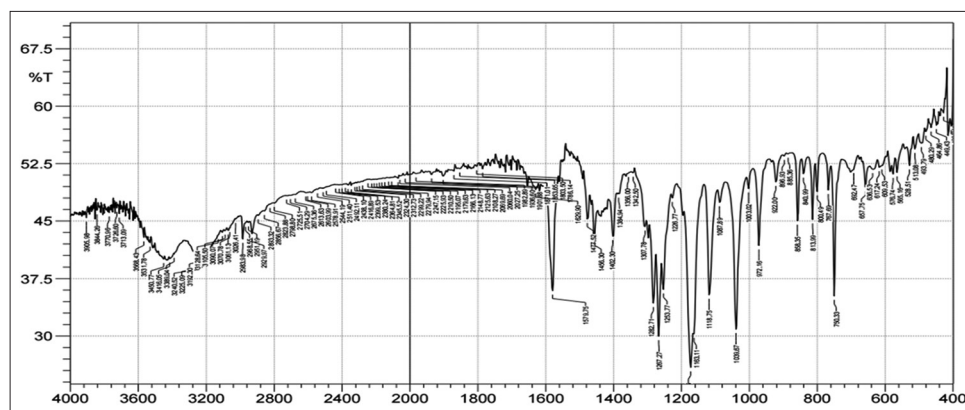


Fig. 5: Fourier transform infrared spectrum of pure drug pirenzepine

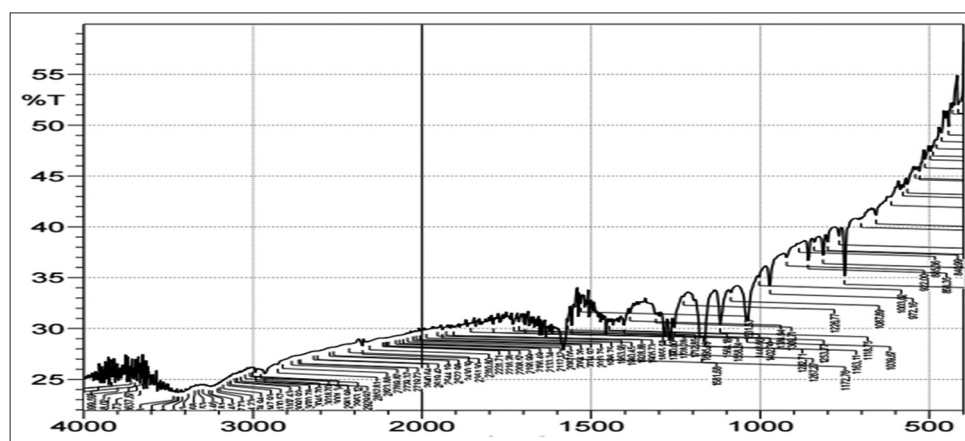


Fig. 6: Fourier transform infrared spectrum of optimized formulation of mucoadhesive microspheres M13

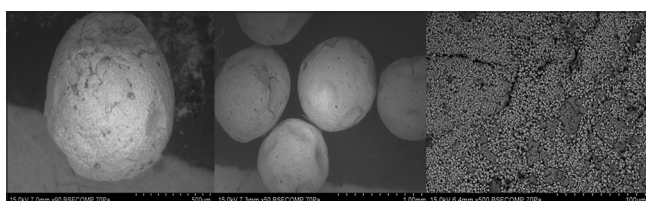


Fig. 7: Scanning electron micrographs of mucoadhesive microspheres at several magnifications

excellent to good flow characteristics. The Carr's index of microsphere formulations was found to be 8.41–14.23% indicating an excellent to good flowability.

The values of percentage yield (75.45–98.90%), entrapment efficiency (76.00–97.07%), swelling index (70.12–96.08%), and mucoadhesiveness (69.00–95.50%) of pirenzepine microspheres were mentioned in Table 3. This showed that the hydroxypropyl methylcellulose K15M possesses good swelling capacity and could retard the release of pirenzepine from the microsphere. The pictorial diagram of mucoadhesive property of pirenzepine microspheres in Chic Intestine was shown in Fig. 2.

#### **In vitro drug release studies**

Drug release profile of pirenzepine microspheres was compared with that of the marketed formulation and results obtained are compared as shown in Table 4 and 5. The optimized formulation M13 drug release was found  $99.07 \pm 0.17\%$  within 12 h whereas the marketed formulation showed the  $95.23 \pm 0.21\%$  within 1 h. Therefore, a sustained drug release pattern was observed for optimized formulation (M13).

#### **Mathematical modeling of optimized formula of mucoadhesive microspheres**

Drug release kinetic data for pirenzepine mucoadhesive microspheres was shown in Table 6. The optimized formulation M13 followed zero-order and Korsmeyer–Peppas release kinetics with regression values ranging from 0.951 to 0.994, respectively. The n-value was 0.812 indicating that the pirenzepine release from the microspheres was followed by erosion and diffusion-controlled.

#### **CHARACTERIZATION**

##### **FTIR**

To develop a stable, optimized mucoadhesive microsphere, the drug-excipient interaction study was performed during the product development stage generally by employing FTIR spectroscopy. The presence of characteristic absorption bands of Pure drug Pirenzepine (Fig. 5) and the optimized formulation (Fig. 6) suggest that there is no interaction taking place between the drug and excipients used in the formulation.

##### **SEM studies**

##### *Pirenzepine mucoadhesive microspheres*

The scanning electron micrographs of pirenzepine microspheres were shown in Fig. 7. From the SEM studies confirmed that the prepared microspheres were spherical in shape with a smooth and porous surface. The porous surface is mainly responsible for drug release from microspheres.

##### *Stability studies*

Stability studies of the prepared pirenzepine mucoadhesive microspheres were carried out by maintaining the best formulation

Table 5: *In vitro* cumulative percentage drug release of pirenzepine mucoadhesive microspheres formulation from M8 to M14

Time (h)	M8	M9	M10	M11	M12	M13	M14
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	14.05±0.15	13.34±0.22	12.70±0.11	12.30±0.21	11.28±0.15	13.31±0.22	13.63±0.13
2	24.80±0.16	24.40±0.12	23.11±0.13	23.40±0.11	23.50±0.16	22.33±0.15	32.01±0.12
4	44.40±0.19	38.20±0.11	38.63±0.16	39.92±0.15	38.60±0.13	37.00±0.18	44.20±0.16
6	51.70±0.16	51.30±0.15	49.92±0.18	51.40±0.13	53.80±0.16	52.84±0.16	57.86±0.12
8	60.30±0.15	63.30±0.16	61.20±0.16	65.20±0.11	68.90±0.18	67.84±0.14	64.03±0.14
10	70.70±0.11	69.91±0.21	70.13±0.15	73.12±0.16	83.90±0.12	82.00±0.11	75.29±0.11
12	80.54±0.21	82.36±0.16	84.06±0.13	88.34±0.11	92.23±0.12	99.07±0.17	85.36±0.13

Table 6: Stability studies of optimized mucoadhesive microspheres

Retest time for optimized formulation	Percentage yield	Entrapment efficiency (%)	<i>In vitro</i> drug release profile (%)
0 days	98.90±0.20	97.07±0.23	99.07±0.17
30 days	97.40±0.02	96.4±0.16	97.20±0.04
60 days	95.22±0.05	94.53±0.15	95.33±0.12
120 days	94.13±0.01	93.55±0.20	94.68±0.25
180 days	93.11±0.23	92.34±0.07	93.45±0.19

Table 7: Comparison of pharmacokinetic parameters of pirenzepine optimized formulation and marketed product

Parameters	Pirenzepine optimized formulation	Marketed product
$C_{max}$ (ng/ml)	2.15±0.01	2.85±0.01
$AUC_{0-t}$ (ng.h/ml)	11.15±1.12	7.21±1.26
$AUC_{0-\infty}$ (ng.h/ml)	14.42±1.16	10.15±1.13
$T_{max}$ (h)	3.00±0.05	1.00±0.04
$t_{1/2}$ (h)	5.85±0.41	3.91±0.01
$Kel$ ( $h^{-1}$ )	1.93±0.11	1.15±0.33

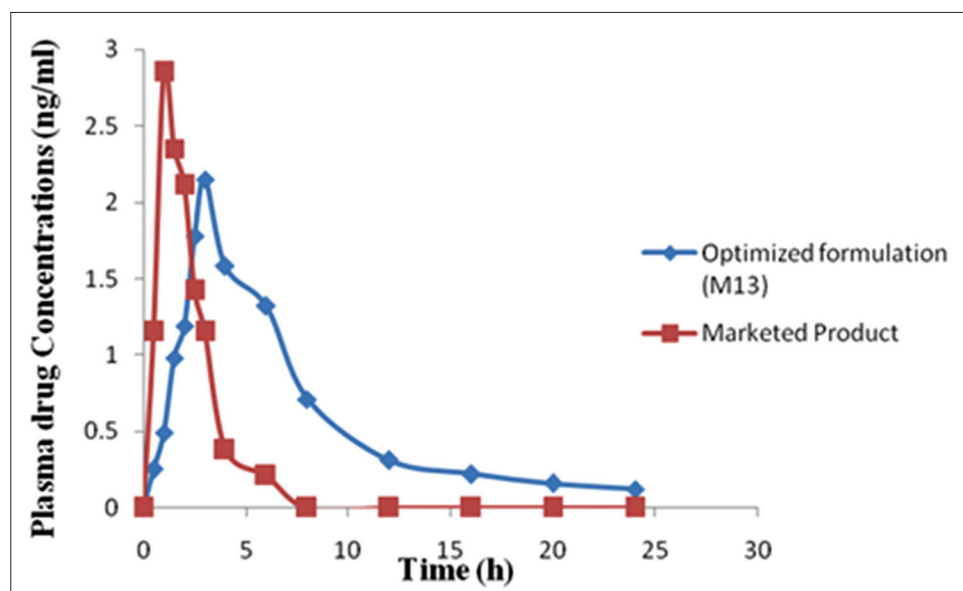


Fig. 8: Plasma concentrations at different time intervals for pirenzepine optimized formulation (M13) and marketed product

M13 at 40±20°C/75±5% RH for 6 months. For optimized formulation, M13 confirmed the negligible change in percentage yield, percentage drug release, and encapsulation efficiency as mentioned in Table 7.

#### *In vivo* studies

##### Pharmacokinetic study

In the present research study, various kinetic parameters of optimized formulation include  $C_{max}$ ,  $t_{max}$ ,  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ , elimination half-life ( $t_{1/2}$ ), and elimination rate constant were calculated and compared with a marketed product, showed in Table 8. The  $C_{max}$  of marketed product (2.85±0.01 ng/ml) was found higher than optimized formulation

(2.15±0.01 ng/ml) due to fast initial drug release; it leads to nonequivalence of the dosage forms. The initial phase drug release rate should be controlled and optimized by the addition of sodium alginate and chitosan so as to reach the target range.

The significant difference in  $T_{max}$ ,  $AUC_{0-t}$ ,  $AUC_{0-\infty}$  and  $Kel$  values of optimized formulation and marketed product were observed this could be due to slow release and prolonged drug absorption from the optimized formulation. Low oral bioavailability (25%) of pirenzepine from the marketed product was due to its narrow absorption window in the upper part of GIT. However, in the optimized formulation, gastric

residence time was increased hence reducing the dosing frequency and sustain the drug release in absorbable regions of the GIT thereby increasing its bioavailability. This observation was supported by ANOVA results ( $p < 0.05$ ). The optimized microsphere formulation was 1.5-fold higher bioavailability than the marketed product.

The optimized pirenzepine microsphere formulation (M13) and marketed product mean plasma concentration profile results were shown in Fig. 8. The optimized formulation sustains the effect for 24 h when compared with a marketed product for 6 h. This shows the optimized mucoadhesive microspheres formulation had prolonged gastric residence time and absorption than that of the marketed product.

## CONCLUSION

In the present research study, stable gastroretentive mucoadhesive dosage form was fruitfully prepared by ionotropic gelation method containing pirenzepine. The technique used was found to be handling easy, inexpensive, and reproducible process. The results of the study revealed that the sodium alginate, chitosan, and calcium chloride considerably affected the drug incorporation efficiency, particle size, percentage yield, and percentage mucoadhesion. The optimized formulation (M13) was found to be efficient with a particle size ( $89.04 \pm 0.21 \mu\text{m}$ ), percentage yield (98.90%), encapsulation efficiency (97.07%), swelling index (96.08%), and mucoadhesion (95.50%). The mucoadhesive property facilitates the microspheres to adhere to the gastric mucosal surface and reside in stomach for prolonged time which eventually leads to better bioavailability. Cumulative percentage drug release studies showed sustained drug release up to  $99.07 \pm 0.17\%$  (12 h). Drug release from pirenzepine microspheres followed zero-order and Korsmeyer–Peppas model suggested that it followed the erosion and diffusion-controlled mechanism. The FTIR studies displayed that drug and excipients were compatible. SEM results revealed that the prepared microspheres were spherical in shape. The stability of optimized formulation (M13) was studied as per the ICH guidelines and found stable for 6 months. The pharmacokinetic study in New Zealand white rabbits revealed the oral bioavailability of pirenzepine in microspheres was enhanced due to the prolonged residence time in GIT in comparison with a marketed product. The optimized formulation was shown much higher bioavailability than the marketed product. Microspheres would be a promising DDS could play a potentially significant role in pharmaceutical drug delivery for gastric therapeutics.

## AUTHOR'S CONTRIBUTION

All the authors have contributed equally.

## CONFLICTS OF INTEREST

No conflicts of interest were raised by the authors.

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