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Formulation development and evaluation of pirenzepine floating microspheres

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ABSTRACT

The current study investigates the development and evaluation of floating microspheres using Pirenzepine as a model drug to prolong gastric retention time. Floating microspheres were prepared by ionotropic gelation method using sodium alginate as polymer, Calcium chloride as cross linking agent, sodium bicarbonate as gas generating agent and HPMCK4M, HPMCK15M as rate retarding agent in concept to optimize the formulation. The FTIR studies indicated no significant interaction observed between drug and excipients. The F7 formulation showed the excellent flow properties. The particle size, % yield, % entrapment efficiency and swelling index of optimized formulation was investigated 55.45±0.09µm, 96.10%, 96.30% and 95.12%, respectively. The %buoyancy was excellent with approximately 98.10% of the microspheres floating upto 24h. The Cumulative % drug released from F7 microspheres was found to be 96.23±0.11% with in 12h and compared with the marketed product 95.23±0.21% with in 1h. The optimized formulation best fitted into zero order and Higuchi kinetics indicating diffusion controlled drug release pattern. SEM studies showed spherical shape and revealed the presence of pores on the floating microspheres surface which was responsible for floating ability. Optimized microspheres (F7) was stable at 40°C ± 2°C/75% RH ± 5% RH for 6 months. The F7 formulation showed the better results with HPMC K4M compared with HPMC K15M as rate retarding polymer. These results indicated that the Pirenzepine-loaded microspheres could potentially be exploited as a delivery system with controlled drug release and improved insitu bioavailability.

Keywords: Floating, Controlled release, Kinetics, Diffusion, HPMC K4M.

INTRODUCTION

The oral route is the ideal and most suitable for drug delivery systems because of its patient compliance, ease of administration [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 a gastric retention and

long-acting release drug products [2]. To achieve this goal a variety of system have been developed including floating, hydrogels etc., among these the gastric floating DDS offers several advantages for those drugs associated with poor bioavailability and narrow absorption window in the GIT upper part [3]. Gastric floating DDS retains the dosage form at the absorption site and consequently enhances bioavailability. Many researcher have published their work on Pirenzepine oral dosage forms but the research on floating microspheres could not be found hence in the present study floating microspheres were developed by ionotropic gelation method [4]. In recent years, microspheres are the attractive carrier for drug delivery. This is because of its controlled drug release, smaller size and deliver drug to a specific site.

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 insitu level [5].

Polymeric drug delivery system display several advantages over the conventional dosage forms and it includes enhanced efficacy, patient compliance, reduced toxicity, and also to control the encapsulated drug release [6]. Sodium alginate is anionic natural polysaccharide, prepared by mixture of D-mannuronic acid and L-glucuronic acid. Sodium alginate is extensively used as carrier for drug delivery due to its biocompatibility and low toxicity [7]. The widely used method for microspheres preparation is an ionotropic gelation method. This technique offers several advantages such as simple method of preparation no need to use of organic solvent, and, also easier to control. Sodium alginate could form gel in the presence of multivalent cations such as Ca^{2+}, Zn^{2+}, Ba^{2+} and Al^{3+} etc., by ionic cross-linking to form microspheres, it has been widely used in sustained drug release [8]. Hence in this study calcium chloride is selected as cross linking agent and also because of its nontoxic and biocompatibility [9]. HPMC K4M and HPMC K15M are the commonly used polymers for the floating microspheres preparation by ionic gelation technique and sodium bicarbonate used as gas generating agent [10].

The aim of present research work was Pirenzepine loaded alginate floating microspheres were developed for GIT specific drug delivery. The prepared microspheres were evaluated for micromeritic properties, particle size, % buoyancy, % yield, encapsulation efficiency, swelling index and characterized by Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and *in vitro* release studies.

MATERIALS AND METHODS

Materials

Pirenzepine was procured as sample of gift from Splendid laboratories, Pune, India. Sodium alginate and Sodium bicarbonate was used as polymer obtained from Pruthvi Chemicals, Mumbai. Calcium chloride was purchased from SD fine chemicals Mumbai, India. HPMC K4M and HPME K15M were purchased from Rubicon Labs, Mumbai, India. All other chemicals used were of analytical grade.

Formulation of Pirenzepine Floating microspheres

HPMC K4M, HPMC K15M as Rate controlling agent, Sodium alginate as Microsphere core forming agent, Sodium bicarbonate as Gas generating agent, and Calcium chloride as Cross linking agent were used for the formulation of Pirenzepine Microsphere.

Table 1: Formulation trials of Pirenzepine Floating microspheres

Formulation code	Pirenzepine (g)	Sodium alginate	HPMCK4M (mg)	Sodium bi carbonate(mg)	Calcium chloride
F1	1	1%	50	25	1%
F2	1	1. 2%	75	50	1%

F3	1	1.4 %	100	75	1%
F4	1	1.6%	150	100	1%
F5	1	1.8 %	175	125	1%
F6	1	2.%	200	150	1%
F7	1	2.2%	200	175	1%
Formulation code	**Pirenzepine (g)**	**Sodium alginate**	**HPMC K15M (mg)**	**Sodium bi carbonate (mg)**	**Calcium chloride**
F8	1	1%	150	25	1%
F9	1	1. 2%	200	50	1%
F10	1	1.4%	250	75	1%
F11	1	1.6%	300	100	1%
F12	1	1.8%	350	125	1%
F13	1	2%	400	150	1%
F14	1	2.2%	450	175	1%

Floating microspheres Preparation

Microspheres containing Pirenzepine as a core material were formulated by ionotropic gelation method showed in Table 1. Initially, Sodium alginate solution was prepared by dissolving in distilled water at a concentration ranges from 1% to 2.2% w/v then stirred thoroughly by magnetically. On complete solution, weighed quantities of Pirenzepine followed by HPMC K4M, HPMC K15M and sodium bicarbonate of different weights were added to the above dispersion. Then the above mixture was stirred at 500rpm, maintained room temperature. The mixture was sonicated for 30min to eliminate air bubbles that may have been formed during the stirring process. The homogenous dispersion was extruded using a 20G needle fitted with a 10 ml syringe into 100ml of 1% of calcium chloride solution, being stirred at 100rpm for 10min into the gelation medium. Then microspheres were collected, washed with distilled water and oven-dried at 60°C [11].

EVALUATION PARAMETERS

Micromeritic properties

The characterization of prepared microspheres were carried out by particle size, angle of repose, bulkdensity, tapped density, and Carr's index.

Determination of swelling index

For estimating the swelling index, the accurately weighed quantities of microspheres were suspended in simulated gastro intestinal fluids (0.1N HCl with pH1.2). The liquid droplets adhered to the surface of microspheres was removed by using blotting paper and then weighed it with the help of a microbalance. The swollen microspheres were dried in oven at 60°C for 5h. The change in weight of dried microspheres was used to calculate the percentage of swelling index [12]. The following equation was used.

Swelling index= (Mass of swollen microspheres - Mass of dry microspheres/mass of dried microspheres) X 100.

% yield of microspheres

The prepared microspheres were collected and weighed. The actual weight of obtained microspheres divided by total weight of added drug and polymer was used for the calculation of % yield and mentioned below.

% yield = [Total weight of microspheres / Total weight of drug and polymer] X 100

Entrapment efficiency

Encapsulation efficiency of Pirenzepine was determined by weighing 10mg of floating microspheres, crushed and dissolved in methanol then transferred in to 100ml conical flask. The above solution was agitated to dissolve the drug and polymers and to extract the drug. Then solution was filtered using membrane filter (0.45µm) to separate shell fragments. The drug was estimated at the λmax of 280nm by using a double beam spectrophotometer (Shimadzu, UV-1800) [13]. The incorporation efficiency was determined using the following equation.

% Drug entrapment = Calculated drug concentration / Theoretical drug concentration X 100

Test for buoyancy

100mg of the microspheres were transferred to a USP type II dissolution test apparatus containing 900ml of simulated gastric fluid (0.1N HCl) and 0.02% of tween 20 was maintained at 37°C. The content of the beakers was stirred at 100rpm. Then microspheres were separated at different time intervals and dried until a constant weight obtained [14]. The percentage of buoyancy is calculated by using following equation.

$$\text{Buoyancy (\%)} = \frac{\text{Weight of floating microspheres}}{\text{Initial weight of floating microspheres}} \times 100$$

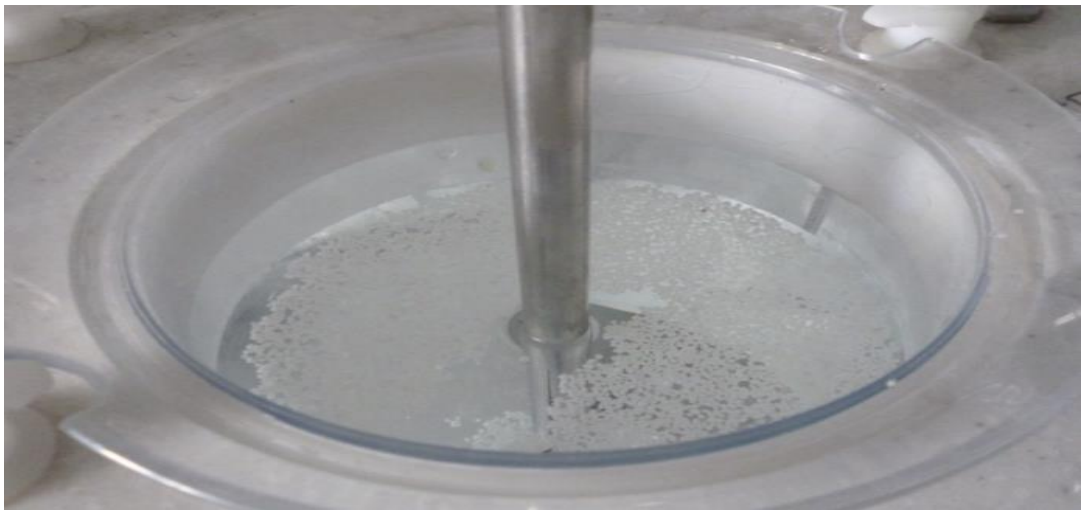

Figure 1: *In vitro* buoyancy study of Pirenzepine floating microspheres

In vitro drug release

In vitro drug release studies of floating microspheres were conducted in 900ml of 0.1N HCl (pH 1.2) at 37±0.5°C by using USP dissolution apparatus II (Paddle type). Accurately weighed quantity of floating microspheres were equivalent to 100mg of drug transferred into 900ml of 0.1NHCl medium and stirring at 100rpm. Aliquots of samples were withdrawn at predetermined time intervals, filtered and diluted with similar medium finally assayed at 280nm using UV-Visible spectrophotometer. The samples withdrawn were replaced with same dissolution medium at predetermined time intervals. All the samples were analyzed in triplicate [15].

Release order kinetics

Drug release data of optimized floating microspheres formulation were fitted to various kinetic models to disclose the mechanism of drug release from the microspheres. Those include Zero order, first order, Higuchi model and Korsmeyer-Peppas exponential equation and r^2 values were calculated [16].

Drug-excipient compatibility studies

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR technique can be used to identify the functional groups in the pure drug and drug-excipient compatibility. Pure Rebamipide FTIR spectra, physical mixtures and optimized formulation were recorded by using FTIR (SHIMADZU). Weighed quantity of KBr and excipients were taken in the ratio 100: 1 and mixed by mortar. The samples were made into pellet by the application of pressure [17]. Then the FTIR spectra were recorded between 4000 - 400 cm^{-1} .

SEM studies

Surface nature of microspheres includes size and shape was examined with the help of Scanning Electron Microscope (HITACHI, S-3700N). The microspheres were dried completely prior to analysis and SEM was carried out at different magnifications of 15.0 kv × 7.6mm, 15 kv x 6.6mm, 15Kv x 7.0mm [18].

Stability studies

Optimized formulation such as F7 floating microspheres were subjected to stability testing at 40°C ± 2°C/75% RH ± 5% RH for 6 months using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals 0, 30, 60, 120, and 180 days period according to ICH guidelines. Various *in vitro* parameters like % yield, entrapment efficiency and *in vitro* release studies were evaluated [19].

RESULTS AND DISCUSSION

Floating microspheres

Figure 2: Pirenzepine Floating Microspheres

The particle size, % buoyancy and flow properties of the microspheres are expressed in terms of bulk density, tapped density, angle of repose and Carr's index results shown in Table 2 and prepared microspheres pictorial diagram depicted in Figure 2. The microspheres ranged in size from 55.45±0.09 to 91.67±0.13, lower particle size was observed in HPMC K4M as rate retarding polymer. The bulk density and tapped density of were ranged from 0.55 to 0.89 g/cc^3 and 0.52 to 0.84 g/cc^3, respectively. The values of the angle of repose was in the range of 21˚.54-30˚.24, which indicates excellent to good flow properties, whereas the Carr's index for all formulations was in the range of 8.95%-13.94%, which indicated excellent to good flow properties. This suggests that the microspheres can be easily handled during processing. The % buoyancies of the microspheres was found highest in F7 this may be due to slow penetration of the dissolution medium in the microspheres, as HPMC K4M is better water swellable polymer than HPMC K15M.

Table 2: Micromeretic properties of Pirenzepine floating microspheres

Formulation code	Particle size (µm)	Bulk density g/cc^3	Tapped density g/cc^3	Angle of repose	Carr's index	Buoyancy%
F1	60.45±0.04	0.59	0.65	25˚.93	13.56%	95.20%
F2	60.12±0.08	0.66	0.59	23˚.74	9.34%	84.50%
F3	65.29±0.13	0.74	0.62	29˚.67	10.34%	83.30%
F4	73.43±0.04	0.76	0.73	25˚.03	14.36%	95.10%
F5	77.35±0.04	0.79	0.75	29˚.74	9.12%	81.64%
F6	79.67±0.09	0.81	0.83	30˚.15	7.23%	89.40%
F7	55.45±0.09	0.55	0.52	21˚.54	8.95%	98.10%
F8	65.23±0.14	0.86	0.63	24˚.91	10.32%	72.50%
F9	61.22±0.11	0.69	0.65	22˚.70	9.03%	75.80%
F10	73.34±0.10	0.71	0.74	30˚.24	12.34%	76.40%
F11	78.45±0.21	0.75	0.76	24˚.91	11.90%	85.30%
F12	85.45±0.09	0.79	0.79	25˚.02	13.90%	93.50%
F13	87.23±0.19	0.85	0.83	25˚.54	10.34%	89.40%
F14	91.67±0.13	0.89	0.84	25˚.91	13.94%	92.20%

Entrapment efficiency, % yield and swelling index

The % yields ranged from 78.09% to 96.10% with the % entrapment efficiency being between 77.09% to 96.30%. The swelling index results from 76.76% to95.12%. These three parameters increased with increasing polymer concentration in the microspheres. The better results were observed in F7 formulation had HPMC K4M as rate retarding polymer.

Table 3: Percentage yield, entrapment efficiency and Swelling index of Pirenzepine microspheres

Formulation code	Percentage Yield	Entrapment efficiency	Swelling index
F1	78.09%	77.09%	76.76%
F2	81.12%	82.23%	79.78%
F3	83.23%	84.56%	83.34%
F4	86.87%	87.30%	85.23%
F5	89.30%	90.20%	88.34%
F6	90.30%	91.10%	89.78%
F7	96.10%	96.30%	95.12%
F8	86.42%	84.30%	82.23%
F9	81.56%	84.89%	84.34%
F10	89.76%	88.78%	88.45%
F11	92.78%	92.78%	89.89%
F12	94.50%	94.56%	91.10%
F13	85.30%	81.30%	83.89%
F14	85.30%	84.88%	87.90%

In vitro drug release studies

The release of drug from the microspheres was controlled over a period of 12h at pH 1.2 as seen in the Tables 4&5 and in Figures 3&4. The cumulative % drug release of optimized formulation F7 was found to be 96.23±0.11% at the end of 12h where as marketed product noted 95.23±0.21 % within 1h.

Table 4: *In vitro* Cumulative % drug release of Pirenzepine floting microspheres from F1 to F7 and Marketed product

Time (h)	F1	F2	F3	F4	F5	F6	F7	Marketed product
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	10.09±0.12	12.08±0.11	12.11±0.22	13.09±0.32	13.1±0.12	13.10±0.18	14.13±0.21	95.23±0.21
2	19.05±0.15	20.07±0.21	23.12±0.11	23.50±0.33	24.11±0.16	24.30±0.14	24.15±0.22	--
4	35.08±0.11	38.20±0.21	38.90±0.21	36.50±0.22	38.20±0.22	39.40±0.21	37.23±0.26	--
6	50.09±0.16	51.30±0.16	49.90±0.15	51.60±0.36	51.30±0.21	53.80±0.22	51.73±0.32	--
8	66.20±0.21	63.30±0.15	61.20±0.16	67.40±0.31	63.30±0.16	68.60±0.24	66.46±0.21	--
10	80.90±0.21	69.90±0.21	71.20±0.14	82.80±0.33	73.30±0.32	73.90±0.12	78.45±0.16	--
12	78.03±0.31	80.52±0.22	83.15±0.21	86.17±0.21	89.54±0.22	91.07±0.21	96.23±0.11	--

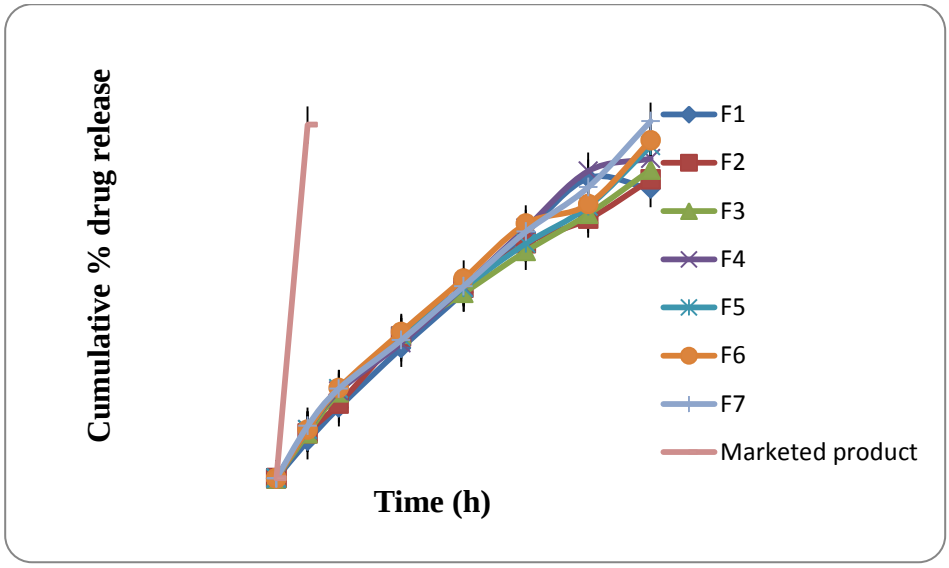

Figure 3: *In vitro* Cumulative % drug release of Pirenzepine floting microspheres F1 to F7 and marketed product (Gastrozepin 100mg IR)

Table 5: *In vitro* Cumulative % drug release of Pirenzepine floating microspheres formulation

Time (h)	F8	F9	F10	F11	F12	F13	F14
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	12.12±0.11	13.12±0.13	13.23±0.16	14.09±0.15	14.09±0.13	11.62±0.11	12.63±0.21
2	22.40±0.16	23.09±0.16	24.80±0.13	25.40±0.22	24.50±0.12	23.01±0.16	22.01±0.15
4	38.20±0.16	38.90±0.11	44.40±0.21	38.20±0.12	37.60±0.19	38.24±0.19	44.83±0.18
6	51.30±0.13	49.90±0.19	51.60±0.13	51.30±0.12	52.80±0.18	52.83±0.13	57.7±0.16
8	63.35±0.19	61.20±0.15	60.30±0.16	63.30±0.26	68.50±0.12	67.03±0.15	64.6±0.21
10	69.90±0.15	70.10±0.16	70.60±0.52	69.91±0.25	83.90±0.11	72.22±0.14	75.56±0.18
12	83.56±0.11	85.56±0.13	89.45±0.13	91.42±0.22	93.11±0.33	81.36±0.11	84.76±0.15

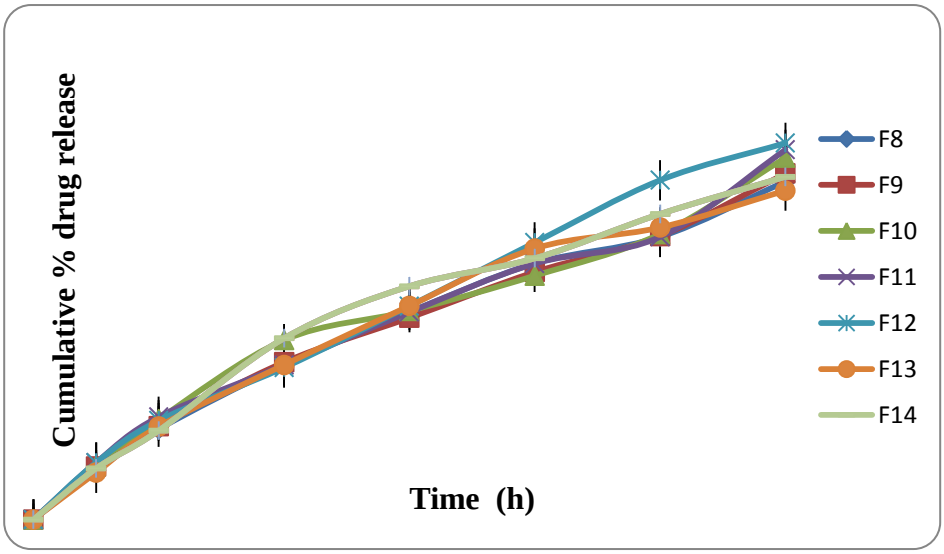

Figure 4: *In vitro* Cumulative % drug release of Pirenzepine floating microspheres from F8- F14

Mathematical modeling of optimized formulation (F12)

The *in vitro* drug release profiles were fitted to several kinetic models and release data followed by their R^2, k and n values shown in the Table 6. The optimized formulation was best fitted in Zero Order and Higuchi indicating control the drug release by diffusion mechanism.

Table 6: Release order kinetics of optimized formulation of floating microspheres

Formula Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
F7	0.997	8.015	0.766	0.131	0.980	35.26	0.559	2.184

Drug excipient compatibility studies

FTIR spectroscopy of Pirenzepine microspheres

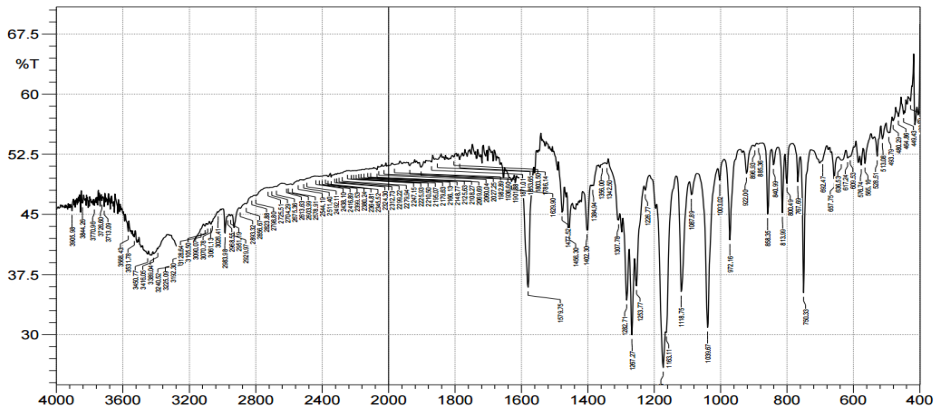

Figure 5: FT-IR spectrum of pure drug Pirenzepine

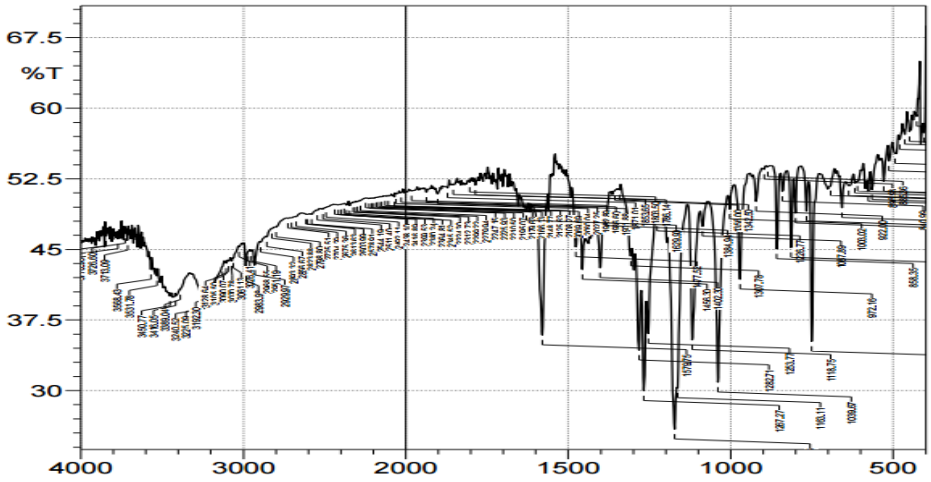

Figure 6: FT-IR spectrum of optimized formulation of Pirenzepine floating microspheres F12

The IR spectra of the Pirenzepine (Figure 5) showed the characteristic band of alkene stretching (═C–H and CH_2) vibration at 3324.32–3016.48 cm^{-1} and alkane stretching (–CH_3, –CH_2 and –CH) vibration at 2853.73 cm^{-1}. Also exhibited C═O stretch at 1738.2 cm^{-1} due to saturated ketone and C═O–NH stretching at 1635.90 cm^{-1}. A selective stretching vibration at 1561.57 cm^{-1} and 1525.80 cm^{-1} for primary and secondary amine was also observed. For functional groups like S═O stretch and –C–S stretch showed vibrations at 1041.78 cm^{-1} and 729.57 cm^{-1}respectively. The optimized formulation (Figure 6) showed characteristic peaks which were close to the principal IR peaks of the drug, confirming the presence of Pirenzepine in the microspheres showing no strong interactions between the drug and the excipients used and also indicating the stability of the drug during the microencapsulation process.

SEM studies of Pirenzepine microspheres

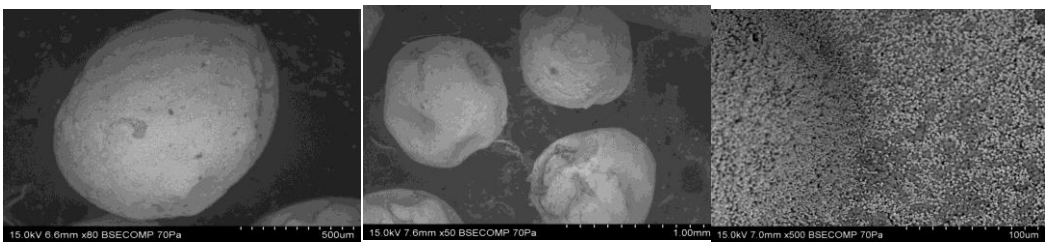

Figure 7: Scanning electron micrographs of Pirenzepine microspheres

The microspheres were smooth and spherical in shape as seen in Figure 7. The surface of the microspheres found smoother and less porous. There was no evidence of aggregation of particles as increasing the polymer concentration

Stability studies

After 6 months at 40^0C / 75 % RH there was no significant difference observed during stability studies in the % yield, entrapment efficiency and *in vitro* drug release of optimized formulation (F7) as shown in Table 7.

Table 7: Stability studies of optimized Floating Microspheres

Retest Time For Optimized formulation	Percentage yield	Entrapment efficiency	*In-vitro* drug release profile (%)
0 days	96.10	96.30	96.23
30 days	95.40	95.4	95.20
60 days	94.22	94.53	94.33
120 days	93.13	93.55	93.68
180 days	92.34	92.22	92.45

CONCLUSION

Pirenzepine loaded floating microspheres were prepared by ionotropic gelation method. From the results it concluded that formulation F7 was found to be satisfactory results in terms of excellent micromeritic properties, particle size (55.45±0.09µm), yield of microsphere (96.10%), incorporation efficiency (96.30%), % buoyancy (98.10%), swelling index (95.12%) and highest *in vitro* drug release of 96.23±0.11% in a sustained manner with constant fashion over extended period of time for 12h compared with marketed product 95.23±0.21 in 1h. The drug and excipients were compatible studied by using FTIR. Drug release from Pirenzepine microspheres followed Zero order and Higuchi model. It was suggested that mechanism of drug release from microspheres was diffusion controlled. The prepared microspheres were spherical in shape studied by SEM studies. The optimized formulation F7 was stable. Hence the formulated and prepared floating Pirenzepine microspheres may establish to be potential candidate for safe and effective sustained drug delivery and improve the bioavailability.

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