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Formulation Development and Evaluation of Floating Microspheres Of Gemifloxacin Mesylate

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Abstract: The present study was aimed to prepare the Gemifloxacin mesylate floating microspheres by Ionotropic gelation technique with different drug to carrier ratio. Gemifloxacin mesylate All the microspheres were characterized for particle size, scanning electron microscopy, FT-IR study, DSC, percentage yield, drug entrapment, % buoyancy, stability studies and found to be within the limits. Among all the formulations F9 were selected as optimized formulation based on the physic chemical and release studies. In the *in vitro* release study of formulation F9 showed 97.58% after 12 h in a controlled manner, which is essential for anti ulcer therapy. The innovator Gemiflox conventional tablet shows the drug release of 96.23% within 1 h. The drug release of F9 formulation followed zero order and Higuchi kinetics indication diffusion controlled drug release.
Key Words : Gemifloxacin mesylate, HPMC, gum kondagogu, floating microspheres.

Introduction:

The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200 micrometer.[1] As the system floats over gastric contents, the drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration.[2] Floating microspheres have emerged as an efficient means of enhancing the bioavailability and controlled delivery of many drugs the increasing sophistication of delivery technology will ensure the development of increasing number of gastro-retentive drug delivery systems to optimize the delivery of molecules that exhibit absorption window, low bioavailability, and extensive first pass metabolism.[3,4]

Gastric emptying of dosage forms is an extremely variable process and ability to prolong and control the emptying time is a valuable asset for dosage forms, which reside in the stomach for a longer period of time than conventional dosage forms[5].

Floating drug delivery system (FDDS) promises to be a potential approach for gastric retention[6]. Floating microspheres have emerged as an efficient means of enhancing the bioavailability and controlled delivery of many drugs the increasing sophistication of delivery technology will ensure the development of increasing number of gastro-retentive drug delivery systems to optimize the delivery of molecules that exhibit absorption window, low bioavailability and extensive first pass metabolism[7].

Gemifloxacin mesylate is histamine H_2-receptor antagonists, which is used to reduce the risk of stomach ulcers in patients treated with nonsteroidal anti-inflammatory drugs, which has less bioavailability

(60%) and lesser half life of 2 hours.[8] The aim of present work is to design and in vitro evaluation of floating microspheres of Gemifloxacin mesylate to enhance its bioavailability and prolonged residence time in stomach.

Materials and Methods:

Materials:

Gemifloxacin mesylate pure drug was generous gift from Hetero Drugs Ltd**,** Hyderabad, India. Sodium alginate was obtained from Pruthvi Chemicals, Mumbai. HPMC K 4 M & HPMC K 15 M was obtained from Rubicon labs, Mumbai. Xanthan gum, Guar gum, Kondagogu gum and sodium CMC were gifted from MSN Labs Ltd. Hyderabad. All other chemicals used were of analytical grade.

Formulation of Gemifloxacin mesylate Floating microspheres – Formulation design:

Gemifloxacin mesylate floating microspheres were prepared using polymers sodium alginate, Calcium chloride, HPMC K4M, HPMCK15M, Xanthan gum, Guar gum, Gum kondagogu and sodium bicarbonate by Ionic gelation method.

Table 1: Formulation trials of Gemifloxacin mesylate floating microspheres:

Formulation code	Gemifloxacin (g)	Sodium alginate	HPMC K4M (mg)	Sodium bi carbonate(mg)	Calcium chloride	Guar Gum	Xanthan Gum
F1	3200	1%	50	25	1%	0.75%	1%
F2	3200	1. 2%	75	50	1%	0.75%	1.2%
F3	3200	1.4 %	100	75	1%	0.75%	1.4%
F4	3200	1.6%	150	100	1%	0.75%	1.6%
F5	3200	1.8 %	175	125	1%	0.75%	1.8%
F6	3200	2.%	200	150	1%	0.75%	2%
F7	3200	2.2%	200	175	1%	0.75%	2.2%
Formulation code	**Gemifloxacin (g)**	**Sodium alginate**	**HPMC K15 M (mg)**	**Sodium bi carbonate (mg)**	**Calcium chloride**	**Guar Gum**	**Gum Kondagogu**
F8	3200	1%	150	50	1%	0.75%	1%
F9	3200	1. 2%	200	75	1%	0.75%	1.2%
F10	3200	1.4%	250	100	1%	0.75%	1.4%
F11	3200	1.6%	300	125	1%	0.75%	1.6%
F12	3200	1.8%	350	150	1%	0.75%	1.8%
F13	3200	2%	400	175	1%	0.75%	2%
F14	3200	2.2%	450	200	1%	0.75%	2.2%

Procedure:

Floating microspheres of Gemifloxacin mesylate were prepared by ionic gelation technique using different proportion of polymers as shown in table. A solution of sodium alginate solution is prepared weighed quantity of drug and HPMC K4 or HPMC K15 was triturated to form fine powder, and then added to above solution. Sodium bicarbonate, a gas forming agent was added to this mixture. Resultant solution was extruded drop wise with the help of syringe and needle into 100ml aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 10 minutes the obtained microspheres were washed with water and dried at 60 degrees -2 hours in a hot air oven and stored in dessicater.[9]

Evaluation of Gemifloxacin mesylate microspheres:

Particle size:

The 100 microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 microspheres were measured randomly by optical microscope.[10]

Angle of repose:

Angle of repose (Θ) of microspheres measures the resistance to particles flow, and is calculated according to fixed funnel standing cone method. Where (Θ) is angle of repose, H/D is surface area of the free standing height of the microspheres heap that is formed on a graph paper after making the microspheres flow from glass funnel.

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

Bulk density: Volume of the microspheres in the measuring cylinder was noted as bulk density.

$$\text{Bulk density} = \frac{\text{Wt of powder}}{\text{Bulk volume of powder}}$$

Tapped density: Change in the microspheres volume was observed in mechanical tapping apparatus.

$$\text{Tapped density} = \frac{\text{Wt of microspheres}}{\text{Tapped volume of microspheres}}$$

Compressibility index:

Also called as Carr's index and is computed according to the following equation.

$$\text{Carr's compressibility index} = \frac{\text{Tapped density - Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio:

Hausner's ratio of microspheres is determined by comparing the tapped density to the fluff density using the equation.[11]

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

Swelling index:

Swelling index was determined by measuring the extent of swelling of microspheres in the given medium. Exactly weighed amount of microspheres were allowed to swell in given medium. The excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed by using microbalance. The hydro gel microspheres then dried in an oven at 60 degrees for 5h until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated by using the formula.[12]

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

Drug entrapment efficiency and % yield:

In order to determine the entrapment efficiency, 10 mg of formulated microspheres were thoroughly crushed by triturating and suspended in required quantity of methanol followed by agitation to dissolve the polymer and extract the drug. After filtration, suitable dilutions were made and drug content assayed spectrophotometrically at particular wavelength using calibration curve. Each batch should be examined for drug content in a triplicate manner.[13]

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

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

Percentage buoyancy of Gemifloxacin mesylate floating microspheres:

In vitro floating ability can be determined by calculating percentage buoyancy and performed in USP type II dissolution test apparatus by spreading the floating microspheres in 0.01N HCL. containing the surfactant .The media is stirred at 100 revolutions per minute (rpm) at 37± 0.5° C. After specific intervals of time, both the fraction of microspheres (floating and settled microspheres) is collected and buoyancy of the floating microspheres is determined by using formula.[14]

$$\% \text{ Floating Microspheres} = \frac{\text{Weight of floating microspheres}}{\text{Initial weight of floating microspheres}} \times 100$$

***In vitro* drug release studies:**

In vitro drug release studies for developed Gemifloxacin mesylate microspheres were carried out by using dissolution apparatus II paddle type (Electrolab TDL-08L). The drug release profile was studied in 900 ml of 0.01 N HCl at 37± 0.5^{0}C temperature at 100 rpm. The amount of drug release was determined at different time intervals of 0, 1, 2, 3, 4, 6, 8, 10& 12 hours by UV Visible spectrophotometer (Shimadzu UV 1800) at 271nm.[15]

Kinetic modeling of drug release:

In order to understand the mechanism and kinetics of drug release, the result of the *in vitro* dissolution study of microspheres were fitted with various kinetic equations, like zero order[21] (percentage release vs. time), first order. (log percentage of drug remaining to be released vs. time) and Higuchi's model. (Percentage drug release vs. square root of time). Correlation coefficient (r^2) values were calculated for the linear curves obtained by regression analysis of the above plots.

Drug excipient compatibility studies

The drug excipient compatibility studies were carried out by Fourier transmission infrared spectroscopy (FTIR) method, Differential Scanning Calorimetry (DSC) and SEM.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The samples were dispersed in KBr and compressed into disc/pellet by application of pressure. The pellets were placed in the light path for recording the IR spectra. The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Samples were accurately weighed and heated in sealed aluminum pans at a rate of 10°C/min between 25 and 350°C temperature rang under nitrogen atmosphere, empty aluminum pan was used as a reference.

SEM studies

The surface and shape characteristics of pellets were determined by scanning electron microscopy (SEM) (HITACHI, S-3700N). Photographs were taken and recorded at suitable magnification.

Stability studies

The stability study of the optimized formulation was carried out under different conditions according to ICH guidelines. The optimized microspheres were stored in a stability chamber for stability studies (REMI make). Accelerated Stability studies were carried out at 40 ^{0}C / 75 % RH for the best formulations for 6 months. The microspheres were characterized for the percentage yield, entrapment efficiency & cumulative % drug released during the stability study period.[16]

Results and Discussion:

Figure 1: Gemifloxacin mesylate floating microspheres

Table 2: Micromeretic properties of Gemifloxacin mesylate 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	0.55	26˚.93	13.56%	95.20%
F2	60.12±0.08	0.66	0.59	27˚.74	9.34%	84.50%
F3	65.29±0.13	0.74	0.62	29˚.67	8.34%	83.30%
F4	73.43±0.04	0.76	0.73	27˚.03	14.36%	96.10%
F5	77.35±0.04	0.79	0.75	29˚.74	8.12%	81.64%
F6	79.67±0.09	0.81	0.83	31˚.15	7.23%	89.40%
F7	85.45±0.09	0.85	0.82	26˚.54	13.95%	98.10%
F8	55.23±0.14	0.86	0.63	26˚.91	10.32%	72.50%
F9	65.23±0.19	0.75	0.73	23˚.54	9.34%	97.00%
F10	73.34±0.10	0.71	0.74	30˚.24	12.34%	76.40%
F11	78.45±0.21	0.75	0.76	26˚.70	11.90%	98.50%
F12	85.45±0.09	0.79	0.79	25˚.02	13.90%	85.30%
F13	87.23±0.19	0.85	0.83	27˚.54	10.34%	89.40%
F14	91.67±0.13	0.89	0.84	25˚.91	13.94%	92.20%

All the formulations were evaluated for their micromeretic properties and physical parameters, found to be within the limits (**Table 2**). From all the above results F9 was found to be best formulation when compared with other formulations. The particle size, bulk density, tapped density, angle of repose, carr's index and % buoyancy was found to be 65.23±0.19, 0.75, 0.73, 23˚.54, 9.34% and 97.00% respectively.

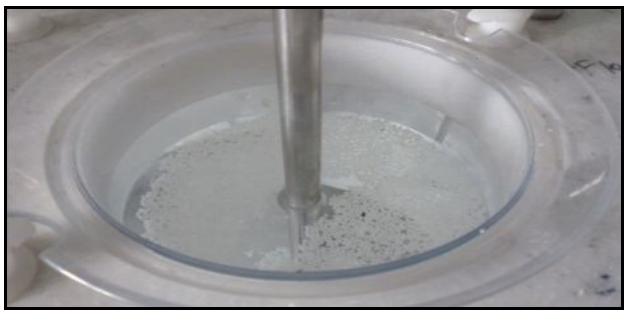

Figure 2: In vitro buoyancy study of Gemifloxacin mesylate floating microspheres

Entrapment efficiency, Percentage yield and Swelling index:

Formulation F9 showed best percentage yield, entrapment efficiency and Swelling Index of 95.50%, 96.56% and 95.10% respectively when compared with other formulations.

Table 3: Percentage yield, entrapment efficiency and swelling index of Gemifloxacin mesylate 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	95.50%	96.56%	95.10%
F10	89.76%	88.78%	88.45%
F11	81.56%	84.89%	84.34%
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**:

Gemifloxacin mesylate microspheres were evaluated for in vitro drug release studies in 0.1N HCL and the results are depicted in **Table 4&5**. The formulation F9 shown best drug release of 97.58% within 12h when compared with other formulations. The drug release of optimized formulation F9 was in controlled manner when compared with innovator product Gemiflox i.e 96.23 within 1h.

Table 4: In vitro cumulative % drug release of Gemifloxacin mesylate floting microspheres F1-F7 and innovator product:

Time	F1	F2	F3	F4	F5	F6	F7	Innovator (Gemiflox) 400mg immediate release
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	16.09±0.22	14.09±0.11	14.09±0.32	16.23±0.66	11.62±0.44	11.31±0.51	13.12±0.23	96.23±0.12
2	23.50±0.98	24.50±0.98	25.40±0.66	24.80±0.32	23.01±0.16	21.15±0.44	23.09±0.98	
3	28.03±0.97	29.20±0.87	30.78±0.18	31.30±0.44	29.11±0.98	28.19±0.32	30.89±0.76	
4	36.50±0.11	37.60±0.52	38.20±0.52	42.40±0.11	38.24±0.99	37.23±0.76	38.90±0.55	
6	53.60±0.32	56.80±0.65	54.30±0.43	51.60±0.42	62.83±0.72	51.73±0.54	46.90±0.65	
8	67.40±0.54	68.50±0.88	63.30±0,42	60.30±0.16	67.03±0.11	66.46±0.14	61.20±0.45	
10	82.80±0.76	83.90±0.76	69.91±0.54	70.60±0.22	72.22±0.14	78.45±0.22	70.10±0.65	
12	86.17±0.18	93.11±0.72	91.42±0.18	89.45±0.42	81.36±0.23	93.23±0.53	85.56±0.13	

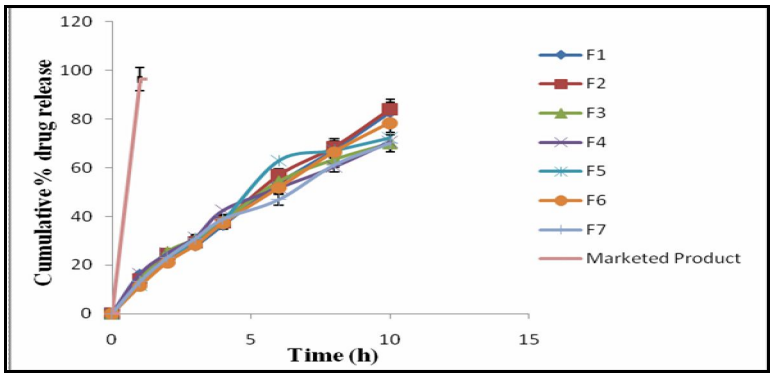

Figure 3: In vitro cumulative % drug release of Gemifloxacin mesylate floting microspheres

Table 5: In vitro cumulative % drug release of Gemifloxacin mesylate floating microspheres formulation F8-F14

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	13.31±0.65	11.12±0.11	10.09±0.19	14.11±0.44	12.08±0.18	13.10±0.88	12.63±0.11
2	24.33±0.87	24.40±0.23	19.05±0.19	26.12±0.63	22.07±0.43	28.41±0.98	22.01±0.21
3	27.11±0.43	34.01±0.32	27.06±0.43	34.13±0.53	30.11±0.33	38.23±0.11	37.11±0.23
4	36.84±0.33	44.20±0.32	35.08±0.19	38.90±0.26	38.20±0.21	38.20±0.76	44.83±0.18
6	52.84±0.23	55.30±0.54	50.09±0.45	49.90±0.87	51.30±0.27	55.30±0.34	57.70±0.11
8	69.84±0.88	68.35±0.76	66.20±0.32	61.20±0.19	63.30±0.73	63.30±0.24	61.60±0.17
10	82.00±0.11	76.90±0.44	80.90±0.11	71.20±0.18	69.90±0.53	73.30±0.55	75.56±0.87
12	91.07±0.11	97.58±0.32	78.03±0.32	83.15±0.63	80.52±0.33	89.54±0.15	84.76±0.16

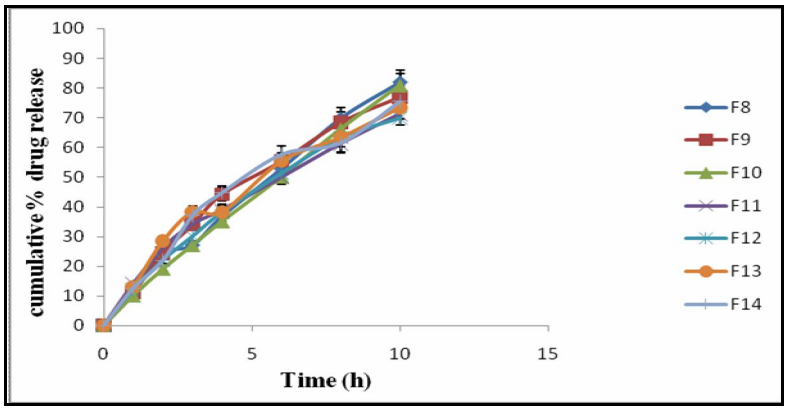

Figure 4: In vitro cumulative % drug release of Gemifloxacin mesylate floating microspheres

Release order kinetics of optimized formulation F9

Table 6: Release order kinetics of optimized formulation of floating microspheres F9

Formula Code	Zero Order		First Order		Higuchi		Korsmeyer Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
F9	0.978	7.624	0.618	0.116	0.962	28.15	0.691	2.135

The *in vitro* release profiles from optimized formulations were applied on various kinetic models. The best fit with the highest correlation coefficient was observed in zero order and Higuchi model, indicating diffusion controlled principle. Further the n value obtained from the Korsmeyer Peppas plots i.e. 2.135 suggest that the drug release from microspheres was anomalous Non fickian diffusion.

Fourier Transform Infrared (FTIR) spectroscopy

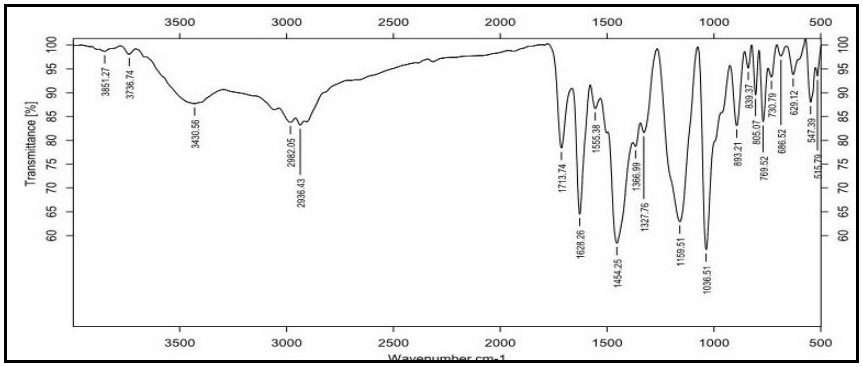

Figure 5: FT-IR spectrum of pure drug Gemifloxacin mesylate

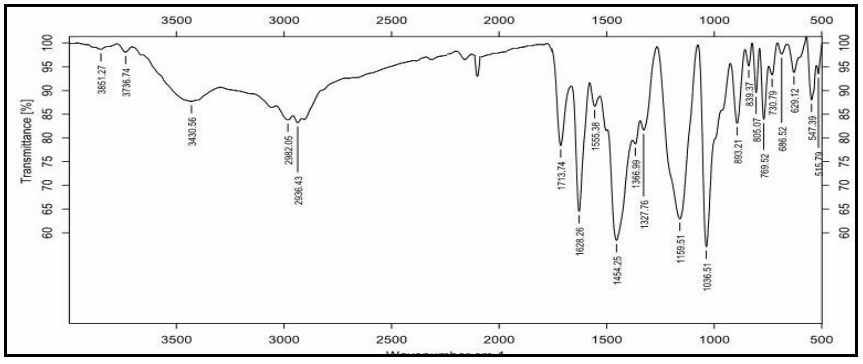

Figure 6:FT-IR spectrum of Gemifloxacin mesylate optimized microspheres (F9)

Drug excipient interaction was checked by comparing the IR spectra of the physical mixture (Figure) of drug with the excipients used with the IR spectrum of pure drug **(Figure 5)** and optimized formulation (F9) (Figure) and results found that there were no possible interaction between drug and polymer **(Figure 6)**.The FTIR spectrum of Gemifloxacin mesylate showed peaks corresponding to (C-F) bending at 1036.51cm-^{1} and O–CH_3 Bending at 1454.25cm-^{1}, R–COOH Stretching at 1159.51cm-^{1}, N–H Scissoring at 1628.26cm-^{1}, Aromatic–C=O Stretching at 1713.74cm-^{1} and C–H Rocking at 730.79cm-^{1}. From the FTIR graphs of drug polymer mixture, it was found that the same peaks of the drug are available. Since it proves that there is no incompatibility with the polymers.

DSC Studies:

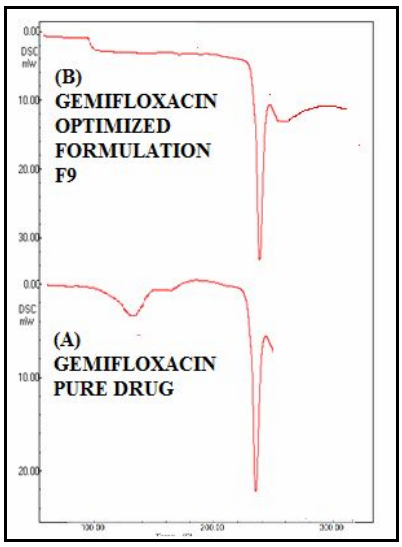

Figure 7: DSC thermogram of Gemifloxacin pure drug (A) and optimized formulatin F9 (B)

DSC thermogram revealed that there is no considerable change observed in Gemifloxacin mesylate melting endotherm of pure drug (232.79) **(Figure 7)** and drug in Gemifloxacin mesylate optimized formulation (F9) (236.36) **(Figure 7)**. It indicates that there is no interaction takes place between drug and other excipients used in the formulation.

Scanning Electron Microscopy

The external and internal morphology of controlled release microspheres were studied by Scanning Electron Microscopy.

Floating microspheres:

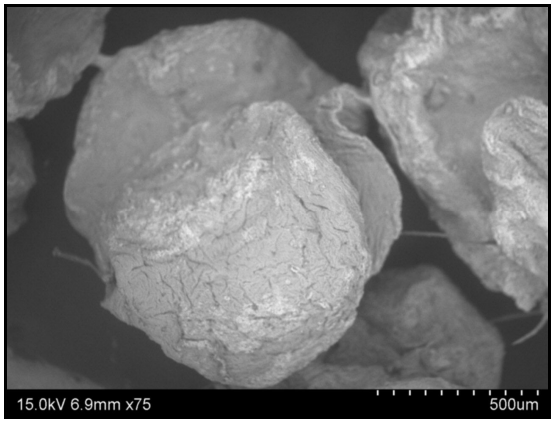

Figure 8: Scanning electron micrographs of floating microspheres F9

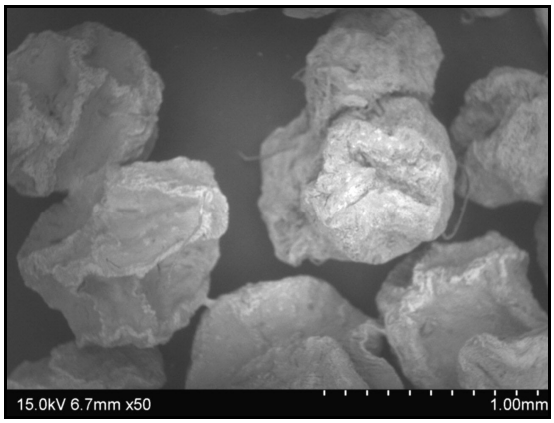

Figure 9: Scanning electron micrographs of floating microspheres F9

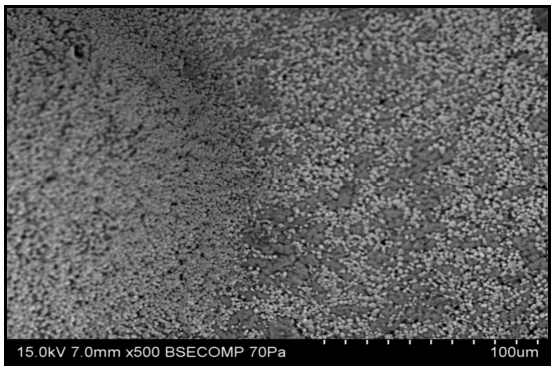

Figure 10: Scanning electron micrographs of floating microspheres F9

The SEM of optimized floating microspheres shows a hollow spherical structure with a rough surface morphology. Some of microsphere showed dented surface structure but they showed good floating ability on medium indicated intact surface (**Figure 8, 9 &10)**. The shell of microspheres also showed some porous structure it may be due to release of carbon dioxide.

Stability studies:

Optimized formulation (F9) was selected for stability studies on the basis of high cumulative % drug release. Stability studies were conducted by performing Percentage yield, %Entrapment efficiency and *In-vitro* drug release profile for 6 months according to ICH guidelines. From these results it was concluded that, optimized formulation is stable and retained their original properties with minor differences.

Conclusion:

From the above data, it could be concluded that Gemifloxacin mesylate floating microspheres exhibited prolonged and controlled release effect compared to Innovator product. All the microspheres were characterized for particle size, scanning electron microscopy, FT-IR study, DSC, percentage yield, drug entrapment, stability studies and found to be within the limits. Among all the formulations F9 were selected as optimized formulations for floating microspheres based on the physic chemical and release studies. In the *in vitro* release study of formulation F9 showed 97.58 respectively after 12 h in a controlled manner, which is essential for disease like peptic ulcer. The innovator Cimetine conventional tablet shows the drug release of 96.23 within 1 h.

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