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Design and evaluation of gemifloxacin mesylate mucoadhesive microspheres

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ABSTRACT

In the present research work mucoadhesive microspheres of Gemifloxacin mesylate was prepared using Ionotropic gelation technique. All the microspheres were characterized for particle size, scanning electron microscopy, FT-IR study, DSC, percentage yield, drug entrapment, stability studies and for in vitro *release kinetics and found to be within the limits. Among all the formulations M11 was selected as optimized formulation based on the physic chemical and release studies.* In vitro *drug release study of optimized formulation M11 showed 98.99% after 12 h in a controlled manner, which is essential for anti ulcer therapy. The innovator Gemiflox conventional tablet shows the drug release of 96.15 within 1 h. The drug release of Gemifloxacin mesylate optimized formulation M11 followed zero order and Higuchi kinetics indicating diffusion controlled drug release.*

Key words: Gemifloxacin mesylate, mucoadhesion, HPMC K100 M, gum kondagogu, xanthan gum.

INTRODUCTION

Sustained and novel delivery envisages optimized drug in the sense that the therapeutic efficacy of a drug is optimized, which also implies nil or minimum side effects. It is expected that the 21st century would witness great changes in the area of drug delivery. The products may be more potent as well as safer. Target specific dosage delivery is likely to overcome much of the criticism of conventional dosage forms. The cumulative outcome could be summarized as optimized drug delivery that encompasses greater potency and greater effectiveness, lesser side effects and toxicity, better stability, low cost hence greater accessibility, ease of administration and best patient compliance [1]. The most desirable and convenient method of drug administration is the oral route due to the ease of administration and patient compliance. One limitation for oral delivery is poor bioavailability and for the drug candidates who show absorption window in the proximal gut and is the major obstacle to the development of controlled release formulation. A number of approaches have been developed to increase the residence time of dug formulation. One of the approaches the formulation of Gastro retentive dosage forms in the form of Mucoadhesive microspheres. Microsphere carrier systems, made from natural polymers are attracting considerable attentions for several years, for sustained drug delivery. Today, those dosage forms which can control the release rates and which are target specific have a great impact in development of novel drug delivery systems. Microspheres are part of such novel delivery systems [2, 3, 4]. The success of these microspheres is limited because due to short residence time at the site of absorption. Therefore, it would be advantageous to provide an intimate contact of the drug delivery systems with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to

microspheres and formulating bioadhesive microspheres. These microspheres provide advantages such as efficient absorption and increased bioavailability of drugs owing to high surface-to-volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site [5, 6, 7, 8]

Controlled release drug delivery systems that can be retained in stomach for long time are important for drug that are degraded in intestine or for drugs like antacids or certain enzymes that should act locally in the stomach. If the drugs are poorly soluble in intestine due to alkaline pH, gastric retention may increase solubility before they are emptied, resulting in improved gastrointestinal absorption of drugs with narrow absorption window as well as for controlling release of drugs having site specific absorption limitation [9]

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 [10]. The aim of present work is to design and in vitro evaluation of mucoadhesive 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, Ethyl cellulose, Guar gum, Xanthan gum, Kondagogu gum and HPMC K100 M were gifted from MSN Labs Ltd. Hyderabad. All other chemicals used were of analytical grade.

Formulation of Gemifloxacin mesylate mucoadhesive microspheres
Gemifloxacin mesylate mucoadhesive microspheres were prepared using different concentrations of polymers mentioned in the formulation table by Ionotropic gelation method. Total 14 formulations are developed using different polymers in different concentrations. In this method weighed quantity of Gemifloxacin mesylate was added to 100ml sodium alginate solution and thoroughly mixed at 500 rpm. 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-2hours in a hot air oven and stored in dessicater [11].

Table 1: Formulation trials for Gemifloxacin mesylate mucoadhesive microspheres

FORMULATION CODE	GEMIFLOXACIN MESYLATE(g)	SODIUM ALGINATE	ETHYL CELLULOSE(mg)	CALCIUM CHLORIDE	GUAR GUM	GUM KONDAGOGU
M1	3200	1 %	100	7%	0.5%	1%
M2	3200	1.2 %	150	7%	0.5%	1.2%
M3	3200	1.4%	200	7%	0.5%	1.4%
M4	3200	1.6%	250	7%	0.5%	1.6%
M5	3200	1.8%	300	7%	0.5%	1.8%
M6	3200	2%	350	7%	0.5%	2%
M7	3200	2.2%	400	7%	0.5%	2.2%
FORMULATION CODE	**GEMIFLOXACIN (g)**	**SODIUM ALGINATE**	**HPMC(K100M) (mg)**	**CALCIUM CHLORIDE**	**GUAR GUM**	**XANTHAN GUM**
M8	3200	1%	100	10%	0.5%	1%
M9	3200	1.2%	150	10%	0.5%	1.2%
M10	3200	1.4%	200	10%	0.5%	1.4%
M11	3200	1.6%	250	10%	0.5%	1.6%
M12	3200	1.8%	300	10%	0.5%	1.8%
M13	3200	2%	350	10%	0.5%	2%
M14	3200	2.2%	400	10%	0.5%	2.2%

Evaluation studies of Gemifloxacin mesylate mucoadhesive 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.[12]

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.[13]

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

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. [14]

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 271nm. Each batch should be examined for drug content in a triplicate manner. [15]

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

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

Mucoadhesiveness:
The In vitro mucoadhesive test was carried out using small intestine from chicken. The small intestinal tissue was

excised and flushed with saline. Five centimeter segment of jejunum were averted using a glass rod. Ligature was placed at both ends of the segment.100 microspheres were scattered uniformly on the averted sac from the position of 2 cm above. Then the sac was suspended in a 50 ml tube containing 40 ml of saline by the wire, to immerse in the saline completely. The sac were incubated at 37^0C and agitated horizontally. The sac were taken out of the medium after immersion for 1, 2, 3, 4, 5, 6, 7 and 8 hrs, immediately repositioned as before in a similar tube containing 40ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation [16].

Mucoadhesion= (No. of microspheres adhered/ No. of microspheres applied) x 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± 0.5^0C 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.

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 [17]. (Log percentage of drug remaining to be released vs. time) and Higuchi's model [18]. (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[24]

RESULTS AND DISCUSSION

Mucoadhesive microspores

All fourteen formulations were evaluated for various micromeretic and physic chemical parameters and the results are tabulated in **Table 2.** Among all the formulations M12 shown best results of particle size, bulk density, tapped density, angle of repose and Carr's index of 68.96±0.11, 0.74, 0.72, 23^o.33 and 08.13% respectively**.**

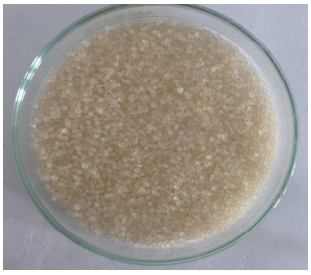


Figure 1: Gemifloxacin mesylate mucoadhesive microspheres

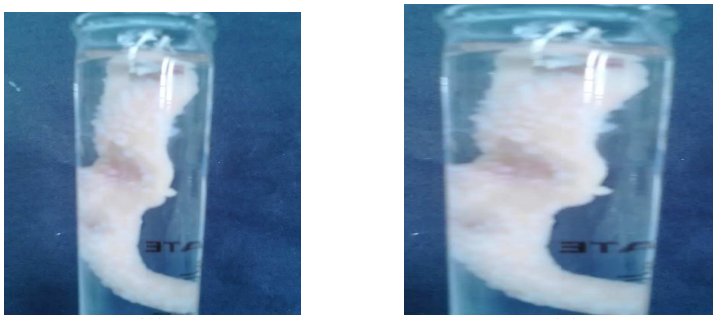
Table 2: Formulated Gemifloxacin mesylate mucoadhesive microspheres

Formulation code	Particle size (µm)	Bulk density (g/cc$^{3)}$	Tapped density (g/cc$^{3)}$	Angle of repose	Carr's index
M1	65.29±0.13	0.63	0.62	29°.67	09.34%
M2	73.43±0.04	0.65	0.69	30°.54	09.12%
M3	78.67±0.09	0.67	0.73	31°.15	09.98%
M4	79.45±0.21	0.69	0.75	26°.91	10.00%
M5	83.42±0.12	0.72	0.79	27°.93	11.00%
M6	85.34±0.09	0.75	0.82	25°.54	13.00%
M7	87.12±0.13	0.76	0.91	24°.91	10.20%
M8	69.43±0.09	0.66	0.61	30°.91	09.34%
M9	72.46±0.09	0.68	0.63	27°.91	09.11%
M10	76.89±0.10	0.72	0.68	30°.24	09.12%
M11	68.96±0.11	0.74	0.72	23°.33	08.13%
M12	88.94±0.11	0.79	0.75	25°.34	11.34%
M13	89.04±0.21	0.81	0.76	25°.54	12.34%
M14	81.45±0.21	0.83	0.83	26°.91	09.45%

Table 3: Percentage yield, entrapment efficiency, swelling index and mucoadhesiveness of Gemifloxacin mesylate mucoadhesive microspheres:

Formulation code	Percentage yield	Entrapment efficiency	Swelling index	Mucoadhesiveness
M1	75.45%	76.00%	72.11%	69.00%
M2	81.38%	82.03%	78.34%	78.00%
M3	82.97%	84.04%	82.89%	71.00%
M4	85.00%	86.00%	84.56%	78.00%
M5	87.02%	88.72%	85.23%	80.00%
M6	96.03%	95.03%	94.12%	95.00%
M7	92.01%	90.01%	84.23%	85.00%
M8	81.08%	80.02%	69.12%	83.00%
M9	83.00%	82.05%	70.12%	82.00%
M10	84.00%	85.00%	75.22%	85.00%
M11	98.70%	97.25%	94.34%	97.00%
M12	92.00%	91.00%	91.09%	92.50%
M13	89.01%	97.07%	96.08%	95.50%
M14	90.72%	89.67%	90.03%	88.00%

The percentage yield and entrapment efficiency of all the formulations were measured by assay method and found to be within the limits. The formulation M12 shows good percentage yield, entrapment efficiency, swelling index and mucoadhesiveness of 98.70%, 97.25%, 94.34% and 97.00% respectively and the results were depicted in **Table 3** and pictorial diagram of mucoadhesive study was shown in **Figure 2.**



(A) (B)

Figure 2: Pictorial diagram showing mucoadhesive property of Gemifloxacin microspheres in Chic Intestine at 0 min (A) & after 8 hr (B)

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

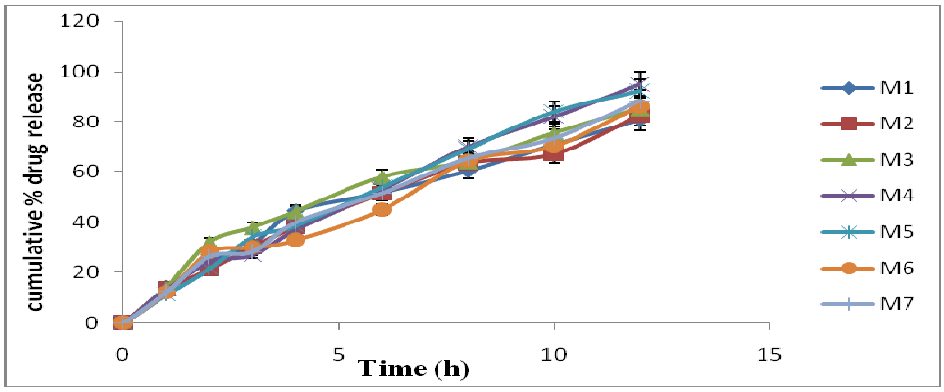


Figure 3: In-vitro cumulative % drug release of Gemifloxacin mesylate Mucoadhesive microspheres

Gemifloxacin mesylate microspheres were evaluated for in vitro drug release studies in 0.01N HCL and the results are depicted in **Table 4&5**. The formulation M11 shown best drug release of 98.99% within 12 h. The drug release of optimized formulation M11 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 mucoadhesive microspheres (M1-M7):

Time (h)	M1	M2	M3	M4	M5	M6	M7
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	14.05±0.11	13.34±0.43	13.63±0.11	13.31±0.16	11.28±0.55	12.12±0.54	12.30±0.12
2	24.80±0.32	21.40±0.21	32.01±0.16	24.33±0.56	21.50±0.65	28.23±0.54	26.40±0.32
3	31.30±0.15	30.23±0.15	37.98±0.32	27.11±0.45	34.20±0.67	30.00±0.43	28.30±0.22
4	44.40±0.42	38.20±0.16	44.20±0.16	37.00±0.16	38.60±0.78	32.90±0.32	39.92±0.16
6	51.70±0.11	51.30±0.32	57.86±0.15	52.84±0.54	53.80±0.54	44.90±0.45	51.40±0.22
8	60.30±0.16	63.30±0.16	64.03±0.21	69.84±0.55	68.90±0.98	64.20±0.67	65.20±0.32
10	70.70±0.17	66.91±0.32	75.29±0.34	82.00±0.45	83.90±0.65	70.10±0.99	73.12±0.16
12	80.54±0.88	82.36±0.52	85.36±0.16	95.07±0.33	92.23±0.12	86.24±0.43	88.34±0.32

Table 5: In-vitro cumulative % drug release of Gemifloxacin mesylate mucoadhesive microspheres (M8-M14) and innovator product

Time (h)	M8	M9	M10	M11	M12	M13	M14	Innovator (Gemiflox) 400mg immediate release)
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	17.89±0.15	15.11±0.44	17.32±0.12	12.70±0.43	13.23±0.66	14.04±0.56	13.63±0.33	96.23±0.22
2	23.40±0.44	26.23±0.15	23.90±0.32	23.11±0.45	22.34±0.76	29.40±0.67	32.01±0.45	--
3	32.08±0.65	30.23±0.14	26.12±0.15	31.62±0.65	28.30±0.77	36.23±0.43	37.98±0.34	--
4	39.20±0.19	37.90±0.55	31.84±0.16	38.63±0.53	36.30±0.87	34.20±0.65	44.20±0.56	--
6	55.30±0.99	47.90±0.43	54.08±0.53	49.92±0.33	50.39±0.76	57.30±0.77	57.86±0.56	--
8	68.30±0.97	61.20±0.15	66.03±0.44	61.20±0.15	62.23±0.54	65.30±0.65	64.03±0.77	--
10	70.98±0.13	74.10±0.18	81.07±0.54	70.13±0.66	73.12±0.65	69.90±0.67	75.29±0.45	--
12	72.30±0.42	81.20±0.32	94.21±0.16	98.99±	88.34±0.16	86.30±0.56	85.36±0.45	--

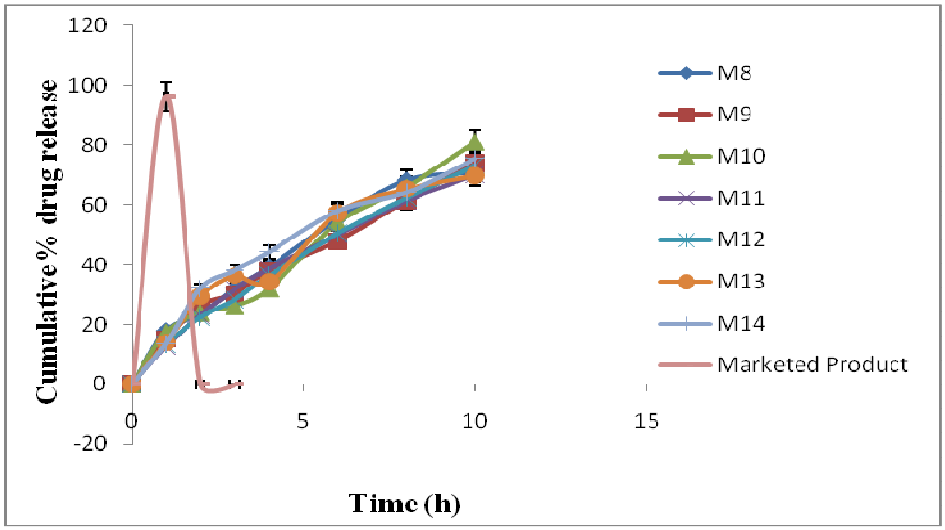


Figure 4: In-vitro cumulative % drug release of Gemifloxacin mesylate mucoadhesive microspheres formulation

Mathematical modeling of optimized mucoadhesive microspheres M11:

Table 6: Release kinetics of optimized formulation (M11) of mucoadhesive microspheres:

Formulation Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
M11	0.975	7.291	0.620	0.113	0.931	26.87	0.701	2.053

The *in vitro* release profiles from optimized formulation M11 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 plots i.e. 2.053 suggest that the drug release from microspheres was anomalous Non fickian diffusion.

DRUG EXCIPIENT COMPATABILITY STUDIES:

Fourier Transform Infrared (FTIR) spectroscopy

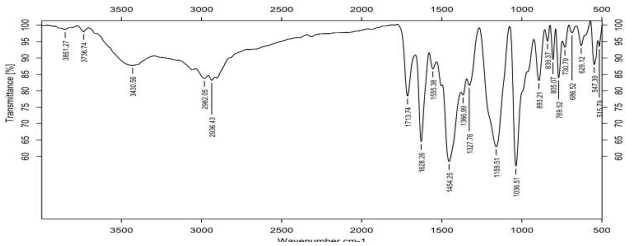


Figure 5: FT-IR spectrum of pure drug Gemifloxacin mesylate

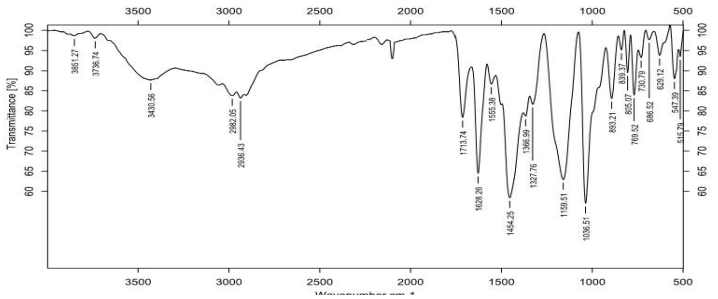


Figure 6:FT-IR spectrum of Gemifloxacin mesylate optimized microspheres (M11)

Drug polymer 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 (M11**) (Figure 6)** and results found that there were no possible interaction between drug and polymer.The FTIR spectrum of Gemifloxacin mesylat 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:

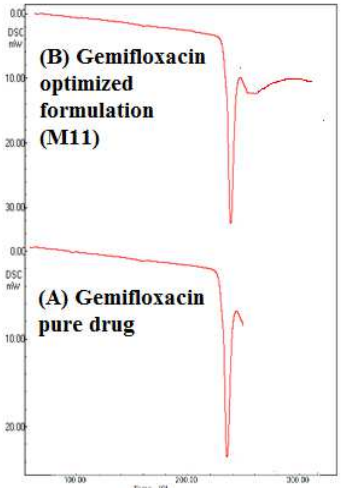


Figure 7: DSC thermogram of Gemifloxacin pure drug (A) and optimized formulatin M11 (B)

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 (M11) (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 studies:

Gemifloxacin mesylate mucoadhesive microspheres:

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

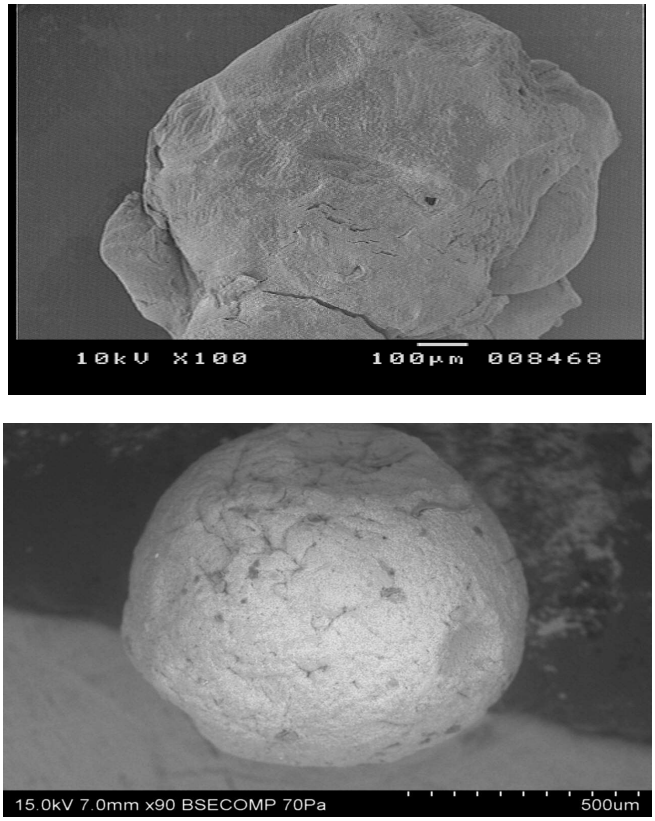


Figure 8: Scanning electron micrographs of Gemifloxacin mesylate optimized mucoadhesive microspheres (M11)

SEM photograph revealed that microspheres were discrete and spherical in shape with outer surface association of drug with polymer. The pores on microspheres surface help in drug release by diffusion mechanism

Stability studies:

Optimized formulation (M11) 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

In vitro data obtained for mucoadhesive microspheres of Gemifloxacin mesylate showed good drug entrapment and % yield. Microspheres of different size and drug content could be obtained by varying the formulation variables. Diffusion was found to be the main release mechanism. Mucoadhesive microspheres exhibited prolonged and controlled release effect compared to Innovator product. Among all the formulations M11 was selected as optimized formulations based on the physic chemical and release studies. *In vitro* drug release study of formulation M11 showed 98.99% after 12 h in a controlled manner, which is essential for disease like peptic ulcer. The innovator Gemiflox conventional tablet shows the drug release of 96.23% within 1 h.

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