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Development and Evaluation of Mucoadhesive Microspheres Containing Gatifloxacin

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ABSTRACT

Present study aims to prepare and evaluate mucoadhesive microspheres by ionotropic gelation method. Among all the formulations M10 was selected as optimized formulation for mucoadhesive microspheres based on the evaluation parameters and drug release studies. *In vitro* release study of formulation M10 showed 97.11% 12 h in a controlled manner, which is essential for disease like peptic ulcer. The release order kinetics for M10 was best fit with the highest correlation coefficient was observed in Higuchi model, indicating diffusion controlled principle. The innovator Abygate conventional tablet shows the drug release of 97.23% within 1 h. FT-IR and DSC analyses confirmed the absence of drug-polymer interaction. The results obtained from evaluation and performance study of Gatifloxacin mucoadhesive microspheres that system may be useful to achieve a controlled drug release profile suitable for peroral administration and may help to reduce the dose of drug, dosing frequency and improve patient compliance when compared with marketed product.

Keywords: Gatifloxacin, mucoadhesiveness, gum olibanum, chitosan, microspheres.

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INTRODUCTION

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[1,2,3]

The term microsphere is defined as a spherical particle with size from 1µm to 1000µm. 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[4]. Microspheres are one of the multiparticulate drug delivery systems and are prepared by Ionotropic gelation method by dropping drug loaded polymeric solution using syringe into the aqueous solution of polyvalent cations to obtain prolonged (or) controlled drug delivery to improve bioavailability or stability and to target drug to specific sites[5].

Mucoadhesive microspheres:

The success of normal 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 absporption site [6,7,8,9.]

Gatifloxacin acetate is a specific and competitive histamine H_2 receptor antagonist, which is used to treat gastric ulcers, Zollinger–Ellison syndrome, erosive esophagitis, gastro-oesophageal reflux disease and gastritis. Gatifloxacin has less bioavailability (80%) and lesser half life of 5 hours[10]. The aim of present work is to design and in vitro evaluation of Gatifloxacin mucoadhesive microspheres to enhance its bioavailability and prolonged residence time in stomach.

MATERIALS AND METHOD

Materials:

Gatifloxacin pure drug was generous gift from Dr. Reddy's Laboratories Ltd., Hyderabad, India. Sodium alginate was obtained from Pruthvi Chemicals, Mumbai. Carbopol 934, Xanthan gum, Gum olibanum, Guar gum and Gum kondagogu was gifted from MSN Labs Ltd. Hyderabad. All other chemicals used were of analytical grade.

Formulation of Gatifloxacin mucoadhesive microspheres

Gatifloxacin mucoadhesive microspheres were prepared using different polymers like Sodium alginate, Calcium chloride, ethyl cellulose, Xanthan gum, Gum olibanum, Guar gum and Gum kondagogu by Ionotropic gelation method.

Table 1: Formulation trials for Gatifloxacin mucoadhesive microspheres

Formulation code	Gatifloxacin (g)	Sodium alginate	Ethyl cellulose(g)	Calcium chloride	Guar gum	Xanthan gum
M1	4	1 %	100	7%	0.4	1%
M2	4	1.2 %	150	7%	0.4	1.2%
M3	4	1.4%	200	7%	0.4	1.4%
M4	4	1.6%	250	7%	0.4	1.6%
M5	4	1.8%	300	7%	0.4	1.8%
M6	4	2%	350	7%	0.4	2%
M7	4	2.2%	400	7%	0.4	2.2%
Formulation code	**Gatifloxacin (g)**	**Sodium alginate**	**Carbopol (940)(mg)**	**Calcium chloride**	**Gum Olibanum**	**Gum kondagogu**
M8	4	1%	100	10%	0.4	1%
M9	4	1.2%	150	10%	0.4	1.2%
M10	4	1.4%	200	10%	0.4	1.4%
M11	4	1.6%	250	10%	0.4	1.6%
M12	4	1.8%	300	10%	0.4	1.8%
M13	4	2%	350	10%	0.4	2%
M14	4	2.2%	400	10%	0.4	2.2%

Procedure for the preparation of Gatifloxacin mucoadhesive microspheres:

The Gatifloxacin mucoadhesive microspheres were prepared by using ion tropic gelation technique. In this method weighed quantity of Gatifloxacin was added to 100 ml sodium alginate, Sodium CMC solution and other polymers, 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 30 minutes the obtained microspheres were washed with water and dried at 60 degrees-4 hours in a hot air oven and stored in dessicater.

Evaluation studies of Gatifloxacin mucoadhesive microspheres:

Micromeretic properties like particle size, angle of repose, bulk density, Tapped density, Compressibility index, Hausner's ratio and evaluation parameters like Swelling index, Drug entrapment efficiency and % yield, mucoadhesive study and In vitro dissolution studies were performed.

Mucoadhesion study:

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[11].

Mucoadhesion= (No. of microspheres adhered/ No. of microspheres applied) X 100

In vitro drug release studies:

In vitro drug release studies for developed Gatifloxacin microspheres were carried out by using dissolution apparatus II paddle type (Electrolab TDL-08L). The drug release profile was studied in 900 ml of 0.1 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 292nm[12].

Kinetic modeling of drug release:

In order to understand the kinetics and mechanism of drug release, the result of the in vitro dissolution study of microspheres were fitted with various kinetic equations like Zero order as cumulative percentage drug released vs time, First order as log percentage of drug remaining to be released Vs time, Higuchi's model cumulative percentage drug released vs square root of time.r^2 and K values were calculated for the linear curves obtained by regression analysis of the above plots.

Drug excipient compatibility studies

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

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 and cumulative % drug released during the stability study period.

RESULTS AND DISCUSSION

Mucoadhesive microspheres

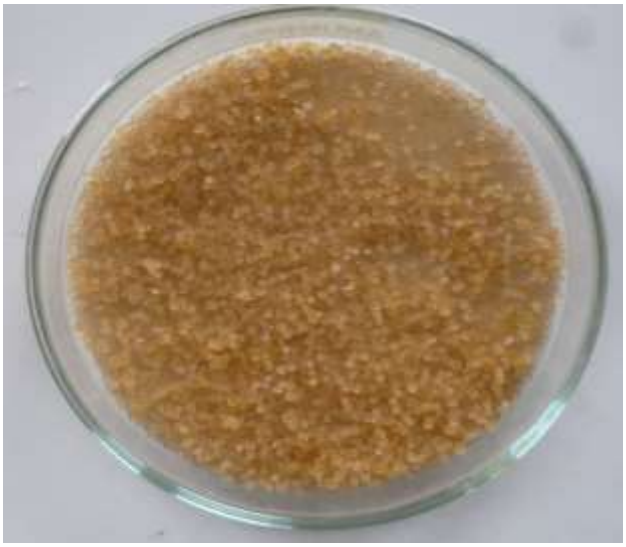


Figure 1: Gatifloxacin Mucoadhesive microspheres

Table 2: Micromeretic properties of Gatifloxacin mucoadhesive microspheres

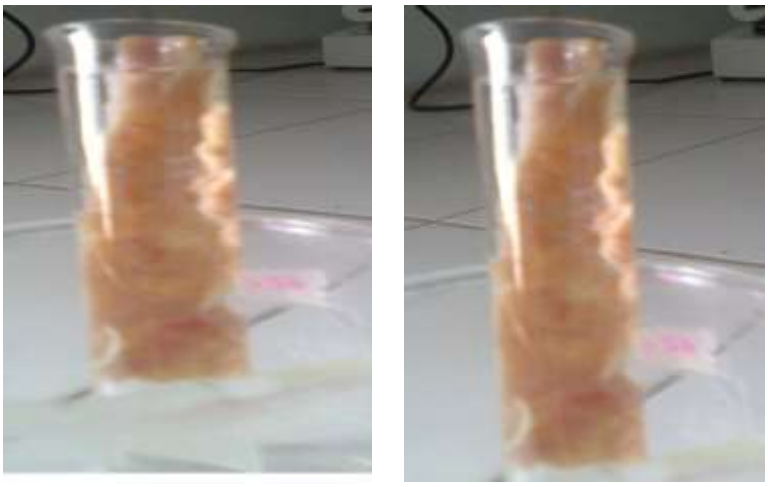
Formulation code	Particle size(µm)	Bulk density (g/cc^{3})	Tapped density (g/cc^{3})	Angle of repose	Carr's index
M1	65.29±0.13	0.63	0.62	$29^{o}.67$	08.34%
M2	73.43±0.04	0.65	0.69	$30^{o}.54$	08.17%
M3	78.67±0.09	0.67	0.73	$31^{o}.15$	07.23%
M4	79.45±0.21	0.69	0.75	$25^{o}.91$	10.00%
M5	83.42±0.12	0.72	0.79	$27^{o}.93$	11.00%
M6	85.34±0.09	0.75	0.82	$25^{o}.54$	13.00%
M7	87.12±0.13	0.76	0.91	$30^{o}.24$	10.20%
M8	69.43±0.09	0.66	0.61	$30^{o}.91$	09.34%
M9	72.46±0.09	0.68	0.63	$27^{o}.91$	09.11%
M10	65.89±0.10	0.72	0.68	$23^{o}.91$	08.12%
M11	85.94±0.11	0.74	0.72	$27^{o}.93$	09.23%
M12	88.94±0.11	0.79	0.75	$25^{o}.34$	11.34%
M13	89.04±0.21	0.81	0.76	$25^{o}.54$	12.34%
M14	91.45±0.21	0.83	0.83	$26^{o}.91$	09.45%

Gatifloxacin microspheres of 14 formulations were prepared by ionotropic gelation method. All the formulations were evaluated for particle size, bulk density, tapped density, angle of repose and carr's index and found to be within the limits, the results were depicted in Table 2.

Table 3: Percentage yield and entrapment efficiency of Gatifloxacin Mucoadhesive microspheres Formulations:

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%	90.00%
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	98.00%	97.40%	95.22%	97.00%
M11	89.00%	88.25%	84.34%	87.00%
M12	92.00%	91.00%	91.09%	92.50%
M13	91.90%	97.07%	91.08%	91.70%
M14	90.72%	89.67%	90.03%	88.00%

Mucoadhesion study:



(A) **(B)**

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

The results of % yield, entrapment efficiency and swelling index was found to be satisfactory which shown in Table 3. The formulation M10 showed the best percentage yield, entrapment efficiency, swelling index and mucoadhesiveness values of 98.00%, 97.40%, 95.22% and 97.00% respectively.

In vitro drug release studies:

Table 4: In-vitro cumulative % drug release of Gatifloxacin mucoadhesive microspheres:

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	17.89±0.21	15.11±0.11	11.07±0.32	14.04±0.21	13.23±0.21	17.32±0.45	12.12±0.54
2	23.40±0.67	26.23±0.18	22.80±0.33	29.40±0.17	22.34±0.18	23.90±0.32	28.23±0.11
3	32.08±0.66	30.23±0.16	34.30±0.42	36.23±0.65	28.30±0.99	26.12±0.76	30.00±0.65
4	39.20±0.45	37.90±0.18	36.40±0.16	34.20±0.11	36.30±0.32	31.84±0.43	32.90±0.54
6	55.30±0.32	47.90±0.33	51.70±0.11	57.30±0.87	50.39±0.33	54.08±0.16	44.90±0.46
8	68.30±0.33	61.20±0.32	64.30±0.32	65.30±0.87	62.23±0.67	66.03±0.13	64.20±0.64
10	70.98±0.18	74.10±0.21	72.40±0.18	69.90±0.88	73.12±0.54	81.07±0.12	70.10±0.53
12	72.30±0.43	81.20±0.17	85.50±0.54	86.30±0.76	88.34±0.43	94.21±0.54	86.24±0.11

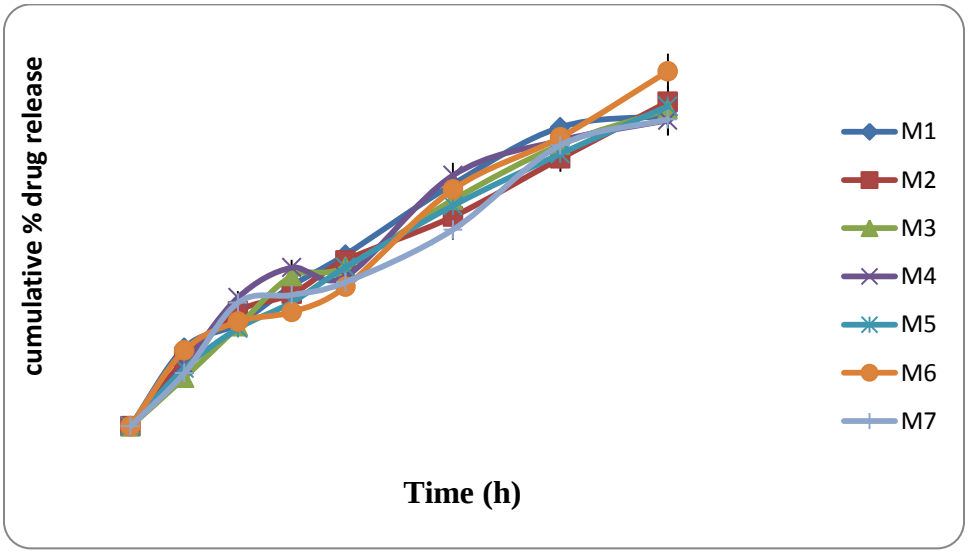


Figure 3: In-vitro cumulative % drug release of Gatifloxacin Mucoadhesive microspheres formulations

Table 5: In-vitro cumulative % drug release of Gatifloxacin mucoadhesive microspheres formulation

Time (h)	M8	M9	M10	M11	M12	M13	M14	Abygate 400mg immediate release)
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	14.05±0.21	13.34±0.15	12.70±0.21	12.30±0.44	11.28±0.32	13.31±0.44	13.63±0.66	97.23±0.16
2	24.80±0.65	21.40±0.16	23.11±0.15	26.40±0.32	21.50±0.43	24.33±0.16	32.01±0.15	
3	31.30±0.22	30.23±0.32	31.62±0.16	28.30±0.43	34.20±0.44	27.11±0.43	37.98±0.32	
4	44.40±0.	38.20±0.	38.63±0.	39.92±0.	38.60±0.	37.00±0.	44.20±0.	

	43	11	43	21	32	98	16
6	51.70±0.44	51.30±0.32	49.92±0.44	51.40±0.16	53.80±0.11	52.84±0.11	57.86±0.17
8	60.30±0.54	63.30±0.15	61.20±0.32	65.20±0.44	68.90±0.32	69.84±0.54	64.03±0.21
10	70.70±0.12	66.91±0.32	70.13±0.17	73.12±0.43	83.90±0.11	82.00±0.64	75.29±0.45
12	80.54±0.15	82.36±0.11	97.11±0.33	88.34±0.65	92.23±0.32	95.07±0.98	85.36±0.43

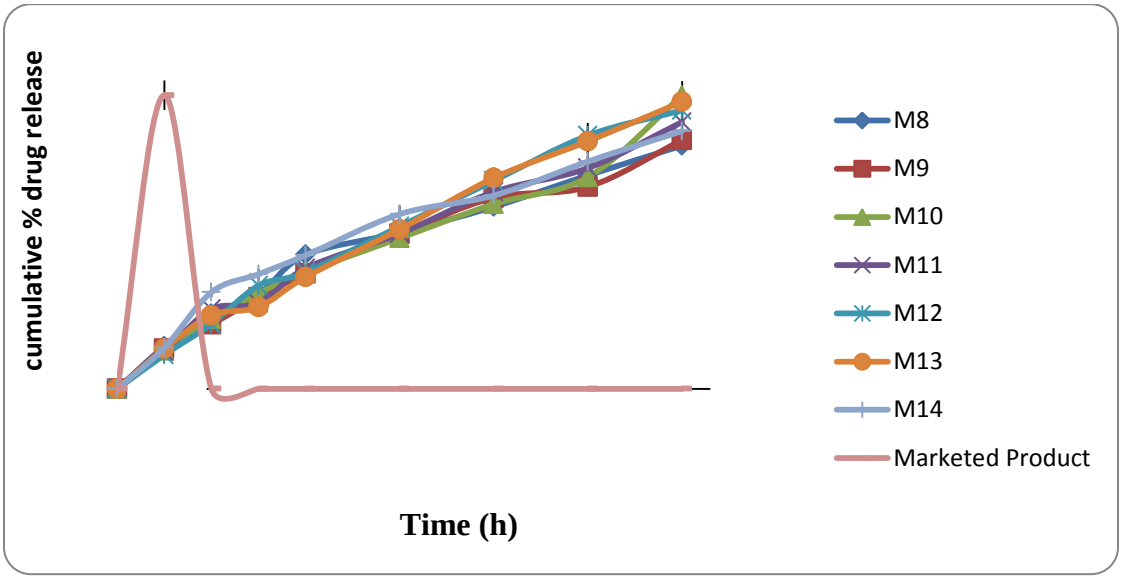


Figure 4: In-vitro cumulative % drug release of Gatifloxacin mucoadhesive microspheres formulation

Dissolution studies were conducted for all Gatifloxacin mucoadhesive microspheres and the % drug release of all the formulations were tabulated in Table 5. The formulation M10 was shown highest % drug release of 97.11% within 12 hrs. The drug release of optimized formulation M10 was in controlled manner when compared with innovator product Abygate i.e 97.23 within 1h.

Mathematical modeling of optimized mucoadhesive microspheres:

Table 6: Release order kinetics of optimized formulation (M10) of Gatifloxacin mucoadhesive microspheres:

Formulation	**Zero Order**		**First Order**		**Higuchi**		**Korsmeyer-Peppas**	
Code	R^2	K	R^2	K	R^2	K	R^2	N
M10	0.975	7.291	0.620	0.113	0.931	26.87	0.575	2.179

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 plots i.e., 2.179 suggest that the drug release from microspheres was anomalous Non fickian diffusion.

Drug excipient compatibility studies:

Fourier Transform Infrared Spectroscopy (FTIR)

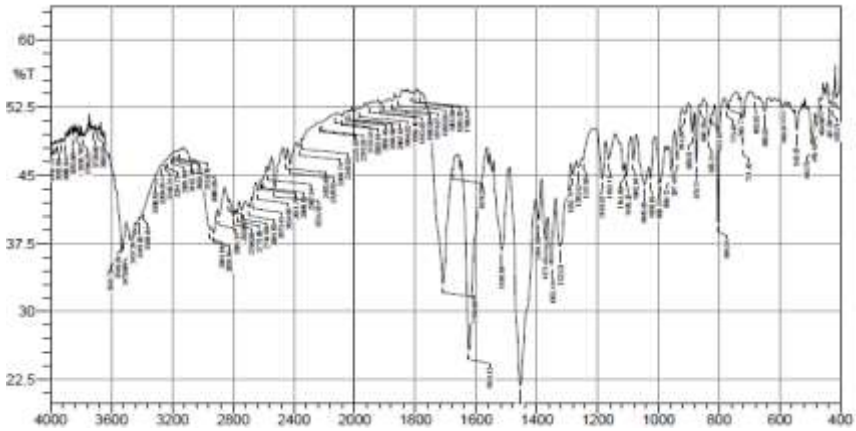


Figure 5: FT-IR spectrum of pure drug Gatifloxacin

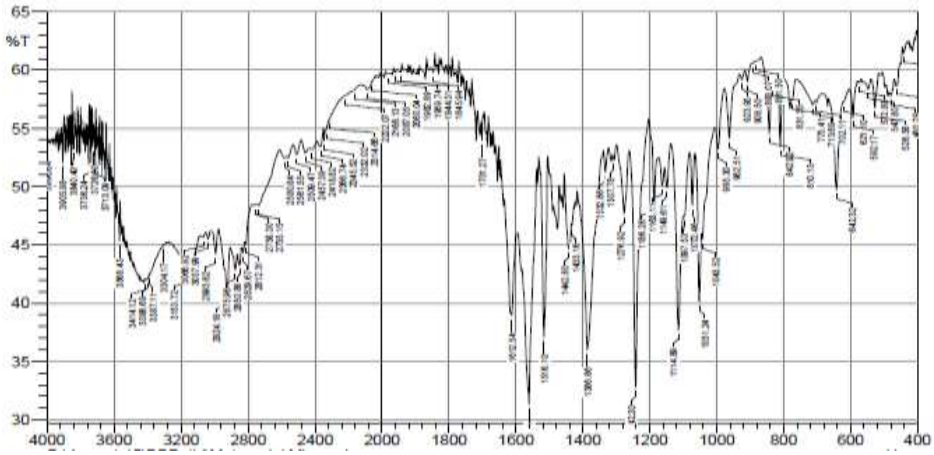


Figure 6: FT-IR spectrum of physical mixture

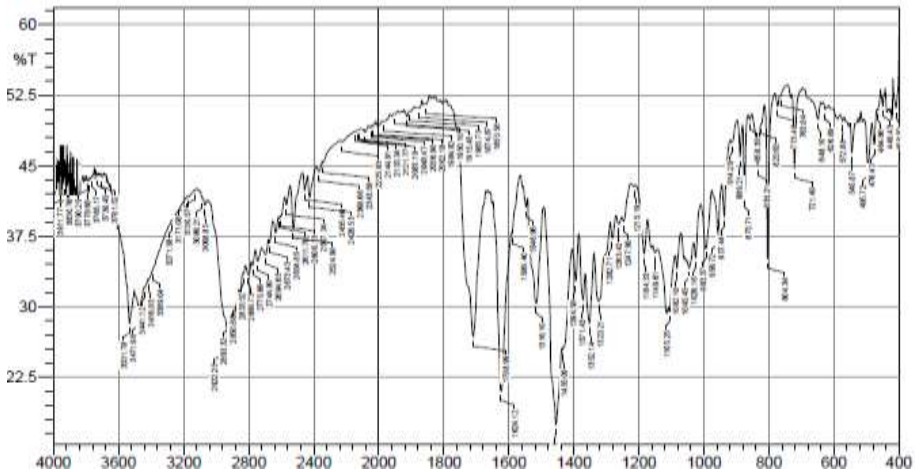


Figure 7: FT-IR spectrum of Gatifloxacin optimized formulation M10

FTIR was carried out to check the drug excipient interaction. The FTIR peak of Gatifloxacin is almost similar to that of the peak obtained with excipient and all the peaks of the functional group is in proper range. Hence, it can be concluded that the drug Gatifloxacin was found to be compatible with the excipient used in the designed formulation.

DSC Studies:

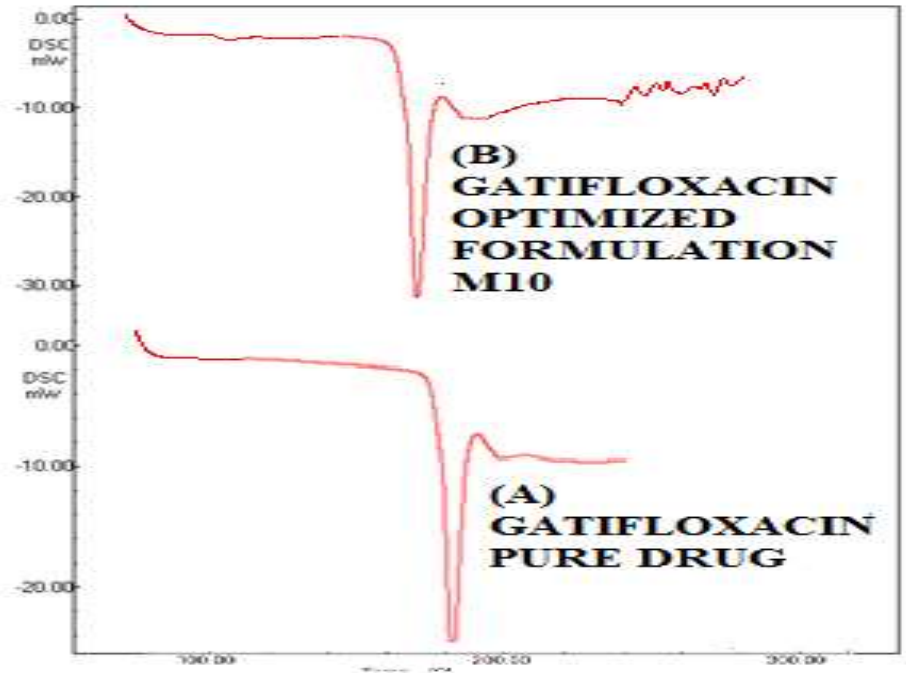


Figure 8: DSC thermogram of Gatifloxacin pure drug (A) and optimized formulatin M10 (B)

DSC was used to detect interaction between Gatifloxacin and excipients. The thermogram of pure Gatifloxacin (Figure) exhibited a sharp endotherm melting point at 181 ^{0}C (Table). The thermogram of optimized microspheres loaded with Gatifloxacin (M10) exhibited a sharp endotherm melting point at179 ^{0}C (Figure 8). The DSC thermogram of sodium alginate was also shown in Figure. The DSC thermogram of microsphere loaded with Gatifloxacin retained properties of pure Gatifloxacin. There is no considerable change observed in melting endotherm of drug in optimized formulation. It indicates that there is no interaction between drug & excipients used in the formulation.

Scanning Electron Microscopy:

SEM of Gatifloxacin normal microspheres

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

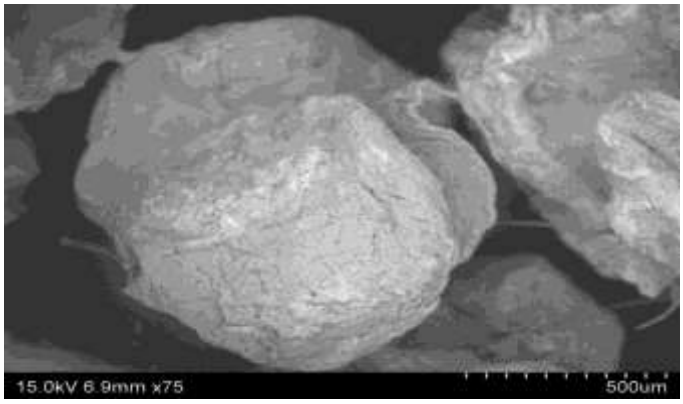


Figure 9: Scanning electron micrographs of Gatifloxacin microspheres

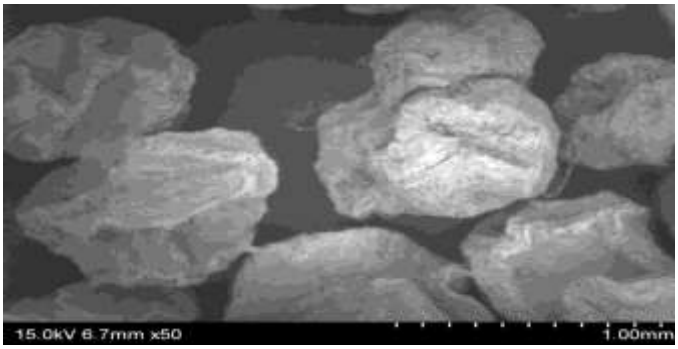


Figure 10: Scanning electron micrographs of Gatifloxacin microspheres

Morphology of the various formulations of Gatifloxacin microspheres prepared was found to be discrete and spherical in shape (Figure 8 & 9). The surface of the Gatifloxacin microspheres was rough due to higher concentration of drug uniformly dispersed at the molecular level in the sodium alginate matrices. There are no crystals on surface which states that is drug is uniformly distributed.

Stability studies:

Optimized formulation M10 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.

CONCLUSION

In vitro data obtained for mucoadhesive microspheres of Gatifloxacin showed good drug entrapment and % yield. In the present study, an attempt was made to prepare mucoadhesive and floating microspheres, which 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 M10 was selected as optimized formulation based on the

physico chemical studies and drug release studies. In the *in vitro* release study of formulation M10 showed 97.11% after 12 h in a controlled manner, which is required for disease like peptic ulcer. The *in vitro* release profiles from optimized formulation M10 was applied on various kinetic models. The best fit with the highest correlation coefficient was observed in Higuchi model, indicating diffusion controlled principle. The innovator Abygate conventional tablet shows the drug release of 97.23 within 1 h. FT-IR and DSC analyses confirmed the absence of drug-polymer interaction. It may be concluded from the result obtained from evaluation and performance study of Gatifloxacin mucoadhesive microspheres that system may be useful to achieve a controlled drug release profile suitable for peroral administration and may help to reduce the dose of drug, dosing frequency and improve patient compliance.

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