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Der Pharmacia Lettre, 2016, 8 (4):361-370
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Design and evaluation of sodium alginate microspheres loaded with gatifloxacin

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ABSTRACT

Present study aims to prepare and evaluate Gatifloxacin microspheres by ionotropic gelation method. Among all the formulations S12 was selected as optimized formulations for based on the physico chemical parameters and drug release studies. In the in vitro *release study of formulation S12 showed 95.21% after 12 h in a controlled manner. In vitro drug release profile from optimized formulation 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 400mg 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 different types of Gatifloxacin 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.*

Key words: Gatifloxacin, SEM, microspheres, sodium alginate, Higuchi.

INTRODUCTION

Controlled drug delivery by encapsulating the drug inside polymeric carriers has made great progress in last two decades as it can enhance the drug release and decrease adverse effects [1, 2, 3, 4] by drug localization at the site of action and by controlling the drug release [5]. Moreover, entrapment inside the polymers can also shield the sensitive drugs (e.g., peptides/proteins) from chemical and enzymatic decomposition. Microspheres developed using biodegradable polymers are widely used to achieve controlled release of drugs [6, 7]. The chief advantage of using biodegradable polymers is that after performing their tasks they break down in a biologically friendly manner.

For the treatment of chronic diseases it is important to take medication several times, this may lead to fluctuating drug level in body. In order to avoid frequent drug administration and maintenance of therapeutic drug level in body it is essential to administer drug by a sustained release system. Drugs with short elimination half life are most suitable for sustained release formulations. Sustained delivery of drugs can be achieved by microspheres formulation [8].

Gatifloxacin is a fourth-generation 8-methoxy fluoroquinolone derivative(1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid) with a broad spectrum of activity encompassing gram-positive and gram-negative pathogens, including *S. epidermidis* , *S. aureus, S. pneumoniae, S. pyogenes* , *H. influenzae* , *E. coli, B. cereus* , *N. gonorrhoeae* , and *P. mirabilis*[9] The aim of present work is to design and in vitro evaluation of Gatifloxacin microspheres to prolong the drug release in a controlled manner throughout the specific period of time.

MATERIALS AND METHODS

Materials:

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

Preparation of Gatifloxacin microspheres:

Gatifloxacin microspheres were prepared with polymers like sodium alginate and calcium chloride by Ionotropic gelation method. Different formulation trials of Gatifloxacin were prepared using different concentration of polymer and cross linking agent. Total 14 formulations are developed using sodium alginate, gelating, pectin and calcium chloride in different concentrations. In this method weighed quantity of Gatifloxacin 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.

Table 1: Preparation of Gatifloxacin microspheres

FORMULATION CODE	GATIFLOXACIN (g)	SODIUM ALGINATE	GELATIN(mg)	CALCIUM CHLORIDE
S1	4	1 %	1000	7%
S2	4	1.2 %	0.858	7%
S3	4	1.4%	0.716	7%
S4	4	1.6%	0.574	7%
S5	4	1.8%	0.432	7%
S6	4	2%	0.290	7%
S7	4	2.2%	0.148	7%
FORMULATION CODE	**GATIFLOXACIN(g)**	**SODIUM ALGINATE**	**PECTIN(mg)**	**CALCIUM CHLORIDE**
S8	4	1%	1000	10%
S9	4	1.2%	0.858	10%
S10	4	1.4%	0.716	10%
S11	4	1.6%	0.574	10%
S12	4	1.8%	0.432	10%
S13	4	2%	0.290	10%
S14	4	2.2%	0.148	10%

Micromeretic properties of Gatifloxacin 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 [11].

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. [12]

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

Evaluation of Gatifloxacin microspheres:
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. [13].

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 292nm using calibration curve. Each batch should be examined for drug content in a triplicate manner. [14]

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

% yield = [Total weight of microspheres / Total weight of drug and polymer] 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. [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 [16](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 [19]

RESULTS AND DISCUSSION

Gatifloxacin normal microspheres:

Figure 1: Gatifloxacin normal microspheres

Table 2: Micromeritic properties of Gatifloxacin microspheres

Formulation code	Particle size (µm)	Bulk density (g/cc³)	Tapped density (g/cc³)	Angle of repose	Carr's index	Swelling index
S1	61.12±0.08	0.66	0.69	27˚.74	9.34%	64%
S2	66.29±0.13	0.74	0.72	29˚.67	8.34%	69%
S3	67.43±0.04	0.76	0.73	30˚.54	8.12%	70%
S4	69.67±0.09	0.79	0.73	31˚.15	9.23%	71%
S5	78.45±0.04	0.89	0.75	27˚.93	14.56%	79%
S6	71.45±0.09	0.92	0.76	27˚.21	13.95%	87%
S7	87.29±0.13	0.94	0.76	26˚.54	10.32%	93%
S8	67.45±0.04	0.66	0.59	27˚.93	14.56%	69%
S9	78.45±0.09	0.67	0.62	26˚.54	13.95%	70%
S10	71.23±0.14	0.69	0.64	27˚.91	10.32%	75%
S11	75.12±0.08	0.71	0.66	26˚.74	9.34%	84%
S12	64.45±0.09	0.72	0.68	24˚.67	8.34%	96%
S13	63.43±0.04	0.76	0.73	26˚.54	8.12%	92%
S14	61.13±0.09	0.87	0.78	29˚.15	7.23%	89%

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, carr's index and swelling index and found to be within the limits, the results were depicted in **Table 2.**

The results of % yield, entrapment efficiency and swelling index was found to be satisfactory which shown in **Table 3**. The formulation S12 showed the best percentage yield and entrapment efficiency values of 96.30% and 95.66% respectively.

Table 3: Percentage drug yield and entrapment efficiency of Gatifloxacin microspheres

Formulation code	Percentage yield	Entrapment efficiency
S1	70.00%	69.00%
S2	71.00%	72.00%
S3	81.00%	80.00%
S4	83.87%	83.30%
S5	86.30%	85.20%
S6	91.30%	91.30%
S7	87.50%	90.10%
S8	76.00%	74.03%
S9	81.00%	82.00%
S10	84.00%	83.00%
S11	86.09%	85.00%
S12	96.30%	95.66%
S13	93.30%	91.03%
S14	85.30%	84.88%

In vitro dissolution studies:

Dissolution studies were conducted for all Gatifloxacin micorspheres and the % drug release of all the formulations were tabulated in **Table 4.** The formulations S12 was shown highest % drug release of 95.21% within 12 hrs and the results are depicted in **Table 5**. The drug release of optimized formulation S12 was in controlled manner when compared with innovator product Abygate i.e 97.23 within 1h.

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

Time (h)	S1	S2	S3	S4	S5	S6	S7	Abygate 400mg immediate release)
0	0±	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	13.09±0.12	17.87±0.66	10.34±0.21	14.23±0.23	12.34±0.76	15.31±0.22	10.10±0.19	97.23±0.98
2	23.09±0.21	23.06±0.67	21.79±0.91	20.12±0.11	25.34±0.45	22.15±0.32	21.30±0.22	
3	28.23±0.11	31.05±0.32	31.85±0.89	32.04±0.98	36.12±0.33	34.19±0.56	35.45±0.32	
4	42.11±0.21	38.20±0.21	39.90±0.21	44.40±0.16	46.20±0.32	45.23±0.32	49.89±0.76	
6	45.39±0.11	49.30±0.16	48.90±0.16	51.00±0.32	52.30±0.19	58.73±0.42	64.80±0.88	
8	54.23±0.21	58.30±0.87	63.31±0.21	62.35±0.88	65.30±0.22	67.46±0.41	73.60±0.54	
10	67.20±0.11	68.9±0.21	72.22±0.11	74.30±0.21	78.58±0.79	81.25±0.22	83.85±0.98	
12	71.34±0.22	73.25±0.22	81.31±0.32	84.50±0.34	86.30±0.18	92.12±0.45	90.41±0.22	

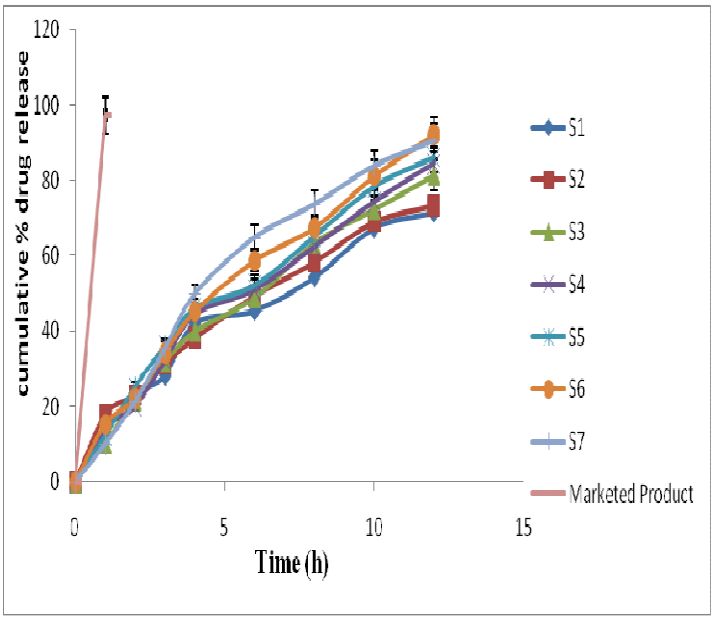

Figure 2: In vitro cumulative % drug release of Gatifloxacin microspheres

Table 5: In vitro cumulative % drug Gatifloxacin sodium alginate release of microspheres formulation

Time (h)	S8	S9	S10	S11	S12	S13	S14
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	11.05±0.16	13.23±0.45	14.23±0.32	15.00±0.98	16.22±0.11	18.62±0.12	14.63±0.11
2	25.40±0.45	23.34±0.33	24.80±0.54	25.40±0.53	24.23±0.19	23.01±0.58	32.01±0.32
3	29.01±0.21	30.08±0.59	31.38±0.65	30.55±0.54	31.96±0.78	29.11±0.87	37.15±0.46
4	44.40±0.16	38.90±0.34	40.10±0.65	38.20±0.31	40.24±0.16	38.24±0.17	44.83±0.18
6	48.30±0.22	49.91±0.77	51.60±0.77	52.30±0.44	54.20±0.11	52.83±0.16	57.76±0.87
8	54.35±0.77	61.20±0.34	60.30±0.75	63.30±0.54	68.24±0.43	67.03±0.22	64.60±0.77
10	67.90±0.64	70.10±0.67	74.60±0.76	69.92±0.44	72.32±0.45	82.62±0.31	75.56±0.45
12	72.30±0.52	80.20±0.77	83.50±0.43	85.42±0.18	95.21±0.11	94.36±0.18	85.00±0.67

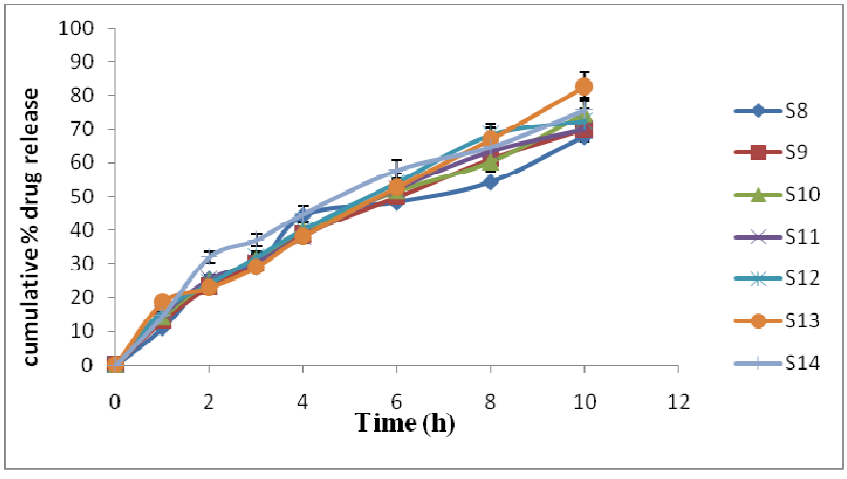

Figure 3: In vitro cumulative % drug release of Gatifloxacin microspheres

Mathematical modeling of Gatifloxacin optimized microspheres (S12):

Table: Release order kinetics of optimized normal microspheres (S12)

Formula Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
S12	0.975	7.291	0.872	0.103	0.950	31.53	0.659	2.123

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., 2.123 suggest that the drug release from microspheres was anomalous Non fickian diffusion.

Drug excipient compatibility studies:
Fourier Transform Infrared Spectroscopy (FTIR)

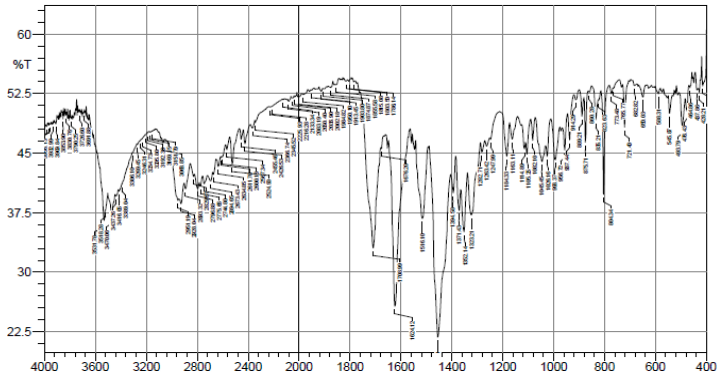

Figure 4: FT-IR spectrum of pure drug Gatifloxacin

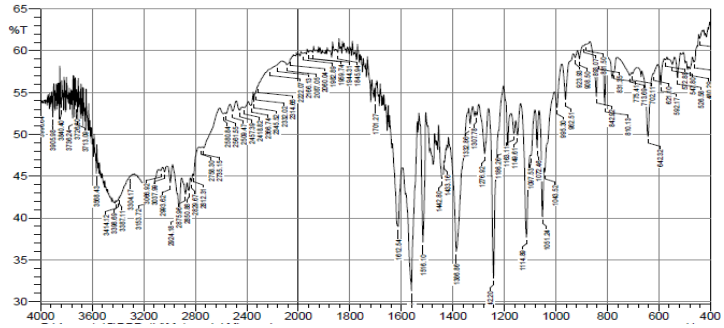

Figure 5: FT-IR spectrum of physical mixture (Gatifloxacin+Sodium alginate+$CaCl_2$)

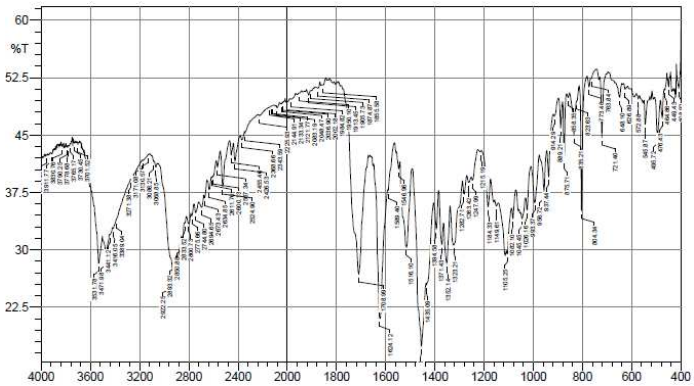

Figure 6: FT-IR spectrum of Gatifloxacin optimized formulation S12

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:

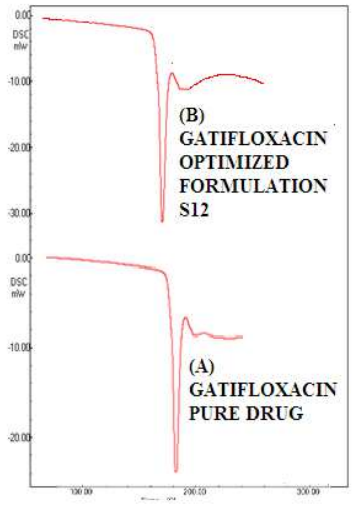

Figure 7: DSC thermogram of Cimetidine pure drug (A) and optimized formulatin S12 (B)

DSC was used to detect interaction between Gatifloxacin and excipients. The thermogram of pure Gatifloxacin **(Figure 7)** exhibited a sharp endotherm melting point at 182 ^{0}C. The thermogram of optimized microspheres loaded with Gatifloxacin (R9) exhibited a sharp endotherm melting point at180 ^{0}C **(Figure 7)**. 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.

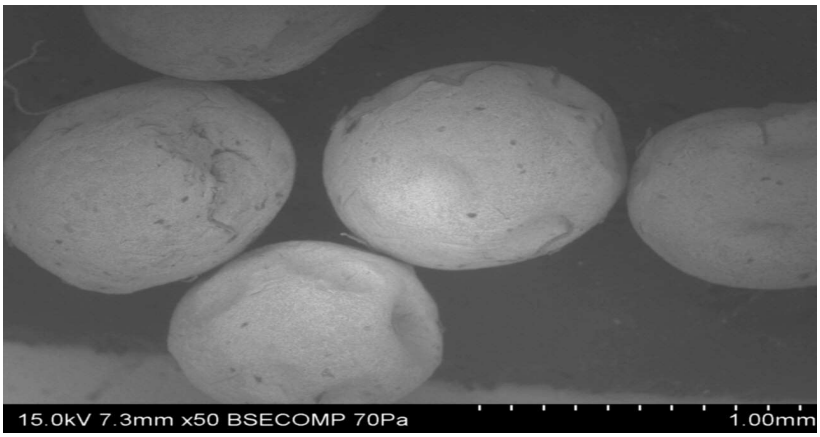

Figure 8: Scanning electron micrographs of Gatifloxacin microspheres

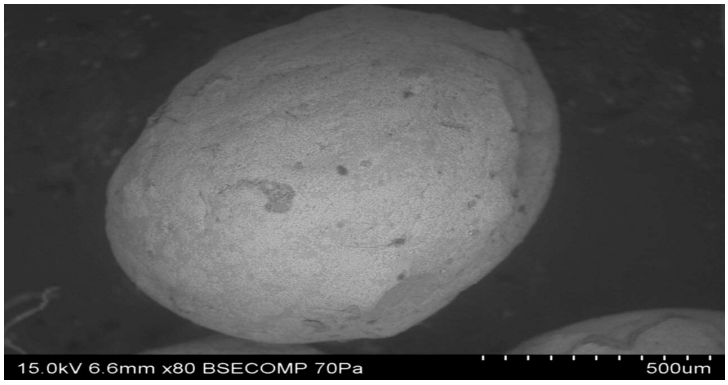

Figure 9: Scanning electron micrographs of Gatifloxacin microspheres

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 S12 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 the present study, an attempt was made to prepare Gatifloxacin 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 S12 was selected as optimized formulations based on the physico chemical studies and drug release studies. In the *in vitro* release study of formulation S12 showed 95.21% of drug release after 12 h in a controlled manner, which is essential for disease like peptic ulcer. 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 Higuchi model, indicating diffusion controlled principle**.** The innovator Abygate 400mg 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 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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