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Formulation and evaluation of ofloxacin microspheres using natural gum polymers

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ABSTRACT

Ofloxacin is a fluoroquinolones antibacterial agent with a broad spectrum of activity against gram positive and negative bacteria. It is used in urinary and respiratory tract infections, gonorrhea and skin. Conventional doses vary from 200-600 mg twice or thrice a day, as per severity of infection and its biological half-life is 5-6 hrs. In present study, Ofloxacin microspheres were formulated using polymer and natural gums such as Guar gum and Xanthan gum as rate controlling material by ionic cross-linking technique with an aim to prolong its release. All formulations prepared by using different drug-polymer ratios, were evaluated for relevant parameters. Depending upon the drug-polymer ratio of Guar gum, the percentage yield and encapsulation efficiency of microspheres were found to range between 89.17±6.02 to 94.61±4.95% and 78.40±5.08 to 83.89±4.17% respectively. Also the drug to polymer ratio of Xanthan gum, the percentage yield and encapsulation efficiency were found to range between 90.12±6.22 to 93.02±5.39% and 65.52±4.27 to 72.73±3.88%, respectively. All prepared Microspheres were spherical, discrete and compact in shape and size distribution was between 44.32-50.17 µm. *In-vitro* studies were carried out at different pH for a period of 12 hours. Guar gum (GG3) formulations was 93.26% and Xanthan gum (XG3) was 82.54% for better sustained release. Stability studies of selected ofloxacin microspheres showed good results. It could be also concluding that the all the formulations were shown satisfactory results.

INTRODUCTION

Many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various conventional dosage forms [1] and it's associated with certain limitations [2-3]**.** Novel drug delivery system (NDDS) can be a major advance for solving the problem related towards the release of the drug at specific site with specific rate and these systems have several advantages over conventional multi dose therapy and the goal of sustained drug delivery are to conserve and maintain effective drug concentration, decrease side effects thus, optimizing drug therapy and also provide a prolonged dosing of the drug from the product by supplying an initial amount (or) loading dose, perhaps one-half of the drugs over the desired time period [4]. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs and these are multiparticulate drug delivery systems to improve bioavailability, stability, therapeutics and it's exhibit strong targeting to concentrate drugs at local tissue and reduce the adverse reactions of drugs [5]. Antimicrobial loaded Microspheres can somehow enhance confronting ability against tolerated bacteria, effectively prolong drug's biological half-life, stabilize blood drug level and benefits offered include reducing dosing frequency with improved patient compliance, better and more uniform clinical effects with lower incidence of side effects and possible enhanced bioavailability and has alleviated the risk of dose dumping *in-vivo.* The present study focused on formulate ofloxacin microspheres using hydrophilic polymer and natural gums and to prolong the release rate and relatively constant effective level in the treatment of infections, so as to decrease the necessity of multiple dosing especially in patients with renal impairment. Generally, ofloxacin is a second generation fluoroquinolone antibiotic and virus reverse transcriptase inhibitor and it has broad spectrum activity against gram negative and gram positive bacteria [6-8].

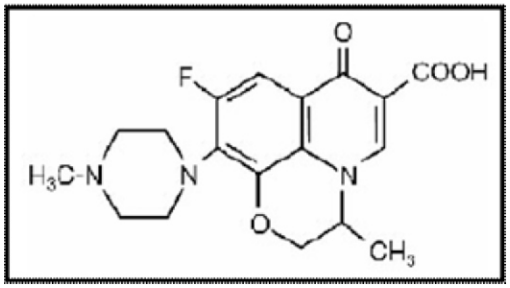


Fig. 1 Structure of Ofloxacin

MATERIALS AND METHODS

Materials

Ofloxacin was obtained as gift sample from Karnataka antibiotics and Pharmaceuticals Limited, Bangalore. Guar Gum from Yarrow Chem Products and Xanthan Gum from Qualigens Fine Chem Pvt Ltd, Mumbai, Sodium Alginate from Rachem Laboratory Chemicals Pvt Ltd, Chennai, Calcium Chloride from Flora Chemicals, Mumbai. All other chemicals and solvents used were of analytical grade.

Method

IR Spectral Analysis

The FT-IR spectrum of ofloxacin and polymers was recorded using KBr mixing method on the FT-IR instrument (Schimadzu FT-IR-8400 S) [9-10]. The drug alone, and in combination with polymers (mixed in the ratio of 1:1) was taken and subjected to FT-IR studies.

Preparation of Ofloxacin Microspheres

Microspheres were prepared by ionic cross-linking technique. The alginate solution comprising 2.5 % sodium alginate, hydrophilic polymer and 100 mg of drug were prepared by dissolving the polymer in 50 % deionized water using gentle heat. On complete dissolution, the weighed quantity of drug was mixed throughly and to this solution of sodium alginate was added to afford homogeneous dispersion. The dispersion was added drop wise via 20 gauge hypodermic needle fitted with a 10 ml syringe into 50 ml 5 % w/v of calcium chloride solution, being stirred at 200 rpm for 10 min. The droplets from the dispersion instantaneously gelled into discrete drug-polymer-alginate matrices upon contact with the solution of cross-linking agent. The formed microspheres were further allowed to stir for an additionally 2 hrs. On expiration, cross-linking agent was decanted and microspheres were washed with deionized water. The microspheres were there after dried at 80°C for 2 hrs in a hot air oven [11-13].

Characterization of Microspheres Formulation

Percentage Yield

The microspheres were evaluated for percentage yield. The yield was calculated as per equation below [14]:

Percentage Yield = The amount of microspheres obtained/The theoretical amount × 100.

Determination of Particle Size

The sample of prepared microspheres was randomly selected and their size was determined using an optical microscope (Olympus, India) [15].

Micromeritic Properties

Bulk density, Tapped density, Carr's (Compressibility) index and angle of repose of each formulation are carried out and the results are analyzed [16-17].

Morphological Studies

Scanning electron microscopy (JEOL JSM-6701F, Japan) of formulations was carried out to study their morphological characteristics of Ofloxacin microspheres [18].

Entrapment Efficiency

The amount of drug was estimated by crushing the microspheres and extracting with aliquots of Phosphate buffer (pH 7.4) repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume made up by using buffer. The solution was filtered and the absorbance is analyzed spectrophotometrically (Shimadzhu UV-1700, Columbia) at 276 nm to determine amount of ofloxacin entrapped in microspheres

Table 1. Composition of various ofloxacin formulations

Formulations Code	Ofloxacin (mg)	Sodium Alginate (%w/v)	Guar gum (%w/v)	Xanthan gum (%w/v)	Calcium chloride (%w/v)
GG1	100	2.5	0.25	-	5
GG2	100	2.5	0.5	-	5
GG3	100	2.5	0.75	-	5
XG1	100	2.5	-	0.25	5
XG2	100	2.5	-	0.5	5
XG3	100	2.5	-	0.75	5

[18].

In-vitro Drug Release Studies

The *in-vitro* drug release studies were conducted in 0.1N HCl for 2 hours and in pH 7.4 buffer for 12 hours using USP XXIII, type-II dissolution apparatus under sink conditions. Accurately weighed samples of the microspheres were added to dissolution medium and temperature was maintained at 37°C ±1°C and fluid was agitated at 100 rpm. Adequate samples was withdrawn every one hour and replaced with equal quantity of the fluid and maintain constant volume. After suitable dilution, the samples withdrawn were analyzed at 276 nm [19-20]. In order to study the exact mechanism of drug release, *in-vitro* release data was analyzed using different kinetics models and mechanism of drug release is determined [21-22].

Stability Studies

Best formulations of sufficient quantity of microspheres was taken in a crucible and placed at 45°C and 75% RH for 45 days, and the microspheres were analyzed at specified days period for their drug content [23].

RESULTS

Percentage yield of all formulations is between the range of 89.17-94.61 % and the average particle size was observed between 44.32-50.17 µm. Entrapment efficiency of guar gum microspheres is between the range of 78.40-83.89 % and xanthan gum formulations was found to be in the range between 65.52-72.73 % (Table-2).

From Table-3, bulk and tapped density values were lies in between 0.416 to 0.535 and 0.526 to 0.652 g/cm^3 and Carr's index values in between 16.12 to 19.72 % using different formulations. Angle of repose of all formulated microspheres is found to be less than 40° indicates acceptable flow properties.

The results obtained from *in-vitro* release studies were plotted in four kinetic models of data treatment as follows: Cumulative percentage drug release Vs Time (Zero order rate kinetics), Cumulative % drug retained Vs Time (First order rate kinetics), Cumulative percentage drug released Vs Square root of Time (Higuchi's classical diffusion), Log cumulative percentage drug release Vs log Time (Korsmeyer-Peppa's exponential).The kinetics data results is shown in Table-4.

From the *in-vitro* results, best formulations (GG3&XG3) were taken and analyzed the stability studies in short period is mentioned in above manner and evaluate the drug content and physical appearances. The results of stability studies data is indicated in Table-5.

FT-IR spectra of prepared samples were taken in the wavelength region was 600-3800 cm^{-1} at ambient temperature and the resolution was 4 cm^{-1} and compared the position and relative intensity of absorption band of physical admixtures and pure drug is illustrated in Figure-2&3. The IR spectrum of both the drug

Table 2. Percentage yield, Average particle size and Drug entrapment of microspheres.

Formulations Code	Percentage Yield (%)	Average Particle Size (µm)	Drug Entrapment (%)
GG1	89.17±6.02	44.32±1.31	78.40±5.08
GG2	91.35±5.24	48.72±2.01	80.45±4.51
GG3	94.61±4.95	48.95±2.55	83.89±4.17
XG1	90.12±6.22	48.63±3.05	65.52±4.27
XG2	91.71±6.19	49.32±3.39	69.40±5.54
XG3	93.02±5.39	50.17±3.36	72.73±3.88

Table 3. Micromeritic properties data of different batches of microspheres

Formulations Code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Angle of repose (θ)
GG1	0.483±0.007	0.576±0.004	16.12±1.01	29^{0}74'
GG2	0.535±0.004	0.652±0.005	17.86±1.06	29^{0}05'
GG3	0.447±0.005	0.555±0.009	19.40±0.95	27^{0}76'
XG1	0.521±0.005	0.621±0.006	16.66±0.94	27^{0}15'
XG2	0.422±0.005	0.526±0.006	19.72±0.99	28^{0}39'
XG3	0.416±0.006	0.517±0.005	19.40±0.94	30^{0}10'

Table 4. Kinetics Analysis Data of Ofloxacin Microspheres

Formulations Code		Release model Zero order	First order	Higuchi's	Korsmeyer Peppas	Mechanism
GG1	R	0.9863	0.9973	0.9670	0.9895	
	S	7.9705	-0.0642	26.222	0.4566	
GG2	R	0.9764	0.9976	0.9676	0.9955	
	S	8.3745	-0.0728	27.559	0.4325	
GG3	R	0.9746	0.9924	0.9889	0.9771	
	S	8.6188	-0.0761	28.484	0.4216	Fickian
XG1	R	0.9594	0.9911	0.9852	0.9894	
	S	6.6360	-0.0494	22.421	0.4259	
XG2	R	0.9717	0.9950	0.9850	0.9812	
	S	6.7633	-0.0503	22.793	0.4785	
XG3	R	0.9794	0.9906	0.9853	0.9872	
	S	6.6450	-0.0496	22.455	0.4872	

Correlation coefficient (r), Slope (s)

Table 5. Stability studies data of Ofloxacin microspheres (GG3 & XG3)

Time in Days	Physical Appearance GG3	XG3	Percentage Drug Content GG3	XG3
0	----	----	87.58 ±1.37	85.08 ± 1.02
15	No change	No change	87.49 ±1.61	87.29 ± 0.90
30	No change	No change	87.36±1.42	86.66± 0.56
45	No change	No change	87.27 ±1.50	87.97 ±1.05

Results are mean S.D of three trials (n=3)

and prepared microspheres there were no considerable changes in comparison between the ratios of percent (%) transmittance.

Scanning electron microscopy for the best formulations was carried out and the scanned images are shown in Photomicrograph-1&2.

The release profile of all batches of microspheres was studied for first two hours in gastric pH followed by intestinal pH 7.4. The percentage drug release of all formulations was calculated by adding the amount of drug released at the end of 2nd hour in gastric pH to the amount of drug released in intestinal pH. The comparative *in-vitro* drug release curve of all batches of microspheres were shown in Figure 4&5.

DISCUSSION

IR Spectral Analysis

In compatibility studies, IR spectrum of pure drug was found to be similar to the standard IR spectrum of which indicates that the obtained sample was pure ofloxacin. The IR spectra of all the pure samples and the ofloxacin physical admixtures of suitable proportion of polymers were subjected to the study and from the results, it was observed that there is no presence of interaction between drug and the polymers.

Percentage yield, Particle size and Drug entrapment

From the results of Table-2, the percentage yield of all the formulations which are directly proportional to number of drop of solution which fall into calcium chloride solution. Without changing any formulation variability, only as the concentration of polymer increases a slight increase in the percentage yield was observed. The average particle size of all the formulations was observed in between 44.32-50.17 µm. Slightly increased particle size was observed with the increase in polymer concentration. A high concentration of polymer produced a more viscous dispersion, which formed larger droplets and consequently larger microspheres. From the results, the particle size of Xanthan gum microspheres are more than that guar gum microspheres. From the results, the entrapment efficiency of guar gum microspheres is more than xanthan gum formulations and it was observed that, the

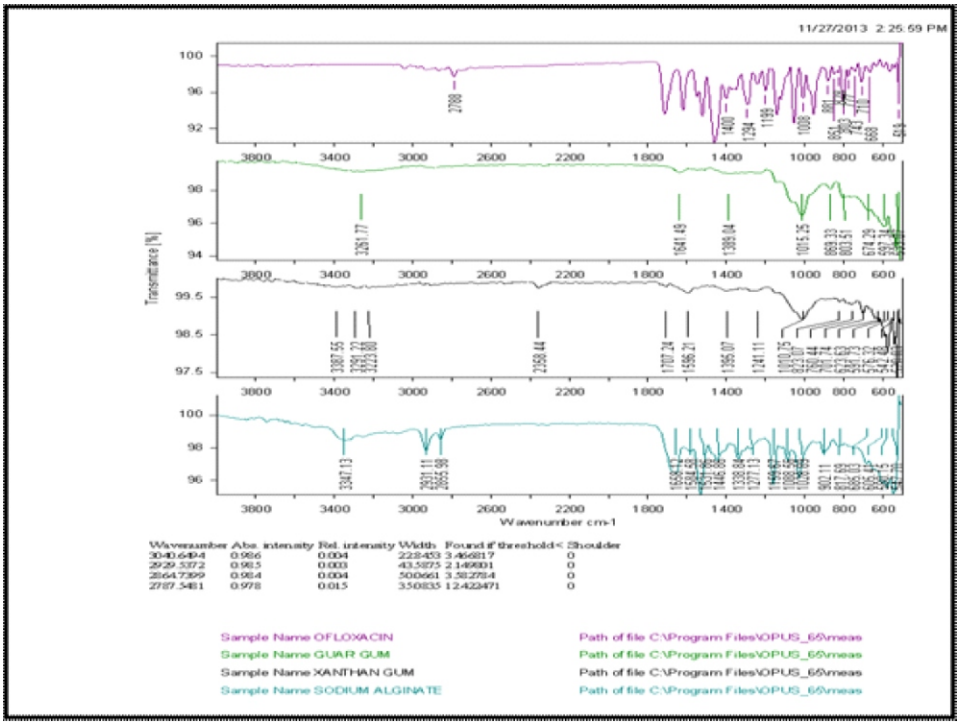


Fig. 2 IR spectra studies of Pure Ofloxacin, Guar gum, Xanthum gum and Sodium alginate

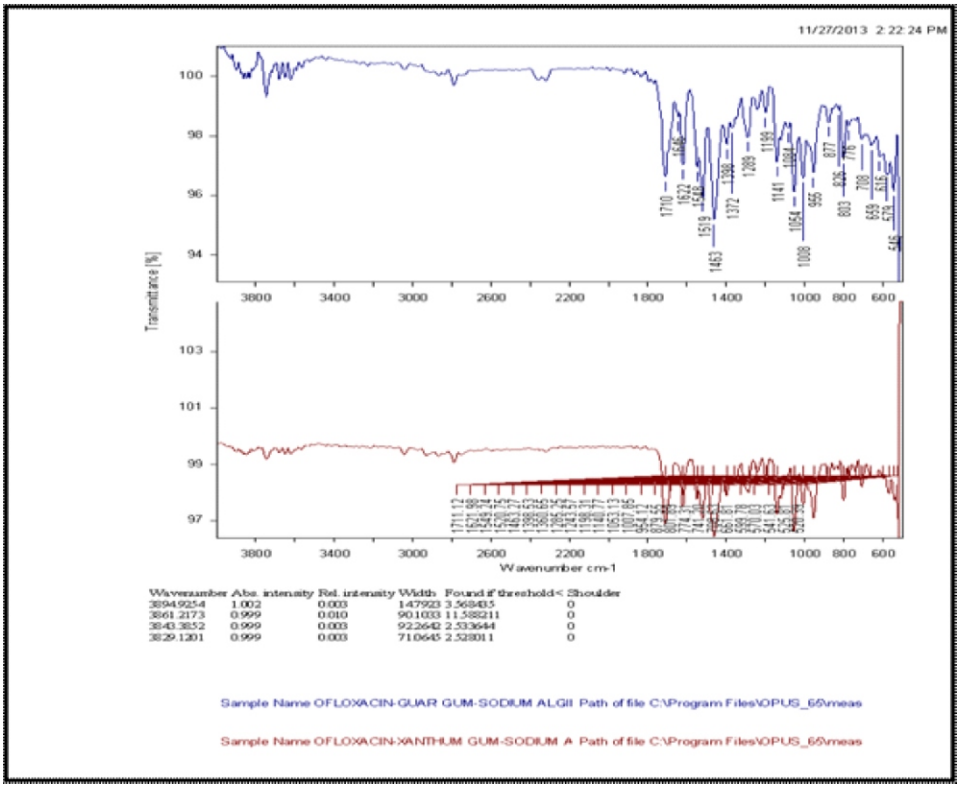
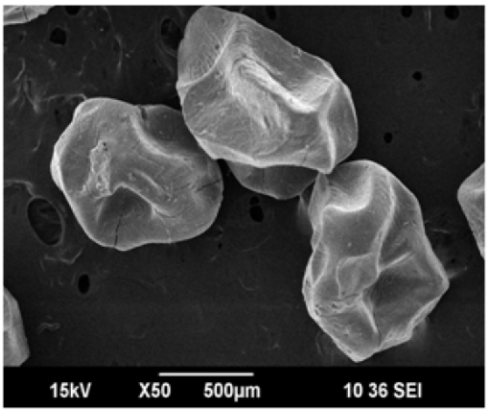


Fig. 3 IR spectra studies of Ofloxacin Physical admixtures using Guar gum and Xanthum gum

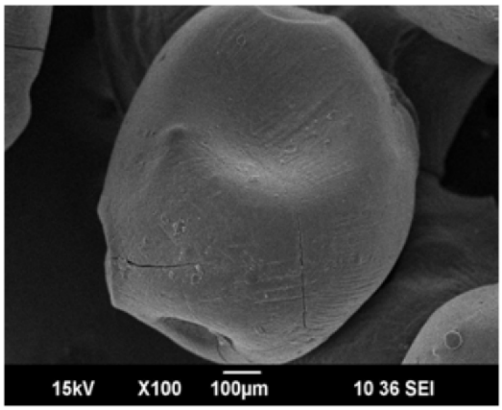
percentage efficiency increases with the increase in polymer concentration.

Micromeritic Properties

The packing properties of the drug and their formulations widely depend upon bulk density. It has been stated that bulk density values less than 1.2gm/cm^3 indicate good flow and values greater than 1.5 gm/cm^3 indicate poor flow. From Table-3 results, it was observed that the bulk density and tapped density values were lies in between 0.416-0.535 g/cm^3 and 0.526-0.652 g/cm^3 indicates good packing. The Carr's index values were lies in between 16.12-19.72 %; indicates good flow characteristics of the microspheres. Angle of repose are less than or equal to 40° indicates free flowing properties and greater than 40° indicates



Photomicrograph-1
SEM image of Ofloxacin microspheres
(Guar Gum-Sodium Alginate)



Photomicrograph-2
SEM image of Ofloxacin microspheres
(Xanthan Gum-Sodium Alginate)

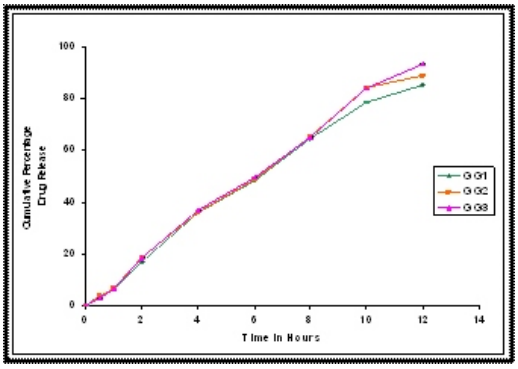


Figure 4 Comparative in-vitro drug release plot of Ofloxacin microspheres (GG)

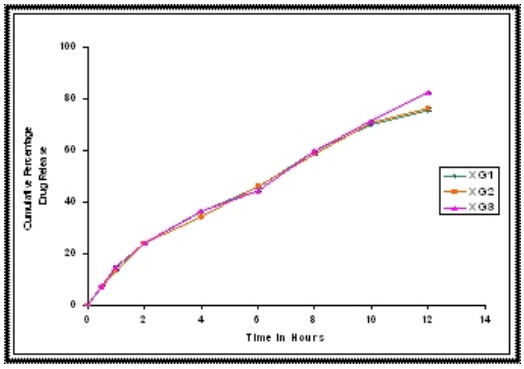


Figure 5 Comparative in-vitro drug release plot of Ofloxacin microspheres (XG)

poor flow of material. It can be observed that, the angle of repose for various batches of microspheres is found to be less than 40° which indicates good flow properties.

Morphological Studies

The surface morphology of best formulations was determined by SEM for characterization of shape and size of microspheres. Photomicrograph results showed that the prepared microspheres showed a good specificity, spherical and uniform in shape with smooth surface and the particles are distributed uniformly without any lumps.

In-vitro Drug Release Studies

The i***n-vitro*** release results (Figure 4&5) indicate more sustain effect with the increase in concentration of polymer. It was observed that the release was achieved after initial lag time and it was directly proportional to the concentrations in both cases. The first phase must be for negligible dissociation of microspheres in stimulated gastric fluid during first two hours and the drug release mainly based on drug diffusion through pores and cracks or may be due to the swelling of polymer. The second phase exhibited a burst-like release pattern, which was accomplished by the polymer disintegration due to enzymatic degradation of polymer. Both the polymers are highly branched and highly branched molecular structure of these polymers resist enzymatic breakdown in digestive tract. The Guar gum showed high rate and extent of drug release. The drug release from guar gum batches (GG1 to GG3) with polymer ratio 0.25 %, 0.5 % 0.75 % was 85.30 %, 88.84 %, and 93.26 % at the end of 12 hours respectively. This indicates that guar gum at minimum concentration was able to sustained drug release. As compare to Guar gum, drug release from Xanthan gum batches (XG1 to XG3), is retardant and it showed 75.49 %, 76.13 % and 82.54 % drug release after 12 hours.

In order to understand the mechanism and kinetics of *in-vitro* drug release studies of all formulations was subjected to goodness of fit test by linear regression analysis according to various kinetics model is mentioned above and coefficient of correlation (r) values were calculated. The formulations GG1, GG2, GG3 and XG1, XG2, XG3 followed first order release as correlation coefficient (r) values were 0.9973, 0.9976, 0.9924 and 0.9911, 0.9950, 0.9906 respectively. So the co-efficient determination indicated that the release data was best fitted with first order kinetics. When the drug release data was put into Higuchi equation, good correlation coefficient (r) values 0.9670 to 0.9889 were obtained, indicating that the drug release was diffusion controlled.

The release data obtained were also put in Korsmeyer-Peppas model in order to find out n values, which describe the drug release mechanism. From the kinetic results data, the n values of microspheres were found in the range of 0.4216 to 0.4872 with correlation coefficient values ranging from 0.9771 to 0.9955, indicating Fickian diffusion mechanism. Hence, the above observations, the drug release from microspheres provide sustained release for a period of sufficient hours and the kinetic

study shows that 'r' values of all formulated batches indicate compliance with Higuchi's plot which reveals that the drug release follows Fickian diffusion**.**

Stability Studies

From the *in-vitro* results, best formulations were taken and analyzed the stability studies is mentioned in above manner. The results shows that there is about 85-87 % of drug is present in the formulations with no-observable physical changes after storage. This indicates a good stability of the ofloxacin microspheres.

CONCLUSION

The objective of the work was to develop the spherical crossed linked ofloxacin microspheres by ionic cross linking technique. The prepared formulations were free flowing, non sticky and evaluated for various parameters like particle size, bulk density, tapped density, Carr's index, angle of repose, entrapment efficiency and *in-vitro* dissolution profile. The percentage yield was directly proportional to the number of drops of the solution which directly fall into calcium chloride solution. All formulations showed sustained action and drug release in all batches of microspheres compliance with Higuchi's plot and follows Korsmeyer mechanism. The best formulations (GG3 & XG3) were showed good stability in short term period. This study also explored polymers and gums in different combination and demonstrated the ability retard drug release effect.

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