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FORMULATION AND EVALUATION OF FLOATING IN SITU GEL BASED GASTRORETENTIVE DRUG DELIVERY OF CIPROFLOXACIN HCL.

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Abstract:
The objective of the present study was to formulate and evaluate a gastro retentive in situ gelling system of ciprofloxacin HCl using HPMC K100M, sodium alginate gelling polymer, calcium chloride and calcium carbonate as a cross linking agent for potentially treating gastric ulcer, associated with Helicobacter pylori. The drug delivery systems of in situ forming polymeric formulation is in sol form before administration in the body, but once administered, that under goes gelation in situ to form a gel. The formulation of gel depends up on factor like temperature modulation, pH changes and presence of ions from which drug gets released in sustained and controlled manner. The floating in situ gelling systems were prepared by dissolving various concentration of sodium alginate in deionized water, to which varying concentrations of drug. The results shows that the formulas which containing a combination of the polymers (sodium alginate and HPMC K 100 M) shows more retardation in drug release than formulas based only with sodium alginate at the same percentage. The increasing gas generating agent calcium carbonate reduces floating lag time gelling integrity and increase floating duration. The formulation batch F6 was most suitable preparation being able to control the drug release for longer duration with administration and physiological suitability in terms of pH and viscosity. The in situ gel exhibited the expected, viscosity, drug content, pH, in vitro gelling capacity, in vitro floating ability, water uptake ability and sustained drug release. A stomach specific in situ gel of ciprofloxacin hydrochloride could be prepared using floating mechanism to increase the residence time of the drug in stomach and thereby increase the absorption.

Key Words: *In situ gel, Gelation, Gastro retentive drug delivery system, H. pylori, Ciprofloxacin HCl.*

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INTRODUCTION

The in situ gel formulation is the most convenient and offers an interesting alternative for achieving systemic drug effects of parenteral routes which can be in convenient or oral routes, which can results in un acceptable low bioavailability and passes the hepatic first pass metabolism, in particular of protein and peptides.

The *in situ* gel dosage form is a liquid before administration but converts into a gel that floats on gastric contents as it comes in contact with it[1] .Such gel conversions is due to one or more mechanisms such as physiological stimuli (e. g., temperature and pH), physical changes in biomaterials (e. g., diffusion of solvent and swelling), and chemical reactions (e. g., enzymatic, ionic and photo-initiated polymerization)[2] .

The development of oral in situ gel forming system also known as stomach specific or raft forming systems have provided a suitable way of providing the controlled drug delivery within stomach with enhanced gastro-retention [3]. Drugs that are easily absorbed from gastro intestinal tract (GIT) and have short half life are eliminated quickly from systemic circulation. Frequent dosing of these drugs is required to achieve therapeutic activity.

Helicobacter pylori (H. pylori) are one of the most common pathogenic bacterial infections. It is associated with the development of serious gastro duodenal disease, including peptic ulcers, gastric lymphoma, and acute chronic gastritis. H. pylori reside mainly in the gastric mucosa or at the interface between the mucous layer and the epithelial cells of the antral region of the stomach. Antibiotics required for eradication of H. pylori are high in dose and in more frequencies [4]. This is because of the low concentration of the antibiotic reaching the bacteria under the mucosa, instability of the drug in the low pH of gastric fluid, and short residence time of the antibiotic in the stomach, leading to incomplete eradication of H. pylori.

Ciprofloxacin is a broad spectrum fluoroquinolone antibiotic. It is approved for the treatment of infectious diarrhea, urinary tract infections, bone and joint infections, lower respiratory tract infections, hospital acquired infections and *meningococcal prophylaxis.* The drug is freely soluble in water and has a short elimination half-life of about 4h; various sustained release preparations were aiming to enhance its antibacterial activity. It has a narrow absorption window and is mainly absorbed in the proximal areas of GIT (Baumgartmer S *et al.*, 2000). Ciprofloxacin Oral Suspension is available in 5% (5 g ciprofloxacin in 100 mL) and 10% (10 g ciprofloxacin in 100 mL) strengths.

An infection of stomach mucosa with *H.pylori*, a gram negative bacillus that causes chronic gastritis, is now generally considered as a risk factor of gastric cancer and duodenal ulcer. Ciprofloxacin is the drug of choice for the treatment of *H.pylori* infection. The drug does not readily cross blood brain barrier (BBB).Considering the above shortcomings, Ciprofloxacin HCl floating systems were developed. The gastro retentive drug delivery systems can be retained in the stomach and assist in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the gastrointestinal tract.

MATERIALS AND METHOD

Material

Ciprofloxacin HCl was obtained as a gift sample of Akums pharmaceutical industry Haridwar India, Hydroxy propyl methyl cellulose (K100M), sodium alginate, sodium citrate; calcium chloride and calcium carbonate were obtained in central drug house of department of pharmaceutical sciences Dehradun. All other reagents and Chemicals used were of analytical grade.

Methods

Preparation of In Situ Gel

SA (sodium alginate) Solution was prepared in distilled water by heating to 60℃ under continuous stirring. After cooling below 40˚ C. Ingredients including drug, gelling agent and other excipients were weighed accurately on butter paper with the help of a stainless steel then Sodium alginate solution of difference concentrations (0.5,1, 1.5, 2 and 2.5gm) were prepared by adding the sodium alginate to distilled water containing difference concentration (0.25gm, 0.5gm) calcium carbonate, and difference concentration (0.25gm, 0.5gm) sodium citrate and heating to 60˚C and after cooling below 40˚C and continuous stirring. Appropriate amounts of Ciprofloxacin hydrochloride 0.25(gm) and calcium carbonate (1 and 2%) were then dissolved in the resulting solution and formulations were prepared. The resulting formulations were finally stored in amber colored bottles until further use [5].

Table 1: Composition of Floating in Situ Gel

S.No	Ingredient	F1	F2	F3	F4	F5	F6
1	Drug	0.25%	0.25%	0.25%	0.25%	0.25%	0.25%
2	Sodium alginate	0.25%	0.5%	1%	1.5%	1.5%	2%
3	HPMC K100M	0.5%	0.8%	1.5%	2%	2.5%	2.5%
4	Calcium carbonate	1%	1%	1.5%	1.5%	2%	2%
5	Sodium citrate	0.225%	0.225%	0.225%	0.225%	0.225%	0.225%
6	Calcium chloride	0.075%	0.075%	0.075%	0.075%	0.075%	0.075%

Evaluation

Determination of UV Absorbance Maxima of Ciprofloxacin HCl

The standard stock solution of ciprofloxacin HCl in water was used to determination the λ max of (0.1 N HCl, pH 1.2) was used as blank for the study. The spectrum was taken between the UV range of 200-400nm. The highest peak obtained from the spectrum analysis was taken as λ max for Ciprofloxacin Hydrochloride.

Preparation of Standard Calibration Curve of Ciprofloxacin HCl in 0.1 N HCl [6]

Ciprofloxacin Hydrochloride (50 mg) was dissolved in 50ml of (0.1 N HCl, pH 1.2) and volume was made up to ml in 50 ml volumetric flask. This solution (100 mcg/ml) was further diluted with (0.1 N HCl, pH 1.2) to obtain solution of 2 to 20 mcg/ml. The absorbance of each solution was measured at 278 nm using UV spectrophotometer. The standard curve was obtained by plotting absorbance v/s. concentration (µg/ml) [7].

Identification of Drug by FTIR

Fourier transform infrared (FT-IR) spectra were obtained using an FTIR spectrometer. The pure ciprofloxacin HCl were mixed thoroughly with KBr, an infrared transparent matrix, at 1:3 (sample: KBr) ratio, respectively. The KBr discs were prepared by compressing the powder at a pressure of 10 tons for 5min in a hydraulic press forty scans were obtained at a resolution of 4cm-1 from 2000 to 400cm-1 [8].

Physical Appearance and pH

All the prepared sodium alginate based *in situ* solutions of Ciprofloxacin HCl were checked for their clarity and the pH of the solutions. After administered of the prepared solutions in 0.1 mol L–1HCl, pH 1.2, the time required for gel formation and consistency of gel formed was checked visually. The pH was also measured in each of the solution of sodium alginate based *in situ* solutions of ciprofloxacin HCl, using a calibrated digital pH meter at 25oC.The measurement of pH of each data were in triplicate [9].

Viscosity of *in Situ* Gelling Solutions – The viscosity of formulations was determined by a Brookfield viscometer DV-III (Brookfield, USA) using spindle number 21 with cup and bob setting at 50 rpm [10].

Floating Behavior – The floating ability of the prepared formulations was evaluated in (0.1N HCl, pH 1.2) Solution. The floating time of the prepared formulation took to emerge on the medium surface (floating lag time) was found to be 60sec.The time the formulation constantly floated on the dissolution medium surface (duration of floating) was evaluated to be 12hrs resulting the formation of thick gel with good floating tendency[11].

***In-vitro* Gelling Capacity** - To evaluate the formulations for their *in-vitro* gelling capacity by visual method, solutions of *in situ* gel forming drug delivery system were prepared. The *in-vitro* gelling capacity of prepare formulations was measured by placing 5 ml of the gelation solution (0.1N HCL, pH 1.2) in a 15 ml borosilicate glass test tube and maintained at 37±1ºC temperature. One ml of formulation solution was added with the help of pipette. The formulation was transferred in such a way that places the pipette at surface of fluid in test tube and formulation was slowly released from the pipette. As the solution comes in contact with gelation solution, it was immediately converted into stiff gel like structure. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel and time period for which they formed gel remains as such. The *in-vitro* gelling capacity was graded in three categories on the basis of gelation time and time period for which they formed gel remains.

(+) Gels after few minutes, dispersed rapidly
(++) Gelation immediate remains for 12 hours

(+++) Gelation immediate remains for more than 12 hours **[12]**

Drug Content:
Ten ml of the solution was added to 900 ml (0.1N HCl, pH 1.2) Solution and sonicate for 75 min. and take over night for 24 hrs. The solution was filtered, suitably diluted with (0.1N HCl, pH 1.2) and the drug concentration was determined by using a UV-visible spectrophotometer a (Shimadzu UV 1700 Pharmazie) at 278 nm against a suitable blank solution [5]**.**

***In vitro* Release Studies:**
An *in vitro* release study was carried out using dissolution test apparatus USP Type II (Paddle Method). The following procedure was followed throughout the study that is shown in (table 2) to determine the *in vitro* dissolution rate for the formulations. The release of ciprofloxacin from the formulations was determined using dissolution test apparatus USP Type II with a paddle stirrer at 50 rpm. The dissolution medium used 900 ml of (0.1N HCL, pH 1.2) solution and temperature was maintained at 37 ± 0.2 °C. Ten ml of the formulation were placed into a Petri dish (4.5 cm i.d.) which was kept in the dissolution vessel and 0.1N HCL solution was carefully added to the Vessel avoiding any disturbance of the Petri dish. At each time interval, a precisely measured sample of the dissolution medium was pipette out and replenished with fresh medium. Ciprofloxacin hydrochloride concentration in the aliquot was determined Spectrophotometrically [13].

Drug Release Kinetic Studies:
The drug release kinetic studies were done by various mathematical models (zero order, first order, Higuchi's square root, Hixson-Crowell cube root law and Pappas equation). The model that best fits the release data is selected based on the correlation coefficient (r) value in various models. The model that gives high 'r' value is considered as the best fit of the release data. The release constant was calculated from the slope of the appropriate plots, and the regression coefficient (r2) was determined [14].

RESULT AND DISCUSSION

Determination of UV Absorbance Maxima of Ciprofloxacin Hydrochloride- The standard stock solution was used to determination the λ max of (0.1 N HCl, pH 1.2) was used as blank for the study. The spectrum was taken between the UV range of 200-400nm. The highest peak obtained from the spectrum analysis was taken as λ max for Ciprofloxacin Hydrochloride that used was found to be 278 nm.

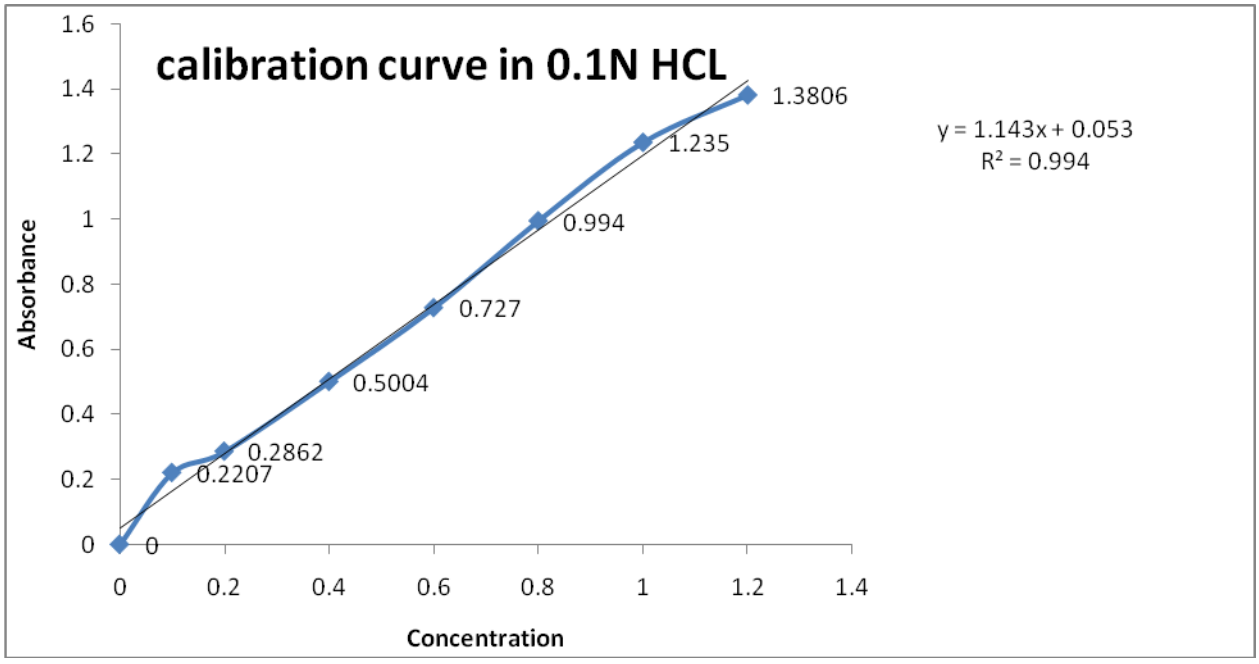

Fig 1: Standard Curve of Ciprofloxacin HCl

Identification of Drug by FTIR

Identification study was performed using FTIR spectrophotometer. The characteristic absorption peaks of ciprofloxacin hydrochloride were obtained at different wave numbers. The peaks obtained in the spectra of pure drug correlates with the peaks of official spectrum of British Pharmacopeia which confirms the purity of drug.

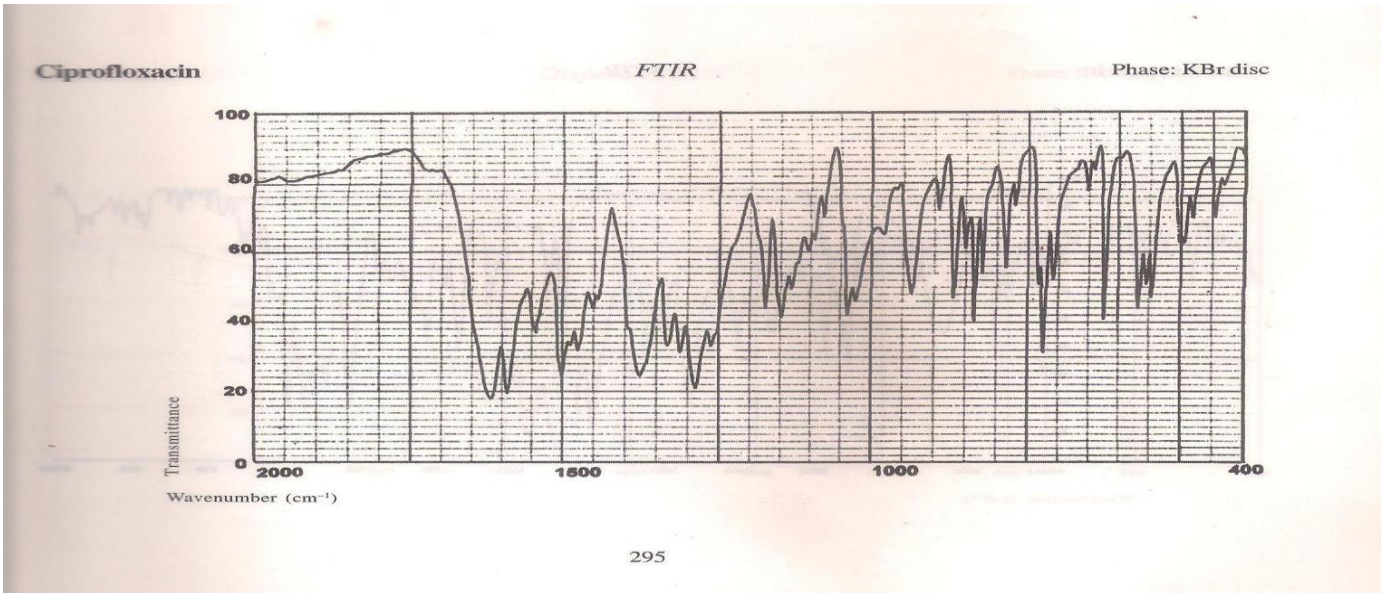

Fig 2: Standard FTIR spectra of Ciprofloxacin HCL (with references of IP-2010)

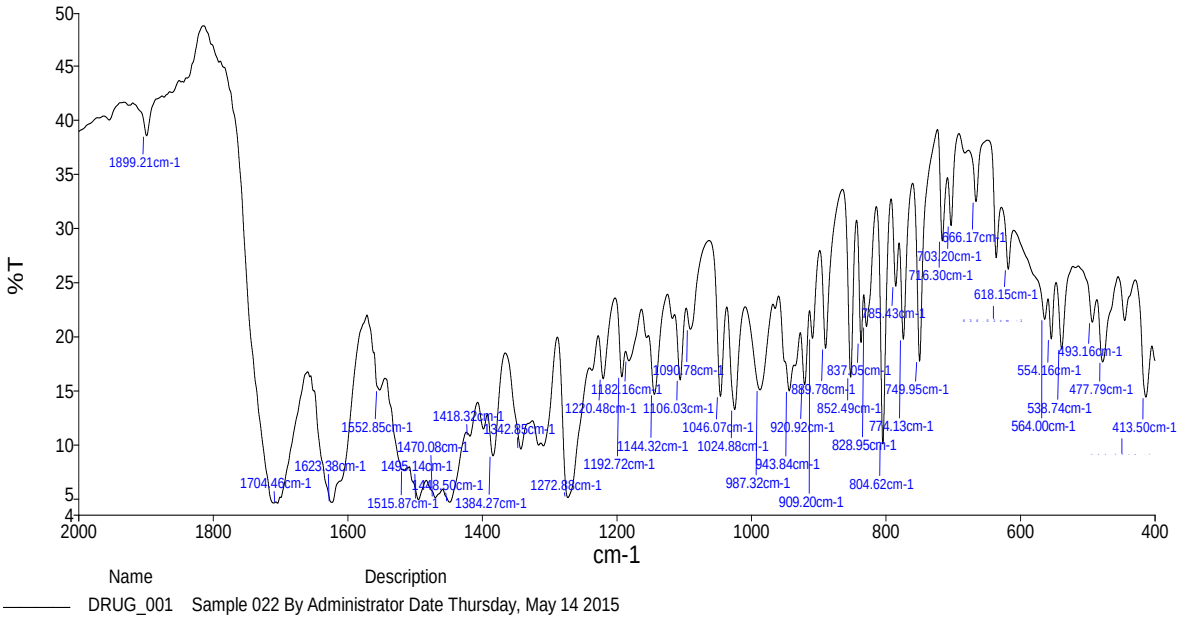

Fig 3: FTIR Spectra of Ciprofloxacin HCl

Physical Appearance and pH

All the prepared sodium alginate based *in situ* solution of Ciprofloxacin hydrochloride was checked for their clarity and the type of the solution. After administration of the prepared solution in (0.1N HCL, ph 1.2) also checked the time required for gel formation and type of gel formed. The pH was measured in each of the solution of sodium alginate based *in situ* solution of Ciprofloxacin hydrochloride, using a calibrated digital pH meter. The measurement of

pH of data were in triplicate and the Average values given in **Table 2.**

Table 2: pH of prepared *In situ* gel formulation

Formulation code	F1	F2	F3	F4	F5	F6
pH	7.3	7.6	6.98	7.4	7.9	8.05

Viscosity

The viscosity of the formulations increased with an increase in sodium alginate concentration. This phenomenon is a consequence of increasing chain interaction with an increase in polymer concentration. Calcium carbonate, which is the source of cations, increased the viscosity of the formulation. This change in viscosity is due to the proportional increase in the amount of dispersed calcium carbonate.

Table 3: Viscosity of Prepared *In situ* gel Formulation

Formulation code	F1	F2	F3	F4	F5	F6
Viscosity (cp)	2177	31174	52123	2304	21728.6	52446

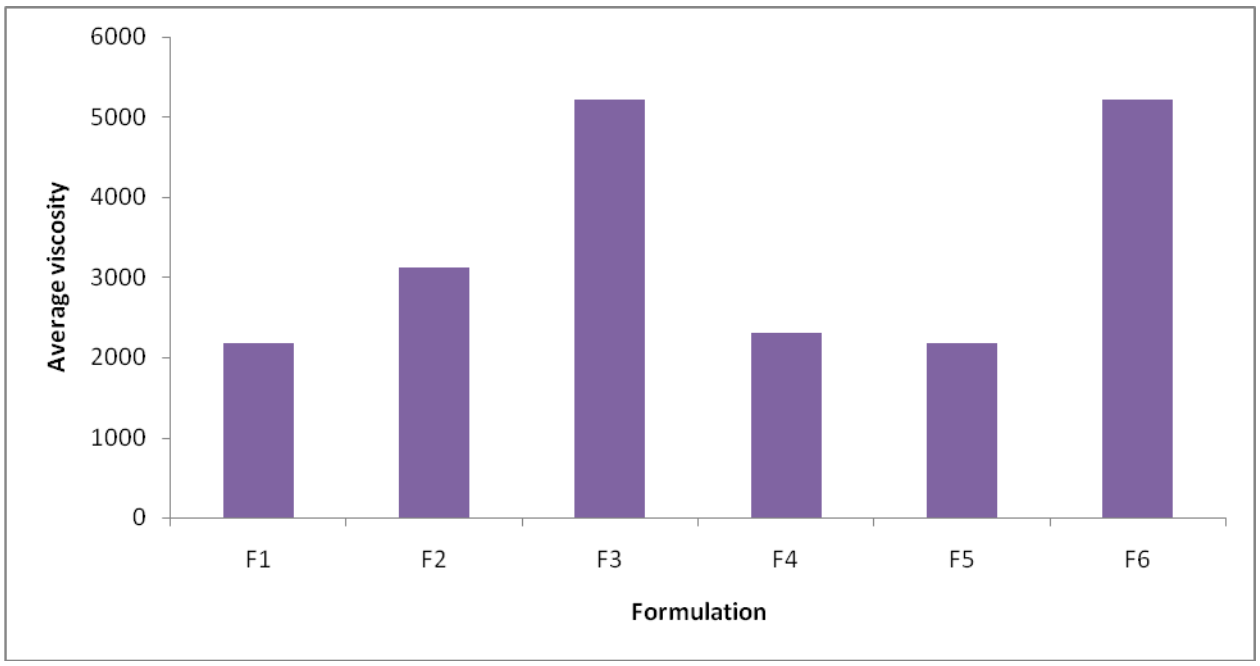

Fig 4: Average Viscosity of Ciprofloxacin HCl

Floating Behavior

The buoyancy lag time varied with the formulation variables. Formulation F3 exhibited the least buoyancy lag time (15 s) while formulation F6 exhibited the highest lag time (209 s). The decrease in the buoyancy lag time of a formulation F6 can be attributed to the availability of an increased the concentration of calcium carbonate was increased, being entrapped in the formed gel to give rapid buoyancy. Irrespective of formulation variables, buoyancy duration was > 12 hours.

Table 4: Floating Behavior of Prepared *In situ* gel Formulation

Formulation code	F1	F2	F3	F4	F5	F6
Floating lag time(sec)	50s	45s	15s	102s	165s	209s
Floating time	>12	>12	>12	>12	>12	>12

Fig 5: Floating Behavior of Floating in situ Gel

Gelling Capacity

In vitro gelling capacity of various formulation of *in situ* floating gel is reported in table 5.

Table 5: Gelling capacity of prepared *In situ* gel formulation

Formulation code	F1	F2	F3	F4	F5	F6
Gelling capacity	++	++	++	++	+++	+++

*****(++) Gelation immediate remains for 12 hours, (+++) Gelation immediate remains for more than 12 hours.

Fig 6: Gelling Capacity of Floating in Situ Gel

Drug Content

The Drug content of all (F1-F6) formulations is given in table no 7; It ranges in between 90.44% - 98.89%. The values are acceptable as per united state pharmacopeia standards.

Table 6: Results of Drug Content of all Formulation of Ciprofloxacin HCL

Formulation code	F1	F2	F3	F4	F5	F6
Drug content uniformity%	90.44±0.23	92.32±0.34	98.75±0.40	96.68±0.33	94.75±0.27	98.89±0.42

***In-Vitro* Drug Release:**

The *in-vitro* drug releases of the in situ floating gel were carried in (0.1N HCl, 1.2 pH) solution from 0 to 8 hrs by using dissolution test apparatus USP Type II (Paddle Method). The samples were withdrawn at different time intervals and analyzed at 278 nm. Percentage Cumulative drug release was calculated on the basis of mean amount of Ciprofloxacin hydrochloride present in the respective solution. The results obtained in the *in vitro* drug release for the formulations F1 to F6 in (Table 8). The plots are shown in (Figure no. 5) for % cumulative drug release VS time. Formulation F1, F2, F3, F4, F5 and F6 released about 93.12 %, 87.87 %, 86.05%, 84.91%, 84.87%, and 79.87% of drug after 8 hrs. Respectively, the results are shown in figure indicate that the formulation, F6 which was prepared by the Sodium alginate (2.5%) with ciprofloxacin HCl showed minimum drug release after 8 hrs. Thus, the formulation (F6) has better result as comparison to others formulations as sustained release.

Table 7: *In-Vitro* Drug release of Ciprofloxacin HCL *in situ* gel Formulations (F1- F6)

%cumulative drug release from various batch						
Time (hrs)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
0	0	0	0	0	0	0
1	15.94	17.77	13.52	16.74	14.52	15.93
2	19.08	18.37	16.86	19.08	19.08	19.77
3	26.91	22.25	22.37	26.89	29.84	21.53
4	41.29	27.97	32.10	31.58	31.07	30.30
5	46.77	32.94	52.50	44.56	43.31	38.07
6	55.22	50.11	69.79	53.10	59.15	51.16
7	69.48	65.89	76.96	64.12	65.16	60.34
8	93.12	87.87	86.05	84.91	84.87	79.87

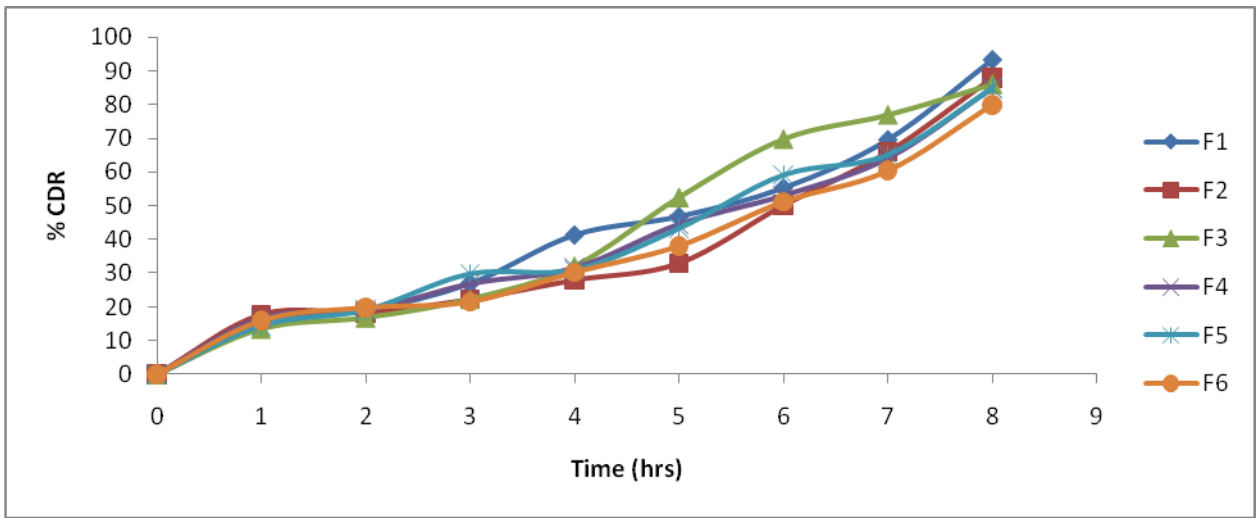

Fig7: *In-vitro* Release Profile of Ciprofloxacin HCL (F1 to F6)

Drug Release Kinetics Studies:

The drug release data of Ciprofloxacin HCl were fitted to models representing Higuchi's, zero order, first order, Hixson crowell and Korsmeyer equation kinetics to know the release mechanisms. The data were processed for regression analysis using Ms Excel statistical function. The results are shown in (Table 9). It was found that the *in vitro* drug release of optimize batch F6 was best explained by zero order as the plots showed the highest linearity (R2 = 0.9954). The formulation code F6 followed the zero order.

Table 8: Kinetic Models Studies of F1 to F6 batch.

Model fitting						
Formulation code	**Zero order**	**First order**	**Higuchi matrix**	**Korsmeyer peppas**	**Hixson crowell**	**N**
F1	0.965	0.726	0.895	0.934	0.833	0.853
F2	0.904	0.724	0.884	0.797	0.797	0.7599
F3	0.965	0.902	0.914	0.920	0.934	0.988
F4	0.963	0.814	0.891	0.909	0.880	0.785
F5	0.970	0.840	0.897	0.942	0.9002	0.848
F6	0.953	0.832	0.889	0.882	0.884	0.770

SUMMARY & CONCLUSION:

The present investigation deal with the formulation, optimization and evaluation of sodium alginate based *in situ* gel of ciprofloxacin hydrochloride. Sodium alginate and calcium carbonate used as a polymer and cross-linking agent respectively. The *in situ* formulations were exhibited well, viscosity, drug content and sustained drug release. This study reports that oral administration of aqueous solution containing sodium alginate result in formation of *in situ* gel. Such formulation are homogenous liquid when administration orally and become gel at the contact site. The evaluation of the formulation is dependent upon accurate results obtained by analytical method used during the study. Accurate results require the use of standard and a calibration procedure. Hence, standard plots of Ciprofloxacin hydrochloride were prepared in (0.1N HCL, pH 1.2) solutions. Ciprofloxacin hydrochloride was analyzed using UV spectrophotometer. Two different were sodium alginate and calcium carbonate used as a polymer and cross-linking agent respectively in the formulation of *in situ* gel. Among different excipients used sodium citrate etc. From the IR studies it may be concluded that the drug and carriers used undergo physical interaction there is no chemical change, and thus the gelling agent, cross-linking agent and other excipients are suitable for formulation of *in situ* gel of ciprofloxacin hydrochloride. Formulation F1, F2, F3, F4, F5 and F6 released about 93.12 %, 87.87 %, 86.05%, 84.91%, 84.87% and 79.87 % of drug after 8 hrs respectively. Indicate that the formulation, F6 which was prepared by the Sodium alginate (2.5 gm) with ciprofloxacin Hydrochloride showed
minimum drug release (sustained drug release) after 8 hrs. Thus, the formulation (F6) has better result as comparison to others formulations. The optimized formulation was found to be stable at room temperature for 90 days showed no significant change in the content of ciprofloxacin hydrochloride. No significant change was observed in the content uniformity, Viscosity and drug release of the *In situ* gel. All other parameters were also observed to be comparable. It could be concluded from study that optimized formulation was stable at room temperature.

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