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Formulation and Development of Alfuzosin Hydrochloride Buoyant In-Situ gel

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ABSTRACT

The aim of the present study was to design a buoyant in-situ gel of Alfuzosin HCl for use in management of Benign Prostrate Hyperplasia (BPH). The attempt was made to provide sustain drug release as well as prolong gastric retention thus improving bioavailability of the drug along with the added advantage of liquid oral dosage forms. The devised phase transition systems are to be administered in liquid form and gels in-situ on coming in contact with gastric fluid. Such liquid formulations are devised taking into consideration the geriatric patients who mostly founds difficult to swallow the solid oral dosage forms. Different prototypes were formulated, using Gellan gum, Sodium alginate and combination of both in-situ gelling polymers. HPMC K100M was incorporated as release retarding agent also used as strength forming polymer. Calcium Carbonate imparts buoyancy and also acts as cross-linking agent. Sodium citrate is used for liquefying solution, which act as sequestering agent by forming a complex with calcium carbonate (Ca^{+2}) and thus delays the gelation until the preparation reaches the acidic environment of stomach. The evaluation was carried out for *In Vitro* parameters and the results substantiated that the optimized formulation (S4A) revealed excellent floating characteristics and gastric retention. The results of dissolution studies was fitted into five different mathematical models and subjected to regression analysis. The drug release from all formulations was found to follow Zero order kinetics and shows to best fit Peppa's equations exhibiting fickian diffusion release mechanism.

Keywords: Buoyant in-situ gel, Alfuzosin HCl, BPH, Peppa's equations.

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INTRODUCTION

A rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamics profiles is to retain the drug reservoir at its absorption site and to release the drug in a controlled manner for prolong period of time. Thus, extensive efforts are being put in by both academia and industry towards the development of gastro retentive dosage forms.

The phenomenon of buoyancy has been introduced and studied extensively in the last decade and is applied to improve the performance of these delivery systems. The newer approach is development of buoyant in-situ gelling systems which has overtaken the conventional liquid oral dosage forms, since they provide the best way to overcome the problems of immediate release and short GI residence which are the characteristics of conventional liquid dosage forms.

The buoyant in-situ gelling systems are phase transition systems where dosage form is originally administered in sol form which transforms itself into gel in acidic environment of stomach and floats on gastric content forming a stable raft so explicitly being termed as "Raft forming systems".

Alfuzosin HCl is a selective alpha(1)-adrenergic blocking agent that exhibits selectivity for alpha(1)-adrenergic receptors in the lower urinary tract. Inhibition of these adrenoreceptors leads to the relaxation of smooth muscle in the bladder neck and prostate, resulting in the improvement in urine flow and a reduction in symptoms in benign prostate hyperplasia. Alfuzosin also inhibits the vasoconstrictor effect of circulating and locally released catecholamines (epinephrine and norepinephrine), resulting in peripheral vasodilation.

Alfuzosin HCl has a short half -life and is rapidly eliminated. Its oral bioavailability is 49% and is intensively absorbed in upper GIT. The therapy of immediate release alfuzosin tablet requires daily dose of 3-4 tablets containing 2.5mg of Alfuzosin HCl so it becomes an ideal candidate for development of gastro retentive delivery systems.[1,2]

In present investigation buoyant in-situ gel has been designed taking Alfuzosin HCl as a model drug as the drug is poorly absorbed from the lower gastrointestinal tract. So the attempt has been made to retain the drug in the upper GIT, mainly in the stomach by applying the concepts of floating. The developed in-situ polymeric systems retains the drug reservoir at its absorption site by forming a buoyant stable raft structure and release the drug in a controlled manner for prolong period of time. In addition floating provides increased absorption of the Alfuzosin at a rate such that effective plasma levels can be achieved and maintained for a prolonged duration in case of

dosage adjustment. The system also offers advantage of reduced frequency of administration and provides ease of swallowing as the drug is particularly meant for treatment of BPH in geriatric patients.

Recently, controlled and sustained drug delivery has become the standard in modern Pharmaceutical design and an intensive research have been undertaken in achieving much better drug product effectiveness, reliability and safety. This interest has been sparked by the advantages shown by in situ forming polymeric delivery systems such as ease of administration and reduced frequency of administration, improved patient compliance and comfort. The formation of gels depends on factors like temperature modulation, pH change, presence of ions and ultra violet irradiation, from which the drug gets released in a sustained and controlled manner.[3]

MATERIALS AND METHODS

Materials

Active pharmaceutical ingredient Alfuzosin HCl was gifted from Unichem laboratories Pilerne Goa. Sodium alginate was purchased from Snap Naturals &Alginate Products Pvt. Ltd, Gellan gum (Kelcogel-CG LA) was purchased from CP Kelco Huber Company Mumbai, HPMC K 100M was gifted by Colorcon laboratory Pvt ltd, Goa. Calcium Carbonate was purchased from Wallace Pharmaceuticals. All other ingredients were used of analytical grade.

Standardization of Alfuzosin HCl

Determination of (λ max) of Alfuzosin HCl

The UV spectrum of Alfuzosin HCl in 0.1N HCl (pH1.2) was recorded by UV spectrophotometer (Lab India analytical/UV3000+) in the UV range of 200-400nm.

Standard Calibration Curve of Alfuzosin HCl

The Standard Calibration Curve of Alfuzosin HCl was carried out in 0.1N HCl (pH1.2)

Preparation of Standard Calibration Curve of Alfuzosin HCl

The Standard Calibration Curve of Alfuzosin HCl was carried out in 0.1N HCl (pH1.2).Stock solution of 100µg/ml was prepared by dissolving accurately weighed quantity of 10mg Alfuzosin HCl in 100ml 0.1NHCl (pH1.2). Aliquots of 0.2-1.6 ml were pipetted out separately into 10ml volumetric flask and made to volume to get concentration in the range 2-16 µg/ml respectively. The absorbance was measured at 244 nm on a UV spectrophotometer (Lab India analytical/UV3000+) using 0.1N HCl as blank.

Drug Excipients Compatibility studies

Drug Excipients Compatibility studies was conducted out in:

1. Solid state IR and DSC
2. Liquid state by TLC

Solid state compatibility study by IR:

FTIR spectra of the pure drug and its physical mixture with the excipients were obtained using FTIR (Shimadzu IR Prestige-21).

Solid state compatibility study by DSC:

The calorimetric characterization of the drug and physical mixture was carried out using a DSC 823einstrument (Mettler Toledo). DSC thermograms were recorded at heating rate of 10^0c/min in the range of 25^0C – 250^0 C.

Liquid state compatibility study by TLC:

All the formulations were chosen for the study keeping in view that this study is decisive factor for the entire project undertaken. Precoated silica plate GF254 were used as stationary phase and the mobile phase used was Methanol: ammonia in ratio of 100:1.2.[12]

Methods of Preparation of Buoyant In-situ gel [7]

The detailed procedure for preparing the in-situ gelling systems of Alfuzosin HCl is as outlined below.

Gellan gum sols **(Formulations G)** of concentrations 0.8%, 1.0%, 1.2% were prepared by adding the gum to distilled water containing 0.5 % w/v sodium citrate and heating it to 90^oCwhile stirring. HPMC K100M was dissolved in around 35% distilled water, then calcium carbonate was added to it while stirring so that there was proper and homogenous dispersion, this solution was then added to above gellan gum solution after cooling it to approximately 40^0C , followed by addition of the drug which was previously dissolved in water. The required quantity of preservatives were then added and mixed well. Finally colour and flavor was incorporated in the formulation and the final volume was made up to 100ml with distilled water.

Sodium alginate sols **(Formulations S)** were also prepared in a similar manner. Sodium alginate solutions of concentrations 1.0%, 1.2%, 1.4%w/v were prepared by adding the alginate to distilled water containing 0.5%w/v sodium citrate and heating it to 60^oC. HPMC K100M was dissolved in around 35% distilled water ,then calcium carbonate was added to it while stirring so that there was proper and homogenous dispersion, this solution was then added to above alginate solution after cooling it to approximately40^0c, followed by addition of the drug which was previously dissolved in water. The required quantity of preservatives was then added and mixed

well. Finally colour and flavor was incorporated in the formulation and the final volume was made up to 100ml with distilled water.

The Sols with combination of gellan gum and sodium alginate **(Formulations SG)**were also prepared in a similar manner, where solution s of the gel forming polymers in total concentration of 1.2% were prepared by adding both this polymers to distilled water containing 0.5%w/v sodium citrate and heating it to 60-90^{o}C. HPMC K100M was dissolved in around 35% distilled water, then calcium carbonate was added to it while stirring so that there was proper and homogenous dispersion, this solution was added to above a solution of gel forming polymers after cooling it to approximately 40^{0}c , followed by addition of the drug which was previously dissolved in water. The required quantity of preservatives were then added and mixed well. Finally colour and flavor was incorporated in the formulation and the final volume was made up to 100ml with distilled water.

All the developed formulation were filled in 100ml capacity amber glass bottles and closed tightly with closure.

Table 2 Composition of Formulations (G)

Ingredients	**Formulation Code**							
(mg)	**G1**	**G2**	**G3**	**G3A**	**G4**	**G5**	**G5A**	**G6**
AlfuzosinHCl	100	100	100	100	100	100	100	100
Gellan Gum	800	800	1000	1000	1000	1200	1200	1200
HPMCK100M	400	600	400	400	600	400	400	600
Calcium Carbonate	1000	1000	1000	1200	1000	1000	1200	1000
Sodium citrate	500	500	500	500	500	500	500	500
Sodium Methylparaben	50	50	50	50	50	50	50	50
Sodium Propylparaben	5	5	5	5	5	5	5	5
Colour-Ponceau 4R CS	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Ruspberry flavour	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Table 3 Composition of Formulations (S)

Ingredients	**Formulation Code**							
(mg)	**S1**	**S2**	**S3**	**S4**	**S4A(OP)**	**S5**	**S6**	**S6A**
AlfuzosinHCl	100	100	100	100	100	100	100	100
Sodium alginate	1000	1000	1200	1200	1200	1400	1400	1400
HPMCK100M	600	800	600	800	800	600	800	800
Calcium carbonate	1000	1000	1000	1000	1200	1000	1000	1200
Sodium citrate	500	500	500	500	500	500	500	500
Sodium Methylparaben	50	50	50	50	50	50	50	50
Sodium Propylparaben	5	5	5	5	5	5	5	5
Colour- Sunset yellow	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Orange Flavour	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Table 4 Composition of Formulations (SG)

Ingredients (mg)	Formulation Code		
	SG1	SG2	SG3
AlfuzosinHCl	100	100	100
Sodium alginate	600	700	800
Gellan Gum	600	500	400
HPMCK100M	800	800	800
Calcium carbonate	1200	1200	1200
Sodium citrate	500	500	500
Sodium Methylparaben	50	50	50
Sodium Propylparaben	5	5	5
Colour– Quinoline yellow	q.s.	q.s.	q.s.
Lemon flavour	q.s.	q.s.	q.s.
Distilled water	q.s.	q.s.	q.s.

Evaluation

The devised oral sol gel formulation of Alfuzosin HCl based on Sodium alginate, Gellan gum with HPMCK100M was evaluated for:

- Physicochemical Evaluation
- *In-vitro* release studies
- Stability Studies

Physicochemical evaluation

- **General appearance:**

  Observe the solution for colour and pourability.

- **Viscosity of the sols:**

  Viscosity of the prepared sols is of significance as it determines the palatability of the preparation and patient acceptance. The viscositiy of the prepared solutions were determined by Brookfield viscometer. The samples were sheared at 30 &60 rpm using LV2 spindle at room temperature. Viscosity measurement for each sample was done by multiplying the dial reading obtained, with the factor corresponding to the respective rpm value given on the chart.

- **In-vitro gelling capacity:**

  The *in-vitro* gelling capacity of prepared formulations was measured by placing 5ml of 0.1N HCl, (pH 1.2) in a 15 ml borosilicate glass test tube and maintained at 37±1ºC temperature. 1 ml of 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 the 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 the formed gel remains.

(+) Gels after few minutes, dispersed rapidly.

(++) Gelation immediate remains for few hours.

(+++) Gelation immediate remains for more than 12hours.[4]

- **Drug Content:**

  10 ml of liquid solution (Containing 10mg of the drug) was added to the 70 ml of 0.1 N HCl, then sample was put on in sonicator for 30 min until clear solution is made, Further make up the volume up to 100ml. Complete dispersion of contents were ensured, visually and filtered using Whattman Filter Paper. From this solution, 1 ml of sample was withdrawn and diluted to 10 ml with 0.1N HCl. Contents of Alfuzosin HCl was determined spectrophotmeterically at the obtained λ max value i.e. 244 nm using UV-Visible spectrophotometer against suitable blank solution.

- **pH Measurement:**

  pH of prepared liquid formulation was measured using digital pH meter (Toshniwal /CL54) at 27^0c. stabilization of the pH meter was done using double distilled water, after stabilization the pH meter was calibrated using 0.1 N HCl and 6.8 pH Phosphate buffer.[8]

- **Density measurement of gel:**

  The prime requirement of the raft formed is that it must have density lesser than gastric contents (~1.004 gcm^{-3}). The density was measured by forming gel of known volume (10ml) in a petri dish containing 0.1N HCl. The weight of this gel was noted and accordingly density was calculated. Density measurement for each sample was done in triplicate.[5]

- **Gel strength Determination:**

  The method as explained by Dettmar et al was modified to measure the gel strength of the gelled mass. The gel strength apparatus was fabricated in house using a measuring cylinder of 2.5 cm radius and a bore of 0.1mm at its base. A needle 2cm in length was used to which a nylon threads was tied as shown in the figure below. Sol (10 ml) was taken in the cylinder with temporarily sealed bore followed by addition of 50ml 0.1 N

HCl for gelation. After gelation the HCl was drained off by opening bore seal leaving the gel mass formed with the needle was rested on to surface of the gel. At the free end of the thread a light weight pan was attached to which the weights were added. The gel strength was reported in N/m^2. Measurement for each sample was done in triplicate. [5]

- **In-vitro buoyancy Study:**

  The *in-vitro* floating study was carried out using 500ml of 0.1N HCl (pH 1.2) in the dissolution vessel. 10ml of the formulation was introduced in a petri dish containing around 10ml of the medium and the formed gel was introduced into the dissolution vessel containing the medium without much disturbance. The time the formulation took to emerge on the medium surface (Buoyancy lag time) and the time the formulation constantly floated on surface of the dissolution medium (duration of floating) were noted.[6]

- **Swelling Index (SI):**

  The % swelling index of the gel of the formulations were determined by a simple method. In this study the in situ gel formed in 40 ml of 0.1 N HCl (pH 1.2) was used. From each formulation the gel portion from the 0.1 N HCl was separated and the excess HCl solution was blotted out with a tissue paper. The initial weight (w_0) of the gel was recorded, to this gel 50 ml of distilled water was added and after specified period of time the water was decanted and the final weight (w_t) of the gel was recorded.[8]

  The %SI for formulations (G) was determined for 8hrs, %SI of G3A &G5A was determined for 9hrs and 10hrs respectively. Similarly, for formulations (S)- %SI was calculated after 12hrs except for S1,S2 and formulation (SG) swelling studies were carried out for 10hrs.All the experiments were carried out in duplicate and %SI for formulations was calculated using the formula,%

  $SI= w_t - w_0 / w_t$

  Where, w_0 =Initial weight of the gel.

  w_t =final weight of the gel.

- **Scanning Electron Microscopy (SEM):**

  Surface morphology of the optimized formulation (S4A) was examined both in sol and gel form using (JEOL/JSM5800LV) SEM. The samples were dried by Lyophilization for around 16 hours using the lyophilizer (Thermoscientific-SAVANT). The dried specimens were mounted on the stub using double sided adhesive tape and coated under vacuum

with gold in an argon atmosphere prior to observation. Finally the image was recorded using Scanning Electron Microscope operated at an accelerated voltage of 20KV. [9,10]

***In-vitro* release study**

The *in vitro* release of Alfuzosin HCl from buoyant in-situ gel was determined using USP type II dissolution test apparatus(Lab India/DS800).10ml of the formulation was placed in a petri dish with an internal diameter of 4.5 cm, already containing 10ml of 0.1N HCl. This was placed in a dissolution vessel containing 490 ml of 0.1 N HCl, (pH 1.2).The dissolution test apparatus was run at 50 rpm for maximum upto12 hours at a temperature 37± 0.5^0C.The speed was slow enough to avoid the breaking of gelled formulation and was maintaining the mild agitation conditions believed to exist in vivo. 10ml samples were withdrawn after every 1 hour by the auto sampler and automatically replenished with 10ml of fresh medium. Alfuzosin HCl concentration in the aliquots was determined Spectrophotometrically at a wavelength of 244nm after suitable dilution.3aliquots were analyzed at each time interval.[4]

Stability studies[11]

The optimized formulation (S4A) which showed promising results in all evaluation parameters was subjected to stability studies. The optimized formulation was stored in a well stoppered amber coloured bottle in the stability chamber (Osworld scientific equipment Pvt. Ltd /OPS - 1081) for 1month at room temperature and accelerated condition. Samples were removed and evaluated for pH, Viscosity, *In-vitro* buoyant properties, *In-vitro* gelation and percent drug content.

Confirmation of the Stability of the API-Alfuzosin HCl in the Optimized Liquid formulation using chromatographic Techniques (TLC/HPLC),after storing it at normal room temperature and accelerated conditions for the period of 1month.

TLC Studies

TLC studies were carried out to indicate the stability of the drug-Alfuzosin HCl in the optimized formulation after exposing it to normal storage conditions and accelerated conditions for the period of 1month.

Procedure: Precoated silica plate GF254 is activated at 120^0c for 30minutes.The developing chamber was lined with filter paper and saturated with the mobile phase (Methanol:ammonia) in ratio of 100:1.2.[12]

Standard(Pure drug) solution and the test solution containing drug from the optimized formulation stored at room temperature(RT) and accelerated conditions(ACC) was prepared in concentration of 10µg/ml using 0.1N HCl and this solutions were spotted on the previously

activated plate, the plate was then developed with the developing solvent system. Further plates were dried in air and the spots were visualized under UV chamber at 254nm.The Rf value was calculated using the following formula.

HPLC Studies

Chromatographic Conditions:

- Column: Phenomenex C_{18} Column (150 x 4.6 x 5µ)
- Flow rate :1.0 ml/min
- Injection volume: 20 µL
- Column temperature: 25^0C
- Run time: 4 minutes
- Detection: 245nm

Composition of the Mobile phase:

Acetonitrile:0.02M potassium di-hydrogen orthophosphate (pH-3) in the ratio of (20:80).[12]

Preparation of Standard and Samples:

Standard solution of pure drug was prepared by dissolving 10mg of the drug in100ml 0.1N HCl, and further working standard was prepared by diluting aliquot of 1ml with 0.1NHCl to obtained final concentration of 10µg/ml. Similarly samples were prepared by taking 10ml of the formulation (equivalent to10mg drug) and diluting with 0.1N HCl to 100ml from this 1ml was further diluted with 0.1NHCL to obtained final concentration of 10µg/ml.

Procedure:

HPLC studies were performed on Waters® HPLC system equipped with Waters 2489 UV/Visible detector and Empower 2 software. First, the column was washed with the HPLC grade water and finally rinse with the prepared degassed mobile phase. The above mentioned conditions were set up and vials containing the standard and samples were loaded in sample tray. The desired sequence was set up and accordingly, the standard was injected for 3 times using auto injector with20 µl loop while the samples were injected twice to obtained the mean retention time with total run time of 4 minutes.[12]

RESULTS AND DISCUSSION

Standardization of Alfuzosin HCl

Determination of λ max of Alfuzosin HCl: λ max of the drug Alfuzosin HCl was found to be 244nm as shown in the figure 1.So Spectrophotometric determination was carried out at this absorbance maxima.

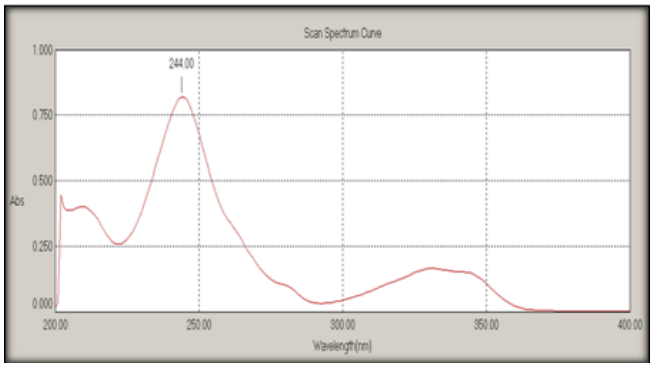


Figure 1: Spectrum curve of Alfuzosin HCl in 0.1N HCl (pH 1.2)

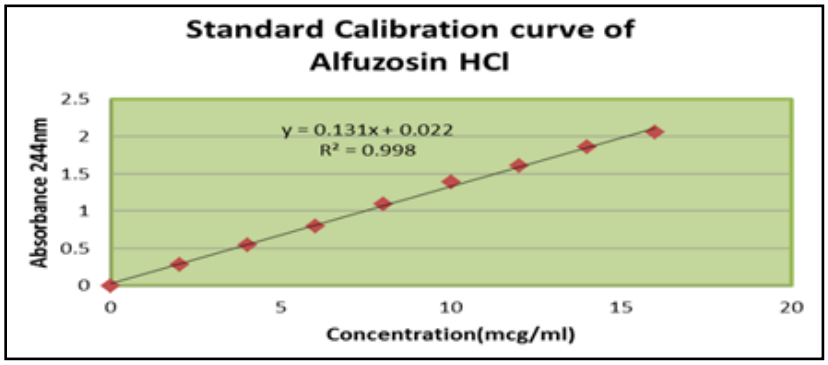


Figure 2: Standard calibration curve of Alfuzosin HCl in 0.1N HCl

Table 1 Calibration table for spectrophotometric determination of Alfuzosin HCl

Sr. no.	Concentration (µg/ml)	Absorbance values (244nm)
1	2	0.282
2	4	0.539
3	6	0.801
4	8	1.091
5	10	1.385
6	12	1.612
7	14	1.866
8	16	2.058

Drug Excipients Compatibility studies

Drug Excipients compatibility studies were carried out on all formulations in solid state using IR and DSC and in liquid state using TLC.

(A) Solid state compatibility

FTIR spectra of pure Alfuzosin HCl drug is shown in figure 3 shows the major characteristic peaks viz.1649.14 cm^{-1},1637.78cm^{-1},1602.85 cm^{-1} which corresponds to C=O stretch. Peak corresponding to secondary N-H appears at 3371.57 cm^{-1}.Aromatic C-H stretching vibration or aliphatic (saturated) C-H stretching band was seen at around 2933.73cm^{-1}.Also prominent bands appears in the fingerprint region from1000-500cm^{-1} such as 873.75$cm^{-1,}$ 993.34cm^{-1}.

The peaks in the physical mixture of drug with the polymers used correlate with drug spectra ranging from 500-4000cm^{-1}. There was neither alteration in characteristic peak nor appearance of any extra peak, which indicates that there is no interaction between drug and the polymers, which confirms the stability of drug.

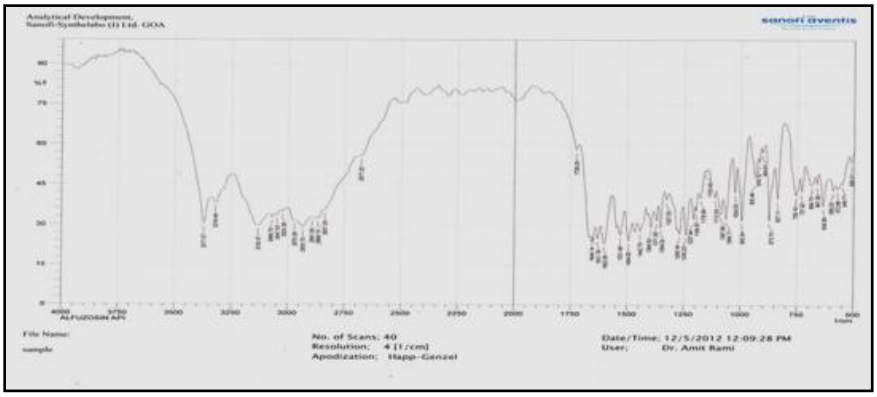


Figure 3: FTIR Spectra of Alfuzosin HCl API

The DSC thermogram of pure Alfuzosin HCl Figure 4a drug shows a sharp endothermic peak at 240^{o}C corresponding to its melting point. DSC thermogram (Figure :4b-4e) of the physical mixture of drug and polymers did not show any significant difference from that obtained for pure Alfuzosin HCl. The obtained result indicates that there was no positive evidence for the interaction between the drug and the selected polymers.

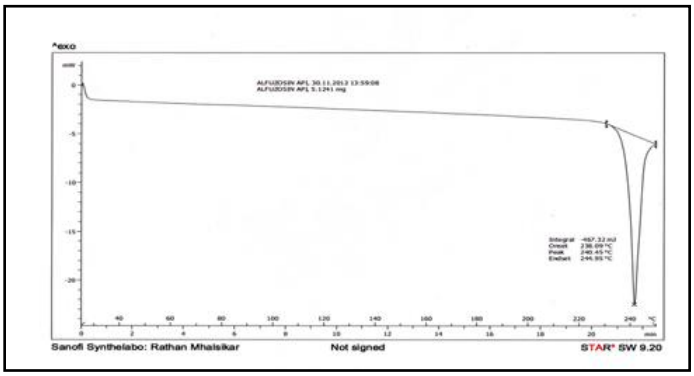


Figure 4a: DSC of Alfuzosin HCl –API

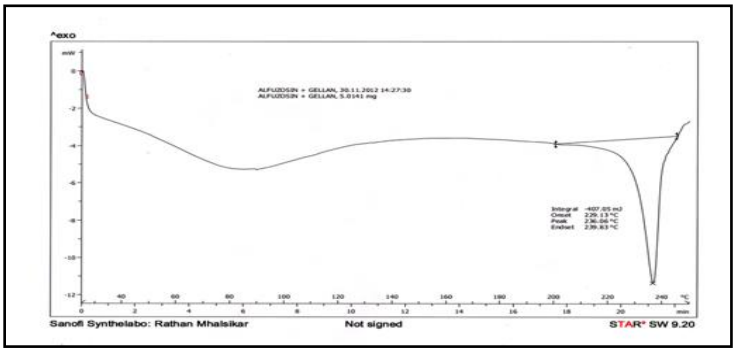


Figure 4b:DSC of Alfuzosin-API + gellan gum

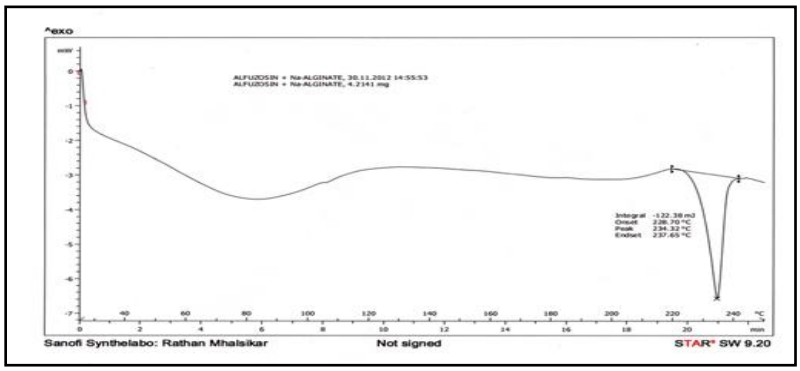


Figure 4c: DSC of Alfuzosin-API +Sodium alginate

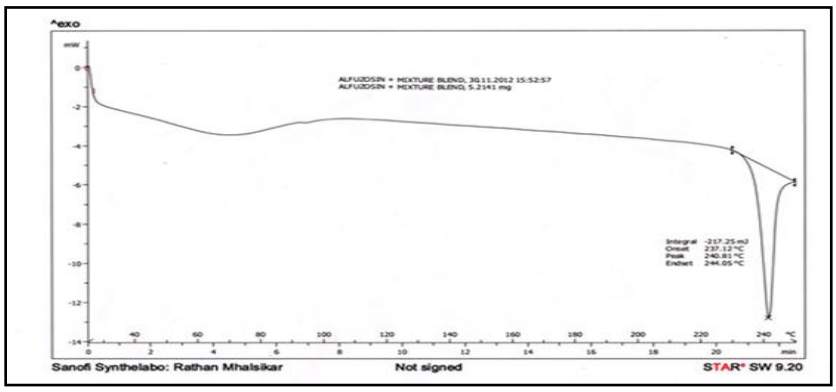


Figure 4d: DSC of Alfuzosin-API +Sodium alginate +gellan gum

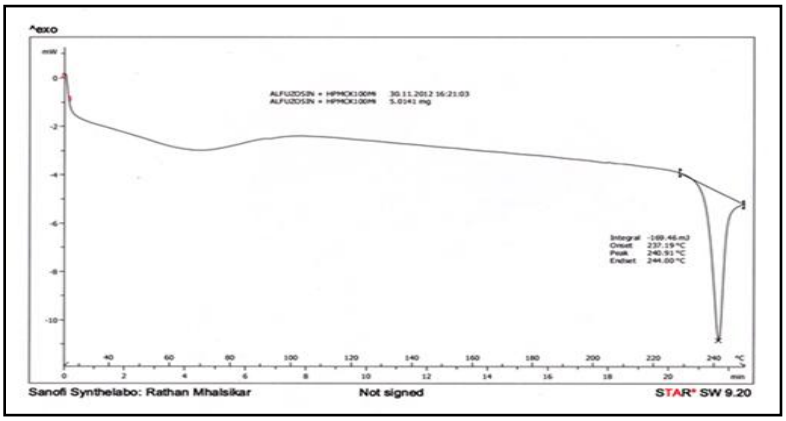


Figure 4e: DSC of Alfuzosin-API +HPMCK100M

(B)Liquid state compatibility

Compatibility studies in solution state was carried out on all formulations along with the pure drug using TLC and Rf values were calculated. The Rf values of the pure drug (standard) and the samples coincides.ie. found to be 0.627 Besides, the developed plate, did not show any other additional spots. This indicates that the chemical form of the drug is not altered in the liquid state.

PHYSICOCHEMICAL EVALUATION

General appearance:

All the formulations were of good pourability wherein-sols prepared with gellan gum were pinkish, those containing sodium alginate were of buff colour and sols prepared with combination of both gelling agent were yellowish in colour.

Viscosity:

Sols prepared with gellan gum were found to be more viscous than those containing sodium alginate. The viscosity of the formulations was found in the range of 510-670 cps (30rpm) and 270-370 cps (60rpm) at a temperature of 25 ± 1^0c.

The rheological properties of the solutions are of importance in view of their proposed oral administration. The two main pre-requisites of in situ gelling systems are optimum viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity that will allow easy swallowing as a liquid, which then undergoes a rapid sol–gel transition due to ionic interaction.

All formulations shows decrease in viscosity with increase in RPM. This decline in viscosity is quite prominent and this may be due to the extension of the polymeric chains on increase in the shear. This signifies the shear thinning behavior of the formulation. Refer figure 5-7

From the observations it can be concluded that the observed increase in viscosity with increase in concentration of gelling agent can be attributed to a consequence of increasing chain interaction with polymer concentration.

Increasing the calcium carbonate content in the formulation simultaneously increased the viscosity at all polymer concentrations studied. Since the calcium carbonate is present in the formulations as insoluble dispersion, an increase in its concentration proportionally increased the number of particles dispersed, thus contributing to the increased viscosity.

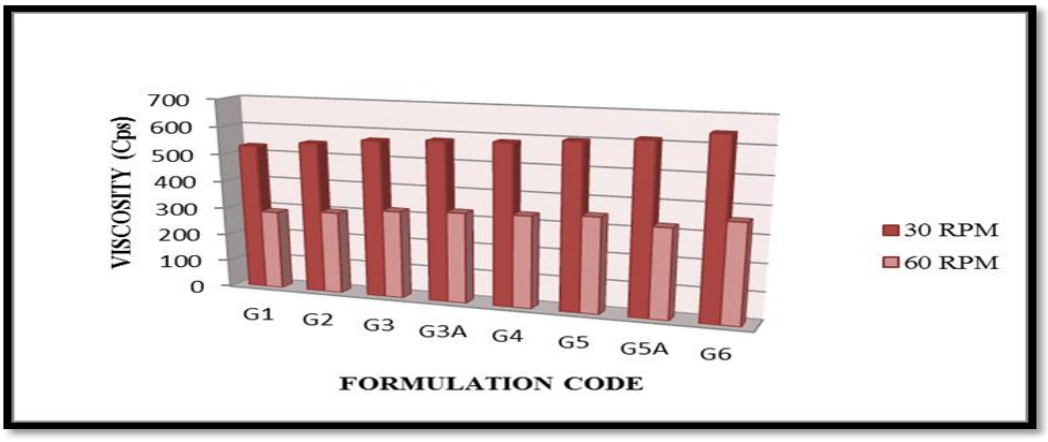


Figure 5: Comparison of viscosities of formulations(G) at 30 and 60 RPM

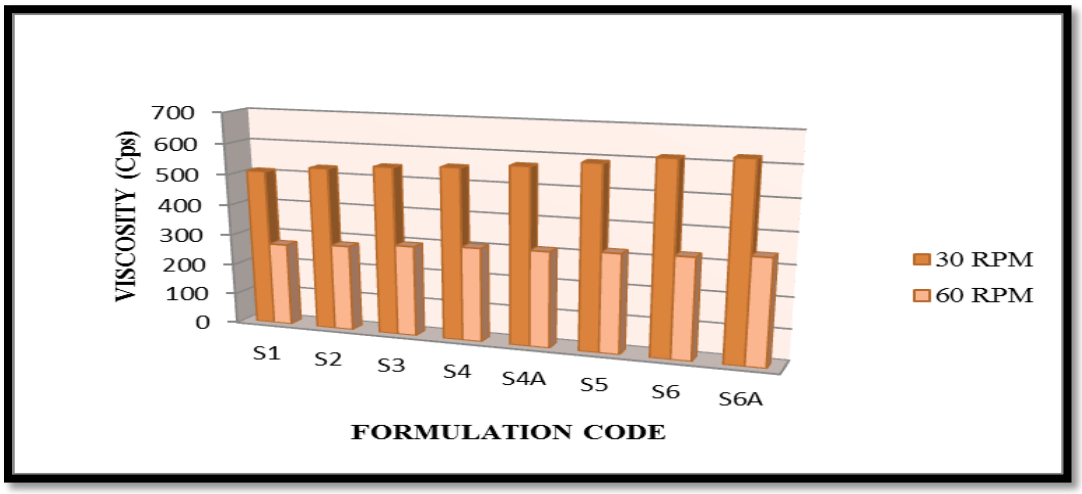


Figure 6: Comparison of viscosities of formulations(S) at 30 and 60 RPM

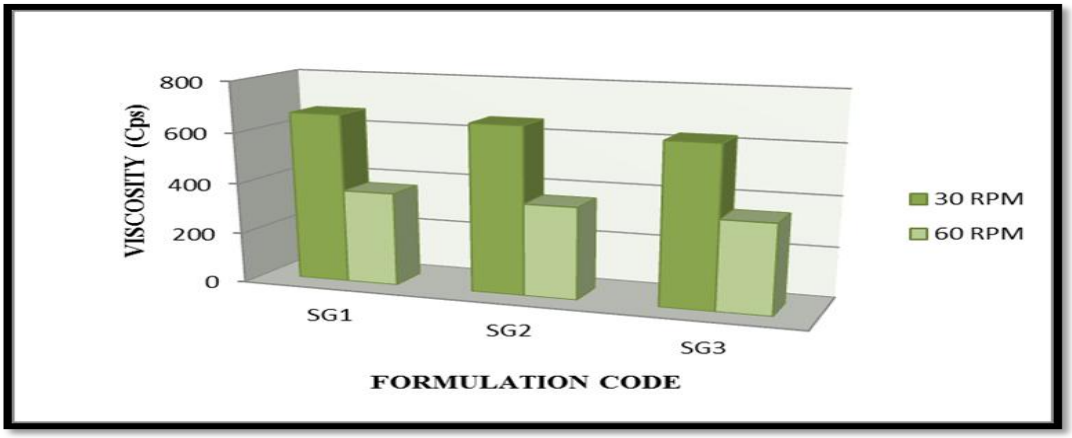


Figure 7 : Comparison of viscosities of formulations(SG) at 30 and 60 RPM

In-vitro gelation capacity:

Gel formation was observed from all of the liquid formulation. The gel formation was immediate and the gel formed remains intact for greater than 12 hours. Refer Table 5

Dug content:

The drug content was calculated for all the formulations, Refer Table 5 For all the formulations, the drug content varies between 98.00 – 101.8%

pH measurement:

The pH of all the formulations was observed in the range of 8.21-8.82. Refer Table 5

Density measurement of gel:

The density value for all the batches was obtained in the range of 0.602-0.712 gcm^{-3}.The density of formulations was found to increase in the following order: SG>G>S. Refer Table 5.

Density is important parameter as far as the floating properties of the gastro retentive dosage form is concerned. Ideally the density of the dosage form, to float on the gastric content must

have density less than or equal to gastric contents (~1.004 gcm^{-3}). All the developed formulations were found to have density lesser than above specified value.

Gel Strength Determination:

Gel strength is indicative of the tensile strength of the gelled mass. It signifies the ability of the gelled mass to withstand the peristaltic movement in-vivo. Table 5 reveals the gel strength of the various polymer combinations.

The gel strength of all the formulation was observed in the range of 21.622-35.454N/m^2. In-situ gels of sodium alginate were found to be more strong and rigid as compared to those of gellan gum.

Gel Strength increased with increase in polymer concentration, Formulations containing low amount of gelling agent shows lower gel strength value. But with increase in $CaCO_3$ content there was slight increase in gel strength. Formulation (S6A) with sodium alginate and HPMCK100M in concentration of 1.4% and 0.8% respectively showed the maximum gel strength.

Table 5 Evaluation Table

Formulation	Percent Drug Content (%)	pH	Density (g/cc)	Gel Strength (N/m^2)	In-Vitro Gelation in 0.1N HCl
G1	99.50	8.63	0.626 ±0.003	21.622 ±0.15	+++
G2	100.0	8.61	0.644 ±0.002	23.270±0.15	+++
G3	99.90	8.63	0.658 ±0.003	26.765±0.15	+++
G3A	99.00	8.68	0.666 ±0.004	29.898±0.25	+++
G4	101.0	8.78	0.665 ±0.006	29.795±0.39	+++
G5	101.8	8.83	0.682 ±0.003	30.794±0.30	+++
G5A	100.0	8.86	0.683 ±0.003	31.631±0.21	+++
G6	101.0	8.82	0.685 ±0.003	31.293±0.21	+++
S1	99.80	8.42	0.626 ±0.019	31.959 ±0.1	+++
S2	100.5	8.40	0.633 ±0.004	32.791±0.15	+++
S3	98.00	8.37	0.636 ±0.005	32.458 ±0.2	+++
S4	100.0	8.27	0.651 ±0.002	33.457 ±0.26	+++
S4A	99.50	8.33	0.661 ±0.005	35.038 ±0.17	+++
S5	100.0	8.25	0.671 ±0.003	34.280 ±0.2	+++
S6	99.70	8.21	0.667 ±0.003	35.121 ±0.2	+++
S6A	101.0	8.28	0.671 ±0.004	35.454 ±0.26	+++
SG1	98.9	8.50	0.712 ±0.019	34.356±0.2	+++
SG2	100.0	8.48	0.692 ±0.007	34.622±0.12	+++
SG3	99.7	8.42	0.686 ±0.005	35.121 ±0.1	+++

In-vitro Buoyancy study:

All the formulations floats instantaneously having Buoyant lag time (BLT) of approximately < 1min and remain buoyant for more than 12 hrs. Refer Table No.6. The floating ability of the in-situ gels formed found to be dependent on the polymer concentration and mainly on the concentration of calcium carbonate (floating agent) present in the formulation. The buoyancy of the in-situ gels having same concentration of gelling agent increased with increase in the concentration of calcium carbonate as seen in optimized formulation (S4A) where floating lag time was reduced to 30sec as compared to formulation (S4) having Buoyant lag time (BLT) of 52sec.

An increase in amount of Ca^{+2} and CO_2 at higher calcium carbonate concentration is responsible for this reduction in Buoyant lag time (BLT) Further, increase in the polymer concentration (i.e. HPMC) showed slight increase in floating lag time, which may be due to slight increase in the density of the system.

Table 6 Results of *In-vitro* Buoyancy Studies

Formulation Code	Buoyant Lag Time (Sec)	Total Floating Duration (Hrs)
G1	52	>12
G2	55	>12
G3	54	>12
G3A	42	>12
G4	59	>12
G5	62	>12
G5A	50	>12
G6	65	>12
S1	45	>12
S2	47	>12
S3	50	>12
S4	52	>12
S4A	30	>12
S5	58	>12
S6	60	>12
S6A	42	>12
SG1	59	>12
SG2	57	>12
SG3	54	>12

Swelling Index:

For formulations (G); % SI at the end of 8hrs was observed in the range of 46.69±1.63 – 69.95±0.47%.

For formulations (S); % SI at the end of 12hrs was observed in the range of 48.07±0.57–61.81±0.80%.

For formulations (SG); % SI at the end of 10hrs was observed in the range of 57.00±1.93–64.59±0.06%.

The results show that water uptake capacity of in-situ gel increases with increase in the polymer concentration and thus % SI increases and found to be proportional to the viscosity of the formulation. Refer Figure 8-10.

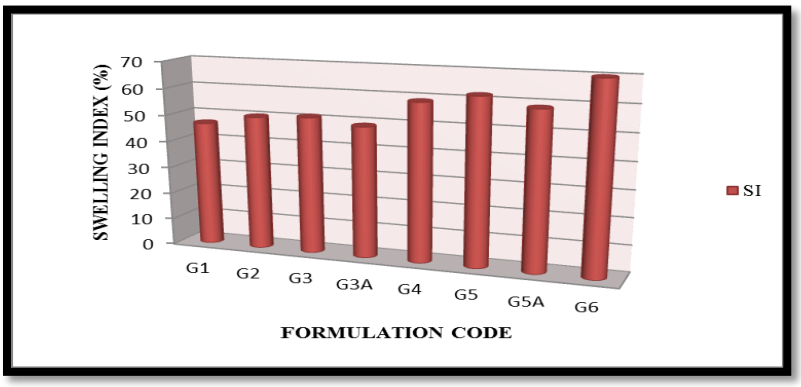


Figure 8: Comparison of Swelling Index of Formulations (G)

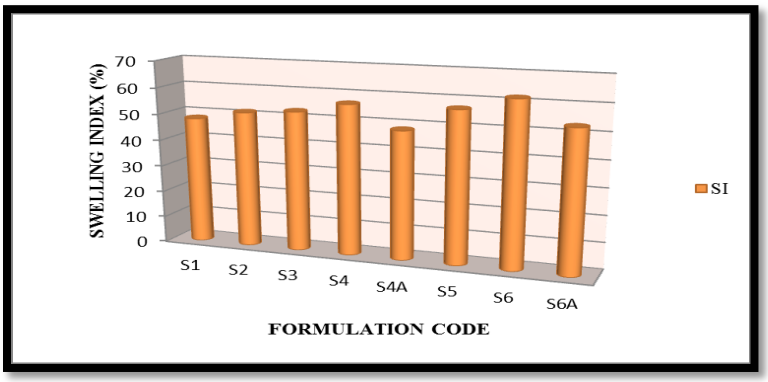


Figure 9 : Comparison of Swelling Index of Formulations (S)

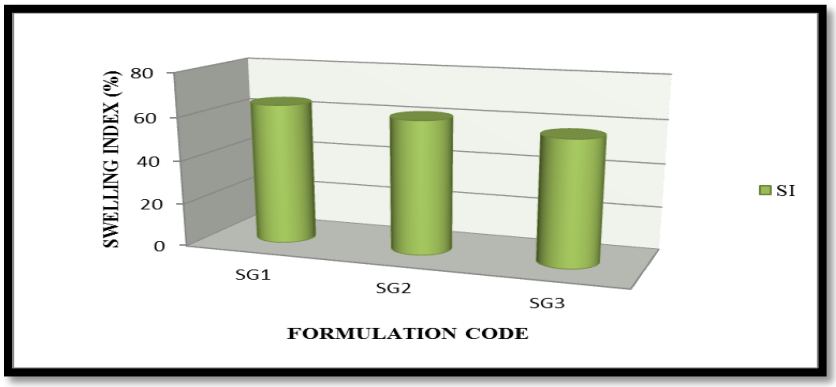
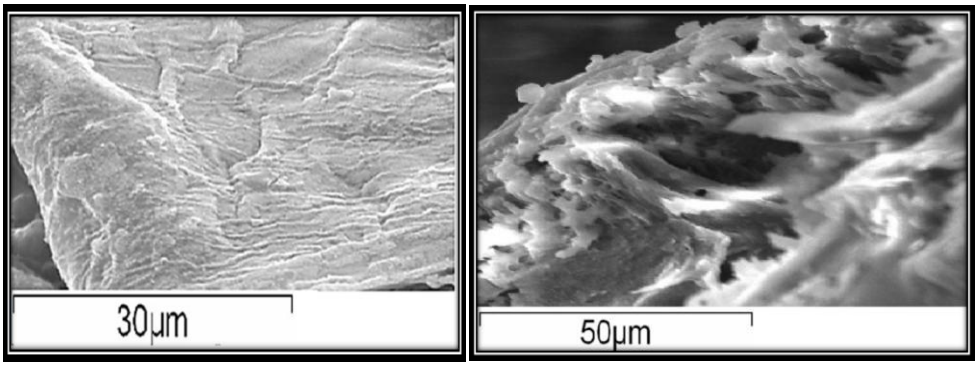


Figure 10: Comparison of Swelling Index of Formulations (SG)

Scanning electron microscopy:

The SEM photograph of optimized batch (S4A) in sol form and as in-situ gel (taken after dissolution studies) are shown in Figure below. The surface of the sol is highly compact whereas distinguishable pores were seen on the surface of in situ gel that further confirms the drug release from the in situ gel was diffusion controlled.



A) **B)**

Figure 11: SEM of Optimized Formulation A) as Sol form B) as In-situ gel

***In-vitro* release study**

In vitro release data shows that Formulations(S), devised using 1.2% and 1.4% of Sodium alginate were highly effective in retarding the drug release upto 12 hours as compared to Formulations(G) and Formulations(SG) devised using varying concentration of Gellan gum and combination of gellan gum + Sodium alginate respectively. In general drug release was decreased with increase in concentration of in-situ gel forming polymers and also with increase in concentration of release retarding polymer-HPMCK100M.

Formulations(G3A,G5A,S4A,S6A) with higher concentration of calcium carbonate i.e.1.2% were found to be more effective in extending the drug release as compare to their counter parts, i.e. Formulations(G3,G5,S4,S6)with 1% concentration of calcium carbonate.

However Formulation S4A(1.2% sodium alginate, 0.8% HPMCK100M and 1.2% calcium carbonate) was considered to be optimized formulation based on the release profile on comparative basis as it could prolong the release for 12 hours showing minimum burst release of 31.45% at first hour and percent cumulative release of 96.66% at the end of 12 hours. Hence it can be concluded that a significant decrease in the rate and extent of drug release is observed with the increase in polymer concentration in the formed buoyant in-situ gels and is attributed to increase in the density of the polymer matrix and also an increase in the diffusional path length which the drug molecules have to traverse.

The drug release from these gels was characterized by an initial phase of high release (burst effect). However, as gelation proceeds, the remaining drug was released at a slower rate.

Also with increase in calcium carbonate concentration in formulations decreased percentage of drug release is observed. This is because the increase in calcium carbonate concentration increases the number of Ca^{2+} ions and their extent of cross linking with the polymeric chains thereby contributing to increase in the density of the polymer matrix and consequent increase in the diffusional path length. In-vitro release data indicating % cumulative drug release has been tabulated in Table 7-9

Table 7 Percent cumulative drug release of formulation G1-G6

Time (hrs)	%CDR							
	G1	G2	G3	G3A	G4	G5	G5A	G6
1	41.42	39.31	35.6	34.92	32.42	30.03	29.00	28.00
2	55.23	53.54	49.85	46.95	46.70	45.47	42.51	44.85
3	66.66	61.83	56.27	56.28	58.27	57.28	54.04	52.11
4	81.74	73.53	69.91	64.54	65.60	63.47	63.58	62.20
5	91.02	82.97	77.74	75.68	74.25	73.97	73.09	73.76
6	93.90	87.40	85.65	83.54	83.32	82.18	80.95	80.22
7	97.97	92.74	91.38	91.98	90.65	90.58	87.25	89.29
8	99.96	98.81	98.18	96.42	98.59	96.95	91.60	96.21
9				98.88			95.95	
10							99.61	

Table 8 Percent cumulative drug release of formulation S1-S6A

Time (hrs)	% CDR							
	S1	S2	S3	S4	S4A	S5	S6	S6A
1	44.36	42.91	35.83	33.39	31.45	35.22	32.04	33.25
2	55.83	54.30	45.25	44.38	42.96	43.74	42.60	41.90
3	63.10	62.13	53.35	51.79	49.80	52.97	50.11	49.88
4	69.79	69.08	60.60	57.86	55.65	59.80	55.89	54.60
5	78.78	76.86	68.04	65.38	63.60	66.03	64.27	62.60
6	82.26	79.41	72.44	69.88	66.24	72.21	69.13	65.98
7	86.54	85.03	78.71	75.15	71.25	79.92	77.17	70.13
8	90.60	89.24	82.37	79.77	77.52	84.69	81.73	77.20
9	94.34	94.22	88.09	84.61	81.20	87.51	86.44	80.15
10	98.40	98.02	92.65	90.57	87.39	92.51	89.92	86.35
11			95.80	93.35	91.64	96.03	94.97	91.52
12			98.76	97.36	96.66	98.96	98.88	94.32

Table 9 Percent cumulative drug release of formulation SG1-SG3

Time (hrs)	%CDR		
	SG1	SG2	SG3
1	34.34	33.58	35.87
2	48.39	43.16	42.30
3	55.34	51.37	50.18
4	67.49	63.54	62.40
5	73.71	70.19	68.79
6	81.00	78.16	75.14

7	89.92	85.76	84.68
8	93.77	91.71	89.96
9	97.09	96.14	94.29
10	99.68	98.47	97.04

Statistical Analysis

The results of the dissolution study of all the batches were fitted to different mathematical models and subjected to regression analysis. The r values of Zero order plots were significantly higher as compare to first order plots which indicates that all formulations best fitted in Zero order kinetics and poorly fitted First order. Therefore it can be concluded that all in-situ formulation of Alfuzosin HCl followed Zero order kinetics as the release pattern of the drug. Refer Table 10-12.

Table 11 Kinetic Model data for formulation S1-S6A

Formulation	**S1**	**S2**	**S3**	**S4**	**S4A**	**S5**	**S6**	**S6A**
Zero Order Plot								
K hr^{-1}	7.404	5.826	5.626	5.587	5.570	5.772	5.938	5.417
r	0.9817	0.9853	0.9854	0.9907	0.9450	0.9875	0.9912	0.9944
First Order Plot								
K hr^{-1}	0.3350	0.3231	0.3058	0.2554	0.2332	0.3175	0.3023	0.2072
r	0.9486	0.9481	0.9394	0.9534	0.9449	0.9357	0.9200	0.9666
Higuchi's Plot								
K hr^{-1}	24.944	25.247	26.268	25.847	26.950	27.630	27.630	25.084
r	0.9981	0.9989	0.9970	0.9982	0.9976	0.9985	0.9986	0.9971
Peppa's Plot								
n	0.3590	0.3437	0.4220	0.4334	0.4446	0.4275	0.4621	0.4358
r	0.9990	0.9987	0.9982	0.9987	0.9975	0.9956	0.9978	0.9972
Erosion Plot								
r	0.9893	0.9872	0.9870	0.9852	0.9820	0.9872	0.9805	0.9891

Table 12 Kinetic Model data for formulation SG1-SG3

Formulation	**SG1**	**SG2**	**SG3**
Zero Order Plot			
K hr^{-1}	7.192	7.246	7.462
r	0.9870	0.9884	0.9908
First Order Plot			
K hr^{-1}	0.4958	0.3924	0.3320
r	0.9678	0.9557	0.9678
Higuchi's Plot			
K hr^{-1}	31.471	32.158	30.831
r	0.9963	0.9967	0.9939
Peppa's Plot			
n	0.4766	0.4976	0.4715
r	0.9976	0.9955	0.9870
Erosion Plot			
r	0.9870	0.9910	0.9870

Table 10 Kinetic Model data for formulation G1-G6

Formulation Code	G1	G2	G3	G3A	G4	G5	G5A	G6
Zero Order Plot								
K hr^{-1}	8.505	8.317	8.829	8.244	9.127	9.276	7.735	9.471
r	0.9594	0.9846	0.9914	0.9889	0.9944	0.9926	0.9793	0.9936
First Order Plot								
K hr^{-1}	0.861	0.474	0.445	0.475	0.462	0.400	0.468	0.379
r	0.8864	0.9288	0.9334	0.9493	0.9039	0.9460	0.9080	0.9506
Higuchi's Plot								
K hr^{-1}	34.059	32.859	34.638	33.983	35.742	36.383	35.670	37.074
r	0.9853	0.9976	0.9974	0.9964	0.9986	0.9985	0.9975	0.9975
Peppa's Plot								
n	0.4489	0.4481	0.4923	0.4966	0.5005	0.5098	0.5073	0.5048
r	0.9919	0.9982	0.9969	0.9968	0.9992	0.9990	0.9983	0.9978
Erosion Plot								
r	0.9899	0.9838	0.9821	0.9886	0.9692	0.9839	0.9854	0.9846

Similarly, the data was treated according to Higuchi's diffusion equation ($Q=Kt^{½}$).The best fit with higher correlation was found with Higuchi 's diffusion equation with all the formulations with highest r value (0.9989) nearly approaching 1.0.This results shows that all formulation exhibit diffusion mechanism in drug release. Kinetic models which best fit zero order and Higuchi's diffusion equation are most suitable for controlled release formulation.

Further, data was subjected to Korsmeyer-Peppa's model where the r value reveals that peppa's model best fit all the formulations. The values of n as derived from peppa's model found to be less than 0.5 for all the Formulations(G),(S) and (SG),hence it can concluded that drug release occurred via fickian diffusion,

Further data was subjected to Erosion model where the r values were not uniform for all the formulations with highest value of r=0.9899, although r values are found to be less than that obtained with Higuchi's diffusion, to some extent there is possibility of the erosion of the polymers taking place. Thus in general it can be concluded that the drug release from almost all formulated matrix is by fickian diffusion and follows Zero order kinetics.

Stability studies

The Stability studies of optimized formulation (OP) were carried out for 1 month at room temperature and accelerated conditions. Stability study revealed that no major changes took place throughout the study for one month. Viscosity was slightly increased due to hydration of polymer. Refer Table 13.

Table 13 Stability study data

Formulation code	Viscosity (cps)	Percent Drug	pH	% Dug Release	Buoyant lag time	Total Floating	In-vitro gelation in

		content (%)		(12hrs)		duration	0.1N HCl
$OP_{(RT)}$	312	98.9	8.32	95.22	32	>12	+++
$OP_{(ACC)}$	311	99.8	8.33	96.17	30	>12	+++

Confirmation of the Stability of the API-Alfuzosin HCl in the Optimized Liquid Formulation (OP) using chromatographic Techniques (TLC/HPLC)

TLC /HPLC results of the optimized formulation (OP) obtained after exposing it to normal room temperature and accelerated storage condition are tabulated below in Table 14.In the developed TLC plates there was no significant difference seen in the R_f value of the pure drug and that of samples $OP_{(RT)}$ and $OP_{(ACC)}$ .The R_f values almost coincides, Besides, the developed plate did not show any other additional spots. This confirms that there was neither chemical degradation of the drug in the solution form nor formation of degradation product even after storing the formulation at accelerated conditions.

The HPLC Chromatograms depicts a sharp principle peak of the drug Alfuzosin HCl, having the mean retention time (tR) of 2.252. The mean retention time (tR) obtained with the standard drug is almost close to the mean retention time(tR) obtained with that of the optimized formulation (OP) after exposing it to normal room temperature (RT) and accelerated storage conditions (ACC) Refer Table 15.Besides there were no major deviations seen in the peak characteristics (area cm^2) nor additional peaks were seen in the chromatograms during the run time of 4 minutes ,confirming that no degradation products are formed thus indicating stability of the drug in the formulation upon its storage at room temperature and accelerated conditions for a period of 1month.

Table 14 TLC Results

Formulation Code	Mean Rf values
Std (Pure Drug)	0.627
$OP_{(RT)}$	0.625
$OP_{(ACC)}$	0.625

Table 15 HPLC Results-Summary of Peak Retention time

Formulation code	Retention Time(tR)	Mean Retention Time (tR)	Peak area (cm^2)	Mean Peak area (cm^2)
STD (Pure drug)		2.252		1565268
Injection 1	2.252		1557319	
Injection 2	2.236		1559945	
Injection 3	2.269		1578540	
OP $_{(RT)}$		2.237		1548280
Injection 1	2.236		1548641	
Injection 2	2.238		1547920	
OP $_{(ACC)}$		2.238		1555695

Injection 1	2.237	1556894
Injection 2	2.238	1554496

CONCLUSION

The liquid formulations were developed to a satisfactory level in terms of their buoyancy, general appearance, viscosity and in-vitro gelation. Formulations containing 1.2% of calcium carbonate were found to be more buoyant than compared to those with 1% of calcium carbonate. The optimized formulation exhibited excellent floating properties with buoyant lag time of only 30 seconds and was highly effective in sustaining the release for 12 hours. The drug release from in-situ gel follows Zero order kinetics and indicates fickian diffusion. The materials utilized in the study are readily available, safe and require simple technology, the study marks successful endeavor.

REFERENCES

1. http://www.drug.com/monograph/Alfuzosinhcl.htm
2. Xatral Product Monograph-Sanofi AventisCanada.2010; 7:84-85
3. Gopinath H, Sowjanya R, Chakaravarthi V, Asma S, Naga S, Khola R. Formulation and evaluation of ofloxacin floating tablets using hydroxypropyl methyl cellulose polymer. J Chem Pharm Sci 2012; 5(4): 144-149.
4. Panwar P, Chourasiya D, Jain G, Sheorey RV .Formulation and evaluation of oral floatable in-situ gel of diltiazem HCl. Int. J. Novel Drug Deliv Tech 2011; 2 (1): 264-270.
5. Gulecha BS, Shahi S, Lahoti SR. Floating In Situ Gelling Drug Delivery System of Verapamil Hydrochloride. Am J PharmTech Res 2012; 2(4):955-969.
6. Bhimani D, Patel R, Patel VP, Delorja CM. Development of a Novel Floating In-situ Gelling System Clarithromycin. Int J Pharm Res Develop 2011; 3 (5):32-40.
7. Debnath S, Babu N, G.Kusuma, NR Sarmika, Reddy A. Formulation and Evaluation of floatable in situ gel as carrier for stomach-specific drug delivery of Metoclopramide HCl. Int J Pharma Frontier Res 2011; 1(1):53-64.
8. Patel MJ, Patel K, Patel M, Patel N. Strategy for Development of pH Triggered Floating Insitu Gel of Levetiracetam. Am. J. Pharm Tech Res 2012; 2(3):828-841.

9. Patel RP, Ladani R, Dadhaniya B, Prajapati BG. In situ gel Delivery systems for Chlordiazapoxide using Artificial Neural Network. Int J Pharm Health Sci 2009; 1 (1): 10-22.
10. Nanjwade BK. A novel pH triggered in-situ gel for sustained ophthalmic delivery of Ketorolac Tromethamine. Asian J Pharma Sci 2009; 4(3) 189-199.
11. http://www.pacifibiolabs,com/testing-stability-testing.asp
12. Ahmed Salah Fayed, Mostafa Adabel-AatyShehata, NagibaYehia Hassan, Soheir Ahmeed EL-Weshahy. Validated HPLC and HPTLC stability indicating methods for determination of AlfuzosinHCl in bulk and pharmaceutical formulations. J Sep Sci 2006; 29(18):2716-2724.