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Formulation, In-Vitro and In-Vivo characterization of Vardenafil Loaded Floating In-Situ Gel: An investigational study For Enhancement of Oral Bioavailability.

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ABSTRACT

The principle purpose of this research work was to enhance oral bioavailability of vardenafil through formulation into gastroretentive in-situ gelling system using different concentrations of sodium alginate as gelling polymer, calcium carbonate as gas providing agent and source of calcium ion necessary for ionic gelation, calcium chloride as cross linking agent and sodium citrate as fluidity maintaining agent. The prepared batches were subjected to various evaluation parameters like viscosity, pH, floating lag time and floating period, gelling capacity and drug content. All prepared batches were clear solutions with pH ranges from 6.9 to 7.9. The viscosity before gelation ranges from 54 to 480 cp and after gelation from 435 to 5321 cp. All prepared batches float after few seconds and remain buoyant for more than 12 hours. They convert into gel 1-2 second or immediately after entering 0.1N Hcl pH1.2. The drug distributed homogeneously within prepared in-situ gel with drug content fall within the range of 94.6 to 100.1%. An increase in the concentration of sodium alginate, calcium carbonate and calcium chloride retard the drug release from the prepared in-situ gel. The formulation batch F4 which showed reasonable viscosity, prolonged floating period and sustained vardenafil release was selected for further in-vivo investigation. Formulation of vardenafil into in-situ gel increased t_{max} and decreased c_{max} thus enhancing its bioavailability.

Keywords: in-situ gel, floating system, vardenafil and sodium alginate.

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INTRODUCTION

The oral drug delivery route remains the most convenient way for drug administration due to increased patient compliance and ease of application[1]. The most serious problem associated with oral route is decreased gastric retention, enhanced emptying and extensive first pass metabolism which may lead to unpredictable and poor bioavailability. There are several approaches have been investigated to solve such problem. Gastro retentive in-situ gel drug delivery system is a promising trend that releases the drug in a sustained manner[2] via increasing gastric residence in the stomach[3].

In-situ gel is a liquid dosage form that undergoes gelation in response to several stimuli like temperature change, solvent exchange, pH change or cation crosslinking[4]. pH induced ionic gelation is the main principle in the formation of the in-situ gel. Sodium citrate interacts with calcium carbonate and gives calcium citrate which maintains the fluidity of the solution before or prior to administration, upon reaching the stomach calcium ion released inducing gelation of sodium alginate and evolved carbon dioxide entrapped within alginic acid helps the gel to float for extended period[5]. vardenafil is highly selective phospho diestrase type 5 inhibitor that increases blood flow to penis and helps to achieve and sustain erection in erectile dysfunction also it has a significant improving impact on pulmonary hypertension. Vardenafil suffers from poor bioavailability and short biological half life that may reach in certain cases to 3 hours.

The present investigational work aims to improve bioavailability of vardenafil through incorporation into floating sodium alginate in-situ gel.

MATERIALS AND METHOD

Vardenafil was kindly supplied by Next Pharma Egypt. Calcium carbonate, sodium alginate, sodium citrate, calcium chloride, Phosphoric acid, Potassium- dihydrogen phosphate and hydrochloric acid were purchased from El Nasr Company Egypt. All other chemicals and solvents are of analytical grade. HPLC grade acetonitrile was purchased from Sigma-Aldrich Chemie, Germany. Diethyl ether was purchased from Fine Chem. Ltd. (India).

Methods

Preparation of in-situ gel

In-situ gel was prepared by dissolving sodium alginate in distilled water at 60^{o}C with continuous stirring. Calcium carbonate, calcium chloride and vardenafil were added after cooling polymer solution to below 40^{o}C and finally sodium citrate was added to maintain the fluidity of prepared solution before administration [6]. Table1 shows the composition of different batches of prepared in-situ gel.

Table 1: composition of different formulations of vardenafil sodium alginate in-situ gel.

Formulation code	Vardenafil (% w/v)	Sodium alginate (% w/v)	Calcium carbonate (% w/v)	Calcium chloride (% w/v)	Sodium citrate (% w/v)
F1	20	0.5	1	0.15	0.5
F2	20	1	1	0.15	0.5
F3	20	1.5	1	0.15	0.5
F4	20	2	1	0.15	0.5
F5	20	2.5	1	0.15	0.5
F6	20	3	1	0.15	0.5
F7	20	2	0.5	0.15	0.5
F8	20	2	1.5	0.15	0.5
F9	20	2	2	0.15	0.5
F10	20	2	1	0.3	0.5
F11	20	2	1	0.6	0.5
F12	20	2	1	1.2	0.5

In-vitro characterization of prepared in-situ gel

Physical appearance

The clarity of the prepared formulations was visually inspected through observation in black back ground [7].

pH

pH of the prepared in-situ gel was determined using pH meter 300 (Jenway LTD, UK) previously calibrated using solution of pH 7.

Viscosity

Viscosity of the developed formulations was determined using visco Star-R viscometer at ambient room temperature with spindle number 6 at 10 rpm before and after gelation [8, 9].

In-vitro gelling capacity

The in-vitro gelling capacity was determined by transferring 1 ml of each formula to 5 ml 0.1 N Hcl PH 1.2 as gelling solution and evaluated visually according to gelation time and the time period for which the prepared gel remains intact [10].

In-vitro buoyancy study

Floating lag time and floating period were determined using type II USP dissolution apparatus. 10 ml of each formula was transferred to 900 ml 0.1N Hcl pH 1.2 maintained at $37^{o}C\pm1$ and 50 rpm [11].

Drug content

Specific volume of each formula equivalent to 10 mg vardenafil was accurately measured and transferred to 100 ml volumetric flask containing 0.1N Hcl pH 1.2. The volumetric flask was then

transferred to thermostatic shaker water bath (Julabo SW-20 C, Germany) maintained at 25°C±1 and 100 rpm till equilibrium. The solution then filtered, suitably diluted and vardenafil concentration was estimated at 272 nm using (UV-Spectrophotometer Shimadzu UV-1201, Japan) against 0.1N Hcl pH 1.2 as a blank[12].

In-vitro release study

The cumulative release concentration of vardenafil was estimated using USP type II dissolution apparatus paddle type. Specific volume of in situ gel equivalent to 10 mg of the drug was applied with syringe to 900 ml 0.1N Hcl pH 1.2 maintained at 37+1°C and stirred at 50 rpm. 5 ml sample was withdrawn hourly for 9 hours and replaced with equivalent volume of fresh sample to maintain sink condition, filtered, suitably diluted and estimated at 272 nm against 0.1N Hcl pH 1.2 as a blank [13].

Kinetic data analysis

The cumulative release data were plotted according to various kinetic models, zero order, first order, Higuchi diffusion, Hixson and Crowell and Korsmeyer-Peppas to determine the kinetic model of drug release[14].

Stability study

Stability study was conducted at 50°C±1, 75%±5 RH for optimized in-situ gel formula. The optimized formula was estimated for physical appearance, pH, drug content, viscosity before and after gelation, gelation capacity, floating lag time, floating period and cumulative release amount after 9 hours at 0, 2 months, 4 months and 6 months [15].

In-vivo studies

The pharmacokinetic parameters of vardenafil were determined in rabbit plasma by simple high performance liquid chromatography (HPLC) (Waters Instrument, Germany) system consisted of HPLC pump (pump A and pump B), mixing pump, multiport computer, HPLC self-injector, and oven controller. Temperature column oven 480, fixed with reverse phase C 18 column (Chromonith® Performance RP-18E, 100x4.6 mm. Merck, Germany). HPLC detector with range of 0.2, resp. time 0.05, remote, monitor and data system computer. With UV detection at 230 nm using fenoprofen as internal standard.

The plasma samples were analyzed using validated HPLC method described by Carlucci et al [16] with slight modification. The mobile phase consists of acetonitrile: potassium dihydrogen phosphate (30:70 v/v) adjusted to pH4.5 with ortho phosphoric acid. The eluent was passed through the column at a flow rate of 1 ml/min.

Stock solutions of vardenafil were prepared by dissolving 10 mg in 100 ml of the mobile phase. Standard solutions were obtained by diluting the stock solution with drug free rabbit plasma in the range of 10-1500 ng/ml. For each solution the internal standard was added at constant level of 100 µl of a 2.5 µg/ml solution. These standards were treated in the same way as the sample to be analyzed. White male rabbits (weighing 1.5-2 kg) were used for the bioavailability study. Animals were housed in the standardized conditions at the animal house of the Faculty of Pharmacy, Zagazig University, Egypt. All animals were acclimatized and kept under constant temperature (25 ± 2^{o}C). All animal procedures were performed in accordance to the approved protocol for use of experimental animals set by the standing committee on animal care of the Faculty of Pharmacy, Zagazig University, Egypt. Animals were divided into three groups, six rabbits each. The study was designed as a single oral dose. All groups received an equivalent of 5 mg/kg vardenafil. Group 1 received vardenafil solution. Group 2 received the optimized formula of the in-situ gel. Group 3 act as control. Blood samples were withdrawn from the sinus orbital into EDTA tubes at 0.5, 1, 2, 4, 6, 8, 24, 48 and 72 hours after each administration. The blood samples were centrifuged immediately at 3000 rpm for 10 minutes and the plasma samples were stored at -20 ^{o}C+0.5 for subsequent assay.

The plasma samples were thawed at room temperature. Vardenafil was extracted from rabbit plasma by liquid-liquid extraction. 1 ml of rabbit plasma was mixed with 100 µl of internal standard solution; vortexed for 15 S then 1ml of di ethyl ether was added. The mixture vortexed for 1 min. and then centrifuged at 3000 rpm for 10 min. the organic phase was transferred into clean tube and evaporated under vacuum to dryness. The residue was reconstituted with 200 µl of the mobile phase, filtered through Millipore filter (0.22 µm). A volume of 20 µl of the re constituted sample was injected into the column of HPLC apparatus.

Statistical analysis

All in-vitro experiments were conducted in triplicate. The resulting data were expressed as mean± standard deviation of the mean. One way analysis of variance (ANOVA) was employed for data analysis using SPSS program, version 17. The data were significant at a level $P \leq 0.05$.

RESULTS AND DISCUSSION

The preliminary study revealed that the optimum concentration of sodium citrate that kept the in situ gel liquefied before administration is 0.5%w/v for all batches, below this concentration the solution gelled before administration and above this concentration stiff gel was formed.

Figure 1: photo micrograph of in situ gel in 0.1N HCL pH 1.2

Appearance

All prepared batches were clear and transparent before gelation and turned into milky white immediately after entering 0.1N Hcl pH1.2 [10].

pH

pH of all prepared batches fall within the range of 6.9±0.3 to7.9±0.3 and this indicates that the prepared formulae are neutral to slightly alkaline so there is no possibility of throat irritation prior to administration[10] (table 2).

Drug content

The drug content of all prepared batches ranges from 94.6±2.8 to 100.1±3.1 (table2)this confirms the homogeneous distribution of the drug within the prepared batches.

Floating lag time

The floating lag time of all prepared batches are illustrated in table 2. It was found that the lag time of all prepared batches ranges from 10 to 45 seconds. The lowest lag time observed in F9 with highest concentration of calcium carbonate and this could be explained on the basis that more calcium carbonate will give more carbon dioxide that entrapped rapidly within the gel and the result is rapid floating[17]. Sodium alginate concentration has a significant impact on floating lag time and the results emphasized that. As the concentration increased from 0.5% to 3% there was a significant increase from 15 to 45 seconds for F1 and F6 respectively. This is in a good correlation with Bharati et al[12] who found that a significant increase in the floating lag time of ofloxacin in situ gel with an increase in calcium carbonate concentration, also there was a significant increase in the lag time with an increase in the concentration of gelling polymer. This might be due to higher concentration of the gelling polymer give higher dense gel mass thus evolved carbon dioxide take long time for penetrating the formed gel.

Floating time

The results in table2 represent that F1 and F7 float for less than 12 hours, F8 and F9 float for more than 24 hours and all other formulae float for more than 12 hours. F1 with the lowest concentration of sodium alginate dispersed rapidly and lost its integrity and this might be the reason for the decreased floating period.

The concentration of calcium carbonate plays a crucial role in keeping the gel float for longer period. F7 with lowest concentration of calcium carbonate (0.5%) float for less than 12 hours. Upon increasing the concentration to 1% as in F2, F3, F4, F5, F6, F10, F11 and F12 the floating period increased to be more than 12 hours further increase to 1.5% and 2%resulted in a corresponding increase in the floating period to be more than 24 hours. More calcium carbonate will give more carbon dioxide and this may account for the longer floating period [10].

Gelling capacity

It is obvious from results depicted in table2 that all prepared batches converted to gel immediately or few seconds upon entering 0.1N Hcl pH1.2. and remain intact for more than 24 hours[10] except for F1 which dispersed and diffused rapidly after few hours and this could ascribed to the low concentration of sodium alginate.

Table 2: various evaluated parameters of vardenafil loaded floating in situ gel.

Formulation code	Appearance	pH±S.D	Drug content (%))±S.D	Floating lagtime (sec)	Floating duration (hr)	Gelling capacity (sec)
F1	clear	7.2±0.1	94.6±2.8	15	<12	5-6
F2	clear	6.9±0.5	97.2±3.4	20	>12	3-4
F3	clear	7.3±0.1	97.5±1.9	26	>12	1-2
F4	clear	7.1±0.8	98.2±2.4	30	>12	Immediate
F5	clear	7.2±0.1	99.1±1.9	37	>12	Immediate
F6	clear	7.1±0.5	100.1±3.1	45	>12	Immediate
F7	clear	7.1±0.5	99.2±2.8	40	<12	Immediate
F8	clear	7.8±0.3	97.5±2.8	20	>24	Immediate
F9	clear	7.9±0.3	95.4±3.4	10	>24	Immediate
F10	clear	6.9±0.3	97.2±3.1	25	>12	Immediate
F11	clear	7.2±0.8	99.3±1.5	25	>12	Immediate
F12	clear	7.3±0.5	98.2±2.8	35	>12	Immediate

*Mean of three determinations ±standard deviation.

Viscosity

The viscosity of all prepared batches was measured before and after gelation. It is clear that the viscosity increased significantly after gelation which considered an evidence for formation of good stable in situ gel. The results reported in table 3 revealed that the lowest concentration of gelling polymer to form gel was 0.5% sodium alginate with viscosity equal 54±5.0 cp but unfortunately it dispersed rapidly few hours later. The viscosity of in situ gel increased to 78±8.3, 143±5.4, 190±6.5, 295±3.5 and 450±7.1upon increasing concentration of sodium alginate to 1%, 1.5%, 21, 2.5% and 3% for F2, F3, F4, F5, and F6 respectively.

Table 3: viscosity of vardenafil loaded floating in situ gel before and after gelation.

Formulation code	Viscosity before gelation (CP)±S.D	Viscosity after gelation (CP))±S.D
F1	54±5.0	435±6.5
F2	78±8.3	810±5.1
F3	143±5.4	2053±7.2
F4	190±6.5	2557±8.7
F5	295±3.5	3210±5.5
F6	450±7.1	4830±5.5
F7	120±5.0	1212±7.8
F8	275±3.4	1827±9.1
F9	392±4.5	4043±5.6
F10	255±3.4	2910±7.8
F11	327±7.8	3150±7.2
F12	480±6.5	5321±8.3

*****Mean of three determinations +standard deviation**.**

The increase in chain interaction associated with the increase in sodium alginate concentration account for the significant increase in viscosity with gelling polymer[17]. It can also observe that an increase in the concentration of calcium chloride from 0.15% (F4) to 0.3 %(F10), 0.6 %(F11) and 1.2 %(F12) resulted in a significant increase from 190±6.5 to255±3.4, 327±7.8 and 480±6.5 respectively. The increase in calcium carbonate concentration from 0.5 %(F7) to 1 %(F4), 1.5 %(F8) and 2 %(F9) associated with an increase in the viscosity from 120±5.0, 190±6.5, 275±3.4, and 392±4.5 respectively.

The increase in the viscosity of in situ gel with the increase in the concentration of either calcium carbonate or calcium chloride might be due to increased calcium ion concentration that increases sodium alginate cross linking and eventually more stiff gel.

Thomas [18]found that a significant increase in the viscosity of metronidazole floating in situ gel resulted from an increase in calcium carbonate concentration.

In-vitro release study

Figure2. illustrates the effect of sodium alginate concentration on cumulative release percentage of vardenafil from in situ gel. It is clear that as the concentration of gelling polymer increased from 0.5% to 1%, 1.5%, 2%, 2.5%, and 3% there was a significant decrease in the released amount after 9 hours from 95.1%±2.5(F1) to 88.2±1.7(F2), 81.5±1.3(F3), 75.6±1.7(F4), 70.3±1.5(F5), and 65.1±1.9(F6)respectively. This could be attributed to the formation of denser polymer mass with increasing polymer concentration which leads to an increase in the diffusional pass length.

Figure3. shows that as the concentration of calcium chloride increased from 0.15%(F4) to 0.3%(F10), 0.6%(F11), and 1.2%(F12) the cumulative released amount decreased from 75.6±1.7 to 70.6±1.2, 65.2±1.5, and 58.1±1.9 respectively.

An increase in calcium chloride concentration results in an increase in calcium ions that increases the extent of crosslinking and consequently increases the viscosity and retards the drug release.

Also an increase in the concentration of calcium carbonate from 0.5 %(F7) to 1 %(F4), 1.5 %(F8), and 2 %(F9) resulted in a significant decrease in vardenafil released amount from 83.3±1.9 to 75.6±1.7, 70.2±2.1, and 63.2±1.9 respectively (figure4). This also may be explained due to increased calcium ions concentration which gives more cross linking and consequently more stiff gel. This is in accordance with Shivaraju et al, [9] who found that a significant decrease in ornidazole released from sodium alginate in situ gel from 96.57±0.34 to 60.24±0.54 with the increase in sodium alginate concentration from 0.5% to 2%.

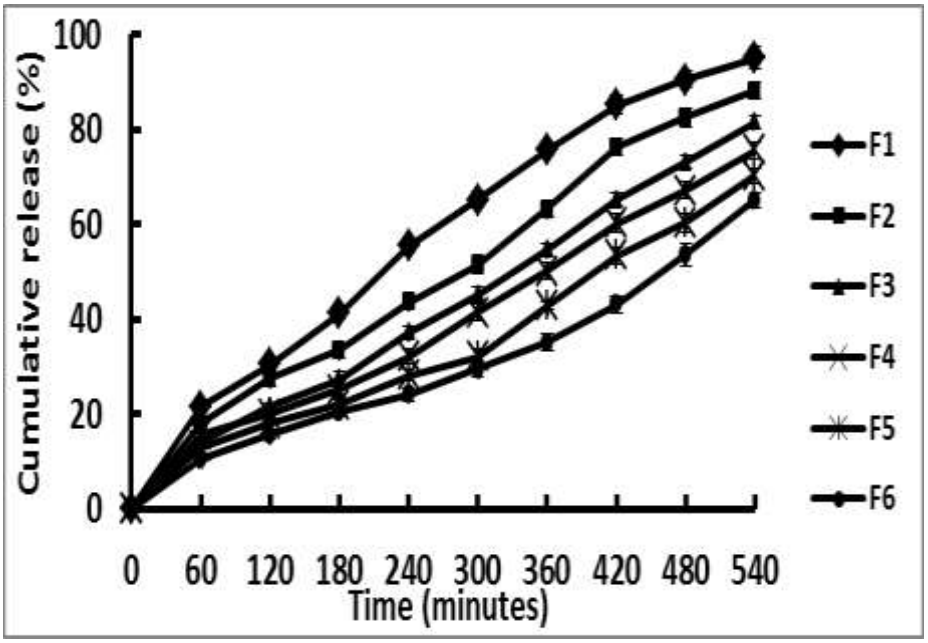

Figure 2: effect of sodium alginate concentration on cumulative release of vardenafil.

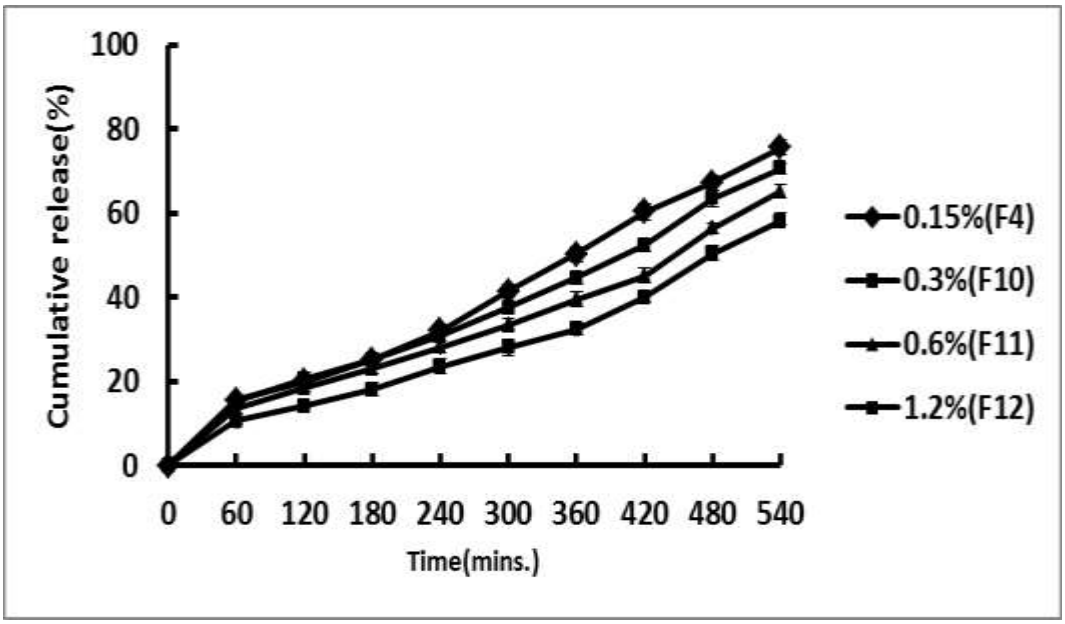

Figure 3: effect of calcium chloride concentration on cumulative release of vardenafil.

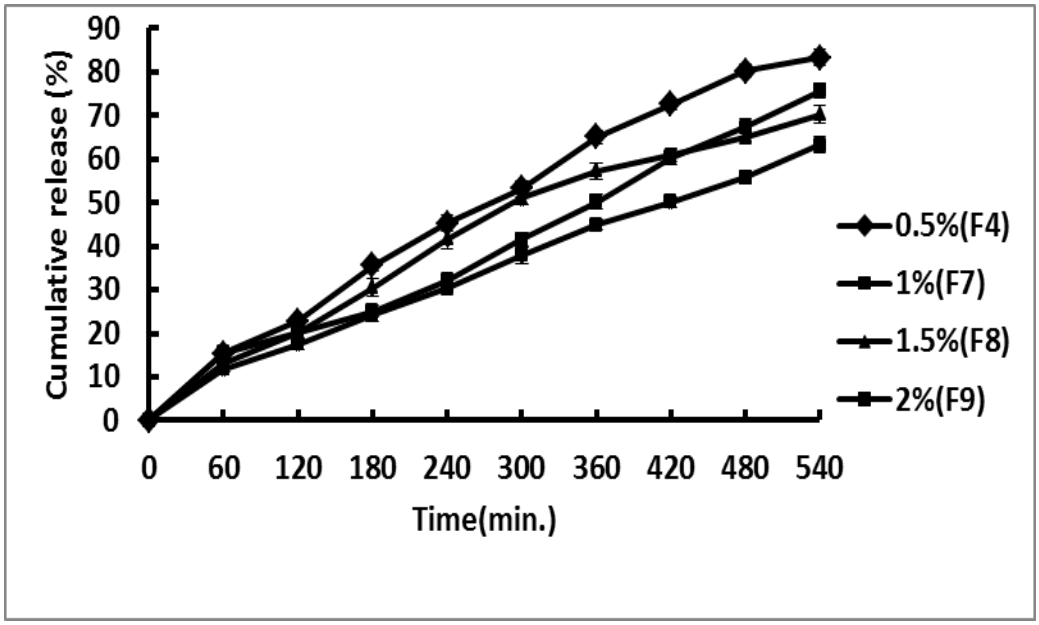

Figure 4: effect of calcium carbonate concentration on cumulative release of vardenafil.

Kinetic data analysis

The in-vitro release profile of all prepared batches follow Higuchi diffusion model.

Stability study

Optimized F4 batch was subjected to accelerated stability conditions. Samples were removed at 0, 2, 4 and 6 months for evaluation of physical appearance, pH, drug content, viscosity before and after gelation, gelation capacity, floating lag time, floating period and cumulative release amount after 9 hours. The selected batch was clear and stable for all evaluated parameters during the duration of the study[6] (table 4).

Table 4: different characterization of F4 under accelerated stability conditions.

Stability duration (month)	pH±S.D	Viscosity before gelation (CP)+S.D	Viscosity after gelation (CP)+S.D	Floating lag time (sec)	Floating duration (hr)	Drug content (%)	Gelling capacity	Cumulative release after 9 hours (%)
0	7.1±0.8	190±6.5	2557±8.7	15	>12	98.2±2.4	immediately	75.6±1.7
2	7.5±0.5	210±7.1	2535±6.1	10	>12	99.4±1.8	immediately	74.2±2.3
4	7.3±0.3	195±6.4	2560±7.6	15	>12	98.5±3.1	immediately	76.8±0.8
6	7.1±0.5	200±4.8	2550±6.5	15	>12	99.1±4.8	Immediately	73.5±1.9

In-vivo studies

Figure 5 shows the chromatogram of rabbit plasma of vardenafil and fenoprofen as an internal standard. It can be noticed that, a typical and well resolved peaks were obtained for plasma, vardenafil and fenoprofen. The retention times of vardenafil and fenoprofen were 6.3 and 11 min, respectively.

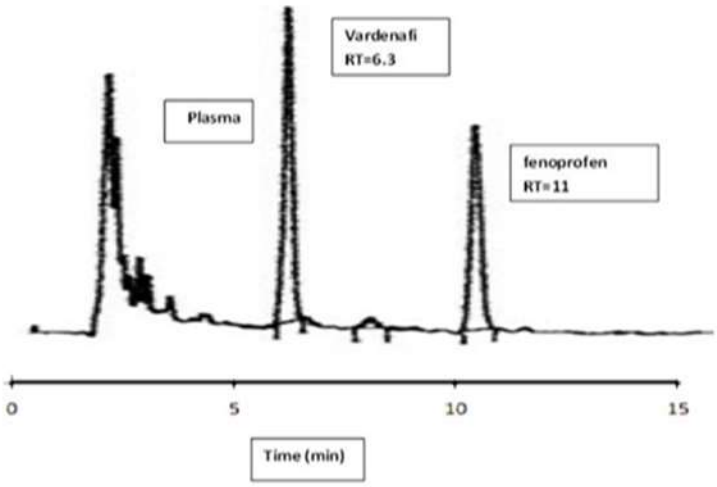

Figure 5: HPLC chromatogram of vardenafil and fenoprofen as an internal standard in rabbit plasma.

The mean plasma concentrations as a function of time for vardenafil after oral administration of plain vardenafil solution and vardenafil in situ gel (F4) are shown in Figure6. From the obtained results, it is obvious that, there was a significant difference between the mean plasma concentrations after oral administration of the tested formulation at all-time intervals compared to the plain drug. Also, there was a clear difference in the tmax between the plain drug and the in situ gel. The mean pharmacokinetic parameters of vardenafil solution and in situ gel represented by the value of Cmax (µg/ml), tmax (h), and AUC0-72 (µg.h.ml-1) are illustrated in table5. From the obtained results, it is observed that, the absorption of plain vardenafil was rapid and reached its

peak plasma concentration after 4 h, whereas, the mean tmax for the F4 was 8 h. The mean peak plasma concentrations (Cmax) were 17.8±0.80 µg/ml and 12.7±0.11 µg/ml for F4 and plain drug respectively. The increase in the mean tmax and the decrease in the mean Cmax of in situ gel in comparison to the plain vardenafil indicate a sustained release effect and a reasonable enhancement of oral bioavailability of vardenafil.

Table 5: pharmacokinetics parameters after oral administration of vardenafil plain solution and in situ gel

Formula	C_{max} (µg/ml)±S.D*	t_{max} (h)	AUC $_{0-72}$(µg. ml .h) ±S.D
In situ gel (F4)	17.8±0.80	8	122.21±9.1
Vardenafil plain solution	12.7±0.11	4	92.78±2.4

***mean of six determinations ± standard deviation**

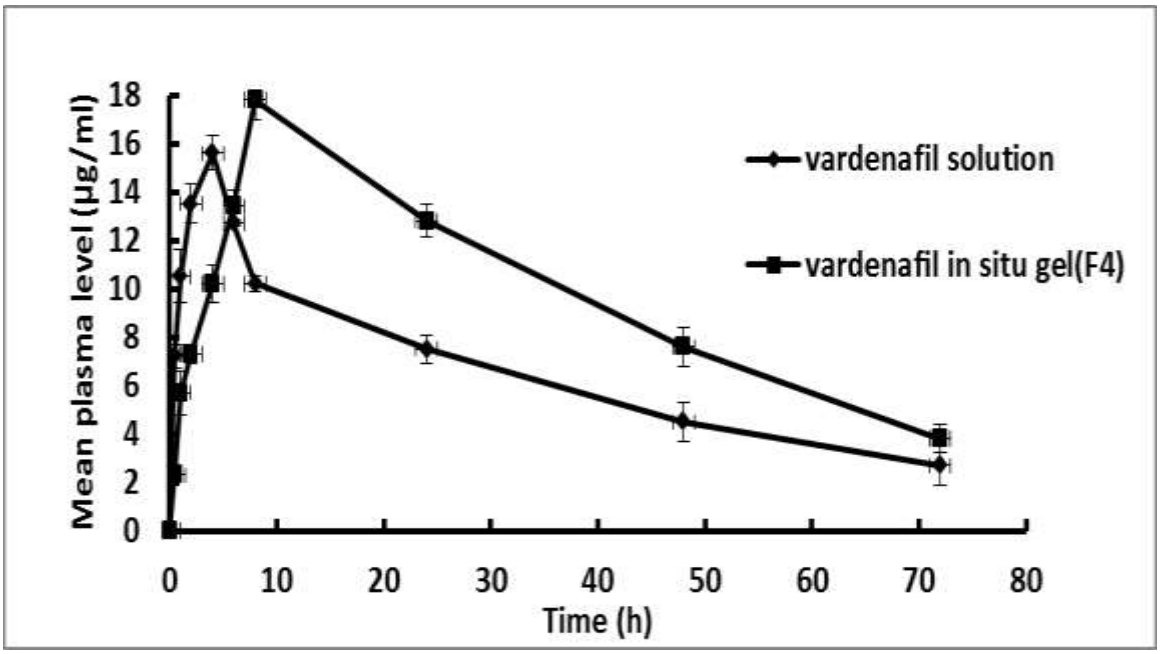

Figure 6: mean plasma levels of vardenafil after oral administration of vardenafil solution and vardenafil in situ gel.

CONCLUSION

It can be concluded from the previously illustrated results that vardenafil was successfully formulated into gastro retentive sodium alginate in-situ gel. The least effective concentration of sodium citrate to maintain fluidity of the gel before administration is 0.5% w/v; 1% w/v calcium carbonate and 0.15% w/v calcium chloride gave gel with reasonable viscosity and immediate floating. The prepared gel showed good stability for more than 6 months with highly improved oral bioavailability. Floating in-situ gel is considered a promising approach to give sustained release effect especially for drug with short duration of action.

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