Controlled Release Ion Sensitive Floating Oral *in situ* Gel of a Prokinetic Drug using Gellan Gum.

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ABSTRACT

There are various approaches commonly used for gastric retention, one of which is raft forming system. Floating oral *in situ* gel is a type of raft forming system. The present work concerns with the formulation, evaluation and optimization of floating oral *in situ* gel of Itopride Hydrochloride for controlled release. Gellan gum has been used as a gel forming polymer and calcium carbonate as cross linking agent and Ca^{2+} ion source, and HPMC K100M as release retardant. The floating oral *in situ* gel undergoes gelation by ion sensitive mechanism. In this formulation 3^2 factorial designs was performed and the effect of variation in concentration of gellan gum and HPMC K100M on drug release at 1 h, 6 h, and viscosity was evaluated. The gel was evaluated for other parameters like floating lag time, floating duration, gel strength, density, pH, *in vitro* drug release, drug content, and *in vitro* gelling capacity. The results of 3^2 full factorial design revealed that the concentration of gellan gum and of HPMC K100M significantly affected the dependent variables i.e. drug release at 1 h, at 6 h, and viscosity. A controlled release profile was observed for these formulations. The drug release mechanism was found to follow Korsmeyer-Peppas model. *In vivo* studies revealed higher T_{max} of gel compared to plain drug which is suggestive of slower absorption. However the $AUC_{0\text{-}12\ h}$ was found to be nearly 90% higher than plain drug.

Keywords: *in situ gel,* CaCO3, Gellan gum, Foating drug delivery system, Ion Sensitive, Prokinetic.

INTRODUCTION

Oral administration is most convenient and preferred means of drug delivery to the systemic circulation. Oral controlled release drug delivery has recently been of increasing interest to achieve improved therapeutic advantages, such as ease of administration, patient compliance and flexibility in formulation. *in situ* gel forming polymeric formulations is in sol form before administration,[1] undergo gelation *in situ* to form a gel. These *in situ* solutions are liquid at room temperature but undergo gelation when in contact with body fluids or change in pH. These have a characteristic property of temperature dependent, pH dependent and cation induced gelation. Compared to conventional controlled release formulations, *in situ* forming drug delivery systems possess potential advantages like simple manufacturing process, ease of administration, reduced frequency of administration, and improved patient compliance and comfort.[2,-4]

Itopride hydrochloride (ITO) is a novel prokinetic agent, widely absorbed from the stomach and upper part of the small intestine and absorption becomes less as the drug passes through the small intestine. These criteria made ITO a suitable candidate for floating drug delivery system. It has half life of 6 h, so there is a need for frequent administration and bioavailability can be improved by making the drug completely absorbed in the stomach and upper part of the small intestine.[5,6] It activates the gastrointestinal motility through synergism of its dopamine D2 receptor antagonistic action and acetylcholine esterase-inhibitory action. In addition to these actions, ITO has an antiemetic action, which is based on its dopamine D2 -receptor antagonistic action.[7,8] ITO is indicated in various diges-

Submission Date :20-08-2014
Revision Date :21-11-2014
Accepted Date :07-01-2015

DOI: 10.5530/ijper.49.2.13

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tive conditions such as heartburn, regurgitation, epigastric pain, and esophagitis. These conditions include gastro esophageal reflux disease, non-ulcer dyspepsia, chronic gastritis and a very important complication seen in diabetics where in the gastric emptying is markedly reduced "i.e"; diabetic gastro paresis. Gellan gum is an anionic deacetylated exocellular polysaccharide secreted by Pseudomonas elodea with a tetrasaccharide repeating unit of one α-L-rhamnose, one β- D-glucuronic acid and two β-D-glucuronic acid residues. It has the tendency of gelation which is cations induced. This gelation involves the formation of double helical junction zones followed by aggregation of the double helical segments to form a three- dimensional network by complexation with cations and hydrogen bonding with water. The formulation consisted of gellan solution with calcium carbonate and sodium citrate complex. When administered orally, the calcium ions are released in acidic environment of stomach leading to gelation of gellan thus forming a gel *in situ*. HPMC K100M has been used as a release retardant.[9]

The present investigation deals with formulation, optimization, and evaluation of gellan gum and HPMC K100M based floating oral *in situ* gel of Itopride Hydrochloride in which calcium carbonate was used as a source of Ca^{2+} ions and as a cross-linking agent. The gel was evaluated for parameters like floating lag time, floating duration, gel strength, density, pH, *in vitro* drug release, drug content, and *in vitro* gelling capacity.

MATERIALS AND METHODS

Materials

Itopride Hydrochloride was obtained as a gift sample from Ami Life Sciences Pvt. Ltd. Baroda Gujarat, India. Gellan gum (Gelrite®) was obtained from Balaji Pharma, Gujarat, India. Calcium carbonate was obtained from Thermo Fisher Scientific Pvt.Ltd Mumbai; India. HPMC K100M was obtained from Vijay Chemicals Pvt. Ltd, Pune, India. All other materials and chemicals used were of either pharmaceutical or analytical grade.

Method

Preparation of ion sensitive floating oral *in situ* gelling solution

The required quantity of gellan gum was dispersed in deionised water followed by addition of sodium citrate (0.25 % w/v). The solution was heated to 90ºC with stirring, and cooled to 40ºC. Calcium carbonate was added after cooling with continuous stirring to form a uniform dispersion after which ITO and HPMC K100M was added. The gellan gum and HPMC K100M were used in the concentration range of (0.25-0.75 %w/v) and (0.4-0.6 % w/v) respectively. To this 10 % w/v sucrose and 0.1 % w/v sodium benzoate was added with continuous stirring using glass rod and volume made up to 100 ml[10] (Table 1).

Experimental Design

Preliminary trials were conducted to identify the concentration of gellan gum and HPMC K100M that formed *in situ* gels of desired strength and floating lag time, floating duration and density. (Table 2). Based on this, a 3^2 simple full factorial design having 9 runs (F1-F9) were selected. Two independent variables, concentration of gellan gum(X1) and HPMC K100M(X2) were selected at 3 levels and dependent variables were percent drug release at 1 h (Y1), at 6h (Y2) and viscosity (Y3). (Table 3). The experimental data was analyzed statistically using Design Expert Software V 9.1 and the main effects and interactions were calculated. The quadratic model was selected. The effect of independent variables

Table 1: Formulation of preliminary trial batches

Ingredients	Formulation Quantity (in% w/v)		
	F1	F2	F3
ITO	1	1	1
Gellan gum	0.25	0.50	0.75
HPMC K100M	0.4	0.5	0.6
Calcium carbonate	1	1	1
Sodium Citrate	0.25	0.25	0.25
Deionised Water(up to)	100 ml	100 ml	100 ml

Table 2: Results of preliminary trial batches (n=3)

Formulation Code	Floating lag time (s)	Gel strength (gm/cm²)	Floating duration (h)	Density (gm/cm³)
F1	98±1.2	18.23±0.7	> 12	0.693±0.54
F2	90±2.0	34.23±1.8	> 12	0.754±0.43
F3	76±1.8	50.28±0.4	> 12	0.635±0.52

Table 3: Formulations as per 3^2 full factorial design for ion sensitive floating oral *in situ* gel

Ingredients	Formulations (Quantity in % w/v)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
ITO	1	1	1	1	1	1	1	1	1
Gellan gum	0.25	0.25	0.25	0.50	0.50	0.50	0.75	0.75	0.75
Coded level(X1)	-1	-1	-1	0	0	0	+1	+1	+1
HPMC K100M	0.4	0.5	0.6	0.4	0.5	0.6	0.4	0.5	0.6
Coded level(X2)	-1	0	+1	-1	0	+1	-1	0	+1
Calcium Carbonate	1	1	1	1	1	1	1	1	1
Sodium citrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sodium benzoate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sucrose	10	10	10	10	10	10	10	10	10
Deionised water(up to)	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

on the response parameters were visualised using 3D response plots. Desirability approach was employed to locate the optimal settings of the formulation variables to obtain desired response. The optimized formulation was evaluated for the responses and the experimental values obtained were compared with those predicted by the mathematical model generated.

Characterization of floating oral *in situ* gel

Physical appearance and pH

All the formulations were visually checked for their appearance and color. The pH of the *in situ* solution was measured using standardized digital pH meter (Deluxe pH meter 101/EI) at room temperature by taking adequate volume in a 50 ml beaker.[11]

In vitro gelling capacity

The *in vitro* gelling capacity of the formulations was measured by placing five ml of the gelation solution (0.1N HCl, pH 1.2) in a 15 ml borosilicate glass test tube maintained at 37 ± 1ºC temperature. The formulation (1 ml) was added slowly by placing the pipette at surface of fluid in test tube. As the solution comes in contact with gelation solution, it is immediately converted into a stiff gel like structure. The gelling capacity of solution was graded in three categories evaluated on the basis of stiffness of formed gel and time period for which the gel retained its rigidity.[12]

(+) Gels after five min, dispersed within 8 h

(++) Gels within 60 sec and retains gel structure for 12 h

(+++) Gels immediately and retains gel structure for more than 12 h.

In vitro buoyancy test

In vitro buoyancy was characterized by floating lag time and total floating duration. This study was carried out using USP dissolution apparatus Type II using 500 ml of 0.1 N HCl (pH-1.2) as the medium. The test was carried out at 50 rpm at 37 ± 0.5ºC. The *in situ* gelling solution (10 ml) was transferred to a petri plate (diameter 2") using a syringe. The plate was then placed on the surface of the medium and plunged in to the medium with the moving paddle. The time required for the gelled mass to rise to the surface of the dissolution medium [floating lag time] and the duration of the time for which the gel constantly floated on the dissolution medium [floating duration] was noted for each formulation.[13-15]

Density

Density of the floating oral *in situ* gel was determined by using water displacement method.[16] To (10 ml) *in situ* solution, 20 ml of 0.1 N HCl (pH 1.2) was added to convert the solution in to gel. Excess of HCl was drained off and the gel so formed was weighed. The gel was then transferred to a 50 ml measuring cylinder and allowed to settle at the base. Distilled water was added up to 50 ml marking of measuring cylinder. Volume of water in the presence of gel was noted. From the difference in the volumes of water with and without gel the volume of gel was obtained i.e. amount of water displaced by the gel was calculated.

Gel strength

The method[17] was modified to measure the gel strength. In house gel strength apparatus was fabricated using a plastic measuring cylinder of 1.2 cm radius and a bore of 0.1 mm at its base. A needle, 2 cm in length was used to which a nylon thread was tied. Sol (15 ml) was taken in the cylinder with temporarily sealed bore followed by addition of 50 ml of 0.1 N HCl (pH 1.2) for gelation. After gelation the HCl was drained off by opening bore seal leaving the gel mass in the measuring cylinder and the needle was rested on the surface of the gel. At the free end of the thread, pan was attached to which

the weights were added. The gel strength was reported in terms of weight required to pass the needle probe through the gel mass.[17] The gel strength was calculated using this formula.[18]

$$Gel\ strength = Mg/a \quad \text{- - - - - - - - - (1)}$$

Where, M = Weight at which needle passes through the formed gel mass. g = gravitational force, taken as 980 cm/s^2; a = Area of surfaces.

Viscosity determination

The viscosities of the solutions were determined by Brook field viscometer (Model RVDV-II+P). The samples (10 ml) were sheared at a rate of 100 rpm using S21 spindle at room temperature. Viscosity measurement for each sample was done in triplicate, with each measurement taking approximately 30s.[19]

Drug content

in situ solution (equivalent to 100 mg of ITO) was taken in a volumetric flask. To this 50 ml of 0.1 N HCl was added and shaken on mechanical shaker for 30 min. This was followed by sonication for 15 min for complete dispersion of contents and filtration using 0.45 µm membrane filters. From this solution, 10 ml of sample was withdrawn and diluted to 100 ml with 0.1 N HCl. Contents of ITO was determined spectrophotometrically at 248 nm using double beam UV-visible spectrophotometer LABINDIA 3000+. [20]

In vitro drug release

The drug release study was carried out using USP type II paddle type apparatus at 37 ± 0.5ºC and at 50 rpm using 900 ml of 0.1 N HCl (pH 1.2). *in situ* gel (10 ml) equivalent to 100 mg of ITO was used for the test. Sample solution (1 ml) was withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, diluted and suitably analyzed by UV spectrophotometric LABINDIA 3000+ at 248 nm. Fresh dissolution medium was replaced immediately after withdrawal of the test sample to maintain sink condition. The dissolution studies were carried out for a period of 12 h.[21,22] The dissolution data was analyzed by DD Solver ™ software for mathematical modeling to predict the release kinetics.[23,24]

In-vivo pharmacokinetic studies

Male Wistar rats, weighing 230–330 g, were fasted for 24 h with free access to water. The rats were divided into three groups of six rats each, viz., first group served as negative control, second group was administered pure ITO solution and third group was administered optimized formulation. The rats were anaesthetized with the help of ether, and the retro-orbital method was used to removal of blood samples. The dose administrated was 10 mg/kg of animal weight. Blood samples were withdrawn from the retro-orbital vane at intervals of 0, 1, 2, 4, 6, 8, 10, 12 h and analyzed by bioanalytical HPTLC method using a mixture of methanol- ammonium acetate (6:4, v/v) as mobile phase and detected at λ_{max} of 288 nm.[25,26] A simple protein precipitation method was employed for extraction of drug from human plasma using 10% perchloric acid. The protocol for the animal experiment was approved by the Animal Ethical Committee ref no. (AISSMS/IAEC/13-14/01-28).

RESULTS AND DISCUSSION

Physical appearance, pH and drug content

All formulations (F1-F9) were found to have creamy appearance and pH was in the acceptable range of 7-8. The percent drug content of all formulations was found to be in the range of 93.03-97.19%, indicating insignificant loss of drug during the formulation (Table 2).

In vitro gelling capacity

In vitro gelling capacity of various formulations of ion sensitive floating oral *in situ* gel is reported in (Table 4). The *in situ* gel should maintain its integrity without dissolving or eroding for an extended time. After ingestion, the liquid polymeric solution of gellan gum undergoes a rapid sol-to-gel transition by means of ionic gelation. The gelation involves formation of double helical junction zone followed by aggregation of the double helical segments to form a three dimensional network by complexation with Ca^{+2} ions and hydrogen bonding with water.[27] It was observed that all formulations showed immediate gelation on contact with acidic environment and retained gel structure for more than 12 h. The rigidity of the gel confers controlled release property to the formulation as the drug molecules have to traverse through the complex network of polymer chains to reach the physiological environment.

In vitro buoyancy test

The floating ability of the formulations was evaluated in 0.1 N HCl (pH 1.2) The time taken by the formulation to emerge on the surface of the medium (floating lag time) and the time for which the formulation constantly floated on the dissolution medium surface (duration of floating) are shown in (Table 4). On contact with acidic medium, the calcium ions react with gellan gum and produce a crosslinked three-dimensional gel network. Further carbon dioxide is released and is entrapped in gel network imparting buoyancy to the formulations.[28,29] Floating lag time for the all formulations showed a

Table 4: Evaluation parameters of ion sensitive floating oral *in situ* gels (n=3)

Formulation code	pH	Drug content (%)	*In vitro* gelling capacity	Floating lag time (s)	Floating duration (h)	Gel strength (gm/cm²)	Density (gm/cm³)	Viscosity Cps
F1	7.04±0.1	95.83±2.0	+++	176±1.2	>20	16.3±1.2	0.694±0.01	139.5±0.07
F2	6.80±0.02	95.52±2.1	+++	151±1.8	>20	23.21±1.5	0.837±0.5	142.7±0.09
F3	7.08±0.1	95.94±0.7	+++	149±1.8	>20	44.93±1.3	0.728±0.65	148.1±0.40
F4	7.50±0.1	97.19±2.1	+++	171±1.5	>20	16.75±1.6	0.862±0.68	164.6±0.08
F5	7.54±0.2	95.1±2.1	+++	155±1.0	>20	23.92±1.8	0.759±0.25	171.4±0.30
F6	7.10±0.4	94.28±1.9	+++	97±1.0	>20	47.09±1.9	0.869±0.30	179.6±0.10
F7	7.02±0.5	97.19±3.6	+++	167±2.5	>20	17.5±0.99	0.670±0.35	193.13±0.18
F8	7.61±0.5	96.77±3.9	+++	124±2.2	>20	24.97±2.0	0.799±0.52	200.7±0.78
F9	7.50±0.5	93.03±2.5	+++	88±1.5	>20	47.94±1.2	0.720±0.01	205.3±0.76

(+++) Gels immediately and retains gel structure for more than 12h.

reduction in floating lag time with increase in polymer concentration. The batches [F3, F6, and F9] containing high concentration of HPMC K100M exhibited lower floating lag time in the range of 88-150 s. The batches [F1, F4, F7] containing low concentration of the polymer displayed higher floating lag time.

Gel strength

Gel strength is indicative of tensile strength of the gelled mass. It signifies the ability of the gelled mass to withstand *in vivo* peristaltic movements. The gel strength of the formulation is an important variable dependent on the concentration of the gelling agent as well as cation source. The high polymer and calcium carbonate combinations demonstrated adequate gel strength when pressed with a pair of fine forceps, indicating that they will withstand the shear forces likely to be encountered in the stomach. The gel strength for all 9 formulations (Table 4) indicated an increase in gel strength with increase in polymer concentration. The batches [F1, F4, and F7] showed low gel strength because of it contains low concentration of polymer. The batches [F3, F6, and F9] showed high gel strength than that of the above. Formulations F2, F5 and F8 comprising different concentrations of gellan gum but same concentration of HPMC K100M were found to have adequate gel strength. Thus the role of HPMC K100 M in maintaining gel integrity cannot be underestimated. We may also presume that gastric residence time of formulations with higher gel strength is also higher.

Density

The prime requirement of any floating system is that it must have density lesser than gastric contents (~1.004 gm/cm³).The density of all floating *in situ* gel formulations were found to be less than that of gastric contents. This can obviously be a pointer to the floatability of the formulations. The average densities of F1-F9 formulations were found to be 0.694 to 0.869 gm/cm³ (Table 4).

Viscosity

The rheological properties of the solutions are of importance in view of their proposed oral administration. The solutions showed a marked increase in viscosity with increasing concentration of gum. (Table 4).Increase in the gellan gum and HPMC K100M concentration in the formulation simultaneously increased the viscosity at all polymer concentration studied. This change in viscosity was due to the proportional amount of dispersed calcium carbonate and HPMC K100M present in the formulation. The average viscosities of all 9 formulations were found to be in the range of 139.5 to 205.3 cps. The formulation [F3, F6, and F9] showed high viscosity due to the high concentration of gellan gum and HPMC K100M than those of the [F1, F4, and F7].

In-vitro drug release

The effect of polymer concentration on *in vitro* drug release from *in situ* gels is depicted in (Figure 1). A significant decrease in rate and extent of drug release was observed with the increase in polymer concentration, and is attributed to increase in the density of the polymer matrix and also increase in the diffusional path length which the drug molecules have to traverse. The release of the drug from these gels are characterized by initial phase of high release (burst effect) followed by a slower release as the gelation proceeds. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics. Since the *in situ* gelling systems are aqueous in nature the matrix formed before the complete gelation cross-linking is already be in a hydrated state there by circumventing the rate limiting step of matrix hydration in the initial stages.[10]

A similar release profile was evident with all the formulations releasing 25-40% drug in the first hour followed by a more gradual release in the next span of studies. It was observed that formulations F3, F6, and F9 with concentration of gellan gum i.e. [0.25, 0.50 &

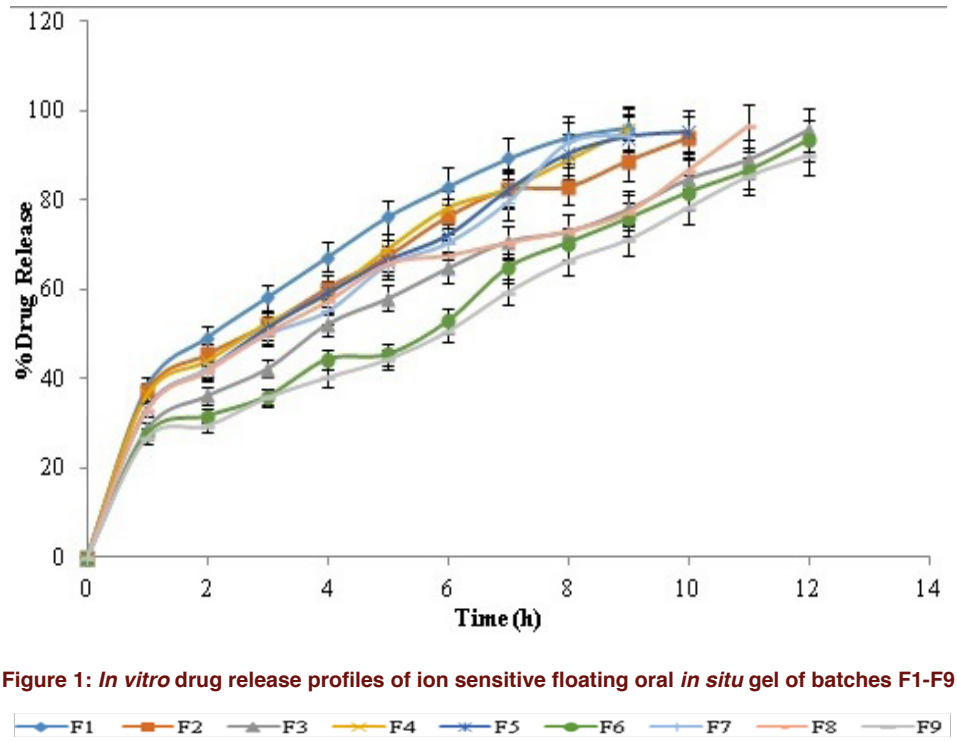

Figure 1: *In vitro* drug release profiles of ion sensitive floating oral *in situ* gel of batches F1-F9

Table 5: Release kinetic data of ion sensitive floating oral *in situ* gels

Batches	Regression value					Parameter's for Peppas equation	
	Zero order	First order	Higuchi	Peppas	Hixson Crowell	n	K
F1	0.6955	0.9776	0.9941	0.9980	0.9568	0.448	49.86
F2	0.6703	0.9603	0.9899	0.9952	0.9276	0.442	54.109
F3	0.7918	0.9706	0.9947	0.9958	0.9553	0.528	55.217
F4	0.7743	0.9630	0.9933	0.9933	0.9466	0.498	40.116
F5	0.7846	0.9704	0.9940	0.9943	0.9608	0.515	35.500
F6	1.0000	0.9206	0.9612	0.9804	0.9334	0.637	35.013
F7	0.8320	0.9533	0.9829	0.9857	0.9460	0.551	27.678
F8	0.6588	0.9336	0.9797	0.9847	0.8905	0.444	30.936
F9	0.8991	0.9470	0.9557	0.9789	0.9511	0.653	24.508

Table 6: Summary of results of regression analysis of ion sensitive floating oral *in situ* gels for responses Y1, Y2 and Y3.

For percent drug release at 1 h					
Model	**Model F value**	**p value**	**R^2**	**Adeq. Precision**	**Std. deviation**
Quadratic	34.86	0.0074	0.9831	16.400	0.89
$Y1 = 34.72 -1.87*X1 - 4.09*X2 - 0.78*X1*X^2 + 0.37* X12 - 2.66*X2^2$					
For percent drug release at 6 h					
Quadratic	25.50	0.0116	0.9770	14.886	2.72
$Y2=71.26-5.93X1-10.58X2-0.41X1X2+1.05X1^2 -5.27X2^2$					
For viscosity					
Quadratic	307.86	0.0003	0.9981	46.498	1.80
$Y3=171.80+28.14X1+5.96X2+0.89X1X2-0.30X1^2 +0.11X2^2$					

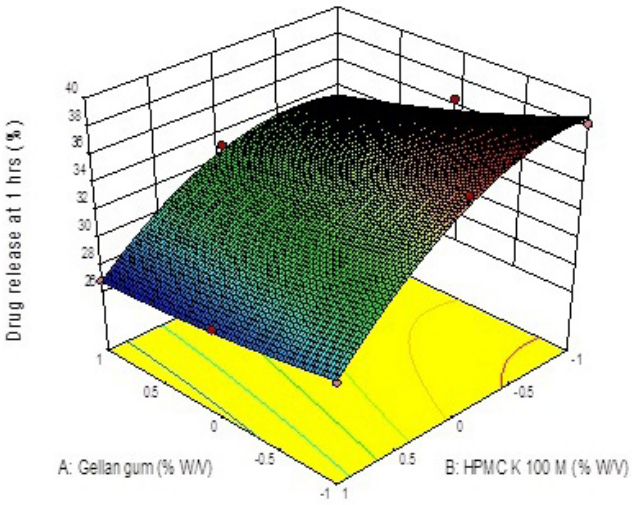

Figure 2: 3-D Response surface plot showing the influence of gellan gum and HPMC K100M concentration on the percent drug release at 1 h

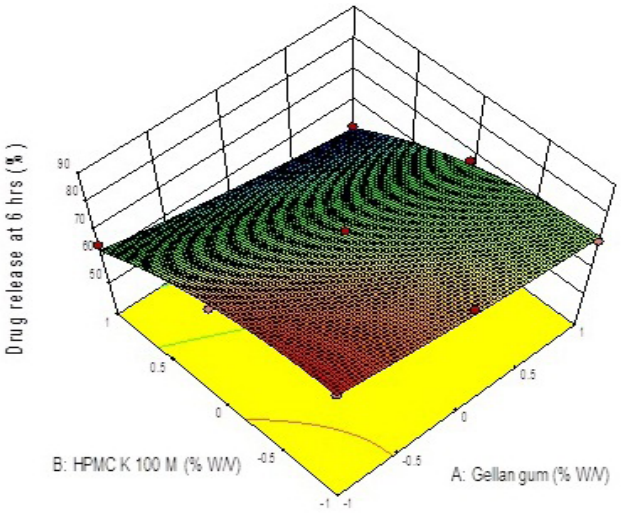

Figure 3: 3-D Response surface plot showing the influence of gellan gum and HPMC K100M concentration on the percent drug release at 6 h

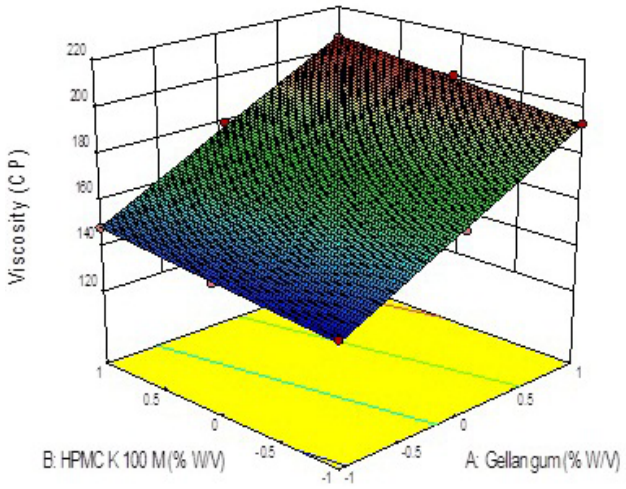

Figure 4: 3-D Response surface plot showing the influence of gellan gum and HPMC K100M concentration on the viscosity.

0.75 %w/v] and HPMC K 100M [0.6 %w/v] show controlled release of up to 90-95% in 12 h. The batches F1, F2 and F4 showed 90 % drug release within 8 h. It was observed that as the concentration of polymer increased, the release retarding effect of the formulation got stronger. The formulations containing higher concentration of gellan gum and HPMC K100M released their contents for longer period of times at slower rates, hence showing controlled release effect.

Release kinetics

The drug release data was fed in DD Solver Disso™ software. The values of regression coefficient (r^2 = 0.9789-0.9980) indicated that Peppas was the best fit model for all 9 formulations. The values of 'n' were in the range of (0.442-0.653).The formulation F1, F2 and F8 signifies Fickian diffusion controlled drug release while other formulation indicative of anomalous (non-Fickian) transport. The results of release kinetic data of factorial batches are given in (Table 5).

Statistical Analysis

Analysis of experimental results was carried out by using Design Expert V 9.1 software. The quadratic model was suggested to run the design. F-values, P-value and model F-value for percent drug release at 1 h, 6 h and viscosity were obtained from ANOVA. The selection of model and polynomial equations are listed in (Table 6). For all responses, the Model F-value implied that the quadratic model was significant. The probability values ($p \leq 0.05$) indicated that all the model terms across all the responses were significant. "Adeq Precision" measures the signal to noise ratio, a ratio greater than 4 is desirable. The obtained ratio of 16.400 for release at 1h indicated an adequate signal and hence the proposed model can be used to navigate the design space. For

Table 7: Evaluation of optimized formulation of ion sensitive floating oral *in situ* gel (n=3)

Percent drug release at 1 h	Percent drug release at 6 h	Viscosity CP	pH	Gel strength (gm/cm^2)	Density (gm/cm^3)
25.99±1.02	49.68±0.99	204.6±1.7	7.09±0.1	68.72±1.5	0.684±0.5

Table 8: Pharmacokinetic parameters for ion sensitive floating oral *in situ* gel (n=3)

Parameters		Pure Drug	Optimized formulation
C_{max}	µg/ml	0.163±0.06	0.183±0.04
T_{max}	h	1±0.68	6±1.02
$AUC_{0\text{-}12\ h}$	µg/ml h	0.80±0.5	1.537±0.72
$K_{ele.}$	h^{-1}	0.198±0.09	0.111±0.08
$t_{1/2}$	h	3.5±1.27	6.25±1.04

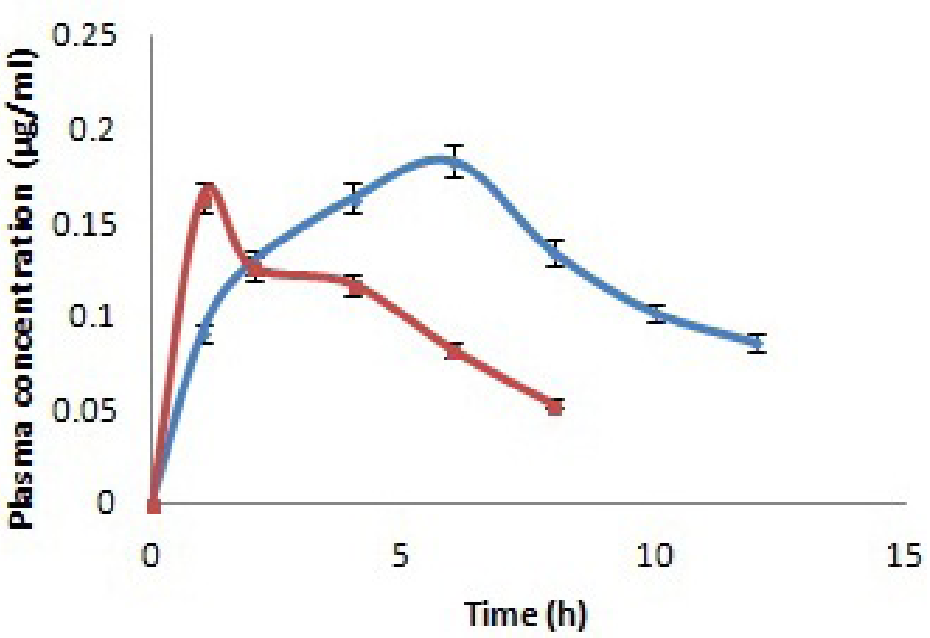

Figure 5: *In vivo* drug release profile of optimum formulation and pure drug ITO of ion sensitive floating oral *in situ* gel

release at 6h and viscosity, the ratios were computed as 14.886 and 46.498, respectively. The polynomial equations for responses Y1, Y2 and Y3 depict the relation between the factors and responses. The coefficients of various terms in the polynomial equations generated by the software give the nature and magnitude of relationship between the variables and the responses (Table 4).The concentration of gellan gum was found to be significantly retard drug release at1 h and 6 h. Both variables were found to have a direct influence on viscosity. The negative coefficients of term (X1X2) for percent drug release at 1 h and 6 h indicated significant interaction between the two independent variables. The same is reflected in the 3D response surface plots which depict the relationship between the response and independent variable across a wider domain. (Figure 2, 3 and 4).

Optimized batch

A numerical optimization technique using the desirability approach using the Design Expert software was employed to develop optimum formulation with the desired responses. Constraints were set for minimizing drug release at 1 h, drug release at 6 h and viscosity to locate the optimum setting of independent variables. Based on the input constraints the optimized *in situ* gel formula was generated by the software which comprised 0.958 % w/v gellan gum and 0.986 % w/v of HPMC K100M.The optimized formulation (SO1) was evaluated for percentage drug release at 1 h, 6 h and viscosity. A low residual error was evident in the observed and predicted responses (0.94, 0.91 and 0.7) with desirability of 0.987 for the optimized formulation. Drug release at 1 h, 6 h and viscosity from optimized

batch was found to be 25.99 %, 49.68 % and 204.6 cp. The optimized batch evaluated further for parameters like pH, gel strength and density (Table 7).

In vivo studies

In vivo studies were performed to quantify plasma concentration of ITO after oral administration of *in situ* gel formulation and pure ITO. The various pharmacokinetic parameters are presented in (Table 8). The plasma concentration time profile of pure drug and formulation is represented in (Figure 5). The elevation of T_{max} for the test formulation represented delayed absorption of the drug due to slow release from the formulations. Increase in $AUC_{0\text{-}12\ h}$ is suggestive of improved bioavailability. For pure drug, peak plasma concentration (C_{max}) was found to be 0.163 µg/ml at 1 h (T_{max}) and $AUC_{0\text{-}12\ h}$ was calculated as 0.80 h µg/ml. For controlled release floating oral *in situ* gel, the (C_{max}) was found to be 0.183 µg/ml at 6 h (T_{max}). The $AUC_{0\text{-}12\ h}$ was found to be 1.537 h µg/ml which was significantly higher than that for plain drug. Though ITO was available in plasma within an hour after its oral administration for plain drug formulation, the plasma profile indicated a continued decrease in 2-8 h whereas for the *in situ* gel a steady increase was evident reaching a peak at 6 h followed by a further gradual decrease in plasma concentration. The elimination rate constant for ITO in the *in situ* gelling system was found to be 0.111/h which was 43% lower than that for pure drug. The relative bioavailability of ITO is 60% due to first-pass effect.[30] It is metabolized in liver by N-oxidation to inactive metabolites by the enzyme flavin-containing monooxygenase.[11] Thus we may infer that formulating ITO as a controlled release formulation using ion sensitive *in situ* gelling system effectively reduced the first-pass effect due to slow release profile and concomitantly increased the bioavailability which could further lead to reduced dosing frequency.

CONCLUSION

This study reports that oral administration of aqueous solutions of gellan gum results in the formation of floating oral *in situ* gel by ion activation mechanism in the highly acidic envirnoment.The results of 3^2 full factorial design revealed that the concentration of gellan gum and concentration of HPMC K100M significantly affected on the dependent variables like percent drug released at 1 h, 6 h and viscosity. The drug release from gel structure followed Korsmeyer-Peppas model and which indicated diffusion-controlled release. Controlled release oral *in situ* gel of Itopride Hydrochloride was prepared successfully improving its bioavailability. *In vivo* study revealed that bioavailability of formulation increased than that of the pure drug. This study has demonstrated that *in situ* gels formed for oral administration can provide controlled release over extended time spans. Floating oral *in situ* gel may improve patient compliance by reducing dosing frequency which will be beneficial for pediatric and geriatric patients.

CONFLICT OF INTEREST

The authors report no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENT

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