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In Situgel: Development, Evaluation and Optimization Using 3^2 Factorial Design

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Abstract
Development of sustained release oral dosage forms is beneficial for optimal therapy regarding the efficacy, safety and patient compliance. Among oral dosage form, liquid dosage forms are more prone to low bioavailability because of their quick transit from the gastrointestinal tract. To produce sustained release formulation of an oral liquid formulation could be successfully augmented substantially through a strategy of liquid in-situ gelling system. The purpose of the present work was to develop oral in situ gelling system using Sodium alginate for *in situ* gelation of ambroxol-HCl. The formulation variables like concentration of polymer and calcium chloride will be optimized using factorial design. The promising formulations will be evaluated for pH, drug content, *in vitro* gelation, *in vitro* drug release, stability.
Key words: Sodium alginate, sustain release, In situ gelation

INTRODUCTION

Oral route is the most convenient and extensively used route for drug administration. This route has high patient acceptability; primarily due to ease of administration.[1] More than 50% of drug delivery systems available in the market are oral drug delivery systems. Drugs that are easily absorbed from the G.I.T and having a short half-life are eliminated quickly from the blood circulation, so multiple dosing is required. To avoid this problem oral controlled release formulations have been developed, as these will release the drug slowly into the GIT and maintain a constant drug concentration in the serum for a longer period of time.[2] Most of the oral controlled drug delivery systems rely on diffusion, dissolution or combination of both mechanisms, to release the drug in a controlled manner to the Gastrointestinal Tract (GIT) and the drug profile data, such as dose, absorption properties and the quantity of drug needed, one can determine the desired release rate of the drug from controlled release dosage form.[3] Many attempts have been made to develop sustained-release preparations with extended clinical effects and reduced dosing frequency. In order to develop oral drug delivery systems, it is necessary to optimize both the release rate of the drug from the system and the residence time of the system within the gastrointestinal tract.[4] The present investigation concerns the development of in situ gelling system using sodium alginate[5] which after oral administration are designed to prolong the gastric residence time, Increase the drug bioavailability, and diminish the side effects of irritating drugs.[6]

MATERIAL AND METHOD

Ambroxol hydrochloride was obtained as a gift sample from Sehat pharma, Himatnagar. All other chemicals were used of analytical grade.

Preparation of sodium alginate in situ gel

Sodium alginate solutions of different concentration were prepared by adding the alginate to ultrapure water containing sodium citrate and different concentration of calcium chloride and heating to 60°C while stirring on a magnetic stirrer. Ambroxol-HCl and Sodium propyl paraben was then dissolved in the resulting solution after cooling to below 40°C. Prepared sols finally stored in amber color bottles until further use. For 3^2 factorial designs, different levels of formulation variables are selected on the bases of preliminary trials.

Calculation of Theoretical release profile

The total dose of ambroxol hydrochloride for twice-daily SR formulation was calculated as per Robinson Erikson equation using available pharmacokinetic data.[7] Pharmacokinetic studies showed that a dose of 30mg of ambroxol hydrochloride produces expected therapeutic effect within 2h with the half- life of 4h.

Thus the elimination rate constant K = 0.693 / t ½ = 0.693 / 4 = 0.1732 mg / h.

Hence the availability rate
R = k D = 0.1732* 30 = 5.2 mg / h,
Where D is the usual dose of the drug
The maintenance dose
Dm = Rh = 5.2* 8 = 41.6 mg,
Where h is the number of hours for which sustained action is desired.
D immediate ,
(Di) = D - RTp = 30 – (5.2* 2) = 19.6mg
Where Tp is the time period required to achieve a peak plasma level,
Therefore, Total dose
Dt = Di + Dm = 19.6 + 41.6 = 61.2 mg (~60 mg).
Hence an oral controlled release formulation of ambroxol hydrochloride should contain a total dose of 61.2 mg (~60mg) and should release 19.6 mg in first 1h like conventional tablets and continuous release of remaining drug till 8 hours.

Optimization by using 3^2 full factorial designs

In the present study, a 3^2 full factorial design was employed for formulation containing two different in situ gelling polymers sodium alginate. Optimization is carried out by studying effect of independent variables, i.e. Concentration of Sodium alginate (X_1) and the concentration of calcium chloride (X_2) on dependent variables. Three factorial levels coded for low, medium, and high settings (−1, 0 and +1, respectively) were considered for three independent variables. The selected dependent variables investigated were "n" value (Y1), percentage of drug released at 30 min (Y2), 4 hours (Y3), and 8 hours (Y4) and viscosity (Y5). Tables 1 show the factors chosen and different factor level settings. The response (Yi) in each trial was measured by carrying out a multiple factorial regression analysis using the quadratic model: A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$

Where, Y is the dependent variables, b_0 is the arithmetic mean response of the nine runs, and b_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. Formulation of desired characteristics can be obtained by factorial design application [8]

EVALUATIONS

Physical appearance and pH

All the prepared in situ solutions of ambroxol-HCl were checked for their clarity and the type of the solutions. The pH was measured in each of the solution of sodium alginate based in situ solutions of Ambroxol-HCl, using a calibrated digital pH meter at room temperature. The measurements of pH of each data were performed in triplicate.

Determination of viscosity

Viscosity of the samples was determined using a Brookfield digital viscometer. The sample temperature was controlled at 25±1°c before the each measurements. The viscosity of the solutions prepared in water was determined at ambient condition. Increasing the concentration of a dissolved or dispersed substance generally gives rise to increasing viscosity (*i.e.* thickening), and also as molecular weight of a solute increases viscosity.

In-vitro gelling capacity

To evaluate the formulations for their *in-vitro* gelling capacity by visual method, colored solutions of *in situ* gel forming drug delivery system were prepared. The *in-vitro* gelling capacity of prepared 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 and maintained at 37±1ºC temperature. One ml of colored formulation solution was added with the help of pipette. The formulation was transferred in such a way that places the pipette at surface of fluid in test tube and formulation was slowly released from the pipette. As the solution comes in contact with gelation solution, it was immediately converted into stiff gel like structure. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel

and time period for which formed gel remains as such. Color was added to give visualized appearance to formed gel. The *in-vitro* gelling capacity was graded in three categories on the basis of gelation time and time period for which formed gel remains.[9]

Determination of drug content

The amount of ambroxol-HCl in each unit dosage form sample was determined by U.V. spectroscopy after sufficient dilution. The UV absorbance of the sample was determined at a wavelength of 245 nm. The drug content for batches was measured in triplicate and the average values are recorded.

FT-IR Spectroscopy

The FT-IR spectrum of the obtained sample of the drug was compared with the standard FT-IR spectra of the pure drug. FT-IR spectroscopy was carried out to check the compatibility between drug and polymer. The FT-IR spectra of drug with polymers were compared with the standard FT-IR spectrum of the pure drug.

In-vitro drug release study

The drug release study was carried out using modified USP XXVI paddle apparatus at 37 ± 0.5 º and at 50 rpm using 900 ml of pH 1.2 buffer as a dissolution medium (n=3) as per modified paddle dissolution test. In situ gels equivalent to 60 mg of ambroxol-HCl were used for the test. 10 ml of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µ membrane filter, dilute suitably and analyzed spectrophotometrically. Equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample. Percent drug dissolved at different time intervals was calculated using the Beer Lambert's equations described above. The amount of drug released in 30 minutes, at 4 hours and at 8 hours was calculated. The values of drug release at 4 hrs & at 8 hrs for in situ gels from batches F1 to F9 are calculated.[10, 11]

Kinetics modeling of drug dissolution profiles

The dissolution profile of all the batches was fitted to Zero order, First order, Higuchi model and korsmeyer to ascertain the kinetic modeling of the drug release. Korsmeyer-Peppas model explains simple relationship which described drug release from a polymeric system equation to find out the mechanism of drug release. The n value is used to characterize different release mechanism for matrices. $0.45 \leq n$ corresponds to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to non-Fickian transport, $n = 0.89$ to Case II (relaxational) transport, and $n > 0.89$ to super case II transport.[12]

Gel integrity test of optimized batch of in situ gels

In vitro gel integrity of optimized batch was checked in simulated gastric condition. In situ gelling solution containing single dose was added in previously weighed china dish containing 0.1 N HCl. Weight of gel formed is recorded after decantation of liquid from china dish. Gel formed in china dish was added in 500 mL beaker containing 0.1 N HCl (1.2 pH). Around 100 plastic beads of 3-mm diameter were incorporated into gastric juice to mimic food particulates in human stomach. Fluid in beaker was rotated by road attached to stirrer at 30-40 rotation per minute. Integrity of gel was checked after 2 hour. Experiment was also performed in absence of polymer beads. At the end of 2 hours difference in weight of gel in both conditions was observed and gel integrity was checked.

RESULT AND DISCUSSION

Appearance and pH

Clarity of all the formulations was found to be satisfactory. The pH of the formulations was found to be satisfactory as depicted in table 1 and was in the range of 6.5 -7.5. The formulations were liquid at room temperature and at the pH formulated.

In-vitro Gelation Studies

Table 2 shows the gelling capacity of all formulations and is depicted as + (gels after few minutes and dissolves rapidly), ++ (gelation immediate, remains for few hours only) and +++ (gelation immediate, remains for extended period). Increase in concentration of sodium alginate and calcium chloride increase in stiffness of gel at optimal concentration. Higher concentration of calcium chloride forms gel without reaction with acidic pH.

Table 1: 3^2 full factorial design layouts for sodium alginate

Batch No.	Variables levels in coded form		Value of n	Viscosity (cp)	% Drug release At 30 minutes	% Drug release At 4 hrs	% Drug release At 8 hrs
	X_1	X_2					
F1	-1	-1	0.307	112	50.3	97.8	97.8
F2	-1	0	0.320	136	47.6	94.38	96.7
F3	-1	+1	0.350	168	42.5	86.03	97.3
F4	0	-1	0.355	250	35.8	71.77	96.09
F5	0	0	0.468	268	21.4	56.72	87.4
F6	0	+1	0.465	286	19.9	52.4	85.2
F7	+1	-1	0.319	299	36.2	65.8	94.4
F8	+1	0	0.449	339	18.1	48.8	80.2
F9	+1	+1	0.457	380	18.2	45.8	76.2

Translation of coded levels in actual units

Variables level	Low (-1)	Medium (0)	High (+1)
Concentration of sodium alginate (X_1)	1.0 %	1.5 %	2.0 %
Concentration of Calcium chloride (X_2)	0.075 %	0.1 %	0.15 %

Note: All the batches contained the constant amount of drug as 60 mg/10 ml, Sodium citrate 0.25%

Drug content

Table 2 **s**hows the percent drug content for formulations. The drug content was found to be in acceptable range for all the formulations indicating uniform distribution of drug.

Table 2: Evaluation parameters of Sodium alginate in situ gel

Formulation	pH	Drug content	In vitro gelation
F1	7.3	96.12	+
F2	7.1	95.65	++
F3	6.9	94.78	++
F4	6.8	95.92	+
F5	7.0	98.72	+++
F6	7.1	95.54	+++
F7	7.2	96.22	+
F8	6.9	97.95	+++
F9	6.9	95.75	+++

(+: poor, ++: good, +++: excellent)

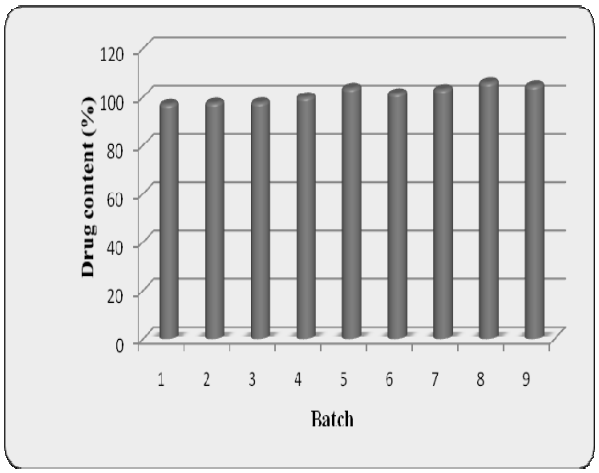


Figure 1: Drug content of sodium alginate based in situ gels batch F1-F9

FT-IR spectroscopy of drug

The IR spectrum of the pure Ambroxol Hydrochloride sample recorded by FTIR spectrometer is shown in Figure 2. Preformulation studies were carried out to study the compatibility of pure drug Ambroxol-HCl with the polymers sodium alginate, Gellan gum and other excipients. The individual IR spectra of the pure drug and combination with polymers are shown in the Figure 2-4.

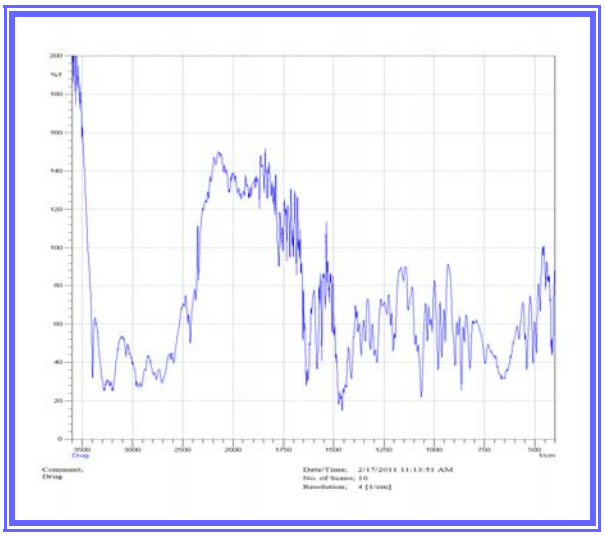


Figure 2: FTIR spectra of drug

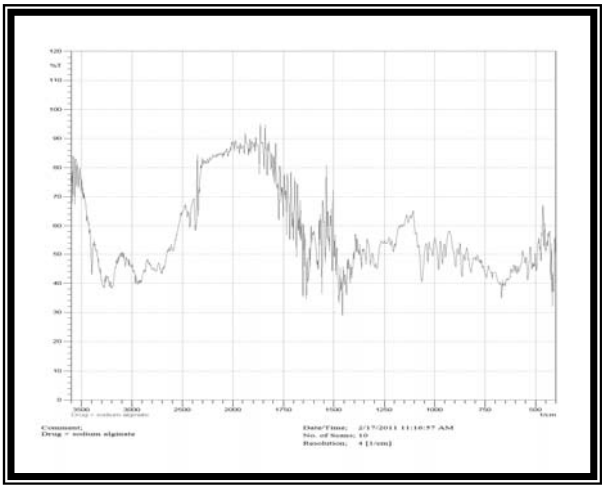
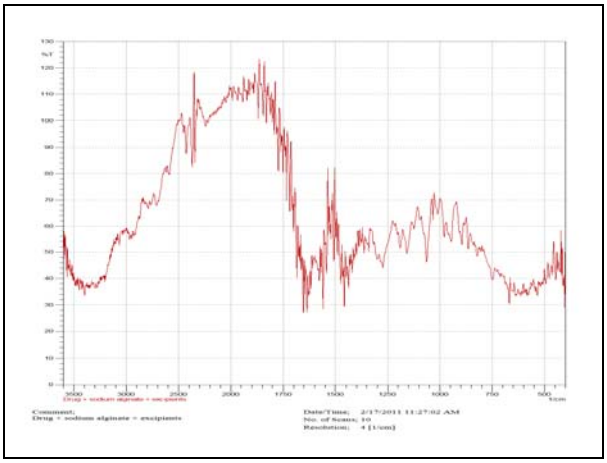


Figure 3: FTIR spectra of drug + sodium alginate



Figue 4: FTIR of Drug+ polymer+ excipients

Optimization of Sodium Alginate Based In Situ Gels

In a 3^2 factorial design, all the factors were studied at all the possible combinations, as it is considered to be most efficient in estimating the influence of individual variables using minimum experimentation. In the present study, effect of independent variables like concentration of gelling agent (X_1) and Calcium chloride(X_2) on dependent variables was studied and is shown in Table 1. Levels of Independent variable are selected on the bases of preliminary trials.

Viscosity

The formulation should have an optimum viscosity that will allow ease of administration as a liquid (drops), which would undergo a rapid sol-to-gel transition. Table 1 also shows the viscosity (cp) of formulations from F1 to F9. The viscosity increased in proportion with gelling agent. This may be attributed to the higher viscosity of sodium alginate.

In vitro drug release study

The release profile of a drug predicts how a delivery system might function and gives valuable insight into its *in vivo* behavior. The drug release data obtained for formulations F1 to F9 is tabulated in Table 3. Figure 5 shows the plot of cumulative percent drug released as a function of time for formulation F1 to F9.

The regression coefficient (r) values of zero order, first order, Higuchi matrix and Peppas are tabulated in Table 4. From the table, it is clear that the drug is released in a controlled manner over a period of time and shows release for all formulations following Peppas model.

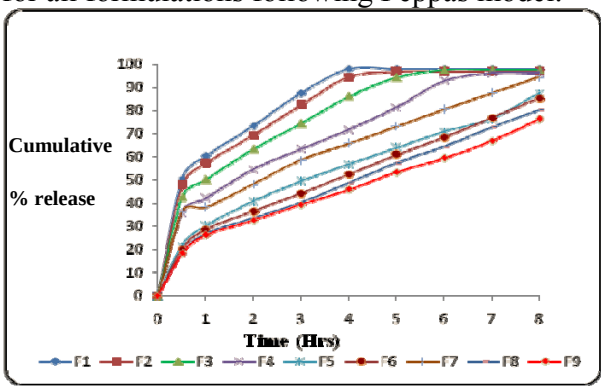


Figure 5: **CPR of insitu gel of Ambroxol-HCl based on sodium alginate batch F1-F9**

Table 3: Cumulative % drug release of in situ gels of Sodium Alginate batches F1 to F9

Time in hr	Cumulative % drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	50.3	47.6	42.5	35.8	21.4	19.9	36.2	18.1	18.2
1	60.3	57.2	50.1	42.1	30.2	28.5	38.1	26.8	26.2
2	73.27	69.36	63.26	54.5	40.76	36.5	48.2	33.5	32.5
3	87.38	82.27	74.36	63.26	49.45	44.2	58.5	40.2	39.2
4	97.8	94.38	86.03	71.77	56.72	52.4	65.8	48.8	45.8
5	97.8	96.7	94.29	81.3	63.86	60.8	73.2	57.3	53.3
6	97.8	96.7	97.3	92.8	70.77	68.4	80.5	64.4	59.4
7	97.8	96.7	97.3	96.09	76.38	76.7	87.5	72.8	66.8
8	97.8	96.7	97.3	96.09	87.4	85.2	94.4	80.2	76.2

Table 4: Release kinetics for sodium alginate based in situ gels of Ambroxol-HCl batches F1-F9

Batch no.	Regression			
	Zero order kinetic	First order kinetic	Higuchi kinetic	Korsmeyer- peppas model
F1	0.653	0.730	0.875	0.997
F2	0.672	0.735	0.900	0.949
F3	0.784	0.827	0.951	0.962
F4	0.879	0.926	0.984	0.981
F5	0.942	0.946	0.995	0.997
F6	0.961	0.978	0.988	0.989
F7	0.894	0.978	0.983	0.957
F8	0.961	0.982	0.982	0.974
F9	0.950	0.986	0.980	0.967

Table 5: Summary of results of regression analysis for sodium alginate based in situ gel of Ambroxol-HCl

Coefficient	B0	B1	B2	B11	B22	B12
Viscosity	248.67	100.33	28.833	-29	-183.4	6.25
n	0.3958	0.0533	0.0365	-0.05	-0.295	0.0058
Drug release in 30 minutes (%)	32.22	-11.32	-6.95	9.7833	-25.22	-2.55
Drug release in 4 hrs (%)	68.83	-19.64	-8.253	12.805	-52.65	-2.058
Drug release in 8 hrs (%)	90.143	-6.833	-4.932	0.87	-69.82	-4.425

Experimental Designing

The factorial design was carried out using the software DESIGN EXPERT® version 7.0.2.8 (Stat-Ease Inc., Minneapolis, USA). A quadratic model was obtained after analyzing data. Values of $p<0.05$ indicate model terms are significant. The statistical model comprising incorporated interactive and polynomial terms was utilized to evaluate the response. Once the uncoded values of factor levels were applied and response quadratic model was performed using DESIGN EXPERT® version 7.0.2, equations were obtained. Values of coefficients are mentioned in table 6. The resulted equations for all five dependent variables—Y1 (Viscosity), Y2 (n), Y3 (X30 min), Y4 (X4 hrs), and Y5 (X8 hrs)—in terms of coded factors are presented below.

$Y_1 = 248.67 + 100.33X_1 + 28.833X_2 - 29x_1^2 - 183.4x_2^2 + 6.25X_1X_2$

$Y_2 = 0.3958 + 0.0533X_1 - 0.0365X_2 - 0.05X_1^2 - 0.295X_2^2 + 0.0058X_1X_2$

$Y_3 = 32.22 - 11.32\ X_1 - 6.95X_2 + 9.7833\ X_1^2 - 25.22X_2^2 - 2.55X_1X_2$

$Y_4 = 68.83 - 19.64X_1 - 8.253X_2 + 12.805X_1^2 - 52.65X_2^2 - 2.058X1X2$

$Y5 = 90.143 - 6.833X_1 - 4.932X_2 + 0.87\ X_1^2 - 69.82X_2^2 - 4.425X_1X_2$

A positive value indicates a synergistic effect that favors optimization, while a negative sign represents an antagonistic effect or inverse effect of the factor on the selected response.

Both variables have positive effect on viscosity and negative effect on drug release study. Optimized batch with desired result of dependant variable can be prepared selecting level of independent variable from above polynomial equation and using design expert softwere.

Gel integrity test of optimized batch

Trial	Initial weight of gel (Gm)	Weight of gel after 2 hour (Gm)	% weight loss
With poymer beads	6	5.5	8.33
Without Polymer beads	6.3	5.5	11.11

(Formulation containing Sodium alginate 1.25%; Calcium chloride 0.1% which pusses optimized condition of dependant variable)

In situ gel formed in stomach is subjected to both hydrodynamic mixing and mechanical force. Mechanical force results from continuous stomach contraction that imposes a considerable force on stomach content. Disintegration is dominated by either fragmentation or erosion, depending on the physical forces acting on and the cohesive forces present within the matrix. Surface erosion is defined as the wearing away of surface of gel by an impinging gastric fluid containing food solids that causes normal impact, friction, and shear forces acting on the gel surface. a mechanical force was created resulting from the beads impacting and grinding the samples that caused erosion of gel, simulating the effect of contraction forces present in human stomach due to peristaltic movement. Using beads mechanical force is created in in vitro system to simulate stomach condition. It was observed that Presence of polymer beads does not show significant attrition effect by hydrodyanamic or mechanical force acting on gel that shows integrity of gel in Gastrointestinal tract.

CONCLUSION

Oral administration of aqueous solutions containing sodium alginate results in formation of in situ gel when comes in contact with stomach pH. Sodium citrate in formulation prevents reaction of calcium chloride with sodium alginate by complexation, at acidic pH Calcium ion becomes free from complex which reacts with sodium alginate to form gel in situ. The results of a 3^2 full factorial design revealed that the concentration of sodium alginate and concentration of calcium chloride significantly affect viscosity and drug release and release exponent.

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