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Floating *in-situ*-gelling gellan formulations of metformin hydrochloride

Linda Nassour[a*], Issa Hasan[a], Mazen El-Hammadi[a] and Habib Abboud[b]

[a]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Damascus University, Damascus, Syria*
[b]*Directorate of Laboratories and Research, Oversight Pharmaceutical, Damascus, Syria*

ABSTRACT

The present study focuses on the development and evaluation of Floating in-situ gelling system of Metformin hydrochloride (FIGC) using 3^2 factorial design experimentation. Metformin, an antidiabetic drug with absorption window limited to the upper part of small intestine was used as a model drug. The formulation contained Gellan Gum as gelling agent and Calcium Carbonate as floating agent. The principle of gelling involves supplying of complexed Calcium ions in form of Calcium Carbonate that are released in the acidic environment of the stomach leading to cross linking of Gellan and hence gelation. The concentrations of Gellan Gum (X_1) and Calcium Carbonate (X_2) were selected as the independent variables, the viscosity of the solution (Y_1), percent drug release at 4 hr (Y_2), and percent drug release at 8 hr (Y_3) were selected as the dependent variables. Differential scanning calorimetry (DSC) was used to check the presence of any interaction between the drug and the excipients. The in-situ gel forms were studied for their viscosity, in-vitro buoyancy ,and in-vitro drug release in acidic medium for about 8hrs.The results of a 3^2 factorial design revealed that the concentrations of Gellan Gum and CaCO3 significantly affected the dependent variables of viscosity, percent drug release at 4hr, percent drug release at 8 hr. Gellan based in-situ gelling system is promising for developing liquid oral delivery system for easy administration for elderly and patients with swallowing difficulties .

Key words: in-situ gelation, Floating, Gellan Gum, Metformin, Factorial design.

INTRODUCTION

The development of in-situ gelling systems has received considerable attention over the past few years. In-situ gel forming formulations present a novel idea of delivering drugs to patients as a liquid dosage, these hydrogels 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[1]. Some of the polysaccharides fall into the class of ion-sensitive ones , such as Carrageenan, Gellan Gum, Pectin, Sodium Alginate, which undergo phase transition in presence of various ions [2]. Gellan Gum is the generic name for extracellular polysaccharide produced by bacterium *Pseudomonas elodea*. It is an anionic deacetylated polysaccharide, with a tetrasaccharide repeating unit of one α-L-rhamnose, one β-D-glucuronic acid and two β-Dglucose residues [3]. It has the characteristic property cation-induced gelation. 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[4]. Metformin is antidiabetic or hypoglycemic agent which acts predominantly by inhibiting hepatic glucose release with an absolute oral bioavailability of 40% to 60%. Gastrointestinal absorptionoccurs mainly in the upper intestine with peak plasma concentrations (Cmax) reaching after 2 to 3 h[5], so it is worth using Metformin as a model drug in development of

gastro retentive systems. Comparative study for xyloglucan, Gellan and Alginate solutions loaded with Cimetidine was performed by Miyazaki et al , Dissolution experiments showed that increasing concentrations of the polymers decreased the drug release rate[6]. Floating in-situ gelling system of Amoxicillin and Clarithromycin for eradication of Helicobacter pylori was assessed by Rajinikanth et al, floating properties were obtained by addition of Calcium Carbonate. After immersion in simulated gastric fluid, immediate gelation with simultaneous production of Carbon dioxide was observed [7, 8] . In the present study we attempted to prepare floating in-situ gel system consists of Gellan solution with Calcium Carbonate (as a source of Ca^{+2} ions) and Sodium Citrate which complexes the free Ca^{+2} ions and releases them only in the highly acidic environment of the stomach, in this way, the formulation remains in liquid form until it reaches the stomach, so ensuring instantaneous gelation.in the acidic medium Calcium Carbonate effervesces, releasing Carbon dioxide and Calcium ions. The released Carbon dioxide is entrapped in the gel network, producing buoyant formulation and Calcium ion reacted with Gellan and produces a cross linked three dimensional gel network [9].

EXPERIMENTAL SECTION

Materials:
Metformin hydrochloride (IPCA laboratories limited, Mumbai), Gellan Gum (Sigma Aldrich, Germany), Calcium Carbonate (GmbH,Germany), Sodium Citrate (Loba Chemicals, Mumbai, India). All other chemicals were purchased and were of analytical grade.

Methods:
Differential scanning calorimetry (DSC) study:
Thermal analysis is an essential analytical method to study the interaction between drugs and excipients .To evaluate the drug-polymer interactions Differential scanning calorimeter (DSC) thermograms of the pure drug and physical mixture of the drug and polymer were studied using DSC apparatus (Mettler Toledo,OH,USA) at a rate of $10C^0$/min in the temperature range of 30-300 C^0.

Experimental Design:
A 3^2 factorial design was applied in these experiments, two factors each at three levels were selected and experimental trials were performed at all possible nine combinations. Selected independent variables studied were the concentration of Gellan Gum (X_1) concentration of Calcium Carbonate(X_2). Each of these independent variables was coded at three levels: low, medium, and high (−1, 0 and +1, respectively). The selected dependent variables were viscosity of the solution(Y_1), percent drug release at 4 hr(Y_2), and percent drug release at 8 hr (Y_3).

Preparation of the in-situ gelling solutions:
The in-situ gelling formulations were prepared as described by Rajinikanth et al [8].Gellan Gum solution of different concentrations were prepared in distilled water containing 0.25 % Sodium Citrate .The Gellan Gum solutions were heated to 90 ^{0}C with stirring. After cooling below 40 ^{0}C, various concentrations of Calcium Carbonate and drug were added and dispersed well with continuous stirring. The resulting Gellan in-situ gel solutions were finally stored in well closed container until further use.

pH Measurement:
The pH of the prepared formulations was measured by digital pH meter (Hanna PH meter, PH 211, USA).

Viscosity measurement of the in-situ gelling solutions:
Viscosity of the samples was determined using a Visco Plus apparatus (Fungi lab, Spain)with suitable spindle . The measurement was performed at room temperature(20-25 ^{0}C) and carried out in triplicate and the results were averaged.

Measurement of drug content:
Accurately, 10ml of in-situ gelling solution was added to 90ml 0.1(N) HCl of 1000 ml volumetric flask followed by sonication for 30 min, then volume was made up to the mark with0.1(N) HCl. The resulting solution was filtered through a membrane filter (0.45 µm). Then drug content of solution was measured at maximum wavelength of Metformin using UV-Visible Spectrophotometer (Hitachi, Spectrophotometer U-1800)after suitable dilution.

In Vitro Gelation Study:
10 ml of the prepared in-situ gel formulations was drawn up using disposable syringe and placed into gelation medium containing 0.1(N) HCl at 37±2 C^0 then gelation was observed visually .

In-vitro buoyancy study:
The in vitro floating study was determined using USP dissolution apparatus II having 900 ml of 0.1(N) HCl at 37±2 C^0 with a paddle speed of 50 rpm.10 ml of in-situ gelling solution was immersed into dissolution apparatus using disposable syringe. The time formulation took to emerge on the medium surface (floating lag time) and the time formulation constantly floated on the dissolution medium surface

(Floating time) were noted by visual observation.
In Vitro Drug Release Study:
The release rate of Metformin from the in-situ gelling sols was determined by using USP dissolution apparatusII (Pharma test PT-DT7,Germany) with a paddle speed at 50 rpm, this speed slow enough to avoid breaking of gelled formulation and ensured low level of agitation.The dissolution medium used was (900 mL 0.1 N HCl, pH=1.2) maintained at 37±2 C^0 . Ten milliliters of the formulation was transferred to the dissolution vessel using a disposable syringe without much disturbance, the needle was wiped clean and excess formulation was removed from the needle end. At a pre-identified time interval, an aliquot was removed and replenished with fresh medium[10]. The samples were assayed for MetforminusingUVspectrophotometer (Hitachi, Spectrophotometer U-1800) at the maximum wavelength after suitable dilution. A concurrent dissolution was performed with preparation devoid of drug to record the interference from excipients, if any. All the studies were conducted in triplicate, and the average was recorded.

Statistical analysis:
The statistical analysis of the factorial design formulations was performed by multiple regression analysis using SPSS14 statistical software . The significance level was considered to be $p < 0.05$.

RESULTS AND DISCUSSION

The DSC thermographs of the pure drug, Gellan Gum, and combination were obtained.The thermal curve of pure Metformin(Figure1) exhibited an initial flat profile followed by a sharp endothermic peak at 231.0°C.This transition is attributed to compound melting(11) .The thermograph of the drug-polymer physical mixture (Figure2) showed that the endothermic peak of the drug was shifted slightly to a lower value (229.81 ^{0}C)which is close to the melting point of Metformin. That means no significant interaction in physical mixture was appeared .

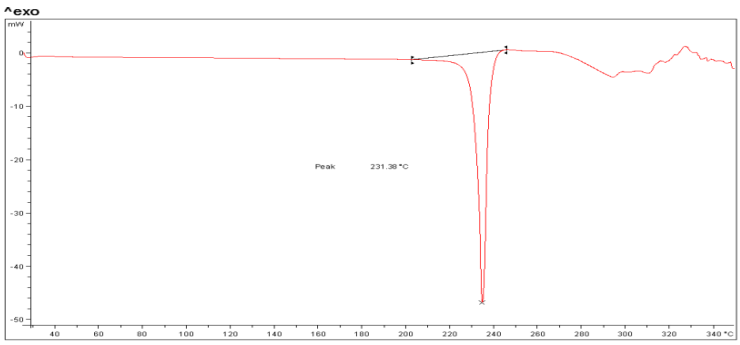

Fig (1):DSC thermographs of Pure Metformin

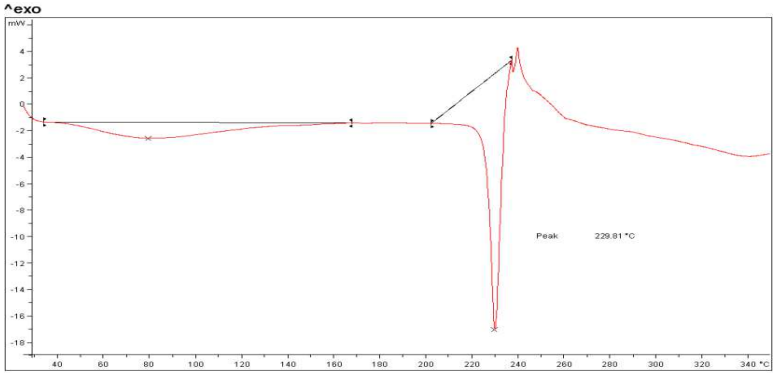

Fig (2): DSC thermographs for physical mixture of Metformin+ Gellan Gum

Evaluation of formulations:

Table (1,2) exhibit Factorial Design of In-situ Forming Formulation .The preparation of in-situ forming system is simple, and reliable, involving dissolving of Metformin in polymeric solution. As the solution comes in contact with gelation solution, it was immediately converted into stiff gel like structure. All the formulation had pH value around neutral or slightly alkali (Table 3), and percentage drug content from 92.66% to 96.06 %.

Table (1): 3^2Factorial Design of In-situ Forming Formulations

Formulation	Real value		Coded value		Dependent variables		
	X_1	X_2	X_1	X_2	Y_1	Y_2	Y_3
G1	0.25	0.5	-1	-1	144	75.13	95.44
G2	0.5	0.5	0	-1	231	73.11	94.27
G3	0.75	0.5	1	-1	347	63.59	83.24
G4	0.25	1	-1	0	183	76.84	93.51
G5	0.5	1	0	0	240	59.67	83.82
G6	0.75	1	1	0	382	57.56	81.83
G7	0.25	1.5	-1	1	196	69.33	84.07
G8	0.5	1.5	0	1	262	59.01	82.34
G9	0.75	1.5	1	1	387	51.74	81.16

Table (2): coded and real values of the in-situ Forming Formulation

	Coded value	Real value
	(x_1) Gellan Gum concentration%	**(x_2)Calcium Carbonate concentration%**
Low -1	0.25	0.5
Medium 0	0.5	1
High +1	0.75	1.5

Viscosity of In-Situ gelling solutions :

The formulation should have an optimum viscosity that allow easy of swallowing as a liquid, which then undergoes a rapid sol-gel transition due to ionic interaction[12]. Figures (3, 4 ,5) show the shear dependency of the viscosity which reduced upon application of the shear on the solutions, all polymer concentrations showed evidence of shear thinning behaviour, the effect being more pronounced at higher concentrations. This shear thinning behaviour provides an advantage for the administration process,shaking of the formulation will enhance its fluidity pour ability[13]. The solutions showed a marked increase in viscosity with increasing concentration of Gellan as shown in

(Table3). The observed increase in viscosity with increase in concentration has been noted previously for Gellan and was attributed to a consequence of increasing chain interaction with polymer concentration [14]. 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 increased viscosity[7].

Table (3):Evaluation of the in-situ gelling formulation

Formulation	pH	Floating time (hrs)	Floating lag time (sec)	Viscosity (cps)
G1	6.78	8	181	144±2.47
G2	7.46	8	165	231±2.36
G3	7.35	8	130	347±2.85
G4	7.03	8	119	183±1.32
G5	7.22	12	104	240±2.45
G6	7.14	12	87	382±2.12
G7	7.12	12	82	196±2.83
G8	7.45	12	75	262±2.43
G9	7.36	12	62	387±2.25

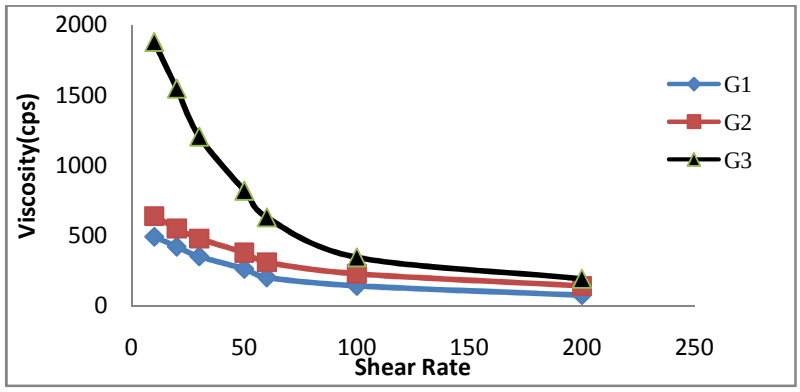

Fig (3):shear dependency of the in-situ gelling formulation (G1-G2-G3)

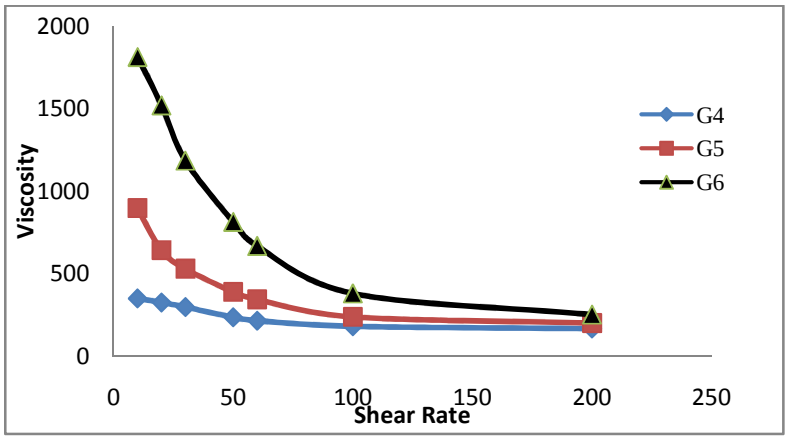

Fig (4): shear dependency of the in-situ gelling formulation (G4-G5-G6)

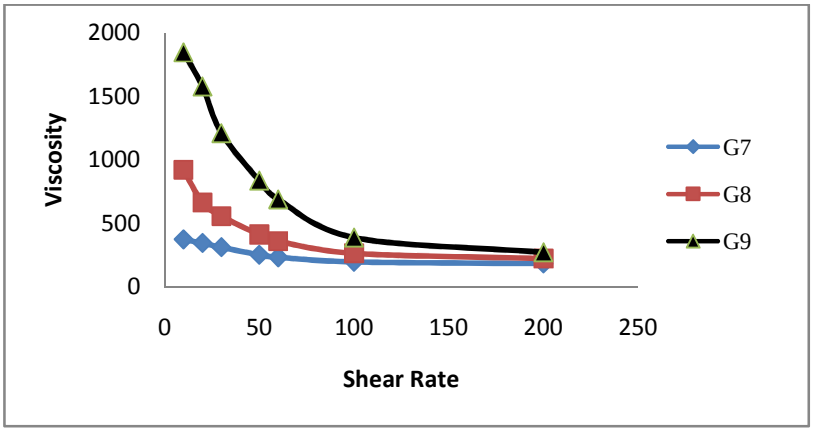

Fig (5): shear dependency of the in-situ gelling formulation (G7-G8-G9)

In-vitro buoyancy:

All prepared formulations of the factorial design were evaluated for their floating properties in simulated gastric fluid. The time for formulation to come to the medium surface (floating lag time) and the time the formulation maintained floated on the medium surface (duration of floating) were determined (Table3) . Upon contact with an acidic medium, Calcium Carbonate effervesced, releasing Carbon dioxide and Calcium ions. Then, gelation and cross-linking by Ca^{+2} ions took place to provide a gel barrier at the surface of the formulation. The released Carbon dioxide was entrapped in the gel network producing a buoyant preparation, which resulted in extended floating[15]. The floating properties of the formulation mainly depend on Calcium Carbonate, on increasing the Calcium Carbonate concentration, the floating lag time was reduced and the duration of floating was extended. The increasing amounts of Ca^{+2} and CO2 resulted from the increase in Calcium Carbonate concentration, are responsible for the observed reduction in floating lag time and increasing duration of floating. Similarly an increase in polymer concentration resulted in decreased floating lag time and increased floating duration of the prepared systems.

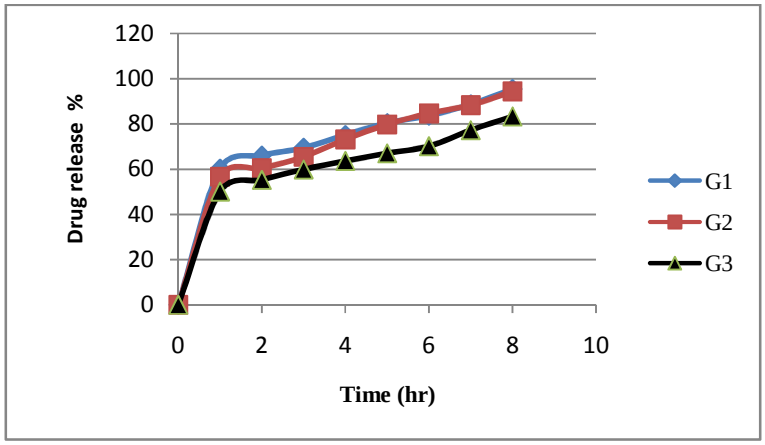

Fig (6): Drug release profiles from the formulations (G1-G2-G3)

In Vitro Drug Release:

Drug release profiles from the formulations at various concentrations of Gellan Gum are shown in Figs(6,7,8). The drug release decreased with increase in the concentration of Gellan Gum 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[9].The released Carbon dioxide is entrapped in the gel network producing buoyant formulation and then Calcium ion reacted with Gellan produced a cross linked three dimensional gel network that might restrict the further diffusion of Carbon dioxide and drug molecules and has resulted in extended period of drug release. The release of drug 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 followed by a second face of moderate release. The initial burst effect was considerably reduced with increase in polymer concentration[16]. As the percentage of Calcium Carbonate increased, the release rate decreased due to the stronger gel formation occurred in presence of increasing concentrations of Ca^{+2}ions, which leads to a slower release of the drug from these gels.

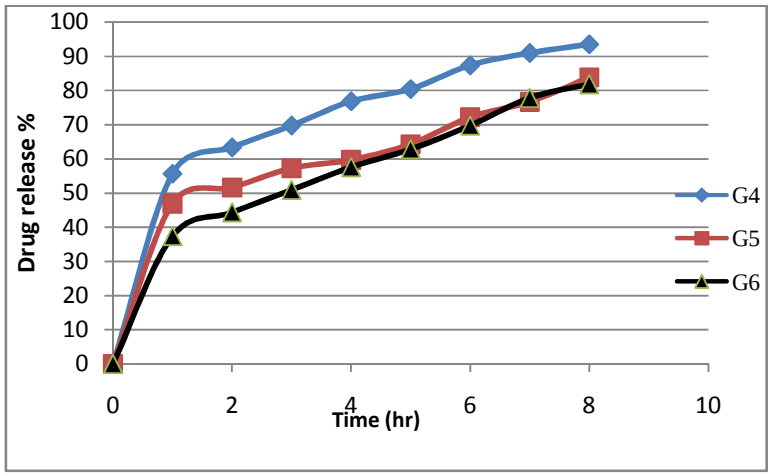

Fig (7): Drug release profiles from the formulations (G4-G5-G6)

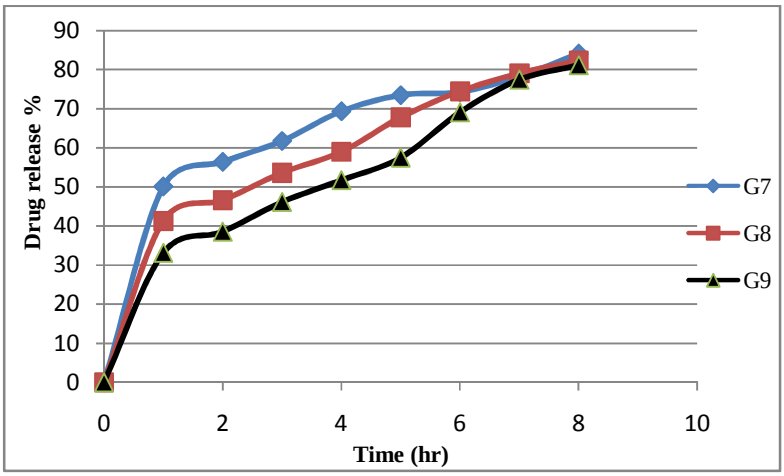

Fig (8): Drug release profiles from the formulations (G7-G8-G9)

Statistical analysis:

To study the effect of independent variables on the selected dependent variables multiple regression analysis, and Analysis of variance (ANOVA) was applied for estimation of significance of the model. Using a 5% significance level, a model was considered significant if the $p<0.05$, it was found that both factors had statistically significant influence on all of the dependent variables ($p < 0.05$)(Table 5). The high values of R^2 indicate clearly that the responses were strongly dependent on the factors studied (Table 3).

The resulted equations for all dependent variables in terms of coded factors are presented in (Table 4)based on this model:

$$Y= b_0+b_1 X_1+b_2 X_2$$

While Y is the dependent variable, b_0 is the arithmetic mean response of the 9 runs,X_1,X_2 is the independent variables, b_1,b_2 are the coefficients of the parameters.

Table (4): Summary of regression output of significant factors for measured responses

Response	coefficients				
	B_0	B_1	B_2	R^2	Equation
Y_1 (viscosity)	24.889	395.333	41.000	0.984	Y_1= - 94.72+96.5(X_1)+26.33(X_2)
Y_2(% drug release at 4h)	91.829	-32.273	-10.583	0.946	Y_2=91.829 - 32.273(X_1) -10.583(X_2)
Y_3(%drug release at 8 h)	104.021	-17.860	-8.460	0.899	Y_3=104.021 - 17.860 (X_1) -8.46(X_2)

Table (5): Results of the ANOVA for the model

Source of variation	DF	SS	MS	F	P value
(Y1)Viscosity					
Regression	2	61129.667	30564.833	88.827	0.000
Residual	6	2064.556	344.093		
Total	8	63194.222			
Y2(% drug release at 4 h)					
Regression	2	558.598	279.299	25.361	0.001
Residual	6	66.077	11.013		
Total	8	624.675			
Y3(%drug release at 8 h)					
Regression	2	226.975	113.487	12.691	0.007
Residual	6	53.652	8.942		
Total	8	280.627			

DF is the degree of freedom, SS is the sum of squares and, MS Mean Square, F is the Fischer's ratio.

CONCLUSION

This study showed the feasibility of in-vitro gel forming from aqueous solutions of Gellan Gum containing Ca^{+2} ions in a complexed form and sustaining the drug release from the in-situ gels over the period of the study (8 h). The results of a 3^2 factorial design revealed that independent variables had significant effect on the selected responses. Hence the in-situ gelling formulation may represent a promising approach of novel liquid oral drug delivery system which may improve the patient compliance especially for elderly and patients with swallowing difficulties.

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