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Design, Optimization & In-Vitro Evaluation of Sodium Alginate Based Gastro-Retentive Floating In-Situ Gel Of Simvastatin in Hyperlipidemia Treatment

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ABSTRACT

Hyperlipidemia (Raised cholesterol levels) increases the potential risks of heart disease and stroke both in the developed and developing world. Drug Simvastatin, is an antihypertensive agent used in treatment of hyperlipidemia. Simvastatin is a derivative of lovastatin and potent competitive inhibitor of 3-hydroxy-3-methyleglutaryl coenzyme is a lipid-lowering medication, with a very short biological half-life of 2 hours, it extensively goes in first pass metabolism. The aim of present investigation is to formulate, optimize & characterized novel gastro-retentive in-situ gel system of simvastatin to avoid challenges associated with rapid gastric transition from stomach & decrease the frequency of dosing with increase enough residence time in stomach. Different polymers like sodium alginate, methyl cellulose were screened at different concentration to finalized optimum formulation based on in-vitro gelling time with drug release behavior. All the batches (ASF1-ASF6) were evaluated for pH, viscosity, gel strength, in-vitro floating ability, drug content determination and in-vitro release studies. The gel strength was conducted in 0.1 N HCl with 1% SLS (pH-1.2). All the formulations showed good gel strength. Formulations ASF1, ASF3, ASF4.and ASF5 were best fitted to Zero order model while formulations ASF2, and F6 were best fitted to Korsmeyer-Peppas modelas evident from correlation coefficients. Formulation ASF3 was found optimized the release as per korsemeyer-peppas model and drug-release from the formulation can be best explained by Higuchi model due to highest value of R-square among all the models. It can be concluded that the In-situ gel was beneficial for delivering the drug which needs sustained release to achieve the slow action.

Key-words: Gastro-retentive in-situ gel system, Simvastatin, Hyperlipidemia, sodium alginate.

Introduction

Unhealthy diet, lack of physical activity, smoking or exposure to tobacco smoke, being overweight or obese and familial hypercholesterolemia are the main causes of hyperlipidemia condition. Hyperlipidemia is a medical term denotes abnormally high lipids (cholesterol & triglycerides) level in the blood. Mostly common type of hyperlipidemia is associate with high cholesterol condition in other term "BAD" cholesterol related. Cholesterol is a fatty material that travels through the bloodstream on protein called lipoproteins. The cholesterol can deposit in blood vessel walls and restrict blood flow.[1] In the United States, people over age 20 years 94 million have elevated total cholesterol levels. The condition associated with hyperlipidemia does not cause any symptoms. This condition is diagnosed by routine blood tests, recommended

for adults in every five years. The primary use of simvastatin is for the treatment of hyperlipidemia and the prevention of cardiovascular diseases. Oral dosage forms pose low bioavailability problems due to their rapid gastric transition from stomach, especially in case of drugs which are less soluble at alkaline pH of intestine. Similarly, drugs which produce their local action in stomach get rapidly emptied and do not get enough residence time in stomach. So, frequency of dose administration in such cases is increased.[1, 2] To avoid this problem floating drug delivery system is developed with available approaches. Different types of gastro-retentive approaches are available like

- High-density system
- Floating system
- Swelling system
- Muco-adhesive system.

Oral gastro-retentive in-situ gel forming system also known as stomach specific or raft forming systems have providing the controlled drug delivery within stomach with increased retention time. The problems with tablets/capsules floating dosage forms compare to liquids as they are stable but need to swallow as whole unit. The formulation shall be design with the objective to retain in stomach for an extended time period to obtain better bioavailability, its increase gastric retention time of the drug.[3]

Materials & Method

Simvastatin was obtained as gift sample from Hiral pvt limited, Uttarakhand. Sodium alginate was obtained from Loba chemicals (Mumbai), Methyl cellulose from Ases chemical work, (Jodhpur), Calcium carbonate from S. D. fine chem ltd (Mumbai), HCl from Merk industry (Mumbai), purified water in house laboratory and all other chemicals and solvents used were of analytical grade.

Compatibility Studies: Analysis of Simvastatin

1. Fourier Transform Infrared Spectroscopy (FTIR)

The IR analysis of the sample was carried out for qualitative compound identification, used to record IR spectra of the prepared disc, to confirm any interaction of Simvastatin with other excipients of dispersion. The pellet of approx. 01 mm diameter of the drug was prepared grinding 3-5 mg of sample with 1-1.5 grams of potassium bromide (KBr) in pressure compression machine. The procedure consisted of dispersing a sample of drug alone or mixture of drug and excipients in KBr and compressing into disc by applying pressure. Then placed the pellet in the light path & obtained the spectrum. The sample pellet was mounted in IR compartment & scanned at wavelength 4000 cm^{-1}-400 cm^{-1}. [4]

2. Ultraviolet Spectroscopy

UV-spectroscopy analysis of drug was carried out for wavelength maxima and absorbance determination and calibration of standard curve of drug. Forming various sample solutions 10-60 mcg/ml in 0.1 N HCl, pH 1.2 of drug & run the spectroscopy in the range 200-400 nm to obtained the absorbance for their relative concentration was measured. [5]

3. Solubility

Determination of solubility was performed according to Higuchi & Connors. The solubility of Simvastatin was determined in different solvents. An excess quantity of the drug was added in 10 ml of each solvent in screw capped glass test tubes and shaken 24 hours at room temperature. The filtered solution was then diluted and the solubility was determined by spectrophotometrically.

Calibration Curve of Simvastatin

Preparation of Buffer 0.1 N HCl (pH-1.2)

0.85 ml concentration of HCl dissolved in a 100 ml of distilled water and sonicated for 10 mins to dissolve the HCl, and then check the pH at 1.2. Finally 0.1 N Solution (pH-1.2) is prepared and used as a blank solution.

Determination of Absorption maxima

A UV absorption maxima was determined by scanning a 10-60 ug/ml solution of Simvastatin in 0.1 N HCl (pH-1.2) between 200-400 nm.

Preparation of Calibration Curve

Simvastatin was found to be soluble in methanol and HCl. A simple reproducible

method of estimation was carried out in methanol and HCl ranging from 10-60 mcg.ml solutions at 242 nm against the blank the standard graph obtained was linear with regression coefficient 0.997. Sample of 10 mg of Simvastatin was weighted accurately and dissolved in 5 ml of 0.1 N HCl in a 100 ml of volumetric flask and volume was made up to with the 0.1 N HCl (pH-1.2) to obtain a stock solution of 100 ug.ml. The calibration curve was plotted between concentration and absorbance.[6]

Preparation of Gastro-retentive Floating In-situ gel of Simvastatin

Simvastatin was passed through sieve no # 60 and excipients were passed through sieve no # 40 and guar gum through #100 to form free flowing powder. In a beaker, aqueous solution of sodium alginate (different concentrations) was prepared and methyl cellulose (1.0%w/v) and Simvastatin (400mg) both were added to it with continuous stirring so that there was a proper and homogeneous dispersion of the drug. (By mixing method)

In another beaker, different concentrations of calcium carbonate solutions were prepared in de-ionized water by heating below 60^0C and cooled to below 40^0C. Both solutions were mixed properly and finally, volume was adjusted with de-ionized water to get the desired preparation.[7]

Table 1: Preparation of Gastro-retentive Floating In-situ gel of Simvastatin

Ingredients	Formulation code and Quantities					
	F1	F2	F3	F4	F5	F6
Simvastatin (mg)	400	400	400	400	400	400
Sodium Alginate (gm)	0.5	1.0	1.5	2.0	2.5	3.0
Guar gum (mg)	250	250	250	250	250	250
Calcium carbonate (mg)	250	250	250	250	250	250
Methyl cellulose (gm)	0.5	1.0	2.0	3.0	4.0	5.0
Distil water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Total weight (ml)	100	100	100	100	100	100

Characterization of Gastro-Retentive Floating In-Situ Gel of Simvastatin

Physical Appearance and pH

All the prepare Gastro-retentive Floating In-situ gel of Simvastatin solution were checked for their clarity and the type of the solution. After administration of the prepared solution in (0.1 N HCl, pH 1.2) also checked the time required for gel formation and type of gel formed. The pH was measured in each of the solution of sodium alginate in-situ solution using a calibrated digital pH meter at 27^oC. The each pH data was measured in triplicate.[8]

Viscosity of In-situ gelling solution

Viscosity of the formulations was determined using Brookfield digital viscometer (DV-III, USA). The formulation was taken in a beaker and maintained at room temperature. For determination of viscosity, spindle no. 63 was selected. Viscosities were determined at 100 rpm at room temperature.[9]

In-vitro Floating behavior

The *in-vitro* floating study was carried out using 0.1N HCl with 1% SLS (pH 1.2). The medium temperature was kept at 37±0.5°C. Ten milliliter formulation was introduced into the dissolution vessel containing medium without much disturbance. The time the formulation took to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on surface of the dissolution medium (duration of floating) were evaluated.[10]

In-vitro gelling capacity

Gel strength is indicative of the tensile strength of the gelled mass. The gel strength of the formulation is an important variable dependent on the concentration of the gelling agent as well as cation source. The gel strength was

determined by placing 10 ml of solution in 50 ml of 0.1N HCl freshly prepared and after gelation the HCl was drained off leaving the gel mass which is then assembled at the weighing balance to measure the penetrating weight. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel and time period for which they formed gel remains as such. [11]

Drug content determination

Accurately, 10 ml of *in situ* gel (equivalent to40 mg of Simvastatin) was measured and transferred to100 ml of volumetric flask. To this 70 ml of 0.1N HCl was added and shaken on mechanical shaker for 30 min and volume was made up to 100 ml and followed by sonication for 15 min. complete dispersion of contents were ensured visually and filtered using What-man filter paper. From this solution, 10 ml of sample was withdrawn and diluted to 100 ml with 0.1N HCl. Content of Simvastatin was determined spectrophotometrically at 242nm using double beam UV-Visible spectrophotometer (Systronics 2203). [12]The estimated drug content of prepared *in situ* gel formulations of Simvastatin

Drug content= Practical content/ Theoretical X100 (%)

In-vitro release studies

The drug release studies were carried out in USP dissolution test apparatus-II (Paddle type apparatus, Lab India DT 8000) at 37 ± 0.5°C at 50 rpm using 900 ml of 0.1N HCl with 1% SLS (pH 1.2) as a dissolution medium (n=6). *In-situ* gel equivalent to 40 mg of Simvastatin (10 ml) was used for test. 10 ml of aliquot was withdrawn at predetermined time intervals of 15, 30, 45, 60, 90, 120, 180, 240 min. and replenished with fresh dissolution medium. The contents were filtered using What-man filter papers (41micron) and analyzed at 242 nm using UV/Visible spectrophotometer. The drug release data of formulations along with theoretical profile are presented. The cumulative percentage drug release was plotted against time to determine the release profile. Consider a constant zero order drug release in 4 hours, a theoretical reference (T.R.) [13]

Drug release kinetics studies

The drug release kinetic studies were done by various mathematical models (zero order, first order, Higuchi's squre root, Hixson-crowell cube root law and Pappas equation). The model that best fits the release data is selected based on the correlation coefficient (r) value in various models. The model that gives high "r" value is considered as the best fit of the release data. The release constant was calculated from the slope of the appropriate plots, and the (r^2) was determined.[14]

Result and Discussion

Determination of UV Absorbance Maxima of Simvastatin

The standard stock solution was used to determination the λ max of (0.1 N HCl, pH 1.2) was used as blank for the study. The spectrum was taken between the UV ranges of 200-400 nm. The highest peak obtained from the spectrum analysis was taken as λ max for Simvastatin.

Table 2: Calibration curve data of Simvastatin

S. No.	Conc. (mcg/ml)	Absorbance	± SD
1	**10**	**0.373**	**±0.011**
2	**20**	**0.487**	**±0.021**
3	**30**	**0.619**	**±0.035**
4	**40**	**0.778**	**±0.050**
5	**50**	**0.930**	**±0.021**
6	**60**	**1.086**	**±0.033**

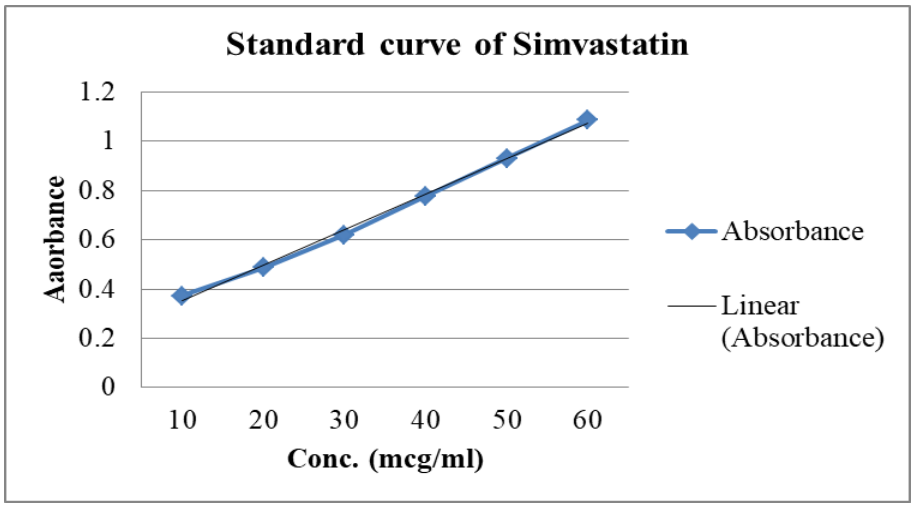

Fig. 1: Standard curve of Simvastatin

Solubility of Simvastatin was determined in different solvents and the observations shown in (Table 3). The maximum solubility was found in Chloroform, methanol and insoluble in water.

Table 3: Solubility profile of Simvastatin

S. No.	Solvents	Solubility
1	Distilled water	+
2	Acetic acid	++++
3	Ethanol	++++
4	0.1 N HCl (pH-1.2)	++++
5	Chloroform	+++++
6	Methanol	++

Insoluble +; freely soluble +++++; soluble ++++; slightly soluble +++

The In-situ gelling system being one among them is a type of raft-forming system principally capable of releasing drug molecules in sustained patterns relative to constant plasma profile.

pH of Simvastatin In-situ gels

Table 4: pH-profile of in-situ gel formulations

Parameter	Formulation code					
	F1	F2	F3	F4	F5	F6
pH*	7.3±0.1	7.3±0.2	7.0±0.3	7.4±0.1	7.4±0.2	7.0±0.3

The pH of the prepared *in situ* gel formulations of Simvastatin was found to be satisfactory and was in the range of 7.0±0.3 to 7.4±0.2. The pH was found to increase with increasing the concentration of sodium alginate and calcium carbonate.

Viscosity of In-situ gelling solution

Table 5: Viscosity-profile of in-situ gel solution formulations

Parameter	Formulation code					
	F1	F2	F3	F4	F5	F6
Viscosity(cps)*	230 ±0.6	271±0.5	310 ±0.1	358 ±0.2	374±0.8	421±0.9

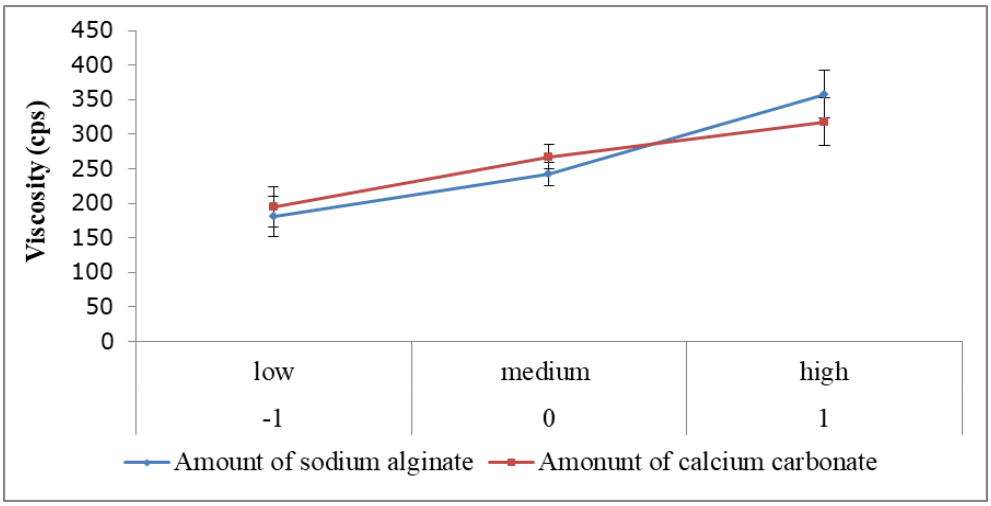

Figure 2: Average effect of sodium alginate concentration on viscosity of Simvastatin *in-situ* gel formulation

The viscosity of prepared *in situ* gel formulations of Simvastatin was found to be in the range of 230 ±0.6 to 421±0.9and the order of increasing viscosity of formulations were F1<F2<F4<F3<F5<F6 respectively.

In-vitro Floating behavior

(a) Floating behaviour of F3 **(b) Gelling behaviour of F3**

Figure 3: Floating and gelling behaviors of *in-situ* gel of Simvastatin formulation F3

Table 6: Floating-time of in-situ gel formulations

Parameters	Formulation code					
	F1	F2	F3	F4	F5	F6
Floating lag time* (sec)	38±0.8	24±0.4	15 ±0.7	36±0.5	26±0.3	22±0.2
Duration of floating (hr)	>12	>12	>12	>12	>12	>12

The floating lag time of prepared *in situ* gel formulations of Simvastatin was found to be in the range of 15±0.7 sec to 38±0.8 sec and duration of floating was found to be greater than 12 hr.

In-vitro gelling capacity

Table 7: In-situ gelling capacity of in-situ gel formulations

Parameter	Formulation code					
	F1	F2	F3	F4	F5	F6
Gel strength(g/cm2)	19.5±0.1	23.6±0.3	40.06±0.2	36.06±0.6	56.46±0.2	69.60±0.1

The gel strength was conducted in 0.1 N HCl with 1% SLS (pH1.2). All the formulations showed good gel strength. The *in situ* gel so formed had good gel strength and thus expected to preserve its integrity without dissolving or eroding so as to localize the drug at absorption site for extended duration.

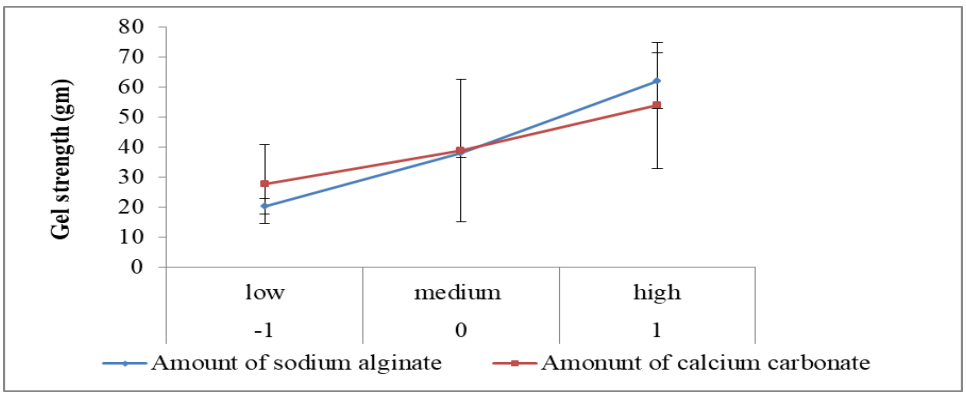

Figure 4: Average effect of independent variables (concentration of Sodium alginate and calcium carbonate) on gel strength of floating *in-situ* gel of Simvastatin.

Drug contents Determination

Table 8: Drug-content determination of in-situ gel formulations

F1	F2	F3	F4	F5	F6
95.2±0.4	95.8±0.5	96.4±0.3	96.9±0.5	97.2±0.4	97.7±0.3

The percentage drug content of prepared *in situ* gel formulations of Simvastatin were found in the range of 95.2±0.4 to 97.7±0.3, indicating homogenous distribution of drug throughout the formulations.

In-vitro drug release study

Table 9: Cumulative percentage of drug release of in-situ gel formulations F1-F4

Time (min)	Cumulative percentage drug release*			
	F1	F2	F3	F4
0	0	0	0	0
15	2.90±0.2	2.46±3.5	2.03±2.1	1.79±0.7
30	4.60±0.8	4.22±2.1	3.78±1.3	3.19±1.2
45	7.35±1.4	7.02±0.6	6.78±0.7	6.22±0.5
60	10.35±0.2	9.87±0.4	8.90±0.5	7.74±0.6
90	13.56±2.4	12.59±0.2	11.37±0.2	10.83±0.3
120	19.89±0.1	18.70±0.1	17.18±0.5	16.22±0.4
180	29.39±0.4	28.52±0.2	27.09±0.1	25.86±0.4
240	32.07±0.2	31.59±0.4	30.92±0.7	28.06±0.1
f_2 value	70.68	71.42	73.83	73.65

Table 10: Cumulative percentage of drug release of in-situ gel formulations F5-T.R

Time (min)	Cumulative percentage drug release*		
	F5	F6	T.R
0	0	0	0
15	1.62±0.1	1.56±0.1	2.08
30	2.93±1.1	2.18±1.1	4.16
45	5.64±0.4	5.09±0.7	6.25
60	7.12±0.6	6.67±0.3	8.33
90	9.85±0.7	9.02±0.4	12.57
120	15.85±0.3	15.09±0.8	16.66
180	24.70±0.4	24.37±0.3	25.67
240	28.06±0.2	27.84±0.3	32.52
f_2 value	71.17	71.98	

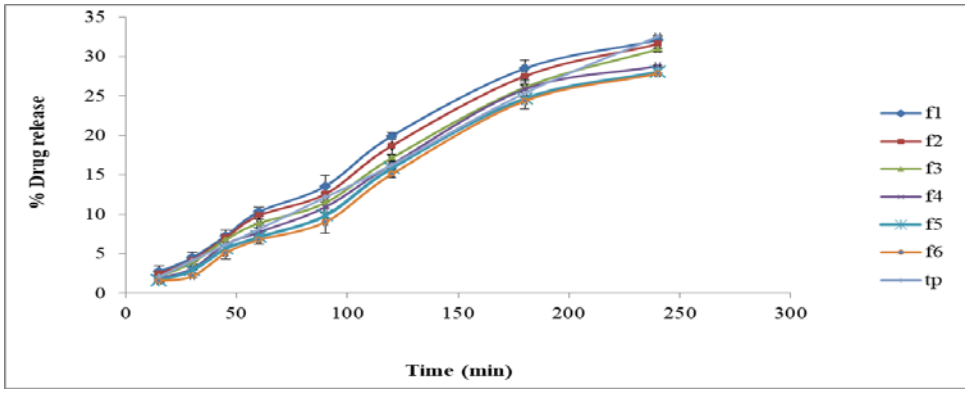

Figure 5: Cumulative in-vitro drug release of formulation F1-TR

The extent of drug release was observed in order of F1>F2>F3>F4>F5>F6. Formulations containing higher amounts of sodium alginate and calcium carbonate released the drug for longer period of time at slower rate.

The release of drug from the *in situ* gel formulations was characterized by an initial phase of high release (burst effect). However, as gelation proceeds, the remainingdrug was released at a slower rate followed by a second phase of moderate release. This biphasic pattern of release is a characteristic feature of matrix diffusion kinetics. The initial burst effect was considerably reduced with increase in polymer concentration.

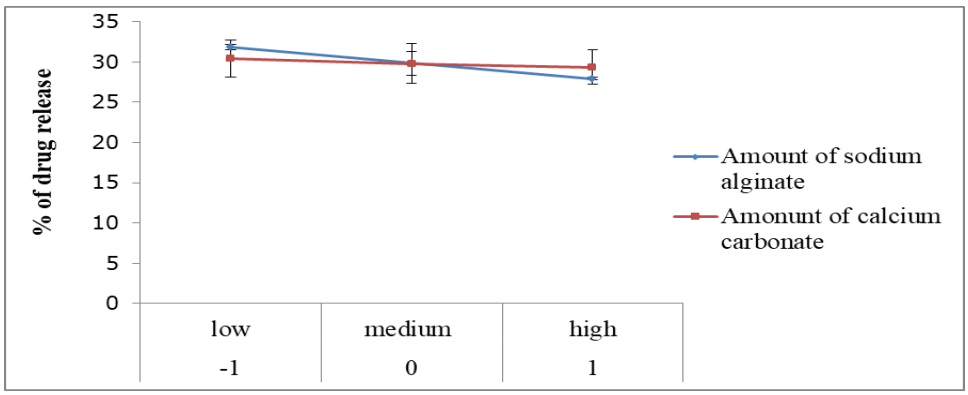

Figure 6: Average effect of independent variables (concentration of sodium alginate and calcium carbonate) on percentage drug release (at 240 min) of floating *in-situ* gel of Simvastatin Model fitting for release kinetics

Table 11: Model-fitting for release kinetics of in-situ gel formulations F1-F6

Formulation code	Zero order	First order	Higuchi Matrix	Hixon-Crowell	Korsmeyer-Peppas			Best Fit model
	R	R	r	r	r	n	K	
F1	**0.995**	0.918	0.954	0.990	0.989	0.001	1.025	Zero order
F2	0.992	0.919	0.958	0.989	**0.996**	0.001	1.008	Peppas
F3	**0.995**	0.920	0.961	0.987	0.993	0.001	1.041	Zero order
F4	**0.991**	0.919	0.960	0.984	0.989	0.000	1.190	Zero order
F5	**0.991**	0.921	0.960	0.983	0.990	0.001	1.243	Zero order
F6	0.990	0.923	0.962	0.979	**0.992**	0.001	1.240	Peppas

Formulations F1, F3, F4.and F5 were best fitted to Zero order model while formulations F2, and F6 were best fitted to Korsmeyer-Peppas model as evident from correlation coefficients. The formulations best fitted to Zero order model suggesting that the drug release from *in-situ* gel was diffusion controlled release which may be due to the swelling nature of polymers. The 'n' value obtained from Peppas equation was less than 0.5, which indicated that the formulation showed drug release by Fickian diffusion mechanism.

Conclusion:

Formulation F3 was found to be the best formulation. The developed formulation because the we used medium value of sodium alginate and high value of calcium carbonate that have provided good result of all evaluation parameter. So sustain release is better and have better bioavailability.

Formulation F3 was found to be the optimized formulation because it showed highest in similairty factor (f_2) was 73.83. Formulation F3 was found to be the best formulation. The developed formulation because the we used medium value of sodium alginate and high value of calcium carbonate that have provided good result of all evaluation parameter. So sustain release is better and have better bioavailability.

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