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Research Article

Formulation and Evaluation of Floating *In-Situ* Gel of Nicardipine Hydrochloride

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Abstract

The present research work aimed to formulate and evaluate a gastro retentive in situ gelling system of Nicardipine Hydrochloride using Sodium Alginate as gelling polymer, HPMC K100M as release retard polymer, calcium carbonate as a cross-linking agent, and tri-sodium citrate as fluidity enhancer agent to treat hypertension. The mechanism for the floatation was based on ionic cross-linking. Several evaluation tests were carried out for pre and final formulation evaluation. Based on the outcomes white-colored, viscous solution of uniform consistency was obtained. Batch B1 was very well prepared for the ability to control long-term drug release. The drug content was found to be > 97 %, the viscosities were in the acceptable range suitable for swallowing, and pH was found to be in the range of 7.33 – 7.68 which was compatible with oral digestion. Design expert 13 Software was used to derive results of interaction and responses based on the concentration of polymer and statistical analysis. The optimized formulation i.e., Batch B1 (0.6 % w/v and 0.5 % w/v HPMC K100M) showed a slow drug release of 96.44 % up to 12 hours. The best fit model for the drug release followed the Higuchi model which explained that the drug release occurred by the Fiskian mechanism i.e., a combination of both diffusion and erosion. The in-situ gel prepared can ultimately provide prolonged release, enhance the bioavailability of the drug and increase patient compliance.

Keywords: - In situ gel, Ionic cross linking, Sodium alginate, HPMC K100M, Nicardipine Hydrochloride.

INTRODUCTION

Administration of drug by the oral route is considered to be the preferred route due to its ease of administration. Gastric retention has received attention in recent years as many of the conventional oral drug delivery systems have some limitations in terms of rapid gastric emptying time. Gastro retentive drug delivery system (GRDDS) is a site-specific drug delivery system that can remain in the gastric area for several hours, thus significantly increasing the gastric residence time of drugs. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves the solubility of drugs that are less soluble in a high pH environment. Various approaches to achieve gastric retention include floating system, bio adhesive system, high-density system, swelling and expanding system, etc. has been developed[1–4].

Table 1: Gastroretentive Drug Delivery Systems Vs. Conventional Drug Delivery Systems.

Sr No.	Factors	Conventional Drug Delivery System	Gastroretentive Drug Delivery System
1.	Toxicity	High risk of toxicity	Low risk of toxicity
2.	Drugs with narrow absorption window in small intestine	Not suitable	Suitable
3.	Drugs having rapid absorption through upper GIT	Not very beneficial	Very beneficial
4.	Drugs which degrade in the colon	Not very beneficial	Very beneficial
5.	Patient compliance	Less	Improves patient compliance
6.	Drugs acting locally in the stomach	Not very beneficial	Very beneficial
7.	Drugs which are poorly soluble at an alkaline pH	Not very beneficial	Very beneficial
8.	Dose dumping	High risk of dose dumping	No risk of dose dumping

Floating drug delivery system

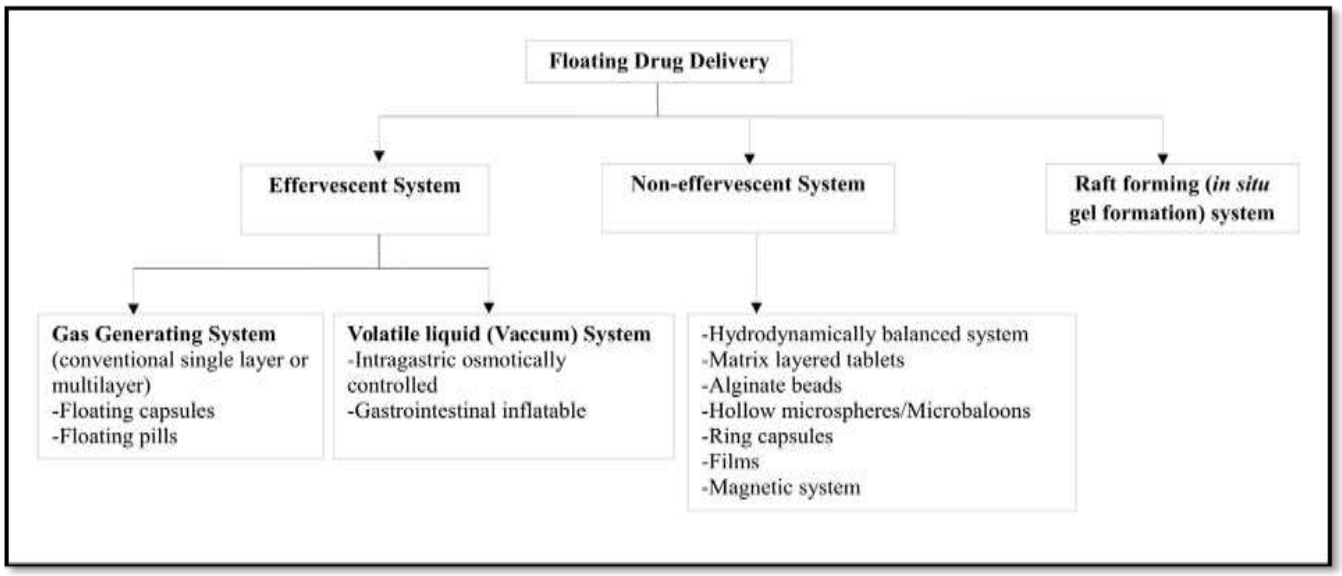

Figure 1: Classification of floating drug delivery system

Floating drug delivery systems float immediately upon contact with the gastric fluid. It is due to its property of low bulk density (< 1.00 g/cm^3) and it provides sufficient buoyancy to remain float over gastric fluid for a prolonged period of time while the drug release at the desired rate at specific site[5–7].

The "in-situ gel" system has been known to be one of the best new drug delivery systems. The in-situ gelling system aids to improve the controlled and sustained release of the drug, patient compliance, and comfort due to the special features of the "sol to gel" transition. An **in-situ gelling system** is a formulation that is in the form of a solution before it enters the body, but it transforms into a gel under one or combinations of a variety of physiological conditions. The sol-to-gel transition depends on various factors, which include pH, temperature changes, solvent exchange, ionic cross linking, etc. The "in-situ gelling system" has several advantages, including ease of dose administration, reduced dosing frequency, etc. Pectin, gellan gum, chitosan, alginic acid, guar gum, carbopol, xyloglucan, xanthan gum, HPMC, poloxamer, etc. are some of the natural polymers used in the in-situ gelling systems[8–11].

Nicardipine HCl is a dihydropyridine calcium channel blocker used for blocking the transmembrane Ca^{+2} channels and cause coronary and peripheral vasodilatation. Its primary use is for the management of angina pectoris, Hypertension and cardiovascular diseases. It is belonging to BCS class-II having 35 - 40% bioavailability on oral administration. It has good solubility at low pH values, but poor solubility at higher pH values. Therefore, Nicardipine HCl is likely to be absorbed only in the stomach and in the upper part of the intestine tract. Because of its short half-life (2-4 h), the drug has to be given frequently (20 mg , 3 times daily).Conventionally, the drug is available as a tablet which is given 3 times a day with a dose of 20 mg for the therapeutic effect. The frequency of this dosage promotes the formulation of twice a day in situ gel to overcome the problem of multiple administrations and reduced patient compliance. Also, formulation of a prolonged release dosage form using Nicardipine HCl can enhance the bioavailability of the drug up to 100 % in comparison to multi dose tablets of Nicardipine HCl, which shows a low bioavailability of 35 – 40 %. Twice in a day can also prevent the fluctuations in the plasma drug concentration. However, it would be easier for them to partake dosage in liquid form who is suffering from hypertension. Every dose of 5 ml in situ will contain 20 mg of drug which will be administered to the patient, to overcome the problem of patient incompliance due to multiple administrations. Nicardipine Hydrochloride in situ floating gel will retain in the gastric area for a prolonged period of time and will release the drug in sustained manner[12–14].

MATERIALS AND METHODS

Method of preparation of in situ polymeric solutions

Using the magnetic stirrer, fluidity enhancer agent was added in 100 ml of distilled water. Gelling agent was added when the temperature reached 70° C, and then release retard polymer was added. The temperature was maintained at 70° C and then stirred continuously to obtain a clear solution. The obtained clear solution was cooled to 40° C and then cross-linking agent was added. The temperature was maintained at 40° C, finally the drug, preservative and sweetening agent were added in the solution along with gas generating agent. The solution was stirred continuously till a uniform solution was obtained. Table 2 represents composition of preliminary formulations prepared.

Table 2: Composition of preliminary formulations.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Nicardipine Hydrochloride (mg)	20	20	20	20	20	20	20	20	20	20	20	20
Sodium alginate (% w/v)	0.25	0.5	0.75	-	-	-	-	-	-	-	-	-
Pectin (% w/v)	-	-	-	0.25	0.5	0.75	-	-	-	-	-	-
Gellan gum (% w/v)	-	-	-	-	-	-	0.25	0.5	0.75	-	-	-

Sodium CMC (% w/v)	-	-	-	-	-	-	-	-	-	0.25	0.5	0.75
Tri-sodium citrate (% w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HPMC K100M (% w/v)	1	1	1	1	1	1	1	1	1	1	1	1
Calcium carbonate (% w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium bicarbonate (% w/v)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Methyl paraben (% w/v)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Saccharin sodium (% w/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Optimization

For the optimization of gelling polymer, four different polymers (Sodium Alginate, Pectin, Gellan gum and Sodium CMC) and three different concentrations (0.25 %, 0.5 % and 0.75 %) were used (Table 2). Amongst the four different gelling polymers, Sodium Alginate was selected for optimization which was having a good pourability, viscosity and floating properties as compared to other gelling polymers. A concentration of 0.75 % of sodium alginate was considered best, based on the floating properties as it took lesser time to emerge on the surface of 0.1 N HCl.

Evaluation parameters[15–18]: -

Pre-formulation Studies:

Organoleptic characteristics: The sample of Nicardipine HCl was studied for organoleptic characteristics such as colour, odour and appearance.

Melting point: The melting point range of Nicardipine HCl was determined by melting point apparatus using an open capillary method. A small amount of drug sample was transferred into the capillary tube. The capillary was placed in melting point test apparatus and the temperature at which drug started and completely melted was noted down.

Solubility: Solubility of drug in two different solvents 0.1N Hcl and distilled water was checked by preparing saturated solutions of drug in respective solvents. Saturated solutions were prepared by adding excess of drug to vehicles, then samples were allowed to shaken in sonicator for 24 hrs overnight. After 24 hours, the solutions were filtered and analyzed spectrophotometrically.

Identification of the drug by UV-Visible Spectroscopy:

(a) Determination of absorption maxima (λ max): The λ max of Nicardipine HCl was determined by scanning the drug solution of 8 µg/ml in the range of 200-400 nm by UV spectroscopy.

(b) Preparation of calibration curve of Nicardipine Hydrochloride:

Preparation of standard solution – Standard stock solution of Nicardipine Hcl was prepared in 0.1 N HCl. 10 mg of Nicardipine HCl was accurately weighed into 10 ml volumetric flask and dissolved in small quantity of 0.1 N HCl. The volume was made up by 0.1 N HCl to get a concentration of 1000 µg/ml (SS - 1). From this 1 ml solution was withdrawn and diluted to 10 ml to get a concentration of 100 µg/ml (SS - 2).

Preparation of working standard solutions – Further, from (SS - 2) aliquots of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml,1.2 ml, 1.4 m1.5,l, 1.6 ml and 1.8 ml were pipette into 10 ml volumetric flasks. The volume was made up with 0.1 N HCl to get the final concentrations of 2, 4, 6, 8, 10, 12, 14, 16 and 18 µg/ml respectively. The absorbance of each concentration was measured at 238 nm.

Drug excipient compatibility study

Study was carried out in which drug and excipient were mixed in ratio of 1:1 and the sample were kept in stability chamber for the period of 15 days at 25°C and 64 % RH. After 15 days the sample was analyzed for FTIR.

Characterization of floating in-situ gel[19–23]:

Physical appearance: General appearance of formulation like colour, odour was observed.

pH determination: The pH of the formulations was determined using digital pH meter which was previously calibrated using standard buffer of pH 4 and pH 7. By bringing the electrode of the pH meter in contact with the surface of the formulation and allowing it to equilibrate for 1 min then readings were noted down.

Viscosity: The viscosity of the formulation was determined by Brookfield viscometer using the spindle no. 63 at 50 rpm at temperature 37 ± 0.5 ° C.

Floating lag time: In this test, 5ml of formulation was added into 500 ml of dissolution vessel containing 0.1 N HCl at 37° C. The time taken for the formulation to emerge at the surface of dissolution media was noted in triplicate.

Total floating time: Total floating time was determined by adding 5 ml of in situ gel into 500 ml of dissolution vessel containing 0.1 N HCl at 37°C. The time for which the formulations took to remain constantly floating on surface of dissolution medium was noted.

Drug content estimation: Drug content of formulations was determined in triplicate by using double beam UV spectrophotometer. 10 ml of formulation was taken in 100 ml volumetric flask and 50 ml of 0.1 N HCl (pH 1.2) was added with continuous shaking. Final volume adjusted up to 100 ml with the help of 0.1 N HCl (pH 1.2) and filtered the solution. Sample was analyzed for determination of drug content spectrophotometrically at λ max 238 nm.

Water uptake study: A simple method was adopted to determine the water uptake by the gel. The insitu gel formed in 0.1 N Hydrochloric acid was used for this study. From each formulation the gel portion from the 0.1 N Hydrochloric acid separated and the excess solution was blotted out with a tissue paper. The initial weight of the gel taken was weighed and to this gel 10 ml of distilled water was added and after every 30 minutes of the interval water was decanted and the weight of the gel recorded and the difference in the weight was calculated and reported.

Gelling strength: The prepared gel was placed in 100 ml measuring cylinder; the probe was placed on the gel and a weight was putted on the probe. The probe was allowed to penetrate at a distance of 5 cm and time required for penetration was noted as a gelling strength.

In-vitro drug release study: The dissolution studies was conducted using a USP type II (paddle method) dissolution apparatus. The 900 ml of dissolution medium of 0.1 N HCl (pH 1.2), maintained at 37˚ C. The stirring rate adjusted to 50 rpm. At predetermined time intervals, 5 ml sample was withdrawn and replaced by fresh dissolution medium, filtered through whatman filter paper, and assayed at maximum absorbance using UV-Visible Spectrophotometer at 238 nm.

Experimental Design

A 3^2 full factorial design was adopted to optimize the variables. In the design 2 factors were evaluated, each at 3 levels. The concentration of Sodium Alginate (X1) and HPMC K100M (X2) were chosen as independent variables, as their marked effect seen on drug release. % Drug release at 6, % drug release at 12 hours were selected as dependent variables. The response (Y) is measured for each trial.

$Y= \beta0+\beta1X1+\beta2X2+\beta12X1X2+\beta11X1\ 2+\beta22X2\ 2$

Where;

Y is the dependent variable,

β0 is arithmetic mean response of the nine runs,

β1 is estimated coefficient for factor X1,

β2 is estimated coefficient for factor X2.

Table 3: Layout of optimization.

Independent variables (factors)	Coded values			Actual values		
	Low	Medium	High	Low	Medium	High
Sodium alginate X1 (%)	-1	0	+1	0.6	0.75	0.9
HPMC K100M X2 (%)	-1	0	+1	0.5	1.0	1.5

Table 4: Independent and dependent variables.

INDEPENDENT VARIABLE		**DEPENDENT VARIABLE**	
X1	**X2**	**Y1**	**Y2**
Conc. of sodium alginate	Conc. of HPMC K100M	% drug release at 6 hr	% drug release at 12 hr

Table 5: Coded and actual values of independent variables.

Formulation code	**Coded values**		**Actual values**	
	X1	**X2**	**Sodium alginate conc. X1 (%)**	**HPMC K100M conc. X2 (%)**
B1	-1	-1	0.6	0.5
B2	0	-1	0.75	0.5
B3	+1	-1	0.9	0.5
B4	-1	0	0.6	1.0
B5	0	0	0.75	1.0
B6	+1	0	0.9	1.0
B7	-1	+1	0.6	1.5
B8	0	+1	0.75	1.5
B9	+1	+1	0.9	1.5

Table 6: Composition of design batches.

Formulation code	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**
Nicardipine Hydrochloride (mg)	20	20	20	20	20	20	20	20	20
Sodium Alginate (% w/v)	0.6	0.75	0.9	0.6	0.75	0.9	0.6	0.75	0.9
HPMC K100M (% w/v)	0.5	0.5	0.5	1.0	1.0	1.0	1.5	1.5	1.5
Tri-sodium citrate (% w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calcium carbonate (% w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium bicarbonate (% w/v)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Methyl paraben (% w/v)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Saccharin sodium (% w/v)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Drug release kinetics

For investigating the mode of drug release from the formulation, the drug release data was analyzed using mathematical models: zero order kinetics, first order kinetics, krosmeyer model, higuchi equations. The zero-order release rate describes the system where the drug release rate is independent of its concentration. The first-order release rate describes the release from the system as concentration-dependent, which shows log cumulative percent drug remaining versus time. Higuchi's model describes the release of the drug from an insoluble matrix as a square root of a time-dependent process based on Fickian diffusion. Higuchi's root kinetics shows the cumulative percentage drug release versus the square root of time. Hixson Crowell model describes the drug release from the system where there is a change in surface area and diameter of particles or tablets. R^2 is a statistical measure of how close the data are to the fitted regression line. The value close to 1 was considered to be the most preferred one[19–21,24].

Short term stability studies

The purpose of stability testing is to provide evidence on how the quantity of a drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and to establish a re-test period for the drug substance or a shelf-life for the drug product and recommend storage conditions. The stability study of the floating in-situ gel was determined by in vitro buoyancy, drug content, invitro drug release study. The selected batch was packed in ambered coloured bottle and kept in a petri dish at room temperature 40˚ C ± 2˚ C and 75 % ± 5 % RH for a period of 30 days[25,26].

RESULTS AND DISCUSSION

Pre formulation studies

Organoleptic properties: The colour and appearance of the drug was found to be greenish-yellow, crystalline powder on visual inspection.

Melting point determination: The melting point range of Nicardipine Hydrochloride was found to be in 168 - 170˚C by capillary tube method within the range i.e. 167 – 171 ˚ C.

Solubility determination

Table 7: Determination of solubility.

Solubility (mg/ml)	Solvent	Observations (n=3, ± S.D.)
	0.1 N HCl	5.32 ± 0.08

Identification of drug by UV spectrophotometer

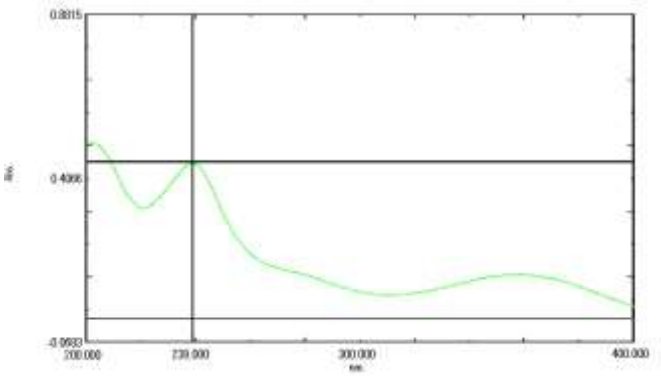

Figure 2: Identification of Nicardipine Hydrochloride by UV spectrophotometer

The absorption maximum (λ max) of drug was found to be 238 nm in 0.1N HCl.

Calibration curve of Nicardipine Hydrochloride in 0.1 N HCl

A calibration curve was plotted between the concentrations versus absorbance as shown in figure. Linearity was obtained within the concentration range of 2-18 µg/ml. This indicates that beer's lamberts law was followed over this range. R^2 value of the curve was found to be 0.993 in 0.1N HCl (pH 1.2).

Table 8: Calibration curve of Nicardipine HCl in 0.1 N HCl.

Concentration (µg/ml)	Absorbance Average (n=3, ± S.D.)
0	0
2	0.1224±0.006
4	0.2193±0.002
6	0.3283±0.008
8	0.4443±0.003
10	0.5438±0.003
12	0.6592±0.005
14	0.7845±0.004
16	0.8829±0.004
18	0.9733±0.004

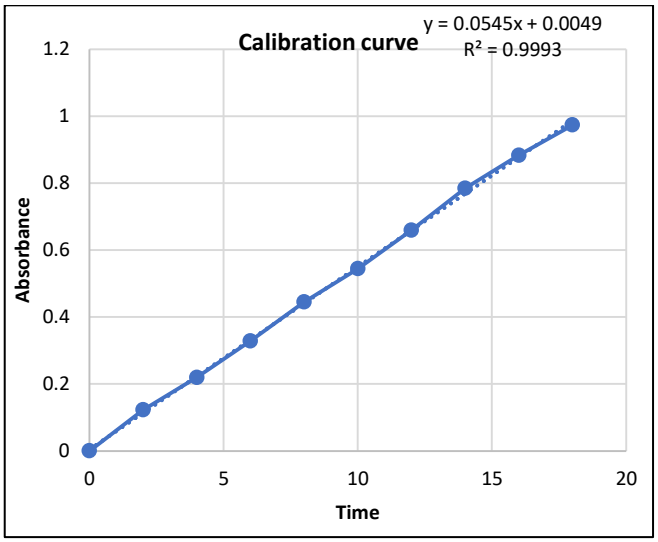

Figure 3: Calibration curve of Nicardipine HCl in 0.1 N HCl.

Identification of drug by FTIR spectroscopy:

Refer figure 5 for IR spectrum of Nicardipine Hydrochloride as per JP 2006 and figure 4 Nicardipine hydrochloride sample and table 9 for interpretation of FT-IR spectrum of Nicardipine hydrochloride. The peaks obtained in the spectra of pure drug correlates with the peaks of official spectrum which confirms the purity of drug.

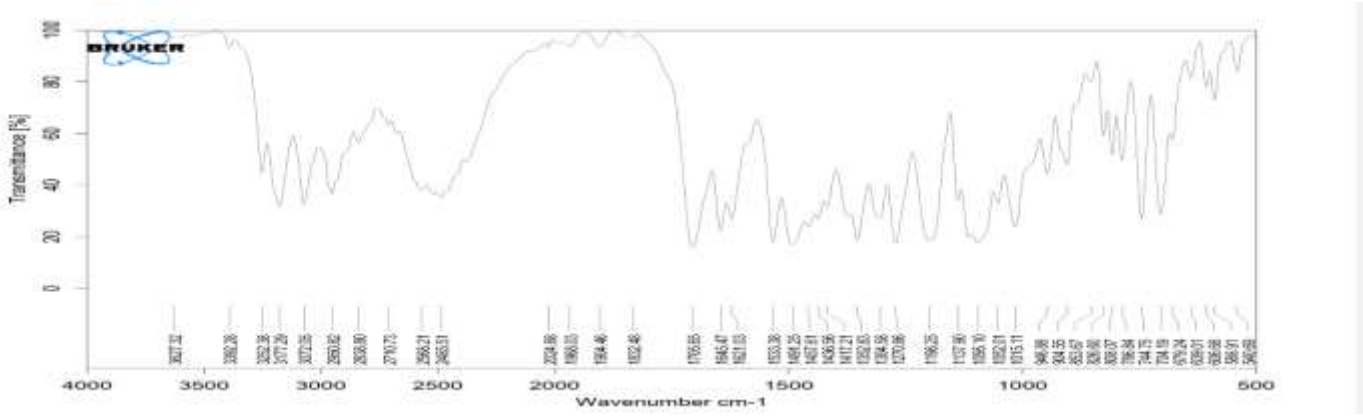

Figure 4: FTIR graph of Nicardipine HCl.

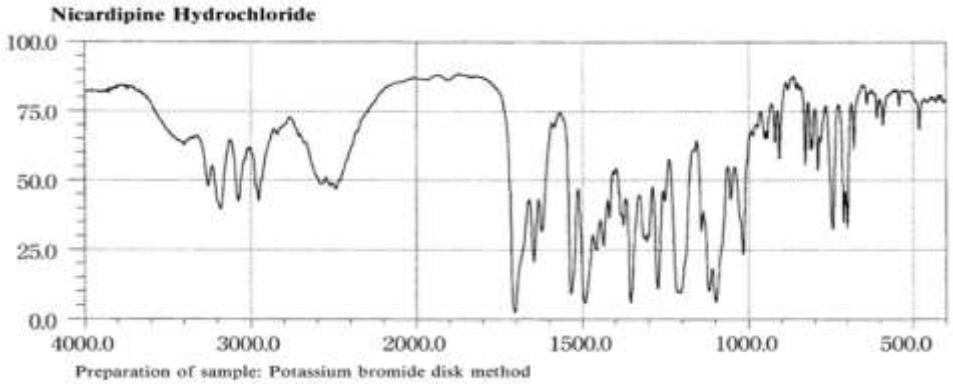

Figure 5: FTIR Identification of Nicardipine HCl from monograph (JP 2006).

Drug Polymer Compatibility study:

It was clearly shown in (Fig 6,7,8 and 9) that peaks of drug were remain present in the physical mixture of drug and polymer in both the temperature condition shows no interaction between the drug and excipient as there is no disappearance of functional group of drugs.

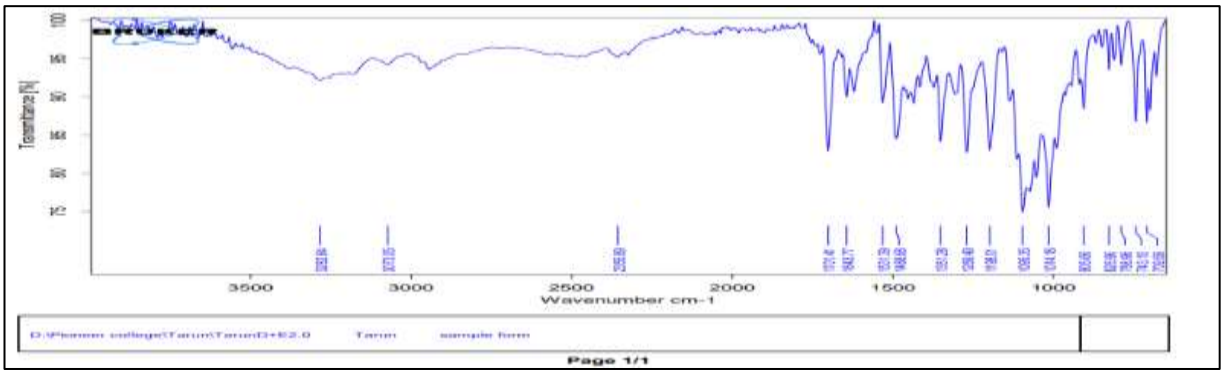

Figure 6. FTIR graph-Nicardipine HCl +Sodium Alginate.

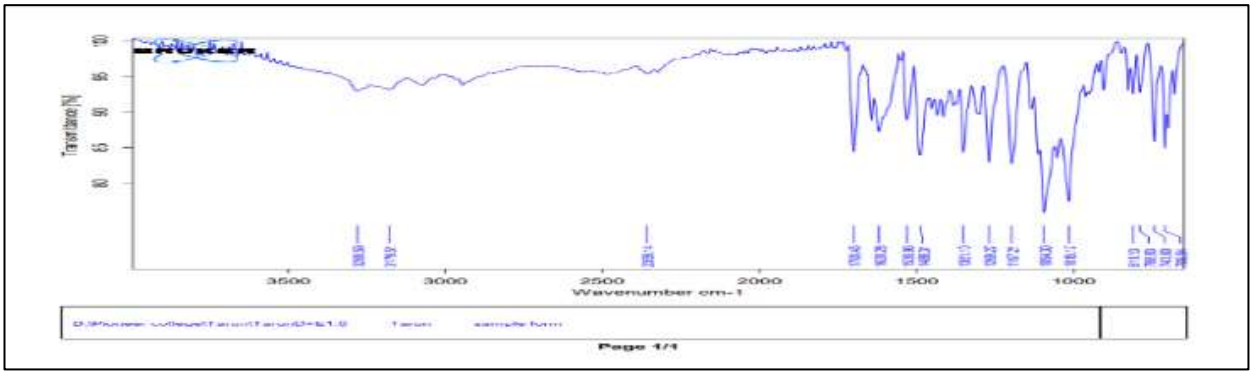

Figure 7. FTIR graph-Nicardipine HCl+Pectin.

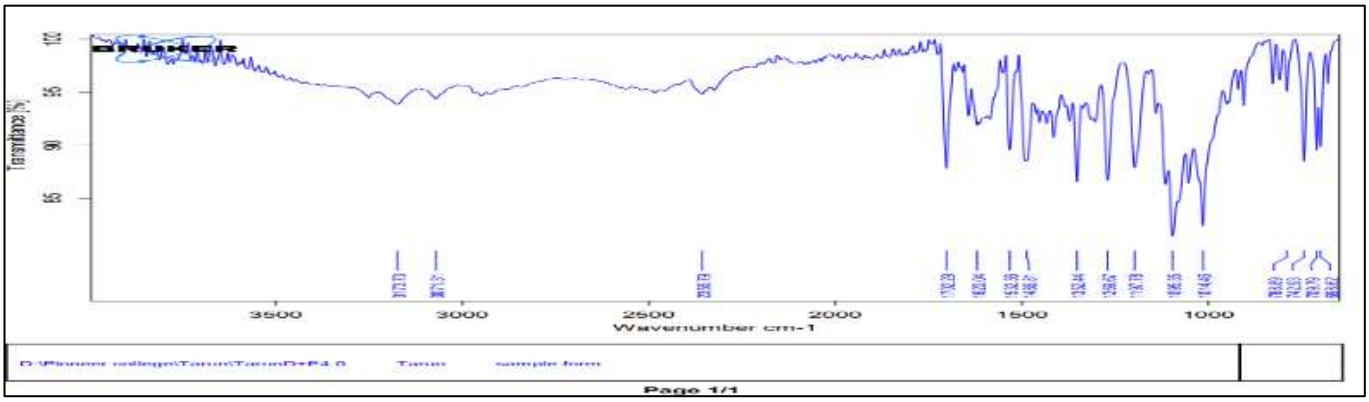

Figure 8. FTIR graph-Nicardipine HCl +Gellan Gum.

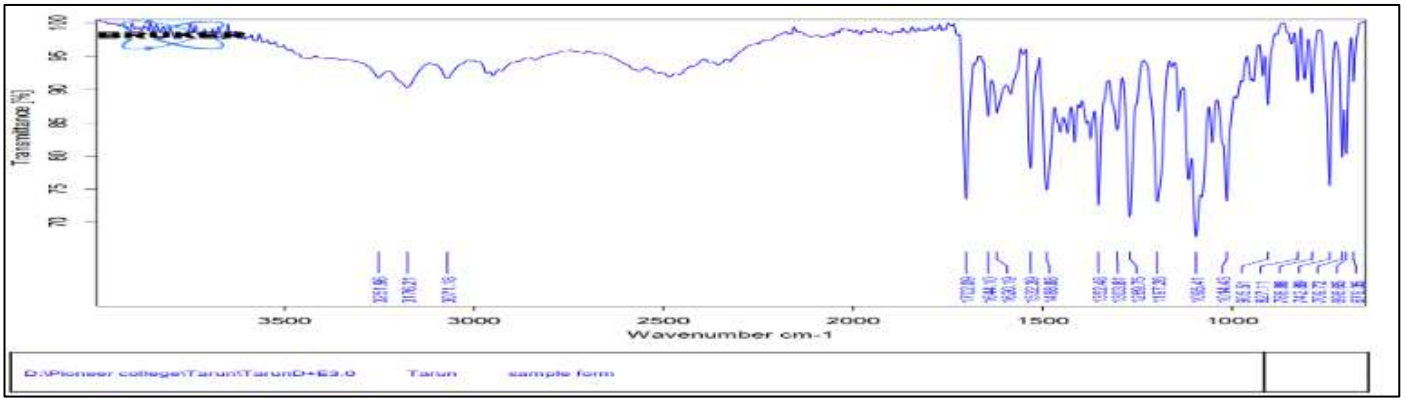

Figure 9. FTIR graph-Nicardipine HCl +Na CMC.

Table 9: Interpretation of Drug and Polymers.

Functional group	Wavenumber cm-1				
	Nicardipine HCl	Nicardipine HCl + Sodium alginate	Nicardipine HCl + Pectin	Nicardipine HCl + Gellan gum	Nicardipine HCl + Na CMC
C-H	1457.61	1488.31	1488.68	1488.88	1486.81
C=C	1645.47	1620.28	1643.77	1644.10	1620.04
Ester group	1705.65	1700.45	1701.41	1702.09	1701.29
Amine group	1015.11	1015.17	1014.16	1014.43	1014.46
Nitro group	1352.83	1351.10	1351.28	1352.48	1352.44

Acid stability of Nicardipine HCl

The acid stability study was conducted for knowing the stability of Nicardipine HCl in acidic environment for a period of 12 hours. From the acid stability study, it was concluded that the drug was found to be stable in 0.1 N HCl and degradation of drug was not occurred.

Table 10: Acid stability of Nicardipine HCl.

Time (hour)	Absorbance
1	0.1571
2	0.1565
3	0.1568
4	0.1567
5	0.1569
6	0.1563
7	0.1565
8	0.1561
9	0.1552
10	0.1554
11	0.1550
12	0.1543

Post formulation studies

Evaluation parameters of preliminary batches (F1-F12)

pH of all the formulations was found to be in the range of 7.23 ± 0.016 to 7.87 ± 0.028. In all the above formulations, viscosity increases as the polymer concentration increases. The viscosity of formulations was found in the range of 247.6 ± 2.98 to 363.23 ± 1.91 cPs for solutions and the viscosity was found to be in the range of 1045.0 ± 1.41 to 1250.3 ± 2.86 for gel. The optimum viscosity was seen in F1 – F3 batch which was containing Sodium Alginate as gelling agent. The lesser viscosity was found in the F4 – F6 batch which was containing pectin as gelling polymer and higher viscosity was found in F7 – F9 batch which was containing Gellan Gum as gelling polymer. Compared to all other batches, the lesser floating lag time was observed in the F3 batch i.e., 3 ± 0.43. Total floating time for all the batches was found to be > 12 hours. The highest % drug content was found to be 99.14 ± 0.63 seen in F3 batch. From the water uptake studies, it was concluded that as the concentration of gelling polymer increases the water uptake by polymer increases. The highest % water uptake was found to be 14.46 ± 0.30 %. From the gelling strength studies, it was concluded that as the concentration of gelling polymer increases the gelling strength also increases. The highest gelling strength was found to be 15.32 ± 0.47.

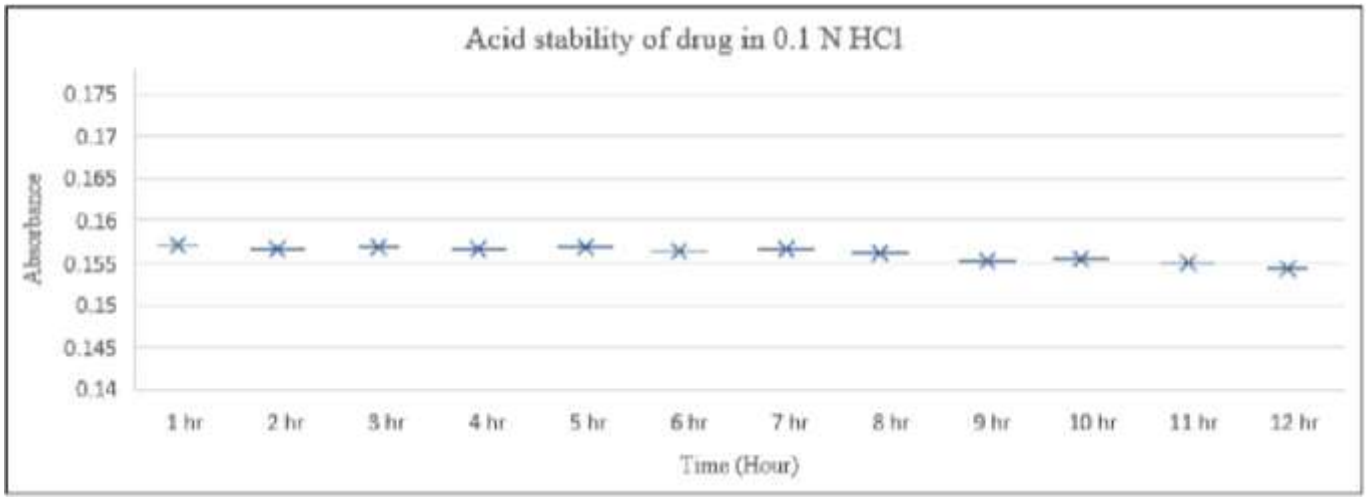

Figure 10. Acid stability of Nicardipine Hydrochloride

Table 11: Evaluation of preliminary formulations F1 – F12.

Formulation Code	pH determination (n=3, ± S.D.)	Viscosity (n=3, ± S.D.) in cPs		Floating Lag time (n=3, ± S.D.) In sec.
		Solution	Gel	
F1	7.56 ± 0.028	265.66 ± 2.04	1353.3 ± 1.69	4.66 ± 0.47
F2	7.59 ± 0.012	288.20 ± 2.33	1450.0 ± 2.16	4 ± 0.81
F3	7.6 ± 0.021	327.26 ± 2.77	1536.6 ± 2.86	3 ± 0.43
F4	7.26 ± 0.028	247.6 ± 2.98	1045.0 ± 1.41	35 ± 3.74
F5	7.23 ± 0.016	265.83 ± 4.01	1152.0 ± 3.74	42.66 ± 2.05
F6	7.30 ± 0.020	296.93 ± 4.04	1224.3 ± 3.39	50.33 ± 2.05
F7	7.66 ± 0.038	312.33 ± 3.23	882 ± 3.74	22.66 ± 1.24
F8	7.61 ± 0.038	333.56 ± 4.56	1008.3 ± 4.64	25.33 ± 2.05
F9	7.69 ± 0.030	363.23 ± 1.91	1250.3 ± 2.86	22 ± 2.44
F10	7.84 ± 0.024	254.03 ± 4.39	942.66 ± 3.39	54.66 ± 3.09
F11	7.86 ± 0.026	283.73 ± 2.90	1135.6 ± 3.29	48.66 ± 2.62
F12	7.87 ± 0.028	318.03 ± 2.77	1216 ± 4.32	56.33 ± 1.24

Table 12: Evaluation of preliminary formulations F1 – F12.

Formulation Code	Total Floating time (hours)	% Drug content (n=3, ± S.D.)	% Water uptake study (n=3, ± S.D.)	Gelling strength (n=3, ± S.D.) in sec.
F1	> 12	98.13 ± 0.11	9.04 ± 2.51	12.09 ± 0.81
F2	> 12	96.85 ± 0.26	11.69 ± 0.75	14.04 ± 1.01
F3	> 12	99.14 ± 0.63	14.46 ± 0.30	15.32 ± 0.47
F4	> 12	94.65 ± 0.67	4.77 ± 1.46	4.11 ± 0.68
F5	> 12	95.29 ± 0.82	8.06 ± 1.48	6.65 ± 0.33
F6	> 12	96.15 ± 0.48	10.31 ± 2.10	8.07 ± 0.43
F7	> 12	89.06 ± 0.15	5.90 ± 1.40	3.31 ± 0.07
F8	> 12	88.08 ± 0.26	8.00 ± 1.10	5.74 ± 0.30
F9	> 12	85.19 ± 0.45	9.13 ± 1.26	6.3 ± 0.74
F10	> 12	87.54 ± 0.14	3.18 ± 0.69	7.45 ± 1.78
F11	> 12	89.37 ± 0.34	6.19 ± 0.67	10.98 ± 0.54
F12	> 12	91.57 ± 0.93	8.42 ± 0.25	12.39 ± 0.84
***Each observation is the mean ± S.D. of three determination**				

Figure 11: 0.75 % Sodium Alginate floating

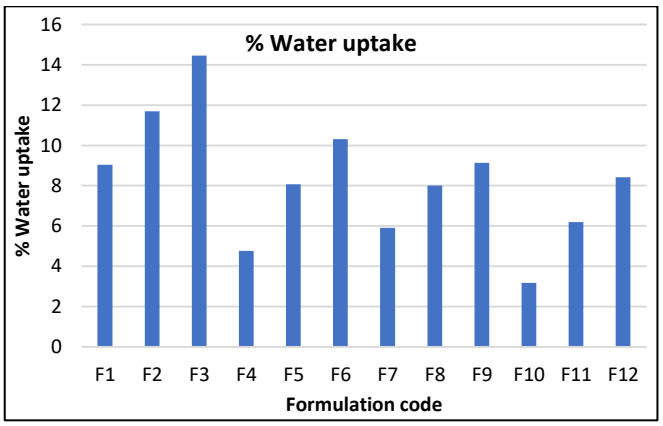

Figure 12: % Water uptake studies.

Table 13: Invitro Drug release of trial formulations.

Time	% CDR											
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**
0	0	0	0	0	0	0	0	0	0	0	0	0
1	14.26	13.09	21.6	11.47	12.6	7.60	14	11.75	15.07	7.42	6.52	5.85
2	26.67	25.49	35.53	17.02	22.52	14.71	25.72	27.56	17.22	13.09	11.60	10.60
3	40.18	40.98	52.74	32.50	27.91	20.68	37.30	47.33	29.65	20.81	17.83	18.27
4	52.19	53.18	57.39	37.14	37.42	39.52	52.94	54.12	48.31	32.17	24.22	23.23
5	54.73	55.63	60.59	47.82	52.21	46.08	56.29	57.56	53.70	42.97	28.32	29.30
6	58.50	58.81	63.98	52.63	57.63	50.65	58.49	59.18	54.90	54.86	37.47	38.95
7	62.28	61.11	67.66	58.05	59.83	54.89	61.69	61.98	58.26	56.74	51.22	53.52
8	65.77	65.09	70.82	60.35	62.81	58.57	66.30	65.87	62.54	60.83	53.62	56.42
9	75.35	73.68	73.85	64.45	65.90	62.53	67.65	71.36	66.66	63.50	58.55	58.98
10	79.40	77.32	78.03	67.77	67.88	64.85	69.5	74.58	69.40	64.56	60.17	62.45
11	80.73	78.81	81.33	72.01	70.22	69.56	72.30	76.87	74.50	66.67	68.51	70.71
12	82.60	80.31	85.50	74.64	72.04	73.09	76.24	77.78	79.58	71.79	77.83	75.55

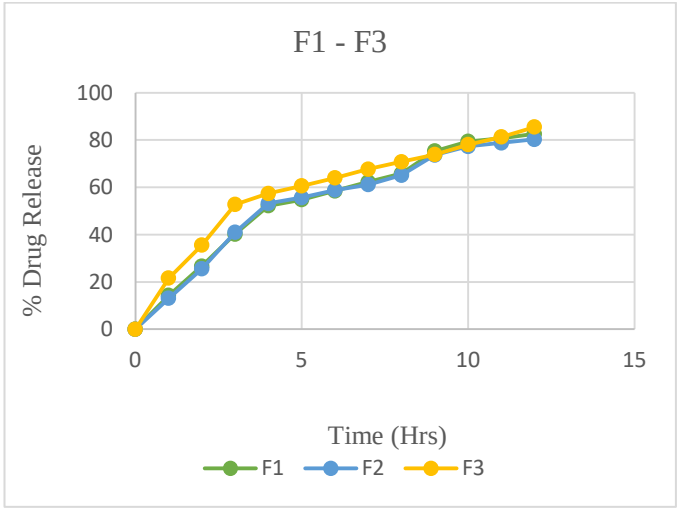

Figure 13: In vitro drug release of formulations F1 – F3

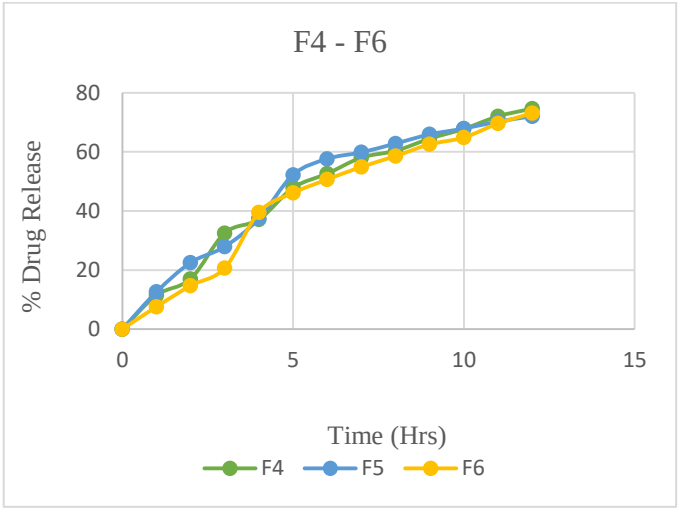

Figure 14: In vitro drug release of formulations F4 – F6

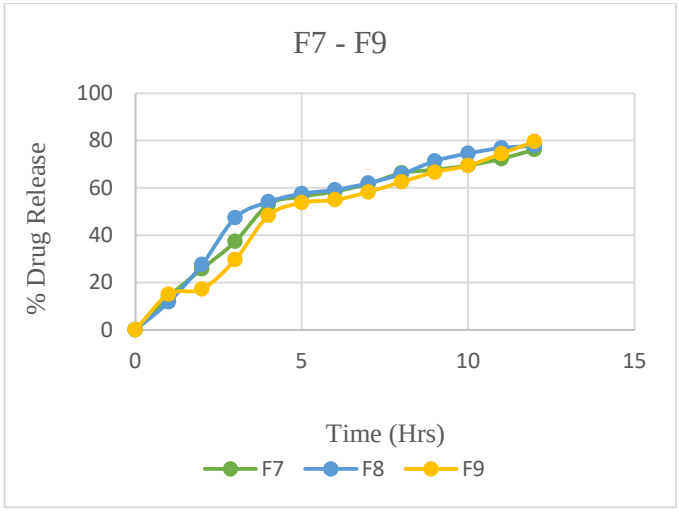

Figure 15: In vitro drug release of formulations F7 – F9

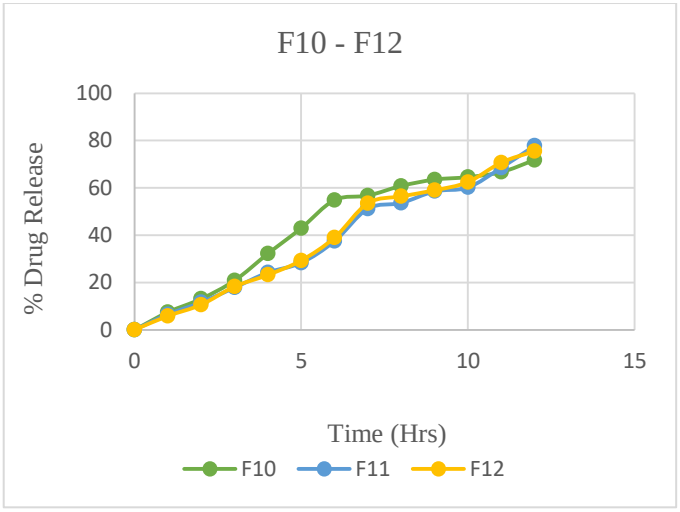

Figure 16: In vitro drug release of formulations F10 – F12

Table 14: Optimization using 3^2 factorial design method

Independent factors		-1	0	+1	Dependent factors
X1 = Concentration of Sodium alginate	Numeric	0.6	0.75	0.9	Y1 = % Drug release at 6 hours
X2 = Concentration of HPMC K100M	Numeric	0.5	1.0	1.5	Y2 = % Drug release at 12 hours

Invitro drug release of trial formulations

The highest % drug release was found in F3 formulation i.e., 85.50 % which was having a 0.75 % of Sodium Alginate in the formulation.

Evaluation of floating in-situ gel prepared by 3^2 factorial design B1-B9:

pH of all formulations was found to be in the range of 7.33±0.03 to 7.68±0.01. In all the above formulations viscosity increases as the polymer concentration increases. The viscosity of formulations was found in the range of 250.90±2.19 to 386.33±2.77 cPs for solutions and the viscosity was found to be in the range of 1337.67±2.05 to 1594.33±3.68 for gel. Floating lag time for all the formulations was found in the range of 3.06±0.09 to 5.6±0.35. B1 batch took lesser time to emerge in the surface of 0.1 N HCl as compared to other batches i.e. 3.06 ± 0.09. % Drug content of all the formulation was found to be in the range of 99.24±0.23 to 97.51±0.42. The highest % drug content was found to be 99.24 ± 0.23 % in batch B1. % Water uptake of all the formulations was found to be in the range of 10.83±0.85 to 18.07±0.60. It was found that as the concentration of polymers increases, the % water uptake of formulations increases. The gelling strength of the formulations was found to be in the range of 12.06±0.02 to 19.67±0.28 s. From the results it can be concluded that as the concentrations of polymers increases the gelling strength also increases.

Table 15: Evaluation parameters of optimized in-situ gel

Batch No.	pH determination (n=3, ± S.D.)	Viscosity (n=3, ± S.D.) in cPs		Floating Lag time (n=3, ± S.D.)
		Solution	Gel	
B1	7.33 ± 0.03	250.90 ± 2.19	1337.67 ± 2.05	3.06 ± 0.09
B2	7.47 ± 0.02	309.73 ± 2.58	1450 ± 2.16	3.2 ± 0.16
B3	7.60 ± 0.02	343.8 ± 3.02	1536.67 ± 2.86	4 ± 0.35
B4	7.36 ± 0.04	275.73 ± 3.29	1348.33 ± 2.86	3.63 ± 0.57
B5	7.50 ± 0.03	333.66 ± 2.80	1468.67 ± 3.68	3.8 ± 0.49
B6	7.64 ± 0.05	362.4 ± 2.38	1557.33 ± 3.39	4.2 ± 0.21
B7	7.41 ± 0.02	291.63 ± 3.80	1374.33 ± 2.86	4.30 ± 0.08
B8	7.56 ± 0.03	356.06 ± 3.96	1485.33 ± 2.62	4.83 ± 0.30
B9	7.68 ± 0.01	386.33 ± 2.77	1594.33 ± 3.68	5.6 ± 0.35

Table 16: Evaluation parameters of optimized in-situ gel

Formulation Code	Total floating time (hours)	% Drug content (n=3, ± S.D.)	% Water uptake (n=3, ± S.D.)	Gelling strength (n=3, ± S.D.)
B1	>12	99.24 ± 0.23	10.83 ± 0.85	12.06 ± 0.02
B2	>12	99.02 ± 0.12	13.42 ± 0.76	13.33 ± 0.22
B3	>12	98.88 ± 0.37	14.27 ± 0.54	15.00 ± 0.63
B4	>12	98.48 ± 0.45	11.38 ± 0.35	13.02 ± 0.45
B5	>12	99.08 ± 0.06	14.45 ± 1.30	15.42 ± 0.69
B6	>12	98.99 ± 0.12	15.93 ± 0.75	16.10 ± 0.44
B7	>12	98.82 ± 0.56	13.02 ± 0.69	15.6 ± 0.29
B8	>12	97.51 ± 0.42	16.49 ± 0.98	17.10 ± 0.37
B9	>12	97.75 ± 0.91	18.07 ± 0.60	19.67 ± 0.28

Table 17: Invitro drug release study of optimized batches

Time (hr)	B1	B2	B3	B4	B5	B6	B7	B8	B9
0	0	0	0	0	0	0	0	0	0
1	14.08	12.46	20.56	13.54	14.17	14.58	14.44	12.6	11.1
2	32.02	25.98	32.78	25.41	24.10	27.08	23.66	20.36	22.03
3	44.85	40.66	41.06	40.17	37.83	36.95	28.11	26.28	27.48
4	49.68	52.00	48.89	45.57	46.41	50.38	40.91	41.00	33.75
5	55.00	55.93	53.03	52.61	50.76	53.36	45.99	45.77	46.90
6	62.48	60.12	58.90	57.61	56.8	55.91	55.29	53.32	52.2
7	65.81	63.95	66.38	62.62	61.34	61.25	60.23	60.36	60.90
8	74.76	73.03	70.20	66.02	71.43	66.71	66.58	65.55	66.36
9	78.27	77.20	74.27	74.89	77.85	73.64	72.66	74.00	75.23
10	80.14	80.10	77.33	78.94	81.24	79.98	80.79	77.28	79.59
11	88.80	85.93	82.78	83.55	84.74	83.65	82.90	80.76	80.43
12	96.44	91.02	90.45	91.36	90.51	89.46	88.11	86.1	84.91

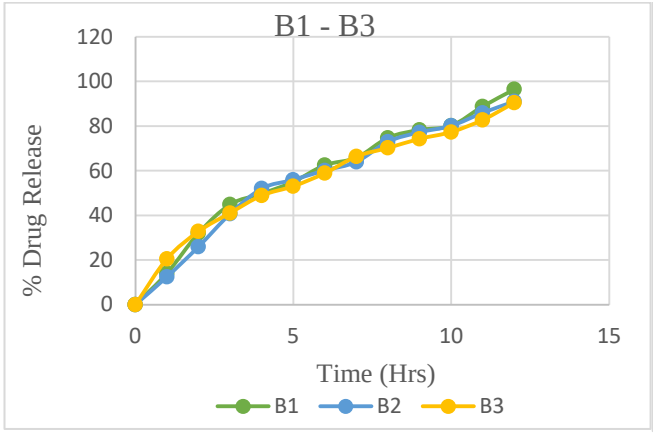

Figure 17: In vitro drug release from B1 – B3

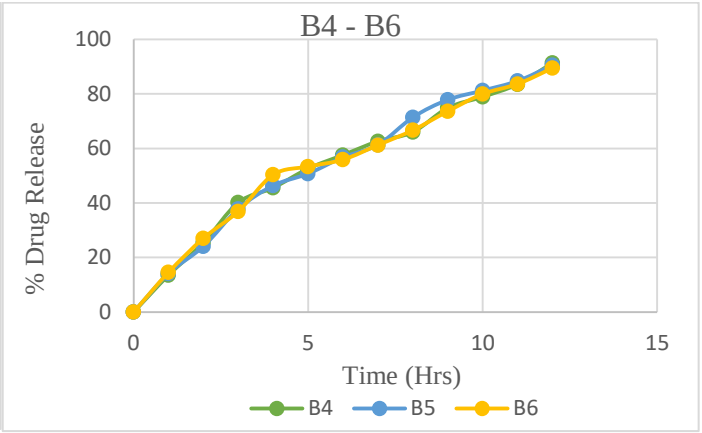

Figure 18: In vitro drug release from B4 – B6

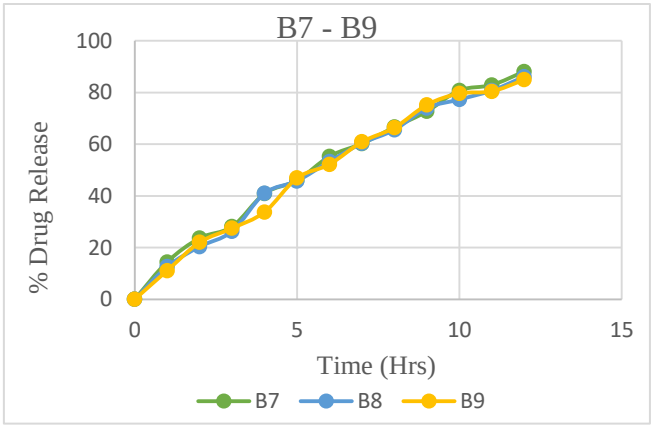

Figure 19: In vitro drug release from B7 – B9

Invitro drug release study of optimized batches

A significant decrease in the rate of drug release was observed with the increase in polymer concentration and it was attributed to increase in the density of the polymer matrix. The release of drug from these gel formulations were 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 phase of moderate release. This biphasic pattern of release is a characteristic feature of matrix diffusion kinetics. As the proportion of Sodium Alginate increased in the gelling systems, a significant decrease in the drug release was observed. The highest % drug release was found to be 96.44 % in batch B1.

Statistical analysis using design expert software

3^2 factorial design was carried using design Expert software. In this concentration of Sodium Alginate and concentration of HPMC K100M were selected as independent variable and % drug release at 6 hr and 12 hr were selected as dependent variable.

ANOVA for % drug release at 6 hours (Response 1)

Table 18: ANOVA table Response 1-% Drug release at 6 hours

Source	Sum of square	Df	Mean square	F - value	P – value Prob > F	
Model	83.44	5	16.69	48.77	0.0045	significant
A – Sodium alginate	11.68	1	11.68	34.12	0.01000	
B – HPMC K100M	71.35	1	71.35	208.52	0.0007	
AB	1	0.060	0.18	0.7035		
A^2	1	0.20	0.59	0.4976		
B^2	1	0.15	0.45	0.5492		
Residual	1.03	3	0.34			
Cor Total	84.47	8				

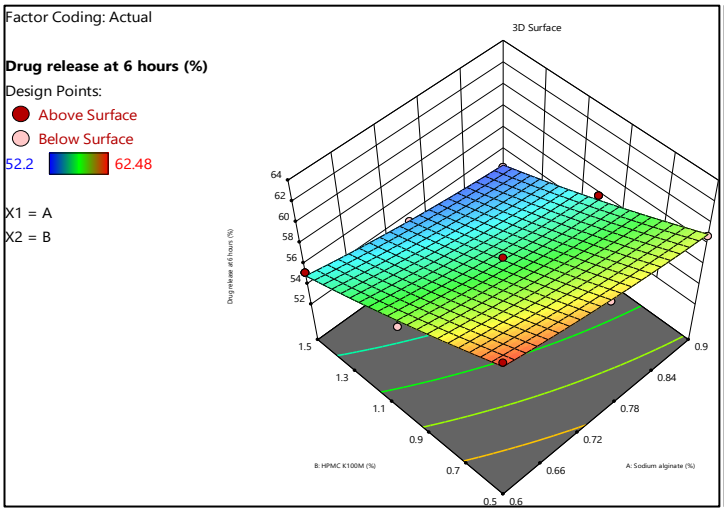

Figure 20: Contour plot of response 1

(% Drug Release at 6 hours)

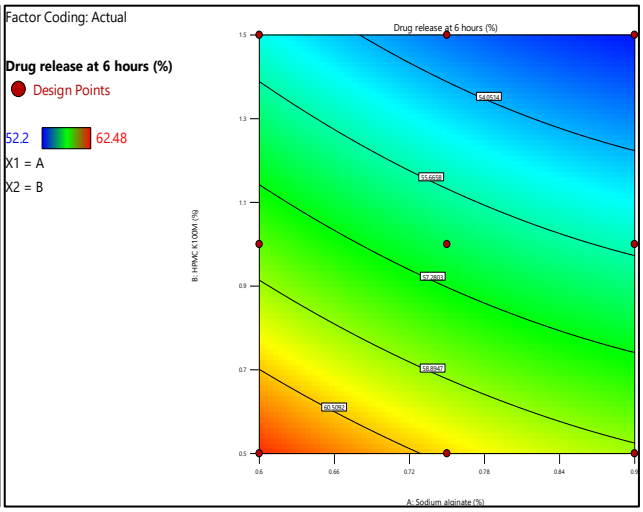

Figure 21: Response surface plot of Response 1

(% Drug release at 6 hours)

Final Equation in terms of actual factors:-

Drug release at 6 hours = + 56.56 - 1.40 * A - 3.45 * B + 0.12 * A * B + 0.32 * A^2 + 0.28 * B^2

ANOVA for % drug release at 12 hours (Response 2):

Table 20: ANOVA table Response 2-% Drug release at 12 hours

Source	Sum of square	Df	Mean square	F - value	P – value Prob > F	
Model	84.71	5	16.94	10.59	0.0401	significant
A – Sodium alginate	20.50	1	20.50	12.82	0.0373	
B – HPMC K100M	58.84	1	58.84	36.79	0.0090	
AB	1	1.95	1.22	0.3505		
A^2	1	1.66	1.04	0.3830		
B^2	1	1.76	1.10	0.3711		
Residual	4.80	3	1.60			
Cor Total	89.51	8				

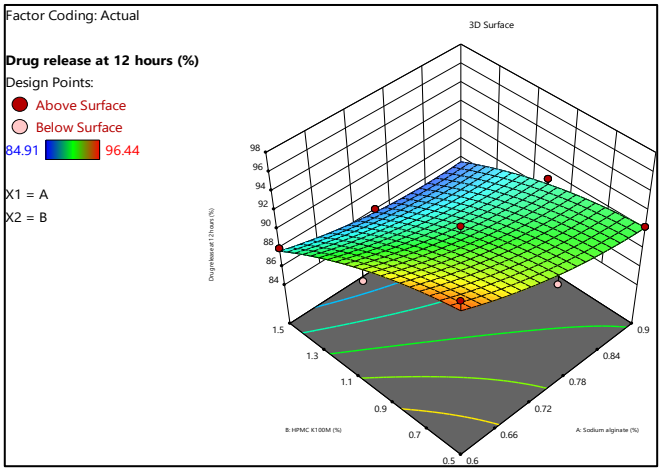

Figure 22: Contour plot of response 2

(% Drug Release at 12 hours)

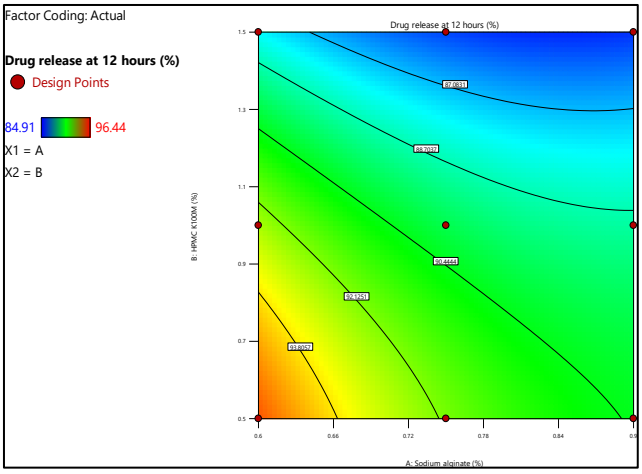

Figure 23: Response surface plot of Response 2

(% Drug release at 12 hours)

Final Equation in Terms of Actual Factors:

Drug release at 12 hours = + 89.84 - 1.85 * A - 3.13 * B + 0.70 * A * B + 0.01 * A^2 - 0.94 * B^2

The result indicates that Y1 and Y2 are affected by the independent variables selected for the study. This negative value indicates that X1 (Conc. Of Sodium Alginate) and X2 (Conc. Of HPMC K100M) has antagonist effect on % drug release. i.e., as X1 (Conc. Of Sodium Alginate) and X2 (Conc. of HPMC K100M) decreases the % drug release increases.

Overlay plot

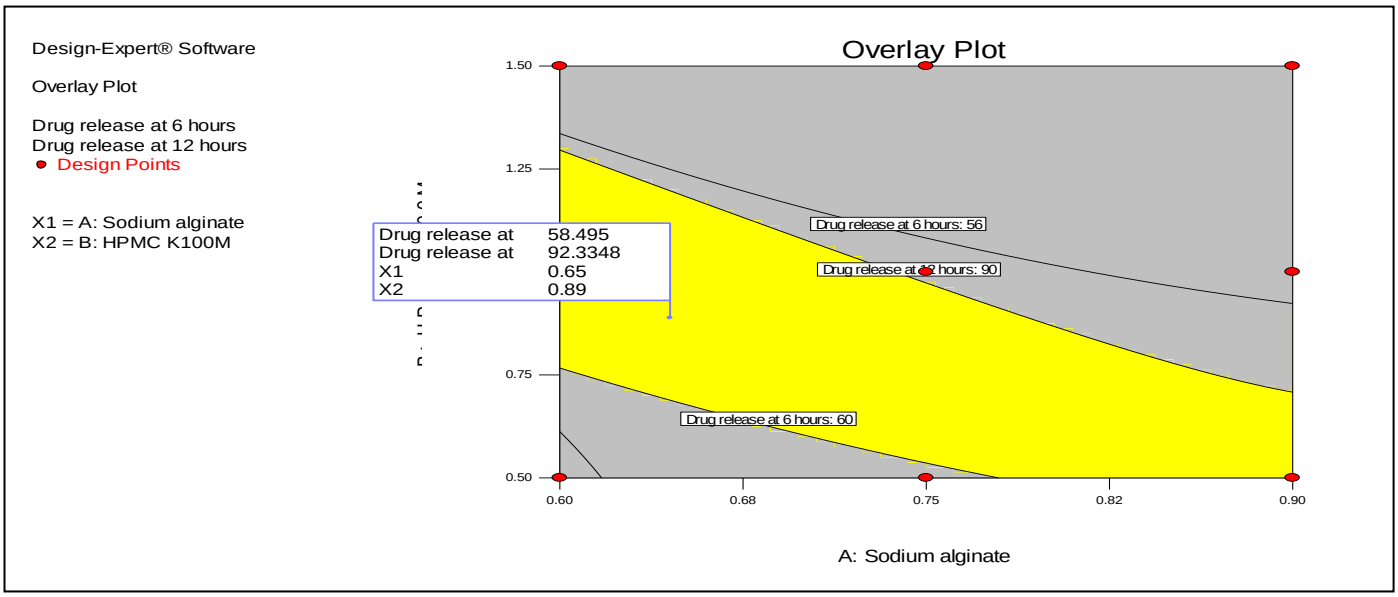

Figure 24: Overlay plot for optimized formulation

Based on the overlay plot, the check point batch (CP1) was selected, which showed that the concentration of 0.65 % w/v for sodium alginate and the concentration of 0.89 % w/v for HPMC K100M to be taken.

Table 21: Suggested formulation by 3^2 factorial design

Batch No.	Parameters	Predicted value	Observed value	% error
CP1	% Drug release at 6 hours	58.49	56.92	1.57
	% Drug release at 12 hours	92.33	91.43	0.90

Released kinetics of optimized formulation:

Drug release data of all the formulation were fitted into different release kinetic model like Zero-order, First-order, Higuchi, Hixson-Crowell and Kors Meyer-Peppas. Results of release kinetic study are shown in the Table 22. From the results, it was concluded that the drug release from the formulation can be best explained by the Higuchi model due to highest R-square value among all the models i.e., Fiskian diffusion and erosion.

Table 22: Released kinetics of optimized formulation

Batch	Zero order	First order	Higuchi	Hixson crowell model	Peppas model
B1	0.9468	0.8738	0.9843	0.958	0.9745

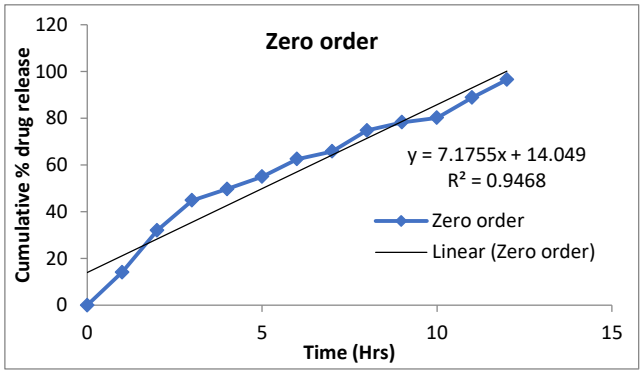

Figure 25: Zero order graph

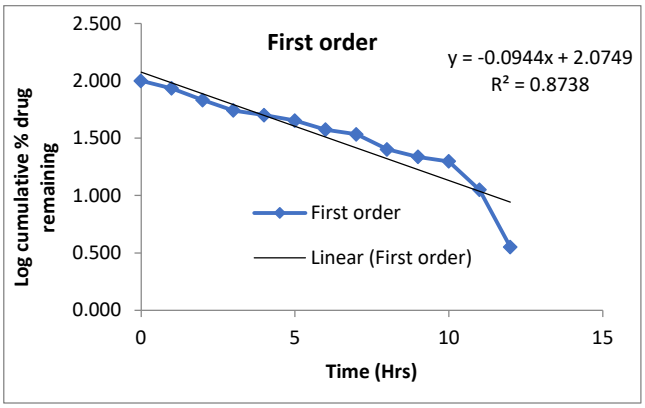

Figure 26: First order graph

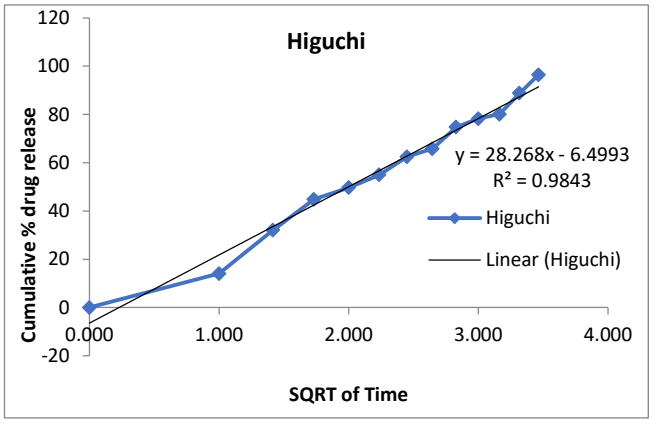

Figure 27: Higuchi graph

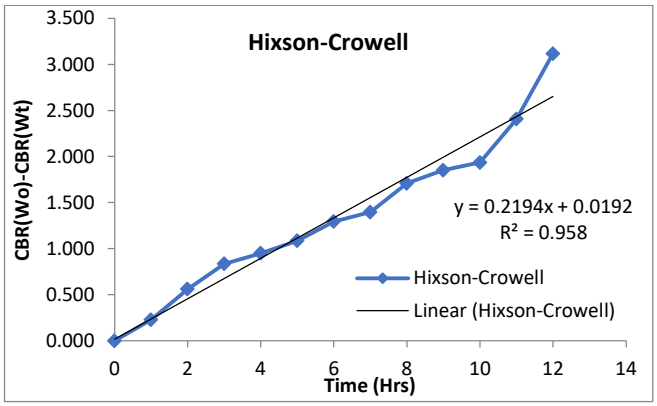

Figure 28: Hixson crowell graph

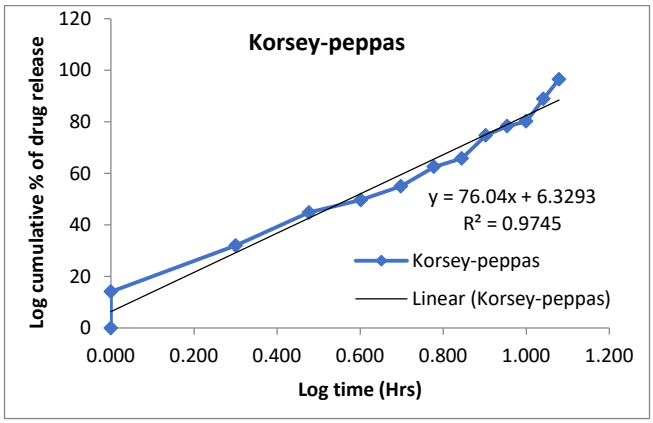

Figure 29: Korsey – peppas graph

Stability studies

On the basis of result of in vitro characteristics of in-situ formulation, B1 formulation was selected for further stability studies. B1 batch was kept for stability study under controlled environment condition (40 ± 2°C and 75 ± 5 %RH). Samples were withdrawn and physical appearance, percent drug content, Floating lag time, Total floating time was determined. After 30 days, there was no significant change in the physical appearance; percent drug content, floating lag time, total floating time was observed in in-situ gel formulation. The graph of the release profile of drug from the optimized formulation does not show any significant change after a stability period of 30 days at 40 ± 2 °C and 75 ± 5 %RH. Thus, the in-situ gel formulation was found to be stable up to 30 days.

Table 23: Stability studies

Parameters	Initial	After 30 days
Floating lag time	3.06 ± 0.09	3.33±0.33
Floating time	>12	>12
% CDR AT 12th hour	96.44	94.98
Drug content	99.24 ± 0.23	98.03±0.09

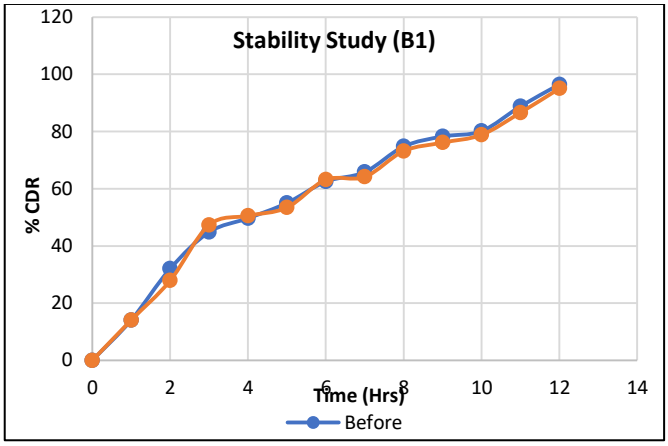

Figure 30: In vitro drug release of B1 formulation before and after stability study

SUMMARY AND CONCLUSION

In present investigation, attempt was made to prepare floating in situ gel of Nicardipine Hydrochloride with different concentrations of gelling agent using ionic cross-linking method. The formulation has prolonged the gastric residence time and released the drug in sustained manner due to the presence of release retard polymer. From the results it was concluded that viscosity, % water uptake and gelling strength increases with increasing the concentration of polymer. Formulation B1 kept for stability studies carried out at 40˚C± 2˚C and 75 % ± 5 % RH for 30 days in order to know the influence of temperature and relative humidity on floating lag time, total floating time, drug content and in-vitro drug release. The study conclusively demonstrated that Nicardipine Hydrochloride can be successfully formulated into floating in situ gel by ionic cross-linking method using HPMC K100M as retard polymer to obtain sustain release over the extended period of 12 hours. Release followed Higuchi model.

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