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Strategy for Development of pH Triggered Floating *In-situ* Gel of Levetiracetam

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ABSTRACT

The aim of this study was to develop a new intra-gastric floating in situ gelling system for controlled delivery of levetiracetam for the treatment of partial onset seizures. High dose of levetiracetam (750 to 1000 mg) is difficult to incorporate in floating tablets but can easily be given in liquid dosage form released. Sodium alginate-based in-situ gelling systems were prepared by dissolving various concentrations of sodium alginate in deionized water, to which drug and calcium carbonate were added. Fourier transform infrared spectroscopies (FTIR) were used to check the presence of any interaction between the drug and the excipients. A 3^2 full factorial design was used for optimization. The concentrations of sodium alginate (X1) and calcium carbonate (X2) were selected as the independent variables. The amount of the drug released after 1 h (Q1) and 6 h (Q6) and 12 h (Q12) and the viscosity of the solution were selected as the dependent variables. The gels were studied for their viscosity, in-vitro buoyancy and drug release. Other ingredient like HPMC K100M used for strength forming polymer, sodium citrate is used for liquefying solution. The drug release from the in-situ gel follows the Higuchi model and Korsemeyer-peppas model, which indicates a diffusion-controlled release.

Key word: In situ gel, Levetiracetam, Floating.

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INTRODUCTION

These are a group of disorders of the CNS characterised by paroxysmal cerebral dysrhythmia, manifesting as brief episode of loss or disturbance of consciousness with or without characteristic body movement (Convulsions), sensory or psychiatric phenomena. Epilepsy has a focal origin in the brain, manifestations depends on the site of focus, regions into which the discharge spread and postictal depression of these regions. Recognised from the drawn of history as 'disease of lightening' it was correctly described by JH Jackson little over a century ago. Epilepsy have been classified variously, major types are described below:

Generalised Seizures

A. Generalised tonic- clonic seizures (major epilepsy, Grand mal)
B. Absence seizure (minor epilepsy, Petit mal)
C. Atonic seizures (Akinetic epilepsy)
D. Myoclonic seizures
E. Infantile spasms (Hypsarrhythmia)

Partial Seizures

A. Simple partial seizures (SPS, Cortical focal epilepsy).
B. Complex partial seizures (CPS, Temporal lobe epilepsy, Psychomotor).
C. Simple partial or complex partial secondarily generalized seizure.[1]
D. **In Situ Gel** [2]

Recently, controlled and sustained drug delivery has become the standard in modern Pharmaceutical design and an intensive research have been undertaken in achieving much better drug product effectiveness, reliability and safety. This interest has been sparked by the advantages shown by in situ forming polymeric delivery systems such as ease of administration and reduced frequency of administration, improved patient compliance and comfort. The formation of gels depends on factors like temperature modulation, pH change, presence of ions and ultra violet irradiation, from which the drug gets released in a sustained and controlled manner.

Approaches of In Situ Gel Drug Delivery

There are four broadly defined mechanisms used for triggering the in situ gel formation of biomaterials: Physiological stimuli (e.g., temperature and pH), physical changes in biomaterials (e.g., solvent exchange and swelling), and chemical reactions (e.g., enzymatic, chemical and photo-initiated polymerization).

A) In situ formation based on physiological stimuli.

- **Thermally trigged system**
- **pH triggered systems**

Another formation of in situ gel based on physiologic stimuli is formation of gel is induced by pH changes. All the pH-sensitive polymers contain pendant acidic or basic groups that either accept or release protons in response to changes in environmental pH. The polymers with a large number of ionizable groups are known as polyelectrolyte. Swelling of hydrogel increases as the external pH increases in the case of weakly acidic (anionic) groups, but decreases if polymer contains weakly basic (cationic) groups. The most of anionic pH-sensitive polymers are based on PAA (Carbopol®, carbomer) or its derivatives. Drug formulated in liquid solutions have several limitations including limited bioavailability and propensity to be easily removed by tear fluid.

B) In situ formation based on chemical reactions

- **Ionic Cross linking.**

Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones .While k-carrageenan forms rigid, brittle gels in reply of small amount of K^+, i-carrageenan forms elastic gels mainly in the presence of Ca^{2+}. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes in situ gelling in the presence of mono- and divalent cations, including Ca^{2+}, Mg^{2+}, K^+ and Na^+. Gelation of the low-methoxy pectin can be caused by divalent cations, especially $Ca2^+$. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations e. g. Ca^{2+} due to the interaction with guluronic acid block in alginate chains.

- **Enzymatic cross-linking.**
- **Photo-polymerisation.**

MATERIAL AND METHOD:

Materials

Active pharmaceutical ingredient levetiracetam was gifted from Zydus Cadila pharmaceutical Ltd. Sodium alginate was purchased from Finar chemical Pvt Ltd, Ahmedabad. HPMC K 100M was gifted by Colorcon laboratory Pvt ltd, Goa. Calcium Carbonate was purchased from Omega laboratory, Vadodara. All other ingredients were used of analytical grade.

Methods

Preparation of floating In situ gel.[3]

Sodium alginate solution was prepared in deionized water with sodium Citrate. HPMC K100M were added in sodium alginate solution and stirred for 30min on magnetic stirrer. In another beaker add 30 ml of deionized water and mixed with Calcium Carbonate and stir on magnetic stirrer for 30 min. mixed both the sample and add API and stir for 30min. add required quantity of preservatives and flavor in formulation. Make up volume with deionized water.

Table 1: Insitu gel composition of different formulations of Levetiracetam tablets Floating in situ gel containing Sodium alginate as extended release polymer using 3^2 factorial.[4]

Ingredient (%w/v)	Formulation Code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Levetiracetam	5	5	5	5	5	5	5	5	5
HPMC K 100M	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Sodium alginate	0.75	1	1.5	0.75	1	1.5	0.75	1	1.5
Calcium Carbonate	0.5	0.5	0.5	0.75	0.75	0.75	1	1	1
Sodium Citrate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium Saccharin	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Orange flavor (AVOSIL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Distilled water Up to	100	100	100	100	100	100	100	100	100

3^2 full factorial designs		
Independent variables		**Dependent variables**
X1	**X2**	**Y**
Concentration of Sodium alginate	Concentration of $CaCo_3$	Drug release at 1,6,12 hrs, Floating Lag time, Viscosity

EVALUATION PARAMETER [5,6 ,7 ,8 ,9]

In Vitro Drug Release Study[10]

The *in vitro* release rate of levetiracetam from sustained release *in situ* gel was performed using USP apparatus (model TDT-08L, Electrolab, Mumbai, India) fitted with paddle over disk (50 r/min) at 37± 0.5^0C using 500 ml of 0.1 N HCl as a dissolution medium. This speed was slow enough to avoid the breaking of gelled formulation and was maintaining the mild agitation conditions believed to exist *in vivo*. At the predetermined time intervals, 5 ml samples were withdrawn, filtered through a whatmann filter paper membrane filter, diluted, and assayed at 205 nm using a Shimadzu UV 1800 double-beam spectrophotometer (Shimadzu, Kyoto, Japan). Cumulative percentage drug release (CPR) was calculated using an equation obtained from a calibration curve.

Appearance of gel

Appearance of gel of suitable formulation by visual determination.

Measurement of Viscosity of In Situ Gelling Solution.

The viscosities of the prepared solutions were determined by brookfield viscometer (Brookfield viscometer, model DV-II proviscometer). The samples (10 ml) were sheared at a rate of 100 r/min using S63 spindle at room temperature. Viscosity measurement for each sample was done in triplicate, with each measurement taking approximately 30 s.

***In Vitro* Floating Study**[11]

Floating study of in situ gelling solution was carried out in 500 ml of 0.1 N HCl (pH 1.2) in a Dissolution jar. Time required for floating on surface after adding solution (floating lag time) and total floating time were measured.

Content uniformity

10 ml of liquid solution (Containing 500mg Lev) add to the 30 ml of 0.1 N HCl for 30 min on magnetic stirrer. After 30 min, sample put on in sonicator for 30 min until clear solution is made. Made up volume up to 100ml. Take 1 ml and dilute up to 100ml and measured the absorption at 205nm in UV spectrophotometer.

pH Measurement

pH of prepared liquid formulation measured using Welltronix digital pH meter. For Measurement pH first stabilization of the pH meter using double distilled water, after stabilization calibrate pH meter using 0.1 N HCl and 6.8 pH Phosphate buffer. Completion of above procedure measured a prepared formulation.

Gel strength determination

A sample of 50 g of the Floating in-situ gel was put in a 100 ml graduated cylinder. A weight of 35g was placed onto the gelled form. The gel strength, which is an indication for the viscosity of the Floating in situ gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel.

Swelling Index

The swelling indexes of the gel of the selected formulations of sodium alginate were determined by a simple method. In this study the in situ gel formed in 40 ml of 0.1 N HCl (pH 1.2) was used. From each formulation the gel portion from the 0.1 N HCl was separated and the excess HCl solution was blotted out with a tissue paper. The initial weight of the gel taken was weighed and to this gel 50 ml of distilled water was added and after 12 hrs the water was decanted and the weight of the gel was recorded and the difference in the weight was calculated and reported.

Drug-polymer compatibility studies:

Studies were carried out using FTIR spectrophotometer (FTIR 8400S Spectrophotometer Shimadzu, Japan) by KBr pellet method.

RESULTS AND DISCUSSION

Drug Excipient Interaction Study

Fourier transform infrared (FTIR) technique has been used to study the physical and chemical interaction between drug and excipients used. FTIR spectrum of levetiracetam, HPMC K100M, sodium alginate and a physical mixture of levetiracetam, HPMC K100M and sodium alginate was recorded using KBr mixing method on FTIR instrument available at central instrument laboratory of the institute.

(FTIR- 8400 S, Shimadzu, Kyoto, Japan).

FTIR of the prepared formulation is done by the following method.

1) Lyophilized the Formulation.
2) Drying under the Sodium lamp.

Lyophilized the sample using EIE instrument Lyophilizer for 4 hrs. After drying the sample measured under the FTIR 8400 S instrument. In another method dry the sample under Sodium lamp for 3.5 hr, then measured under the instrument.

Table 2: Standard Calibration Curve of Levetiracetam in 0.1 N HCl

Sr. No	Concentration (µg/ml)	Absorbance			Average
		I	II	III	
1	0	0.000	0.000	0.000	0.000
2	4	0.142	0.135	0.14	0.139
3	8	0.269	0.262	0.264	0.265
4	12	0.377	0.384	0.382	0.381
5	16	0.5	0.499	0.516	0.505
6	20	0.637	0.636	0.635	0.636
7	24	0.75	0.761	0.757	0.756
8	28	0.842	0.852	0.856	0.85
Absorbance(y) = 0.0306 × Concentration(x) + 0.0135					
Regression coefficient (R^2) = 0.9987					

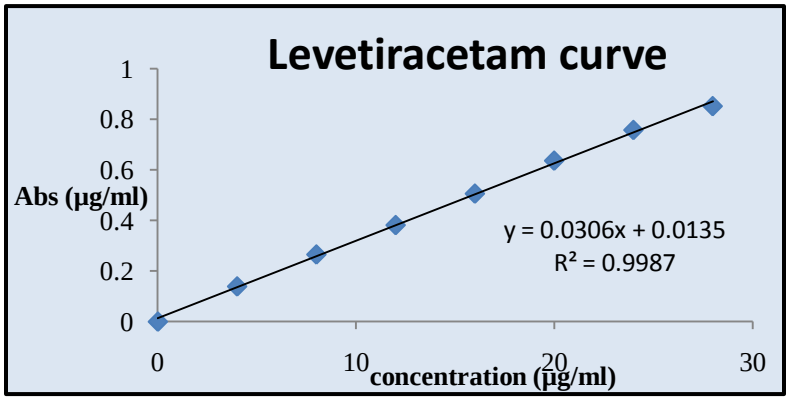

Figure 1: Calibration curve of levetiracetam in 0.1 N HCl

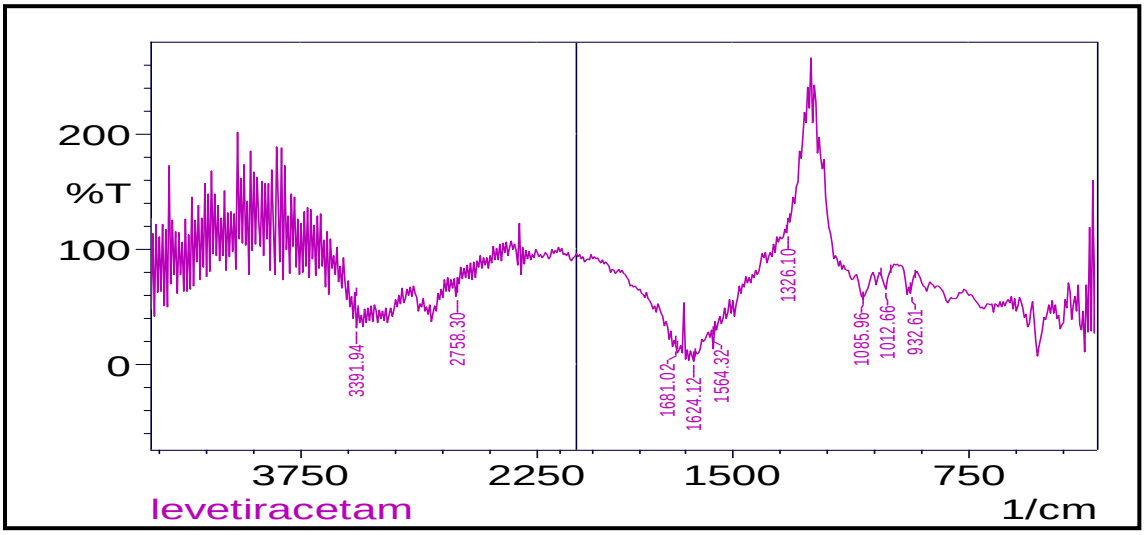

Figure 2: FTIR of pure Levetiracetam

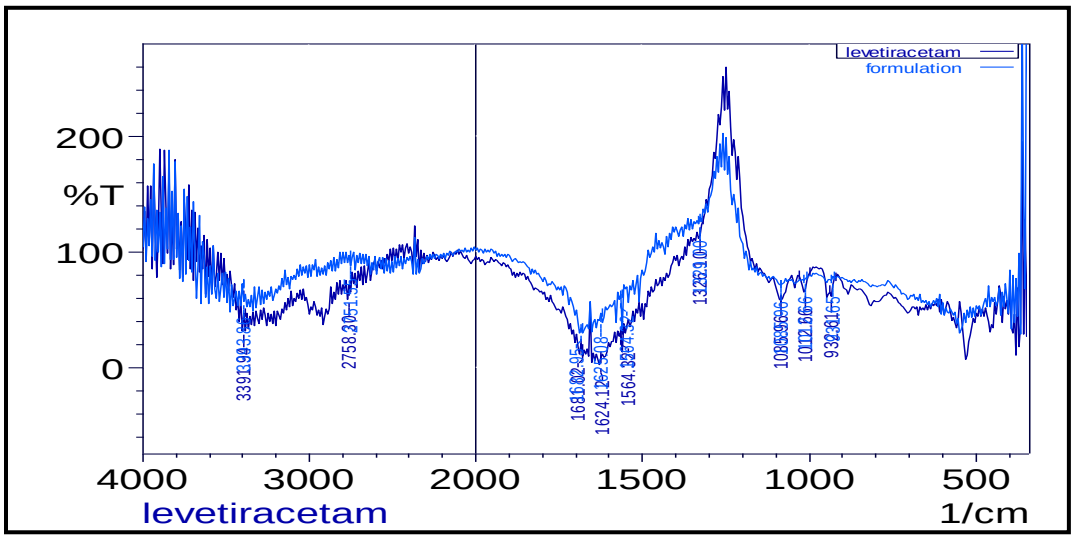

Figure 3: FTIR spectrum of formulation

Table 3: Evaluation Parameter of batch F1- F9.

Batch Code	pH	Gel Strength(Sec)	Viscosity (Cps)	Swelling Index (%)	Drug Content (%) (Mean±S.D.)
F1	8.36	15.62 ± 1.55	112.2	34.15 ± 1.55	99.04 ± 2.21
F2	8.62	18.48 ± 0.24	156	84.59 ±1.86	99.48 ± 1.33
F3	8.42	33.89 ± 1.87	220.1	96.4 ± 2.87	99.76 ± 1.96
F4	8.42	17.34 ± 2.21	240	44.99 ± 1.09	98.67 ± 1.12
F5	8.42	35.29 ± 1.50	259.4	55.34 ± 1.61	100.11 ± 1.15
F6	8.45	55.59 ± 1.35	302	70.45 ±1.81	98.84 ± 1.38
F7	8.40	35.09 ± 1.40	320	24.49 ± 1.23	98.91 ± 1.02
F8	8.36	58.16 ± 1.31	345	45.78 ±2.28	99.24 ± 1.38
F9	8.49	78.67 ± 1.99	405	67.78 ± 2.43	97.77 ± 1.31

Table 4: Effect of Shear on viscosity of formulation

Batch Code	Viscosity (Cps)	
	50 RPM	**100RPM**
F1	112.2	100.8
F2	156	140.3
F3	220.1	197.6
F4	240	210
F5	*259.4*	*237.4*
F6	302	276
F7	320	290
F8	345	329.2
F9	405	317.9

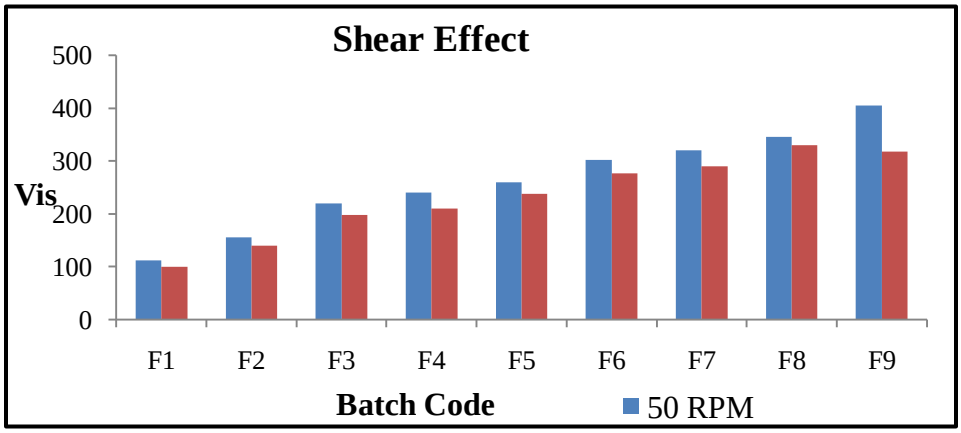

Figure 4: Comparison of Rheological profile of Batch F1- F9.

Table 5: Percentage drug release of formulations F1-F9

Time (Hr)	CPR								
	F1	**F2**	**F3**	**F4**	***F5***	**F6**	**F7**	**F8**	**F9**
0.5	53.88	51.02	47.96	51.82	48.25	45.48	49.01	46.36	44.09
1	56.98	53.72	51.25	55.49	53.06	50.39	51.72	50.77	48.19
2	60.02	57.40	55.96	59.44	56.61	53.41	57.91	54.17	51.98
3	65.94	61.24	60.25	63.38	60.15	56.91	61.33	57.06	55.90
4	71.04	66.29	64.23	68.70	64.81	60.62	65.04	61.69	59.84
5	77.73	71.29	68.05	73.90	69.09	64.86	69.07	65.47	62.52
6	84.31	76.81	73.57	80.49	74.03	67.91	73.05	68.62	66.06
7	89.78	82.24	79.14	85.63	79.74	72.51	78.19	73.14	70.13
8	96.68	88.15	84.39	89.59	83.31	76.51	82.38	76.77	73.95
9	98.77	92.25	87.12	93.00	86.90	80.97	86.68	80.68	78.50
10	-	96.81	90.87	96.81	90.49	85.08	94.25	83.66	81.51
11	-	-	94.02	-	95.40	90.37	97.61	88.09	86.29
12	-	-	-	-	100.86	91.67	-	91.21	88.98

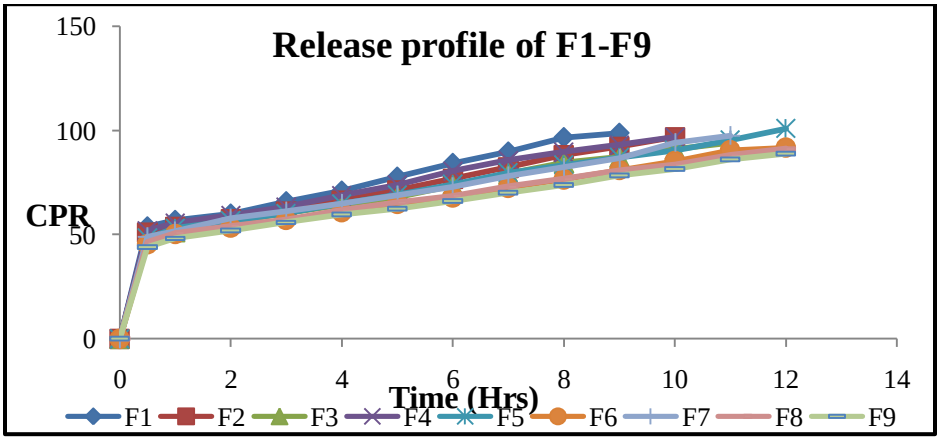

Figure 5: In-vitro release profile for Batches F1-F9.

Table 6: Results for ANOVA.

	DF	SS	MS	F	R^2	
Viscosity						
Regression						
FM	5	114020.6	22804.12	28.248	0.9792	
RM	3	110037.9	36679.31	28.6354	0.9449	F_{cal} = 2.467
Error						F_{tab} = 9.55
FM	3	2421.871	807.2904			(DF=2.,3)
RM	5	6404.539	1280.908			
Floating Lag time						
Regression						
FM	5	346.9167	69.38333	67.50811	0.99119	
RM	4	306.4167	76.60417	7.030593	0.8755	F_{cal} = 39.41
Error						F_{tab} = 10.13
FM	3	3.083333	1.027778			(DF=1,3)
RM	4	43.58333	10.89583			
Q1						
Regression						
FM	5	55.71759	11.14352	74.051	0.9959	
RM	4	55.69776	13.92444	118.18	0.9958	F_{cal} =0.131
Error						F_{tab} = 10.13
FM	3	0.451456	0.150485			(DF=1,3)
RM	4	0.471296	0.117824			
Q6						
Regression						
FM	5	253.7624	50.75248	23.15125	0.9747	
RM	3	253.5174	84.5058	61.93967	0.9738	F_{cal} = 0.056
Error						F_{tab} = 9.55
FM	3	6.576641	2.192214			(DF=2,3)
RM	5	6.821623	1.364325			
Q12						
Regression						
FM	5	52.421	10.484	1.912193	0.8724	
RM	4	16.63905	4.159762	0.318569	0.4915	F_{cal} = 6.526

Error				F_{tab} = 10.13
FM	3	16.44848	5.482827	(DF=1,3)
RM	4	52.23056	13.05764	

FM – full model, RM – Reduced model

Table 7: Kinetic Model data of factorial Batches[12]

	F1	F2	F3	F4	F5	F6	F7	F8	F9
Zero Order Model									
R^2	0.9971	0.9966	0.9981	0.9977	0.9988	0.9988	0.9978	0.9990	0.9988
a	5.566	5.142	4.873	4.917	4.413	3.992	4.52	3.808	3.803
b	50.18	47.03	45.41	49.73	47.39	44.86	47.15	46.06	43.80
Higuchi Model									
R^2	0.9773	0.9702	0.9774	0.9840	0.9845	0.9822	0.9793	0.987	0.9859
a	20.67	19.87	19.78	19.26	18.76	16.94	18.37	16.22	16.19
b	34.13	31.09	28.74	33.97	30.80	29.92	31.61	31.67	29.45
Korsemeyer-Peppas Model									
n	0.2190	0.2246	0.2384	0.2182	0.2318	0.2234	0.223	0.2146	0.2222
R^2	0.9445	0.9361	0.9496	0.9549	0.9576	0.9552	0.9542	0.9600	0.9617
b	-0.246	-0.275	-0.296	-0.261	-0.290	-0.315	-0.287	-0.308	-0.329
First Order Model									
R^2	0.9961	0.9988	0.9966	0.9934	0.9928	0.9937	0.9956	0.9926	0.9925
a	0.0324	0.0308	0.0297	0.0292	0.0265	0.0256	0.028	0.025	0.025
b	1.721	1.697	1.683	1.715	1.698	1.673	1.70	1.682	1.662
Hixon Crowell Model									
R^2	-0.997	-0.996	-0.998	-0.997	-0.998	-0.998	-0.997	-0.999	-0.999
a	-1.855	-1.714	-1.624	-1.639	-1.471	-1.330	-1.51	-1.269	-1.268
b	16.60	17.65	18.19	16.75	17.53	18.37	17.62	17.98	18.73

R^2- regression coefficient, a- Slope, b- Intercept, n- Diffusion coefficient.

Comparison Of In Vitro Release Profile For Selection Optimum Batch [13]

The values of similarity factor (f_2) for batches F1 to F9 were shown in Table 8. The batch F_5 showed maximum value of f_2 (72.83), hence was selected as optimum batch.

Table 8: Result of Similarity factor *f2*

Batch Code	f_2
F1	50.46
F2	61.69
F3	69.84
F4	56.82
F5	*72.83*
F6	69.25
F7	68.62
F8	70.16
F9	66.48

RESPONSE SURFACE PLOT OF DEPENDENT VARIABLES[14]

(Using Statistica Software)

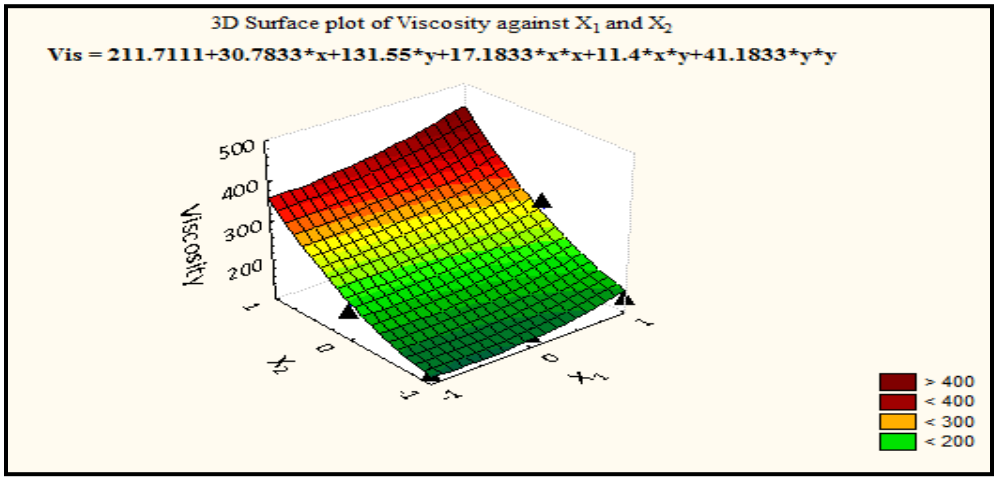

Figure 6 Response surface plot of Viscosity

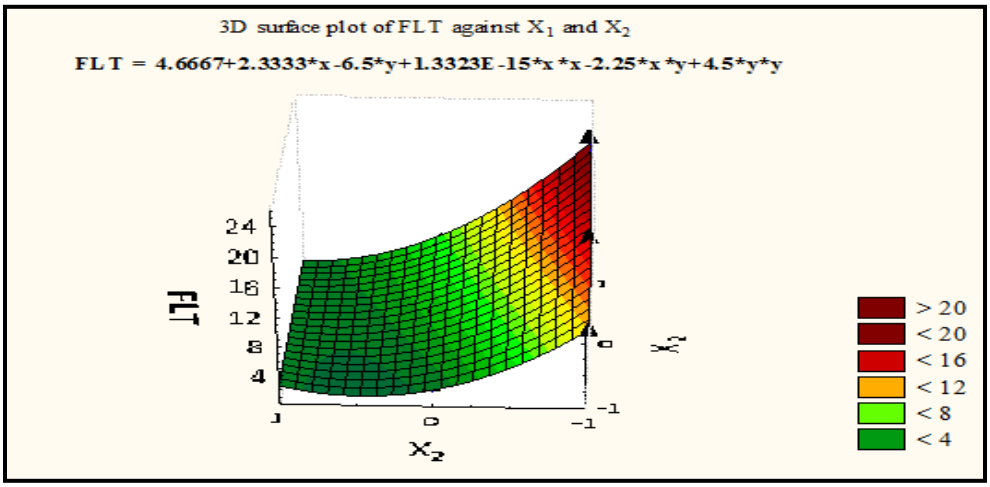

Figure 7 Response surface plot of Floating lag time

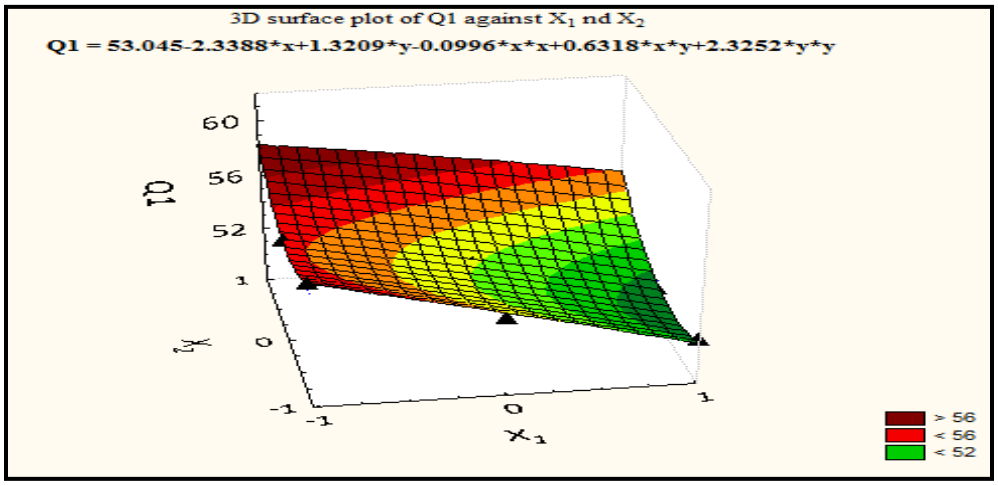

Figure 8 Response surface plot of Q1

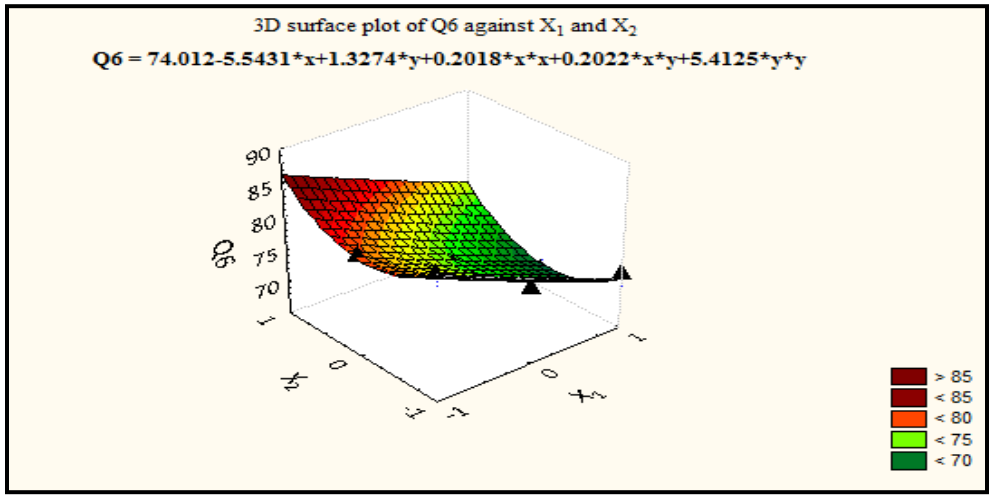

Figure 9 Response surface plot of Q6

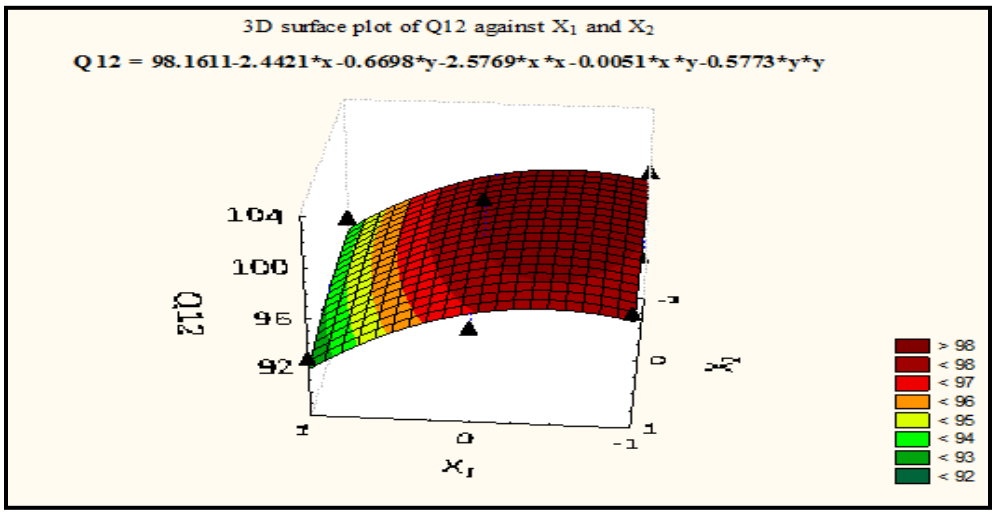

Figure 10 Response surface plot of Q12

STABILITY STUDY[15]

- The optimized formulation (F5) sealed in vial with rubber cap and kept in humidity chamber maintained 40 ± 2 °C / 75 ± 5 % RH for 1 month.
- The optimized formulations stored at 40 ± 2 °C / 75 ± 5 % RH were found stable.
- At the end of studies, samples were analyzed for the drug content, in vitro drug release, pH and viscosity were carried out which was shown in Table 5.15 and Table 5.16.
- There was not any change in morphological condition during stability study and also not any measurable change in the remaining parameter as shown in Table 5.15.
- Similarity factor of the batch after stability study was 84.57 comparable to initial drug release profile.
- Viscosity was slightly increased due to hydration of polymer.

Table 9: Comparison of evaluation parameter after stability study

	pH	Viscosity(Cps)	Drug Content
Initial	8.42	259.4	100.11 ± 1.15%
After storage at 40 ± 2°C / 75 ± 5 %RH	8.40	267.3 Cps	99.34 ± 2.09%

Table 10: Comparison of in vitro drug release study after stability study

Time (hr)	CPR at Initial	CPR at 40 ± 2°C / 75 ± 5 %RH
0.5	48.25	46.37
1	53.06	50.94
2	56.61	53.65
3	60.15	58.87
4	64.81	64.91
5	69.09	68.23
6	74.03	73.09
7	79.74	75.45
8	83.31	80.20
9	86.90	85.59
10	90.49	88.34
11	95.40	94.27
12	100.86	98.87

CONCLUSION

Levetiracetam was successfully formulated as a pH triggered floating in situ gelling system using Sodium alginate as a natural polymer. The optimized formulation F5 provided sustained *in vitro* release of drug over an extended period of 12 hrs. The drug release from gel structure follows a zero order release and release pattern from structure was diffusion type, Fickian release. The optimized formulation can be a competent alternative to conventional oral Solid dosage form. As in oral solid dosage form the dose of Levetiracetam dose is higher and this drug is generally prescribed in children and elder patient so having the difficulty in swallowing problem. That was overcome by formulating pH triggered Floating in situ gel which having drug release of extended period of time and less toxicity, compete the extended release oral dosage form.

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