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Research Article

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Formulation and evaluation of oral floating *In situ* gel of Tramadol hydrochloride

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Abstract *In situ* gel forming systems have been widely investigated as vehicles for sustained drug delivery. *In situ* gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. So, *In situ* gelling system via different route such as oral, nasal, ophthalmic etc can be formulated. In the present research work Oral Floating Insitu gel of Tramadol Hydrochloride was formulated using Eudragit L100, Eudragit S100, Eudragit RSPO, Ethyl cellulose, HPMC K100M. The optimized batch gave drug release for 12 hrs in the polymer combination of Sodium Alginate 2% and Ethyl Cellulose 2.5%.
In vivo study was performed by providing the formulation to rabbit and then X-ray was taken for the confirmation of formation of gel in stomach and floating of dosage form for 12 hrs. And it was found to be floating for more than 12 hrs. The batch was found to be stable for 6 months in accelerated stability study.

Keywords Tramadol hydrochlotide, *in situ* Gel, *in vivo* study

Introduction

In situ gel forming systems have been widely investigated as vehicles for sustained drug delivery. 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. *In situ* gel formation occurs due to one or combination of different stimuli like pH change, temperature modulation and solvent exchange. So, *In situ* gelling system via different route such as oral, nasal, ophthalmic etc can be formulated. Various natural and synthetic polymers such as gellan gum, alginic acid, xyloglucan, pectin, chitosan, poly (DL lactic acid), poly (DL-lactide-co-glycolide) and polycaprolactone are used for formulation development of *In situ* forming drug delivery systems. Gastro retentive *In situ* gelling system helps to increase bioavailability of drug compared to conventional liquid dosage form. The gel formed from *In situ* gelling system, being lighter than gastric fluids, floats over the stomach contents or adhere to gastric mucosa due to presence of bioadhesive nature of polymer and produce gastric retention of dosage form and increase gastric residence time resulting in prolonged drug delivery in gastrointestinal tract [1].

These *In situ* gel preparations can be easily formulated in bulk, it gives site specific drug delivery and sustained action when compared to other conventional suspensions. The polymers which are used to prepare *In situ* gels can be termed as smart polymers. They are having the ability to change their physicochemical properties in response to the altered environmental conditions.

The *In situ gel* formation occurs due to one or combination of different stimuli like PH change, temperature change, ionic activation etc.

The *In situ* gelling systems can be applied in different routes like oral, nasal, ophthalmic, injectable, and vaginal route. Various natural and synthetic polymers can be used in the preparation of *In situ* gels like alginates, gellan gum, xyloglucan, pectin, chitosan, PLA and carbopol. The *In situ gel* forming polymeric formulations are having several advantages like sustained and prolonged action compared to conventional drug delivery system, ease of administration, deliverance of accurate dose as well as to prolong residence time of drug, reduced frequency of administration, improved patient compliance and comfort. This system is also suitable from the manufacturing point of view as the productions of them are less complex and lowers investment and manufacturing cost [2].

Stomach Specific Floating *in situ* Gel

In situ gel forming systems have been widely studied, for their capability of producing the sustained and controlled drug delivery. Such systems offer the advantage of easy administration. It along with improved patient compliance. In recent few years, lots of work on development of *In situ* gelling the formulation has been done and delivery of drug via popular routes like oral, nasal, ophthalmic along with other routes like vagina has been studied, which has shown the promising result, for the use of system as a potential way of producing the controlled drug delivery. The system basically utilizes polymers which undergo transformation from solution to gel like consistency, due to change in their physicochemical properties. *In situ* gel formation can be stimulated by change in the temperature, change in pH, change in the solvent medium, by radiation exposure or by combination of any of these.

Principle of *in situ* gel formation

Formulation of gastro retentive *In situ* gel system involves the use of gelling agent which can form a stable sol/suspension system to contain the dispersed drug and other excipients. The gelling of this sol/suspension system is to be achieved in gastric environment, triggered by ionic complexation due to change in pH. The formulation adopted is a sodium alginate solution containing calcium carbonate (as a source of Ca^{2+}) and releases them only in the acidic environment of the stomach.

Sodium alginate acts as a gelling agent. The free Ca^{2+} ions gets entrapped in polymeric chains of sodium alginate thereby causing cross linking of polymer chains to form matrix structure. This gelation involves the formation of double helical junction zones followed by reaggregation of the double helical segments to form a three-dimensional network by complexation with cations and hydrogen bonding with water [3].

Sodium citrate + Ca. carbonate ($CaCO_3$)

Ca. citrate complex in acidic environment gives Ca + CO_2

In this way, the formulation remains in liquid form until it reaches the stomach, where gelation of sodium alginate is instantaneous.

The gelling agent serves as a dispersion medium in the form of aqueous solution to contain the drug in dispersed form along with the cross linking agent.

The gas forming agent employed serves two functions

a) Act as source of divalent cation that triggers gelation at gastric pH.
b) Produces CO_2 that get entrapped in the gelled matrix to impart buoyancy.

The gelling agent employed in this case is the sodium alginate, It is one of the widely used polymer in cases where ion triggered gelation of *In situ* gelling agent is desired. Its aqueous solution serves as the medium for containing the drug in dispersed form along with the gas forming agent.

Calcium carbonate is incorporated as the gas forming agent that act as source of divalent cat ions and produces CO^2 at gastric pH. Anhydrous Calcium chloride is employed as a source of Ca^{2+} which is added to the sol just to impart sufficient viscosity to the solution, so as to form uniform dispersion [2].

Material and Methods

Material

Drug -Tramadol HCl, Polymers- Eudragit L100, Eudragit S100, Eudragit RSPO, Ethyl cellulose, HPMC K100M Excipients: Sodium Bicarbonate, calcium carbonate, Calcium chloride, Sodium citrate etc.

Method

The formulation was prepared as given in table no 1 by heating polymer at 60 °C in deionized water with continuous stirring. After cooling below 40 °C, gas forming agent Calcium Carbonate, Cross linking agent Calcium Chloride, Buoyancy enhancer Sodium Bicarbonate, and drug (Tramadol HCl) was added with continuous stirring. Finally Sodium Citrate was added to maintain fluidity of formulation.

Evaluation of Formulation

Characterization of oral *in situ* Gel of Tramadol HCl

a) Appearance: The developed formulations were inspected visually for clarity of sol by observing in white and black background [4].

b) pH measurement: The pH of the each formulation was determined by using pH meter. The pH meter was first calibrated using solutions of pH 4 and pH 7 [4].

c) Measurement of viscosity: The viscosity of formulations was determined by a Brookfield viscometer DV-III (Brookfield, USA) [5].

d) Gelling time: It was graded in three categories on the basis of gelation time and time period for which the formed gel remains as it is a) gel after few minutes, b) dispersed rapidly, c) gelation immediate, remain for 12h. Gelation immediate, remain for more than 12 h.

Table 1: Formulation of oral floating *in situ* gel of Tramadol Hydrochloride (weights in %)

Name of Ingredients	Formulation Code											
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**	**T12**
Tramadol HCl (mg)	100	100	100	100	100	100	100	100	100	100	100	100
Sodium alginate	-	-	-	-	2.5	1	2.5	1.5	0.5	3.5	2	2
HPMC K 100M	1	1	-	-	-	-	-	-	-	-	-	-
Eudragit L100	-	-	-	2								
Eudragit S100	-	-	2	-	-	-	-	-	-	-	-	-
Eudragit RSPO	2	-	-	-	-	-	1.5	2.5	-	0.5	2	-
Ethyl cellulose	-	2	2	2	1.5	3	-	-	3.5	-	-	2.5
Calcium carbonate	2	2	2	2	2	2	2	2	2	2	2	2
Calcium chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Sodium bicarbonate	2	2	2	2	2	2	2	2	2	2	2	2
Sodium citrate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Flavoring agent	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs
Distilled water (up to ml)	100	100	100	100	100	100	100	100	100	100	100	100

Figure 1: Gelation of in situ Gel formulation.

e) Floating lag time: In this test 10 ml of *in situ* formulation was added into the 900 ml dissolution vessel containing 0.1 N HCl at 37 °C. It is the time the formulation took to emerge on surface of dissolution medium is referred as floating lag time [6].

f) Floating duration: In this test 10 ml of *in situ* formulation was added into the 900 ml dissolution vessel containing 0.1 N HCl at 37 °C. The time that formulation took to remain constantly floating on surface of dissolution medium is referred as duration of floating [6].

g) Drug content estimation: The prepared *in situ* gel formulations were analyzed for drug content by transferring 1 ml of formulation in 100 ml volumetric flask and add 50 ml of 0.1 N HCl with pH 1.2 with continuous shaking. Final volume was adjusted upto 100 ml with the help of 0.1 N HCl of pH 1.2 and filtered the solution. Drug

concentration in filtrated solution was determined spectrophotometrically at respective wavelength of drug using UV-Visible spectrophotometer (Shimadzu 1800, Japan) [6].

h) Drug Interaction Studies (Compatibility Studies)

It's important to check any kind of interaction between drug candidate and polymer. The polymers which are to be incorporated into formulation should be compatible with the drug. This compatibility study or interaction study was done using Fourier transformed infrared spectroscopy.

IR spectra of pure Tramadol hydrochloride and polymers viz. Sodium Alginate, Ethyl cellulose, Eudragit RSPO were taken separately. Then to know if there is any interaction between drug and polymer, IR spectra of Tramadol hydrochloride and other polymers were taken in combination (figure 2-5).

***In vitro* dissolution study**: An *in vitro* release study was carried out using dissolution test apparatus USP Type II (Paddle Method). Volume of dissolution media was 900 ml, of Hydrochloric acid buffer solution of pH 1.2, temperature 37 ± 0.2 °C, RPM was 50. 1 ml of sample was removed each h and it was diluted to 10 ml with 0.1 N HCl at 305 nm. And 1 ml of sample was replaced in dissolution media to maintain sink condition [7].

***In vivo* studies**

An *in vivo* release study was carried out in healthy rabbits. Using oral feeding tube and syring gels (Tramadol HCl) were feeded to rabbits and X-ray was taken of rabbit abdomen to check the floating ability of gel formulation.

For this barium sulphate (15%) loaded oral floating *in situ* gel was prepared, Healthy rabbit weighing approximately 2.3 Kg was used to assess *in vivo* floating behaviour. Ethical clearance for the handling of experimental animals was obtained from the institutional animal ethical committee (IAEC) of the institute. The animal was fasted for 12 hrs. The rabbit was made to swallow barium sulphate loaded insitu gel with water. During the experiment, rabbit was not allowed to eat but water was provided. At predetermined time intervals, (empty stomach, immediate after feed, after 1 h of feed and after 8 hr of feed) the radiograph of abdomen was taken using an X-ray machine [8].

Drug Kinetic study

The release data obtained from various batches were studied with respect to effect of drug: polymer ratio. To analyze the mechanism of drug release from the formulation, the dissolution profile of optimized batches was fitted to zero-order, first-order, Higuchi, Hixson-Crowell, Korsemeyer and Peppas, models to ascertain the kinetic modeling of drug release [9].

Statistical Analysis

It comes under Planned versus posteriori (unplanned) comparisons in ANNOVA. Bonferroni method is often used to control the alpha level for multiple comparisons for an overall level of alpha, the level is set at α/k for each test, while k is the number of comparisons planned. For the planned data comparisons at an overall level of 0.05 it show there is significant difference or non significant different in the data.

In this study the Bonferroni method was applied to dissolution study to check there was significant difference or non significant difference in release of drug in formulated formulations [10].

Stability testing

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. FDA and ICH specifies the guidelines for stability testing of new drug products, as a technical requirement for the registration of pharmaceuticals for human use. The ICH Guidelines have established that long term stability testing should be done at 25°C/60% RH; stress testing should be done at 40°C/75%RH for 6 months. If significant change occurs at these stress condition, then the formulation should be tested at an intermediate condition i.e. 30°C/65%RH [11].

Results and Discussion

Characterization of oral *in situ* gel of Tramadol HCl

For the Characterization of oral *in situ* gel of Tramadol HCl pH, viscosity, Gelling time, Floating lag time, Floating duration and Content uniformity tests were performed and the results were as follows:

a) Appearance: All the prepared batches was found to be clear in appearance

b) pH: The pH was measured of each of the polymer formulation based *in situ* solution using a calibrated digital pH meter at 27 °C. The pH of all the prepared batches was found in the range of 9.2 to 10.8. The optimized batch T12 showed pH 10.8 (Table 2).

c) Viscosity: The viscosity of all formulations was determined by a Brookfield viscometer DV-III (Brookfield, USA) using spindle number 62 with cup and bob setting at 100 rpm. All the prepared formulations showed viscosity in the range of 8.75 to 9.86 cps. The optimized batch T12 show viscosity of 9.86 cps (Table 2).

d) Gelling time: The gelling capacity of prepared formulations was observed by visual examination. All the prepared batches show gelling time from 4-5 second to immediate after entering in 0.1 N HCl. The optimized batch T12 showed immediate gelling after getting in contact with 0.1 N HCl and remain in the form of gel for more than 12 h (Table 2).

e) Floating lag time: Floating lag time of all the prepared formulations was observed by visual examination. All the prepared formulations show Floating lag time from 4-5 sec to immediate. And the optimized batch T12 show immediate floating after entering in 0.1 N HCl and show floating for more than 12 h (Table 2).

f) Floating Duration: All prepared formulation show floating duration more than 12 h (Table 2).

g) Drug Content Uniformity: All the prepared formulations show drug content uniformity in the range of 97.96% to 100.06 % (Table 2). The values are acceptable as per Indian pharmacopeia standards

h) FT-IR Spectroscopy (Tramadol hydrochloride)

It's important to check any kind of interaction between drug candidate and polymer. The polymers which were to be incorporated into formulation should be compatible with the drug. This compatibility study or interaction study was done using Fourier transformed infrared spectroscopy. IR spectra of pure Tramodol HCl and polymers viz. Sodium Alginate, Ethyl cellulose, Eudragit RSPO etc were taken separately. Then to know if there is any interaction between drug and polymer, IR spectra of Tramadol HCl and other polymers were taken in combination (Table 3-6 & Figure 2-5). There was no interaction in drug and polymer since the peaks of pure drug and polymers retains in combination.

Table 2: Various characteristics of oral *in situ* gel of Tramadol HCl

Formulation code	pH	Viscosity in cps	Gelling time (sec)	Floating lag time (sec)	Floating Duration in hr	Drug content (%)
T1	9.5	8.99	2-5	4-5	> 12	97.96 ± 0.63
T2	9.2	8.75	3-4	3-4	> 12	98.08 ±0.53
T3	9.4	9.12	4-5	3-4	> 12	98.77 ±0.55
T4	9.5	9.07	3-6	4-5	> 12	100.06 ±0.60
T5	9.6	9.58	3-5	2-3	> 12	99.70 ±0.45
T6	9.3	9.38	3-4	2-3	> 12	98.90 ±0.55
T7	9.8	9.77	2-5	1-2	> 12	99.3 ±0.52
T8	9.7	9.67	2-3	1-2	> 12	99.1 ±0.66
T9	10.2	9.55	1-2	Immediate	> 12	98.12 ±0.49
T10	10.5	9.85	Immediate	Immediate	> 12	98.8 ±0.44
T11	10.2	9.71	5-6	4-5	> 12	99.12 ±0.59
T12	10.8	9.86	Immediate	Immediate	> 12	98.5 ±0.37

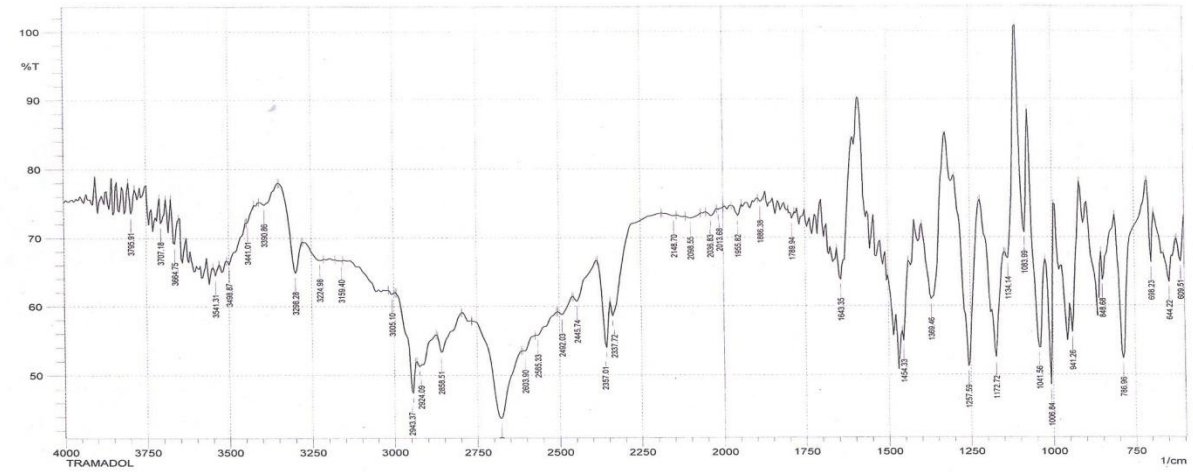


Figure 2 : IR of Tramadol Hydrochloride

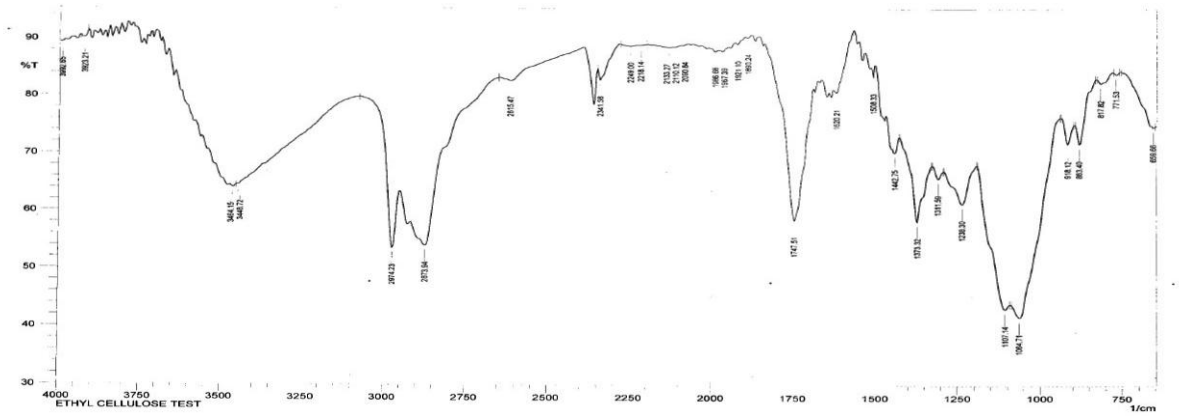


Figure 3 : IR of Ethyl Cellulose

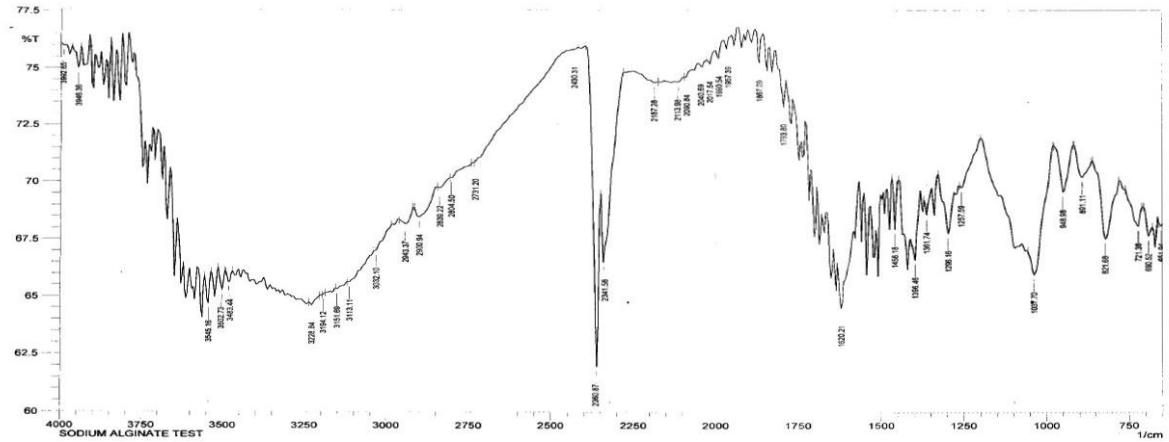


Figure 4 : IR of Sodium Alginate

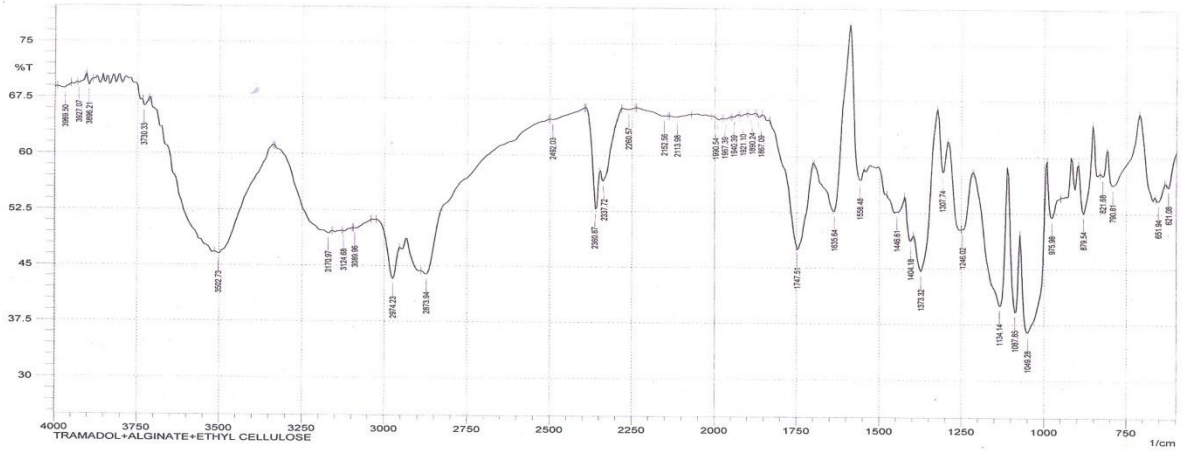


Figure 5 : IR of Tramadol Hydrochloride + Sodium Alginate + Ethyl Cellulose

Table 3: Principle peaks and chemical groups present in IR spectrum of pure Tramadol HCl

S. No.	Functional group	Peak values (cm^{-1})
1.	-OH	3541.31 or 3298.28
2.	=C-H	3008
3.	-C-H	2943.37
4.	-C-O	1006.84
5.	C=C	1643
		1789.94

Table 4: Principle peaks and chemical groups present in IR spectrum of Sodium Alginate.

S. No.	Functional group	Peak values (cm^{-1})
1.	-OH Stretching	3545
2.	-C-H Stretching	2943.27
3.	-C=O Stretching	1620
4.	C-O Stretching	1037.70

Table 5: Principle peaks and chemical groups present in IR spectrum of plane Ethyl cellulose

S. No.	Functional group	Peak values (cm^{-1})
1.	C-H	2974.23
2.	C-O	1107.14
		1064.71

Table 6: Principle peaks and chemical groups present in IR spectrum of Tramadol HCl, Sodium Alginate and Ethyl cellulose

S. No.	Functional groups in Tramadol HCl	Peaks values (cm^{-1}) in Tramadol HCl	Peaks values (cm^{-1}) in Sodium Alginate	Peaks values (cm^{-1}) in Ethyl Cellulose	Peaks values (cm^{-1}) in Constitution Combination	Interpretation
1.	-OH	3541.31 or 3298.28	-	-	3502.73	No Interaction
2.	=C-H	3008	-	-	3089.96	No Interaction
3.	-C-H	2943.37	-	-	2943.37	No Interaction
4.	-C-O	1006.84	1037	1064	1049	No Interaction
5.	-C=C	1643 1789.94	1747.84	-	-	No Interaction
6.	-C=O	-	1620	-	1643	No Interaction

***In vitro* Dissolution Study for Oral Floating *In situ* Gel of Tramadol HCl**

In situ gel forming polymeric formulations are the drug delivery system that are in solution or suspension form before administration in body, but once administered, undergo gelation *in situ*, to form gel. *In situ* gel forming system have been widely investigated as vehicle for sustain drug delivery system.

- The developed formulations were to meet all the pre-requisites to become an *in situ* gelling floating system, gelled and floated instantaneously at the pH conditions of the stomach. The calcium carbonate present in the formulation as insoluble dispersion was dissolved and releases carbon dioxide on reaction with acid of the stomach and the *In situ* released calcium ions results in formation of gel with floating characteristics. The released carbon dioxide was entrapped in the gel network of the formulation, and gel rises to the surface of the dissolution medium (*in vitro*) or the stomach fluid (*in vivo*).
- On this basis various formulations were tried to formulate and evaluate as various natural as well as synthetic polymers in the combination of Natural: Natural, Natural: Synthetic, Synthetic: Synthetic were used in different ratio.
- Different formulations were developed with various polymers like guar gum, xanthan gum, pectin, various grads of carbapol like carbapol 934, carbapol 940, carbapol 971, various grades of eudragits like Eudragit L100, Eudragit S 100, various grades of HPMC like HPMC K4 M, K15 m, etc. but these polymers in different proportion fails to sustain the drug release for 12 h, they show burst release within 2-3 h so they fails to show gastric retention.
- The formulated batch T1 (HPMC K100 M 1% : Eudragit RSPO 2%) show *in vitro* release for 4 h, batch T2 (HPMC K100 M 1% : Ethyl cellulose 2%) show *in vitro* release for 4 h, batch T3 (Eudragit S100 % : Ethyl cellulose 2%) show *in vitro* release for 3 h, batch T4 (Eudragit L100 2% : Ethyl cellulose 2%) show *in vitro* release for 4 h, batch T5 (Sodium Alginate 2.5% : Ethyl cellulose 1.5%) show *in vitro* release for 8 h, batch T6 (Sodium Alginate 1% : Ethyl cellulose 3%) show *in vitro* release for 10 h, batch T7 (Sodium Alginate 2.5% : Eudragit RSPO 1.5%) show *in vitro* release for 9 h, batch T8 (Sodium Alginate 1.5% : Eudragit RSPO 2.5%) show *in vitro* release for 6 h, batch T9 (Sodium Alginate 0.5% : Ethyl Cellulose 3.5%) show *in vitro* release for 7 h, batch T10 (Sodium Alginate 3.5% : Eudragit RSPO 0.5%) show *in vitro* release for 6 h. As all these formulation did not retard the release of drug for 12 h it did not show

gastric retention. In the batches T1-T4 there was no use of *in situ* gelling polymer *i.e.* sodium alginate, so though there was used of combination of sustain release polymers, viscosity increasing agent etc. (combination of symthetic and semisynthetic) polymers was unable to retard the release of drug. In the batched T5–T10, there was use of *in situ* gelling agent but there combination with other polymers in different ratio fail to retard the release. This can be because the interaction between polymers was not sufficient to retard the release.

- In the formulated batch T11 (Sodium Alginate 2%: Eudragit RSPO 2%) show *in vitro* release for 12 h. Though it show release for 12 h it gave floating lag time as 5-6 sec and floating duration 4-5 sec, than optimized batch show floating lag time and floating time immediate. It also show significant different than optimized batch.
- The formulate batch T12 (Sodium Alginate 2%: Ethyl cellulose 2.5%) in the ratio 4:5 gave *in vitro* drug release for 12 h (98.45±0.97), as the matrix formation between sodium alginate and ethyl cellulose (Natural: Semisynthetic) sodium alginate having use as gelling agent use in *in situ* gelling formulation and ethyl cellulose as viscosity increasing agent were strong to retard the drug release. Hence it show gastric retention it floats immediately also gets converted in to gel immediately after incorporation in 0.1 N HCl. It also floating duration was found to be more than 12 h. Kinetically this batch follow Korsmeyr-pappas model. So batch T12 was optimized.

Table 7: % Drug Release of Oral Floating *in situ* Gel of Tramadol HCl.

Time (in hr)	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	68.89 ±0.66	66.21 ±0.77	53.96 ±0.93	56.57 ±1.03	37.39 ±0.95	33.29 ±1.01	42.88 ±0.67	37.4 ±1.06	40.17 ±0.75	47.07 ±0.74	38.8 ±0.88	29.81 ±0.91
2	78.56 ±0.86	72.61 ±0.67	69.22 ±1.02	71.69 ±0.99	45.66 ±0.79	41.54 ±0.67	49.77 ±0.81	55.2 ±0.67	51.18 ±0.90	56.57 ±0.84	45.55 ±0.70	38.8 ±0.81
3	90.97 ±0.78	88.97 ±0.79	92.57 ±1.5	78.77 ±0.76	51.19 ±0.91	51.18 ±0.99	58.64 ±0.82	60.74 ±0.61	56.71 ±0.73	71.77 ±0.67	49.82 ±0.75	47.06 ±0.72
4	100.65 ±0.90	97.61 ±0.95		91.3 ±0.85	59.46 ±0.84	56.71 ±0.71	66.32 ±0.93	69.02 ±0.71	66.37 ±0.77	78.69 ±0.98	55.35 ±0.85	55.33 ±0.82
5					66.37 ±0.88	64.99 ±0.98	71.87 ±0.74	77.32 ±0.80	74.8 ±0.98	85.63 ±0.90	60.89 ±0.96	59.9 ±0.93
6					76.03 ±0.99	70.54 ±0.83	76.06 ±0.84	91.02 ±0.95	84.52 ±0.91	92.57 ±0.87	65.07 ±0.94	66.41 ±0.83
7					82.95 ±0.78	76.09 ±0.78	82.99 ±0.91		91.3 ±1.01		70.61 ±0.78	73.33 ±0.94
8					92.64 ±1.02	83.03 ±0.96	88.56 ±0.83				74.08 ±0.87	78.89 ±0.60
9						87.23 ±1.0	95.95 ±0.76				77.62 ±0.77	85.82 ±0.98
10						96.91 ±0.91					80.45 ±0.80	90.03 ±0.79
11											88.75 ±0.70	95.6 ±0.88
12											97.06 ±0.77	98.45 ±0.97

N=3

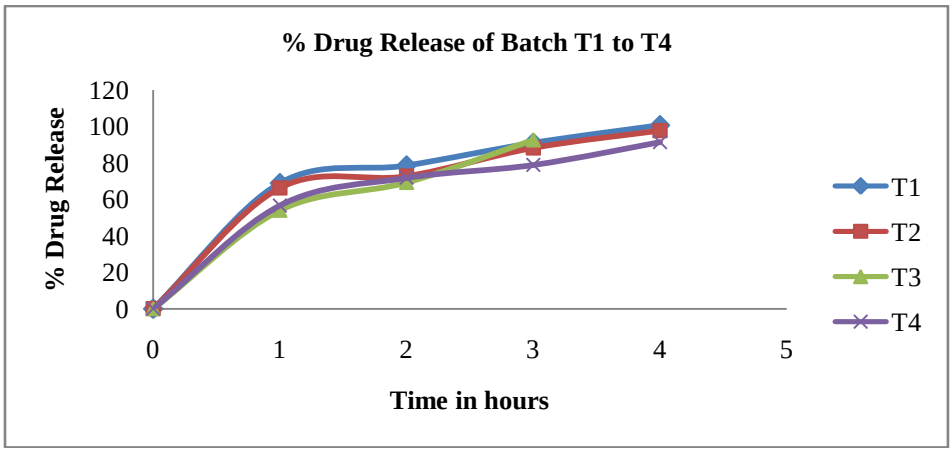


Figure 6: % Drug release of Batches T1 to T4

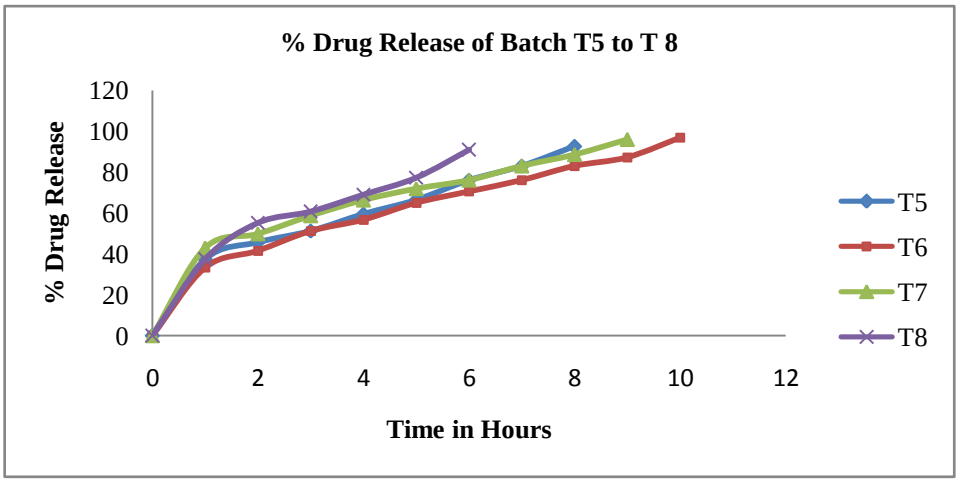


Figure 7 : % Drug release of Batches T5 to T8

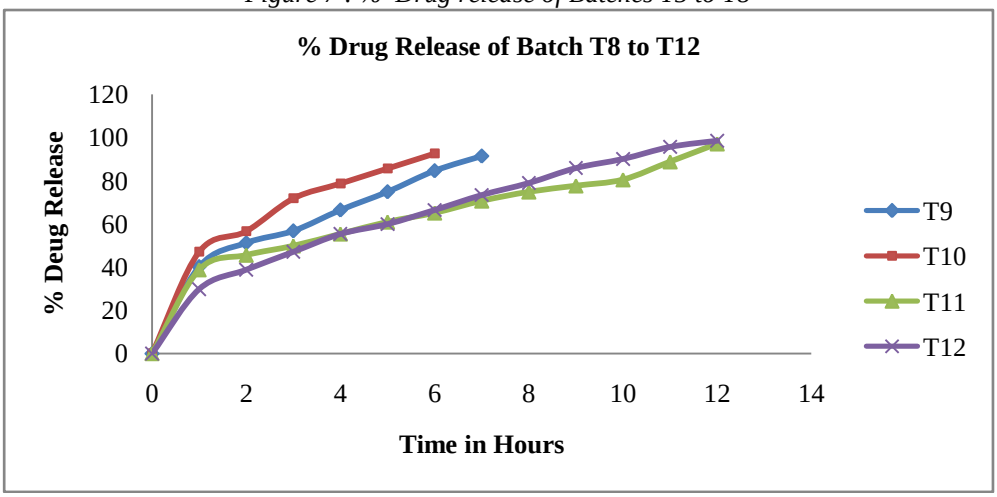
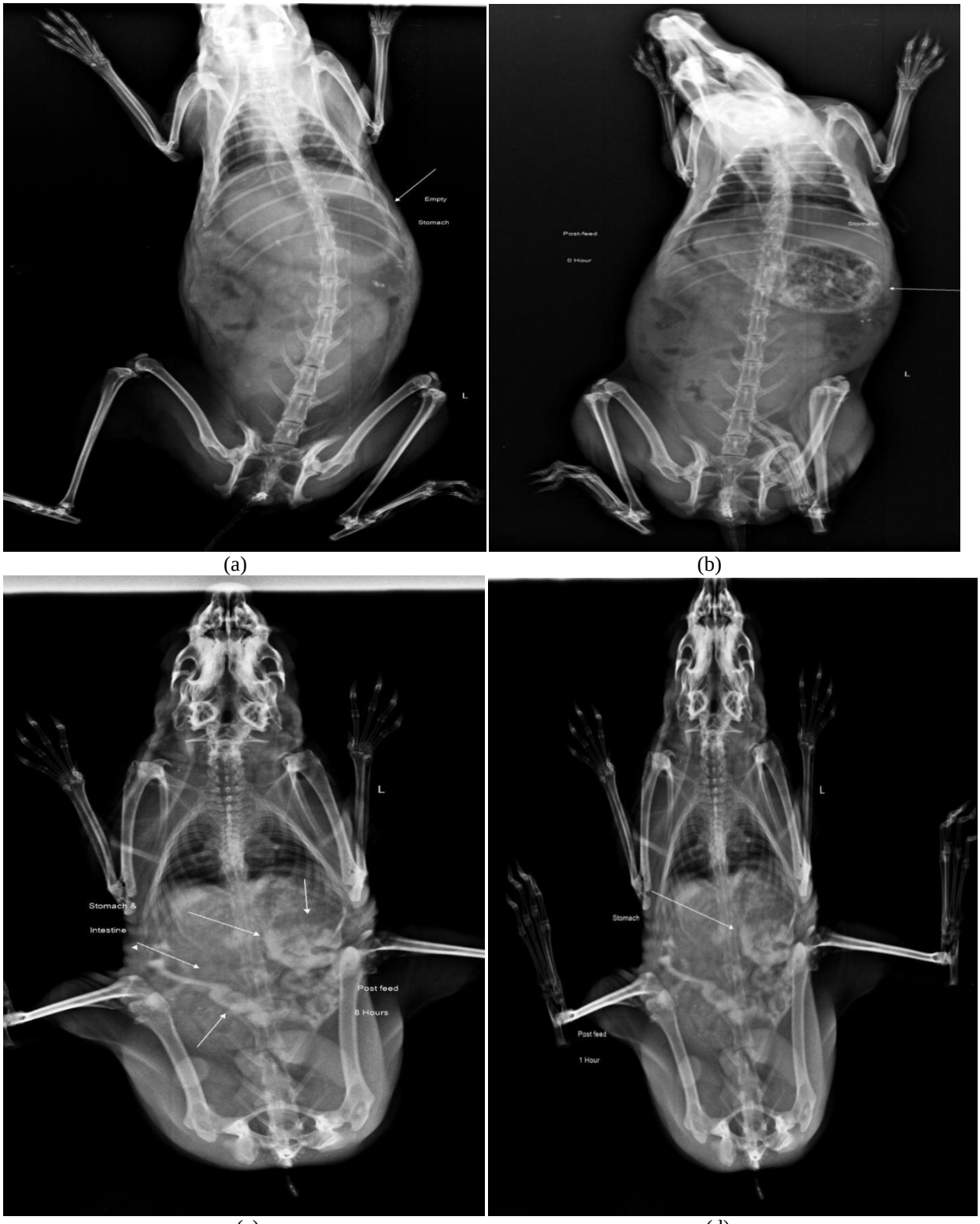


Figure 8 : % Drug release of Batches T9 to T12

***In vivo* studies:** An *in vivo* release study was carried out in healthy rabbits. Using oral feeding tube and needle gel of Tramadol HCl was administered to rabbits and X-ray was taken of rabbit abdomen to check the floating ability of gel formulation. The X-ray were taken as a) X-ray of empty stomach b) X ray immediate after feeding of gel c) X-ray after 1 h. of feeding of gel d) X-ray after 8 h of feed. It was found that the oral floating *in situ* gel was float immediately after feeding to rabbit and it was found to be floating in the stomach more than 6 h. The following X-

ray shows a) the X-ray of empty stomach, b) show X-ray immediate after feeding of gel to rabbit, c) X-ray after 1 h of feeding d) X-ray after 8 h of feeding. Images are as follows:



(a) (b)

(c) (d)

Figure 9: In vivo study of Oral Floating In situ gel of Tramadol HCl

Optimization

In the present study various natural as well as synthetic polymers in the combination of Natural: Natural, Natural: Synthetic, Synthetic: Synthetic were used in different ratio. But in the formulated batches T1 to T10 did not gave *in vitro* release for 12 h. In batch T11 though it float for 12 h (98.45 ±0.97), it gave floating lag time as 5-6 sec and floating duration 4-5 sec, than optimized batch show floating lag time immediate and floating time immediate. It also show significant different than optimized batch so this batch did not optimized. The formulate batch T12 (Sodium Alginate 2%: Ethyl cellulose 2.5%) in the ratio 4:5 gave *in vitro* drug release for 12 h. Hence it show gastric retention it can be because sodium alginate is gel formulating agent and use mostly in *in situ* gel formulation and ethyl cellulose use to increase the viscosity of sol that form strong gel matrix formulation in 0.1 N HCl. Also the floating lag time and floating time were immediate, also it get converted in to gel immediately after incorporation in 0.1 N HCl. It also floating duration was found to be more than 12 h. Also it was found stable in 6 months of accelerated stability study. So batch T12 was optimized.

Kinetic Studies

The release data obtained from various batches was studied with respect to effect of drug: polymer ratio, diluents ratio. Dissolution data of drug from prepared *in situ* gel at different time periods was plotted as cumulative % drug release v/s time. The dissolution data so obtained was fitted to various kinetic models like Zero Order, First order, Higuchi, Korsmeyer-Peppas models. It was found that the optimized batch T12 follow Korsmeyr-pappas model.

The drug release kinetics from all the batches were calculated, which was illustrated as follows:

Table 8: Kinetic study of Oral Floating Insitu gel of Tramadol HCl

Batch	Zero order	First order	Matrix	Peppas	Hixon crowell	Best Model Fit
T1	0.8019	0	0.9764	0.9871	0.941	Peppas
T2	0.8151	0.967	0.9774	0.9552	0.969	Matrix
T3	0.9444	0.9704	0.9821	0.9971	0.9874	Peppas
T4	0.8442	0.9813	0.9876	0.9937	0.9594	Peppas
T5	0.887	0.9546	0.9929	0.98	0.9738	Matrix
T6	0.8811	0.916	0.9974	0.9942	0.9706	Matrix
T7	0.7846	0.9567	0.9894	0.99	0.9653	Peppas
T8	0.8866	0.9711	0.9961	0.9913	0.9734	Matrix
T9	0.8704	0.9768	0.9954	0.9884	0.9776	Matrix
T10	0.8333	0.9896	0.9929	0.9934	0.9732	Peppas
T11	0.7628	0.8945	0.9841	0.9811	0.9409	Matrix
T12	0.8968	0.9111	0.9975	0.998	0.9815	**Peppas**

Statistical Analysis

In this study the Bonferroni method was applied to dissolution study to check there was significant difference or non significant difference in release of drug in formulated formulations. In this formulation the optimized batch show significant difference from all formulated batch except batch T11. The details are given in table no 40.

Table 9: Statistical Analysis of Dissolution parameters of Oral Floating *In situ* gel of Tramadol HCl

Sr. No	Between batches	t-test	P-value (<0.05)	Significance
1	T12 vs T1	3.961	0.0167	S
2	T12 vs T2	3.922	0.0172	S
3	T12 vs T3	2.643	0.0195	S
4	T12 vs T4	3.896	0.0176	S
5	T12 vs T5	5.235	0.0008	S
6	T12 vs T6	5.673	0.0002	S
7	T12 vs T7	8.548	0.0001	S
8	T12 vs T8	4.530	0.0040	S
9	T12 vs T9	5.769	0.0007	S
10	T12 vs T10	5.516	0.0015	S
11	T12 vs T11	0.7505	0.7505	NS

Stability Study

Stability studies were carried out as per ICH guidelines. The optimized formulation T12 was exposed to accelerated stability conditions as 40°C±2°C 75%RH±5% RH for the period of 6 months. In between the stability studies the formulation was removed at 3 month and 6 month to check all the evaluation tests as pH, viscosity, gelling time, floating lag time, floating time, Floating duration, Dissolution studies etc. During the stability studies, the product

was exposed to normal conditions of temperature and humidity. The optimized batch was found to be stable for all the evaluation tests in this time period of stability testing.

Table 10: Characteristics of oral Floating *In situ* gel of Tramadol HCl after stability study

Stability Duration	pH	Viscosity in cps	Gelling time (sec)	Floating lag time (sec)	Floating Duration in h	Drug content (%)
0 month	10.8	9.86	Immediate	Immediate	> 12	98.5 ±0.37
After 3 Month	10.7	9.86	Immediate	Immediate	> 12	98.5 ±0.41
After 6 Month	10.7	9.86	Immediate	Immediate	> 12	98.5 ±0.44

Table 11: % Drug Release of Oral Floating *In situ* gel of Tramadol HCl

Time (h)	0 Month	3 Month	6 Month
1	29.81 ± 0.91	27.31 ± 0.95	25.92 ± 0.92
2	38.8 ± 0.81	37.06 ± 0.84	35.67 ± 1.2
3	47.06 ± 0.72	45.44 ± 0.92	44.04 ± 0.90
4	55.33 ± 0.82	53.28 ±0.71	51.43 ± 0.60
5	59.9 ± 0.93	57.65 ± 0.77	55.35 ± 0.93
6	66.41 ± 0.83	64.06 ± 0.82	62.66 ± 0.72
7	73.33 ± 0.94	71.7 ± 0.94	69.86 ± 0.81
8	78.89 ± 0.60	77.17 ± 0.95	75.31 ± 0.78
9	85.82 ± 0.98	84.47 ± 1.02	83.34 ± 0.86
10	90.03 ± 0.79	89 ± 0.61	87.59 ± 0.66
11	95.6 ± 0.88	94.65 ± 0.92	93.25 ± 0.76
12	98.45 ± 0.97	97.34 ± 0.86	95.34 ± 0.92

N=3

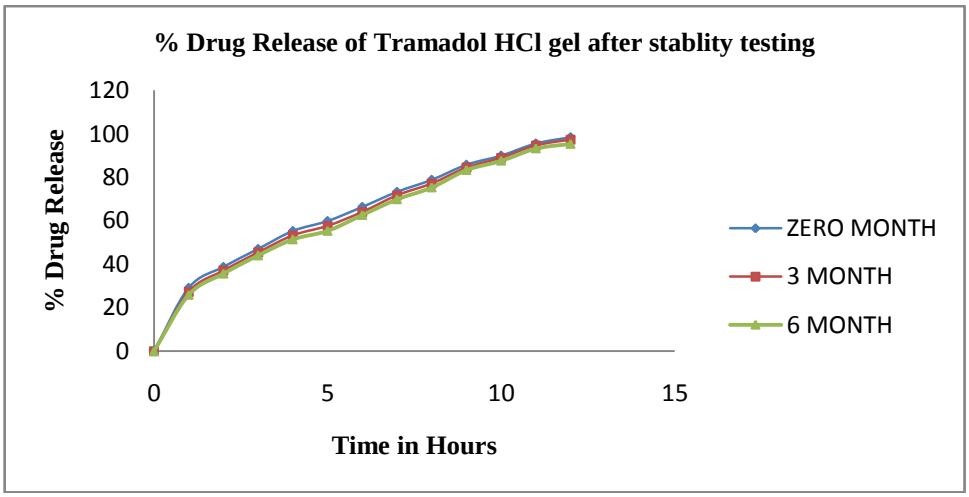


Figure 10: % Drug Release of oral flaoting Insitu Gel of Tramadol Hcl after stability testing.

Table 12: Kinetic study of Tramadol gel after stability study

Time/Model	Zero order	First order	Matrix	Peppas	Hixon crowell	Best Model fit
0 Month	0.8968	0.9111	0.9975	0.998	0.9815	**Peppas**
3 Month	0.9056	0.9440	0.9874	0.9974	0.9876	**Peppas**
6 Month	0.9141	0.9592	0.9970	0.9986	0.9905	**Peppas**

Summary

In the present study various natural as well as synthetic polymers in the combination of Natural: Natural, Natural: Synthetic, Synthetic: Synthetic were used in different ratio. But in the formulated batches T1 to T10 did not gave *in vitro* release for 12 h. In batch T11 though it float for 12 h. (97.06 ± 0.77) it gave floating lag time as 5-6 sec and floating duration 4-5 sec, than optimized batch show floating lag time immediate and floating time immediate. It also show significant different than optimized batch so this batch did not optimized. The formulate batch T12 (Sodium Alginate 2%: Ethyl cellulose 2.5%) in the ratio 4:5 gave *in vitro* drug release for 12 h (98.45 ±0.97). The optimized batch show floating of *in situ* gel of Tramadol Hydrochloride for more than 8 h in *in vivo* study. The batch

was found to be stable for 6 months in accelerated stability study. In kinetic study the optimized batch follows peppas model.

Conclusion

- From formulation and evaluation studies of oral floating *in situ* gel it was concluded that gas forming agent sodium bicarbonate and calcium carbonate 2% each (1:1) proportion gives floating of gel for >12 h.
- For Tramadol Hydrochloride Matrix formation between Sodium Alginate and Ethyl cellulose gives maximum drug retardation for 12 h.
- *In vivo* study was performed by providing the formulation to rabbit and then X-ray was taken for the confirmation of formation of gel in stomach and floating of dosage form for 12 h and it was found to be floating for 12 h.
- Formulations was found to be stable for 6 Months in accelerated stability studies,
- The objective of increase of residence time in stomach, reduce in dosing frequency, safety profile etc had achieved.

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