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DESIGN, DEVELOPMENT AND CHARACTERIZATION OF FLOATING *IN-SITU* GELLING SYSTEM FOR LISINOPRIL DIHYDRATE

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ABSTRACT

The aim of this study was to develop a novel gastro retentive floating in-situ gelling system for controlled delivery of lisinopril dihydrate for treatment of hypertension. Gellan gum based lisinopril dihydrate floating in situ gelling systems was prepared by dissolving varying concentrations of gellan gum in de-ionized water containing sodium citrate and calcium chloride. This mixture was then heated to 90^0C and cooled to 40^0C followed by addition of mixture of lisinopril dihydrate coated with 2% solution of TPP, than $CaCO_2$ was added to form a uniform dispersion. The formulation variables like gellan gum and calcium carbonate significantly affected the *in-vitro* drug release, viscosity, *in-vitro* buoyancy and floating lag time. Lisinopril dehydrate floating in-situ gel (LFIG) showed significant anti-hypertensive effect. The results further showed that the prepared LFIG has feasibility of forming rigid gels in the gastric environment and gives anti-hypertensive effect from gastro- intestinal tract. Lisinopril dihydrate floating in-situ gel was found more effective than the lisinopril dehydrate suspension because of the prolonged gastro-intestinal residence time of the formulation.

KEYWORDS: Gastro Retentive Drug Delivery System, In-situ gel, lisinopril dehydrate, Hypertension, Floating drug delivery system.

1) INTRODUCTION

There are different approaches of Novel Drug Delivery Systems. One of the most important approaches is Gastro Retentive Drug Delivery System (GRDDS). Floating is one of most popular GRDDS. Different dosage form are developed in gastro retentive floating system as microspheres, micro beads, tablets, capsules, films etc. *In-situ* gelling system is a new trend in floating DDS. In-situ gelling system have its application in different routes of administration like oral, nasal, ophthalmic, peroral, rectal, vaginal and also parenteral route. In-situ forming polymeric drug delivery systems has many advantages such as ease of administration, increased local bioavailability, reduced dose frequency, improved patient compliance and has less complex method of production and so is cost effective. This is an approach in which dosage form get retained in stomach and release drug slowly to obtain sustained drug delivery. This approach is important for drugs with narrow therapeutic index and it also show the local as well as systemic effect of drug and avoids the gastric emptying of drug within 2 hrs. Generally drugs with small half life are used in GRDDS to maintain its plasma peak concentration of drug. There are different approaches of Gastro Retentive Drug Delivery System including floating system, high density system, swelling and expanding system, modified shape system, bioadhesive system. In *in-situ* gel forming preparation are 'stimuli-responsive' drug delivery system, that is given conveniently orally in liquid form, following sol to gel transition when it come in contact with gastric fluid in stomach. *In-situ* gelling system is a novel idea of delivering drugs to patient in liquid form. It gives controlled or sustained release of drug for desire duration of time. These systems are used for drugs which are having absorption site i.e. upper GIT including upper part of jejunum. This form may remain buoyant (8-10 hours) on gastric contents without affecting the rate of gastric empting. Different polymer systems are used in floating drug delivering dosage forms. Among those some are polysaccharides, polymethacrylates, hydrocolloids etc. in this cellulose ether polymers are most popular, especially HPMC. The formulation of floating in situ gelling solution may sustain and prolong drug action, improve patient compliance and reduce frequency of administration of the drug in comparison to conventional drug delivery system.

Lisinopril dihydrate is an ACE inhibitor used as antihypertensive having average half life of multiple dosing 12 hrs. It is a class III drug i.e. having high solubility but low permeability. It is absorbed from upper part of jejunum. It is also used in Hypertension caused in paediatrics (between ages 6 to 15). The absorption of lisinopril dihydrate is 25% and it does not bind to other serum proteins except ACE.

The *in-situ* gel of Lisinopril Dihydrate was developed because as it has narrow therapeutic index and is absorbed from specific site only. And drugs with narrow therapeutic index are best suited for floating drug delivery system. Gellan gum along with TPP and calcium chloride were used as cross-linking agent. And calcium carbonate as effervescent agent.

2) MATERIALS

Lisinopril dihydrate was purchased from Taj pharmaceuticals, Mumbai. Gellan gum was supplied by Research Lab fine chem. Industries, Mumbai. Sodium tripolyphosphate was supplied by Loba Chemie, Mumbai, India. Sodium citrate, calcium carbonate and all other chemicals were supplied by Research Lab. Mumbai.

3) METHOD

3.1 Melting point determination

Melting point of the drug was determined by the Capillary method.

3.2 UV spectrophotometry

3.2.1 λ_{max} determination

The 10mg drug was dissolved in sufficient quantity of distilled water. This stock solution (i.e. 100 µg/ml) was used further to make dilutions. According to the range obtain the dilutions where made between 10-30 µg/ml. This solutions where then scanned from 200-400 nm on the double beam UV visible spectrophotometer (Shimadzu, UV-1650, Japan).

3.2.2 Calibration Curve

Calibration of the drug was done on UV visible spectrophotometer (Shimadzu, UV-1650, Japan) by scanning the diluted solutions prepared in the range 10-30 µg/ml as discussed earlier. And absorbance was noted. As shown in table No.

3.3 Experimental Design (3^2 factorial design)

The 3^2 full factorial design was constructed and the concentration of gellan gum (X_1) and calcium carbonate (X_2) where the two factors selected. The concentration of two factors was

selected on basis of preliminary studies carried out during experimental work and the other parameters where kept constant throughout the experimental work.

Table No.1: The factor combination as per experimental design for Lisinopril dehydrate.

Formulation batches	Coded valves	
	X1	X2
F1	-1	-1
F2	0	-1
F3	1	-1
F4	-1	0
F5	0	0
F6	1	0
F7	-1	1
F8	0	1
F9	1	1

3.4 Preparation of floating *in-situ* gelling solution

Gellan gum, at varying concentrations of 0.25-1% w/v were dissolved in deionized water previously containing sodium citrate (0.25%w/v) and calcium chloride (0.016% w/v). The above mixture is then heated to 90^0C and after cooling at 40^0C the drug which is coated with 2% solution of TPP and kept for 15 min was then added and stirred properly. To this mixture $CaCO_2$ is added with continuous stirring to form a uniform dispersion.

3.5 Measurement of viscosity of *in-situ* gelling solutions

The rheological properties preferably viscosity is most important if it deals with the oral administration of drug or dosage form. The viscosity of prepared solutions was measured on Brook field viscometer. Viscosity shown in table

3.6 *In-vitro* gelation study

The gelation property of in-situ gel was observed visually. The study was done in USP dissolution apparatus. The formulated viscous liquid was placed in Petri plate and as soon as it was placed in 1.2 pH solution the timer was started and the gelation time of the gel was recorded.

3.7 *In-vitro* buoyancy study

In-vitro floating study was determined using dissolution apparatus II having 500 ml simulated gastric fluid (pH 1.2). The temperature was set 37^oC and at 50 rpm. The 10ml of prepared in-

situ gelling solution was placed into Petri dish by using disposable syringe (4.5mm internal diameter). Then place the Petri plate in dissolution vessel containing medium without much disturbance. The time the formulation emerged in the medium (floating lag time and time the formulation constantly floated on the dissolution medium surface (duration of floating) were noted.

3.8 *In-vitro* drug release study

The release of lisinopril dihydrate from the in-situ gel preparation s was determined as described by Zatz and Woodford (1987) with some modification using dissolution test apparatus (USP 24) with a paddle stirrer at 50 rpm. This speed was slow enough to avoid the breaking of gelled formulation and was maintaining with the mild agitation conditions. The dissolution medium used was 500ml of 0.1 N HCl and temperature was maintained at 37^{o}C. 10ml formulation was drawn up using disposable syringe, the needle was wiped clean and excess formulations removed from the needle end. The end was then placed into the Petri dish (4.5 mm internal diameter) and the syringe plunger depressed slowly to extrude 10ml and finally the Petri dish containing formulation was kept in the dissolution vessel containing dissolution medium without much disturbance. At each time interval, a pre warmed of Lisinopril dihydrate in withdrawn samples was measured at 206nm on double beam UV visible Spectrophotometer (Shimadzu, UV-1650,Japan). Interference from the excipients was negligible. Each study was conducted in triplicate till 8 hrs.

3.9 Stability Studies

The optimized formulation was sealed in the vials and kept in humidity chamber maintained $40+2^{0}$C/ 75+5% RH for 3 months. After 30, 60, 90 days samples were retrieved and analyzed for drug content, viscosity, pH, in-vitro drug release etc.

4) RESULT AND DISCUSSION

4.1 Drug Excipients Compatibility Studies

4.1.1 Melting point determination

Melting point was determined by capillary method. Melting point was found to be 166-167^{0}C.

4.1.2 Determination of λ_{max} of Lisinopril Dihydrate

λ_{max} of Lisinopril dihydrate was found to be 206 nm.

4.1.2 Calibration curve of Lisinopril Dihydrate

The UV absorption data at the wavelength 206 nm is shown in fig.no. It was observed that lisinopril dihydrate showed good linearity (r^2- 0.9987) over the range of 10-30 ppm. Calibration curve of lisinopril dihydrate obey Beer-Lambert's law over this range. Fig.no.1

Table.no.2 Calibration readings.

Sr. No.	Concentration (ppm)	Absorbance (206nm)
1	0	0
2	10	0.284
3	15	0.431
4	20	0.600
5	25	0.711
6	30	0.874

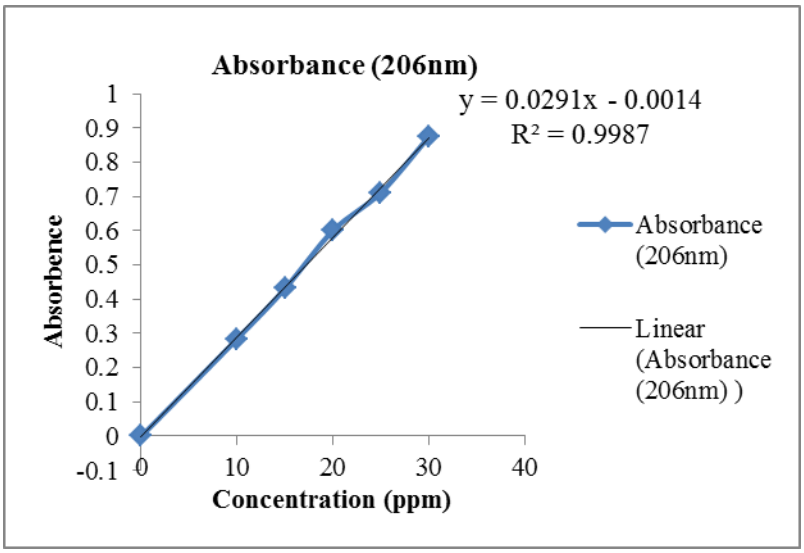

Fig 1: Calibration curve of lisinopril in 0.1N HCl.

4.1.3 FT-IR study

The FT-IR study showed that there are no significant changes observed in the position of specific absorption bands of groups and bonds. The spectra of pure drug (fig.no 3) and mixture showed slight changes but there is no change in the absorption bands of drug which indicate that there is no interaction between drug and excipients. So, lisinopril dihydrate, gellan gum and TPP were found to be compatible with each other. Results are shown in fig.no:4.

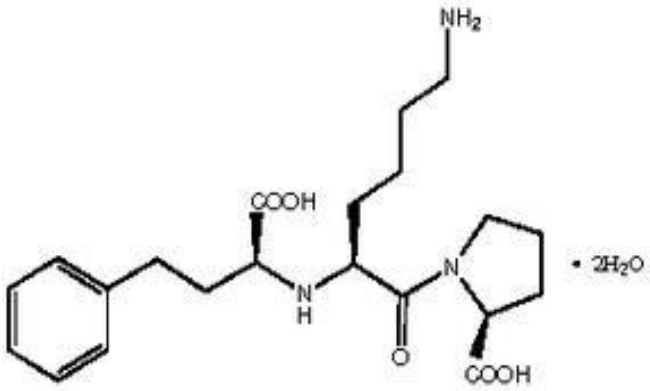

Fig.no. 2 Structure of Lisinopril Dihydrate.

Table: 3 IR ranges for pure drug.

Sr. No.	Groups	Ranges cm^{-2}
1	NH- stretch	3292.88
2	NH_2 stretch	3554.85
3	C=N stretch	1655.41
4	COOH stretch	1343.08
5	C=C stretch	2965.48

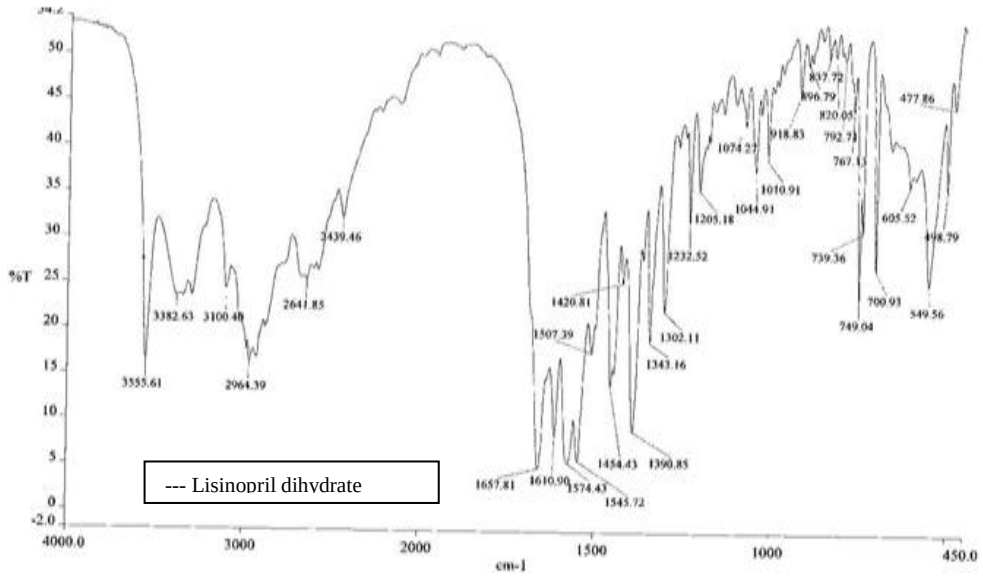

Fig.no.3: IR of Lisinopril dehydrate.

Table 4: IR ranges for mixture.

Sr. No.	Groups	Ranges cm^{-2}
1	NH- stretch	3284.88
2	NH_2 stretch	3529.85
3	C=N stretch	1643.41
4	COOH stretch	1381.08
5	C=C stretch	2970.48

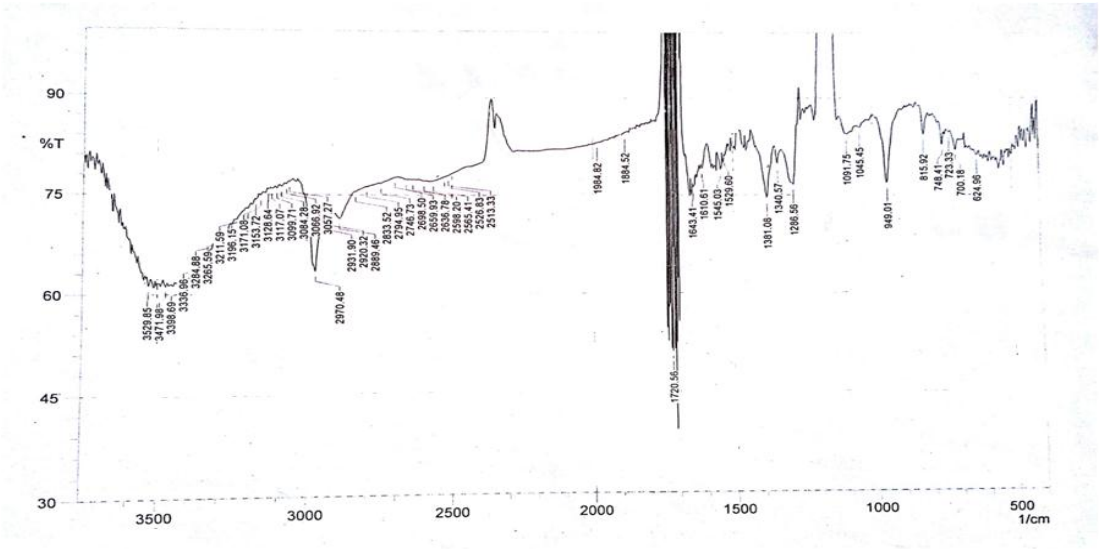

Fig.no. 4: IR spectra of Drug+ gellan gum.

4.2 Evaluations of floating in-situ gels

4.2.1 Viscosity

Rheological determination of in-situ floating gel by using Brookfield Viscometer. The viscosity details are given in the table no. 6

4.2.2 Physical appearance and pH

All prepared gellan based in-situ gelling solutions of Lisinopril dihydrate were checked for their clarity and the time required for gel formation. The pH of in-situ solution of lisinopril was measured by using a calibrated digital pH meter at 25^0C All the measurements where done in triplicate.

Fig no. 5: Floating in-situ gelling solution.

4.2.3 Determination of floating lag time

The floating lag time for all the formulation batches are given below in table no. Gellan gum showed the instantaneous floating when came in contact with simulated gastric fluid. The basic mechanism behind floating is calcium carbonate present in the formulation as insoluble dispersion and gets solubilised in acidic medium. This causes release of carbon dioxide and ca^{++} ions which cause gelation of polymer and released gas get entrapped in gel matrix which leads to floating of gel matrix on gastric fluid. Floating gel is shown in fig. no. 7

Fig.no 6: Floating in-situ gel.

4.2.4 Determination of duration of floating

The duration of floating for all formulation were studied in 0.1N HCl maintained at 37.5^0C temperature and at 100 RPM are depicted in table no. Upon contact with acidic medium calcium carbonate effervescent and release CO_2 and calcium ions, which causes gelation and cross-linking by Ca++ ions occurred to provide a gel barrier at the surface of the formulation. The released carbon dioxide get entrapped in the gel matrix producing buoyant formulation, these three-dimensional gel matrix and drug molecules and has resulted in extended period of floating lag time and duration of floating. Similarly an increase in the polymer concentration resulted in decreased floating lag time and an increase in floating duration of the prepared systems.

4.2.5 Determination of drug content

The amount of lisinopril dihydrate in each sample was determined by spectrophotometer. The UV absorbance of the sample was determined at a wavelength of 206nm. The drug content for batches F1-F9 is depicted in the table no.6.

Table.no: 5 Optimization by 3^2 factorial design.

Batch	Gellan Gum	CaCO2	TPP	Drug
B1	0.25	0.5	2	0.1
B2	0.5	0.5	2	0.1
B3	1	0.5	2	0.1
B4	0.25	1	2	0.1
B5	0.5	1	2	0.1
B6	1	1	2	0.1
B7	0.25	2	2	0.1
B8	0.5	2	2	0.1
B9	1	2	2	0.1

4.2.6 In-vitro drug release study

The release of lisinopril dihydrate from floating *in-situ* gel was analysed by plotting the % Cumulative drug release against Time (in hours). The effect of gellan gum concentration on *in vitro* drug release from *in-situ* gels is shown in figure no. A significant decrease in the rate and extent of drug release was observed with the increase in gellan gum concentration in *in-situ* gels. The effect of calcium carbonate concentration on in vitro drug release from in-situ gels is shown in figure no. With increase in calcium carbonate concentration in formulations decreased percentage of drug release.

Table.no.6: Evaluations of floating in-situ gel for batch B_1-B_9.

Batch	Viscosity (Cps)	gelation	Floating lag time (min)	Duration of floating (hrs)	Drug content (%)	Drug Release (%)
B1	135	++	>2	>20	90.26	74.11
B2	185	+++	>2	>24	97.11	93.72
B3	255	+++	>2	>24	96.10	68.11
B4	130	++	<1	>20	93.34	69.57
B5	190	+++	<1	>24	95.56	80.59
B6	260	+++	<1	>24	96.20	65.66
B7	140	++	<1	>22	92.12	65.67
B8	180	+++	<1	>24	95.52	75.12
B9	265	+++	<1	>24	96.61	61.85

Where, (+++) excellent gelation, (++) good gelation, (+) poor gelation

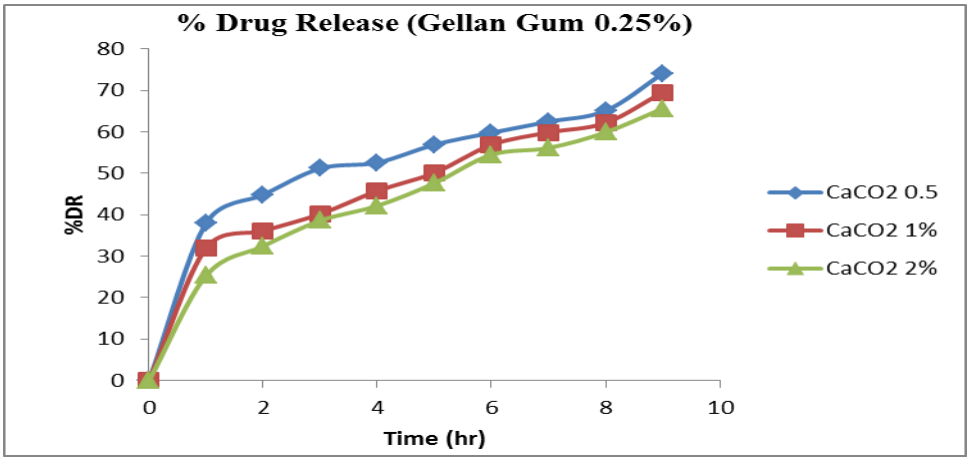

Fig no. 7: Drug release profile where gellan gum (0.25%) and different conc. Of $CaCO_2$ (0.5%, 1%, 1.5%)

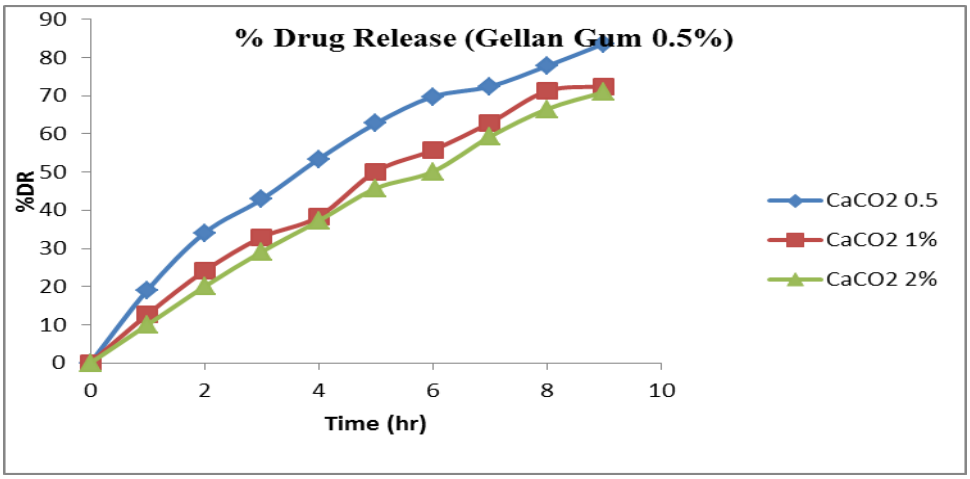

Fig no. 8: Drug release profile where gellan gum (0.5%) and different conc. Of $CaCO_2$ (0.5%, 1%, 1.5%)

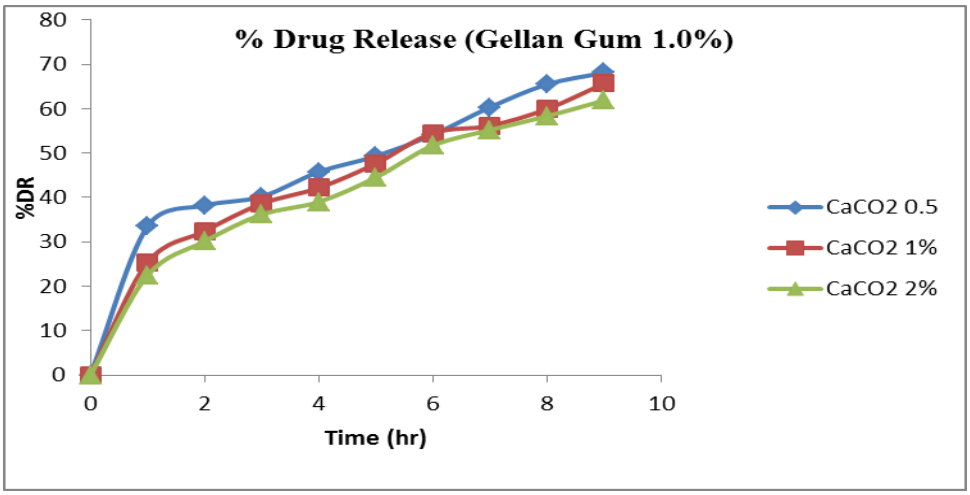

Fig no. 9 Drug release profile where gellan gum (1%) and different conc. Of $CaCO_2$ (0.5%, 1%, 1.5%)

4.2.7 Drug Release Kinetics

The model fitting was done using PCP Disso software and the result of model fitting for optimized batch (B_2) are given in figure no. and the valves of regression for all models are given in the fig.no.

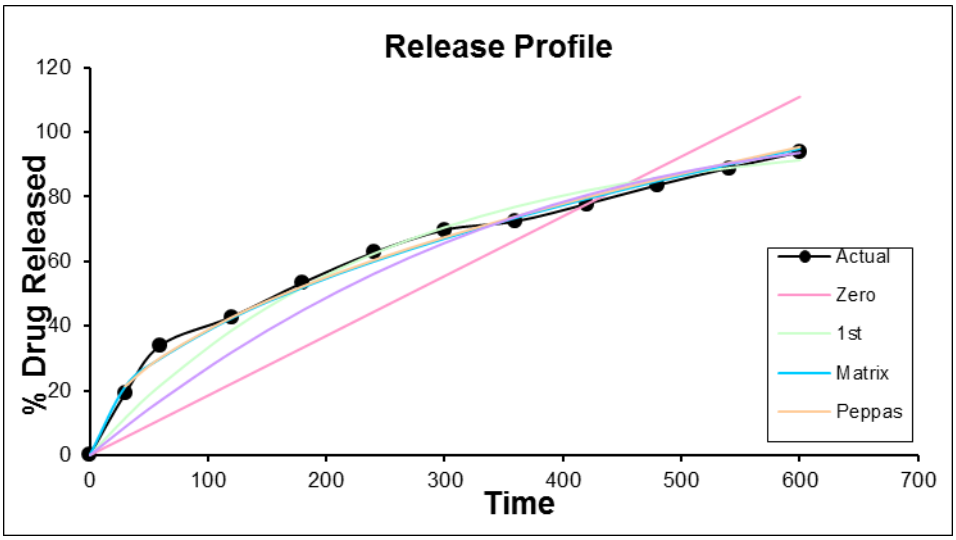

Fig.no: 10 Model fitting for optimized batch.

Table.No. 7: Model fitting for optimized batch B_2.

Sr. No.	Parameters	Zero order	First order	Higuchi	Korsmeyer-Peppas	Hixson-Crowell	Release pattern
1	Regression coefficient	0.8524	0.9831	0.9976	0.9931	0.9793	Non-fickian (n=0.4994)

4.2.8 Stability study

The optimized formulation was sealed in vial with rubber cap and kept in humidity chamber maintained 40+2^{0}C temperature and 75+5% RH for 3 months. After 30, 60, 90 days samples retrieved and analyzed for the drug content, *in vitro* drug release, pH and viscosity. There was no significant change in morphological condition and also in the remaining parameter during stability study.

Table. No. 8: Results of optimized batch on storage up to 3 months.

Days	% Drug release	Viscosity (Cps)	% Drug content
Before storage			
0	93.72	185	97.11
After storage			
30	92.78	180	96.96
60	92.21	175	96.10
90	91.67	170	95.85

4) CONCLUSION

According to results obtained the *in-situ* gelling property depends on concentration of gelling agent and concentration of calcium carbonate. The concentration of gelling agent increases hardness of gel increases and floating lag time also increases. Also as concentration of polymer and calcium carbonate increases the drug release decreases while the viscosity of formulation increases. TPP places an important role in cross-linking of drug as it provides coating to the drug.

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