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Research Article

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Study of effect of sodium alginate and calcium carbonate composition difference on *in situ* gelling gastroretentive amoxicillin liquid formulation

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ABSTRACT

The present investigation deals with the formulation and evaluation of sodium alginate based In situ gel of Amoxicillin. Sodium alginate was used as a polymer and $CaCO_3$ was used as a cross-linking agent. The objective of this study was to Study of Effect of Sodium Alginate & Calcium Carbonate Composition Difference on In Situ Gelling Gastro retentive Amoxicillin Liquid Formulation. Gelling capacity was evaluated based on a graded response which indicates rapidity of gelation and time taken by the gel to dissolve. All the formulations showed instant gelation but with regard to integrity, all formulations except the one with lowest SA level formed stiff gels maintaining integrity for at least 12 hr. With respect to floating behavior, all formulations except the one with the lowest SA level floated for more than 12 hr irrespective of their composition. Most of the formulations took less than 1 min to float but those with the lowest SA and $CaCO_3$ levels floated after about 2 min. The formulations showed satisfactory content uniformity and pH ensuring their safe use. The rheological studies showed that the formulations possessed optimal viscosity which can facilitate easy administration of the required dose. Drug release study revealed release retarding behavior of the formulations and noticeable burst release. This effect was reduced at higher concentration of SA and $CaCO_3$. Release retarding effect of SA was only marginal at higher concentrations. $CaCO_3$ showed a similar effect but at higher levels insignificant change in release was observed. The study demonstrated that release rate of amoxicillin was depends on the amount of sodium alginate and $CaCO_3$ used.

Key words: Amoxicillin, Sodium alginate, Calcium Carbonate, Floating *In-Situ* Gel.

INTRODUCTION

The present investigation deals with the formulation and evaluation of sodium alginate based *In situ* gel of Amoxicillin. Sodium alginate was used as a polymer and $CaCO_3$ was used as a cross-linking agent. *Helicobacter pylori* (*H. pylori*) is one of the most common pathogenic bacterial infections. It is associated with the development of serious gastro duodenal disease, including peptic ulcers, gastric lymphoma, and acute chronic gastritis. *H. pylori* resides mainly in the gastric mucosa or at the interface between the mucous layer and the epithelial cells of the antral region of the stomach. Antibiotics required for eradication of *H. pylori* are high in dose and in more frequencies. This is because of the low concentration of the antibiotic reaching the bacteria under the mucosa, instability of the drug in the low pH of gastric fluid, and short residence time of the antibiotic in the stomach, leading to incomplete eradication of *H. pylori* [1, 2].Amoxicillin is a semi synthetic, orally absorbed, broad-spectrum antibiotic. It is widely used in a standard eradication treatment of gastric *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent [3]. As conventional drug delivery systems do not remain in the stomach for prolonged periods, they are unable to deliver the amoxicillin to the site of infection in effective concentrations. Therefore, it is necessary to design drug delivery systems that not only alleviate the shortcomings of conventional delivery vehicles but also deliver amoxicillin to the infected cell lines. Some researchers had prepared and reported new amoxicillin formulations, such as floating tablets, mucoadhesive tablets, and mucoadhesive microspheres, which were able to reside in stomach for an extended period for more effective *H. pylori* eradication [4, 5]. Amongst the described

formulations, the floating tablet is preferred for better and less variable gastric retention, but it has a limitation of incorporation of high dose of the drug. The drug with high dose like amoxicillin can be easily incorporated in liquid *in situ* gelling formulation that upon oral administration can float for a prolonged period of time in the stomach [6, 7]. The objective of this study was to Study of Effect of Sodium Alginate & Calcium Carbonate Composition Difference on *In Situ* Gelling Gastro retentive Amoxicillin Liquid Formulation.

EXPERIMENTAL SECTION

Materials:

Amoxicillin Was Received As a Gift Sample From Doctors Life Sciences Pvt Ltd (India). Sodium Alginate (SA) Were Purchased From Loba chemie (P) Ltd. Mumbai, Calcium Carbonate, Sodium Citrate, Saccharin sodium Were Purchased From S d FINE-CHEM Limited, Mumbai. All Other Chemicals Used In The Study Were of Analytical Grade.

Method:

Preparation of Amoxicillin *In-Situ* Gelling Solution (F1-F4)

Sodium alginate (SA) solution at different concentrations (0.5%W/V, 1.5%W/V, 2.5%W/V, 3.5%W/V) were prepared in Deionized water containing $CaCl_2$ (0.075%w/v) and Sodium citrate (0.25%w/v) with continuous stirring. Sodium Alginate solution was heated to 70^0C and then cool to below 30^0C. After cooling, Calcium Carbonate ($CaCO_3$) Solution (1.0%w/v) and drug was added and dispersed well with stirring. Add Citric Acid (0.2%w/v) and Saccharin Sodium (0.05 %w/v) to the above solution with stirring. The resulting Sodium Alginate In-Situ gel solution containing Amoxicillin was finally stored in Amber coloured bottles until Evaluated [8, 9].

Preparation of Amoxicillin *In-Situ* Gelling Solution (F5-F8)

Sodium alginate (SA) solution (1.5%W/V) was prepared in Deionized water containing $CaCl_2$ (0.075%w/v) and Sodium citrate (0.25%w/v) with continuous stirring. Sodium Alginate solution was heated to 70^0C and then cool to below 30^0C. After cooling, $CaCO_3$ Solution at different concentrations (0.5%w/v, 0.75%w/v, 1.5%w/v, 2.0%w/v) and drug was added and dispersed well with stirring. Add Citric Acid (0.2%w/v) and Saccharin Sodium (0.05 %w/v) to the above solution with stirring. The resulting Sodium Alginate In-Situ gel solution containing Amoxicillin was finally stored in Amber coloured bottles until Evaluated [10].

Table 1: Composition of Amoxicillin Floating in situ gel

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8
Amoxicillin	7.5%	7.5%	7.5%	7.5%	7.5%	7.5%	7.5%	7.5%
Calcium Chloride	0.075%	0.075%	0.075%	0.075%	0.075%	0.075%	0.075%	0.075%
Sodium Alginate	**0.5%**	**1.5%**	**2.5%**	**3.5%**	1.5%	1.5%	1.5%	1.5%
Calcium Carbonate	1%	1%	1%	1%	**0.5 %**	**0.75%**	**1.5%**	**2 %**
Citric Acid	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Saccharin Sodium	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
Sodium Citrate	0.25%	0.25%	0.25%	0.25%	0.25%	0.25%	0.25%	0.25%
DI Water	Upto 100ml	Upto 100ml	Upto 100ml	Upto 100ml	Upto 100ml	Upto 100ml	Upto 100ml	Upto 100ml

Evaluation of In-Situ Gelling Solution

Determination of pH

The pHs of all formulations were measured using a calibrated digital pH-meter (LABTRONICS, India) at room temperature and results were recorded as average of three measurements [11].

Viscosity

Viscosity of the samples was determined using Brookfield Digital Viscometer (Model. DV-E Viscometer). The formulation (100ml) was taken in a beaker and maintained at room temperature. Viscosities were determined at different shear rates from 0.5-100rpm at room temperature by using Spindle No.62 [12].

Determination of In Vitro Gelling Capacity

To assess the in vitro gelling capacity, the method described by Rathod *et al* was employed. Gelling capacity was determined by placing 1 ml of each formulation into 5 ml of the gelation solution (0.1 N HCl) in a 15 ml borosilicate glass test tube maintained at 37±1 0C. Each formulation was added with a pipette; placing the pipette at the surface of liquid and slowly releasing the content. The *in vitro* gelling capacity of solution was evaluated and graded on the basis of stiffness of formed gel and the time taken for the gel to dissolve [13].

Floating lag time (buoyant time)

Time taken by the gel to reach the top from the bottom of the dissolution flask is defined as the floating lag time (buoyant time)**.**The floating lag time of gel determination was performed by visual inspection in a USP type II dissolution test apparatus containing 500 ml of 0.1 N HCl (pH 1.2) at 37±0.5^{0}C[14].

Water uptake

In the present study a simple method has been adopted to determine the water uptake by the gel. The *In situ* gel formed in 40 ml of 0.1N HCl (pH 1.2) was used for this study. The gel was separated from the buffer solution and blotted out with tissue paper; all the formulations were done in the same way and weighed. It is considered as an initial weight of the gel. To this gel 10 ml of distilled water was added. After 30 minutes decanted the water and re weighed the gel. It was considered as final weight of the gel. By using the following formula water uptake was calculated [15].

$$Water\ uptake\ (\%) = \frac{Final\ Weight - Initial\ Weight}{Initial\ Weight} \times 100$$

Drug content

10 ml of the solution was added to 900ml (0.1N HCL) solution and stirred for 1 hour on a magnetic stirrer. The solution was filtered, suitably diluted with (0.1N HCL) and the drug content was determined by using U.V Visible Spectrophotometer at 294 nm against a suitable blank solution [16]**.**

In- Vitro Drug Release

The release of drug from the formulations was determined using a USP/24 dissolution test apparatus with a paddle stirrer at 50 rpm. The dissolution medium used was 900 ml of simulated gastric fluid (0.1N HCl, pH 1.2) and temperature was maintained at 37 ± 0.2 °C. Ten ml of the formulation was added into the dissolution vessel containing simulated gastric fluid avoiding any disturbance using test tube. At each time interval, a precisely measured sample of the dissolution medium (5ml) was pipette out and replenished with fresh medium (5ml). Drug concentration in the aliquot was determined spectrophotometrically at 272 nm using a Shimadzu UV 1800 double-beam spectrophotometer [17] (Shimadzu, Kyoto, Japan).

RESULTS AND DISCUSSION

Determination of UV Absorbance Maxima of Amoxicillin: The standard stock solution was used to determination the λmax of (0.1 N HCl, pH 1.2) was used as blank for the study. The spectrum was taken between the UV range of 200-400nm. The highest peak obtained from the spectrum analysis was taken as λmax for Amoxicillin that used was found to be 272nm.

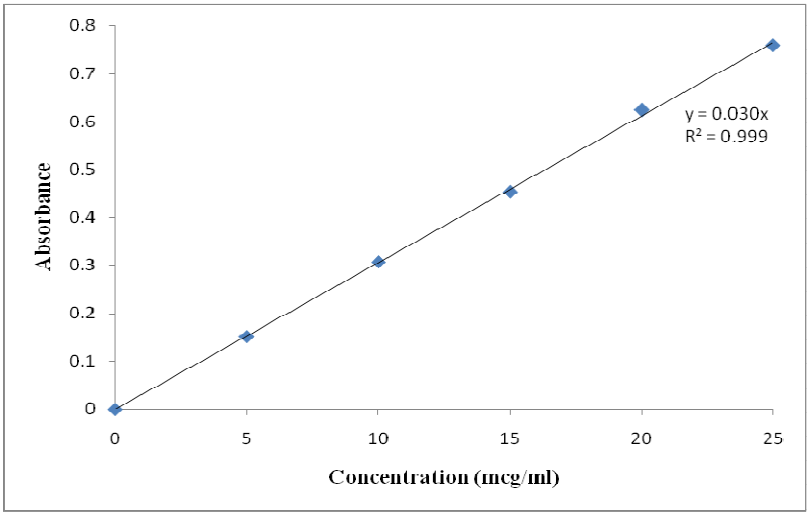


Fig.1: Standard curve of Amoxicillin in 0.1N HCl (pH1.2)

Physical Appearance and Clarity

Physical characterization parameters are reported. All the formulations had off white to pale yellow coloured solution. Clarity of the all formulations was found to be satisfactory.

pH of the Formulations

Table 2: pH of the In-Situ Gelling Formulations

Formulation Code	pH
F1	7.72
F2	7.79
F3	7.91
F4	7.93
F5	8.03
F6	8.1
F7	8.27
F8	8.41

The formulations possessed satisfactory pH value ranging from 7.72 to 8.41**(Table 2)** which is suitable to maintain the formulations in a liquid state. Aqueous solutions of sodium alginate are more stable at pH range of 4-10. Below pH 3, alginic acid is precipitated from the alginate solution making the formulations unsightly containing gel and liquid phases.

Viscosity of In-situ Gelling solutions:

Table 3: Viscosity of the In-Situ Gelling Formulations

Formulation Code	Viscosity (cps)
F1	172
F2	289
F3	383
F4	461
F5	224
F6	258
F7	294
F8	311

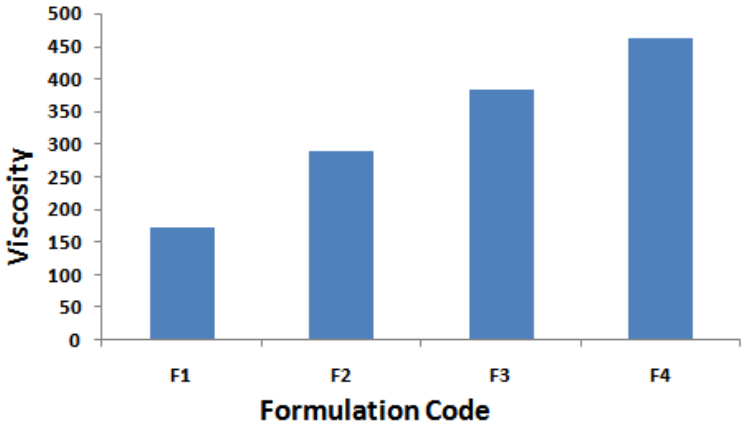


Fig.2: viscosity of Amoxicillin Oral Floating In-Situ Gel (F1-F4)

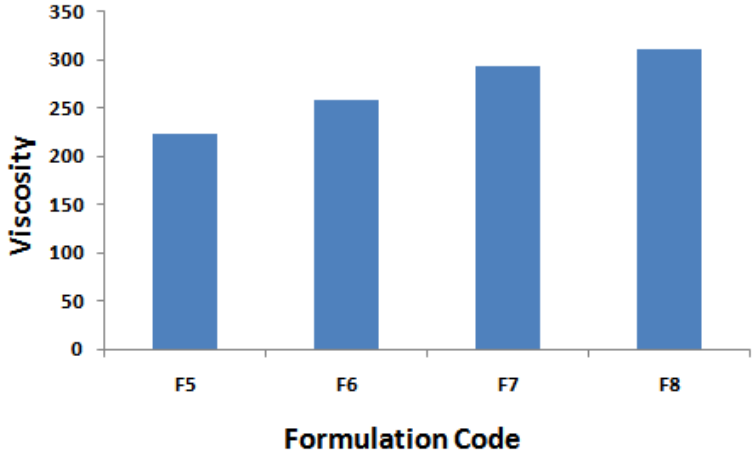


Fig.3: viscosity of Amoxicillin Oral Floating In-Situ Gel (F5-F8)

The viscosity of the formulations increased with an increase in sodium alginate concentration. The phenomenon is a consequence of increasing chain interaction with an increase in polymer concentration calcium carbonate which is the source of cations, increased the viscosity of the formulation. This change is viscosity is due to the proportional increase in the amount of dispersed calcium carbonate.

Floating Behaviour

Table 4: Floating lag time & Floating Duration of the In-Situ Gelling Formulations

Formulation Code	Floating Lag Time (sec)	Floating Duration (hr)
F1	122	10
F2	38	>12
F3	31	>12
F4	28	>12
F5	108	>12
F6	35	>12
F7	25	>12
F8	19	>12

a) Floating lag time

Regarding floating lag time, higher polymer concentrations shorten the time taken to float completely over the surface of the dissolution medium in agreement with other reports. This may be due to the higher cross-linking density at higher polymer concentrations which could effectively trap the $C0_2$ bubbles so that density of the gel is reduced rapidly to induce buoyancy. Formulations (F2-F4) float to the surface.

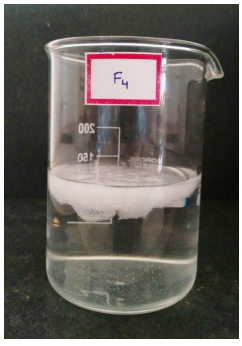
b) Floating Duration

The total floating time of the prepared formulations were performed in 0.1N HCL (P^H-1.2). Results of in-vitro total floating time of formulation F1 to F8 were described in **(Table 4)**.

In-situ gel containing
0.5% SA & 1.0% $CaCO_3$ (F1)

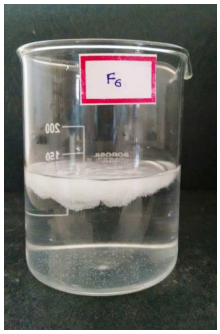
In-situ gel containing
1.5% SA & 1.0% $CaCO_3$ (F2)

In-situ gel containing
2.5% SA & 1.0% $CaCO_3$ (F3)



In-situ gel containing
3.5% SA & 1.0% $CaCO_3$ (F4)

In-situ gel containing
1.5% SA & 0.5% $CaCO_3$ (F5)



In-situ gel containing
1.5% SA & 0.75% $CaCO_3$ (F6)

In-situ gel containing
1.5% SA & 1.5% $CaCO_3$ (F7)

In-situ gel containing
1.5% SA & 2.0% $CaCO_3$ (F8)

Fig.4: Floating Behaviour of Amoxicillin In-Situ Gel

Gelling Capacity of the Formulations

Table 5: Gelling Capacity of the In-Situ Gelling Formulations

Formulation Code	Gelling Capacity
F1	+
F2	++
F3	+++
F4	+++
F5	++
F6	++
F7	+++
F8	+++

+ = gelation immediate (< 10 s), weak gels dissolve after few hours (6 hr)
++ = gelation immediate (< 10 s), stiff gels remaining for 12 hr
+++ = gelation immediate (< 10 s), more stiff gels remaining for more than 24 hr

As a prerequisite, an Oral in-situ gelling gastro retentive formulation should undergo rapid sol to gel transition when it comes in contact with the gastric fluid. Moreover, to facilitate sustained drug release, the *in situ* formed gel should preserve its integrity without dissolving for a prolonged period of time. In all the formulations, sol-to-gel transition occurred instantaneously at the formulation/simulated gastric fluid interface as the formulations were dropped from pipette.

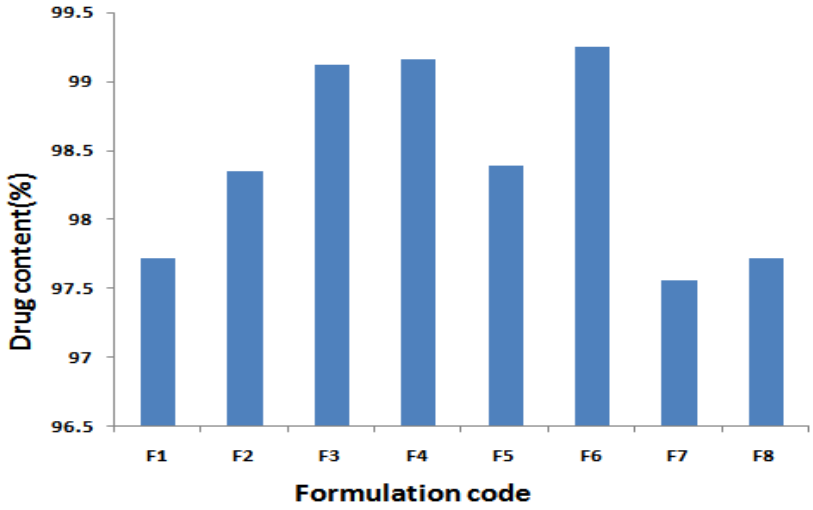
However, though gelation occurred instantaneously, the nature of the gels formed was dependent upon the polymer and $CaCO_3$ concentration. Low sodium alginate concentration (F1) formed weak gels **(Table 5)** which would not be able to withstand peristaltic waves of the GI tract, and might be propelled to the intestine with stomach contents. F1 could not last longer than 10 hr, even during this period it appeared very weak gel full of bubbles. F2 showed an intermediate gelling capacity, with stiffness lower than F3 and F4. Therefore, lower concentration of sodium alginate as in F1 could be of no value for sustained and site specific release of drugs such as Amoxicillin. The observed increase in gel strength with increased SA concentration is presumably due to increased polymer chain interaction. At lower $CaCO_3$ concentration, the gels did not go beyond 12 hr because at the $CaCO_3$ levels used, the available sodium alginate chains might not be sufficiently cross linked. In other words, the formed gels might not be of pure calcium-alginate gels which were reported elsewhere, to be stronger than the alginic acid gels.

Drug Content

Table 6: Drug content of the In-Situ Gelling Formulations

Formulation Code	Drug Content (%)
F1	97.72
F2	98.35
F3	99.12
F4	99.16
F5	98.39
F6	99.25
F7	97.56
F8	97.72

All the batches formulated exhibited drug content uniformity ranging from 97.72% to 99.25%, indicating homogenous distribution of drug through -out the gel.



Figu.5: Drug content of Amoxicillin Floating In-Situ Gel (F1-F8)

Water uptake

Table 7: Water Uptake of the In-Situ Gelling Formulations

Formulation Code	Water Uptake (%)
F1	18.63
F2	33.29
F3	50.36
F4	62.65
F5	30.78
F6	39.63
F7	47.31
F8	58.52

Prepare all formulations exhibited water uptake which is observed in the range of 18.63% to 58.52%. Release of the drug from the polymer matrix depends on the amount of water extended period of floating lag time and drug release respectively. The release of the drug may involve the penetration of water into the matrix and simultaneously release of the drug via diffusion or dissolution. Increase in the concentration of sodium alginate from 0.5% and 3.5% resulted in the increasing of water uptake capacity of sodium alginate based insitu gelling solution. Similarly, $caco_3$ concentration was found to have an effect on water uptake capacity of insitu gel.

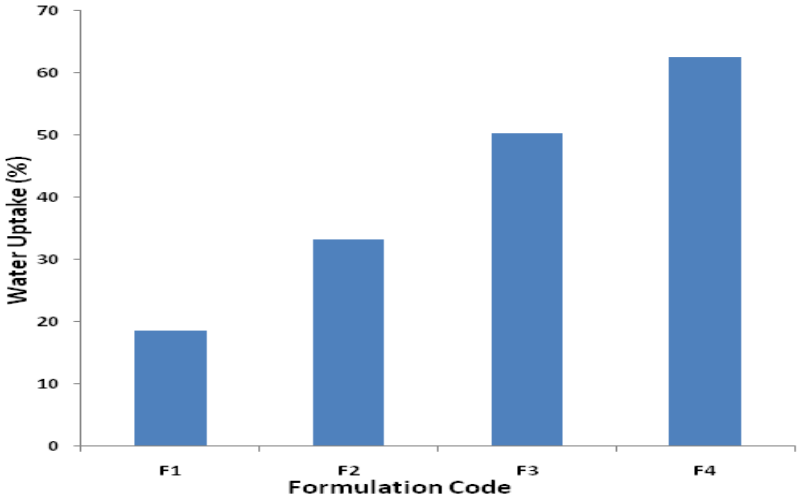


Fig.6: water uptake by Amoxicillin Floating In-Situ Gel (F1-F4)

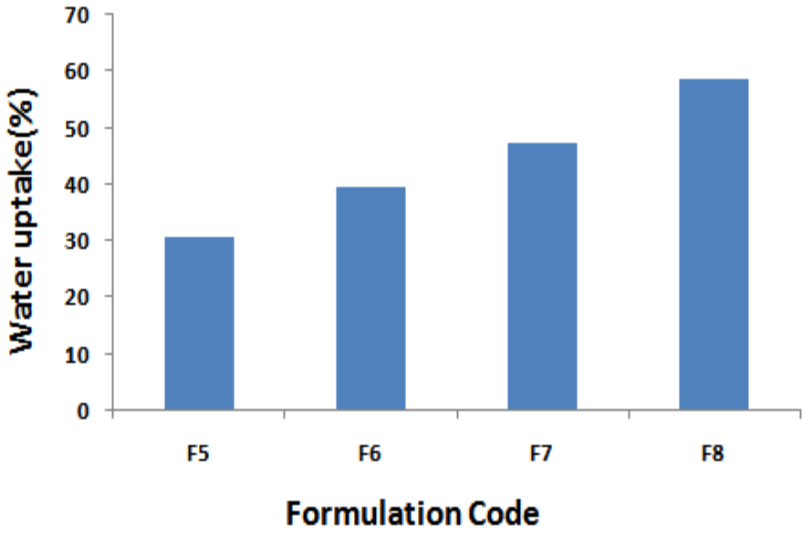


Fig.7: water uptake by Amoxicillin Oral Floating In-Situ Gel (F5-F8)

***In vitro* Drug Release Studies:**

Table 8: In-vitro Drug release of the In-Situ Gelling Formulations

TIME (Hr)	FORMULATION CODE							
	F1	F2	F3	F4	F5	F6	F7	F8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	44.11	45.87	43.11	41.98	49.34	47.13	42.76	41.07
2	66.24	64.16	61.43	60.76	67.43	63.59	60.44	61.23
3	79.54	71.06	66.54	64.98	78.56	72.67	66.65	63.11
4	90.57	78.02	73.32	69.44	86.89	78.56	71.32	70.16
5	98.23	84.12	79.43	75.81	94.99	83.23	76.54	77.54
6	98.52	91.10	84.65	79.09	98.14	89.34	82.82	83.12
7		96.07	88.32	83.10	98.33	97.04	87.81	88.65
8		98.08	94.47	87.12		97.23	91.23	92.45
9		98.41	97.57	92.69			95.19	96.62
10			97.86	96.40			96.74	97.23
11				96.56			96.96	97.51
12				96.73				

All the formulations showed significant burst release where approximately, 41-50% of amoxicillin was released within the first hr. This burst release might be attributed to the dissolved drug present at the surface of the formed immediately upon contact with the 0.1N HCL. In addition to this, some lag time is required for the release of ca^{2+} ions from $caco_3$ and cross linking of the guluronate residues of sodium alginate which plays a major role in the formulation of barrier gel. The release profiles also depicted that all formulations release 60% or more of

amoxicillin within the first 2hr and the remaining amount released at a steady rate that declined with time till the end of the dissolution study.

Increasing concentration of SA from 0.5% - 3.5% **(Fig.8)** resulted in the reduction of amoxicillin release rate. Higher polymer concentration levels reduced the release rate presumably due to the higher polymer density formed that could serve as an effective barrier across which the drug had to diffuse. Though higher SA contents resulted in slower release of amoxicillin compared to the formulation with the lowest level of SA.

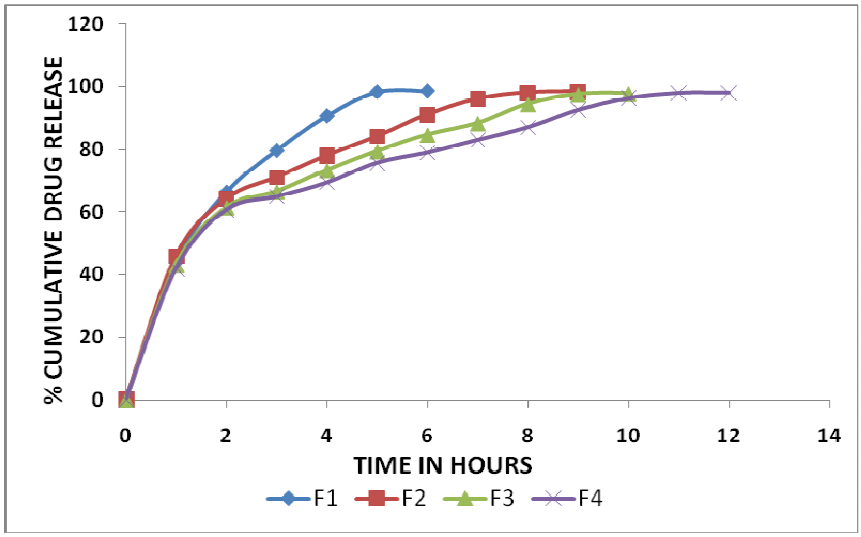


Fig.8: In-Vitro Drug Release of Amoxicillin Floating In-Situ Gel (F1-F4)

The effect of concentration of the cross-linking agent, $CaCO_3$, on the release of Amoxicillin is shown in **(Fig.9).** As it can be observed, the higher CO_2 released, due to increase in the concentration of $CaCO_3$ might make the gels more porous with undesirable weakening effect of gel integrity. This finding is similar with that reported by Nagarwal *et al*[49] where they found that increasing $CaCO_3$ concentration beyond 1.5% did not show appreciable release retardation compared to the formulations with lower concentration.

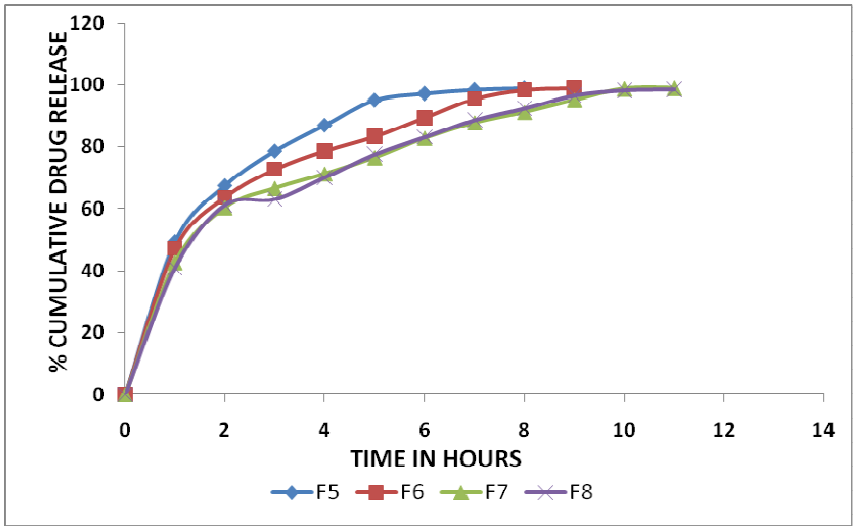


Fig.9: In-Vitro Drug Release of Amoxicillin Floating In-Situ Gel (F5-F8)

CONCLUSION

This study showed the feasibility of in-vitro gel forming from aqueous solution of sodium alginate containing Ca^{2+} ions in a complexed form. The aqueous solution of sodium alginate based in-situ gelling formulations showed different properties based on their difference in composition. Except for the formulation with lowest level of sodium alginate, all systems preserved their integrity for the 12 hours study period. Furthermore, all these formulations, regardless of their composition, remained floating for the study period. Therefore, in-vivo, the preparations are

expected to remain in stomach long enough without being emptied into the intestine to provide a sustained release of amoxicillin to be efficiently observed within its absorption window. The formulations showed satisfactory content uniformity and pH ensuring their safe use. The rheological studies showed that the formulations possessed optimal viscosity which can facilitate easy administration of the required dose. *In-vitro* drug release study proves that release rate of amoxicillin was depends on the amount of sodium alginate and $CaCO_3$ used.

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