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Development and Evaluation of a Novel Oro-Sustained Stomach Specific Floating *in Situ* Gelling System of Azithromycin Dihydrate

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Abstract: Floating drug delivery system provides the advantage of sustained release of drugs over a prolonged period of time thereby maximising the oral absorption of drugs with narrow absorption window, thus, it overcomes the drawback of conventional oral drug delivery system. *In situ* gelling system is a novel approach in this regard. The aim of the present work was to develop an oral sustained stomach specific novel floating *in situ* gelling system of Azithromycin dihydrate. *In situ* gelling system were prepared by dissolving different concentrations of gelling polymers like sodium alginate, xanthan and combination of sodium alginate and xanthan gum in deionized water at 80^0C. After cooling to 40^0C fixed amount of drug and calcium carbonate were dispersed in it with continuous stirring. Compatibility between drug and polymers were confirmed by FTIR studies. All formulations showed pH in the range of 6.5 to 6.9, drug content was found to be in the range of 97.92% to 99.27%, floating lag time was less than 1 min, duration of floating was more than 24 h for all the prepared formulations. Gelling capacity, viscosity and water uptake by the gel increased with the increase in polymer concentration. *In vitro* drug release was found to be in between 68.81% to 89.49%, up to 8 h with formulation F1 showing the maximum drug release. Combination polymers showed better sustained release of drug over a period of 8h. Drug release was found to decrease with the increase in polymer concentration and water uptake by gel. The release kinetics of all the formulations followed Higuchi diffusion mechanism. Hence, an oral sustained stomach specific floating *in situ* gelling system of Azithromycin dihydrate could be prepared using different concentrations of combination polymers to increase the patient compliance with reduced dosing frequency and increased residence time of drug in the stomach.
Keywords: Floating drug delivery system, Azithromycin dihydrate, *H. pylori*, *In situ* gelling system.

Introduction:

Helicobacter pylori are among the most common bacteria's which are capable of colonizing the harsh environment of the human stomach and are capable of causing peptic ulcers. Many other micro organisms responsible for serious stomach specific infections include Klebsiella, Salmonella, some harmful strains of E.coli, Streptococcus etc[1]. There are many antibacterial which are used for the treatment of infections caused by above pathogens, but studies reveals that eradication of such bacterium are not efficient due to some of the

basic hindrance like instability of drug in low pH of the gastric fluid, very low concentration of drug reaching the bacteria under the mucosa, short residence time oh the antibiotic in the stomach[2.] Patient compliance, side effect and bacterial resistance are other secondary problems in the delivery of the desired concentration of drug for the complete eradication of the bacterium. One way to develop the efficacy of an antibiotic for better eradication of *H. pylori* and other pathogens is to deliver the antibiotic locally in the stomach with better stability and longer residence time which will allow more of the active drug to penetrate through the gastric mucus layer to act on infectious organism[3].

Floating drug delivery system is a novel approach to achieve gastric retention to obtain sufficient drug bioavailability. These systems are capable of maintaining their buoyancy in the stomach for a prolonged period of time due their less bulk density than the gastric fluids, thus with a desired rate drug is released slowly in the stomach. After the drug is released, the residual system is emptied from the stomach[4].

In situ gelling system is a novel approach in the FDDS. *In situ* gels are in solution form which when comes in contact with gastric fluids forms gels. The phase transition of *in situ* gels may be attributed to one or combination of different stimuli like ionic interaction/pH change/ temperature modulation and solvent exchange[5]. The advantages of *in situ* gelling systems are ease of administration, reduced frequency of administration, improved patient compliance and comfort[6]. As the gelling capacity of the *in situ* gels increases, their residence time in the stomach increases which eventually leads to sustaining the delivery of the drug.

Azithromycin is a macrolide antibiotic used in the treatment of many gram negative and gram positive infections. It is chosen as the active drug in the present study to be incorporated in the *in situ* gelling system due to its low bioavailability following oral administration[7]; their tablet form shows low bioavailability around 34±19% and their stability in the gastric environment. Since, with a low oral bioavailability they are needed to be taken for several days to completely eradicate the infection. But the novel floating *in situ* gelling system of Azithromycin will provide the advantage of increased gastric residence time resulting in prolonged drug delivery with better patient compliance and lesser dosing frequency.

Materials and Method:

Method:

Azithromycin dihydrate was obtained as a gift sample from Glow Pharma, Mumbai. All other materials used were of analytical grade.

Preparation of *in situ* gelling system:

Sodium alginate, xanthan gum and combination of sodium alginate and xanthan gum in different concentrations (1.0-2.0 % w/v) were prepared in deionized water containing sodium citrate (0.45% w/v). The gelling polymers were dispersed in deionized water, heated to 60^0C to 90^0C with stirring and then cooled below to 40^0C. Fixed quantity of drug (250 mg) and calcium carbonate (0.05%w/v) was added after cooling the solution below 40^0C with continuous stirring to form uniform dispersion. These prepared sols were stored at room temperature until further use. The composition of various formulations of Azithromycin dihydrate floating *in situ* gels is tabulated in table 1.

Table 1: Formulation table of Azithromycin dihydrate *in situ* gelling system

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Azithromycin dihydrate	250 mg	250 mg	250 mg	250 mg	250 mg	250 mg	250 mg	250 mg	250 mg
Sodium Alginate	1.0% w/v	1.5% w/v	2.0% w/v	-	-	-	-	-	-
Xanthan gum	-	-	-	1.0% w/v	1.5% w/v	2.0% w/v	-	-	-
Sodium alginate +	-	-	-	-	-	-	1.0% w/v	1.5% w/v	2.0% w/v

Xanthan gum**									
Sodium Citrate	0.45% w/v	0.45% w/v	0.45% w/v	0.45% w/v	0.45% w/v	0.45% w/v	0.45% w/v	0.45% w/v	0.45% w/v
Calcium Carbonate	0.50% w/v	0.50% w/v	0.50% w/v	0.50% w/v	0.50% w/v	0.50% w/v	0.50% w/v	0.50% w/v	0.50% w/v
Water (q.s)	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml

(**sodium alginate is 0.50%w/v and xanthan gum is 0.50%w/v, 1.0%w/v, 1.5%w/v to make up final percentage of 1.0%w/v, 1.5%w/v, 2.0%w/v of formulation F7, F8 and F9 respectively**)**.

Evaluation:

Compatibility studies:

The drug polymer interaction studies were carried by FTIR technique by KBr pelletization method. The physical mixture of drug and polymers were taken in a ratio of 1:1 and mixed uniformly with demoisturized KBr[8]. The mixture was then compressed to a thin transparent pellet by subjecting to hydraulic press, which is then placed in the path of IR rays using a sample holder to record the spectra from 400 – 4000cm^{-1}.

***In vitro* gelation study[9]:**

The *in vitro* gelling capacity of prepared formulations was measured by placing 5 ml of the gelation solution (0.12N HCl, pH 1.2) in a 15 ml borosilicate glass test tube and maintained at 37±1 ^{0}C temperature. 1 ml of formulation solution was added with the help of pipette. The formulation was transferred in such a way that places the pipette at surface of fluid in test tube and formulation was slowly released from the pipette. As the solution comes in contact with gelation solution, it was immediately converted into stiff gel like structure; the gelling capacity of solution was evaluated in the basis of stiffness of formed gel and time period for which the formed gel remains as such. The *in vitro* gelling capacity was graded in three categories on the basis of gelation time and time period for which the formed gel remains.

(+) Gels after few minutes, dispersed rapidly.
(++) Gelation immediately remains for few hours.
(+++) Gelation immediate remains for an extended period.

***In vitro* floating studies[10]:**

The *in vitro* floating study was determined using USP dissolution apparatus having 500 ml of simulated gastric fluid (pH 1.2). The petri dish containing 5 ml of *in situ* gelling solution was immersed into dissolution apparatus (USP24) containing 500 ml of SGF (pH 1.2) at 37 ^{0}C. The time the formulation took to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on the dissolution medium surface (duration of floating) were noted by visual observation.

Determination of viscosity[9,11]:

Viscosity of the samples was determined using a Brookfield digital viscometer with spindle number 2. The sample temperature was controlled at 25±1^{0}C before each measurement. Viscosities of gels were determined at different shear rates from 10 to 100 rpm at room temperature.

Drug Content[12]:

5 ml of the solution was added to 900 ml of simulated gastric fluid (0.1 mol/l HCl, pH 1.2) and stirred for 1 hr on a magnetic stirrer. The solution was filtered, suitably diluted with simulated gastric fluid and the drug concentration was determined by using a UV-visible spectrophotometer at 272 nm against a suitable blank solution.

Measurement of water uptake by the gel[13]:

The water uptake by the gel of all formulations can be determined using a thermo-gravimetric analyzer. But in this 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 gastric acid buffer (pH 1.2) was used for this study. From each formulation the gel portion from the buffer was separated and the excess buffer was blotted out with a tissue paper. The initial weight of the gel taken was weighed and to this gel 10 ml of distilled water was added and after every 30 mins of the interval water was decanted and the weight of the gel was recorded and the difference in the weight was calculated and reported.

Microbiological studies:

Four petridish were prepared with nutrient agar medium using micro pipette, 0.2 ml of seeded broth containing test organism were spreaded uniformly. With an aluminium bore of 5mm diameter four wells were cut out on the agar media. Each well was filled with equal quantity of test and standard alternatively and in a zig zag manner. The plates were then incubated at 37±1 ^{0}C for 24 h. After the incubation period the mean diameter of zone of inhibition in cm obtained around the well were measured. Strict aseptic conditions were followed for the entire process[9].

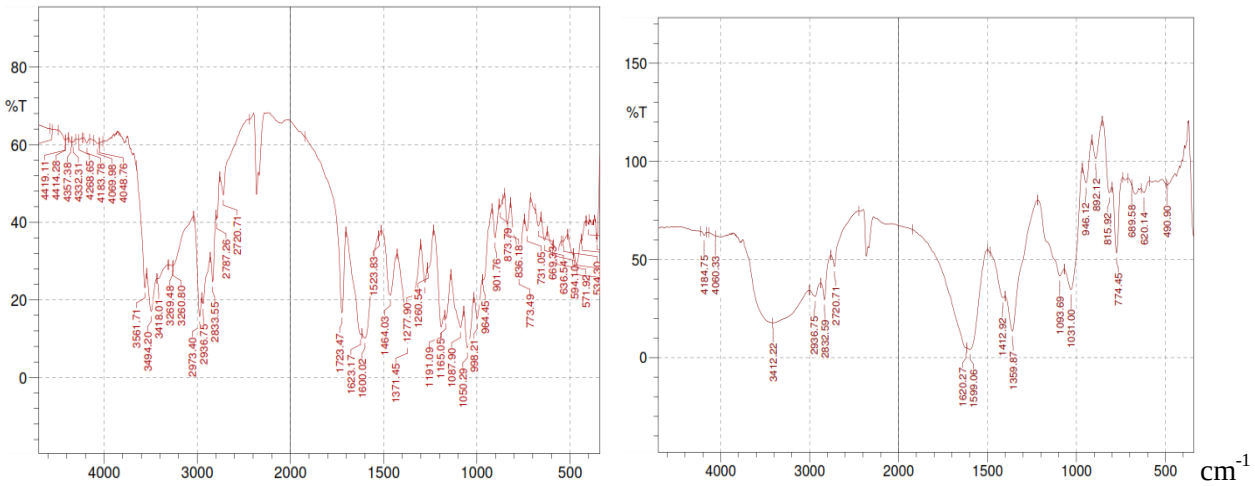
***In vitro* drug Release[13]:**

The release of Azithromycin dihydrate was studied using USP type II dissolution apparatus containing 500 ml of 0.1N HCl maintained at 37±0.5^{0}C and stirred at 50 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. These samples were analyzed for the drug present in them with help of UV spectrophotometer at 272 nm. Further the *in vitro* drug release for the drug release kinetics like zero order (cumulative %drug release against time), first order release (log cumulative %drug release against time), Higuchi equation (cumulative %drug release against square root of time and Korsmeyer peppas model (log cumulative %drug release against log time) were also studied.

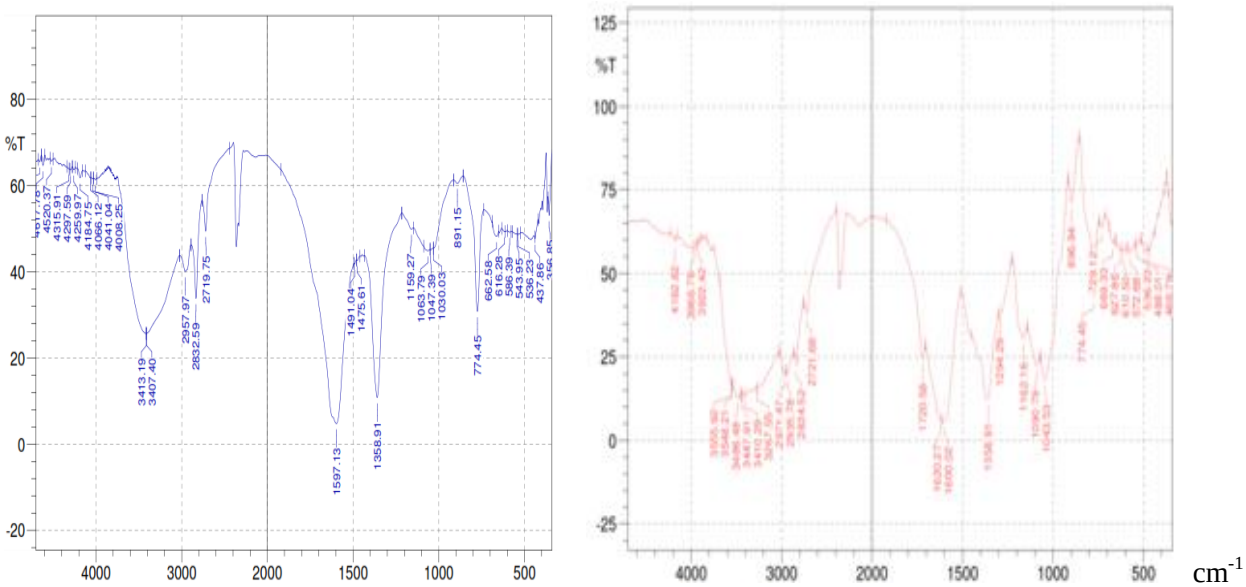
Results and Discussion:

FTIR studies:

The identification characterization of Azithromycin dihydrate and compatibility with different polymers were carried out using infrared spectroscopy from 400 to 4000cm^{-1} using KBr pellets. As there were no major changes in the peaks, indicates that there was no incompatibility of the drug with the polymers used in the formulations fig 1.



(1) (2)



(3) (4)

Figure 1: FTIR compatibility studies: (1) Azithromycin dihydrate (2) sodium alginate (3) xanthan gum (4) drug +polymers

There were almost no change in the peak regions of pure drug like aromatic primary amine group stretch at 3494 cm^{-1}, Methyl –CH symmetric stretch at 2973 cm^{-1} and –C=O stretch at 1718 cm^{-1}, when compared with the mixture of drug and polymer[12].

The concentration of polymers used affect the viscosity as well as gelling capacity. As the concentration of the gelling polymers were increased the viscosity of the formulation also increased with an increase in the gelling capacity[9] as shown in table 2. Formulation F9 showed better gelling than all other formulations.

Table 2: Viscosity, Floating time, Floating lag time and Gelling capacity of Azithromycin dihydrate *in situ* gel formulations

Formulation	Viscosity of sol (cps)	Viscosity of gel (cps)	*In vitro* floating lag time (min)	*In vitro* floating time (h)	Gelling capacity
F1	3.5	80.6	<1	>24	++
F2	5.7	84.8	<1	>24	+++
F3	8.2	91.1	<1	>24	+++
F4	5.4	87.2	<1	>24	++
F5	7.8	89.3	<1	>24	+++
F6	9.2	92.4	<1	>24	+++
F7	8.4	91.2	<1	>24	+++
F8	8.8	91.4	<1	>24	+++
F9	9.5	92.7	<1	>24	+++

Drug content:

The amount of drug present in all formulations was evaluated by spectrophotometrically at 272nm. The results are tabulated in table 3. The drug content ranges from 97.92% to 99.27%.

Table 3: Drug content of Azithromycin dihydrate *in situ* gels in 0.1N HCl

Formulations	Drug Content (%)
F1	98.52
F2	98.84

F3	99.16
F4	97.92
F5	97.99
F6	99.27
F7	97.82
F8	98.99
F9	99.19

Percentage water uptake by gel:

The water uptake by gel was directly proportional to the polymer concentration. As the concentration of the gelling polymers was increased the water uptake by the gel also increased. The % of water uptake by gel also influenced the drug release from the gel[13]. As the % of water uptake by gel increased the drug release was found to decrease. Formulation F7-F9 showed higher % of water uptake by gel than formulation F1-F6. The % of water uptake by gel is graphically shown in fig 2

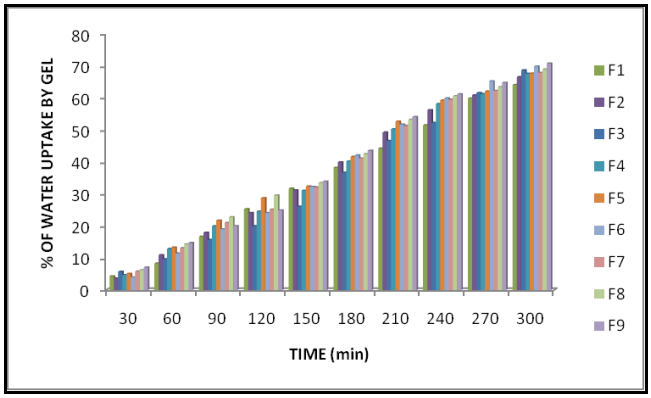


Figure 2: % of water uptake by Azithromycin dihydrate *in situ* gel formulations (F1-F9)

Microbial studies:

For the microbial studies *E.coli, Klebsiella, Streptococcus* and *Staphylococcus* were selected. The effect was studied by comparing the zone of inhibition of the microbial growth as tabulated in table 4. The oral floating *in situ* gelling system of Azithromycin dihydrate was found to be effective in eradication of bacteria causing stomach infection[12].

Table 4: Microbial studies of various formulations of Azithromycin dihydrate floating *in situ* gels

Formulations	Zone of inhibition (cm)			
	E.coli	***Klebsiella***	***Streptococcus***	***Staphylococcus***
Standard drug	1.75	1.80	1.60	1.55
F1	1.71	1.65	1.26	1.39
F2	1.69	1.66	1.29	1.47
F3	1.67	1.70	1.41	1.47
F4	1.65	1.71	1.40	1.42
F5	1.69	1.68	1.39	1.42
F6	1.68	1.66	1.38	1.49
F7	1.63	1.70	1.40	1.48
F8	1.61	1.67	1.42	1.50
F9	1.62	1.70	1.37	1.47

Cumulative percentage drug release:

The concentration of gelling polymers on *in vitro* drug release from floating *in situ* gels is showed in fig 3. A significant decrease in rate and extent of drug release is seen with increase in polymer concentration this pattern

is attributed to increase in the density of the polymer matrix and also an increase in the diffusional path length which the drug molecules have to travel. With the increase in the concentration of gelling polymer decreased rate and extent of drug release with sustained effect was observed table 5 and fig 3. Formulation F7, F8, F9 showed greater decrease in drug release than formulation F1-F6. The release of drug from these gels was characterized by an initial phase of high release which is actually the burst effect. As the concentration of gelling polymers was increased the burst effect was found to decrease[9].

Table 5: *In vitro* cumulative % drug release of Azithromycin dihydrate *in situ* gel

Time (h)	Cumulative % drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	40.34	38.02	34.22	41.11	40.40	39.21	34.31	32.21	30.11
2	50.12	48.32	41.77	52.21	51.12	48.75	39.97	38.51	36.31
3	58.88	56.21	49.91	59.85	59.88	56.63	45.72	44.65	41.11
4	67.91	64.32	57.85	68.16	68.19	64.89	50.21	49.29	47.72
5	75.99	73.10	66.91	75.13	76.90	73.70	57.63	56.62	53.34
6	79.56	77.21	74.32	81.01	80.50	77.80	61.21	59.98	56.49
7	83.92	81.15	80.22	82.89	81.97	80.16	68.33	66.73	62.79
8	89.49	85.54	83.59	84.06	83.61	82.96	73.21	70.01	68.81

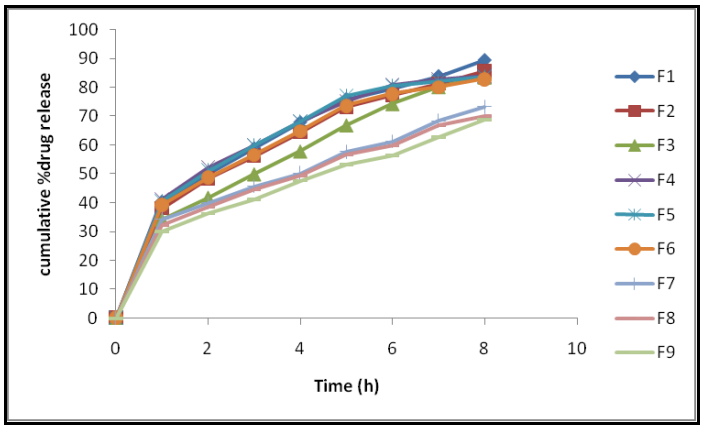


Fig 3: *In vitro* drug release of Azithromycin dihydrate *in situ* gel from different gelling polymers in different concentrations

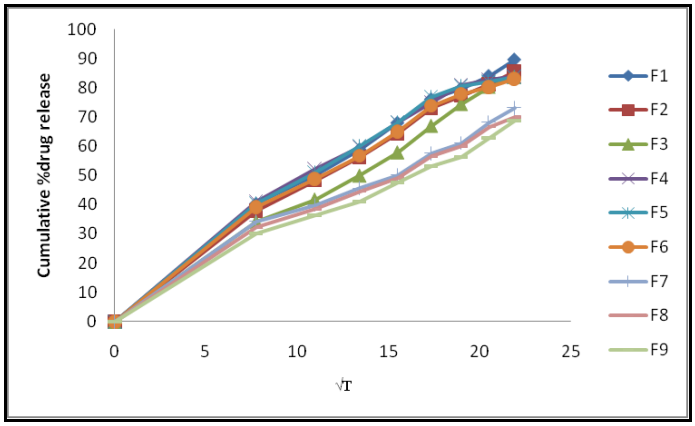


Figure 4: Higuchi plot of Azithromycin dihydrate *in situ* gels formulations (F1-F9)

Release kinetics:

In vitro drug release study for all nine prepared formulations showed sustained drug release for longer period of time and followed Higuchi release pattern[12] as tabulated in table 6.

Table 6: Release mechanism of Azithromycin dihydrate floating *in situ* gels

Formulations	Zero Order (R^2 value)	First Order (R^2value)	Higuchi Model (R^2value)	Peppas model (n values)
F1	0.853	0.979	0.989	0.656
F2	0.858	0.986	0.991	0.660
F3	0.910	0.977	0.994	0.660
F4	0.813	0.975	0.976	0.655
F5	0.811	0.970	0.975	0.658
F6	0.835	0.980	0.983	0.659
F7	0.866	0.981	0.977	0.661
F8	0.869	0.980	0.981	0.660
F9	0.883	0.986	0.987	0.658

(a) (b)

Figure 5: *In situ* formed gel of formulation F9 in SGF pH 1.2 (b) Photograph of formed gel of formulation F9

Conclusion:

Based on the results obtained and experimental conditions used in the study, F9 formulation showed sustained release of drug from the gel. The formed gel by formulation F9 is shown in fig 5. The developed formulations met all prerequisites to become an *in situ* gelling floating system, formed and floated instantaneously in the pH conditions of the stomach. This study demonstrated that *in situ* gels formed by oral administration of solutions of gellan gum and release of Azithromycin dihydrate is sustained over a period of time for at least 8h. It was observed that the feasibility of *in vitro* gel was formed form aqueous solutions of different gelling polymers. It can be concluded from the study that with the increase in concentration of polymers, the viscosity and % of water uptake by gel also increased but the drug release from the *in situ* gel decreased. It was revealed from the study that use of combination polymer provides a better sustained release of drug from the *in situ* gelling systems. Thus, it can be concluded that the release of Azithromycin dihydrate could be targeted to stomach and sustain the drug release over a period of time. Further investigation is needed to prepare the best and most successful formulation of Azithromycin dihydrate *in situ* gelling system.

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