Development and optimization of smart raft forming in-situ gel for gastroretentive drug delivery of anthelmintic drug

***Dhawal Dorwal. [1]Ravikant Gupta**

**Research Scholar Faculty of Pharmacy, Oriental University, Indore 453555 MP India*

[1]Faculty of Pharmacy, Oriental University, Indore 453555 MP India

KEYWORDS

In-situ gel, Sodium alginate, albendazole, Floating, Drug release

ABSTRACT

The main objective of the research was to formulate an oral raft-forming in situ gelling system of Albendazole (ABZ) to improve gastric retention and drug release in a controlled manner and remain floating in the stomach for a prolonged time. In this study in situ gelling system was developed and optimized by a two-factor at three-level (3^2) factorial design. It was analyzed to study the impact of two independent variables viz sodium alginate [A] and HPMC K4M [B] on the responses, such as floating lag time, percentage (%) water uptake at 2 h, and % drug release at 6 h and 12 h. An in vitro gelation study of the in situ gel formulation showed immediate gelation and was retained for a longer period. From the obtained results of 3^2 factorial designs, it was observed that all the selected factors had a significant effect on the chosen response, supporting the precision of design employed for optimization. Thus, the developed oral raft-forming in situ gelling system of ABZ can be a promising and alternate approach to enhance retention in the stomach and to attain sustained release of drug by floating, thereby augmenting the therapeutic efficacy of ABZ.

1. Introduction:

Improved drug delivery and the creation of restricted substances have drawn more attention during the past three decades. Since in situ gel systems have shown benefits such ease of application, decreased frequency of use, and enhanced human pain compliance and comfort, their development has drawn a lot of attention in recent years.

Before being administered, gel dosage forms are liquid, but they become a gel that floats on the stomach when they come into touch with stomach contents. One or more mechanisms, including physiological cues (like temperature and pH), physical alterations in the biomaterial (like solvent

transport and swelling), and grafts (like vaccinations), are responsible for this gel shift. Numerous production challenges affect the biodegradable polymers used to create in situ gels, In contrast to natural polymers, there are occasionally batch differences, explosive effects, non-reproducible drug release kinetics, operational problems, and the utilization of organic solvents. [1–2]

Albendazole is an unoriginal benzimidazole used to treat a variety of parasitic worm infections. It works well against roundworms, tapeworms, and flukes and is a broad-spectrum anti-helminthic.

It is used to treat a wide range of illnesses caused by parasitic worms, including neuro-cysticercosis, giardiasis, trichuriasis, and filariasis.[3,4,5,6,7,8]

Methods

Preformulation studies were formed for Albendazole as per the received COA of the drug and it was found to be in line with the COA

Preparation of in-situ gel[11-13]

All the additives used in the preparations were passed from a No. 60 sieve (250 microns). Required ingredients for the preparation, like sodium alginate, HPMC K4M, sodiumbicarbonate, and sodium citrate, were accurately weighted as per the formulation chartdepicted in Table 2.HPMC K4M was dissolved using 40 mL of deionized water.Therequired quantity of sodium bicarbonate and sodium citrate were incorporated in it whilestirring to attain complete homogenous dispersion. 30 mg Albendazole drug was dissolved in the solution.Sodium alginate was dissolvedusing deionized water (30 mL) taken in a beaker pre-heated to around 60 ˚C on a hot platewith continuous stirring. The sodium alginatesolution was cooled to 40 ˚C and added to the HPMC K4M solution. The total amount ofthepreparationfinallyreached100mL,makinguseofdistilledwaterafteraddingmethylparabena sapreservativeandmixedthoroughly. 3^2 factorial design for the oral raft-forming in situ gelling system of Albendazole, obtained using Design-Expert software (version 130.2.0) from Stat-Ease Inc., Minneapolis, MN, USA.[1]

Table 1 Formulation of *in-situ* gel

S.No.	Ingredients	Quantity taken								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Albendazole (mg)	30	30	30	30	30	30	30	30	30
2	Sodium alginate (g)	1	2	3	1	2	3	1	2	3
3	HPMC K4M (g)	1	1	1	1.5	1.5	1.5	2	2	2
4	Calcium carbonate (mg)	50	50	50	50	50	50	50	50	50
5	Sodium bicarbonate (mg)	50	50	50	50	50	50	50	50	50
6	Sodium citrate (mg)	25	25	25	25	25	25	25	25	25
7	Methyl paraben (mg)	10	10	10	10	10	10	10	10	10

Experimental Design[01]

The interaction and relationship between dependent and independent variables can be studied using a scientific and systemic approach, i.e., experimental design. The optimization of the formulations was performed using 3^2 factorial design. The effect of the independent variables and their interactions can be determined from the chosen experimental design, which can provide a satisfactory degree of freedom. Two independent factors (variables), sodium alginate (A) and HPMC K4M (B), were selected and evaluated at two levels, i.e., higher level (1), medium level (0), and lower level (+1). The responses (independent variables) chosen to know the effect of the factors were floating lag time, % water uptake at 2 h, and % drug release at 6 h and 12 h. The analysis of the obtained data was carried out employing Design-Expert software (version 130.2.0) offered from Stat-Ease Inc., Minneapolis, MN, USA. Table 3 enlist the factors and their levels for preparing the oral raft-forming in situ gelling system of Albendazole

Table 2 Composition of independent variables and their levels for the preparation of the oral raft-forming in situ gelling system of Albendazole

Variables	Actual value (g)			Code value		
	Low	Medium	High	Low	Medium	High
Sodium alginate	1	2	3	-1	0	1
HPMC K4M	1	1.5	2	-1	0	1

Evaluation

1. **Physicochemical properties**

The colour, odour and taste of the formulated in-situ gel of albendazolewere determined as per the senses.[13]

2. **Determination of drug content**

Accurately, 10 ml of the formulation (containing the equivalent of 30 mg albendazole) from different batches was measured and transferred to 100 ml volumetric flasks. To this 50-70 ml of 0.1 N HCl was added and sonicated for 30 min. Volume was adjusted to 100 ml. Complete dispersion of the contents was ensured visually and the dispersion was filtered using Whatman's filter paper. From this solution, 10 ml of sample was withdrawn and diluted to 100 ml with 0.1N HCl. Contents of albendazolewere measured at a maximum absorbance at 235 nm using UV-Visible spectrophotometer.[14]

3. **pH measurement**

pH of the prepared formulations was measured using a calibrated digital pH meter at 27 ˚C [6].

4. **In vitro gelation study**

To evaluate the formulation for their in vitro gelling capacity accurately measured 1 ml of the colored formulation were added to 5 ml of the gelation solution (0.1 N HCl, pH 1.2) at 37 ˚Cin a test tube with mild agitation that avoids breaking of formed gel. The in vitro gelling capacity was graded in three categories on the basis of the stiffness of the formed gel, gelation time and time period for which they formed gel remains as such (+) gels after few minutes, dispersed rapidly; (++) gelation immediate remains for few h; (+++) gelation immediate remains for an extended period.[15-17]

5. **In vitro floating study**

The in vitro floating study was carried out by introducing 10 ml of the formulation into a beaker containing 900 ml of 0.1 N HCl (pH 1.2) at 37 ˚C without much disturbance. The time the formulation took to emerge on the medium surface (floating lag time) and the time the formulation constantly floated on the surface of the dissolution medium (Duration of floating) were recorded.[19]

6. **Measurement of water uptake**

The water uptake by the gel of selected formulation of sodium alginate was determined by a simple method. In this study, the in- situ gel formed in 40 ml of 0.1 N HCl (pH 1.2) was used for each formulation the gel portion from the 0.1N HCl was separated, and the excess HCl solution

was blotted out with a tissue paper. The initial weight of the gel taken was weighed, and to this gel, 10 ml of distilled water was added and after every 30 min of the interval water was decanted and the weight of the gel was recorded and the difference in the weight was calculated.[18]

7. **Viscosity**

   The formulation should have an optimum viscosity that will allow ease of administration and swallowing as a liquid and produce satisfactory gel strength for use as a delivery vehicle. Results of viscosity for formulationsare shown in table. The formulations showed an increase in viscosity with increasing the concentration of gel forming polymer sodium alginate as a consequence of the increase in chain interaction.

   Measurement of gel strength

   A sample of 50g of the gel formed in 0.1 N HCl (pH 1.2) was introduced into a 100ml graduated cylinder. A weight of 35g was placed onto the center of the surface of the gel and allowed to penetrate through the gel. The time taken by the 35 g weight to penetrate 5 cm down through the gel was noted for all formulations. The same procedures were followed for each fresh formulation in triplicate and average time was determined

8. **Swelling index**

   The gel swelling index of the selected formulation is determined by a simple method. In this study, an in-situ gel formed in 40 ml of 0.1NHCl(pH1.2)wasused.Separatethe0.1NHClgelfractionfromeach formulation, and remove the excess HCl solution with papertowels.Weightheinitialweightofthegel,add50mlofdistilledwa-tertothegel,pouroutthewaterafter12hours,recordtheweightof the gel, calculate and report the weight difference

9. **In vitro drug release study**

The dissolution studies were performed in triplicate using type I (basket method) dissolution apparatus. The dissolution medium used was 900 ml of 0.1 N HCl maintained at 37 ˚C. The stirring rate was adjusted to 50 rpm. This speed was believed to stimulate the in vivo existing mild agitation and was slow enough to avoid the breaking of gelled formulation. At predetermined time intervals 1 ml samples were withdrawn and replaced by fresh dissolution medium, filtered through what Mann’s filter paper, diluted and assayed at a maximum absorbance at 235 nm using double beam UV-Visible spectrophotometer.[20]

10. Kinetics of Drug Release Studies

To determine the kinetics of the drug release, the dissolution profile of each batch was adapted for different models, including first-order, zero-order, Hixon and Crowell, Higuchi, and KorsemeyerPeppas (KP). The KP equation describes the method to explain the drug release mechanisms

9. In vivo study[21]

The animal experiment was carried out in compliance with the protocol of the Institutional animal ethical committee (IAEC: 1877/PO/Re/S/16/CCSEA/2024/021).

Six White rabbits with mean weight of 2.5 ± 0.3 kg were used. The rabbits were accommodated to the dosing for 1 month before the study to prevent withdrawal and defense reaction that may lead to inac- curate dosing. The rabbits were kept in a single cage and fasted for 12 h before the study with free access to water during the experiments. A cannula was inserted into the marginal ear vein for blood sampling and flushed with heparinized normal saline solution.

Study design- [21]

White rabbits were selected as an experimental model because they provide a well controlled animal model for screening the oral absorption potential of oral formulations. In a cross-over study with 1 week apart as a wash out period,l gm oral in situ gel ABZ (equivalent to 10 mg ABZ) was deposited in oral cavity. The animals also received 5 ml of oral drug solution ABZ SO (equivalent to 10 mg ABZ).

Sample preparation:[21]

Blood samples were centrifuged and the plasma was separated from the cells. All plasma samples were stored at –20 Absolute Eosinophil Count (AEC) until analysis. Plasma was extracted by placing 200 IL of plasma sample in a glass tube. Sodium metabisulphite (100 ÌL) and 2 ml of ethyl acetate were added. Tube was vortexed for 10 min and then placed on a shaker, shaking gently for another 20 min. After centrifugation at 2000 rpm for 10 min, 2 ml of the organic layer of tube was removed, placed in a new labelled 12x75 mm glass tube and evaporated in a Thermo Savant rotary vacuum chamber and Refrigerated The residue was re-suspended in 200 ÌL of methanol for HPLC analysis. Before analyzing plasma samples, ABZ and ABZ-SO standard solutions prepared in methanol were run on HPLC to optimize analysis and standardize calibration. Standard drug and metabolite concentrations were chosen on the basis of

levels found in plasma samples. ABZ standard solutions used were in the range of 0.05 to 0.4 Ìg/ml while those for ABZ-SO was 0.5 to 25 Ìg/ml.

HPLC analysis-[21]

The HPLC system consisted of a CMB Controller,Pump, Auto Sampler, Degasser and a Fluorescence Detector set at Ex 280 nm,. A reverse-phase, Luna 5-Ìm C18 (2) column, 250x4.6 mm was used with a Shimadzu Column Oven set at 45AEC. The mobile phase was a mixture of 0.01M phosphoric acid in deionized water and acetonitrile (80:20 v/v) containing 5 M tetra-butyl-ammonium hydrogen sulfate, pH 2.2.

Statistical analysis-

All statistics were performed using GraphPad In Stat on Prism The Student's t-test was used to compare the data of two groups. A p-value of less than 0.05 was considered to represent a statistically significant difference. All values are presented as mean±SE.

Stability Study as per ICH guidelines:[12-14]

The optimized formulation ABZ was initially stored in glass bottles and subjected to a three-month stability study. Subsequently, the in-situ gels were monitored for stability over a period of 6 months under accelerated conditions. At regular intervals of 0, 3 and 6 months, samples were taken for analysis as per ICH

Long term stability studies:[12-14]

The results of the long term stability studies of the optimized formulation ABZ, conducted under conditions of 30±2°C/65±5%RH, are presented in Table

a. Physical Appearance:

Throughout the 6-month stability period, there were no observable changes in the physical appearance of the optimized formulation. The in-situ gel maintained its original color without any discoloration.

b. % Drug Content:

The drug content of the optimized formulation was found to be highly consistent over the 6-month period. At the start of the study (0 month), the drug content was determined to be 99.94±0.21%. Subsequent testing at 3 months and 6 months showed minor variations, with drug contents of 99.37±0.18% and 99.15±0.03%, respectively. These results indicate that the

optimized formulation remained stable with minimal drug degradation or loss during the storage period.

c. Floating Time:[12-14]

The floating time of the optimized formulation also remained constant throughout the 6-month stability study. The in-situ gel consistently exhibited a floating time of 12 hours at each time point (0 month, 3 months, and 6 months). This demonstrates that the buoyancy of the formulation was retained, ensuring prolonged residence time in the stomach for sustained drug release.

d. Viscosity:

The viscosity of the optimized formulation remained relatively stable during the long term stability study. There were minimal changes in viscosity values over the 6-month period, with recorded values of 443±0.10, 442±0.15, and 441±0.20 cps at 0 month, 3 months and 6 months, respectively. This suggests that the rheological properties of the in-situ gel were well-preserved throughout the storage period.

e. Gel Strength:[12-14]

The gel strength of the optimized formulation was consistently maintained during the 6-month stability study. The recorded gel strength values were 10.82±0.12 N/m2 at 0 month, 10.78±0.15 N/m2 at 3 months and 10.73±0.17 N/m2 at 6 months. These results indicate that the gel maintained its structural integrity and mechanical strength during the storage period.

Accelerated stability studies:[12-14]

The results of the accelerated stability studies of the optimized formulation ABZ, conducted under conditions of 40±2°C/75±5%RH, are presented in Table.

a. Physical Appearance:

Similar to the long term stability results, the physical appearance of the optimized formulation remained unchanged throughout the 6- month accelerated stability study. There were no observable colour changes or any other visual alterations, indicating the preservation of the formulation's integrity and stability.

b. % Drug Content:

The drug content of the optimized formulation showed good stability during the accelerated stability study. At the beginning of the study (0 month), the drug content was measured to be

99.94±0.01%. At 3 months and 6 months, the drug content slightly decreased, with values of 99.14±0.07% and 99.03±0.21%, respectively.

c. Floating Time: [12-14]

The floating time of the optimized formulation was consistent during the accelerated stability study. The in-situ gel exhibited a constant floating time of 12 hours at each time point (0 month, 3 months and 6 months).

d. Viscosity:

The viscosity of the optimized formulation also showed good stability over the 6-month accelerated stability study. There were negligible changes in viscosity values, with recorded values of 443±0.10 cps at 0 month, 441±0.28 cps at 3 months and 440±0.36 cps at 6 months.

Gel Strength:

The gel strength of the optimized formulation remained constant during the accelerated stability study. The measured gel strength values were 10.82±0.12 N/m2 at 0 month, 10.75±0.23 N/m2 at 3 months and 10.68±0.28 N/m2 at 6 months. These results indicate that the gel retained its structural integrity and mechanical strength even under the accelerated storage conditions.

In vitro drug release:

The results of % drug release for the ABZ formulation under both long term (30±2°C/65±5% RH) and accelerated (40±2°C/75±5% RH) stability
conditions at 0, 3, and 6 months are presented in Table and figures

a. Under Long Term Stability (30±2°C/65±5% RH):

The % drug release from the ABZ formulation at each time point (0, 3, and 6 months) remained consistent. At the initial time point (0 months), the % drug release was 0% as expected, as no release occurred immediately after preparation. As the time progressed, the drug release increased, reaching 99.85±0.09% at 12 hours, which indicates the complete release of the drug from the formulation. There were minor variations in drug release values at 3 and 6 months (ranging from 99.83±0.28% to 99.82±0.12%), but overall, the formulation exhibited a sustained and stable drug release profile.

b. Under Accelerated Stability (40±2°C/75±5% RH):

Similar to the long term stability results, the % drug release from the ABZ formulation at accelerated conditions showed consistency. At 0 month, the drug release was indicating no immediate release upon preparation. The drug release increased with time and at 12 hours, the %

drug release reached 99.85±0.09%. Throughout the 3-month and 6-month time points, the drug release values showed minor fluctuations but remained within the range of 99.81±0.09% to 99.80±0.09%, demonstrating the maintenance of drug release performance under accelerated stability conditions.

Results and Discussion

1. **Organoleptic characterization**

Albendazole is white crystalline to pale yellowish powders with a characteristic odor, consistent with the characteristics described in the F. Bras. V (2010) and DMF.

2. **Formation of calibration curve:**

Table 3 Readings obtained from UV interpretation

Conc. In µg/ml	Absorbance
5	0.3917
10	0.7045
15	0.9374
20	1.3934
25	1.6244

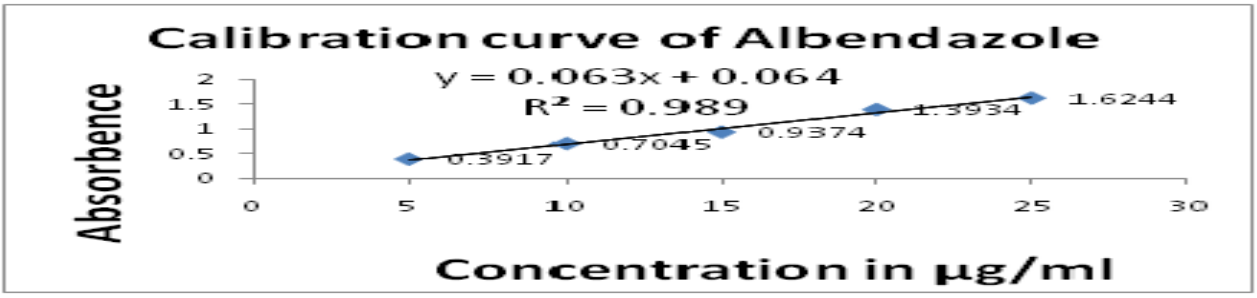

Fig. 1: Calibration curve of albendazole pure drug

3. Preformulation parameters:

(i) Bulk density: It is a important parameter used to determine the bulk flow property of powder blend at the time of feeding powder blend in dies from the hopper. It was found to be 0.3gm/ml

(ii) Tapped density: It is the parameter used to calculate the tapping property of powder blend by which the flow property of the blend can be determine.It was found to be 0.42

(iii) Angle of repose: It is determine for determining the flow property of the blend.It was found to be 24.60. So by interrelating from the table the flow is good.

Table 4 Angle of Repose as an Indicate the Powder Flow Properties

S.No.	Angle of repose(º)	Type of flow
1	< 20	Excellent
2	20-30	Good
3	30-34	Passable
4	>34	Very poor

(iv) Carr's compressibility index: It is determine because it detect the flow ability of the powder blend.It was found to be 28.57. So by interrelating from the table the flow ability is poor.

Table 5 Relationship between % compressibility and flow ability

% Compressibility	Flow ability
5-12	Excellent
12-16	Good
18-21	Fair passable
22-33	poor
35-38	Very poor
>40	Very very poor

(v) Hausner ratio: It is useful in determining the flow property of the powder blend.It was found to be 1.4.

4. Infrared spectroscopy

The infrared spectra of ABZ confirm the authenticity of the API. The infrared spectrum from ABZ is represented below

Functional group	Wave number (cm-1)	
	Reference Value	Observation
N-H stretching	3328	3325
Aromatic C-H stretching	3151	3142
CH3/CH2 C-H stretching	2810	2797
C-H stretching	2666	2647
Amide I band	1717	1712

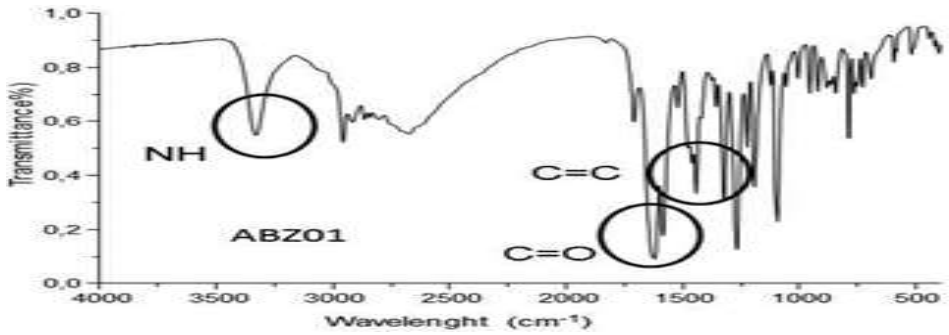

Fig. 2: Infrared spectrum of Albendazole and its absorption bands.

5. Meltingpoint

The measured melting points of the 3 Samples, ABZ01,ABZ02,andABZ03,were208°C,208°C,and209 °C, respectively, and based on DTA analysis, these values were 209.27 °C, 209.89 °C, and 209.60 °C, respec- tively; both techniques yielded results in accordance with the F. Bras. V (2010) and DMF (Table I).

6. Assay

The pharmacopeial method was not reproducible because it presented differing results with variations above 5.0%; therefore, it was necessary to make changes in the method to achieve better results. These changes resulted in a good method with reproducible results, in accordance with the F. Bras. V (98% to 102%)

Table 6 Result for determination of melting point and Assay of pure drug

S.No.	Melting Point (°C)			Assay
	Sample	Method F. Bras. V	DTA	
1	ABZ01	208	209.27	99.74% ± 0.21%
2	ABZ02	208	209.89	100.01% ± 0.12%
3	ABZ03	209	209.6	99.89% ± 0.59%

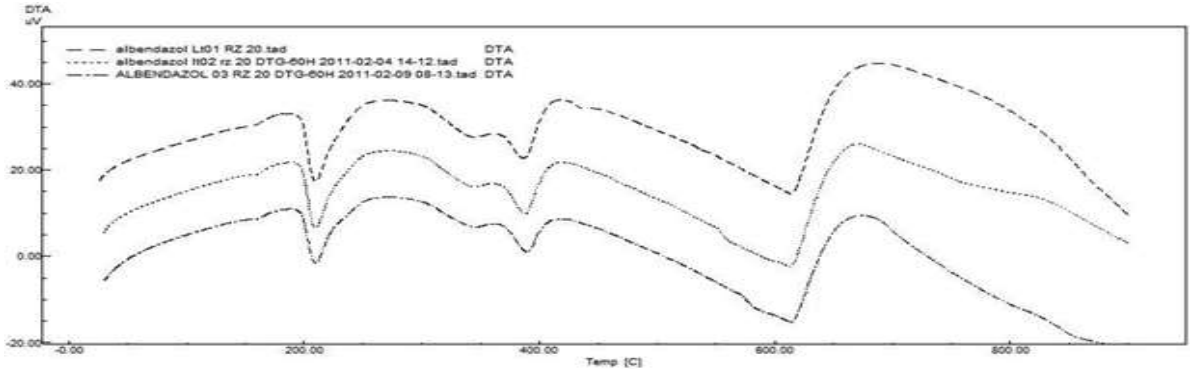

Fig. 3: Thermal profile of the 3 samples ABZ01, ABZ02, ABZ03 based on DTA curves

7. Solubility

The 3 samples ABZ01, ABZ02, and ABZ03 showed aqueous solubilities of 5.07, 4.27, and 4.52 µg/mL (classified as insoluble), and in 0.1 M HCl solution presented solubilities of 55.10, 56.90, and 6170 µg/mL, respectively (classified as very slightly soluble).

Table 7 Result for determination of Pka of pure drug

S.No.	Sample	Pka
1	ABZ01	3.5
2	ABZ02	3.8
3	ABZ03	3.6

8. Drug excipient incompatibility study

Table 8 Drug excipient incompatibility study

S. No.	Parameter	Initial	After 4 week	Observation
1	Albendazole (Pure Drug)	White	No change	No change
2	Drug + HPMC	White	No change	No change
3	Drug + Sodium alginate	White	No change	No change
4	Drug + Calcium carbonate	White	No change	No change
5	Drug + Sodium bicarbonate	White	No change	No change
6	Drug + Sodium citrate	White	No change	No change
7	Drug + Methyl paraben	White	No change	No change

agent. In addition sodium, bicarbonate was also included in the formulations as an additional gas generating agent to enhance floating behaviour of the In situ gelling system of albendazole.

Physicochemical properties

The formulated oral In situ gelling system of albendazolewas found to be off white in colour with characteristic odour and a bland taste.

Drug content

The percentage drug content for all formulation was determined and shown in table 3. The drug content was found to be in the range of 96-98% for all the formulations indicating a uniform distribution of the drug.

pH measurement

The measurement of pH is very important for oral preparations. Otherwise, it leads to irritation to the throat. All the formulations had a slightly alkaline pH. The pH of formulations was found in the range of 7.1- 7.8 as shown in table 4

Table 9: Evaluation parameters of the oral raft-forming in situ gelling system of ABZ

Formulations	pH	Drug Content %	In Vitro Gelation	Floating Lag Time * (min)	Total Floating Time (h)	Water Uptake at 2h*%	% Drug Release * (6 h)	% Drug Release * (12 h)
F1	7.2 ± 0.44	98.7 ± 0.52	+++	2.47 ± 0.4	>24	44 ± 0.76	73.1 ± 0.42	99.89 ± 0.27
F2	7.8 ± 0.24	99.3 ± 0.32	+++	3.12 ± 0.2	>12	6.8 ± 0.42	65.2 ± 0.53	87.54 ± 0.65
F3	7.8 ± 0.32	97.8 ± 0.41	+++	6.27 ± 0.2	>12	42 ± 0.71	58.3 ± 0.21	84.32 ± 0.14
F4	7.1 ± 0.21	97.4 ± 0.22	+++	30 ± 0.8	1	13 ± 0.18	70.2 ± 0.02	84.33 ± 0.17
F5	7.4 ± 0.52	98.8 ± 0.16	+++	42 ± 0.5	>12	17.3 ± 0.22	45.6 ± 0.62	74.31 ± 0.02
F6	7.3 ± 0.12	99.2 ± 0.51	+++	49 ± 0.4	>12	15.7 ± 0.32	30.2 ± 0.76	55.44 ± 0.87
F7	7.1 ± 0.52	97.8 ± 0.46	+++	74 ± 0.6	<3	10.82 ± 0.43	42.3 ± 0.29	69.91 ± 0.54
F8	7.2 ± 0.52	98.4 ± 0.31	+++	86 ± 0.2	<3	30.87 ± 0.21	34.2 ± 0.71	59.16 ± 0.22
F9	7.2 ± 0.52	97.7 ± 0.61	+++	89 ± 0.7	<3	7.2 ± 0.11	29.4 ± 0.63	50.73 ± 0.87

Mean±SD,*n*=3.

+++ Indicates good In vitro gelation capacity

Table 10: Evaluation parameters of the oral raft-forming in situ gelling system of ABZ

Formulations	Gel Strength(Sec)	Viscosity (Cps)	Swelling Index(%)
F1	20.32 ± 1.50	200	28.15 ± 1.50
F2	25.62 ± 2.45	240	43.50 ± 1.35
F3	31.45 ± 1.22	300	76.34 ± 2.25
F4	32.56 ± 1.56	380	36.60 ± 1.86
F5	40.34 ± 0.89	396	49.10 ± 1.36
F6	49.54 ± 2.21	420	67.12 ± 1.60
F7	55.20 ± 1.87	460	24.26 ± 1.49
F8	60.75 ± 1.35	480	50.12 ± 2.35
F9	72.35 ± 1.45	560	84.50 ± 2.39

In vitro gelation study

All the prepared formulations resulted in immediate gelation that was retained for an extended period. The systems that resulted in instantaneous gel formation upon exposure to biological fluids and body temperature are ideal in situ gelling systems. As the concentration of HPMC K4M increased, gelation was observed to be enhanced. Formulations with the lowest concentration of polymer resulted in weak gel formation, which may not be able to withstand peristaltic waves of the GIT. Hence, an optimum polymer concentration was required to get an ideal gelling system.

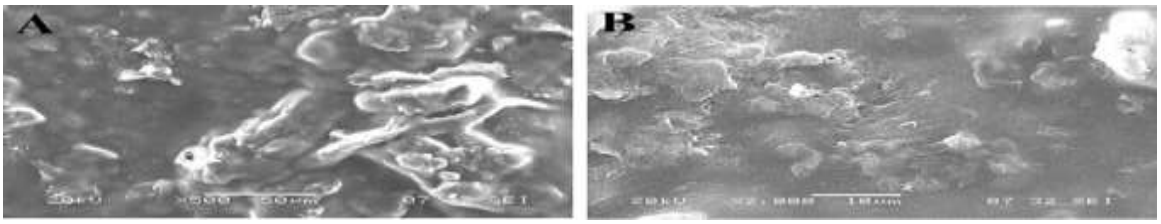

Fig. 4: Scanning electron micrographs of (A) ABZ (pure drug) at 500× magnification and (B) ABZ Gel at 2000× magnification

In vitro floating study

. In the present study, the previously optimized concentration of sodium bicarbonate (1%) was used along with sodium citrate (1%), which was used to maintain fluidity of the formulation prior to administration. Sodium alginate and HPMC K4M were used as gelling agents. All the developed formulations were shown to float on the surface of 0.1N HCL (simulated gastric

fluid), but formulations with a higher concentration of HPMC K4M (F4–F9) showed a floating lag time of more than 30 min and the formulations F1, F2, and F3 showed a floating lag time of 2.4, 3.1, and 6.2 min, respectively. The results of floating duration were different for all the formulations. Formulation F1 remained floating for more than 24 h, whereas F2, F3, F5, and F6 remained buoyant for less than 12 h. The floating duration of formulations F7 to F9 was less than 3 h, which may have been due to the higher concentration of HPMC K4M. F4 showed a floating duration of just 1 h, after which the gel formed was settled at the bottom. Hence, it can be concluded from the results of the floating study that formulations with a higher HPMC concentration are not be an ideal composition for an in situ gelling system. Among all the formulations, F1, F2, and F3 exhibited desirable floating on the surface of the medium.

Measurement of water uptake by the gel

The formulation exhibited water uptake which is observed in the range of 10-107% as shown in table 4. The release of the drug from the polymer matrix depends on the amount of water associated with the system. The release of the drug may involve the penetration of the water into the matrix and simultaneously release the drug via diffusion or dissolution. The water associated with the formulation at any point in the time can be determined by the simple test for all the formulation of sodium alginate based in- situ gel of diltiazem hydrochloride. From the study, it was concluded that formulation F5 Containing 250mg and F6 containing 300 mg of the sodium alginate resulted in 100% water uptake, in turn, a good release of the drug from the polymer.

In vitro drug release study

A combination of different polymer (HPMC K4M and sodium alginate) concentrations was used to sustain the drug release from the prepared in situ gel formulations. In vitro drug release profile studies were performed on all formulations (F1–F9), as shown in Figure 4. All prepared formulations showed a sustained drug release. F1 (% HPMC and sodium alginate 1:1) showed nearly 100% drug release, and F2, F3, and F4, showed nearly 85% drug release at 12 h. The in vitro drug release study revealed that as the polymer concentration increased there was a considerable decrease in the rate and extent of drug released from the formulation, which was due to an increase in the density of the polymer matrix as well as an increase in the diffusional path length of the drug molecules. F9 showed the least drug release, at 50.73% at 12 h; this was due to the high concentration of both polymers, which formed thick sol–gel formations that

retarded the drug release from the formulation. F5, F6, F7, and F8 showed 74.31%, 55.44%, 69.91%, and 59.16% drug release at 12 h, respectively.

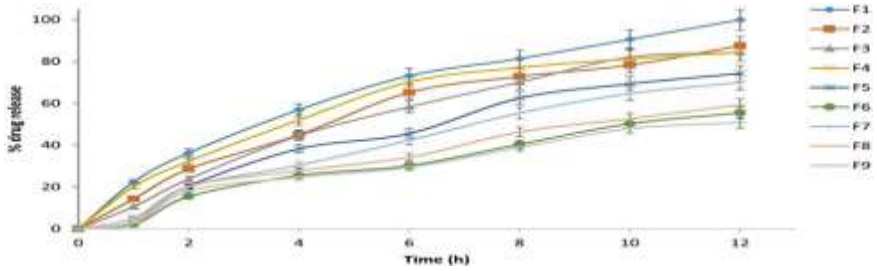

Fig. 5: In vitro drug release profile of the oral raft-forming in situ gelling systems of Albendazole viz. formulations (F1–F9).

Kinetics of Drug Release Studies

According to the regression coefficients, The formulations F3, F5, F6, F8, and F9 were found to have non-Fickian release, whereas F1, F2, F4, and F7 were found to have Fickian release. F2, F2, F4, F5, F7, and F8 were best suited to Peppas release, and F6 and F9 were found to be Hix Crow models, respectively. However, F1 showed zero-order release and F3 showed matrix release, as shown in Table 5. The optimized formulation F1 showed Fickian diffusion with zero-order drug release.

Table 11: Kinetic studies of the dissolution profile of NTB matrix tablets (values of R2, k, and n) and mechanism of drug release.

Formulation	Zero Order		Hix Crow		Higuchi Matrix		1st Order		Korsmeyer–Peppas			Mechanism of Release	Drug Release Kinetics
	R2	K	R2	K	R2	K	R2	K	R2	K	n		
F1	0.994	10.845	0.968	25.48	0.979	13.179	0.971	11.194	0.982	10.052	0.385	fickian	Zero order
F2	0.918	10.539	0.973	15.678	0.871	12.802	0.945	11.986	0.996	12.983	0.417	fickian	Peppas release
F3	0.974	6.132	0.835	10.605	0.997	19.769	0.994	16.897	0.987	18.529	0.564	Nonfickian	Higuchi matrix
F4	0.942	12.195	0.899	11.481	0.934	11.983	0.992	10.631	0.995	14.777	0.399	fickian	Peppas release
F5	0.891	10.523	0.971	10.771	0.909	16.911	0.919	12.752	0.979	11.65	0.672	Nonfickian	Peppas release
F6	0.917	15.225	0.993	17.232	0.781	17.668	0.984	11.981	0.843	21.811	0.685	Nonfickian	Hix Crow
F7	0.932	13.809	0.789	10.765	0.927	21.286	0.912	10.098	0.998	16.659	0.391	fickian	Peppas release
F8	0.874	17.22	0.985	13.723	0.801	10.096	0.951	13.231	0.992	10.526	0.772	Nonfickian	Peppas release
F9	0.909	10.448	0.987	11.791	0.923	11.776	0.926	11.875	0.975	15.433	0.566	Nonfickian	Hix Crow

Mean±SD,*n*=3.

Data Analysis and Optimization

The effect of independent variables such as the concentration of sodium alginate (A) and HPMC K4M (B) on responses such as floating lag time, percentage water uptake, and percentage drug release was analyzed using 3^2 factorial designs. When different concentrations of factors were loaded at three levels (high, medium, and low), nine different formulations were obtained from the software. The formulations and their responses are depicted in Table.

Table 12: Observed responses in 3^2 full factorial design for the oral raft-forming in situ gelling system of ABZ

Formulations	Variables		Responses			
	A (Sodium Alginate) g	B (HPMC K4M) g	Floating Lag Time * (min)	Water Uptake at 2 h * (%)	% Drug Release * (6 h)	% Drug Release * (12 h)
F1	1	1	2.47 ± 0.4	44 ± 0.76	73.1 ± 0.42	99.89 ± 0.27
F2	2	1	3.12 ± 0.2	6.8 ± 0.42	65.2 ± 0.53	87.54 ± 0.65
F3	3	1	6.27 ± 0.2	42 ± 0.71	58.3 ± 0.21	84.32 ± 0.14
F4	1	1.5	30 ± 0.8	13 ± 0.18	70.2 ± 0.02	84.33 ± 0.17
F5	2	1.5	42 ± 0.5	17.3 ± 0.22	45.6 ± 0.62	74.31 ± 0.02
F6	3	1.5	49 ± 0.4	15.7 ± 0.32	30.2 ± 0.76	55.44 ± 0.87
F7	1	2	74 ± 0.6	10.82 ± 0.43	42.3 ± 0.29	69.91 ± 0.54
F8	2	2	86 ± 0.2	30.87 ± 0.21	34.2 ± 0.71	59.16 ± 0.22
F9	3	2	89 ± 0.7	7.2 ± 0.11	29.4 ± 0.63	50.73 ± 0.87

*Mean±SD,*n*=3.*

The obtained results show that the independent variables had a significant impact on the dependent variables selected, such as floating lag time (ranging from 2.4 to 89 min), percentage water uptake (ranging from 7.2 ± 0.11 to 44 ± 0.76 %), and percentage drug release (ranging from 47.9 ± 0.63% to 90.5 ± 0.42%). For given levels of each independent variable, the equation in terms of coded factors can be utilized to made predictions about the response. The high levels of the factors are coded as +1 and the low levels of the factors are coded as −1 by default. By comparing the factor coefficients, the coded equation can be used to determine the relative impact of the components. A positive value in the factorial equation indicates a direct relationship with the independent variable. The particular response and a negative value denote inverse correlation between independent variables, and the response is depicted in Table.

Table 13: Multiple regression output for dependent variables, showing the intercept, relationship between the factor and variables, and p-value obtained from the software.

	Intercept	A[1]	A[2]	B[1]	B[2] R_2
Floating lag time	42.4289	−6.93889	1.27778	−38.4756	−2.09556 0.9924
***p*−value**		0.053	0.053	<0.0001	<00001
% Water uptake at 2 h	20.8444	1.75556	−2.5444	10.0889	−5.511 0.9111
***p*−value**		0.059	0.059	0.0517	0.0517
% Drug release at 6 h	49.82	−3.09	12.01	3.78	+6.68 0.8977
***p*−value**		0.0748	0.0748	0.0197	0.0197
% Drug release at 12 h	73.96	−3.10	10.75	4.35	+3.05 0.9312
p−value		0.0523	0.0523	0.0528	0.0528

Impact of Independent Variables on Floating Lag Time Response

Formulations F1, F4, and F7, with the same concentration of sodium alginate but different concentrations of HPMC K4M, showed a variation in floating lag time. It was observed that as the concentration of HPMC K4M increased, floating lag time also increased. The quantitative effect of the formulation factors on the dependent variables are represented in Equation (2) and also shown in Table 7. Factor A (sodium alginate) showed a positive effect on floating lag time, which means that as the concentration of sodium alginate increased, floating lag time also increased. However, factor B (HPMC K4M) showed a negative impact on floating lag time, indicating an inverse relationship between factor and response.. The ANOVA results for predicting floating lag time are shown in Table.

Floating lag time (min) = + 42.43 − 6.94 A[1] + 1.28 A[2] − 38.48 B[1] − 2.10 B[2]

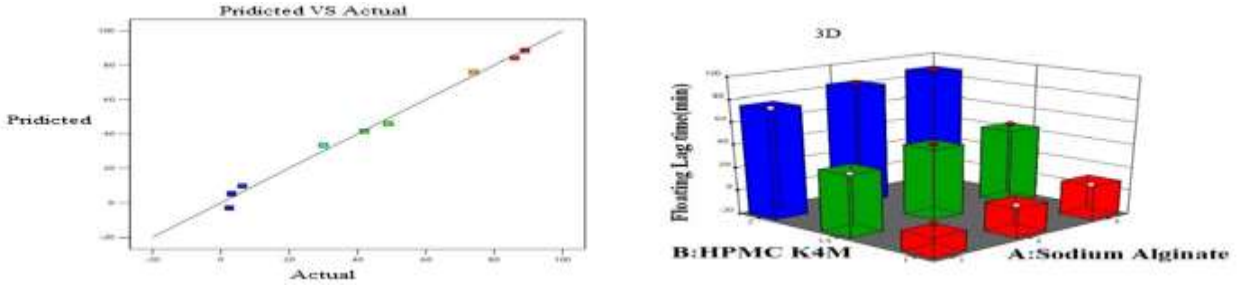

Fig. 6: Predicted and actual value plots and 3D response surface plots showing the effect of independent variables, i.e., factor A (sodium alginate) and factor B (HPMC K4M), on response floating lag time.

Impact of Independent Variables on Percentage Water Uptake Response

From the results obtained for percentage water uptake study, it was observed that all formulations behaved differently at a different time interval. At 60 min, the percentage water uptake for F1, F2, and F3 was 33, 23.5, and 16%, respectively, showing that % water uptake decreased with increasing sodium alginate concentration. At 120 min, the % water uptake by F1 increased to 44% and F3 increased to 42%, but for F2 there was no increase in % water uptake. When formulations F1, F4, and F7, which had had the same concentrations of sodium alginate and different concentrations of HMPMC K4M, were compared with each other, it was observed that as the amount of HPMC K4M increased from 1 to 2 g, the percentage water uptake decreased from 44% to 10% at 120 min. Also evident from the results is that formulations F3, F6, and F9 showed 42, 15, and 7.2% water uptake, respectively, at 120 min. The ANOVA results for predicting % water uptake are shown in Table 8. The effect of factors on % water uptake is represented by the fitted linear regression in Equation (3) given by the software.

$$\% \text{ Water uptake at 2 h} = + 20.84 + 1.76\ A[1] - 2.54\ A[2] + 10.09\ B[1] - 5.51\ B[2] \quad (3)$$

Table 14: Percentage water uptake study results of the oral raft-forming in situ gelling system of NTB

S.No.	Formulations	%Water Uptake		
		At 30 min	At 60 min	At 120 min
1	F1	7.5 ± 0.61	33 ± 0.49	44 ± 0.76
2	F2	15.6 ± 0.31	23.5 ± 0.28	6.8 ± 0.42
3	F3	16 ± 0.22	16 ± 0.12	42 ± 0.71
4	F4	7.4 ± 0.34	7.5 ± 0.18	13 ± 0.18
5	F5	0 ± 0.76	15.3 ± 0.08	17.3 ± 0.22
6	F6	4.5 ± 0.28	14.79 ± 0.32	15.7 ± 0.32
7	F7	0.57 ± 0.11	2.2 ± 0.63	10.82 ± 0.43
8	F8	5.26 ± 0.37	12.5 ± 0.42	30.87 ± 0.21
9	F9	13 ± 0.28	5.6 ± 0.28	7.2 ± 0.11

* Mean ± SD, n = 3.

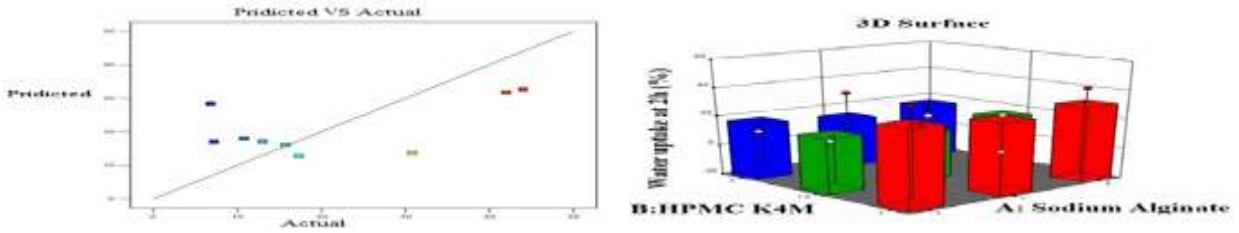

Fig. 7: Predicted and actual value plots and 3D response surface plots showing the effect of independent variables, i.e., factor A (sodium alginate) and factor B (HPMC K4M), on % water uptake response at 2 h.

Impact of independent variables on % drug release response at 6 h and at 12 h.

In the developed oral raft-forming in situ gel formulation, the gelling agents employed were sodium alginate and HPMC K4M. The gel formation takes place at an acidic pH; when the formulation is administered orally the sol-to-gel transition occurs, and due to the release of CO2 because of the presence of sodium bicarbonate in the formulation, it helps the gel formed by the polymer to float on the surface of the gastric medium, thereby preventing direct contact of the drug with the mucosal layer and leading to sustained release of the drug.. The predicted and actual value plots and 3D response surface plots showing the effect of independent variables, i.e., factor A (sodium alginate) and factor B (HPMC K4M), on percentage drug release at 6 h and 12 h are shown in Figure 8.

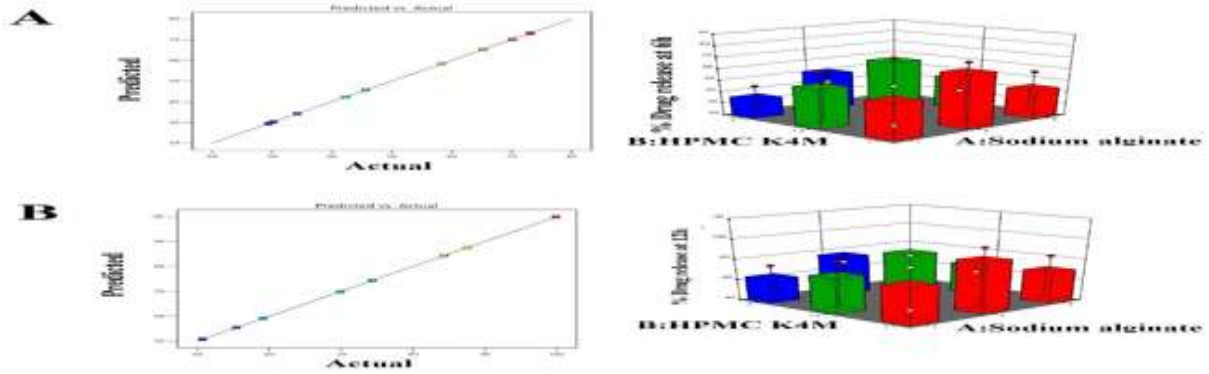

Fig. 8: Predicted and actual value plots and 3D response surface plots showing the effect of the independent variables, i.e., factor A (sodium alginate) and factor B (HPMC K4M), on response % drug release at 6 h (A) and % drug release at 12 h (B).

The ANOVA results for predicting % drug release at 6 h and 12 h are shown in Table 8. The fitted linear regression equation showing a significant effect on the % drug release response at 6 h and 12 h are shown below.

% Drug release at 6 h = + 49.823.09 A[1] + 12.01 A[2] + 3.78 B[1] + 6.68 B[2] (4)

% Drug release at 12 h = + 73.96 − 3.10 A[1] + 10.75 A[2] + 4.35 B[1] + 3.05 B[2] (5)

Optimization: The effect of various levels of independent variables on the responses can be analyzed by desirability and optimization approaches. Constraints were applied to the dependent variables to achieve an optimized formula by generating desirability plots, as shown in Figure 8.

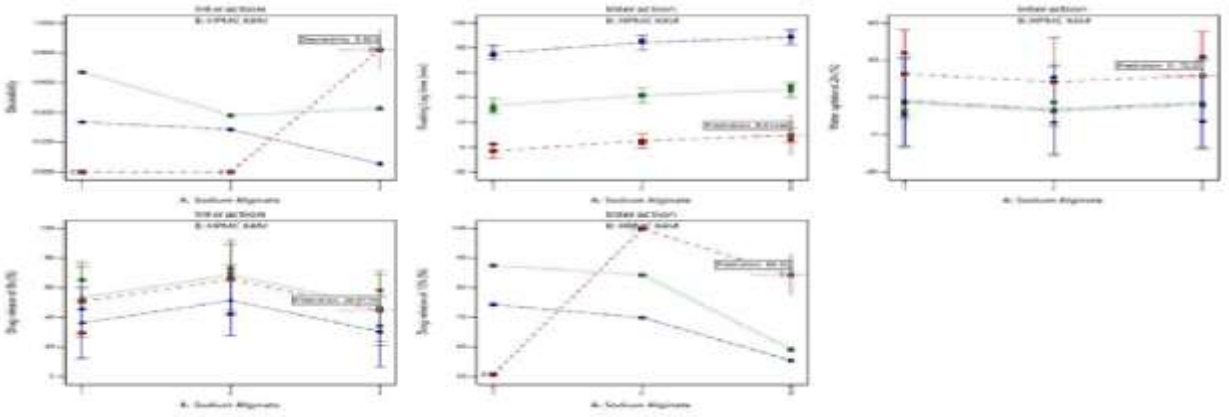

Fig. 9: Optimizationoftheoralraft-forminginsitugellingsystemof ABZ,representedby desirability plots and interactions.

Oral Gel (ABZ) plasma concentrations.

The parent drug ABZ, was detected in the plasma of both orall gel and oral solution treated groups. In the 12 h samples, ABZ levels were significantly($p<0.001$)higherafteroraladministration.TheAUC values of both groups were very low and did not show significantdifferences.TheT_{max}ofABZwassimilarinthe 2 groups, while the C_{max} of the oral group was higher than that of the i.p. group but not significantly.

Solution Oral (ABZ-SO) plasma concentration. The concentration of the major metabolite, ABZ-SO in plasma from the oral groupwas greater than that of the other. group (Figure 3). Oral administration resulted in significantly higher plasma concentration($p<0.05$)at12hand16h.ThemeanAUC inthe solutionoral .groupwasmuchlowerthanthatoftheoral group; the AUC of the SO group was 528.33 Ìg/ml/hwhereastheoralgroupwas1010.43Ìg/ml/h.TheC_{max}ofSOgroupwasat16.84Ìg/mlcompa redwith theoralgroupat41.86Ìg/ml,theoralgroup was more than 2-fold higher than the SO group. In the oral group, there was a trend for C-max being reached soonerthan in the SO group.

Fig. 10: Standard chromatogram of ABZ and metabolites using HPLC fluorescence detection: ABZ (16.1. min), ABZ-SO (4.05 min)

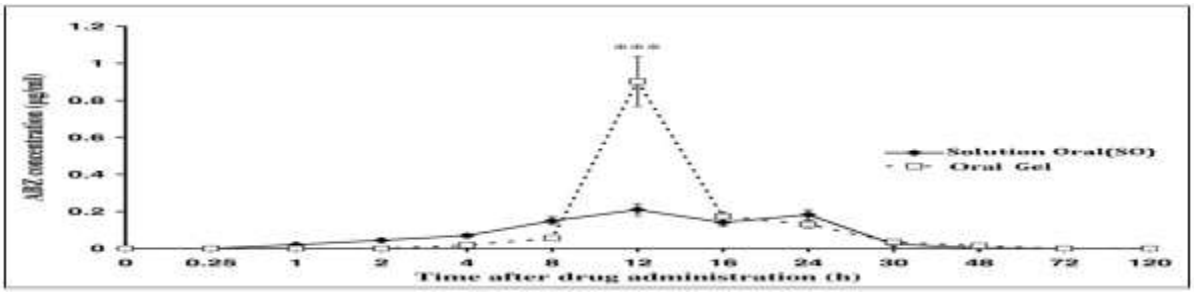

Fig. 11: Plasma ABZ concentrations following solution oral or oral administration of the drug. Each point (n=4) represents the mean±SE. At 12 h time point, ABZ plasma concentration was much higher in the orally administered group (*p<0.001 by Student's t-test).**

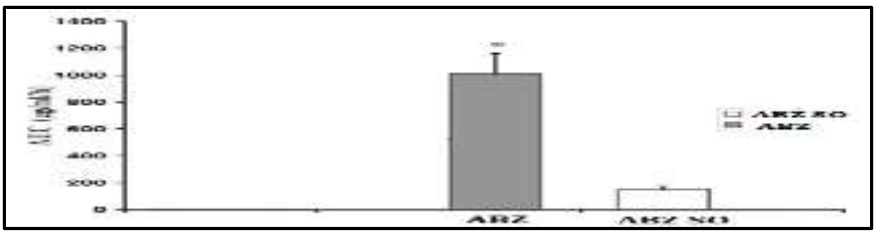

Fig. 12: AUC of ABZ, ABZ-SO orally administered groups (*p<0.05). There were significant differences in AUCs of ABZ or ABZ-SO in these groups.

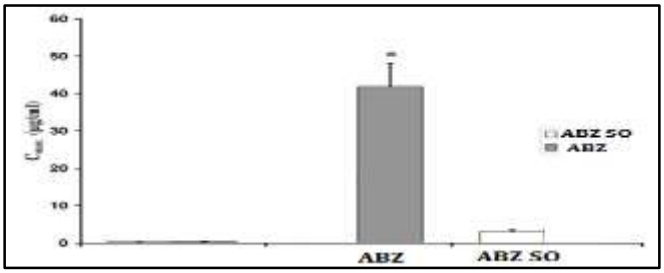

Fig. 13: Cmax of ABZ, ABZ-SO in oral administered groups,Cmax of ABZ was significantly higher in the oral group (*p<0.05).

Table 15: Stabilitydataofoptimizedformulation ABZ under long term stability conditions (30±2°C/65±5%RH)

S.No	Stability testing on	Long term stability (30±2°C/65±5%RH)		
		0 month	3months	6months
1	Physical appearance	No colour change	No colour change	No colour change
2	% Drug Content	99.94±0.21	99.37±0.18	99.15±0.03
3	Floating time in hrs	12±0.01	12±0.23	12±0.33
4	Viscosity in cps	443±0.10	442±0.15	441±0.20
5	Gel strength in N/m^2	10.82±0.12	10.78±0.15	10.73±0.17

Note: ± SD (n=3)

Table 16: StabilitydataofoptimizedformulationABZ Accelerated stability conditions (40±2°C/75±5% RH)

		Accelerated stability (40±2°C/75±5% RH)		
S.No	Stability testing on	0 month	3months	6months
1	Physical appearance	No colour change	No colour change	No colour change
2	% Drug Content	99.94±0.21	99.14±0.17	99.03±0.35
3	Floating time in hrs	12±0.01	12±0.37	12±0.51
4	Viscosity in cps	443±0.10	441±0.28	440±0.36
5	Gel strength in N/m^2	10.82±0.12	10.75±0.23	10.68±0.28

Note: Mean ± SD (n=3)

Table 17: % DrugreleaseofABZ formulationinlongtermand accelerated stability conditions for 0, 3 and 6 months of time interval

	% Drug release of ABZ (long term stability)			% Drug release of ABZ (accelerated stability)		
Time (h)	(30±2°C/65±5% RH)			(40±2°C/75±5% RH)		
	0 months	3 months	6 months	0 months	3 months	6 months
1	25.85±0.01	25.74±0.11	25.71±0.21	25.85±0.01	25.70±0.31	25.69±0.12
2	36.19±0.06	36.17±0.06	36.13±0.06	36.19±0.06	36.11±0.04	36.20±0.06
3	43.18±0.15	43.14±0.14	43.11±0.16	43.18±0.15	43.10±0.13	43.19±0.15
4	50.75±0.17	50.65±0.11	50.64±0.18	50.75±0.17	50.62±0.17	50.68±0.17
5	58.26±0.04	58.24±0.24	58.23±0.03	58.26±0.04	58.21±0.02	58.29±0.04
6	67.12±0.07	67.10±0.57	67.09±0.17	67.12±0.07	67.06±0.15	67.05±0.07
7	73.26±0.09	73.22±0.29	73.21±0.28	73.26±0.09	73.20±0.09	73.19±0.09
8	80.12±0.02	80.11±0.32	80.10±0.12	80.12±0.02	80.09±0.02	80.07±0.02
9	85.26±0.03	85.25±0.02	85.22±0.01	85.26±0.03	85.21±0.03	85.20±0.03
10	93.56±0.01	93.53±0.31	93.52±0.11	93.56±0.01	93.51±0.01	93.50±0.01
11	97.62±0.10	97.61±0.16	97.60±0.13	97.62±0.10	97.61±0.10	97.60±0.10
12	99.85±0.09	99.83±0.28	99.82±0.12	99.85±0.09	99.81±0.09	99.80±0.09

All the formulation values ±SD (n =6)

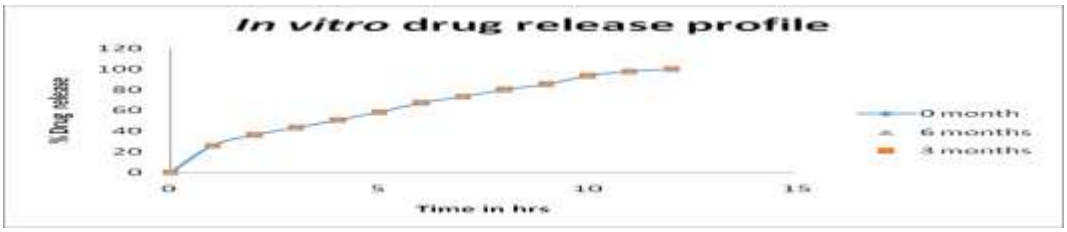

Fig. 17 % Drug release of ABZ (long term stability)

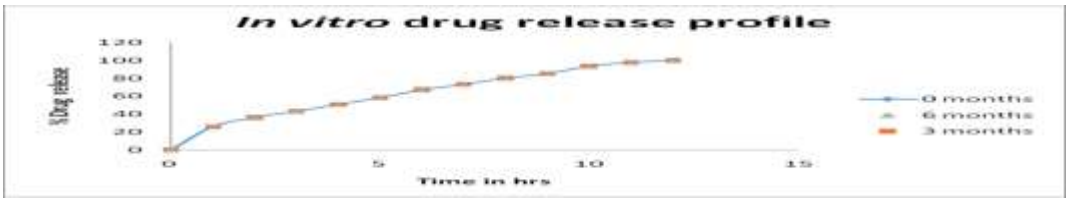

Fig. 14: % Drug release of ABZ (accelerated stability)

Conclusion

The oral raft-forming in situ gelling system of antuihelmentic drug ABZ was successfully developed using 3^2 factorial designs. The developed in situ gel formulations exhibited a pH value near 7. The in vitro gelation of an in situ gel formulation showed immediate gelation, and the gel was retained for an extended period of time. From the obtained results of the three-level (3^2) factorial design analyzing the impact of two independent variables viz. sodium alginate [A] and HPMC K4M [B], it was evident that both the selected factors had a significant effect on the chosen responses, such as floating lag time, water uptake (%), and percentage release of drug, supporting the precision of the design employed for optimization. Thus, the developed oral raft-forming in situ gelling system of ABZ may be a favorable and alternative strategy to enhance gastric retention and sustained release of the drug by letting it remain floating in the stomach, thereby augmenting the therapeutic efficacy of ABZ. The optimized formulation was subjected to different temperature and relative humidity conditions as specified by the ICH guidelines. For long term stability studies, the formulation was stored at 30±2°C with a relative humidity of 65±5%. For accelerated stability studies, the storage conditions were set at 40±2°C with a relative humidity of 75±5%. Throughout the stability period, the optimized formulation was monitored for any significant changes in physical appearance, drug content, drug release percentage, viscosity, in vitro gel response, and in vitro drug release at different time intervals (0

month, 3 months, and 6 months). The stability data revealed that there were no notable changes observed in any of these parameters, indicating that the optimized formulation remained stable under the specified stability storage conditions as per the ICH Guidelines.

References

1. Hani U, Rahamathulla M, Osmani RA, Begum MY, Wahab S, Ghazwani M, Fatease AA, Alamri AH, Gowda DV, Alqahtani A. Development and characterization of oral raft forming in situ gelling system of neratinib anticancer drug using 32 factorial design. Polymers. 2022 Jun 21;14(13):2520.
2. Peppas N, Langer R. New challenges in biomaterials. Science 1994;263:1715-20.
3. Pande SD, Vaidya KP, Gulhane KP. Floating drug delivery system (fdds): a new way for oral drug delivery system. Int J PharmClinSci 2013;3:1-13.
4. Nidhi D. In-situ gel: a novel approach of gastro retentive drug delivery. Asian J PharmSci Res 2013;3:1-14.
5. Plourde F, Motulsky A, Couffin-Hoarau AC, Hoarau D, Ong H, Lerour JC. The first report on the efficacy of l-alanine based In- situ-forming implants foe the long-term parenteral delivery of drugs. J Controlled Release 2005;108:433-41.
6. Khan AD, Meenakshi B. Floating drug delivery system: an overview. Int J PharmTech Res 2010;2:2497-505.
7. Miyazaki S, Endo K, Kawasaki N, Kubo W, Watanale H, AttwoodD. Oral sustained delivery of paracetamol from in situ gelling xyloglucan formulations. Drug Dev IndPharm 2003;29:113-9.
8. Modi SA, Gaikwad PD, Bankar VH, Pawar SP. Sustained release drug delivery system: a review. Int J Pharm Res Dev 2011;2:147-60.
9. MoinAfrasim, Shiva kumar HG. Formulation of sustained release Diltiazem matrix tablet using hydrophilic gum blends. Top J Pharm Res 2010;9:283-91.
10. NayakAmitkumar, MajiRuma, Das Biswarup. Gastroretentive drug delivery system: a review. Asian J PharmClin Res 2010;3:1-10.
11. Madan M, Bajaj A, Lewis S, Udapa N, Baig JA. In-situ forming polymeric drug delivery. Indian J PharmSci 2009;71:242-51.

12. Thomas LM. Formulation and evaluation of floating oral in-situgel of metronidazole. Int J PharmPharmSci 2014;6:265-9.
13. Hasan MJ, Kamal BA. Formulation and evaluation of ranitidine hydrochloride are floating In situ gel. Int J PharmPharmSci 2014;6(Suppl 2):401-5.
14. Shivaraju, Parthiban S, Senthilkumar SK. Preparation and evaluation of ornidazole in situ gelling system for gastro retentive drug delivery. Int J Pharm 2013;3:62-9.
15. Al-Mamun MA, Rahman MR, Biswas S, Kundu SK, Rayhan J. Formulation and bioequivalence evaluation of an extended release solid drug delivery system for metronidazole using usingeudragit nm30d and methocel premium K4m as retardant material. Am J Adv Drug Delivery 2014;2:39-51.
16. Patel DM, Patel CN. Formulation and evaluation of floating oralin-situ gelling system of amoxicillin. ISRN Pharm 2011;1:1-8.
17. Patel JK, Chavda JR, Modasiya Mk. Floating in-situ gel based on alginate as a carrier for stomach specific drug delivery of famotidine. Int J PharmSci Nanotech 2010;3:1092-104.
18. Choi BY, Park HJ, Hwang SJ, Park JB. Preparation of alginate boads for floating drug delivery system: effects of CO2 gas- forming agents. Int J Pharm 2002;239:81-91.
19. Google J, Vandrbist F, Amighi K. Development and evaluation of new multiple-unit levodopa sustained–release floating dosage forms. Int J Pharm 2007;334:35-41.
20. Rajinikanth PS, Mishra B. Floating in-situ gelling system for stomach site-specific delivery of clarithromycin to eradicate Helicobacter pyroli. J Controlled Release 2008;125:33-41.
21. Cai ZY, Galettis P, Lu Y, Morris DL, Pourgholami MH. Pharmacokinetics of albendazole in New Zealand white rabbits: oral versus intraperitoneal administration. Anticancer research. 2007 Jan 1;27(1A):417-22.