

Optimization, In-Vitro and In-Vivo Evaluation of Floating Tablet of Tetrabenazine

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Abstract

The present studies discuss about the optimization and in-vitro and in-vivo evaluation of floating tablet of tetrabenazine. For designing a dosage form with consistent and controlled residence in the stomach, an important strategic consideration is selection of suitable excipient. Floating properties of dosage forms is anticipated to be improved by using high molecular weight and less hydrophilic polymers. Non-effervescent systems can be developed by using number of polymers including swellable and gel forming cellulose derivative hydrocolloids as well as matrix formers (e.g. hydroxypropyl methylcellulose, hydroxyethyl cellulose, and Ethyl Cellulose). For creation of a suitable dosage form excipients and APIs are mixed to form powder blends followed by compressing in to solid oral (tablet) dosage forms.

Key words: Optimization, Floating tablet, *in-vitro* evaluation, *in-vivo* evaluation.

Introduction

In the course of drug release the outer layer of the formulation gets hydrated and swell as it come in contact with the gastric fluid due to diffusion of the aqueous medium inside the delivery system from gastric region. The average density of the preparation decreases as the air entraps in the gel forming gelatinous layer. A relative integrity of shape and bulk density less than that of the medium can be maintained by the structure. The diffusion of medium into the preparation decreases as the thickness of the swollen layer increases (Ju et al., 1995). During the process, active ingredient from one layer to another is dissolved and is released via diffusion controlled mechanism. Thus, coherent gel structure is created which acts as reservoir for the sustained release of the API (Sheth et al., 1984). The first hydrated swollen layer gets dissolved during hydration then further layers get hydrated after its detachment. This technology is the key of Hydrodynamically Balanced Systems (HBS™) and several commercially available products work on the basis of this system. Technologically non-effervescent floating dosage forms are of following types: microporous systems, alginate beads and hollow tablets.

During development of multiple-unit floating system alginate beads and hollow tablets plays significant role. Microballoons another terminology of tablets are prepared by dissolving suitable polymer(s) in organic phase in which drug is either dispersed or dissolved followed by emulsification in aqueous solution containing surfactant(s). Organic solvent is removed by evaporation and an emulsion of O/W type is prepared. A cavity is formed by precipitation of polymer onto the surface of remaining drops resulting in formation of hollow spheres.

Among several polymers used for formulation of FDDS, ethylcellulose (EC) and hydroxypropyl methylcellulose (HPMC) have number of applications. Floating tablets using EC and HPMC recently have received much attention (Mastiholmath et al., 2008; Vaghani et al., 2010) for delivering drug in controlled manner in the stomach. EC is non polar dissolves in several organic solvents, biocompatible, non-toxic, non-biodegradable and non-irritant cellulose derivative (Das et al., 2006). EC can clearly slow down the rate of drug release rate, and is especially effective for water-soluble drugs.

Evaluation Parameter

Preparation of standard calibration curve of Tetrabenazine

10 mg of drug was dissolved in 0.1 N HCl solution and final volume was made up to 100 ml in 100 ml volumetric flask. The stock solution concentration was 100 mcg/ml obtained. It was diluted with 0.1 N HCl solution to obtain solutions of different concentrations range from 10 to 100 µg/ml. Absorbance of each solution was measured at 228 nm by using Shimadzu UV-1601 UV/Vis double beam spectrophotometer and 0.1 N HCl solution was used as reference standard.

Drug excipient compatibility study

The FTIR 8400S model instrument was used for the compatibility study of drugs with other excipients which were used in formulation of tablets.

Pre Compression parameter

Angle of repose

Funnel method was more suitable for determination of angle of repose. In the funnel accurately weight amount of powder blend was taken. Funnel height was adjusted in a manner where the funnel tip just touched to the apex of the powder blend. The powder blend was flow by the funnel freely, on to the surface. The powder cone diameter was measured, then by using the following equations calculated the angle of repose.

$$(\tan \theta = h/r)$$

* h was height and r was radius of the powder cone.

Bulk density

The loose bulk density, (LBD) and tapped bulk density, (TBD) were determined. 2 gm of powder blend from each category, which were previously shaken for breaking agglomerates if formed, then it was poured in to calibrated measuring cylinder (10 ml), and noted down the initial volume and the cylinder was allowed to tapped on hard surface about the height of 2.5 cm at second interval. There no further change in volume noted then discontinued the tapping. By using the following equations loose bulk density, (LBD) and tapped bulk density (TBD) were determined:

LBD= Total Weight of the powder blend/Total Untapped Volume of the packing
TBD= Total Weight of the powder blend/ Total Tapped Volume of the packing.

Compressibility Index

Compressibility Index of the powder blend was determined of by using Carr's compressibility index. It is an easy method for evaluate Compressibility Index by LBD and TBD. The equation for calculation of Carr's Index is given as:

$$\text{Carr's Index (\%)} = [(TBD-LBD) \times 100] / TBD$$

Total Porosity

It was determined as total porosity in behalf of measured the volume which was occupied by a true volume of the powder blend and selected weight of powders (V_{bulk})

$$\text{Porosity (\%)} = \frac{V_{bulk} - V}{V_{bulk}} \times 100$$

Post Compress Parameter

Weight variation test

Weight variation study of formulated tablets from sample of 20 tablets was weighed by using electronic balance and the official method was used for the test.

Hardness

Pfizer hardness tester was used for determination of hardness. Five tablets were used from different formulation and the average values were calculated.

Thickness

Vernier caliper was used for determination of thickness of the tables from all formulations, and average values were calculated.

Drug Content

An accurately weight amount of Tetrabenazine powder blend (100 mg) was extracted with 0.1 N HCl solution and the solution was filter through 0.45 μ m membrane. The absorbance was measured at 228 nm after suitable dilution using a Shimadzu UV-1601 UV/Vis double beam spectrophotometer.

Buoyancy studies of batch F-13 (In-vitro)

According to FLT method given by Rosa et al.²⁰ in-vitro buoyancy was determined. 0.1 N HCl solution was taken in beaker and placed the tablet in it. The results of in vitro buoyancy study of the best batch was shown in study, which clearly indicated the floating lag time, stable and persistent buoyancy and swelling characteristic of tablet.

Swelling index (SI):

0.1 N HCl solution having pH 1.2 was used to determination of the swelling index of tablets at room temperature. Weight of the tablets after the swollen was determined at specific time intervals.

The given equation was used for calculation of the swelling index.

Swelling index $W_t - W_0 / W_0$

Where W_0 = initial weight of tablet,

W_t = weight of the tablet in t (time)

Dissolution studies (In-Vitro)

According to United States Pharmacopoeia (USP) XXIV, dissolution testing apparatus-II (paddle type) was used for dissolution study of Tetrabenazine for floating tablet. The Test was performed by use of 0.1 N HCl solution (900ml) at temperature $37 \pm 0.5^{\circ}\text{C}$ and speed was set at 75 rpm.⁹ Sample was withdrawn (quantity 10 ml) at specific time interval, and 10 ml of 0.1 N HCl solution was added to dissolution medium. Drug samples were filtered through 0.45 μ membrane filter and dissolution medium was used for dilution purpose. Shimadzu UV-1601 UV/Vis double beam spectrophotometer was used for measurement of the absorbance at 228 nm and Cumulative % release of drug was calculated by help of the equation, which was obtained from standard Calibration Curve.

Drug Content (Assay)

Ten tablets were finely powdered; quantities of the powder equivalent to 50 Mg of Tetrabenazine were accurately weighted and transferred to a volumetric flask then 0,1 N HCL (PH 1,2 Buffer) solutions in flask were used with and mixed and volume was make upto 100 ml of the resulting solution to 100 ml with 0.1 N HCl and absorbance of the resulting solution were measured at 228 nm using UV- -visible spectrophotometer. The linearity equation obtained from calibration curve was used for estimation of Tetrabenazine in the tablet formulations.

In vivo study

Barium sulphate was used as x ray opaque substance for optimized formulation. For initial formulation trials drug was replaced with barium sulphate. Relative high density, aqueous insolubility and poor buoyancy properties were the limiting factors to achieve in vitro buoyancy of the prepared formulation. Hence this formulation was further selected for in vivo evaluation.

The study was carried out by administering the prepared optimized gastro retentive tablets to rabbit. The tablet was administered in the fasting state. The x ray opaque formulation was administered along with 25 ml of water. Position of subject was in sitting or up-right position. A light meal was given to rabbit, 2 hour after administration of the tablet, to evaluate effect of food of gastro retentive property. The position of tablet was monitored by X-Ray screening technique. X-Ray photographs were taken after 6 hours and 12 hours to monitor tablet position in human gastrointestinal tract. The X-Ray photographs was observed for buoyancy time/retention time of prepared tablet in stomach after specific time.

RESULTS AND DISCUSSION

Estimation of Tetrabenazine

0.1 N HCl solution was used for preparation of Tetrabenazine solution and absorption were measure by Shimadzu UV-1601 UV/Vis double beam spectrophotometer, then λ_{max} of Tetrabenazine was found at 228 nm.. The result was showed in the table no. 1 and figure no. 1:

Table 1: Standard calibration curve of Tetrabenazine in 0.1 N HCl Solution

Sr.	Concentration	Absorbance			Average	Calculated
No.	(g/ml)	1	2	3	Absorbance	Absorbance
1	0	0	0	0	0	0.002
2	10	0.042	0.041	0.042	0.042	0.038

3	20	0.078	0.079	0.079	0.079	0.074
4	40	0.149	0.149	0.150	0.149	0.148
5	60	0.212	0.214	0.215	0.214	0.216
6	80	0.268	0.269	0.268	0.268	0.270
7	100	0.365	0.366	0.366	0.366	0.370

Correlation Coefficient: 0.9984

Absorbance= 0.0038x conc. + 0.0021

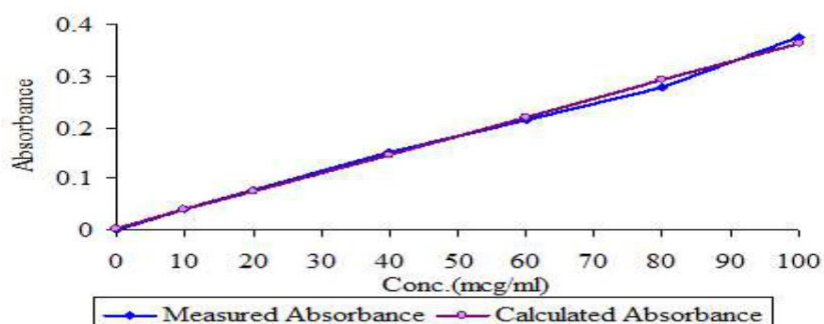


Fig. 1: Standard calibration curve of drug in 0.1 N HCl Solution.

Table 2: Comparison of natural and synthetic polymers

Ingredients	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9	B-10
Tetrabenazine	50	50	50	50	50	50	50	50	50	50
Microcrystalline Cellulose	30	30	30	30	30	30	30	30	30	30
HPMCK4M	110	-	-	-	-	-	-	-	80	-
HPMC K 15 M	-	110	-	-	-	-	-	-	-	80
HPMC K 100 M	-	-	110	-	-	-	-	-	-	-
Ethocel	-	-	-	110	-	-	-	-	30	30
Sodium alginate	-	-	-	-	110	-	-	-	-	-
Sesbania gum	-	-	-	-	-	110	-	-	-	-
Guar gum	-	-	-	-	-	-	110	-	-	-
Gum Acacia	-	-	-	-	-	-	-	110	-	-
Magnesium Stearate	5	5	5	5	5	5	5	5	5	5
Talc	5	5	5	5	5	5	5	5	5	5

Comparison of different sustained release floating matrix tablets (floating drug delivery system) formulated using natural and synthetic polymers: Floating matrix tablets were prepared using natural & synthetic polymers as above mentioned. Comparison of different polymers (natural and synthetic) was done as matrix forming polymer for formulating a sustained release matrix floating drug delivery system. Then effect of different matrix forming polymers on drug release was determined by dissolution (paddle) method.

Table 3: Determination of effect of different matrix forming polymers on drug release by paddle method

Time (hr)	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	B-9	B-10
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	2.91	3.18	2.51	6.65	5.12	4.15	4.87	5.16	3.85	4.52
2	5.23	6.19	5.26	8.26	7.11	6.72	6.98	7.13	5.55	7.55
3	9.69	10.22	9.56	12.15	9.56	9.24	10.21	10.85	10.11	12.47
4	15.02	16.57	14.26	17.38	13.04	13.14	15.32	16.04	16.48	19.88
5	21.12	23.26	19.98	20.26	18.01	17.01	20.09	21.93	21.88	27.69
6	26.32	28.54	24.52	25.53	26.59	21.14	24.95	26.32	28.92	35.96
7	31.99	33.36	29.89	30.88	29.31	27.19	29.87	31.97	33.32	41.45
8	33.94	38.81	32.06	34.04	33.33	33.72	34.86	36.56	36.98	48.27
9	39.99	41.21	37.88	39.16	38.79	38.55	39.78	42.61	42.51	58.62
10	44.89	46.69	41.50	43.78	42.45	44.13	45.72	47.91	48.74	66.74
11	50.71	53.17	48.71	50.52	48.92	49.28	51.09	53.31	53.21	78.24
12	56.22	59.95	53.13	55.59	54.77	55.11	56.84	58.79	59.66	87.21
13	61.12	64.27	59.03	61.99	59.91	60.74	61.42	64.04	65.34	92.35
14	69.99	72.13	67.65	68.22	65.04	66.33	67.56	69.46	71.18	98.64
15	76.89	78.54	74.12	76.02	71.52	73.35	75.02	78.02	78.85	99.28
16	82.91	84.15	80.11	83.65	80.01	80.89	81.68	85.21	83.64	99.35

By comparing in vitro dissolution drug release studies it was found that maximum drug 99.35% was released by B-10 formulation in 16 hrs, however more than 85% drug release happens in more than 12 hrs.

Final formulation was prepared from different composition of drug polymers as mentioned in experimental work section.

Evaluation Parameters

Pre compression parameters

The prepared blend of the formulations was evaluated for the parameters like angle of repose, bulk density, tap density, compressibility index and hausner's ratio. After addition of glidants, angle of repose was improved.

- Hausner's ratio was found to be 1.2 (or) less than 1.2.

- Carr's index was found to be in the range of 12 – 16.

For uniform tablet compression, good flow property is the major criteria. All these values indicated that the powder have good flow property.

***Values mentioned are average of 3 determinations**

Table 4: Preformulation study data of the pure drug

Table 6: Post Compression Parameter

S. No.	Parameters	Values obtained		
1.	Angle of repose	21°50'± 0.1216		
2.	Bulk density (gm/ml)	0.448± 0.007		
Formulation code	Weight Variation (mg)±S.D	Hardness (Kg/cm)±S.D	Friability (%)±S.D	Thickness (mm) ±S.D
	Tap density (gm/ml)	Hausner's ratio	Carr's index	
B1	200±1.10	5.2±0.218	0.31±0.06	2.88±0.011
B2	200±1.52	5.4±0.225	0.33±0.04	2.45±0.013
B3	201± 1.08	6.1±0.363	0.36± 0.08	2.37±.012
B4	201±1.32	5.9±0.106	0.38± 0.09	2.65±0.018
B5	200±1.48	5.5±0.167	0.32± 0.03	2.11±0.013
B6	200±1.56	6.2±0.412	0.37± 0.07	2.54±0.016
B7	200±1.22	5.7±0.332	0.36± 0.11	2.44±0.012
B8	202± 1.07	5.9±0.423	0.28± 0.13	2.75±0.075
B9	201±1.23	5.6±0.154	0.29± 0.06	2.45±0.19
B10	201±1.03	5.1±0.213	0.35± 0.15	2.68±0.017

Table 5: Physical Characteristics of powder blend

Formulation code	Angle of Repose (o)	Bulk Density (gm/ml)	Tap Density (gm/ml)	Hausner's Ratio	Carr's Index (%)
B1	20°09±0.4580	0.351±0.014	0.410± 0.003	1.17±0.002	14.41±0.603
B2	21°13±0.4670	0.356±0.012	0.420± 0.004	1.18±0.001	15.20±0.520
B3	20°22±0.2654	0.361±0.015	0.434± 0.005	1.18±0.002	15.35±0.109
B4	20°36±0.3564	0.371±0.018	0.421± 0.001	1.19±0.003	16.69±0.211
B5	21°09±0.8547	0.358±0.019	0.442± 0.004	1.17±0.003	15.80±0.309
B6	20°18±0.5226	0.353±0.011	0.424± 0.002	1.19±0.004	14.78±0.408
B7	20°54±0.6548	0.364±0.014	0.438± 0.006	1.20±0.001	15.92±0.554
B8	22°16±0.5547	0.376±0.016	0.415± 0.002	1.18±0.001	16.55±0.612
B9	20°26±0.6321	0.366± 0.012	0.428± 0.005	1.19±0.001	14.69±0.469
B10	20°28±0.4568	0.374±0.017	0.446± 0.002	1.18±0.002	15.23±0.532

IN-VIVO STUDIES

The effectiveness of any formulation prepared can be justified by performing its *in-vivo* evaluation. Thus, the results of *in-vitro* performance can be validated by conducting the experiments in living organism usually animals including human beings, microorganisms, cells or biological molecules. Results of *in-vitro* experiments cannot usually be transposed to predict the reaction of an entire organism *in-vivo*. Constitution of a reliable extrapolation method from *in-vitro* results to *in-vivo* is at most important. Two approaches are useful to solve certain problems:

Floating dosage forms can be evaluated *in-vivo* by number of techniques. The *in-vivo* drug release, *in-vivo* buoyancy, pharmacokinetic and pharmacodynamic profile of floating systems can be generated using rat, rabbit, dog or human volunteers. Floating dosage forms required to validate for proper *in-vivo* buoyancy study to ascertain the proper release of drug. Release of drug from floating system in controlled manner will also display its pharmacological response for longer period of time.

However radiation exposure during imaging is harmful to human beings whereas, it is of less concern for laboratory rats. Therefore radiology method was selected to visualize the fate of tablets through the GI tract of the rat. Based on *in-vitro* studies among all the formulations B10 (HPMC: EC) has shown best and satisfactory results. Hence, this formulation was selected for *in-vivo* evaluations such as *in-vivo* floating behavior, followed by bioavailability study.

Experimental

In-vivo floating behaviour Tablet

Preparation of radiopaque Tablet

Even though there are number of studies reported, radiological technique given by Tanwar et al., with modification was used for the determination of anatomical location of the selected formulation in the gastrointestinal tract. X-ray contrast material barium sulphate (BaSO_4) is used for localization and detection of floating tablets. Concentration of BaSO_4 was varied from 1 – 5% w/v and on the basis of floating behaviour optimum concentration was selected. Radiopaque placebo floating tablets (RB10) were prepared by incorporating 3% barium sulphate in polymeric solution using the optimised process parameters.

B. Procurement of animals and *in-vivo* floating behaviour experimental procedure

Healthy albino rats, weighing 150-200 gm were used for the investigation. Rats were placed individually in polypropylene cages where a standard condition (12-h light and 12-h dark cycle; 25-30°C) was maintained. For about 12 hours rats were fasted and initially exposed to X-ray to check the absence of any radio opaque substance in the stomach. During the study animals were not allowed to ingest food but sufficient quantity of water was provided. After ingestion of radiopaque tablets, rats were exposed to X-ray photography (Siregraph-B, Siemens, Karlsruhe, Germany) in the abdominal part at different intervals of 0, 2, 4, 6, 8 and 12 hours and observed for the nature and position of the formulation up to 12 hours.

Results and discussion

In-vivo floating behaviour

Radiopaque placebo floating tablets RB10 (HPMC : EC) were successfully prepared by the same method as described in experimental section using barium sulphate. For *in-vivo* radiographical contrast a sufficient quantity of barium sulphate is required to be encapsulated in tablets. But at the same time, a higher quantity of barium sulphate affects the floatability of tablets. Therefore optimized concentration of 3 % was used to attain the same. The prepared radiopaque tablets were of particle size $219 \pm 3.1 \mu\text{m}$ ($n=3$) which is almost similar to size of tablets of normal B10 formulation $208.27 \pm 2.7 \mu\text{m}$ ($n=3$). The radiopaque tablets were found to float over selected medium, which indicates that their density is <1 , although some particles settled down due to high density than normal B10 formulation.

The characterized radiopaque tablets were administered to rat by intragastric tube with sufficient quantity of water. Since gastrointestinal motility under fasting conditions has characteristic housekeeping waves, which occurs after every 1.5-2 hours approximately and may sweep away undigested substances from the stomach independent of its shape, size and density. Therefore working of floating device will be better when sufficient quantity of water is present in the stomach to avoid the effect of such waves. Thus, administration of formulation is done with approximately 100 ml of water. Series of X-ray photographs of radiopaque tablets in the stomach of rat are shown in Fig. 3. Image at 0 hour clearly indicates absence of any material before dosing of radiopaque tablets. At the initial stage, within 4 hours after taking dose, in the upper part of the stomach, clouds of particles were observed (Figs. 3 b, c) which indicates uniform distribution of formulation over the fluid.

At later stage 8 hours after dosing, contents of the stomach surface was very much roughened owing to peristaltic movement as compared to initial condition, but still some tablets remained in such condition. Few tablets in the form of clouds were seen in upper portion of stomach, even in the presence of peristaltic waves (Fig. 3 d). The prolonged residence of tablets may be understood owing to floating characteristic and also by random emptying effect as it is a multiple-unit system. As floating tablets due to their low density always spread and reside in the upper part of the stomach and may have less chance of “fortuitous emptying”. As shown in Fig. 2 (e) almost complete emptying of tablets from the stomach was observed at the 12th hour after dosing. As the density of drug loaded tablets, being more lighter than barium sulphate loaded formulation, their residence is prolonged further the gastric emptying time. Thus, results show that the mean GRT of optimized formulation was more than 8 hours in rats; it means that formulation with stands the different phases without emptying.

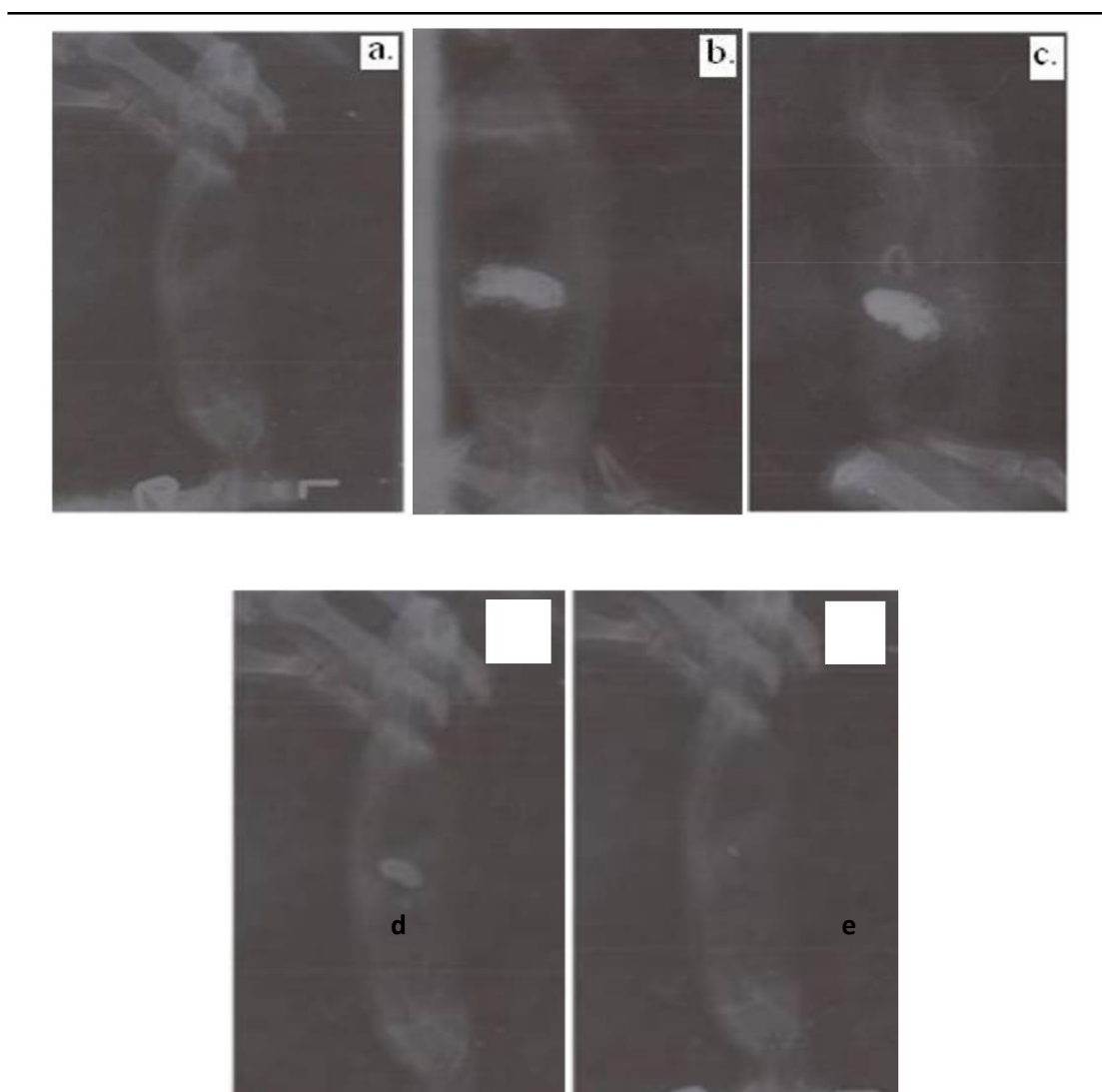


Fig. 3 X-ray images of formulation in gastric region of rat: (a) before dosing, (b) 2 hours, (c) 4 hours, (d) 6 hours and (e) 8 hours after dosing.

Conclusion:

In-vitro drug release characteristics of the final formulation B-10 was showing more than 85% release in 12 hours. In-vivo floating behavior was found promising till 12th hour. Base on the In-vitro drug release and in-vivo floating behavior, it is concluded that the finalized formulation will improve bioavailability with comparison to the existing available immediate release dosage form. Also dosing frequency will be reduced with reduced side effects.

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