

## DESIGN AND OPTIMIZATION OF MICROPARTICULATE RESERVOIR MATRICES FOR COMBINATION THERAPY

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Received: 12 Dec 2019, Revised and Accepted: 28 Feb 2020

### ABSTRACT

**Objective:** The purpose of this investigation was to design and develop controlled release floating beads of Ketoprofen and Pantoprazole sodium. To determine the interaction between excipients used and to find out the nature of drug in the formulation, X-ray diffraction (XRD) and Differential Scanning Colorimetry (DSC) studies were performed.

**Methods:** The beads were prepared by ionotropic gelation technique. Sodium alginate and HPMC E5LV was dissolved in deionized water (9:1 sodium alginate: HPMC E5LV) at a concentration of 1-3 % w/v using gentle heat on the water bath. After getting a clear solution, an accurately weighed quantity of Pantoprazole sodium was added and dispersed uniformly into the solution. In a separate beaker, Ketoprofen and calcium carbonate (1:1, Sodium alginate: CaCl<sub>2</sub>) was dispersed in water and mixed with sodium alginate solution containing Pantoprazole sodium. The bubble-free sodium alginate-drug dispersion (20 ml) was added dropwise via a 22-gauge hypodermic needle fitted with a 10 ml syringe into 100 ml of calcium chloride solution (1-2 % w/v) containing 10 % glacial acetic acid and stirred at 400 rpm for 15 min.

**Results:** From the experimental study, it was concluded that optimized batch F9 showed good micromeritic properties, entrapment efficiency and releases the drug slowly and completely for 12 h as beads remain in floating condition throughout dissolution study that assures prepared formulation remain floated in the stomach without its early passing to lower Gastro-Intestinal Tract (GIT) side. The percentage of drugs release of Ketoprofen and Pantoprazole Sodium was 96.56% and 97.74%, respectively at 12 h.

**Conclusion:** Combination of different polymer provide sustained release pattern in different concentration. Formulation F9 gives good floating behavior using sodium alginate and HPMC hydroxypropyl methylcellulose in different ratios. In the present study, a satisfactory attempt has been made to formulate gastro retentive floating beads of Ketoprofen and Pantoprazole sodium.

**Keywords:** Anti-inflammatory, Ketoprofen, Pantoprazole sodium, Alginate beads, Ionotropic gelation

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### INTRODUCTION

Gastro Retentive Drug Delivery (GRDD) devices are primarily site-specific drug delivery systems, which gets retained in the stomach for a longer period of time, thus helping in the absorption of drug for the intended duration of time [1, 2]. Floating Drug Delivery System (FDDS) reside in the stomach for a longer period of time than conventional dosage forms after the release of drugs; the residual system is emptied from the stomach [3]. The contribution of floating drug delivery system includes; gastric retention time is increased because of buoyancy, site-specific drug delivery to stomach [4]. The real challenge in the development of an oral controlled-release drug delivery system is not just to sustain the drug release but also to prolong the presence of the dosage form within the Gastro-Intestinal Tract (GIT) until all the drugs is completely released at the desired period of time [5]. To overcome these limitations, various gastroretentive systems have been designed to be retained in the gastric region for a prolonged time. These systems include a) Floating systems, b) Bioadhesive systems, c) Swelling and expanding systems, d) High-density systems, e) Modified systems [6]. The anti-inflammatory effects of Ketoprofen are believed to be due to inhibition of cyclooxygenase-2 (COX-2), an enzyme involved in prostaglandin synthesis via the arachidonic acid pathway.

Ketoprofen is a non-specific cyclooxygenase inhibitor, and inhibition of cyclooxygenase-2 (COX-2) is thought to confer some of its side effects, such as GI upset and ulceration. Pantoprazole sodium is a Proton Pump Inhibitor (PPI) that suppresses the final step in gastric acid production by covalently binding to the (H<sup>+</sup>, K<sup>+</sup>)ATPase enzyme system at the secretory surface of the gastric parietal cell. This effect leads to inhibition of both basal and stimulated gastric acid secretion, irrespective of the stimulus. The binding to the (H<sup>+</sup>, K<sup>+</sup>)ATPase results in a duration of antisecretory effect that persists longer than 12 h. for all doses tested. Response Surface Methodology (RSM) as a tool of designing of experiments is a widely practiced approach in the development and optimization of drug delivery devices [7-12]. The technique requires minimum experimentation and less time.

### MATERIALS AND METHODS

#### Materials

Ketoprofen and pantoprazole sodium were provided by Milan Laboratories (India) Pvt. Ltd. Maharashtra. Sodium alginate, hydroxypropyl methylcellulose (HPMC) and calcium chloride were obtained from MolyChem Thane-421503 (India) and all other ingredients used were of pharmaceutical grade.

Table 1.1: Trial batches of formulation

Formulation code	Sodium alginate (% w/v)	Calcium chloride (% w/v)	HPMC K15M,	HPMC K4M	HPMC E5LV	HPMC E6LV	HPMC K100
C1	2	2	1	-	-	-	-
C2	2	2	-	1	-	-	-
C3	2	2	-	-	1	-	-
C4	2	2	-	-	-	1	-
C5	2	2	-	-	-	-	1

Pantoprazole Sodium (40 mg) and Ketoprofen (100 mg) was used in all the above formulations

### Methods: Preparation of drug-loaded beads by manual syringe method

The beads were prepared according to the composition presented in table 1.1. Sodium alginate was dissolved in distilled water at 60 °C in a water bath for 1 hour in order to obtain a clear polymer solution with a concentration of 2% (w/w). Different grade of HPMC was dispersed in the alginate solution. Cool down the solution at room temperature and Pantoprazole Sodium and Ketoprofen was added with continued stirring with a glass rod in a bath sonicator. Accordingly, 10 ml syringes fitted with a needle having a size of 22 G were employed in the preparation of all formulation. Above solution was introduced dropwise into the gelling solution (2% w/v) aqueous calcium chloride while being gently stirred at 25 °C. The stirring was continued for 15 min and the calcium alginate beads were harvested by filtration.

### Formulation of floating beads

The beads were prepared by ionotropic gelation technique. Sodium alginate and HPMC E5LV was dissolved in deionized water (9:1 sodium alginate: HPMC E5LV) at a concentration of 1-3 % w/v using gentle heat on a water bath. After getting a clear solution, an accurately weighed quantity of Pantoprazole sodium was added and dispersed uniformly into the solution. In a separate beaker, Ketoprofen and calcium carbonate (1:1, Sodium alginate: CaCl<sub>2</sub>) was dispersed in water and mixed with sodium alginate solution containing Pantoprazole sodium. The bubble-free sodium alginate-drug dispersion (20 ml) was added dropwise via a 22-gauge hypodermic needle fitted with a 10 ml syringe into 100 ml of calcium chloride solution (1-2 % w/v) containing 10% glacial acetic acid and stirred at 400 rpm for 15 min. The droplets from the dispersion instantaneously gelled into discrete matrices upon contact with the solution of the gelling agent. Finally, beads were filtered, washed and dried at room temperature. For bead formation,

20 ml of a 1-3 % w/v aqueous solution of sodium alginate containing Pantoprazole sodium and Ketoprofen was introduced dropwise from a glass syringe with a size-22 needle into 100 ml of an aqueous 2 % W/V calcium chloride solution being stirred at 400 rpm. The stirring was continued for one hour and the calcium alginate beads were harvested by filtration, washed with distilled water, and air-dried overnight [13].

### Experimental design

A number of preliminary trials were conducted before the application of the design as shown above. The levels of the factors were also determined randomly by evaluating the dependent variables (responses) of trial batches. A 3<sup>2</sup> factorial design was used for optimizing the formulations using Design-Expert Software (Version-8.0.7.1). The concentration of sodium alginate (X1) and the concentration of calcium chloride (X2) were classified as low, medium and high values for independent variables as given in table 1.2. These studied factors along with their levels and the corresponding responses are summarized in table 1.3.

### Methods

A response surface method 3<sup>2</sup> factorial designs were applied to evaluate the relationship between the independent variables and their responses. Two variables and one response were involved in the experimental design. The dependent response factor variables measured were entrapment efficiency and % Floating. The independent variables are the concentration of alginate (X1) and the concentration of calcium chloride (X2). The formulation variables and the high and low levels of each variable were defined based on preliminary experiments. Center points were repeated three times to estimate the experimental error. The independent and dependent variables are shown in table and process variables and their levels for full-factorial design are given in table.

Table 1.2: Various independent and dependent variables

S. No.	Independent variables	Dependent variables
1	X1-Sodium alginate concentration (% W/V)	Y1-%Entrapment efficiency
2	X2-Concentration of calcium chloride (% W/V)	Y2-% Floating

Table 1.3: Process variables and their levels for full-factorial design

S. No.	Factors	Low level	Middle level	High level
1	Concentration of Sodium alginate (% W/V)	1	2	3
2	Concentration of Calcium chloride (% W/V)	1	1.5	2

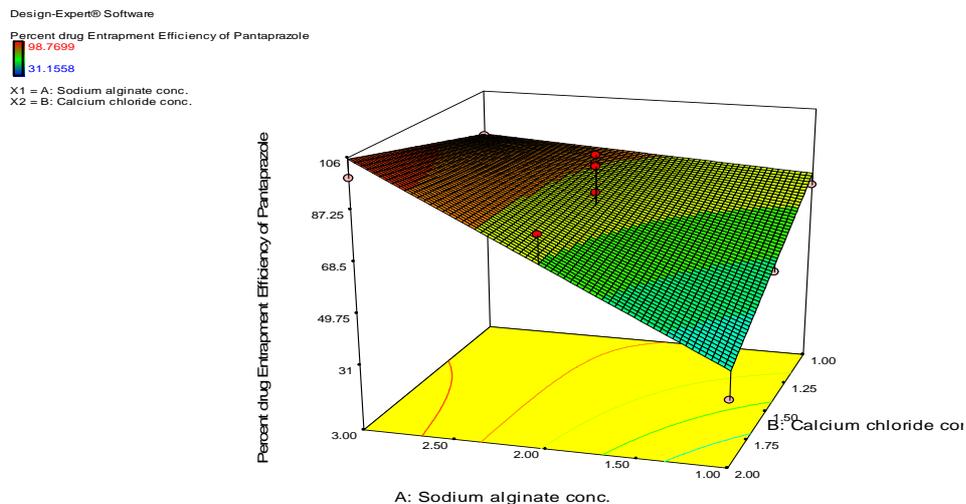


Fig. 1.1: Percentage drug entrapment efficacy pantoprazole sodium

## RESULTS AND DISCUSSION

Table 1.4: Evaluation parameters of different floating alginate beads

Formulation code	% Yield	% Entrapment efficiency		% Floating	Physical appearance
		Pantoprazole sodium (PTS)	Ketoprofen (KT)		
C1	86.35±0.12	63.16±0.41	36.26±0.23	20±0.16	Oval
C2	76.75±0.32	66.99±0.31	38.39±0.34	25±0.33	Oval
C3	81.89±0.30	97.29±0.13	91.27±0.43	55±0.28	Round
C4	77.97±0.16	77.37±0.22	73.29±0.31	30±0.34	Round
C5	78.37±0.34	73.92±0.17	70.29±0.25	27±0.33	Oval

Data from each response is presented in mean±SD (n=3)

Among of the above formulations (trial batches), formulation C3 having Hydroxy Propyl Methyl Cellulose [HPMC] E5LV polymer shows good entrapment efficiency 97.29% and 91.27% for

Pantoprazole Sodium and Ketoprofen respectively. Round appearance but buoyancy is not good so the different ratio of calcium chloride was tried for better buoyancy.

Table 1.5: Experimental design and results for the various measured responses of Pantoprazole Sodium and Ketoprofen beads

Run	Variables		Response				% Yield	Physical appearance
	Formulation code	SA Conc. (%) W/V (X1)	Cal. C Conc. (W/V) (X2)	% Drug EE (PTS)	% Drug EE (KT)	% Floating		
1	F1	1	2	31.15±0.19	23.63±0.21	65±0.32	44.40±0.32	Irregular
2	F2	2	1.5	95.85±0.37	74.48.93±0.16	97±0.21	58.94±0.22	Round
3	F3	3	2	98.76±0.31	68.20±0.22	95±0.42	47.6±0.25	Oval with tailing
4	F4	2	1.5	93.72±0.26	63.62±0.17	98±0.22	46.6±0.13	Round
5	F5	1	1.5	58.58±0.32	40.67±0.19	85±0.31	37.28±0.27	Irregular
6	F6	2	1.5	93.17±0.21	56.06±0.16	95±0.24	32.69±0.25	Round
7	F7	2	1.5	55.47±0.41	64.14±0.18	97±0.11	47.78±0.26	Round
8	F8	3	1.5	86.51±0.34	62.01±0.23	100±0.32	47.11±0.23	Oval with tailing
9	F9	2	1	97.74.18±0.15	96.5763.66±0.18	95±0.20	46.63±0.26	Round
10	F10	2	1.5	83.48±0.15	70.76±0.22	85±0.14	46.15±0.19	Round
11	F11	3	1	87.96±0.26	70.81±0.35	96±0.23	39.32±0.16	Rod Shaped
12	F12	1	1	76.37±0.14	65.65±0.16	95±0.41	39.49±0.31	Irregular
13	F13	2	2	84.01±0.31	65.60±0.17	100±0.22	57.98±0.25	Round

Data from each response is presented in mean±SD (n=3), 3D Surface Response Graph between Sodium alginate (X<sub>1</sub>), Calcium chloride (X<sub>2</sub>) with % drug entrapment efficiency of pantoprazole sodium (Y<sub>1</sub>)

Table 1.6: Actual Vs predicted value by ANOVA analysis

Standard Order	Actual Value	Predicted Value	Residual
1	76.3734	80.96774	-4.59434
2	83.184	84.81986	-1.63586
3	87.9686	88.67199	-0.70339
4	58.5852	61.36478	-2.77958
5	83.48053	79.22163	4.258892
6	86.517	97.07848	-10.5615
7	31.1558	41.76183	-10.606
8	84.01093	73.6234	10.38752
9	98.7699	105.485	-6.71508
10	55.47	79.22163	-23.7516
11	93.72117	79.22163	14.49954
12	97.47394	79.22163	18.2523
13	93.17078	79.22163	13.94915

Table 1.7: ANOVA for response surface factorial model: analysis of variance table of Ketoprofen drug entrapment efficiency

Anova	SS	DF	MS	F-value	P-value	
Model	1533.693	3	511.231	5.518152	0.0199	significant
Sodium Alginate Conc. (X1)	841.8526	1	841.8526	9.086831	0.0146	
B-Calcium Chloride Conc.(X2)	303.5476	1	303.5476	3.276448	0.1037	
AB	388.2929	1	388.2929	4.191176	0.0709	
Residual	833.8081	9	92.64534			
Adjusted R <sup>2</sup>	0.530415					
Standard Deviation	9.625245					
Coefficient of Variance (%)	15.82291					

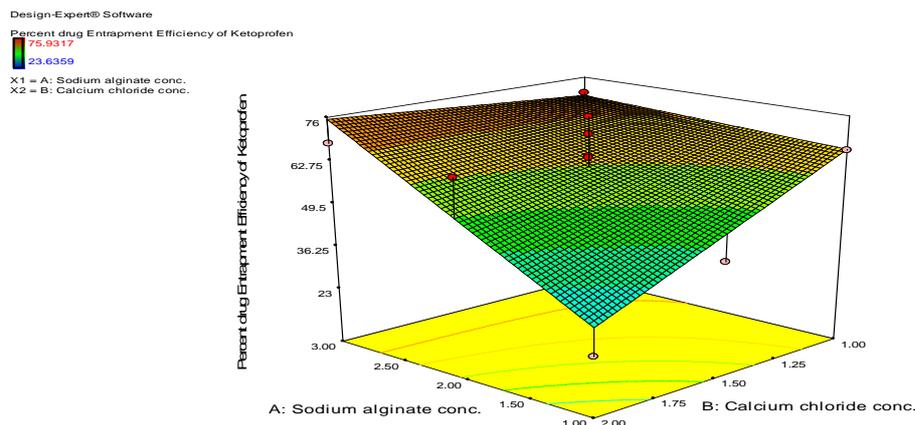


Fig. 1.2: Percentage drug entrapment efficacy of Ketoprofen

#### Final factorial equation for the process

$$Y = \beta_0 + \beta_1 \cdot X_1 - \beta_2 \cdot X_2 + \beta_3 \cdot X_1 \cdot X_2$$

$$\begin{aligned} \text{\% Drug Entrapment Efficiency of Ketoprofen} \\ = 60.831 + 11.8452 \cdot X_1 - 7.112 \cdot X_2 \\ + 9.852 \cdot X_1 \cdot X_2 \end{aligned}$$

3D Surface Response Graph between Sodium alginate ( $X_1$ ), Calcium chloride ( $X_2$ ) with % Drug Content of Ketoprofen ( $Y_1$ )

#### Determination of percent yield and drug entrapment efficiency

Beads (100 mg) were crushed in a glass mortar and suspended in 20 ml of 0.1N HCl. After 24 h, the solution was filtered through 0.45 mm membrane filter, and the filtrate was analyzed for drug content [14].

$$\text{Drug entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

#### Buoyancy test

The buoyancy of the beads is study at  $37 \pm 0.5$  °C, soaking 100 beads in 100 ml of 0.1 HCl at pH 1.2. The number of floating beads is evaluated at fixed time intervals. The floating time is considered as the time at which the 100 % of beads floated. All the data are the average of at least three determinations [15]

#### Scanning electron microscopy

The samples were coated with a thin gold layer by a sputter coater unit (SPI, sputter, USA). Then, SEM photographs were taken by a scanning electron microscope (Joel JSM 5400LV SEM, Japan) operated at an acceleration voltage of 15 kV[16]. The morphological evaluation of the optimized beads formulation was done by scanning electron microscopy. SEM study revealed that the microspheres were almost spherical in shape with a rough outer surface.

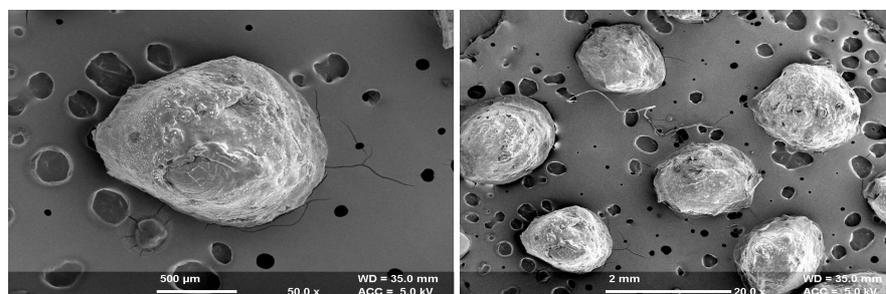


Fig. 1.3: Shape and size of the beads

The scanning electron microscopy (SEM) image of the beads has been used to examine surface topography, the texture and morphology of fractured surfaces are compared to hypothesize the mechanism of drug release and floating.

#### Differential scanning calorimetry (DSC) studies

Differential Scanning Calorimetry (DSC) is a useful tool to monitor the effect of additives on the structure of a material and to obtain information about the physicochemical interaction between drug and polymer. Differential Scanning Calorimetry (DSC) of drugs, polymer and statistically optimized formulation were carried out using a Mettler Toledo Star SW 8.10, Model no: DSC 822 instrument. In this process, samples (8-10 mg) were weighed into aluminum pans and heated under nitrogen from 5 to 250 °C [17].

#### X-ray diffraction (XRD) studies

To understand the crystalline state of the drug in the polymer matrix, the X-ray diffraction pattern of drug-loaded beads was

performed and compared with that of pure drug. Powder forms of the samples were exposed to Cu radiation ( $30\text{kV} \times 15\text{mA}$ ) in a wide-angle X-ray diffractometer (Miniflex gonio Meter, Japan). The instrument was operated in continuous mode in increments of  $1^\circ/\text{min}$  and scanned over a  $2\theta$  range of 10 to 90 °C [18].

#### In vitro drug release studies

The *in vitro* drug release studies of different formulations (Formulation-2 and Formulation-9) and pure drugs were conducted to ensure the effect of sodium alginate concentration and calcium chloride concentration on the release of Pantoprazole Sodium and Ketoprofen from the formulations. The *in vitro* dissolution studies of the floating formulations were carried out using USP dissolution test apparatus I (basket method). The basket of USP dissolution test apparatus I, each containing a number of beads equivalent to 40 mg Pantoprazole Sodium and 100 mg Ketoprofen, were rotated at 100 rpm in 900 ml of 0.1N HCl maintained at  $37 \pm 0.5$  °C. An aliquot of 5 ml of the solution was withdrawn at predetermined time intervals.

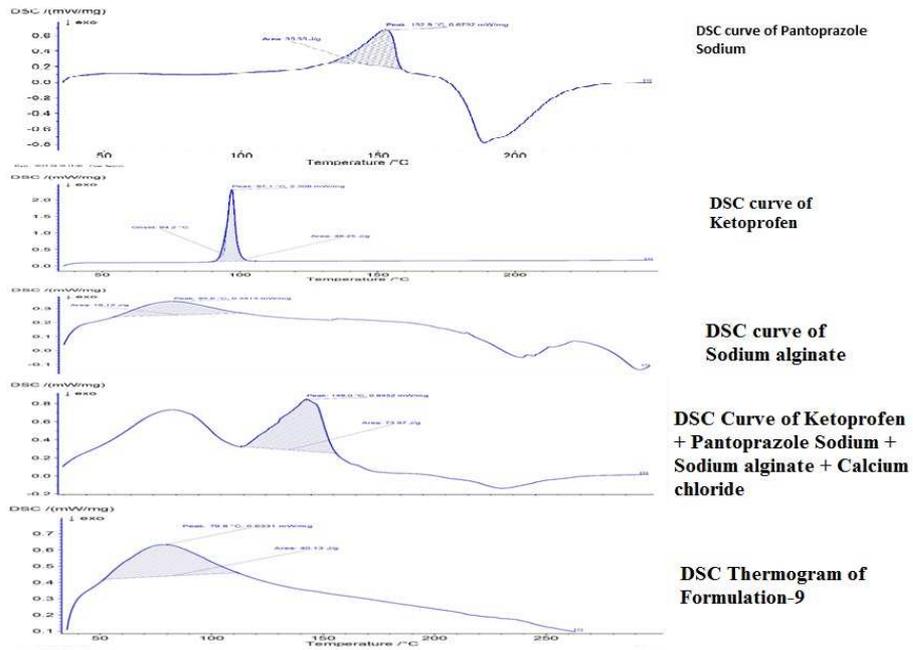


Fig. 1.4: DSC curve of pantoprazole sodium, DSC curve of Ketoprofen, DSC curve of Sodium alginate, DSC curve of Ketoprofen+Pantoprazole Sodium+Sodium Alginate+Calcium chloride, DSC Thermogram of optimized formulation

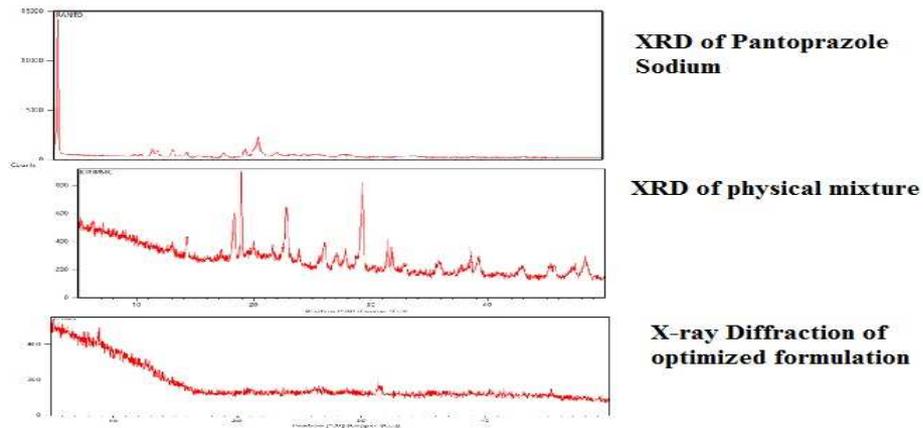


Fig. 1.5: XRD of pantoprazole sodium, XRD of the physical mixture, X-ray diffraction of optimized formulation

Table 1.8: Percentage drug release of formulations

Time (H)	Pure drug		Formulation-2 (F2)		Formulation-9 (F9)	
	%DR pantoprazole sodium	% DR ketoprofen	%DR pantoprazole sodium	% DR ketoprofen	%DR pantoprazole sodium	% DR ketoprofen
0.25	29.79±0.24	28.66±0.31	16.29±0.20	14.10±0.31	15.82±0.40	11.51±0.39
0.5	41.89±0.32	39.24±0.17	25.04±0.14	23.35±0.15	31.64±0.13	21.62±0.36
1	68.86±0.13	52.63±0.18	35.37±0.18	30.08±0.16	40.01±0.12	28.43±0.27
2	75.39±0.21	68.61±0.27	43.74±0.16	37.36±0.22	48.40±0.23	44.88±0.28
3	77.25±0.11	69.08±0.32	53.98±0.15	46.29±0.32	61.43±0.15	53.34±0.14
4			62.35±0.37	52.63±0.41	69.81±0.22	64.38±0.23
5			67.01±0.26	60.62±0.22	76.33±0.26	72.37±0.15
6			79.11±0.41	67.67±0.27	82.85±0.28	84.11±0.26
7			84.69±0.20	70.72±0.28	86.57±0.21	85.76±0.33
8			87.48±0.38	72.60±0.29	90.29±0.11	88.34±0.25
9			88.41±0.11	73.07±0.21	93.09±0.32	91.40±0.13
10			93.99±0.15	73.78±0.23	96.81±0.22	95.86±0.11
11			93.99±0.7	74.01±0.32	96.81±0.21	96.57±0.21
12			95.85±0.37	74.48±0.16	97.74±0.15	96.57±0.18

DR: Drug release, Data from each response is presented in mean±SD (n=3)

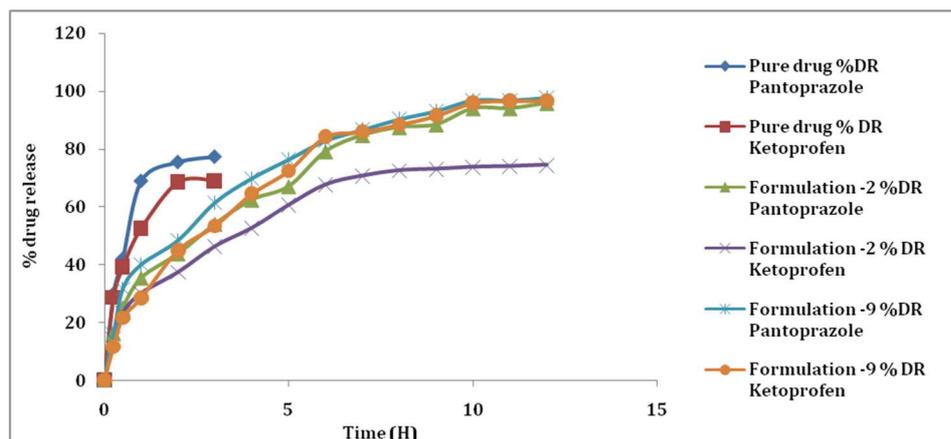


Fig. 1.6: *In vitro* drug release of different optimized formulations and pure drug in 0.1 N HCl

Discussion: Prepared floating beads shows almost spherical in shape with the rough outer surface, but high loaded beads were not smooth as low drug-loaded beads as their surface was covered by drug crystals. Differential Scanning Calorimetry (DSC) thermograms showed relatively broad melting endothermic peak, indicate that only a small fraction of drugs substance existed in the crystalline state. A small sized particle leads to high surface energy, which creates an energetically suboptimal state causing a decrease in the melting point. X-ray diffraction analysis showed the stable character of drug in the drug-loaded hollow beads and revealed the absence of any drug-polymer interactions. X-ray diffraction (XRD) indicates the amorphous dispersion of the drug after entrapment into microbeads may increase the bioavailability of drugs. The optimized formulation F9 showed 97.74% and 96.57% drug release for Pantoprazole sodium and ketoprofen, respectively, optimized formulation indicated good floating characteristics. The *in vitro* dissolution studies of the formulation formulation-9 shows maximum percent cumulative release with in 12 h. This shows that Formulation-9 was having the good sustained release of the Pantoprazole Sodium and Ketoprofen up to the 12 h.

#### CONCLUSION

In the present study, a satisfactory attempt has been made to formulate gastroretentive floating beads of Pantoprazole sodium and Ketoprofen. From the experimental study result, it was concluded that optimized batch showed good micromeritic properties, entrapment efficiency and releases drug slowly and completely for 12 h as beads remain in floating condition throughout dissolution study that assures prepared formulation remain floated in stomach without its early passing to lower GIT side. This will help to increase the residence time of Pantoprazole sodium and Ketoprofen in stomach and achieve sustained release thereby increase the bioavailability of drugs. Finally the prepared floating beads may prove to be potential gastroretentive delivery system for safe and effective controlled release for an extended period of time.

#### FUNDING

Nil

#### AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

#### CONFLICT OF INTERESTS

Declared none

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