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Original Article

STATISTICAL OPTIMIZATION AND CHARACTERIZATION OF MICROBALLOONS FOR INTESTINAL DELIVERY OF ACID LABILE DRUG UTILIZING ACRYLIC POLYMER

PRAVIN GUPTA*[1], MANISH KUMAR[2], NARSINGH SACHAN[3]

[1]Department of Pharmaceutics, [2]Department of Pharmaceutics, Sir Madanlal Institute of Pharmacy, Etawah (U. P) 206001, India, [3]Department of Pharmaceutical chemistry, BBD University, Lucknow (U. P) 226001, India
Email: pravin.mpharm75@gmail.com

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ABSTRACT

Objective: The present work was to formulate buoyant system in order to protect the drug from gastric acid degradation, increasing the gastric residence time, control the drug leaching by use of enteric polymers, prolong the half life for longer duration of action, furthermore to prevent dosage form adherence to the mucous wall in order to avoid incomplete drug release. Moreover the side effects of effervescent dosage form could be well controlled.

Methods: Employed was emulsion solvent diffusion, using 2^3factorial design with Eudragit®L100 and RS100 in solvent mixture dichloromethane and ethanol whereas the optimization and validation were carried out through Design-Expert 9.0.3 software.

Result: Optimized formulation (LRS-O) showed buoyancy (B %) of 78.88±0.23 %, entrapment efficiency (EE %) of 71.12±0.04 % and drug release over 12 h (CDR12 h %) of 99.50±0.08 % in PB pH 6.8. Whereas in PB pH 7.4 the actual values for B %, EE % and CDR12 h % was 87.35±0.68 %, 89.30±0.05 % and 98.68±0.37 %. Smaller error values for both showed that actual responses were within the predicted range. Particles were in the size range 80-100 µm with spherical, rough, porous and internal hollow cavity. Drug-polymer interactions were negligible and showed zero order patterns over 12 hrs.

Conclusion: Enhanced bioavailability of the drug was achieved with excellent responses of B %, EE % and CDR12 h % and the software used for the optimization and validation of formulation design, was economical and reduces the number of trials.

Keywords: Emulsion solvent diffusion method, Gastro retentive drug delivery, Non effervescent, Pantoprazole sodium, 2^3 factorial design.

INTRODUCTION

Intestinal absorption occurs through passive transport and follows Fick's law of diffusion from higher concentration in the gastrointestinal tract (GIT) to the lower one in systemic circulation. Diffusion through intestinal cells depends on drug ionization, solubility, concentration and permeability. Drug diffuses in its molecular form from the lipophilic mucosal wall of intestinal membranes in to the systemic circulation because of the larger partition coefficient between membranes and gastrointestinal fluids. For absorption to occur both drug solubility and permeability play a vital role in its transport from intestinal mucosa and in to the systemic circulation [1].

Different types of response surface methodology (RSM) designs include 3-level factorial design, central composite design (CCD), box-Behnken design and D-optimal design. RSM is used when only a few significant factors are involved in optimization. It is a computer based optimization technique utilizing a polynomial equation [2-4].

The gastric retention time (GRT) of the dosage form could be increased by providing buoyancy has been reported. Buoyancy could be achieved by effervescent as well as non-effervescent techniques. They provide better drug absorption at the proximal small intestine as well as in the stomach [5]. Effervescent technique has been reported with the side effects of violent gas generation; disintegration of the dosage form; burst release; dose dumping and alkaline microenvironment, these drawbacks could be well controlled by non-effervescent technique [6].

The single unit product has the disadvantages of uneven distribution in the gastrointestinal tract and may also cause local damage by dose dumping effect [7]. The use of oral conventional dosage form was limited due to their inability to increase their residence time in the stomach and proximal portion of the small intestine [8]. In contrast the multiple unit products like microballoons are less affected by the pH and the gastric transit time, attain more constant plasma levels, give higher accuracy in reproducibility and achieve a sustain-release effect [9].

Pantoprazole sodium (PAN) is a proton pump inhibitor [10] and belongs to biopharmaceutical classification system (BCS) class I with high solubility and permeability, degrades in the acidic environment hence for restoring its efficacy it is available for administration as lyophilized powder and as enteric coated gastric resistant tablets [11]. It is administered to treat gastric ulcers, gastro-esophageal reflux disease and to treat infections caused due to *Helicobacter pylori* [12]. It is chemically, sodium 5-(difluoromethoxy)-2-{[(3, 4-dimethoxy 2-pyridinyl) methyl] sulfinyl}-1H-benzimidazole. It consists of two heterocyclic moieties-a pyridine and a benzimidazole moiety-linked via methyl sulfinyl group, inhibits the gastric H^+/K^+-ATPase via covalent binding to cysteine residue of the proton pump [13]. PAN degrades faster at low pH due to its shorter elimination half life of 1 h and bioavailability of 77 %, so it requires encapsulation with enteric polymers has been reported to enhance its duration of action in case of oral dosage forms.

The glass transition temperature of Eudragit®RS100 could be lowered by adding small amount of dibutyl phthalate (DBT) (10-25 %) that fits between the glassy molecules, gives them mobility and thus increases its solubility whereas Eudragit®E100 do not require the addition of plasticizer for solubilization [14]. Magnesium stearate in concentration 0.25 to 5 % w/w is of low bulk density and hydrophobic in nature. Polyvinyl alcohol (PVA) (0.75 % w/v) was used as a dispersing agent, sodium citrate (0.3-2.0 %) as a buffering agent for the medium. Sodium chloride serves as channeling agent. When the coated particles come in contact with aqueous medium, sodium chloride may leach out creating channels within the film coat [15].

In previous reported works, it has been successfully encapsulated with Eudragit®S grades by emulsion solvent evaporation and spray drying methods, for its protection in the gastric mucosa [16, 17]. Our aim in the present work was to give porous nature as well as sustained release characteristics to the formulation, which could be achieved by Eudragit®L100 and RS100 combinations. Due to the presence of ammonium group Eudragit®RS100 provides porosity to the formulation and also shows pH independent dissolution. The

encapsulation was achieved through enteric coating polymer Eudragit®L100 that dissolves above pH 6 in the jejunum.

In order to achieve the objectives of buoyancy, controlling the rate of drug release, increase in the GRT and controlling the fluctuations of drug level in plasma, we formulated hollow microspheres (microballoons) by emulsion solvent diffusion method [18], employing non-effervescent technique with 2^3 factorial designs. These systems may also be advantageous for drugs that have narrow absorption window, irritant to gastric mucosa and with stability problems. The microballoons formed were evaluated for its percentage swelling, *in vitro* buoyancy and entrapment efficiency. They are characterized by particle size analysis, SEM, FTIR, *in vitro* release, mechanism of release and for stability studies. Stability testing of optimized formulation provides the evidence on how the quality shows variations with time, predicts the shelf life and suggests the optimal storage conditions [19].

MATERIALS AND METHODS

Materials

Pantoprazole sodium (PAN) was obtained as a gift sample from Akum Drugs (Haridwar, India); Eudragit®L100 and RS100 from Evonic industries (Mumbai, India). Magnesium stearate, dichloromethane, DBT and PVA were purchased from LOBA Chemicals (Mumbai, India). All other chemicals used were of analytical grades. Differential UV-spectrophotometric studies were carried out using double-beam UV-spectrophotometer-2203, Systronics Pvt. Ltd. (Ahmedabad, Gujarat, India).

Methods

Preparation of PAN-loaded microballoons

Microballoons were prepared by emulsion solvent diffusion method [20]. In this method Eudragit®L100 (600-900 mg) and RS100 (600-900 mg) were dissolved in ethanol-dichloromethane mixture each 8 ml. PAN (40 mg) was mixed with sodium chloride (0.09 g) separately with the help of mechanical stirrer which serves as channeling agent. This drug mixture was added to above prepared polymer solution with continuous stirring at 300 rpm, than DBT (20 % w/v) was incorporated and stirred for 1 h. This drug-polymer mixture was slowly introduced into aqueous solution of PVA (0.75 % w/v in 200 ml distilled water) containing sodium citrate (1 % w/v) as buffering agent to get the desired pH of the medium. The solution was maintained at 40 °C on a magnetic stirrer at 300 rpm for 1 h and the prepared microballoons were collected by filtration, washed three times with distilled water, dried at room temperature and kept in desiccators.

Experimental design

A 2^3 Full Factorial Designs (FFD) was used for the optimization of sustained release PAN formulations. Magnesium stearate (X_1 % w/w), Eudragit®L100 (X_2, mg) and Eudragit®RS100 (X_3, mg) were the three factors (independent variables) studied. The responses (dependent variables) studied were buoyancy (Y_1, %), drug entrapment efficiency (Y_2, %) and amount of drug released in 12 h (Y_3, %) in both phosphate buffer (PB) pH 6.8 and 7.4. The experimental design was evaluated using Design-Expert 9.0.3 software (Stat-Ease Inc., USA) and the effect of three factors, two factor levels and their interaction on three basic responses was investigated and evaluated for all batches LRS 1-8 (table 1).

Table 1:2^3 full factorial design layouts for different formulations in PB pH 6.8 and 7.4

Code	Independent variables (factors, *X*)			pH	Dependent variables (responses, *Y*)		
	Magnesium stearate (X_1, % w/w)	Eudragit® L100 (X_2, mg)	Eudragit®RS 100 (X_3, mg)		B % [a, e]	EE % [b, e]	CDR12 h % [c, e]
LRS-1	2.5 (-1)	600(-1)	600(-1)	6.8	28.97±0.021	10.88±0.045	75.05±0.017
				7.4	33.82±0.032	46.39±0.008	74.64±0.028
LRS-2	5.0 (+1)	600(-1)	600(-1)	6.8	78.88±0.043	71.12±0.008	99.50±0.015
				7.4	87.35±0.012	89.30±0.043	98.68±0.073
LRS-3	2.5 (-1)	900(+1)	600(-1)	6.8	41.03±0.024	26.67±0.021	95.92±0.026
				7.4	49.35±0.067	61.86±0.084	94.37±0.031
LRS-4	5.0 (+1)	900(+1)	600(-1)	6.8	75.59±0.011	77.09±0.012	71.55±0.018
				7.4	81.42±0.078	66.76±0.006	63.18±0.046
LRS-5	2.5 (-1)	600(-1)	900(+1)	6.8	C_d	C_d	C_d
				7.4	C_d	C_d	C_d
LRS-6	5.0 (+1)	600(-1)	900(+1)	6.8	46.87±0.025	40.71±0.046	66.54±0.072
				7.4	51.82±0.098	59.29±0.061	72.75±0.079
LRS-7	2.5 (-1)	900(+1)	900(+1)	6.8	61.05±0.034	30.86±0.063	64.08±0.084
				7.4	67.92±0.033	55.39±0.068	69.09±0.062
LRS-8	5.0 (+1)	900(+1)	900(+1)	6.8	88.46±0.009	62.13±0.031	77.01±0.064
				7.4	93.37±0.065	73.75±0.041	84.21±0.067

(+1) = higher values; (-1) = lower values; [a] B % = percentage buoyancy; [b]EE % = percentage entrapment efficiency; [c]CDR12 h % = cumulative percentage drug release over 12 h; [d]C_d = collapsed; [e]mean±S. D.: n = 3.

Evaluation of microballoons

Percentage swelling (Ps)

The swelling kinetics of microballoons was carried out by the gravimetric method in triplicate. For the study 50 mg of microballoons were immersed in PB pH 6.8 and 7.4 (100 ml, 37±0.5 °C) in a beaker, maintained at 100 rpm on a magnetic stirrer.

After fixed intervals of 1 h they were removed and weighed immediately. The difference in weights gave the amount of water uptake by the microballoons. *Ps* was calculated by following equation in triplicate [21]:

$$Ps = \left\{\frac{Ws - Wd}{Wd}\right\} \times 100 \quad (1)$$

Where, *Ws* is weight of swollen and *Wd* is the weight of the dried microballoons.

Percentage *in vitro* buoyancy (B %)

The study was carried out by spreading 50 mg of dried microballoons over the surface of PB pH 6.8 and 7.4 (100 ml, 37±0.5 °C) containing 0.02 % w/v Tween 80 in the dissolution apparatus (type II) and stirred at 100 rpm on a magnetic stirrer for 12 h. After 12 h the microballoons were collected, dried, weighed and then kept in desiccators until constant weight was achieved. Percentage buoyancy was calculated using the following equation in triplicate [22]:

$$B\% = \left\{\frac{W_F}{W_F + W_{NF}}\right\} \times 100 \quad (2)$$

Where, W_F is weight of floated and W_{NF} is the weight of the non-floated microballoons.

Percentage entrapment efficiency (EE %)

For the study dried microballoons equivalent to 40 mg of PAN was crushed and dissolved in 100 ml of PB pH 6.8 and 7.4. The contents

were shaken on vortex mixture for 1 h to extract the drug and then filtered through whatman filter paper 1. The drug concentration was determined spectrophotometrically at λ_{max} 215 nm and 288 nm in triplicate. The percentage drug entrapped was calculated by following equation [23]:

$$\text{EE \%} = \frac{\text{Calculated drug content (x)}}{\text{Theoretical drug content}} \times 100 \quad (3)$$

Optimization and validation of experimental design

On the basis of evaluation results of the response parameters the formulation with maximum B %, EE % and highest CDR 12 h % over 12 h was selected. For validating the design polynomial equations were generated for each response using the software. The statistical model equation consisting of interactive as well as polynomial terms for each response parameters for the evaluation:

$$y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad (4)$$

Where b_0, the intercept is the arithmetic mean was found out with the help of table 1. The main effects (regression coefficients) b_1, b_2, b_3, b_{12}, b_{13}, b_{23} and b_{123} were calculated by use of signs in the columns, by adding or subtracting the value of the obtained responses, *Y*. Finally the values are summed up and divided with the number of formulations [24].

Model matrix method was used for the optimization process. The interaction effects were calculated in the same way as that of the main effects. The signs of the interaction effects such as: X_1X_2, X_1X_3, X_2X_3 and $X_1X_2X_3$ were calculating by multiplying the signs of the corresponding main effects and separate columns are constructed for each effect. To validate the polynomial equation model, one check point formulation LRS-O was also formulated and evaluated in both the mediums, the significance of model design and individual response parameters (p<0.05) was estimated by one-way ANOVA method. The percentage error was calculated with the help of following equation:

$$\text{Percentage error (\%)} = \frac{(\text{predicted value} - \text{experimental value})}{\text{predicted value}} \times 100 \quad (5)$$

Characterization of optimized microballoons

Determination of particle size

Particle size analysis of the optimized formulation was carried out by optical microscopy method. For the study sizes of 200 particles was determined by calibrating the eyepiece micrometer with the help of stage micrometer. The particles are arranged on the basis of size ranges. The number of particles in each size range are then counted and tabulated. Percentage in each range was calculated using following equation:

$$\text{\% in each range} = \frac{\text{Number of particles}}{200} \times 100 \quad (6)$$

Scanning electron microscopy (SEM)

The external and internal morphology of the optimized formulation was analyzed by SEM (JEOL 5400, Kyoto, Japan). For the study sample was prepared by mounting it over the metal grid with the help of adhesive tape, the grid was than coated with gold ion and visualized under 1x, 150 x and 500 x magnifications.

Fourier transform infrared spectroscopy (FTIR)

The study was performed (IR-Thermo Nicklet 380 US) for the identification of specific functional groups within the samples of: PAN, Eudragit®L100, Eudragit®RS100 and optimized formulation. For the analysis about 3-5 mg of sample was ground in pestle and mortar with 100 mg KBr and transformed in to transparent discs with the help of pellet press then spectra were obtained in the range 4000-500 cm^{-1}. Resulting spectra's between transmittance and wave number (cm^{-1}) were used to identify drug-polymer interactions.

***In vitro* release studies**

The study was carried out at 37 °C in PB pH 6.8 and 7.4 which were prepared by taking 50 ml of 0.2M KH_2PO_4 and 39.1 ml of 0.2 N NaOH in volumetric flask to make volume 200 ml with distilled water. 0.2 M KH_2PO_4 was prepared by dissolving 27.218 g of KH_2PO_4 in distilled water to make volume 1000 ml. Each batch of microballoons equivalent to 40 mg of PAN was added to 250 ml of both mediums in an iodine flask and shaken for 1 h at 50 rpm maintained at 37 °C. The samples were withdrawn at regular intervals of 1 h up to 12 hrs. Each withdrawn sample was immediately replaced with the fresh sample to maintain the sink conditions. Sample was then analyzed at λ_{max} 215 nm and 288 nm in triplicate using UV-spectrophotometer [25]. For confirming the accuracy, the release profiles of check point formulation LRS-O was compared with the marketed formulation Pantop-40 (Aristo pharmaceutical Pvt. Ltd. Mumbai, India).

Drug release kinetics and mechanism

To predict and correlate the release kinetics of PAN loaded microballoons in PB pH 6.8 and 7.4, the obtained data were fitted in to a suitable mathematical models. In zero-order, first-order, Higuchi and Korsmeyer-peppas model plot were made between *T Vs % CDR*, *T Vs log % CDR*, $\sqrt{T}$ *Vs % CDR* and *log T Vs log % CDR*. In Korsmeyer-peppas model the competing release mechanism of the formulations was distinguished as Fickian release (diffusion-controlled with $n \leq 0.43$), Non-Fickian release (anomalous transport; $0.43 \leq n \leq 0.85$) and case-II transport (relaxation-controlled; $*n \geq 0.85$) [26-28].

Stability study

Microballoons after packing in market capsule kept in vials at 40±2 °C/75±5 % RH for a period of six months and sampled each month for physical changes, % buoyancy and % entrapment efficiency and the data obtained was tabulated for both PB pH 6.8 & 7.4. The stability protocol was in compliance with that of the world health organization (WHO) guidelines. One-way analysis of variance (ANOVA) *F*-test was applied for checking the statistical significance (*p>0.05).

RESULTS

The optimal preparative conditions found out were stirring speed of 300 rpm, temperature of 40 °C and the stirring time of 1 h. It was cleared from the results of B %, EE % and CDR12 h % that the optimum concentrations for independent variables was 5 % w/w of Magnesium stearate, 600 mg of Eudragit®L100 and 600 mg of Eudragit®RS100 in order to get the desired responses.

In the experimental designs a total of eight formulations (LRS 1-8) were made. In LRS-2 the percentage swelling in PB pH 6.8 and 7.4 was found to be 0.011-0.045 g/g and 0.016-0.048 g/g for 10 h study. The percentage buoyancy in PB pH 6.8 and 7.4 was between 28.97-88.46 % and 33.82-93.37 % and the percentage entrapment was found out to be 10.88-77.09 % in PB pH 6.8 and 46.39-89.3 % in PB pH 7.4.

The formulation with higher responses for percentage swelling, buoyancy and entrapment efficiency was selected as the best. Formulation LRS-2 gave best results for percentage swelling, buoyancy and entrapment efficiency in both PB pH 6.8 and 7.4 which was found to be 0.045 g/g and 0.048 g/g, 78.88±0.043 % and 87.35±0.012 %, 71.12±0.008 % and 89.30±0.043 %. More over the CDR12 h % was found as 99.50±0.015 % for PB pH 6.8 and 98.68±0.073 % for PB pH 7.4 as shown in table1.

Optimization

For the optimization suitable polynomial equations were generated with the help of Design-Expert 9.0.3 software for all the three dependent variables such as B %, EE % and CDR12 h % for both PB pH 6.8 and 7.4. The equations were analyzed with statistical parameters such as multiple correlation coefficients (R^2) and the predicted residual sum of squares (PRESS) as presented in table 2. The value of PRESS serves as the measure of fitness of the model with the data points. The ANOVA results showed that the PRESS values were smaller for all the models and thus the data points were

better fitted with the model and all the response models were significant with the response parameters.

Process optimization in PB pH 6.8

$$B\% = 60.12 + 22.67X_1 + 15.91X_2 - 4.01X_3 - 4.97X_1X_2 - 1.45X_1X_3 + 13.41X_2X_3 - 0.58X_1X_2X_3 \quad (7)$$

$$[R^2 = 0.9970; \text{F value} = 478.82; {}^{*}p < 0.05]$$

$$EE\% = 45.63 + 26.09X_1 + 10.57X_2 - 7.43X_3 - 2.75X_1X_2 - 5.52X_1X_3 + 4.36X_2X_3 + 0.05X_1X_2X_3 \quad (8)$$

$$[R^2 = 0.9989; \text{F value} = 51186; {}^{*}p < 0.05]$$

$$CDR12h\% = 78.52 + 11.36X_1 + 9.63X_2 - 19.19X_3 - 14.63X_1X_2 + 11.34X_1X_3 + 11.66X_2X_3 \quad (9)$$

$$[R^2 = 0.9990; \text{F value} = 380.63; {}^{*}p < 0.05]$$

From the ANOVA results for B %, EE % and CDR12 h % as the dependent responses, the model equation for B % showed that coefficients b_3, b_{12} and b_{13} had no static significance (*p>0.05) with the model F-value of 380.63 and R^2 value of 0.9970, the model equation for EE % had all coefficients significant (*p<0.05) with model F-value of 51186 and R^2 value of 0.9989, whereas for the model equation for CDR12 h% (PB pH 6.8) it was evident that all the coefficients had static significance (*p<0.05) with the model F-value of 380.63 and R^2value of 0.9990 (table 2).

For model simplification the non-significant terms p>0.05 were eliminating from all the polynomial equations obtained from the multiple regression analysis, so the final equation becomes:

$$B\% (Y_1) = 60.12 + 22.67X_1 + 15.91X_2 + 13.41X_2X_3 - 0.58X_1X_2X_3 \quad (10)$$

$$EE\% (Y_2) = 45.63 + 26.09X_1 + 10.57X_2 - 7.43X_3 - 2.75X_1X_2 - 5.52X_1X_3 + 4.36X_2X_3 + 0.05X_1X_2X_3 \quad (11)$$

$$CDR12\ h\% (Y_3) = 78.52 + 11.36X_1 + 9.63X_2 - 19.19X_3 - 14.63X_1X_2 + 11.34X_1X_3 + 11.66X_2X_3 \quad (12)$$

Table 2: ANOVA summary for response parameters in PB pH 6.8 and 7.4

Source	Sum of squares		d. f. [a]	Mean square		*F*-value		*P*-value (Prob>F)	
pH	**6.8**	**7.4**		**6.8**	**7.4**	**6.8**	**7.4**	**6.8**	**7.4**
(a) B % [b]									
Model	6066.24	6817.90	6	1011.04	1136.32	478.82	377.07	0.0350 (S)	0.0394 (S)
X_1	3150.20	3315.83	1	3150.20	3315.83	1491.91	1100.32	0.0165 (S)	0.0192 (S)
X_2	1551.52	1772.21	1	1551.52	1772.21	734.79	588.09	0.0235 (S)	0.0262 (S)
X_3	98.63	188.47	1	98.63	188.47	46.71	62.54	0.0925 (NS)	0.0801 (NS)
X_1X_2	151.47	285.96	1	151.47	285.96	71.73	94.89	0.0748 (NS)	0.0651 (NS)
X_1X_3	12.98	8.67	1	12.98	8.67	6.15	2.88	0.2441 (NS)	0.3391 (NS)
X_2X_3	1101.45	1246.75	1	1101.45	1246.75	521.64	413.72	0.0279 (S)	0.0313 (S)
(b) EE % [c]									
Model	5543.51	4809.74	6	923.92	801.62	51186.63	752.13	0.0034 (S)	0.0279 (S)
X_1	4169.67	1967.53	1	4169.67	1967.53	2.310E+005	1846.06	0.0013 (S)	0.0148 (S)
X_2	685.24	492.67	1	685.24	492.67	37963.45	462.25	0.0033 (S)	0.0296 (S)
X_3	338.78	719.72	1	338.78	719.72	18769.00	675.29	0.0046 (S)	0.0245 (S)
X_1X_2	46.37	778.94	1	46.37	778.94	2568.89	730.85	0.0126 (S)	0.0235 (S)
X_1X_3	187.02	111.30	1	187.02	111.30	10361.10	104.43	0.0063 (S)	0.0621 (NS)
X_2X_3	116.43	739.59	1	116.43	739.59	6450.63	693.93	0.0079 (S)	0.0242 (S)
(c) CDR12 h% [d]									
Model	6549.90	6592.89	6	1091.65	1098.82	380.63	1526.13	0.0392 (S)	0.0196 (S)
X_1	791.03	814.46	1	791.03	814.46	275.81	1131.20	0.0383 (S)	0.0189 (S)
X_2	569.03	524.56	1	569.03	524.56	198.40	728.55	0.0451 (S)	0.0236 (S)
X_3	2257.58	1373.40	1	2257.58	1373.40	787.16	1907.51	0.0227 (S)	0.0146 (S)
X_1X_2	1311.49	1592.17	1	1311.49	1592.17	457.28	2211.35	0.0297 (S)	0.0135 (S)
X_1X_3	787.85	1128.60	1	787.85	1128.60	274.70	1567.50	0.0384 (S)	0.0161 (S)
X_2X_3	832.93	1159.69	1	832.93	1159.69	290.42	1610.68	0.0373 (S)	0.0159 (S)

X_1, X_2 and X_3 represents amount of Magnesium stearate (% w/w), Eudragit® L 100 (mg) and Eudragit® RS 100 (mg) respectively. X_1X_2, X_1X_3 and X_2X_3 are the interaction effects; S and NS indicate significant and not significant respectively.

[a]d. f. indicate degree of freedom, [b]B % = percentage buoyancy, [c]EE % = percentage entrapment efficiency, [d]CDR12 h % = cumulative percentage drug release over 12 h.

Process optimization in PB pH 7.4

$$B\% = 66.43 + 23.26X_1 + 17.01X_2 - 5.54X_3 - 6.83X_1X_2 - 1.19X_1X_3 + 14.26X_2X_3 - 0.70X_1X_2X_3 \quad (13)$$

$$[R^2 = 0.9986; \text{F value} = 377.07; {}^{*}p < 0.05]$$

$$EE\% = 64.67 + 17.92X_1 + 8.96X_2 - 10.84X_3 - 11.27X_1X_2 + 4.26X_1X_3 + 10.98X_2X_3 - 0.41X_1X_2X_3 \quad (14)$$

$$[R^2 = 0.9988; \text{F value} = 752.13; {}^{*}p < 0.05]$$

$$CDR12\ h\% = 79.56 + 11.53X_1 + 14.97X_2 - 14.97X_3 - 16.12X_1X_2 + 13.57X_1X_3 + 13.76X_2X_3 - 0.34X_1X_2X_3 \quad (15)$$

$$[R^2 = 0.9996; \text{F value} = 1526.13; {}^{*}p < 0.05]$$

The results obtained from ANOVA analysis showed that in the model equations for B % the coefficients b_3, b_{12} and b_{13} had no static significance (* p>0.05) with F-value of 377.07 and R^2 value of 0.9986, the polynomial equation for the response EE % showed only one coefficient b_{13} that has no static significance (*p>0.05) with the model F-value of 752.13 and R^2 of 0.9988, while evaluating the polynomial equation for the response CDR12 h %, all coefficients were statistically significant (*p<0.05) with F-value of 1526.13 and R^2 value of 0.9996 (table 2).

After eliminating the non-significant terms (* p>0.05) the final model equation becomes:

$$B\% (Y_1) = 66.43 + 23.26X_1 + 17.01X_2 + 14.26X_2X_3 - 0.70X_1X_2X_3 \quad (16)$$

$$EE\% (Y_2) = 64.67 + 17.92X_1 + 8.96X_2 - 10.84X_3 - 11.27X_1X_2 + 4.26X_1X_3 + 10.98X_2X_3 - 0.41X_1X_2X_3 \quad (17)$$

$$CDR12\ h\% (Y_3) = 79.56 + 11.53X_1 + 14.97X_2 - 14.97X_3 - 16.12X_1X_2 + 13.57X_1X_3 + 13.76X_2X_3 - 0.34X_1X_2X_3 \quad (18)$$

The results of investigated responses such as B %, EE % and CDR12 h % for all batches of formulations (LRS 1-8) for both the mediums PB pH 6.8 and 7.4 was found within the limits. Linear correlation plots between the actual and the predicted responses are depicted in Figure1 (PB pH 6.8) and fig. 2 (PB pH 7.4). Further we have elucidated the main and the interaction effects of independent variables over the responses through response surface method using Design-Expert 9.0.3 software, as it is considered as the best for the development and optimization of formulations.

In PB pH 6.8 the response surface plots for B % showed an increase in response with increase of both Magnesium stearate (X_1) and Eudragit® L100 (X_2), the response surface plots related to EE % predicts an increase of response with an increase of both Magnesium stearate (X_1) and Eudragit® L100 (X_2) and decrease of Eudragit® RS 100 (X_3), whereas the plot for CDR12 h % showed an increase with increasing Magnesium stearate (X_1) and Eudragit® L100 (X_2) and decreasing Eudragit® RS100 (X_3) fig. 3(a-c). On the other hand the response surface plots obtained in case of PB pH 7.4 depicts increase of B % with increasing Magnesium stearate (X_1) and Eudragit® L100 (X_2) whereas increase in EE % as well as CDR12 h % was due to increase of both Magnesium stearate (X_1) and Eudragit® L100 (X_2) and decrease of Eudragit® RS 100 (X_3) fig. 4 (a-c).

Optimized formulation with the desired response was obtained by numerical optimization technique using the desirability approach. The desirable values of independent variables (factors) in PB pH 6.8 were: X_1 = 5.00 %, X_2 = 601.01 mg and X_3 = 600.00 mg, whereas the desirable ranges of dependent responses were restricted to 75 ≤ B % ≤ 80 , 70 ≤ EE % ≤ 75 and 95 ≤ CDR12 h % ≤ 100. The obtained predicted values for dependent variables were B % = 79.37, EE % = 71.09 and CDR 12 h % = 99.99. In PB pH 7.4 the desirable values of independent variables are: X_1 = 5.00 %, X_2 = 600.00 mg and X_3 = 600.00 mg while the desirable ranges of dependent responses were restricted to: 85 ≤ B % ≤ 90, 85 ≤ EE % ≤ 90 and 95 ≤ CDR12 h % ≤ 100. The predicted values for the responses were B % = 87.96, EE % = 89.66 and CDR12 h % = 98.98 (table 3).

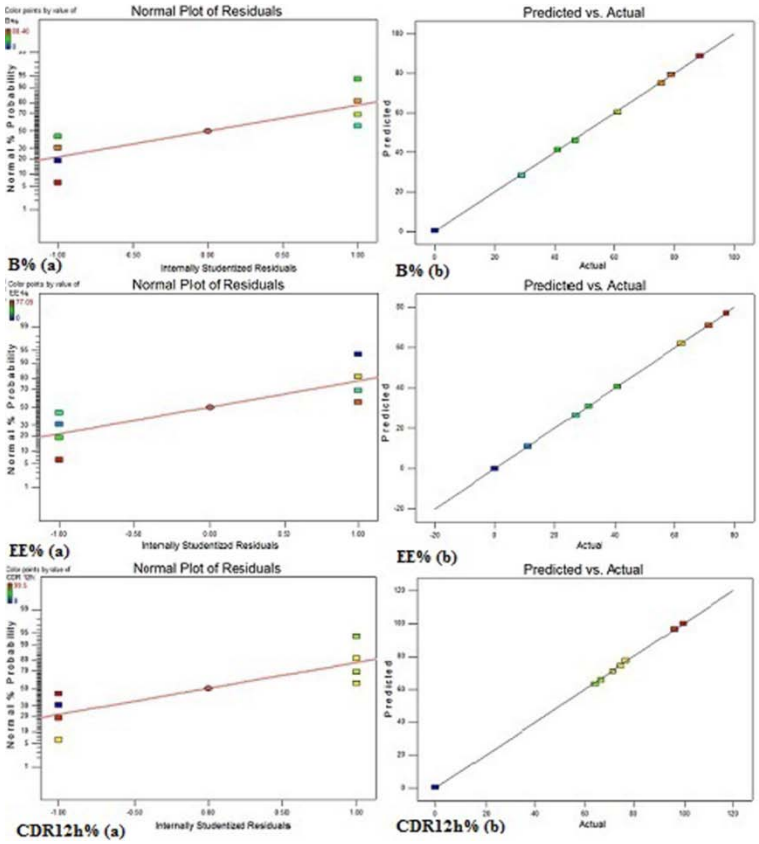

Fig. 1: Residual plot showing scatter of residuals versus predicted values (a), and linear correlation plot between the actual and predicted values (b) in phosphate buffer pH 6.8 for B, EE and CDR12 h %

Table 3: Confirmation of optimization capability

Code	Composition				Predicted value	Experimental value	Percentage error[d]
	X_1 (Magnesium stearate, % w/w)	X_2 (Eudragit® L100, mg)	X_3 (Eudragit® RS100, mg)	Response (Y, %)			
LRS-O (pH 6.8)	5.00	601.01	600.00	B[a]	79.37	78.88±0.23	0.617
				EE[b]	71.09	71.12±0.04	-0.042
				CDR12h[c]	99.99	99.50±0.08	0.490
LRS-O (pH 7.4)	5.00	600.00	600.00	B[a]	87.96	87.35±0.68	0.693
				EE[b]	89.66	89.30±0.05	0.363
				CDR12h[c]	98.98	98.68±0.37	0.303

[a]B = buoyancy; [b]EE = entrapment efficiency; [c]CDR12 h = cumulative drug release over 12 h; [d]Percentage error (%) = (predicted value−experimental value)/predicted value x 100.

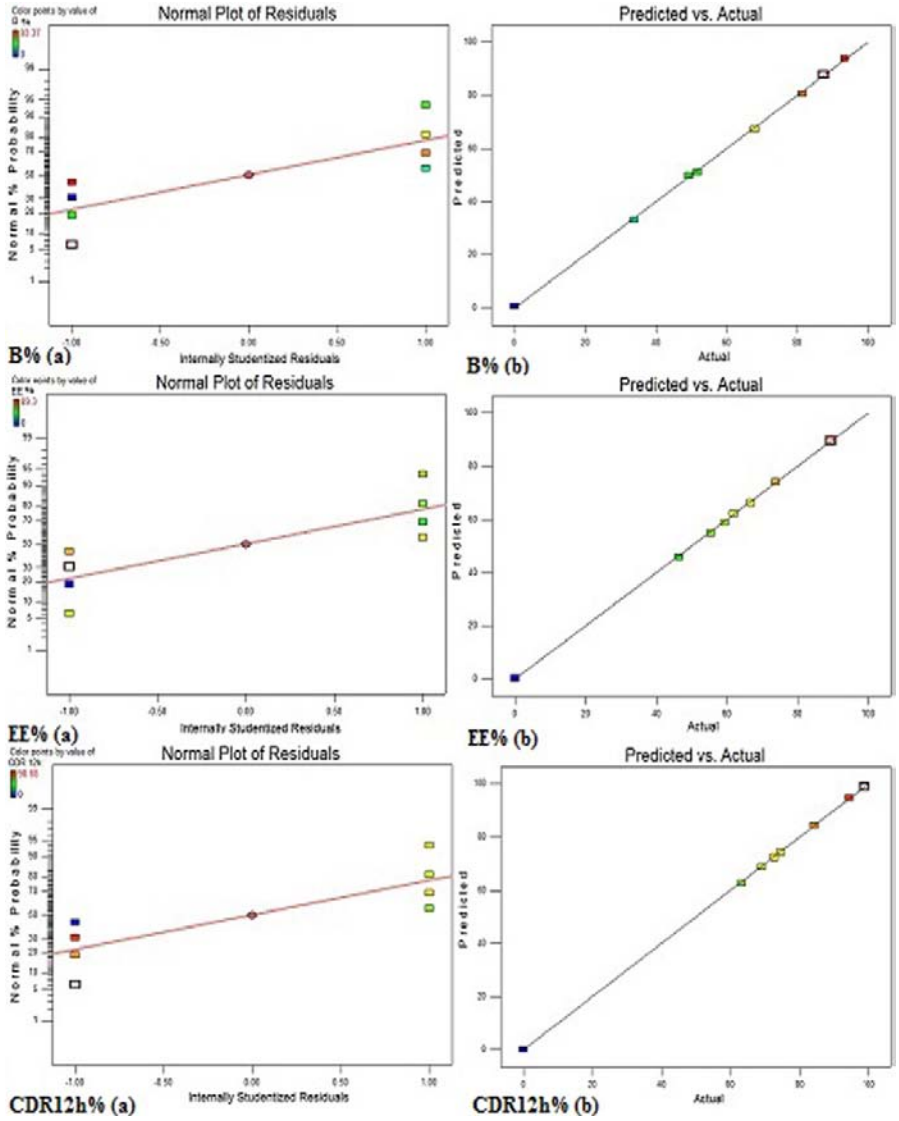

Fig. 2: Residual plot showing scatter of residuals versus predicted values (a), and linear correlation plot between the actual and predicted values (b) in phosphate buffer pH 7.4 for B, EE and CDR12 h %

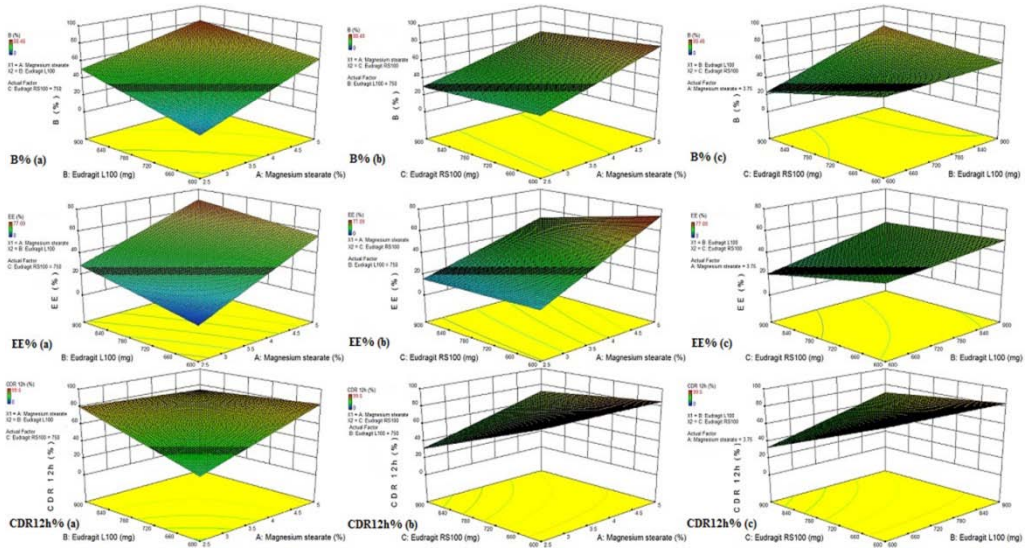

Fig. 3:(a-c) Response surface plot predicting mutual effects of amount of Magnesium stearate (% w/w), Eudragit®L100 (mg) and Eudragit®RS100 (mg) on B, EE and CDR12 h % in phosphate buffer pH 6.8

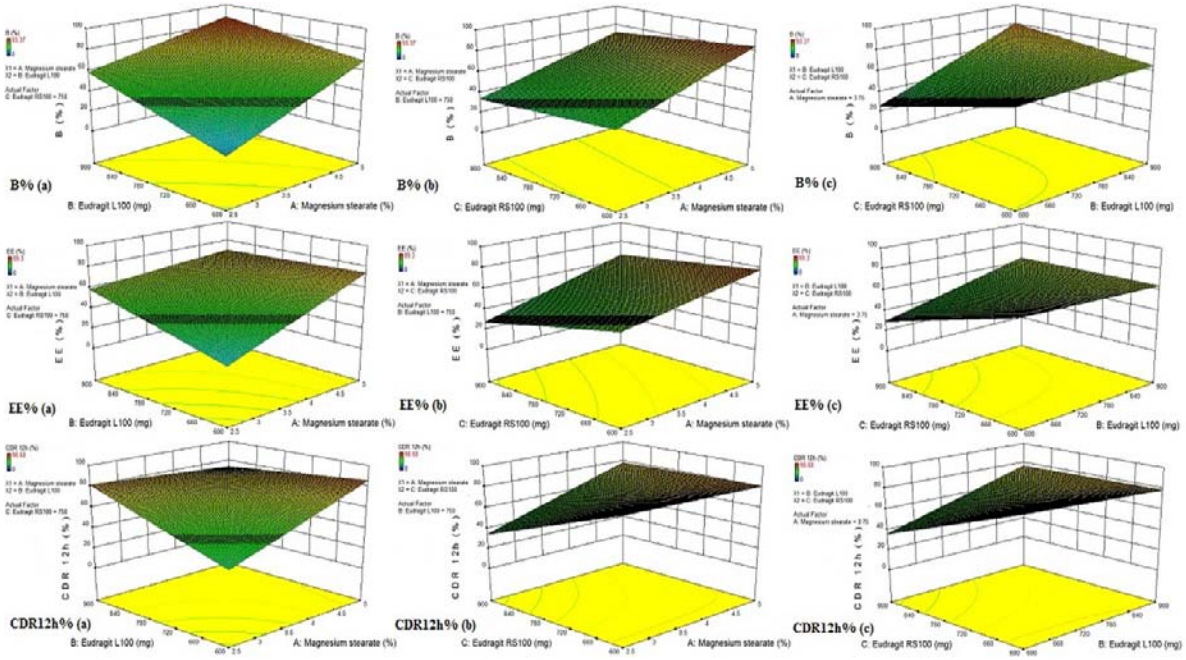

Fig. 4:(a-c) Response surface plot predicting mutual effects of amount of Magnesium stearate (% w/w), Eudragit®L100 (mg) and Eudragit®RS100 (mg) on B, EE and CDR12 h % in phosphate buffer pH 7.4

Validation of factorial design

Further for validating the optimization capability an extra check point formulation LRS-O was formulated using the optimal process variables and evaluated for B %, EE % and CDR12 h % for both the mediums. The experimental and the predicted responses by the mathematical model were presented in table 3 and the percentage error was calculated. Optimized formulation (LRS-O) showed buoyancy of 78.88±0.23 %, entrapment efficiency of 71.12±0.04 % and drug release in 12 h of 99.50±0.08 % in PB pH 6.8 with smaller error values (0.617, -0.042 and 0.490). Whereas in PB pH 7.4 the actual values for B %, EE % and CDR12 h % was 87.35±0.68 %, 89.30±0.05 % and 98.68±0.37 % with smaller error values (0.693, 0.363 and 0.303) respectively. Hence this validation approach confirms that the mathematical models obtained from 2^3 FFD were well fitted.

Characterization of optimized microballoons

Particle size analysis

The sizes of dried PAN-loaded microballoons for all LRS formulations (LRS 1-8) were measured by optical microscopy method and the obtained average particle size ranges from 20-120 µm. While analyzing the optimized formulation (LRS-O) it was found that the maximum frequency of particles (27.5±0.07 %) was in the range 80-100 µm.

Surface morphology

SEM of the optimized PAN-loaded microballoon (LRS-O) showed rough surface, spherical shape with internal hollow cavity. No drug crystals were found on the surface of microballoons, indicates that the drug was homogeneously dispersed within the polymer blend (fig. 5).

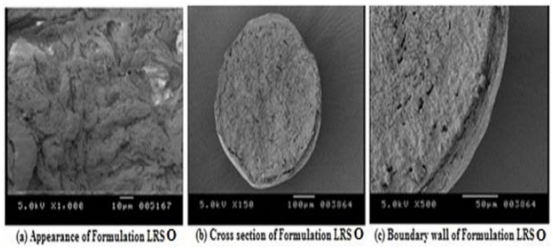

Fig. 5: Scanning electron micrographs (a) surface appearance (b) cross-sectional view and (c) porous boundary wall of formulation LRS-O

Fourier transform infrared spectroscopy

Eudragit®L100 gave characteristic bands at 3613, 2950, 1708, 1450, 1153, 839 and 753 cm^{-1} which was due to the presence of $[O–H]_{str}$; $[C–H]_{str}$; $[C=O]_{str}$; $[C–H]_{Bend\ in\ plane}$; $[C–O]_{str}$; $[C–C]_{str}$ and $[C–H]_{Rocking}$. Eudragit®RS100 showed the bands at 2951, 1724, 1448, 1238, 1143, 988, 848 and 752 cm^{-1}which corresponds to $[C–H]_{str}$; $[C=O]_{str}$; $[C–H]_{Bend\ in\ plane;}$ $[C–N]_{str}$; $[C–O]_{str}$; $[C–C]_{str}$; $[C–H]_{Rocking}$ and $[C–Cl]_{str}$. The principle FTIR peaks of pure drug PAN were observed at 2942, 1588, 1376, 1303, 1040, 983, 838 and 796 cm^{-1} which was due to $[C–H]_{str}$; $[C=N]_{str}$; $[S=O]_{str}$; $[C–H]_{def}$; $[C–F]_{str}$; $[C–O]_{str}$; $[C–C]_{str}$ and $[N–H]_{Rocking}$. While LRS O showed peaks at 3466, 2955, 1724, 1473, 1438, 1449, 1271, 1144 and 1040 which was due to $[O–H]_{str}$; $[C–H]_{str}$; $[C=O]_{str}$; $[C=N]_{str}$; $[S=O]_{str}$; $[C–H]_{Bend\ in\ plane}$; $[C–N]_{str}$; $[C–O]_{str}$ and $[C–F]_{str}$ respectively (fig. 6).

***In vitro* drug release**

The study for all the formulated PAN-loaded microballoons (LRS 1-8 and LRS-O) and marketed formulation (LRS-M) were carried out in PB pH 6.8 and 7.4 (fig. 7). All the formulations and the marketed formulation sustained the PAN release over 12 hrs. This is attributed due to Magnesium stearate which lowers the density in turn provides buoyancy to the system thus retards and sustains the release up to 12 hrs [29].

The use of polymer mixture along with plasticizer increases the density of the polymer matrix and thus increases the diffusion path length, which favors in prolonged drug release characteristics. Moreover Eudragit®E100 dissolution occurs above pH 6 and hence prolongs the release whereas Eudragit®RS100 is pH independent polymer and the ammonium group present favors channel formation for the dissolution medium to enter thus initiates dissolution and diffusion of drug [30].

Kinetics of drug release

The *in vitro* drug release data of formulations (LRS-2, O and M) for both mediums PB pH 6.8 and 7.4 were fitted in to kinetic models such as zero order, first order, Higuchi, and Korsmeyer-Peppas and the results obtained was given in table 4. When respective correlation coefficients of these formulations in both the mediums were compared, the PAN release from formulations LRS-2, O and M in PB pH 6.8 follows zero order over a period of 12 hrs, with initial burst release in first hour than sustained it for the rest of the period. Whereas in PB pH 7.4 the formulations LRS-O and M follows zero

order release rate while LRS-2 follows Korsmeyer-Peppas model with release exponent n = 0.1, follows fickian release mechanism (diffusion-controlled with $n \leq 0.43$), thus offers diffusion-controlled PAN release from microballoons.

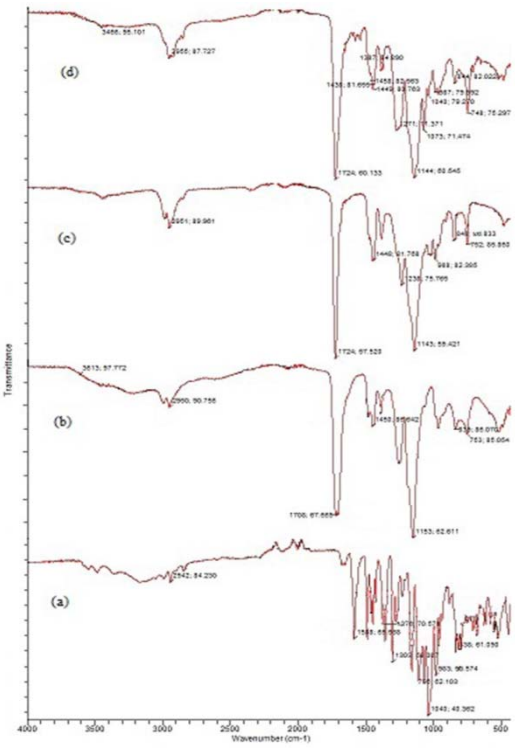

Fig. 6:FTIR spectra of (a) PAN, (b) Eudragit®L100, (c) Eudragit®RS100 and (d) Optimized formulation LRS-O containing PAN

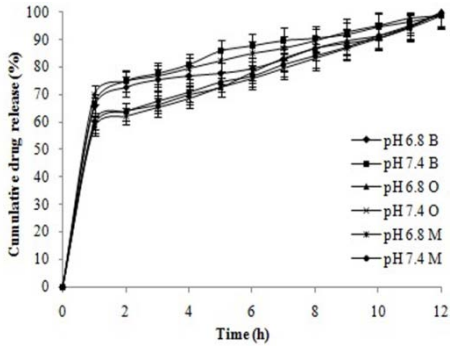

Fig. 7: Comparative cumulative percentage *in vitro* drug release profiles of Best (B), Optimized (O) and Marketed (M) LRS formulations in PB pH 6.8 and 7.4 for 12 h (37±0.5 °C, 300 rpm)

Stability study

The results of stability study of the optimized formulation carried out for a period of six months showed no physical change among themselves. The ANOVA table values for *F* at 5 % level of significance for B % and EE % was 16.29 and 15.16 in PB pH 6.8 and 9.08 and 28.29 in PB pH 7.4.

DISCUSSION

The floating buoyant system for the administration of acid labile drugs through oral route using acrylic polymers was successfully formulated employing 2^3 factorial designs by emulsion solvent diffusion method. The conventional pulsatile release enteric dosage forms usually shows a drug release lag period of 5-6 h in large intestine affecting the efficacy [31, 32]. Moreover in small intestine the higher viscosity of contents may sometimes cause hindrance to drug diffusion and degradation by enzymes, so an effective enteric coat was recommended for the intestinal delivery [33]. The present study clearly demonstrates how effectively concentration of polymer mixture could sustain and enhance the gastric retention to increase the bioavailability [34]. Since the drug used was highly soluble and permeable so in order to retard its release and to protect its degradation from acidic environment we have used Eudragit®L100 and RS100 in definite proportions.

In the design Magnesium stearate in higher concentration 5 % w/w provides hydrophobicity to the formulation thus reduces the density and provides floatability. Also it may inhibit the hydration of the polymer matrices and thus sustains the drug release profile of PAN from the buoyant systems [35]. Eudragit®L100 in low concentration encapsulates the drug and retards its release in stomach as it shows pH dependent dissolution in jejunum whereas Eudragit®RS100 in low concentration decreases the drug release and increases the entrapment efficiency as the pore formed will be less and shows pH independent dissolution after swelling in the GIT. In the swollen state it is permeable to water and dissolves actives [36]. The swelling depends on the relative rate of diffusion of medium through pores inside the polymer matrix and on the rate of polymer chain relaxation [37]. In order to enhance the solubility of Eudragit®RS100 in the solvent mixture, plasticizer DBT was used that reduces its glass transition temperature. The B % was correlated to the use of equal amount of solvents ethanol and dichloromethane for the dissolution of polymers, they evaporates forming cavity inside when stirred at 40 °C in PVA solution containing sodium citrate buffer. Thus both dissolution and disruption of microballoons in acidic environment could be prevented that may be advantageous in prolonging the GRT.

Increase in the size was found with increase in the polymer ratios in the formulations. This could be attributed due to the increased viscosity of the emulsion when cross-linking agent (DBT) was added that in turn increases the droplet size when poured in to the PVA solution. The porosity or channels on the boundary wall was due to the porous nature of the polymer Eudragit®RS100 and also due to channeling effect of sodium chloride. The rough appearance of LRS-O may be attributed to the use of plasticizer (DBT) that results in higher cross-linking. In case of LRS-O formulation all characteristic bands of PAN appeared with slight variations in wave numbers that is due to the higher cross-linking, indicates that there were no chemical interactions between the pure drug and the polymers used.

Passive diffusion of drug from microballoons showed to occur in two steps firstly, the leaching out of drug through pores in to the polymer matrix, secondly diffusion from matrix in to the dissolution medium [38]. As dissolution medium enters the formulation the pH sensitive polymer swells, the swollen particles forms closely packed network, which hinders further entry of dissolution medium thus results in retardation of drug release over 12 h study [39, 40]. The optimized LRS-O follows zero order release model, independent of the concentration of polymers used whereas LRS-2 follows relaxation-controlled mechanism [41].

To restrict the number of trials for optimization, RSM was successfully used that saves time, more effective and economical when compared to the conventional methods [42, 43]. For the reliability of the model design dissolution test was performed for both, the formulations with predicted optimum polymer concentrations and for the additional check point formulation covering the entire experimental domain [44]. Design Expert software provides the ANOVA provision for the statistical validation of polynomials. Subsequently, the feasibility and grid searches were performed to locate the composition of optimum formulations [45]. Model simplification was carried out by eliminating the non-significant terms [46]. Statistically significant difference between *in vitro* drug releases of PAN from the formulations was defined as $p<0.05$ [47]. During the stability studies since the calculated value for *F* was found to be less than the tabulated (F_{Tab} = 225), the difference was not significant and we conclude that the means do not differ among themselves only a slight decrease in buoyancy and entrapment efficiency was observed that was insignificant.

Table 4: Different drug release models as applied to percentage drug release profiles of LRS-2, O and M formulations in PB pH 6.8 and 7.4

Code	PB	Evaluation parameters	Zero order	First order	Higuchi model	Peppas model	Best fit model
LRS-2	pH6.8	r^2	0.973	0.970	0.948	0.914	Zero order
		A	65.20	1.822	52.87	1.805	
		B	2.674	0.014	12.19	0.150	
	pH7.4	r^2	0.938	0.909	0.984	0.991	Korsmeyer-Peppas
		A	69.61	1.846	56.73	1.823	
		B	2.561	0.013	12.11	0.152	
LRS-O	pH6.8	r^2	0.993	0.985	0.976	0.934	Zero order
		A	55.72	1.762	38.60	1.737	
		b	3.675	0.020	16.82	0.218	
	pH7.4	r^2	0.999	0.996	0.972	0.928	Zero order
		A	54.27	1.752	37.55	1.728	
		b	3.634	0.020	16.55	0.217	
LRS-M	pH6.8	r^2	0.994	0.986	0.987	0.947	Zero order
		A	68.85	1.844	56.58	1.827	
		b	2.603	0.013	11.97	0.143	
	pH7.4	r^2	0.998	0.997	0.968	0.922	Zero order
		A	56.68	1.769	40.65	1.747	
		b	3.494	0.019	15.89	0.204	

PB: phosphate buffer; LRS-O: Optimized formulation; LRS-M: Marketed formulation

CONCLUSION

The study demonstrates the potential of PAN-loaded microballoons prepared by emulsion solvent diffusion method employing non-effervescent technique with 2^3factorial designs for the intestinal delivery. This technique may be advantageous in achieving enhanced bioavailability of acid labile drugs for the gastro retentive delivery with excellent responses of B %, EE % and CDR12 h % following zero order patterns. The Design Expert software used for the optimization and validation of formulation design was economical and reduces the number of trials. SEM confirms the spherical shape with rough surface, porous boundary wall and internal hollow cavity moreover the FTIR results showed that there were no drug-polymer interactions found.

CONFLICT OF INTERESTS

The authors report no conflicts of interest

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CONFLICT OF INTERESTS

Declared None

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