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Development, Optimization and *In Vitro* / *In Vivo* Evaluation of Pantoprazole Sodium Loaded Eudragit Microballoons for Stomach Specific Delivery

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ABSTRACT

In the present study we have developed a non-effervescent gastro retentive dosage form containing pantoprazole sodium by emulsion solvent diffusion method. A 2^3 full factorial design was used for the optimization process and the various responses obtained were evaluated by fitting in the binomial equations. Formulations ERS 1-8 were made utilizing three independent variables such as amount of crospovidone (%), Eudragit®E100 (mg) and Eudragit®RS100 (mg) which were varied at low and high levels and are evaluated for percentage buoyancy, entrapment efficiency and cumulative drug release after 5 h in buffer pH 2.2. The experimental values of the optimized formulation ERS-O coincides well with the predicted values obtained from optimization technique. Design expert 9.0.3 predicts responses as % buoyancy of 75.04±0.05, % entrapment efficiency of 87.57±0.21 and cumulative drug release after 5 h of 97.93±0.15 which were close to the actual values validates the design. The optimized formulation were in size range 20-120 µm with spherical shape, internal hollow cavity and porous boundary wall, moreover only physical cross-linking occurred with zero order release pattern and the *in vivo* analysis through ethanol induced ulceration method gave better ulcer healing orally than the intravenous route.

Keywords: Emulsion solvent diffusion method; Non effervescent; Gastro retentive drug delivery; Gastric residence time; Anti-ulcer activity

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INTRODUCTION

Various dosage forms developed reported were microballoons, granules, powders, pills and laminated films[1, 2]. In FDDS (Floating Drug Delivery Systems) two technological developments reported were non-effervescent and effervescent systems. In non-effervescent systems drug is mixed with gel forming excipients such as hydrocolloids, polysaccharides and matrix forming polymers: polycarbonate, polyacrylate, polymethacrylate and polystyrene, as they are harmless and inert compounds, not absorbed in the gastrointestinal tract (GIT) and are resistant to body fluids[3]. They stay for a limited time in the GIT, are excreted unchanged, and do not produce degradation products[4]. The gel forming polymers swells when comes in contact with the gastric fluid, imparts buoyancy and also acts as drug reservoir for sustaining the release[5]. In previous reported works pantoprazole sodium (PAN) has been successfully encapsulated with Eudragit®S grades by emulsion solvent evaporation and spray drying methods for its protection in the gastric mucosa[6,7]. Effervescent technique has been reported with the side effects of violent gas generation; disintegration of dosage form; burst release; dose dumping and produces alkaline microenvironment[8]. Response surface methodology (RSM) reduces the number of trials in formulation design. It is more effective and economical when compared to conventional method for optimization[9-11]. PAN suffers from incomplete absorption in GIT with oral bioavailability of 77 % and elimination half life of 1 h. It has lower affinity for cytochrome P 450 than omeprazole and lansoprazole that lowers the risk of drug interaction and also the low dose of 40 mg OD makes it a suitable drug candidate for the study[12, 13]. It acts by inhibiting the gastric H^+/K^+ ATP ase via covalent binding to cysteine residues of the proton pump and converts to its active form upon accumulation in the gastric parietal cells (Figure 1). Also it is considered as the drug of choice in case of ulceration by ethanol as its pharmacokinetics showed negligible interaction with ethanol when compared with other anti-ulcer drugs like cimetidine [14]. To avoid its degradation in the gastric environment it is administered using enteric-coated dosage forms [15] or through intravenous (IV) route. Enteric coating of famotidine with Eudragit® L100 was reported to target the stomach utilizing PEG 6000 as pore former results in good buoyancy (> 10 h) and release characteristics with lower entrapment efficiency up to 33-56 % [16], in contrast an attempt have been made to formulate it with Eudragit® E100, utilizing crospovidone and Eudragit® RS100 with release studies up to 5 h, which would protect the drug through encapsulation further a protective alkaline buffer like sodium citrate increases the bioavailability and release it in sustained manner. Eudragit® E100 targeted drug release area is stomach, soluble in gastric pH below 5 in solvents

like acetone-alcohols, dichloromethane and ethyl acetate[17]. In Eudragit® RS100 the ammonium groups are present as salts that make the polymer permeable[18]. Crospovidone acts as a swelling agent, capable of swelling greater than its original volume when comes in contact with an aqueous fluid, such as gastrointestinal fluid[19]. Sodium chloride was incorporated in the coating dispersion to serve as channeling agent. When the coated particles come in contact with aqueous medium, it leaches out creating channels within the film coat[20]. Sodium citrate was used as buffering agent to get the desired pH of the medium[21]. The microballoons formed were evaluated for percentage swelling, buoyancy and entrapment efficiency. Microballoons were characterized for particle size, SEM, FTIR, *in vitro* release behavior, the release mechanisms and diffusion coefficients in release medium, at different reaction parameters and for the *in vivo* anti-ulcer activity. The aim of the present study was to protect the drug from gastric acid degradation, provide effective anti-ulcer activity by 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. For achieving the above mentioned objective 2^3 full factorial design was used and validated via Design expert 9.0.3 software (Stat-Ease Inc., USA).

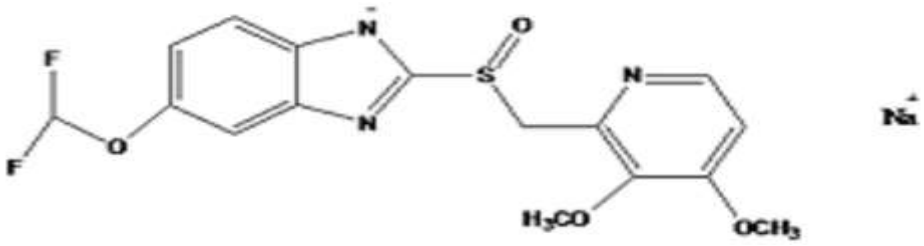

Figure 1: Chemical structure of Pantoprazole sodium (PAN).

METERIALS AND METHOD

Pantoprazole sodium (PAN) was provided as a gift sample from Akum Drugs (Haridwar, India); Crospovidone was obtained from Cachet pharma (bhiwadi, Raj. India); Eudragit® E100 and RS100 were received from Evonic Pvt. Ltd. (Mumbai, India). Ethanol and dichloromethane from s.d Fine-chemicals Pvt. Ltd. (Mumbai, India); Polyvinyl alcohol from Qualikems Fine Chemicals Pvt. Ltd. (New Delhi, India); sodium citrate and sodium chloride from Loba-Chemie Pvt. Ltd (Mumbai, India). All other chemicals used were of analytical grades. Differential spectrophotometric studies were carried out using double-beam UV spectrophotometer -2203, Systronics Pvt. Ltd. (Ahmedabad, Gujarat, India).

Synthesis of Pantoprazole sodium (PAN) loaded microballoons

They were prepared by emulsion solvent diffusion method. For the preparation Eudragit® E100

(600-900 mg) and RS100 (600-900 mg) were dissolved in ethanol (8 ml) - dichloromethane (8 ml) solution. Drug (40 mg) mixed with sodium chloride (0.09 g) was added to crospovidone (2-5 %) solution in ethanol-dichloromethane mixture, drug may get adhered over the crospovidone particles. This drug mixture was added to above prepared polymer solution with continuous stirring at 300 rpm. This polymer solution containing drug was slowly introduced into aqueous solution of PVA containing sodium citrate (1 % w/v) maintained at 40 °C on a magnetic stirrer at 300 rpm for 1 h to get oil in water type of emulsion [22]. The dispersed droplets get solidified in the aqueous phase by evaporation of the solvents and the prepared microballoons were collected by filtration, washed three times with distilled water, dried at room temperature until constant weight and kept in desiccators.

Experimental design

For the optimization of immediate release PAN formulation 2^3(three-factor and two-level) full factorial design was employed. Amount of crospovidone ($X_1, \%$), Eudragit® E100 (X_2, mg) and Eudragit® RS100 (X_3, mg) were selected as independent variables, which were varied at two levels (low and high). The responses (dependent variables) studied were buoyancy ($Y_1, \%$), drug entrapment efficiency ($Y_2, \%$) and amount of drug released in 5 h ($Y_3, \%$) in buffer pH 2.2. Design-expert 9.0.3 software was used for the generation and evaluation of experimental design. In the experimental design the effect of three factors, two factor levels and their interaction on three basic responses were investigated. So the factorial design will consist of 2^3 (8) formulations as shown in Table 1.

Table 1: Full factorial design layouts (2^3) for different formulations containing pantoprazole sodium

	Variables					
Code	**Independent variables (factors, *X*)**			**Dependent variables (responses, *Y*)**		
	Amount of crospovidone ($X_1, \%$)	Eudragit® E 100 (X_2, mg)	Eudragit® RS 100 (X_3, mg)	*B %* [a,e]	*EE %* [b,e]	*CDR 5h %* [c,e]
ERS-1	2 (-1)	600(-1)	600(-1)	Cd	Cd	Cd
ERS-2	5 (+1)	600(-1)	600(-1)	40.92±0.057	64.54±0.247	76.67±0.035
ERS-3	2 (-1)	900(+1)	600(-1)	43.64±0.043	58.62±0.311	80.03±0.037
ERS-4	5 (+1)	900(+1)	600(-1)	58.28±0.035	82.13±0.449	84.85±0.029
ERS-5	2 (-1)	600(-1)	900(+1)	34.26±0.057	51.81±0.414	61.32±0.049
ERS-6	5 (+1)	600(-1)	900(+1)	74.46±0.035	87.27±0.432	97.93±0.043
ERS-7	2 (-1)	900(+1)	900(+1)	69.48±0.043	67.87±0.566	78.13±0.029
ERS-8	5 (+1)	900(+1)	900(+1)	86.90±0.043	66.91±0.470	47.98±0.037

(+1) = higher values and (-1) = lower values. [a] *B %* = percentage buoyancy. [b] *EE %* = drug percentage entrapment efficiency. [c] *CDR 5h %* = cumulative percentage drug release. [d] Cd = collapsed. [e] Mean ± S.D.; n = 3

EVALUATION OF MICROBALLOONS

Percentage swelling (P_s)

The effect of pH of the medium on swelling kinetics of microballoons was carried out by gravimetric method in triplicate [23]. For the study 50 mg of microballoons were immersed in buffer pH 2.2 (100 ml, 37 ± 0.5 °C) in a beaker, maintained at 100 rpm on a magnetic stirrer. After fixed intervals of 30 min they were removed and weighed immediately. The difference in weight gave the amount of water uptake by the microballoons. The P_s of microballoons were calculated by following equation:

$$Ps = \left\{\frac{Ws - Wd}{Wd}\right\} \times 100 \qquad \text{Eq. (1)}$$

Where, W_s is weight of swollen and W_d is the weight of the dried microballoons.

In vitro buoyancy (*B %*)

50 mg of microballoons were placed in buffer pH 2.2 (100 ml, 37 ± 0.5 °C) containing 0.02 % w/v Tween 80 in a beaker. The mixture was stirred at 100 rpm on a magnetic stirrer. After 5 h, the microballoons that floated (W_F) and those settled or not floated (W_{NF}) were collected and weighed. Formulations were dried in desiccators until constant weight is achieved. Percentage buoyancy (*B %*) was determined by using the following equation [24] :

$$B\% = \left\{\frac{W_F}{W_F + W_{NF}}\right\} \times 100 \qquad \text{Eq. (2)}$$

Entrapment efficiency (*EE %*)

Prepared dried microballoons equivalent to 40 mg of PAN was mechanically busted and dissolved in 100 ml of buffer pH 2.2, than filtered through whatman filter paper 1. The dissolved drug amount was measured spectrophotometrically at λ_{max} 290 nm. The amount of drug entrapped was calculated using the following formula.

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

Selection and validation of experimental design

The optimized formulation was selected on the basis of evaluation results of the response parameters. The formulation with maximum % buoyancy, entrapment efficiency and *in vitro* drug release after 5 h was selected. In order to validate the design polynomial equations were

generated for each response parameters manually as well as through software. The different responses were fitted in the following binomial equation for the evaluation:

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

The main effects and the mean value, b_0 intercept 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} are 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 [25]. For the optimization of the formulation process so that the responses could be maximized model matrix method was used. 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 were constructed for each effect. To validate the polynomial equation model one check point formulation ERS-O was also formulated and evaluated. The relationship between the variables was studied by placing the values of the estimated effects in the polynomial model. For estimating the significance of model design and individual response parameters ($p < 0.05$) one-way ANOVA was applied.

Characterization of optimized microballoons

Particle size analysis

The optimized formulation was analyzed by optical microscopy method. The microballoons were immersed in distilled water and the eye piece micrometer was calibrated with the help of stage micrometer and size of 200 particles was analyzed. From the data obtained particle size distribution was plotted and average size was found out.

Scanning electron microscopy (SEM)

Surface appearance and the cross-sectional analysis of the optimized formulation were carried out with scanning electron microscope (JEOL 5400, Kyoto, Japan) at different magnifications. Gold coated microballoons were mounted on metal grids for the analysis.

Fourier transform infrared (FTIR) spectroscopy

About 3-5 mg of sample was added to approximately 100 mg of KBr. The mixture was than ground to a fine powder using mortar and pestle, and transparent discs were formed using a pellet press. The discs were placed in the FTIR spectroscopy apparatus and spectra were collected in the range 4000-500 cm^{-1}.

***In vitro* release studies**

The *in vitro* release dynamics of the optimized formulation was carried out by placing dried and loaded microballoons equivalent to 40 mg of drug in definite volume of releasing medium (buffer pH 2.2, 900 ml) at 37 °C. Buffer pH 2.2 was prepared by mixing 50 ml of 0.2 M KCl with 7.8 ml of 0.2 N HCl in 200 ml volumetric flask, final volume was adjusted with distilled water [26]. The amount of drug release was analyzed at λ_{max} 290 nm using UV-spectrophotometer after fixed interval of 1 h up to 5 h and the release profile of the drug was calculated [27]. The release profiles of the check point formulation ERS-O was also determined and compared with the marketed formulation Pantop-Fast (Aristo pharmaceutical Pvt. Ltd. Mumbai, India).

Drug release kinetics and mechanism

Drug release mechanism for ERS formulations were found out by plotting the dissolution profiles of formulations according to various kinetic models [28]. The highest value for coefficient of determination (R^2) and the release exponent (n) was determined for finding out the mechanism involved. Fickian diffusion is related to $n < 0.5$, whereas n = 1 indicates case II transport (zero order or time dependent drug release) and $n > 1$ super case II transport. And the values of n between 0.5-0.1 identify anomalous (Non-Fickian) diffusion, corresponding to coupled diffusion (diffusion and erosion) such that more than one type of release mechanism.

Stability study

The optimized formulation ERS-O after packing with market capsule was placed in vials and kept at 40 ± 2 ^{o}C/ RH 75 ± 5% for 6 months in case of the accelerated stability examination and sampled each month for physical changes, % buoyancy and % EE. The samples were withdrawn after 1, 2, 3, 4, 5 and 6 months and reanalyzed for % buoyancy and entrapment efficiency. The results obtained were checked for statistical significance using the one-way analysis of variance (ANOVA) *F*-test for testing the equality of several means, the *p*-value < 0.05 was considered statistically insignificant.

***In-vivo* anti-ulcer activity**

The cytoprotective activity was performed by dividing male wistar rats in to four groups each containing eight animals, weighing between 150-200 g, are deprived of food 18 h prior to the experiment but are allowed to have free access to water and kept in straining cages to prevent coprophagy. Each group was administered ethyl alcohol (5 ml. kg^{-1}). After 1 h of ethyl alcohol administration each group receives respective doses of drug or the formulation orally. Group-1 represents the control received ethyl alcohol only, Group-2 was designated as standard-1 with sodium bicarbonate solution (4.2 %) and Group-3 was standard-2 received the cytoprotective drug PAN dissolved in distilled water IV (2 mg. ml^{-1}). Group-4, the treatment received ERS-O

suspended in 0.1 % gum acacia (equivalent to 2 mg. ml^{-1} of PAN). Two hour after the administration of appropriate doses, the animals were anesthetized with diethyl ether, sacrificed and their stomachs were removed, cut at greater curvature, gently rinsed under tap water, stretched on a piece of thermo coal with mucosal site up and analyzed for gastric lesions. Ulcer index (*UI*) was found out using equation below [29]:

$$UI = \frac{10}{x} \qquad \text{Eq.(5)}$$

Where, *x* represents the total mucosal area (*TM*) divided by the total ulcerated area (*TU*).

MEAN ± SEM was found out for each groups and a graph showing the *UI* for the respective groups was made using graph pad prism® version-6.05. The experimental protocols were approved by International Animal Ethics Committee (SMGI/SMIP/IAEC/2015/011) Sir Madanlal institute of pharmacy, Etawah, Uttar Pradesh, India.

RESULTS AND DISCUSSION

The microballoons were successfully prepared by emulsion solvent diffusion method using 2^3 full factorial design. A total of eight formulations (ERS 1-8) were formulated and evaluated for maximum % buoyancy, entrapment efficiency and drug release characteristics. It was observed that the concentrations of polymers used had profound effect on the obtained responses. The percentage yields were higher in case of ERS-8 and ERS-6, might be attributed due to higher concentrations of crospovidone and Eudragit® RS100, as it decreases with increasing polymer ratios due to instant diffusion of the solvent [30]. The *B %* was higher in case of ERS-6 (74.46±0.035 %) and ERS-8 (86.90±0.043 %) whereas the *EE %* and *CDR5 h %* was maximum in ERS-6 such that 87.27±0.432 % and 97.93±0.043 %. Moreover the % swelling which was carried out in buffer pH 2.2 gave best results in ERS-6 (86±0.036 %). The evaluation results of prepared formulations showed best in ERS-6. The composition crospovidone (5 %), Eudragit® E100 (600 mg) and Eudragit® RS100 (900 mg) along with PAN (40 mg) and the 40 °C temperature with 300 rpm stirring speed was the optimum for constant drug release (Table 1.). Higher concentration of Eudragit® RS100 showed good floating ability due to its insolubility in buffer pH 2.2. Optimization was done by design expert software and the responses were evaluated by fitting it in to the binomial model equations. With the help of software the response parameters was easily modified and the optimized formulation ERS-O with higher *B %*, *EE %* and *CDR 5 h %* was formulated [31].

Selection of optimized formulation and process optimization

The % swelling and buoyancy study which was carried out in buffer pH 2.2, gave best results in ERS-6 formulation. The *EE %* and the *CDR 5h %* were also higher in ERS-6. The data obtained were analyzed using Design expert software. The responses of *B %, EE %* and *CDR5 h %* were optimized by considering the three independent variables: amount of crospovidone $(X_1, \%)$, Eudragit® E100 (X_2, mg) and Eudragit® RS100 (X_3, mg) and by calculating the main effects as well as the interaction effects. The obtained values were fitted in the polynomial equation and analyzed. Relationship between the variables was obtained by placing the values of the effects in the polynomial model:

% Buoyancy

$$B(\%) = 58.27 + 16.16X_1 + 15.52X_2 + 17.46X_3 - 7.05X_1X_2 - 0.29X_1X_3 - 1.90X_2X_3$$

$$[R^2 = 0.9980; F\ value = 573.51; p < 0.05]$$

After eliminating the non-significant terms $p > 0.05$ the final equation becomes:

$$B(\%) = 58.27 + 16.16X_1 + 15.52X_2 + 17.46X_3 - 7.05X_1X_2 \qquad \text{Eq. (6)}$$

If the concentration of variable 3 is increased that will result in an increase in buoyancy by 17.46%.

% Entrapment efficiency:

$$EE(\%) = 68.45 + 17.50X_1 + 10.27X_2 + 9.79X_3 - 11.06X_1X_2 - 7.65X_1X_3 - \qquad 11.50X_2X_3$$

$$[R^2 = 0.9963; F\ value = 315.57; p < 0.05]$$

After eliminating the non-significant terms $p > 0.05$ the final equation becomes:

$$EE(\%) = 68.45 + 17.50X_1 + 10.27X_2 + 9.79X_3 - 11.06X_1X_2 - 11.50\mathrm{X}_2X_3 \quad \text{Eq. (7)}$$

If the concentration of variable 1 is increased that will result in an increase of EE by 17.50 %.

% CDR 5h (Buffer pH 2.2)

$$CDR(\%) = 75.27 + 12.56X_1 + 7.86X_2 + 6.25X_3 - 19.80X_1X_2 - 10.71X_1X_3 - \qquad 17.33X_2X_3$$

$$[R^2 = 0.9965; F\ value = 336.16; p < 0.05]$$

After eliminating the non-significant terms $p > 0.05$ the final equation becomes:

$$CDR(\%) = 75.27 + 12.56X_1 - 19.80X_1X_2 - 10.71X_1X_3 - 17.33X_2X_3 \qquad \text{Eq. (8)}$$

If the concentration of variable 1 is increased that will result in an increase of CDR by 12.56%.

From the polynomial equations it was showed that by increasing the concentration of variable-3 (Eudragit® RS100) the % buoyancy could be increased and by increasing the concentration of variable-1 (amount of crospovidone) the *EE %* as well as *CDR 5 h %* could also be increased. The results of the model matrix showed that the response (*Y*) was directly influenced by the variable

interactions. Corresponding increase in the responses (percentages) was achieved with an increase of the variable concentrations. In statistical optimization through ANOVA method as shown in Table 2 confirms the significance $p < 0.05$ of all models investigated for all the three response parameters. The polynomial equations of the dependant variables were used in generating the 3D response surface graphs, which predicts the responses (Y) of the dependent variables at intermediate levels of independent variables$(X_1, X_2$ and $X_3)$. The response variable data was fitted in the software and analyzed. A linear correlation plots was obtained when actual values are plotted against the predicted and corresponding residual plot showed the scatter of residual versus predicted as presented in Figure 2.

Table 2: ANOVA summary for response parameters

Source	Sum of squares	d.f.[a]	Mean square	*F*-value	*P*-value (Prob> F)
(a) *B %* [b]					
Model	5269.16	6	878.19	573.51	0.0320 (S)
X_1	1601.21	1	1601.21	1045.69	0.0197 (S)
X_2	1475.87	1	1475.87	963.84	0.0205 (S)
X_3	1868.44	1	1868.44	1220.20	0.0182 (S)
X_1X_2	300.86	1	300.86	196.48	0.0453 (S)
X_1X_3	0.53	1	0.53	0.35	0.6613 (NS)
X_2X_3	22.24	1	22.24	14.53	0.1633 (NS)
(b) *EE %* [c]					
Model	5029.92	6	838.32	315.57	0.0431 (S)
X_1	1481.82	1	1481.82	557.81	0.0269 (S)
X_2	1706.41	1	1706.41	642.35	0.0251 (S)
X_3	1412.41	1	1412.41	531.68	0.0276 (S)
X_1X_2	749.81	1	749.81	282.25	0.0378 (S)
X_1X_3	358.45	1	358.45	134.93	0.0547 (NS)
X_2X_3	810.23	1	810.23	305.00	0.0364 (S)
(c) *CDR 5h%* [d]					
Model	6531.91	6	1088.65	336.16	0.0417 (S)
X_1	966.90	1	966.90	298.56	0.0368 (S)
X_2	379.09	1	379.09	117.06	0.0587 (NS)
X_3	239.91	1	239.91	74.08	0.0736 (NS)
X_1X_2	2401.59	1	2401.59	741.57	0.0234 (S)
X_1X_3	703.69	1	703.69	217.29	0.0431 (S)
X_2X_3	1840.73	1	1840.73	568.39	0.0267 (S)

X_1, X_2 and X_3 represents amount of crospovidone (*%*), Eudragit®E 100 (*mg*) and Eudragit®RS100 (*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] *CDR 5h%* = cumulative percentage drug release after 5h

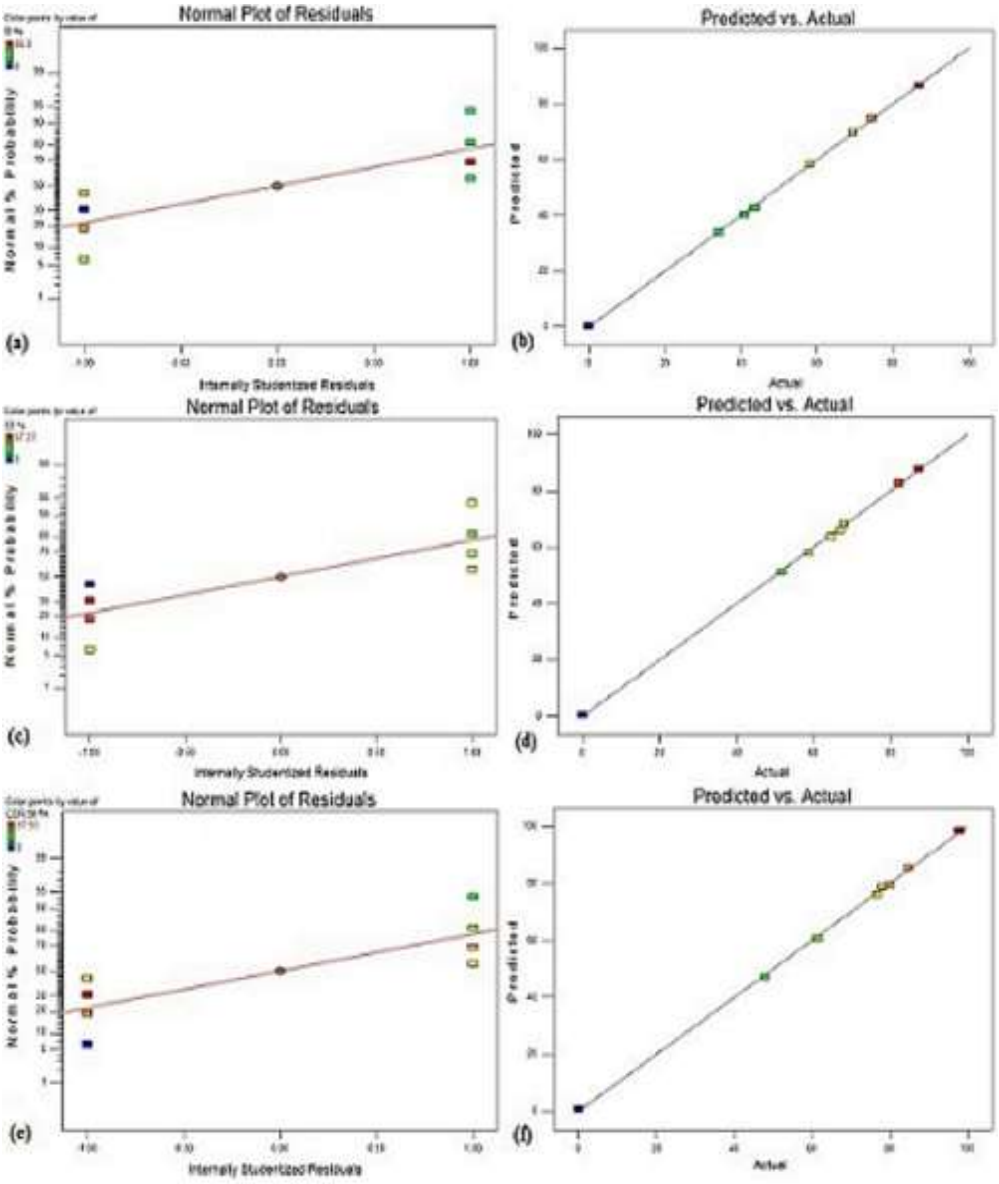

Figure 2: Residual plot showing scatter of residuals versus predicted values (a, c, e), and linear correlation plot between the actual and predicted values (b, d, f) for *B*, *EE* and *CDR* 5 h (%).

The 3D surface plots for percentage buoyancy, entrapment efficiency and drug release after 5 h were generated and presented in Figure 3. Desirability approach was employed for numerical optimization in order to develop a new optimized formulation with the desired responses. The selected desirable values for independent variables were: $X_1 = 5.00$ %, $X_2 = 603.72$ mg and $X_3 = 900.00$ mg, whereas the desirable ranges for dependent responses were $75 \leq B\ \% \leq 80$, $85 \leq EE\ \% \leq 90$ and $95 \leq CDR\ \%\ 5\ h \leq 100$. The obtained predicted values for dependent variables were *B %* = 75.04; *EE %* = 87.57 and *CDR %* 5 *h* = 97.93.

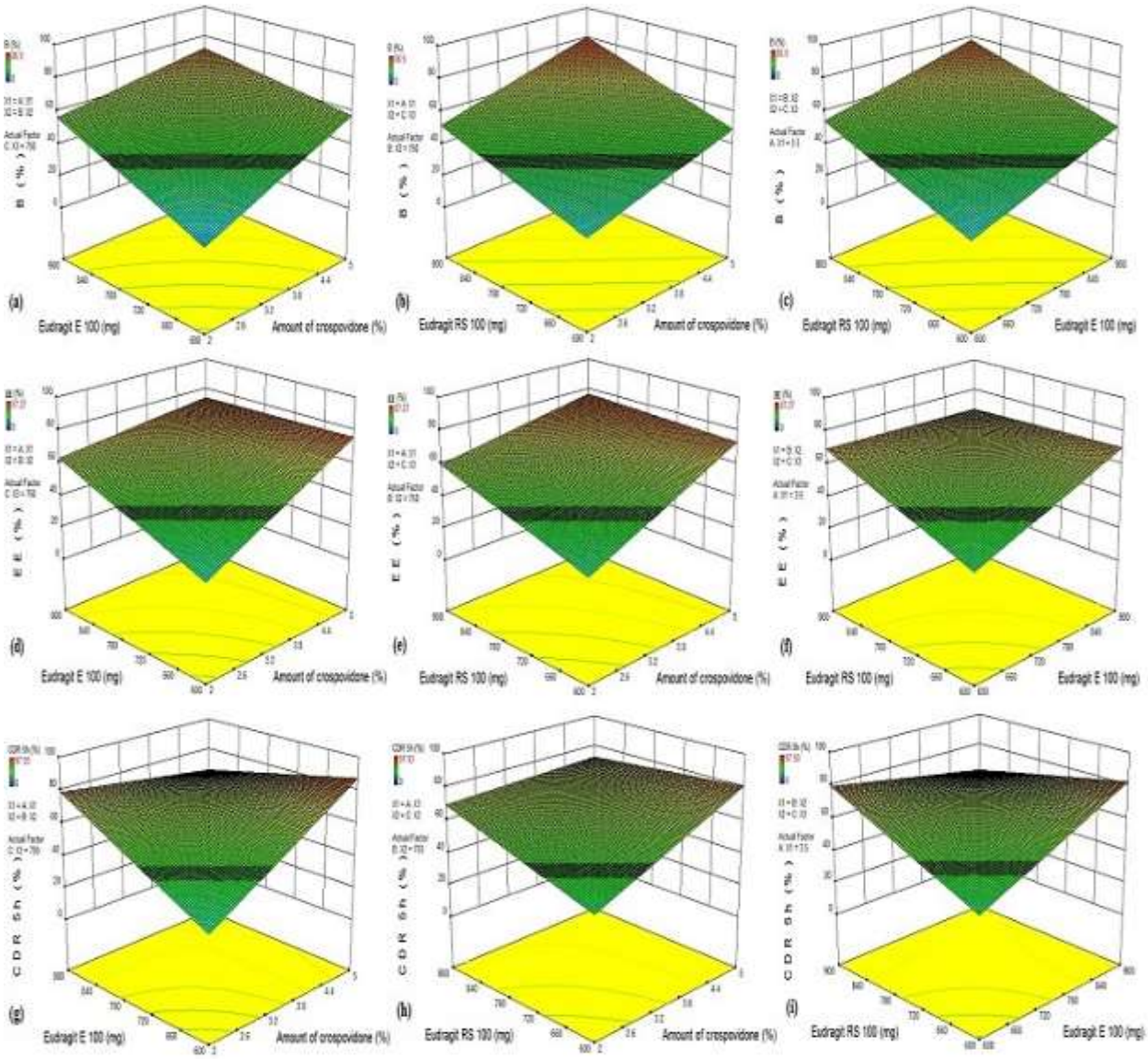

Figure 3: Response surface plots (a-i) showing the combined effect of amount of crospovidone (%), Eudragit®E100 (mg) and Eudragit®RS100 (mg) on *B, EE* and *CDR 5 h* %.

Validation of factorial design

On the basis of the 3D surface plots the predicted responses were easily found out and validated by comparing with that of the actual values. The design was validated by formulating an extra check point formulation ERS-O and evaluating the responses for percentage buoyancy, entrapment efficiency and cumulative drug release after 5 h. It was observed that the experimental values of the responses were close to the software predicted values. The experimental and the predicted values are shown in Table 3 and the percentage error was calculated as:

$$Percentage\ error\ (\%) = \frac{(predicted\ value - experimental\ value)}{predicted\ value} \times 100 \quad \text{Eq. (9)}$$

When experimental values are compared with that of the predicted values, it was observed that the value of percentage error lies in the range 0.00 - 0.77. As the magnitude of error was low, this confirms the validation of 2^3 full factorial designs [32].

Characterization of optimized microballoons

Particle size analysis

It was observed that the mean size of microballoons increases with increase in the concentrations of polymers ratios (Eudragit® E100: Eudragit® RS100). The size of the optimized formulation ERS-O ranges from 20-120 µm and the maximum particles were in the range 60-80 µm.

Table 3: Confirmation of optimization capability

Code	Composition				Predicted value	Experimental value	Percentage error[d]
	***X*₁ (Amt. of crospovidone, %)**	***X*₂ (Eudragit® E 100, *mg*)**	***X*₃ (Eudragit® RS 100, *mg*)**	**Response (*Y*, %)**			
ERS-O	5.00	603.72	900.00	*B*[a]	75.04	74.46	0.77
				EE[b]	87.57	87.27	0.34
				CDR 5h[c]	97.93	97.93	0.00

[a] B = buoyancy; [b] EE = entrapment efficiency; [c] CDR-5h = cumulative drug release after 5h; [d] Percentage error (%) = (predicted value − experimental value)/ predicted value x 100

Surface morphology

The spherical shape, smooth surface and the internal hollow cavity of the formulation was confirmed by scanning electron microscopic study. Hollow cavity was due to the escape of the solvents ethanol and dichloromethane during the preparation. Porosity may be contributed to the leaching of sodium chloride and also due to Eudragit® RS100. The smooth surface of ERS-O was due to the hydrating and gel effect of crospovidone. Internal pores were also observed which may be due to the capillary effect of crospovidone (Figure 4). Moreover the lower density of polymers also makes contribution in attaining buoyancy and internal hollow cavity [33].

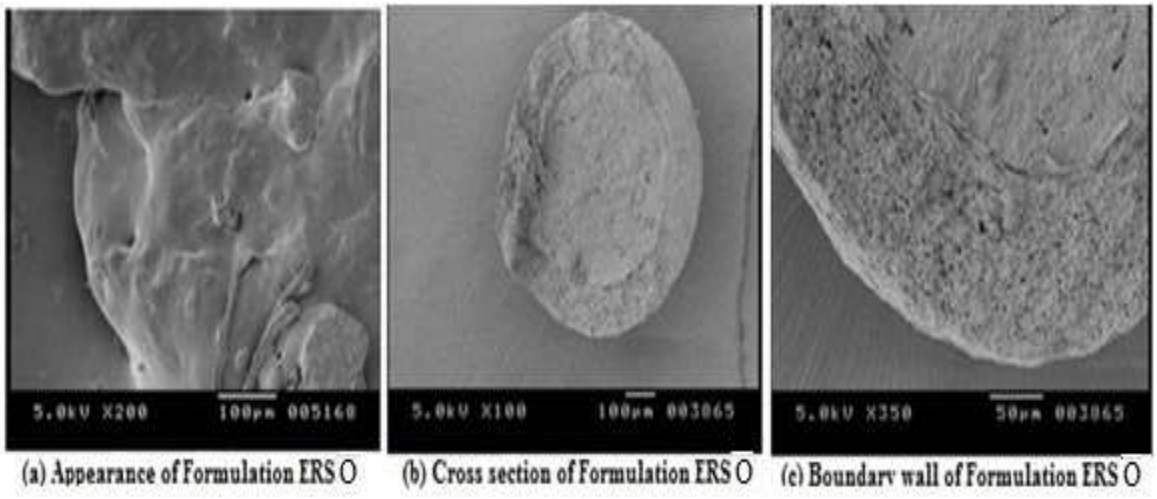

Figure 4: Scanning electron micrograph of optimized microballoon containing pantoprazole sodium showing: (a) rough surface, (b) cross-sectional view and (c) porous boundary wall (ERS-O).

Fourier Transform Infrared Spectroscopy

The FTIR spectra of Eudragit® E100, Eudragit® RS100, PAN and the optimized formulation ERS-O were analyzed. The principle peaks of pure drug PAN were observed at 2942, 1588, 1376, 1303, 1040, 983, 838 and 796 cm^{-1} which are due to $[C\text{-}H]_{str}$; $[C{=}N]_{str}$; $[S{=}O]_{str}$; $[C\text{-}H]_{def}$; $[C\text{-}F]_{str}$; $[C\text{-}O]_{str}$; $[C\text{-}C]_{str}$ and $[N\text{-}H]_{Rocking}$ respectively. Spectra of Eudragit® E100 showed the band around 2949, 1722, 1451, 1144, 966, 749 cm^{-1} which was due to $[C\text{-}H]_{str}$; $[C{=}O]_{str}$; $[N\text{-}H]_{def}$, $[C\text{-}O]_{str}$; $[C\text{-}C]_{str}$ and $[N\text{-}H]_{Rocking}$. And the common polymer Eudragit® RS100 showed the bands at 2951, 1724, 1448, 1238, 1143, 988, 848 and 752 cm^{-1} which corresponds to $[C\text{-}H]_{str}$; $[C{=}O]_{str}$; $[C\text{-}H]_{Bend\ in\ plane;}$ $[C\text{-}N]_{str}$; $[C\text{-}O]_{str}$; $[C\text{-}C]_{str}$; $[C\text{-}H]_{Rocking}$ and $[C\text{-}Cl]_{str}$. While ERS-O showed peaks at 2952, 1952, 1722, 1450, 1384, 1040, 1072, 965, 780 and 748 which was due to $[C\text{-}H]_{str}$; $[C{=}O]_{str}$; $[C{=}N]_{str}$; $[C\text{-}H]_{Bend\ in\ plane}$; $[S{=}O]_{str}$; $[C\text{-}F]_{str}$; $[C\text{-}O]_{str}$; $[C\text{-}C]_{str}$; $[C\text{-}Cl]_{str}$ and $[N\text{-}H]_{Rocking}$ respectively (Figure 5.a-d). In the FTIR spectra of optimized formulation ERS-O, various characteristic peaks of Eudragit® E100, Eudragit® RS100 and pure PAN had appeared without any significant changes of bands, only shifting of $[C{=}N]_{str}$ band towards higher wave number appeared due to cross-linking. This showed that spectra of ERS-O formulation prepared by emulsion solvent diffusion method had significant characters of PAN and confirms that there were no chemical interactions between PAN and other excipients.

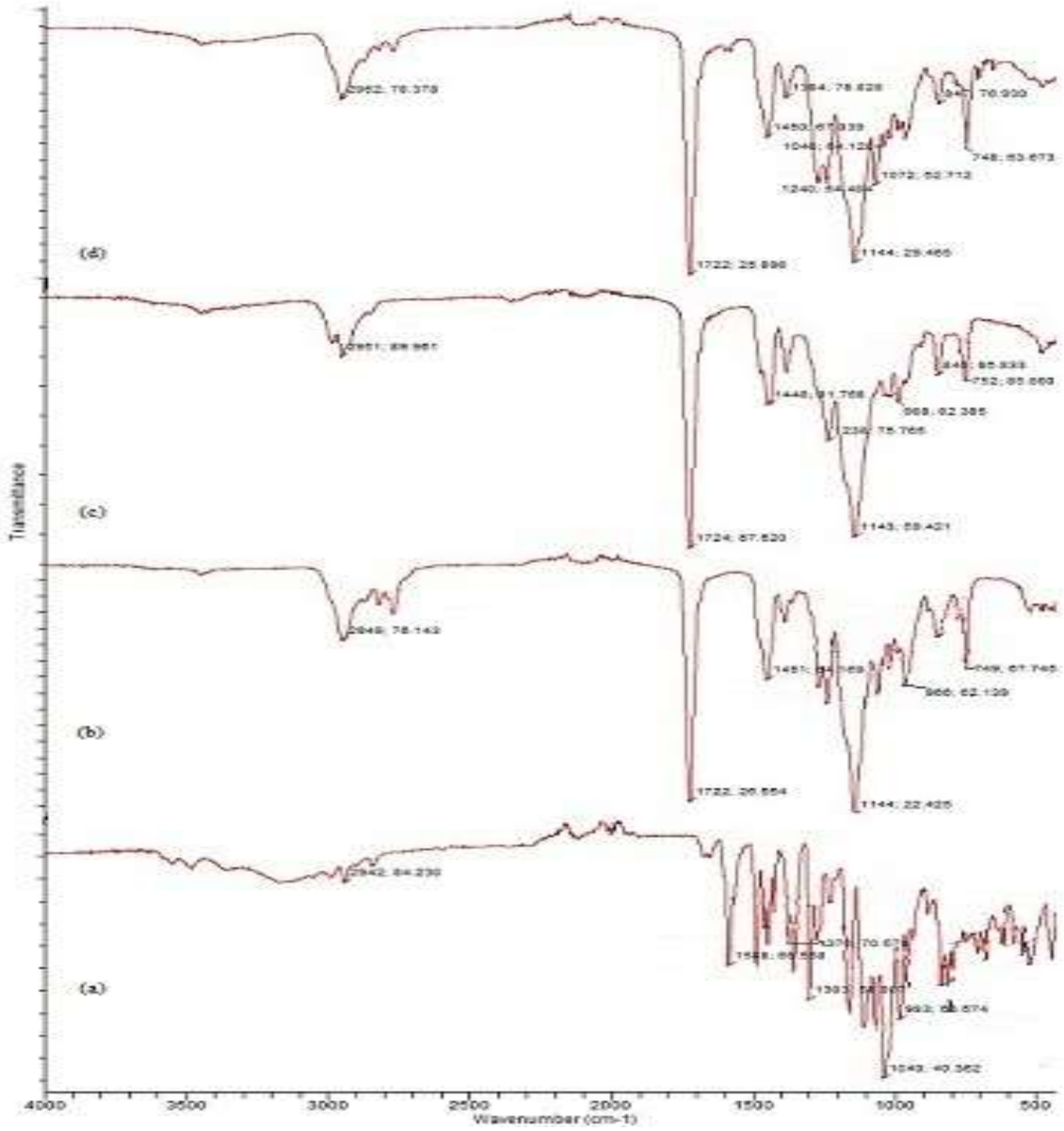

Figure 5: FTIR spectra of (a) Pantoprazole sodium, (b) Eudragit®E100, (c) Eudragit®RS100 and (d) Optimized formulation ERS-O containing pantoprazole sodium.

***In vitro* drug release**

The *in vitro* study of the optimized formulation ERS-O was performed in buffer pH 2.2 and it was observed that the release after 5 h was 97.93±1.22 % whereas marketed formulation Pantop-Fast showed 94.64±0.12 % as depicted in Figure 6. ERS-O prolongs the release rate much better in comparison with that of the marketed formulation due to the presence of enteric polymers Eudragit®E and RS100 and also due to the shorter transit time of stomach the study was restricted to 5 h. The drug release increases in acidic environment due to basic nature of the drug as it shows higher solubility in lower pH values. 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. 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 [34].

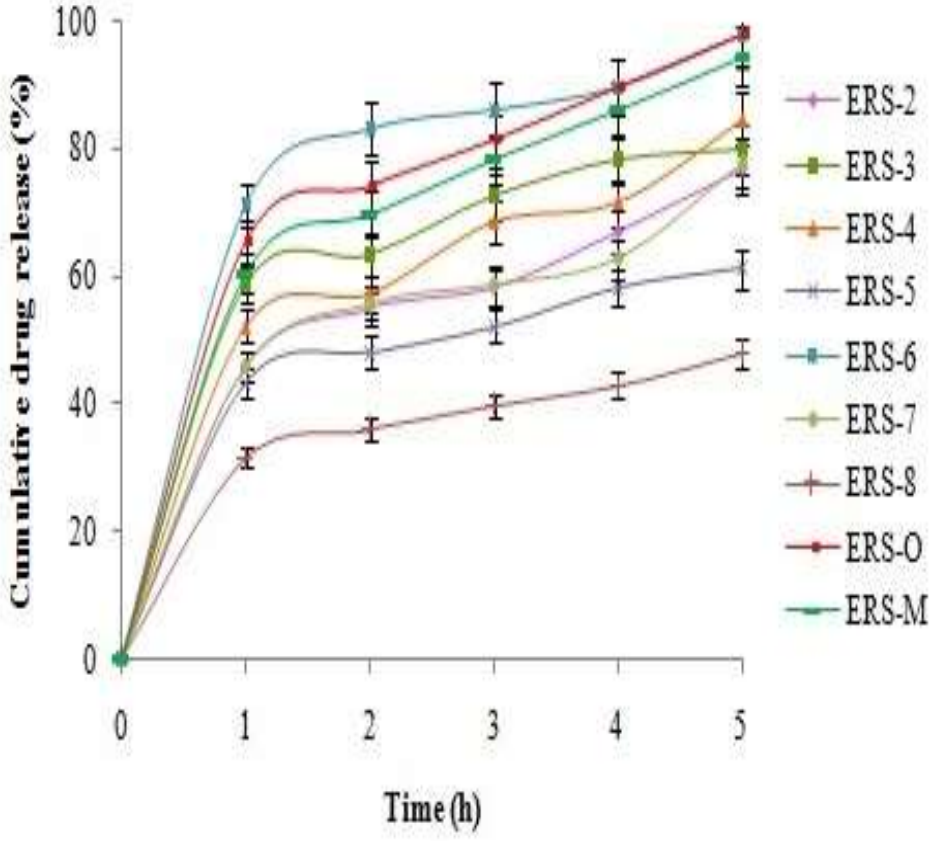

Figure 6: Comparative cumulative percentage *in vitro* drug release profiles of different ERS formulations in buffer pH 2.2 for 5 h (37 ± 0.5 °C, 300 rpm).

Kinetics of drug release

In buffer pH 2.2 the best fit model was Korsmeyer peppas indicated for formulation ERS-6, showing anomalous (Non-Fickian) diffusion corresponding to coupling of both diffusion and erosion with *n* values between 0.5-0.1, as crospovidone increases the release rate through erosion. Best fit model for ERS-3 was Higuchi with $n > 1$ showed super case II transport whereas ERS-5, ERS-8, ERS-O and Pantop-Fast showed zero order release indicating that the release was independent of the concentration of polymers with r^2 values 0.999 as shown in Table 4. ERS-2, ERS-4 and ERS-7 showed first order release mechanism depending on the concentration of one of the reactants [35].

Table 4: Various drug release mechanisms for PAN loaded microballoons in buffer pH 2.2

Code	Evaluation parameters	Zero order	First order	Higuchi	Peppas	Best fit model
ERS-2	r^2	0.981	0.984	0.956	0.947	First order
	a	38.73	1.618	21.31	1.653	
	b	7.331	0.052	23.51	0.296	
ERS-3	r^2	0.959	0.952	0.970	0.959	Higuchi
	a	53.41	1.738	39.20	1.759	
	b	5.738	0.035	18.74	0.206	
ERS-4	r^2	0.966	0.975	0.936	0.919	First order
	a	43.10	1.664	24.37	1.699	
	b	7.933	0.051	25.37	0.287	
ERS-5	r^2	0.991	0.987	0.983	0.967	Zero order
	a	38.90	1.603	27.71	1.628	
	b	4.611	0.038	14.92	0.217	
ERS-6	r^2	0.941	0.926	0.958	0.964	Peppas
	a	67.66	1.837	52.72	1.855	
	b	5.987	0.030	19.62	0.180	
ERS-7	r^2	0.918	0.936	0.886	0.893	First order
	a	39.05	1.621	22.37	1.655	
	b	7.093	0.050	22.64	0.284	
ERS-8	r^2	0.996	0.994	0.982	0.969	Zero order
	a	27.90	1.464	18.40	1.492	
	b	3.947	0.043	12.73	0.246	
ERS-O	r^2	0.999	0.995	0.986	0.971	Zero order
	a	57.85	1.78	38.54	1.807	
	b	8.002	0.042	25.83	0.243	
PANTOPFAST	r^2	0.999	0.991	0.992	0.980	Zero order
	a	52.56	1.742	32.05	1.722	
	b	8.45	0.047	27.35	0.273	

Stability study

An accelerated stability study which was carried out for a period of six months and the data obtained after analysis showed insignificant differences among themselves. The ANOVA values of *F* at 5% level of significance for % buoyancy and % EE were 15.74 and 7.51.

***In vivo* study**

The *in vivo* evaluation study was successfully performed for determining the ulcer indexes and percentage protection for various treated groups using curative approach by ethanol induced ulcer method. The ulcer index values for the various treated groups were 7.81±0.20 for control, 0.5±0.06 for standard-1, 0.20±0.06 for standard-2 and 0.13±0.03 for the treatment group respectively when compared to control. From the values of the ulcer indexes for various groups percentage protection was determined. Treatment group (ERS-O formulation) showed the maximum percentage

protection of 98.33 %. The percentage protection in case of Standard-1 and 2 groups were 93.59 % and 97.43 % (Table 5). The ERS-O formulation clearly demonstrates its higher inhibition effect in comparison to the sodium bi carbonate and PAN treated groups. The control group with oral administration of 95 % ethanol clearly showed the hemorrhagic streaks in the glandular portion of the stomach. The control group with hemorrhagic streaks was due to stasis of gastric mucosa that in turn producing hemorrhage and necrosis may be attributed due to formation of free radical and lipid per oxidation products. After 1 h, the standard-1 group with sodium bi carbonate solution showed healing but red coloration was observed whereas group treated with standard PAN IV showed remarkable healing of ulcerated area with slight spot ulcers left respectively. Stomachs of ERS-O were normal colored without any hemorrhagic streak clearly demonstrates the effectiveness of the dosage form orally (Figure 7). The statistical analysis clearly demonstrates that the ulcer index values for treatment groups were lower than that of the standard groups with $P < 0.001$ (Figure 8). In comparison with IV route, ERS-O oral therapy gave sustained release characteristics with higher patient compliance as it is non-invasive and economical. Moreover a sudden rise in PAN concentration in plasma through IV route is followed by rapid degradation results in lowering of therapeutic effective concentration due to its shorter half-life. So use of pH responsive biodegradable inert non-toxic polymers for encapsulation was proved to be successful in enhancing the PAN bioavailability and to achieve more rapid onset of anti-secretory effect.

Table 5: Anti ulcer activity of PAN loaded microballoons

Groups Treated	Ulcer Index (UI)*	Protection (%)
Control	7.81±0.20	-
Standard 1	0.5±0.06	93.59
Standard 2	0.20±0.06	97.43
Treatment	0.13±0.03	98.33

*All value expressed as MEAN ± SEM, P< 0.001 when compared to control

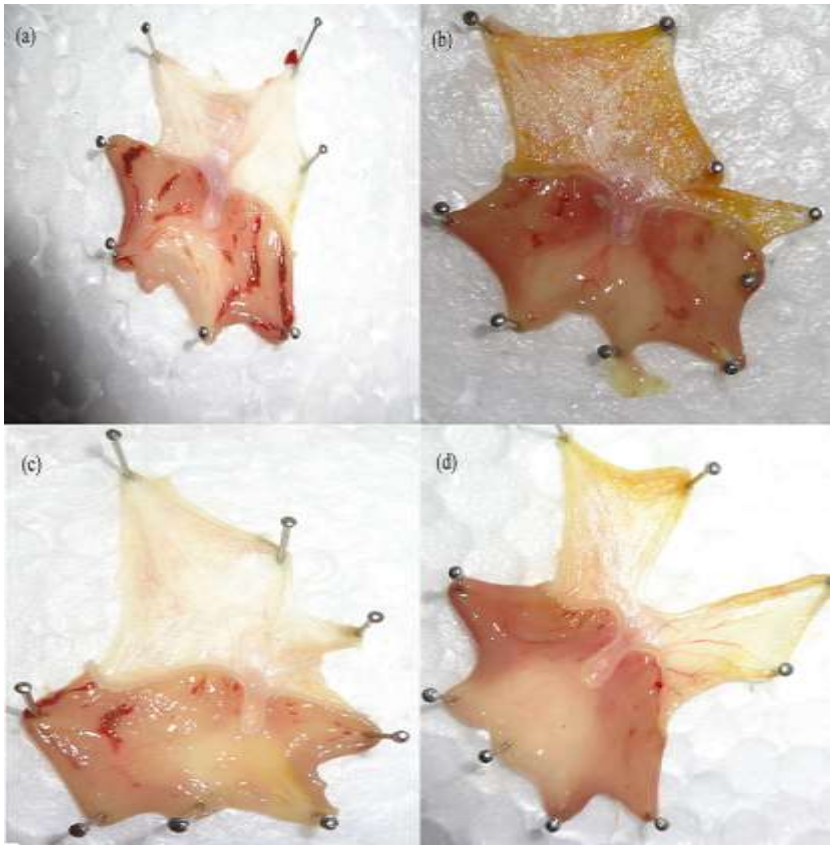

Figure 7: Photographs of the inner stomach wall of animals treated with: (a) absolute alcohol, (b) sodium bi carbonate aqueous solution, (c) standard pantoprazole sodium solution and (d) optimized microballoons aqueous suspension.

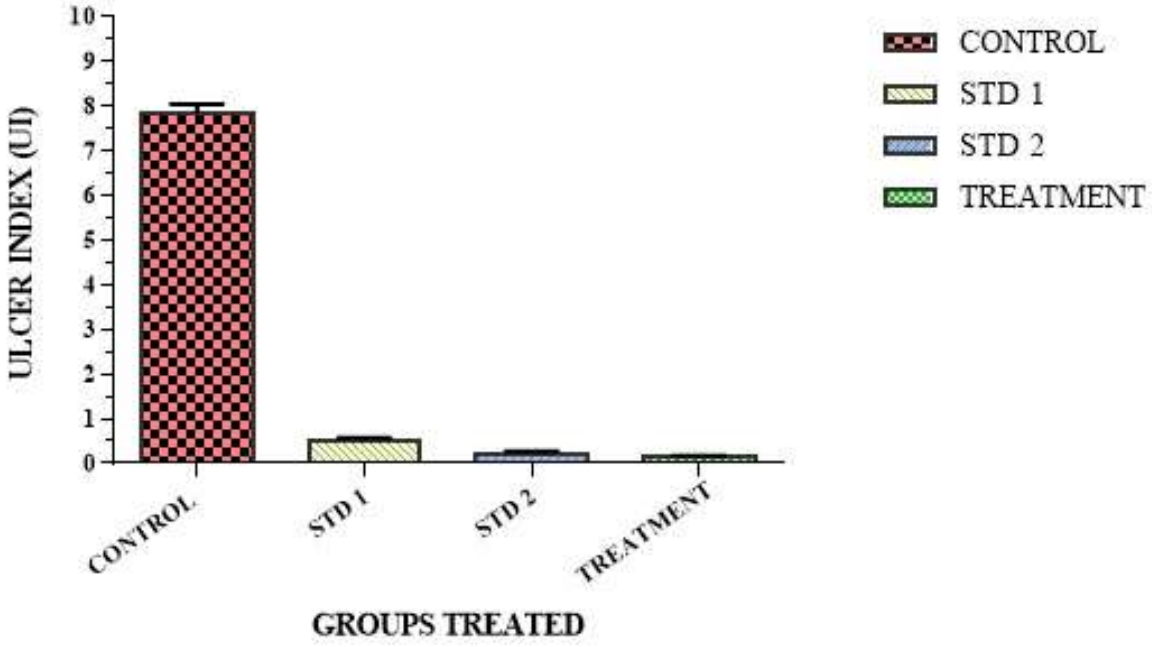

Figure 8: Showing ulcer indexes for various treated groups

CONCLUSION

The buoyant system for PAN by non-effervescent technique employing emulsion solvent diffusion method was successfully made with the help of 2^3 full factorial design using crospovidone, Eudragit® E and RS100 polymers. The *in vitro* result confirms their potential and zero order mechanism showed their capability of controlling the fluctuations in plasma levels and reducing the dose frequencies. The optimized formulation gave excellent results for particle size, percentage swelling, buoyancy, entrapment efficiency, moreover the prolonged drug release via gastric retention might be advantageous for enhancing the bioavailability of the drug in stomach specific approach. The results of *in vitro* and *in vivo* studies were well correlated and confirm the effectiveness of the formulation design.

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