

Formulation, In-vitro and In-vivo Evaluation of Enteric Coated Pellets of Substituted Benzimidazole Proton Pump Inhibitor

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Abstract: The objective of present work was to design, formulate and characterize the enteric coated pellets of Rabeprazole using dry powder layering method and also study the ulcer protecting effect of pellets against the gastric lesions induced by 70 % ethanol in albino wistar rats. The evaluation of pellets was done using DSC, FT-IR and SEM study. The kinetic release study was also done on all formulations. In vitro drug release study data was fitted on the different kinetic models and formulation F5 showed the Higuchi drug release. In vivo antiulcer activity was done on formulation F5 showed that the formulation was able to protect the rat stomach against ulcers formation. The levels of thiobarbituric acid, estimation of lipid peroxidation, estimation of reduced glutathione, estimation of catalase activity and protein determination was also done.

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I. Introduction

Rabeprazole sodium is used in the treatment of peptic ulcer, gastroesophageal reflux disease and Zollinger-Ellison syndrome. It is also used in combination with appropriate antibacterial therapeutic regimens, which are used in eradication of *H. Pylori* in patients with peptic ulcer disease (PUD). It inhibits the final step in gastric acid secretion. It is inactive at neutral pH but as the pH is less than 5 it rearranges to two charged cationic forms that reacts covalently with the SH groups of H⁺K⁺ATPase enzyme and inactivate it irreversibly. Charged form after diffusing into parietal cells it gets concentrated in the canaliculi because the charged form at acidic pH are unable to diffuse back. Rabeprazole is well absorbed (at least 30%) and can be detected in the plasma within 1 hr. Food delays its absorption. It is extensively and rapidly metabolized and this regulated by CYP2C19 isoenzyme in the liver and to some extent by CYP3A4. The primary metabolite is rabeprazolethioether and other metabolites are sulfone, E 3810, desmethylatedthioether E 3810 and thioether carboxylic acid E 3810. About 90% of drug is excreted in urine with 30% as thioether carboxylic acid metabolite and its glucuronide. No unchanged rabeprazole has been recovered in urine or faeces. There is no accumulation following multiple dosing. Many approaches for various formulations like enteric coated tablets, sustained release mucoadhesive tablets, drug coated beads, microspheres and gastroretentive systems were made to protect the gastric degradation along with controlled release. Multiparticulate drug delivery systems like granules, pellets, microspheres, nanoparticles, minitabets and various mini depots were acquired central position in pharmaceutical drug development. It gives the various opportunities in elaborating the first step in future pharmaceutical development. Pellets are used in the form of hard gelatin capsules or disintegrating tablets which are quickly liberate their contents and get distributed throughout the gastrointestinal tract without loss of depot effect. Pellets have the ability to pass the pylorus even in close state and disperse freely throughout the gastrointestinal tract, thus increase the absorption. Layering is the type of Pelletization technique in which powdered drug is layered onto the starter seed materials in powdered or suspension form with the help of binder. The concentration of binder is based on the choice of drug because it has the ability to influence the physical and chemical properties of pellets and also the release behavior of drug. Powder layering of drug or excipients or both is done by spraying the binding liquid which results in deposition of successive layers on nuclei or cores. The equipments used in powder layering are conventional coating pan, tangential/spray/centrifugal/rotary fluidized bed granulator etc. Formation of pellets involves adhering of solid particles or fine powder to each other when they are brought together due to attractive forces i.e. molecular forces, electrostatic forces and rarely magnetic forces.

Therefore the above research work entails the formulation of pellets of Rabeprazole sodium which is a proton pump inhibitor using powder layering method in which PEG-4000 was used as binding solution and Eudragit RS-100 was used as enteric polymer for enteric coating of pellets with varying the concentration of triethyl citrate as plasticizer. The pellets showed the complete protection of Rabeprazole sodium in gastric acid environment of rats stomach to increase the systemic availability of drug with desired sustained release action and to improve the patient compliance.

II. Material and Methods

Pure Rabeprazole Sodium was obtained as gift sample from Metrochem API Limited (Hyderabad). Evonik India Private Limited, Mumbai provided the gift sample of Eudragit RS-100 and Goa Antibiotics and pharmaceutical limited supplied a gift sample of sugar spheres

Preparation of Enteric Coated Pellets of Rabeprazole Sodium

Powder layering method was used during formulation of pellets of rabeprazole sodium. PEG-4000 was used as the binding solution and it was dissolved in the isopropyl alcohol for making binding solution. In the conventional coating pan desired size (mesh size: 30) of sugar spheres were loaded and charged. Rabeprazole sodium and lactose were sieved through a sieve having mesh size 90 and mixed properly to prepare a powder blend. After charging of sugar spheres powder blend was loaded on to sugar spheres with simultaneously spraying the binding solution. After smooth coating of powder blend the drug loaded pellets were dried at 60 °C for 6 hours in hot air oven. After drying the pellets was sieved through sieve having mesh size 20 and 24 to get the desired mesh size i.e. 20/24.

Enteric Coating of Pellets

The enteric coating of pellets was done using Eudragit RS-100 as enteric polymer. The coating suspension was made using triethyl citrate as plasticizer and purified talc. The concentration of plasticizer was 5%, 10%, 15%, and 20% (w/w of Eudragit RS-100 on a dry basis). The drug loaded pellets were put into the coating pan and coating suspension was sprayed uniformly on the pellets for smooth coating. After completion of coating of pellets, the pellets were dried in the hot air oven for 6 hr at 60°C. Tried pellets were sieved through 18 and 24 mesh size to get the desirable size of pellets i.e. 18/24. .

Table 1: Formulation of Rabeprazole Sodium Pellets

Sr. No.	Name of ingredients	Batch code				
		F1(mg)	F2(mg)	F3(mg)	F4(mg)	F5(mg)
1	Rabeprazole sodium	20	20	20	20	20
2	Sugar spheres	25.5	25.5	25.5	25.5	25.5
3	PEG – 4000	11.5	11.5	11.5	11.5	11.5
4	Lactose	3.5	3.5	3.5	3.5	3.5
5	Isopropyl alcohol	Up to 50 ml.	Up to 50 ml.	Up to 50 ml.	Up to 50 ml.	Up to 50 ml.
Coating materials						
1.	Eudragit RS - 100	25	25	25	25	25
2.	Purified talc	1	1	1	1	1
3.	Triethyl citrate	-	1.25	2.5	3.75	5.0
4.	Ferric oxide	0.4	0.4	0.4	0.4	0.4
5.	water	up to 50 ml.	up to 50 ml	up to 50 ml	up to 50 ml	up to 50 ml

Evaluation of enteric coated pellets of Rabeprazole sodium

In-vitro Drug Release Study

In-vitro drug release study of Rabeprazole sodium was carried out in triplicate employing USP XXIII paddle (Apparatus 2) using 900 ml. 0.1 N HCl at 37 ± 0.5 °C with rotation of 100 rpm for 2 hrs. After 2 hrs the dissolution medium was removed and replaced by 900 ml of phosphate buffer saline pH 7.4 and rotated at 100 rpm at 37 ± 0.5 °C for 10 hrs. An aliquots sample of 10 ml was periodically withdrawn at suitable time intervals and volume replaced with equivalent amount of dissolution medium. The absorbance of samples was analyzed on UV- visible spectrophotometer. The dissolution study of free Rabeprazole sodium incorporated into capsule shell was also performed simultaneously.

Characterization of enteric coated pellets of Rabeprazole sodium

Differential Scanning Calorimetry (DSC)

The physical state of drug inside the hydrogel beads was investigated by DSC thermogram. A small amount (2-5 mg) of sample was sealed in the aluminium pan and the temperature was raised at 20⁰C/min from 40 to 300⁰C.

FTIR (Fourier Transform Infrared Spectroscopy)

Infrared spectroscopy of the selected formulation was carried out to confirm the drug loading and drug-excipient interaction using KBr pellet method

Scanning Electron Microscopy (SEM)

The surface morphology of hydrogel beads was investigated by scanning electron microscopy. Samples were coated with gold and palladium using a vacuum evaporator and examined using at 10 kV accelerating voltage.

Drug release kinetics studies

The mechanism of drug release from the optimized formulations of enteric coated alginate beads and enteric coated pellets were studied by treating the data according to first order equation (log cumulative percentage of drug remaining v/s time) Higuchi's equation (cumulative percentage drug release vs. square root of time) and Korsmeyer equation (log cumulative percentage drug release v/s log time) along with zero order (cumulative amount of drug release v/s time).

Animal studies on pellets of Rabeprazole sodium

The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of the Department of Pharmaceutical Sciences GJUS&T, Hisar. The antiulcer activity was carried out on ethanol induced ulcers. The animals were divided into four groups each having six animals.

Model: Ethanol Induced Gastric Ulcers

- | | |
|--|---------|
| 1. Control group (distilled water) | 1 group |
| 2. Ethanol group (70% ethanol) | 2 group |
| 3. Pure drug control group | 3 group |
| 4. Pellets formulation treatment group | 4 group |

Ethanol Induced Ulcers

The animals were randomly divided into 4 groups with 6 animals in each group. During the fasting period rats were given to avoid excessive dehydration a nutritive solution of 8% sucrose in 0.2% NaCl. Control group received only water and group 2 received 70 % ethanol at the dose of 10 ml /kg body weight. Pure Rabeprazole sodium at the dose of 20 mg/kg body weight was given to the group 3 animals. The group 4 received the aqueous solution of pellets + ethanol at the dose of 20 mg / kg body weight. The animals were sacrificed after 6 hrs by cutting the stomach along the greater curvature, washed carefully with 0.9% sodium chloride and the ulcers were scored.

The ulcers were scored as given below

Scoring of severity of ulcers

0= Normal colored stomach

0.5= Red coloration

1= ulcers in spots

Mean ulcer score for each animal was expressed as ulcer index .The ulcer index was determined using the following formula

Ulcer index = 10/x

Where X = total mucosal area/total ulcerated area

The results were presented as mean \pm SD. The statistical significance was determined using one way ANOVA followed by Dunnett's multiple comparison test. Stomachs were collected and were subjected to macroscopic evaluation and biological estimation of antiulcer activity.

Histopathological Evaluation

For macroscopic evaluation, a portion of stomach from each experimental group was fixed in 10% formalin and then immersed in the paraffin. Sections of stomach of 5mm were made with a standard microtome and were stained with hematoxylin and eosin. The sections of stomach were examined for edema/erosion/necrosis and ulceration. Histopathological analysis was done using homogenized tissue of stomach in 9 ml of 0.1 mol/L potassium phosphate buffer (pH 7.4).

Macroscopic Evaluation

The stomach was opened from greater curvature and washed with 0.9% NaCl to study the lesions using dissecting microscope. The grading was assigned to severity of lesions to calculate the ulcer index.

Biochemical Estimation for Antiulcer Activity

Estimation of Lipid Peroxidation

The estimation of lipid peroxidation was done by method given by Karmakar and Chatterjee making reaction mixture prepared by mixing the homogenate (0.2 ml) with the 1.5 ml of aqueous solution of thiobarbituric acid, 8.1% dodecylsulfate, 1.5 ml of 20% acetic acid having pH 3.5 and 0.6 ml of distilled water. The incubation of mixture was done at 95 °C by keeping in water bath. After cooling under tap water to the mixture 1.0 ml distilled water and 5 ml. of butanol: pyridine (15:1) was added. The mixture was shaken and centrifuged at 4000 rpm for 10 minutes. After centrifugation the organic was layer obtained and the absorbance of this layer was measured at 532nm. The results were calculated as n moles of malondialdehyde per minute per mg protein¹¹.

Estimation of Reduced Glutathione

The tissue homogenate was mixed with the 10 % w/v of trichloroacetic acid in ration of 1:1 and centrifuged at 4°C for 10 minutes at 5000rpm. The 0.5 ml. of supernatant was obtained and it was mixed with 2.0 ml of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 ml of double distilled water. Then 0.25 ml of 0.001 M freshly prepared DNTB [5, 5- dithiobis (2- nitro benzoic acid) was dissolved in 1% sodium citrate solution and this was added to the above mixture. The reaction mixture was incubated for 10 minutes and absorbance of yellow colored complex was noted using spectrophotometer at 412 nm. The results were calculated as n moles of GSH per mg of protein.¹²

Estimation of Catalase Activity

From the homogenate 50 µl the supernatant was added to 1.95 ml of 50 mM phosphate buffer having pH 7.0 placed in 3.0 ml cuvette. To this mixture 1.0 ml of 30 Mm hydrogen peroxide was added and change in absorbance was noted at 240 nm. The results were calculated as n moles of H₂O₂ used / min / mg of protein.

Protein Determination

The protein concentration in stomach was determined using Lowery's method. This method is used widely for quantitative determination of protein concentration. Folin-Ciocalteu reagent contains phosphomolybdic/tungstic acid and it produces blue/purple colour on reaction with phenolic moiety of tyrosine present in protein at 660 nm. In the above mixture copper reagent is added to enhance the colour formation by chelating with the peptide bond and helps in electron transfer to the chromophore formed.

Table 1: Assay procedure for protein determination (Lowery's Method)

Sr. No.	Pipette in marked tubes	Blank Solution	Standard Solution	Test Solution
1	Lowery's Reagent	5 ml.	5 ml.	5 ml.
2	Distilled Water	1 ml.	0.9 ml.	..
3	Standards	..	100 µl.	..
4	Test	100 µl.
Incubation for 10 min.				
5	Folin-Ciocalteu reagent	0.5 ml.	0.5 ml.	0.5 ml.

After adding Folin-Ciocalteu reagent incubate the above mixture for 30 min at 37 °C. The absorbance of standard and test solution was taken at 750 nm against reagent blank.

Statistical Analysis

The all values were expressed as mean ± SEM and data were analyzed using one way ANOVA followed by Dunnet's multiple comparison test. A level of p<0.05 was considered as statistically significant.

III. Results

Dissolution Studies

The formulation containing no plasticizer i.e. F1 showed 18.22% drug release in acidic medium (pH 1.2) after 2 hours and above 90% of drug release was observed in the phosphate buffer (pH 7.4) after 4 hours. Formulations F2, F3, F4 and F5 showed 0.9 %, 0.7%, 0.4% and 0.2% respectively, of drug release in acidic medium (pH 1.2) after 2 hours, which indicated good acid resistance. The formulation containing no plasticizer (F1) showed the highest drug release in phosphate buffer (pH 7.4) as compared to other formulations.

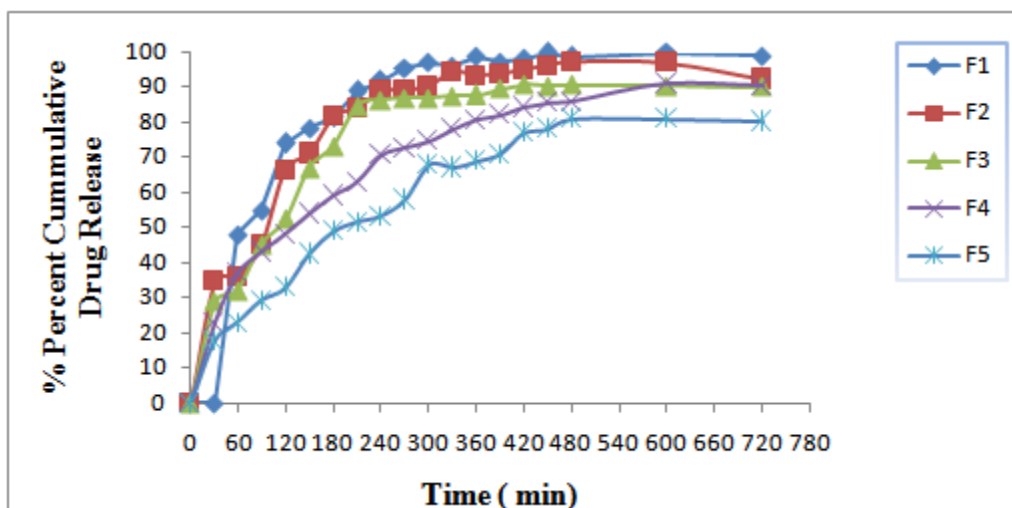


Figure no 2: Dissolution Profiles of Enteric Coated Pellets of Rabeprazole Sodium in Phosphate Buffer Saline (pH 7.4)

Moreover, the formulation F5 showed a sustained release profile which could be attributed to the formation of a denser and smoother film. The level of TEC in the films significantly ($p < 0.5$) influenced the rate of drug release. The release behavior could be further explained based on the fact that the polymer particles did not coalesce completely to form a uniform film in the absence of plasticizer TEC. The drug release was found to slightly decrease with the increase in the concentration of plasticizer. The drug release profile from formulation F5 was compared with the marketed formulation of drug and it was observed that the drug release followed a sustained release pattern. Thus it could be concluded that the release rate of Rabeprazole Sodium can be modify by changing the amount of TEC in the formulation, keeping the concentration of polymer constant.

Drug Release Kinetics of Enteric Coated Pellets

There are a number of drug release kinetic models, which describe the overall release of drug from the dosage forms, because qualitative and quantitative changes in a formulation may alter drug release and *in vivo* performance. Correlation coefficient (R^2) was determined for kinetics models (Zero order, First order, and Higuchi and Korsmeyer Peppas model) and compared with each other, the model which showed the highest correlation coefficient (≈ 1) was taken as the best fit model. The drug release kinetics data for all formulations is given in Table 4 and Figs 4-8.

Table no 4: Drug Release Kinetics Data for the Pellets of Rabeprazole Sodium

Batch Code	Correlation Coefficient (R^2)				
	Zero Order	First Order	Higuchi	Korsmeyer-Peppas	Best Fit Model
F1	0.691	0.882	0.928	0.932	Korsmeyer-Peppas
F2	0.762	0.962	0.925	0.934	First Order
F3	0.761	0.881	0.923	0.934	Korsmeyer-Peppas
F4	0.872	0.980	0.987	0.915	Higuchi
F5	0.952	0.931	0.931	0.899	Higuchi

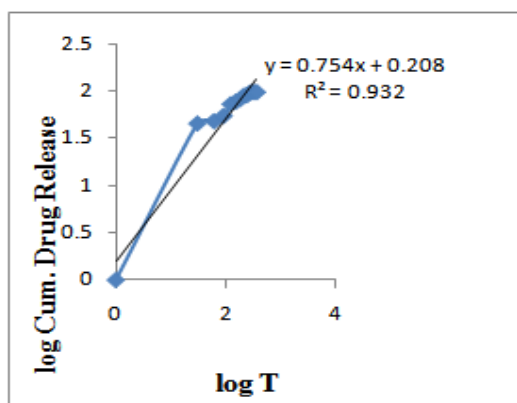


Fig no 4: Best Fit Model for Formulation F1

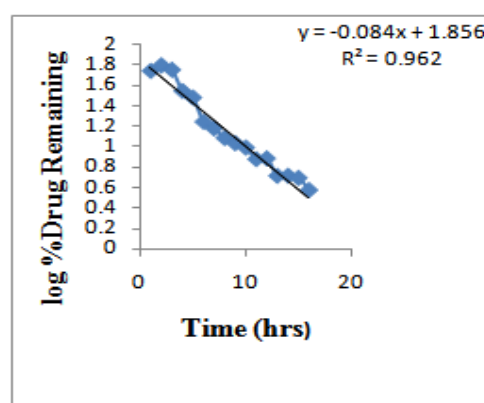


Fig no 5: Best Fit Model for Formulation F2

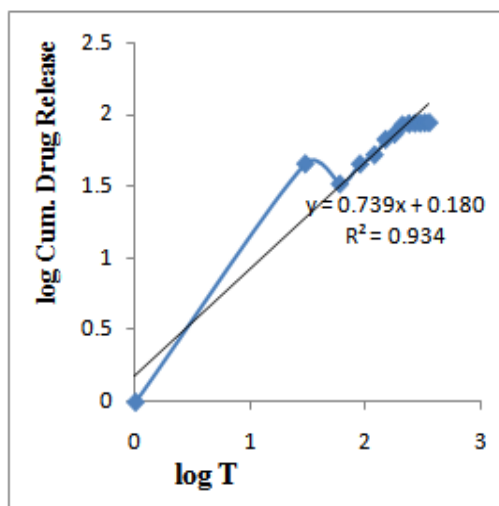


Fig 6: Best Fit Model for Formulation F3

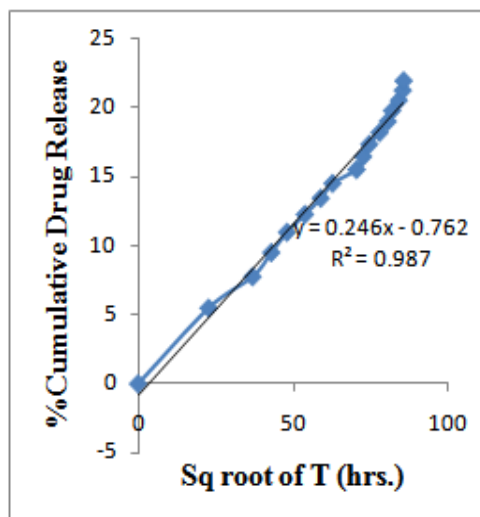


Fig 7: Best Fit Model for Formulation F4

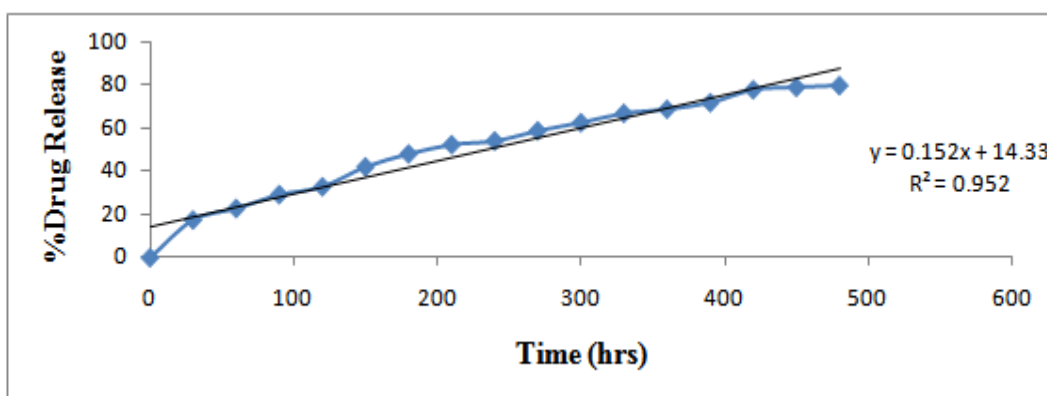


Fig 8: Best Fit Model for Formulation F5

Characterization of enteric coated pellets

Differential Scanning Calorimetry (DSC)

A small amount (2-5 mg) of sample (F5) was sealed in the aluminium pan and the temperature was raised at 20°C/min from 40 to 300°C. From the DSC spectrum of optimized formulation, it was observed that there was no interaction between drug and excipients. The results of DSC showed that the identity of drug was retained in the coated pellets and additional peaks were due to other components present in the formulation.

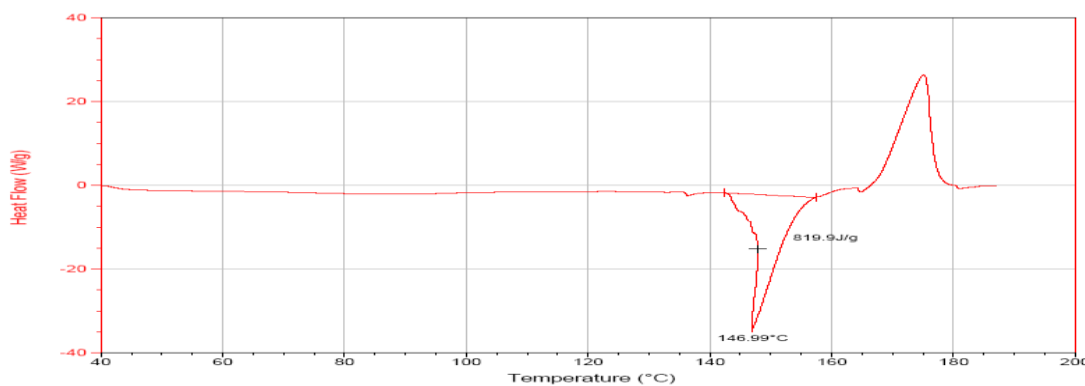


Fig 9: DSC Thermogram of Formulation (F5)

FT-IR (Fourier Transform Infrared) Spectral Analysis

Infrared spectroscopy of the formulation (F5) was carried out to confirm the drug loading and drug-excipient interaction using KBr pellet method. The FTIR spectrum of optimized batch is given in Figure below.

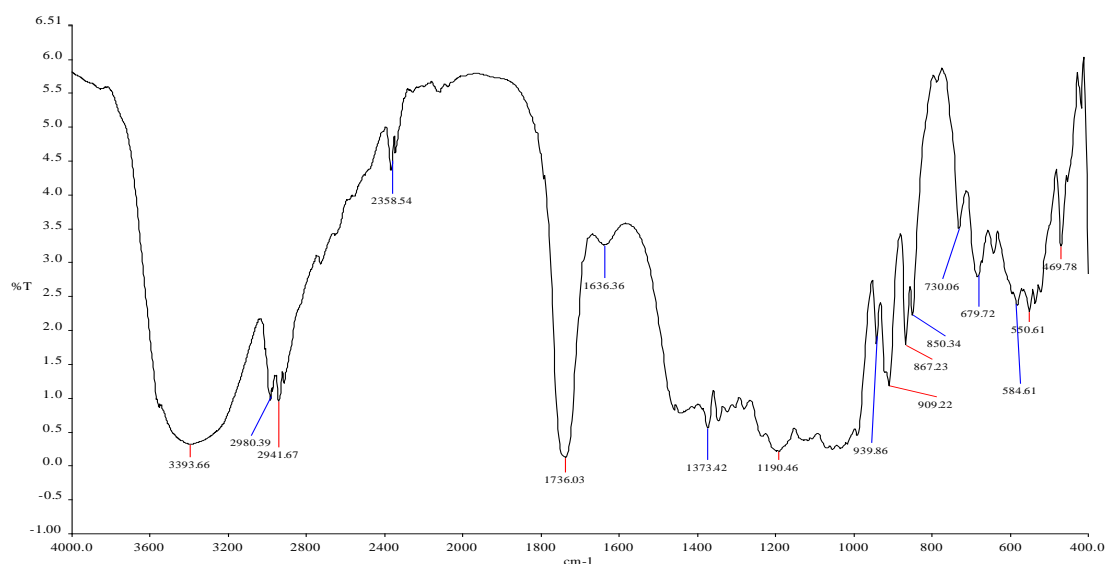


Fig 10: FT-IR Spectrum of the Formulation (F5)

Scanning Electron Microscopy

The surface morphology of pellets was investigated by scanning electron microscopy (SEM). The coated pellets showed the smooth and porous surface due to the application of consecutive layers applied on it.

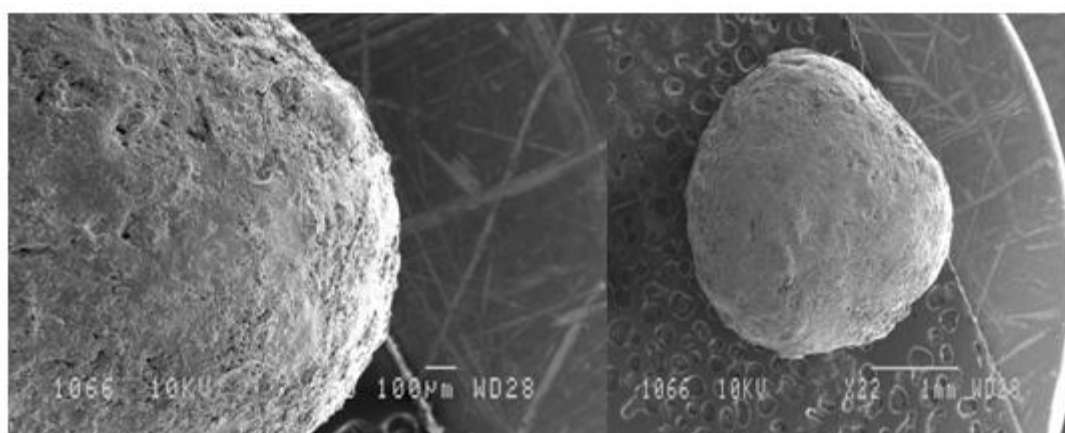


Fig 11: SEM Micrograph of Formulation (F5)

In-vivo study

The antiulcer activity of the selected formulations was studied on albino Wistar rats.

Ulcer Index

For calculation of ulcer index the stomach of rats were opened from the greater curvature and washed with 0.9% NaCl. The stomach was studied on the dissecting microscope and Further grading was assigned to the lesions to calculate the ulcer index as shown in Table 5.

Table 5: Ulcer Index for Ethanol Induced Ulcers in Rats

Treatment Group	Ulcer Index ± S.D.
Group 1 (Normal group)	
Group 2 (ethanol group)	0.84 ± 0.615
Group 3 (Pure drug group)	0.216 ± 0.119
Group 4 (Pellets group)	0.455 ± 0.17

N=3, (S.D.= Standard Deviation)

Macroscopic Analysis

There was presence of ulcerative hemorrhage due to 70% ethanol in group 2 animals. It was attenuated by prior and administration of pure drug in group 3 and pellets in group 4. Furthermore, the animals treated with pellets were able to prevent the damage induced by ethanol as with the normal group. Due to administration of ethanol there was increase in the ulcer index.

Histopathological Evaluation

The results of histopathological evaluation revealed that due to ethanol administration a deep alteration of glandular epithelium and loss of the histological structure of stomach was seen. There was severe swelling in the tissue structure, loss of continuity epithelial and pronounced infiltration of neutrophils. Furthermore, the group 3 showed protection against ethanol induced ulcers and swelling.

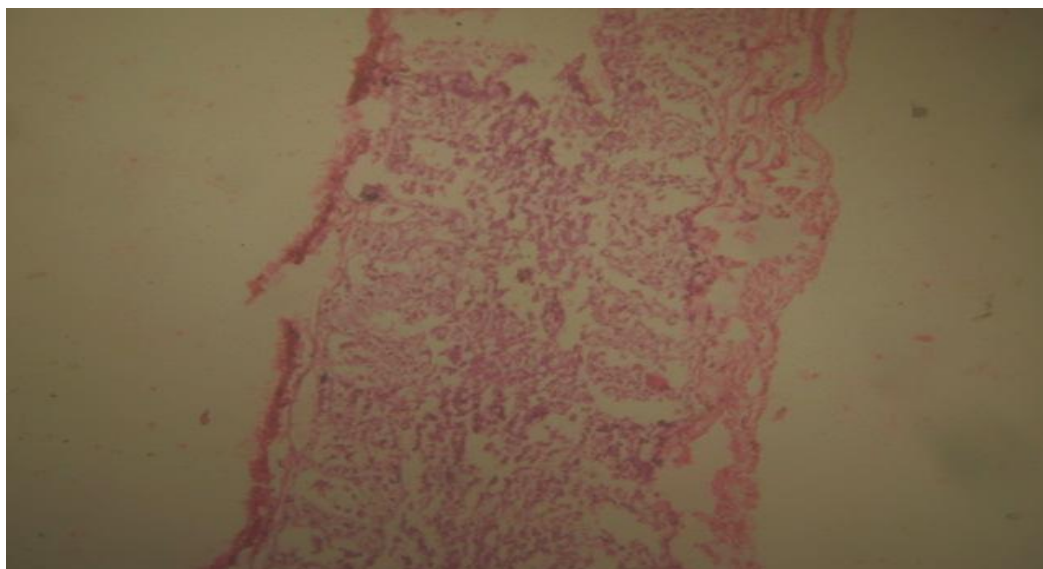


Fig 12: Histological Section of Gastric Mucosa of Rat (ethanol group). There is severe disruption of the surface epithelium (H&E stain, 10 X)

The lesions of stomach were characterized by many granulation tissues and intense inflammation. Pre treatment with RAB 20 pellets prevented the hemorrhage, edema, necrosis and deep ulcerations induced by ethanol with an appearance of intact mucosal layer was observed in the healed tissues of stomach.

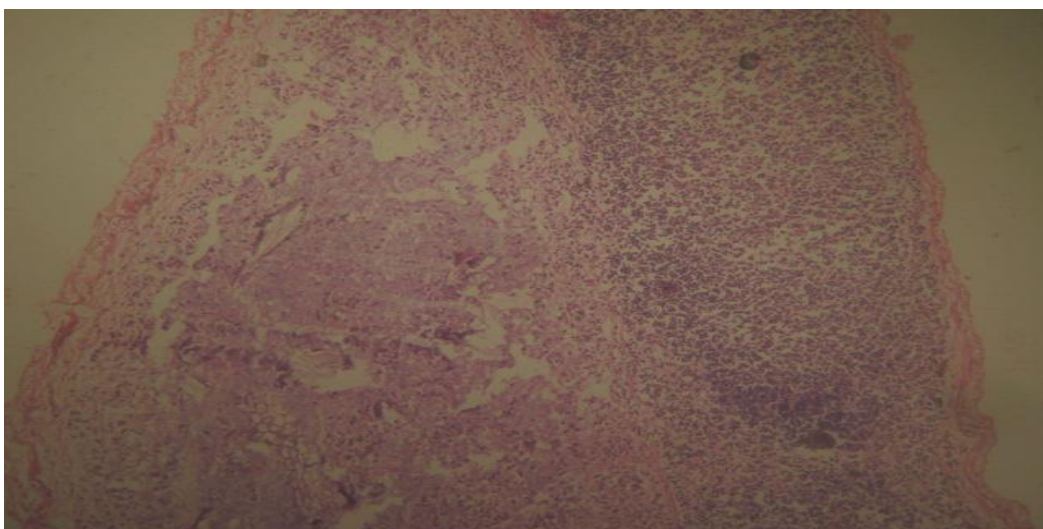


Fig 13: Histological Section of Gastric Mucosa of Rat Pre-treated Rabeprazole pure 20 mg/kg). There is no disruption of surface epithelium (H&E stain, 10 X)

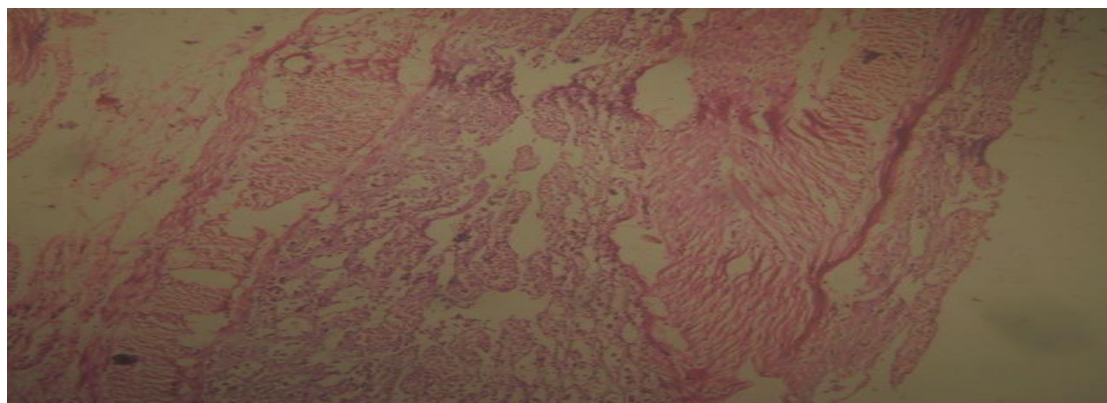


Fig 14: Histological Section of Gastric Mucosa of Rat Pre-Treated with Rabeprazole sodium pellets. There is mild disruption of the surface of epithelium (H&E stain, 10 X)

Biochemical estimation of antiulcer activity

Reduced Glutathione (GSH) Estimation

The ethanol group showed significant changes on oxidative markers with a decrease in GSH level (GSH = 3.152 ± 0.6760 n mol/mg protein). However, the animals receiving enteric coated enteric coated pellets of Rabeprazole sodium (RAB 20 PEL) (20 mg/kg) completely attenuated the damage induced by ethanol as compared to ethanol group as shown in Table 6.

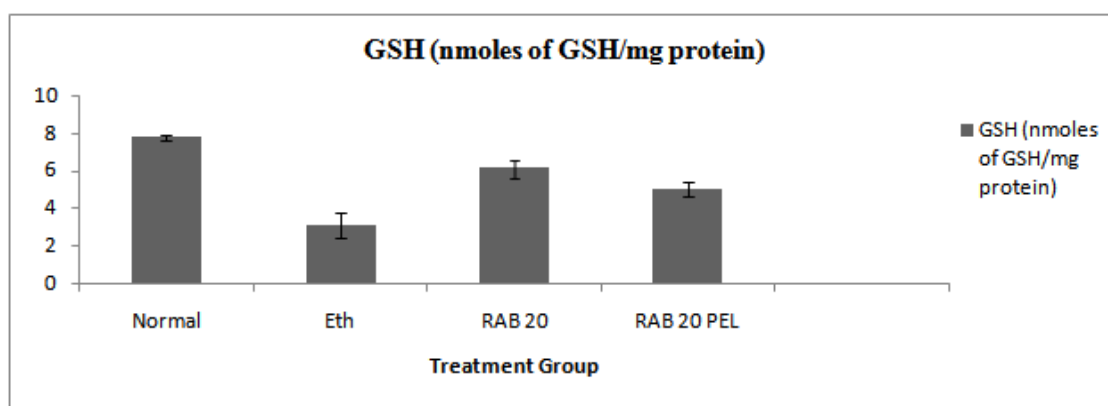


Fig 15: Effect of Formulations on Antioxidant Parameter Reduced glutathione (GSH) in Ethanol Induced Ulcers in Rats. Values are as mean \pm SEM; N = 6

Lipid Peroxidation (LPO) Estimation

Ethanol administration resulted in marked lipid peroxidation estimated by increased level of TBARS (MDA = 4.524 ± 0.369). However, treatment with enteric coated pellets of Rabeprazole sodium (RAB 20 PEL) (20 mg/kg) markedly attenuated the TBARS level as shown in Table 6

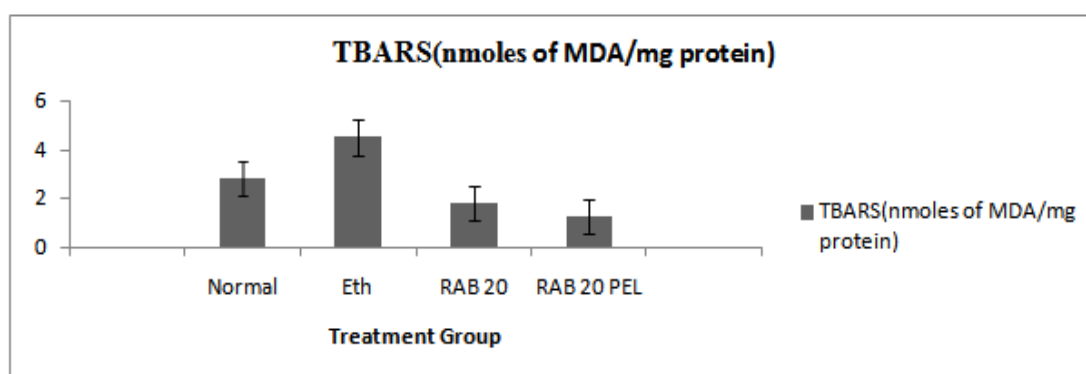


Fig 16: Effect of Formulations on Antioxidant Parameter Lipid Per oxidation (LPO) in Ethanol Induced Ulcers in Rats. F value= 8.635. Values are mean \pm SEM; N = 6

Catalase Activity Estimation

Ethanol induced the depletion of antioxidant enzyme Catalase thus in ethanol group of animals the catalase activity decreased (CAT = 1.571 ± 0.2634) and this decrease in Catalase activity was increased on administration of Rabeprazole formulation i.e. enteric coated pellets of Rabeprazole sodium (RAB 20 PEL) (20 mg/kg). (CAT = 4.66 ± 0.3984, CAT = 4.142 ± 0.2803 respectively).

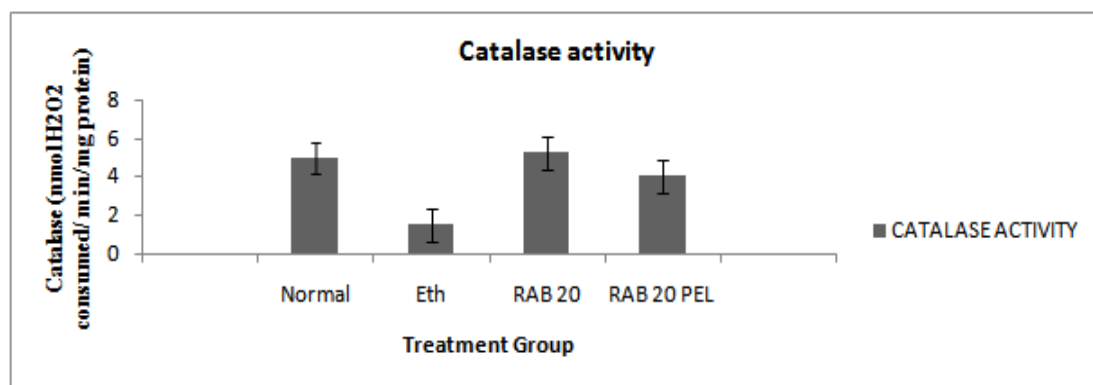


Fig 17: Effect of Formulations on Antioxidant Parameter (Catalase Activity estimation) in Ethanol Induced Ulcers in Rats. F value=7.048. Values are mean ± SEM; N = 6

Table 6: List of antioxidant parameters

Antioxidant Parameters	Groups			
	Group 1 (Normal group)	Group 2 (Ethanol group)	Group 3 (Pure Drug Group)	Group 4 (pellets group)
Reduced glutathione (GSH)	7.781 ± 0.1441	3.152 ^a ± 0.6760	6.143 ± 0.4836*	5.029 ± 0.4094*
Lipid peroxidation (LPO)	2.853 ± 0.285	4.524 ^a ± 0.369	1.827 ± 0.610*	1.331 ± 0.424***
Catalase activity	5.0 ± 0.8690	1.571 ^a ± 0.2634	5.261 ± 0.7156*	4.071 ± 0.2803***

Values are presented as mean ± SEM (Standard Error of Mean); N=6 in each group. One way ANOVA followed by Dunnet's test ^ap < 0.01 v/s normal group; *P < 0.01, ***P < 0.05 V/S ethanol group.

IV. Conclusion

This study was carried out to develop oral modified release delivery systems for Rabeprazole sodium using enteric coated pellets. Dry powder coating of pellets was found to be less time consuming as well as easy and reliable method compared to other coating methods. *In vivo* studies also showed that damage induced by ethanol causes the production of marked gross mucosal lesions and long hemorrhage bands. Animals pretreated with Rabeprazole pellets showed very less/mid lesions due to hemorrhage and sometimes few lesions.. Thus, this study confirmed that enteric coated formulation can be used to protect degradation of Rabeprazole sodium from the acidic environment of stomach. The therapeutic potential of the formulations can be further explored with the help of long term pharmacokinetic and pharmacodynamic studies in clinical settings.

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