AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

FORMULATION AND EVALUATION OF ENTERIC COATED MICROSPHERES OF PROTON PUMP INHIBITOR: *IN-VIVO* CHARACTERIZATION

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ABSTRACT

Pantoprazole Sodium sesquihydrate is a proton pump inhibitor that belongs to group benzimidazole, used for the treatment of ulcers in duodenum and gastric, which was degraded in stomach .Thus, the purpose of the study is to formulate a dosage form which is coated by coating polymer(s) which passed the acidic medium and exhibit significant effect in intestine and cures ulcers. An attempt was made to formulate micro particles with two coating polymers: Eudragit L-100 & Cellulose acetate phthalate as well as using of mucoadhesive polymers will release drug in controlled manner. Thus, these different types of micro particles was characterized in terms of Particles size, Mucoadhesive efficiency, Entrapment efficiency, *In-vitro* as well as *In-vivo* studies.

Key words: - Pantoprazole, Coating polymers, Mucoadhesive polymers

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Received 15 August 2011, Accepted 31 August 2011

Please cite this article in press as: Patel HJ *et al.*, Formulation and evaluation of Enteric Coated Microspheres Of Proton Pump Inhibitor: *In-Vivo* Characterization. American Journal of PharmTech Research 2011.

INTRODUCTION

Pantoprazole is a proton pump inhibitor that has been widely used in the treatment of gastric, duodenal ulcer and also in gastro esophageal reflux disease (GERD), Zollinger-Ellison syndrome. This the most popular drug used in cure and maintenance therapy of peptic ulcer along with drugs such as metronidazole, Clarithromycin or amoxicillin. It suppresses the acid production by inhibiting the Na+ K+ ATPase. The pantoprazole is an acid labile drug, which can be degraded in the stomach. Therefore, the dosage form should be should be coated so as to bypass the stomach release drug in intestine and exhibit desired pharmacological action. The drug has less half-life so an attempt was made to formulate core micro particles with mucoadhesive polymers which release drug in delayed form. This drug was the first water soluble benzimidazole, 5- (difluoromethoxy)-2-[[(3, 4-dimethoxy-2-pyridinyl) methyl] sulfinyl]-Benzimidazole, which can be administered intravenously in the form of sesquihydrate sodium pantoprazole.[1, 2]

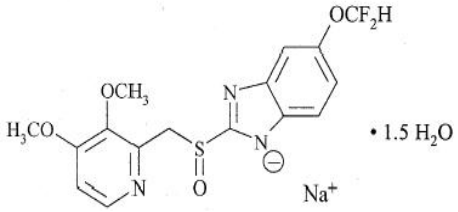


Figure 1 Chemical structure of sodium Pantoprazole

In order to administer Pantoprazole by the oral route, polymeric microspheres appear to be an interesting device. Despite the more complex and onerous production of the multiple-unit systems, microspheres have several advantages in relation to the single-unit products, including ready and uniform distribution in the gastrointestinal tract, minimizing the risk of local damage caused by a dose dumping effect. Furthermore, microspheres are also less affected by the pH and the gastric transit time, attain more constant plasma levels, give higher accuracy in reproducibility dose by dose and achieve a slow-release effect. Pantoprazole microspheres were pre pared by ion-gelation and solvent evaporation method. Firstly core microspheres was prepared along with mucoadhesive polymers and then subjected for coating.[3, 4, 5]

The microspheres were evaluated for Entrapment efficiency, Mucoadhesive property, Micromeritics property, *In-vitro* study as well as *In-vivo study*. The flow characteristics of the microspheres were assessed by determining their angle of repose and Carr's Index. The values of compressibility index and angle of repose signify good flow ability of the microspheres for all the batches. The stability study indicated that the prepared formulation was stable and retained

their pharmaceutical properties at room temperature and 40°C/75% RH over a period of 1 month. The Cellulose acetate phthalate coated as well as Eudragit l-100 microspheres did not release the drug in hostile acidic environment (pH 1.2) due to protective polymer coating and released the drug in the intestinal environment (pH 7.4).[1,3]

The antiulcer activity of the Cellulose acetate phthalate & Eudragit l-100 coated microspheres of pantoprazole formulations were evaluated by using ethanol immersion stress induced ulcer model.[6]

MATERIALS AND METHODS

Pantoprazole was obtained as a gift sample from Ipca, Mumbai. Eudragit L-100 and Cellulose acetate phthalate, Sodium alginate, HPMC K 14 were procured from Degussa and High Media Mumbai respectively and all the other polymers used are of analytical grade.[7, 8, 9, 10, 11]

Preparation of microspheres

Coating polymers were previsiously shoaked overnight in acetone in a beaker at room temperature, for complete dissolutionand in another beaker others polymers was placed on magnetic stirrer and prepared slurry, afterwhile solution of drug was added and finally stirred at 300-400 rpm for 40 -50 min so as to reched uniformity. This slurry was pour dropwise in 7 % w/v $CaCl_2$ solution through 26 or 24 # guage needle. Finally, filter ,washed three times with distilled water and dried over night in incubator at 37^0 C. These prepared microspheres were placed in coating polymer solution and stirred for 2 hrs at 50 -100 rpm and finally filtered, washed with organic solvent and dried at room temperature.[12, 13]

Table 1 formula for different batches

Batch	Ratio of Sodium alginate: HPMC K-14	Drug	Eudragit L-100	CAP
M_1	2:1	100 mg	100mg	-
M_2	1.5:1	100 mg	150mg	-
M_3	0.5:1.5	100 mg	200mg	-
M_4	1:0.5	100 mg	150mg	-
M_5	2:1	100 mg	-	100mg
M_6	1.5:1	100 mg	-	150mg
M_7	0.5:1.5	100 mg	-	200mg
M_8	1:0.5	100 mg	-	150mg

Evaluations:-

1. **Micromeritics characterization**[14]

Bulk density is determined from bulk volume and the weight of dry powder in a graduate cylinder.

$$\text{Bulk density} = \frac{\text{weight of powder}}{\text{bulk volume}}$$

Tapped density is obtained by mechanically tapping the measuring cylinder containing powder.

$$\text{Tapped density} = \frac{\text{weight of powder}}{\text{tapped volume}}$$

Angle of repose

It is measured by fixed funnel and free standing cone methods. In fixed funnel and free standing cone, a funnel is fixed with its tip at given height (2 cm) and below it, placed a graph paper flat surface. Powder is carefully poured through a funnel until the apex of the conical pile touches the tip of the funnel. Then, put this values in the following equation,

$$\theta = \tan^{-} \frac{h}{r}$$

h = Height of pile

r = Radius of pile

Carr's index

It is measured by the following equation,

$$\text{Carr's index} = \frac{\text{tapped density - bulk density}}{\text{tapped density}}$$

Hausner's ratio

It is measured by the following equation,

$$\text{Hausner's ratio} = \frac{\text{tapped density}}{\text{bulk density}}$$

Porosity

It is measured by the following equation,

$$\text{Porosity} = \frac{\text{Void volume}}{\text{bulk volume}}$$

$$\text{but Void volume } V = Vb - Vt$$

$$\text{Porosity} = \frac{Vb - Vt}{Vb}$$

2. Photo stability[15]

Pantoprazole-loaded microspheres and pure pantoprazole were exposed to UVA light for 96 h. The light source was a fluorescent lamp UVA, 130 V, 30 W fixed to a chamber in a horizontal position 22 cm from the samples. The chamber was internally coated with mirrors in order to distribute light homogeneously. Pure pantoprazole powder and the microspheres powder were put in a very fine layer in watch glasses and placed inside the chamber. Samples were collected at 6, 24 and 96 h and analyzed for the pantoprazole contents by UV-VIS spectrophotometer. Protected samples, completely covered with aluminum foil, were used as dark controls in order to evaluate the influence of thermally induced drug content the total change.

3. Mucoadhesive Efficiency[16]

The in vitro mucoadhesive test was carried out using small intestine from chicken. The small intestinal tissue was excised and flushed with saline. Five centimeter segment of jejunum were everted using a glass rod. Ligature was placed at both ends of the segment. Desired microspheres were scattered uniformly on the everted sac from the position of 2 cm above. Then the sac was suspended in a 10ml tube containing 8 ml of saline by the wire, to immerse in the saline completely. The sac were incubated at 370C and agitated horizontally. The sac were taken out of the medium after immersion for 0.5, 1, 1.5, 2, and 2.5 hrs, immediately repositioned as before in a similar tube containing 8ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation.

Mucoadhesion= (no. of microspheres adhered/no. of microspheres applied) ×100

4. Particle Size, Shape & Morphology

The size distributions in terms of average diameter of the microspheres were determined by an optical microscope method. A compound microscope fitted with a calibrated ocular micrometer and a stage micrometer slide was used to count at least 100 particles. Scanning electron microscope was performed to characterize the surface morphology of the formed microspheres .The parameter of SEM were an acceleration voltage of 20 kv, a chamber pressure of 0.6 mmHg and an original magnification of X 80.

5. Drug And Polymers Interactions [17,18]

The FT-IR spectra acquired were taken from dried samples. An FTIR-8400S (SHIMADZU, IR Prestige-21) spectrometer was used for the analysis in the frequency range between 4000 and 400 cms-1. This was carried out to find out the compatibility between the drug pantoprazole sodium sesquihydrate and the polymer hydroxyl propyl methylcellulose (HPMC), Eudragit L-

100, Sodium alginate and Cellulose acetate phthalate. 10 mg of the sample and 400 mg of KBR were taken in a mortar and triturated. A small amount of the triturated sample was taken into a pellet maker and was compressed at10 kg/cm2 using a hydraulic press. The pellet was kept onto the sample holder and scanned from 4000 cm-1 to 400 cm-1 in Shimadzu FT-IR spectrophotometer. Samples were prepared for drug pantoprazole sodium sesquihydrate, polymer HPMC, Eudragit L-100, Sodium alginate and Cellulose acetate phthalate and physical mixture of drug and polymer. The spectra obtained were compared and interpreted for the functional group peaks.

6. *In-Vitro* Drug Release Study[19,20,21]

The prepared formulation was evaluated for *in-vitro* release by USP dissolution apparatus 1 at 50 rpm and at 37^0 C in order to determine 100% drug release. To evaluate gastro-resistant microspheres containing Pantoprazole were exposed to 300ml of 0.1M HCl. After 1 hr, a NaOH (2.6gm) and KH_2PO4 (6.12gm) aqueous solution (600ml) was added into the medium in order to reach pH 7.4. The samples were collected in pre-determined time intervals from 0 up to 720 min (12 hrs). Pantoprazole concentrations were determined by UV at 289 nm.

Release Kinetics

Data obtained from the *in-vitro* release studies of cellulose acetate phthalate & Eudragit l-100coated pantoprazole sodium sesquihydrate microspheres formulations were fitted to various kinetic equations such as zero order, first order, Higuchi model, Korsmeyer- Pappas model and Hixon-Crowell model.

Zero order equation

$Q_t = Q_0 + K_0t$

First order equation

$\text{Log } Q_t = \log Q_0 + Kt/2.303$

Higuchi equation

$Q = K_Ht^{1/2}$

Korsmeyer - Peppas equation

$F= (M_{t/} M) =K_m t^{n}$

Hixon-Crowell equation

$\sqrt[3]{Q_o} - \sqrt[3]{Q_t} = K_{HC}.t$

Where, Q_t = Cumulative drug release

Q_0 =Initial amount of drug

K_0, Kt, K_H, K_{HC} & K_m are respective constant of Zero, first, Higuchi, Hixon – Crowell & Korsmeyer-Peppas,

F = fraction of drug released at time, t

M_t = amount of drug released at time, t

M = total amount of drug in dosage form

N = diffusion or release component

t = time in hrs

If n < 0.5, Fickian mediated drug release occurs. Anomalous release (i.e. diffusion coupled with polymers relaxation) occurs 0.5 < n > 1.0 and erosion (i.e. complete matrix relaxation) mediated release occurs in n = 1.

7. *In- Vivo* Anti-Ulcer Activity[6]

Ulcers were induced by the oral administration of absolute ethanol (5 mL.kg-1) to 24 h fasted Wistar Female rats (n = 4), weighing. Formulations (20 mg.kg-1 of drug) were administered orally 1 h before the administration of ethanol. Prior to the oral administration, rats were anesthetized with diethyl ether. After 2 h of ethanol administration, animals were sacrificed; the stomachs were removed, opened along the greater curvature and examined for lesion measurements.

Table 2 Grouping of animals for the treatment

Group No.	Treatment	No. of animals
1	Ethanol (5 ml/kg) + distilled water	**04**
2	Ethanol (5 ml/kg) + Pantoprazole (20 mg/kg) dissolved in distilled water	**04**
3	Ethanol (5 ml/kg) + Pantoprazole microspheres (equivalent to pantoprazole 20 mg/kg)	**04**

An ulcer index U_1 is calculated as,

$U_1 = U_N+U_S+U_P * 100^{-1}$

Where, U_N = average of number of ulcers per animal

U_S = average of severity score

U_P = percentage of animals with ulcers

Specifications for ulcer count

0 = no ulcer

1 = superficial ulcers

2 = deep ulcers

3 = perforations

Biostatical interpretation

All data were analyzed by one way ANOVA followed by Benferroni's test

RESULTS AND DISSCUSION

Micromeritics characterizations:-

Carr's index: - Batch M_4, M_5, M_7 is of excellent flow

Batch M_1 is fair to passable

Batch M_2, M_3, M_6, M_8 are of good flow

Angle of Repose: - Batch M_1 M_2, M_3, M_4, M_5, M_6 are of excellent flow

Batch M_7 & M_8 is fair to passable

Hausner's ratio: - All formulation is of good flow

Photo stability

Photo stability was evaluated for 96 h (Table 3). After 6 h of light exposure no difference in the drug content was observed between pure pantoprazole or drug-loaded microspheres (93.50 and 96.54%, respectively). After 24 h, dark controls showed that pure pantoprazole was affected by temperature (drug content of 45%), but the microspheres were not (drug content of 92%). When exposed to light, pure pantoprazole was degraded almost 70%. However, drug-loaded microspheres exposed to light present a degradation of 23%. The dark controls after 96 h of experiment showed decay in pure pantoprazole concentration of 60% and in the drug-loaded microspheres of 20%. Exposing pure pantoprazole and the pantoprazole-loaded microspheres to 96 h of UVA light, pure pantoprazole was unstable and the sample presented only 18.58% of non-degraded pantoprazole (Table 3). On the other hand, when exposed to light, the drug loaded microspheres were able to protect 58.63% of the initial pantoprazole content.

Table 3 Photo stability studies of drug & formulations after exposure in UVA light

Drug concentration(%) after exposure period in UV chamber			
Samples	**6 (hrs)**	**24(hrs)**	**96 (hrs)**
Drug	93.50	36.74	18.58
Microspheres	96.54	78.29	58.63

Mucoadhesive, Entrapment Efficiency and Particle Size

From the above data it was concluded that on decreasing the concentration of Sodium alginate entrapment efficiency and mucoadhesive efficiency decreases but particles size reduces. Formulations M_1, M_2 shows significant entrapment & Mucoadhesion efficiency while that of M_3, M_7 shows smaller particles size. Alternatively, CAP coated microspheres shows better

entrapment & Mucoadhesion efficiency as compared to Eudragit L-100 coated which show better particles size in comparison to CAP coated.

Table 4 shows mucoadhesive, entrapment efficiency and particle size of different batches

Batch	Mucoadhesion	Entrapment	Particle size
M_1	83.33	87.04	565.20 ±12
M_2	86.66	89.80	469.26 ±05
M_3	73.33	79.01	329.90 ±07
M_4	83.33	73.33	512.31 ±09
M_5	83.33	78.90	516.48 ±15
M_6	66.66	66.64	509.86 ±10
M_7	66.66	34.38	305.94 ±08
M_8	50	58.53	455.59 ±12

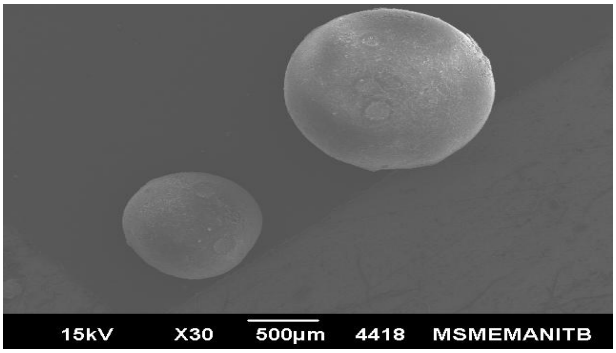


Figure 2 Particles size of microspheres by SEM

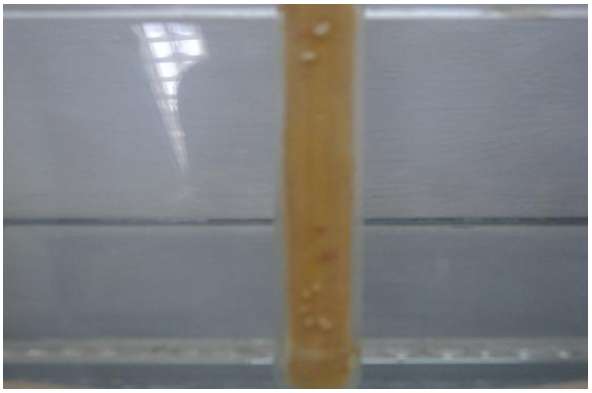
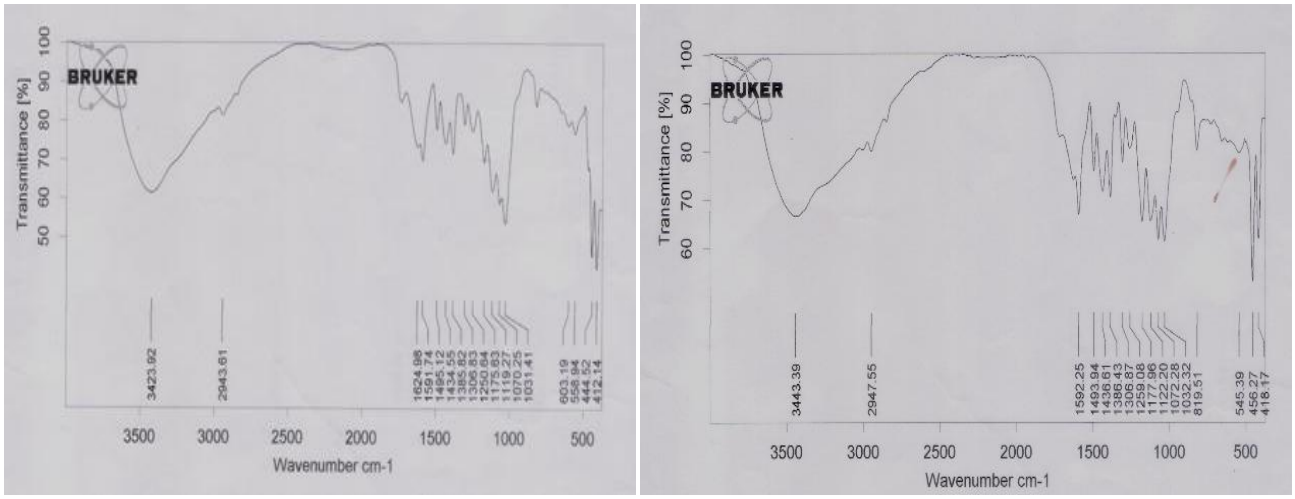


Figure 3 Microspheres adhered on chicken intestine showing Mucoadhesion property

Drug-Excipients study

From the FT-IR graph it was concluded that none of the either drug or polymers exhibits similar peaks at various stretching or deformation. Thus, it was concluded that no polymers interact with drug or itself. Hence formulation with these drugs in combination with these polymers should be possible and maintain stability and also not resulting in any kind of toxicity due to interaction, if it exits.



M4 **M6**

Figure 4 FT-IR graph of M_4 & M_6

***In-Vitro* Drug Release Study**

Table 5 Parameters for dissolution studies

Batch No	M_1 –M_8
Dissolution medium	7.4 Phosphate buffer
Volume of D.M.	900 ml
Dilution factor	3
Amount of drug	200 mg
RPM	50
Temperature	37.5^0 C

Table 6 Drug release profile (%CDR) of various formulations of Pantoprazole Sodium sesquihydrate microspheres

Time (Hrs.)	M1	M2	M3	M4	M5	M6	M7	M8
0	0	0	0	0	0	0	0	0
1	5.78	6.03	1.46	2.79	1.56	2.75	5.76	2.49
2	16.39	18.42	9.05	16.14	9.27	6.46	15.74	6.12
3	32.1	37.83	16.64	39.15	21.39	20.28	30.74	10.73
4	49.94	64.68	28.57	64.4	38.04	36.36	47.93	17.53
5	69.77	99.02	44.94	92.06	59.28	54.89	67.16	26.82
6	91.39	140.86	65.85	121.84	84.56	75.5	88.15	38.16
7	114.97	189.94	90.86	163.41	114.11	107.89	111.12	60.66
8	140.26	245.55	120.2	206.99	148.21	142.29	135.83	85.22
9	167.35	306.87	154.13	252.62	186.49	178.73	162.32	111.61
10	198.91	372.66	192.23	302.72	229.24	219.63	193.28	142.46
11	234.46	442.44	234.8	356.81	275.98	264.52	228.23	177.3
12	274.01	516.22	281.36	414.9	326.72	313.42	267.18	216.15

***In-vivo* Study**

Table 7 Shows treatment for ulcer

Group No.	Treatment *	Ulcer Index
1	Ethanol (5 ml/kg) + distilled water	1.118±0.032
2	Ethanol (5 ml/kg) + Pantaprazole (20 mg/kg) dissolved in distilled water	1.1±0.018
3	Ethanol (5 ml/kg) + Pantaprazole microsphere (equivalent to pantoprazole 20 mg/kg)	0.518±0.014 [a]

* n=4, a – $p < 0.05$ as compared to vehicle treated group

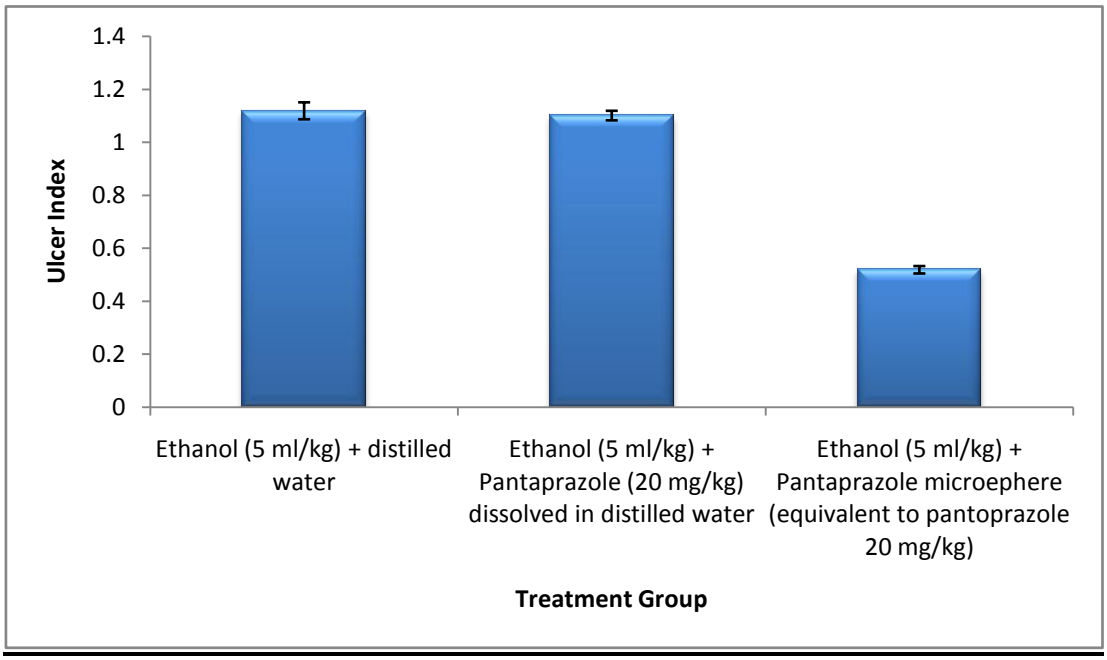


Figure 7 Biostatics graph of ulcer index

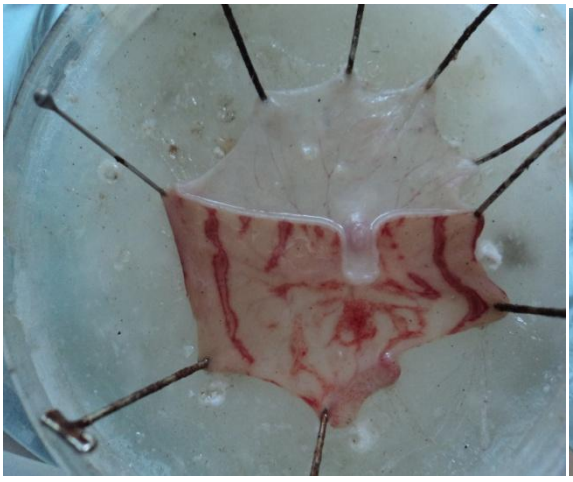


Figure 8– Ulcer group 1

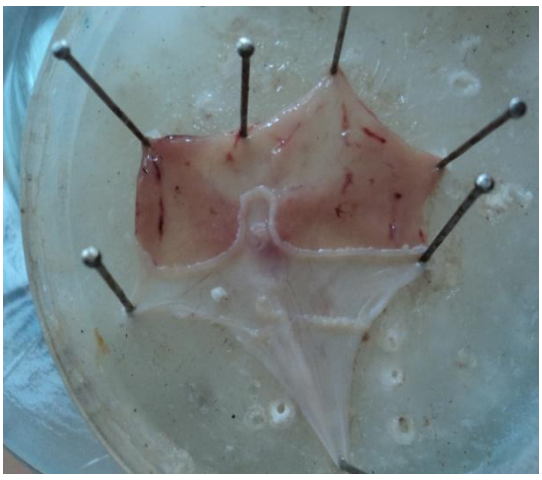


Figure 9 – Ulcer group 2

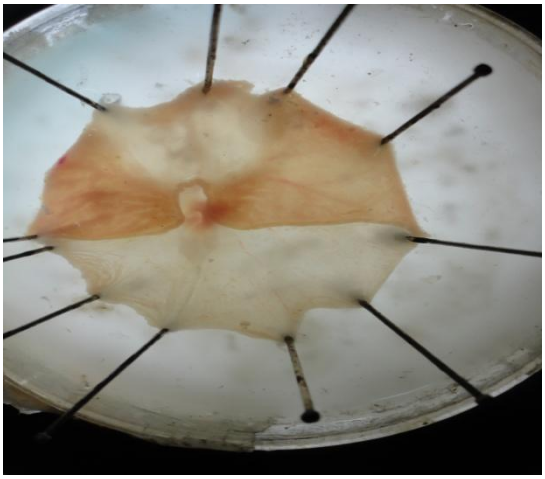


Figure 10 Ulcer groups 3

From the above *in-vivo* studies it was concluded that the ethanol induced ulcer was treated more superficially than that of the drug which was degraded in acidic medium. Hence, purpose of preparation of enteric coated microspheres was exhibit significantly effect on ulcer.

CONCLUSION

The aim of the present study was to formulate and evaluate controlled release drug delivery system of Pantoprazole sodium sesquihydrate microspheres by using HPMC, CAP, Eudragit L-100, Sodium alginate. FT-IR study was carried out to check any possible interactions between the drug and the polymers HPMC, CAP, Eudragit L-100, Sodium alginate, which confirmed that no interaction between the selected drug and the polymers. Pantoprazole sodium sesquihydrate microspheres were prepared by ion-gelation method using different concentration of HPMC, CAP, Eudragit L-100, Sodium alginate, enteric coated as well as mucoadhesive polymers.

The microspheres were evaluated for Entrapment efficiency, Mucoadhesive property, Micromeritics property, *In-vitro* study as well as *In-vivo study*. The flow characteristics of the microspheres were assessed by determining their angle of repose and Carr's Index. The values of compressibility index and angle of repose signify good flow ability of the microspheres for all the batches.

The *in-vitro* dissolution studies were carried out for coated microspheres using USP dissolution apparatus type I. The cumulative percentage of drug release from the microspheres varied and depends on the type of polymer used and its concentration. All formulations, M_5, were subjected to release kinetics, stability studies and M_3 & M_6 were subjected for animal studies. The drug releases from the formulations were zero order diffusion controlled and release mechanism was super case-II transport.

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