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Research Article

DEVELOPMENT AND EVALUATION OF GASTRORESISTANT MICROSPHERES OF PANTOPRAZOLE

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ABSTRACT

The present aim of the work was undertaken with one objective to develop gastroresistant drug delivery system for pantoprazole. Pantoprazole is an acid labile drug, which can be degraded in the stomach. Therefore, the drug should be targeted to intestine; to bypass the stomach the gastroresistant double walled microspheric drug delivery system was adopted. The formulations were developed consisting of double wall. The primary wall composed of mucoadhesive polymer sod. CMC and a release controlling polymer sod. alginate. The second wall coating the primary microspheres was composed of eudragit S-100.The effect of polymer concentration on the particle size, shape drug entrapment efficiency, mucoadhesive property, release study of core microspheres were evaluated.

Key word: Gastroresistant, Enteric coated, w/o emulsification/solvent evaporation, Pantoprazole, Acid labile, Microspheres.

INTRODUCTIION

Pantoprazole is a proton pump inhibitor that has been widely used in the treatment of gastric, duodenal ulcer and also in gastroesophageal reflux disease (GERD), Zollinger-Ellison syndrome. This the most popular drug used in cure and maintenance therapy of peptic ulcer along with antibiotics. 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.[1, 2, 3, 4] Therefore, the drug should be targeted to intestine; to bypass the stomach. The gastro resistant drug delivery system is developed for the drugs which are acid labile due to the necessity to pass intact through the stomach for reaching the duodenum for absorption. The dosage form is formulated to bypass the stomach by formulating solution for intravenous administration (lyophilized powder for reconstitution) or as gastric-resistant tablets (oral delayed-release dosage form).[5] In the case of oral administration, the enteric coating prevents the drug from degradation in the gastric juice (at pH 1–2, for few minutes.[6, 7, 8] therefore the enteric coating, on the acid labile drug, is necessary, thus they are less affected by pH. Thus the concept of gastroresistant drugs was generated. The gastroresistant delivery system is used for targeting the release of the drug in the gastrointestinal tract and recommended for application or therapy reasons, gastroresistant drug delivery system in which the drug could targeted in the intestine with the help of enteric coated or pH sensitive coating. *Raffin et al. 2006;* prepared and characterized gastro-resistant Pantoprazole-loaded microparticles prepared using an O/O emulsification/solvent evaporation technique. The *in-vivo* activity of the Pantoprazole loaded Eudragit S-100 microparticles was carried out in rats. Furthermore, tablets containing the microparticles were also investigated.[9] *Pollaufa et al. 2006;* prepared double-walled microspheres, with drug localized to the particle core, presented a promising route for control of drug release.[11] *Rahman et al. 2006;* prepared colon-specific microspheres of 5-fluorouracil for the treatment of colon cancer. They prepared core microspheres of alginate by the modified emulsification method and coated the core microspheres with eudragit S-100, to prevent drug release in the stomach and small intestine. They performed release studies of coated microspheres in a pH progression medium mimicking the conditions of the gastrointestinal tract. They evaluated that the release was sustained for up to 20 hours in formulations with core microspheres to a eudragit s-100 coat ratio of 1:7. [12]

Eudragit s-100 is a gastroresistant polymer used for colonic delivery, protecting drug from pH of upper gastrointestinal tract. Taking into account, this study concerns the characterization of gastroresistant double wall microspheres containing pantoprazole prepared by w/o emulsification/ solvent evaporation technique for successful encapsulation of acid labile drug, resulting in a gastroresistant and reduced initial burst as well as sustain release profile suitable for the care of peptic ulcer.

MATERIAL AND METHOD

Pantoprazole sodium was gift sample from Finecure (P Ltd.)U. S. Nagar. Uttrakhand Eudragit S-100 was gift sample from Degussa (Mumbai), sod.carboxymethyl cellulose, sod. alginate, liquid paraffin, isopropyl alcohol, sodium hydroxide, acetone and dichloromethane was purchased from Central Drug House(New Delhi) all the chemical were of analytical grade and double distilled water used throughout the experiment.

Preparation of double walled microspheres

The double walled microspheres were prepared by two step process. In first step the core microspheres of sod. alginate and sod. CMC were formulated. The microspheres then dispersed in the organic phase. The organic phase containing polymer in which drug was dissolved then the organic phase was emulsified with liquid paraffin. The solvent was allowed to evaporate and double walled microspheres were collected.

Formulation of core sodium alginate and sodium CMC microspheres with drug

Microspheres were prepared by water in oil emulsification solvent evaporation technique. A 3% polymeric aqueous solution was made in which the drug was dispersed and then the solution poured into 200 ml of light liquid paraffin containing 0.5% span-20 as an emulsifying agent. The aqueous phase was emulsified in oily phase by stirring the system in a 500ml beaker. Constant stirring at 500-1000 rpm was carried out using magnetic stirrer. The beaker and its content were heated at 50^0C, stirring and heating were maintained for 4.5 hrs. The aqueous phase was evaporated. The microspheres were washed with n-hexane, separated and dried at room temperature.

Table 1: Shows various core formulations using sod. alginate and sod. CMC polymer

S. No	Formulation	Drug	Sod. CMC	Sod.alginate
1	A1	1	1	3
2	A2	1	1.5	2.5
3	A3	1	2	2
4	A4	1	2.5	1.5
5	A5	1	3	1

Formulation of double walled microspheres

The previously formulated microspheres were dispersed in the organic phase (methanol: dichloromethane 1:4). Pantoprazole and the second polymer eudragit s-100 were dissolved in the same organic phase. The resulting organic phase solution was emulsified in liquid paraffin. 1% span-80 solution was used as emulsifying agent. Above emulsion was stirred at 500-1000 rpm for 4 hrs for complete evaporation of the organic solution. After complete evaporation of the organic solution the double walled microspheres were collected by vacuum filtration and washed with 3-4 times with n-hexane. The resulted double walled microspheres were freeze dried for 24 hrs.

Table 2: Showing various formulations of coated microspheres

S. No	Formulation	Core to coat ratio (w/w)
1	B1	1: 0.5
2	B2	1: 0.75
3	B3	1: 1
4	B4	1: 1.5

Morphology and Particle size Determination:

The size was measured using an optical microscope, and the mean particle with the help of a calibrated ocular meter.

Surface morphology /Scanning Electron Microscopy (SEM)

The external morphology of the microspheres was studied by scanning electron microscopy using apparatus Philip 505.

Drug entrapment efficiency or incorporation efficiency

$$Incorporation\ efficiency = \frac{b}{a} \times 100$$

To determine the drug entrapment efficiency or incorporation efficiency the microspheres were crushed in glass mortar and powered, then suspended in 10 ml of methanol, after 24 hrs the solution was filtered and filtrate was analyzed for drug content. The drug incorporation efficiency was calculated by the following formula:

b = calculated amount of drug present in the formulation,
a = theoretical amount of drug present in the formulation

Mucoadhesion study

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. 100 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 37^0C 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

***In-vitro* drug release of core microspheres**

The prepared formulation was evaluated for *in-vitro* release by USP dissolution apparatus 1 at 50 rpm and at 37^0 C temperatures in order to determine 100% drug release. To evaluate microspheres containing pantoprazole were exposed to 900ml of phosphate buffer (pH 7.4). The samples were collected in pre-determined time intervals from 0 upto 480 min (8 hrs). Pantoprazole concentrations were determined by UV at 289 nm.

***In-vitro* drug release of coated microspheres**

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 gastroresistant microspheres containing pantoprazole were exposed to 300ml of 0.1M HCl. After 1 hr, a NaOH (2.6gm) and KH_2PO_4 (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 upto 720 min (12 hrs). Pantoprazole concentrations were determined by UV at 289 nm.

RESULTS AND DISCUSSION

Particle size of the drug loaded microspheres

The particle size and surface morphology was determined with the help of optical microscope and Scanning Electron microscope. Spherical shaped microspheres were observed with optical microscope and particle size between 30.61µm to 33.5µm.

Table 3: Showing particle size, percentage drug entrapment and percentage mucoadhesion,

S. No	Formulation	Particle size (µm)	Percentage drug entrapment	Percentage mucoadhesion
1	A1	33.5±1.43	52±1.43	80±2.4
2	A2	33.1±1.54	56±1.43	82±0.98
3	A3	32.3±1.65	64±1.43	83±1.45
4	A4	31.4±1.23	68±1.43	86±0.97
5	A5	30.6±0.98	72±1.43	88±1.20

*Results shown are the mean ±S.D. n=3

Surface morphology

Surface morphology of the core microspheres was examined by scanning electron microscopy (SEM) (PHILIP 505). It was observed that surface of the A1 microspheres were some rough, in comparison to A2, A3, A4 and A5 because it have the higher concentration of sod. alginate. As the sod. c.m.c. concentration increased the smoothness in shape of microspheres was observed, as shown in Fig.1. A5 showed the least particle size 30.61µm because it contains higher proportion of sod. CMC which was due to spherical nature of the microspheres. A1 had the largest proportion of sod. alginate, showed the largest particle size of 33.51µm. On increasing the proportion of sod.cmc the decrease in size of microspheres was observed, that was 33.5, 33.1, 32.32, 31.46 and 30.61 µm for formulation A1, A2, A3, A4 and A5 respectively. This may be due to of increase in availability of the, polymer for entrapment of drug particles. A3 shows the particle size in between A4 and A1 because A3 contains the equal proportion of the sod.cmc and sod. alginate polymer, The rank order of size A5> A4> < A3>A2> A1. As given in table -3.

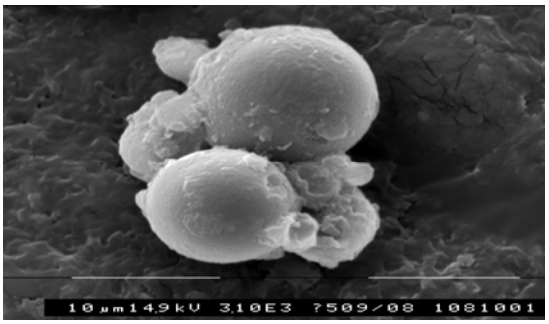

Fig. 1: SEM photograph of microspheres

Drug Entrapment Efficiency

In case of core microspheres, on increasing the concentration of sod. c.m.c. polymer, the amount of drug entrapment will increase as it was observed maximum 72% in A5 and less 52% in A1 where the polymer to polymer ratio is 3:1 and 1:3 for sod.cmc and sod. alginate, respectively. This was due to the sod. CMC shows good entrapment efficiency then the polymer sod. alginate, as given in table 3.The rank order of entrapment efficiency A5> A4> A3>A2> A1.

Effect on mucoadhesion

To assess the mucoadhesivity of the microspheres in-vitro wash off test was performed for all the formulations. At the end of 405 min (4hrs 15 min) the percent mucoadhesivity was found 10, 15, 18, 23, 26 for formulation A1, A2, A3, A4 and A5 respectively, shown in table 3. Formulation A5 showed the highest mucoadhesivity due to the presence of higher proportion of sod. c.m.c. polymer, due to the anionic nature of the polymer, and A1 showed the lowest mucoadhesivity due to higher proportion of sod. alginate due to the irregular surface was increased.

***In-vitro* drug release profile of core microspheres**

These studies show the effect of environment of the body on the drug release pattern from the prepared microspheres. The *in-vitro* release was observed in phosphate buffer (pH 7.4) for 8 hrs. It was found that the release rate from the all formulation was found to be different for the different polymer proportion used in the formulation 76.3%, 79.4%, 84.0%, 86.0% and 93.0% for formulation A1, A2, A3, and A4 and A5 respectively. The A5 has highest proportion of polymer sod. CMC, showed maximum release. While the A1 shows the least drug release after 8 hrs. Due to less swelling action and irregular surface as compared to A5, as given in table 3 and fig.3.

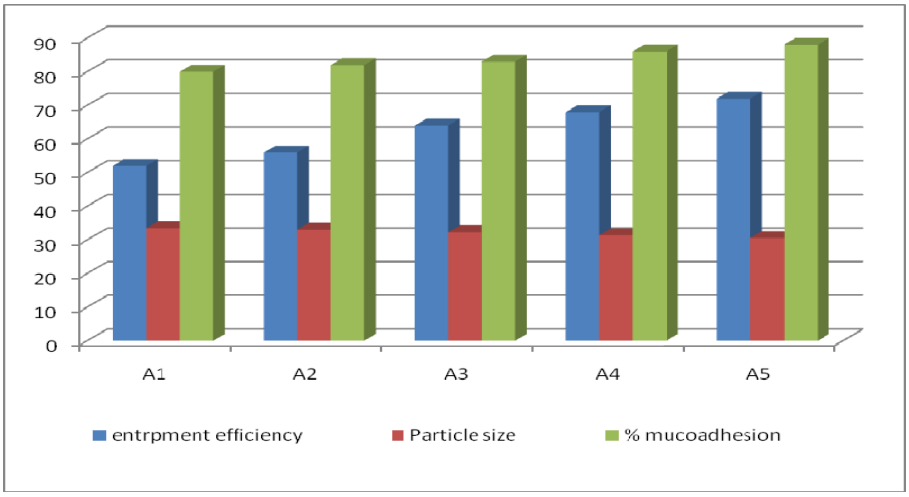

Fig.2: Showing entrapment efficiency particle size and percentage mucoadhesion

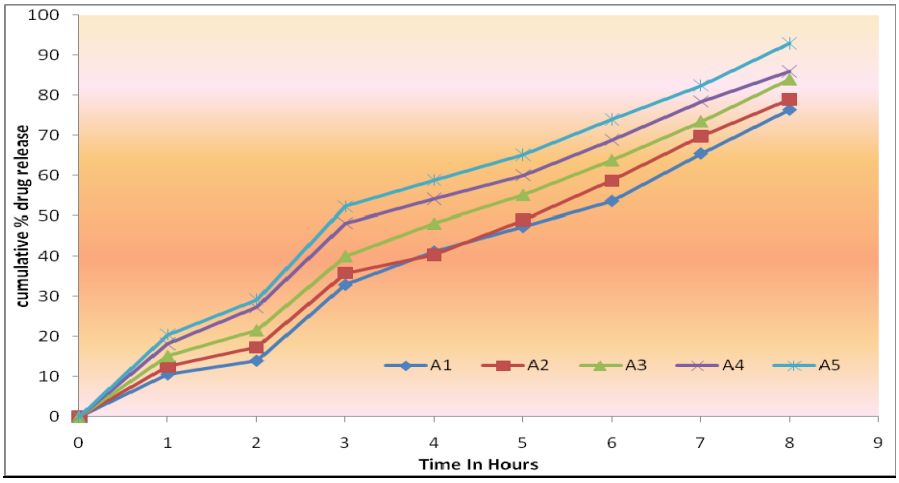

Fig.3: In-vitro drug release profile of different formulations showing the effect of drug and polymer on drug release from core sod.alginate and sod. CMC microspheres

Evaluation of double walled microspheres

Particles size and surface morphology

The particle size and surface morphology was determined with the help of optical microscope and scanning electron microscope. Smooth spherical shaped microspheres were observed with optical microscope and particle size between 30.61µm to 33.5µm (fig.4)

The change in particle size was observed only for some extent. Results are given in table -4.

Table 4: For particle size, percentage drug, entrapment and percentage mucoadhesion

S. No.	Formulation	Core : Coat	Particle size(µm)	Percentage drug release (12 hrs)
1	B1	1;0.5	61.952±1.31	94.352±0.93
2	B2	1:0.75	65.552±0.97	92.452±1.13
3	B3	1:1	75.252±0.79	89.252±1.63
4	B4	1.2	78.452±1.25	80.152±1.03

*Results shown are the mean ±S.D. n=3

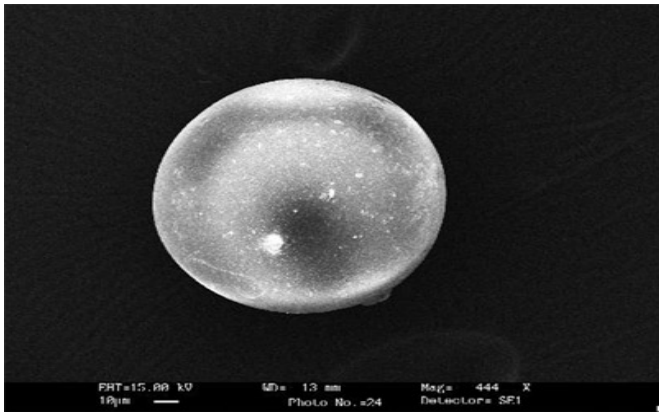

Fig.4: SEM photograph of coated microspheres

***In -vitro* drug release profile of double walled microspheres**

These studies show the effect of environment of the body on the drug release pattern from the prepared microspheres. The *in-vitro* release first determined in the pH 1.2 for 2 hrs, all formulation shows no drug release at this pH. Then the pH was increased to 7.4 Phosphate buffer (pH 7.4) for 12hrs. It was found that the release rate from the all formulation was found to be different for the different polymer proportion used in the formulation 94.3%, 92.4%, 89.2% and 80.1% for formulation B1, B2, B3, and B4 respectively. This may be due to of increase in availability of the polymer for entrapment of drug particles. The B1 has lower proportion of polymer eudragit s-100 showed maximum release, while the B4 shows the least drug release after 12 hrs due to less swelling action and irregular surface as compared to B1 as shown in table no 4 and fig.5.

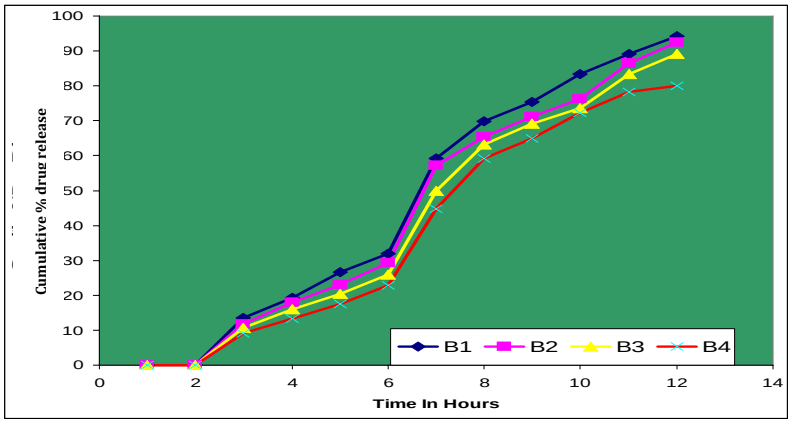

Fig.5: in-vitro drug release profile of different formulations showing the effect of polymer on drug release from coated microspheres.

After evaluating all the formulation, the formulation A5 which is containing the higher percentage of sod CMC showed the good entrapment efficiency, mucoadhesion, good drug release profile. Therefore it was selected as the best formulation. Then the walled

microspheres was formulated by varying concentration of eudragit s-100, there four formulation was formulated B1, B2, B3 and B4 from A5, on analyzing the all the formulations, B1 was found as best formulation.

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