Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2011, 3 (6):82-89
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0974-248X
USA CODEN: DPLEB4

Synthesis, characterization and antiulcer study of pH-sensitive microspheres

Rama Nargund[2] and A R Kulkarni[1]

[2]*Nargund Research Foundation, Nargund College of Pharmacy, Bangalore, Karanataka, India*
[1]*Soniya Education Trust's College of Pharmacy, Sangolli Rayanna Nagar, Dharwad, Karnataka, India*

ABSTRACT

Omeprazole, a proton-pump inhibitor used in peptic ulcers, gastro-esophageal-reflux disorder, Zollinger-Ellison syndrome and in H. pylori infections. The omeprazole is unstable at acidic pH, undergoes degradation in stomach. To prevent the degradation in stomach, dosage forms are supplied as enteric-coated tablets or granules encapsulated in gelatin capsules. The efficiency of such dosage forms depends upon the - extent of coating, solubility of coating material; type of dosage form etc. Recently, pH-sensitive polymer are utilized to deliver drug to intestine. The polymer swells only in alkaline pH and releases the drug as it enters the intestine. In this research, pH-sensitive formulations were developed to deliver the omeprazole effectively. Using the stimuli-responsive polyacrylamide-g-sodium alginate polymer, microspheres were prepared by coaservation followed by cross-linking with gluteraldehyde. Omeprazole drug were loaded in microspheres. All microspheres were evaluated for size distribution, content uniformity, in vitro dissolution and pulsatile swelling study. Pharmacological screenings were done for antacid and antiulcer activities of different omeprazole containing formulations. Results indicated that the pH, free acidity, total acidity, and ulcer-index in both non-lighted and ligated ulcer models were comparatively reduced in rats treated with the omeprazole containing pH-sensitive microspheres than enteric-coated granules than neat omeprazole. Thus, developed formulation release was superior in intestine and thus produced superior action.

Key words: Antiulcer activity, Omeprazole, Polyacrylamide, Microspheres, enteric coating.

INTRODUCTION

Omeprazole, a proton-pump inhibitor is widely prescribed for the treatment of peptic ulcer, Zollinger-Ellison syndrome, Gastro-esophageal reflux disease (GERD), H.Pylori infection and NSAID associated ulcers. Its oral bioavailability (40-50%) in humans is poor due to acid sensitivity and first pass metabolism. Attempts were made earlier to improve the bioavailability by formulating it as enteric-coated granules encapsulated in gelatin shell and enteric-coated

tablets. The efficiency of such dosage forms depends upon the number of parameters such as extent of coating, solubility of coating material and type of dosage form etc.

In the present study, omeprazole was encapsulated in acrylamide and that was crosslinked with gluteraldehyde. These microspheres are sensitive to pH changes and release the drug in an alkaline pH undergoing swelling. Microspheres were prepared by coaservation followed by crosslinking with gluteraldehyde. They were then evaluated for content uniformity, size analysis, in-vitro dissolution and pulsatile swelling. Absence of interaction between omeprazole and polymer was determined using IR spectral data. Pharmacological screening was carried out for antacid and antiulcer activities using pylorus ligation and non-ligation methods in rats. The parameters used to evaluate where change in pH, total acidity and ulcer index. The results were compared against data generated for neat omeprazole, and two marketed formulation viz. OMEZ, OCID.

MATERIALS AND METHODS

Omeprazole was a kind gift from Dr. Reddy's Laboratories, Hyderabad, India. Sodium alginate, Ceric ammonium nitrate (CAN), gluteraldehyde, tween-80, acetone, methanol, alcohol, phenolphthalein, hydrochloric acid (HCl), sodium hydroxide (NaOH) was purchased from s.d. Fine Chemicals, Mumbai, India. Marketed enteric- coated omeprazole granules containing capsules were obtained commercially (Omez capsules, Batch No. Y4125 made in India by Dr. Reddy's Laboratories, Hyderabad and Ocid capsules, Batch No. JD1059 made in India by Cadila Pharma).

Development of formulation and evaluation

Grafted polymer synthesis

Sodium alginate (Na-alg) was hydrated for 24 hr with constant stirring. Acrylamide (AAm) solution was then added followed by (0.005mol) of ceric ammonium nitrate (CAN). The mixture was allowed to polymerize at 60°C under continuous stirring for 6 hours. The grafted polymer was precipitated by acetone and washed with methanol. It was then dried overnight under vacuum (60 mm Hg) at 40°C.

Production of pH-sensitive microspheres

The grafted polymer solution was added drop wise into a solution of gluteraldehyde in a mixture of ethanol and HCl to obtain microspheres. These were then stirred for 1 h, decanted and washed with water. They were made pH-sensitive by stirring in 1 M NaOH for 4 h, and dried at 60°C. Drug was loaded into the microspheres by stirring them in drug solution for 4 h, decanting them, followed by drying at 40°C.

FTIR studies

Neat drug, empty microspheres and drug loaded microspheres were crushed and subjected to IR studies in the form of KBr pellets (Nicolet, Model Impact 410, USA at USIC, Karnataka University, Dharwad). Spectra were recorded over the range 400-3000^{-1} .

Swelling studies

The pH-dependent swelling of the microspheres were studied both in simulated gastric (0.1N pH-1.2 HCl) and intestinal (0.1M pH 7.4 phosphate buffer) respectively. Microspheres were then allowed to swell for 12 hours to attain equilibrium at 37° C. The swollen microspheres and dried microspheres (60° C for 5 h) were weighed. Then the degree of swelling Q was measured as follows-

$$Q = \frac{\text{Mass of Swollen Microspheres} - \text{Mass of Dry Microspheres}}{\text{Mass of Dry Microspheres}} \text{ ---------- Equation No.1}$$

Drug Content analysis

The amount of omeprazole loaded in the microspheres was estimated by refluxing 20 mg of the microspheres in 20 ml of 0.1N NaOH for 5 h, crushed and heated for 2 h. The resulting solution was analyzed by UV spectrophotometer (Thermospectronic, Genesys 6 USA) for omeprazole at 305 nm.

Drug Release studies

In vitro drug release studies were carried out, by using Dissolution tester (USP-XXIII, Electro lab), at 100 rpm. The dissolution media gastric fluid (0.1 M HCl) and intestinal fluid (pH 6.8 phosphate buffer) at 37° C were used to simulate the conditions. The drug release was measured in UV spectrophotometer (Thermospectronic, Genesys 6 USA) at 305 nm.

Antiulcer Studies

Pyloric ligation method

Wistar strain albino rats of either sex weighing 200-250 g were fed on regular diet with water ad libitum and used for the experiment.

Rats were divided into various groups on the bases of receiving neat omeprazole, OMEZ, OCID and pH-sensitive microspheres containing omeprazole at a dose of 10 mg/kg oral. Pyloric ligation (PL) was performed as described by shey et al. the PL was carried out on 50% of animals in each group at 30 min to obtain gastric juice before drug absorption and 4 h after the drug administration in the remaining to obtain gastric juice after the drug administration. 4 h after PL animals were sacrifice, their, gastric contents collected and subjected to centrifugation (3000 rpm for 10 min). The samples were then analysed for gastric acid volume, pH, and total acidity. The stomach was examined for severity of ulceration according to the following scale.

0= Normal grey coloured stomach, 0-5 = Pink to red coloration of stomach,
1= Spot ulcer, 1.5= Hemorrhagic streak, 2= Number of ulcers <5,
3= Number of ulcers >5, 4= ulcers with bleeding.
Ulcer index was calculated by adding the total number of ulcers and the severity of ulcer.

Pyloric non-ligation method

After 30 min and 4 h of the drug administration, the animals were sacrificed. Then gastric volume, pH, free, total acidity and ulcer index was determined as mentioned above.

RESULTS AND DISCUSSION

Development of formulation and evaluation

Grafted polymer synthesis and production of pH-sensitive microspheres

The grafted polymer obtained with different ratio of Na-alg and AAm are shown in (Table 1).

FTIR studies

The FTIR spectra of omeprazole, Na-alg, Na-alg-g-pAAm, microspheres containing omeprazole are displayed in (Figures 1 - 4). The peak at 1616 cm^{-1} shows the CN imine group present in the microspheres by the crosslinking between the gluteraldehyde and CAN and polymers. The microspheres containing omeprazole shown peak at 1700 cm^{-1} indicated the presence of pendent gluteraldehyde in the microspheres. The spectra results that there is no interaction between omeprazole and polymer. And it shows the presence of all peaks, which were present in the cross-linked polymer and pure omeprazole.

Swelling studies

The % water uptake Q was 108 at acidic pH and 1552 at alkaline pH and shown in (Table 2). This indicates that pH sensitive microspheres were highly swellable at alkaline medium, hence facilitates the drug release in intestine. The swelling was displayed in (fig 5).

Drug release studies

In-vitro drug release studies were carried out in simulated gastric solution for 12 h and the release of drug in acidic pH was very low where as it increases in intestinal pH and shown in (fig 6). The release data were fitted with Peppas equation, and the values of k, n, t calculated and is displayed in (Table 3). The calculated values of n were found to be 0.4 to 0.6 and the values of k ranges from -1 to 5 min 10^{-2} .

Antiulcer studies

Antiulcer and anatacid properties were estimated for neat omeprazole, OMEZ,OCID and microspheres containing omeprazole. The pyloric ligation and non-ligation methods results were tabulated in (Table 4 - 7) for 30 min and 4 h after the drug pyloric ligation and non-ligation respectively. The ulcer protection of pH-sensitive microspheres was superior to neat omeprazole and marketed formulation.

Figure-1 FTIR sspectrum of OMEPRAZOLE

Figure 2- FTIR spectrum of SODIUM ALGINATE

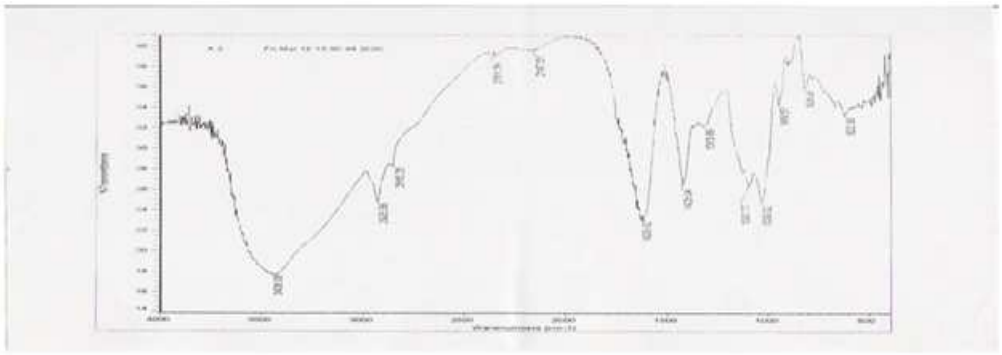

Figure 3- FTIR spectrum of sodium alginate-g-poly acryl amide

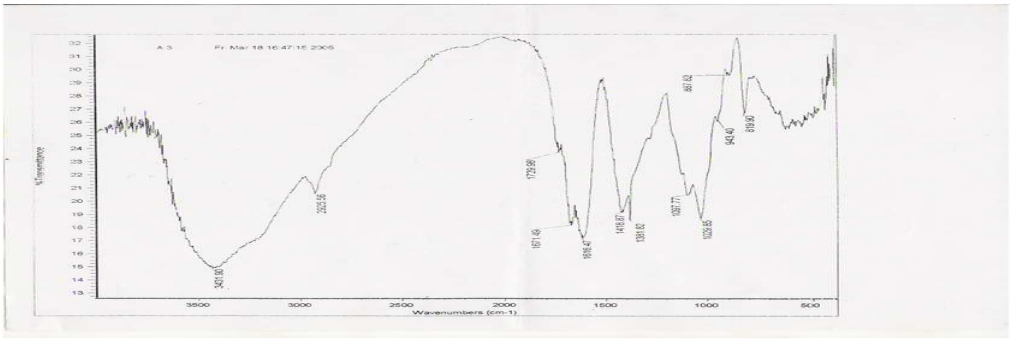

Figure 4- FTIR spectrum of pH sensitive microspheres containing omeprazole

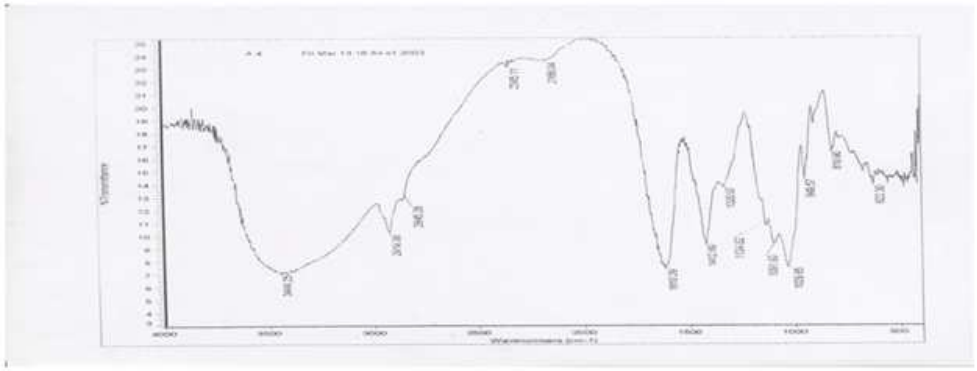

Table –1. % grafting, drug content, and microspheres size

Sl. No.	Polymer (Na-alg-g-pAAm)	% of Grafting	Drug content	microspheres size (in µm)
1	60 : 40	20	16.5	
2	50 : 50	30	23.4	780.5
3	40 : 60	40	29.96	

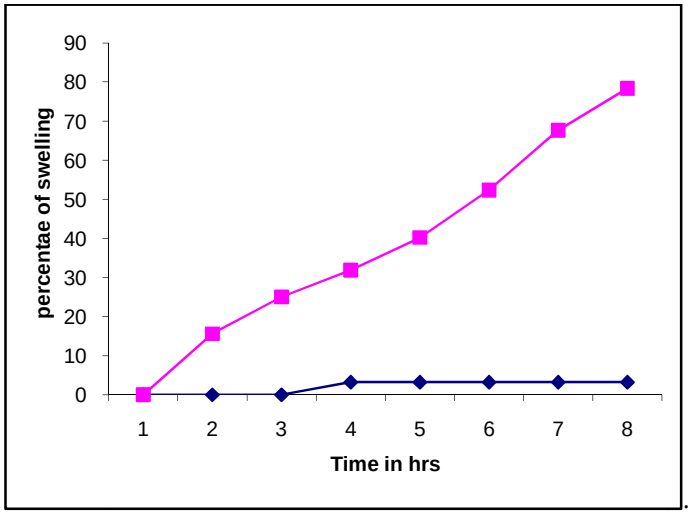

Fig-5. Swelling study of pH sensitive microspheres

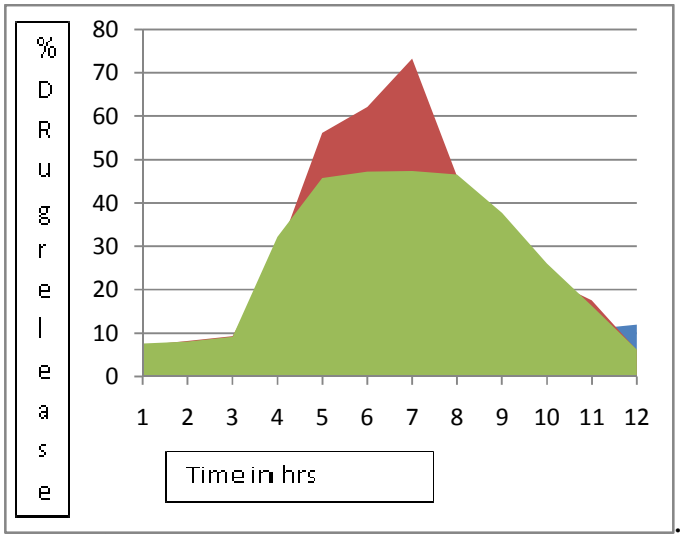

Fig - 6 Invitro drug release studies of pH-sensitive microspheres

Table 1- % water uptakeof pH sensitive microspheres

	HCl 0.1N	NaOH 0.1 M	Distilled Water
Mass of dry microspheres	50 mg	50 mg	50 mg
Mass of swollen microspheres	104 mg	826 mg	540 mg
Q	108	1552	980

Table 3- *In vitro* drug release kinetics of pH sensitive microspheres

Sl. No.	Mtrix type	K(min)10^2	n	r
1	Omeprazole -20	1.3	0.61	0.99
2	Omeprazole -30	2.7	0.53	0.98
3	Omeprazole -40	4.9	0.42	0.98

Table 4- Mean ulcer index of various omeprazole formulations

Sl. No.	Treatment	U I (30 min)	U I (4 hr)
1	Control	6.58 ± 0.15	6.5 ± 0.18
2	omeprazole	6.5 ± 0.35	6.33 ± 0.21
3	Omez	6.16 ± 0.28	2.58 ± 0.35 ***
4	Ocid	6.25 ± 0.11	2.75 ± 0.34 ***
5	Ome micro	6.08 ± 0.24	2.42 ± 0.35 ***
F		0.81 (4/25)	50.53 (4/25)
P		P < 0.53	P < 0.0001

*Significantly different from control at *** P< 0.0001*

Table 5- Mean ulcer index of various omeprazole formulations

Sl. No.	Treatment	U I (30 min)	U I (4 hr)
1	Control	6.42 ± 0.15	6.58 ± 0.15
2	omeprazole	6.25 ± 0.11	5.83 ± 0.21
3	Omez	6.25 ± 0.11	2.5 ± 0.38 ***
4	Ocid	6.08 ± 0.15	2.58 ± 0.35 ***
5	Ome micro	6.16 ± 0.11	2.33 ± 0.31 ***
F		0.95 (4/25)	48.89 (4/25)
P		P < 0.0001	P < 0.0001

*Significantly different from control at *** P< 0.0001*

Table 6- Gastric juice, pH and total acidity of various omeprazole formulations

Sl. No.	Treatment	30 min after drug administration			4 hr after drug administration		
		gastric juice (in ml)	pH	Total acidity	gastric juice (in ml)	pH	Total acidity
1	Control	5.05 ± 0.29	2.47 ± 0.04	76.5 ± 2.95	5.02 ± 0.07923	2.47 ± .04	78.5 ± 2.91
2	Ome	4.95 ± 0.06	6.45 ± 0.04 ***	70.17 ± 0.70 *	4.95 ± 0.05	7.38 ± 0.03***	69.5 ± 1.80 *
3	Omez	4.83 ± 0.08	7.35±0.02 ***	60.33± 1.2 ***	2.95 ± 0.07 ***	7.82 ± 0.04 ***	47.83 ± 0.79 ***
4	Ocid	4.86 ± 0.07	7.32±0.05 ***	64.5 ±0.12 **	3.02 ± .08 ***	7.8 ± 0.04 ***	55 ± 0.93 ***
5	Ome micro	3.02 ± 0.08	7.8 ±0.04 ***	55 ± 0.93 **	2.9 ± 0.13 ***	7.83± 0.03 ***	52.33 ± 1.61 ***
F		0.37	118.92	33007	173.9	52.27	1.31
P		P<0.8294	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.9999

*Significantly different from control at *** P< 0.0001*

Table 7- Effects of different omeprazole formulations in rats (observed after 30 minutes of drug administration)

Sl. No.	Treatment	gastric juice (in ml)	pH	Total acidity	gastric juice (in ml)	pH	Total acidity
1	Control	1.75 ± 0.04	2.63 ± 0.05	76.67± 1.43	1.52± 0.05	2.62± 0.06	85.33 ± 0.98
2	Ome	1.38 ± 0.03 ***	3.28 ± 0.06 ***	66 ± 0.63 ***	1.33 ± 0.03 *	3.55 ± 0.04 ***	69.5 ± 1.34 ***
3	Omez	1.3 ± 0.04 ***	6.7 ± 0.04 ***	57.5 ± 0.43 ***	0.92 ± 0.05 ***	7.88 ± 0.05***	45.83 ± 0.83 ***
4	Ocid	1.35 ± 0.02 ***	6.63 ± 0.05 ***	58.17 ± 0.72 ***	0.93 ± 0.04 ***	7.9 ± 0.04 ***	45 ± 0.30***
5	Ome micro	1.36 ± 0.03 ***	6.68 ± 0.08 ***	57.67 ± 1.62 ***	0.8 ± 0.05 ***	7.97 ± 0.08 ***	44.17 ± 1.4 ***
F		28.92	1249.2	60.185	50.48	2201.3	315.19
P		P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001

*Significantly different from control at *** P< 0.0001*

CONCLUSION

Analysis of the data reveals that the microspheres containing omeprazole in pyloric ligation and non-ligation ulcer models, when compared with the neat omeprazole or the two marketed formulations. The drug release follows the relaxation-controlled release, which is related to the

swelling of the miccrospheres in response to the pH changes. Thus drug release from microspheres in intestine, and was found to be superior to enteric-coated granules or neat omeprazole resulting in better bioavailability.

Acknowledgement
We sincerely thank Department of Science and Technology, New Delhi, for financial support SP/S1/H-50/2001 to purchase sophisticated instruments used in this research. I thank Dr. V.I. Hukkeri, Dr.A.R Kulkarni and for providing the necessary facilities, support and encouragement.

REFERENCES

[1] K Takeuchi, K Akira, *Journal of Gastroenterology and Hepatology.*,**1999**, 142, 251.
[2] JD Korwin, P Ducrotte, T Vallot, *Presse Med*., **2004**, 33, 11, 746.
[3] J Bateman, B Teusher, R Telford, *Pharmaceutics I (PSCI)*, **2002**, 423.
[4] A Farinha, A Bica, *Eur J Pharm Sci*., **1999**, 7, 4, 311.
[5] S Bozdang, S Calis, M Sumnu, *Farm Vestn*., **1997**, 48, 119.
[6] A Pilbrant, C Cederberg, *Scand J Gastroenterol*., **1995**, 20, 336.
[7] C Cederberg, T Anderson, I Skanberg, *Scand J Enterol*., **1989**, 166, 33.
[8] ML Buck, *Pediatric Pharmacotherapy*., **1999**, 5: 4A.
[9] VP Pandey, A Phanindrudu, *Boll Chim Farm*., **2002**, 141, 6, 419.
[10] M Turkoglu, H Varol, M Celikok, *Eur J Pharm Biopharm*., **2004**, 57, 2, 279.
[11] MA Navarro, N Raei, *Gastroenterol Hepatol*., **1998**, 21, 2, 63.
[12] AR Kulkarni, KS Soppimath, TM Aminabhavi, *J Controlled Release.*, **2001**, 75, 331.
[13] RC. Ganorkar, F Liu, M Baudys, SW Kim, *J Controlled Release*., **1999**, 59, 287.
[14] AR Kulkarni, KS Soppimath, WE Rudzinski, TM Aminbhavi, *Drug Met Rev*., **2000**, 33, 149.