Formulation Development and Evaluation of Microparticles of Pantoprazole

[1]Vaibhav Saxena, [2]Raj Kumar Mishra, [3]Amrita Singh, [4]Bhanu P. S. Sagar

IEC Department of Pharmacy, IEC College of Engineering & Technology, IEC Group of Institutions, Greater Noida, Uttar Pradesh, India

Abstract

The present research investigation was undertaken with the objective to develop gastrointestinal drug delivery system of pantoprazole – a well known proton pump inhibitor and widely used in the treatment of gastric, duodenal ulcer and also in gastro-esophageal reflux disease (GERD), Zollinger-Ellison syndrome. A controlled release system for pantoprazole is designed to enhance the stability of the pantoprazole in stomach and to by-pass the stomach by making a gastro-resistant double walled microspheres drug delivery system. The formulations were developed consisting of double wall. The primary wall composed of mucoadhesive polymer like sodium CMC and a release controlling polymer sodium alginate. There were many batches formulated to optimize the formulation. The first batch was formulated without drug and observed to optimize the effect of stirring speed (500 rpm and 1000 rpm) for the preparation of the microspheres, the 500 rpm speed was selected due to reproducible result and a good particle size. The microspheres with the drug were analysed for the mucoadhesion and drug release. Five formulations were formulated like A1, A2, A3, A4 and A5. Formulation A5 contains the higher percentage of Sodium CMC showed the good drug entrapment efficiency, mucoadhesion, good drug release profile. Therefore it was selected as best formulation. Then the double walled microspheres was formulated by varying the concentration of Eudragit RS-100, there four formulations were formulated like formulation B1, B2, B3 and B4 which were analysed for particle size and drug release study, and finally formulation B1 shown good drug release among all the formulations analyzed.

Keywords: Drug entrapment efficiency double walled microspheres, Gastro-esophageal reflux disease (GERD), mucoadhesion, mucoadhesive polymers, Eudragit, Sodium CMC.

Introduction

Microspheres

Varde *et al*, 2004, summarized that microspheres or microparticles based drug delivery system has gained substantial attention in the modern era and are typically 1 µm to 1000 µm. Microspheres used for delivering small molecule drugs, vaccines, gene therapy agents and protein therapeutics because of their biocompatibility, easiness of administration and potential for lasting sustained release. Microspheres are formulated so as to provide constant drug concentration in blood thereby increasing patent compliance, decrease dose and toxicity. Microspheres are spherical empty particles with size varying from 50 nm to 2 mm. The microspheres are characteristically free flowing powers consisting of synthetic powder, which are biodegradable in nature ideally having a particle size less than 200 µm. Biodegradable microspheres are used to control drug release rates, conserve the stability of drugs such as proteins and peptides and to target drugs to specific sites in the body, thereby optimizing their therapeutic response, decreasing toxic side effects, and eliminating the inconvenience of repeated injections. They are also used in gene delivery and in diagnostic materials. Examples of polymers used in microspheres CDDS are chitosan MS, gelatine MS, polyadipic anhydride MS, gellan- gum MS, polypeptide MS, albumin MS, poly lactic acid (PLA) MS, poly lactic-co- glycolic acid (PLGA) MS and eudragit MS (Pillai & Panchagnula, 2001).

Double Walled Microspheres

Present microsphere delivery system technology consisting of a single drug dispersed within a polymer matrix has several drawbacks. One is the problem of the so-called “burst effect”. By exploiting the phenomenon of phase separation between two immiscible polymers dissolved in a mutual solvent, a double-walled microsphere could be manufactured with the second polymer coating the polymer/drug matrix. This one-step process would give a consistent coating of even very small microspheres not achievable via normal, two-step coating processes and would help to smooth out the release curve by lessening the “burst effect”.

Along with solving the problem of the “burst effect”, this concept of double-walled microspheres could be used to achieve constant release of the drug over long periods of time. So far, this has only been achieved with a limited number of geometric configurations. Since every polymer has its own characteristic release rate, the release could be kept much more constant by changing the polymer type and/or properties. By combining these layers so that the release rate of one layer would complement the slowing of release due to decreased surface area or increased diffusion distances.

Advantages of Double Walled Microspheres

a. Reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects.
b. Solid biodegradable microspheres have the potential throughout the particle matrix.
c. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumor.

d. The size, surface charge and surface hydro-philicity of microspheres have been found to be important in determining the fate of particles *in vivo.*
e. Studies on the macrophage uptake of microspheres have demonstrated their potential in targeting drugs to pathogens residing intra-cellularly.

Limitation of Double Walled Microspheres

Some of the disadvantages were found to be as follows:

a. The modified release from the formulations.
b. The release rate of the controlled release dosage form may vary from a variety of factors like food and the rate of transit though gut.
c. Differences in the release rate from one dose to another.
d. Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form.
e. Dosage forms of this kind should not be crushed or chewed.

Methods to Formulate Microspheres (Wani *et al*., 2020)

S. No.	Methods to Formulate Microspheres
1.	Simple emulsion-based method
	(i) Heat cross-linking
	(ii) Cross-linking by chemical agents
	(iii) Double emulsion-based method
2.	Polymerization technique
	(i) Normal polymerization
	(ii) Interfacial polymerization
3.	Spray drying and congealing method
4.	Wax coating and hot melt
5.	Ionotropic gelation method
6.	Solvent evaporation method
7.	Coacervation phase separation method

Gastro-Esophageal Reflux Disease (GERD)

Rudolph *et al*., 2001 illustrated that Gastro-esophageal reflux disease **(**GERD**)** / also known as acid reflux is a highly prevalent digestive disorder which results from the reflux of stomach contents into the esophagus. GERD shows oesophageal and extra oesophageal syndromes.

- Oesophageal syndromes are reflux chest pain syndrome, typical reflux syndrome, reflux stricture, reflux esophagitis, oesophageal adenocarcinoma and Barrett's oesophagus.
- Extra oesophageal syndromes are reflux cough syndrome, reflux asthma syndrome, reflux ental erosion syndrome, reflux laryngitis syndrome, pharyngitis, and sinusitis.

Pathogenesis of GERD

The pathogenesis of GERD is complex, resulting from an imbalance between defensive factors protecting the oesophagus and aggressive factors refluxing from the stomach (gastric acidity, volume, and duodenal contents).

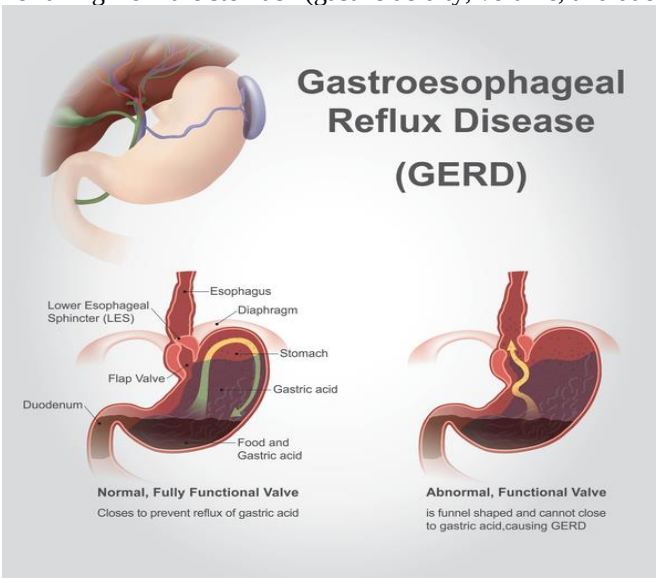

Figure 1 : Gastro-Esophageal Reflux Disease (GERD)

Treatment of GERD

➢ Antacids
➢ Histamine antagonists (ranitidine, famotidine, cimetidine, and nizatidine)
➢ Lifestyle changes : Weight loss; Raise the head of the bed six to eight inches; Avoid acid

	reflux inducing foods; Quit smoking; Avoid large and late meals; Avoid tight fitting clothing; Chew gum or use oral lozenges;
➢	Proton pump inhibitors (e.g. omeprazole, esomeprazole, lansoprazole, dexlansoprazole, pantoprazole, and rabeprazole)
➢	Surgical treatment

The aim of the present work was to formulate spherical gastro-resistant micro particles by using solvent evaporation method. The drug selected for the study is pantoprazole sodium. It is an antiulcer drug used to reduce the acid, secreted by the stomach and cause lesions. Pantoprazole sodium is a proton pump inhibitor and it is acid labile drug which can be degraded in the acidic medium. In the case of oral administration, the enteric coating prevents pantoprazole sodium from degradation in the gastric juice (at pH1-2, pantoprazole degrades in few minutes). Up to now, no multiple-unit pharmaceutical dosage forms containing pantoprazole sodium has been developed. As a general rule, the multiple–unit products show large and uniform distribution; they are less affected by pH and there is a minor risk of dose dumping.

Besides, these new drug delivery systems, as the polymeric microparticle microsphere, are also proposed to improve absorption, distribution, and bioavailability of the acid labile drugs. As they rapidly disperse in the gastrointestinal (GIT) tract, they can maximize drug absorption, minimize side effects, and reduce variation in gastric emptying rates and inter-subject variability. Eudragit RS-100 is a gastro-resistant polymer used for colonic delivery, protecting drugs from pH of upper GIT tract. It is insoluble in acids and pure water, whereas it is soluble in alkaline aqueous solution (having pH more than 7) offering the advantages of the particulate-controlled release dosage forms. Taking all the above in consideration, this study was undertaken for the formulation and characterization of gastro-resistant double walled microspheres of pantoprazole sodium using w/o emulsification / solvent evaporation technique. In which the primary microsphere made containing polymer with drug is then coated again with an enteric coated polymer Eudragit RS100.

Materials and Methods

The all materials used in current investigation were analytical grade or the best available AR (Analytical Reagent) as supplied by the different commercial sources / manufacturer. Drug: Pantoprazole sodium complimentary sample from Fine Cure Pharmaceuticals Ltd. Polymer : Eudragit – RS100 (SD Fine Chemicals), Polymer : Sodium Carboxymethyl Cellolose, Sodium alginate (Qualigen Chemical), Chemical : liquid paraffin, octanol, isopropyl alcohol, sodium hydroxide, potassium bromide, pot.dihydrogen phosphate buffer (pH 7.4), methanol, chloroform and Span 80 (CDH Chemicals / SD Fine Chemicals / Qualigen Chemical / Merck Chemicals).

Pre-formulation Studies

Identification of Drug

Infrared Spectrum

The sample of pantoprazole sodium was procured from Fine Cure Pharmaceuticals Ltd., (Uttrakhand, India) and identified and characterized as per norms of official standards. Accurately weighed 1 mg of pantoprazole sodium powder was mixed with 100 mg of potassium bromide (Spectroscopic grade) in a glass mortar-pestle. The mixture was compressed into transparent discs with the help of compressor and disc was placed in FTIR (8400 S Shimadzu Corporation, Japan) instruments for scanning between 4000-400 cm^{-1}. Characteristic peaks attributable to functional groups were observed in the drug sample to establish the identity of drug pantoprazole sodium.

Determination of λ max

To determine the λmax of drug pantoprazole sodium, stock solutions in water and phosphate buffer solution were prepared. Samples were analyzed using UV Visible spectrophotometer (Model 2202, Systronic, India) and scanned for absorbance between 200-400 nm. The λmax peak of pantoprazole sodium was observed at 288.5 nm.

Identification of Pantoprazole sodium by Melting point determination

Because of gradual degradation of pantoprazole sodium during heating, its melting point could not be determined.

Identification of Pantoprazole sodium by Appearance

Colour	**:**	Pale Yellow / Yellowish white Powder
Nature	**:**	Amorphous Powder
Odour	**:**	No Odour / Odorless

Identification of Pantoprazole sodium by Partition coefficient (PC)

The partition coefficient of Pantoprazole sodium was determined in solvent system octanol/0.1 N HCl. Accurately weighed quantity of drug (10 mg) taken in a glass vial containing 5 ml of octanol, 5ml of 0.1 N HCl was added to the vial. After appropriate dilutions, the aqueous phase was analyzed for pantoprazole sodium against blank solution using double beam UV spectrophotometer at 288.5 nm. The drug concentration in octanol phase was determined by subtracting the amount in aqueous phase from the total quantity of drug added to the vials. The partition coefficient value P was calculated by the following equation.

$$\mathbf{Po/w = (C_{organic} / C_{aqueous}) Pw/o = (C_{aqueous} / C_{organic})}$$

Po/w is the partition coefficient of the oil in water.

Pw/o is the partition coefficient of the water in oil.

$C_{aqueous}$ is the concentration of drug in the aqueous phase

Purity determination of drug by standard curve

- Pantoprazole sodium solution in water and in phosphate buffer pH 7.4 was prepared and absorbance was measured on UV spectrophotometer at 288.5 nm.
- The method obeys Beer's law in the concentration range 1-10 µg/ml. The standard curve of pantoprazole sodium was prepared.

- 100 mg of pantoprazole sodium was dissolved in 50 ml of phosphate buffer (pH 7.4) and volume was made upto 100 ml (Stock solution A).
- 1 ml of stock solution A was diluted up to 100 ml with phosphate buffer (pH 7.4) (Stock solution B).
- 1,2,3,4,5,6,7,8,9,10 were taken from stock solution B then volume made upto 10 ml with phosphate buffer solution (pH 7.4).
- Absorbance was measured at 288.5 nm by using UV Visible spectrophotometer (Model 2202, Systronic, India) against blank.

Purity determination of drug by Solubility Method

Purity of drug can also be determined by solubility analysis. In solubility study a saturated solution of drug is made in solvent system (water, alcohol, methanol, chloroform etc. and the concentration of the drug was measured by UV spectrophotometer at 288.5 nm.

Preparation of Double walled Microspheres

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

Method of Preparation of Core Microspheres

Three batches of microspheres were prepared for the purpose of assessing the reproducibility of drug loading, particle size and *in-vitro* drug release by this method. First the core microspheres without drug were formulated at different stirring speed like 500 and 1000 rpm. Composition of microspheres of formulation using sodium CMC and sodium alginate were shown in Table 1-2below.

Table 1 : Composition of microspheres without drug (S1, S2, S3, S4, S5)

S. No.	Formulation Code	Drug	Sod. CMC	Sod. Alginate
1.	S1	----	1.0	3.0
2.	S2	----	1.5	2.5
3.	S3	----	2.0	2.0
4.	S4	----	2.5	1.5
5.	S5	----	3.0	1.0

Table 2 : Composition of drug loaded microspheres (A1, A2, A3, A4, A5)

S. No.	Formulation Code	Drug	Sod. CMC	Sod. Alginate
1.	A1	1	1.0	3.0
2.	A2	1	1.5	2.5
3.	A3	1	2.0	2.0
4.	A4	1	2.5	1.5
5.	A5	1	3.0	1.0

Method of Preparation of Double Walled Microspheres

Table 3: Composition of core and polymer in preparation of double walled microspheres.

S. No.	Formulation Code	Core to Coat Ratio
1.	B1	1:0.5
2.	B2	1:0.75
3.	B3	1:1
4.	B4	1:1.5

Characterization of Microspheres Core and Coated Microspheres

Micromeritics Properties

The microspheres were characterized by their micromeritics properties such as particle size and surface morphology. The particle size was measured using an optical microscope, and the mean particle with the help of a calibrated ocular meter.

Surface Morphology

The surface morphology and structure were visualized by scanning electron microscopy (EVO-40, Zeiss, Germany) at advanced instrumentation research facility JNU, New Delhi. The samples were prepared by lightly sprinkling the microspheres powder on a double side adhesive tape which already shucked to on aluminum stubs. The stubs were then placed into fine coat ion sputter for gold coating. After gold coating samples were randomly scanned for particle size and surface morphology.

Particle Size Analysis

The mucoadhesive microspheres were examined by optical microscope. The freshly prepared suspension of microspheres was examined on an optical microscope and size of the microspheres was measured by using a pre-calibrated ocular micrometer and stage micrometer. Around 100 particles of each formulation were observed and counted.

Drug Entrapment Efficacy (DEE)

25 mg of dried microsphere were weighed accurately and drug was extracted from microspheres by digesting for 24 hrs with 10 ml of methanol. During this period the suspension was agitated. After 24 hrs the suspension was centrifuged at 2000 rpm for about 3 minutes. The solution was filtered through 0.45 mm membrane filter and the filtrate was analysed for drug content at 288.5 nm. Entrapment efficacy was calculated.

***In-vitro* Drug Release of Core Microspheres**

The prepared formulations were evaluated for *in-vitro* release by USP dissolution apparatus at 50 rpm at 37ºC in order to determine 100% drug release. To evaluate microspheres containing pantoprazole sodium were exposed to 900 ml of phosphate buffer pH 7.4. The sample were collected in pre-determined time intervals from 0 up to 480 min (8 hrs). Pantoprazole concentrations were determined by UV at 288.5 nm.

***In-vitro* Drug Release of Coated Microspheres**

The prepared formulations were evaluated for *in-vitro* release by USP dissolution apparatus at 50 rpm at 37ºC in order to determine 100% drug release. To evaluate gastro resistant microspheres containing pantoprazole were exposed to 300 ml of 0.1M HCl. After 1 hr, a 2.6 gm of NaOH and 6.12 gm of KH_2PO_4 in 600 ml aqueous solution (phosphate buffer) was added into the medium in order to reach pH 7.4. Pantoprazole concentrations were deterimed by UV analysis at 288.5 nm.

Stability Studies

The final coated microspheres were packaged in clear glass vials and placed on stability at accelerated conditions 25ºC, 30ºC and 40ºC. The stability was monitored for 3 months. The potency and dissolution results were observed.

Results and Discussions

UV Spectroscopy

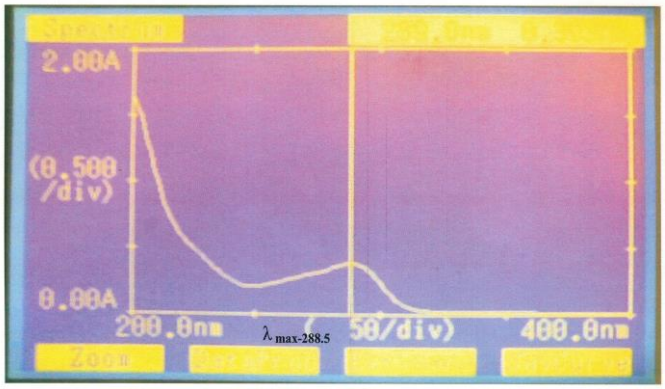

Figure 2: UV Spectra of Pantoprazole.

It was found that the drug was confirming standards with respect to melting point, wavelength of maximum absorption (λmax) and the characteristic IR peaks. The estimation of purity of drug by plotting standard curve was given in Figure 2. UV absorption spectra of drug showed λmax at 288.5 nm whereas the reported value is 290 nm.

FTIR Spectroscopy

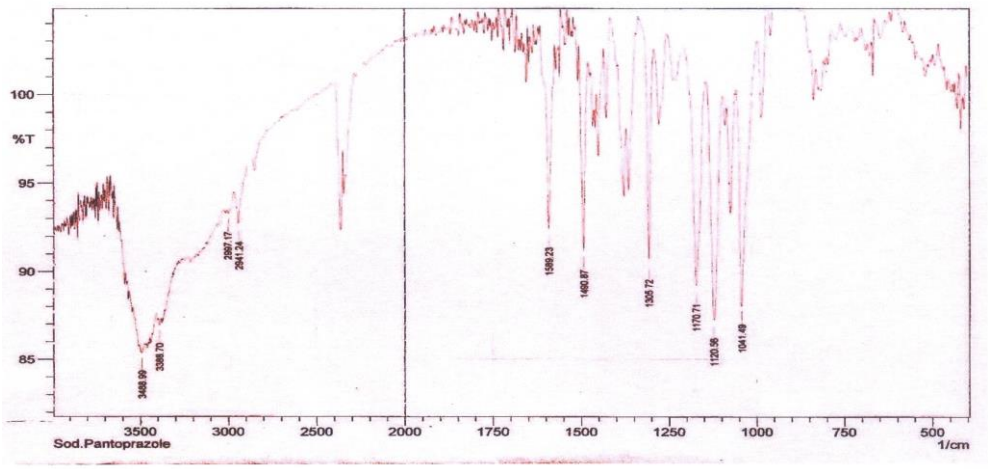

Figure 3: FTIR Spectra of Pantoprazole sodium.

Table 4: Interpretation of FTIR spectra of Pantoprazole.

S. No.	Peak	Functional Group
1	3488.99	NH = Aromatic stretching
2	1589.23	C=N stretching
3	1305.72	C-N stretching
4	1120.56	C-O stretching
5	1170.71	C-F stretching
6.	1041.49	S=O stretching

The peaks of the FTIR spectra of the drug sample were found to be similar with standard FTIR spectrum of pure pantoprazole as reported. The FTIR spectrum of mixture of drug and polymer indicated no incompatibility between drug and polymers, hence sodium CMC and sodium alginate were chosen as polymers for further investigations.

Solubility Profile

The solubility was observed by only visual inspection. Pantoprazole showed good solubility in water, ethanol, methanol; slightly soluble in n-hexane and insoluble in chloroform, ether and acetone.

Partition Coefficient

Table 5 : Partition Coefficient value of Pantoprazole.

S. No.	Solvent System	Reported	Observed
1	n-Octanol / Water	0.266	0.262

The partition coefficient of pantoprazole, in n-octanol : water system was found to be 0.262 which indicated hydrophilic behavior of the drug.

Calibration Curve

Table 6 : Calibration curve for Pantoprazole.

S. No.	Concentration (µg/ml)	Absorbance	Regression coefficient
1	1	0.050	r^2=0.9992
2	2	0.092	
3	3	0.138	
4	4	0.176	
5	5	0.216	
6.	6	0.260	
7.	7	0.300	
8.	8	0.336	
9.	9	0.377	
10`	10	0.415	

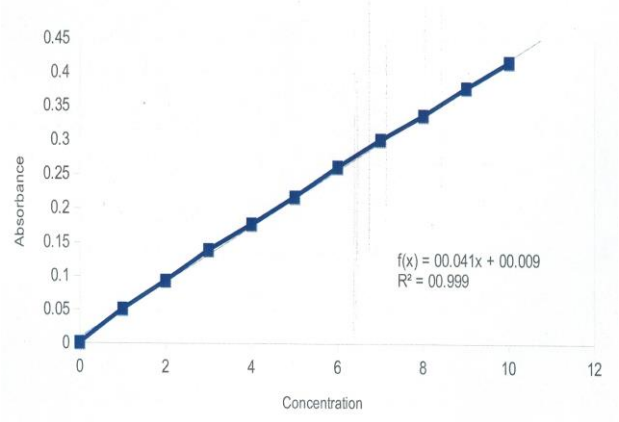

Figure 4: Calibration curve of Pantoprazole in water.

Table 7: Calibration curve of Pantoprazole in phosphate buffer (pH 7.4).

S. No.	Concentration (µg/ml)	Absorbance	Regression coefficient
1	1	0.039	r^2=0.9998
2	2	0.079	
3	3	0.111	
4	4	0.150	
5	5	0.190	
6.	6	0.230	
7.	7	0.268	
8.	8	0.305	

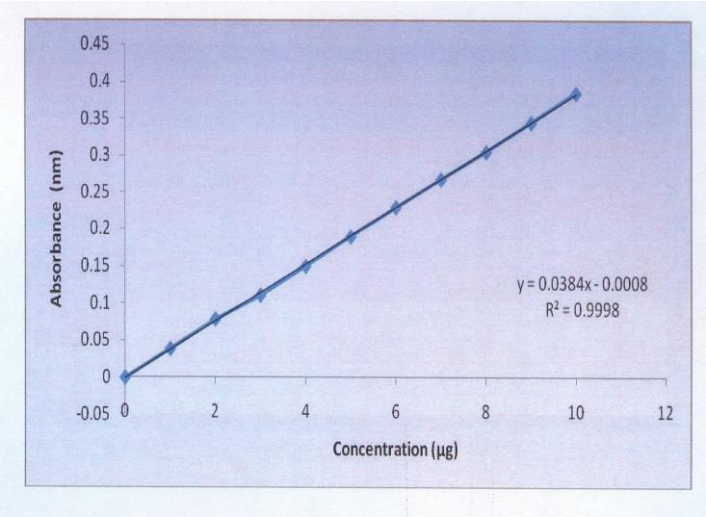

Figure 5: Calibration curve of Pantoprazole in phosphate buffer (pH 7.4)

FTIR Spectroscope

Table 8 : FTIR spectra of Pantoprazole with sodium alginate.

S. No.	Peak	Pure drug + sodium alginate peaks
1	3488.99	3483.20
2	1589.23	1588.23
3	1305.72	1305.72
4	1170.71	1170.71
5	1120.56	1120.56
6.	1041.49	1041.49

Table 9: FTIR spectra of Pantoprazole with Sodium CMC.

S. No.	Peak	Pure drug + sodium alginate peaks
1	3488.99	3487.99
2	1589.23	1588.23
3	1305.72	1305.72
4	1170.71	1168.76
5	1120.56	1120.56
6.	1041.49	1041.49

Scanning Electron Microscopy (SEM)

Surface morphology of the mucoadhesive microspheres was examined by scanning electron microscope (SEM) analytical technique. Microspheres of sodium CMC alone produced smooth surface, spherical shaped microspheres. While soudium alginate microspheres were irregular in shape with a rough surface morphology due to less water solubility and non-uniform evaporation of water from the surface of microspheres.

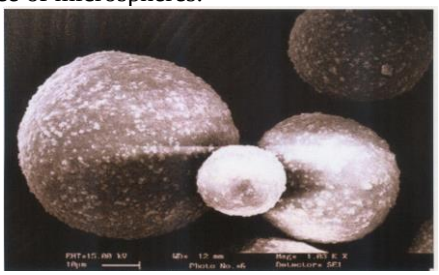

Figure 6: Scanning electron micrograph of A1.

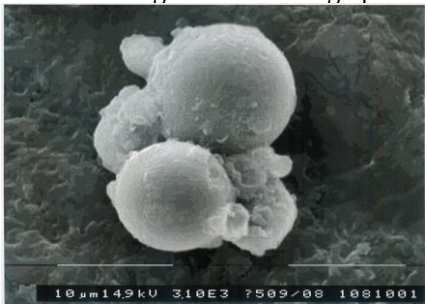

Figure 7: Scanning electron micrograph of A3.

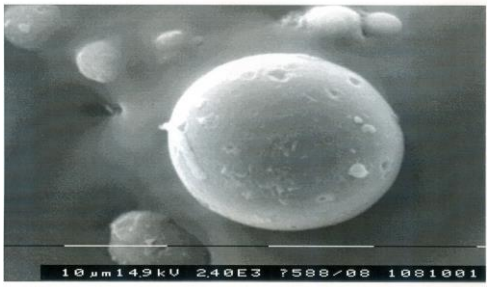

Figure 8: Scanning electron micrograph of A5.

Particle Size Analysis

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 was found between 30.61 µm to 33.51 µm. Formulation A5 showed the least particle size i.e. 30.61 µm because it contains higher portion of sodium CMC which was due to spherical shape / nature of the microspheres. Formulation A1 had the largest portion of sodium alginate, showed the largest particle size of 33.51 µm. All formulations were prepared at 3% polymer concentration and 500 rpm stirring speed.

Table 10: Particle size of microspheres without drug.

S. No.	Formulation Code	Particle Size (µm) at speed 500 rpm
1	S1	31.9 ± 1.2
2	S2	30.5 ± 1.34
3	S3	29.2 ± 0.98
4	S4	28.4 ± 2.1
5	S5	27.3 ± 1.9

**values are represented as mean ± standard deviation (n=3)

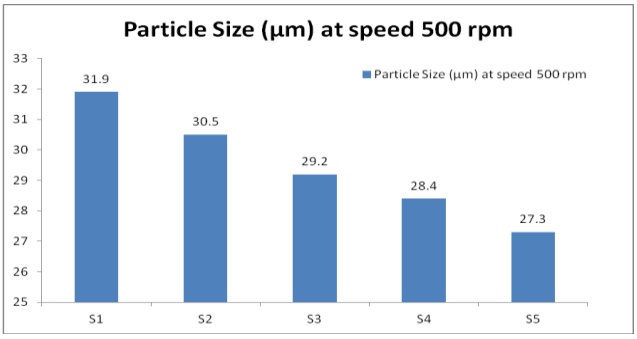

Figure 9: Particle size of S1, S2, S3, S4, and S5 microspheres without drug.

Table 11: Effect of stirring speed on particle size.

S. No.	Formulation Code	Particle Size (µm) at speed 500 rpm	Particle Size (µm) at speed 1000 rpm
1	S1	31.9 ± 1.2	28.2 ± 2.13
2	S2	30.5 ± 1.34	27.1 ± 2.2
3	S3	29.2 ± 0.98	26.2 ± 0.99
4	S4	28.4 ± 2.1	25.2 ± 1.87
5	S5	27.3 ± 1.9	24.1 ± 1.2

**values are represented as mean ± standard deviation (n=3)

Table 12 : Particle size of microsphere with drug.

S. No.	Formulation Code	Particle Size (µm) at speed 500 rpm
1	A1	33.5 ± 1.43
2	A2	33.1 ± 1.54
3	A3	32.3 ± 1.65
4	A4	31.4 ± 1.23
5	A5	30.6 ± 0.98

**values are represented as mean ± standard deviation (n=3)

On increasing the proportion of sodium CMC the decrease in size of microspheres was observed, that was 33.51 µm, 33.1 µm, 32.32 µm, 31.46 µm, and 30.61 µm formulation A1, A2, A3, A4 and A5 respectively. This may be due to increase of availability of the polymer for entrapment of drug particles. The rank order of size was A5>A4> <A3>A2>A1. Formulation A3 showed the particle size in between A4 and A1 because A3 contains the equal proportions of the sodium CMC and sodium alginate polymers.

Percent Drug Loading

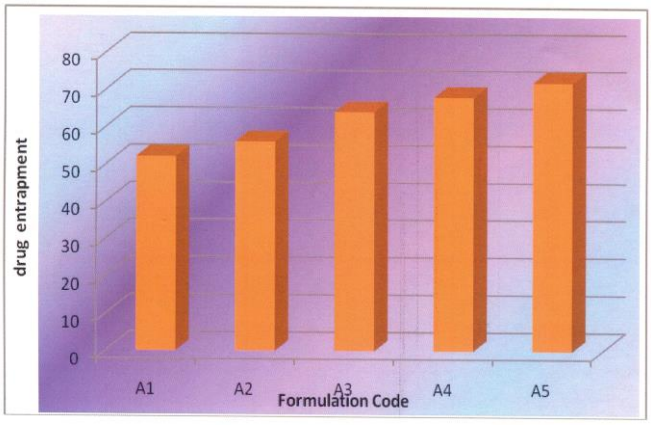

Figure 10: Percent drug loading or drug entrapment of A1, A2, A3, A4 and A5.

In case of core microspheres, on increasing the concentration of sodium CMC polymer, the amount of drug entrapment was increased as it was observed maximum 72% in formulation A5 and less in formulation A1 where the polymer to polymer ratio was 3:1 and 1:3 for sod. CMC and sod. alginate respectively. This was due to the sodium CMC showed good entrapment efficiency them polymer sodium alginate. The rank order of drug entrapment efficiency was A5>A4>A3>A2>A1.

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 (4 hrs 15 min) the percent mucoadhesivity was found 10, 15, 18, 23, and 26 for formulation A1, A2, A3, A4 and A5 respectively.

Table 13: Percent of microspheres adhering to tissue at 7 times (min) distilled water.

Formu-lation	Percent Microspheres adhering to tissue at 7 times (min) distilled water.						
	45	90	135	180	225	360	405
A1	80 ±2.4	60 ±1.44	52 ±0.98	38 ±1.68	28 ±1.54	18 ±2.4	8 ±0.86
A2	82 ±0.98	61 ±1.2	54 ±1.66	41 ±1.62	32 ±0.98	23 ±1.08	15±1.96
A3	83 ±1.46	63 ±1.58	55 ±0.64	44 ±1.54	35 ±2.92	26 ±1.02	18 ±0.62
A4	86 ±0.96	65 ±1.24	57 ±2.24	47 ±1.36	38 ±0.96	29 ±1.6	23±1.04
A5	88 ±1.2	67 ±1.54	59 ±0.76	50 ±2.4	43±1.04	32 ±1.2	26 ±0.64

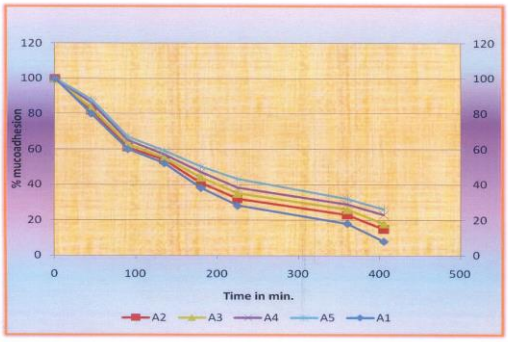

Figure 11: Microspheres (%) adhering to tissue at 7 times (min) in distilled water.

Table 14: Drug entrapment, Particle size, & Microspheres (%) adhering to tissue.

S. No.	Formulation Code	Drug Entrapment (%)	Particle size (µm)	Mucoadhesion (%)
1	A1	52	33.5 ±1.42	80±2.4
2	A2	56	33.1 ±1.54	82±0.98
3	A3	64	32.3 ±1.64	83±1.45
4	A4	68	31.4 ±1.22	86±0.96
5	A5	72	30.6 ±0.98	88±1.2

Percent Cumulative Drug Release

Table 15: % cumulative drug release from Formulation A1, A2, A3, A4 & A5.

S. No.	Time (hrs)	A1	A2	A3	A4	A5
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	1	10.6 ±1.32	12.5 ±1.32	15.2 ±1.42	18.2 ±1.72	20.5 ±1.52
2	2	14.0 ±0.98	17.31±1.88	21.6±1.32	27.32±1.4	29.3±1.8

3	3	32.8±1.2	35.8±1.98	40.1±0.98	48.3±1.08	52.3±1.32
4	4	41.2±1.22	40.35±0.96	48.2±1.6	54.2±0.88	58.9±1.4
5	5	47.3±1..5	49.2±2.0	55.2±1.7	60.1±0.96	65.21±0.98
6	6	53.6±1.08	58.72±1.4	63.82±1.4	68.88±1.6	74.03±1.64
7	7	65.4±0.96	69.8±1.4	73.5±1.6	78.5±1.8	82.4±1.36
8	8	76.3±1.4	79.0±0.88	84.0±1.08	86.0±1.2	93.0±2.10

These studies showed the effect of environment on the body of 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 all the formulations were fund to be different for different polymer proportions used in the formulation like 76.3%, 79.4%, 84.0%, 86.0% and 93.0% for formulation A1, A2, A3, A4 and A5 respectively. The formulation A5 had highest proportion of polymer sodium CMC showed maximum release while formulation A1 showed the least drug release after 8 hrs due to less swelling action and irregular surface as compared to formulation B1.

Particle Size Analysis of Coated Formulation

Table 16: Particle size of Formulation B1, B2, B3 and B4 microspheres.

S. No.	Product Code	Particle Size (µm) at speed 500 rpm
1	B1	61.9 ±1.2
2	B2	65.5±1.34
3	B3	75.2±0.98
4	B4	78.4±2.1

Cumulative Drug Release (%) of Coated Formulation

Table 17 : Cumulative *in-vitro* drug release of Formulation B1, B2, B3 and B4.

S. No.	Time (hrs)	pH	B1	B2	B3	B4
			Mean±SD	Mean±SD	Mean±SD	Mean±SD
1.	1	1.2	0	0	0	0
2.	2	1.2	0	0	0	0
3.	3	7.4	13.5 ±1.2	11.7 ±1.9	10.56±1.76	9.2 ±1.3
4.	4	7.4	19.3 ±1.5	17.9±2.2	15.89±1.8	13.2±1.8
5.	5	7.4	26.5±1.8	23.1±2.01	20.5±0.96	17.5±1.9
6.	6	7.4	31.9±1.3	29.2±0.87	25.9±2.1	22.9±1.8
7.	7	7.4	59.3±0.98	57.3±0.98	49.9±2.4	44.7±1.6
8.	8	7.4	69.8±2.1	65.4±1.07	63.2±1.7	59.1±1.76
9.	9	7.4	75.4±1.3	71.2±1.54	69.1±1.9	65.0±2.4
10.	10	7.4	83.4±2.1	76.3±1.9	73.7±2.2	72.2±2.1
11.	11	7.4	89.2±1.9	86.5±1.65	83.3±1.4	78.2±2.9
12.	12	7.4	94.3±1.7	92.4±1.70	89.2±1.7	80.1±1.98

Stability Studies

The final coated microspheres were packaged in clear glass vials and placed on stability at accelerated conditions 25ºC, 30ºC and 40ºC. The stability was monitored for 3 months and the potency and dissolution results were observed. The microspheres were stable over three months period and a 40mg equivalent dose of microspheres was tested for the stability.

Table 18: The stability data of the coated microspheres at 25ºC.

S. No.	Time (in months)	% Drug Retained
1	Initial (zero month)	100
2	1 month	98.2
3	2 month	96.1
4	3 month	93.8

Table 19: The stability data of the coated microspheres at 35ºC.

S. No.	Time (in months)	% Drug Retained
1	Initial (zero month)	100
2	1 month	97.4
3	2 month	95.5
4	3 month	92.3

Table 20: The stability data of the coated microspheres at 40ºC.

S. No.	Time (in months)	% Drug Retained
1	Initial (zero month)	100
2	1 month	97.1

3	2 month	93.4
4	3 month	91.1

Double Walled Microspheres

(A) Particle Size and Surface Morphology

The particle size and surface morphology was determined with the help of optical microscope and scanning electron microscope. Spherical shaped microspheres of sod. alginate were observed with optical microscope and particle size was found between 30.61 µm to 33.51 µm. On increasing the concentration of polymer, there was no significant effect on the size of the microspheres was observed, that was 126.19 µm, 129.4 µm, 131.61 µm and 134.81 µm for formulation B1, B2, B3 and B4 respectively. This was due to increase of availability of the polymer for entrapment of drug particles.

(B) Effect on Percentage Drug Entrapment

In case of double walled microspheres, on increasing the concentration of polymer, the amount of drug entrapment will increase as it was observed maximum in formulation B4 with 76.40% and less in formulation B1 with 68.50% where the drug polymer ratio was 1:1.5 and 1:0.05 respectively.

(C) Effect on Percentage Yield

On increasing concentration of polymer the increase in % yield of microspheres was observed. This was due to the formation of large number of aggregates due to availability of more polymers concentrations. As drug concentration increased % yield was also increased.

(D) *In-vitro* Drug Release Profile of Core Microspheres

The *in-vitro* release first determined in the pH 1.2 for 2 hrs, all formulations showed no drug release at this pH. Then the pH was increased to 7.4 in phosphate buffer (pH 7.4) solution for hrs. It was found that the release rate from all formulation was found to be different for different polymer proportions used in the formulation like 94.3%, 92.4%, 89.2% and 80.1% for formulation B1, B2, B3 and B4 respectively. The B1 has lower proportion of polymer Eudragit RS 100 showed maximum release. While the formulation B4 showed the least drug release after 12 hrs due to less swelling action and irregular surface as compared to formulation B1.

Conclusions

Formulation A5 contains the higher percentage of Sodium CMC showed the good drug entrapment efficiency, mucoadhesion, good drug release profile. Therefore it was selected as best formulation. Then the double walled microspheres was formulated by varying the concentration of Eudragit RS-100, there four formulations were formulated like formulation B1, B2, B3 and B4 which were analysed for particle size and drug release study, and finally formulation B1 shown good drug release among all the formulations analyzed.

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