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Formulation and Evaluation of Colon Targeted Microspheres Of Pantoprazole

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A B S T R A C T : The objective of the study was to formulate and evaluate colon targeted microspheres of pantoprazole. Colon is capable of absorbing some drugs efficiently which are unstable or poorly absorbed from upper GI tract. Microspheres of pantoprazole which were meant to be targeted on colon were prepared by solvent evaporation method by using polymers Eudragit Rs100 and ethyl cellulose in different ratios. The formulated microspheres were evaluated for percentage yield, particle size, entrapment efficiency, scanning electron microscopy and drug release and the performances of these formulation were evaluated and the effect of various formulation variables were studied. The results showed that percentage yield and microsphere's particle size were affected by mainly polymer concentration and its type.

KEYWORDS: Microspheres, colon Targeted, Pantoprazole ,Eudragit RS 100, Targeted drug delivery.

I. INTRODUCTION

Conventional dosage form is the convenient form among other formulation types. In this, Targeted drug delivery enables maximum therapeutic activity by preventing degradation or inactivation of drug during transit to targeted or desired site. It also minimizes adverse effects because of inappropriate disposition and minimize toxicity of potent drugs by reducing dose. [(1,2)] The colon targeted microspheres of pantoprazole also prevent the drug from degradation due to acidic environment in the upper part of gastrointestinal tract by using colon specific polymers i.e., Eudragit RS 100 and ethyl cellulose. [(3,4,5)]

The microspheres of Pantoprazole were prepared by solvent evaporation method using colon specific polymers which remain intact in stomach and gets degraded in the colon pH. One of the mechanism of colon targeting is to coat the drug using specific polymers which remain intact or undisturbed in stomach's acidic environment and only releases drug at specific or desired pH. [(6,7)]

Pantoprazole is used for short term treatment of erosive esophagitis associated with gastroesophageal reflux disease (GERD), maintenance and healing of erosive esophagitis, and pathological hypersecretory conditions including zollinger-ellison syndrome.It is a proton pump inhibitor drug that inhibits the gastric acid secretion. It works on gastric parietal cells to irreversibly inhibit the production of Gastric acid.It was first sold in 1994 in germany and became available as a generic medication in 2010. Pantoprazole may also be used in combination with antibiotics to treat ulcers caused by helicobacter pylori.

Pantoprazole is an acid labile drug which degrades in the acidic pH of stomach by virtue of secretion of hydrochloric acid secreted by parietal cells of stomach. As drug move from stomach to small intestine then to large intestine , the pH level increases and colon has a neutral pH .Also in case of high acid secretion in stomach the pH of small intestine also somewhat increase.Therefore Pantoprazole is made to target in colon for better bioavailability and absorption and to protect it from degradation from acid.

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II. MATERIALS AND METHODS

MATERIALS USED

S.NO.	Chemicals/Materials	Specification/grades	Sources
1.	Dichloromethane	99%	Thermo Fisher scientific Pvt.Ltd., India
3.	Membrane filters(syringe)	0.22µ	Pall Corporation, USA
4.	Syringe	2ml,5ml	Hindustan Syringe & Medical Devices Ltd
5.	Sodium chloride	99.8%	Himedia Lab. Pvt. Ltd.,India
6.	Laboratory Film	Parafilm "M"	Parafilm, Chicago
7.	Aluminium Foil	Freshwrap	Hindalco, India
8.	Ethyl Cellulose	Avg.M.W.454.50g/mol	Aglomed pharma,Roorkee
9.	Eudragit RS 100	Avg.M.W.399.52g/mol	Aglomed pharma,Roorkee
10.	$NaHPO_4$	Reagent grade	S.D. Fine Chem. Ltd., India
11.	Na_2HPO_4	Reagent grade	Sarabhai M. Chemicals
12.	Sodium phosphate	Reagent grade	Sarabhai M. Chemicals
13.	Pantoprazole	Pure API	Akums pharmaceuticals and drug limited

EQUIPMENTS USED

S.No	Equipment	Model/Specification	Company
1.	Micropipette	200µm, 1000µm	Eppendrof,Germany
2.	Electronic Weighing Balance	Mettler AE63,XR25 SM-FR	MettlerToledo,USA
3.	Homogeniser	Model RQ-1400/D-FP	Remi, Mumbai, India
4.	UV visible spectrophotometer	UV-1700 Pharmaspec	Shimadzu Corp., Japan
5.	Centrifuge	Sigma 3-18k	Sigma,USA
6.	pH Meter	Orion 2 star pH Benchtop	Thermoscientific,USA
7.	Triple distilled water assembly	Milliq, Solpe Tank, Prefilkit	Millipore,USA
8.	Dialysis Membrane	Cut off 7000 Daltons	Himedia Lab Pvt. Ltd.,India
9.	Sonicator(probe)	Vibra	Sonic & Materials,Inc
10.	Differential Scanning	Diamond DSC	Perkin Elmer, USA

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	Calorimetry		
11.	Solvent Filteration Assembly-Vaccum Pump	0.22 micron filter	Millipore,USA
12.	Masterisezer	Mastersizer 2000	Malvern Instruments, UK
13.	Dissolution Apparatus	USP Type 2, Disso 2000	Labinda, India

METHOD

1.Preformulation study

Preformulation studies are done to characterize properties of raw materials including their physico-chemical,biopharmaceutical and mechanical properties ,as well as compatibility.

Drug-excipient Compatibility studies by Fourier transform infra red spectroscopy

Infra red spectra of pantoprazole and physical mixtures of all polymers are shown in table. From this it is observed that there is no interaction between drugs.

IR SPECTRA OF PANTOPRAZOLE

IR PEAKS OF DRUG (API)

S.No	Reference peak(cm^{-1})	Obtained peak(cm^{-1})	Functional group
1.	677	682	S-C Stretching
2.	815	828	N-H waging bond peak Stretching
3.	1042	1042.5	Strong –S=O Stretching
4.	1073	1070.42	-C-O-C-Asymmetrical Stretching
5.	1120	1116.42	Aryl fluoride stretching
6.	1378	1364	Symmetrical C-H Bending
7.	1654	1590	C=C Stretching
8.	2942	2940	Methyl C-H Stretching

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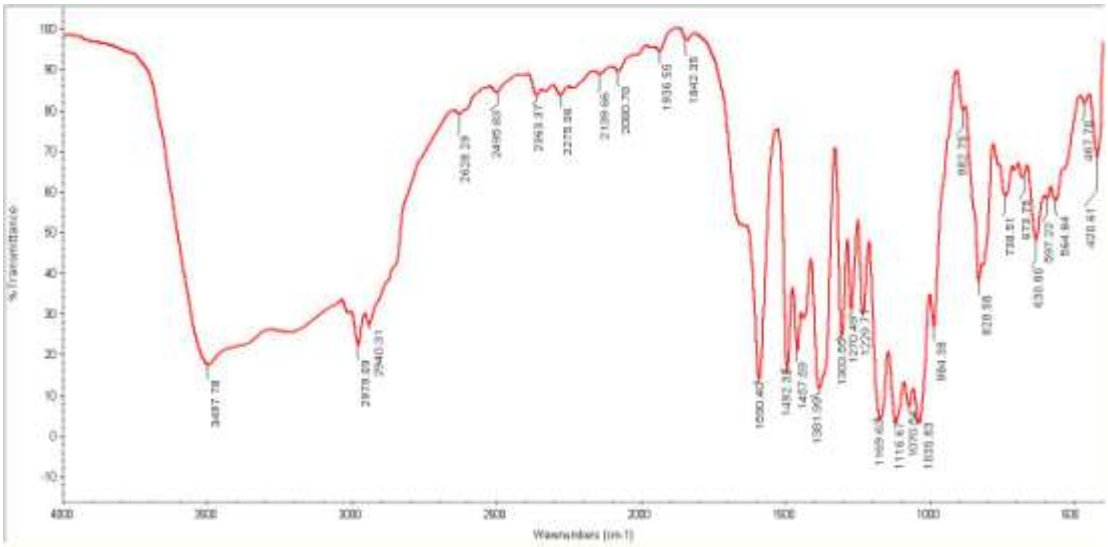
9.	3184	3013.90	O-H Stretching

IR PEAK SHOWING DRUG INTERACTION WITH ETHYL CELLULOSE



IR PEAKS OF ETHYL CELLULOSE WITH PANTOPRAZOLE

S.NO	Reference peak(cm^{-1})	Obtained peak (cm^{-1})	Functional group
1.	677	673.75	S-C Stretching
2.	815	828.68	N-H waging bond Stretching
3.	1042	1035.83	Strong –S=O Stretching
4.	1073	1070.54	-C-O-C-asymmetrical Stretching
5.	1120	1116.67	Aryl fluoride Stretching
6.	1378	1381.09	Symmetrical C-H Stretching
7.	1654	1642.54	C=C Stretching
8.	2942	2940.31	Methyl C-H Stretching
9.	3184	2979.01	O-H Stretching

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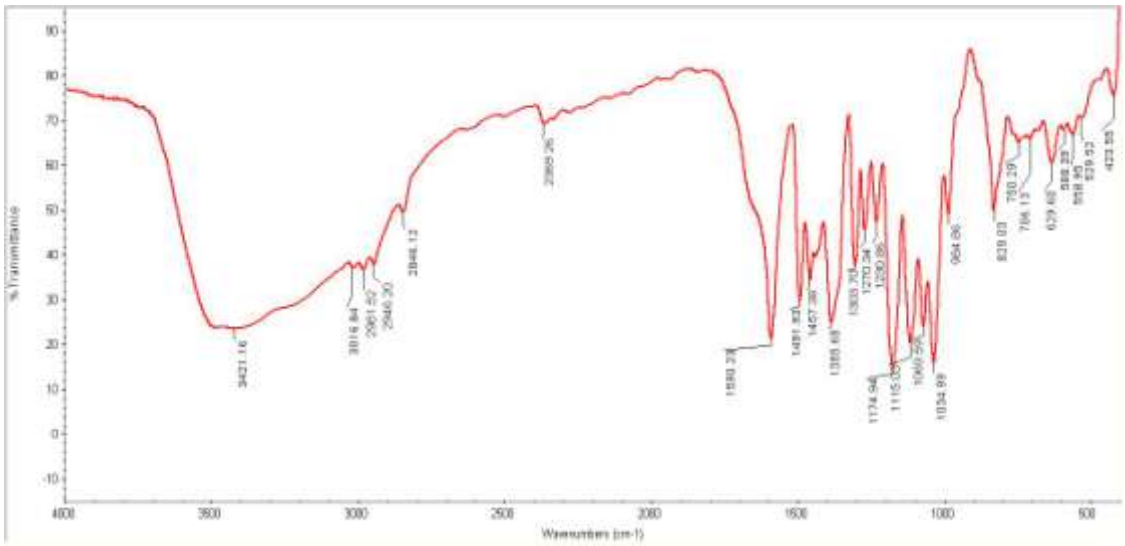
IR PEAK SHOWING DRUG INTERACTION WITH EUDRAGIT



IR PEAKS OF EUDRAGIT WITH PANTOPRAZOLE

S.No	Reference peak(cm^{-1})	Observed peak(cm^{-1})	Functional group
1.	677	629.60	S-C Stretching
2.	815	829.03	N-H waging bond Stretching
3.	1042	1034.99	Strong –S=O Stretching
4.	1073	1069.59	-C-O-C-asymmetrical Stretching
5.	1120	1115.03	Aryl fluoride Stretching
6.	1378	1358.68	Symmetrical C-H Stretching
7.	1654	1590.23	C=C Stretching
8.	2942	2946.20	Methyl C-H Stretching
9.	3184	3016.84	O-H Stretching

2.FORMULATION OF MICROSPHERES

The microspheres were prepared by solvent evaporation technique using ethyl cellulose and Eudragit RS 100 as polymers. Nine formulations were prepared using different ratios of these two polymers. To the mixture of ethanol and dichloromethane (1:1), the polymers in various ratios were added. The drug was dispersed in above solution of polymers. This dispersion was added slowly with stirring into the distilled water containing 0.01% Tween 80 maintained at the temperature between 30-40^{0}C. Stirring was continued for 20 minutes, which allowed the evaporation of dichloromethane and ethanol

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completely. After evaporation of solvents, the microspheres formed were collected by filtration, washed 3 to 4 times with distilled water and dried at room temperature for 24 hrs. The prepared microspheres were stored in desiccators. [8,9]

PREPARED MICROSPHERES

3. DRUG FORMULATION DESIGN

INGREDIENTS	**FORMULATIONS**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Pantoprazole	100mg	100 mg	100mg	100mg	100mg	100mg	100mg	100mg	100mg
Ethyl cellulose	500mg	450 mg	400mg	350mg	300mg	250mg	200mg	150mg	100mg
Eudragit RS 100	500mg	550 mg	600mg	650mg	700mg	750mg	800mg	850mg	900mg
Ethanol	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml
Dichloromethane	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml
Distilled water	500ml	500 ml	500ml	500ml	500ml	500ml	500ml	500ml	500ml
Tween 80	0.50µl	0.50 µl	0.50µl	0.50µl	0.50µl	0.50µl	0.50µl	0.50µl	0.50µl

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4.EVALUATION OF MICROSPHERES

Physical evaluation

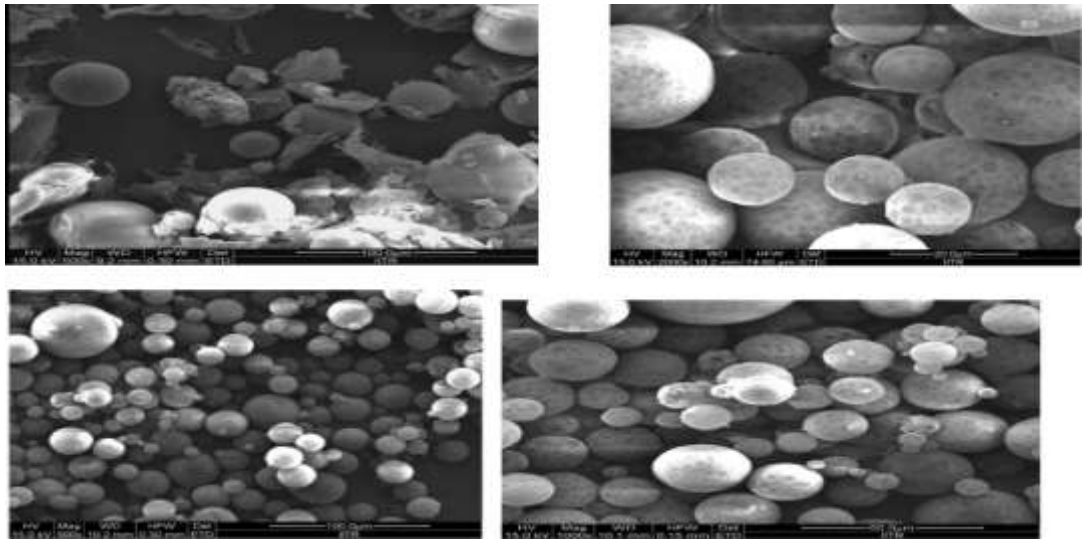
1.Shape and colour of microspheres

S.NO	Formulation	SHAPE	COLOUR
1.	F1	Spherical	Whitish yellow
2.	F2	Spherical	Pale yellow
3.	F3	Spherical with tail	Pale yellow
4.	F4	oval	Light yellow
5.	F5	Roughly spherical	Whitish yellow
6.	F6	Spherical	Whitish yellow
7.	F7	Spherical	Light yellow
8.	F8	Roughly spherical	Pale white
9.	F9	Roughly spherical	Pale white

2.SCANNING ELECTRON MICROSCOPY

The shape and surface morphology of microspheres were investigated by using scanning electron microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300 A^0 under an argon atmosphere using a gold sputter module is a high vacuum evaporator. [10]

SHOWING RESULT OF SEM



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3.MICROMERITIC PROPERTIES

Bulk density and Tapped density

S.NO	Formulation	Bulk density (gm/cm^3)	Tapped density
1.	F1	0.270	0.322
2.	F2	0.294	0.344
3.	F3	0.300	0.364
4.	F4	0.378	0.322
5.	F5	0.289	0.337
6.	F6	0.272	0.315
7.	F7	0.280	0.335
8.	F8	0.289	0.334
9.	F9	0.289	0.336

Prepared microspheres were evaluated for bulk density, hausner ratio,Car's index, percentage yield, particle size, entrapment efficiency, shape, scanning electron microscopy and in- vitrodrug release.

Angle of repose

It was observed that angle of repose decreases as the quantity of Eudragit RS100 increases. The decreases in the angle of repose with the increment of Eudragit RS 100 and Ethyl cellulose amount may be due to the increase in the particle size and it tend to decreases the angle of repose.

Angle of repose for determination of flow properties

S.NO.	BATCH NO.	ANGLE OF REPOSE (DEGREE)
1.	F1	19.98
2.	F2	20.80
3.	F3	21.28
4.	F4	21.45
5.	F5	23.14
6.	F6	23.41
7.	F7	17.38
8.	F8	19.45
9.	F9	21.80

PARTICLE SIZE DETERMINATION

Optical microscope was used to determine the size of the microspheres. This method involves the calibration of eye piece micrometer for which the stage micrometer is used. In stage micrometer one mm is divided into 100 equal divisions and hence, each division is equal to 10 mm and the particles are measured chosen fixed line across the center of the particle.The average diameter of was calculated using following formula. **Average**

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diameter = Σnd/n×C.F. The particle size of microspheres tend to be roughly spherical as the amount of eudragit increases.

Percentage yield

Percentage yield of all the formulation were performed . Percentage yield of different formulation was determined by weighing the microspheres after drying The results are given in the table listed below.

$$\text{Percentage yield} = \frac{\text{PRACTICAL YIELD}}{\text{THEORTICAL YIELD}} \times 100$$

SHOWING RESULT OF PARTICLE SIZE AND % YIELD

S.No	Formulation	Particle size(µm)	% Yield
1.	F1	192	78
2.	F2	200	77.33
3.	F3	250	78.33
4.	F4	258	77
5.	F5	240	78.66
6.	F6	220	79.01
7.	F7	229	79.90
8.	F8	215	82.5
9.	F9	245	81.6

ENTRAPMENT EFFICIENCY AND HAUSNER'S RATIO [9]

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula:

$$\text{ENTRAPMENT EFFICIENCY} = \frac{\text{PRACTICAL DRUG LOADING}}{\text{THEORTICAL DRUG LOADING}} \times 100$$

Showing result of drug entrapment efficiency and hausner's ratio

S.NO	FORMULATION	% DEE	HAUSNER RATIO
1.	F1	70.85±1.38	16.14
2.	F2	64.55±1.53	14.53
3.	F3	69.35±1.29	17.58
4.	F4	58.45±1.58	17.30
5.	F5	68.12±1.45	14.24
6.	F6	72.89±1.30	13.65

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7.	F7	68.10±1.50	16.41
8.	F8	73.56±1.45	13.47
9.	F9	69.54±1.35	13.98

In-vitro dissolution studies

In vitro drug release studies were performed in 0.1 N HCL ,PBS 6.8 and PBS 7.4.The cumulative drug release study was carried out as per the prescribed method indicated in Indian pharmacopoeia. The observation is given below.All the microspheres showed good release of drug over 12 hrs. The percentage drug released from Eudragit and ethyl cellulose containing Pantoprazole microspheres in 12 h was within the range of 76.89 ± 2.75% (F-1) to 67.81 ± 1.99% (F-9), and this was found to be lower with the increasing of both eudragit and ethyl cellulose in the polymer-blend used. In –vitro drug release study shows that the formulations F1,F4 and F6 shows the highest drug release among all the formulations.

RESULT SHOWING CUMMULATIVE DRUG RELEASE

S.NO	TIME (hr)	% CUMMULATIVE DRUG RELEASE								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	0.5	11.39	10.40	9.99	10.14	10.89	11.85	10.44	9.13	9.99
2.	1	15.49	12.59	11.11	13.26	13.19	12.93	12.11	11.22	11.02
3.	2	19.654	14.68	13.25	16.35	17.26	16.99	15.64	14.45	13.21
4.	3	25.034	22.64	16.65	24.49	22.36	21.32	19.92	18.03	17.06
5.	4	30.657	28.48	25.73	28.59	27.62	25.59	24.97	23.65	22.98
6.	5	38.70	32.69	29.58	34.99	34.48	36.45	34.99	32.67	29.49
7.	6	41.92	37.51	33.59	39.10	40.63	40.56	38.94	37.63	35.44
8.	7	46.57	41.03	35.01	45.94	45.15	44.59	43.68	41.98	39.72
9.	8	52.21	45.84	40.01	50.89	49.76	47.48	46.97	45.99	42.07
10.	9	58.69	50.66	44.37	54.12	55.92	54.64	53.09	52.98	50.95
11.	10	64.84	56.04	54.10	62.66	61.54	60.38	59.95	58.72	57.40
12.	11	70.55	63.21	60.80	70.19	67.60	66.45	65.89	64.66	63.42
13.	12	76.89	69.50	69.58	74.79	73.51	72.72	72.00	68.78	67.81

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STABILITY STUDIES

Stability studies revealed that the microspheres kept at room temperature (25ºC) and 40ºC /75% RH showed the maximum stability. The values of drug content and drug release studies were close to that of the initial data suggesting that it has an acceptable shelf life. The stability data showed that all formulation was no change in the appearance of the microspheres indicating that the formulations were stables at all the condition to which they were exposed.

In-vitro drug release studies for all the formulations were carried out at the end of 90 days and did not reveal any significant change in the drug release from all the formulation. Thus, we may conclude that the drug does not undergo degradation on storage.

Drug entrapment stability studies of Pantoprazole microspheres

Stability condition	Sampling Day	Drug Entrapment F1	F5	F6	Physical Appearance
25°C /60%RH	30	70.52	68.12	72.89	No change
	60	70.54	68.15	72.71	No change
40°C /75% RH	30	70.51	68.20	72.25	No change
	60	70.40	68.18	72.15	No change

Drug release stability studies of Pantoprazole microspheres

Stability Condition	Sampling Day	Drug Release F1	F5	F6	Physical Appearance
25°C/60°C%RH	30	76.89	73.51	72.72	No change
	60	76.55	73.46	72.47	No change
40°C /75% RH	30	76.40	73.16	72.24	No change
	60	76.45	73.29	72.14	No change

III. DISCUSSION

In the present study nine different formulations were prepared. The main polymers used were Eudragit RS 100 and Ethyl Cellulose. Microspheres of different size and drug content were obtained by altering the formulation variables. The dichloromethane and ethanol were used as solvent. Surfactant used was Tween 80. Preformulation studies were conducted. FTIR study results showed that there were no incompatibility between drug and polymers. Solubility study was performed and results were noted which were within the specification. A calibration data was obtained by using UV spectrometer and calibration curve was plotted. The preformulation studies which were conducted revealed that the drug was pure and has no interaction or incompatibility with excipients. Evaluation studies such as Bulk density and tapped density was calculated. Percentage yield, particle size analysis, drug entrapment efficiency and in vitro drug release were conducted. All the nine formulation gave good results within the limits.

Percentage yield was calculated and it was in the range of 68%-86.5%. The entrapment efficiency was in the range of 73.56±1.45 % to 58.45±1.58 . Scanning electron microscopy showed that microspheres were spherical in shape and porous in nature. In vitrodissolution studies showed that formulation F1 has the release of 76.89%at the end of 12 hours. Stability study shows that there

was no significant change in the properties of microspheres when subjected to stress conditions.

IV. SUMMARY AND CONCLUSION

The aim of present study was to formulate colon targeted microspheres of Pantoprazole with different proportions of polymers such as Eudragit RS 100 and ethyl cellulose for oral drug delivery. From the results obtained by preformulation studies, it can be concluded that there was no incompatibility between drug and polymers. The evaluation studies such as particle size analysis, percentage drug entrapment, in vitro drug release and stability studies showed that all the formulations gave good results within limits Percentage yield was calculated and it was in the range of 68%-86.5%. The entrapment efficiency was in the range of 73.56±1.45 % to 58.45±1.58 . Scanning electron microscopy showed that microspheres were spherical in shape and porous in nature. In vitro dissolution studies showed that formulation F1 has the release of 76.89% at the end of 12 hours.. Although formulation F1, F5 and F6 is the best formulation as per the study. Stability studies showed that there were no significant changes in the formulation after certain time period and temperature condition.Thus the aim of the study to formulate colon targeted microspheres of Pantoprazole Sodium was achieved. In future this system can be developed by using various colon specific polymers in various proportions for more better results.

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