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HOLLOW MICROSPHERES OF PANTOPRAZOLE SODIUM SESQUIHYDRATE: GASTRORETENTIVE CONTROLLED DELIVERY SYSTEM

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Abstract: The objective of the present investigation was to design controlled release gastroretentive hollow microspheres of Pantoprazole sodium sesquihydrate using polymer; cellulose acetate by using the emulsion solvent evaporation method. The effect of drug-polymer interaction and effect of drug-excipients interaction was studied by using FTIR analysis. Preformulation studies like solubility analysis, partition coefficient, UV spectra of drug, IR spectrum of pantoprazole were performed to check the drug purity and standards. Dummy microspheres of cellulose acetate was prepared by optimizing various formulation parameters like effect of polymer concentration, effect of stirring speed, effect of PVA concentration and surface morphology characteristics of dummy microspheres. Encapsulation efficiency, the yield, particle size, floating capability, flow properties, morphology of microspheres was evaluated. Production yield, loading efficiencies, and particle size of S4 were found to be 69.88%, 68.94% and 194.22 micron respectively. Microsphere prepared with cellulose acetate showed the best floating ability (85.54 ± 0.03% buoyancy) in 0.1 N HCl for over 12 hours. Scanning electron micrographs of formulations indicated that the microspheres were smooth spheres without crystals on surroundings. The particles were spherical and hollow. Regarding the drug content during the accelerate stability study, samples showed complete encapsulation efficiency and were considered stable.

Keywords: Controlled Release, Hollow microspheres, Pantoprazole sodium, cellulose acetate, Emulsion solvent evaporation method.

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INTRODUCTION

Pantoprazole is a proton pump inhibitor (PPI's) used in the treatment of gastric, duodenal ulcer and also in gastro esophageal reflux disease (GERD), Zollinger-Ellison syndrome[1]. Pantoprazole has several advantages compared to its analogues (e.g. omeprazole and lansoprazole) such as specific site of binding, greater stability in neutral pH environment and longer duration of action. This drug was the first water soluble benzimidazole 5 (difluoromethoxy)-2-[[(3, 4-dimethoxy-2-pyridinyl) methyl] sulfinyl] - sesquihydrate, which can be administered intravenously in the form of sesquihydrate sodium pantoprazole[4].

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[4]. Various attempts have been made to retain the dosage form in the stomach as a way of increasing the retention time[7]. Among all FDDS systems can remain in the stomach for long periods and hence can release the drug over a prolonged period of time. The problem of short gastric residence time encountered with an oral CR formulation hence can be overcome with these systems. These systems have a bulk density of <1 as a result of which they can float on the gastric contents. These systems are relatively large in size and passing from the pyloric opening is prohibited[8]. Gastro retentive drug delivery is an approach to prolong gastric residence time, thereby targeting site-specific drug release in the upper gastrointestinal tract (GIT) for local or systemic effects[9].

Taking all above into account, the aim of this work was to formulate the gastroretentive controlled delivery system to protect the drug from rapid degradation in gastric fluid. Additionally, the work was also consecrated to determine the drug release profiles from microspheres.

MATERIALS AND METHODS-

The gastroretentive controlled microspheres of Pantoprazole sodium sesquihydrate were prepared using Cellulose acetate by o/w emulsion solvent evaporation method[10, 11]. Major advantages of the suggested novel preparation technique include short processing times, no exposure of the ingredients to high temperatures, the ability to avoid toxic organic solvents and high encapsulation efficiencies (close to 100%)[12]. It is preferred to other preparation methods such as spray-drying, sonication and homogenization, etc., because it requires only mild conditions such as ambient temperature and constant stirring. Thus, a stable emulsion can be formed without compromising the activity of the core Materials[13]. These microspheres were

characterized by means of their morphology, flow properties, buoyancy % and dissolution kinetics.

Experimental Work- Preparation of pantoprazole sodium sesquihydrate floating microspheres: Microspheres were prepared by the emulsion solvent evaporation method. Five different ratios (S1 [1:1], S2 [1:2], S3 [1:3], S4 [1:4], S5 [1:5]) of floating microspheres of Pantoprazole sodium sesquihydrate were prepared by using cellulose acetate as the polymer. Calculated quantities of Cellulose acetate and Glyceryl monostearate were dissolved in 20 ml mixture of ethyl acetate and acetone (1:1) to get a homogenous polymer solution. Pantoprazole sodium sesquihydrate was dispersed uniformly in the polymer solution and then it was poured slowly into 100 ml of 0.45% w/v polyvinyl alcohol in distilled water. The emulsion formed was stirred continuously for 3 hrs using a mechanical stirrer at 650 rpm. The temperature was maintained at 40^0C. The microspheres were formed and filtered and washed repeatedly with distilled water. The microspheres were air dried overnight and stored at room temperature[10-11].

RESULTS AND DISCUSSION-

Effect of Stirring Speed on the Size of Dummy Microsphere:

Effect of stirring speed on size of dummy microspheres: In the present study, a dramatic decrease of microsphere size from 189.15µm to 132.60µm occurred when the stirring speed in the w/o emulsion increased from 300 to 1000 rpm as shown in table 1.

Table 1: Effect of Stirring Speed on the Size of Dummy Microsphere

Formulation code	Stirring speed (rpm)	Particle size (µm)			Mean ± SD
		1	2	3	
R1	300	189.54	188.37	189.54	189.15 ± 0.68
R2	400	176.67	175.50	174.33	175.50 ± 1.17
R3	650	166.14	166.14	164.97	166.14 ± 1.17
R4	700	150.93	150.93	149.76	150.93 ± 1.17
R5	1000	132.21	132.21	132.21	132.60 ± 0.68

All values are given as Mean ± SD; n= 3

Effect of PVA Concentration on the Size of Dummy Microsphere:

Effect of PVA concentration on size of dummy microspheres: The mean diameter sizes of microsphere are represented in table 2. The mean size of the microspheres decreased as the concentration of PVA increased from 0.1% to 0.6%.

Table 2: Effect of PVA Concentration on the Size of Dummy Microspheres

Formulation code	PVA Conc. (%)	Particle size (µm)			Mean ± SD
		1	2	3	
A1	0.1	169.65	167.31	17082	169.26 ± 1.79
A2	0.3	159.12	160.29	159.12	159.51 ± 0.68
A3	0.45	147.42	148.59	149.76	148.59 ± 1.17
A4	0.6	145.08	146.25	145.08	145.47 ± 0.68

All values are given as Mean ± SD; n= 3

Effect of Polymer Concentration on the Size of Dummy Microsphere:

The mean size of the microspheres increased as the concentration of polymer increased from concentration 1% to 5% as shown in table 3.

Table 3: Effect of Polymer Concentration on the Size of Dummy Microspheres

Formulation code	Polymer Conc. (%)	Particle size (µm)			Mean ± SD
		1	2	3	
P1	1	149.76	148.59	147.42	148.59 ± 1.17
P2	1.25	163.80	161.46	160.29	161.85 ± 1.79
P3	2.5	175.50	170.82	173.16	173.16 ± 2.34
P4	3.75	186.03	189.54	187.20	186.42 ± 1.79
P5	5	202.41	204.75	203.58	203.58 ± 1.17

All values are given as Mean ± SD; n= 3

Morphology characteristic of dummy microspheres: From all above experiments the A3 formulation was selected for SEM and evaluation of their surface morphologies. The SEM photographs of A3 formulations are shown in fig. 1.

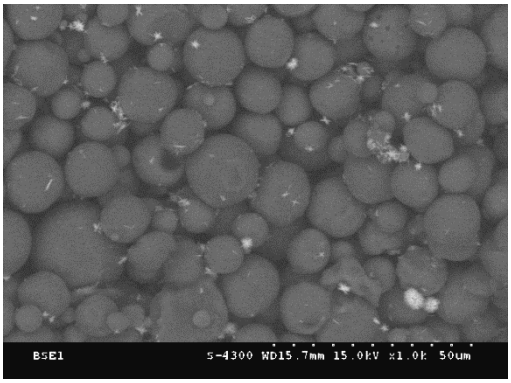

Fig. 1: SEM photograph of dummy microsphere (cellulose acetate 250mg) of formulation code A3.

The microspheres were generally spherical in shape. The SEM images showed smooth spheres without crystals on surroundings. Particles were spherical and hollow. Because of the high concentration of solids in the liquid feed, solids will come out of solution at the surface of the droplet first, leading to the formation of a crust around a hollow particle. It is also observed that pilot-scale powders presented large holes due to the rapid evaporation of water. We can classify the microspheres as hollow microspheres[4].

Preparation of Pantoprazole loaded microspheres: Microspheres were prepared by the emulsion solvent evaporation method. The Ingredients which were used for microspheres are given in table 4.

Table 4: List of Ingredients Used For Final Drug Loaded Formulation

Sr no.	Ingredients	S1(1:1)	S2(1:2)	S3(1:3)	S4(1:4)	S5(1:5)
1	Pantoprazole (mg)	250	250	250	250	250
2	Cellulose acetate (mg)	250	500	750	1000	1250
3	Glyceryl monostearate (mg)	250	250	375	500	500
4	PVA (mg)	450	450	450	450	450
5	Ethyl acetate (ml)	10	10	10	10	10
6	Acetone (ml)	10	10	10	10	10
7	Distilled water (ml)	100	100	100	100	100

Production yield and Entrapment efficiency:

Table 5: % Production Yield and Entrapment efficiency

Formulation code	Drug: Polymer ratio	Production yield Mean ± SD	Entrapment Efficiency Mean ± SD
S1	1:1	72.88 ± 0.20	72.14 ± 0.34
S2	1:2	70.70 ± 0.26	69.81 ± 0.23
S3	1:3	69.27 ± 0.13	67.53 ± 0.81
S4	1:4	69.88 ± 0.09	68.94 ± 0.79
S5	1:5	70.30 ± 0.13	68.66 ± 0.57

All values are given as Mean ± SD; n= 3

The maximum production yield was 72.88% for formulation S1 and minimum was found to be 69.27% for formulation S3. The production yield of formulation S2, S4 and S5 was found 70.70%, 69.88% and 70.30%. The maximum entrapment efficiency was 72.14% at 250mg of drug for S1 (1:1) and minimum entrapment efficiency was 67.53% for S3 (1:3). The entrapment efficiencies of formulation S3 (67.53%) were found to be less as compared to S4 formulation

(68.94%). This may be due to the ability of drug to partition in to aqueous phase prior to microspheres solidification[35].

Measurement of particle size:

Table 6: Particle Size of Drug Loaded Microspheres

Formulation code	Drug : Polymer ratio	Particle size(µm) Mean ± SD
S1	1:1	154.05 ± 1.79
S2	1:2	161.46 ± 2.34
S3	1:3	183.30 ± 1.79
S4	1:4	194.22 ± 1.17
S5	1:5	218.40 ± 1.79

All values are given as Mean ± SD; n= 3

The increase in particle size of the microspheres with increase in polymer/drug ratio was observed.

Buoyancy Percentage:

Table 7: *In Vitro* Buoyancy %.

Formulation code	D:P Ratio	Buoyancy % Mean ± SD
S1	1:1	77.56 ± 0.46
S2	1:2	73.33 ± 0.77
S3	1:3	68.87 ± 0.82
S4	1:4	65.97 ± 0.81
S5	1:5	62.48 ± 0.29

All values are given as Mean ± SD; n= 3

The percentage buoyancies of formulations S1 to S5 at the end of 12 hour were founded to be 77.56%, 73.33%, 68.87%, 65.97% and 62.48%. The results indicate that with increase in the concentration of cellulose acetate decreases the floating time as seen in table 7.

Flow properties:

Table 8: Results of Flow Properties of Microspheres.

Formulation Code	Evaluation Parameters				
	Angle of Repose (θ)	Bulk Density (gm/cm^3)	Tapped Density (gm/cm^3)	Carr's Index (%)	Hausner's Ratio
S1	26.28	0.54	0.65	16.43	1.19
S2	22.10	0.50	0.57	12.54	1.14
S3	23.48	0.47	0.51	6.27	1.06
S4	21.08	0.44	0.48	9.27	1.10
S5	18.81	0.39	0.41	5.26	1.05

The Flow properties were determined Pantoprazole sodium sesquihydrate of formulations S1-S5. The evaluation parameters of formulations showed good flow properties.

FTIR analysis: The FTIR spectra of the free drug (Fig. 2), cellulose acetate (Fig. 3) and the drug loaded microspheres were recorded (Fig. 4). The identical peaks corresponding to functional groups and polymer confirms that neither the polymer nor the method of preparation has affected the drug stability. Pantoprazole sodium sesquihydrate had characteristic bands at 3180 (N-H stretches), 2983 (C-H stretches), 1589 (C=N, C stretches), 1453 (CH_2 bending), 1267 (S=O bending), 1165 (Sp^2 C-O aromatic ether stretches), 1038 (CH-O stretches), 823 (C-H bending of pyridine ring hydrogen's). These bands are shown in FTIR spectrum of Pantoprazole sodium sesquihydrate loaded microspheres along with the spectrum bands of Cellulose acetate (Fig. 4) which indicated that Pantoprazole sodium sesquihydrate was physically entrapped in the polymer matrix and there was no chemical interaction between Pantoprazole sodium sesquihydrate and polymer.

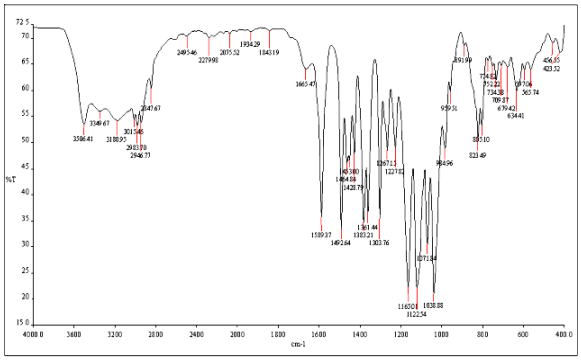

Fig. 2: FTIR spectrum of pantoprazole sodium sesquihydrate

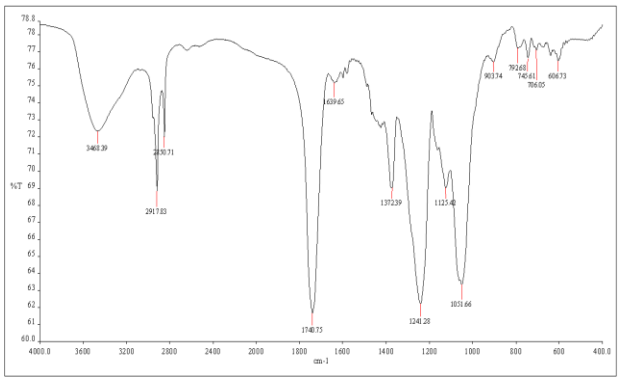

Fig. 3: FTIR Spectrum of Cellulose Acetate

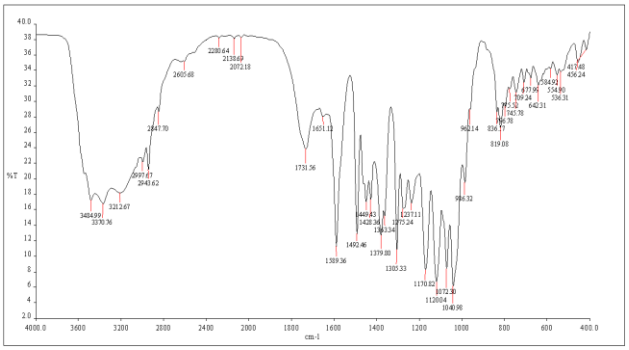

Fig. 4: FTIR Spectrum of Pantoprazole Sodium Sesquihydrate Loaded Microspheres

Surface morphology: The morphology of the microspheres (S1- S5) was studied using scanning electron microscopy (SEM). Particles were spherical and hollow. Because of the high concentration of solids in the liquid feed, solids will come out of solution at the surface of the droplet first, leading to the formation of a crust around a hollow particle. It is also observed that pilot-scale powders presented large holes due to the rapid evaporation of water. We can classify the microspheres as hollow microspheres as shown in fig. 5[4].

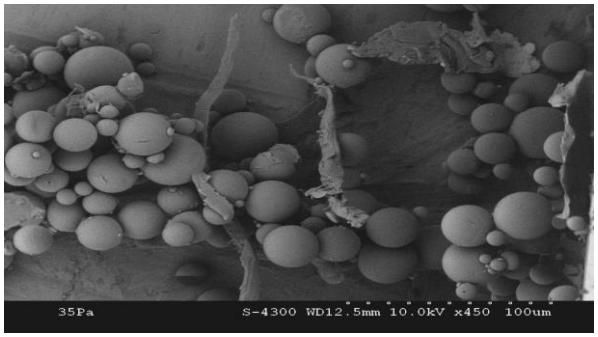

Fig. 5: SEM of drug loaded cellulose acetate microspheres of S4 (1:4)

***In vitro* drug release studies of drug loaded floating microsphere:** The *in vitro* drug release data of pantoprazole sodium sesquihydrate loaded microspheres in simulated gastric fluid followed by intestinal fluid after 10 hours are given in table 9. The *in vitro* drug release data of 72.14%, 69.81%, 67.53%, 68.94% and 68.66% drug loaded floating microsphere for 2 hours in simulated gastric fluid are shown in fig. 6.

Table 9: *In Vitro* Drug Release Data for Formulation S1- S5 after 10 Hrs.

Cumulative % drug Release				
S1	S2	S3	S4	S5
84.54 ± 0.03	77.93 ± 0.04	70.61 ± 0.97	68.67 ± 0.52	66.57 ± 0.61

All values are given as Mean ± SD; n= 3

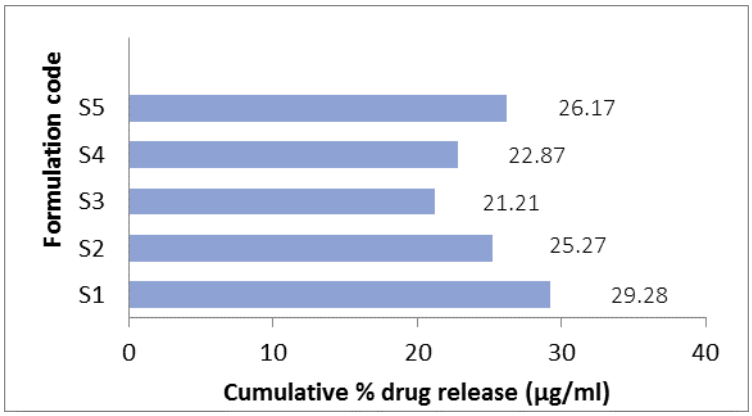

Fig. 6: comparison of *in vitro* drug release data of S1, S2, S3, S4 and S5 drug loaded floating microsphere for 2 hours in simulated gastric fluid.

The Cumulative % drug release from formulation S1 to S5 was as follows S1, S2, S3, S4, S5 show percentage drug release 84.54%, 77.93%, 70.61%, 68.67%, 66.57% at end of 10 hour. It was observed that cumulative % drug release significantly decreased with increase in the amount of polymer. The increase in cellulose acetate concentration leads to the increased density of polymer matrix into the microspheres which result in an increased diffusional path length. This may decrease the overall drug release from polymer matrix. Furthermore smaller microspheres are formed at lower polymer concentration and have larger surface area exposed to dissolution medium, giving rise to faster drug release[10, 11, and 22]. The S1 formulation higher release rates 84.54% at all time-points compared with the other formulation. It is possible that in the S2, S3, S4, and S5 formulation, the majority of the drug particles were discrete and isolated within the polymer matrix and that drug release was limited by the diffusional barrier of the polymer matrix. In S1 formulation particles may have been in physical contact forming a particle network which connects the inner part of the network to particles located near the surface of the microspheres. This would have allowed penetration of solvent and dissolution and diffusion of

Pantoprazole sodium sesquihydrate from one particle site to another without any limitation of a polymer barrier, resulting in a greatly enhanced release rate[39].

Fig. 6 showed the cumulative release of pantoprazole sodium sesquihydrate of five different loadings in gastric fluid at 37°C. There was no significant burst release of drug from any of the preparations. The release was faster from microspheres because of having a higher drug payload. The drug/polymer ratio of microspheres having high drug payload is large and therefore hindrance to diffusion of drug from the polymer matrix would be rather low making the diffusion facile at high drug loading which was then reflected in faster release. At all loading tested the amount released increases with respect to time for about 10 hours[23].

This experiment was performed because FDDS are known to prolong the gastric residence time in the stomach. The exact residence time of the dosage form in the gastric and intestinal regions has not been determined, but the data obtained from *in vitro* dissolution readings demonstrated that only about a 1/4^{rth} of the drug would be released in gastric tract depending upon the gastric residence time and the rest would be available for release in the intestinal fluid. The total absorptive area of the small intestine is about 200 m^2 while an estimate for stomach is only 1 m^2. Therefore the absorption of drug is more in intestine than in stomach region[23].

***In vitro* release kinetics:**

Table 10: The Values of R2, K, and N for Pantoprazole Loaded Formulations

Formulation code	Zero order		First order		Higuchi model		Korsmeyer Peppas	
	R	K (mg/hr)	R	K (hr^{-1})	R	K ($mg/hr^{-1/2}$)	R	'n'
S1	0.9749	7.5185	0.9686	0.0716	0.9777	26.385	0.9662	0.5289
S2	0.9832	7.3086	0.9783	0.0621	0.9657	25.382	0.9703	0.5951
S3	0.9686	6.9108	0.7238	0.1063	0.9719	24.259	0.9543	0.9600
S4	0.9639	7.2276	0.6999	0.1094	0.9694	25.401	0.9399	0.9971
S5	0.9517	6.1575	0.9894	0.0449	0.9895	22.003	0.9797	0.6419

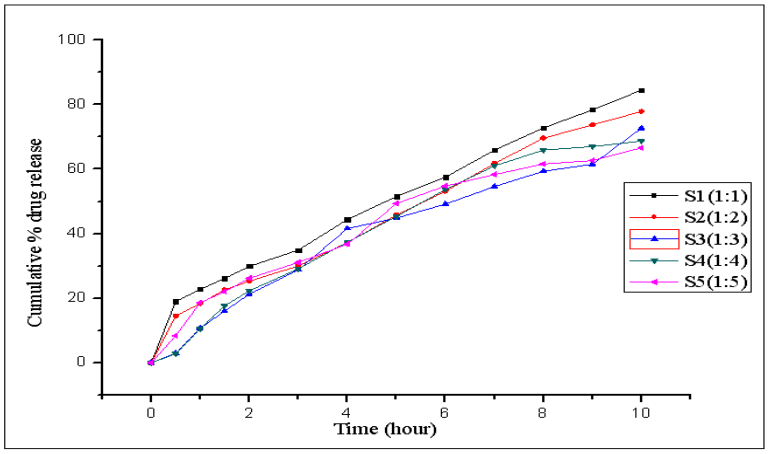

Fig. 7: Zero Order Plots Of Different Formulations (S1-S5) Of Pantoprazole Sodium Sesquihydrate

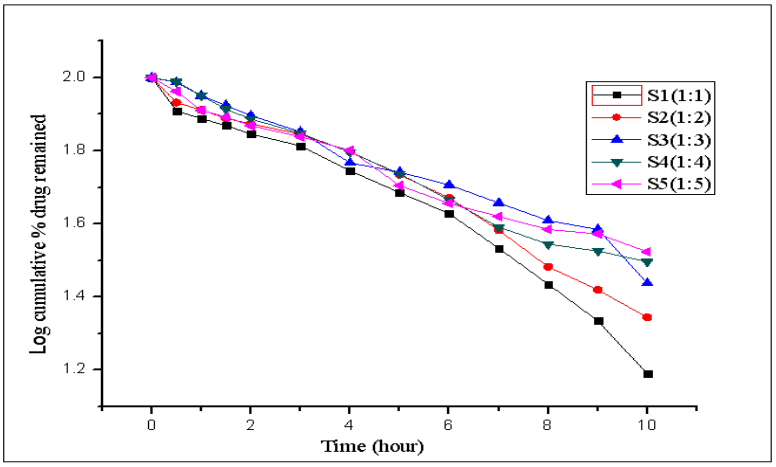

Fig. 8: First order plots of different formulations (S1-S5) of pantoprazole sodium sesquihydrate

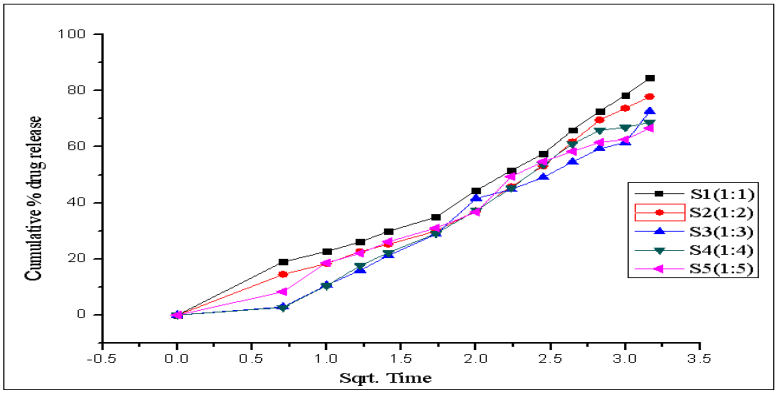

Fig. 9: Higuchi Plots Of Different Formulations (S1-S5) Of Pantoprazole Sodium Sesquihydrate

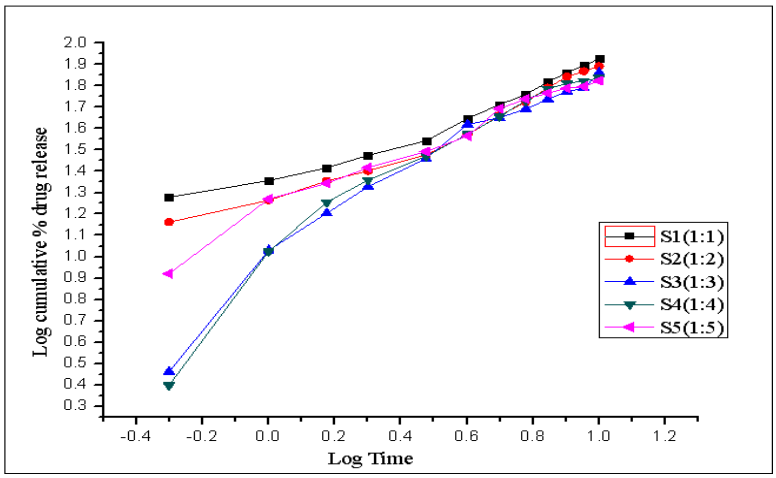

Fig. 10: Korsemeyer Peppas Plots Of Different Formulations (S1-S5) Of Pantoprazole Sodium Sesquihydrate

The regression coefficient values of different microspheres formulations namely S1-S5 was found to be between 0.9749 - 0.9517 respectively for zero order model, 0.9686 - 0.9894 respectively for first order model and 0.9777 - 0.9895 respectively for Higuchi model. The R values were much closer to one for the Higuchi kinetics.

The n values for formulations S1 - S5 was found to be between 0.5289 - 0.6419, respectively. Concerning the mathematical modeling fitting the Korsmeyer-Peppas model for spherical particles, the exponent n of 0.43 indicates that the release mechanism is governed by Fickian diffusion and n higher than 0.85 it is governed by swelling of polymer (Case-II transport or super Case-II transport). The value of n between 0.43 and 0.85 for spherical particles indicates that the mechanism is governed by both phenomena (anomalous transport). From the above kinetics data (table 10) exponent n of 0.52 for S1, 0.59 for S2 and 0.64 for S5 indicated that pantoprazole release mechanism was based on anomalous transport (non-Fickian mechanism) and exponent n of 0.96 for S3 and 0.99 or S4 indicated that pantoprazole release was based on swelling of polymer (Case-II transport or super Case-II transport). The drug release can be explained by the superposition of swelling, relaxation and dissolution of the polymer[4].

Statistical analysis

One way ANOVA analysis was applied on final *in vitro* dissolution readings. Microsoft excel 2007 software was used to calculate the value of F-ratio. The calculated value of F was 0.33 which is less than the table value of 2.52 at 5% level with d.f. being $v_1 = 4$ and $v_2 = 60$ and hence could have arisen due to chance. This analysis supports the null – hypothesis of no differences is sample means.

Stability Studies

The stability studies were performed for the formulation (S4) at 25°C ± 2°C / 60% ± 5% RH, 35°C ± 2°C / 60% ± 5% RH and 40°C ± 2°C / 75% ± 5% RH. The samples were analyzed for drug content. The results indicated in table 11 showed that the formulation were stable, and did not show any significant difference in drug content.

Table 11: Stability Results of S4 at Various Storage Temperatures

Sampling intervals (days)	Storage conditions		
	25 ± 2^0C	35 ± 2^0C	40 ± 2^0C
	Drug content	Drug content	Drug content
01	63.34 ± 0.27	63.19 ± 0.46	63.03 ± 0.7
30	62.88 ± 0.27	60.73 ± 0.70	60.11 ± 0.55
60	62.57 ± 1.35	60.42 ± 1.22	59.96 ± 0.80
90	59.65 ± 0.96	59.34 ± 0.70	59.19 ± 0.53

All values are given as Mean ± SD; n= 3

SUMMARY AND CONCLUSION: The aim of the present study was to formulate and evaluate controlled release drug delivery system of Pantoprazole sodium sesquihydrate floating microspheres by using Cellulose acetate.

The pantoprazole sodium sesquihydrate solubility decreases in acidic environment and increases with increase in pH. Pantoprazole degraded in Gastric pH with a half-life of 1 hour only. Hence such a drug requires a novel approach of gastroretentive drug delivery system which can provide extended period of time in stomach. The floating hollow microspheres are formed to achieve control release of Pantoprazole sodium sesquihydrate in acidic environment followed by release in Intestinal pH so as to protect it from rapid degradation and prolong its half-life.

The Microspheres are formed by o/w emulsion solvent evaporation method. FT-IR study was carried out to check any possible interactions between the drug and the polymers which confirmed that there were no interaction between the selected drug and the polymers are seen. The microspheres were evaluated for Entrapment efficiency, Micromeritics property, buoyancy%, *In-vitro* study and stability 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 formulation codes.

The *in-vitro* dissolution studies were carried out for microspheres using USP dissolution apparatus type I. Results of the in vitro drug release indicated that the controlled drug release

upto 10 hours were obtained from the so prepared carrier backbone. The cumulative percentage of drug release from the microspheres varied and depends on the type of polymer used and its concentration. All formulations were subjected to release kinetics. The drug releases from the formulations were zero order followed by diffusion mechanism of drug release.

From the results it was observed that maximum production yield was achieved for S1 formulation 72.88% with maximum entrapment efficiency of 72.14% but with high cumulative % drug release of 85.54% in 10 hours. The production yield for S4 formulation was achieved to 69.88% with entrapment efficiency of 68.94%. About 65.97% buoyancy was achieved for 12 hours of S4 formulation. The cumulative % drug released for S4 formulation is least i.e. 68.67% and release pattern follows zero order indicates drug release in a controlled manner and Higuchi's model indicating diffusion to be a predominant mechanism of drug release. The S4 formulation meets the target requirement to increase the half-life of pantoprazole sodium sesquihydrate in a controlled manner.

The Presence of cellulose acetate efficiently protects the acid labile drug from highly acidic environment of stomach. In conclusion the performed studies suggested that cellulose may be a promising candidate for oral controlled drug delivery system because of its low density than gastric fluid as a result microspheres float over a prolonged period of time and sustaining the release of a drug.

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