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INVITRO EVALUATION STUDIES OF CROSSLINKED CHITOSAN MICROSPHERES CONTAINING RABEPRAZOLE SODIUM

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ABSTRACT

For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms including tablets, capsules, pills, suppositories, creams, ointment, liquids, aerosols and injectables as drug delivery systems are the primary pharmaceutical products commonly seen in the market. In the past two and a half decades several advancements has been made in the pharmaceutical products. They have resulted in the development of new techniques for drug delivery[1]. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of drug. A series of three different trial formulation of Rabeprazole Sodium, Microsphere using Chitosan an aminopolysaccharide, so as to optimize the sustained conditions. In the case of microsphere, the initial formulation was done with 0.5% Chitosan solution and drug. Then the concentration of chitosan had been increased to 1.5%. It showed that the percentage release was maximum 65.2% for the chitosan microsphere in the formulation F3, when compared with other formulation of microsphere containing Rabeprazole sodium, the 1.5% of chitosan showed better release than the other formulations.

INTRODUCTION: For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms including tablets, capsules, pills, suppositories, creams, ointment, liquids, aerosols and injectables as drug delivery systems are the primary pharmaceutical products commonly seen in the market. In the past two and a half decades several advancements has been made in the pharmaceutical products. They have resulted in the development of new techniques for drug delivery [1]. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of drug. The range of techniques for the preparation of microsphere offers a variety of opportunities to control aspects of drug administration. The term "control" may be used broadly and includes phenomena such as protection and masking, reduced dissolution rate, facilitation of handling, and spatial targeting of the active ingredients.

Microsphere can be defined as solid, approximately spherical particles ranging in size from 1 to 1000µm. They are made of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products such as starches, gums,

proteins, fats, and waxes. The natural polymers include albumin and gelatin [2, 3]; the synthetic polymers include polylactic acid and polyglycolic acid [4, 5].

Chitin is the major polysaccharide of the shells of crustaceans and exoskeletons of insects. Chitosan is a non- acetylated or partially chitin derivative. Crustacean's shells are the usual raw material of chitin. Chitin is (1-4-) 2-amino-2-deoxy-D-glucan. It has similar structural characteristics as that of glycosamino glycans. It is tough, biodegradable and non-toxic. Chitosan is used for making absorbable and digestible surgical sutures. It is also used as hypocholesterolemic and hypolipidemic agent, liposomes stabilizer and for making contact lenses chitosan microsphere are used in delivering anticancer and antibacterial agents and for developing orthopaedic materials. Chitosan is an efficient substrate for peripheral nerve regeneration and its immunological reaction in peripheral nervous system [6]. Oxygen permeability property of chitosan might have helped in the nerve regeneration by preventing oxygen deprivation of tissues.

Rabeprazole is a proton pump inhibitor with anti secretory properties. It has 2 to 10 fold greater anti-secretory activity than omeprazole. Rabeprazole is a benzimidazole proton pump inhibitor, the chemical structure of which differs from that of omeprazole duo to substitutions on the pyridine and benzimidazole rings. It is partially reversible inhibitors of H^+, K^+-ATPase and is activated in the acidic lumen of gastric parietal cells.

MATERIALS AND METHODS: The materials used for the study were Rabeprazole Sodium, Chitosan, Gacial acetic acid, Glutaraladehyde, Sonicator and UV Spectrometer. The reagents for the study were Phosphate buffer- pH 7.4 I.P and 0.9% w/v Sodium Chloride saline I.P.

Formation of cross linked Chitosan microspheres of Rabeprazole Sodium: Three formations of 0.5 %, 1 % and 1.5 % solution of chitosan was prepared in aqueous acetic acid. Rabeprazole sodium (10 mg) was dispersed in this solution and mixed well. This solution was added to the liquid paraffin to form water-in-oil (w/o) emulsion. The dispersion was stirred at 710 rpm for 30 minutes after the addition of glutaraldehyde solution. The product was filtered and washed with chloroform, several times and finally with water and dried at 50°C. All batches were prepared at least three times.

Standard curve of Rabeprazole Sodium: A 10 mg of Rabeprazole Sodium was dissolved in 10 ml of Phosphate buffer saline pH 7.4. From that stock solution 0.1 to 0.8 ml of the solution was withdrawn and diluted to 100 ml with phosphate buffer saline pH 7.4 in a 100 ml standard flask to produce 1 to 8 mcg/ml concentrations. Then the sample were assayed spectrophotometrically Elico SL282 nm using buffer 7.4 as blank.

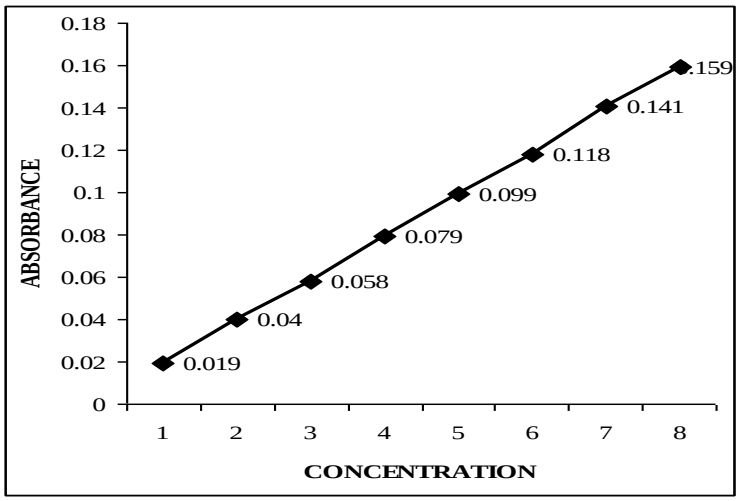

FIG. 1: STANDARD CURVE OF RABEPRAZOLE SODIUM

Assay of Rabeprazole Sodium: Rabeprazole Sodium raw material was analyzed for its percentage purity by spectrophotometer at 282 nm.

TABLE 1: ANALYTICAL PROFILE OF CHITOSAN

Tests	Analytical values
Viscosity	5.60
Nitrogen content	6.25% w/w
Degree of acetylation	87.15% w/w
Ash content	1.43% w/w
Molecular weight	6.7×10^5

Drug Expedient Interaction Study:

Particle size determination: Optical microscope was used to determine the size of the particle that lies within a range from 0.2 mm to 100 mm. 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 along an orbitarily chosen fixed line across the center of the particle. The particle size is a factor to be considered important in formulation of microspheres.

Determination of drug content: A weighed quantity of microspheres was extracted with ethanol for 4 hours, and then the drug content was spectrophotometrically assayed at 282 nm. Each determination was carried out in triplicate.

Stability studies of Microspheres: The formulations F1, F2 and F3 were studied profile for 1 month at different environmental conditions such as room temperature, 45^0C, 82.5% RH. 45^0C environment is produced by keeping the microspheres in hot air oven. 82.5% relative humidity was maintained in dessicator by keeping 13.1 ml of H_2SO_4/100 ml of water.

Evaluation: A weighed amount of microspheres was suspended in phosphate buffer pH 7.4 I.P. (50 ml) contained in a 100 ml glass bottle. This dissolution medium was stirred atn100 rpm in a horizontal laboratory shaker and maintained at constant temperature (37^oC ± 0.1^oC) in a water bath. Samples were periodically removed and analyzed spectrophometerically at 282 nm using medium a blank.

RESULTS AND DISCUSSION:

Analysis of Rabeprazole Sodium: The procured sample was analyzed for drug content. The percentage purity of the used samples lies within the limits of I.P. calculated on the dried basis (99.8%).

Release studies: This work describes the study of drug release from various chitosan microspheres containing 0.5%, 1% and 1.5% of chitosan and Rabeprazole Sodium respectively and compares them with the pure drug. In this work an attempt was also made to study the release of Rabeprazole Sodium from various concentrations of Microspheres using Chitosan in buffer pH 7.4. The observations have been shown in **Tables 2, 3** and **4**.

- When the amount of drug released from the formulation F1, F2 and F3 was plotted against time and the observed study period was 12 hours.
- Overall release of drug Rabeprazole Sodium from the microsphere were compared, among all the formulation (F3) 2% Chitosan microspheres showed much release retardant behavior. It may be explained by the fact of high proportion of chitosan incorporation. The rate of dissolution was faster in the case of Albumin microspheres. T90% for (F1) 0.5% Chitosan based microsphere formulation were 720 min. The plot of percentage of drug release Vs time is linear in cases of 1.5%, 2% Chitosan based microspheres. The lag time was not observed in the formulation. Microspheres did not form effectively at low concentrations of Chitosan in acetic acid solution.
- 0.5% w/v volume solution of chitosan containing rabeprazole Sodium showed entrapment efficiency 52.68%. These results are parallel with the report of Akulya J., *et. al.,* [7]. The burst effect was observed prominently in all the formulations during initial phase of drug release.

TABLE 2: INVITRO RELEASE STUDIES OF 0.5% CROSS LINKED CHITOSAN MICROSPHERE IN BUFFER PH 7.4 (FORMULATION F1)

Sl. No.	Time in hours	Absorbance	Concentration ug/ml	Amount present in 50 ml (mg)	% release
1	½	0.919	58.46	2.923	29.33
2	1	0.930	59.16	2.958	29.58
3	2	0.982	62.46	3.123	31.23
4	3	0.049	66.73	3.336	33.36
5	4	0.098	69.84	3.492	34.92
6	5	0.348	85.74	4.287	42.87
7	6	0.602	101.90	5.095	50.95
8	7	0.916	121.88	6.094	60.94
9	8	0.082	132.44	6.622	66.22
10	9	0.169	137.97	6.898	68.98
11	10	0.564	163.10	8.155	81.55
12	11	0.824	179.64	8.982	89.82
13	12	0.988	190.07	9.503	95.03

TABLE 3: INVITRO RELEASE STUDIES OF 1% CROSS LINKED CHITOSAN MICROSPHERE IN BUFFER PH 7.4 (FORMULATION F2)

Sl. No.	Time in hours	Absorbance	Concentration ug/ml	Amount present in 50 ml (mg)	% release
1	½	0.928	59.03	2.951	29.51
2	1	1.054	67.04	3.352	33.52
3	2	1.198	76.20	3..810	38.10
4	3	1.278	81.29	4.064	40.64
5	4	1.302	82.82	4.141	41.41
6	5	1.398	88.93	4.446	44.46
7	6	1.492	94.91	4.745	47.46
8	7	2.021	128.56	6.428	64.28
9	8	2.084	132.56	6.628	66.28
10	9	2.106	133.96	6.698	66.28
11	10	2.194	139.56	6.978	69.78
12	11	2.204	140.20	7.010	70.10
13	12	2.204	140.20	7.010	70.10

TABLE 4: INVITRO RELEASE STUDIES OF 1.5% CROSS LINKED CHITOSAN MICROSPHERE IN BUFFER PH 7.4 (FORMULATION F3)

Sl. No.	Time in hours	Absorbance	Concentration ug/ml	Amount present in 50 ml (mg)	% release
1	½	0.616	39.185	1.959	19.59
2	1	0.665	42.302	2.115	21.15
3	2	0.714	45.419	2.270	22.70
4	3	0961	48.409	2.420	24.20
5	4	0.772	49.109	2.422	24.22
6	5	0.801	50.954	2.547	25.47
7	6	0.920	58..524	2.926	29.26
8	7	0.953	60.63	3.031	30.31
9	8	1.080	68.70	3.435	34.35
10	9	1.090	64.440	3.222	32.22
11	10	1.118	71.119	3.555	35.55
12	11	1.377	87.595	4.379	43.79
13	12	2.050	130.402	6.520	65.20

Stability studies: The observations have been shown in **table 5, 6** and **7**.

- Rabeprazole Sodium microspheres stored at room temperature, 45^0C and 82.5% R.H. for one month revealed no significant degradation.
- At 45^oC the degradation rate was accelerated. F1 formulation showed 52.32% degradation, F2 showed 40.23% of degradation, F3 formulation showed 31.11% of degradation. F3 formulation is more stable than F1 formulation. This may be the protective role Chitosan in higher concentration.

TABLE 5: STABILITY STUDIES OF 0.5% CROSS LINKED CHITOSAN MICROSPHERE CONTAINING RABEPRAZOLE SODIUM

Environmental conditions	1st week	2nd week	3rd week	4th week
Room Temperature	0.000	0.015 ± 0.012	0.022 ± 0.011	0.042 ± 0.122
45^0C	0.038± 0.05	6.58 ± 0.110	27.9 ± 0.002	52.32 ± 0.132
82.5% R.H.	0.012 ± 0.072	1.35 ± 0.082	1.58 ± 0.052	1.78 ± 0.092

TABLE 6: STABILITY STUDIES OF 1% CROSS LINKED CHITOSAN MICROSPHERE CONTAINING RABEPRAZOLE SODIUM

Environmental conditions	1st week	2nd week	3rd week	4th week
Room Temperature	0.000	0.010 ± 0.012	0.020 ± 0.123	0.034 ± 0.045
45^0C	0.025	5.25 ± 0.052	18.22± 0.032	40.23 ± 0.048
82.5% R.H.	0.010 ± 0.682	0.98 ± 0.782	1.22 ± 0.078	1.56 ± 0.058

TABLE 7: STABILITY STUDIES OF 1.5% CROSS LINKED CHITOSAN MICROSPHERE CONTAINING RABEPRAZOLE SODIUM

Environmental conditions	1st week	2nd week	3rd week	4th week
Room Temperature	0.000	0.008 ± 0.122	0.018 ± 0.236	0.032 ± 0.325
45^0C	0.021± 0.542	4.85 ± 0.542	14.11± 0.054	31.11 ± 0.322
82.5% R.H.	0.09 ± 0.542	0.85 ± 0.642	1.02 ± 0.336	1.46 ± 0.125

Physicochemical evaluation:

- Drug content uniformity of formulation F1, F2 and F3 were within the limits of ±2% variability.
- The size diameter of microspheres ranged between 5µm to 13 µm. This was confirmed by the optical microscopy method using calibrated eye piece micrometer. This size range was closely parallel with the work reported by Maria Jose Priesto *et al.,* [8], by controlling the rpm a better formulation can be standardized.

SUMMARY AND CONCLUTION: A series of three different trial formulation of Rabeprazole Sodium, Microsphere using Chitosan an aminopolysaccharide, so as to optimize the sustained conditions. In the case of microsphere, the initial formulation was done with 0.5% Chitosan solution and drug. Then the concentration of chitosan had been increased to 1.5%. It showed that the percentage release was maximum 65.2% for the chitosan microsphere in the formulation F3, when compared with other formulation of microsphere containing Rabeprazole sodium, the 1.5% of chitosan showed better release than the other formulations.

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