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FORMULATION AND EVALUATION OF ENTACAPONE MICROSPHERES BY IONOTROPIC GELATION TECHNIQUE

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Abstract:

In this present research study, an attempt was made to develop novel Entacapone microspheres and consider the influence of various concentrations of sodium alginate and calcium chloride on particle size, entrapment efficiency, and drug release of the same. These microspheres were prepared by ionotropic gelation technique for oral delivery in the treatment "wearing-off" symptoms of Parkinson's disease. The formulated microspheres were evaluated regarding entrapment efficiency, percentage yield, drug release and characterization was studied using FTIR, DSC, SEM analysis. Among the total S14 formulations, S13 formulation was optimized at 2% of sodium alginate, 10% of calcium chloride with pectin. The in vitro dissolution showed sustained release of Entacapone up to 97.89±5.05 by diffusion mechanism over 12h, which followed the zero order and Higuchi model (R^2 = 0.982, 0.979) respectively, the drug release from microspheres was anomalous Non fickian diffusion. The marketed product displayed the drug release of 95.12±5.01within 1 hr. From these results it was concluded that, optimized formulation is stable and retained their original properties with minor differences. Entacapone alginate microspheres is an effective drug delivery system that offers more predictable and extensive drug release with enhanced shelf-life in the treatment of acute Parkinsonism.

Key Words: *Entacapone, Microspheres, Parkinson's disease, Cross-linking agent.*

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INTRODUCTION:

Sustained release systems include any drug delivery system that achieves slow release of drug over an extended period. More precisely, sustained drug delivery can be defined as "sustained drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects.**[1]**A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug.to achieve maximum therapeutic efficacy it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period thereby causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion.one such approach is using microspheres as carrier for drug. Microspheres have potential to deliver drug in a controlled fashion. Microspheres are small spherical particles, with diameters in the micrometer range (1 µm to 1000 µm). **[2]** Microspheres can be manufactured from various natural and synthetic polymers. Microspheres are used to achieve sustained and controlled drug release. **[3]** Microspheres based formulation can be formulated to provide a constant drug concentration in the blood, target drug to specific cell and to treat disease that require sustained concentration at anatomical site.

Parkinsonism is a neurological disability, characterizes by rigidity, tremor and hypokinesia with secondary symptoms like defective posture, mask like face, sialorrhea (increase saliva secretion), dementia. The motor symptoms are tremor at rest, stiffness, slowing of movement and postural instability. **[4]** The symptoms of Parkinsonism are largely related to progressive loss of dopamine neurotransmitter in brain. Parkinsonism is a result of the loss of specific types of brain cells (neurons) that generate a chemical called dopamine. The motor symptoms come from the slow, progressive degeneration and death of these neurons in an area of the brain called the substantia nigra, in the brain stem. **[5]** These brain cells begin to die may be due to genetic abnormalities. The earliest symptoms do not appear for several years because there is plenty of dopamine left in reserve to compensate for the declining supply**. [6]**

Entacapone is a selective, reversible catechol-O-methyl transferase (COMT) inhibitor. It is a member of the class of nitrocatechol. The chemical name of Entacapone is (2E)-2-cyano-3-(3, 4- dihydroxy-5-nitrophenyl)-N, N-diethylprop-2- enamide**. [7]** Entacapone is rapidly absorbed (approx. 1 hour) and has an absolute oral bioavailability of 35%. **[8]**

The aim of the present study is to develop Entacapone loaded microspheres by ionotropic gelation method to obtain an extended retention in the upper GIT, which may result in increased absorption and thereby improved bioavailability. The prepared microspheres were evaluated for particle size, shape, % yield, incorporation efficiency, and *in vitro* release study.

MATERIALS AND METHODS:

Materials:

Entacapone procured from Hetero Drugs Ltd**,** HYD. Sodium alginate from Pruthvi Chemicals, Mumbai. Calcium chloride from SD Fine ltd, Mumbai. Pectin was purchased from India Citrus Products ltd. Hyderabad. All other chemicals and solvents were of analytical grade.

Methods:

Formulation of Entacapone microspheres:

Table 1: Formulation trials for Entacapone microspheres:

FORMULATION CODE	ENTACAPONE (mg)	SODIUM ALGINATE	PECTIN(mg)	CALCIUM CHLORIDE
S1	200	1 %	1000	7%
S2	200	1.2 %	858	7%
S3	200	1.4%	716	7%
S4	200	1.6%	574	7%
S5	200	1.8%	432	7%
S6	200	2%	290	7%
S7	200	2.2%	148	7%
S8	200	1%	1000	10%
S9	200	1.2%	858	10%
S10	200	1.4%	716	10%
S11	200	1.6%	574	10%
S12	200	1.8%	432	10%
S13	200	2%	290	10%
S14	200	2.2%	148	10%

Procedure:

Sodium alginate microspheres of Entacapone were prepared by ionotropic gelation technique using different proportion of polymers as shown in table 1. A solution of sodium alginate is prepared, weighed quantity of drug and pectin was triturated to form fine powder, and then added to above solution. Resultant solution was extruded drop wise with the help of syringe and needle into 100ml aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 10 minutes the obtained microspheres were washed with water and dried at 60 degrees for 2 hours in a hot air oven and stored in desiccator. **[9].**

Evaluation of Entacapone Microspheres:

Particle size:

The 100 microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 microspheres were measured randomly by optical microscope**. [10]**

Angle of repose:

Angle of repose (Θ) of microspheres measures the resistance to particles flow, and is calculated according to fixed funnel standing cone method. Where (Θ) is angle of repose, H/D is surface area of the free-standing height of the microspheres heap that is formed on a graph paper after making the microspheres flow from glass funnel. **[11]**

$$\theta = \tan^{-1}(h/r)$$

Bulk density:

Volume of the microspheres in the measuring cylinder was noted as bulk density. **[12]**

Tapped density:

Change in the microspheres volume was observed in mechanical tapping apparatus. **[12]**

Compressibility index:

Also called as Carr's index and is computed according to the reported equation. **[13]**

Hausner'sratio:

Hausner's ratio of microspheres is determined by comparing the tapped density to the fluff density using the reported method. **[14]**

Swelling index:

Swelling index was determined by measuring the extent of swelling of microspheres in the given medium. Exactly weighed number of microspheres could swell in given medium. The excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed by using microbalance. The hydro gel microspheres then dried in an oven at 60 degrees for 5hr until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated by using the formula. **[15]**

Swelling index= (Mass of swollen microspheres - Mass of dry microspheres/mass of dried microspheres) 100.

Drug entrapment efficiency and %yield:

To determine the incorporation efficiency, 10 mg of formulated microspheres were thoroughly crushed by triturating and suspended in required quantity of methanol followed by agitation to dissolve the polymer and extract the drug. After filtration, suitable dilutions were made and drug content assayed spectro-photometrically at 377nm. Each batch should be examined for drug content in a triplicate manner. **[16]**

(% Drug entrapment = Calculated drug concentration /Theoretical drug concentration x 100

% yield = [Total weight of microspheres / Total weight of drug and polymer] x 100

In vitro drug release studies:

Release rate of Entacapone from sodium alginate microspheres was carried out using USP type II dissolution apparatus with 0.1N HCl (pH 1.2) of 900ml as dissolution medium. Accurately weighed number of microspheres from each batch was subjected to dissolution studies in triplicate manner. At appropriate intervals up to 12 h, specific volume of aliquots was withdrawn and analyzed spectrophotometrically. The withdrawn volume was replaced with an equivalent volume of fresh dissolution medium to maintain the volume of dissolution medium constant. The sample solutions were analyzed for the concentration of drug by UV spectrophotometer at 377nm. The amount of drug released was calculated from the calibration curve of the same dissolution medium. **[17]**

Kinetic modeling of drug release:

In order to understand the kinetics and mechanism of drug release, the result of the in vitro dissolution study of microspheres were fitted with various kinetic equations like Zero order as cumulative percentage drug release Vs. time, first order as log percentage of drug remaining to be released Vs. time, Higuchi's model cumulative percentage drug released Vs. square root of time. r^2 and K values were calculated for the linear curves obtained by regression analysis of the above plots.

To analyze the mechanism of drug release from the tablets the in vitro dissolution data was fitted to zero order, first order, Higuchi's release model and Korsmeyer – Peppas model.

Drug excipient Compatability Studies [12]

The drug excipient compatibility studies were carried out by Fourier Transmission Infrared Spectroscopy (FTIR) method, SEM and Differential Scanning Colorimetry

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The samples were dispersed in KBr and compressed into disc/pellet by application of pressure. The pellets were placed in the light path for recording the IR spectra. The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

SEM studies

The surface and shape characteristics of pellets were determined by scanning electron microscopy (SEM) (HITACHI, S-3700N). Photographs were taken and recorded at suitable magnification.

Stability studies

The stability study of the optimized formulation was carried out under different conditions according to ICH guidelines. The optimized microspheres were stored in a stability chamber for stability studies (REMI make). Accelerated Stability studies were carried out at 40 ^{0}C / 75 % RH for the best formulations for 6 months. The microspheres were characterized for the percentage yield, entrapment efficiency & cumulative % drug released during the stability study period.

RESULTS AND DISSCUSSION:

Formulation of Microspheres of Entacapone:

Fig.1: **Entacapone microsphere**

Micromeretic parameters:

Microspheres of Entacapone were formulated by ionotropic gelation method (Figure 1), using different polymers like sodium alginate, and calcium chloride in different concentrations. All the formulations were evaluated for their various physical parameters and found to be in limits.

Table 2: Micromeretic properties of Entacapone microspheres

Formulation code	Particle size (µm)	Bulk density(g/cc^3)	Tapped density (g/cc^3)	Angle of repose	Carr's index (%)	Swelling index (%)
S1	72.13±0.09	0.52±0.02	0.69±0.09	26 ˚.20±0.06	11.99	74
S2	78.45±0.04	0.50±0.01	0.66±0.08	25 ˚.45±0.04	12.26	75
S3	72.65±0.05	0.54±0.03	0.67±0.08	24 ˚.30±0.04	11.25	82
S4	79.13±0.10	0.56±0.05	0.60±0.02	28 ˚.21±0.05	10.01	73
S5	78.25±0.10	0.58±0.07	0.68±0.08	29 ˚.19±0.05	14.45	75
S6	71.98±0.08	0.55±0.05	0.63±0.05	32 ˚.15±0.06	11.66	78
S7	79.99±0.10	0.54±0.03	0.66±0.08	31 ˚.75±0.06	13.89	91
S8	75.15±0.04	0.52±0.02	0.68±0.08	28 ˚.25±0.05	14.13	90
S9	70.40±0.05	0.61±0.01	0.69±0.07	26 ˚.10±0.05	11.65	78
S10	73.69±0.03	0.59±0.07	0.64±0.05	25 ˚.90±0.02	10.15	93
S11	74.89±0.05	0.60±0.02	0.61±0.02	22 ˚.55±0.04	13.99	76
S12	72.33±0.09	0.55±0.05	0.65±0.04	23 ˚.65±0.04	12.88	82
S13	68.12±0.10	0.49±0.08	0.58±0.03	21˚.45±0.05	9.25	96
S14	75.01±0.11	0.58±0.07	0.60±0.01	22 ˚.85±0.02	11.12	80

Particle size was measured by using optical microscopy. All the formulations S1 to S14 varied from 68.12±0.10µm to 79.99±0.10µm. The bulk densities of all the formulations S1 to S14 were measured and they are ranged from 0.49±0.08g/cc³ to 0.61±0.01g/cc³. The tapped densities of all the formulations S1 to S14 were measured and they are ranged from 0.58±0.03g/cc³ to 0.69±0.09g/cc³. The compressibility index values were found to be in the range of 9.25 to 14.45 %. These findings indicated that all the batches of formulations exhibited good flow properties. Angle of repose of all the formulations was found satisfactory result. The angle of repose of formulation S13 was found to be 21^0. 45±0.05, it is having good flow property. The percentage swelling obtained from the water uptake studies of the formulations is shown in table 2. All the formulations S1 to S14 showed the swelling of microspheres. The swelling index of the formulation S13 was found to be 96%.

Table 3: Percentage drug yield and entrapment efficiency of Entacapone microspheres.

Formulation code	Percentage yield (%)	Entrapment efficiency (%)
S1	84.21	77.42
S2	85.74	79.01
S3	78.29	80.28
S4	70.38	82.17
S5	76.16	85.49
S6	72.98	86.22
S7	73.39	88.49
S8	74.44	76.44
S9	80.79	92.49
S10	81.24	94.55
S11	85.39	93.17
S12	72.88	81.87
S13	97.88	96.64
S14	75.47	91.77

The percentage yield and entrapment efficiency of all the formulations found to be within the limits and shown in Table 3. The formulation S13 shows better percentage yield and entrapment efficiency of 97.88% and 96.64 respectively.

***In vitro* drug release studies**

In vitro drug release studies were carried out and the results are depicted in Table 4 & 5 and Figure 2 & 3. The formulation S13 shown highest drug release of 97.89% in 12 hrs.

Table 4: In vitro cumulative % drug release of Entacapone sodium alginate microspheres formulations:

Time (h)	S1	S2	S3	S4	S5	S6	S7
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	15.26±0.95	14.98±0.94	17.89±0.97	18.36±0.98	13.85±0.92	16.89±0.96	18.99±0.98
2	20.78±1.01	25.36±1.33	28.18±1.89	24.69±1.32	27.18±1.45	26.45±1.35	29.96±1.90
4	41.25±2.46	43.18±2.64	44.89±2.64	39.98±2.44	42.19±2.47	39.99±2.44	42.13±2.47
6	55.46±2.68	52.18±2.61	56.39±2.69	54.58±2.66	51.89±2.60	53.86±2.61	58.16±2.95
8	61.12±3.10	60.23±3.09	62.99±3.11	60.11±3.09	62.95±3.11	60.98±3.10	62.13±3.11
10	79.99±3.95	85.96±4.68	86.98±4.69	79.98±3.95	84.23±4.66	75.99±3.85	80.12±4.90
12	90.25±5.00	93.22±5.03	91.45±5.01	89.56±4.99	90.16±5.01	89.65±4.99	90.05±5.01

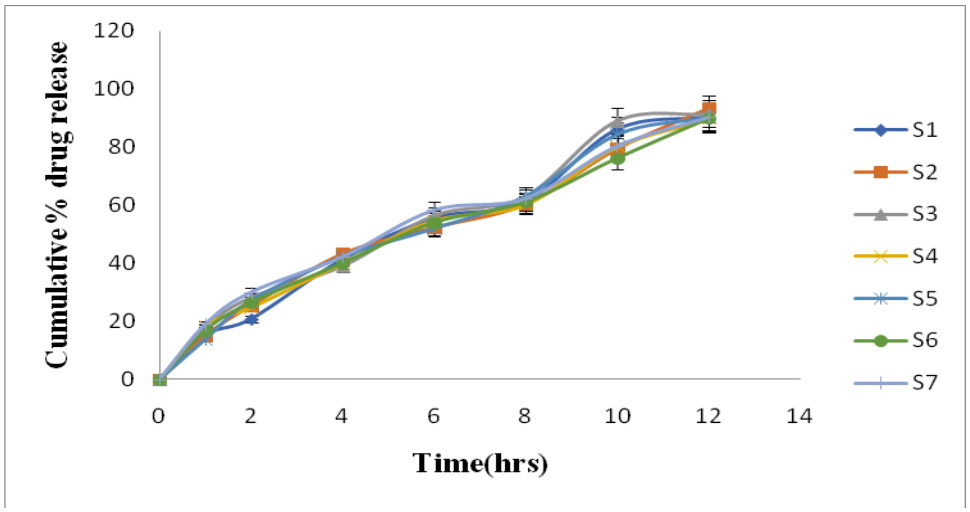

Fig. 2: In vitro cumulative % drug release of Entacapone sodium alginate microspheres formulation

Table 5: In vitro cumulative % drug Entacapone sodium alginate release of microspheres formulation:

Time (h)	S8	S9	S10	S11	S12	S13	S14	Marketed product
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	18.16±0.98	19.98±0.99	17.25±0.97	15.28±0.95	16.55±0.96	20.25±1.01	19.05±0.99	95.12±5.01
2	31.26±2.00	30.18±1.90	33.45±2.22	30.99±1.90	34.89±2.23	35.26±2.25	32.16±2.10	
4	39.99±2.44	42.58±2.62	43.56±2.63	41.25±2.46	44.96±2.64	45.96±2.65	40.96±2.45	
6	53.16±2.58	55.18±2.68	55.89±2.68	54.32±2.66	51.98±2.61	58.12±2.95	52.18±2.60	
8	62.13±3.11	72.19±3.81	71.89±3.80	72.67±3.82	65.16±3.15	69.80±3.70	62.19±3.11	
10	75.69±3.81	88.51±4.97	82.13±4.55	85.98±4.68	78.98±3.94	88.96±4.97	80.12±4.90	
12	89.99±4.99	91.2±5.01	90.08±5.00	92.25±5.02	89.25±4.99	97.89±5.05	90.12±5.00	

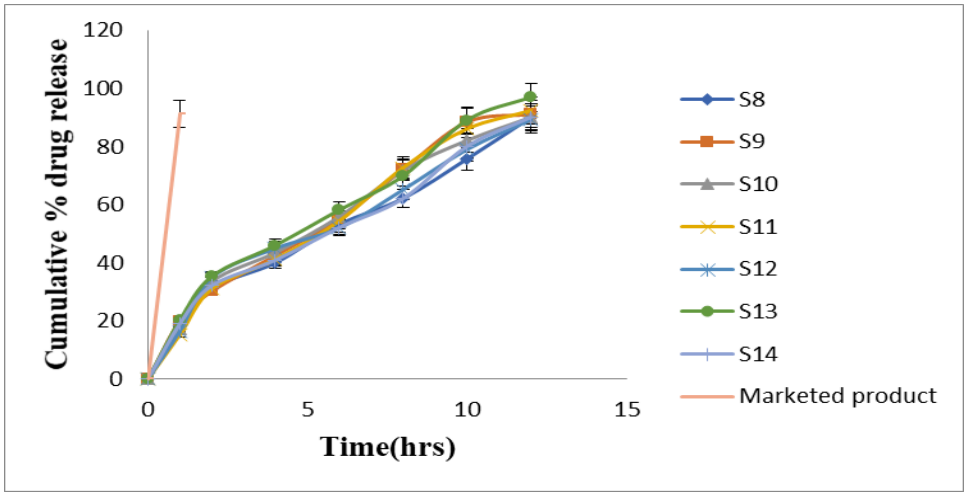

Fig. 3: In vitro cumulative % drug Entacapone sodium alginate release of microspheres formulations

Mathematical modeling of optimized formula of microspheres:

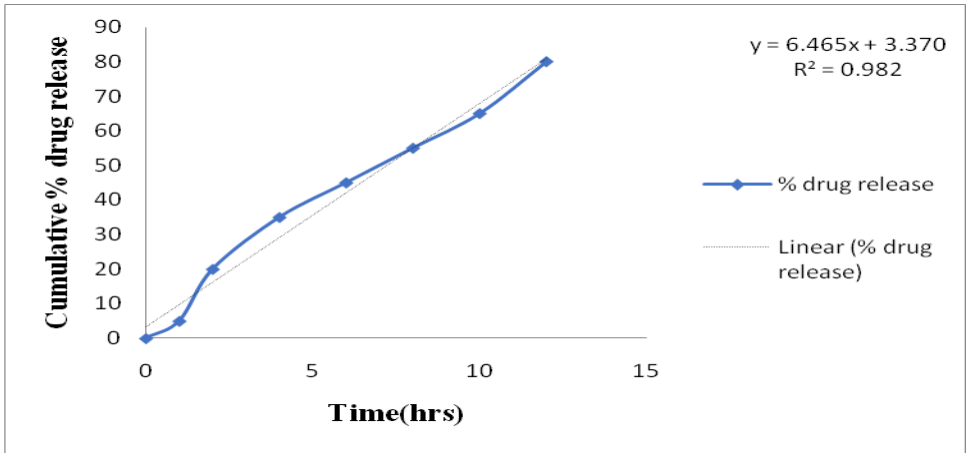

Fig. 4: Zero order plot for the optimized formulation of Entacapone micropsheres S13

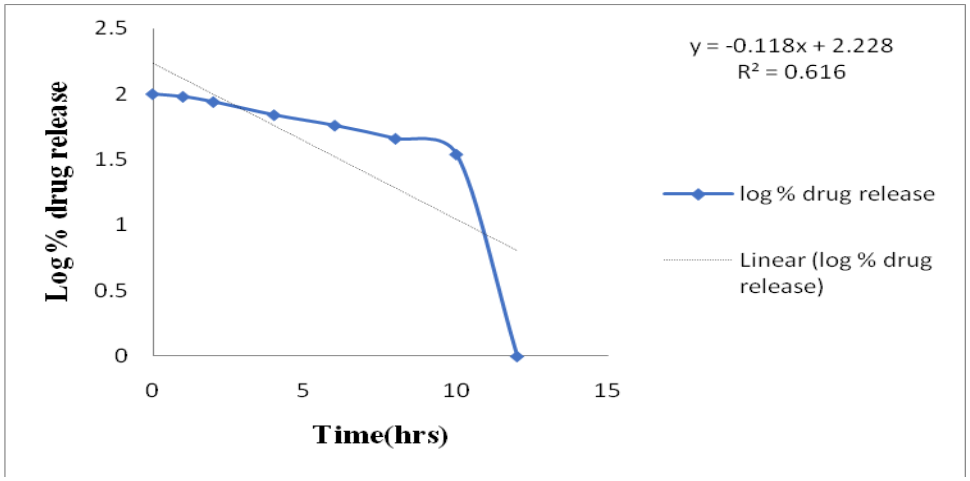

Fig. 5: First order plot for the optimized formulation of Entacapone microspheres S13

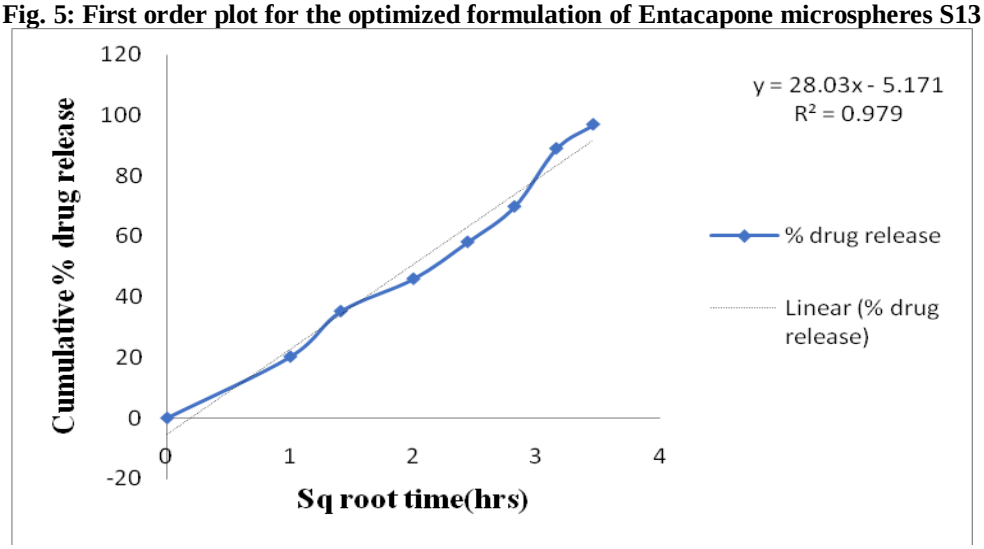

Fig. 6: Higuchi plot for the optimized formulation of Entacapone microspheres S13

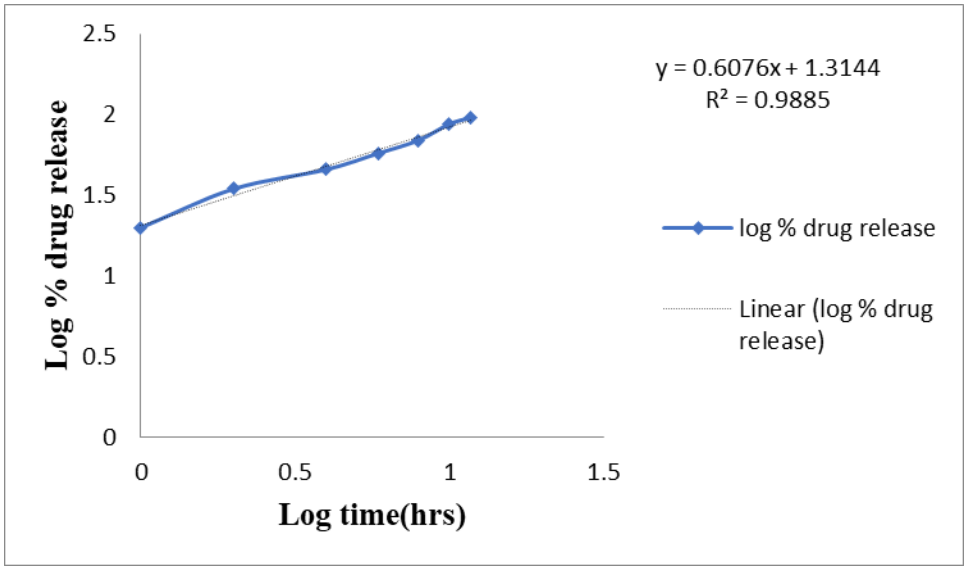

Fig. 7: Korsmeyer-peppas plot for the optimimized Entacapone microspheres S13

Table 6:Release order kinetics of optimized microspheres (S13)

Formula Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
S13	0.9825	6.465	0.616	0.118	0.979	5.17	0.988	0.607

From the above results it is apparent that the regression coefficient value closer to unity in case of zero order plot i.e.0.982 indicates that the drug release follows a zero-order mechanism (Figure 4). This data indicates a lesser amount of linearity when plotted by the first order equation. Hence it can be concluded that the major mechanism of drug release follows zero order kinetics. First order plot was showing in Figure 5. Further, the translation of the data from the dissolution studies suggested possibility of understanding the mechanism of drug release by configuring the data in to various mathematical modeling such as Higuchi and Korsmeyer plots. The mass transfer with respect to square root of the time has been plotted, revealed a linear graph with regression value close to one i.e. 0.979 starting that the release from the matrix was through diffusion (Figure 6). Further the n value obtained from the Korsmeyer plots i.e. 0.607 suggest that the drug release from microspheres was anomalous Non fickian diffusion (Figure 7).

Drug excipient compatibility Studies:

FT-IR:

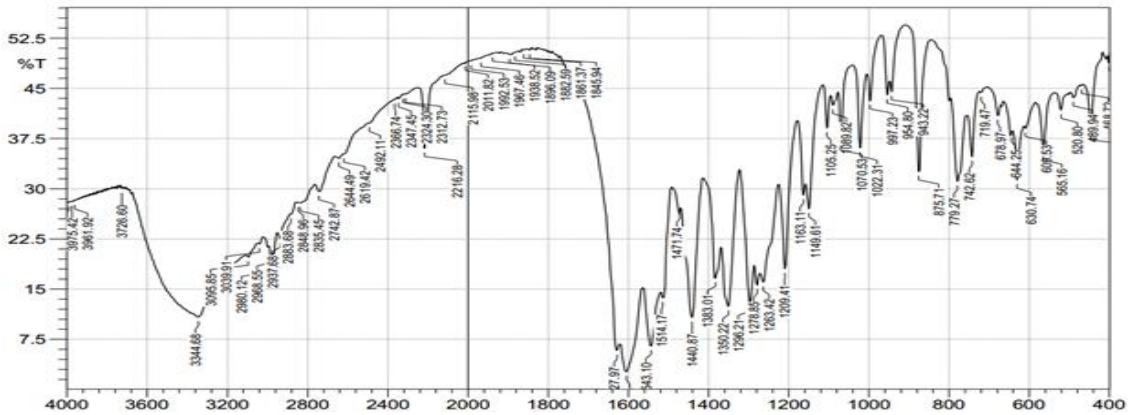

Fig. 8: FT-IR spectrum of pure drug Entacapone

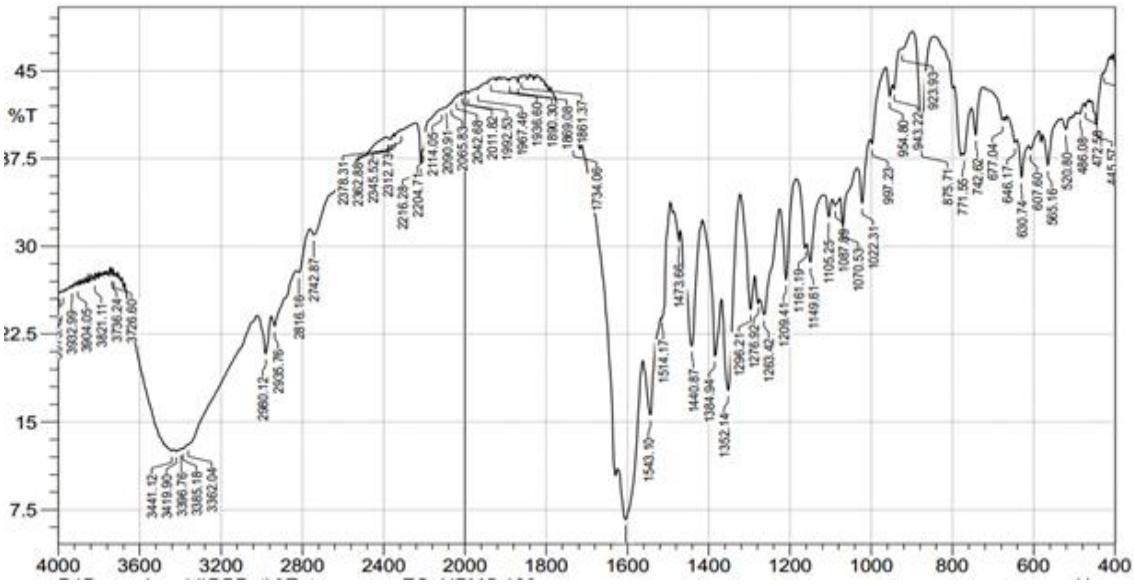

Fig. 9: FT-IR spectrum of physical mixture

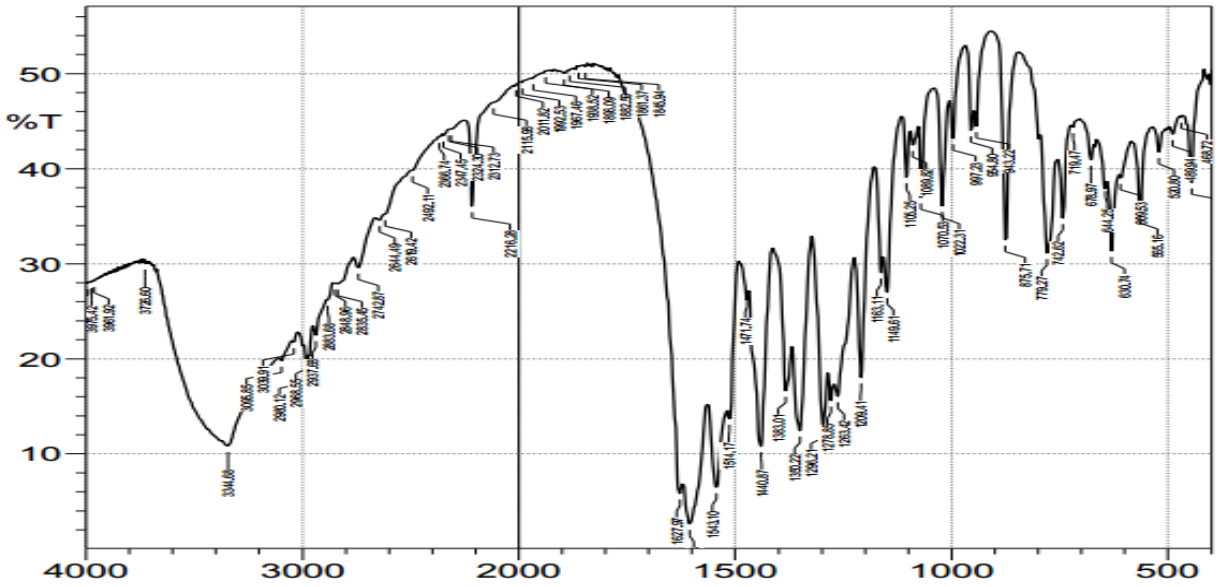

Fig. 10: FT-IR spectrum of Entacapone optimized formulation

Overall there was no alteration in peaks of Entacapone pure drug (Figure 8), physical mixture (Figure 9) and optimized formulation (Figure 10), suggesting that there was no interaction between drug & excipients. There are additional peaks appeared or disappeared hence no significant changes in peaks of optimized formulation was observed when compared to pure drug, indicating absence of any interaction.

SEM of Entacapone microspheres

The external and internal morphology of controlled release microspheres were studied by Scanning Electron Microscopy.

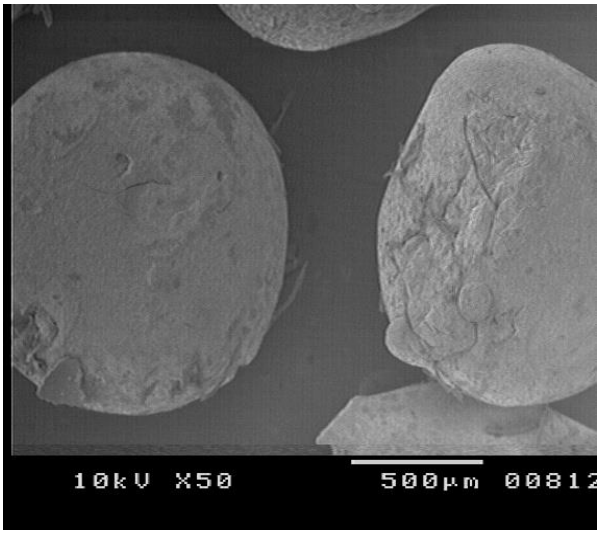

Fig. 11: Scanning electron micrographs of Entacapone microspheres

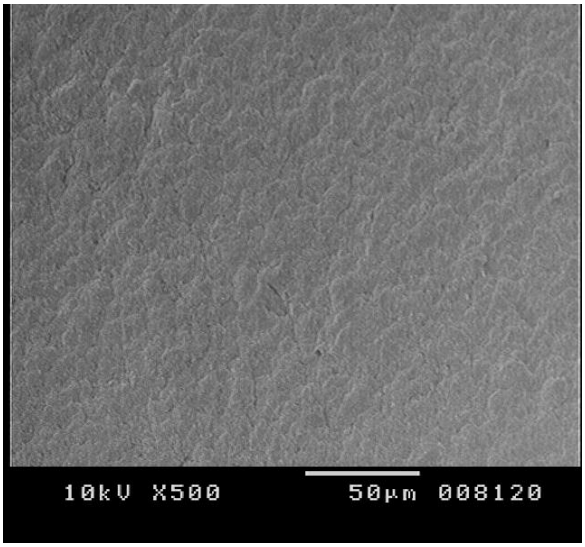

Fig. 12: Scanning electron micrographs of Entacapone microspheres

Morphology of the various formulations of Entacapone microspheres prepared was found to be discrete and spherical in shape. The surface of Entacapone microspheres was rough due to higher concentration of drug uniformly dispersed at the molecular level in the sodium alginate matrices. There are no crystals on surface which states that drug is uniformly distributed (Figure 11 & 12).

Stability studies:

Optimized formulation S13 was selected for stability studies based on high cumulative % drug release. Stability studies were conducted for 6 months according to ICH guidelines. From these results it was concluded that, optimized formulation is stable and retained their original properties with minor differences which depicted in Table 7.

Table 7: Staility studies of optimized microspheres

Retest Time for Optimized formulation	Percentage yield	Entrapment efficiency	*In-vitro* drug release profile (%)
0 days	97.88	96.64	97.89±5.05
30 days	94.29	94.16	95.27±4.09
60 days	93.44	93.17	94.17±3.05
120 days	91.13	92.77	92.19±4.88
180 days	90.34	91.47	91.77±2.11

SUMMARY AND CONCLUSION:

Entacapone microspheres of 14 different formulations were prepared with an aim to increase the gastric retention time of the drug which can enhance its oral bioavailability. Alginate microspheres of Entacapone were formulated by ionotropic gelation method, using different polymers like sodium alginate, calcium chloride and pectin in different concentrations. The flow properties of all the formulations were found to be within the limits. All formulations were evaluated for their various physical parameters and found to be within the limits. Formulation S13 was found to be optimized one based on the different evaluation parameters like swelling index and dissolution studies. The amount of drug release was found to be 97.89% upto 12h. Entacapone alginate microspheres is an effective drug delivery system that offers more predictable and extensive drug release with enhanced shelf-life in the treatment of acute Parkinsonism.

REFERENCES:

1.Chavanpatil M, Jain P, Chaudhari S, Shear R, Vavia P. Development of sustained release gastro retentive drug delivery system for Ofloxacin. Int J Pharm, 2005; 304:178-84.

2.Junzi Wu, Gareth RW, Christopher BW. Liraglutide-loaded poly (lactic-co-glycolic acid) microspheres. Preparation and in vivo evaluation. Eur J Pharm Sci, 2016; 92: 28–38.

3.Ghosh SK, Weinheim 2006. Wiley-VCH GmBH, Co. KGaA. Functional Coatings and Microencapsulation. A general perspective.

4.Gibb WR. Neuropathology of Parkinson's disease and related syndromes. Neurol Clin, 1992; 10:361-376.

5.Fearnley J, Lee A 1994. Pathology of Parkinson's disease. In Neurodegenerative Diseases. Calne DB. Saunders, Philadelphia.

6.Lang AE, Lozano A M. Parkinson's disease. First of two parts. New Engl J Med,1998; 339:1044-1053.

7.Najib J. Entacapone a catechol-O-methyltransferase inhibitor for the adjunctive treatment of Parkinson's disease. Clin Ther, 2001; 23(6):802-32.

8.Chong BS, Mersfelder TL. Entacapone Ann Pharmacotherapy, 2000; 34(9):1056-65.)

9.Manoj K, Das ,Vanshika Lumb, Neeru Singh, Vas Dev,Wajihullah Khan, Yagya D. SharmaAntimicrob Agents Chemother, 2011; 55(6): 2813–2817.

10.Trivedi parul. Preparation and characterization of Aceclofenac microspheres. Asian journal of pharmaceutics, 2008; 110-115.

11.Caroter SJ 1986. Tutorial pharmacy. Power flow and Compaction, CBS publishers and distributors, New Delhi, India 1st edition. Chapter 19

12.Slobbe L. A prolonged immune response to antigen delivered in poly (epsilon-caprolactone) micro particles, Immunol. Cell Biol,2003; 81:185-191

13.Banker G. S, Rhodes C.T. Modern Pharmaceutics. 3rd edition. New York: Marcel Dekker Inc; 2002; 333-394.

14Rockville 1980. The United States Pharmacopoeia XX/ National Formulary XV. 15th edition 1980. US Pharmacopoeial Convention, MD; p. 958-990.

15.Rajput GC, Majumdar FD, Patel JK, Patel KN, Thakor RS, Patel BP, Rajgor NB. Stomach Specific Mucoadhesive Tablets as Controlled Drug Delivery System. A Review Work. Int. J. Pharma, 2010.

16.Pradeesh T, Sunny M, Varma HAND Ramesh P. Preparation of microstructured hydroxyapatite microspheres using oil in water emulsions. Indian academy of sciences, 2005; 28(5):383-390.

17.Fundeanu G, Esposito E and Mihai D. Preparation and characterization of calcium alginate microspheres by a new emulsification method. International Journal of Pharmaceutics, 1998; 170:11– 21.