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Development and characterization of alginate microspheres containing tolcapone by ionotropic gelation method

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ABSTRACT

The present study was designed to formulate and evaluate the Tolcapone sodium alginate microspheres. Fourteen formulations batches of microspheres (S1-S14) were prepared by using ionotropic gelation method. Sodium alginate, calcium chloride and gelatin were selected in the formulations. The effect of polymer and cross-linking agent on particle size, shape, % yield, entrapment efficiency, and drug release were studied. From the evaluation parameters S13 was found to be optimized and the drug release was highest of 97.55% with in 12 h in controlled manner when compared with other parameters. From release order kinetics the drug release from microspheres was followed zero order with anomalous Non fickian diffusion. The prepared microspheres were further preceded for compatibility studies and morphological studies, found to be compatible with the polymers. Thus, Tolcapone microspheres could be the best choice in the effective management of Parkinsonism with better patient compliance for prolonged period of time.

Keywords: Tolcapone, Sodium alginate microspheres, Ionotropic Gelation technique, Release order kinetics.

INTRODUCTION

A controlled release drug delivery system is usually designed to deliver the drug at rate-controlled release properties can also be imparted to oral dosage formulations through the formation of resin-drug complexes coated with polymers [1]. As multiarticulate drug delivery lead to wide and uniform distribution throughout GIT, a localized high concentration at a specific point may be avoided. In addition, multiparticulate delivery systems spread out more uniformly in the gastrointestinal tract. This results in more reproducible drug absorption and reduces local irritation when compared to single unit dosage form [2]. Microspheres are one of the microparticulate systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites [3]. Microspheres are one of the multiple unit dosage

forms. Eventually the total dose and few adverse reactions may be reduced since a steady plasma concentration is maintained. Microspheres are potential drug delivery carrier systems in the segment of novel drug delivery and are prepared using assorted polymers [4, 5].

Tolcapone is a selective, reversible inhibitor of peripheral and central catechol-O-methyl transferase (COMT). Tolcapone is used to treat patients with Parkinson's disease. Parkinson's disease is a progressive brain disorder that causes shaking, slow movement and muscle stiffness [6]. Tolcapone is eliminated quickly, with an elimination half-life of 1.6 to 3.4 h [7].

Polymeric drug delivery system displays several advantages over the conventional dosage forms and it includes enhanced efficacy, patient compliance, reduced toxicity, and to control the encapsulated drug release. Sodium alginate is an anionic natural polysaccharide, prepared by mixture of D-mannuronic acid and L-glucuronic acid [8].

Sodium alginate is extensively used as carrier for drug delivery due to its biocompatibility and low toxicity. The widely used method for Tolcapone microspheres preparation is an ionotropic gelation method. This technique offers several advantages such as simple method of preparation no need to use of organic solvent, and, also easier to control. Sodium alginate could form gel in the presence of multivalent cations such as Ca2+, Zn2+, Ba2+ and Al3+ etc... by ionic cross-linking to form microspheres, it has been widely used in sustained drug release. Hence in this study calcium chloride is selected as cross-linking agent and also because of its nontoxic and biocompatibility [9]. The short half-life of tolcapone necessitated for fabricating extended release microspheres to provide a therapeutic amount of drug and maintain the desired drug concentration. The objective of the present study was to develop tolcapone microspheres by ionotropic gelation method. The results indicate that the optimized formulation of tolcapone microspheres can be successfully used for the treatment of Parkinson's disease.

MATERIALS AND METHODS

Materials

Tolcapone was gifted by R A Chem Pharma Ltd, Hyderabad. Sodium alginate was purchased from Pruthvi Chemicals, Mumbai. Calcium chloride and Gelatin was obtained from Matrix Exports, Bangalore. All other chemicals and solvents were of analytical grade.

METHODS

FORMULATION OF TOLCAPONE MICROSPHERES

Table 1: Formulation trials for Tolcapone microspheres

Formulation Code	Tolcapone (g)	Sodium Alginate	Gelatin(mg)	Calcium Chloride
S1	200	1.0 %	1000	7%
S2	200	1.2 %	918	7%
S3	200	1.4%	888	7%
S4	200	1.6%	627	7%
S5	200	1.8%	430	7%
S6	200	2.0%	250	7%
S7	200	2.2%	111	7%
S8	200	1.0%	1000	10%
S9	200	1.2%	918	10%
S10	200	1.4%	888	10%
S11	200	1.6%	627	10%
S12	200	1.8%	430	10%
S13	200	2.0%	250	10%
S14	200	2.2%	111	10%

Procedure

The microspheres of sodium alginate were prepared by using ionotropic gelation technique. In this method weighed quantity of Tolcapone and other polymers listed in Table 1 was added to 100ml sodium alginate solution and thoroughly mixed at 500 rpm. 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-2hours in a hot air oven and stored in desiccators [10].

EVALUATION OF TOLCAPONE 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 diameter was measured randomly by optical microscope [11].

Angle of repose [12], Bulk density, tapped density [13], Compressibility index [14] and Hausner's ratio [15] were evaluated according to the reported procedure.

Swelling index

Swelling index was determined by measuring the extent of swelling of microspheres in the given medium. Exactly weighed amount 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 hydrogel microspheres then dried in an oven at 60 degrees for 5h until there was no change in the dried mass of sample. The swelling index of the microsphere was calculated by using the formula [16].

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 spectrophotometrically at 257 nm. Each batch should be examined for drug content in a triplicate manner [17].

% 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 drug from sodium alginate microspheres was carried out using USP type II dissolution apparatus with 900 ml of 0.1N HCl (pH 1.2) as dissolution medium. Accurately weighed amount of microspheres from each batch were subjected to dissolution studies in triplicate manner. At appropriate intervals up to 12 h, specific volume of aliquots was withdrawn and the same volume was replaced analyzed for the concentration of drug by UV spectrophotometer at 257 nm [18].

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 [19, 20,21].

DRUG EXCIPIENT DRUG COMPATABILITY STUDIES

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}.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Samples were accurately weighed and heated in sealed aluminum pans at a rate of 10°C/ min between 25 and 350°C temperature rang under nitrogen atmosphere. Empty aluminum pan was used as a reference.

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 DISCUSSION

Formulation of microspheres of tolcapone

Figure 1: Sodium alginate microspheres of Tolcapone

Table 2: Micromeritic properties of formulated Tolcapone sodium alginate microspherese

Formulation code	Particle size (µm)	Bulk density(g/cc^3)	Tapped density (g/cc^3)	Angle of repose	Carr's index	Swelling index
S1	76.20±0.05	0.57±0.07	0.65±0.04	27˚.67±0.07	12.26%	72%
S2	72.58±0.02	0.62±0.02	0.64±0.03	26˚.56±0.07	11.38%	79%
S3	74.13±0.03	0.55±0.04	0.65±0.04	29˚.20±0.08	15.28%	85%
S4	73.15±0.02	0.61±0.02	0.63±0.03	30˚.51±0.02	13.96%	91%
S5	78.54±0.06	0.60±0.01	0.66±0.05	28˚.62±0.08	15.65%	82%
S6	80.16±0.01	0.62±0.02	0.64±0.03	25˚.75±0.06	12.56%	90%
S7	79.89±0.06	0.58±0.07	0.65±0.04	26˚.72±0.07	14.35%	83%
S8	69.19±0.08	0.59±0.08	0.62±0.02	27˚.93±0.07	11.36%	58%
S9	70.44±0.01	0.61±0.02	0.61±0.01	29˚.22±0.08	13.25%	65%
S10	77.60±0.05	0.59±0.08	0.60±0.01	30˚.91±0.02	12.85%	72%
S11	74.18±0.02	0.60±0.01	0.66±0.05	28˚.53±0.08	14.15%	81%
S12	75.16±0.03	0.55±0.04	0.64±0.03	26˚.14±0.07	12.54%	75%
S13	67.12±0.04	0.52±0.03	0.58±0.06	22˚.53±0.02	9.15%	96%
S14	72.14±0.01	0.57±0.07	0.62±0.02	25˚.72±0.04	15.45%	82%

Micromeritic properties of formulated Tolcapone sodium alginate microspheres (Figure 1) were shown in Table 2, all the formulations were evaluated and found to be within the IP limits.

Table 3: Percentage drug yield and entrapment efficiency of Tolcapone microspheres

Formulation code	Percentage yield (%)	Entrapment efficiency (%)
S1	81.01	75.46
S2	85.20	82.13
S3	75.22	79.85
S4	82.10	81.50
S5	72.12	72.31
S6	70.25	85.89
S7	65.36	71.59
S8	76.21	85.05
S9	82.15	81.09
S10	89.50	89.26
S11	84.62	90.12
S12	79.55	86.22
S13	95.30	96.12
S14	88.56	82.13

Percentage yield and entrapment efficiency of all the fourteen formulations were evaluated and depicted in Table 3. The formulation S13 shown the good percentage yield and entrapment efficiency when compared with other formulations of 95.30 % and 96.12%.

In vitro drug release studies

In vitro drug release studies were carried out and depicted in Table 4 & 5 and Figure 2 & 3. Among all the formulations S13 showed best drug release of 97.55% within 12 h when compared with other formulations.

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

Time (h)	S1	S2	S3	S4	S5	S6	S7
1	11.20±0.91	15.27±0.95	18.27±0.98	17.25±0.97	16.22±0.96	13.25±0.93	19.12±0.99
2	19.26±0.99	20.25±1.28	22.18±1.32	21.25±1.30	23.28±1.31	24.89±1.32	26.13±1.34
4	25.13±1.33	33.18±2.19	29.96±1.40	30.16±2.01	32.18±2.10	36.98±2.16	35.18±2.24
6	48.96±2.74	49.96±2.75	35.28±2.24	48.18±2.69	45.88±2.65	47.92±2.68	46.25±2.65
8	68.27±3.25	58.16±2.95	55.26±2.90	59.98±2.96	56.96±2.89	58.12±2.95	59.98±2.96
10	72.18±3.82	79.27±3.95	68.96±3.26	75.96±3.88	79.99±3.95	78.68±3.95	69.28±3.32
12	90.02±5.00	89.99±4.98	85.98±4.88	91.25±5.01	89.58±4.98	92.01±5.03	90.03±5.00

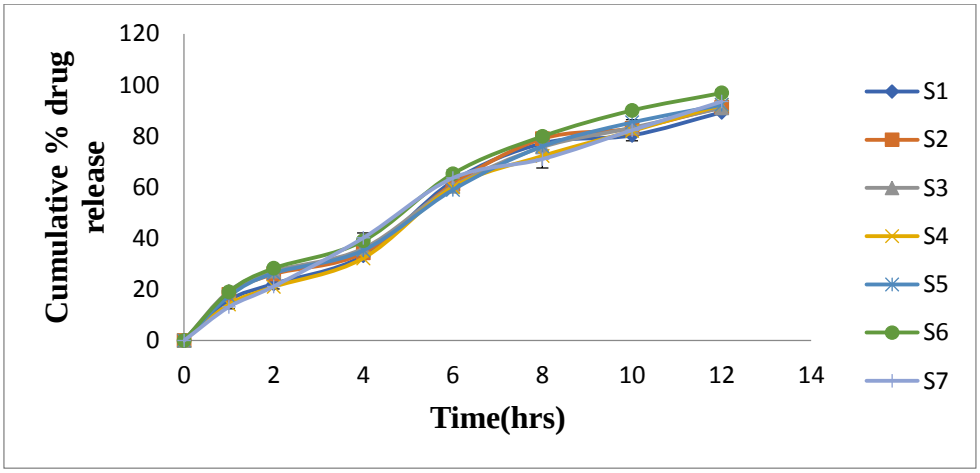

Figure 2: In vitro cumulative % drug release of Tolcapone sodium alginate microspheres formulation

Table 5: In vitro cumulative % drug Tolcapone sodium alginate relase 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	15.67±0.95	18.01±0.98	19.22±0.99	14.12±0.93	17.26±0.97	18.98±0.98	13.12±0.92	91.25±5.00
2	22.18±1.32	25.99±1.33	27.12±1.38	20.98±1.28	26.35±1.36	28.26±1.39	21.18±1.30	
4	33.16±2.19	34.29±2.23	35.98±2.25	32.19±2.10	35.26±2.15	38.96±2.40	40.12±2.45	
6	62.27±3.11	61.13±3.10	60.26±3.10	59.95±2.95	58.92±2.95	65.22±3.15	63.56±3.12	
8	77.12±3.90	78.93±3.92	75.67±3.88	72.18±3.82	75.96±3.81	79.85±3.95	70.99±3.79	
10	80.26±4.50	82.96±4.55	83.22±4.59	81.96±4.50	85.29±4.68	89.99±4.99	82.26±4.58	
12	89.26±4.99	91.26±5.01	90.98±5.02	92.13±5.03	92.19±5.03	97.55±5.05	93.45±5.01	

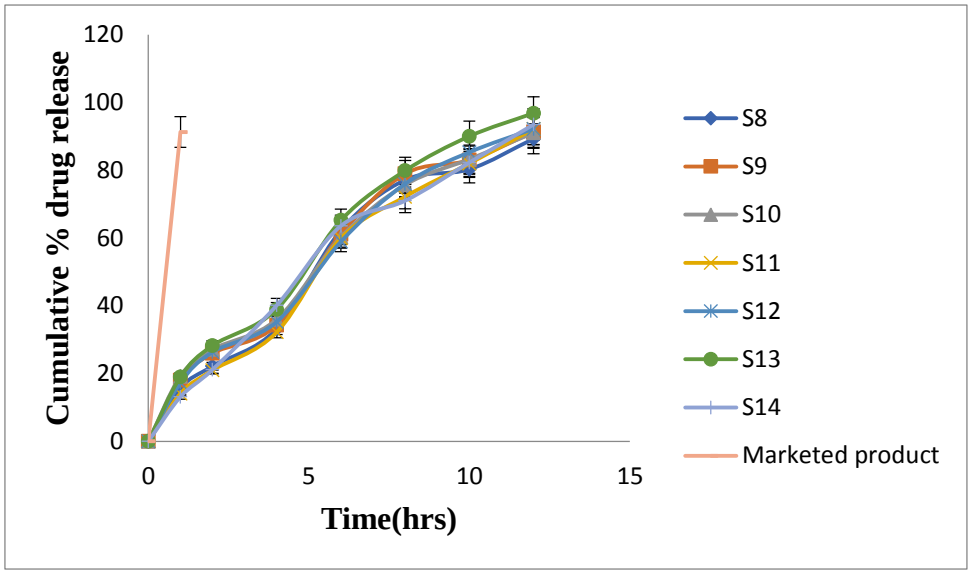

Figure 3: In vitro cumulative % drug Tolcapone sodium alginate release of microspheres formulations

Mathematical modeling of optimized formula of microspheres

In the view of establishment of release mechanism and quatitatively interpreting and translate mathematically the dissolution date being plotted (Figure 4, 5, 6 & 7 and Table 6).

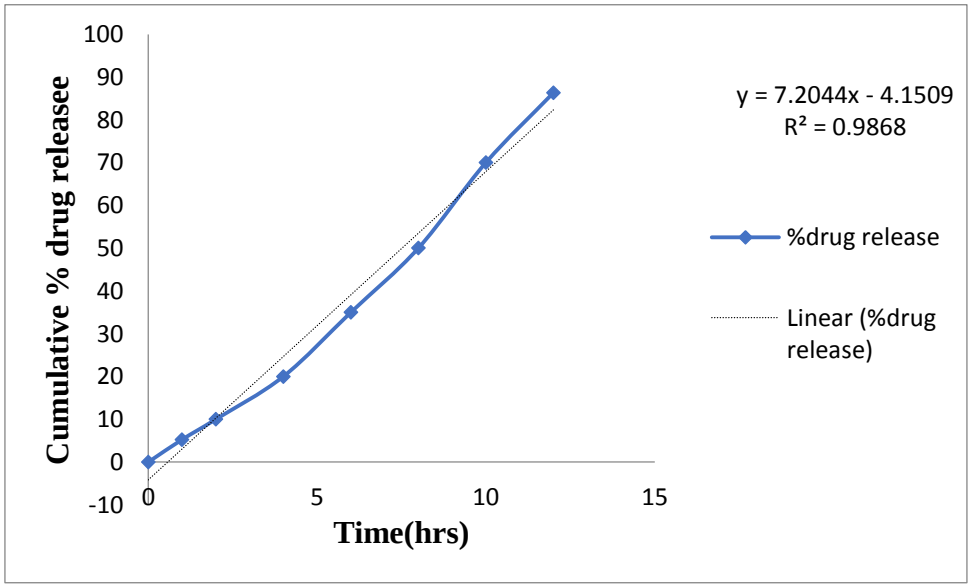

Figure 4: Zero order plot for the optimized formulation of Tolcapopne micropsheres S13

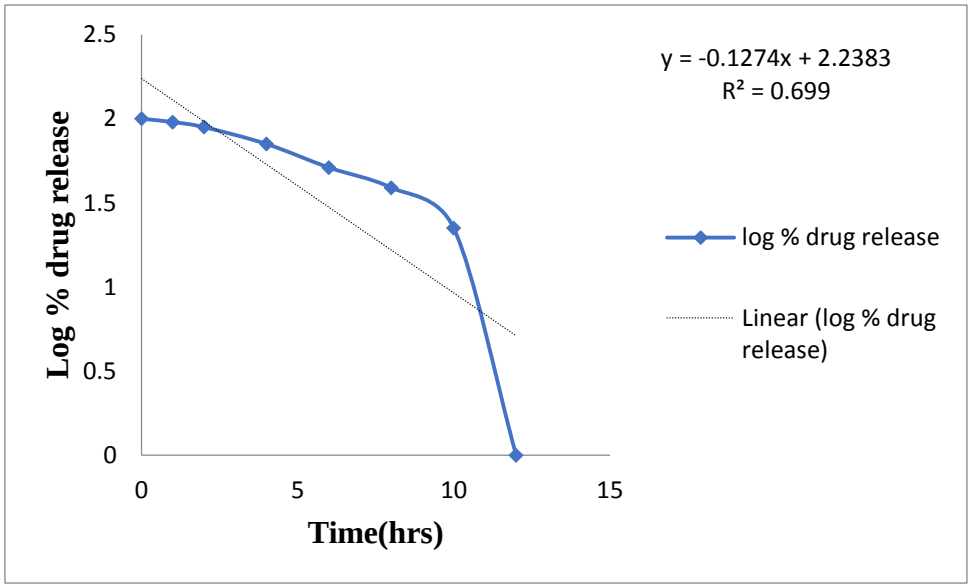

Figure 5: First order plot for the optimized formulation of Tolcapopne microspheres S13

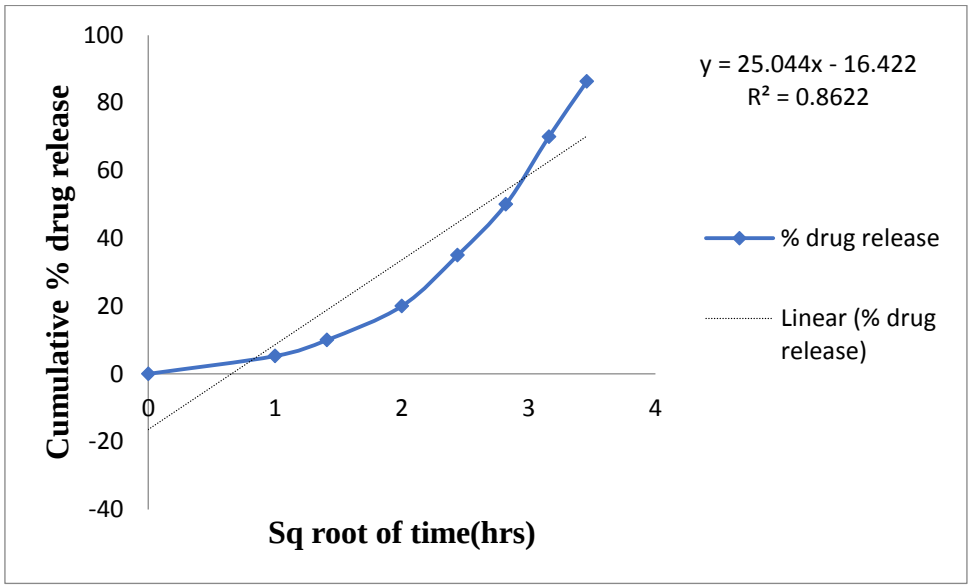

Figure 6: Higuchi plot for the optimized formulation of Tolcapopne microspheres S13

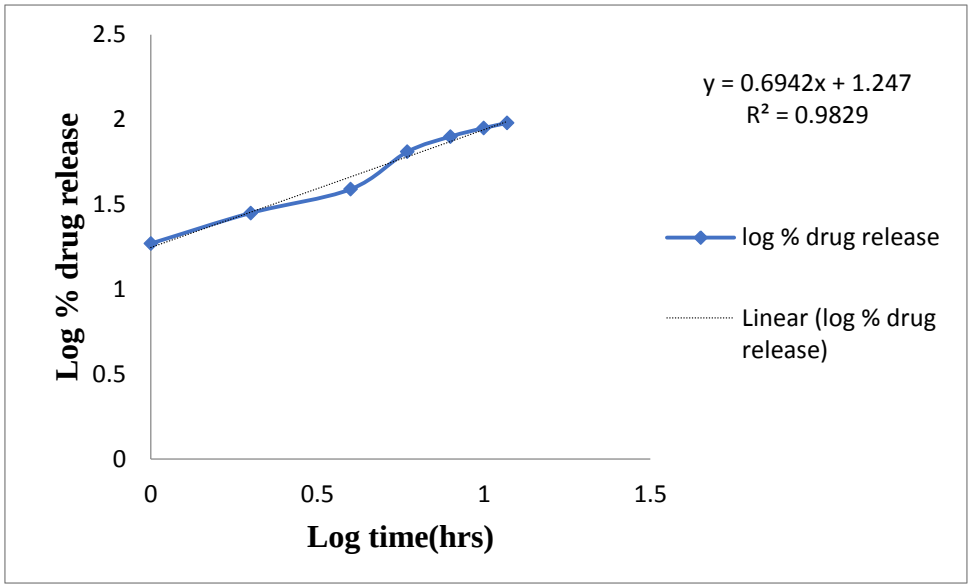

Figure 7: Korsmeyer-peppas plot for the optimimized Tolcapopne microspheres S13

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	0.986	7.204	0.699	0.127	0.862	25.04	0.982	0.694

From the above results it is apparent that the regression coefficient value closer to unity in case of zero order plot i.e.0.986 indicates that the drug release follows a zero-order mechanism. 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.

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. 0.862 starting that the release from the matrix was through diffusion. Further the n value obtained from the Korsmeyer plots i.e. 0.694 suggest that the drug release from microspheres was anomalous Non fickian diffusion.

DRUG EXCIPIENT COMPATABILITY STUDIES

FT-IR studies

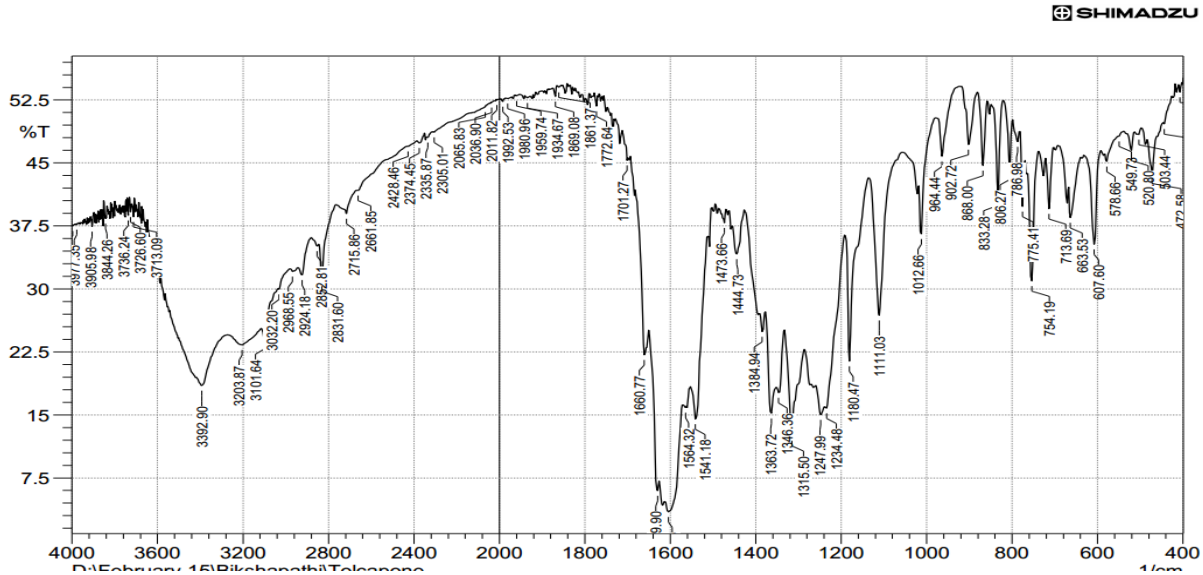

Figure 8: FT-IR spectrum of pure drug Tolcapone

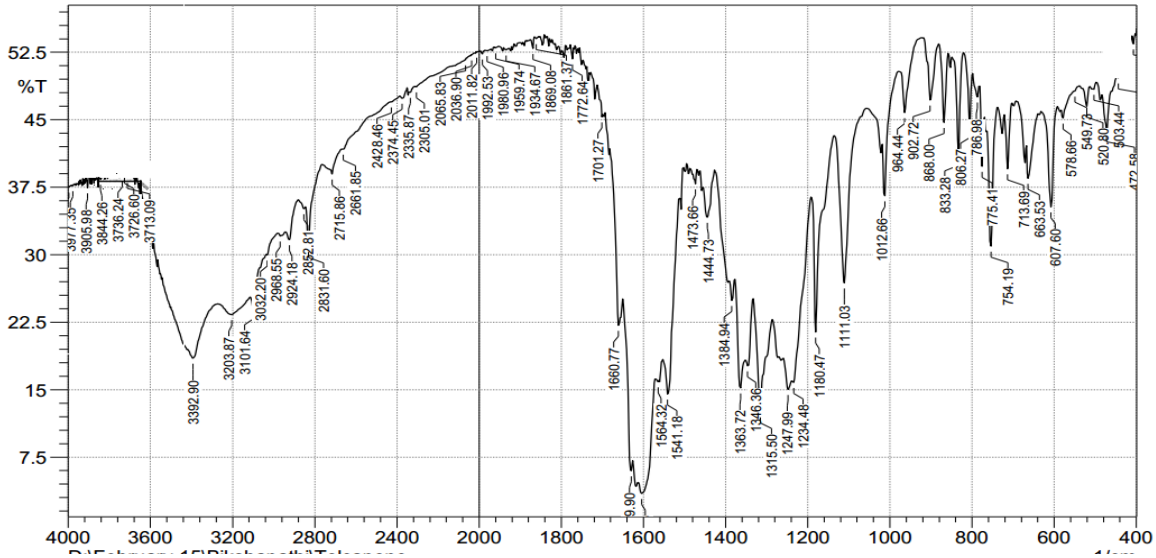

Figure 9: FT-IR spectrum of Tolcapone physical mixture

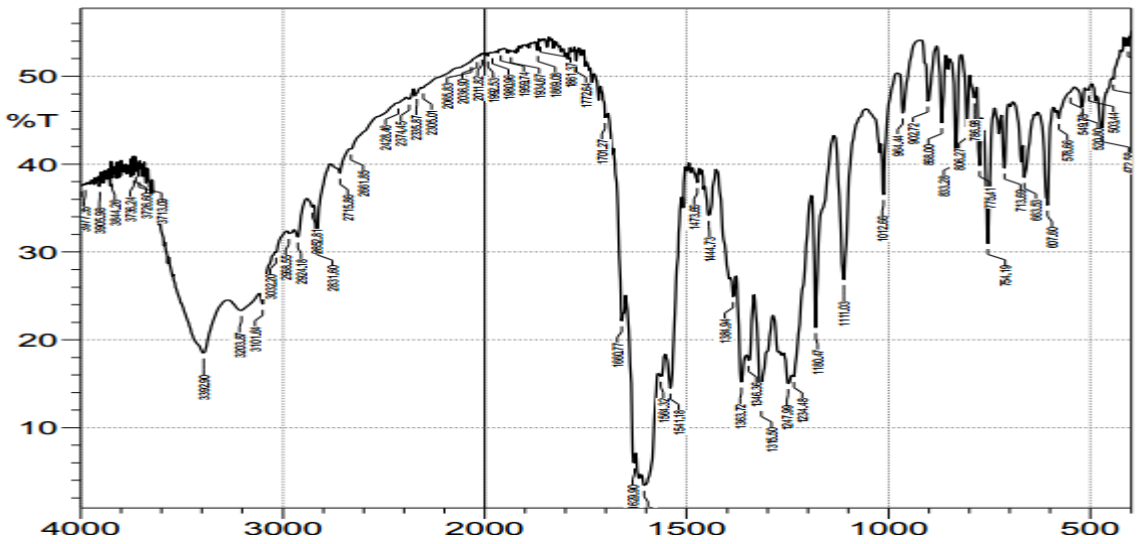

Figure 10: FT-IR spectrum of Tolcapone optimized formulation

There was no alteration in peaks of Tolcapone pure drug (Figure 8) and optimized formulation (Figure 10), also shown tolcapone physical mixture in Figure 9, 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.

Scanning Electron Microscopy

SEM of Tolcapone microspheres

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

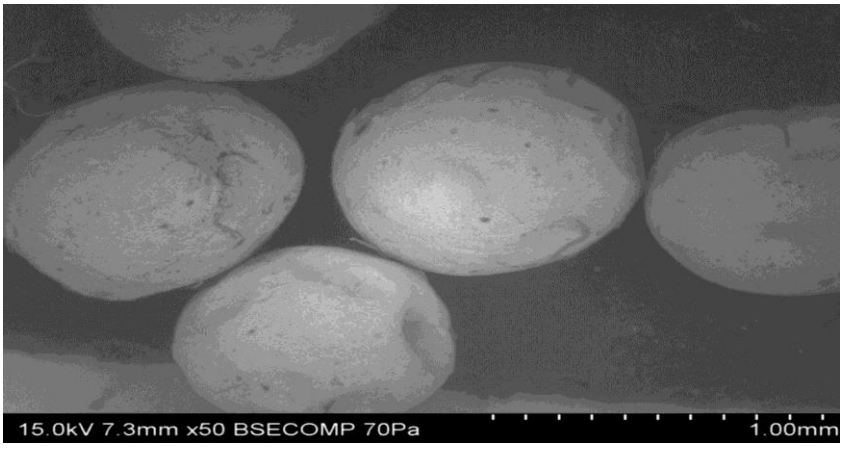

Figure 11: Scanning electron micrographs of Tolcapone microspheres

Figure 12: Scanning electron micrographs of Tolcapone microspheres

Morphology of the various formulations of Tolcapone microspheres prepared was found to be discrete and spherical in shape (Figure 11 & 12). The surface of the Tolcapone 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 is drug is uniformly distributed.

Stability studies

Optimized formulation was selected for stability studies since 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	95.30	96.12	97.55
30 days	94.12	95.10	96.42
60 days	93.68	94.25	95.51
120 days	92.19	93.88	95.02
180 days	91.22	93.32	94.09

CONCLUSION

Tolcapone loaded microspheres were prepared by ionotropic gelation method. From the results it concluded that formulation S13 was found to be satisfactory results in terms of excellent Micromeretic properties, particle size (67.12±0.04µm), yield of microsphere (95.30%), entrapment efficiency (93.12%), swelling index (96%) and highest *in vitro* drug release of 97.55±5.05% in a sustained manner with constant fashion over extended period for 12h compared with marketed product 91.25±5.00 in 1 h. The drug and excipients were compatible studied by using FTIR. Drug release from Tolcapone microspheres followed Zero order and Higuchi model. It was suggested that mechanism of drug release from microspheres was diffusion controlled. The prepared microspheres were spherical in shape studied by SEM studies. The optimized formulation S13 was stable. Hence the formulated and prepared floating Tolcapone microspheres may establish to be potential candidate for safe and effective sustained drug delivery and improve the bioavailability.

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