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Formulation and evaluation of alginate microspheres of pramipexole HCL

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ABSTRACT

The present work was designed to formulate and evaluate the Pramipexole microspheres. Drugs with short half-life frequent administration of dosage form is required, so the possible way by which this can be overcome by preparing sustained release microspheres of Pramipexole which can also improve patient compliance. The alginate microspheres were prepared by ionotropic gelation technique. Sodium alginate and chitosan were used as polymers, calcium chloride as cross-linking agent, and Olibanum gum is also used. Microspheres were characterized for the Micromeritic properties, incorporation efficiency, Swelling Index, SEM analysis, FTIR, and *in vitro* release studies. Among all formulations S14 was optimized on the basis of different evaluation parameters. The % yield of S14 formulation was found to be 96.41 %, entrapment efficiency and swelling index of S14 formulation was 95.26%, 97% respectively. The Cumulative % drug release of S14 formulation was 97.19±5.10% in 12h when compared with marketed product 90.45±5.10 in 12hrs. SEM studies showed the particles were in spherical shape. The method developed in the present study can be effectively utilized to achieve the formulation with desired release characteristics in the effective management of Parkinson's disease.

Keywords: Pramipexole, Ionotropic gelation technique, Parkinson's disease

INTRODUCTION

Some conventional dosage forms can provide poor management of plasma drug concentrations. Drug-level fluctuations due to frequent administration and variations in their absorption and/or metabolism can result in toxic effects or render the drugs ineffective [1]. A microsphere is well known method to delay and modify drug release characteristics. For oral use, it has been employed to sustain the drug release and to reduce or eliminate gastrointestinal tract irritation [2]. It is a process of enclosing micron size particles of solid or liquid or gases in an inert shell resulting in the formation of Microparticles or microcapsules or microspheres. [3] As multiparticulate 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.

Microspheres have potential to deliver drug in a controlled fashion. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance. They spread out more uniformly in the GI tract, thus avoiding exposure of the mucosa to high concentration of drug and ensuring more reproducible drug absorption. The risk of dose dumping also seems to be lower than with a single unit dosage form. [4, 5]

Pramipexole has been a widely used dopamine agonist for the last decade. Recently an extended release formulation of Pramipexole has been introduced as both monotherapy for patients with early Parkinson's disease as well as for patients with more advanced disease, as an adjunct to L-DOPA. Along with the enhanced patient compliance seen with once a day dosing, there are other potential advantages of extended release preparations of dopamine agonists. Patients initiated on Pramipexole have a lower incidence of developing motor fluctuations including dyskinesia than those initiated on L-DOPA. Pramipexole requires a prolonged dose titration compared to L-DOPA, and generally does not have the efficacy of L-DOPA. [6].

MATERIALS AND METHODS

Materials

Pramipexole procured from Sun Pharmaceutical Industries Ltd. Sodium alginate from Pruthvi Chemicals, Mumbai. Calcium chloride from SD Fine ltd, Mumbai. Ethyl cellulose from Aay Cee Enterprises, Roorkee. Chitosan from Yarrow chemical limited Mumbai. Olibanum Gum from Nutriroma, Hyd.

Methods

Formulation of pramipexole alginate microspheres

Table 1: Formulation trials for Pramipexole Alginate microspheres:

F. CODE	PRAMIPEXOLE (mg)	SODIUM ALGINATE	CHITOSAN (mg)	CALCIUM CHLORIDE	OLIBINUM GUM
S1	0.5	0.25%	0.04	7%	0.5
S2	0.5	.5 %	0.06	7%	0.75
S3	0.5	1%	0.08	7%	1
S4	0.5	1.25%	0.1	7%	1.5
S5	0.5	1.5%	0.12	7%	1.75
S6	0.5	2%	0.14	7%	2
S7	0.5	2.5%	0.16	7%	2.5
S8	0.5	0.25%	0.04	10%	0.5
S9	0.5	.5 %	0.06	10%	0.75
S10	0.5	1%	0.08	10%	1
S11	0.5	1.25%	0.1	10%	1.5
S12	0.5	1.5%	0.12	10%	1.75
S13	0.5	2%	0.14	10%	2
S14	0.5	2.5%	0.16	10%	2.5

PROCEDURE

The microspheres of sodium alginate were prepared by using ion tropic gelation technique. In this method weighed quantity of Pramipexole 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 dessicater [7].

EVALUATION OF PRAMIPEXOLE ALGINATE MICROSPHERES

Particle size

The particle size of microspheres was determined using an optical microscopy method. Approximately 100 microspheres were counted using calibrated microscope [8].

Angle of repose

Angle of repose (Ɵ) of microspheres measures the resistance to particles flow, and is calculated according to fixed funnel standing cone method. Where (Ɵ) 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 [9].

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

Bulk density

Bulk density was determined by the following formula [10].

Bulk density= Sample weight/Sample volume

Tapped density

The tapped density was determined by tapping method, in which the cylinder containing known amount (M) of microspheres was subjected to a fixed number of taps (approximately 100) until the bed of microspheres had reached the minimum [11]. The final volume after tapping 'Vo' was recorded and the tapped density was calculated by the following equation:

Tapped density (Pp) = M/Vo

Compressibility index (CI), Hausner's ratio

Carr's index (% compressibility index), Hausner ratio and were determined to predict flowability and thesecan be determined by following equations.

CI= (Tapped density- Bulk density) × 100
Tapped density

Hausner's ratio = Tapped density/Bulk density

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 liquid drops adhered to surface were removed by blotting and the swollen microspheres were weighed. The microspheres were then dried in hot air oven at 40^0C for 60 hrs until there was no change in dried mass of sample. The swelling index was calculated from the following equation [12].

Swelling index= (Mass of swollen microspheres - Mass of dry microsphere/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 particular wavelength using calibration curve. Each batch should be examined for drug content in a triplicate manner [13].

% 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 263 nm [14].

Kinetic modeling of drug release

Data obtained from in-vitro release studies were fitted to various kinetic equations to find out the mechanism of drug release from the ethyl cellulose microsphere. Various kinetic models used were [15]. Determining the correlation coefficient assessed fitness of the data into various kinetic models. The rate constants for respective models were also calculated fromslope.

Drug excipient drug compatability studies

The drug excipient compatibility studies were carried out by Fourier Transmission Infrared Spectroscopy (FTIR) method and SEM [17].

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

Selected formulations were kept tapped with vials in an incubator maintained at 40±2°C and 75±5% RH for three months [18]. Changes in the appearance, particle size, drug content and release profile of these stored microspheres were investigated at regular time intervals (1-6 months).

RESULTS AND DISCUSSION

Alginate microspheres of pramipexole

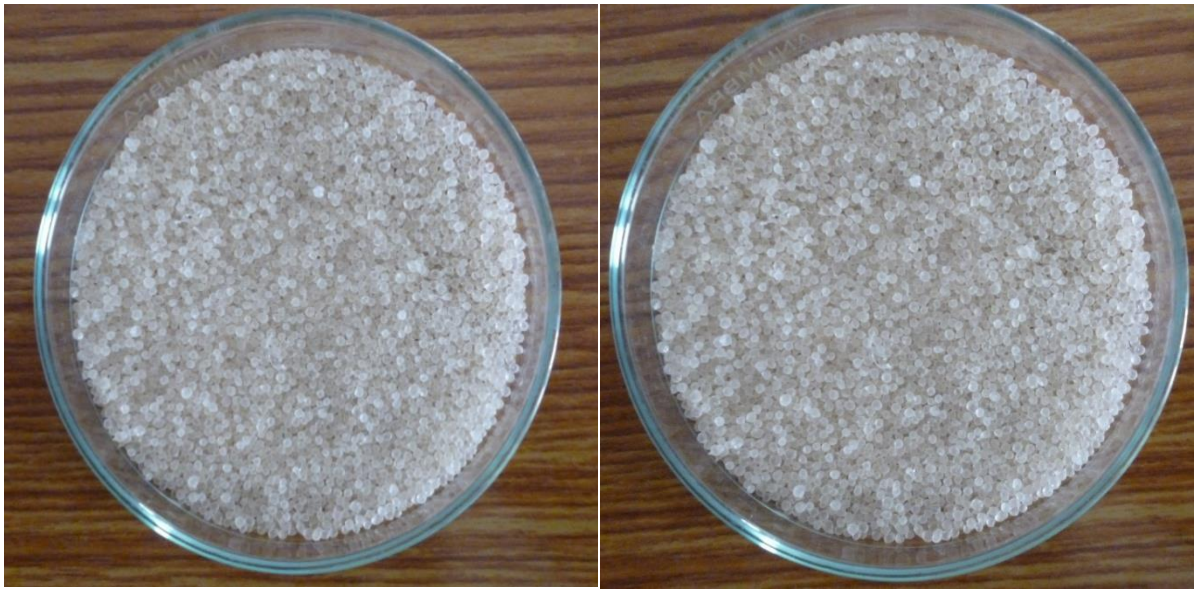

Figure 1: **Pramipexole Alginate microspheres**

Table 2: Formulated Pramipexole sodium alginate microspheres micromeritic properties:

Formulation code	Particle size (µm)	Bulk density(g/cc³)	Tapped density (g/cc³)	Angle of repose	Carr's index	Swelling index
S1	67.05±0.09	0.52±0.01	0.58±0.05	24˚.15±0.03	15.22%	70%
S2	70.14±0.01	0.51±0.01	0.56±0.03	26˚.25±0.05	14.98%	73%
S3	68.25±0.10	0.52±0.01	0.59±0.06	25˚.55±0.05	12.90%	50%

S4	70.50±0.01	0.58±0.05	0.58±0.05	28˚.60±0.06	13.18%	82%
S5	72.46±0.01	0.55±0.03	0.56±0.03	29˚.85±0.07	12.12%	90%
S6	73.46±0.02	0.56±0.03	0.59±0.06	30˚.70±0.01	16.98%	83%
S7	68.14±0.10	0.54±0.03	0.57±0.05	27˚.65±0.06	15.21%	92%
S8	67.23±0.10	0.52±0.01	0.58±0.05	28˚.95±0.06	11.45%	81%
S9	71.27±0.01	0.53±0.03	0.57±0.05	27˚.25±0.06	14.55%	54%
S10	69.18±0.10	0.55±0.03	0.56±0.03	24˚.65±0.03	11.67%	64%
S11	72.15±0.01	0.52±0.01	0.59±0.06	26˚.80±0.05	12.81%	73%
S12	70.16±0.01	0.54±0.03	0.57±0.05	24˚.25±0.03	13.82%	79%
S13	68.14±0.10	0.51±0.01	0.56±0.03	25˚.55±0.05	12.56%	74%
S14	65.26±0.06	0.48±0.05	0.54±0.02	20˚.45±0.02	9.40%	97%

Alginate microspheres of Pramipexole were formulated by ionic gelation method, using different polymers like sodium alginate, calcium chloride in different concentration and the formulation codes S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13 and S14 were prepared. All the formulations were evaluated for their various physical parameters.

Particle size was measured by using optical microscopy. All the formulations S1 to S14 varied from 65.26±0.06 to 73.46±0.02µm. the formulation S14 shows the particle size 65.26± 0.06µm.

The bulk densities of all the formulations S1 to S14 were measured and they are ranged from 0.48±0.05g/cc³ to 0.58±0.05g/cc³.

The tapped densities of all the formulations S1 to S14 were measured and they are ranged from 0.54±0.02g/cc³ to 0.59±0.06g/cc³.

The compressibility index values were found to be in the range of 9.40 to 16.98 %. These findings indicated that the 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 S14 was found to be 20^0. 45±0.02 it is having good flow property.

The percentage swelling obtained from the water uptake studies of the formulations is shown in table. All the formulations S1 to S14 showed the swelling of microspheres. The swelling index of the formulation S14 was found to be 97%.

The other formulation S8 to S14 showed better swelling index, and entrapment efficiency. The drug release was very less due to more concentration of sodium alginate and calcium chloride.

The percentage release and entrapment efficiency of all the formulations were measured by assay method. The formulation S1 to S14 shows the percentage yield values ranges from 65.12% to 96.41%.

The formulation S14 shows the good percentage yield and entrapment efficiency the values were 96.41% and 95.26% with better release profile.

Table 3: Percentage drug yield, entrapment efficiency, in vitro cumulative % drug release of normal Pramipexole microspheres.

Formulation code	Percentage yield	Entrapment efficiency
S1	85.12	68.19
S2	80.20	70.15
S3	72.15	60.30
S4	71.13	81.66
S5	65.12	72.22
S6	81.40	81.02
S7	81.44	75.40
S8	79.18	79.86
S9	81.40	72.66

S10	82.35	65.84
S11	75.45	61.28
S12	91.06	92.16
S13	82.18	85.42
S14	96.41	95.26

The samples are drawn at the specified time intervals and the absorbance was noted using UV-visible spectrophotometer at 263 nm. The cumulative percentage drug release was calculated.

Figure 2: In vitro dissolution study

An attempt is being made in designing controlled release dosage form of Pramipexole normal microspheres. The study began by designing a normal microsphere using different excipients like sodium alginate and calcium chloride in different concentration.

Initially seven formulations were developed S1,S2,S3,S4,S5,S6 and S7 having Pramipexole sodium alginate concentration 1%, 1.2%, 1.4%, 1.6%, 1.8%, 2%, and 2.2% and calcium chloride concentration 7%.These formulations were evaluated for in vitro release studies in 0.1N HCL. Drug release studies of formulations S1 to S7 shows that the release profile were 64% , 69%,70%, 71%, 79% , 87% and 69% in 12h respectively.

The next seven formulations were developed S8 to S14 having Pramipexole sodium alginate concentration 1% to 2.2% and calcium chloride concentration 10% .These formulations were evaluated for in vitro drug release studies in 0.1N HCL. Drug release studies of formulations S8 to S14 shows that the release was 70% to 96% respectively.

The formulations S14 was developed using Pramipexole, sodium alginate in concentration of 2.2%. And calcium chloride 7%. Results revealed that the % drug Release of 97.19% in 12 hrs.

Table 4: *Invitro* cumulative % drug release of Pramipexole microspheres formulations:

Time	S1	S2	S3	S4	S5	S6	S7
0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
1	11.16±0.92	13.45±0.94	12.18±0.93	11.98±0.92	14.52±0.93	15.89±0.95	16.17±0.96
2	19.25±0.99	25.60±1.35	18.67±0.98	22.14±1.32	26.19±1.36	21.50±1.30	20.79±1.29
4	30.12±2.08	32.19±2.10	31.67±2.09	33.66±2.02	34.67±2.02	35.67±2.05	33.58±2.02

6	42.18±2.46	54.72±2.90	45.89±2.50	55.49±2.89	56.18±2.90	55.44±2.89	46.21±2.50
8	50.35±2.83	68.74±3.19	66.19±3.16	62.15±3.09	67.19±3.18	62.16±3.10	59.88±2.96
10	72.21±3.82	85.18±4.89	75.60±3.81	70.60±3.82	74.50±3.80	74.32±3.80	77.60±3.93
12	87.60±4.93	90.12±5.01	89.11±4.99	91.35±5.01	92.19±5.02	89.12±4.99	85.89±4.95

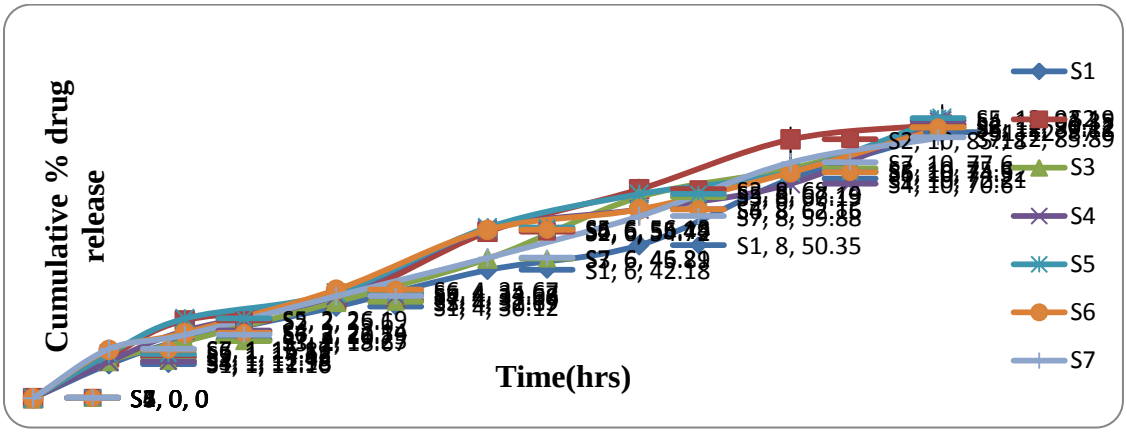

Figure 3: In vitro cumulative % drug release of Pramipexole microspheres formulation

Table 5: In vitro cumulative % drug Pramipexole release of microspheres formulation:

Time	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	16.12±0.96	14.35±0.93	17.45±0.99	15.22±0.95	18.16±0.98	13.25±0.93	19.18±0.99	10.12±0.85
2	23.15±1.33	24.18±1.34	21.40±1.30	20.54±1.29	25.18±1.35	22.19±1.32	28.96±1.39	19.18±0.99
4	34.58±2.02	32.20±2.01	35.18±2.05	30.19±2.08	33.45±2.02	32.16±2.01	36.81±2.06	28.16±1.39
6	44.89±2.48	46.82±2.59	45.16±2.49	42.16±2.46	40.60±2.42	43.65±2.47	48.60±2.67	35.26±2.05
8	50.13±2.82	53.19±2.85	54.87±2.88	52.19±2.84	55.98±2.87	58.19±2.95	63.35±3.10	45.89±2.49
10	81.12±4.57	70.60±3.80	73.45±3.85	72.20±3.84	75.11±3.81	76.44±3.82	82.32±4.82	69.30±3.21
12	92.88±5.02	91.12±5.01	93.05±5.03	90.16±5.01	88.98±4.97	89.99±4.99	97.19±5.10	90.45±5.01

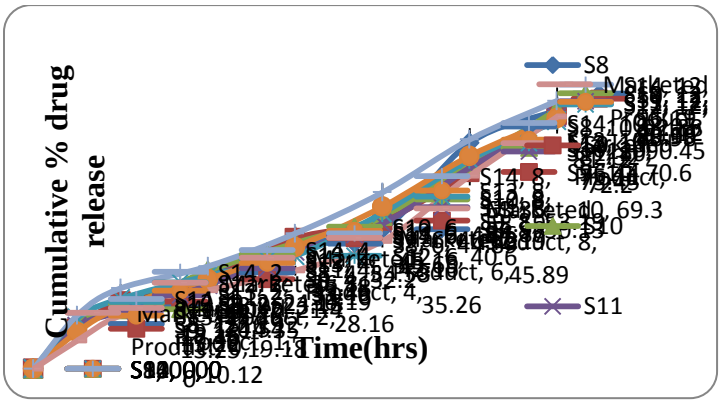

Figure 4: In vitro cumulative % drug Pramipexole release of microspheres formulations

Mathematical modeling of optimized formula of Alginate microspheres

In the view of establishment of release mechanism and quatitatively interpreting and translate mathematically the dissolution date being plotted.

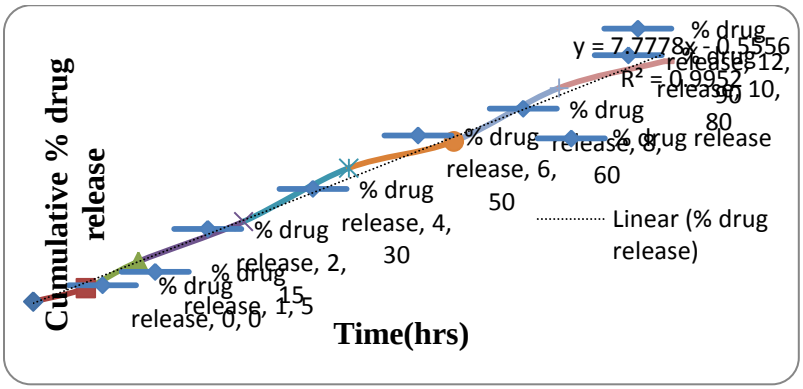

Figure 5: Zero order plot for the optimized formulation of Pramipexole normal micropsheres S14

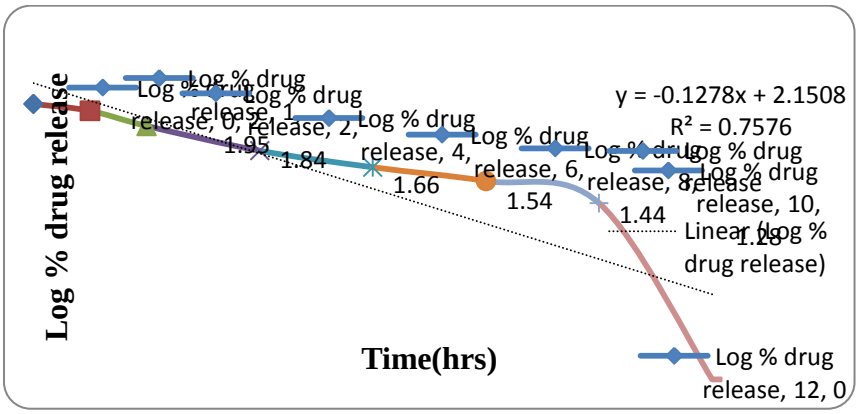

Figure 6: First order plot for the optimized formulation of Pramipexole Alginate microspheres S14

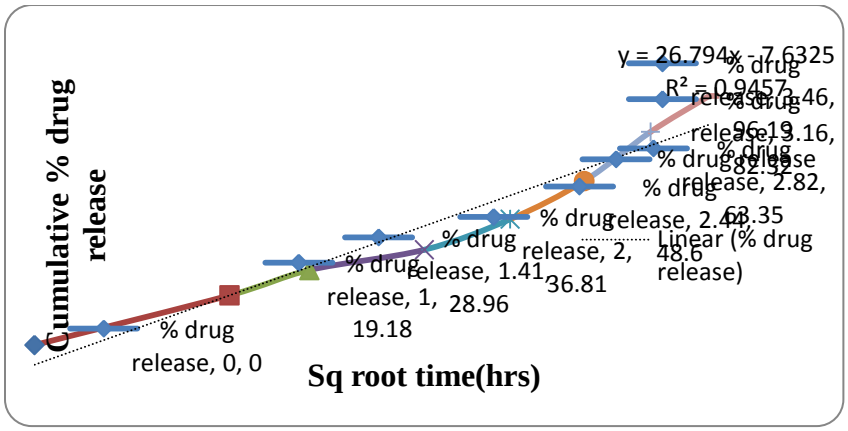

Figure 7: Higuchi plot for the optimized formulation of Pramipexole Alginate microspheres S14

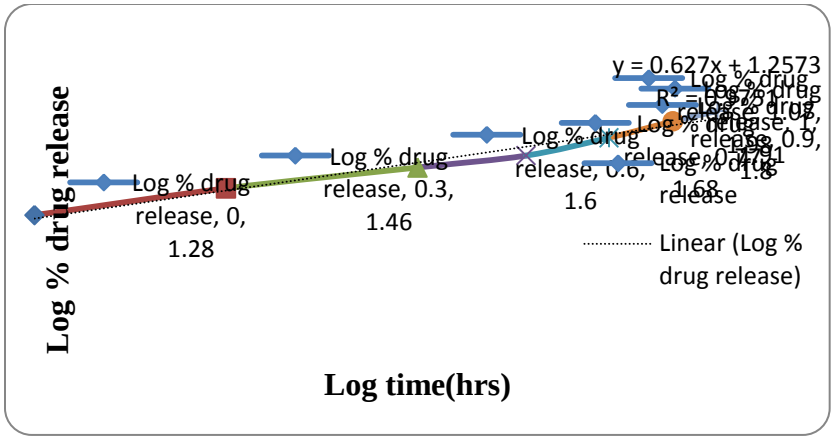

Figure 8: Korsmeyer-peppas plot for the optimimized of Pramipexole Alginate microspheres S14

MARKETED PRODUCT

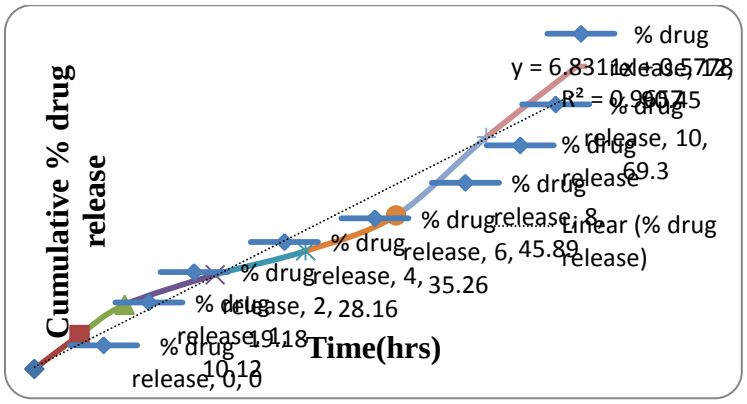

Figure 9: Zero order plot for the Marketed product

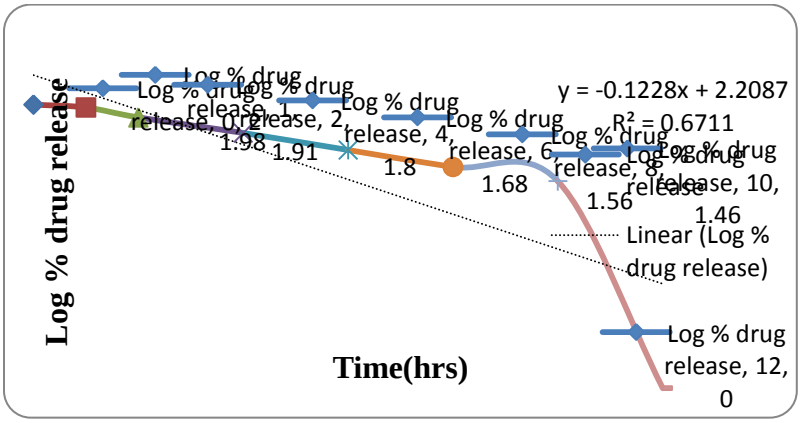

Figure 10 : First order plot for the Marketed product

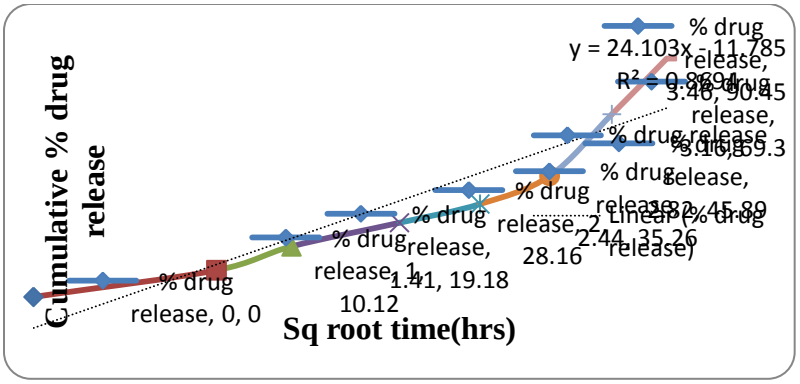

Figure 11 : Higuchi plot for the Marketed product

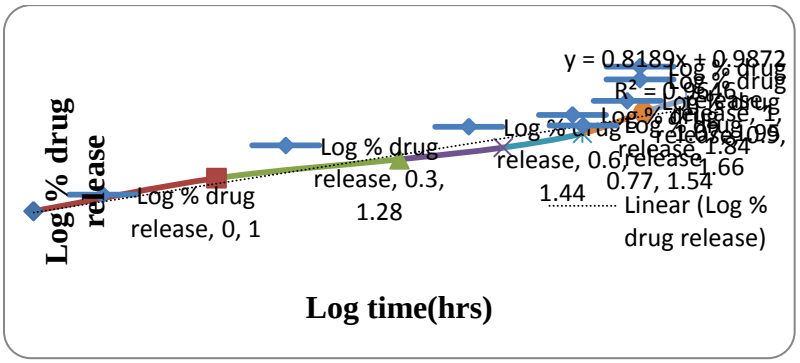

Figure 12: Korsmeyer-peppas plot for the Marketed product

Table 6:Release order kinetics of optimized Alginate microspheres (S14)

Formula Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
S14	0.995	7.777	0.757	0.127	0.945	26.79	0.975	0.627
Marketed product	0.965	6.831	0.671	0.122	0.869	24.103	0.964	0.818

From the above results it is apparent that the regression coefficient value closer to unity in case of zero order plot i.e.0.995 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. 0.945 starting that the release from the matrix was through diffusion. Further the n value obtained from the Korsmeyer plots i.e. 0.627 suggest that the drug release from microspheres was anomalous Non fickian diffusion.

DRUG EXCIPIENT COMPATABILITY STUDIES

Stability studies

Optimized formulation was selected for stability studies on the basis of 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**

Table 7 : Stability studies of optimized Alginate microspheres

Retest Time For Optimized formulation	Percentage yield	Entrapment efficiency	*In-vitro* drug release profile (%)
0 days	96.41	95.26	97.19
30 days	95.61	94.18	95.20
60 days	94.19	93.46	94.18
120 days	92.06	92.15	93.44

CONCLUSION

Alginate microspheres of Pramipexole were formulated by ionotropic gelation method, using different polymers like sodium alginate, calcium chloride in different concentrations and the formulation codes S1 to S14 were prepared. All formulations were evaluated for their various physical parameters and S14 was found to be optimized formulation. All the formulations of particle size varied from 65.26±0.06µm to 73.46±0.02µm. The swelling index of the formulation S14 was found to be 97%. The percentage release and entrapment efficiency of all the formulation S14was found to be 95.26%. The formulation S14 shows the good percentage yield and entrapment efficiency the values were 96.41% and 95.26% with better release profile. The *in vitro dissolution studies of* formulations S14 was found to be %drug release of 97.19±5.10 in 12hrs. The method developed in the present study can be effectively utilized to achieve the formulation with desired release characteristics in the effective management of Parkinson's disease.

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