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Research Article

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In vitro & *in vivo* Evaluation of Pramipexole HCl Mucoadhesive Microspheres

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ABSTRACT

The study was aimed to design and develop the novel gastro retentive mucoadhesive Pramipexole microspheres using ionotropic gelation technique. Based on the results of Micromeretic properties confirmed that microspheres were free flowing with good pack ability. The optimized M13 formulation displayed the % entrapment efficiency 96.07%, % yield 98.01%, swelling index 96.08% and Mucoadhesiveness was 95.42%. The *in vitro* drug release showed the sustained release of Pramipexole up to 99.16 ± 5.12% within 12 h. FTIR studies revealed incompatibility was not found between drug and excipients. SEM confirmed the particles were of spherical in shape. Optimized formulation (M13) were stable at 40°C ± 2°C/75% RH ± 5% RH for 6 months. *In vivo* studies were performed and kinetic parameters like C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and $t_{1/2}$ were calculated. The marketed product C_{max} (2.19 ± 0.01 ng/ml) was higher than optimized formulation (2.0 ± 0.01 ng/ml). The optimized formulation AUC_{0-t} (20.15 ± 1.12 ng.hr/ml), $AUC_{0-\infty}$ (27.42 ± 1.16 ng.hr/ml) was significantly higher than that of marketed product AUC_{0-t} (13.21 ± 1.26 ng.hr/ml) and $AUC_{0-\infty}$ (19.15 ± 1.13 ng.hr/ml) respectively. Which indicated the optimized formulation bioavailability was higher than marketed product. Microspheres would be a promising drug delivery system which plays potentially significant role in pharmaceutical drug delivery in the efficient management of Parkinson's disease.

Keywords: Pramipexole, Mucoadhesive microspheres, *In vivo* bioavailability studies, Parkinson's disease.

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INTRODUCTION

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1µm to 1000µm or 1 mm). Microspheres are sometimes referred to as Microparticles. Microspheres are defined as "the monolithic spheres or therapeutic agents distributed throughout the matrix either as a molecular dispersion of particles". [1] One of the approaches the formulation of Gastro retentive dosage forms in the form of carrier

systems, made from natural polymers are attracting considerable attentions for several years, for sustained drug delivery. Today, those dosage forms which can control the release rates, and which are target specific

have a great impact in development of novel drug delivery systems. [2] Mucoadhesive system had selected in the present research work. From the scientific and patent literature and due to advancements in controlled DDS, it is marked that if gastro retentive dosage form retains in GIT for a particular time period then the drug is released slowly over a long period of time. [3] It clearly indicated that these dosage forms can control the drug release at gastric region without getting cleared from the GIT hence it avoids the fluctuations and reducing the requirement of several administrations. [4] Parkinson's disease is a chronic and disabling illness. There is still some uncertainty in its diagnosis, particularly in the early stages, as some other neurological conditions present with similar clinical features. There has been wide variation in the management of Parkinson's disease due to a lack of consensus on the best approach. [5] Pramipexole dihydrochloride is a well-known antiparkinsonism drug. It has less bioavability and only a minimal amount of the drug is crossing the blood brain barrier. The polymer as a carrier plays an important role in transport the drug across the blood brain barrier which may be effective in producing the therapeutic effect. [6-7] The use of biodegradable natural polymer controlled drug delivery has shown significant therapeutic potential suggested by many reports and most promising approaches for CNS drug delivery. [8] Their drug loading efficiency may be limited of their conjugation sites in the polymer leads to target active site. Depending upon the method of preparation of nanoparticles also influence in the penetration of drug across blood brain barrier which can be evidence by more entrapment efficiency of the drug by *in vitro.* [9-10] Due to this the drug can able to penetrate the blood brain barrier easily for targeting the brain disorder with increased bioavailability. [11] Hence the present study is to develop nanoparticles of a hydrophilic drug pramipexole dihydrochloride and improve the entrapment efficiency for treating Parkinson's disease.

MATERIALS AND METHODS

Materials

Pramipexole procured from Sun Pharmaceutical Industries Ltd. Sodium alginate from Pruthvi Chemicals, Mumbai. Calcium chloride obtained from SD Fine Ltd, Mumbai. Ethyl cellulose and chitosan were from Aay Cee Enterprises, Roorkee. Olibanum Gum obtained from Nutriroma, Hyd. All other materials and solvents were of HPLC grade.

Formulation of Pramipexole mucoadhesive microspheres

The Mucoadhesive microspheres were prepared by using ion tropic gelation technique. In this method weighed quantity of Pramipexole was added to 100 ml sodium alginate, Chitosan and Ethyl cellulose solution and thoroughly mixed at 500 rpm. Resultant solution was extruded drop wise with the help of syringe and needle into 100 ml aqueous calcium chloride solution and stirred at 100 rpm. After stirring for 30 minutes the obtained microspheres were washed with water and dried at 60 degrees 4 hours in a hot air oven and stored in desiccator. [12]

Table 1: Formulation trials for Pramipexole mucoadhesive microspheres

Formulation Code	Pramipexole (mg)	Sodium Alginate	Ethyl Cellulose (mg)	Calcium Chloride	Olibanum Gum	Chitosan
M1	0.5	1%	100	6%	0.5	-
M2	0.5	1.2%	150	6%	0.75	-
M3	0.5	1.4%	200	6%	1	-
M4	0.5	1.6%	250	6%	1.5	-
M5	0.5	1.8%	300	6%	1.75	-
M6	0.5	2.0%	350	6%	2.0	-
M7	0.5	2.2%	400	6%	2.5	-
M8	0.5	1%	-	10%	0.5	10
M9	0.5	1.2%	-	10%	0.75	15
M10	0.5	1.4%	-	10%	1	20
M11	0.5	1.6%	-	10%	1.5	25
M12	0.5	1.8%	-	10%	1.75	30
M13	0.5	2.0%	-	10%	2.0	35
M14	0.5	2.2%	-	10%	2.5	40

Evaluation studies of Pramipexole Mucoadhesive Microspheres

Micromeritic properties

Micromeritic properties were used for the assessment of flow ability and characterization of microspheres such as angle of repose, bulk density, tapped density, compressibility index, and Hausner's ratio. [13]

Swelling Index

The swelling index of drug loaded microspheres was determined by suspending the accurately weighed quantities of microspheres in simulated gastro intestinal fluids (0.1 N HCl with pH 1.2) and allowed to swell for the specified time. The excess surface adhered liquid drops of swollen microspheres were removed by using blotting paper and then weighed it with the help of a microbalance. The swollen microspheres were dried in oven at 60°C degrees for 5 hours or until showed the constant weight. The swelling index was determined using the initial weight of microspheres with respect to the weight of microspheres after drying (final weight) as per the formula below mentioned. [14]

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

% yield

The prepared microspheres were collected, dried and weighed. The percentage yield is calculated by taking the weight of dried microspheres divided by the total weight of drug and all excipients used in the microspheres preparation. [15] It was determined using the following formula.

% yield = [Total weight of Microspheres/Total weight of drug and polymer] × 100

Entrapment efficiency

The prepared microspheres of Pramipexole (equivalent to 10 mg of drug) was transferred in a mortar and crushed. The crushed microspheres were dissolved in 50 ml of methanol then transferred in to 100 ml conical

flask and made the volume up to the mark using methanol. The above solution was agitated to dissolve the drug, all excipients and to extract the drug. The solution was filtered through membrane filter (0.45µm) to separate shell fragments. The solution was diluted suitably, and the absorbance was estimated at the λmax of 263 nm by using a double – beam spectrophotometer (Shimadzu, UV-1800). [15] The amount of drug incorporated was determined using the following equation.

% Drug entrapment = Calculated drug concentration / Theoretical drug concentration × 100

Ex-vivo Mucoadhesion study

The microspheres mucoadhesive property was assessed by *ex-vivo* mucoadhesion method using chicken small intestinal tissue. The mucosal membrane was excised and washed with saline. 5 cm of jejunum portion was separated and averted with a glass rod. About 100 microspheres were spread uniformly on the tissue specimen. Then both ends of the segment were tied using a thread. The tissue specimen was suspended in a 50 ml tube containing 40 ml of saline at 37°C and stirred horizontally. The tissue specimen was removed from medium at specified time periods such as 1, 2, 3, 4, 5, 6, 7 and 8 h, then immediately immersed into tube containing 40 ml of fresh saline and unbound microspheres were counted. [16] The adhering percent was calculated using the formula shown below.

Mucoadhesion= (No. of microspheres adhered/ No. of microspheres applied) × 100

In vitro drug release studies

The *in vitro* drug release from formulated and prepared mucoadhesive microspheres was studied using USP dissolution apparatus II. Accurately weighed quantity of microspheres equivalent to 5 mg of drug was transferred into 900 ml of 0.1N HCl (pH 1.2) medium maintained at 37±0.5°C and stirring at 100 rpm. Aliquots of samples were withdrawn at specified time intervals, filtered and diluted with similar medium finally assayed at 263 nm using UV-Visible spectrophotometer. [17] The samples withdrawn were replaced with same dissolution medium at predetermined time intervals. All the samples were analyzed in triplicate.

Analysis of *in vitro* drug release kinetics and mechanism

The *in vitro* release data from several microspheres formulations containing Pramipexole were determined kinetically using different mathematical models like Zero order, First order, Higuchi and Korsmeyer–Peppas model. [17]

Drug-excipients compatibility studies

Fourier transform infrared spectroscopy (FTIR)

The spectral analysis can be used to identify the functional groups in the pure drug and drug-excipients compatibility. Pure Pramipexole FTIR spectra, physical mixtures and optimized formulation were recorded by using FTIR (SHIMADZU). Weighed quantity of KBr and drug-excipients were taken in the ratio 100: 1 and mixed by mortar. The samples were made into pellet by the application of pressure. [18] Then the FTIR spectras were recorded in the wavelength region between 4000 and 400 cm^{-1}.

SEM studies

Surface nature of microspheres includes size and shape was examined with the help of Scanning Electron Microscope (HITACHI, S-3700N). The microspheres were dried completely prior to analysis and SEM was carried out at different magnifications of 15.0 kv × 7.0 mm, 15 kv × 7.3 mm, 15 kv × 6.4 mm. [19]

Stability studies

Stability testing was conducted at 40°C ± 2°C/75% RH ± 5% RH for 6 months using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals 0, 30, 60, 120, and 180 days period according to ICH guidelines. [20] Various *in vitro* parameters like % yield, entrapment efficiency and *in vitro* release studies were evaluated.

In-vivo study Pramipexole

Animal Preparation

Twelve New Zealand white rabbits of either sex rabbits were (weighing 2-3 Kg) selected for this study, all the animals were healthy during the period of the experiment. Animals were maintained at room temperature 25°C, RH 45% and 12 h alternate light and dark cycle with 100% fresh air exchange in animal rooms, uninterrupted power and water supply and rabbits were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee.

In vivo Study design [21]

Rabbits were randomly divided into two groups each group contains six animals. Group A rabbits were fed with Pramipexole mucoadhesive microspheres (optimized formulation M13), group B fed with Marketed Product (0.5 mg) product with equivalent dose to animal body weight. Blood samples (approximately 0.5 ml) were obtained with syringes by marginal ear vein at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 20 and 24 h post dose. During collection, blood sample has been mixed thoroughly with heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5min and stored frozen at −20°C until analysis.

Preparation of Plasma Samples for HPLC Analysis

Rabbit plasma (0.5 ml) samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was re suspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000-6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200µ1 of 70% of acetonitrile and 30% water was injected for HPLC analysis.

Table 2: Formulated Pramipexole Mucoadhesive microspheres-Micromeritic properties

Formulation code	Particle size (µm)	Bulk density(g/cc³)	Tapped density (g/cc³)	Angle of repose	Carr's index (%)
M1	71.29 ± 0.01	0.57 ± 0.09	0.63 ± 0.02	27 °.61 ± 0.06	13.67
M2	75.42 ± 0.06	0.54 ± 0.05	0.60 ± 0.01	29 °.45 ± 0.10	11.27
M3	77.60 ± 0.06	0.57 ± 0.09	0.66 ± 0.05	24 °.15 ± 0.05	14.12
M4	71.86 ± 0.01	0.56 ± 0.05	0.64 ± 0.05	26 °.35 ± 0.05	15.27
M5	74.12 ± 0.05	0.54 ± 0.05	0.63 ± 0.02	25 °.34 ± 0.05	15.86
M6	77.24 ± 0.06	0.58 ± 0.09	0.60 ± 0.01	24 °.32 ± 0.05	11.62
M7	73.26 ± 0.04	0.56 ± 0.05	0.61 ± 0.01	26 °.46 ± 0.05	14.67
M8	72.18 ± 0.03	0.54 ± 0.05	0.64 ± 0.05	28 °.18 ± 0.06	12.18
M9	76.22 ± 0.06	0.55 ± 0.05	0.62 ± 0.02	27 °.35 ± 0.06	13.62
M10	74.12 ± 0.05	0.57 ± 0.09	0.61 ± 0.01	28 °.15 ± 0.06	11.95
M11	75.18 ± 0.05	0.58 ± 0.09	0.63 ± 0.02	29 °.25 ± 0.10	13.12
M12	71.28 ± 0.01	0.55 ± 0.05	0.61 ± 0.01	25.°19 ± 0.05	12.48
M13	69.16 ± 0.08	0.52 ± 0.03	0.58 ± 0.07	20. °67 ± 0.01	10.60
M14	70.12 ± 0.01	0.56 ± 0.05	0.60 ± 0.01	24°.75 ± 0.05	11.19

Table 3: Percentage yield and entrapment efficiency of Pramipexole Mucoadhesive microspheres Formulations

Formulation code	Percentage yield (%)	Entrapment efficiency (%)	Swelling index (%)	Mucoadhesiveness
M1	72.45%	75.00%	72.11%	68.14%
M2	81.38%	82.03%	78.34%	78.84%
M3	70.77%	73.04%	82.89%	71.02%
M4	85.00%	86.00%	84.56%	77.67%
M5	85.02%	87.72%	85.23%	80.94%
M6	96.03%	95.03%	94.12%	90.36%
M7	92.01%	90.01%	83.23%	85.12%
M8	81.08%	80.02%	68.12%	83.62%
M9	83.00%	81.05%	70.12%	82.18%
M10	84.00%	85.00%	75.22%	85.20%
M11	96.90%	88.25%	84.34%	87.98%
M12	92.00%	91.00%	91.09%	92.50%
M13	98.01%	96.07%	96.08%	95.42%
M14	88.72%	87.67%	89.03%	93.12%

Determination of Pramipexole in Rabbit plasma by HPLC method

For HPLC an Inertsil ODS 3V, 250 × 4.6 mm, column with 5µm particle size and in this method, chromatographic separation was achieved using a LiChrospher 60 RP column at 25°C, with a flow rate of 1.0 mL/min at 263 nm. The eluent comprised 0.01 mol/L ammonium acetate (pH 4.4) and acetonitrile (35:65 by volume) tamsulosin HCl was used as internal standard. Pramipexole and tamsulosin HCl retention times are 2.063 and 3.2 respectively. [22]

Pharmacokinetic Analysis

The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24 h, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity.

The $AUC_{0-\alpha}$ was calculated using the formula AUC_{0-t} + [C_{last}/K] where C_{last} is the concentration in µg/ml at the last time point and K is the elimination rate constant.

Various pharmacokinetic parameters like area under the curve [AUC], elimination half life ($t_{½}$). Volume of distribution (V_d), total clearance (Cl_T) and mean residence time for each subject using a non-compartmental pharmacokinetic program. The pharmacokinetic parameters were performed by a non-compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ± SD. Statistical analysis was performed with Graph Pad InStat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant.

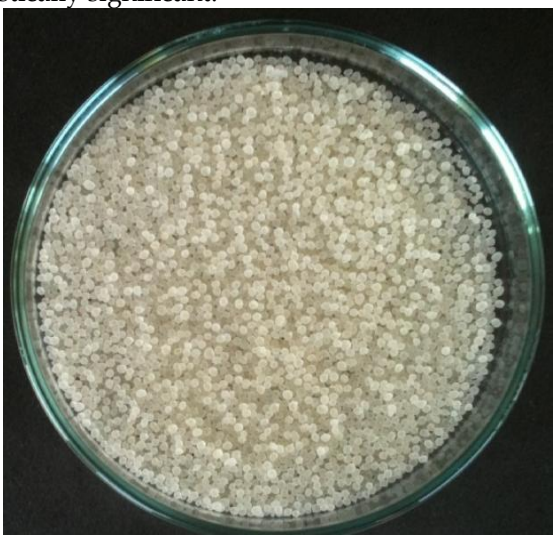

Fig. 1: Pramipexole Mucoadhesive microspheres

Fig. 2: Pictorial diagram showing mucoadhesive property of mucoadhesive microspheres in Chic Intestine

Table 4: *In-vitro* cumulative % drug release of Pramipexole Mucoadhesive microspheres Formulations

Time	M1	M2	M3	M4	M5	M6	M7
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1	12.15 ± 0.94	18.67 ± 0.97	19.89 ± 0.99	13.60 ± 0.94	16.33 ± 0.96	11.90 ± 0.92	20.18 ± 1.29
2	20.35 ± 1.29	22.98 ± 1.31	28.67 ± 1.40	21.56 ± 1.30	25.99 ± 1.35	23.45 ± 1.33	26.35 ± 1.36
4	32.18 ± 2.01	35.98 ± 2.05	33.98 ± 2.02	36.45 ± 2.06	38.98 ± 2.10	37.85 ± 2.10	39.64 ± 2.40
6	48.16 ± 2.59	46.13 ± 2.50	45.88 ± 2.50	48.94 ± 2.59	42.90 ± 2.46	44.56 ± 2.48	48.36 ± 2.59
8	59.67 ± 2.96	62.19 ± 3.10	65.67 ± 3.15	55.49 ± 2.89	64.90 ± 3.12	58.93 ± 2.95	66.49 ± 3.17
10	78.83 ± 3.94	75.60 ± 3.81	79.80 ± 3.95	75.96 ± 3.81	80.19 ± 4.82	79.62 ± 3.98	81.25 ± 4.82
12	88.32 ± 4.98	90.69 ± 5.01	91.80 ± 5.01	89.99 ± 4.99	92.61 ± 5.02	85.75 ± 4.95	93.49 ± 5.03

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

Time	M8	M9	M10	M11	M12	M13	M14	Marketed product
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1	20.18 ± 1.29	19.45 ± 0.99	15.60 ± 0.95	18.99 ± 0.98	21.50 ± 1.30	25.67 ± 1.35	22.67 ± 1.32	10.12 ± 0.85
2	30.16 ± 2.08	29.15 ± 2.07	25.75 ± 1.35	26.19 ± 1.36	32.19 ± 2.03	36.19 ± 2.06	33.84 ± 2.02	19.18 ± 0.99
4	43.25 ± 2.47	42.36 ± 2.46	38.46 ± 2.25	35.67 ± 2.05	45.98 ± 2.51	48.19 ± 2.58	41.60 ± 2.47	28.16 ± 1.39
6	50.16 ± 2.83	52.98 ± 2.88	55.67 ± 2.89	56.98 ± 2.90	54.09 ± 2.90	58.96 ± 2.92	53.62 ± 2.78	35.26 ± 2.05
8	68.19 ± 3.19	65.96 ± 3.15	64.98 ± 3.15	69.02 ± 3.20	70.90 ± 3.82	79.60 ± 3.95	75.18 ± 3.81	45.89 ± 2.49
10	82.15 ± 4.80	85.96 ± 4.95	79.60 ± 3.95	81.29 ± 4.79	83.99 ± 4.92	88.19 ± 4.98	82.15 ± 4.89	69.30 ± 3.21
12	90.35 ± 5.00	92.18 ± 5.02	89.46 ± 4.99	91.66 ± 5.01	93.69 ± 5.03	99.16 ± 5.12	94.45 ± 5.04	90.45 ± 5.01

Table 6: Release kinetics of optimized formulation of mucoadhesive microspheres

Formulation Code	Zero Order		First Order		Higuchi		Korsmeyer -Peppas	
	R^2	K	R^2	K	R^2	K	R^2	N
M13	0.993	4.873	0.824	0.142	0.986	28.308	0.987	0.551
Marketed product	0.965	6.831	0.671	0.122	0.869	24.10	0.964	0.818

Table 7: Stability studies of optimized mucoadhesive microspheres

Retest Time for Optimized formulation	Percentage yield	Entrapment efficiency	*In-vitro* drug release profile (%)
0 days	98.01	96.07	99.16
30 days	96.23	95.16	95.18
60 days	95.20	94.18	94.25
120 days	94.67	93.20	93.67
180 days	92.18	92.14	92.18

Table 8: Comparison of pharmacokinetic parameters of Pramipexole optimized formulation and Marketed Product

Parameters	Pramipexole Optimized formulation	Marketed Product
C_{max} (ng/ml)	2.0 ± 0.01	2.19 ± 0.01
AUC_{0-t} (ng hr/ml)	20.15 ± 1.12	13.21 ± 1.26
$AUC_{0-\infty}$ (ng hr/ml)	27.42 ± 1.16	19.15 ± 1.13
T_{max} (hr)	4.00 ± 0.05	3.00 ± 0.04
$t_{1/2}$ (hr)	8.85 ± 0.41	6.91 ± 0.01

RESULTS AND DISCUSSION

Formulation of Mucoadhesive microspheres

Mucoadhesive microspheres of Pramipexole were formulated by ionic gelation method, using different polymers like sodium alginate, chitosan and calcium chloride in different concentrations and the formulation code M1, M2, M3, M4, M5, M6, M7, M7, M8, M9, M10, M11, M12, M13 and M14 were prepared. All the formulations were evaluated for their various physical parameters.

Particle size was measured by using optical microscopy. All the formulations M1 to M14 varied from 69.16 ± 0.08µm to 77.24 ± 0.06µm.

The bulk density and tapped density of all the formulations M1 to M13 were measured and they are ranged from 0.52 ± 0.03 g/cc³ to 0.57 ± 0.09 g/cc³ and 0.58 ± 0.07 g/cc³ to 0.66 ± 0.05 g/cc³.

Angle of repose of all the formulations was found satisfactory results. And the formulation M13 was found to be 20˚.67 ± 0.01 having good flow property.

The compressibility index values were found to be in the range of 10.60 to 15.86%. These findings indicated that the all batches of formulation exhibited good flow properties. All the formulations M1 to M13 showed the swelling of microspheres. The swelling of the formulation M13 was found to be 96.08%.

Mucoadhesion study

The *in vitro* mucoadhesive test was carried out using chicken small intestine. The small intestinal tissue was excised and flushed with saline. A five-centimeter segment of jejunum was averted using a glass rod. Ligature was placed at both ends of the segment. 100 microspheres were scattered uniformly on the averted sac from the position of 2 cm above. Then the sac was suspended in a 50 ml tube containing 40 ml of saline by the wire, to immerse in the saline completely. The sac was incubated at 37°C and agitated horizontally. The sac was taken out of the medium after immersion for 1, 2, 3, 4, 5, 6, and 8 h, immediately repositioned as before in a similar tube containing 40 ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation.

Mucoadhesion = (No. of microspheres adhered/No. of microspheres applied) × 100

All the 14 formulations of mucoadhesive microspheres were exposed to mucoadhesion test. The formulation M13 shows the high percentage of mucoadhesive property it shows 95.42% of adhesion nature

The percentage release and entrapment efficiency of all the formulations were measured by assay method. The mucoadhesive microspheres of formulation M1 to M13 shows the percentage release values ranges from 70.77% to 98.01%.

The entrapment efficiency values of all the 14 formulations were ranges from 73.04% to 96.07%.

The formulation M13 shows the good percentage yield and entrapment efficiency the values were 98% and 96%.

In vitro drug release studies

The *in vitro* drug release from the prepared microspheres was studied (M1- M14) and showed in the Table 4 & 5 and Figure 3 & 4. The drug release from the microspheres was found to decrease with increase in the polymer concentration. Among all the formulations M13 showed maximum drug release was 99.16 ± 5.12% within 12 h.

Mathematical modeling of optimized formula of mucoadhesive microspheres

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

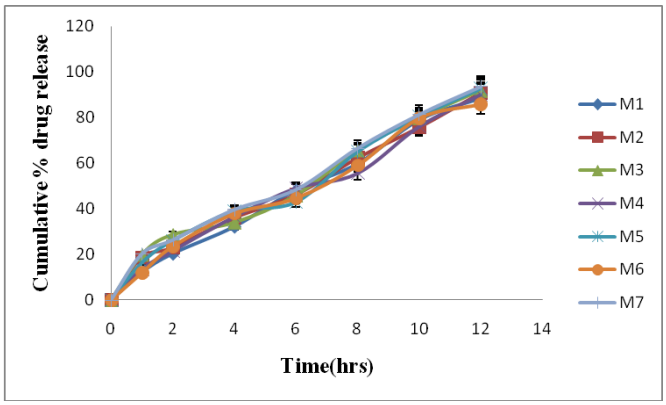

Fig. 3: *In-vitro* cumulative % drug release of Pramipexole Mucoadhesive microspheres formulations

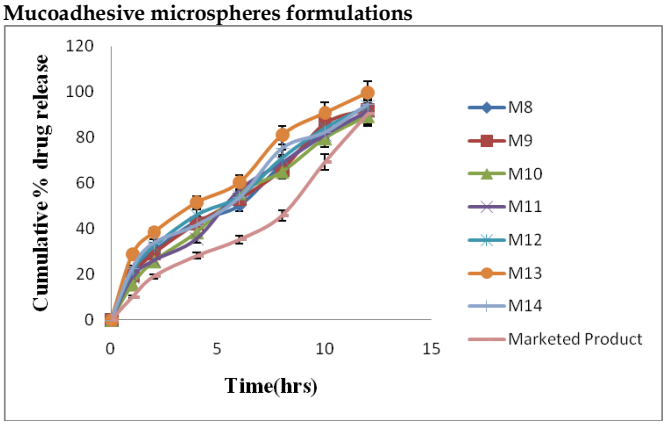

Fig. 4: *In-vitro* cumulative % drug release of Pramipexole mucoadhesive microspheres formulation

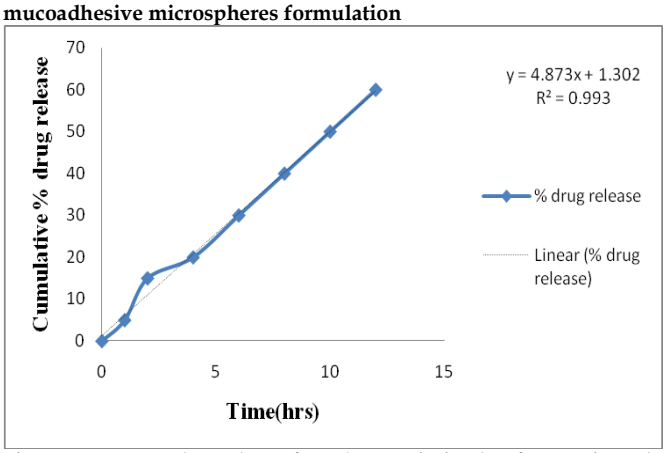

Fig. 5: Zero order plots for the optimized of Pramipexole Mucoadhesive microspheres M13

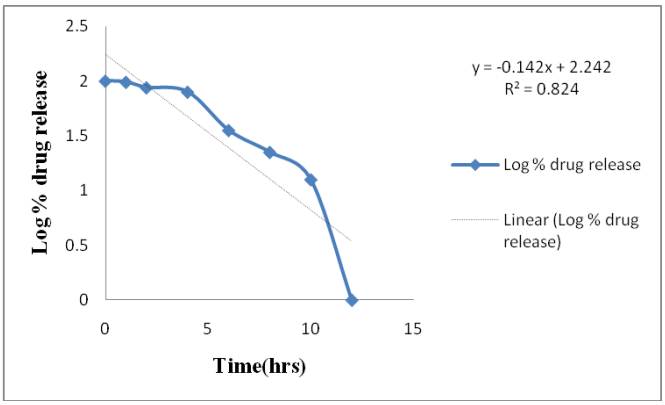

Fig. 6: First order plot for the optimized of Pramipexole Mucoadhesive microspheres M13

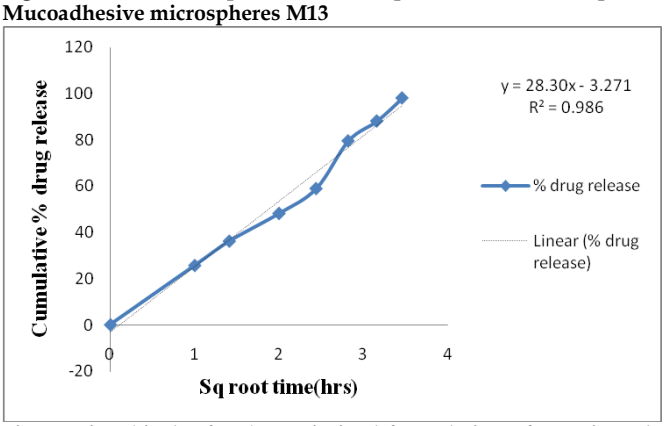

Fig. 7: Higuchi plot for the optimized formulation of Pramipexole Mucoadhesive microspheres M13

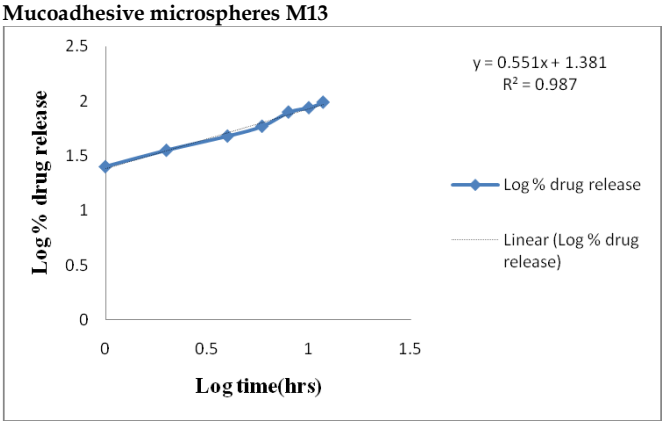

Fig. 8: Korsmeyer-Peppas plot for the optimized formulation of Pramipexole Mucoadhesive microspheres M13

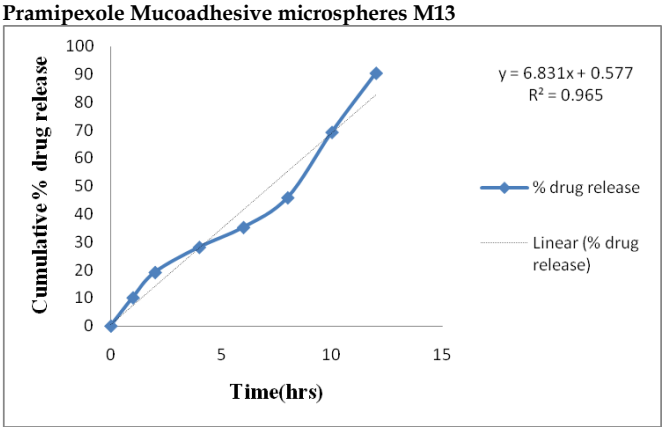

Fig. 9: Zero order plots for the Marketed product

From the results it is apparent that the regression coefficient value closer to unity in case of zero order plot i.e.0.993 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. 0.986 starting that the release from the matrix was through diffusion. Further the n value obtained from the Korsmeyer plots i.e. 0.551 suggest that the drug release from floating microspheres was anomalous Non fickian diffusion. The release order kinetics of marketed product was also shown in Table 6 and Figure 5-12.

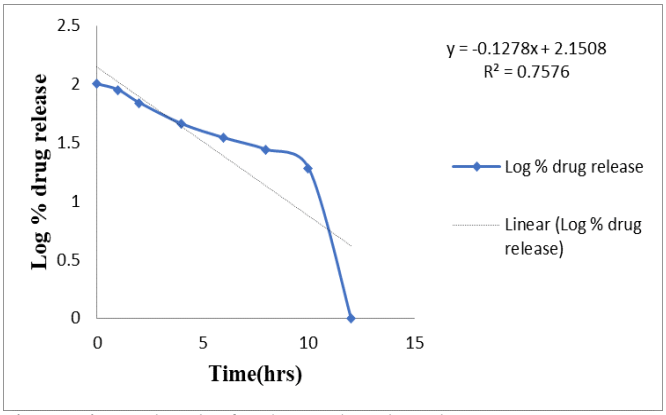

Fig. 10: First order plot for the Marketed product

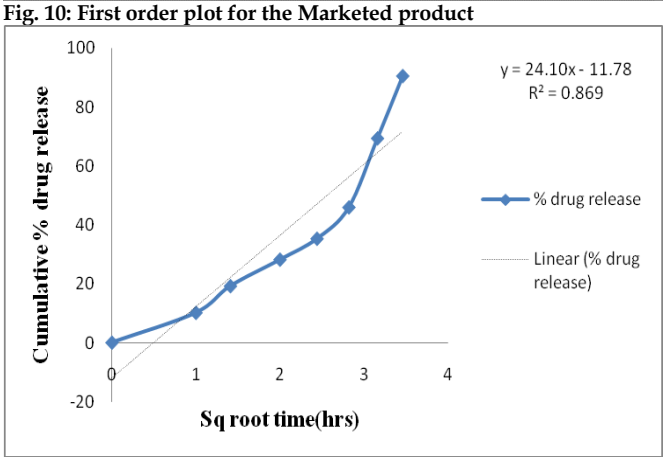

Fig. 11: Higuchi plot for the Marketed product

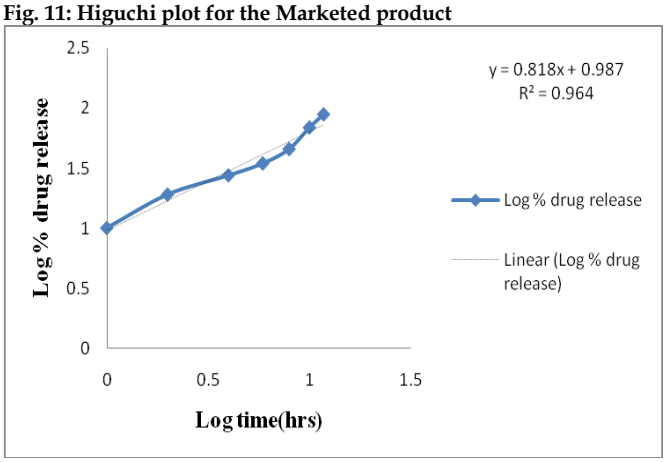

Fig. 12: Korsmeyer-Peppas plot for the Marketed product

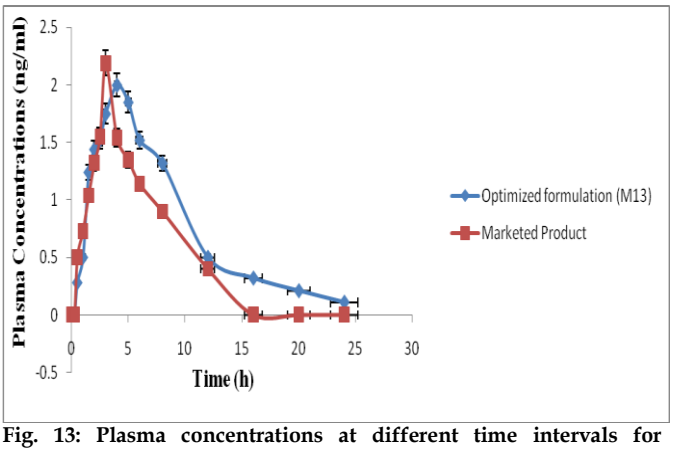

Fig. 13: Plasma concentrations at different time intervals for Pramipexole optimized formulation (M13) and Marketed Product

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 7.

In vivo study

Bioavailability parameters

Mean plasma concentration profiles of prepared Pramipexole Optimized formulation and marketed product are presented in Figure 13. Pramipexole Optimized formulation exhibited as sustained release *in vivo* when compared with innovator tablet. All the pharmacokinetics parameters displayed in Table 8. The release pattern of both marketed and prepared formulation was not significantly different. The Cmax of test formulation and marketed formulations was 2.0 ± 0.01 and 2.19 ± 0.01 respectively. The T_{max} of marketed formulation was 3.00 ± 0.04 h, and Pramipexole Optimized formulation was 4.00 ± 0.05. This delayed absorption of test and marketed preparation was most likely due to the sustained release of the drug. The optimized formulation AUC_{0-t} (20.15 ± 1.12 ng.hr/ml), $AUC_{0-\infty}$ (27.42 ± 1.16 ng.hr/ml) was significantly higher than that of marketed product AUC_{0-t} (13.21 ± 1.26 ng.hr/ml) and $AUC_{0-\infty}$ (19.15 ± 1.13 ng.hr/ml) respectively. The results indicated that the test formulation could increase the bioavailability of Pramipexole in rabbits effectively than that of a marketed product.

In the Current study, we successfully prepared stable gastroretentive mucoadhesive dosage form (using ionotropic gelation method) containing Pramipexole. The preparation process was found to be easy, economical, and reproducible process. The optimized formulation (M13) was found to be efficient with % yield (98.01%), entrapment efficiency (96.07%), swelling index (96.08%) mucoadhesion (95.42%), and an adequate particle size (69.16±0.08µm). Further, the *in vitro* mucoadhesive results suggested that the fabricated formulation possess mucoadhesive property.

This property facilitates the mucoadhesive microspheres to adhere to the gastric mucosal surface and reside in stomach for delayed time period which eventually leads to better bioavailability. *In vitro* release studies showed better extent of drug release up to 99.16 ± 5.12% (12 h). Drug release from Pramipexole microspheres followed zero order and Higuchi model suggested that it followed the diffusion-controlled mechanism. The FTIR studies showed that drug and excipients were compatible. SEM results revealed that the prepared microspheres were spherical in shape. The stability of optimized formulation (M13) was investigated as per ICH guidelines thoroughly and found stable for 6 months. *In vivo* studies were performed and kinetic parameters like C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and $t_{1/2}$ were calculated. The marketed product C_{max} (2.19 ± 0.01 ng/ml) was higher than optimized formulation (2.0 ± 0.01 ng/ml). The optimized formulation AUC_{0-t} (20.15 ± 1.12 ng.hr/ml), $AUC_{0-\infty}$ (27.42 ± 1.16 ng.hr/ml) was significantly higher than that of marketed product AUC_{0-t} (13.21 ± 1.26 ng.hr/ml) and $AUC_{0-\infty}$ (19.15 ± 1.13 ng.hr/ml) respectively. Which indicated the optimized formulation bioavailability was higher than marketed product. Microspheres would be a promising drug delivery system which plays potentially significant role in pharmaceutical drug delivery in the efficient management of Parkinson's disease.

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