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FORMULATION AND EVALUATION OF CHITOSAN NANOPARTICLES OF ROPINIROLE HCL TO TARGET BRAIN IN THE TREATMENT OF PARKINSON'S DISEASE

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ABSTRACT

The aim of the current research is to develop chitosan nanoparticles of Ropinirole HCl to target the brain. Drug characterization was done by UV, Melting point analysis, DSC and FTIR. Drug excipient compatibility showed no possible interaction between drug and excipients. Ten formulations (CNP 1 to CNP 10) were prepared by varying the ratios of Chitosan (0.2%, 0.5%) and STPP (0.2%). The % encapsulation efficiency was found to be ranging from 14.35±0.29 to 69.14±0.23 %. The loading capacity was found to be ranging from 0.18±0.004 to 0.86±0.003 mg/ml and showed a linear relationship with encapsulation efficiency. The mean particle size was found to be ranging between 275.5 nm and 601.3 nm and result quality report was found to be good for all formulations. All formulations were found to be homogenous (less polydisperse) with polydispersity index values ranging from 0.147 to 0.274 (closer to zero). The zeta potential of all formulations was found to be positive with values ranging from 15.97 to 35.27 mV. *In vitro* diffusion studies were carried for all formulations and the drug release ranged from 29.70±1.24 to 80.56±1.97 after 24 h. From the kinetic studies, nanoparticles were found to be following Higuchi model indicating drug release mechanism was by diffusion. The n value from korsemeyer peppas plot was below 0.5 for all formulation indicating fickian diffusion. Results of *in vivo* BBB crossing study showed that when compared with pure drug, the formulated nanoparticles (CNP 2) carried the drug to brain effectively. Stability studies performed at room temperature (25±2° C) and refrigerator (3-5±2° C) showed no significant changes upon storage.

KEYWORDS: Brain targeting, Chitosan, Ropinirole HCl, BBB.

INTRODUCTION

Parkinson's disease

Parkinson's disease is a progressive disorder of the nervous system that affects movement. It happens due to death of cells in the substantia nigra, a region of the midbrain resulting in low levels of dopamine. The cause of Parkinson's disease is generally unknown, but believed to involve both genetic and environmental factors.[1] The reason for this cell death is poorly understood, but involves the build-up of proteins into Lewy bodies in the neurons. It develops gradually, sometimes starting with a barely noticeable tremor in just one hand.[2] In the early stages of Parkinson's disease, your face may show little or no expression, or your arms may not swing when you walk. Your speech may become soft or slurred. Parkinson's disease symptoms worsen as your condition progresses over time. Although Parkinson's disease can't be cured, medications may markedly improve your symptoms.[3]

Initial treatment is typically with the antiparkinson medication levodopa (L-DOPA), with dopamine agonists being used once levodopa becomes less effective. As the disease progresses and neurons continue to be lost, these medications become less effective. Surgery to place microelectrodes for deep brain stimulation has been used to reduce motor symptoms in severe cases where drugs are ineffective. Evidence for treatments for the non-movement-related symptoms of PD, such as sleep disturbances and emotional problems, is less strong.[2]

Several dopamine agonists that bind to dopamine receptors in the brain have similar effects to levodopa. These were initially used as a complementary therapy to levodopa for individuals experiencing levodopa complications (on-off fluctuations and dyskinesias); they are now mainly used on their own as first therapy for the motor symptoms of PD with the aim of delaying the initiation of levodopa therapy and so delaying the onset of levodopa's complications. Dopamine agonists include bromocriptine, pergolide, pramipexole, ropinirole, piribedil.[2]

For development of new medicines for PD, an obstacle is BBB that acts as a barrier for the absorption of drugs. Hence, material transported from blood to CNS is restricted. Because of this BBB drug transport restriction mechanism, drug delivery to the PD is difficult. The drug in the current research Ropinirole HCl is hydrophilic in nature which cannot pass through lipophilic BBB.

Nanotechnology

Nanotechnology is one of the more promising and efficient technologies for enhancing drug delivery to brain (brain targeting). The nanoparticles are the drug carrier system (ranging from 1-100 nm) which is made from a broad number of materials such as poly (alkyl cyanoacrylates), poly acetates, polysaccharides, copolymers and colloidal biodegradable polymeric particles likePoly (D,L-lactide-co-glycolide) (PLGA) etc., and can be used as drug delivery vehicles to deliver such drugs to brain by infiltrating BBB.[4]

Polymer based nanoparticles are made from natural & biodegradable polymers such as Chitosan, polylactic acid (PLA), and polycyanoacrylate (PCA) etc. The mechanism for the transport across the BBB has been characterized as receptor-mediated endocytosis by the brain capillary endothelial cells. Transcytosis then occurs to transport the nanoparticles across the tight junction of endothelial cells and into the brain. Surface coating of the nanoparticles with surfactants such as polysorbate 80 or poloxamer 188 were shown to increase uptake of the drug into the brain.[4]

NpDDS offer numerous advantages over conventional dosage forms, including improved efficacy, reduced toxicity, improved patient compliance and also sustains the drug effect. The advantage of Nano technological approach is that it carries the active form of drug to the brain in nanoparticle size. So it provides the active form of drug to be delivered for maximal efficacy.[5]

So the current research is focused on developing chitosan nanoparticles of Ropinirole HCl to target the brain.

MATERIALS AND METHODS

Materials

Ropinirole Hydrochloride drug) and Sodium Tripolyphosphate (TPP) were purchased from Yarrow Chem Pvt. Ltd, Mumbai. Chitosan was purchased from Sigma Aldrich. HPLC grade Acetonitrile, Water, Methanol were purchased from S.D Fine Chem. HPLC grade Potassium Dihydrogen Phosphate and Ortho phosphoric acid were purchased from Finar Chemicals, Ahmedabad. All other chemicals used are of analytical grade.

Drug-Excipient compatibility studies

Compatibility studies were carried out by using FTIR spectroscopy & DSC.

- FTIR has been used to quantify the interaction between the drug and the carrier used in formulation. Spectra were recorded for pure drug and for drug and polymers (1:1) physical mixture, on Bruker tensor-27 Spectrophotometer.
- DSC is a technique in which the difference in heat flow between the sample and a reference is recorded versus temperature. DSC thermal analytical profile of a pure chemical represents its product identity. By comparing the DSC curves of a pure drug sample with that of formulation, the presence of an impurity can be detected in a formulation.

Ionic gelation method[6,7]

This method involves an ionic interaction between the positively charged amino groups of chitosan and the polyanion TPP, which acts as chitosan crosslinker. Chitosan solution (0.2% and 0.5%) is prepared using 2% Glacial Acetic Acid. 0.2% TPP solution was prepared in distilled water. Calculated amount of drug was dissolved in 0.2% chitosan solution. Nanoparticles formation takes place immediately after the drop wise addition of TPP solution to the solution of chitosan and drug, under mild stirring, at room temperature. The solution becomes turbid after complete formation of nanoparticles. The ratio of Chitosan:TPP is varied in various formulations to optimize the best formulation. Stirring should be maintained for approximately 10 minutes to allow particle stabilization. NPs were collected by centrifugation at 15,000 rpm for 45 min at 4°C and the supernatant will be used to determine encapsulation efficiency (EE) and loading capacity (LC). The resultant pellet of nanoparticles was then resuspended in water.

Table 1: Formulation chart of Chitosan Nanoparticles.

Formulation Code	Ratio	
	Chitosan (0.2 %)	**Sodium Tripolyphosphate (0.2%)**
F1	1	1
F2	1	1.5
F3	1	2
F4	1.5	1
F5	2	1
	Chitosan (0.5 %)	**Sodium Tripolyphosphate (0.2%)**
F6	1	1
F7	1	1.5
F8	1	2
F9	1.5	1
F10	2	1

The prepared nanoparticles are evaluated for following parameters:

Entrapment Efficiency & Loading Capacity[8]

Ropinirole-loaded chitosan nanoparticles were centrifuged by at 15,000 rpm and 4°C for 45 min using REMI Ultra Centrifuge. The non-entrapped drug (free drug) was determined in the supernatant solution.

Entrapment efficiency is calculated by equation:

$$\% \text{ Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Free drug}}{\text{Total amount of drug}} \times 100$$

$$\text{Loading capacity} = \frac{\text{Total amount of drug} - \text{Free drug}}{\text{Suspension volume}}$$

Particle size determination

The particle size of the chitosan nanoparticles was determined by Malvern Zeta sizer ZS90. It performs size measurements using a process called Dynamic Light Scattering (also known as PCS - Photon Correlation Spectroscopy).

Zeta potential measurement

A potential exists between the particle surface and the dispersing liquid which varies according to the distance from the particle surface – this potential at the slipping plane is called the zeta potential. The Zetasizer Nano series measures Zeta potential using a combination of the measurement techniques: Electrophoresis and Laser Doppler Velocimetry, sometimes called Laser Doppler Electrophoresis.

***In vitro* diffusion studies**[6]

The *in vitro* release profile of Ropinirole chitosan nanosuspension was performed using dialysis sacs. The drug loaded chitosan nanosuspension (containing about 2 mg of drug) was placed in pretreated dialysis sacs which were immersed into 100 ml of phosphate buffer saline, pH 7.4, at 37±0.5°C and magnetically stirred at 50 rpm. Aliquots were withdrawn from the release medium at intervals 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h and replaced with the same amount of phosphate buffer. The samples were analyzed at 249 nm.

***In vivo* drug release study & *In vivo* blood brain barrier crossing study[9,10]**

Healthy adult Wistar rats weighing 180-220 g were used as animal model. The rats were randomly divided into different groups. Group 1 served as the control, Group 2 was injected with drug solution and Group 3 was intravenously injected with CNP 2 formulation in tail vein. After time intervals of 0.5, 2, 4 and 8 h, they were sacrificed by decapitation.

Blood samples were also collected before sacrifice. The selected organs, i.e., the brain, lungs, kidneys, spleen, heart and liver will be quickly dissected and stored at -20°C. Internal standard is externally spiked to each organ before homogenization. The homogenate is centrifuged at 8000 rpm, 4°C for 30 min (Methanol is added to precipitate the proteins) and clear supernatant is collected for HPLC analysis.

By estimating the amount of drug present in brain, the ability of formulated nanoparticles to pass BBB and target brain was estimated.

RESULTS AND DISCUSSION

Identification of Drug

i. Melting Point Determination

The melting point of Ropinirole HCl was determined using digital melting point apparatus and was found to be 244°C. The reported melting point for the drug was 243-250°C.[26]

ii. Fourier Transform Infra-Red Spectroscopy

The FT-IR spectrum of drug was taken by using Bruker Tensor 27 which uses ATR technique. The characteristic peaks of drug were spotted in the spectra which upon comparison with reference standard confirmed it as Ropinirole HCl[31]

Results were shown in fig 1 and table 2.

Drug-Excipient compatibility studies

FTIR study showed that all the characteristic peaks of drug are present in the spectra of physical mixture of drug and excipients thus indicating there was no interaction between them. Results of the compatibility studies were shown in the fig 1 and table 2.

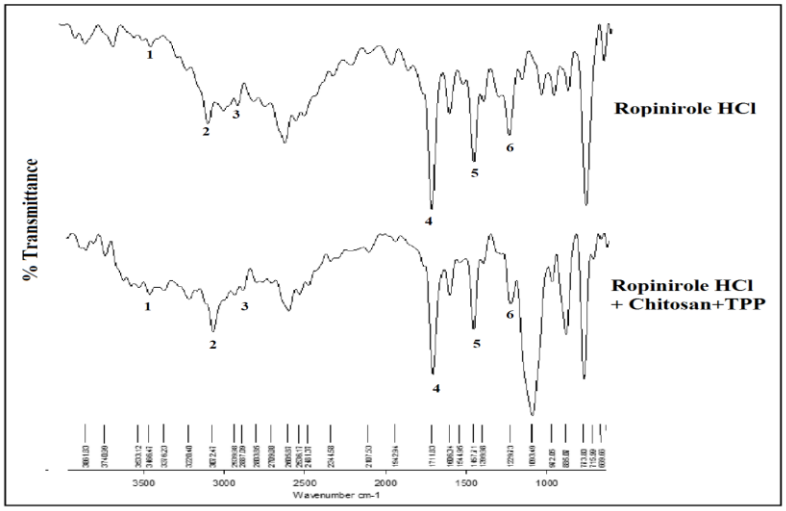


Figure 1: Drug-Excipient compatibility study by FTIR.

Table 2: Drug-Excipient compatibility study by FTIR- Peak picking.

Frequency (cm-1)				
S. No	**Ropinirole HCl**	**Drug+ Chitosan+ TPP**	**STANDARD FREQUENCY RANGE**	**DESCRIPTION**
1	3415	3466	3500-3100	N-H tretch (2° Amine)
2	3067	3072	3100-3000	C=C stretch
3	2974 2886	2939 2887	3000-2850	Alkyl CH Stretch
4	1710	1711	1755-1650	C=O (Ketone)
5	1603	1606	1700-1500	Aromatic C=C bending
6	1240	1229	1340-1020	C-N Stretch

DSC analysis of Ropinirole HCl reported an endotherm at 248°C. The melting point range of Ropinirole HCl standard was 243-250°C. DSC study identified the drug Ropinirole HCl.

DSC analysis of drug:chitosan:STPP (1:1:1) physical mixture revealed no possible interaction between them with drug endotherm reported at 247.86°C. Results are shown in fig 2 and 3.

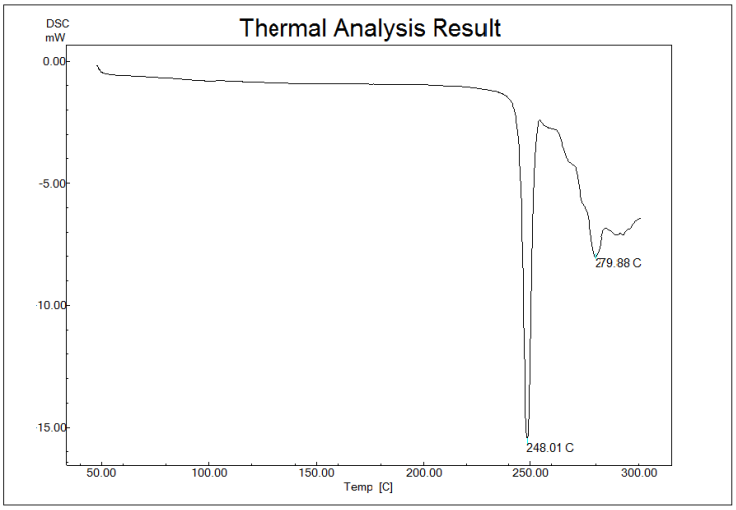


Fig 2: DSC Thermogram of Ropinirole HCl.

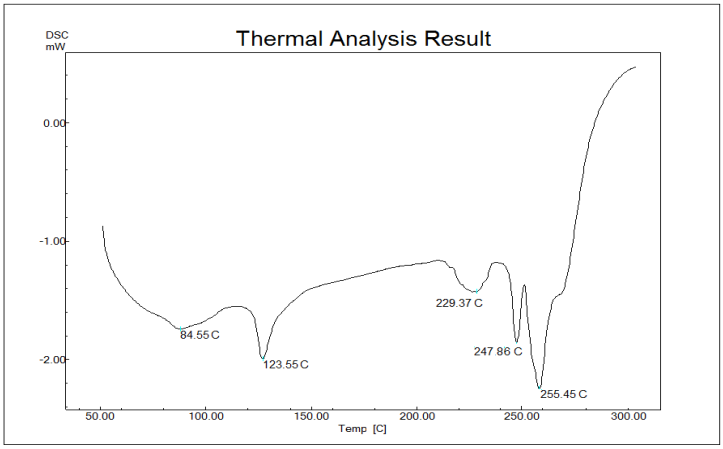


Fig 3: DSC Thermogram of Ropinirole HCl Chitosan Nanoparticles.

Encapsulation efficiency & loading capacity

Ten formulations were prepared by varying the ratios of Chitosan (0.2%, 0.5%) and STPP (0.2%). From the observed results, it was found that formulations with high STPP and low concentration of chitosan showed higher encapsulation compared to rest. The formulations with higher concentration of chitosan and low STPP showed poor encapsulation probably due to the reason that amount of STPP in the formulation is not sufficient to completely crosslink the chitosan. CNP 3 with 1:2 ratio of Chitosan (0.2%):STPP(0.2%) showed higher encapsulation efficiency of 62.69±0.11%. The results are shown in the table 2.

The volume of nanosuspension was kept constant. The loading capacity was found to follow linear relationship with encapsulation efficiency. CNP 3 showed higher loading capacity with 0.86±0.003 mg/ml. The results are shown in table 2.

Table 3: Encapsulation efficiency of Chitosan nanoparticles.

Formulation Code	Encapsulation efficiency (%)	Loading capacity (mg/ml)
CNP 1	57.41±0.41	0.72±0.005
CNP 2	62.69±0.11	0.78±0.001
CNP 3	69.14±0.23	0.86±0.003
CNP 4	47.72±0.57	0.60±0.007
CNP 5	39.54±0.12	0.49±0.002
CNP 6	34.37±0.21	0.43±0.003
CNP 7	46.41±0.09	0.58±0.001
CNP 8	51±0.26	0.64±0.003
CNP 9	22.29±1.36	0.28±0.017
CNP10	14.35±0.29	0.18±0.004

Particle size analysis & Polydispersity index

The particle size of formulations CNP 1 to CNP 10 was determined by Malvern Zetasizer - Nano ZS 90. The size of the particles ranged between 275.5 nm to 601.3 nm. CNP 2 showed good particle size of 275.50±13.08. The results are shown in table 3 and the size distribution graph of CNP 2 is shown in fig 4.

Polydispersity index ranged from 0.147 to 0.274. All formulations were found to be less polydisperse (more homogenous) with values closer to zero. The result quality for all formulations was found to be good as reported by instrument. The results are shown in table 4.

Table 4: Particle size & Polydispersity index of formulations CNP 1 to CNP 10.

Formulation Code	Particle size (nm)*	Polydispersity index*
CNP 1	371.57±20.12	0.148±0.037
CNP 2	275.50±13.08	0.274±0.003
CNP 3	359.03±7.32	0.147±0.010
CNP 4	390.73±22.50	0.179±0.008
CNP 5	448.70±24.00	0.178±0.021
CNP 6	427.90±19.79	0.162±0.015
CNP 7	447.33±14.12	0.247±0.025
CNP 8	349.63±25.80	0.197±0.003
CNP 9	576.77±61.42	0.262±0.027
CNP 10	601.30±26.79	0.241±0.007

n = 3

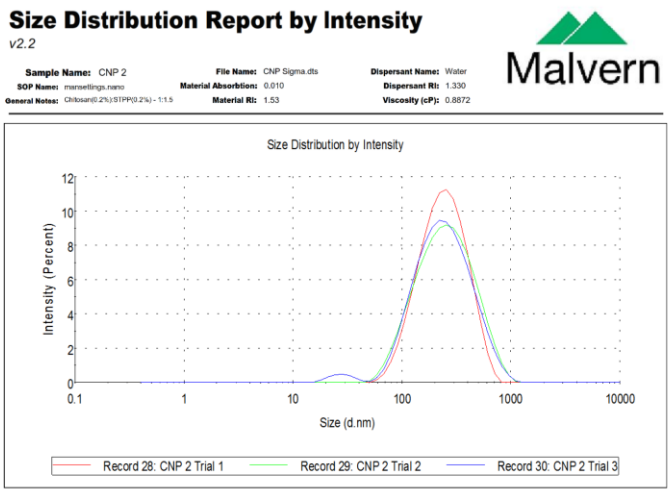


Fig 4: Size distribution report of CNP 2.

Zeta potential measurement

Zeta potential was determined for formulations CNP 1 to CNP 10 by Malvern Zetasizer - Nano ZS 90 and found to be positive ranging from 15.97 to 35.27 mV. From the observed results, it was found that as the chitosan concentration in formulation increased (when concentration of STPP was kept constant) the zeta potential value increased due to cationic nature of chitosan as evident in CNP 4, 5, 9 and 10. When the concentration of STPP was increased keeping concentration of chitosan constant, zeta potential decreased, probably due to higher crosslinking of cationic groups by STPP as evident in CNP 1-3 (0.2% chitosan) and CNP 6-8 (0.5% chitosan) respectively. The results are shown in table 5 and the zeta potential distribution graph of CNP 2 is shown in fig. 5.

Table 5: Zeta potential of formulations CNP 1 to CNP 10.

Formulation Code	Zeta potential (mV)*
CNP 1	22.77±0.25
CNP 2	17.90±0.26
CNP 3	15.97±0.23
CNP 4	25.07±0.74
CNP 5	28.90±1.06
CNP 6	27.97±1.01
CNP 7	25.73±0.40
CNP 8	23.13±0.40
CNP 9	29.97±0.31
CNP 10	35.27±2.59

All values are positive (+); n=3

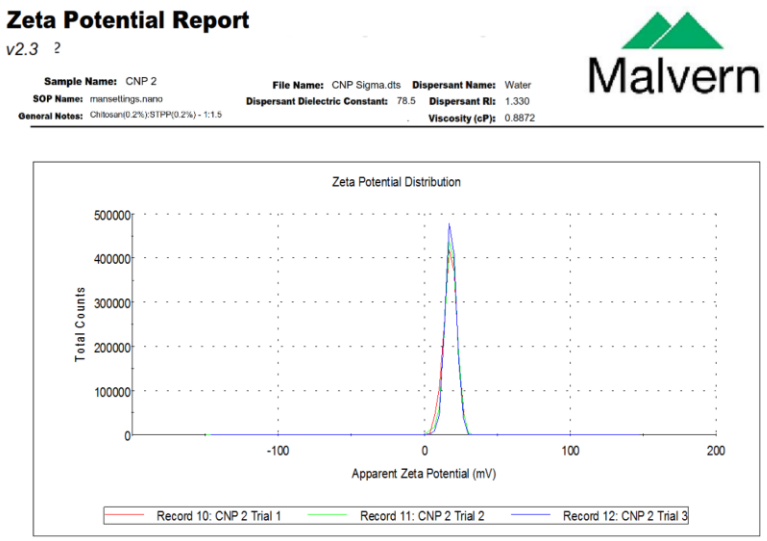


Fig 5: Zeta potential report of CNP 2.

In vitro diffusion study

In vitro diffusion was performed for formulations CNP 1 to CNP 10 in PBS pH 7.4 at 50 rpm and 37±0.5°C for 24 h. All formulations showed initial burst release due to release of drug that is present on the surface of nanoparticles. CNP 1 showed higher release of 80.56±1.97% after 24 h. From the observed results it was found that the nature of crosslinking directly affected the drug release from nanoparticles. Higher the cross linking, tighter the polymeric matrix and slower is the drug release as found in CNP 3 and CNP 8. It was also observed that higher concentration of chitosan resulted in slow drug release as evident in CNP 5 and CNP 10. The results are shown in fig 6 and 7.

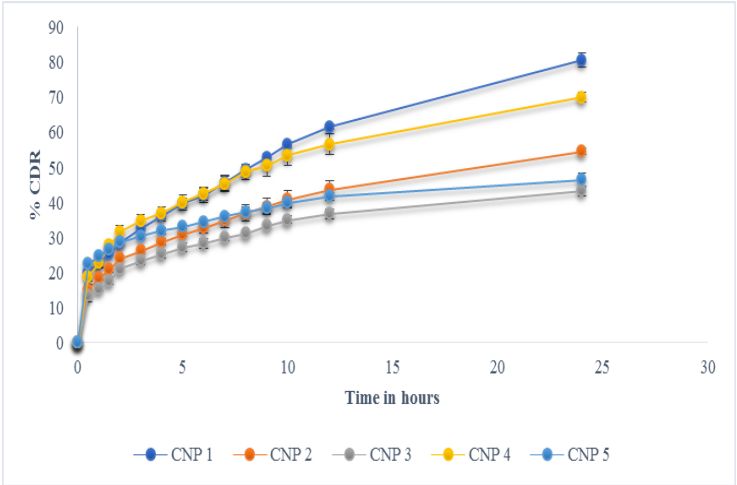


Fig 6: % CDR of formulations CNP 1 to CNP 5.

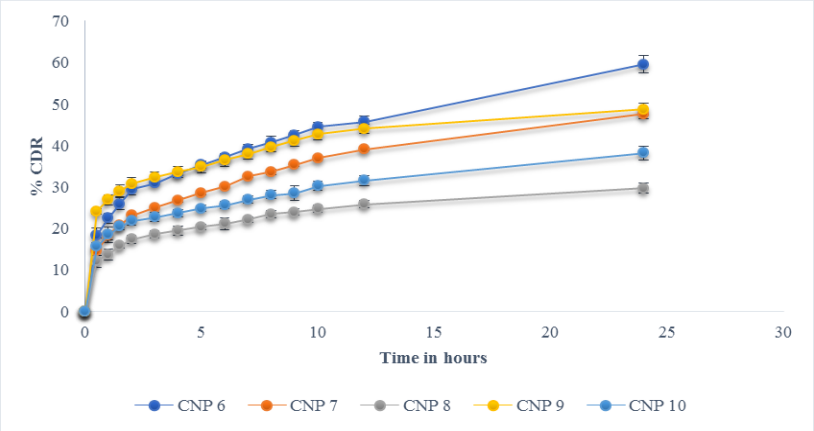


Fig 7: % CDR of formulations CNP 6 to CNP 10.

Kinetic modelling of *in vitro* drug diffusion profiles

The drug diffusion profiles of all formulations were fitted into various kinetic models. From the results it was evident that all the formulations (CNP 1 to CNP 10) were more linear towards Higuchi model with R^2 value ranging from 0.967 to 0.994 indicating that drug release mechanism is by diffusion. In korsemeyer-peppas plot the n value for all formulations was found to be less than 0.5 indicating fickian diffusion. The results are shown in Table 7.

Table 7: Kinetic modelling of formulations CNP 1 to CNP 10.

Formulation	Model			
	Zero order	1st order	Higuchi	Korsemeyer- peppas
	R^2	R^2	R^2	n value (slope)
CNP 1	0.939	0.809	0.994	0.38
CNP 2	0.879	0.926	0.993	0.33
CNP 3	0.900	0.961	0.974	0.32
CNP 4	0.897	0.908	0.988	0.34
CNP 5	0.835	0.968	0.974	0.19
CNP 6	0.878	0.882	0.990	0.29
CNP 7	0.878	0.939	0.988	0.30
CNP 8	0.839	0.950	0.969	0.23
CNP 9	0.903	0.972	0.967	0.18
CNP 10	0.829	0.913	0.988	0.21

SEM Studies

SEM analysis of CNP 2 revealed near spherical and little aggregation among chitosan nanoparticles. Result is shown in fig 8.

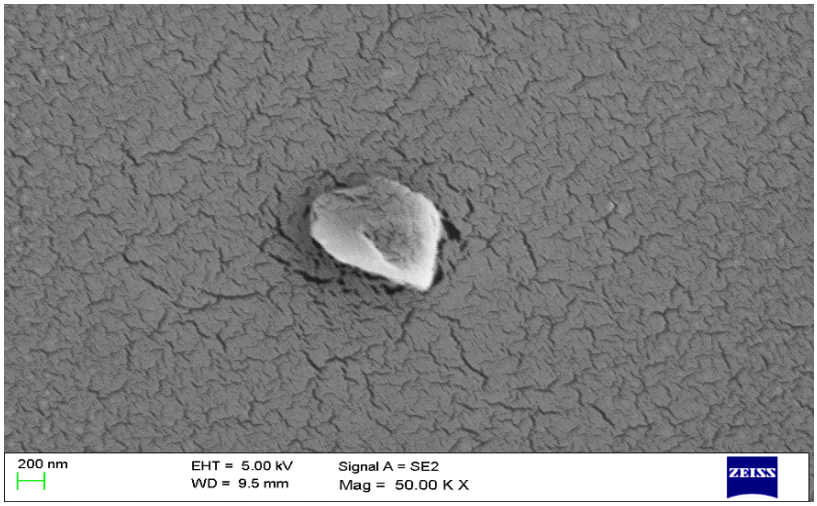


Fig 8: Sem of CNP 2.

***In Vivo* Studies**

Results of *in vivo* BBB crossing study showed that when compared with pure drug, the formulated nanoparticles (CNP 2) carried the drug to brain effectively. A two-way ANOVA was employed for statistical analysis of Blood Brain Barrier crossing study. When compared with pure drug at different time intervals, CNP 2 found to be statistically significant with p value<0.05 (*). Result is shown in table 8.

Table 8: Blood Brain Barrier crossing study (% of drug present in brain after I.V).

Frequency	Pure drug	CNP 2
30 min	2.48±0.82	9.44±1.39
3 hours	4.77±1.15	26.48±3.71
6 hours	8.32±1.32	39.92±4.88
12 hours	5.81±1.08	27.35±4.11

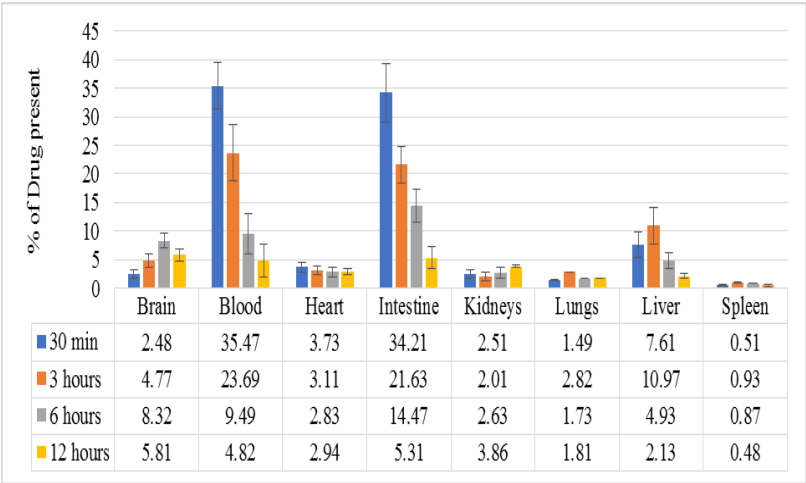
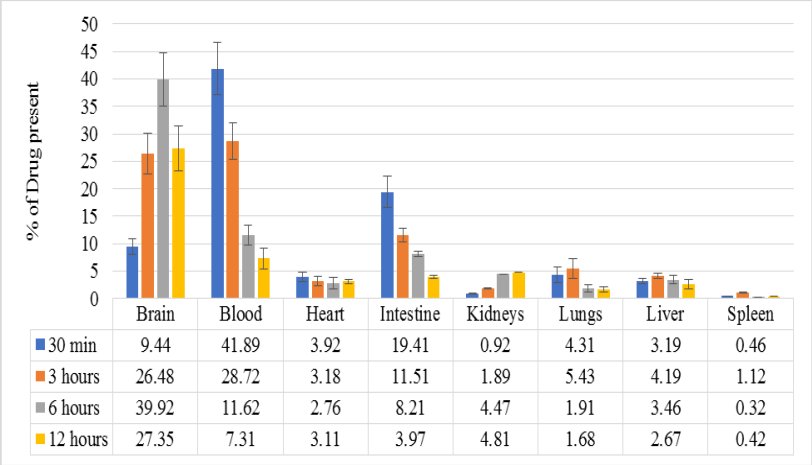


Fig 15: Bio distribution study of Pure drug (I.V).



	Brain	Blood	Heart	Intestine	Kidneys	Lungs	Liver	Spleen
30 min	9.44	41.89	3.92	19.41	0.92	4.31	3.19	0.46
3 hours	26.48	28.72	3.18	11.51	1.89	5.43	4.19	1.12
6 hours	39.92	11.62	2.76	8.21	4.47	1.91	3.46	0.32
12 hours	27.35	7.31	3.11	3.97	4.81	1.68	2.67	0.42

Fig 16: Bio distribution study of CNP 2 (I.V).

Stability studies

The results of stability study suggested no significant changes upon storage. The results are shown in table 9.

Table 9: Stability studies of Optimized formulations.

Frequency	CNP 2			
	Particle Size (nm)	PDI	Zeta Potential (mV)	*In vitro* release (24 h)
0^{th} day	275.50±13.08	0.274±0.003	27.90±0.26	54.5±0.9
1 Month	279.01±7.15	0.275±0.017	27.61±0.73	51.86±3.42
3 Months	285.17±11.32	0.277±0.009	25.11±0.45	52.31±0.11
6 Months	286.45±14.89	0.277±0.014	24.90±0.19	55.72±1.34

CONCLUSION

The aim of the current research is to develop chitosan nanoparticles of Ropinirole HCl to target the brain. The prepared chitosan nanoparticles were found to pass through BBB and effectively carry the hydrophilic drug to brain. The developed technology can be employed for the effective treatment of other neurodegenerative disorders.

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