WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 12, 455-469. Research Article ISSN 2277– 7105

FABRICATION OF PLGA NANOPARTICLES OF ROPINIROLE HCL BY EMULSION SOLVENT EVAPORATION TECHNIQUE TO TARGET BRAIN

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Article Received on
24 April 2018,

Revised on 14 May 2018,
Accepted on 04 June 2018

DOI: 10.20959/wjpr201812-12485

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ABSTRACT

The aim of the current research is to develop PLGA 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. A general full factorial design was constructed using Minitab 17 statistical software. Nine formulations were prepared with two factors PLGA and PVA with 3 levels each. The % encapsulation efficiency was found to be ranging from 57.31±0.82 to 85.13±0.29%. The loading capacity was found to be ranging from 0.96±0.014 to 1.42±0.005 mg/ml and showed a linear relationship with encapsulation efficiency. The mean particle size was found to be ranging between 224.3 nm to 581.9 nm and result quality report was found to be good for all formulations except F5 and F9. All formulations except F9 & F5 (PDI value of 1 and 0.643 respectively) were found to be homogenous (less polydisperse) with polydispersity index values ranging from 0.052 to 0.382 (closer to zero). The zeta potential of all formulations was found to be near neutral with values ranging from -3.64 to -0.14 mV. *In vitro* diffusion studies were carried for all formulations and the drug release ranged from 35.17±1.63 to 79.46±1.08 after 48 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 (F2) carried the drug to brain effectively. Stability studies performed at refrigerator conditions (3-5±2°C) showed no significant changes upon storage.

KEYWORDS: Brain targeting, PLGA, PVA, Emulsion solvent evaporation, 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, Poly (D,L-Lactide-co Glycolide) (PLGA), 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 PLGA nanoparticles of Ropinirole HCl to target the brain.

MATERIALS AND METHODS

Materials

Ropinirole Hydrochloride was purchased from Yarrow Chem Pvt. Ltd, Mumbai. PLGA was purchased from Lactel – Durect Corporation, USA. PVA was purchased from Central Drug House (p) ltd, New Delhi. 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.

Formulation of PLGA – polyvinyl alcohol (PVA) NPs by W/O/W emulsion solvent evaporation method[6]

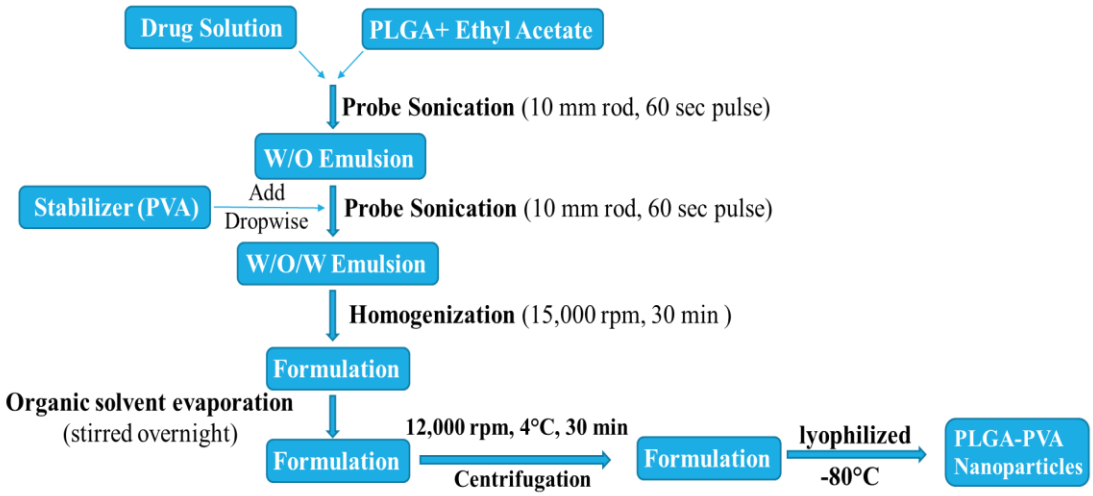

Fig. 1: W/O/W emulsion solvent evaporation method.

Nine formulations were prepared by using general full factorial design (Qsutra Minitab 17 software) with two factors PLGA and PVA with 3 levels each. Refer to Table 1.

Table 1: Formulation chart of PLGA-PVA NPs (F1 – F9).

Formulation	PLGA (mg)	PVA (%)
F1	500	0.5
F2	100	0.5
F3	1000	1
F4	1000	0.5
F5	1000	0.1
F6	100	1
F7	500	1
F8	500	0.1
F9	100	0.1

The prepared nanoparticles are evaluated for following parameters:

Entrapment Efficiency & Loading Capacity[7]

Ropinirole-loaded PLGA 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 PLGA 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 PLGA Nanoparticles was performed using dialysis sacs. The drug loaded PLGA 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 blood brain barrier crossing study[8,9]

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 F2 formulation in tail vein. After time intervals of 0.5, 2, 4 and 8 h, they were sacrificed by decapitation.

Brain was 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.[10]

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.[11]

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 2 and table 2.

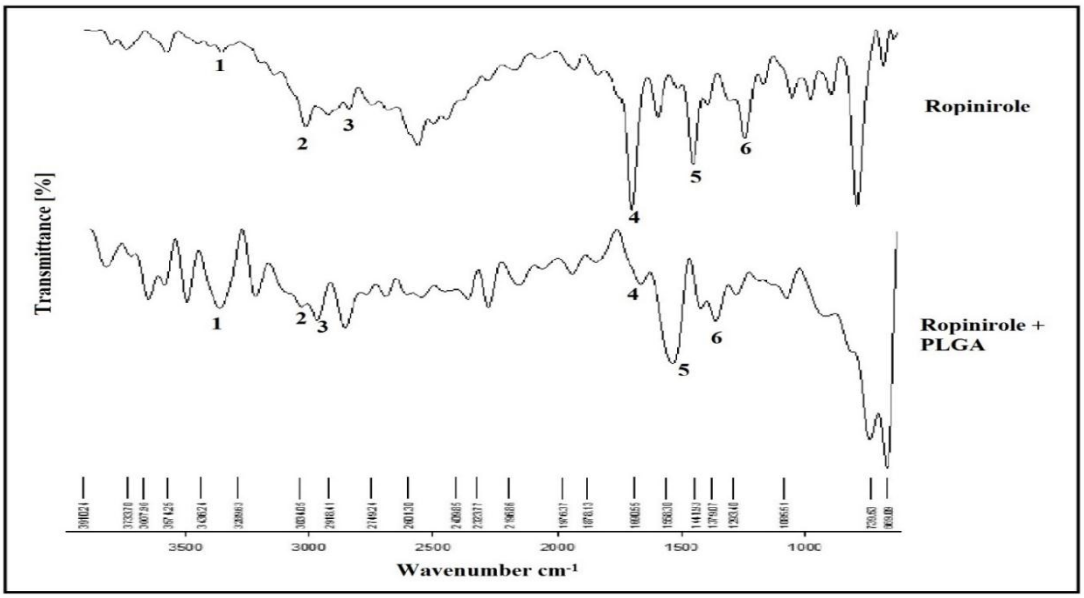

Fig. 2: Drug-Excipient compatibility study by FTIR.

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

FREQUENCY (cm^{-1})				
S. No	**Ropinirole HCl**	**Drug+ PLGA**	**STANDARD FREQUENCY RANGE**	**DESCRIPTION**
1	3415	3436	3500-3100	N-H Stretch (2° Amine)
2	3067	3034	3100-3000	C=C stretch
3	2974 2886	2918	3000-2850	Alkyl CH Stretch
4	1710	1690	1755-1650	C=O (Ketone)
5	1603	1558	1700-1500	Aromatic C=C bending
6	1240	1293	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:PLGA (1:1) physical mixture revealed no possible interaction between them with drug endotherm reported at 247.86°C. Results are shown in fig 3 and 4.

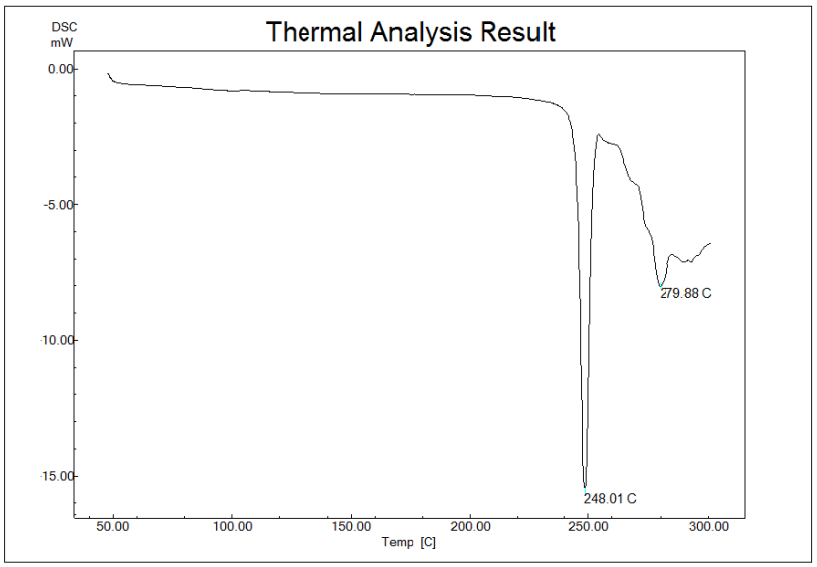

Fig. 3: DSC Thermogram of Ropinirole HCl.

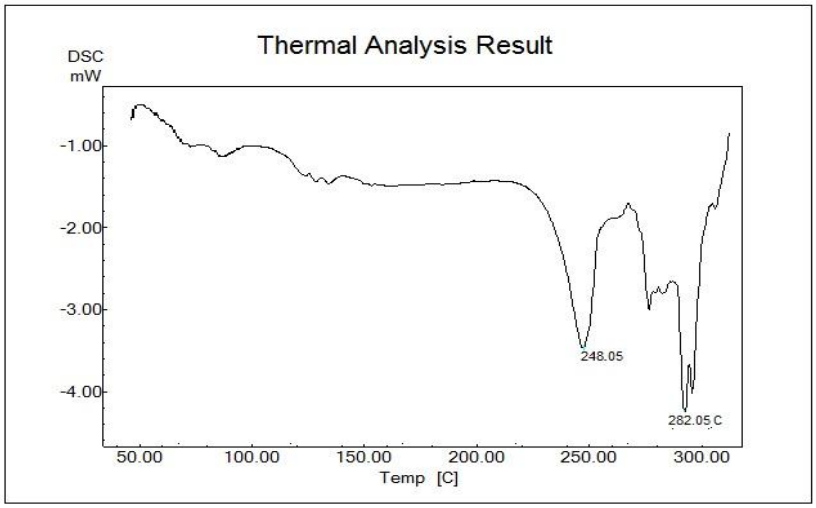

Fig. 4: DSC Thermogram of Ropinirole HCl PLGA Nanoparticles.

% Encapsulation efficiency & loading capacity

Nine formulations were prepared by solvent evaporation technique using different quantities of PLGA and PVA. From the results, it was observed that the formulations containing high concentration of PLGA showed higher encapsulation as in F4, F5 (84.72% and 85.13% respectively). The results are shown in table 3.

The volume of nanosuspension was kept constant. The loading capacity was found to follow linear relationship with encapsulation efficiency. F5 showed higher loading capacity with 1.42±0.005 mg/ml. The results are shown in table 3.

Table 3: % Encapsulation efficiency and loading capacity of F 1 – F 9.

Formulation Code	Encapsulation efficiency* (%)	Loading capacity* (mg/ml)
F1	81.31±0.69	1.36±0.012
F2	78.65±0.64	1.31±0.011
F3	76.28±0.35	1.27±0.006
F4	84.72±0.13	1.41±0.002
F5	85.13±0.29	1.42±0.005
F6	80.54±0.14	1.34±0.002
F7	66.88±0.19	1.11±0.003
F8	57.31±0.82	0.96±0.014
F9	68.39±0.30	1.14±0.005

***n=3**

Particle size analysis & Polydispersity index

The particle size of formulations F1 to F9 was determined by Malvern Zetasizer -Nano ZS 90. The size of the particles ranged between 224.3 nm to 581.9 nm. F2 (100 mg PLGA & 0.5% PVA) showed lowest particle size of 224.3±8.90 nm among all formulations. Formulations F5 and F9 showed multiple scattering, high multi-modal fit and poor data quality. From the results, it was observed that as the concentration of PLGA increases a gradual increase in particle size was observed. The results are shown in table 4 and the size distribution graph of F2 is shown in fig 5.

Polydispersity index (PDI) ranged from 0.052 to 1. Concentration of PVA showed considerable effect towards PDI. From the results, it was observed that formulations containing low concertation of PVA (0.1%) were found to be highly polydisperse with PDI values closer to 1. It was also observed that as the concentration of PVA increases the PDI value decreased indicating homogenous nature. F7 (500 mg PLGA & 1% PVA) was found to be less polydisperse with PDI value of 0.052±0.07. The results are shown in table 4.

Table 4: Particle size & polydispersity index of F 1 to F 9.

Formulation Code	Particle size* (nm)±SD	Polydispersity index*±SD
F1	356.4±23.12	0.140±0.06
F2	224.3±8.90	0.074±0.04
F3	247.8±21.32	0.107±0.01
F4	408.0±63.51	0.243±0.07
F5	477.1±98.41	0.643±0.11
F6	334.9±14.92	0.063±0.05
F7	321.1±27.52	0.052±0.07
F8	581.9±57.83	0.382±0.09
F9	333.5±88.10	1

***n=3**

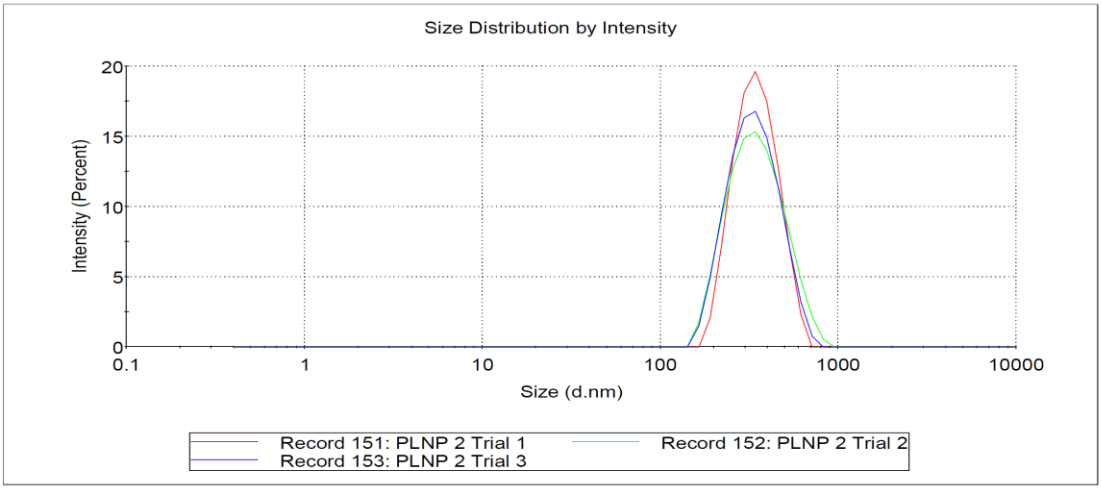

Fig. 5: Size distribution report of F2.

Zeta potential measurement

Zeta potential for formulations F1 to F9 was determined by Malvern Zetasizer -Nano ZS 90 and found to be negative ranging from -0.364 to -0.14 mV. The results indicate poor to moderate stability. F9 showed highest zeta potential of -3.64±0.26 mV. The possible reason for zeta potential values closer to zero may be due to presence of residual PVA on surface of nanoparticles which was found to act as shield between nanoparticles and its surrounding medium by masking the charged groups present on the surface. The results are shown in table 5 and the zeta potential distribution graph of F9 is shown in fig 6.

Table 5: Zeta potential analysis of F1 to F9.

Formulation Code	Zeta potential (mV)*
F1	0.42±0.03
F2	1.63±0.22
F3	0.18±0.07
F4	0.90±0.11
F5	3.34±0.70
F6	0.14±0.98
F7	0.62±0.05
F8	1.71±0.45
F9	3.64±0.26

*** n=3 & All values are negative (-)**

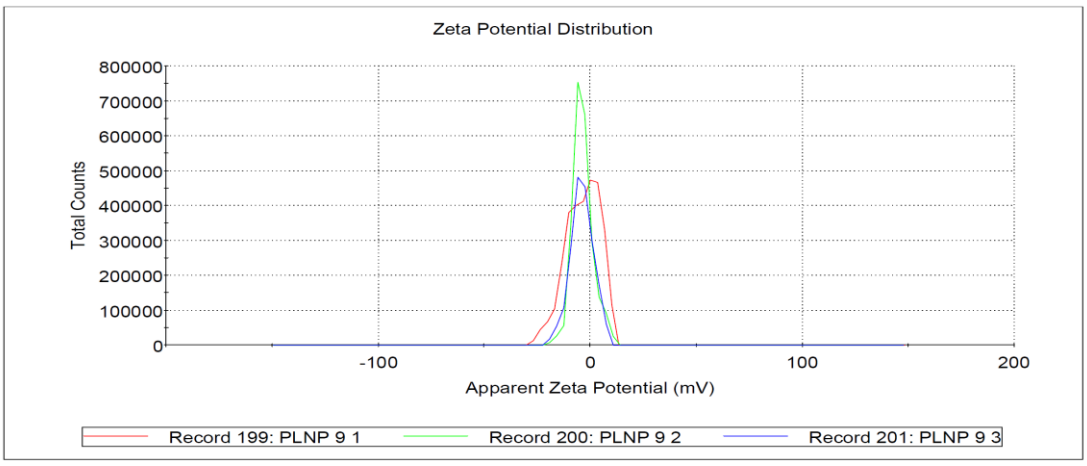

Fig. 6: Zeta potential report of F9.

5.3.4 *In vitro* diffusion study

In vitro diffusion for F1 to F9 was performed in PBS pH 7.4 at 37±0.5°C for 48 h. A biphasic release was observed in all formulations with initial burst release (due to release of drug that is present on the surface of nanoparticles) followed by sustained release. Formulations with poor %EE showed highest burst release compared formulations with higher encapsulation. F2 showed higher release of 79.46±1.08% after 48 h. After initial burst release the drug is released by hydrolyzing the polymer followed by diffusion and erosion. From the observed results, it was found that higher the concentration of PLGA slower is the drug release due to tight polymer matrix. It was also observed that formulation with highest concentrations of both PVA and PLGA resulted in slowest drug release of 41.94±1.16% after 48 h as found in F3. The results are shown in fig 7.

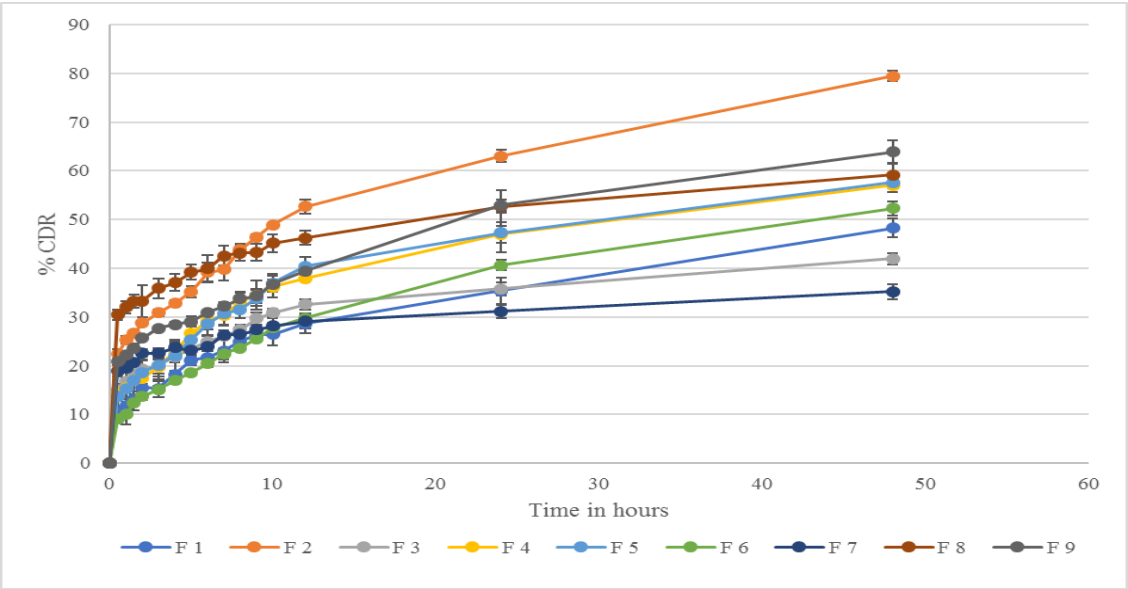

Fig. 7: % CDR of F1 to F9.

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 (F1 to F9) were more linear towards Higuchi model with R^2 value ranging from 0.838 to 0.851 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 6.

Table 6: Kinetic modelling of F1 to F9.

Formulation	Model			
	Zero order	1st order	Higuchi	Korsemeyer- peppas
	R^2	R^2	R^2	n value
F1	0.890	0.940	0.848	0.35
F2	0.849	0.665	0.851	0.29
F3	0.774	0.645	0.842	0.24
F4	0.817	0.687	0.849	0.34
F5	0.815	0.640	0.843	0.35
F6	0.802	0.660	0.846	0.41
F7	0.781	0.654	0.848	0.14
F8	0.828	0.620	0.838	0.15
F9	0.832	0.640	0.841	0.25

Optimization by Minitab 17

The evaluation data of all formulations was analyzed by Minitab 17 response optimizer. Encapsulation efficiency, particle size, PDI, zeta potential and *in vitro* drug release were selected as responses. d-value (individual desirability) on a scale of 0 to 1 indicates the possibility of obtaining the desired results with the selected combination. Values closer to 1 indicates good desirability and vice versa. D value (composite desirability) is calculated taking all individual responses in to consideration. Higher the value of D higher is the possibility for optimal results. The results from the response optimizer suggested F2 with D value of 0.688 as the optimized formulation among all. The results are shown in table 7.

Table 7: Response optimization of F1 to F9.

Formulation	d-value (individual desirability or goal)					Composite Desirability
	%EE	Particle Size	PDI	Zeta Potential	*In vitro* release	
F1	0.586	0.310	0.933	0.075	0.202	0.303
F2	0.498	1	0.991	0.458	0.683	**0.688**
F3	0.450	0.871	0.953	0	0.104	0
F4	0.647	0.376	0.725	0.227	0.338	0.423
F5	0.654	0.556	0.025	1	0.345	0.316
F6	0.540	0.889	0.993	0.193	0.264	0.475
F7	0.234	0.358	1	0.138	0	0
F8	0	0	0.806	0.483	0.370	0
F9	0.273	0.890	0	0.981	0.443	0

SEM Studies

SEM analysis of F 2 revealed spherical and no aggregation among PLGA nanoparticles. Result is shown in fig 8.

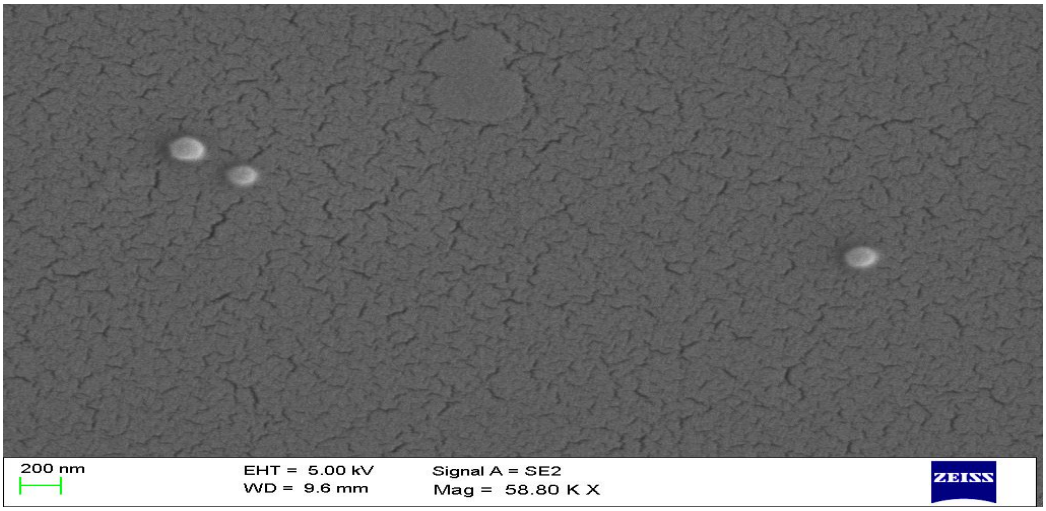

Fig. 8: SEM image of F2.

***In Vivo* Studies**

Results of *in vivo* BBB crossing study showed that when compared with pure drug, the formulated nanoparticles (F2) 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, F2 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	F2
30 min	2.48±0.82	7.31±1.24
3 hours	4.77±1.15	24.52±2.75
6 hours	8.32±1.32	46.31±3.61
12 hours	5.81±1.08	25.27±3.56

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	F2			
	Particle Size (nm)	PDI	Zeta Potential (mV)	*In vitro* release (48 h)
0^{th} day	224.3±8.90	0.052±0.07	3.64±0.26	79.46±1.08
1 Month	227.2±7.78	0.064±0.06	3.71±0.35	78.27±3.15
3 Months	225.7±9.14	0.079±0.09	3.77±0.21	76.71±2.15

CONCLUSION

The aim of the current research is to develop PLGA nanoparticles of Ropinirole HCl to target the brain. The prepared PLGA 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.

ACKNOWLEDGEMENTS

The authors thank management of Acharya & B. M Reddy College of Pharmacy for the facilities & Lady Tata Memorial Trust, Mumbai for funding the research.

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