

RESEARCH ARTICLE

Formulation of PLGA Polymeric Nanosuspension containing Pramipexole Dihydrochloride for improved treatment of Parkinson's Diseases

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ABSTRACT:

Aim and Objectives The present investigation was undertaken to develop nanosuspension of a hydrophilic drug pramipexole dihydrochloride and improve the entrapment efficiency of the drug.

Materials and Methods: Nanosuspension of pramipexole dihydrochloride was prepared with PLGA and Pluronic F68 by the process of modified nanoprecipitation technique. The particle size, zeta potential, SEM, TEM and *in vitro* drug release were performed. The cell viability study were performed by using SH-SY5Y cells.

Result and Discussion: Nano-formulations are prepared with different concentrations of PLGA. The formulation variables such as polymer concentration (PLGA) were found to possess significant effect on the particle size and entrapment efficiency of drug in nanosuspension. The maximum entrapment efficiency, least particle size and optimal *in vitro* drug release profile were exhibited with 1:3 ratio having 1% concentration of Pluronic F 68. The least particle size of 195 nm and maximum zeta potential value 34.8 mv were observed with PPNP 3 formulation. The SEM, TEM and *in vitro* drug release of PPNP 3 were performed shows spherical shape with controlled release when compared to other formulations. Further the MTT assay were performed by using SH-SY5Y cells which shows more cell viability with PPNP 3 nanoformulation when compared to pure drug.

Conclusion: PPNP 3 can be selected as best formulation among the other formulations. Thus, the biodegradable poly mer influences a better delivery in brain for the treatment in Parkinson's disease.

KEYWORDS: Pramipexole dihydrochloride; PLGA; Zeta potential; MTT assay; Parkinson's disease

INTRODUCTION:

The development of polymer based drug carriers has attracted increased attention over the last years. Nanoparticles are the forefront of the rapidly developing field with several potential applications in drug delivery, clinical medicine and research, as well as in other various sciences⁽¹⁾. Other drug delivery systems for brain disease have a low therapeutic effect due to insufficient bioavailability in the targeted site⁽²⁾. Nanoparticles may overcome this problem due to its smaller particle size and may produce therapeutic effect in a particular site which offers the possibility to develop new therapeutics⁽³⁾.

The development of new drug alone is not sufficient to provide the base for the progress in drug therapy and the *in-vitro* data obtained from various experiments are very often followed by disappointing results *in vivo* due to poor absorption, rapid metabolism and elimination lead to insufficient drug concentration at the specific site, high fluctuation of the plasma levels due to unpredictable bioavailability after oral drug administration with poor drug solubility.⁽⁴⁻⁶⁾

The nanosuspension is a newer drug delivery system in the pharmaceutical field to overcome all these above problems^(7,8,9). The aim of the present study is to develop polymeric nano suspensions of pramipexole dihydrochloride for treating Parkinson's disease. Pramipexole is a well known antiparkinsonism drug which produces toxicity as a side effect with frequent administration in dose regimen only a minimal amount

of the drug crosses the blood brain barrier^(10,11). Pramipexole dihydrochloride is having manageable bio availability due to its high first pass metabolism and poor penetrability through blood brain barrier due to its hydrophilic nature. Therefore more amount of the drug is required to achieve the required therapeutic activity. But in nanosuspension with small amount of drug which can achieve the better therapeutic activity. Nanosuspension is a novel drug delivery system which makes the drug lipophilic and protect the drug from degradation.^(12,13) Due to this the drug can able to penetrate the blood brain barrier easily for targeting the brain disorder with increased bioavailability^(14,15). Hence the present study is to formulate and evaluate nano suspension of Pramipexole dihydrochloride for treating Parkinson's disease.

MATERIALS AND METHODS:

Materials:

Pramipexole dihydrochloride, PLGA and Pluronic F68 are purchased from Sigma Aldrich, Bangalore. Other chemicals were purchased from Hi media and Sisco Research Laboratories, India. All the chemicals which were used in the formulation and evaluation were analytical grade.

Compatibility studies:

Fourier Transform Infra Red Spectroscopy (FTIR) studies:

The Fourier transform infra red analysis was conducted for the structure characterization. FTIR spectra of the Pramipexole dihydrochloride, polymer and in combination were recorded. FTIR spectra were recorded on Bruker Alpha – T Spectrophotometer. Test samples were mixed with KBr, pressed into a pellet and scanned from 400 to 4000 cm⁻¹.

Differential Scanning Calorimetry (DSC):

Samples were analyzed by differential scanning calorimetry (DSC), with a Shimadzu DSC T-60 using nitrogen gas. The sample was poured in an Al crucible, which was then sealed. The sample was kept at 25°C for 10 min, and heated from 25 to 250°C at a scan rate of 5°C/min.

Preparation of Pramipexole dihydrochloride nanosuspension:

Preparation of Pramipexole dihydrochloride nanosuspension is carried by modified nano precipitation method^(16,17). In modified nanoprecipitation method, phosphate buffer with pH 9.0 was used as external medium instead of aqueous phase. Various concentration ranging 10- 50 mg of PLGA and 10 mg pramipexole were accurately weighed and dissolved in 5 ml acetone. This organic solution was added slowly to Pluronic F 68 (1%) in phosphate buffer (pH 9.0) solution. The organic

solvent was then allowed to evaporate for 2 h with continuous stirring on a magnetic stirrer (Remi). The NP suspension was then centrifuged at 15,000 rpm for 30 min at 4°C using high-speed centrifuge (Remi). Supernatant was taken for further evaluation. Formula for the pramipexole nanosuspension is given in the table-1.

Table-1: Formula for Pramipexole dihydrochloride nanosuspension

	PPNP 1	PPNP 2	PPNP 3	PPNP 4	PPNP 5
Premipexole (in mg)	10	10	10	10	10
PLGA (in mg)	10	20	30	40	50
F 68 (in %)	1				
SPEED (in rpm)	1000				
TIME (in hr)	2				

Characterization of nanoparticles:

The optimized nanoparticles containing Pramipexole dihydrochloride were characterized by studying various physico-chemical properties.

Particle size and Zeta potential:

The size of the prepared nanoparticles was analyzed by using malvern apparatus. All samples were diluted with ultra purified water and the analysis was performed at a scattering angle of 90° and at a temperature of 25°C. The mean diameter for each sample and mean hydrodynamic diameter was generated by cumulative analysis in triplicate. Nanoparticles were characterized with Zeta potential using a Zeta Sizer. The measurements were performed using an aqueous dip cell in an automatic mode by placing diluted samples in the capillary measurement cell and cell position is adjusted.

Scanning Electron Microscopy (SEM):

The surface morphology of the particles were studied using Scanning Electron Microscopy Quanta 200 FEG scanning electron microscope (FEI Quanta FEG 200) set at 200 kV by placing an air dried nanoparticle suspension on copper electron microscopy grids and the image was captured at desired magnification.

Transmission electron microscopy (TEM):

TEM analysis of the prepared formulations was carried out to understand the morphology of nanoparticles. A drop of NPs suspension containing 0.01% of phosphotungstic acid was placed on a carbon film coated on a copper grid for transmission electron microscopy. TEM studies were performed at 80 kV. The copper grid was fixed into sample holder and placed in vacuum chamber of the transmission electron microscope and observed under low vacuum, and the images were recorded.

Drug content:

Drug content was determined by taking 1ml of the PLGA Nanoparticles loaded Pramipexole dihydrochloride. To this formulation 1ml of aqueous potassium dihydrogen phosphate solution (30mM) was added and the mixture was centrifuged at 33,000 xg at 15°C. The clear supernatant was removed and analysed spectrophotometrically and drug content was calculated.

Drug entrapment efficiency:

The drug loaded nanoparticles are centrifuged at 13000xg for 30 min and the supernatant is assayed for non-bound drug concentration by spectrophotometer. Entrapment efficiency was calculated as follows:

$$DEE\% = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

In vitro release studies:

In vitro release studies were performed using diffusion test apparatus USP-II at 50rpm⁽¹⁸⁾. 10ml of the nanoformulation was placed in dialysis membrane having molecular weight cut-off from 12000 to 14000 daltons. Membrane was soaked in phosphate buffer saline for 12 hours before using. Pramipexole dihydrochloride formulation in dialysis membrane was placed in the bowl containing 100 ml of phosphate buffer saline pH 7.4 at fixed time intervals, 1 ml of the aliquot was withdrawn and fresh phosphate buffer saline pH 7.4 was replaced to maintain constant volume.

In- Vitro Studies:

Analysis of Cell Viability:

To evaluate the activity of Pramipexole dihydrochloride nano-suspension protection against Parkinson's disease MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) assay was performed as an in vitro model⁽¹⁹⁾. The MTT assay where performed by using SH-SY5Y cells lines, the cells are plated separately in 96 well plates at a concentration of 1×10^5 cells/well. After 24 hours cells were washed twice with 100 µl of serum-free medium and starved for an hour at 37°C. After starvation, cells were treated with the test material for 24 hours. At the end of the treatment period the medium was aspirated and serum free medium containing MTT (0.5 mg/ml) was added and incubated for 4 h at 37°C in a CO₂ incubator. The MTT containing medium was then discarded and the cells were washed with PBS (200 µl). The crystals were then dissolved by adding 100 µl of DMSO and this was mixed properly by pipetting up and down.

**RESULTS AND DISCUSSION:
COMPATIBILITY STUDIES:**

Fourier transforms infra red spectroscopy (FTIR):

The FTIR spectrum of Pramipexole dihydrochloride, polymer, and the mixture of both drug and polymer were carried out by KBr pellet technique using FTIR spectrophotometer.

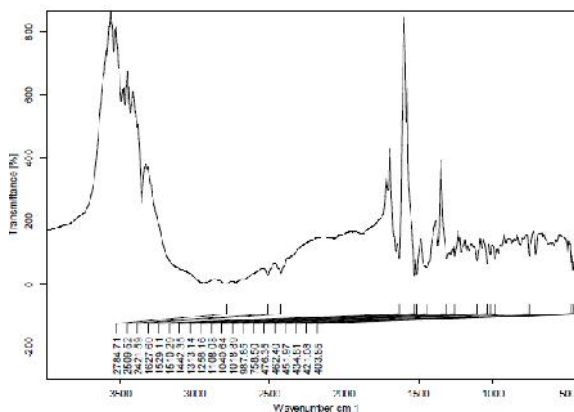


Fig 1: FTIR spectrum of Pramipexole

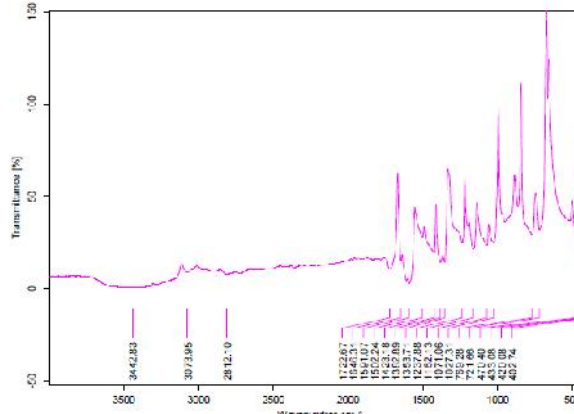


Fig 2: FTIR spectrum of PLGA dihydrochloride

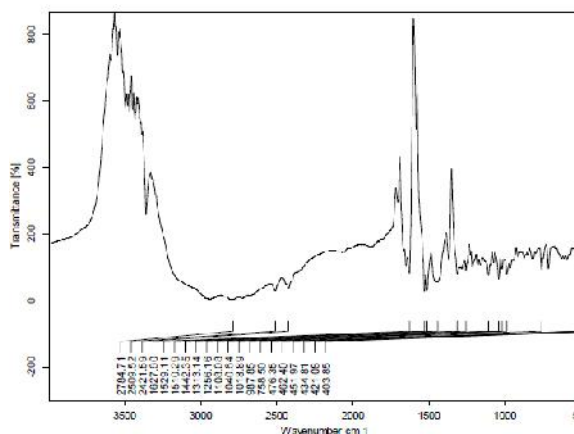


Fig 3: FTIR spectrum of Pramipexole dihydrochloride and PLGA

A sharp peak obtained at 3489cm^{-1} showed an NH stretching of Pramipexole dihydrochloride, peak obtained at 1256cm^{-1} showed a C-O stretching of PLGA and similar peaks were observed in combination mixture. There is no significant changes observed in Pramipexole dihydrochloride and PLGA mixture spectra and it will conclude that there was no incompatibility and significant interaction between drug and polymer.

Differential Scanning Calorimetry (DSC):

The DSC studies were carried out by Differential Scanning Calorimeter.

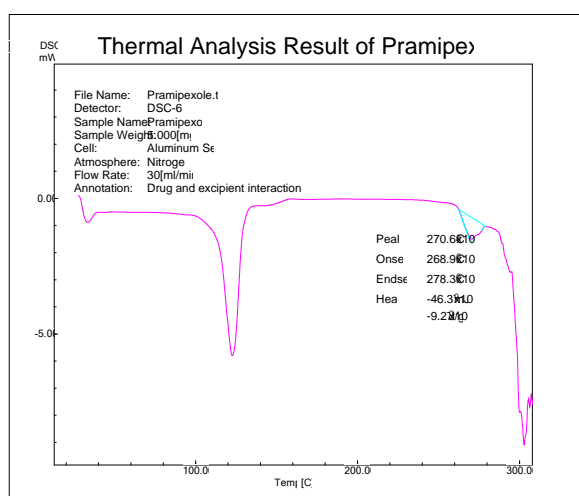


Fig 4: DSC of Pramipexole dihydrochloride

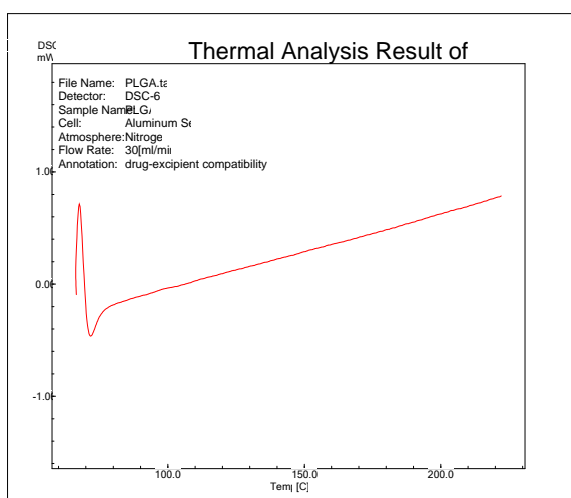


Fig 5: DSC of PLGA

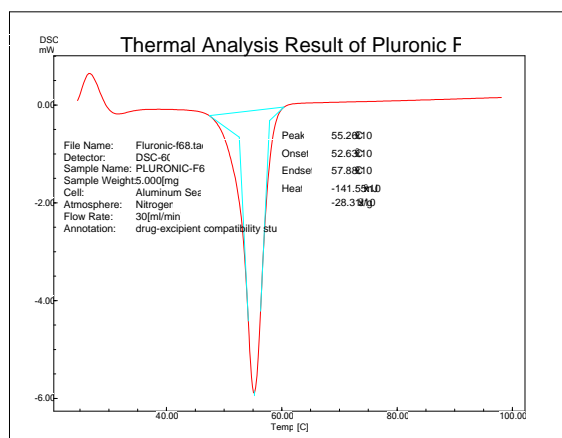


Fig 6: DSC of Pluronic F68

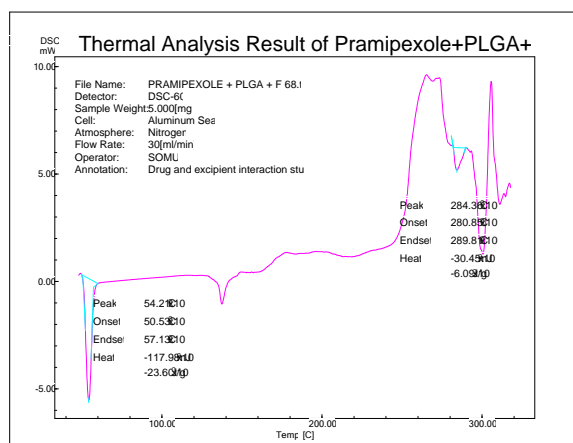


Fig 7: DSC of a mixture of Pramipexole

Dihydrochloride PLGA-Pluronic F68:

A sharp exothermic peak for Pramipexole dihydrochloride and PLGA was obtained at 270.64°C, 55.26°C and similar exothermic peaks was observed in combination of both drug and polymer. There is no significant interaction observed between drug and polymer.

Particle size and Zeta Potential analysis:

The particle size analyses were carried out for the nanoformulations prepared with different concentration of polymer. The particle sizes were shown in the following table 3 which describes increase in polymer concentration having an impact on particle size. The pramipexole dihydrochloride loaded PLGA nanoformulations of 1:3 shows 195 nm in the table is chosen as better particle size because other ratio of 1:1, 1:2 having less polymer so that more drug may not be encapsulated. On the other hand 1:4, 1:5 are having more amount of polymer with high zeta potential, hence the 1:3 ratio with 195 nm particle size with 34.8 mv zeta is chosen due to the higher value of zeta potential implies

more stable The size and stability may be compromised to achieve a better bioavailability.

Table- 2: Particle size and Zeta Potential analysis of pramipexole dihydrochloride nanosuspension

S. NO.	FORMULATION	RATIO	PARTICLE SIZE (nm)*	ZETA POTENTIAL (mV)*
1.	PPNP1	1:1	160±1.5	27.2± 1.7
2.	PPNP2	1:2	183±1.6	30.4±1.1
3.	PPNP3	1:3	195±2.8	34.8±1.5
4.	PPNP4	1:4	223±2.3	34.4± 1.4
5.	PPNP5	1:5	240± 2.4	34.2±2.3

* Values indicated in the results of triplicate trials ± s.e.m

Scanning Electron Microscopy (SEM):

The SEM analysis was performed for the polymeric nanosuspension after selecting appropriate field and magnification. The SEM photographs are shown in fig 8 & 9 shows the morphological characters of PPNP 3 nanoformulation. It is conferred that the particles are in nano sizes and the particle shape was found to be spherical shaped in appearance.

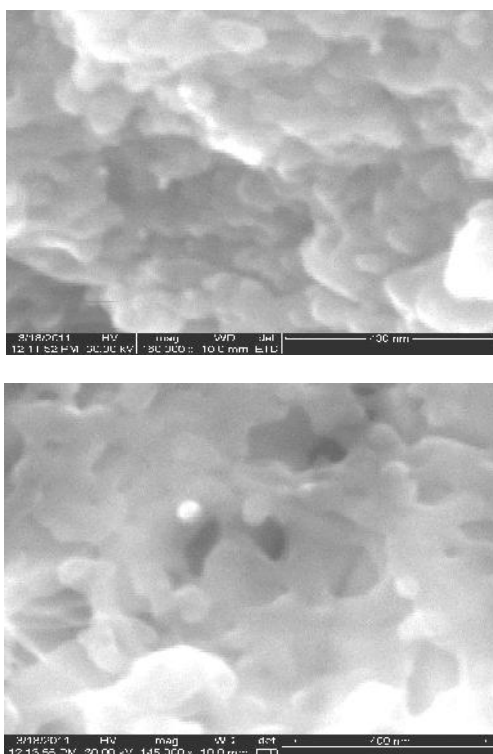


Fig 8: SEM photos of pramipexole dihydrochloride nanosuspension (PPNP3)

Transmission electron microscopy (TEM):

TEM image shows internal composition such as morphology, crystallization, stress or even magnetic domains The fig 10 and 11 shows that the polymeric nanoparticles of PPNP3 were smooth, spherical and uniform.

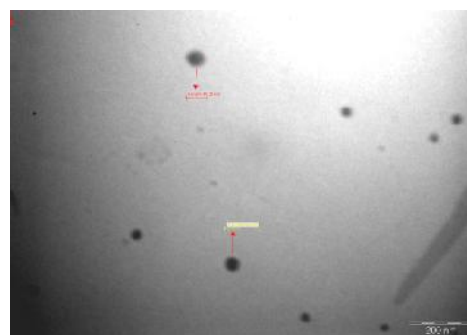
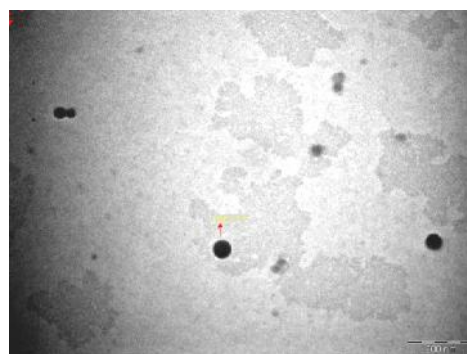


Fig 9: TEM photos of pramipexole dihydrochloride nanosuspension (PPNP3)

Drug content and entrapment efficiency:

The drug content of the prepared nanoformulations where determined and the results shows 0.57 mg/ml with 86.21% entrapment efficiency in PPNP3 with higher drug content having more entrapment efficiency which was shown in table 4, since other formulation shows less drug content when compare to PPNP3 with varying entrapment efficiency. Thus based upon result PPNP3 may be best formulation among the remaining nano suspension.

Table 3: Drug content and entrapment on efficiency of pramipexole dihydrochloride nanoparticle formulations

Formulation	Average drug content mg/ml*	Average entrapment efficiency (%)*
PPNP1	0.35±0.03	67.28% ± 2.2
PPNP2	0.49±0.05	75.53% ± 1.2
PPNP3	0.57±0.02	86.21% ± 2.6
PPNP4	0.55±0.04	86.13% ± 1.8
PPNP5	0.54±0.03	85.89% ± 2.1

* Values indicated in the results of triplicate trials ± s.e.m

In vitro release studies:

The table shows *in vitro* drug release of all formulations; in that, PPNP1, PPNP2 shows the highest drug release but was rejected as the particle size was very high PPNP 4 and PPNP 5 were not selected despite low particle size because they act as release retardants due to a high concentration of polymer. So PPNP 3 is selected as the best formulation due to its optimum drug release 95.2 in 24hrs . The selected formulation was taken for further cell viability studies and *in vivo* studies.

Table - 4: *In vitro* release studies of pramipexole dihydrochloride nanosuspension

S. No.	TIME (HOURS)	% CUMULATIVE DRUG RELEASE*				
		PPNP1	PPNP2	PPNP3	PPNP4	PPNP5
1.	0	0	0	0	0	0
2.	1	12.3± 1.1	11.2± 1.1	7.2± 1.5	6.7± 0.7	6.1± 0.6
3.	2	36.1± 1.8	28.7± 2.2	10.8± 1.3	11.1± 1.2	9.2± 1.2
4.	4	58.2± 3.3	45.1± 2.4	21.2± 2.5	22.7± 3.2	19.9± 2.6
5.	6	75.9± 2.2	59.5± 3.3	33.5± 3.3	35.4± 2.6	30.7± 2.1
6.	8	77.4± 3.1	74.3± 3.2	46.1± 3.4	52.1± 2.3	43.4± 3.1
7.	12	99.9± 1.1	88.4± 2.3	62.6± 1.2	61.3± 3.4	59.3± 2.9
8.	16	100± 0.3	97.9± 3.4	71.3± 3.6	73.8± 2.8	60.5± 2.7
9.	20	-	100± 0.5	85.7± 1.5	75.3± 3.1	65.7± 2.2
10.	24	-	-	89.2± 2.4	76.8± 2.6	72.1± 1.8

* values indicated in the results of triplicate trials ± s.e.m

Cell Viability studies:

Cell viability studies were performed by MTT assay. It depends on mitochondrial dehydrogenases for MTT conversion and these enzymes are also inhibited by MPP. As shown in Figure MPP significantly decreased cell viability. The protection of pramipexole dihydrochloride NPs suspension on MPP was assayed at various concentrations from 1 to 50 µg/ml. It showed dose-dependent protection at concentrations of 5, 10 and 50 µg/ml. The protections of cells at 50 µg/ml of pramipexole dihydrochloride NPs suspension was nearly equal to control.



Fig 10: The plates of control

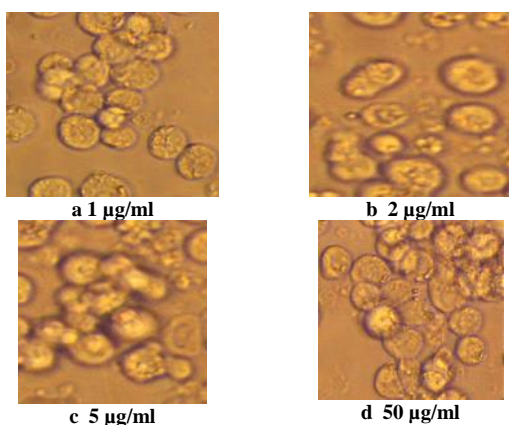


Fig 11: The plates of MPP and pramipexole dihydrochloride pure drug in different concentration

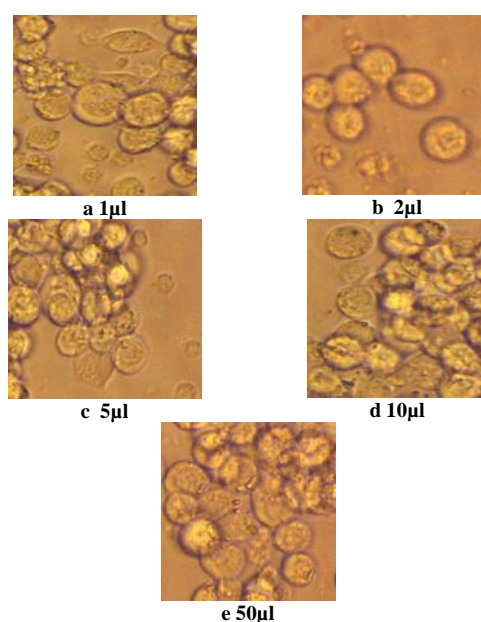


Fig 12: The plates of MTT assay of MPP and pramipexole dihydrochloride nanosuspension in different concentration

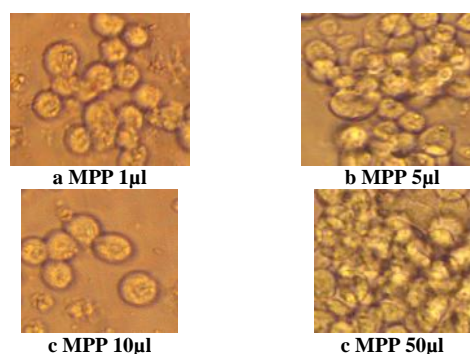
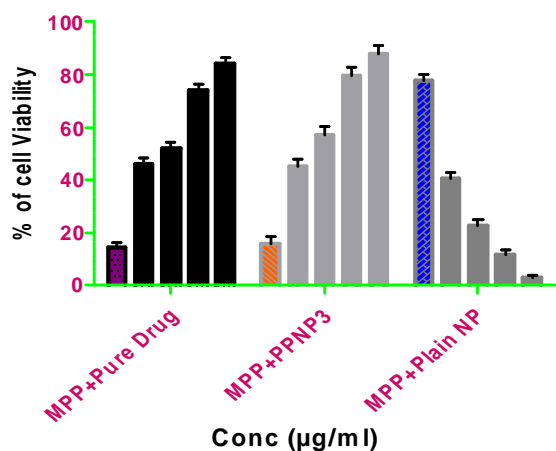


Fig 13: The plates of MTT assay of MPP in different concentration



Graph 1: The graph of MTT assay of MPP, pramipexole dihydrochloride nanosuspension and pure drug in different concentration

CONCLUSION:

There was no incompatibility observed between drug and polymer. The particle size was found to be 195 nm for formulation PPNP3. The entrapment efficiency was found to be 86.21% for formulation PPNP3. More than 85% drug release was observed in PPNP3. The zeta potential of PPNP3 was found to be 34.8mV. PPNP4 were rejected due to high content of polymer. Therefore, PPNP3 were chosen as the best formulation as they has better release than other formulations. The cell viability in SH-SY5Y cells were performed in that the protections of cells at 50 µl/ml of pramipexole diHydrochloride NPs suspension was nearly equal to control.. MPP caused a significant decrease in cell viability and NPs suspension showed dose-dependent protection. Thus, the biodegradable polymers which help in production of more controlled release dosage form for treatment in Parkinson's disease.

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