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Formulation And In - Vitro Evaluation Of Ropinirole Nano Suspension

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ABSTRACT:

Ropinirole (RR) is a non-ergoline dopamine agonist and is the drug of choice in the treatment of patients with Parkinson's disease and restless leg syndrome. The drug is practically insoluble in water and poses formulation challenges when conventional techniques are attempted in the preparation of a dosage form.Keeping in view of itslow oral bioavailability,the present study was designed and undertakento prepare and characterize anano-Suspension (NS) to enhance its solubility and dissolution characteristics.NS was prepared by homogenization followed by ultrasonication method. Prepared NS was characterized forsolubility, drug content, particle size, surface morphology, sedimentation, stability and in-vitro drug release.The Scanning electron microscopy(SEM) image revealed a change in appearance of the surfaceof the dispersed drug particles. The in vitro drug release studies indicated a significant increasein the dissolution rate as compared with pure drug. The Fourier Transform Infra red spectroscopy (FTIR) has suggested no evidence of interaction of drug and excipient.The present study

establishes that the initial crystal structure of RR is reduced when its particle size is reduced and the dissolution properties were improved so that it can be further explored to formulate in the form of a NS with substantial therapeutic gains.

Keywords: Ropinirole, Solubility, FTIR, Scanning Electron Microscopy, Nano- suspension

INTRODUCTION:

A large proportion of new drug candidates coming out of drug discovery programmeshave beenpoorly water-soluble[1]Poor aqueous solubility – an industry wideproblem in drug discovery[2]. These drug candidates were previously called 'brickdust' candidates and can pose formulation and therapeutic difficulties due to their erratic and often variable absorption profiles leading tolow oral bioavailabilities.The performance of the orally administered drug generally depends on its solubility and absorption through thegastrointestinal tract. Therefore, a drug that exhibits poor aqueous solubility and/or dissolution-rate limited absorption possesses low and/or highly variable oral bioavailability.An increase in oral bioavailability can lead to a subsequent reduction in drug dose, making the therapy cost-effective and avoiding any undue drug dumping in the body.

Numbers of formulation approaches are available and employed to resolve the problem of solubility. Traditional strategies, such as micronization,solubilization, use of permeation enhancers and surfactant dispersions have limited use [3]Nanotechnology can be used to overcome the problems associated with the conventional approaches by formulating them into crystalline nanosuspensions (NS). Over the last few decades, nanoparticle engineering has been developed and reported for pharmaceutical applications[4].Nanosuspensionscan tackle the problems associated with the delivery of poorly water-soluble and poorly water- and lipid-soluble drugs, and they are unique due to of their simplicity and the advantages for over other strategies.

A NS can be defined asa submicron colloidal dispersion of drug particles stabilized by surfactants[5].A NS consists of poorly water soluble drug without any matrix material suspended in dispersion[6]NS formulation approach is most suitable to the active pharmaceutical ingredients having high log p value and high melting point. NS, when compared to the coarse suspensions, are more stable because of their uniform particle size.Physical modifications often aim to increase the surface area,solubility and wettability of the powder particles and are therefore focused on particle size reduction or generation of amorphous states[7]

The absence of particles with large differences in their size in NS prevents the existence of different saturation solubilities and concentration gradients, consequently preventing the Oswald ripening

effect.Ostwald ripening is caused by the differences in dissolution pressure/saturation solubility between small and large particles.Drug particle size reduction leads to an increase in surface area and consequently in the rate of dissolution asdescribed by the Nernst–Brunner and Levich modification of the Noyes–Whitney equation [9]

Reducing the particles of the drug to nanosize and employing highly soluble excipients can increase the solubility and dissolution efficiency[10]. Additionally, these formulations can offer the possibility of intravenous administration without the risk of blockade of narrow blood vessels.In recent times, different kinds of strategies have been undertaken to overcome the formulation-related problems associated with the delivery of poorly water soluble drugs in order to improve their therapeutic efficacy and outcomes. The present study was such an effort to formulate NS of RN in order to overcome its solubility and bioavailability problemsRopinirole is a non-ergoline dopamine agonist which is practically insoluble in water. It is the drug of choice for patients with Parkinson's disease and restless leg syndrome.

MATERIALS AND METHODS:

Materials: Ropinirole gift sample procured from Dr.ReddyLaboratories,Hyderabad. Poly Ethylene Glycol (PEG) 400; Tween 80 purchased from S.D.Fine chemicals, Hyderabad.

Methods:

Preparation of ropinirolenanosuspensions (R-NS)Homogenization followed by ultrasonication:Ropinirole powder (0.2%) was dispersed and added to various concentrations (2.0%-8.0%) of PEG-400(stabilizer) and Tween-80 (surfactant) (1.0%-4.0%) and were triturated in a mortar and pestle by using water as vehicle to form a normal suspension. The resultant suspension was transferred into a beaker and subjected to homogenization at 50-100 rpm for 1 hr[11].The homogenized suspension was ultra -sonicated by using bath sonicator for 15-20 minutes to obtain nanosuspension. The ingredients and their quantity used in the formulation of R-NS are given in table -1.

Table no.1 Formulation design for the preparation of Ropinirolenano suspension

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Ropinirole	0.2 %	0.2 %	0.2 %	0.2 %	0.2 %	0.2 %	0.2 %	0.2 %	0.2 %	0.2 %	0.2 %	0.2 %

Tween-80	1.0 %	2.0 %	3.0 %	4.0 %	1.0 %	2.0 %	3.0 %	4.0 %	1.0 %	2.0 %	3.0 %	4.0 %
PEG-400	2.0 %	2.0 %	2.0 %	2.0 %	4.0 %	4.0 %	4.0 %	4.0 %	8.0 %	8.0 %	8.0 %	8.0 %
Double distilled water	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml	20ml

Effect of homogenization time and speed on particle size:

In order to observe the effect of homogenization time and speed on particle size, homogenization was carried out for different time periods (30min, 45min and 60min) at different speeds i.e, 50 and 100 rpm. The obtained coarse suspensions were suitably diluted and the particle size was measured microscopically.

Effect of ultrasonication time on particle size:

The nanosuspensions obtained after homogenization were subjected to ultrasonication for10,15 and 20 minutes using a bath sonicator. Particle size of the resulted dispersions was measured microscopically by diluting the formulation with aqueous phase.

Effect of stabilizer and surfactant concentration on particle size:

12 R-NS formulations were formulated using 3 different concentrations of PEG (2%, 4%, 8%) and 4 different concentrations of Tween 80 (1%, 2%, 3%, 4%) preparation of R-NS formulations. The particle size of the formulations was measured microscopically to determine the effect of stabilizer and surfactant.

Preparation of optimized formulation:

Taking into account the parameters of optimized process and formulation variables, an optimized formulation was selected. The optimized formulation was then subjected to evaluation.

CHARACTERIZATION OF R-NS:

Measurement of size of optimized R-NS formulations:

Average particle size of optimized formulations of R-NS was measured microscopically using eye piece and stage micrometer. The samples were diluted 1 in 10 with filtered, deionised water and observed

under an optical microscope. At least 100 particles were measured and the average particle size was calculated.

Drug solubility:

A solution, conventional suspension and nanosuspension formulation were compared for the drug solubility in water. 1mg/ml of R-sol, R-susp and R-NS were formulated using water and the solubility of ropinirole in all the three systems were calculated and compared.

Drug content:

A measured quantity of the nanosuspension that is equivalent to 50 mg of ropinirole was taken and mixed with 30 ml of methanol. The mixture was allowed to stand with intermittent sonication to ensure complete solubility of drug. Further the resulting solution was passed through 0.45 µm membrane filter followed by adding ofmethanol to obtain a stock solution of0.5mg/ml. An aliquot of this solution (0.5 ml) was transferred to a volumetric flask and made up to a sufficient volume with methanol to get desired concentration of 25µg/ml. Drug content in the nanosuspension was calculated by measuring the absorbance in Elico UV-Spectrophotometer at 250 nm.

In vitro release of ropinirole from R-NS:

In vitro dissolution study was performed using USP dissolution test apparatus-I (basket assembly). The dissolution was performed using 500ml of 0.1N HCl as dissolution medium maintained at 37 ± 0.5°C at 100rpm speed for drug suspension and drug nanosuspension formulation. Samples (5ml) were withdrawn at regular intervals of 5min for 60mins and replaced with fresh dissolution medium. Samples were filtered and analyzed by measuring the absorbance at 250nm using Elico UV-Spectrophotometer. Dissolution for each formulation was performed in triplicate and mean of absorbance was used to calculate cumulative percent of drug release.

Scanning Electron Microscopy (SEM):

The sample for the SEM analysis were prepared by applying a monolayer of the R-NS dispersion on to one side of double adhesive stub and the stubs were then coated with platinum using the auto fine coater (JFC-1600, JEOL, Japan). The scanning electron microphotographs of R-NS were taken using (JSM-6360, JEOL, Japan) scanning microscope.

Sedimentation rate:

The sedimentation of suspended particle of ropinirolenanosuspension was determined by measuring the changes in nephloturbidimetric units using a digital nephloturbity meter at regular time intervals for a period of 12 hrs.

Storage stability:

To assess the stability of the R-NS formulation, the optimized R-NS was stored at room temperature and at 4^{o}C. The particle size calculated for the formulations on day0, day 30, day 60 & day 90 and was compared as the measure of stability. The formulations which were stored at room temperature turned turbid by the end of 60 days.

RESULTS AND DISCUSSION

Preparation Of Ropinirolenano Suspensions (R-NS)

Homogenization followed by ultrasonication method was used for the preparation of R-NSPolysorbate 80 (Tween 80) was used as non-ionicand PEG- 400 was includedas the stabilizer, as it is a readilyavailable, hydrophilic polymer. The nanosuspensions obtained by this method were found to be quite stable and clear in appearance.

Optimization of process variables:

Effect of homogenization time and speed on particle size: The effect of homogenization time and speed on average particle size of R-NS is investigated and reported in table-2. As the homogenization time was increased, the particle size was found to be decreased and least particle size (2.17 μm) was obtained at 45 min of homogenization at 100 rpm. There was no further decrease in particle size with increase in homogenization time to 1hr at 100 rpm and hence, 45min and 100rpm were considered as optimum time of speed of homogenization.

Table 2: Effect of homogenization time on particle size of R-NS formulations

Homogenization speed	**50rpm**	**100rpm**
Homogenization time	**Average particle size (μm)**	

30 min	4.74	4.03
45 min	2.42	2.17
60 min	2.31	2.18

Effect of Ultrasonication time on particle size:

To reduce the particle size to below 1 micron, bathultrasonicator was used. The effect of ultrasonication time on average particle size of R-NS is shown in Table 3. As the sonication time increased, the particle size was decreased. At the end of 20 min of sonication, least particle size (946 nm) was obtained. Further increase in sonication time did not show reduction of the particle size. Therefore sonication time was optimized as 20 minutes.

Table 3: Effect of ultrasonication time on particle size of R-NS formulations

Ultra sonication time	Average particle size (nm)
10 min	1174
15 min	1035
20 min	946
25 min	948

Formulation of optimized R-NS:

From the optimization of the process variables it was concluded that 45minutes of homogenization at 100 rpm and 20 minutes of ultrasonication were optimum for the production of R-NS. Twelve formulations were prepared, designated as F1 to F12 with varying surfactant (1.0%, 2.0%, 3.0% and 4.0%) and stabilizer (2.0%, 4.0% and 8.0%) concentrations. All the formulations were characterized for their particle size and the values are mentioned in table-4.Based on the result, F7 was selected as the optimized formulation which contained 3.0% surfactant and 4.0% stabilizer concentration.

Table 4 Formulation of optimized R-NS

Conc. Of PEG	2%	4%	8%
Conc. Of Tween 80	Average particle size (nm)		
1%	(F1) 1292	(F5) 1167	(F9) 1079
2%	(F2) 1035	(F6) 996	(F10) 942
3%	(F3) 983	(F7) 880	(F11) 885
4%	(F4) 960	(F8) 904	(F12) 901

Characterization of F7-R-NS:

Measurement of particle size of optimized R-NS:

Dynamic light scattering is a technique used for particle sizing of samples, typically in the sub-micron range. Photon Correlation Spectroscopy (PCS) determines hydrodynamic diameter of the nanoparticles via Brownian motion by employing light scattering technique. The technique measures the time-dependent fluctuations in the intensity of scattered light from a suspension of particles undergoing random, Brownian motion. Analysis of these intensity fluctuations allows for the determination of the diffusion coefficients, which in turn yield the particle size. Average particle size of a conventional ropinirole suspension (R-susp) optimized R-NS formulations was calculated and the values are tabulated in table-5. The particle size distributions are given in Fig 1 and Fig 2.

Table 5 - Measurement of particle size of Ropinirole-NS

Formulation	Average particle size (nm)	Poly dispersity index
R-susp	14238.0 ± 14.53	2.150 ± 0.074
R-NS (F7)	798.0 ± 7.84	0.902 ± 0.010

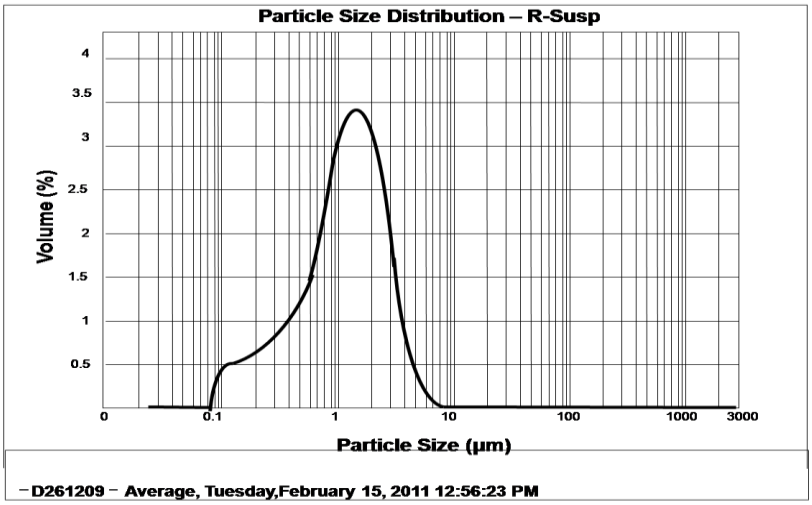

Figure 1

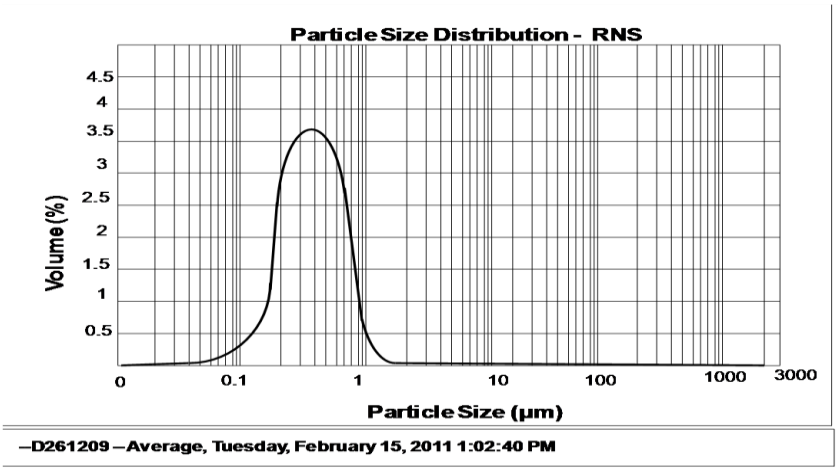

Figure 2

Drug content:

A stock solution of F7 R-NS containing 0.5mg/ml was prepared using methanol. From this stock, 0.5ml was taken and further diluted to 50ml to obtain 25µg/ml. The absorbance was measured by UV spectrophotometer at 250 nm. The amount of drug present in optimized R-NS calculated in terms of mg/ml. The concentration of ropinirole in the optimized formulation F7 was found. The average standard equation y = 0.0132x - 0.0135 (R^2 = 0.9952) was used for calculation. The % drug content was found to be 97.24%.

Estimation of solubility of ropinirole in optimized R-NS

The solubility of ropinirole in the optimized R-NS was determined by the assay method mentioned above. The absorbance of the resultant solution was measured by UV spectrophotometry at 250nm. To know the advantage of formulating nanosuspension, the optimized R-NS was compared to a solution R-sol and a suspension R-Susp regarding solubility. The concentration of all the three, R-sol, R-susp and R-NS were made to 100µg/ml. The solubility of ropinirole when formulated as R-NS was found to increase approximately twice as compared to R-susp and by four times when compared to R-sol.

Measurement of particle size of optimized R-NS

The in vivo performance of nano suspension is related to particle mean size and particle size distribution which will affect saturation solubility and dissolution rate .Dynamic light scattering is a technique used for particle sizing of samples, typically in the sub-micron range. Photon Correlation Spectroscopy (PCS) determines hydrodynamic diameter of the nanoparticles via brownian motion by employing light scattering technique. The technique measures the time-dependent fluctuations in the intensity of scattered light from a suspension of particles undergoing random, Brownian motion. Analysis of these intensity fluctuations allows for the determination of the diffusion coefficients, which in turn yield the particle size. Average particle size of a conventional ropinirole suspension (R-susp) optimized R-NS formulations was calculated and the values are tabulated and particle size distributions are given in Table 6.

Table 6- Measurement of particle size of Ropinirole-NS

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R-susp	14238.0 ± 14.53	2.150 ± 0.074
R-NS (F7)	798.0 ± 7.84	0.902 ± 0.010

Scanning Electron Microscopy (SEM):

The SEM analysis was performed for the SLN after selecting appropriate field and magnification. The SEM image reveals a change in appearance of the surface upon formulating the nanosuspension. The

altered shape might be due to coating of ropinirole with a surfactant/stabilizer layer and creation of an amorphous surface layer due to the high attrition and shearing rate. (Figure -3)

Figure 3 - SEM photomicrograph of R-NS

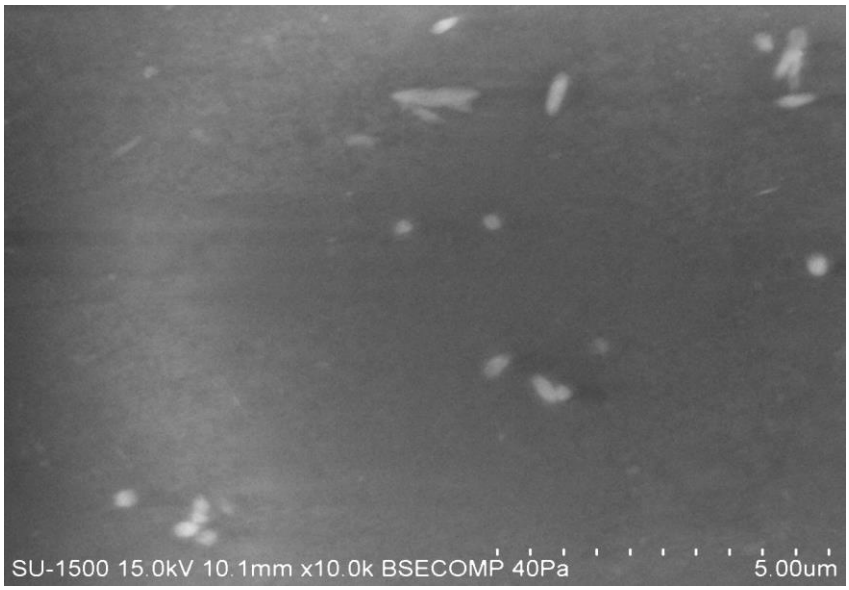


Table 7: Drug content and entrapment efficiency of optimized R-NS formulations

Formulation	Absorbance	Drug content (µg/ml)	Percent drug content
R-NS (F7)	0.321	24.31	97.24 %

Estimation of solubility of ropinirole in optimized R-NS:

The solubility of ropinirole in the optimized R-NS was determined by the assay method mentioned above. The absorbance of the resultant solution was measured by UV spectrophotometry at 250nm. To know the advantage of formulating nanosuspension, the optimized R-NS was compared to a solution R-sol and a suspension R-Susp regarding solubility. The concentration of all the three, R-sol, R-susp and R-NS were made to 100µg/ml. The solubility of ropinirole when formulated as R-NS was found to increase

approximately twice as compared to R-susp and by four times when compared to R-sol. The results were tabulated in table-8.

Table 8 Estimation of solubility of Ropinirole-NS

Formulation	Absorbance	Concentration(µg/ml)
R-sol	0.310	23.48
R-susp	0.562	42.57
R-NS (F7)	1.129	85.53

In vitro release of ropinirolefrom optimized R-NS formulation:

The in vitro release of ropinirole from the optimized R-NS formulation was studied using USP dissolution test apparatus-I (basket assembly). The dissolution was performed using 500ml of 0.1N HCl as dissolution medium maintained at 37 ± 0.5°C at 100rpm speed for R-NS (F7) and R-susp formulations. Samples (5ml) were withdrawn at regular intervals of 5min for 60 mins and replaced with fresh dissolution medium. Samples were filtered through 0.2µ filter paper and assayed spectrophotometrically on Elico UV-Visible spectrophotometer at 250 nm wavelength. Dissolution for each formulation was performed in triplicate and mean of absorbance was used to calculate cumulative percent of drug release table-9. The drug release profile of ropinirole from the optimized R-NS and R-susp is shown in fig 4

Fig 4: in vitro release profile of optimized R-NS formulation

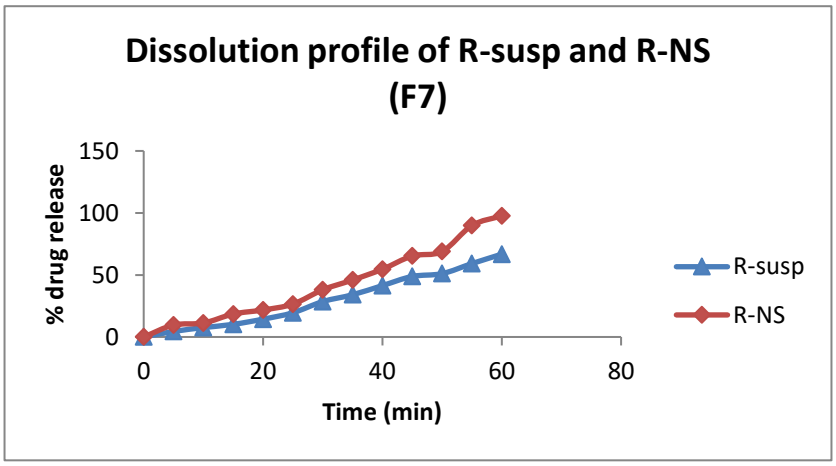


Table 9- In vitro drug release study of R-NS

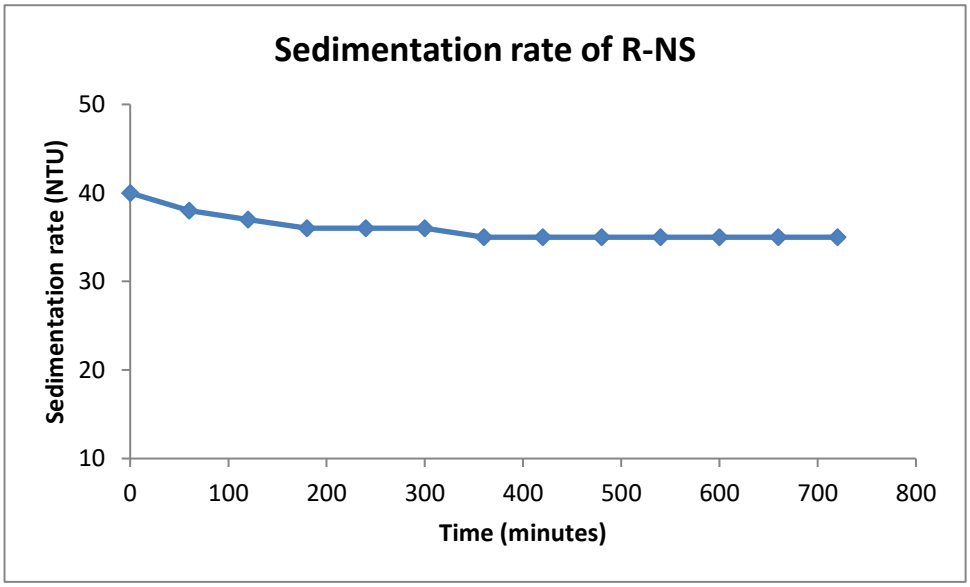
Time (min)	Cumulative % drug release	
	R-Susp	R-NS (F7)
5	4.43 ± 0.04	9.64 ± 0.02
10	7.58 ± 0.05	11.23 ± 0.01
15	10.17 ± 0.02	18.51 ± 0.02
20	14.36 ± 0.03	21.76 ± 0.01
25	19.54 ± 0.01	26.51 ± 0.02
30	28.54 ± 0.01	38.05 ± 0.05
35	34.05 ± 0.06	45.93 ± 0.01
40	41.43 ± 0.04	54.65 ± 0.02
45	48.82 ± 0.03	65.31 ± 0.07
50	51.08 ± 0.01	68.93 ± 0.01
55	59.17 ± 0.08	89.87 ± 0.03
60	66.77 ± 0.01	97.72 ± 0.01

Mean ± Standard deviation (n = 3)

Sedimentation:

The sedimentation of suspended particle of ropinirolenanosuspension was determined by measuring the changes in nephloturbidimetric units using a digital nephloturbidy meter at regular time intervals for a period of 12 hrs. The ropinirolenanosuspension formulation was found to be stable and not much sedimentation of suspended particle could be observed during the period of 12 hours as shown in fig. 5

Figure-5- sedimentation rate of R-NS



Storage Stability of R-NS:

To assess the stability of the R-NS formulation, F7 was stored at room temperature and at 4°C. The particle size calculated for the formulations on day 0, day 30, day 60 & day 90 and was compared as the measure of stability. The formulations which were stored at room temperature turned turbid by the end of 60 days. The impact of storage on the stability at different time intervals is shown in table 10.

Table 10 Storage stability of R-NS

R-NS (F7)	Average particle size (nm)	
	RT	4 ± 1°C
Day 0	880	880

Day 30	983	888
Day 60	1035	896
Day 90	1154	904

Figure-6 FTIR spectra -Ropinirole

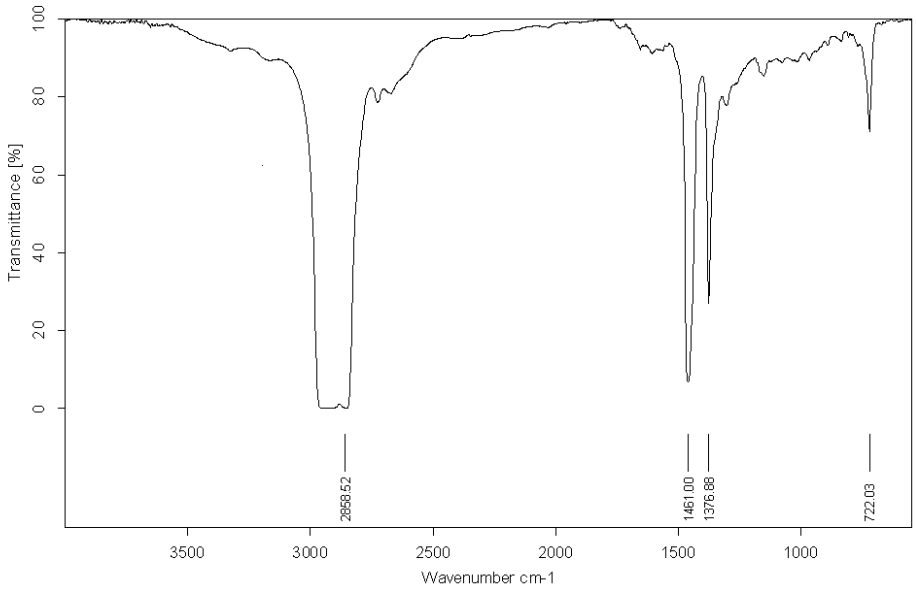


Figure-7 FTIR spectra –Ropinirole+PEG400

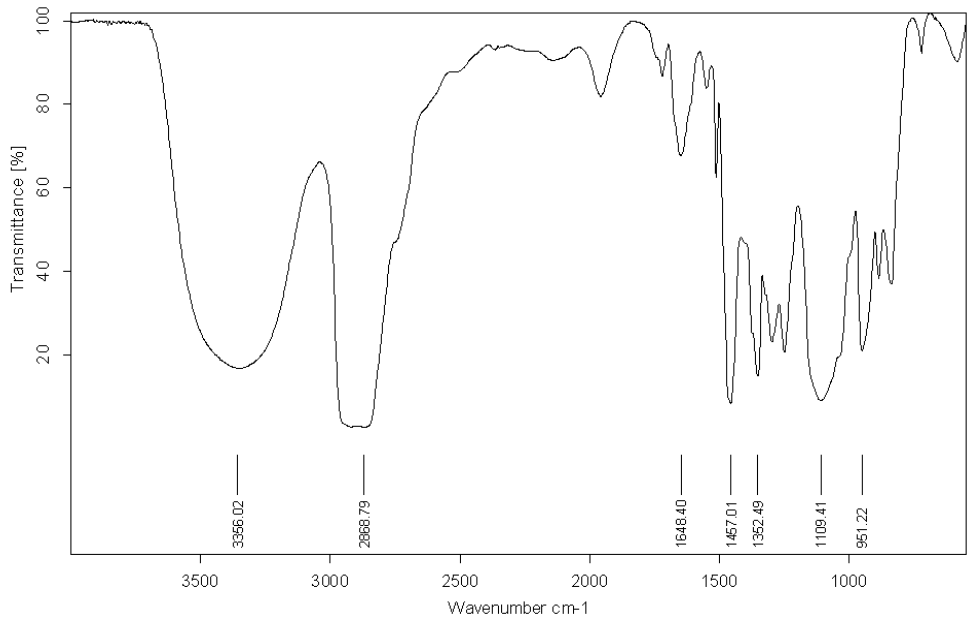
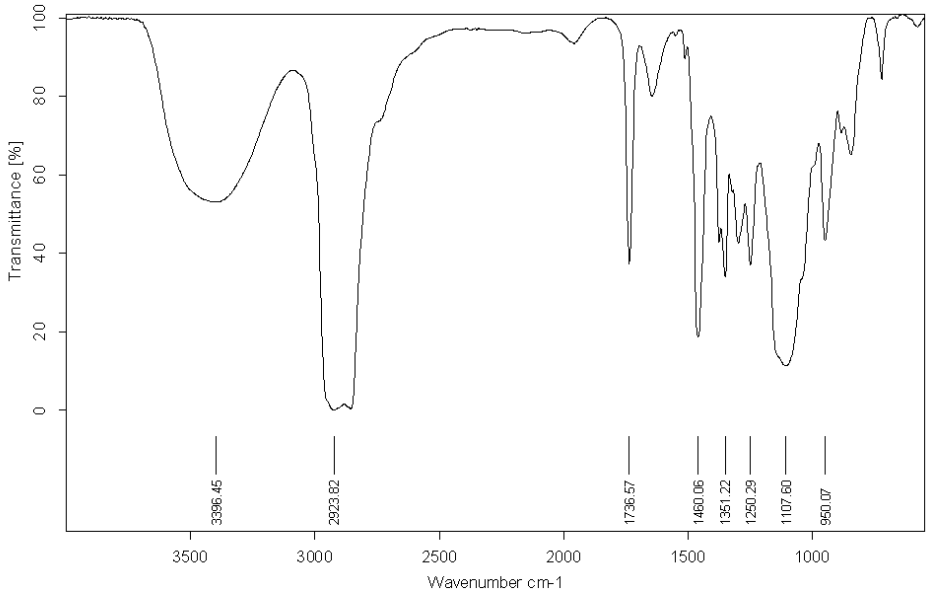


Figure-7 FTIR spectra –Ropinirole+TWEEN 80



Optimization of process variables

Effect of homogenization time and speed on particle size

The effect of homogenization time and speed on average particle size of R-NS is investigated and reported in table 6. As the homogenization time was increased, the particle size was found to be decreased and least particle size (2.17 μm) was obtained at 45 min of homogenization at 100 rpm. There was no further decrease in particle size with increase in homogenization time to 1hr at 100 rpm and hence, 45min and 100rpm were considered as optimum time of speed of homogenization (Table-11)

Table 11: Effect of homogenization time on particle size of R-NS formulations

Homogenization speed	**50rpm**	**100rpm**
Homogenization time	**Average particle size (μm)**	
30 min	4.74	4.03
45 min	2.42	2.17
60 min	2.31	2.18

CONCLUSION:

In this study, an attempt was made to formulate ananosuspensionof ropinirole for oral delivery in order to increase its solubility and consequently itsbio- availability.The results of the % in vitrodrug release studies indicated that there was more than two fold increase in the solubility compared to the solution of the drug and conventional suspension.The results of the stability studies demonstrated that the NS formulations retained their original composition and it was observed that there was no sedimentation.R-NS drug delivery in the form of a nano suspension is a convenient, patient- compliant, novel delivery that could offer many advantages over conventional oral dosage forms.

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