

Rasagiline-Loaded PLGA Nanoparticles: Design and Evaluation

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Abstract

Purpose: In present research work, MAO-B inhibitor rasagiline mesylate (RAS) was loaded in PLGA nanoparticles (PNPs) to improve its oral drug delivery.

Method: To achieve this goal, various formulation trials were generated by Box-Behnken statistical design, prepared and evaluated for entrapment efficiency (EE), particle size (PS) and drug release (DR). These responses were analyzed in terms of statistical which indicate significance of each factor and their best optimum level by using ANOVA.

Result: Optimization studies revealed most significant factor for PNPs and the best combination of A, B & C factor levels for optimized formulation (Fopt) was found to be -1, +1 & +1 respectively. Fopt has shown good EE & PS with well defined, smooth surface particles. The drug was transformed from crystalline to amorphous form when entrapped in nanoparticles, indicating better and improved physicochemical properties of drug for absorption. The Fopt has shown sustained drug release which was eight times higher than control (drug solution). Finally, the formulation was found to be stable (shelf life of 362.53) days at storage condition mentioned by ICH guidelines.

Conclusion: The RAS loaded polymeric nanoparticles were successfully and achieved significant prolonged delivery which can be consider for neurodegenerative disorder, further.

Keywords: RAS; PLGA; Design; Nanoparticles; Optimization; Drug delivery

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Introduction

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder that affects the movement and expression up-to 3% of population in the age range over 65 years of all races. The burden of Parkinson's disease (PD) is likely to increase in the years to come as many countries, particularly in Asia wiz face an ageing population. As such, it has been estimated that the number of individuals suffering PD in the world will double by the year 2030. Due to this increasing prevalence of PD it is essential to device a formulation which shall provide more effective management as well patient compliance with sustained effect to decrease the frequency of dosing [1-3].

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FDA approved (2006) Rasagiline (RAS) is a novel, potent, irreversible and highly selective MAO-B inhibitor that provides a new option in the oral treatment of early and advanced PD [4]. RAS [5-7] is most likely to exert its primary effect in PD by MAO-B inhibition leading to slower metabolism of endogenous and exogenous dopamine thereby eventually causing symptomatic benefits. The selection of the model drug (RAS) rationale is photo stable, having low oral bioavailability (only 36%), at low dose (1mg/daily) showed almost 80% inhibition of MAO-B activity, well tolerated, safe does not produce any toxic metabolites like selegiline (e.g. amphetamine and methamphetamine) [8].

In current research objectives are fabrication of polymeric nanoparticulate [9-11] of RAS intended for oral delivery to improve bio-availability of drug by achieving optimum drug concentration in systemic circulation and provide sustained effect to inhibiting MAO-B enzymes. Therefore, in order to improve this drug therapy of PD, a modified and novel drug delivery system (polymeric nanoparticles; PNP) [12-16] of RAS will be designed in such a manner that it can release drug for prolonged time & consequently cross BBB in order to provide better therapeutic sustained effect through oral route.

Material and Methods

RAS & Poly-(D, L-lactide-co-glycolide; PLGA) was received as a free sample from IntasPharma, Ahmedabad, India and Boehringer Ingelheim, Germany respectively. Polyvinyl alcohol (PVA), Mannitol and disodium hydrogen orthophosphate were purchased from Central Drug House Pvt. Ltd, India. Potassium dihydrogen orthophosphate were obtained from Qualigens fine chemicals, Mumbai, India.

Analytical Methodology

An analytical method of RAS was developed for its quantification [17] in phosphate buffer saline (PBS) pH 7.4 using a double beam UV Visible Spectrophotometer (JASCO V 630). A stock solution (I) of RAS was prepared by dissolving 100 mg RAS in 100 ml PBS pH 7.4 to obtain a concentration of 1000 µg/ml. Further, stock solution (II) was prepared by taking 10 ml of stock solution (I) and diluting it to 100 ml PBS pH 7.4 to obtain a concentration of 100 µg/ml. Aliquots of 2, 6 ml from stock solution (II) and 1.4, 1.8, 2.2, 2.6 ml from stock solution (I) were withdrawn respectively and volume was made up to 10 ml to get concentrations of 20, 60, 100, 140, 180, 220 and 260 µg/ml. These solutions were analyzed using UV-visible Spectrophotometer at 271 nm and absorbances were recorded. The procedure was followed in triplicate to minimize the chances of error.

Preliminary trials

To select the factors and their experimental ranges (process variables & excipient having major influence on PNP) beginning trials were done by characterized EE & PS. Polymer concentration (A), stabilizer concentration (B) and sonication time (C) were found to be the major factors affecting efficiency of PNP. These factors and their ranges were fitted in factorial design to give formulations with their best possible combinations.

Design

A Box-Behnken design was selected for statistical optimization of formulation parameters by investigating effects of factors on encapsulation efficiency (A), particle size (B), drug release (C) of RAS loaded nanoparticles. It was used to determine optimal condition of all factors by estimating experimental conditions having minimum variability using analysis of variance (ANOVA) (18-20). The factors for design were selected on the basis of literature and preliminary trials performed and used at three different levels. The levels of factors and responses are coded as shown in Table 1.

Preparation of RAS loaded PNP

The PLGA nanoparticles (PNP) of RAS were prepared using "Double Emulsion Method" [21]. The procedure was carried out in two stages by preparing primary and secondary emulsions. The first step i.e. primary emulsion was prepared by dissolving of drug (10 mg) in aqueous phase (1 ml) and adding it to the solution of poly-(d, l-lactide-co-glycolide) (PLGA in 4ml dichloromethane) and sonicated (5

minutes) in a probe sonicator on an ice bath for complete preparation of the emulsion of the two phases. Further, preparation of secondary emulsion was done by adding primary emulsion to a known concentration of aqueous solution (16 ml) of PVA and again sonicated for a specified amount of time on an ice bath.

Factors				Levels		
Independent	Agent	Codes	Unit	-1 (Low)	0 (Central)	+1 (High)
Polymer	PLGA	A	mg	40	60	80
Stabilizer	PVA	B	% w/v	0.6	0.9	1.2
Sonication time	C	minutes	1.0	3.0	5.0	
Response	Abbreviation	Codes	Unit	Constraints		
Entrapment efficiency	EE	Y1	%	Maximize		
Particle size	PS	Y2	nm	Minimize		
Drug release	DR	Y3	%	Optimum		

Table 1: Design coded levels of factors and responses.

The following double emulsion was then kept on a magnetic stirrer at 300 rpm for 4 hrs for complete removal of organic phase. The drug loaded nanoparticles were collected after centrifugation at 13400 rpm for half an hour and lyophilized using mannitol (2.5% as cryoprotectant) and stored at 4°C. All 20 formulation trails were prepared in triplicate and evaluated for EE, PS & drug release.

Evaluation

The samples were analyzed for physicochemical interaction between drug and polymer by FT-IR analysis. The surface morphology of drug loaded PNPs was analyzed using Transmission Electron Microscopy (TEM). For TEM analysis, a drop of nanoparticles dispersion diluted with distilled water (dH₂O) was negatively stained with phosphor-tungstic acid (2% w/v) and placed on a 400-mesh TEM copper grid coated with carbon film and dried at room temperature. Then, the slide was examined under transmission electron microscope using TEM CM-10 Philips, operating at 60-80 KV at different magnifications.

EE and PS Analysis

The entrapment efficiency (EE) of drug loaded PNPs-1 to 20 (Table 2) was determined by "Indirect Method" by estimating total amount of free drug present [21-22]. In this, aqueous dispersion of drug loaded nanoparticles was centrifuged at 13400 rpm for half an hour and supernatant was withdrawn carefully without disturbing the cake left at bottom of Eppendorf.

The supernatant was diluted sufficiently and analyzed spectrophotometrically at 271 nm for estimation of free drug present in nanoparticles dispersion. The PS of all formulations of RAS loaded PNPs were determined by Photon Correlation Spectroscopy using Particle Size Analyzer (Malvern Zeta SizerNano ZS, UK). The samples were appropriately diluted with dH₂O before analysis and the analysis was performed at a scattering angle of 90° and a temperature of 25°C. The total amount of drug encapsulated was calculated using below formula equation;

$$\% \text{ Encapsulation Efficiency} = \frac{\text{Total amount of drug added in formulation} - \text{Amount of free drug present in supernatant}}{\text{Total amount of drug added in formulation}} \times 100$$

In vitro Release [23-24]

Preparation of dialysis Bag

The dialysis tubing was cut into small pieces of desired length and immersed & soaked in warm dH₂O (250 ml) for half an hour. After that, they were immersed in sodium sulphide (0.3%, 250 ml) and boiled for 5 min. The tubings were then rinsed thoroughly with warm dH₂O and immersed in sulphuric acid (2%, 250 ml) and boiled again for 5 min. The dialysis tubings were rinsed thoroughly again with fresh warm dist. H₂O for 10 min to get rid of any chemical residue. Afterwards, dH₂O was decanted and tubings were submerged completely in freshly prepared phosphate buffer saline (PBS pH 7.4 250 ml). The tubings were stored at 4°C till 24 hrs before use.

Release study

In vitro release study was performed in PBS pH 7.4 for 48 hrs over drug solution as control and nanoparticles dispersion (equivalent to 2 mg drug contained in dialysis bag was placed in a beaker containing 50 ml of fresh PBS pH 7.4 kept on a magnetic stirrer at 37°C). At fixed time 0.5, 1, 2, 4, 6, 12, 18, 24, 36 and 48 hrs intervals; 5 ml samples were withdrawn from receptor compartment and same volume of fresh PBS pH 7.4 was replaced in receptor medium immediately after each withdrawal to maintain sink condition. The samples were diluted sufficiently and analyzed using UV Visible Spectrophotometer at 271 nm.

Mechanism of drug release

The drug release kinetic of was determined by fitting the release data in kinetic models; Zero, First, Higuchi order models Korsmeyer-Pappas, Hixson Crowell and assessing the line of best fit. The mechanism of drug release from formulation was investigated by incorporating 60% release data of $t_{0.6}$ in equations of kinetic model to calculate exponent value of n from slope of straight line graph and interpreted release mechanism.

Accelerated stability studies

The stability study of optimized formulation was performed as per ICH guidelines Q1A (R2) [25-27]. The optimized formulation packed in an ambered colored tightly closed glass vial was stored at normal and accelerated storage conditions i.e. $5 \pm 3^\circ\text{C}$ and $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{RH}$ respectively for two months and analyzed after specific time intervals 0, 15, 30 and 60 days for any change in physical and chemical characteristics.

Result and Discussion

The absorption maxima (λ_{max}) of RAS in PBS pH 7.4 was found to be $271 \pm 1.0 \text{ nm}$ and obeyed linearity between 20-240 $\mu\text{g/ml}$ (Beer's Range) with regression coefficient of 0.999 and a straight line of $y=0.0039x-0.0085$ (Figure 1). Another main variable (other than API) of PNPs include polymer, stabilizer and solvent system. The first two variables were selected on the basis of literature wherein PLGA was selected as a polymer due to its predictable degradation behavior, biodegradability, biocompatibility, and sustained release pattern of delivery system & ease of fabrication. The experimental range of polymer concentration was selected by evaluating the batches of PNPs prepared with different polymer concentration, maintaining all other parameters constant. Polymer concentration below 40 mg showed poor EE with small PS, whereas above 80 mg showed good EE but large PS. Therefore, 40-80 mg was selected as an experimental range of polymer concentration.

Polyvinyl alcohol (PVA) was selected as a stabilizer due to its excellent stabilizing capacity & biocompatibility. Various concentrations of polyvinyl alcohol (PVA) was tried to prepare PNPs. The EE of PNPs having less than 0.6% w/v stabilizer concentration was comparatively low due to insufficient amount of stabilizer present to encapsulate the amount of drug used. In contrast, more than 1.2% w/v of stabilizer concentration showed very low EE and PS due to presence of excess stabilizer leading to break down of intact particles into smaller fragments resulting into drug leakage. Therefore, a range of 0.6-1.2% w/v of stabilizer concentration was selected for the formulation of PNPs.

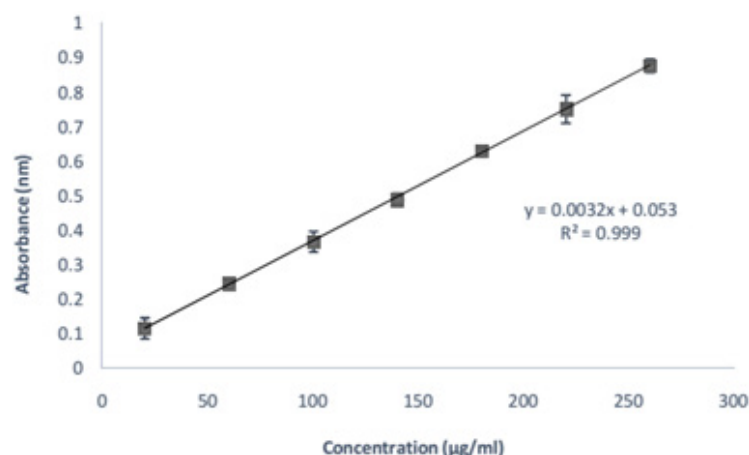


Figure1: Standard Calibration Curve of RAS in PBS pH 7.4.

However, selection of the sonication time, batches were prepared by altering time to obtain the secondary emulsion. Significant variation in particle size was observed by taking various time limits. Sonication time less than one minutes resulted in larger PS where as more than 5 minutes again resulted into larger PS due to agglomeration of the very small fragments of PNPs. Therefore, a limit of 1-5 min of sonication time was selected best fit to obtain PNPs of favorable PS. From preliminary trials, it could be concluded that the polymer, stabilizer concentration & sonication time critically influence the performance of PNPs and their experimental ranges were found to be 40-80 mg, 0.6-1.2% & 1-5 min respectively.

Box-Behnken Design

As revealed by preliminary trials, polymer, stabilizer concentration and sonication time were found to be the critical parameters for PNPs and thus, were taken into consideration as independent variables (factors) and were coded as A, B, C respectively. Total 20 experimental runs/trials (formulations) were generated by design expert with all the possible combinations of factors, as shown in Table 1. Based on design, formulations were prepared using double emulsion method and analyzed in terms of encapsulation efficiency (EE), particle size (PS) and drug release (DR) as dependent variables and were coded as Y1, Y2& Y3 respectively (Table 2).

Std	Run	A:PLGA (X ₁)	B:PVA (X ₂)	C:ST (X ₃)	%EE (Y ₁)	PS (Y ₂)	DR (Y ₃)
		Experimental					
		mg	%	rpm	%	Nm	%
13	1	60	0.9	1	37.60	402.70	97.80
3	2	40	1.2	1	46.90	443.50	96.31
15	3	60	0.9	3	46.30	378.70	97.50
12	4	60	1.2	3	42.70	378.50	97.87
1	5	40	0.6	1	35.10	415.70	97.10
19	6	60	0.9	3	43.60	378.70	97.80
7	7	40	1.2	5	62.10	338.60	94.70
8	8	80	1.2	5	47.40	360.50	96.32
20	9	60	0.9	3	43.60	412.30	97.37

10	10	80	0.9	3	53.60	495.20	94.85
6	11	80	0.6	5	47.70	347.80	96.46
11	12	60	0.6	3	35.80	341.10	98.21
17	13	60	0.9	3	43.60	378.70	97.80
14	14	60	0.9	5	44.70	270.40	97.23
2	15	80	0.6	1	54.80	539.80	95.61
9	16	40	0.9	3	53.80	423.90	95.10
16	17	60	0.9	3	43.60	378.70	97.80
4	18	80	1.2	1	48.10	540.60	96.62
5	19	40	0.6	5	47.30	298.80	96.58
18	20	60	0.9	3	43.60	378.70	97.80

Table 2: Formulation trials with Responses (EE, PS & DR).

Data Analysis: Equations and Models

A three factor, three levels Box-Behnken statistical experimental design as the RSM requires 20 runs. The total 20 runs with triplicate center points were generated and the responses so observed are shown in (Table 2). All the responses obtained for 20 formulations prepared were simultaneously fitted to first order, second order and quadratic models using Design Expert. The best-fitted model was quadratic and the comparative values of R², SD and %CV are given in along with the regression equation generated for each response. All statistically significant ($p < 0.05$) coefficients are included in the equations. A positive value represents an effect that favors the optimization, while a negative value indicates an inverse relationship between factors and the response.

Polynomial equation

Response 1 (Y1): Entrapment efficiency

The model purports the following polynomial equation for entrapment efficiency of PNPs $Y_1 = 43.66 + 0.64A + 2.65B + 2.67C - 4.20AB - 4.40AC - 4.40BC + 10.62A^2 - 3.83B^2 - 1.93C^2$ Where Y₁ is the entrapment efficiency of PNPs, A is the polymer conc., B is the stabilizer conc. and C is the sonication time. The model F-value of 56.18 implies that the model is significant ($p < 0.0001$). The lack of fit F-value of 1.56 implies that the lack of fit is not significant. In this case X₁X₂, X₁X₃ & X₂X₃ are significant model terms and X₁X₂ had a more pronounced effect on entrapment efficiency of PNPs than any other parameters. The predicted R-squared of 0.8844 is in reasonable agreement with the adjusted R-squared of 0.9632. Adequate precision is within desirable limit. A ratio of 31.576 indicates an adequate signal. Therefore this model can be used to navigate the design space. The contour plots (Figure 2) which showed the effect of different independent variables on entrapment efficiency of PNPs (Y₁). The EE of PNPs for all batches was found to be in the range of 35.10-62.10%.

From the experimental data it is inferred that the polymer conc. of variable A at a level 0 (60 mg), the EE was found to be more as compared to the polymer conc. at +1 level (80 mg). An optimum level of EE was obtained at level -1. %EE is bound to increase with increase in polymeric conc. owing to the presence of sufficient polymer in the formulation but further increase in conc. leads to decrease in the %EE. These effects are attributed to increased resistance against emulsification of nanoparticles in external aqueous phase due to increasing viscosity of polymeric solution which reduces its dispersibility in aqueous phase resulting in formation of larger particles. It leads to formation of particles having higher EE due to low diffusion of drug from polymeric solution to external phase. But after certain level, encapsulation reduces with polymer concentration due to increase in intrinsic viscosity of polymeric solution during double emulsification which restricts diffusion of solvent towards the non-solvent i.e. external phase.

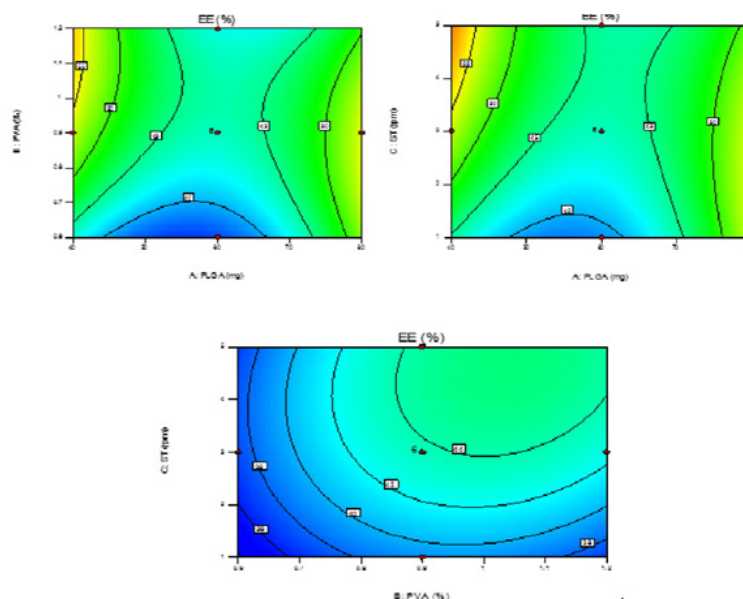


Figure 2: Contour Plots depicting the effect of factors (A, B & C) on Y1 (EE, %).

As for the effect of stabilizer conc. (B) on the EE; was found to be optimum on level +1 (1.2%). Though the effects were very unpredictable i.e. increasing stabilizer conc. resulted in either increase or decrease in EE. The effect is attributed to presence of insufficient stabilizer available to stabilize nanoparticles formed at very low conc. thus, forming aggregates and encapsulating high drug concentration. In contrast, at high stabilizer conc., small size nanoparticles are produced with low EE due to reduced interfacial tension between particles caused by stabilizer adsorbed at surface of particles. Sonication time (C) was found to have an inverse relationship with entrapment efficiency of PNPs.

Response 2 (Y2): Particle size

The following polynomial equation was projected by the model for particle size of the PNPs. $Y_2 = 380.90 + 36.34A + 11.85B - 72.62C - 6.76AB - 18.79AC + 2.99BC + 83.75A^2 - 16.00B^2 - 39.25C^2$ Where Y2 is the particle size of the PNPs; the negative coefficients for the sonication time show that the particlesize decreased with increase in sonication time. In totality, the model is significant (F-value = 71.85; $p < 0.0001$). The lack of fit is 0.49 not significant relative to pure error. The predicted R-square of 0.9572 is in reasonable agreement with the adjusted R-square of 0.9711; i.e. the difference is less than 0.2. Adequate Precision measures of 32.272 indicate it's an adequate signal. It was observed that response Y2 i.e. PS was significantly affected by altering the polymeric conc. (A; Figure 3 showed the effect of different independent variables on PS).

There was a remarked increase in PS with increasing polymer conc. This was probably caused by the increasing viscosity of dispersed phase (organic phase), resulting a low dispersibility of PLGA solution into aqueous phase. Increase in polymer conc. leads to increase in the viscous forces resisting the droplet breakdown by sonication. These forces oppose the shear stresses in the organic phase and the final size of particle depends on the net shear stress, which is available for droplet breakdown, therefore, level -1 (40 mg) was found optimum for smaller PS. For a better stabilization (B), the surfactant molecules must cover the organic/aqueous interfacial area of all the droplets.

It was observed that, as the concentration of stabilizer was increased, the size of PNPs first decrease then increased due to increased viscosity of the medium as already discussed. So the size decreased due to the enhanced interfacial stabilization while it increases due to the increased aqueous phase viscosity. Therefore optimum amount of conc. plays an important role, which was found to be level 1 (1.2%). Reverse effect was observed for sonication time, particle size decreased with increase in sonication time (C) i.e. level 1 (5 min)

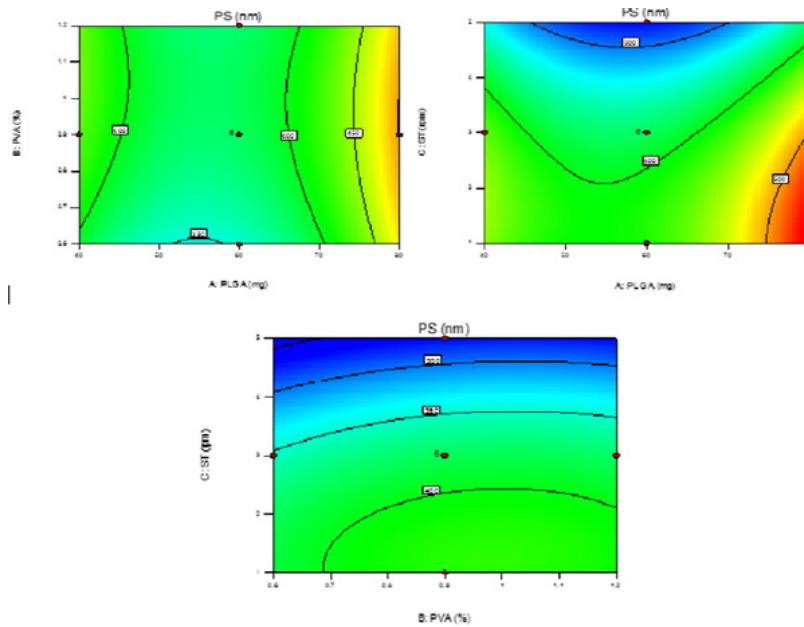


Figure 3: Plots showed the effect of factors (A, B & C) on Y2 (PS, nm).

Response 3 (Y3): Drug Release

The model proposed constant, the regression coefficients and the statistical parameters for each response variable, as follows: $Y3=97.53+7.000E-003A-0.21B-0.22C+0.44AB+0.34AC-0.28BC-2.33A^2+0.74B^2+0.21C^2$; Where Y3 is the drug release from PNPs. This model was found to be significant (F-value = 33.49; $p < 0.0001$). The lack of fit is not significant (F-value = 2.86) relative to the pure error. The predicted and adjusted R-squared values (0.8799 and 0.9390) are in reasonable agreement. Adequate precision of 20.147 indicates an adequate signal to navigate the design space. The DR of PNPs was found to be in the range of 94.70-98.21% (Figure 4) after 48 hrs of study via dialysis sac method.

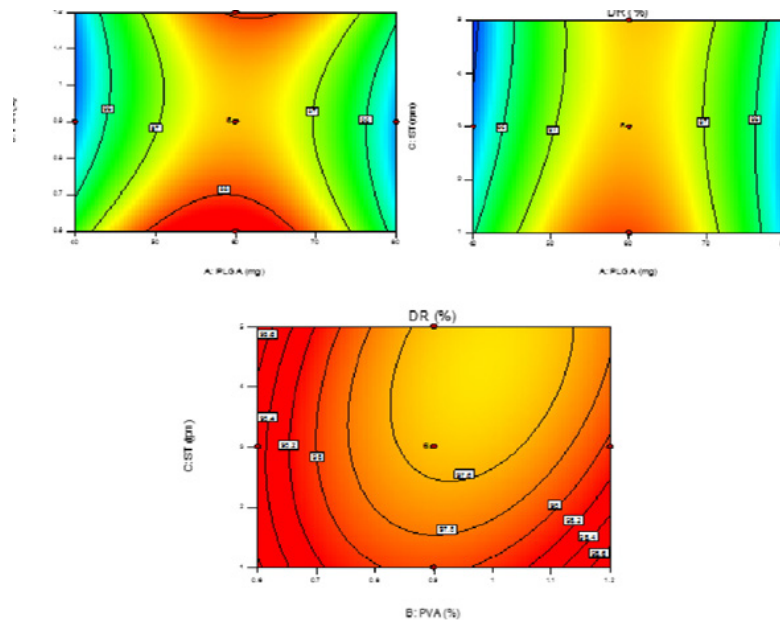


Figure 4: The two dimensional plots showed the effect of factors (A, B & C) on Y3 (DR, %).

It was observed (Figure 4) that entirely DR was dependent on the proper formulation and intact formation of PNPs as well as the amount of drug encapsulated. The effect of polymer conc. (A) was found to be critical as the polymer degradation is the major factor affecting the intact formation of PNPs. Increase in PLGA content directly affected the polymer degradation resulted in increase in DR, thus, level -1 (40 mg) showed significant DR and sustained effect. As for the factors B & C which were more of inter-related in affecting DR of the PNPs, it was observed that higher range of level +1 (1.2%) and level +1 (5 min) of B & C produced slower DR which was due to the fact that smaller size PNPs had higher surface area resulting in increased DR. Moreover, smaller sized PNPs have more surface-associated PVA which presents a significant barrier to the outward diffusion.

Models & Validation

Two dimensional contour plots were prepared for all the three responses shown in (Figure 2, 3 & 4) on Y_1 , Y_2 & Y_3 . These plots are used for studying the interaction effects of the factors on the responses as well as are useful in studying the effects of two factors on the response at one time. For validation of RSM results, the experimental values of the response were compared with the anticipated values. The linear correlation plots drawn between the predicted and experimental values demonstrated high values of R^2 for all three responses. The R^2 value (Table3) for response Y_1 , Y_2 and Y_3 was observed to be 0.9806, 0.9848, and 0.9679, respectively. It indicates excellent goodness of fit at $p < 0.0001$. Thus, the low magnitudes of error as well as the significant values of R^2 in the present investigation prove the high predictive ability of the RSM. The plots between actual and predicted value are shown in (Figure 5).

Quadratic model	R^2	Adjusted R^2	Predicted R^2	S.D	%CV
Response (Y_1)	0.9806	0.9632	0.8844	1.25	2.71
Response (Y_2)	0.9848	0.9711	0.9572	11.84	3.00
Response (Y_3)	0.9679	0.9390	0.8799	0.27	0.28

Table 3: Comparative Values of R^2 , SD & %CV.

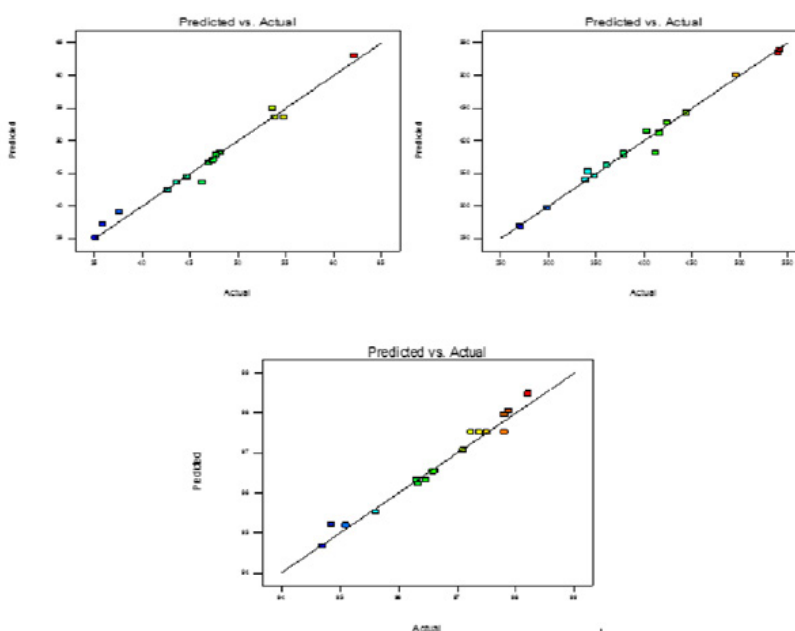


Figure 5: A linear correlation between actual and predicted values of the responses (Y_1 , Y_2 & Y_3).

Optimization

From the above discussion it is clear that, best results were concluded with consideration of all the levels of factors and relating them with the response so generated. The optimized formulation was selected on the basis of independent variable i.e. %EE, PS and DR, obtained for all 20 trials. When the values of all the above mentioned factors were compared, trial number 7 obtained the most appropriate results and hence was selected as optimized formulation

Characterization of Optimized Formulation (Fopt)

Morphology

The TEM analysis of Fopt showed formation of homogenous, smooth, spherical and well-formed nanoparticles. No aggregation was observed between particles, indicating their integrity as discrete entities. Therefore, it could be concluded that the integrity of PNPs formed by double emulsion were good indicating suitability of method for preparation of PNPs.

EE, PS and Zeta potential

The encapsulation efficiency (EE) of Fopt was determined by "indirect method" and was found to be 62.10 ± 1.1 . The particle size (PS) and polydispersity index (PDI) of Fopt determined by Particle size analyzer (Malvern Zeta SizerNano ZS, UK) as depicted from (Figure 7a). The PS of Fopt was found to be 338.60 ± 13 nm with PDI of 0.191 (observed range 0.193 to 0.187), indicating a narrow size distribution and good homogeneity of PNPs. As depicted from (Figure 7b) the zeta potential of Fopt was found to be -35.9 (observed range was -34.8 to -37.1), indicating better physical stability of formulation as particles with ζ -potential more than +30 mV and lower than -30 mV are considered to be stable.

The higher stability of nanoparticles at high zeta potential value is attributed to presence of high surface charge. It reduces the chances of agglomeration due to electrostatic repulsion between particles bearing same electric charges and makes the system stable for longer time which is easy to redisperse. A high zeta potential also shows better absorption via Peyer's patches and their uptake by liver for more sustained effect.

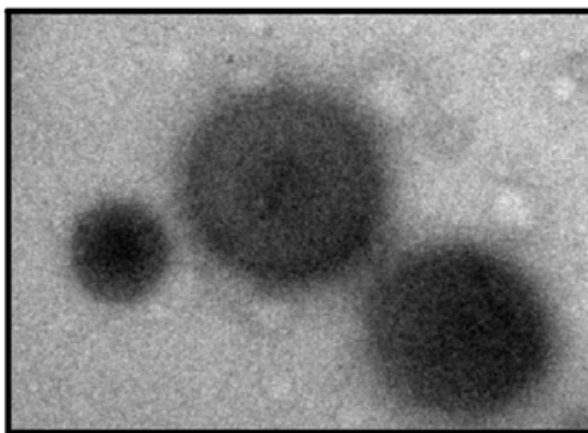


Figure 6: The transmission electronic image of optimized formulation (Fopt).

Release and Kinetics studies

The in vitro drug release profile of control (drug solution) was rapid, consistent and completed within 6 hrs ($97.78 \pm 1.55\%$) whereas Fopt showed a sustained release profile ($94.70 \pm 0.28\%$) for 48 hrs, indicating sustained release behavior of PNPs over free drug. The Fopt have shown biphasic release pattern of RAS, consisting of an initial burst effect of 6 hrs followed by a sustained release phase up to 48 hrs (Figure 8). The initial burst effect is characterized by $43.97 \pm 1.22\%$ release of drug within 6 hrs, attributed to dissolution and diffusion of drug adhered on or located near the surface of PNPs or entrapped poorly in polymer matrix.

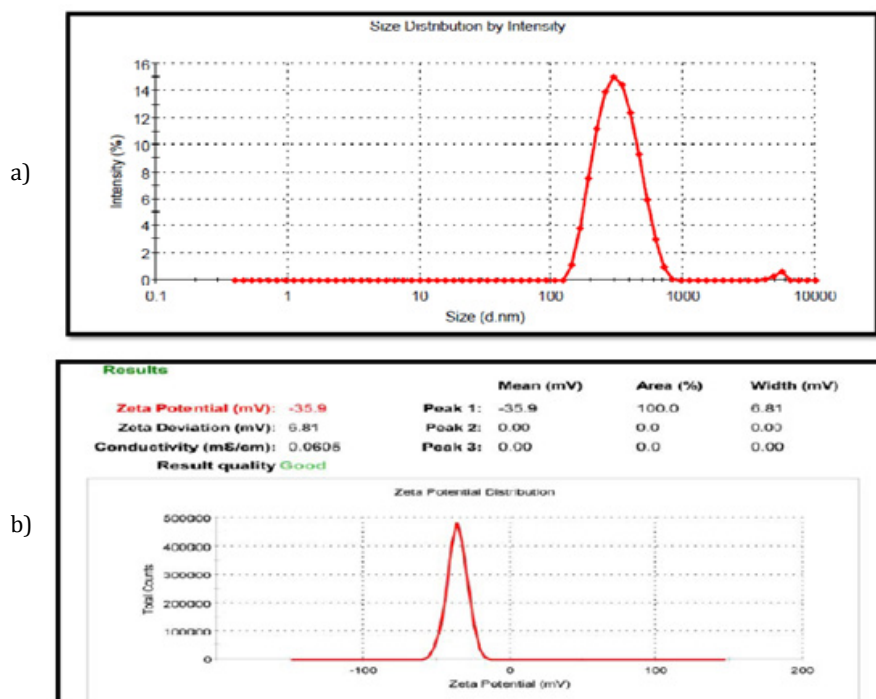


Figure 7: The optimized formulation; a) particle size b) zeta potential.

It was followed by sustained release profile ($94.70 \pm 0.28\%$) of drug for 48 hrs due to diffusion of drug located within polymer core of nanoparticles. However, the burst effect is considered to be beneficial for RAS loaded PNPs as it helps in achieving therapeutic concentration of drug in very less time to produce quick action & thereby, relieving the pathological condition quickly. The burst effect is followed by a constant release phase which helps in maintaining concentration of drug in therapeutic window for longer duration of time. It could be concluded that PLGA nanoparticles are efficient sustained release carrier for RAS. The drug release mechanism was best explained by Korsmeyer-Peppas release model with regression coefficient of 0.9873. The n-value obtained for Fopt using Korsmeyer-Peppas equation was found to be 0.3531. It indicates that the formulation followed “Non-Fickian Diffusion Transport” mechanism of drug release of controlled drug release.

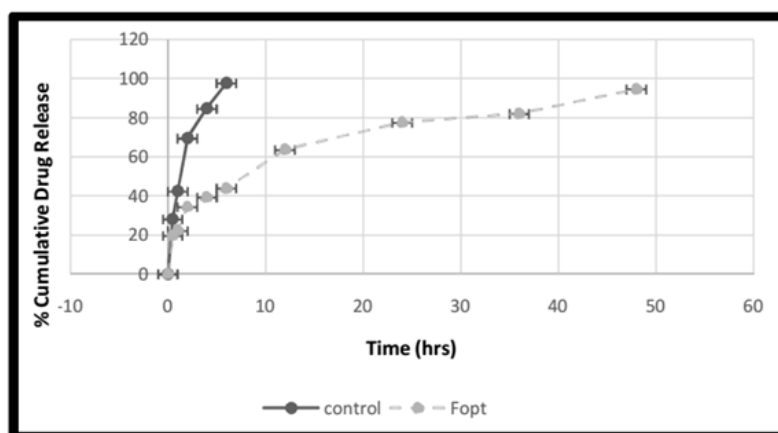


Figure 8: The drug release profile of optimized formulation as compared with control.

Stability studies

The samples of FoPt at both storage conditions were observed for physical changes at specific time interval during stability studies and results are summarized in Table 4. No physical change was observed in FoPt during stability study at normal and accelerated storage conditions of temperature and humidity, indicating good physical stability of formulation in terms of color, odour, particle aggregation and redispersibility of nanoparticles in water. Moreover, the FoPt was observed for chemical changes in terms of encapsulation efficiency and drug content at accelerated storage conditions and results are summarized in Table 5. The change in drug content observed at accelerated storage conditions i.e. $25\pm 2^\circ\text{C}/60\pm 5\% \text{RH}$ was plotted against time using software, Sigma Plot 13.0; calculated shelf life of FoPt was found to be 362.53 (Figure 9).

S.No	Time (days)	Physical Changes at							
		$5\pm 3^\circ\text{C}$				$25\pm 2^\circ\text{C}/60\pm 5\% \text{RH}$			
		Colour	Odour	Particle Aggregation	Redispe rsibility	Colour	Odour	Particle Aggregation	Redispe rsibility
1.	0	White	Odour- less	X	Good	White	Odour- less	X	Good
2.	7	√	√	X	√	√	√	X	√
3.	15	√	√	X	√	√	√	X	√
4.	30	√	√	X	√	√	√	X	√
5.	60	√	√	X	√	√	√	X	√

√(Ok), X(No)

Table 4: Accelerated analysis for Physical Stability.

S.No.	Time (days)	Chemical Changes at			
		$5\pm 3^\circ\text{C}$		$25\pm 2^\circ\text{C}/60\pm 5\% \text{RH}$	
		% EE \pm S.D	% Drug Content	% EE \pm S.D	% Drug Content
1.	0	62.10 \pm 1.13	100.0 \pm 1.33	62.10 \pm 1.13	100.0 \pm 1.55
2.	7	62.07 \pm 1.67	99.96 \pm 1.38	61.98 \pm 2.10	99.82 \pm 1.89
3.	15	61.91 \pm 0.46	99.73 \pm 1.57	61.77 \pm 1.45	99.51 \pm 1.67
4.	30	61.71 \pm 0.92	99.48 \pm 1.90	61.50 \pm 2.09	99.12 \pm 1.44
5.	60	61.54 \pm 1.25	99.26 \pm 1.24	61.22 \pm 1.76	98.71 \pm 1.28

Table 5: Accelerated analysis for Chemical Stability.

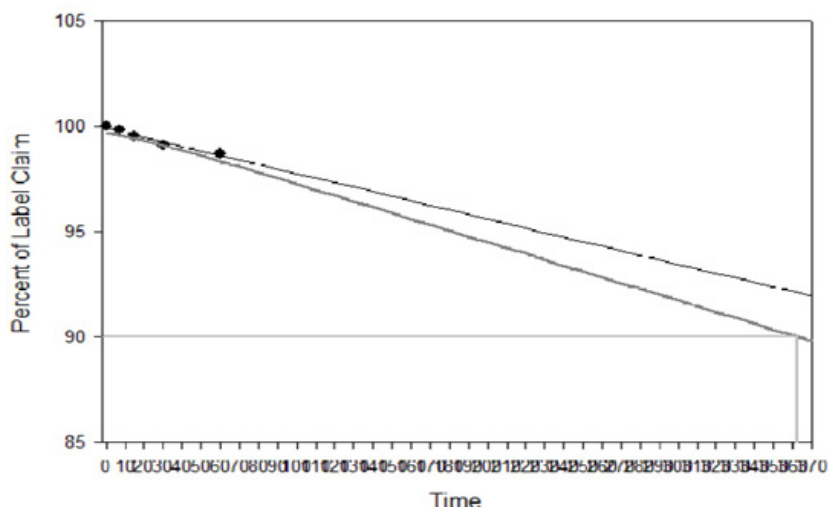


Figure 9: Shelf life of Fopt.

Conclusion

Box-Behnken statistical design was used for optimization of factors with minimum number of formulation trials. The formulation trials generated by design expert were prepared and evaluated for EE, PS & DR were analyzed critically and polynomial equation was generated, which represented both the responses simultaneously. Formulation trial efficient results indicated optimal parameter for representation of EE, PS & DR which also suggests optimum level of factors was found to be factor A at -1, factor B at +1 and factor C at +1 by fitting experimental data to the statistical model. An optimized formulation (Fopt) was prepared using above combination of factors and their optimum level.

The Fo_{opt} was characterized for EE, PS, DR, PDI, zeta potential and their values were found to be $62.10 \pm 1.13\%$, 338.60 nm , 0.191 & -35.9 mV respectively. TEM analysis of Fo_{opt} revealed formation of spherical, smooth surfaced, well-formed nanoparticles without aggregates. The X-ray diffraction of formulation confirmed conversion of drug from crystalline to amorphous phase when dispersed in PNPs. In vitro drug release studies indicated sustained release ($94.70 \pm 0.28\%$ in 48 hrs) behavior of Fo_{opt} compared to control (free drug solution) where drug release ($97.78 \pm 1.55\%$ release) was rapid and completed within 6 hrs. The stable biodegradable polymeric nanoparticles of Rasagiline Mesylate were productively prepared by double emulsion method for significant antiparkinsonian activity for neurodegenerative disorder with targeted action for drug delivery.

Conflict of Interest

None to declare

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