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FORMULATION, OPTIMIZATION, AND EVALUATION OF RIVASTIGMINE TARTRATE LOADED SOLID LIPID NANOPARTICLE BY MICROEMULSION TECHNIQUE

Padmapreetha, Nithish. K* and Rajesh B

Department of Pharmaceutics, KMCH College of Pharmacy, Coimbatore

*Corresponding author E-mail: nithishboj@gmail.com

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ABSTRACT

Solid lipid nanoparticle (SLN) having an attracting importance for drug developer to produce intellectual property through innovations in drug delivery. The objective of present investigation was to develop, optimize and evaluate Rivastigmine tartrate loaded solid lipid nanoparticle (RT SLN) by using central composite design. SLN were prepared by microemulsion method using a systemic approach of design of experiments and evaluated. Various preliminary experiments were performed for selection of suitable excipients. Various lipids and surfactant are selected on the basis of solubility and particle size and thus glyceryl monostearate selected as lipid, and poloxamer 188 as surfactant. Further High-speed homogenizer stirring speed and time was optimized HSH is operated at 15000 rpm for 5minutes. Characterization of RT SLN was carried out by infrared spectroscopy (FTIR), scanning electron microscopy (SEM), particle size and zeta potential, differential scanning calorimetry (DSC), entrapment efficiency, *in vitro* drug release and kinetics. Particle size of optimized formulation is 152.4 nm and PDI value 0.198 indicates mono dispersion. Optimized formulation with zeta potential -31.4 mV was found to be stable. Entrapment efficiency of optimized formulation is 76.1%. This condition has shown an improved response values in comparison with the previously optimized formulation. SEM photographs of RT SLN indicate particles have uniform loose aggregates, spherical in shape with a smooth surface and they are uniformly distributed. On comparison of *in vitro* drug release studies of RT solution and RT SLN shows that RT SLN has higher release 87.74% at 12 hrs compared to RT solution RT Solution 61.59%. From the obtained results it was concluded that the Rivastigmine Tartrate SLNs can be employed for controlled delivery of drug in the treatment of Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease most often characterized by initial memory impairment and cognitive decline that can ultimately affect behaviour, speech, visuo-spatial orientation and the motor system. [1] Alzheimer's disease (AD) and other forms of Dementia is a growing public health problem among the elderly people in developed and developing countries, whose aging population is increasing. The aging population size is now bigger for all the countries due to sustainable development in health care system around the globe. AD is epidemic with an estimated 33.9

million people worldwide having the disease. The incidence rate increases exponentially with aging so that at age 90 about 12% of people have AD, but about 40% of those over age 100 have it. Factors that put persons at increased risk of AD are a history of head injury, obesity, histories of smoking, diabetes mellitus, hypertension, renal disease and traumatic brain injury, or depression. Since these factors are occurring more commonly, the incidence of AD may increase even more. Clearly, interventions that prevent, stabilize, remediate, or cure AD are desperately needed. [2] Nanoparticles are solid polymeric, submicron colloidal system range between 5-300 nm consisting of macromolecular substances that vary in size 1 nm to 1000 nm. The drug of concern is dissolved, entrapped adsorbed, attached or encapsulated into the nanoparticle matrix. Depending upon the method of preparation, nanoparticle, nanosphere or nanocapsule can be obtained with different properties and release characteristics for the encapsulated therapeutic agent. [3] Solid lipid nanoparticles (SLNs) were introduced in 1991 with the objective to provide biocompatibility, storage stability and to prevent the incorporated drug from degradation. SLNs that is colloidal carriers of nanoscopic size (50–1000 nm) are made up of solid lipids (high melting fat matrix) and are developed to conquer the weaknesses (e.g., polymer degradation and cytotoxicity, lack of a suitable large scale production method, inferior stability, drug leakage and fusion, phospholipid degradation, high production cost, and sterilization problems) of traditional colloidal carriers, like polymeric nanoparticles and liposomes. SLNs show various distinctive features such as low toxicity, large surface area, prolonged drug release, superior cellular uptake as compared to traditional colloidal carriers as well as capability to improve solubility and bioavailability of drugs. The release of drug from SLNs depends on matrix type and drug location in the formulation. The SLNs fabricated from biodegradable and biocompatible ingredients are able to incorporate both hydrophilic and lipophilic bioactive and thus turning out to be a viable option for controlled and targeted drug delivery. The solid core of SLNs is hydrophobic with a monolayer coating of phospholipids and the drug is usually dispersed or dissolved in the core

Advantages of SLNs

1. The cells of reticulo endothelial system (RES) are unable to take up SLNs because of their nano size range, thus enabling them to bypass spleen and liver filtration

2. Provide high stability to incorporate drugs

3. Feasibility of incorporating both hydrophilic and lipophilic drugs

4. Improve bioavailability of poorly water-soluble molecules

5. Ease in sterilization and scale up

6. Immobilizing drug molecules within solid lipids provides protection from photochemical, oxidative, and chemical degradation of sensitive drugs, with reduced chances of drug leakage

7. Drying by lyophilization is achievable

8. Provide opportunities for targeted and controlled release of drug

9. Biocompatible and biodegradable compositional ingredients

Disadvantages of SLNs

1. SLNs are compactly packed lipid matrix networks (ideal crystalline structure) having low space for drug encapsulation, leading to poor drug loading capacity

2. Various factors affect the loading or encapsulation of drugs in SLNs, such as interaction of drug and lipid melt, nature or state of lipid matrix, drug miscibility with lipid matrix, and the drug being dispersed or dissolved in the lipid matrix

3. Chances of drug expulsion following polymeric transition during storage

4. The dispersions have high (70–90%) water content

Microemulsion based SLN preparation

Gasco and coworkers (1997) developed SLNs based on the dilution of micro emulsions. These are made stirring an optically transparent mixture at 65-70°C which is typically composed of a low melting fatty acid like stearic acid, an emulsifier (e.g.

polysorbate 20, polysorbate 60, soya phosphatydyl choline and tauro deoxy cholic acid sodium salt), co-emulsifiers (e.g. butanol, sodium mono octyl phosphate) and water. The hot micro emulsion is dispersed in cold water (2-3°C) under stirring. Typical volume ratios of the hot micro emulsion to cold water are in the range of 1:25 to 1:50. The dilution process is critically determined by the composition of the microemulsion. The SLN dispersion can be used as granulation fluid for transferring in to solid product like tablets and pellets by granulation process, but in case of low particle content too much of water need to be removed. The nanoparticles were produced only with solvents which distribute very rapidly into the aqueous phase (acetone), while larger particle sizes were obtained with more lipophilic solvents.

Aim: The aim of the study was to develop, optimize and evaluate Rivastigmine tartrate loaded solid lipid nanoparticle (RT SLN) by using central composite design.

Materials used: Rivastigmine Tartrate(gift sample from Alembic pharmaceuticals limited), Poloxamer 188 (Sigma Aldrich), Tween 80 (RFCL Ltd), Stearic acid (FINAR), Glcerylmonosterate, Glyceryl tristate, Coco mono ethanolamide (Mohini organics Private limited).

EXPERIMENTAL PROTOCOL

Formulation Development, Optimization and Evaluation of Solid Lipid Nanoparticles (SLNs) For Rivastigmine Tartrate

Solid lipid nanoparticles were developed by using strategy of quality by design (QBD) which includes steps of preliminary screening of lipid, surfactant, homogenization speed and time followed by optimization of nano formulation.

Formulation Strategy: Preparation of RT-loaded solid lipid nanoparticles: The solid lipid nanoparticle was prepared using the microemulsion technique. A microemulsion was spontaneously obtained as recognized by a clear solution after adding the heated water phase into the oil phase of the same temperature. Addition of a hot microemulsion to cold water led to precipitation of the lipid phase forming fine particles. High-temperature gradients facilitate rapid lipid crystallization and prevent lipid aggregation. Briefly, the drug (10mg) was dispersed in melted lipid, and then the mixture was dispersed in a hot aqueous solution with surfactant concentration ranging from 50 to 150mg, by high speed stirring, using a High speed homogenizer at 15000 rpm for an appropriate period of time. The resulting dispersion was then cooled and each sample was diluted with water before the particle size was measured. [4]

Risk analysis of SLN: Risk variables were categorised in initial drug and excipient related risk, processing equipment related risk, processing variable risk and product profile related risk. Justification of all variables was studied based on literature survey and further it was defined with low, medium and high level with possible justification. These factors were studied from past published data and implemented with aim to implement at each stage of formulation preparation. However, it is purely related to multiple trials based on suitable conditions.

SCREENING OF COMPONENTS FOR THE PREPARATION OF SLNS

For the SLNs development, selection of suitable excipients (lipid and surfactant) is vital. Excipients should be pharmaceutically acceptable, non-irritant and non-sensitizing in nature. They should be generally regarded as safe.

Screening of Lipids: As equilibrium solubility study was not possible due to the solid nature of the lipids, an alternative method was adopted to measure solubility of drug in the solid lipids. Briefly, 10 mg Rivastigmine Tartrate was weighed accurately and placed in a screw capped glass bottle covered with aluminum foil. About 100 mg of lipid was added in the bottle and heated at 80 ◦C under continuous stirring. Then additional lipid was added in portions under continuous stirring and heating at 80 ˚C until a clear solution was formed. Total amount of lipid added to get a clear solution was recorded.[5]

Screening of Surfactants: The lipid selection was made on the basis of solubility of drug in lipid. The surfactant was selected here on the basis of HLB value of selected lipid i.e. GMS. GMS has the HLB value 3.8 and therefore the

HLB value of surfactant needed to emulsify the GMS for a stable emulsion should be around or more than 3.8. Hence surfactant or co-surfactant or solvent should be chosen in such a concentration as to have a required combined value of 3.8 or more than 3.8. In this research work several surfactants like tween 80 and poloxamer 188 alone or in combination were applied for the preparation of SLNs. The stability of prepared SLN dispersion was observed visually after 24 h. [6]

Screening of High-Speed Homogenizer rpm and time: During the process of stirring, organic solvents diffuse into the aqueous phase, leading to the synthesis of SLNs. The speed and the time of stirring may influence the particle size as well as the drug entrapment. In the present study, the stirring speed was kept constant at 15000 rpm, and the time of stirring was optimized. Three points of time were used for the optimization 5, 10 and 15 minutes along with screened surfactant and lipid.

Central composite design: Present experimental design is based on variables including the Lipid concentration (A) and Surfactant concentration (B) (Table 2). In this model, 13 random experiments were selected to minimize the effect of uncontrolled variables. The purpose of an experimental design is to plan and conduct experiments in order to extract the maximum amount of information from the collected data in a minimal number of experimental runs. Central composite design, based on the response surface method, is applied to design formulations. [7]

Characterization of Optimized Rivastigmine tartrate SLN: Particle size, PDI and zeta potential analysis: RT SLN (1 ml) was dispersed in distilled water (10 ml), and then its particle size range was determined using Malvern Mastersizer (MAL 1021384 Malvern Instruments, Worcestershire, UK). The mean particle size, polydispersity index (PI) and zeta potentials were determined.

Encapsulation efficiency: Entrapment efficiency was determined by determining the amount of free drug spectrophotometrically at 264 nm in the supernatant after centrifugation of the known amount of nanoparticulate dispersion at 10000 rpm using REMI centrifuge for 15minutes.The entrapment efficiency was calculated using the equation. [8]

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Total drug taken} - \text{Free drug}}{\text{Total drug taken}} \times 100$$

Scanning electron microscopy (SEM*):* The surface morphology of drug substance and prepared formulations of RT SLN were examined using scanning electron microscopy (Jeol, JSM 6390). [9]

***In vitro* drug release studies:** *In vitro* release studies were performed using the dialysis bag method, modified to maintain a sink condition and achieve satisfactory reproducibility. The dialysis bag (molecular weight cut off 12000–14000) was soaked in deionised water for 12h before use. 1mL of RT loaded SLN dispersion was first poured into the dialysis bag with the two ends fixed by thread and placed into the preheated dissolution media (phosphate buffer pH 7.4) placed in beaker. The beaker was placed on a magnetic stirrer. At fixed time intervals of 0.5,1,2,3,4,6, and 8hrs a sample was removed for analysis and equal volume of fresh dissolution medium was added. The sample was analyzed by using a UV Spectrophotometer at a λmax of 263nm against a blank of phosphate buffer of pH 7.4. [10, 11]

***In vitro* drug release kinetics:** In order to understand the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles were fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs time), Higuchi's model (cumulative % drug release vs. square root of time). r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots. [12]

RESULTS AND DISCUSSIONS

Formulation Development and Optimization of Solid Lipid Nanoparticles (SLNs) For Rivastigmine Tartrate:

Minimumparticle size is one of the most important CQA along with minimum PDI (monodispersity), maximum entrapment efficiency, maximum drug loading, minimum zeta potential of ± 30 mV for stability and no

residual solvent for avoiding toxicity and ensuring safety. In the present investigation, risk analysis and different preliminary experiments were carried out on the basis for selection of suitable excipients/materials which may directly or indirectly influence critical quality attributes. The compatibility of the drug-excipient was checked before the optimization of various process and formulation variables. Risk analysis data was implemented in to the formulation preparation.Finally, the formulation variables were optimized using central composite design in design expert software further analyzing the data statistically and graphically using response surface plots.

Risk Analysis: The initial risk assessment of the overall formulation process is shown in table 11 and possible justifications are provided is given based on literature survey. Existing experience with these process steps was used to determine the degree of risk associated with each process step and its potential to impact the CQAs of the finished product in form of nano formulation. Process variables that could have potentially impact on product CQAs were identified and their associated risk was evaluated. Categorisation of variable (i.e.: drug and excipient related risk, processing equipment related risk, processing variable risk and product profile related risk) were defined with low, medium and high level with possible justification.

Screening of Components forthe Preparation Of SLNS

Screening of Lipids: RT is highly hydrophobic and lipophilic drug. Solubility study was performed to evaluate the solubility profile of drug against various lipids as it is one of the essential steps for formulation development. The solubility of the drug was determined in four different lipids – Glyceryl mono stearate, Glyceryl tri sterate, Stearic acid, and Coco mono ethanolamine. Among them, GMS (48 mg) showed highest solubilisation capacity followed by GTS (55 mg). Amount of coco mono ethanolamide (67 mg) and Stearic acid (83 mg) required to solubilize 10 mg tretinoin was significantly higher than GMS (48 mg). This study indicated that Rivastigmine tartrate loading capacity along with GMS is found to be higher than GTS, coco mono ethanolamide, and Stearic acid. GMS is selected as lipid for formulation.

Screening of surfactants: The surfactant was selected here on the basis of HLB value. SLN was prepared with two different surfactants alone or combination using GMS as lipid and were evaluated for particle size and PDI. The results obtained are as shown in Table 4.

Screening of HSH rpm and time: The HSH time was selected here on the basis of particle size and PDI value. SLN was prepared with poloxamer 188 as surfactant on using GMS as lipid and were evaluated for particle size and PDI. The results obtained are as shown in table 5.

This study indicates that HSH time with 5 minutes (158.0) having smaller particle size when compared to 10 minutes (246.40) and 15 minutes (277.53)

Compatibility Study of Selected Excipients with Drug

FT-IR Spectroscopy: FT-IR spectra of drug, selected lipid, and surfactants were analyzed to check the interactions between them. The spectra and major peaks of individual compounds and their combinations were studied. The spectra showed that there is no interaction between the drug, selected lipids and surfactants. Hence, the selected stabilizer was found to be compatible with drug and other components without any mutual interactions.

Optimization of Formulation: The design selected was Central composite design by employing design expert software. Two input factors were studied at five different levels including central point 0, +1, -1, ±α throughout the preparation process to determine their effect on two responses, namely mean particle size and encapsulation efficiency. The input factors being selected are the following: Lipid concentration (100,200 and 300), concentration of surfactant (50,100,150) shown in table 16. The response values were subjected to multiple regression analysis to find out the relationship between the input factors used and the response values obtained. Data reported in table 6 provides the 13 runs of experimental design where the compositions of the lipid and surfactant are indicated. Thirteen formulations (F1-F13)

were prepared accordingly and analyzed for their physical characteristics.

Determination of Particle Size, PDI and %EE: Particle size of the formulation is found in the range between 121 to 279 nm shown in table 7. Formulation factors were found to influence the particle size significantly.

Data Analysis: The data analysis was carried out using design expert software. The summary of design is described in table 8. This summary describes the overview of entire resultant data for total 32 runs. Summary describes the particle size of the formulation is found in the range between 121 to 279 nm. A mean for size of particle is found 162.73. Similarly, encapsulation efficiency size of the formulation is found in the range between 36.45 to 76.95%. The mean value for %EE is 62.74%.

Analysis of Particle Size: The particle size range in all the formulation was found from 121 nm as minimum size to 279 nm as maximum size. The result of p-values is indicated for each model. The data presented in table 16 suggest the p-value for all sources i.e.: linear, 2FI, quadratic, and cubic. The p-value was found 0.0065 for linear, 0.3757 for 2FI, <0.0001 for quadric and 0.2826 for cubic model source. From this quadratic model is suggested for study further summary of ANOVA generated from the software is presented in table 9.

The **Model F-value** of 1110.11 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. **P-values** less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The **Lack of Fit F-value** of 0.89 implies the Lack of Fit is significant. There is only a 51.93% chance that a Lack of Fit F-value this large could occur due to noise. The **Predicted R^2**of 0.9952 is in reasonable agreement with the **Adjusted R^2** of 0.9978; i.e. the difference is less than 0.2. **Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 88.763 indicates an adequate signal. This model can be used to navigate the design space. Full model equation for Particle Size in terms of coded factors was obtained as

$$PS = +124.72 + 35.16*A - 41.46*B - 17.30*AB + 24.65*A^2 + 37.12*B^2$$

PS represents the particle size, and A, B are coded values for the lipid concentration and surfactant concentration, respectively. Terms with p-values of less than 0.0001 were considered as significant. The p-values of all other terms were less than 0.0001 (Table 19); thus they possibly have significant effects on the EE of drug. The coefficient of determination (R^2) and adjusted R^2 were 0.9987 and 0.9978, respectively, which means that there is a good fit between independent variables and the particle size of drug. The statistical analysis of data showed that the obtained model was significant ($p < 0.0001$), which confirms that the coefficient of determination (R^2) and adjusted R^2 are statistically significant. The lack of fit was insignificant ($p = 0.5193$), indicating that the quadratic model accurately explains the variations of experimental data. Since one purpose of this study was to reduce size of SLNs, the terms with positive coefficients are considered favorable for increasing particle size. Conversely, negative coefficients which have a decreasing effect on particle size. The lipid concentration (A) and surfactant concentration (b) have a positive and negative coefficient, respectively. Accordingly, a linear increase in A and B leads to a decreasing and increasing effect on particle size, respectively. The normal probability distribution diagram implies that the residues are mainly located on a straight line and follow a normal distribution, as shown in Fig. 6.

Analysis of Encapsulation Efficiency: The particle size range in all the formulation was found from 36.45 to 76.95. The result of p-values is indicated for each model. The data presented in table 20 suggest the p-value for all sources i.e.: linear, 2FI, quadratic, and cubic. The p-value was found 0.0518 for linear, 0.1394 for 2FI, 0.0115 for quadric and 0.3594 for cubic model source. From this quadratic model is suggested for study further summary of ANOVA generated from the software is presented in table 12.

Design of Experiment

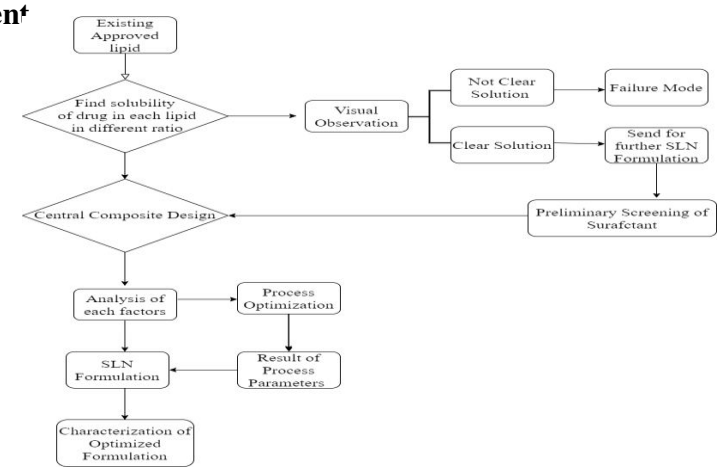


Figure 1: Strategical overview of methodology

S.No	Variable	Risk category
1	Drug particle distribution	Drug and excipient related risk
2	Drug and lipid solubility	Drug and excipient related risk
3	High speed homogenizer	Processing equipment related risk
4	Dissolution of lipid	Processing variable related risk
5	Homogenization speed	Processing variable related risk
6	Drug and lipid ratio	Processing variable related risk
7	Surfactant concentration	Processing variable related risk
8	Homogenization time	Processing variable related risk
9	Particle size	Product profile related risk
10	Encapsulation efficiency	Product profile related risk

Table 1: Process variable and associated category of risk

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High
A	Lipid concentration	Mg	Numeric	100.00	300.00	-1 ↔ 129.29	+1 ↔ 270.71
B	Surfactant concentration	Mg	Numeric	50.00	150.00	-1 ↔ 64.64	+1 ↔ 135.36

Table 2: Factors used in formulation design

SI.NO	VARIABLE	RISK ASSESSMENT	JUSTIFICATION
DRUG AND EXCIPIENT RELATED RISK			
1	Drug particle distribution	Low	SLN processing step involves the solubilisation of drug in solvent and ultimately in lipid. There is no direct effect in any response. Hence this can be categorised as low risk factor.
2	Drug and lipid solubility	High	This variable is directly related to solubility of drug in lipid which is an important step in formulation as well as in drug loading. So this falls under the category of high risk.
PROCESSING EQUIPMENT RELATED RISK			
3	High speed homogeniser	Low	HSH is selected based on the accessibility and easiness. This can be

			categorised under low risk.
PROCESSING VARIABLE RELATED RISK			
4	Dissolution of lipid	High	It is an initial step of SLN preparation undissolved or partially dissolved in lipid fraction and may directly effect the product yield.
5	Homogenisation speed	Medium	Lower or higher speed may result inappropriate drug encapsulation. However, this variable is controllable.
6	Drug and lipid ratio	Medium	This factor indirectly effect on encapsulation efficiency as well as PDI and particle size.
7	Surfactant concentration	Medium	This factor indirectly effect on encapsulation efficiency as well as PDI and particle size.
8	Homogenization time	Medium	Particle aggregation may form due to static force for prolonged time of homogenisation. However this variable is controllable.
PRODUCT PROFILE RELATED RISK			
9	Particle size and PDI	Medium	Homogenously distributed particles provides a uniform drug release from the entire formulation .
10	Encapsulation efficiency	High	Encapsulation efficiency is accepted with maximum level upto 90 to 95%. This variable directly affects the drug content.

Table 3: Variable related risk assessment

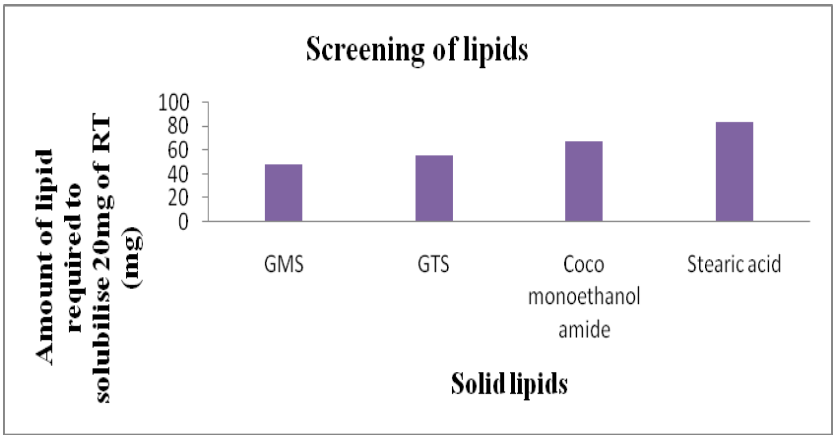


Figure 2: Solubility of Rivastigmine tartrate in different lipids

Surfactants	Particle size (nm)	PDI
Poloxamer 188	381.1	0.056
Tween 80	458.3	0.013
Poloxamer 188/ Tween 80	498.8	1.000

. Table 4: Selection of Surfactant on the basis of Particle size and PDI

This study indicates that Poloxamer 188 (381.1) having smaller particle size and stable when compared to Tween 80 (458.3) and combination of poloxamer 188/Tween 80 (498.8)

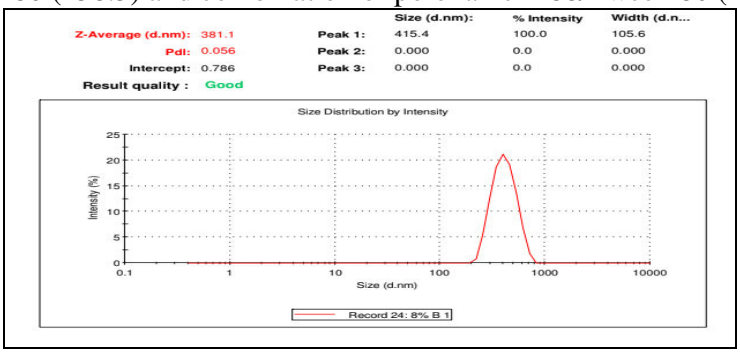


Figure 3: Particle size distribution of RT SLN using poloxamer 188

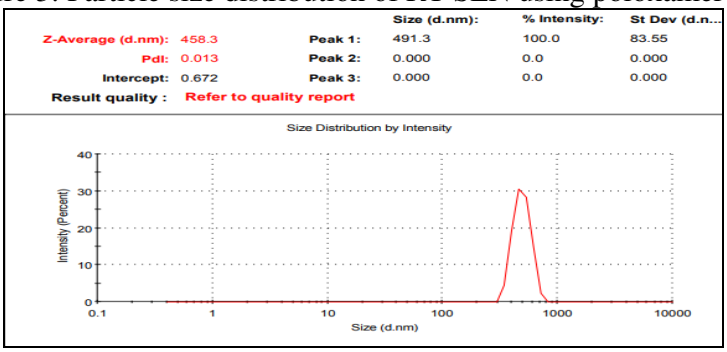


Figure 4: Particle size distribution of RT SLN using Tween 80

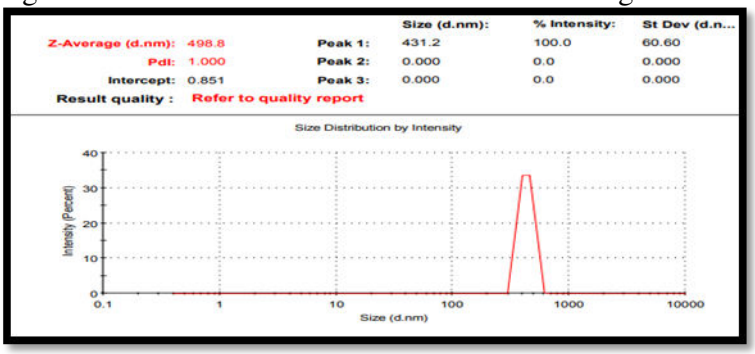


Figure 5: Particle size distribution of RT SLN using poloxamer 188 and tween 80 (combination)

HSH speed (rpm)	HSH time (min)	Particle size (nm)	PDI
15000	5	158.0	0.375
	10	246.40	0.389
	15	277.53	0.397

Table 5: Selection of HSH time on the basis of Particle size and PDI

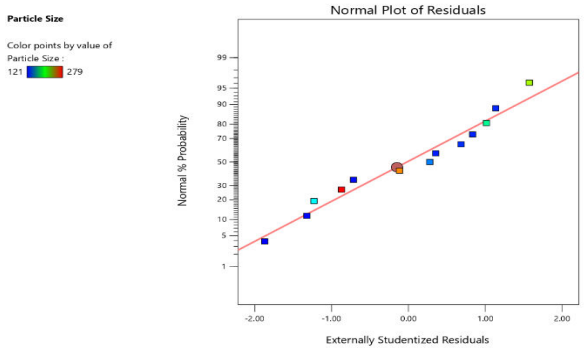


Figure 6: Normal probability distribution plot for particle size

Formulation code	Factor 1	Factor 2
	A:Lipid concentration mg	B:Surfactant concentration Mg
F1	270.711	64.6447
F2	129.289	135.355
F3	200	150
F4	200	100
F5	100	100
F6	200	100
F7	200	100
F8	300	100
F9	270.711	135.355
F10	200	50
F11	129.289	64.6447
F12	200	100
F13	200	100

Table 6: Runs using CCD

Formulation code	Factor 1	Factor 2	Response 1	Response 2
	A:Lipid concentration Mg	B:Surfactant concentration mg	Particle Size Nm	Encapsulation Efficiency %
F1	270.711	64.6447	279	36.45
F2	129.289	135.355	128.3	68.21
F3	200	150	140.8	73.72
F4	200	100	125.6	76.95
F5	100	100	122.3	70.98
F6	200	100	123	73.26
F7	200	100	126.7	75.11
F8	300	100	226	66.03
F9	270.711	135.355	161	68.31
F10	200	50	257.4	54.03
F11	129.289	64.6447	177.1	63.58
F12	200	100	127.3	75.9
F13	200	100	121	71.62

Table 7: Particle size, and Encapsulation efficiency of all SLNs formulations (F1-F13)

Response	Nam-e	Unit	Observation	Minimum	Maximum	Mean	Std. Dev.	Ratio
R1	PS	nm	13.00	121	279	162.73	55.71	2.31
R2	EE	%	13.00	36.45	76.95	67.24	11.11	2.11

Table 8: Result summary from formulation trials (±=SD, n=3)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0065	< 0.0001	0.5617	0.3950	
2FI	0.3757	< 0.0001	0.5558	0.2626	
Quadratic	< 0.0001	0.5193	0.9978	0.9952	Suggested
Cubic	0.2826	0.8905	0.9982	0.9986	Aliased

Table 9: Summary of ANOVA for particle size

Response 1 - PS (Particle Size)						
ANOVA for Response Surface Quadratic Model						
Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	37199.67	5	7439.93	1110.11	< 0.0001	significant
A-Lipid concentration	9887.97	1	9887.97	1475.38	< 0.0001	
B-Surfactant concentration	13752.89	1	13752.89	2052.06	< 0.0001	
AB	1197.16	1	1197.16	178.63	< 0.0001	
A^2	4225.65	1	4225.65	630.51	< 0.0001	
B^2	9586.00	1	9586.00	1430.32	< 0.0001	
Residual	46.91	7	6.70			
Lack of Fit	18.77	3	6.26	0.8889	0.5193	not significant
Pure Error	28.15	4	7.04			
Cor Total	37246.59	12				

Table 10: Response surface quadratic model for particle size

Fit Statistics	
R^2	0.9987
Adjusted R^2	0.9978
Predicted R^2	0.9952
Adeq Precision	88.7632

Table 11: Fit statistics for particle size

Factor coding is **coded,** Sum of square is **TYPE III – Partial**

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0518	0.0029	0.3361	-0.0214	
2FI	0.1394	0.0035	0.4291	-0.4122	
Quadratic	0.0115	0.0190	0.7951	0.2182	Suggested
Cubic	0.3594	0.0094	0.8095	-3.3147	Aliased

Table 12: Summary of ANOVA for encapsulation efficiency

Response 1 - EE (Encapsulation efficiency)						
ANOVA for Response Surface Quadratic Model						
Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	1304.99	5	261.00	10.32	0.0040	significant
A-Lipid concentration	144.76	1	144.76	5.72	0.0480	
B-Surfactant concentration	517.39	1	517.39	20.45	0.0027	
AB	185.37	1	185.37	7.33	0.0303	
A^2	159.92	1	159.92	6.32	0.0402	
B^2	351.63	1	351.63	13.90	0.0074	
Residual	177.11	7	25.30			
Lack of Fit	158.96	3	52.99	11.68	0.0190	significant
Pure Error	18.14	4	4.54			
Cor Total	1482.09	12				

Table 13: Response surface quadratic model for encapsulation efficiency

Fit Statistics	
R^2	0.8805
Adjusted R^2	0.7951
Predicted R^2	0.2182
Adeq Precision	9.0739

Table 14: Fit statistics for encapsulation efficiency
Factor coding is **coded,** Sum of square is **TYPE III – Partial**

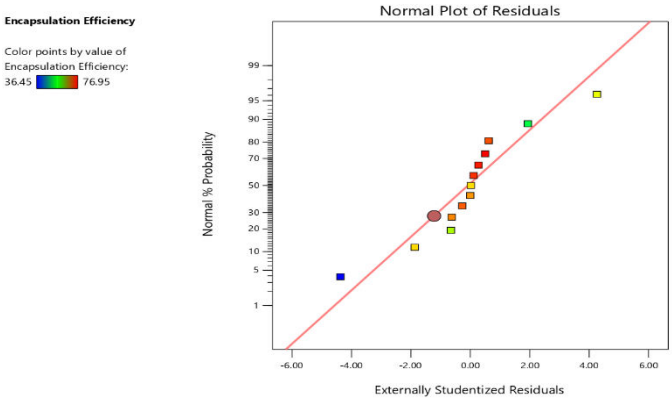


Figure 8: Normal probability distribution plot for encapsulation efficiency

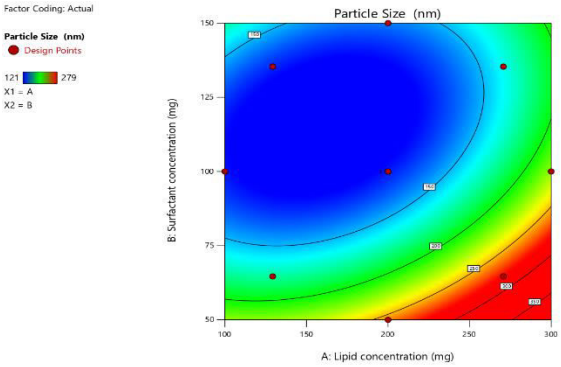


Figure 9: Contour plot for particle size analysis

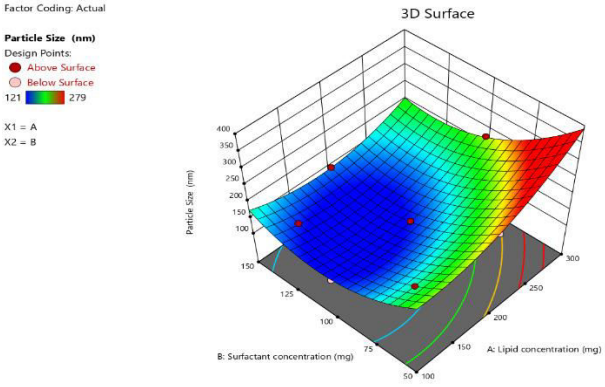


Figure 10: 3D plot for particle size analysis

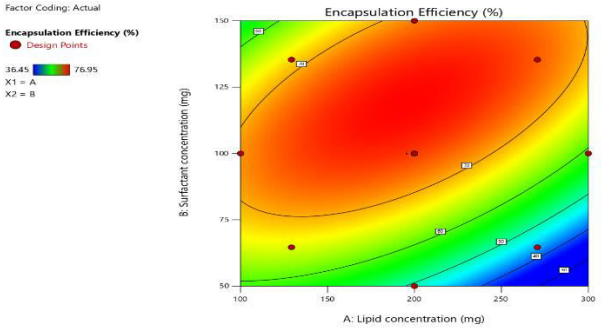


Figure 11: Contour plot for particle size analysis

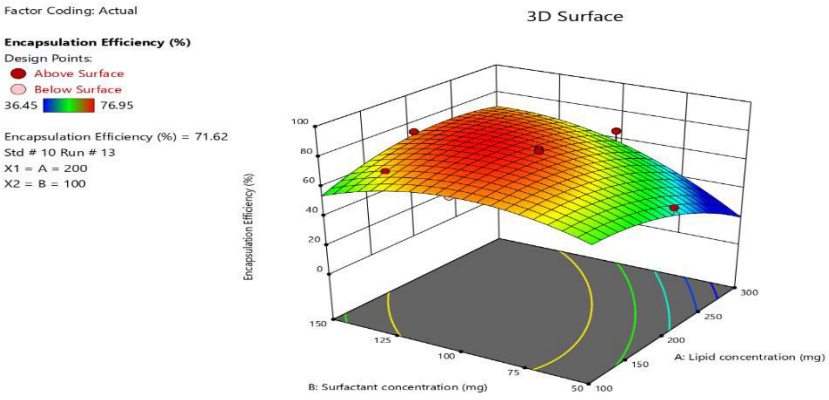


Figure 12: Contour plot for particle size analysis

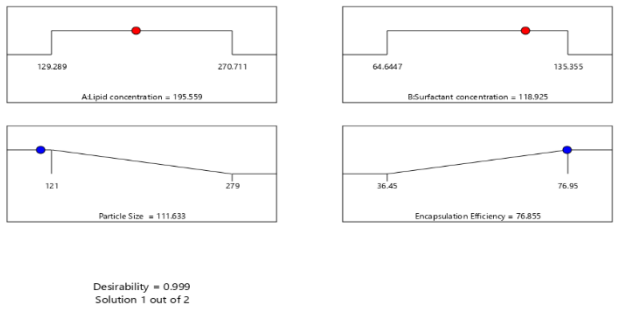


Figure 13: Ramp model of optimized formulation

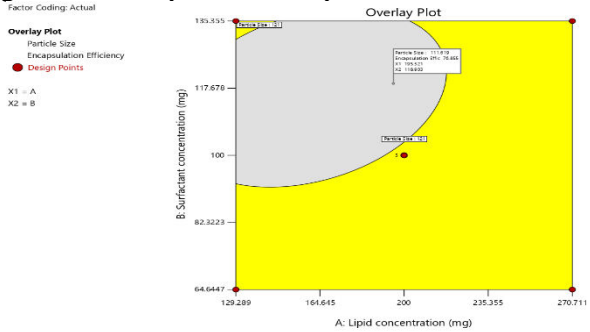


Figure 14: Over lay plot for optimized formulation

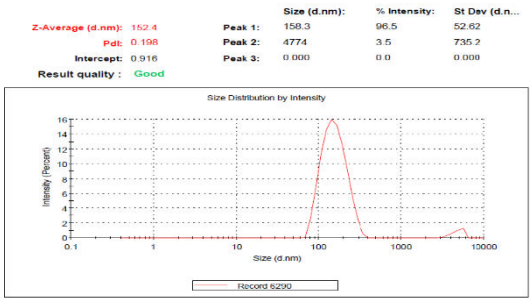
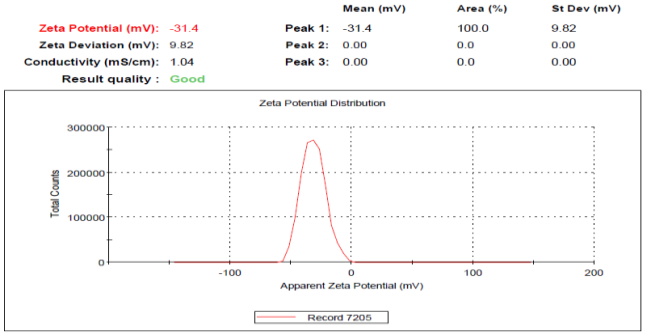


Figure 15: Particle size distribution of Optimized RT SLN



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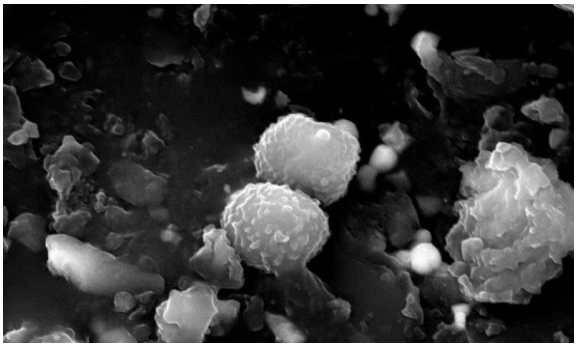

Figure 17: SEM image of Optimized RT SLN

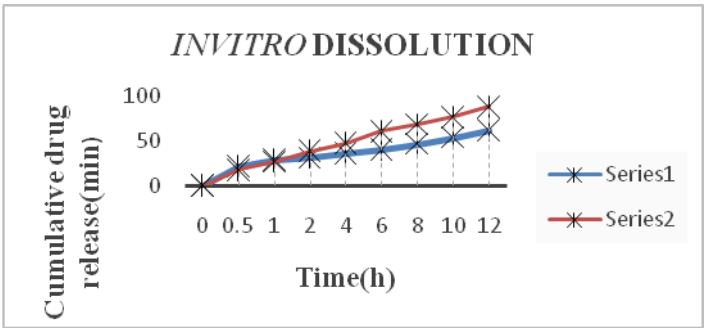

Figure 18: *In vitro* drug release of RT solution and RT SLN
Series 1 – RT solution, Series 2 – RT SLN solution

Code	Zero order	First order	Higuchi	Korsemeyerpeppas	
	r^2	r^2	r^2	N	r^2
Formulation	0.9627	0.9853	0.9946	0.481	0..9976

Table 15: *In vitro* release kinetics

The **Model F-value** of 10.32 implies the model is significant. There is only a 0.40% chance that an F-value this large could occur due to noise. **P-values** less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The **Lack of Fit F-value** of 11.68 implies the Lack of Fit is significant. There is only a 1.90% chance that a Lack of Fit F-value this large could occur due to noise. The **Predicted R^2** of 0.2182 is not as close to the **Adjusted R^2** of 0.7951 as one might normally expect; i.e. the difference is more than 0.2. **Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. Ratio of 9.074 indicates an adequate signal. This model can be used to navigate the design space. According to the statistical analysis, the quadratic equation of entrapment efficiency is significantly fitted to the experimental data and presented in the following equation:

$EE = +74.57 - 4.25 * A + 8.04 * B + 6.81 * AB - 4.79 * A^2 - 7.11 * B^2$

EE represents the entrapment efficiency, A and B are coded values for the lipid concentration and surfactant concentration, respectively. Terms with p-values of less than 0.0040 were considered as significant. Except for the main term of Lipid concentration (A), all other terms were less than 0.0040 (Table 20); The coefficient of determination (R^2) and adjusted R^2 were 0.8805 and 0.7951, respectively, which means that there is a good fit between independent variables and the EE of drug. The statistical analysis of data showed that the obtained model was significant ($p < 0.0040$), which confirms that the coefficient of determination (R^2) and adjusted R^2 are statistically significant. Since one purpose of this study was to increase the amount of entrapped drug in SLNs, the terms with positive coefficients are considered favorable for increasing entrapment efficiency. Conversely, negative coefficients which have a decreasing effect on entrapment efficiency must be kept at lower levels. The Lipid concentration (A) and surfactant concentration (B) have a negative and positive coefficient, respectively. Accordingly, a linear increase in A and B leads to a decreasing and increasing effect on entrapment efficiency, respectively. The normal probability distribution diagram implies that the residues are mainly located on a straight line and follow a normal distribution, as shown in Fig. 8. Describes the healthy correlation for the data in of predicted results and actual results. Each value points are located closer to the center line which indicates the relations with strong prediction.

Response Surface Analysis: The effect of the formulation variables on a response was assessed by studying the three-dimensional response surface plots.

Effect on Particle Size: The three-dimensional response surface plots and contour plots for particle size are presented in Figure 9, 10. As shown in Figure particle size increased with increasing in lipid concentration. Furthermore, increasing the particle size as a result of higher content of lipid might occur due to increased collision and aggregation of the nanoparticles, or relatively lack of enough surfactant for covering the surface of the particles. However, surfactant concentration showed negative effect on particle size and by increasing B, particle size decreased, while increasing the concentration of surfactant.

Effect on Encapsulation Efficiency: Figure 11, 12 describes the response surface model and contour plots for EE in response to the investigated variables. As shown in Figure 11, 12 EE improved with increases in lipid and surfactant concentration to an optimal maximum value. The possible reason could be that higher content of lipid afforded more space to accommodate the drug.

Experimental Validation of Design Space: The overlay plot has suggested a maximum possibility for predicted response values and concluded the possible values of responsible factors for it. Based on this conclusion, the trial run was carried out by taking the values reference from overlay plot. Overlay plot for optimization is given in figure 33. The predicted values for respective factors are 111.633 and 76.885 for particle size and %EE respectively

1. Characterization of Rivastigmine tartrate SLN

Particle size, PDI and Zeta potential analysis: The average particle size and the poly dispersity index of optimized

formulations were analysed using Malvern zeta sizer is found to be 152.4nm

The most important characterization parameter, also called Polydispersity Index (PI) which governs the physical stability of nanoparticles and should be as low as possible for the long-term stability of nanoparticles. a) PI value of 0.1–0.25 indicates a fairly narrow size distribution b) PI value greater than 0.5 indicates a very broad distribution.

Polydispersity index of optimized formulation is 0.198 and it indicates fairly narrow size distribution. Zeta potential is affected by the charge of the groups present on the MWCNTs. Nanoparticles with a zeta potential above ±30 mV have been shown to be stable, as the surface charge prevents aggregation of the particles. Optimized formulation with zeta potential -31.4 mV was found to be stable

Encapsulation efficiency: The result of entrapment efficiency of optimized formulation is 76.1%. This condition has shown an improved response values in comparison with the previously optimized formulation.

Scanning electron microscopy (SEM): SEM image describes that the particles have uniform loose aggregates, spherical in shape with a smooth surface and they are uniformly distributed

***In vitro* drug release studies:** The *in vitro* release of RT-SLN and RT solution was studied by the dialysis bag technique. The release study was performed in Phosphate buffer pH 7.4. The results are shown in table 25. The results showed that the during initial time points, slight increase in percentage drug release in drug solution because of high aqueous solubility of RT in aqueous medium. But in case of RT SLN, the time required for drug to leach out from lipid core was high compared to RT solution. Later increase in drug release is due to decrease in particle size. The drug release was plotted in a graph and given in figure 18

***In vitro* release kinetics:** The kinetics and mechanism of drug release were studied by release kinetics, r^2 and n values are indicated in the table 15. Results shows Higuchi model have high linearity compared to first and zero order. The exact mechanism of the release kinetics was determined by korsemeyerpeppas model. Results indicated that the SLN formulations followed Non-fikian model of release kinetics

SUMMARY AND CONCLUSION:

The objective of present investigation was to develop, optimize and evaluate rivastigmine tartrate loaded solid lipid nanoparticle (RT SLN) by using central composite design. SLN were prepared by microemulsion method using a systemic approach of design of experiments and evaluated. From the characterization of active drug it was concluded that the drug sample Rivastigmine tartrate was authentic, pure and confirming to the standards. The sample showed maximum absorbance at wavelength 263 nm. The concentrations of RT (10-60 µg/ml) showed good linearity with R2 value of 0.999 which suggests that it obey Beer-Lamberts law. Various preliminary experiments were performed for selection of suitable excipients. Various lipids and surfactant are selected on the basis of solubility and particle size and thus glyceryl monosterate selected as lipid, and poloxamer 188 as surfactant. Further High-speed homogenizer stirring speed and time was optimized HSH is operated at 15000 rpm for 5minutes. The design selected was Central composite design by employing design expert software. Two input factors were studied at five different levels including central point 0, +1, -1, ±α throughout the preparation process to determine their effect on two responses, namely mean particle size and encapsulation efficiency. The input factors being selected are the following: Lipid concentration (100, 200 and 300 mg), concentration of surfactant (50, 100, 150 mg). Characterization of all formulation particle size found to be in range of 121 to 279 nm and encapsulation efficiency in range of 36.45 to 76.95%. The overlay plot has suggested a maximum possibility for predicted response values and concluded the possible values of responsible factors for it. Based on this conclusion, the trial run was carried out by taking the values reference from overlay plot. The predicted values for respective factors are 111.633 and 76.885 for particle size and %EE respectively. Characterization of optimized RT SLN particle size found to be 152.4nm and

particle distribution index value is 0.198 indicates fairly narrow distribution. Optimized formulation with zeta potential -31.4 mV was found to be stable. SEM photographs of RT SLN indicate particles have uniform loose aggregates, spherical in shape with a smooth surface and they are uniformly distributed. On comparison of *in vitro* drug release studies of RT solution and RT SLN shows that RT SLN has higher release 87.74% at 12hrs compared to RT Solution 61.59%. From the obtained results it was concluded that the Rivastigmine tartrate SLNs can be employed for controlled delivery of drug in the treatment of Alzheimer's disease.

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Conflict of Interest: The authors declare no conflict of interest.

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