



International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: www.ijrpns.com



BRAIN TARGETED DELIVERY OF RIVASTIGMINE POLYMERIC NANOPARTICLES THROUGH ORAL ADMINISTRATION TO TREAT ALZHEIMER'S DISEASE

Natarajan Tamilselvan¹, Chellan Vijaya Raghavan*¹, Krishnamoorthy Balakumar¹, Siram Karthik¹

*¹Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore-641044, Tamil Nadu, India.

ABSTRACT

The aim of the present study was to formulate and characterize polysorbate-80 coated PLGA nanoparticles to target brain upon oral administration. Rivastigmine tartrate (RT) loaded PLGA nanoparticles were prepared by adopting modified nanoprecipitation method and were later coated with polysorbate 80 (PS80) and characterized for particle size, morphology physical state, drug entrapment and *in vitro* release. Further, *in vivo* pharmacokinetics studies conducted in rat exhibited the AUC₀₋₂₄ of RT in rivastigmine nanoparticle (R-NP) was (4825.9ng/ml) which was higher when compared with the AUC₀₋₂₄ of rivastigmine solution R-sol (224.483ng/hr). Tissue distribution studies of RT in brain after oral administration of polysorbate 80 coated nanoparticles (RNP-PS-80) was found to be 60.99ng/ g tissue and 93.5ng/g tissue as compared with the uncoated R-NP 30.0ng/g tissue and 41.33 ng/g tissue at 1st and 4th hour respectively. Histopathology of the brain sections treated with R-sol, RNP and RNP-PS-80 indicated no morphological changes and toxicity. The brain to blood ratio of RNP-PS-80 was indicates a greater ability of RNP-PS-80 to reach the brain and can be a promising approach for targeted delivery of RT to brain through oral route for the treatment of Alzheimer's disease.

KEYWORDS

Blood Brain Barrier, polymeric nanoparticles, Alzheimer's disease, Oral delivery and Brain targeted delivery.

Author of correspondence:

Chellan Vijaya Raghavan,
Department of Pharmaceutics,
PSG College of Pharmacy,
Coimbatore-641 044, Tamil Nadu, India.

Email: drvijayaragha@gmail.com.

INTRODUCTION

Neurodegenerative diseases represent a crucial and exponentially increasing challenge to the health care systems all over the world. Alzheimer's disease (AD) is the most common form of dementia and currently affects 35 million patients across the world which is expected to double in the next 20 years¹. Targeting of drugs to the brain is not only a challenging issue, but an equally important issue for

pharmaceutical research, as many hydrophilic drugs and neuropeptides fail to cross the blood–brain barrier (BBB)². It is estimated that more than 98% of the small new molecules do not cross the BBB, and hence fail to achieve the therapeutic concentration within the brain parenchyma cells³.

Many approaches have been developed to circumvent this problem using liposomes⁴ magnetic nanoparticles⁵ solid lipid nanoparticles⁶ and polymeric nanoparticles. Among these, polymeric nanoparticles are advantageous when compared with the aforementioned drug delivery systems in terms of obtaining a sustained release and better stability during storage⁷. Drug delivery to the brain using polymeric nanoparticles opens a new pathway to treat neurodegenerative diseases like Alzheimer's, Parkinson's, and multiple sclerosis. Polymeric nanoparticles using a biodegradable carrier are one of the best known carriers for controlled and sustained release and one among them is poly lactide-co-glycolide (PLGA)⁸. Further, coating the nanoparticles using a surfactant has also attracted attention as a potential means to enhance the drug uptake to the brain. It has been reported that nanoparticles coated with polysorbate 80 enhance brain targeting by acting as an anchor for apolipoproteins and facilitating uptake by the brain endothelial cells via receptor mediated endocytosis⁹.

Rivastigmine tartrate (RT) is a reversible cholinesterase inhibitor used in the treatment of Alzheimer's disease¹⁰. Which does not cross the blood brain barrier (BBB) owing to its hydrophilic nature. Further, a particle size below 200nm is a very important prerequisite for crossing BBB. Several methods have been developed to prepare polymeric nanoparticles of hydrophilic drugs like nanoprecipitation and emulsion solvent evaporation. In spite of its low entrapment efficiency values for hydrophilic drugs nanoprecipitation offers an easy and reproducible technique with narrow unimodal particle size distribution (100-300nm) and is widely used by several research groups to prepare polymeric nanoparticles¹¹. Owing to the ability of RT to be soluble in both water and organic solvents its incorporation into NP makes it an interesting

challenge, as the drug should not leak out from its aqueous phase during the preparation of nanoparticles¹².

Hence, the present study was aimed to formulate RT loaded PLGA nanoparticles, coated with polysorbate 80 and to study the influence of pH on aqueous phase during the preparation of nanoparticles. These nanoparticles were characterized for its physicochemical properties and finally, *in vivo* pharmacokinetic studies and tissue distribution studies were conducted in rats to check for the efficiency of these nanoparticles to reach brain through oral administration.

MATERIALS AND METHODS

PLGA resomer 502 H were purchased from Sigma-Aldrich, rivastigmine tartrate was received as a gift sample from alembic pharmaceutical (Vododra Gujarat), Potassium dihydrogen phosphate, tetra butyl methyl ether, ortho phosphoric acid Pluronic F-127, acetone, sodium hydroxide, D-mannitol, acetonitrile were analytical Grade.

Preparation of nanoparticles by modified nanoprecipitation method

RT loaded poly lactide-co-glycolide (PLGA) NPs were prepared by modified nanoprecipitation method using phosphate buffer (pH 6.0 to 9.0) as an aqueous phase¹³. RT and PLGA in the ratio of 1:1,1:2,1:3 and 1:4 were dissolved in acetone and acetonitrile. This organic phase was added slowly to 10 ml of phosphate buffer containing 1% pluronic F127 and the organic solvent was then allowed to evaporate for 4 hours with continuous stirring (50 rpm) on magnetic stirrer (Remi). The NP suspension was then centrifuged at 13,000 rpm for 1hr at 4° C using high speed centrifuge (Eppendorf) and the sediment comprising NPs was freeze dried for 24 hours using freeze drier (Lyodel, India) using 2% D-mannitol as a cryoprotectant.

Coating of RT nanoparticles with Polysorbate 80

The coating of nanoparticles was performed according to the procedure described by Kreuter *et al.*¹⁴. The freeze dried product was resuspended in phosphate buffered saline (PBS) pH 7.4 at a concentration of 20 mg/ml under constant stirring of

50 rpm (Remi magnetic stirrer). Polysorbate 80 at concentrations of 1% and 2% (w/v) was then added to the solution containing nanoparticles and the final mixture was incubated for 30 minutes. The freeze dried nanoparticles were subjected to drug entrapment and *in vitro* release studies.

Physicochemical characterization of nanoparticles

Particle size distribution, zeta potential and polydispersity index (PDI) of the formulated nanoparticles were determined by dynamic light scattering (DLS) analysis using the Zetasizer Nano ZS90 (Malvern Instruments Ltd, UK), the samples were placed in disposable cuvettes for particle size measurements. Each experiment was conducted in triplicate¹⁵.

Morphological studies

The surface morphology of the prepared NPs was determined by using high resolution transmission electron microscopy (HRTEM) and atomic force microscopy (AFM). A drop of nanosuspension was placed on a carbon film coated copper grid for TEM. And the studies were performed at 80kv using JOEL JEM 2100, Japan equipped with a selected area electron diffraction pattern (SAED)¹⁶. AFM images were captured using (Multimode Scanning probe microscope (NTMDT, NTEGRA prima, Russia)¹⁷.

Drug entrapment

Freeze-dried nanoparticles 50mg were dissolved in acetonitrile and the drug concentration was measured by ultraviolet spectroscopy at 220nm¹⁸. Drug entrapment efficiency was estimated using the following equation

$$\text{Drug entrapment (\%)} = \frac{\text{Mass of drug in nanoparticles} \times 100}{\text{Mass of drug used in formulation}}$$

Differential Scanning Calorimetry

The physical state of entrapped RT was analysed using Differential Scanning Calorimetry (DSC, Universal V4.7A TA Instruments). The sample (5mg) was placed on a standard crimped aluminium pan and heated at a rate of 10°C/min from 4 to 450°C with continuous purging of nitrogen gas (20ml/min). An empty aluminium pan was used as a reference¹⁹.

In vitro drug release studies

In vitro release studies were performed using dialysis sac method²⁰. The freeze dried nanoparticles (2mg) were suspended in phosphate buffer pH 7.4 and then placed in a dialysis membrane (molecular weight cut off 10,000-12,000Da) with its ends closed using membrane clips. The dialysis membrane was then placed in a beaker containing 50ml of 0.1 M phosphate buffer (pH 7.4), and maintained at 37 °C with continuous magnetic stirring. At specified time intervals of 1, 2, 4,8,12 and 24 hours 2ml of aliquots were withdrawn from the medium and replaced with the equal volume of phosphate buffer. The concentration of RT was assayed spectrophotometrically at 220 nm.

Stability of drug in stimulated gastric fluid

The stability of RT was evaluated in SGF, prepared according to British Pharmacopoeia (BP). Briefly; SGF was prepared by dissolving 2 g of NaCl and 3.2 g of pepsin in 80 ml of 1M HCl and finally made up to 1000 ml with adjustment of final pH to 1.2. 50 mg of each formulation was suspended in 15 ml of SGF and placed in screw capped tubes. Te tubes were kept in the water shaker bath maintained at 37°C. At specific time interval of incubation in SGF for 3hours, suspensions were centrifuged at 13000rpm for 40min and the supernatants were discarded. The sediment was dispersed in the solvent and the drug content is determined in UV.

Bio analytical Method

The concentration of RT in samples were determined by HPLC (Waters 2475) equipped with a fluorescence detector and a reversed phase column (Gemini C18, 5µ, 50×30mm, Phenomenox). A mixture of phosphate buffer (20mm) and acetonitrile (80:20) adjusted to pH 3.1 using orthophosphoric acid was used as mobile phase. The flow rate was maintained at 0.3 ml/min and the column temperature was set to 36°C. RT was analysed by fluorimetric detector with an excitation and emission wavelengths of 220 and 293nm respectively.

Extraction of RT from the plasma and tissue samples was carried out using a modified liquid-liquid extraction method ²¹. Rat plasma or tissue homogenate (100µl) was mixed with 20µl of 1M

sodium hydroxide solution and vortexed for 60seconds. To this mixture, 0.7 ml of tetra butyl methyl ether was added and centrifuged at 10000 rpm for 5 min followed by separation and evaporation of organic layer in a turbo vap LV (Biotage USA) at 40°C under a nitrogen gas stream. Finally, the dried residue was reconstituted with 100µl of mobile phase and 20µl of the reconstituted mobile phase was subjected for HPLC analysis.

***In vivo* pharmacokinetic studies**

In vivo pharmacokinetic studies were carried out in male wistar rats weighing about 200-250g. The protocol for the animal experiments was reviewed and approved by PSG Institutional Animal Ethics Committee (Proposal No: 197/2013/IAEC), Coimbatore. The animal house was well ventilated and the animals were provided with food and water ad libitum and maintained on a standard diurnal cycle in a cage with sterile paddy husk. The animals were allowed to fast overnight before the study was performed. The animals were divided in to 3 groups and each group contains 6 animals. A dose equivalent to 1mg/kg was administered orally to the animals using a gastric lavage needle. Group 1 received RT solution suspended in PBS 7.4, (R-sol) Group 2 received uncoated RT nanoparticle(R-NP) and Group 3 received polysorbate-80 coated R-NP(RT-PS-80). The blood samples (500µL) were collected in to micro centrifuge tubes containing sodium citrate by retro-orbital venous plexus puncture using heparinised capillary tubes at 60, 120, 240, 480, 720 and 1440 min post dosing. The blood samples were centrifuged at 4000rpm for 10 min and the plasma was separated and stored at 20°C until analysis²².

Tissue distribution study

The tissue distribution experiments were conducted on male Wistar rats weighing 200-250 g after oral administration. The rats were divided in to three groups with 6 each group. Group 1 received pure RT, Group 2 received uncoated R-NP and Group 3 received polysorbate-80 coated R-NP at the dose of 1mg/kg. After 1 hour and 4th hour post administration, 3 animals from each group were sacrificed and the target tissue of interest, brain and

non target tissues of interest, lungs, liver and kidney were removed and homogenised using tissue homogenizer (Remi) with ice cold PBS 7.4²³. The drug content in the organs was analyzed using HPLC waters 2475 coupled with FLD.

Histopathological analysis

Histopathological analysis was carried out in male Wistar rats weighing 200-250g. The animals were divided in to three groups, group -1 received R-sol, group-2 received R-NP and group-3 received RNP-PS-80 nanoparticles via oral administration. Animals were sacrificed after 24 hours and brains were removed. Brain tissue was fixed in 10% formaldehyde solution and embedded in paraffin. Paraffin sections of thickness 7µm were prepared for microscopy study. Finally the tissues were dipped in water and are stained with haematoxylin and eosin for 10 min at 60 °C. The stained sections were washed in running water to remove the excess stain and then upgraded for dehydration through different grades of alcohol. Finally the slides were then cleared with xylene and mounted with DPX to make them permanent. The sections were observed for the presence of vacuolisation/spongiosis, lipofuscin deposits and neuronal degeneration²⁴.

Stability studies

Stability studies were carried out as per ICH guidelines to assess the stability of RT loaded PLGA nanoparticle(R-NP) and polysorbate 80 coated nanoparticles (RNP-PS-80). The formulations were stored in a glass vial at refrigerated conditions (5°C ± 3°C) and room temperature (30°C ± 2°C and 65% RH ± 5% RH) over a period of 6 months. At definite time intervals (0, 1, 2, 4 and 6 months), the samples were evaluated for physical appearance, particle size and drug entrapment²⁵.

RESULTS AND DISCUSSION

Preparation of RT nanoparticles

RT- loaded PLGA nanoparticles were prepared by modified nanoprecipitation method by varying the pH of the aqueous phase and the organic solvent to obtain better particle size and drug entrapment. Due to the high solubility of RT in the aqueous phase, it partitions into the external aqueous phase during the

preparation here by decreasing the amount of drug entrapped in the polymer. Initially organic solvents like acetonitrile and acetone were selected for the preparation of nanoparticles because of its water miscibility nature. Table No.1 shows the particle size of formulations with various solvents. The formation of nanoparticle can be is mainly due to the marangoni effect, solvent flow, diffusion and surface tensions of the organic solvent. When acetone was used in the preparation of nanoparticles, it diffused out quickly from the droplets which lead to the formation smaller particle size of less than 200nm. But in case of acetonitrile it diffuses slowly due to the lower surface tension of the solvent which leads to the formation of larger droplets in size range of 400nm. A particle size below 200nm is a very important prerequisite to cross the BBB, hence for the preparation of nanoparticles acetone was selected as the organic phase As RT is a hydrophilic drug poor entrapment efficiencies are observed. So in order to achieve better entrapment efficiency the effect aqueous phase pH from 6to9 was studied. Since RT is strong base, studies were performed at the pH level greater than 6 so that it can be absorbed faster in that basic pH of the intestine. As the pH of the medium increased from 6 to 9 the amount of RT that partitioned into the organic phase also increased. At pH 9, RT showed a high affinity towards the organic phase. Further the NP is coated with polysorbate 80 will enhance drug uptake by the brain microvessel endothelial cells.

Influence of aqueous phase pH on the preparation of nanoparticles

The pH of the aqueous phase influences the ionisation of the drug, there by its solubility. Formulations were prepared with the pH of 6, 7, 8 and 9, which showed an enhanced drug entrapment in the polymer with an increase in the pH of the aqueous phase, as shown in Figure No.1. The affinity of RT towards the organic phase increased when the pH of the aqueous phase was increased from pH 6 to 9 with strong augmentation at pH 9. The formulations with pH 6, 7 and 8 showed only 30.2, 35.6 and 53.5% of drug entrapment respectively. At pH 9 a dramatic increase in drug

entrapment of 79.1% was observed. This increased drug entrapment was related to the ionic state of RT in the aqueous phases which lead to a decrease in the solubility of drug in aqueous phase and an increase in the drugs affinity toward the organic phase. Hence, the substitution of the aqueous phase with pH 9 probably decreased the leakage of the drug and improved drug entrapment.

Physiochemical characterization of the nanoparticles

Table No.2 shows the composition of the formulations particle size, polydispersity index and drug entrapment based on a drug to polymer ratio. An increase in the amount of the polymer concentration increases the viscosity of the solution, resisting the breakdown of the particles. Thus the particle size tends to increase with an increase in the amount of polymer. When the amount of polymer was same, an increase in the amount of drug added from 6 to 12 mg in the formulation did not show any difference in particle size. For the formulation containing drug to polymer ratio 1:3 a mean particle size of $115 \pm 5\text{nm}$ was observed (Figure No.2A) and a zeta potential of $-31.9 \pm 4.2\text{mV}$ as shown Figure No.2B before coating. Coating of nanoparticles with surfactant polysorbate 80 slightly enhanced the particle size and reduced the zeta potential (Table No.3). An increase in the concentration of polysorbate 80 from 1 to 2% leads to an increase in the particle size above 200nm because the high viscosity of polysorbate 80 has lead to adsorption of excess amount polysorbate 80 by masking the surface charge of nanoparticles^{26, 27}.

TEM images of RT loaded NPs were roughly spherical and sub spherical in shape. The morphology of nanoparticles for formulation F3 was investigated by TEM and AFM. TEM image in Figure No.2C revealed that the particle size is 50 nm. This is more than the particle size obtained by photon correlation spectroscopy (PCS)²⁸. TEM yields a number average size while Zetasizer® measures the hydrodynamic diameter which reflects on how a particle diffused within a fluid. The hydrodynamic diameter depends not only on the size of the particle core, but also on its surface structure,

concentration and type of ions in the medium. Therefore, the size determined by a Zetasizer® could be larger than the size measured by TEM. The topographic images were found out using AFM (Figures No.3A and 3B) which indicated a smooth surface, without any noticeable pinholes or cracks. All particles were spherical in shape and separated from each other, suggesting possible stabilization of the NPs due to the presence of Pluronic F127. The particle size observed by AFM correlated well with that observed by TEM.

DSC and SAED experiments were carried out for inspecting the crystalline properties and to scrutinize for any possible interactions between the drug and polymer in the nanoparticles. DSC is one of the most general methods to find out the physicochemical interaction between drug and polymer in a formulation. DSC thermograms of pure RT, PLGA, physical mixtures and RT nanoparticles are demonstrated in Figure No.4A. The DSC thermogram of the polymer showed an endothermic peak corresponding to the glass transition (T_g) at 51.2°C. Melting point corresponding to the drug was not observed in the thermogram of formulation, indicating the presence of drug in amorphous state. The DSC curves of the RT and PLGA physical mixture showed peaks resulting from the simple superposition of the DSC curves of the individual components and the RT containing nanoparticles showed the decreased melting point peak of RT which indicates that the encapsulated drug is in an amorphous form. This fact is further supported by Selected Area Electron Diffraction (SAED) pattern in Figure No.4B which reveals that encapsulated RT is in an amorphous state or is molecularly dispersed in the polymer matrix.

The drug entrapment values increase with in polymer concentration. Drug entrapment for formulations (F1 to F4) containing 6mg of RT with various drug: polymer ratios of 1:1 to 1:4 range from 68.1% to 80.5% and for formulations F4 to F8 containing 12 mg of the RT with various polymer concentrations the entrapment values ranged from 62.4 % to 85.9 %. An increase in the amount of RT in the formulation did not show much difference in

drug entrapment. Higher drug entrapment efficiency is due to the change of pH of the aqueous phase and increased polymer concentration. Formulations F1, F2, F3, F5 and F6 with a particle size less than 200 nm and better drug entrapment efficiency were evaluated for drug release.

***In vitro* drug release**

The rate of release of drug from PLGA NPs is dependent upon the molecular weight and lactide content of the polymer. The rate of release of drug reduced with an increase in molecular weight and lactide content of the polymer²⁹. A low molecular weight PLGA (7000-17000) with a lactide: coglycolide ratio of 50:50 was used to obtain a sustained release of the drug. The release profile of the RT loaded formulations F1, F2, F3, F5 and F6 shown in Figure No.5. The initial burst release of drug is associated with the drug adsorbed on to the nanoparticles surface, which easily gets dispersed in the initial 60 minutes. The formulations (F1 to F6) initially showed a rapid release in two hours followed by sustained release over a period of 24hours. After 24 hours, the percentage of RT release was 44.2 %, 45.4 % and 51.2 % for formulations F1, F2 and F3, respectively and for formulation F5 and F6 it was 48.4 % and 49.8%. The coating of nanoparticles with polysorbate 80 slightly reduced the amount of RT released from the nanoparticles which can be attributed to the slow diffusion of drug from the polymer coating and also from the polysorbate 80 coating, which could lessen the percentage of drug release. The effect of drug entrapment (when the amount of polymer is same) on release pattern of RT was also studied. When the amount of drug concentration increased, the initial burst release of RT also increased because of the higher amounts of free drug in the aqueous phase and increased amount of drug adsorbed on the nanoparticle.

The stability of drug in stimulated gastric fluid was also studied it was observed that the percentage of drug remaining was ranged from 68 ± 1.5 to 70.3 ± 2.1 %. These results suggest that the drug was protected from degradation from gastric enzymes up to 3hrs. It may due to the long chains of polysorbate-80 which

forms a protective brush which prevents from degradation of the drug and surface density with long chains able to provide the protective effect on drug.

In vitro release kinetics data were computed using DD solver, which is an excel plug-in module³⁰ and the resultant data were fitted to the Korsmeyer-Peppas exponential equation to establish the mechanism of drug release. The 'n' values were in range of (0.232 to 0.293) with r² values of 0.9917 to 0.9983 which suggest that drug release follows fickian diffusion controlled mechanism.

***In vivo* pharmacokinetic studies**

In vivo pharmacokinetic and tissue distribution studies were performed in male Wister rats and the plasma and tissue concentration of RT at different time points following oral administration of R-sol, R-NP and RNP-PS-80 were determined. The pharmacokinetic parameters were calculated using non compartmental analysis using WinNonlin (version 5.1) and pharmacokinetic parameters like C_{max}, t_{max}, AUC₀₋₂₄, t_{1/2} and MRT (hr) were summarized in Table No.4. The concentrations vs. time profile of different formulations in plasma were plotted in Figure No.6. The concentration of RT in plasma was higher in rats which were administered with R-NP than those administered with free RT. The C_{max} of RT after administration with R-NP was significantly higher than the C_{max} obtained after administration of R-Sol (P < 0.05) and a 5.2 fold increase was observed. An increase in C_{max} after administration through nanoparticles indicated an increase in the amount of nanoparticles absorbed and a delayed T_{max} demonstrates a sustained release pattern of RT. This showed that R-NP facilitates better absorption by reducing the first pass metabolism leading to improved bioavailability. Plasma area under curve (AUC) of RT sol and R-NP was found to be 224.48 and 4826.00 ng.hr/ml respectively. A higher amount of RT was observed in plasma when administered as nanoparticles at 24 hrs than that of RT sol. which indicates a sustained release pattern of PLGA nanoparticles. The plasma drug concentration profile obtained after administering polysorbate -80 coated nanoparticles

(RNP-PS-80) indicated a lower amount of RT in blood when compared with the plasma concentration profile of R-NP indicating the presence of RT in brain.

Tissue distribution

At 1st and 4th hour after oral administration, the target tissue of interest, brain and non target tissues like liver, lungs and kidney were collected to determine the drug concentration by HPLC. Figure No.7 shows the concentration of RT (ng/g) achieved in different organs such as brain, liver, lungs and kidney at different time points. A slight higher drug concentration was observed for R-NP in liver and lungs in comparison with drug solution at 1st and 4th hr probably due to the combined activity of circulating blood passing through the organs and uptake of cells of the endothelial system. The concentration of RT in liver and lungs at 1st hr when administered as R-Sol and R-NP was found to be 129.80, 123.50ng and 129.8, 180.07ng respectively. The drug accumulation in liver, lungs and kidney was reduced for RNP-PS-80 and can be attributed to polysorbate 80 coating which prevented RES uptake, which was also supported by the earlier findings^{31,32} indicating the role of surface properties in biodistribution of nanoparticles. An increase in the amount of RT was observed in brain for RNP-PS-80 when compared to drug solution and uncoated nanoparticles. Apart from the size of nanoparticles the surface properties and hydrophobicity also influence the amount of opsonization and biodistribution pattern of nanoparticles. Opsonin proteins are present in blood stream and bind with conventional non stealth nanoparticles allowing macrophages of the reticuloendothelial system to easily recognise and remove the drug before the therapeutic concentration is achieved. To prevent opsonization hydrophilic coating is used as it repels the adsorption of opsonin protein via steric repulsion force there by promoting longer circulation. Since polysorbate-80 is a hydrophilic surfactant, it inhibits the P-glycoprotein efflux pump and further, it enhances uptake by brain endothelial cells by covalently coupling with apolipoprotein E, A-1. Thus, in the present study after oral administration

of RN-PS-80, a higher drug concentration in brain was observed when compared to the uncoated R-NP and R-sol.

Figure No.8 represent the brain to plasma ratio of RT when administered with R-sol, R-NP, and RNP-T80. It is clear that the brain to plasma ratio of RNP-PS-80 was higher than that of the drug in solution and uncoated nanoparticles after oral administration which clearly indicates the accumulation of accumulation of RT in brain tissue. A possible mechanism of transport of nanoparticles across BBB and increased retention of nanoparticles in the brain is the creation of higher concentration by the blood capillaries which would enhance the transport across endothelial cells. Secondly the surfactant can solubilise the endothelial cell membrane lipids leading to membrane fluidisation. Thirdly the nanoparticle could promote opening of tight junctions between the endothelial cells. Finally the polysorbate-80 coated nanoparticles possibly adsorb apolipoprotein B or E which interacts with brain capillary endothelial cells via receptor- mediated endocytosis and help in permeation across the blood brain barrier. The therapeutic availability of RT from RNP-PS-80 was 3.76 and 2.03 folds higher than that of R-sol and R-NP at 1st hr. The availability of RT at 4th hr was found to be 6.50 and 2.26 folds higher than that of drug in solution and uncoated nanoparticles. These data collectively supports the delivery of polysorbate-80 coated nanoparticles to brain through oral route and this enhanced concentration of RT in brain will be beneficial in treating Alzheimer’s disease.

Histopathological analysis

Photomicrographs of rat brain sections of control showed normal nerve cells (Figure No. 9A). and drug in solution. (Figure No.9B). Brain sections of uncoated R-NP were evaluated for any morphological changes, including inflammation of neurons after oral administration of nanoparticles in

cerebral region. The results indicate absence of abnormalities after 24 hrs. Figure No.9 C shows that the nanoparticles did not produce any toxicity in the brain. Sections of brain (Figure No.9D) after administering RNP-PS80 did not show any morphological changes like vacuolisation / spongiosis and neuronal degeneration. Hence, it can be concluded that the polymeric nanoparticles are safe to the brain.

Stability studies

Stability studies of RNP-PS-80 were carried our as per ICH guidelines. The formulations were evaluated for any changes in physical state, particle size and drug entrapment Table No.5 shows the stability studies data of PLGA nanoparticles. The formulation had good physical appearance over the period of time. The particle size was analyzed at the time interval of 0, 1, 2, 4, 6 months of storage at refrigerator (3-5°C) and room temperature 37°C (RH=75%). Indicated an increase in particle size at different time intervals. The initial particle size at day 0 was 125±5 nm and the size after 6 months was 165±10nm. The slight increase in particle size may be due to the weak vander waals force that holds the particles together which leads to the formation soft agglomerates. The drug content after 6 months storage at different conditions was similar which indicates that there was no drug leakage during storage. These results demonstrate that the developed PLGA nanoparticles are stable and retain its physical appearance at various storage conditions with slight increase in particle size over a period of 6 months.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out by two way analysis of variance and subsequent bonferroni post test. P values less than 0.05 were considered significant.

Table No.1: Effect of the solvent on nanoparticle size (n=3±SD)

S.No	Solvent	Particle size(nm)	Zeta potential(mV)
1	Acetonitrile	400±15	-31± 2.4
2	Acetone	120±10	-32±4.2

Table No.2: Compositions, size and drug loading of RT loaded PLGA nanoparticles

S.No	Formulation code	Drug (mg)	Drug: Polymer Ratio	Particle size (nm)	PDI	Drug Entrapment (%)
1	F1	6	1:1	110±12	0.090±0.04	68.1±2.2
2	F2	6	1:2	112±7	0.120±0.08	77.8±1.8
3	F3	6	1:3	125±5	0.151±0.034	79.1±0.8
4	F4	6	1:4	221±10	0.201±0.004	80.5±1.5
5	F5	12	1:1	123±6	0.148±0.065	62.4±1.5
6	F6	12	1:2	165±9	0.189±0.045	74.2±0.7
7	F7	12	1:3	214±8	0.204±0.023	82.5±2.4
8	F8	12	1:4	240±5	0.225±0.054	85.9±1.8

(n = 3 ± SD)

Table No.3: Physicochemical parameters of surfactant coated PLGA nanoparticles

S.No	Formulation code	Size after coating with PS-80	Zeta Potential(mV)	
			Before coating with PS-80	After coating with PS-80
1	F1	120±10	-21.2±3.2	-18.6±2.4
2	F2	125±12	-25.5±5	-20.2±3.2
3	F3	135±8	-40.5±2.4	-31.9±4.2
4	F4	260±8	-22.6±6.4	-18.6±4.2
5	F5	145±6	-26.4±4.6	-22.4±2.2
6	F6	190±9	-27.8±3.4	-21.2±6.2
7	F7	235±12	-26.1±4.2	-24.6±4.2
8	F8	275±10	-18.4±4.6	-10.4±5.4

(n = 3 ± SD)

Table No.4: Pharmacokinetic parameters of R-sol, R-NP and RNP-PS-80 after oral administration of rivastigmine a4 1mg/kg in rats

S.No	Formulation code	C _{max} (ng/ml)	t _{max} (hr)	t _{1/2} (hr)	AUC ₀₋₂₄ (ng/hr/ml)	AUC 0-Inf (ng/hr/ml)
1	R- Sol	53.53 ±13.0	1	2.31±0.3	224.48±42.5	234.247±42.3
2	R-NP	278.85±17.6	4	15.37±2.6	4826.00±325.7	8786.098±1390.3
3	RNP-PS-80	157.57±10.6	4	17.49±2.8	2737.87±113.6	5142.292±803.3

Table No.5: Stability studies of PLGA nanoparticles of the Rivastigmine

S.No	Temperature	Evaluation parameter	Observation(month)				
			0	1	2	4	6
1	30°C (RH=65%)	Physical appearance	Pale white powder	No change	No change	No change	No change
		Particle size(nm)	135±4	145±5	158±4	160±9	178±6
		Drug loading (%)	79.1±0.8	79.0±0.6	79.2±0.2	79.18±0.8	79.4±0.5

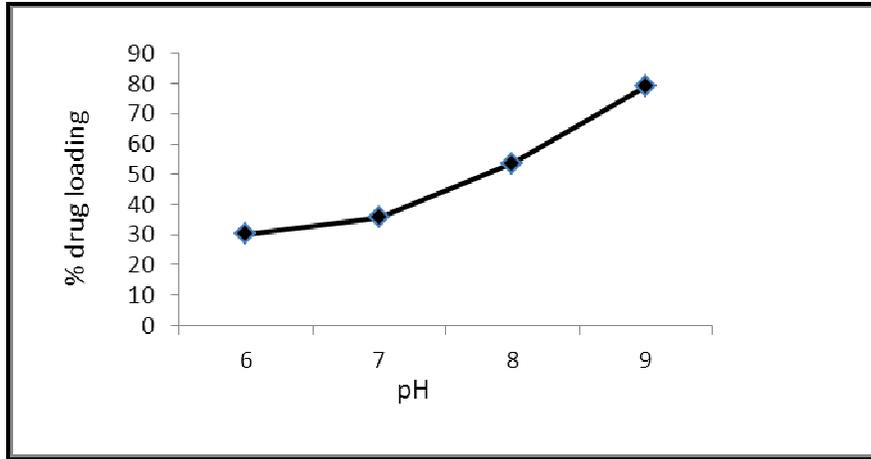


Figure No.1: Influence of aqueous phase pH on drug entrapment efficiency

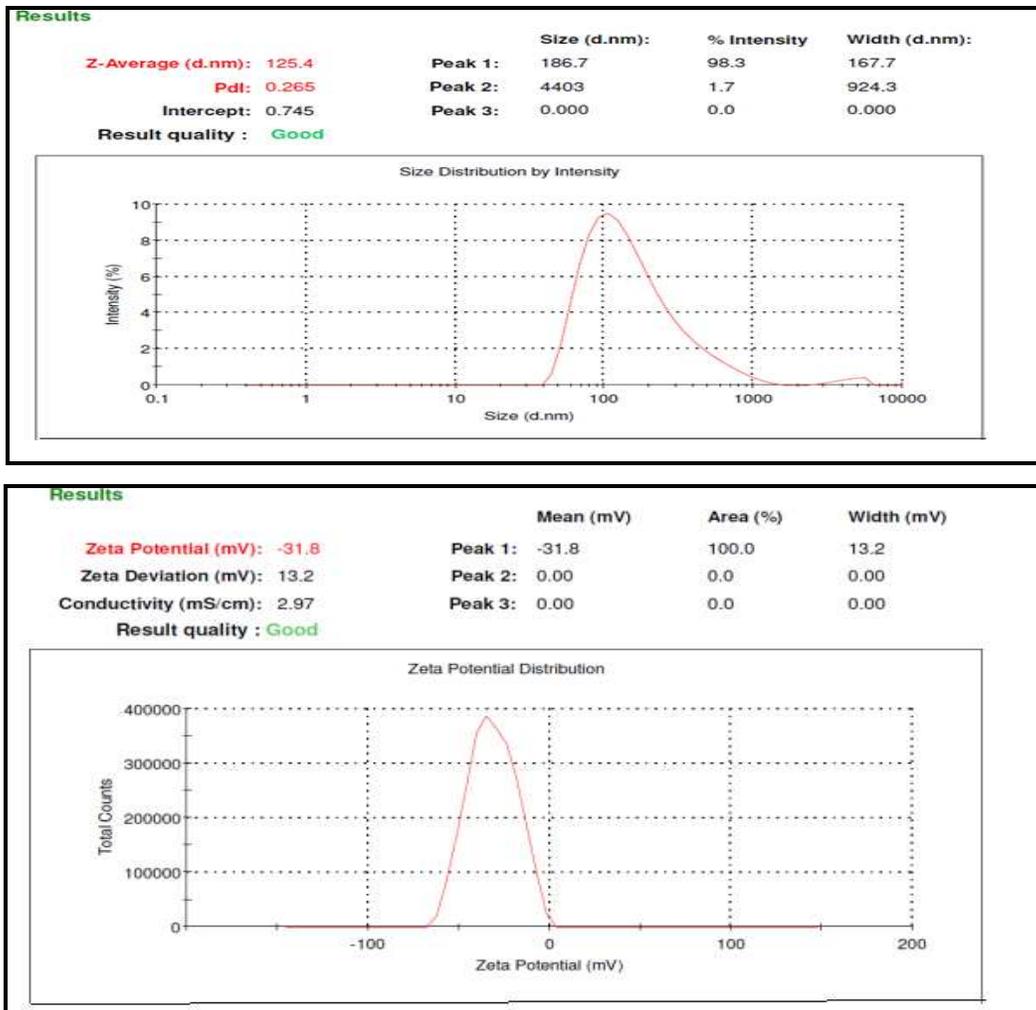


Figure No.2 (a and b): DLS measurement to determine particle size for RT loaded Nanoparticle (A) and Zeta Potential analysis of RT loaded nanoparticles

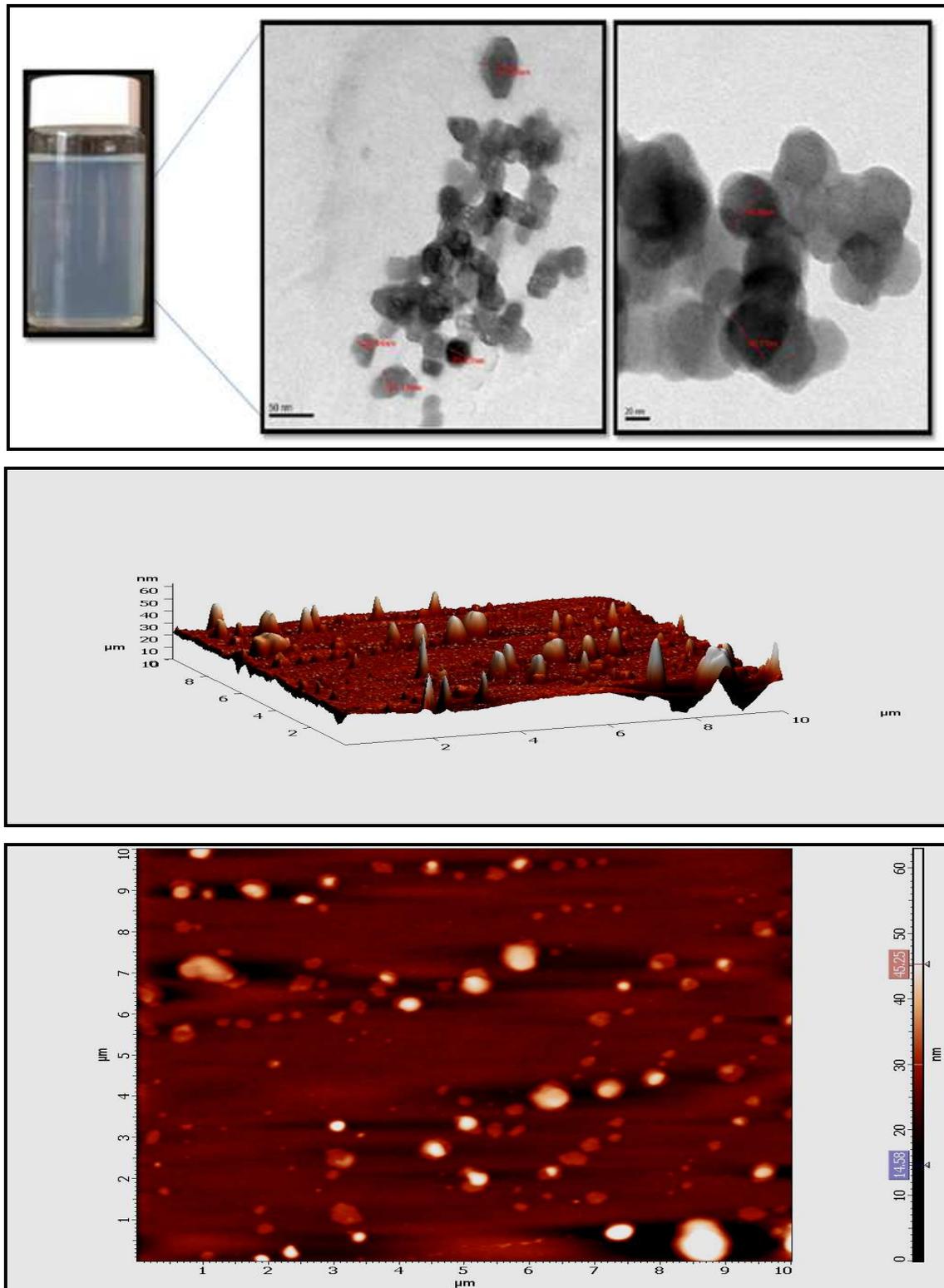


Figure No.3: TEM images of RT loaded nanoparticles (A) AFM image of Rivastigmine loaded PLGA nanoparticle (B) Three-dimensional image showing multiple particles (C)

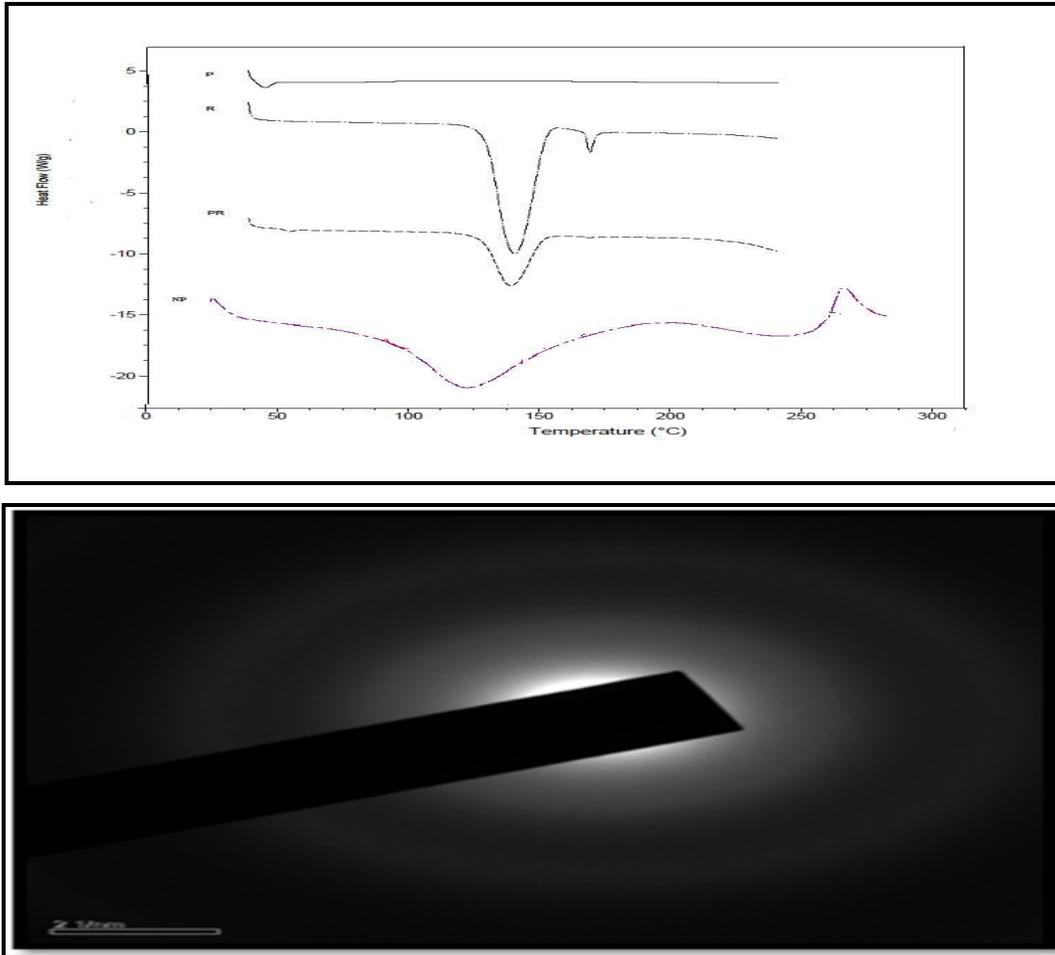


Figure No.4: DSC Thermograms of RT, PLGA, Physical Mixture RT/PLGA and R-NP (A)
Selected area electron diffraction pattern of RT nanoparticle (B)

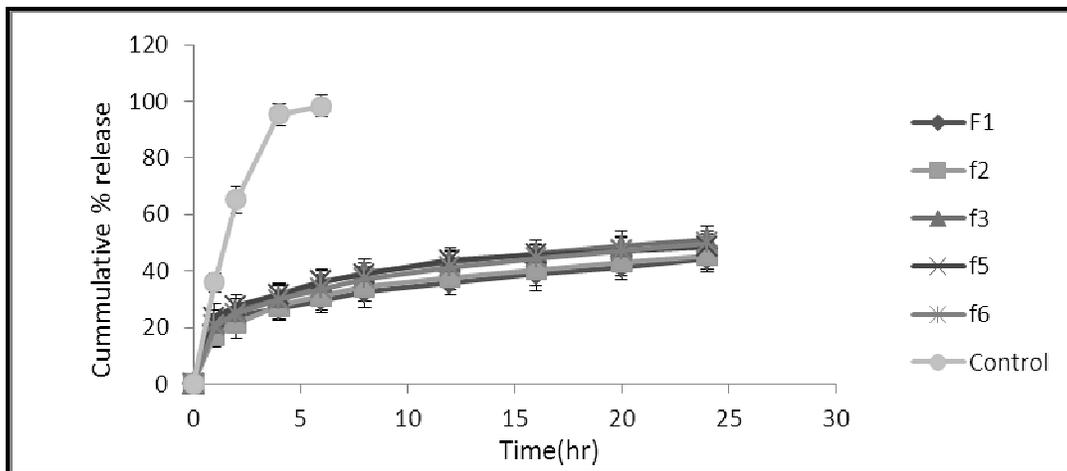


Figure No.5: *In vitro* release profile of rivastigmine nanoparticles with various drug-to-polymer ratios (DP)

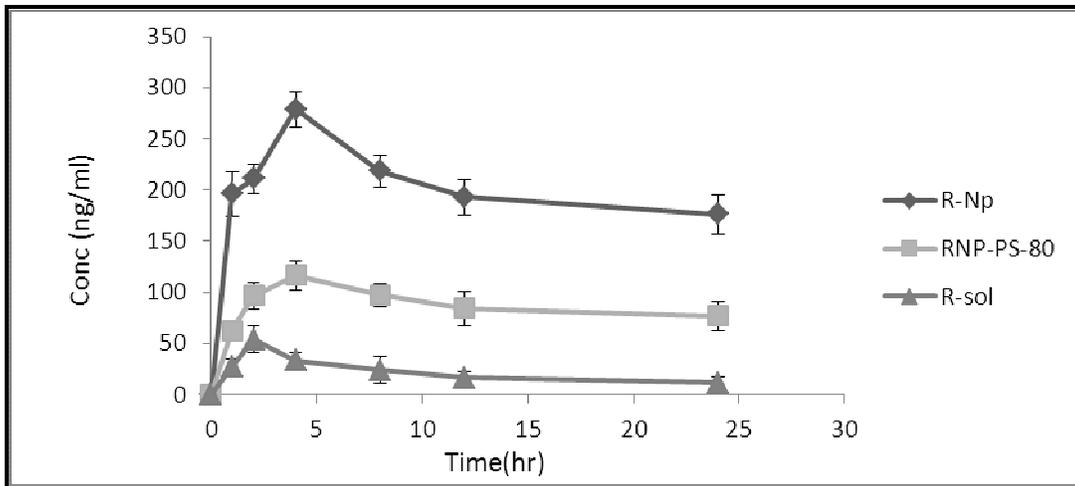


Figure No.6: Plasma concentrations vs. time profile of R-Sol, RNP-PS-80 and R-NP after oral administration at different time intervals

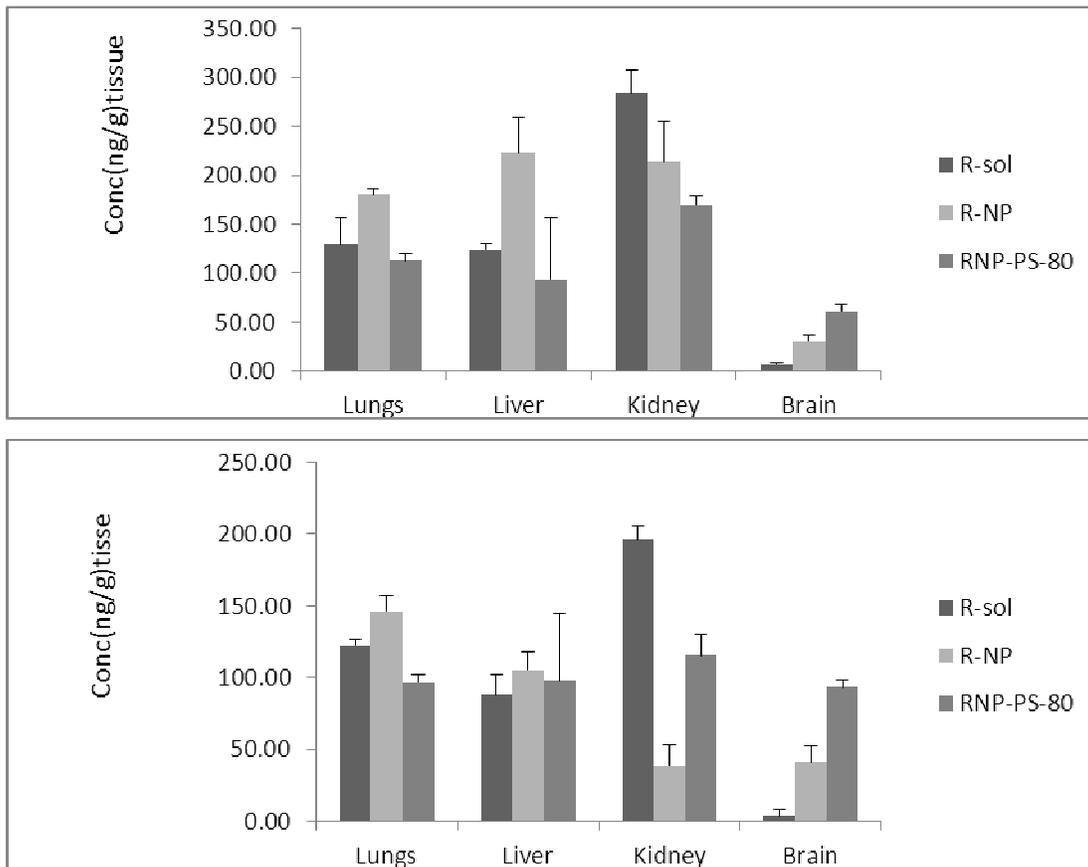


Figure No.7: Rivastigmine concentration (ng/g) in different organs brain, kidney, liver and lungs at 1st hr (A) rivastigmine concentration (ng/g) in different organs brain, kidney, liver and lungs at 4th hr after oral administration of R-sol, RNP and RNP-PS-80(B)

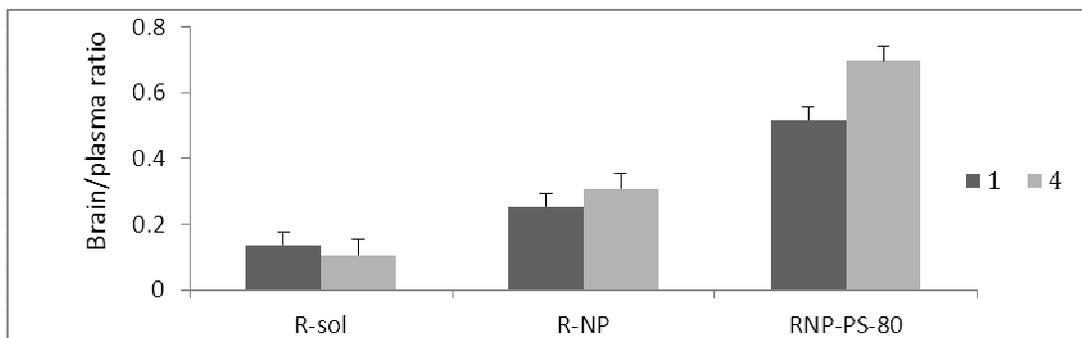


Figure No.8: Brain to plasma ratio of R-sol, RNP and RNP-PS-80 formulations at different time intervals after oral administration

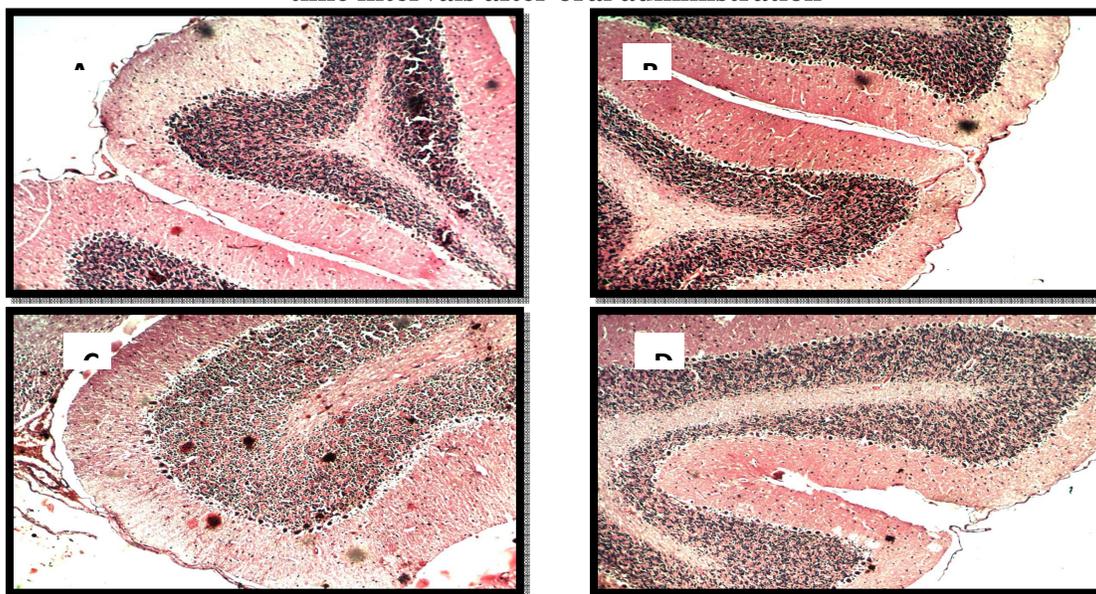


Figure No.9: Microscopic images of cerebral sections of rat brain (10x) Histological sections of brain were stained with haematoxylin & eosin A) control rat brain (B) rat exposed to rivastigmine drug solution(1mg/kg) (C) uncoated rivastigmine nanoparticles (D) polysorbate-80 coated rivastigmine nanoparticles

CONCLUSION

Polysorbate 80 coated RT-loaded PLGA nanoparticles were prepared by adopting modified nanoprecipitation method. The optimized formulation F3 showed better particle size >200nm and a better drug entrapment of 79.1±0.8 at pH 9. The pharmacokinetic data suggested an initial burst release and final sustained release of RT for 24 hours with improved RT uptake in plasma when compared to R-sol. The tissue distribution study revealed that the enhanced RT to brain with polysorbate-80 coated nanoparticles when compared that of the uncoated nanoparticles after oral

administration. It could be effective for brain targeting to treat Alzheimer’s disease.

ACKNOWLEDGEMENT

The authors thank Indian Council of Medical Research (ICMR), New Delhi for providing a Senior Research Fellowship. The authors thank Dr. K. Balakumar and Mr. Siram karthick for the assistance in characterization of nanoparticles. We also thank Mr. Siva selva Kumar, Mr. Hari Prasad for the assistance in HPLC analysis and Mr. Rangith Kumar for the assistance in animal study.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Vijaya Raghavan C, Tamilselvan N, Balakumar K and Dineshkumar B. Blood Brain Barrier: Surface Modified Nanocarriers and *In Vitro* Models to Treat Alzheimer's disease - A Review, *International Journal of Advances in Pharmaceutical Research*, 3, 2012, 798 - 804.
2. Tamai I, Tsuji A. Drug delivery through the blood brain barrier, *Adv. Drug Deliv. Rev*, 19, 1996, 401-424.
3. Partridge W M. Alzheimer's disease drug development and the problem of the blood-brain barrier, *Alzheimer's and Dementia*, 5, 2009, 427-432.
4. Mori N, Kurokouchi A, Osonoe K, Saitoh H, Ariga K, Suzuki K, Iwata Y. Liposome entrapped phenytoin locally suppresses amygdaloid epileptogenic focus created by db-CAMP/EDTA in rats, *Brain Res*, 703, 1995, 184-190.
5. Shuting K, Feng Y, Ying W, Yilin S, Nan Y, Ling Y. The blood-brain barrier penetration and distribution of PEGylated fluorescein-doped magnetic silica nanoparticles in rat brain, *Biochem. Biophys. Res*, 394, 2010, 871-876.
6. Zara G P, Cavalli R, Bargoni A, Fundaro A, Vighitto D, Gasco M R. Intravenous administration to rabbits of non-stealth and stealth doxorubicin loaded solid lipid nanoparticles at increasing concentration of stealth agent: pharmacokinetics and distribution of doxorubicin in brain and in other tissues, *J. Drug Target*, 10, 2002, 327-335.
7. Tian X H, Lin X N, Wei F, Feng W, Huang Z C, Wang P, et al., Enhanced brain targeting of temozolomide in polysorbate-80 coated polybutylcyanoacrylate nanoparticles, *Int. J. Nanomedicine*, 6, 2011, 445-452.
8. Panyam J, Dali M M, Sahoo S K, Ma W, Chakravarthi S S, Amidon G L et al., Polymer degradation and *in vitro* release of a model protein from poly (D, L-lactide-co-glycolide) nano- and micro-particles, *J. Control. Rel*, 92, 2003, 173-187.
9. Kreuter J, Shamenkov D, Petrov V, Ränge P, Cychutek K, Koch-Brandt C, et al., Apolipoprotein-mediated Transport of Nanoparticle-bound Drugs across the Blood-Brain Barrier, *J. Drug Target*, 10(4), 2002, 317-325.
10. Raschetti R, Albanese E, Vanacore N, Maggini M. Cholinesterase inhibitors in mild cognitive impairment: a systematic review of randomised trials, *PLoS Med*, 4, 2007, 338.
11. Bilati U, Allemann E, Doelker E. Development of a nanoprecipitation method intended for the loading of hydrophilic drugs into nanoparticles, *Eur. J. Pharm. Sci*, 25, 2005, 67-75.
12. Cheow W S, Hadinoto K. Enhancing encapsulation efficiency of highly water-soluble antibiotic in poly (lactic-co-glycolic acid) nanoparticles: Modifications of standard Nanoparticle preparation methods, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 370, 2010, 79-86.
13. Joshi S A, Chavhan S S, Sawant K K. Rivastigmine-loaded PLGA and PBCA nanoparticles: Preparation, optimization, characterization, *in vitro* and pharmacodynamic studies, *Eur. J. Pharm. Biopharm*, 76, 2010, 189-199.
14. Kreuter J, Ränge P, Petrov V, Hamm S, Gelperina S E, Engelhardt B et al., Direct evidence that Polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanism requiring prior binding of drugs to the nanoparticles, *Pharm Res*, 20, 2003, 409-16.
15. Siram K, Vijaya Raghavan C, Tamilselvan N, Balakumar K, Habibur Rahman S M, Vamshi Krishna K, et., al, Solid lipid nanoparticles of diethylcarbamazine citrate for enhanced delivery to the lymphatics: *in vitro* and *in vivo* evaluation, *Expert Opin. Drug Deliv*, 11(8), 2014, 1-15.
16. Ruiz A, Suarez M, Martin N, Albericio F, Rodriguez H. Morphological characterization of

- fullerene-androsterone conjugates, *Beilstein J. Nanotechnol*, 5, 2014, 374-379.
17. Shevtsov M A, Yakovleva L Y, Nikolaev B P, Marchenko Y Y, Dobrodumov A V, Onokhin K V. Tumor targeting using magnetic nanoparticle Hsp70 conjugate in a model of C6 glioma, *Neuro-Oncology*, 3(3),(2013), 1-12.
 18. Govender T, Stolnik S, Garnett M C, Illum L, Davis S S. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug, *J. Control. Rel*, 57, 1999, 171-185.
 19. Mainardes R M, Gremiao M P D, Evangelista R C. Thermo analytical study of praziquantel-loaded PLGA nanoparticles, *Braz. J. Pharm. Sci*, 42, 2006, 524-530.
 20. Hua Y, Jianga X, Dinga Y, Zhanga L, Yanga C, Zhang J. Preparation and drug release behaviours of nimodipine-loaded poly (caprolactone)-poly (ethyleneoxide)-poly lactide amphiphilic copolymer nanoparticles, *Biomaterials*, 24, 2003, 2395-2404.
 21. Amini H, Ahmadiani A. High performance liquid chromatographic determination of rivastigmine in human plasma for application in pharmacokinetic studies, *Iran. J. Pharm. Res*, 9(2), 2010, 115-121.
 22. Arumugam K, Subramanian G S, Mallayasamy M R, Averineni R K, Reddy M S, Udupa N A. Study of rivastigmine liposomes for delivery in to the brain through intranasal route, *Acta Pharm*, 58, 2008, 287-297.
 23. Prabhakar K, Afzal S M, Kumar P U, Rajanna A, Kishana V. Brain delivery of transferrin coupled indinavir submicron lipid emulsions - Pharmacokinetics and tissue distribution, *Colloids and Surfaces B: Biointerfaces*, 86, 2011, 305-313.
 24. Vandita K, Indu Pal K. Evaluating potential of curcumin loaded solid lipid nanoparticles in aluminium induced behavioural, biochemical and histopathological alterations in mice brain, *Food Chem. Toxicol*, 49, 2011, 2906-2913.
 25. ICH harmonised tripartite guideline stability testing of new drug substances and products Q1A (R2). 2003.
 26. Reddy L H, Murthy R S. Pharmacokinetics and biodistribution studies of doxorubicin loaded poly (butylcyanoacrylate) nanoparticles synthesized by two different techniques, *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, 6, 2004, 148-161.
 27. Yesim A, Karine A, Maria J A, Pilar C, Gursoya R N, Patrick C, et al., Preparation and *in vitro* evaluation of chitosan nanoparticles containing a caspase inhibitor, *Int. J. Pharm*, 298, 2005, 378-383.
 28. Douglas S J, Davis S S, Illum L. Nanoparticles in drug delivery, *Crit Rev Ther drug*, 3, 1987, 233-261.
 29. Zhou S B, Deng X M, Li X H. Investigation on a novel core-coated microspheres protein delivery system, *J Control Release*, 75, 2001, 27-36.
 30. Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C et al., "DD Solver: an add-in program for modelling and comparison of drug dissolution profiles.", *AAPS Journal*, 12(3), 2010, 263-271.
 31. Wilson B, Samanta M K, Santhi K, Sampathkumar K P, Ramasamy M, Suresh B. Chitosan nanoparticles as a new delivery system for the anti-Alzheimer's drug tacrine, *Nanomedicine nanotechnology biology and medicine*, 6, 2010, 144-152.
 32. Yung C K, Chun Y, Shih H. Solid lipid nanoparticles with surface antibody for targeting the brain and inhibiting lymphatic phagocytosis, *J Taiwan Inst Chem*, 4(1), 2014, 1-9.

Please cite this article in press as: Chellan Vijaya Raghavan et al., Brain Targeted Delivery of Rivastigmine Polymeric Nanoparticles through Oral Administration to Treat Alzheimer's Disease, *International Journal of Research in Pharmaceutical and Nano Sciences*, 3(6), 2014, 536- 551.