



## IMPROVED ANTI-AMNESIC ACTIVITY OF TACRINE LOADED PLA POLYMERIC NANOPARTICLES

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### ABSTRACT

Tacrine (9-amino-1,2,3,4-tetrahydroacridine monohydrochloride) a reversible cholinesterase inhibitor, for the treatment of mild to moderate Alzheimer disease. The objective of this study was to formulate and characterize tacrine loaded poly (lactic acid) (PLA) nanoparticles (TPLN) for improving the therapeutic activity and to reduce adverse drug reactions by targeting to brain. TPLN with varying polymer ratios were prepared by nanoprecipitation method and subjected to various physico-chemical evaluations like determination of drug content, particle size, zeta potential, entrapment efficiency and *in vitro* release of tacrine from the polymeric nanoparticles. The particle size of various trials ranged from 219nm to 251nm. The PLA nanoparticles were able to entrap tacrine up to  $74.53 \pm 1.3\%$ . The release of tacrine from TPLN-3 at the end of 24hrs was found to be 92.59%. The *in vitro* cytotoxicity of the TPLN indicates that the  $IC_{50}$  of the tacrine shown an improvement in the reduction of the  $IC_{50}$ , whereas, plain nanoparticles had not shown any severe cytotoxicity. In cellular uptake studies, the optimized formulation TPLN-3, showed increased intracellular accumulation when compared with pure drug and the control. The results further suggest the increase in extent of transport of tacrine in TPLN treated mice with a remarkable improvement of the therapeutic efficacy.

**KEYWORDS :** Tacrine Hydrochloride, Poly (lactic acid), MTT assay, Alzheimer disease and Brain targeting.



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Received on: 26-12-2016

Revised and Accepted on : 06-03-2017

DOI: <http://dx.doi.org/10.22376/ijpbs.2017.8.2.p117-129>

## INTRODUCTION

Alzheimer's disease is characterized by marked atrophy of the cerebral cortex and loss of cortical neurons. The neuropathology hallmarks of the disorder that are generally noted on post-mortem brain examination; amyloid rich senile plaques, neurofibrillary tangles, and neuronal degeneration.<sup>1</sup> Impairment of short-term memory are the first clinical feature. As the condition progresses, additional cognitive abilities are impaired.<sup>2</sup> Tacrine hydrochloride (9-amino-1,2,3,4-tetrahydroacridine mono hydrochloride) (TH) a reversible cholinesterase inhibitor, a drug approved by the USFDA in 1993 for the treatment of mild to moderate Alzheimer disease.<sup>3</sup> Tacrine was available in the market as oral capsule dosage forms. Since it possess aqueous solubility, per oral administration of tacrine is related with low bioavailability (i.e.17%). Hepatic first-pass effect, gastrointestinal side effects and reversible dose-dependent hepatotoxicity are the other major reasons lead to the withdrawal of tacrine from the market.<sup>4</sup> The treatment of AD becomes more complicated not only because of lesser choice of drugs for the treatment but also due to the limited entry of drugs in to the brain. The drug delivery to the brain is a challenge, because this tissue is considered as a very effective protective barrier. The BBB is the major barrier to the passage of active molecules from the blood compartment to the brain.<sup>5,6</sup> Therapeutic strategies are limited by the restrictive tight junctions at the endothelial cells of the blood brain barrier (BBB). It can be overcome by the polymeric nanoparticles, these are the promising candidates to probe the central nervous system because of capillary to cross the restrictive tight junctions at the endothelial cells of the blood brain barrier (BBB).<sup>7</sup> Nanoparticles deliver the drugs for therapeutic application in neurological disorders, particularly AD.<sup>8</sup> Various synthetic polymers have been intensively studied as a delivery carrier due to their well-

known therapeutic benefits, such as biocompatibility, biodegradability, and long-term safety of drugs.<sup>9</sup> In the nanoparticle formulation, particular interest has been focused on the use of polyesters materials such as poly (D,L-lactide) (PLA), which undergo scission in the body to monomeric units of lactic acid, as a natural intermediate in carbohydrate metabolism.<sup>10</sup> The degradation rate is dependent on several parameters, such as crystallinity, molecular weight, pH, ionic strength, temperature and particle morphology.<sup>11,12</sup> Hence in the present study sustained release TPLN were formulated and evaluated with an aim to minimize dosing frequency and to reduce the overall dose and to improve the therapeutic efficacy by increasing the bioavailability in brain, thereby reducing the adverse effects.

## MATERIALS AND METHODS

TH, PLA,  $\beta$ -amyloid and scopolamine hydrobromide were procured from Sigma Aldrich, Mumbai, India. Polyvinyl alcohol, Disodium hydrogen phosphate and sodium hydroxide were purchased from S.D fine chemicals, Mumbai, India. All other chemicals, solvents and reagents used in the study were of analytical grade.

### Preparation of PLA Nanoparticles

TH loaded PLA nanoparticles with were prepared by the modified nanoprecipitation method specified by Mehrotra et al.<sup>13</sup> In this method PLA and tacrine were dissolved in acetone, and were homogenized with homogenizer (Ika Labortechnik) at 19,000 rpm for 5 min. This solution was then added drop wise to 1% w/v aqueous polyvinyl alcohol (PVA) solution, by continuous homogenization for 20 min. Then, organic solvent was removed using rotary evaporator (B-480 Buchi, Switzerland). The final volume of suspension was adjusted to 10 ml, with deionized water.

### Various formulations of Tacrine loaded PLA nanoparticles

S.No.	Ingredients	Tacrine HCl	PLA	1% PVA	Acetone
1	TPLN-1	100mg	100mg	50ml	20ml
2	TPLN-2	100mg	200mg	50ml	20ml
3	TPLN-3	100mg	300mg	50ml	20ml
4	TPLN-4	100mg	400 mg	50ml	20ml

## CHARACTERIZATION

### Fourier Transform Infrared Spectroscopy (FT-IR)

F-TIR studies were carried out to confirm the interaction between the drug and the excipients. The spectra of TH loaded PLA nanoparticles were recorded on Fourier Transform Infrared Spectrophotometer (Bruker, India). Test samples were mixed with KBr, pressed into disc and scanned from  $400\text{cm}^{-1}$  to  $4000\text{cm}^{-1}$ .

### Differential Scanning Calorimetry (DSC)

The physical mixture of TH entrapped nanoparticles was characterized by Differential scanning calorimetry (DSC-60 Shimadzu, Japan) Each sample was sealed in standard aluminium pan with lids and purged with air at a flow rate of 40 ml/min. A temperature range of 30 – 300 °C under inert nitrogen atmosphere. Thermogram

were taken for TH, PLA, PVA and TH loaded PLA and PVA.

### Determination of Particle Size, Zeta Potential and Percentage Drug Entrapment

The particle size and zeta potential of TPLN were determined by photon correlation spectroscopy using particle size analyser (Malvern zeta sizer Nano Zs, UK). The amount of drug entrapped in the nanoparticles was estimated by UV spectrophotometer (Shimadzu 1700, Japan). The nanoparticles suspension was subjected to centrifugation at 25,000 rpm for 1 hr at 4 °C.

### Entrapment Efficiency

The nanoformulations were centrifuged and the supernatant containing free drug was collected which was further analysed by UV at 240 nm. This gives the amount of drug that is untrapped in the nanoparticles.

Amount of drug found in the supernatant was subtracted from the total amount of drug added to the formulation gives the amount of drug entrapped in the nanoparticles.<sup>14</sup>The formulations were evaluated for entrapment efficiency by the following formula

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Weight of the drug in the nanoparticles}}{\text{Weight of the drug used in formulation}} \times 100\%$$

#### **Drug content**

Drug content was determined by taking 1 ml of the PLA nanoparticles loaded with tacrine. To this formulation 1ml of aqueous potassium dihydrogen phosphate was added and the mixture was centrifuged at 33,000 at 15 °C. The clear supernatant was removed and analysed spectrophotometrically and drug content was calculated.

#### **Transmission electron microscopy (TEM)**

TEM analysis of the prepared formulations was carried out to understand the morphology of nanoparticles. A drop of nanoparticle suspension containing 0.01% of phosphotungstic acid was placed on a carbon film coated on a copper grid for TEM. TEM studies were performed at 80 kV using PHILIPS TECHNAI-20, Japan. The copper grid was fixed into sample holder and placed in vacuum chamber of the Transmission electron microscope and observed under low vacuum, and TEM images were recorded.

#### **In vitro release studies**

The *in vitro* drug release of tacrine loaded PLA nanoparticles was measured in PBS at pH 7.4. The nanoparticulate dispersion equivalent to 1 mg of Tacrine HCl was placed in the dialysis bag, which was sealed at both ends. The dialysis bag was immersed in 25 ml of the receptor phase, which was stirred at 100 rpm and maintained at 37±5 °C. The receptor compartment was covered to prevent the evaporation of release medium. Samples were withdrawn at regular time intervals (0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24h), and the same volume was replaced by fresh release medium. The acceptor phase was changed every day to maintain sink condition. The samples were analysed UV Spectrophotometer at 240 nm. All the experiments were performed in triplicate, and the average values were taken.<sup>15</sup>

#### **In vitro cytotoxicity of nanoparticles**

SH-SY5Y cells were obtained from National Centre for Cell Science, Pune, India. The cells were grown in Dulbecco's modified Eagle medium (DMEM) with 10% foetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) in a humidified incubator under the conditions of 37 °C and 5% CO<sub>2</sub>. Dissolve MTT in Dulbecco's Phosphate Buffered Saline, pH=7.4 (DPBS) to 5 mg/ml. Filter-sterilize the MTT solution through a 0.2 µm filter into a sterile, light protected container. Store the MTT solution, protected from light, at 4 °C for frequent use or at -20 °C for long term storage. Prepare cells and test compounds in 96-well plates containing a final volume of 100 µl/well. Incubate for desired period of exposure. Add 10 µl MTT Solution per well to achieve a final concentration of 0.45 mg/ml. Incubate 1 to 4 hours at 37 °C. Add 100 µl Solubilisation solutions to each well to dissolve formazan crystals. Mix to ensure

complete solubilisation and record absorbance at 570 nm.

#### **Cellular Uptake of drug loaded nanoparticles**

For qualitative study using laser scanning fluorescence microscope, SH-SY5Y cells were seeded onto 96-well plates with glass cover slips at a density of 50,000 cells per well, incubated for 24h, treated with tacrine and plain nanoparticle formulation at a concentration of 200mg/ml for 4hr. Then washed with PBS and fixed using 4% formaldehyde at room temperature for 15 min. Subsequently, the cells were washed with PBS for three times and stained with Hoechst dye (1mg/ml) for 30 min. The cells were washed with PBS for three times before the cover slips were mounted onto microscope slides and visualized using fluorescence microscope.

#### **Invasive Assay**

SH-SY5Y cells were grown to 80% confluence then serum starved overnight before setting up the experiment. Cells were washed twice in Dulbecco's PBS and harvested from the plate using 0.5 mol/L EDTA (pH 6.8). The cells were collected and re suspended in starvation medium. We used 24-well trans well chambers (BD Bio Coat Control Inserts from BD Biosciences) with 8.0-µm pore size polycarbonate membrane for this experiment. The cells were plated at a density of 5 ×10<sup>4</sup> per well in 0.5 ml in the upper well, which was placed into a lower well containing one of the following conditions: complete medium and drug at different concentrations or complete growth medium (10% PBS), After 24 hr at 37 °C, 5% CO<sub>2</sub> incubator for 24 hr, the experiment was stopped by wiping the cells from the cell with a cotton swab and fixed and stained using the Diff-Quik kit. Migration was quantified by counting 12 fields at a magnification of 400. Each experiment was repeated in triplicate and the results were averaged.

#### **Pharmacodynamic studies**

To evaluate the influence of developed formulation on learning and memory capacities, Morris water maze test, step down Inhibitory avoidance were performed in Scopolamine-induced amnesia in mice model.<sup>16</sup>The animals (n=24) were divided into four different groups of six animals per each group. Scopolamine hydrobromide (1.5mg/kg of body weight) was administered to all groups through intraperitoneal route (i.p) after drug administration to all the groups except normal control group. The treatment details of the animal groups were as follows: *Control* group received normal saline (0.5 ml), *Tacrine* group received a solution of tacrine in normal saline (5 mg/kg b.w.p.o), *PNP* group received plain nanoparticles of PLA (2.5 mg/kg.b.w.p.o) *TPLN-3* group received tacrine loaded PLA nanoparticles dose equivalent to 5mg/kg b.w of tacrine by per oral route. The above treatment were repeated for 9 days. This protocol has been approved by the Institutional animal ethics committee for pursuing animal studies via, approval No:XVI/VELS/PCOL/03/2000/CPCSEA/IAEC/25.11.14.

#### **Morris water maze test<sup>17</sup>**

The Morris water maze was performed as described previously. The experimental apparatus consists of a

circular water tank (diameter= 100cm; height= 35cm), containing water at 28 °C to a depth of 15 cm and rendered opaque by adding powdered milk. A platform (diameter = 4.5cm; height= 14.5cm) was submerged 0.5 below the water surface and placed at the midpoint of one quadrant. After several trials, the test was conducted on the 5<sup>th</sup> day after the injection of A $\beta$ . In each training trial, the time required to escape on to the platform was recorded.

#### Step down Inhibitory avoidance<sup>18</sup>

The apparatus is a 50.0cm×25.0cm×25.0cm poly (methyl methacrylate) box, whose floor was a series of parallel 0.2-cm calibre bronze bars spaced 1.0 cm apart. A 7.0 cm wide, 2.5 cm high, 25.0cm long platform occupied the centre floor. In the training session, immediately after stepping down, placing their four paws on the grid of the animals received 0.4-mA, 2.0-s scrambled foot shock. In test sessions no foot shock given and step-down latency is measured with a cut off time of 300s. One trial step down inhibitory avoidance in mice involves the activation of two separate memory types, a short term memory (STM) system, and a long-term memory (LTM) system. Therefore, retention tests will be carried out 90 min after training to evaluate STM.

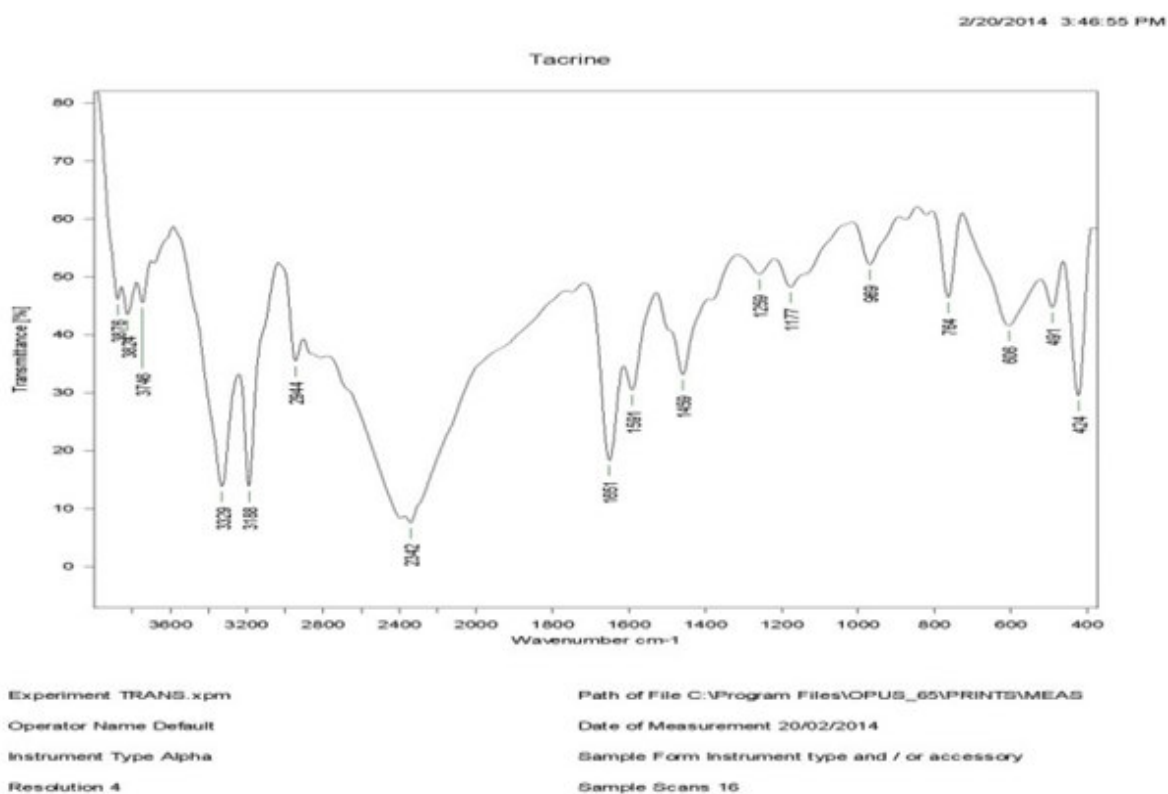
#### Acetylcholinesterase (AChE) Activity<sup>19</sup>

The esterase activity is measured by providing an artificial substrate, acetylthiocholine (ATC). Thiocholine released because of the cleavage of ATC by AChE is allowed to react with the -SH reagent 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maxima at 412 nm. The extinction coefficient of the thionitro benzoic acid is  $1.36 \times 10^4$ /molar/centimeter. The concentration of thionitrobenzoic acid detected using a UV spectrophotometer is then taken as a direct estimate of the AChE activity.

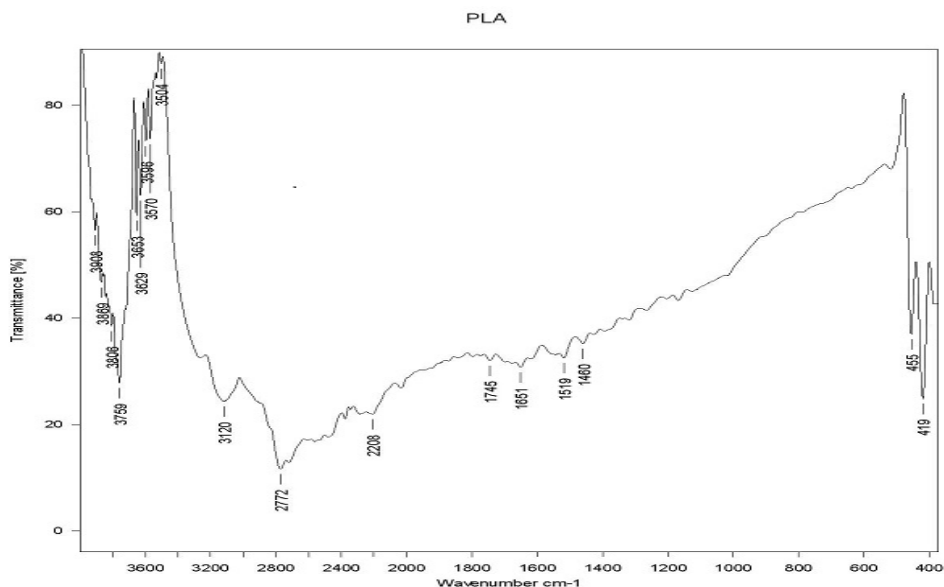
## RESULTS AND DISCUSSION

#### FT-IR Spectroscopic Studies

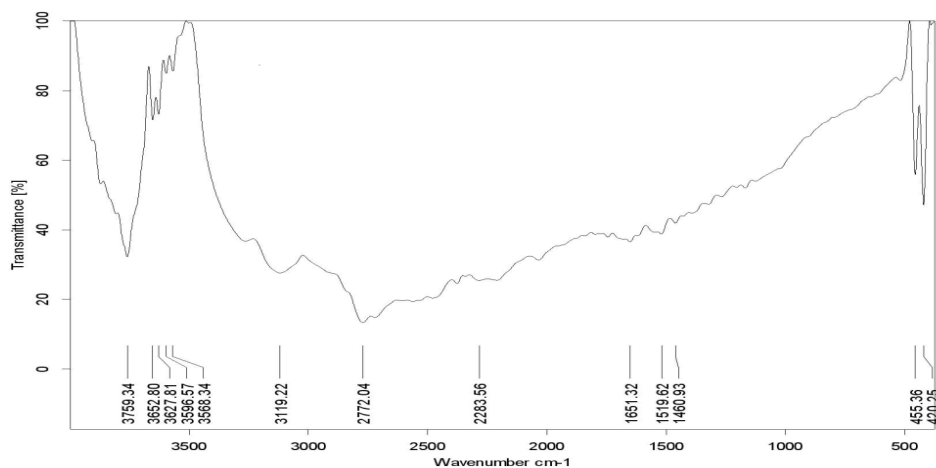
FT-IR study was carried out to confirm the compatibility between the selected polymers PLA, drug tacrine and the nanoparticles. The spectra obtained from the FT-IR studies are over the range from  $400\text{cm}^{-1}$  to  $4000\text{cm}^{-1}$ . The FT-IR spectra of Tacrine hydrochloride exhibited distinctive peaks at  $3336.25\text{cm}^{-1}$  N-H stretching,  $2938.98\text{cm}^{-1}$  due to C-H stretching,  $1265.07\text{cm}^{-1}$  N-H stretching,  $1461.78\text{cm}^{-1}$  aromatic ring present. It was confirmed that there are no major shifting of peaks between the spectra of drug, polymer and drug loaded nanoparticles. The Fourier Transform Infrared Spectra of PLA nanoparticles are shown in Fig No:1



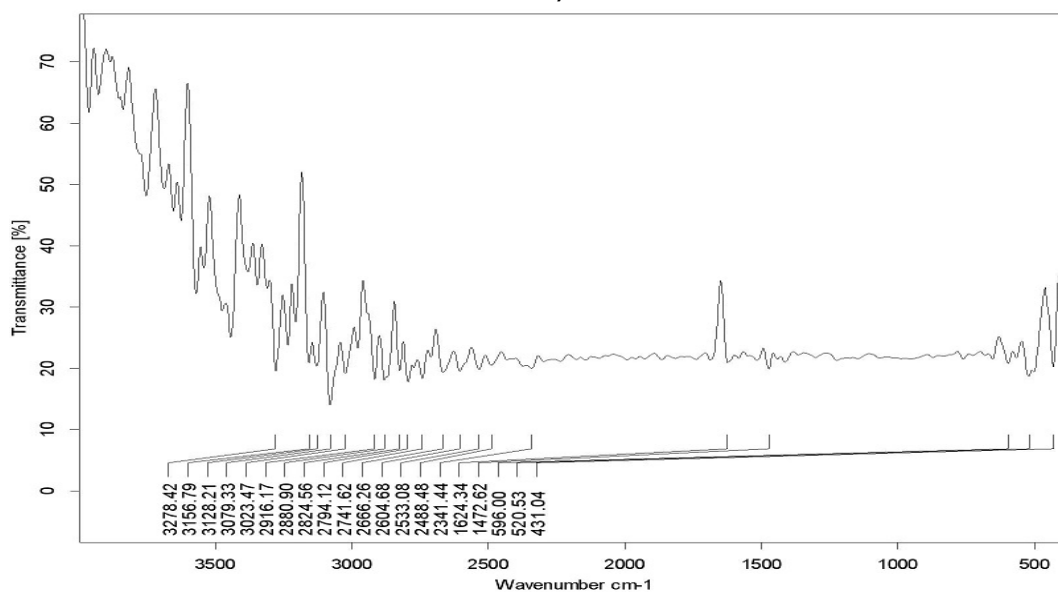
a)



b)



c)



d)

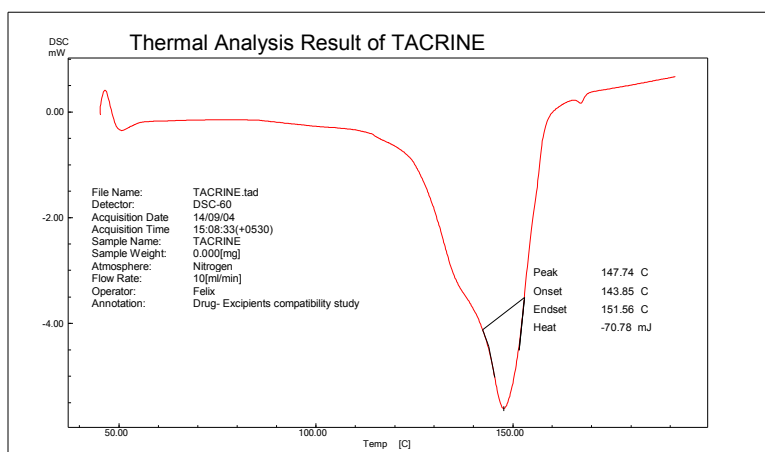
Figure 1

FTIR spectrum of a) Tacrine b) PLA c) PVA d) combination of Tacrine, PLA and PVA.

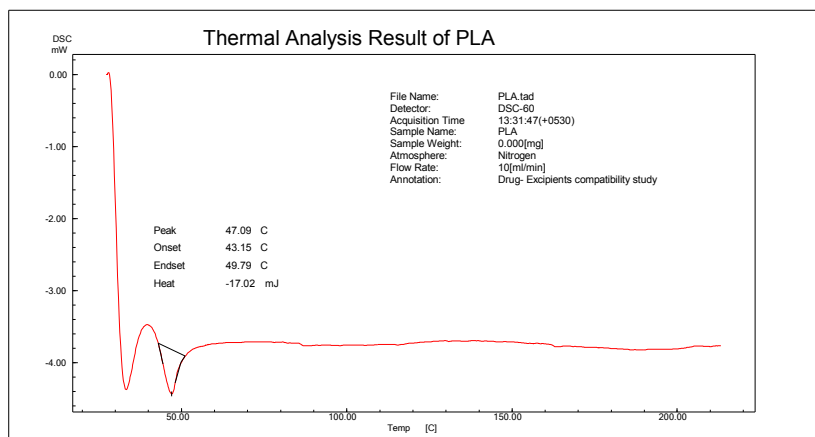
**Differential Scanning Calorimetry**

DSC thermogram of tacrine, polymer, poly vinyl alcohol and tacrine loaded with PLA and PVA are shown in

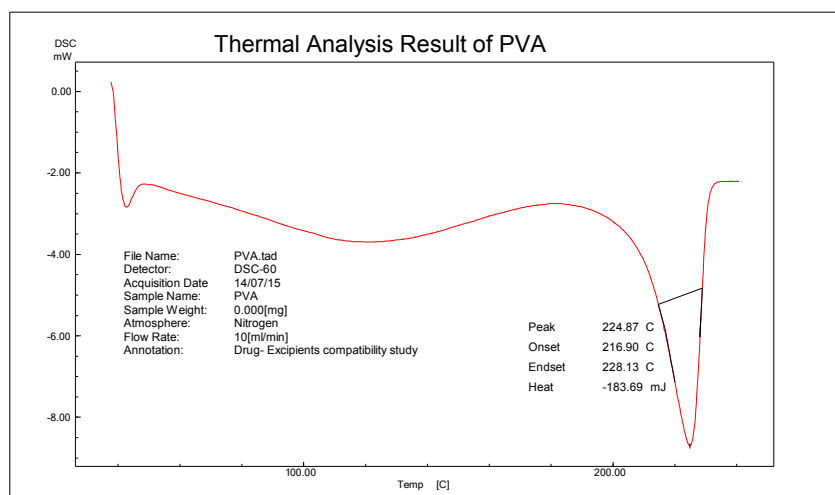
Figure No1 to 4. Pure drug tacrine shows the peak at 147 °C.so the peak shows that there is no interaction between the drug and polymer



a)

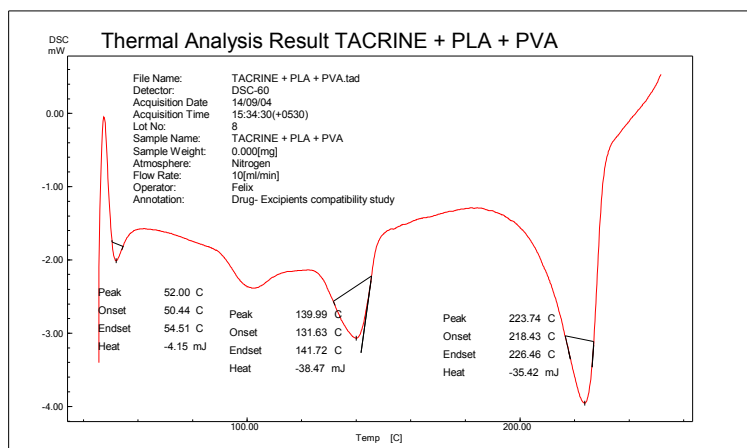


b)



c)





d)

Figure 2

### DSC of a) Tacrine b) PLA c) PVA d) combination of Tacrine, PLA and PVA

DSC studies were performed to assess the nature of drug present in the formulations and also to study the interaction between excipients used, DSC studies were performed to find out the interaction between the formulations and also to determine the form in which the

drug is entrapped in the nanoparticles. The drug tacrine shows a characteristic endothermic peak at 147.7 °C which is the melting point/transition temperature for the drug which is shown in Fig. 2. DSC thermo gram of physical mixture of pure drug, polymer and PVA showed endothermic peaks at 223.7 °C respectively.<sup>20</sup>

Table 2

### Particle size, zeta potential, percentage of drug entrapment and percentage of drug content and in vitro drug release of tacrine loaded PLA nanoparticles

Sl.No	Formulation Code	Particle Size (nm)	Zeta Potential (mV)	Drug content (mcg/ml)	Entrapment Efficiency (%)	In vitro drug release (% CDR)
1	TPLN-1	219±1.2	-20±2.1	0.855±1.2	62.53±2.5	75.39±3.2
2	TPLN-2	232±2.3	-22.6±1.5	0.983±1.3	67.25±1.3	78.73±2.4
3	TPLN-3	245±1.4	-22.4±1.8	1.205±1.6	74.53±1.3	95.59±1.8
4	TPLN-4	251±3.1	-21.3±1.2	0.857±1.4	73.23±2.4	90.15±1.5

### Particle Size, Zeta Potential and Percentage Drug Entrapment

TPLN had an average particle size of 219 nm to 251nm. The particle size distribution curves for all samples are unimodal. The nanoparticle size was 219nm, 232nm, 245nm and 251nm for TPLN-1, TPLN-2, TPLN-3 and TPLN-4 respectively. The particle size of the nanoparticle is dependent on polymer concentration. The data suggest that an increase in polymer concentration increase the particle size. Particle size is an essential parameter for brain targeting because particles lesser than 200nm are required for targeting to the brain. Studies have showed particles lesser than 100nm are more effective in delivering drugs to the brain.<sup>21</sup> The concentration of PVA will influence the particle size of the prepared nanoparticles which was in accordance with the results obtained which described that nanoparticles with a smaller range and narrow size distribution are obtained only when there is sufficient surfactant concentration. This proves that polymer and stabilizer blend have well suitably in nanoprecipitation technique.<sup>22</sup> Zeta Potential of the optimized formulation TPLN-3 was found in the range of -20.5 to -22.4mV. Samples were diluted using Millipore water before the measurement of zeta potential. Zeta potential value for TPLN-3 was found to be -22.4mV. Zeta potential is an important parameter which influences

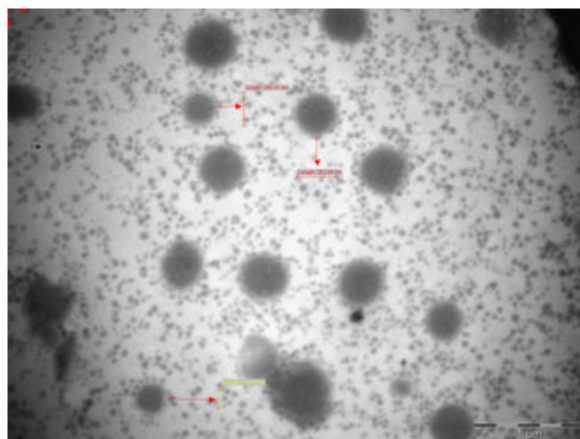
formulation stability, which prevents settling of nanoparticles.

### Drug content

The total drug content of the prepared nanoparticles varied from 0.855±1.2 to 1.205±1.6 mcg/ml. The drug content increased with increase in concentration of PLA. The Entrapment efficiency for TPLN-3 was found to be in the range of 62.53% to 74.53%. The increase in PLA concentration increases the encapsulation efficiency. Entrapment efficiency was found to be influenced by the amount of polymer and pH of the aqueous phase. Formulation TPLN-3 containing 100mg drug and 300mg polymer possess high entrapment efficiency.<sup>23,28</sup>

### Transmission electron microscopy (TEM)

The external morphological characteristics of nanoparticles were observed using TEM. The studies revealed that TPLN were spherical in shape and correlated with the particle size distribution measured by mastersizer. The images of TPLN are shown in Fig No:3. Surface morphology of the prepared formulations was evaluated by obtaining TEM images. TEM images were taken for formulation TPLN-3 and the images proved that nanoparticles are smooth and spherical in shape as shown in Fig.3.

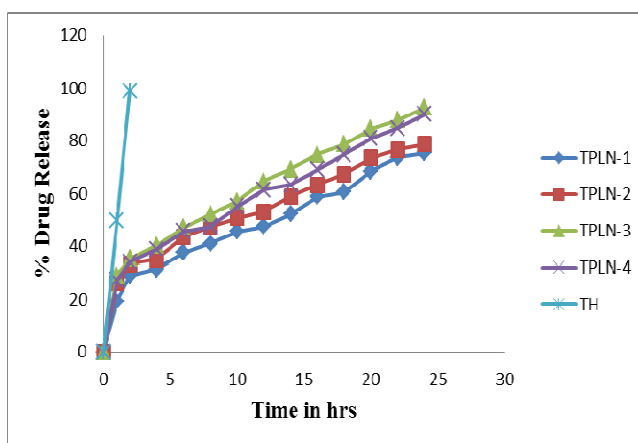


**Figure 3**  
*TEM images of TPLN-3 for optimized formulation*

**In vitro release studies**

The nanoparticle shows initial burst effect occurs within 30 min and the remaining amount of drug was found to be released in sustained manner, over a period of 24 hrs. After 24hrs of dialysis in PBS pH 7.4 the percentage

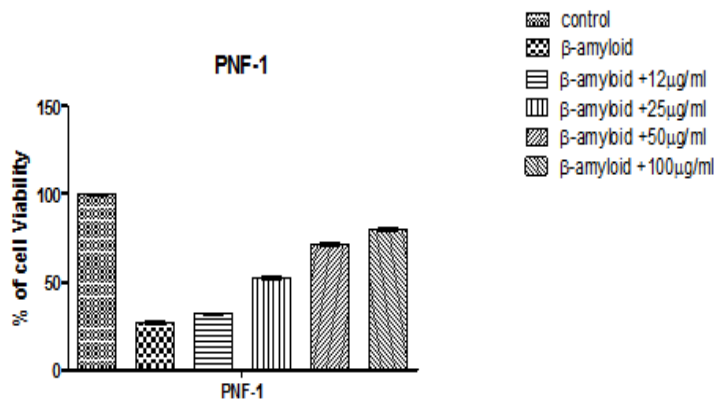
of drug release were found to be 75.39%, 78.73%, 92.59% and 90.15% for formulations (TPLN-1 to TPLN-4) and for pure drug shows that 99.01% drug release in 2hrs



**Figure 4**  
*In vitro drug release profiles of Tacrine loaded PLA nanoformulations in phosphate buffer pH 7.4*

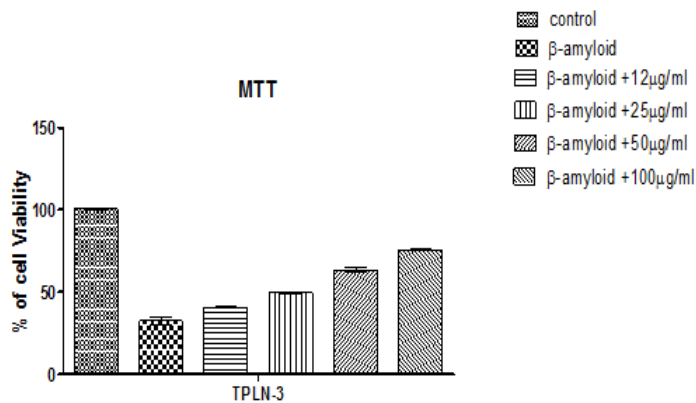
The cumulative percentage release of formulations TPLN-1 to TPLN-4 ranges from 75.39%, to 92.59%. All formulations showed burst release initially followed by sustained release; burst release was due to drug molecules adsorbed in the surface of nanoparticles. The drug particles at the surface dissolve when it comes in

contact with the medium. Release of drug from the nanocarriers is influenced by parameters that are drug related, polymer related and environment related. The drug related parameters are its molecular weight, concentration, interaction with carrier, diffusion, ion exchange and physicochemical properties.<sup>24</sup>



**Figure 5**  
*MTT assay for Plain nanoformulation*

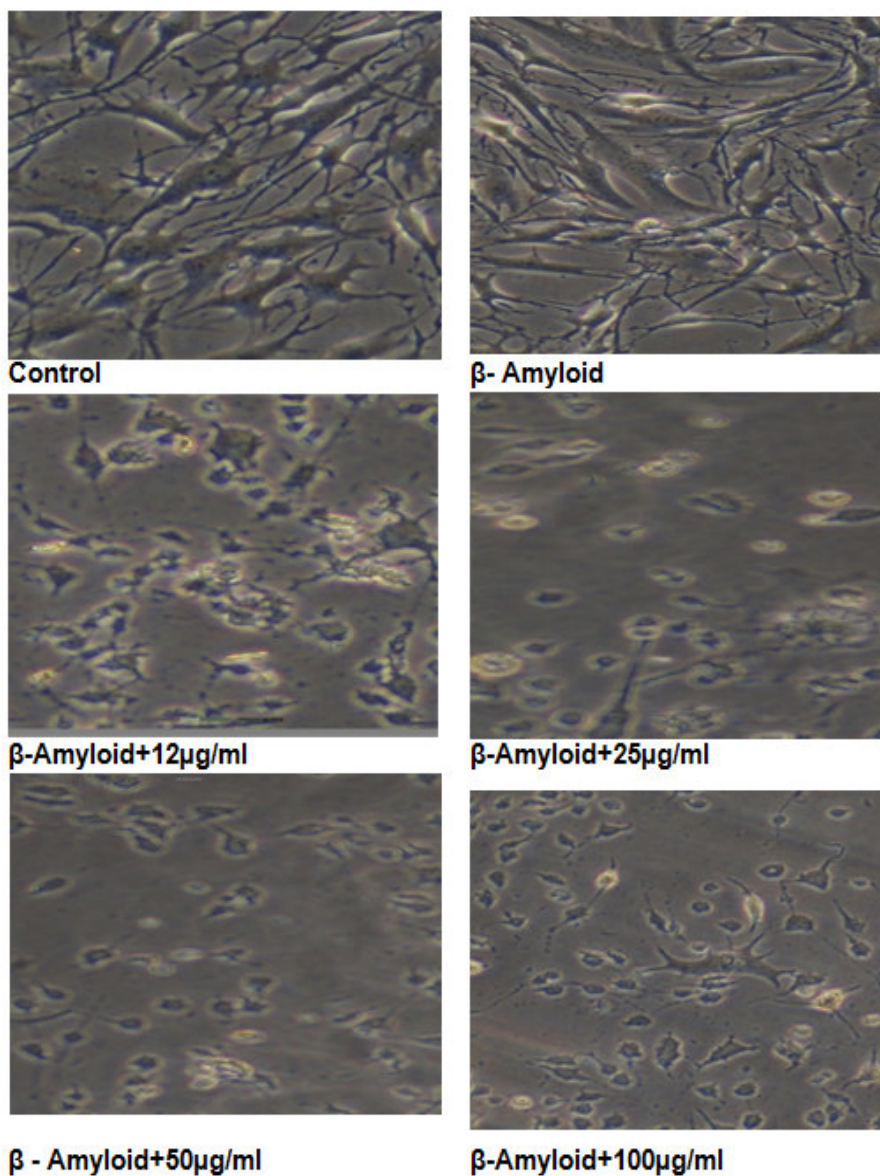




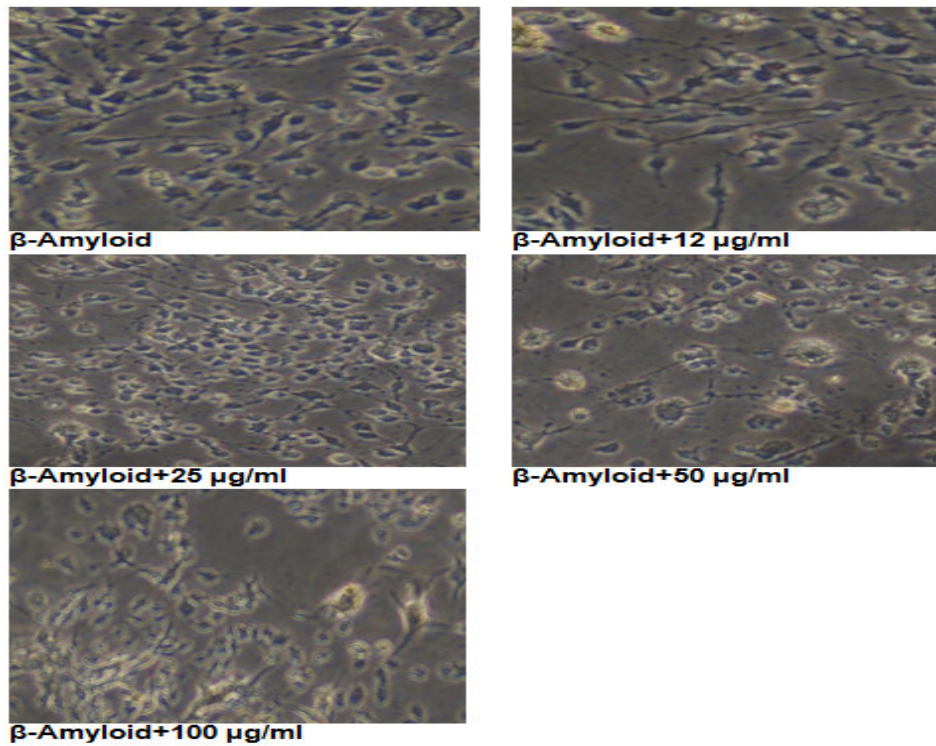
**Figure 6**  
**MTT assay for TPLN-3 optimized formulation**

MTT cell viability assay was done to determine the cytotoxicity of tacrine loaded nanoparticles was observed under in SH-SY5Y cells under various conditions. The  $IC_{50}$  of the tacrine loaded nanoparticles, shown improved in the reduction of  $IC_{50}$  of the nanoparticle was also observed. The plain nanoparticles did not show severe cytotoxicity in which cell viability of more than 80% was achieved in SH-SY5Y cells. The *in*

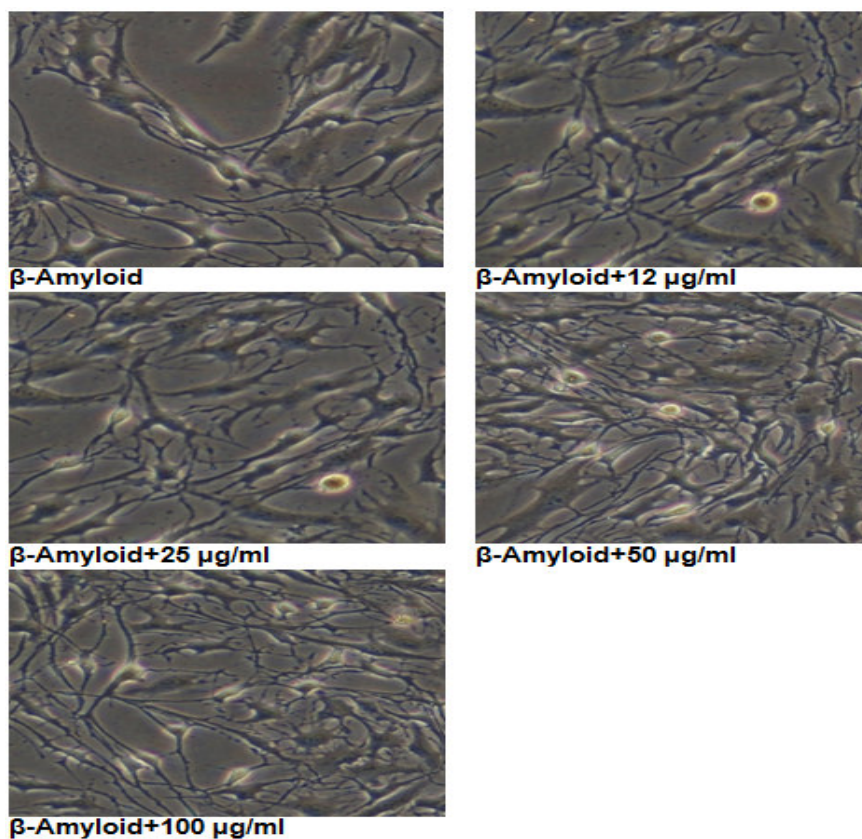
*vitro* cytotoxicity of the nanoparticles was detected using SH-SY5Y cells after 24hr incubation using MTT. At every concentration studied, the viability of cell was above 80% for TPLN-3 formulations and no significant difference was found between the formulations, indicating they had low toxicity.



**Figure 7**  
***Invasive Study of pure drug Tacrine***



**Figure 8**  
***Invasive Study of Plain nanoformulation***



**Figure 9**  
***Invasive Study for TPLN-3 formulations***

***Invasive Assay***

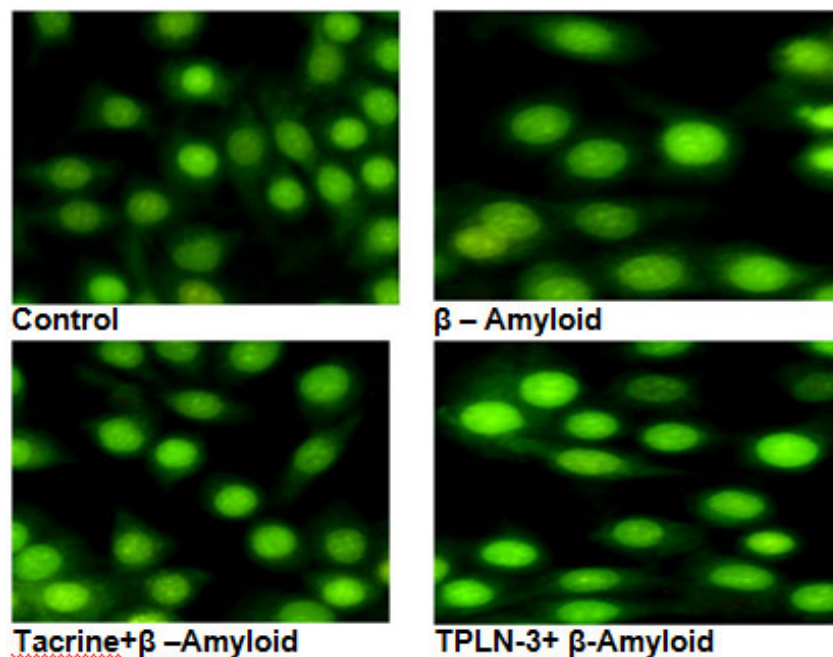
It was observed that the tacrine loaded TPLN-3 nanoparticles inhibits the cell migration significantly

when compared with pure drug. Further significant was not observed in control and plain nanoparticles.

**Cellular Uptake of drug loaded nanoparticles**

The cellular uptake of tacrine loaded nanoparticles was examined by Fluorescence microscopy. Cells treated

with tacrine loaded TPLN-3 showed higher intracellular accumulation



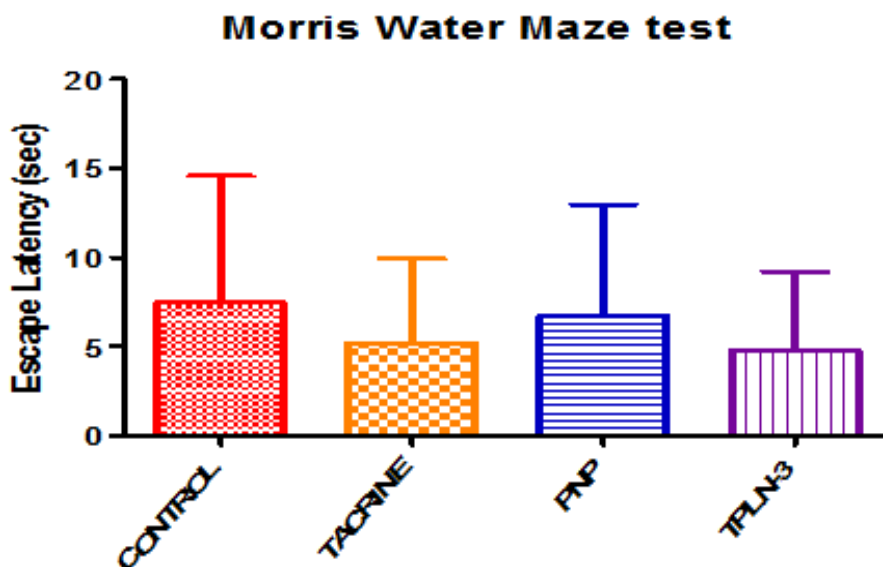
**Figure 10**  
**Intracellular Uptake of Tacrine Nanoparticles**

The *in vitro* cytotoxicity of the nanoparticles was detected using SH-SY5Y cells after 24hr incubation using MTT. At every concentration studied, the viability of cell was above 80% for TPLN-3 formulations and no significant difference was found between the formulations, indicating they had low toxicity. The cellular uptake of nanoparticles in SH-SY5Y cell lines showed that TPLN-3 formulations exhibited greater uptake in comparison to pure tacrine.

**Pharmacodynamic studies**

**Morris Water maze**

The Control significantly delayed mean latency and retention mean latency, which indicates induction cognitive impairment. The polymeric nanoformulations shows significant improvement performance (increased memory retention)



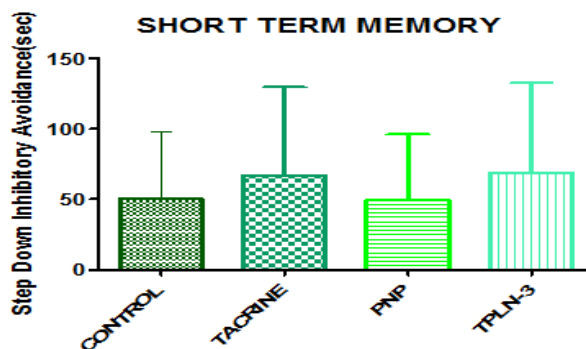
**Figure 11**  
**Scopolamine induced memory deficits in Morris Water maze task**

**Inhibitory step down avoidance**

The step-down condition avoids response produced by the memory improvement. Polymeric nanoformulations,

and then followed by pure tacrine and control the number of errors is higher





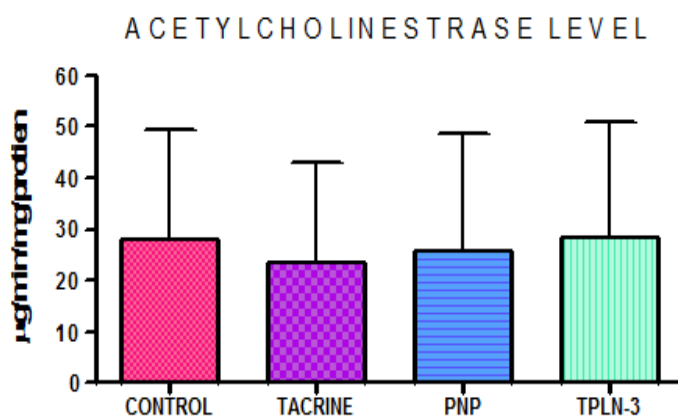
**Figure 12**  
*Scopolamine induced memory deficits in Inhibitory step down avoidance*

In order to confirm the effects of TPLN-3 on other types of memory we performed the Morris water maze test on spatial learning. Scopolamine-treated mice showed more prolonged escape latency than mice from the control group. TPLN-3 and tacrine groups significantly reduced escape latency prolonged by scopolamine during training sessions. In the probe test, TPLN-3 treatment lowered the escape latency comparable to the control group and significantly increased the number of times of crossing over the platform site. So the Morris water maze test on spatial learning and memory has been used to detect the changes in cholinergic system.<sup>25</sup> The Scopolamine induced memory impairment was assessed by exploratory behaviour, step-down inhibitory avoidance, one step trial step-down inhibitory

avoidance involves the activation of two separate memory types, an STM and LTM.<sup>26</sup> The effect of TPLN-3 exhibited increased memory retention in STM.

#### **Acetylcholine Esterase**

The brain homogenate produce the absorbance's/min is higher in control and followed by standard and then the Nano formulations produced significant decrease in Acetylcholine Esterase level in brain homogenate. For memory enhancing effects of TPLN-3, activities of AChE as cholinergic markers were assessed using brain homogenates. Neurochemical studies suggested that the cholinergic system play an important role in learning and memory.<sup>27</sup>



**Figure 13**  
*Scopolamine induced Acetylcholine Esterase level in brain*

## **CONCLUSION**

TPLN indicated improved anti-Alzheimeric activity when compared with the activity produced by pure tacrine. This may help to improve the therapeutic efficacy of the patients with Alzheimer's disease. The prepared nanoformulation indicates better physical stability which may yield long shelf life of the formulations. Hence it can be concluded that, tacrine loaded PLA, nanoparticles can serve as a potential formulation for the treatment of

AD, but further pharmacokinetic studies are needed to confirm the prepared drug delivery system.

## **ACKNOWLEDGEMENTS**

The authors wish to express their heartfelt gratitude to Dr. Ishari K. Ganesh, Hon'ble Chancellor of VISTAS, Vels University, Pallavaram, Chennai-600074, for providing their unrestricted support for carrying out this research.

## **CONFLICT OF INTEREST**

Conflict of interest declared none.

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We sincerely thank the above reviewers for peer reviewing the manuscript