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RESEARCH ARTICLE

Pharmacokinetics of Tacrine Loaded MPEG-PCL Polymeric Nanoparticles

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ABSTRACT:
The Tacrine loaded mPEG-PCL nanoparticles were prepared by emulsion polymerization and solvent evaporation method, to improve the bioavailability of the drug in brain. The nanoparticulate formulation was characterized for particles size, encapsulation efficiency, zeta potential and *in vitro* release study. The pharmacokinetic studies of Tacrine-loaded mPEG-PCL nanoparticles were carried out in Sprague dawley rats. The study revealed C_{max} and T_{max} were significantly altered and Clearance was less in brain and blood when compared to that of plain Tacrine solution. These results suggest that mPEG-PCL nanoparticles had considerably increased the transport of Tacrine across the BBB.

KEYWORDS: Alzheimer's disease, Tacrine, Blood Brain Barrier (BBB), Emulsion Polymerization method, Nanoparticles.

INTRODUCTION:

Alzheimer's disease (AD), a Neurodegenerative disorder characterized by its neurofibrillary tangles, senile plaques, oxidative stress and neuro inflammation[1]. AD is epidemic with 339 million worldwide having the disease.[2] The BBB is a host of specific and selective transporters that supply the CNS with glucose, free fatty acid, amino acids, minerals, vitamins and electrolytes.[3] It also play an role in communicating transport between the blood and CNS informational molecules such as regulatory proteins and peptides.[4,5,6] The diffusion of drugs from the blood to the brain depend upon the ability of biologically active molecules to transverse lipid membranes. Therefore, many drugs do not have adequate physicochemical parameters such as lipid solubility, low molecular size and positive charge, which is necessary for crossing the BBB.

Hence, there has was strategies to overcome the BBB including invasive and non-invasive techniques.[7] Among the non-invasive techniques, use of colloidal drug carriers includes emulsions, nanoparticles (Nano capsules and Nano spheres) micelles and liposomes.Amongthese nanoparticles and liposomes have been exploited for brain drug delivery. The purpose of using colloidal carriers is to increase the specificity towards the cells or tissues, to improve the bioavailability of drugs by diffusion through the biological membranes and to protect them against enzyme inactivation. Nanoparticles have a size around 200 nm and the drugs may be dissolved, entrapped, encapsulated or adsorbed or attached. Nanoparticles are used because of method of preparation are generally simple and easy to scale up. Nanoparticles are made from number of materials such as poly (alkylcyanoacrylates), polyacetates, polysaccharides and copolymers. The advantage of using nanoparticles for delivery is due to small size, nanoparticles penetrate in small capillaries and taken by cells and allowing efficient drug accumulation at the target site. The Biodegradable polymers used for the preparation of nanoparticles allows sustained drug release at the targeted site over a period of days or even weeks after injection.[8]

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Tacrine, the first FDA approved cholinesterase inhibitor for the treatment of dementia of the Alzheimer's type, is a centrally acting noncompetitive, reversible inhibitor of acetylcholinetrase and butyrylcholinestrase. It is chemically 9-amino-1,2,3,4-tetrahydroacridine. The Chemical formula is $C_{13}H_{14}N_2$.HCl and molecular weight is 198.26 g/mol.The melting point is ranged between 183 and 187^0C.[9] Methoxy poly(ethylene glycol) poly(caprolactone), approved by the united states Food and Drug administration as a biomedical material, has been used in the applications in the field of drug delivery and tissue engineering. mPEG-PCL has been used because of its excellent permeability to drugs, non-cytotoxicity, thermal properties, biocompatibility and biodegradability. And it is known that mPEG-PCL is hydrophobic and slow degradation for the needs of drug delivery.[10,11,12]Moreover, the PCL NPs could be rapidly removed in the liver and captured by the reticuloendothelial system (RES) when they are injected into the blood stream.[13] Methoxy poly(ethylene glycol) (MPEG) based modification has attracted increasing attention in the field of drug delivery due to its relatively low melting point that enables it to be fabricated by existing melt processing techniques and the ability to stabilize the delivery vehicle against undesirable aggregation and non-specific electrostatic interactions with its surroundings.[14] Sustained-release formulation for Tacrine-loaded mPEG-PCL NPs administered by direct intravenous injection.We have determined the physicochemical characterization of Tacrine loaded mPEG-PCL NPs using transmission electron microscopy (TEM), and evaluated them for particles size, zeta potential, drug entrapment, drug loading, and *in vitro* drug release and pharmacokinetic parameters in the rat model.

MATERIALS AND METHODS:

mPEG-PCL and PVA were purchased from Sigma Aldrich (St. Louis, MO, USA), Tacrine Hydrochloride (TH) were purchased from Sigma Aldrich (St. Louis, MO, USA). The solvents used were analytical grade and was purchased from Sigma Aldrich (St. Louis, MO, USA).

Preparation of MPEG-PCL Nanoparticles:

mPEG-PCL nanoparticles were prepared by single O/W emulsion and solvent evaporation method according to the description of Tweeset al.[15] with modification. Briefly, 20mg of mPEG-PCL and a certain amount of tacrine were dissolved in 1mL of methylene chloride. The mixture was emulsified in 3 ml of aqueous poly (vinyl alcohol) (PVA) solution at 5% (w/v) by sonication (XL2000, Misonix, USA) for 30 s (17.5 W) to obtain an O/W emulsion. This emulsion was then diluted in 8 ml of aqueous solution containing 1% (w/v) PVA and left under mechanical stirring for 2 hours to remove methylene chloride. The resulted solution was filtered to remove non-incorporated drug. Blank nanoparticles were produced in a similar manner without adding drug. Finally, nanoparticle suspensions were freeze dried and stored at 4°C. The prepared Tacrine loaded mPEG-PCL nanoformulation was characterized for particle size, zeta potential, TEM, drug content, entrapment efficiency and *in vitro* drug release. The protocol involved in this study were submitted to IAEC and duly approved by Vels University, i.e, XV/VELS/PCOL/04/2000/CPCSEA /IEAC/30/10/14.

Pharmacokinetic Studies:

Adult male Sprague Dawley rats weighting 230 and 250 gm were selected for the study. One day before the study the right jugular vein of five rats were cannulated with a polyethylene tubing (0.5 mm ID, 1 mm OD) for blood collection via, a light surgery under anesthesia by intramuscular injection of a mixture containing Ketamine (80mg/kg) and Xylazine (8mg/kg). After a 24 hr recovery and overnight fasting, rats were orally administered with 1ml of TH solution (1mg/ml) prepared in saline (2mg/kg dose). Around 200 µl of blood was collected at predetermined time points (30 min, 1hr, 2hr, 3hr, 4hr, 6hr, 8hr, 12hr, 16hr, 20hr and 24hr) post dosing. Plasma was obtained by centrifugation of blood at 16,000×g for 5min. rats were allowed for free access to food and water 4hr after drug administration. For Brain distribution study, three rats were orally administered with 1ml of TH solution (3g/ml) prepared in saline (2mg/kg dose). After 1hr after administration, the rats were sacrificed under anesthesia. Blood sample was collected by cardiac puncture followed by centrifugation to obtain plasma. The whole brain tissue was then placed on ice pack and dissected into olfactory bulb, frontal cortex, cerebral cortex, hippocampus, striatum and other remaining (midbrain, thalamus, hypothalamus and cerebellum) regions. Blood vessels and meninges were carefully removed with forceps and each brain region was accurately weighed. All samples were stored at -80 °C for overnight and analyzed the next day. Pharmacokinetic parameters were calculated using win Nonlin software (Pharsight Corp., version 2.1, Mountain View, CA) employing a non-compartmental model.[16]

RESULTS AND DISCUSSION:

Characterization Studies of Tacrine Loaded mPEG-PCL Nanoparticles:

The tacrine loaded mPEG-PCL nanoparticles were prepared by using emulsification method and solvent evaporation method. Optimization studies were carried out by varying the polymer concentration. Characterization studies were carried out to identify the suitable nanoformulations (data not shown). Selected nanoformulation (TMPCN-3) was used for

pharmacokinetic studies. The mean Particle size, zeta potential, percentage of drug entrapment and percentage of drug content and *invitro* drug release of the selected Tacrine loaded mPEG-PCL nanoparticles was shown in the following Table.1.

Table.1 Particle size, zeta potential, percentage of drug entrapment and percentage of drug content and *invitro* drug release of Tacrineloaded mPEG-PCL nanoparticles

Characterization Studies of Nanoparticles	Results
Particle Size	292±2.4 nm
Zeta potential	-26±1.6mV
Drug content	1.253µg/ml
Entrapment Efficiency	82.76 %

The mean particle size of plain nanoparticles and TH loaded nanoparticles exhibited 292±2.4 nm.The greater the zeta potential values of the nanodispersion higher the stability due to repulsive force between the like charged particles. The zeta potential value of the plain nanoparticles and TH loaded nanoparticles were recorded as -26±1.6, which indicates a higher stability. TEM image exhibits that TH loaded mPEG-PCL nanoparticles were spherical and homogeneity In the present study the TH content in the mPEG-PCL nanoparticles was found to be 1.253µg/ml and the entrapment efficiency was found to be 82.76 %. The *In vitro* release of TH from the polymeric NPs was compared with the plain drug. The release of TH from mPEG-PCL NPs for 24 hrs was shown in Fig.1. At the end of 24 hrs 94.52 % TH was released from the NPs, whereas 98.5 % TH was released within 2hrs. However, a burst release of TH was exhibited by the TH NPs within 1 hr, which suggests the release of TH adhered on to the surface of the NPs. Further the sustained release of the drug occurred up to 24hrs.

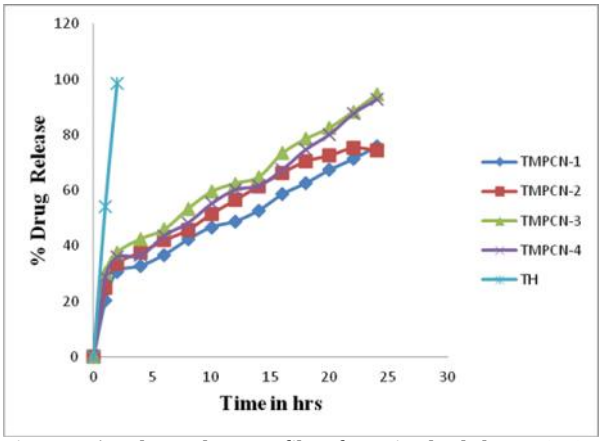

Fig.1. *In vitro* drug release profiles of Tacrine loaded MPEGPCL nanoformulations in phosphate buffer pH 7.4

Pharmacokinetic Studies:

The different pharmacokinetic parameters of Tacrine-loaded mPEG-PCL nanoparticulate suspension and drug solution were carried out in SD rats by iv, administration. The concentration of drug in plasma and brain homogenate was determined by LCMS/MS technique and shown in Table.2 and Figures 2 to 10. The pharmacokinetic parameters of TMPCN-3 were compared with that of drug (TH) solution administered by intravenous route.

C_{max}:

It was observed that there was a increase of drug in blood (1967) and brain (1257) was higher in case of TH loaded mPEG-PCL nanoparticles (TMPCN-3) as compared with that of the drug solution (Tacrine) administered by intravenous route as shown in figure.2.This, indicates the increased transport across BBB of TH in NP's when compared that of plain drug.

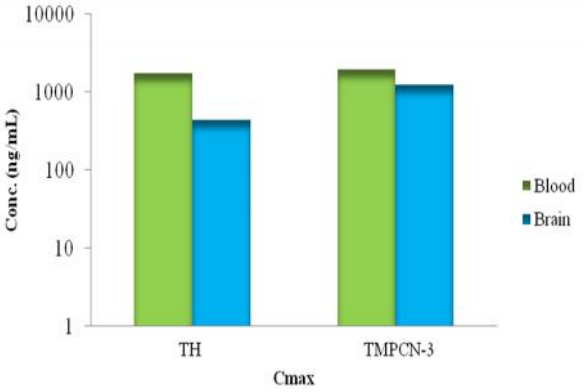

Fig.2. Comparison of C_{max} values for TH and TMPCN-3

AUC_{0-24}:

It was observed that the AUC_{0-24} of TH in blood (9597) and brain (3120) was less when compared to TH loaded mPEG-PCL nanoformulation and the results were shown in following figure.3.

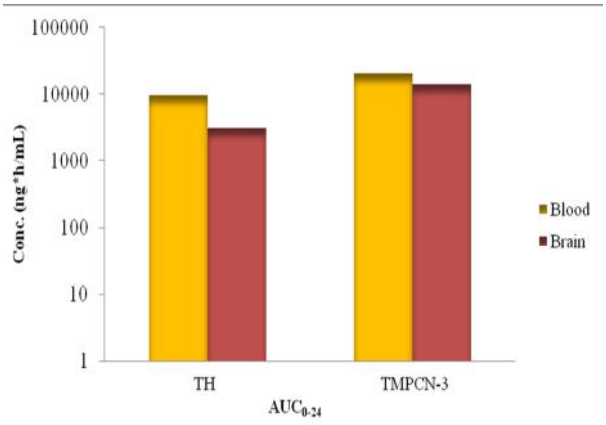

Fig.3. Comparison of AUC_{0-24} value for TH and TMPCN-3

$AUC_{0-\alpha}$:

A graph was plotted with the plasma drug concentration versus time, then the area under this curve, or $AUC_{0-\alpha}$ represents the total amount of drug absorbed. It was observed that the $AUC_{0-\alpha}$of TH in blood (9855) and brain (3337) was significantly lower when compared to TH loaded mPEG-PCL Nano formulation and the results were shown in figure.4.

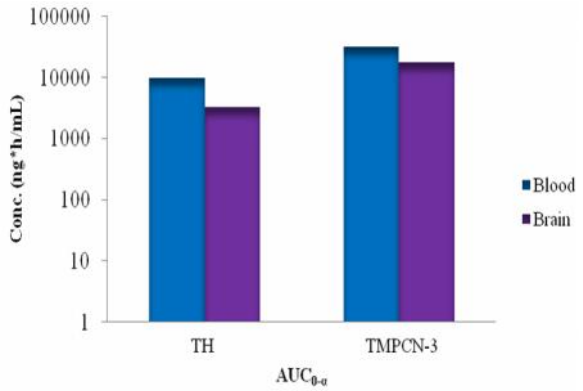

Fig.4. Comparison of $AUC_{0-\alpha}$value TH and TMPCN-3.

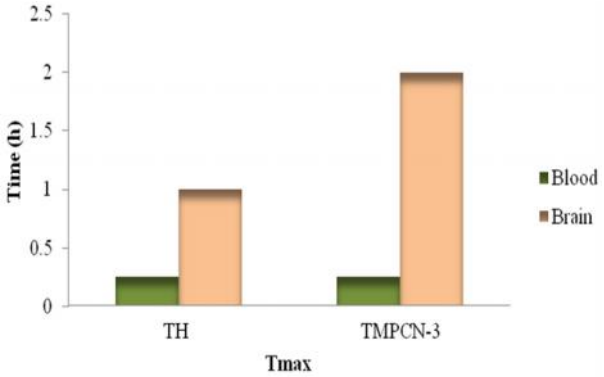

Fig.5. Comparison of T_{max}value for TH and TMPCN-3

T_{max}:

The time at which maximum concentration was observed was significantly high in TH loaded mPEG-PCL nanoformulation when compared to Tacrine solution. The $T_{max}$of TH was found in blood (0.25) and in brain was (2.0). Thus, TH loaded mPEG-PCL nanoformulation was found to provide a higher concentration of drug in brain compared to TH solution.

Clearance:

The maximum rate of elimination of drug of TH loaded mPEG-PCL nanoformulation in blood (1.03) and brain (1.89) was significantly less when compared to TH solution. Thus, TH loaded mPEG-PCL nanoformulation can sustain the release of drug for prolong period of time without elimination of drug in brain when compared to the TH solution.

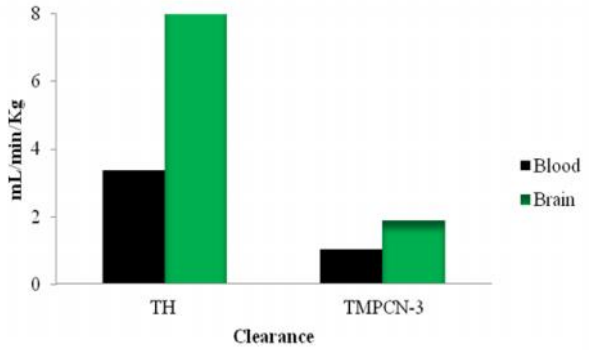

Fig.6. Comparison of CL values for TH and TMPCN-3

Volume of Distribution:

The volume of distribution represents the degree to which the drug is distributed in the body rather than blood. The volume of distribution of tacrine in blood (1.47) and brain (5.50) was significantly higher when compared to tacrine loaded mPEG-PCL nanoparticulate formulation and the results were shown in following figure.7.

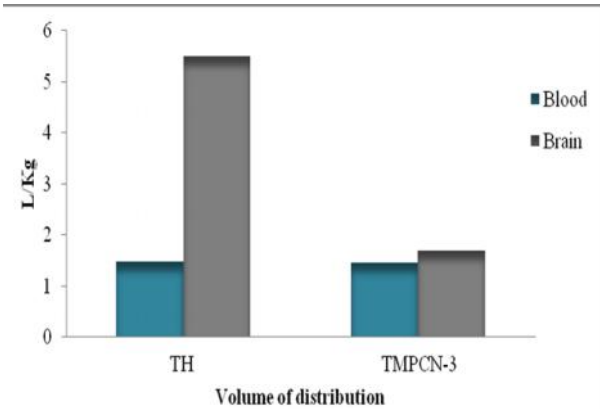

Fig.7. Comparison of Vd values for TH and TMPCN-3

Mean Residence Time

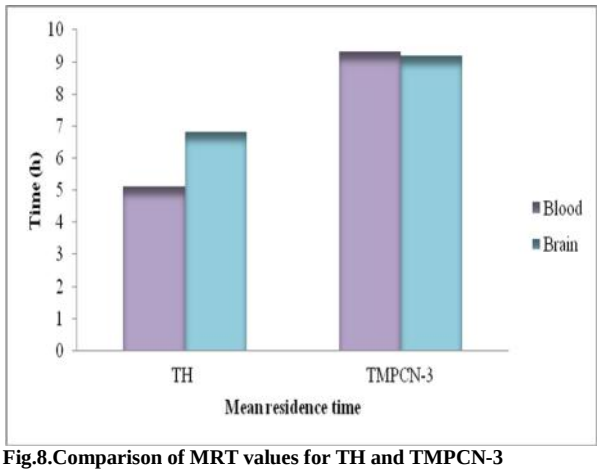

Fig.8.Comparison of MRT values for TH and TMPCN-3

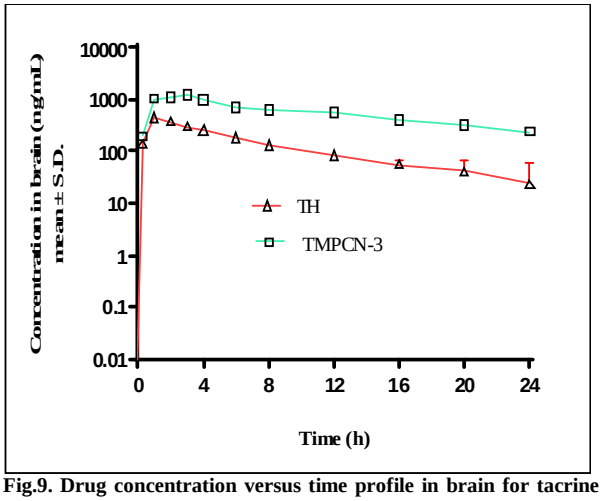

Fig.9. Drug concentration versus time profile in brain for tacrine via, intravenous Administration

The average time of the TH loaded mPEG-PCL nanoformulation was significantly high when compared to TH solution which is shown in figure.8.The maximum mean residence time, this leads to longer residence and lowers the elimination of drug. The concentrations of TH and TH loaded mPEG-PCL nanoformulation in brain and blood was recorded periodically in different time intervals and the results were shown in figure.8.

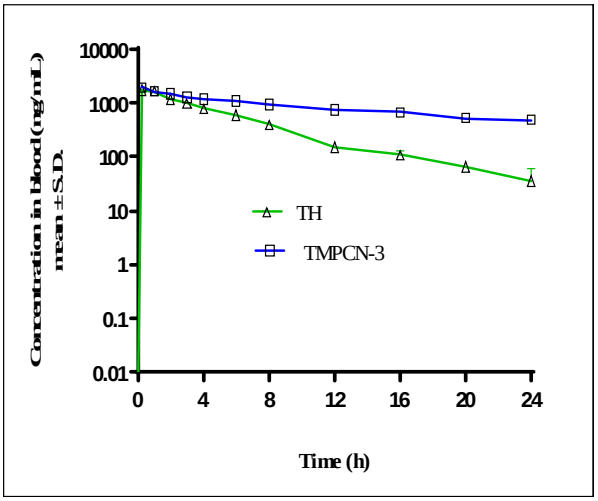

Fig.10. Drug concentration versus time profile in plasma for TMPCN-3 via, intravenous administration.

Table.2.Mean Pharmacokinetic parameters of TH and TMPCN-3 nanoparticles via, intravenous administration for plasma and brain in SD rats

Samples tested		C_{max}(ng/mL)	T_{max}(h)	AUC_{0-24} (ng*h/mL)	$AUC_{0-\alpha}$ (ng*h/mL)	CL (mL/min/Kg)	Vd (L/Kg)	MRT (hr)
TH	**Blood**	1734.23±0.06	0.25	9597±1.0	9855±1.0	3.37	1.47	5.13±0.2
	Brain	440±2.6	1.0	3120±0.5	3337±0.9	9.99	5.50	6.83±0.5
TMPCN-3	**Blood**	1967.39±28.4	0.25	20953±2.1	32503±1.8	1.03	1.46	9.34±0.8
	Brain	1257.34±47.6	2.0	13972±1.7	17627±1.3	1.89	1.70	9.2±1.3

The different pharmacokinetic parameters of TMPCN-3 nanoformulation and drug solution when administered by intravenous route were calculated by determining the concentration of drug in plasma and brain homogenate, as shown in Table and figures. The pharmacokinetic parameters of TMPCN-3 Nano formulation were compared with that of drug solution administered by intravenous route. It was observed that the C_{max} of the drug in blood (1967ng/mL) and brain (1257 ng/mL) was higher in TMPCN-3 administered by intravenous route. Thus, TMPCN-3 Nano formulation was found to provide a higher concentration of drug in brain compared to the tacrine solution. The NPs can open the tight junctions in the BBB. The NPs may be endocytosis/ transcytosed by the endothelial cells followed by the release of drugs within these cells and delivery to the brain. The

surfactant effect results in solubilisation of the endothelial cell membrane lipids that would lead to membrane fluidization and enhanced drug permeability through the BBB.

CONCLUSION:

The polymeric nanoparticles plays a major role for targeting drugs to the brain due to their great potential for the transport of anti alzeimereric drugs to the brain which are normally unable to cross the tight junctions of BBB. The research work shows that the tacrine loaded mPEG-PCL nanoformulation had significantly transported the drug tacrine in comparison with the free drug solution (tacrine) to the brain. The higher concentrations of tacrine achieved in the brain may be a significant improvement for treating the Alzheimer's disease. But further more extensive clinical studies are needed to confirm the efficacy of the prepared drug delivery system.

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REFERENCES:

1. Corrada MM, Brookmyer R, Paganini-Hill A, Berlau D, Kawas CH. Dementia incidence continues to increase with age in the oldest old: the 90 + study. Ann Neurol.(67)2010:114-121.
2. Barnes DE, Yaffe K. The projected effect of risk factor reduction on Alzheimer's disease prevalence. Lancet Neurol.(10); 2011: 819-828.
3. Davson H, Segal MB. Special aspects of the blood-brain barrier. Physiology of the CSF and blood-brain barriers, CRC press, Boca raton; 1996:303-385.
4. Banks WA, Kastin AJ. Passage of peptides across the blood-brain barrier pathophysiological perspectives. Life Sci; 59;1996:1923-1943.
5. Kastin AJ, Pan W. Feeding peptides interact in several ways with the blood-brain barrier. Curr Pharm Res;(9)2003:789-794.
6. Pan W, Kastin AJ. Cytokine transport across the injured blood-spinal cord barrier. Curr Pharm Res;(14)2008:1620-1624.
7. Garcia-Garcia E, Andrieux K, Gil S, Couvreur P. Collodial carriers and blood-brain barrier translocation: a way to deliver drugs to the brain. Int J Pharm.298(2);2005:274-292.
8. Vinogradov SV, Bronich TK, Kabanov AV, Nanosized cationic hydrogels for drug delivery: preparation, properties and interactions with cells. Adv Drug Deliv Rev.(54)2002:135-147.
9. Kumar V, Becker RE, Pharmacology of Tetrahydroaminoacridine: A possible therapeutic agent for Alzheimer's disease, Int.J. Clin. Pharmacol. Ther.Toxicol. 27 ; 1989:478-485.
10. Tao W, Zeng XW, Zhang JX, et al. Synthesis of cholic acid core poly (epsilon-caprolactone-ran-lactide)-b-poly(ethylene glycol) 1000 random copolymer as a chemotherapeutic Nano carrier for liver cancer treatment. Biomater Sci.(2);2014:1262-1274.
11. Lale SV, Aswathy RG, Aravind A, Kumar DS, Koul V. AS1411 Aptamer and folic acid functionalized pH-responsive ATRP fabricated pPEGMA-PCL-pPEGMA polymeric nanoparticles for targeted drug delivery in cancer therapy. Biomacromolecules. (15), 2014:1737-1752.
12. Tamboli V, Mishra GP, Mitra AK. Novel pentablock copolymer (PLAPCL-PEG-PCL-PLA) based nanoparticles for controlled drug delivery: effect of copolymer compositions on the crystallinity of copolymers and in vitro drug release profile from nanoparticles. Colloid PolymSci.291;2013:1235-1245.
13. He W, Zhao Y, Zhang C, et al. Rad9 plays an important role in DNA mismatch repair through physical interaction with MLH1. Nucleic Acids Res.(36);2008:6406-6417.
14. Tanaka K, Kanazawa T, Shibata Y, Suda Y, Fukuda T, Takashima Y, et al. Development of cell penetrating peptide modified mPEG-PCL diblockcopolymeric nanoparticles for systemic gene delivery. Int J Pharm. 396;2010:229-38.
15. Tewes F, Munnier E, Antoon B, NgaboniOkassa L, Cohen-Jonathan S et al. Comparative study of doxorubicin-loaded Poly(lactide-co-glycolide) nanoparticles prepared by single and double emulsion methods. Eur J Pharm Biopharm.66;2007:488-492.
16. Shuai Q, Siu K.W, Zhong Z. Pharmacokinetics and brain dispositions of tacrine and its major bioactive monohydroxylated metabolites in rats. J. Pharm & Biomed . Anal, 61; 2012:57-63.