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Research paper

Targeted delivery of tacrine into the brain with polysorbate 80-coated poly(*n*-butylcyanoacrylate) nanoparticles

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Abstract

The purpose of the present study was to investigate the possibility of targeting an anti-Alzheimer's drug tacrine in the brain using polymeric nanoparticles. Rats obtained 1 mg/kg of tacrine by intravenous injection in the form of three preparations: (1) a simple solution in phosphate buffered saline, (2) bound to poly(*n*-butylcyanoacrylate) nanoparticles, and (3) bound to poly(*n*-butylcyanoacrylate) nanoparticles coated with 1% polysorbate 80 (Tween® 80). After 1 h of post injection the rats were killed by decapitation and tacrine concentration in brain, liver, lungs, spleen and kidneys was analyzed by HPLC. A higher concentration of drug tacrine was observed in liver, spleen and lungs with the nanoparticles in comparison to the free drug. The accumulation of drug tacrine in the liver and spleen was reduced, when nanoparticles were coated with 1% polysorbate 80. In the brain a significant increase in tacrine concentration was observed in the case of poly(*n*-butylcyanoacrylate) nanoparticles coated with 1% polysorbate 80 compared to the uncoated nanoparticles and the free drug. In conclusion, the present study demonstrates that the brain concentration of intravenously injected tacrine can be enhanced by binding to poly(*n*-butylcyanoacrylate) nanoparticles, coated with 1% the nonionic surfactant polysorbate 80.

Keywords: Brain targeting; Blood–brain barrier; Tacrine; Nanoparticles; Poly(*n*-butylcyanoacrylate)

1. Introduction

Targeting of drugs in the brain is one of the most challenging issues for the pharmaceutical research, as many hydrophilic drugs and neuropeptides are unable to cross the blood–brain barrier (BBB) [1]. Despite enormous advances in brain research, brain and central nervous system (CNS) disorders remain the world's leading cause of disability, and account for more hospitalizations and prolonged care than almost all other diseases combined [2]. Brain disorders may be treated with better pharmacodynamic effects using targeted drug strategies. Drug delivery to the brain requires advances in both, drug delivery technologies and drug discovery [3]. Drugs that are effective against diseases in the CNS and reach the brain via the blood compartment must pass the BBB. The management of brain related diseases with present available therapeutic system is very difficult, as insufficient amount of drug reaches to the brain, due to highly lipophilic nature of BBB. The BBB has been called "the problem behind the problem" of CNS drug development. The BBB prevents the entry of >98% of small molecules and ~100% of large molecules [4]. The BBB constitutes an insurmountable barrier for the entry of many drugs and blood-borne substances into the brain [5–7].

The blood–brain barrier represents one of the hurdles for drugs including anti-Alzheimer, antibiotics, antineo-

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plastic agents and a variety of neuroleptic drugs. The BBB is formed by the capillary endothelial cells lining the microvessels, which are coupled by much tighter junctions (zonulae occludentes) than found in peripheral vessels [8]. Many strategies have been developed to overcome this problem which includes chemical delivery systems, magnetic drug targeting, or drug carrier systems such as antibodies, liposomes, or nanoparticles [9–12]. Among that polymeric nanoparticles have attracted great attention as potential drug delivery systems in the brain recently. Nanoparticles may be defined as a submicron drug carrier system, generally of polymeric nature. The drugs or other molecules may be dissolved into the nanoparticles, entrapped, encapsulated and/or adsorbed or attached. These systems are attractive because the methods of preparation are generally simple and easy to scale-up [13]. Due to their small size, nanoparticles penetrate into even small capillaries and are taken up within cells, allowing an efficient drug accumulation at the targeted sites in the body. The use of biodegradable materials for nanoparticles preparation, allows sustained drug release at the targeted site over a period of days or even weeks after injection [14].

Many authors have shown that therapeutic agents which are normally can not cross the BBB can be transported across this barrier into the brain by binding them to poly(butylcyanoacrylate) nanoparticles coated with polysorbate 80. Drugs that have been successfully delivered into the brain using the carrier poly(butylcyanoacrylate) include the hexapeptide dalargin [15], the dipeptide kytorphin [16], loperamide [17], tubocurarine [18], the NMDA receptor antagonist MRZ 2/576 [19], and doxorubicin [20]. The delivery of drugs into the brain by using nanoparticles may open a new era for treating diseases such as Alzheimer's, multiple sclerosis and brain tumors. Tacrine is a cholinesterase inhibitor used for treating mild to moderate Alzheimer's disease [21]. It is chemically 9-amino-1,2,3,4-tetrahydroacridine. The chemical formula is $C_{13}H_{14}N_2$ and molecular weight is 198.26. The melting point is ranged between 183 and 187 °C. Tacrine hydrochloride is yellow colored and needle shaped. It has a bitter taste and soluble in water. The pH of 1.5% solution is 4.5–6.0 [22]. It has a pK_a value of 9.85 [23]. The solubility of tacrine in water is 0.25 ± 0.02 mg/ml [24].

Alzheimer's disease is the most common brain disease of adulthood. It has recently received lot of attention, especially in areas related to novel treatments. It is a progressive and fatal disorder characterized by neuronal deterioration that results in loss of cognitive functions, such as memory, communication skills, judgments and reasoning [25]. Alzheimer's disease is 1.5× more common than stroke or epilepsy and is as common as congestive heart failure [26]. It affects 15 million people worldwide. It affects 10% of people over the age of 65 and 50% of people over the age of 85 [27]. Moreover, Alzheimer's disease has a tremendous negative economic impact amounting to over $100 billion a year. Treatment of Alzheimer's disease in the US reportedly costs more per patient than management of other major age associated disease [28].

Alzheimer's disease is characterized by marked atrophy of the cerebral cortex and loss of cortical neurons. The neuropathologic hallmarks of the disorder that are generally noted on postmortem brain examination: amyloid rich senile plaques [29], neurofibrillary tangles [30], and neuronal degeneration. Impairment of short-term memory is the first clinical feature. As the condition progresses, additional cognitive abilities are impaired [31]. The classic clinical features of Alzheimer's disease are an amnesic type of memory impairment [32], deterioration of language [33], and visuospatial deficits [34]. Death, most often from a complication of immobility such as pneumonia or pulmonary embolism [31]. The average time elapsed between diagnosis of Alzheimer's disease and death is 10 years, with a range of 3–20 years [35].

The explosion of interest of biological aspects of Alzheimer's disease in recent years has led lot of improvements in the understanding as well as the treatment of the disease. Though many studies have been carried out to treat the disease still it remains a chronic and debilitating disorder for many patients. Targeting of drugs in the brain with maximum benefits and minimum or no side effects, may be a break through in the treatment of Alzheimer's disease as well as other neurodegenerative disorders. Hence in the present study, the possibility of targeting of an anti-Alzheimer's drug tacrine with polysorbate 80-coated nanoparticles to the brain was investigated.

2. Materials and methods

2.1. Materials

Tacrine (9-amino-1,2,3,4-tetrahydroacridine), Dextran, D-(+)-glucose and polysorbate 80 (Tween 80) were purchased from Sigma, St. Louis, USA. The monomer *n*-butylcyanoacrylate was gifted by Loctite, Dublin, Ireland. All other materials and reagents used in the study were Analytical/HPLC grade.

2.2. Preparation of poly(n-butylcyanoacrylate) nanoparticles

The poly(*n*-butylcyanoacrylate) nanoparticles of tacrine was prepared according to the method described by Kreuter et al. [15]. Briefly, the monomer *n*-butyl cyanoacrylate was added drop by drop under magnetic stirring to 10 ml of acidic polymerization medium (0.1 N HCl) containing 1% dextran 70,000 and the drug tacrine (drug polymer ratio 1:1, 1:2, 1:3, 1:4 and 1:5). The monomer, *n*-butylcyanoacrylate, is a clear liquid with sharp characteristic odor. It undergoes polymerization in contact with water or in the presence of moisture [36]. The mixture was then stirred magnetically at 500 rpm for 4 h to facilitate nanoparticles formation. The resulting suspension was neutralized with 0.1 N sodium hydroxide solution, and filtered through a sintered glass filter (pore size 10 μm) to remove any agglomerates [15,37]. Anhydrous glucose (1%) was added

to improve redispersibility of the nanoparticles after lyophilization. The nanoparticles suspension was then lyophilized using a lyophilizer (Christ, Germany). The drug-free nanoparticles were prepared in the same manner to that of drug containing nanoparticles except for omitting the drug.

2.3. Coating of poly(n-butylcyanoacrylate) nanoparticles with 1% polysorbate 80

Coating of poly(*n*-butylcyanoacrylate) nanoparticles formulations was performed as per the procedure described by Kreuter, et al. [38]. Poly(*n*-butylcyanoacrylate) nanoparticles which contains the drug tacrine (drug polymer ratio 1:1) were resuspended in phosphate buffered saline at a concentration of 20 mg/ml under constant stirring. Then (relative to total suspension volume) polysorbate 80 was added to give a final solution of 1% polysorbate 80, and the mixture was incubated for 30 min and finally lyophilized.

2.4. Determination of process yield

The process yield of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine was determined; as the weight percentage of the final product after drying, with respect to the initial total amount of drug, polymer and other materials used for the preparation [39].

2.5. Determination of drug loading capacity

The drug loading to the poly(*n*-butylcyanoacrylate) nanoparticles was calculated as the difference between total amount of drug in suspension and amount of unbound drug [20]. For this purpose, the unbound drug was separated by filtration through a membrane filter (Minisart, Sartorius, Germany) and its concentration in the filtrate was measured spectrophotometrically at 240 nm.

2.6. Particle size analysis

The particle size of the nanoparticles (drug polymer ratio 1:1) was analyzed by Scanning Probe Microscope. The samples were dispersed and mounted on the cover glass and allowed to dry in air. This was mounted directly on the specimen metal disc using Scotsch double adhesive tape. Samples were scanned at various scan areas using Shimadzu SPM 9500-2J Scanning Probe Microscope. For high resolution, contact mode cantilever was used for all analyses.

2.7. Zeta potential measurement

Zeta potential of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine coated with 1% polysorbate 80 and without coating (drug polymer ratio 1:1) were separately measured in deionized distilled water [40] using Zetasizer 3000 HSA (Malvern Instruments, U.K.).

2.8. In vitro release studies

The release of drug tacrine from poly(*n*-butylcyanoacrylate) nanoparticles were studied separately according to the method described by Marchal-Heussler et al. [41] in pH 7.4 phosphate buffer. Nanoparticles coated with polysorbate 80 (drug polymer ratio 1:1) were also subjected to the *in vitro* release studies. Nanoparticles equivalent to 1 mg of drug was placed in a cellulose dialysis bag (cut-off 5 kDa, Himedia), and to this a little amount of dissolution media was added, which was then sealed at both ends. The dialysis bag was dipped into the receptor compartment containing the dissolution medium, which was stirred continuously at 100 rpm maintained at 37 °C. The receptor compartment was closed to prevent evaporation of the dissolution medium. Samples were withdrawn at regular time intervals and the same volume was replaced with fresh dissolution medium. The samples were measured by UV Spectrophotometer at 240 nm against dummy nanoparticles as reagent blank which had also been prepared and treated similar to the drug loaded nanoparticles.

2.9. Release kinetics

Data obtained from the *in vitro* release studies of poly(*n*-butylcyanoacrylate) nanoparticles (drug polymer ratio 1:1) without coating and coated with polysorbate 80 were fitted to various kinetic equations such as first order, Higuchi model and Korsmeyer–Peppas model [42].

2.10. Stability studies

A study was carried out to assess the stability of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine (drug polymer ratio 1:1). This was carried out as per the procedure described by Zhang et al. [43]. The samples were stored in room temperature (15–20 °C), refrigerator (3–5 °C) and 37 °C (RH = 75%) over a period of 3 months. Samples were evaluated at 0, 1, 2 and 3 months for their drug content as well as any changes in their physical appearance. FT-IR studies were also carried out to find out any changes in the IR spectra after 3 months of storage.

2.11. Animal testing

The targeting efficacy of selected poly(*n*-butylcyanoacrylate) nanoparticles which contains the drug tacrine (drug polymer ratio 1:1) was carried out on rats [20,44]. Healthy adult Wistar rats weighing 180–220 g were obtained from the animal house, J.S.S. College of Pharmacy, Ootacamund, India after getting approval from CPCSEA (JSSCP/IAEC/PH.D/PH.CEUTICS/02/2006-07). The animal house was well ventilated and the animals were main-

tained on a 12:12 h light/dark cycle in large spacious cages throughout the experimental period. The animals were provided with food and water *ad libitum*. All efforts were made to minimize animals suffering and to reduce the number of animals for the study. The animals were divided into four groups and each group contained 6 rats.

The group one was given tacrine pure drug, group two was administered tacrine bound with poly(*n*-butylcyanoacrylate) nanoparticles, group three was received tacrine bound with poly(*n*-butylcyanoacrylate) nanoparticles coated with 1% polysorbate 80, and group four was served as control. For the *in vivo* experiments the formulations were resuspended in phosphate buffered saline. For surfactant coating 1% polysorbate 80 was added and the suspension was incubated for 30 min under stirring prior to administration. All the formulations were given in a dose level equivalent to 1 mg/kg body weight [45]. The formulations were administered intravenously in the tail vein of rats. After 1 h of post injection [44] the rats were killed by decapitation. The brain, liver, lungs, spleen and kidneys were quickly removed and weighed, and stored at −20 °C. The drug content in the organs was analyzed by using Shimadzu LC 2010A HT HPLC.

2.12. Statistical analysis

The results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out by one way ANOVA and subsequent post hoc Tukey comparison. A *p*-value less than 0.05 was considered as significant.

3. Results

3.1. Nanoparticles

Poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine was prepared by emulsion polymerization. The process yield, drug loading and particle size of poly(*n*-butylcyanoacrylate) nanoparticles are shown in Table 1. The process yield of poly(*n*-butylcyanoacrylate) nanoparticles contain tacrine with different drug polymer ratio was ranged between 81.67 ± 3.35% to 89.40 ± 1.17%. The drug loading was varied from 8.80 ± 0.41% to 17.18 ± 0.26% depends upon the drug polymer ratio. The size of drug loaded nanoparticles (drug polymer ratio 1:1) was 35.58 ± 4.64 nm. The drug loading capacity of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine (drug polymer ratio 1:1) after coating with 1% polysorbate 80 was 14.68 ± 0.38% w/w. The mean zeta potential of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine coated with 1% polysorbate 80 and without coating was −39.5 ± 1.0 mV and −41.8 ± 1.4 mV respectively (Table 2). The cumulative percentage release of tacrine from poly(*n*-butylcyanoacrylate) nanoparticles were varied from 73.16 ± 1.65% to 86.44 ± 1.73% depends upon the drug polymer ratio for 24 h. But the cumulative percentage release of tacrine from poly(*n*-butylcyanoacrylate) nanoparticles (drug polymer ratio 1:1) coated with polysorbate 80 was 79.71 ± 1.47 for 24 h (Fig. 1). The results of stability studies at different storage conditions are given in Table 3.

3.2. Animal testing

Tacrine concentrations (ng/ml) in brain, liver, spleen, lungs and kidneys after intravenous injection of poly(*n*-butylcyanoacrylate) nanoparticles which contains tacrine are shown in Table 4 and Fig. 2. The concentration of tacrine differs in the brain, liver, spleen, lungs and kidneys depending on in which form it was administered. The concentration of tacrine, after the intravenous administration of pure drug alone, in the liver, spleen, lungs and kidneys was 316.88 ± 35.13, 275.68 ± 16.68, 779.94 ± 57.46 and 1063.13 ± 96.22 ng/ml, respectively. But when it was bound with poly(*n*-butylcyanoacrylate) nanoparticles the concentration in the liver, spleen, lungs and kidneys was 596.60 ± 52.50, 580.07 ± 56.09, 814.24 ± 59.84 and 974.76 ± 94.47 ng/ml, respectively. The concentration of tacrine in the liver, spleen, lungs and kidneys, after the intravenous administration of tacrine bound to nanoparticles coated with polysorbate 80, was 515.81 ± 40.49, 547.04 ± 38.87, 844.06 ± 68.05 and 1045.52 ± 138.96 ng/ml, respectively.

In the brain a different picture was observed, the polysorbate 80 coating significantly increased the uptake of tacrine into the brain in comparison with the free drug alone and drug bound with nanoparticles. The concentration of tacrine achieved in the brain when tacrine was administered alone, it was bound to nanoparticles, and the drug bound nanoparti-

Table 1
Process yield, particle size and percent drug loading of poly(*n*-butylcyanoacrylate) nanoparticles formulations

Dp ratio	Process yield	Percent drug load (w/w)			Particle size in nm
		Theoretical	Practical	After coating	
1:1	89.40 ± 1.17	20.00	17.18 ± 0.26	14.68 ± 0.38	35.58 ± 4.64
1:2	87.33 ± 2.39	16.67	14.09 ± 0.31	nt	nt
1:3	85.71 ± 3.71	14.29	11.57 ± 0.40	nt	nt
1:4	84.50 ± 3.92	12.50	9.94 ± 0.25	nt	nt
1:5	81.67 ± 3.35	11.11	8.80 ± 0.41	nt	nt

($n = 3 \pm$ SD).
Dp, drug polymer.
nt-not tested.

Table 2
Release kinetics and zeta potential of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine (drug polymer ratio) without coating and coated with polysorbate 80

Formulation	Zeta potential[a] (mV)	Release kinetics			
		First order r^2	Higuchi r^2	Korsmeyer–Peppas	
				r^2	n
Tac-Np	-41.8 ± 1.4	0.0357	0.9296	0.9730	0.17
Tac-Np + Ps 80	-39.5 ± 1.0	0.0044	0.9691	0.9903	0.25

Tac-Np, tacrine bound to nanoparticles.
Tac-Np + Ps 80, tacrine bound to nanoparticles coated with 1% polysorbate 80.
[a] ($n = 3 \pm$ SD).

cles coated with polysorbate 80 was 61.79 ± 4.98, 78.15 ± 5.86 and 251.49 ± 17.25 ng/ml, respectively. The poly(*n*-butylcyanoacrylate) nanoparticles coated with 1% polysorbate 80 increased the concentrations of tacrine in the brain by 4.07-fold when compared to the free drug tacrine.

4. Discussion

4.1. Preparation of poly(n-butylcyanoacrylate) nanoparticles

Poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine was prepared by emulsion polymerization. Emulsion polymerization has been the selected procedure to obtain cyanoacrylate nanoparticles [46]. Dextran has long been used as a steric stabilizer for obtaining poly(alkylcyanoacrylate) nanoparticles [47]. In the presence of dextran, following a period of equilibrium, colloidally stable particles form, but in the absence of dextran, particles are colloidally unstable and rapidly coalesce [48]. Hence dextran was used as the stabilizer in the preparation of poly(*n*-butylcyanoacrylate) nanoparticles. The preparation of nanoparticles is not complicated [49] and storage in a lyophilized form normally ensures long durability [50].

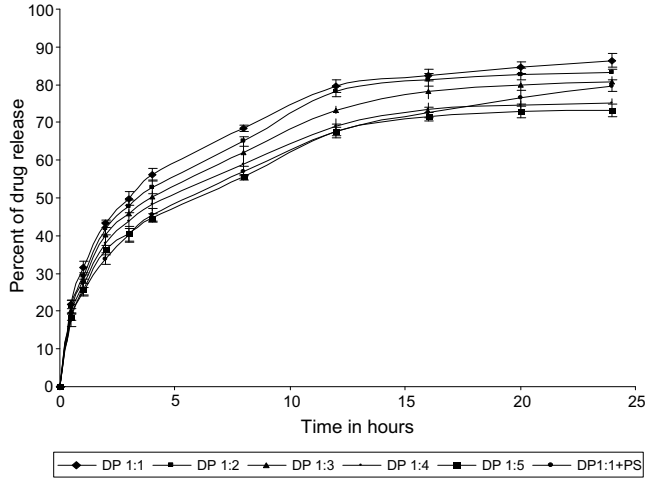

Fig. 1. *In vitro* release profile of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine with different drug polymer ratio. Dp, drug polymer ratio; Ps, Polysorbate 80.

4.2. Particle size

The mean size of drug loaded nanoparticles was 35.58 ± 4.64 nm (Table 1). Nanometer range particles have easy accessibility in the body, being transported via the circulation to different body sites. Extremely small particles, of <100 nm diameter with hydrophilic surface, have been found to have longer circulation in blood [51]. Such systems should allow the control of the rate of drug administration that prolongs the duration of the therapeutic effect, as well as the targeting of the drug to specific sites [52]. Sizes above 100 nm will tend to restrict their biodistribution, contributing to an increase in their capture by Küpffer cells or other phagocytic cell populations within the mononuclear phagocytic system [53]. It has already been claimed the possibility of obtaining small-sized nanoparticles down to as low as 30–50 nm in diameter [52,54].

4.3. Drug loading capacity

Drug payload for any carrier system should be high to minimize the quantity of delivery system used per ml of the solvent. The prepared nanoparticles showed a high drug loading capacity in comparison with the theoretical drug loading of the nanoparticles. The drug loading capacity of poly(*n*-butylcyanoacrylate) nanoparticles was ranged between 8.80 ± 0.41 to 17.18 ± 0.26% w/w against a theoretical drug loading of 11.11 to 20% w/w depends on the drug polymer ratio. Where as the mean drug loading capacity of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine (drug polymer ratio 1:1) after coating with 1% polysorbate 80 was 14.68 ± 0.38% w/w, and it was found that coating with 1% polysorbate 80 reduced the drug loading of the nanoparticles. This was probably due to the leakage of drug into the medium during the process of coating.

4.4. Zeta potential

The zeta potential is a measure of the charge of the particles, as such the larger the absolute value of the zeta potential the larger the amount of charge of the surface. In a sense, the zeta potential represents an index for particle stability [55]. For the case of charged particles, as the zeta potential increases, the repulsive interactions will be

Table 3
Stability studies of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine with drug polymer ratio of 1:1

Temp.	Evaluation parameter	Observation (month)			
		0	1	2	3
15–20 °C	Physical appearance	White	No change	No change	No change
	FT-IR	Performed	–	–	nsc
	Drug content[a] (% w/w)	17.18 ± 0.26	17.17 ± 0.34	17.15 ± 0.20	17.12 ± 0.18
3–5 °C	Physical appearance	White	No change	No change	No change
	FT-IR	Performed	–	–	nsc
	Drug content[a] (% w/w)	17.18 ± 0.26	17.18 ± 0.33	17.16± 0.17	17.14 ± 0.24
37 °C (RH = 75%)	Physical appearance	White	No change	No change	No change
	FT-IR	Performed	–	–	nsc
	Drug content[a] (% w/w)	17.18 ± 0.26	17.16 ± 0.26	17.14 ± 0.19	17.11 ± 0.32

–. not performed.
nsc, no significant change.
[a] ($n = 3 \pm$ SD).

Table 4
Tacrine concentrations (ng/ml) in different organs after intravenous injection of poly(*n*-butylcyanoacrylate) nanoparticles formulations

S. No.	Organ	Formulation		
		Tac	Tac-Np	Tac-Np + Ps 80
1	Brain	61.79 ± 4.98	78.15 ± 5.86	251.49 ± 17.25[b,d]
2	Liver	316.88 ± 35.13	596.60 ± 52.50[a]	515.81 ± 40.49[c]
3	Spleen	275.68 ± 16.68	580.07 ± 56.09[a]	547.04 ± 38.87[b]
4	Lungs	779.94 ± 57.46	814.24 ± 59.84	844.06 ± 68.05
5	Kidneys	1063.13 ± 96.22	974.76 ± 94.47	1045.52 ± 138.96

($n = 6 \pm$ SD).
Tac, tacrine solution; Tac-Np, tacrine bound to nanoparticles; Tac-Np + Ps 80, tacrine bound to nanoparticles coated with 1% polysorbate 80.
[a] $p < 0.001$ Tac vs Tac-Np.
[b] $p < 0.001$ Tac vs Tac-Np + Ps 80.
[c] $p < 0.01$ Tac vs Tac-Np + Ps 80.
[d] $p < 0.001$ Tac-Np vs Tac-Np + Ps 80.

larger leading to the formation of more stable particles with a more uniform size distribution. A physically stable nanosuspension solely stabilized by electrostatic repulsion will have a minimum zeta potential of ±30 mV [56]. This stability is important in preventing aggregation. The mean zeta potential of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine coated with 1% polysorbate 80 and without coating was −39.5 ± 1.0 mV and −41.8 ± 1.4 mV, respectively (Table 2). The values are highly sufficient to form stable nanoparticles suspension. The polysorbate 80 coating slightly reduced the zeta potentials. This was probably due to masking of surface charge of nanoparticles by the adsorbed polysorbate 80, as reported elsewhere for other surfactants, which were used for surface modification [57,58].

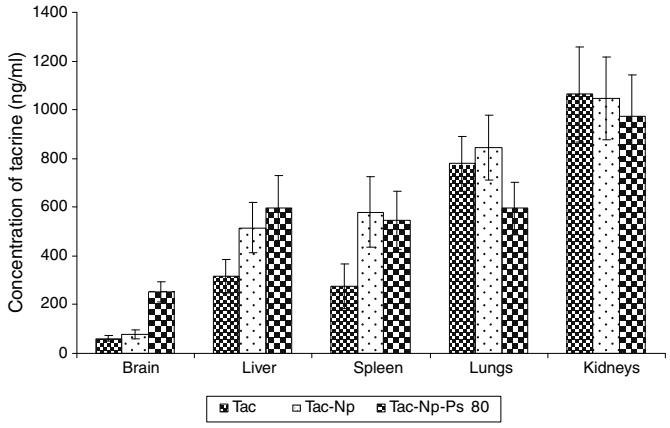

Fig. 2. Tacrine concentrations (ng/ml) in different organs after intravenous injection of poly(*n*-butylcyanoacrylate) nanoparticles formulations. Tac, tacrine solution; Tac-Np, tacrine bound to nanoparticles. Tac-Np + Ps 80, tacrine bound to nanoparticles coated with 1% polysorbate 80.

4.5. In vitro release studies

The cumulative percentage release of tacrine from poly(*n*-butylcyanoacrylate) nanoparticles were varied from 73.16 ± 1.65% to 86.44 ± 1.73% depends upon the drug polymer ratio for 24 h (Fig. 1). It can be seen from the figure that all the drug loaded batches of poly(*n*-butylcyanoacrylate) nanoparticles showed a biphasic release pattern. The initial burst effect occurs within 30 min and the remaining amount of drug was found to be released in a sustained manner, over a period of 24 h. The burst release of drug is associated with the drug molecules entrapped in the surface layer of the particles instantaneously dissolves

when it comes in contact with the release medium [59]. It has been reported that nanoparticles exhibited a biphasic release pattern with an initial burst effect followed by a sustained release [58,60,61]. It was also found that the release of drug tacrine increased with increased drug loading of the nanoparticles. It has been reported that drug release is faster from microparticles with higher drug content [62,63]. The cumulative percentage release of tacrine from poly(*n*-butylcyanoacrylate) nanoparticles (drug polymer ratio 1:1) after coating with 1% polysorbate 80 was $79.71 \pm 1.47\%$ for 24 h. It was found that coating of poly(*n*-butylcyanoacrylate) nanoparticles which contain tacrine with 1% polysorbate 80 slightly decreased the release of drug tacrine from the nanoparticles.

4.6. Release kinetics

Data obtained from *in vitro* release studies of poly(*n*-butylcyanoacrylate) nanoparticles (drug polymer ratio 1:1) without coating and coated with polysorbate 80 were fitted to various kinetic equations and the results are presented in Table 2. The release of drugs from poly(*n*-butylcyanoacrylate) nanoparrticles were diffusion controlled as indicated by the higher r^2 values in Higuchi model. Since, the n values obtained from the Korsmeyer–Peppas model were less than 0.45, the mechanism of drug release from the poly(*n*-butylcyanoacrylate) were Fickian [42].

4.7. Stability studies

One of the major criteria for any rational design of a dosage form is its stability. Drug instability in pharmaceutical formulations may be detected in some instances by the changes in the physical appearance, color, odor, taste, or texture of the formulation. Where as, in other cases, chemical changes may occur which are not self evident and may only be ascertained through chemical analysis. The results of stability studies of poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine (drug polymer ratio 1:1) are shown in Table 3. There were no changes in their physical appearance. It was observed that the initial drug content and the drug contents of the samples analyzed after 1, 2 and 3 months of storage at various conditions were similar indicating there were no significant changes in the physical as well as chemical characteristics of the formulations. No significant changes were observed in the IR spectra of the formulations after 3 months of storage. Based on the observations, it was concluded that the developed poly(*n*-butylcyanoacrylate) nanoparticles of drug tacrine physically and chemically stable and retain their pharmaceutical properties at various temperature and humidity conditions over a period of 3 months.

4.8. Animal testing

Particulate substances or drug carriers with an average size below 7 μm are normally taken up by the reticuloendothelial system, particularly by the Kupffer-cells of the liver, after intravenous injection [64,65]. This was also evident in the study for the drug tacrine bound to poly(*n*-butylcyanoacrylate) nanoparticles. A higher concentration of tacrine was observed in the liver with the nanoparticles in comparison to the free drug in phosphate buffered saline. The concentration of tacrine in the liver when administered as free drug and tacrine bound with the nanoparticles was 316.88 ± 35.13 ng/ml and 596.60 ± 52.50 ng/ml, respectively. Similar results and trends were observed in the spleen and lungs. In spleen the concentration of tacrine achieved after the administration of free drug and tacrine bound with nanoparticles was 275.68 ± 16.68 ng/ml and 580.07 ± 56.09 ng/ml, respectively, whereas in lungs it was 779.94 ± 57.46 ng/ml and 814.24 ± 59.84 ng/ml, respectively. This was in accordance with previously reported data for other drugs [44,66]. The accumulation of tacrine in the liver and spleen was reduced, when nanoparticles were coated with 1% polysorbate 80. This result supports the findings reported earlier [44,67]. But a somewhat different picture was seen in the case of kidneys. Coating of nanoparticles with polysorbate 80 increased the accumulation of tacrine in the kidney. This result was in accordance with findings reported by Löbenberg el al. [44].

The results showed that polysorbate 80 coated poly(*n*-butylcyanoacrylate) nanoparticles were able to deliver the drug tacrine in the brain significantly in comparison to the free drug and the drug bound uncoated nanoparticles. It was reported that polysorbate 80 coated nanoparticles enabled the hexapeptide dalargin as well as loperamide to cross the blood–brain barrier and exhibited a strong dose dependent pharmacological effect [15,17]. Both drugs normally do not cross the blood–brain barrier. The maximum effect with the drug bound nanoparticles coated with polysorbate 80 was observed by these authors after 45 min. But Löbenberg et al. [44] reported that the maximum concentration of azidothymidine in brain was achieved after 1 h of administration of azidothymidine bound to nanoparticles coated with polysorbate 80. One hour as cited by Löbenberg et al. [44] and 45 min as cited by Kreuter et al. [15] and Alyautdin et al. [17] were similar enough time periods to exert the maximum effects. Hence, in the present study the animals were sacrificed after 1 h of drug administration and the organs were collected and the drug content was analyzed.

The poly(*n*-butylcyanoacrylate) nanoparticles coated with 1% polysorbate 80 increased the concentrations of tacrine in the brain by 4.07-fold when compared to the free drug tacrine. The transport of drugs across the blood–brain barrier with polymeric nanoparticles has been explored by various studies. It was observed that coating of ^{14}C-labelled poly(methylmethacrylate) nanoparticles with various surfactants including polysorbate 80 significantly increased the concentrations of nanoparticles in the brain after intravenous injection in rats [68]. Later it was confirmed by *in vitro* bovine microvessel

endothelial cell cultures that polysorbate 80 coating increased the uptake of poly(methylmethacrylate) nanoparticles into the brain and reported that polysorbate 80 was the most efficient agent for delivering drugs across the blood–brain barrier and was identified as a potential "lead substance" for brain targeting [69]. Later, studies showed that drugs that do not cross the blood–brain barrier, the leu-enkephalin analogue dalargin [15], loperamide [17], the quaternary ammonium compound tubocurarine [18] and the chemotherapeutic agent doxorubicin [20], were successfully delivered into the brain by binding the drugs with poly(butylcyanoacrylate) nanoparticles and coating these particles with polysorbate 80. Drugs bound with nanoparticles without polysorbate 80 coating showed no pharmacological effects indicating that the polysorbate 80 coating is essential for the delivery of drugs into the brain. Many other studies also support that polysorbate 80 play a specific role for the delivery of drugs, which are unable to cross the BBB, into the brain. Recently, Sun et al. [70] studied the specific role of Tween 80 (polysorbate 80) coating for the delivery of nanoparticles into the brain. They reported that polysorbate 80 was necessary for the delivery of model nanoparticles into the brain. It appeared that brain targeting of nanoparticles was concerned with the interaction between polysorbate 80 coating and brain micro-vessel endothelial cells. And they confirmed the specific role of polysorbate 80 coating on nanoparticles in brain targeting. Hence, the present study also suggests that the higher concentration of tacrine was observed in the brain may be due to polysorbate 80 coating.

Several mechanisms have been proposed for the transport of nanoparticles coated with polysorbate 80 across the blood–brain barrier [12,15,20]. Among them the mechanism of endocytosis [12,15,20] was supported by many studies. Poly(butylcyanoacrylate) nanoparticles coated with polysorbate 80 adsorb apolipoprotein B and/or E after injection into the blood stream. The polysorbate acts mainly as an anchor for the apolipoprotein-overcoated nanoparticles thus would mimic lipoprotein particles and could interact with and then be taken up by the brain capillary endothelial cells via receptor-mediated endocytosis [71].

A slightly increased concentration of tacrine was also observed in the case of uncoated poly(*n*-butylcyanoacrylate) nanoparticles compared to the free drug tacrine. It is well known that after intra vascular injection uncoated nanoparticles are rapidly captured by the reticuloendothelial system resulting in an accumulation of the particles in the liver, the spleen, the lungs and the bone marrow [64]. It is possible that uncoated tacrine loaded nanoparticles indeed were captured by the reticuloendothelial system, and the particles continuously released the drug into the blood stream resulting in elevated plasma and brain levels of tacrine (because tacrine crosses the blood–brain barrier). This result is in accordance with the previous data reported for other drug [19].

5. Conclusion

The high concentrations of tacrine achieved in the brain in the present study may be useful for treating Alzheimer's disease. The developed formulations may also reduce the total dose required for the therapy with concurrent reduction in dose related toxicity. But it requires further studies as a drug delivery system.

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