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Development and Characterization of Anti-alzheimer Drug-loaded Chitosan Nanoparticles for the Enhanced Penetration of Blood Brain Barrier

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAGR is the principal investigator who designed the study, conducted research and contributed in manuscript writing. Author MWA helped in physicochemical characterizations and in vitro characterization. Author NMA contributed in manuscript writing. All authors read and approved the final manuscript.

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ABSTRACT

Nanotechnology facilitated drug delivery has been used to enhance the drug bioavailability, efficacy, reduce toxicity and improve patient compliance aiming to targetthe cells and tissues to produce anticipated pharmacological action. The aim of the present study was to formulate and evaluate rivastigmine (RT) loaded chitosan (CS) nanoparticles for sustained release. RT is a short actingreversible acetylcholinesterase inhibitor used for the treatment of mild to moderate Alzheimer's and Parkinson's disease. In current research RT loaded CS-tripolyphosphate (TPP) nanoparticles were prepared by usingionic gelation method in fourdifferent polymer concentrations (0.1%,0.2%,0.3%,0.4%). The prepared nanoparticles were evaluated by Zeta sizer in order to determine particle size, PDI and zeta potential. Further, drug entrapment efficiency and in vitro release studies were carried out. The results showed that particle size decreased by loading drug within nanoparticles when compared with unloaded nanoparticles. The particle size of RT loaded CS nanoparticles ranged from 125.9 ± 2.5 to 356.0 ± 7.9 by varying CS concentration from 0.1% to

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0.4% w/v. Among different ratios studied, 0.4% ratio showed highest drug entrapment efficiency (80%). In vitro release studies showed that RT loaded CS nanoparticles could sustain release the drug.In conclusion, the current research results showed that the chitosan nanoparticles can be used as a potential carrier for providing sustained delivery of RT.

Keywords: Chitosan (Cs), nanoparticles, rivastigmine (RT), sodium tripolyphosphate (TPP).

1. INTRODUCTION

The neurodegenerative disorders comprise ofdifferent medical states that slowly damages patient memory and cognition in the geriatric population. Alzheimer's disease (AD) affects approximately 24.3 million people globally and considered as one of the most progressive disease socially and economically [1]. AD is the most common neurodegenerative disorders in this century. The etiology of the disease is still not clear, however some of the factors that play important role in its pathogenesis include abnormal proteins, oxidative stress, and disproportionate accumulation and reduced acetylcholine levels [2]. One of the major aspects that hinderthe effective drug development and discovery of drug delivery system for thetreatment and the prevention of AD is due to the presence of blood brain barrier (BBB)which impedesthe delivery of anti-Alzheimer's drug to brain [3]. In order to overcome this difficulty several strategies have been employed to enhance the availability of drugs in the brain.

In recent years, various polymeric carriers like liposomes [4], polymeric nanoparticle [5],solid lipid nanoparticle [6],and dendrimers [7] are used to develop drug delivery system to target brain. Among the various nanocarriers used, polymeric nanoparticles(NPs) are promising carrier because of their ability to open tight junctions of BBB [8].Nanoparticles which are made up of hydrophilic polymers for example chitosan(CS) have better advantages in terms of prolonged circulation and decrease particle size in the range of 200 nm.CS is a natural hydrophilic polysaccharide comprised of glucosamine and N-acetylglycosamine units. It is natural polymer and safe to use due to it biocompatibility, biodegradability and lack of toxicity properties [9].Furthermore,due to its cationic in nature and mucoadhesive property it will enhance the cellular uptake by ionic interaction. Recently it has gained massiveinterest in drug delivery as well as biomedical applications including wound healing ointment and dressings, [10]. artificial membranes [11].contact lenses and bandages [12].

Rivastigmine (RT) is a reversible acetylcholinesterase inhibitor used for the treatment of mild to moderate Alzheimer's and Parkinson's disease [13]. However, because of its poor penetration across BBB, it is often necessary to take higher doses of drug resulting in cholinergic side effects. Furthermore, a particle size below 200nm is also a crucial prerequisite for crossing BBB. Several methods have been developed to prepare polymeric nanoparticles of hydrophilic drugs like nanoprecipitation, emulsion solvent evaporation and ionic gelation methods [14]. Among all the other methods, ionic gelation was chosen for this study because it is a simple method and have more advantages including smaller particle size and higher encapsulation efficiency, as compared to the other methods. Owing to the solubility of RT in both aqueous and organic solvents, its incorporation into NP comes out to be an interesting challenge. Different CS nanoparticles played an important role to cross the BBB including CS-TPP, Trimethylated chitosan, antibody coated chitosan, and alkylglyceryl modified chitosan [15]. Hence the present study was aimed at formulation RTloaded CS-Tpp nanoparticlesby ionic gelation methodand characterized on the basis of physicochemical properties including particle size, PDI, zetapotential,encapsulation efficiency, and in vitro release.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

RT, CS (Medium Mol.Wt, Viscosity of 200 cps) TPPwere purchased from Sigma Aldrich USA, glacial acetic acid, Dialysis membrane with molecular weight cut off of 12,000-14,000 daltons, phosphate buffer (0.1 M, pH 7.4) were obtained from Fishier scientific.

2.2 Preparation of Loaded Nanoparticles

CS nanoparticles were prepared via ionic gelation method with some modifications [16]. Different concentration of CS solutions (0.1%, 0.2%, 0.3%, and 0.4%, w/v) was prepared by dissolving the required amount of CS in 2% v/v glacial acetic acid. TPP solution (0.1% w/v) was

prepared by separately dissolving them in deionized distilled water. 10 mg of RTwill be added to the CS solution under constant magnetic stirring, followed by addition of aqueous TPP solution in a drop wise manner. Then the solution will be kept on constant magnetic stirring for 30 min and sonicated using probe sonicator. The RT loaded CS nanoparticle suspension will be centrifuged at 13,000 rpm and 4ºC for 30 min using Ultracentrifuge to remove excessive amounts of TPP and unencapsulated drug. The pellets will be dispersed in deionised water. Finally, NPs will be lyophilized for 24 h using freeze dryer for storage in powdered form.

2.3 Physiochemical Characterization of Nanoparticles

2.3.1 Determination of particle size and PDI

The average hydrodynamic diameter and polydispersity index (PDI) of the formulated RT loaded CS nanoparticles will be determined by dynamic light scattering (DLS) analysis using Zetasizer (Malvern Instruments, Worcestershire, UK). 1milliliter(ml) of sample of nanoparticles dispersion will be placed in disposable cuvettes for particle size measurements. Each experiment will be conducted in triplicate.

2.3.2 Determination of zeta potential

The mean surface charge (zeta potential) of freshly prepared RT loaded CS nanoparticles were determined by photon correlation spectroscopy (PCS) using ZS-90 Zetasizer (Malvern Instruments, Worcestershire, UK). Each sample was measured in triplicate at 25°C, and the data was reported as mean ± SD.

2.3.3 Encapsulation efficiency

RT loaded CS nanoparticles will be separated from aqueous phase by ultracentrifugation at 13000 rpm and 4°C for 45 minutes. The supernatants will be collected and evaluated for Rivastigmine residue by UV-visible spectrophotometer. The encapsulation efficiency (EE) will be determined indirectly by measurement of the amount of free RT in the supernatant after ultracentrifugation and was calculated according to the following equation:

$$EE = Amount\ of\ total\ drug - Amount\ of\ free\ drug\ in\ supernatant\ x\ \frac{100}{Amount\ of\ total\ drug}$$

2.3.4 *In vitro* release

A modified dialysis method will be used to evaluate the in vitro release of RT loaded CS nanoparticles [17]. 2 ml of nanoparticles suspension (corresponding to 2 mg of drug) will be placed in a dialysis bag (cellophane membrane, molecular weight cut off 10,000–12,000),which will be tied and placed into 20 ml of phosphate buffer (0.1 M, pH 7.4) maintained at 37ºC with continuous magnetic stirring. At selected time intervals, aliquots will be withdrawn from the release medium and replaced with the same amount of phosphate buffer. The sample will be assayed spectrophotometrically for drug [17].

3. RESULTS AND DISCUSSION

In the current study a nanoparticulate system which is composed of hydrophilic polymer chitosan having various advantageswas developed including 1) formulating NP spontaneously by mild agitations 2) formulating NP with positive charge which could enhance the cellular uptake. CS produces low to high positive charge nanoparticles resulting in improving the cellular uptake and mucoadhesive property.

3.1 Conditions for Formation RT-Loaded CS Nanoparticles

CS nanoparticles were prepared by simple ionic gelation method. The mean particle size of CS-TPP nanoparticles (unloaded NPs) ranged from 179.9 ± 3.9 to 506.0 ± 8.1 was increased significantly by increasing the CS concentration from 0.1% to 0.4% w/v, as shown in Table 1. This phenomenon was expected as lower viscosity of lower CS concentration resulted in a better solubility and subsequently, better interaction between CS and cross-linkers, and thus, produced smaller particle size [18,19].

PDI values for these CS nanoparticles ranged from 0.3 to 0.6.The PDIvalues increased as CS concentration varied from 0.1% to 0.4% w/v (Table 1).

Similar increasing trend was observed for the the surface charge of CS-TPP nanoparticles which rangedfrom+30.1±1.0 to +59.7 ± 0.5 mV by varying CS concentrations from 0.1% to 0.4% w/v.

The CS-TPP ratio is rate limiting step and controls the size anddistribution of nanoparticles.In order to obtain nanoparticles under 200 nm, the effect of the CS/TPP ratio on the formation of nanoparticleswas studied. Our results of this study exhibited that particle size depend on both CS and TPP concentration that the specific concentration of CS/TPP can only form the nanoparticles with smaller size.

3.2 Particle Size, PDI and Zeta Potential of RT Loaded CS Nanoparticles

The mean particle size of RT loaded CS nanoparticles was increased significantly by increasing the CS concentration from 0.1% to 0.4% w/v, as shown in Table 2. Moreover, RT loaded CS nanoparticles exhibited decrease in particle size as compared to the unloaded nanoparticles. The decrease in particle size after loading might be due to good interaction with CS and TPP resulting in small particle size. Moreover, no significant difference is observed in PDI after loading nanoparticles with RT.

The results are in accordance with the previously reported study [20] that the zeta potential of RT-loaded CS nanoparticles increased as the concentration of CS was increased because a greater number of excess free positive charges was increasingly available that did not counteract with the negatively charged RT. Moreover, the decrease in zeta potential after RT loading demonstrated the successful association of RT with the nanoparticles.

3.3 Encapsulation Efficiency

RT entrapment efficiency in the range of 65% to 80% was achieved for CS-TPP nanoparticles. In general, the entrapment efficiencies of all formulations increased when CS concentration was increased as shown in Fig. 1.

3.3.1 *In vitro* release

The cumulative percentage release of optimized RT loaded CS nanoparticles (0.2%) was selected for study due to its small particle size and high entrapment efficiency. The release characteristics were studied in phosphate buffer pH 7.4 and shown in Fig. 2. The release profile of RT loaded CS nanoparticles exhibited initial burst release of approximately15% of the drug in 1 hour followed by the sustained release of 40% of drug for following 24 hrs. The observed burst effect was due to the dissociation of drug molecules that were loosely bound to the surface of the CS nanoparticles. The second part of the release was slow and sustained due to the entrapped RT within CS nanoparticles owing to strong binding with the nanoparticles [20].

Table1. Particle size, PDI, and zeta potential of CS-TPP unloaded nanoparticles prepared at different CS concentrations, *n*=3

CS concentration (% w/v)	Tpp concentration(%w/v)	Particle size (nm) ± SD	PDI ± SD	Zeta potential (mV) ± SD
0.1	0.1	179.9 ± 3.9	0.3 ± 0.10	+30.1 ± 1.0
0.2	0.1	272.3 ± 9.0	0.3 ± 0.29	+35.5 ± 0.5
0.3	0.1	342.0 ± 7.0	0.4 ± 0.03	+42.5 ± 1.0
0.4	0.1	506.0 ± 8.1	0.6 ± 0.08	+59.7 ± 0.5

Table 2. Particle size, PDI and zeta potential of RT Loaded CS nanoparticles prepared at different CS concentrations, *n*=3

CS concentration (% w/v)	Particle size (nm) ± SD	PDI ± SD	Zeta potential (mV) ± SD
0.1	125.9 ± 2.5	0.3 ± 0.10	+18.1 ± 0.5
0.2	195.3 ± 6.1	0.3 ± 0.09	+22.5 ± 0.5
0.3	272.0 ± 7.0	0.4 ± 0.03	+27.5 ± 1.0
0.4	356.0 ± 7.9	0.5 ± 0.08	+30.7 ± 0.5

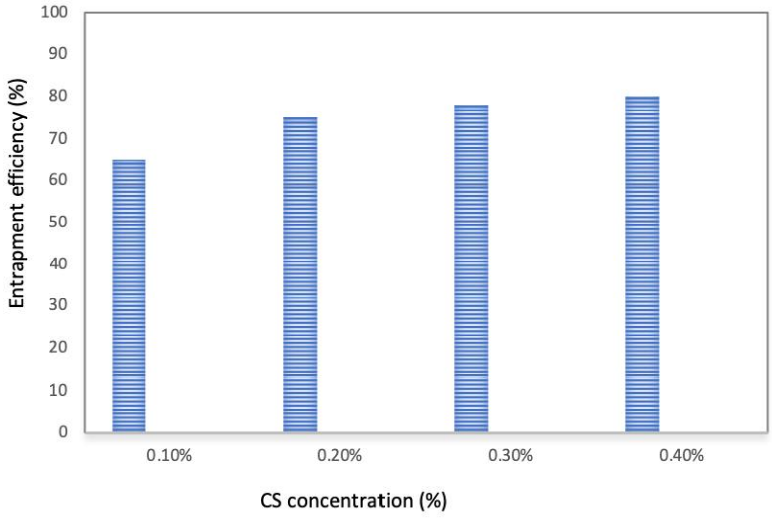

Fig. 1. Graphical representation showing entrapment efficiency against CS concentration

***In vitro* release**

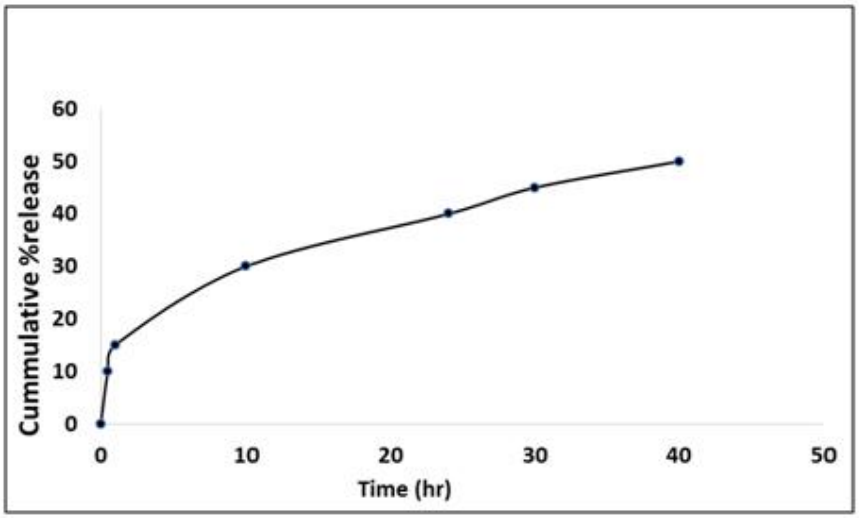

Fig. 2. Graphical representation showing cumulative percentage release

4. CONCLUSION

RT loaded CS nanoparticles were successfully prepared by ionic gelation method. CS nanoparticle loaded with RT showed smallest particle size with 0.1%w/v CS concentration. The entrapment efficiency observed was high and sustained the release of RT from CS nanoparticles. The concentration of CS and TPP alsoaffects the particle size formation of the CS-NP. Further *in vitro* cytotoxicity and *in vivo*evaluationswill confirm the targeting efficiency of CS-NP across blood brain barrier to treat Alzheimer's disease.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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