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Research Article

Preparation and Characterization of Levodopa-Selegiline Loaded Chitosan Nanoparticles

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ABSTRACT

Targeting to brain is a challenging aspect for the treatment of parkison's disease (PD). Chitosan polymer based nanoparticles have been well developed and reported for their brain targeting ability. Levodopa and selegiline are widely used drugs for the treatment of PD. In the present manuscript we have developed and reported levodopa-selegiline loaded chitosan nanoparticles by ion gelation technique. Various formulation variables were optimized to obtained highest drug entrapment efficiency. The optimized formulation (CNP-C3S3P3T2) had 66.02±2.09% entrapment efficiency and 62.18 nm particle size range. The *in vitro* drug release profile of optimized formulation showed that both drugs were released within 2 hrs in PBS pH 5.5 solutions. These results showed that the prepared chitosan nanoparticles have the ability to carry both drugs at a time and can be utilized in the treatment of PD.

INTRODUCTION

Nanoparticles are solid colloidal particles, utilizes for drug delivery, ranging in size from 1 to 1000 nm. A huge number of medications, including antibiotics, anti-neoplastics, and a range of CNS active agents, are unable to cross the blood-brain barrier. The use of nanoparticles to transport medications to the brain across the blood-brain barrier could provide a major advantage over current approaches. The fundamental advantage of nanoparticle carrier technology is that nanoparticles cover the blood-brain barrier, restricting the properties of treatments and drug molecules, as well as minimizing peripheral toxicity by generating gradual drug release in the brain. Physiological factors including phagocytic activity of the reticuloendothelial system and opsonization may restrict the amount of medication transported to the brain [1]. Nanoparticle formulations can be delivered through numerous methods, including parenteral, oral, cutaneous, ophthalmic, pulmonary, and rectal [2].

Chitosan is a natural polymer formed by the deacetylation of chitin. It is a biosafe, non-toxic, biocompatible, and biodegradable polysaccharide. Chitosan nanoparticles have acquired popularity as drug delivery carriers due to their superior stability, low toxicity, simple and mild manufacturing procedure, and capacity to provide many routes of administration. Their submicron size makes them suited for mucosal routes of administration, such as oral, nasal, and ocular mucosa, which are non-invasive. Chitosan

nanoparticles shown efficacy as a vaccination adjuvant [3]. Chitosan molecules are relatively big polymers. High molecular weight (HMW) chitosan can contain more water in a hydrogel than low molecular weight (LMW). Higher molecular weight chitosan can be prepared at lower quantities while maintaining the same viscosity. Even chitosans from the same manufacturer have been found to differ in molecular weight from batch to batch [4].

Parkinson's disease (PD) is a progressive neurodegenerative condition of the extrapyramidal nerve system that impairs skeletal muscle movement and control [5]. Resting tremor, rigidity, and bradykinetic movement are signs of Parkinson's disease, which is caused by dopamine depletion in the corpus striatum [6]. However, dopamine injection is ineffective in treating Parkinson's disease because it cannot pass the blood-brain barrier. However, levodopa, a metabolic precursor of dopamine, crosses the blood-brain barrier and is likely converted to dopamine in the brain. Selegiline inhibits the enzyme monoamine oxidase-B (MAO-B), which breaks down dopamine in the brain, boosting dopamine levels around the synapse region. Selegeline is one of the first medications approved for the treatment of Parkinson's disease, and it was originally thought to have neuroprotective properties. Nasal administration appears to be a good technique to circumvent the hurdles for the blood-brain barrier (BBB), allowing direct medication delivery in the biophase of CNS active substances [7].

The aim of present study is to formulate levodopa and selegiline loaded chitosan nanoparticle. This combination levodopa and selegiline nanoparticles is used to treat symptoms of parkinson's (such as treatment of memory and motor function). Levodopa is converted to dopamine in the brain, helping to control movement and selegiline can also reduce 1/4th levodopa dose, irreversible and selectively inhibits MAO-B inhibitor the oxidative metabolism of dopamine. This approach will maximize drug utilization and thus reduce dose frequency. The present approach will also minimize drug associate side effects by prevention of its absorption at nasal mucosa.

MATERIALS AND METHODS

Materials

Drugs levodopa and selegiline HCl was received as a gift sample from Mylan, Nasik, (Maharashtra) India. Chitosan was purchased from Central Drug House (Mumbai, India). All other chemicals were of analytical reagent grade.

Methods

Preparation of Levodopa and Selegiline HCl loaded chitosan nanoparticle.

Chitosan nanoparticle were prepared by the method reported through Singh et al (2015) with slight modification. In brief chitosan nanoparticle was prepared by ionic gelation method [8]. Levodopa and selegiline HCl loaded CS-NPs were prepared using an ionic gelation method. Determinate weights of chitosan were dissolved in glacial acetic acid 1% (v/v). The drug levodopa and selegiline HCl was added to the above solution under constant magnetic stirring, followed by adding of aqueous tripolyphosphate (TPP) solution in a drop wise mode. Then the solution was kept on constant magnetic stirring for 30 min. The nanoparticle deferment was centrifuged at 13,000 rpm and 4ºC for 30 min using Eppendrof Ultracentrifuge to remove excessive amounts of TPP and unencapsulated Levodopa and Selegiline HCl.

Evaluation Parameters of Noparticles

Shape and Surface morphology

Nanoparticles were suspended in water; a drop was placed on a glass slide, covered with a cover slip and viewed under the optical microscope to examine their size and shape. In order to examine the surface morphology, the formulations were viewed under scanning electron microscope [9].

Size and Size Distribution

Nanoparticles were studied for their size and size distribution using zeta Seizer (Malvern 1 instrument) Nipper, Mohali, Punjab, India. Effect of drug concentration, stirring rate and stirring time on a particle size, shape and size distribution were studied on chitosan nanoparticles [10].

Drug entrapment efficiency

The entrapment efficiency of prepared nanoparticles formulations was observed by centrifugation method [11]. Nanoparticles were centrifuged at 20000 rpm for1 hour at controlled temperature. Above phase obtained as supernatant

containing unentrapped drug was separated and measured by UV spectrophotometer at λmax 260 nm against phosphate buffer (pH 5.5). The remaining entrapped drug in nanoparticles was measured after rupturing the nanoparticles using triton X. The amount of drug entrapped in nanoparticles was determined.

Optimization

There are various formulation variables such as Levodopa and Selegiline concentration, polymer concentration, TPP concentration and process variable. These variables like stirring speed and stirring time, could affect the preparation and properties of nanoparticles were identified and studies [12].

Drug Content

A film of required area (1*1cm) was cut, put this small piece of film into 50ml buffer (*pH* 5.5) and kept for 24hrs. Then the whole solution was ultra sonicated for 15min. After filtration the drug was estimated spectrophotometrically at 232.34nm and the drug content was determined.

***In vitro* drug release**

The *in vitro* release of levodopa and selegiline from optimized nanoparticles formulation (CU-G2) and the amount of drug that was permeated through cellophane membrane using the diffusion apparatus. The donor cell was filled with 300 mg of nanoparticles formulation. The receptor compartment was filled with phosphate buffer *pH* 5.5. The temperature of the receptor compartment was maintained at 37 ± 0.5ºC by magnetic stirrer. The samples were removed at predetermined intervals at 0, 0.5, 10, 15, 30, 40, 50, 60, 1, 2, 3, 4 hours and replaced immediately with equal volume of receptor solution to maintain sink conditions. The removed samples were analyzed levodopa drug release at 280 nm and selegiline drug release at 223nm on UV spectrophotometer.

RESULTS AND DISCUSSION

The FT-IR of provided drug sample was found to be concordance with the reference FT-IR of levodopa and selegiline HCl. From the various drug identification tests, it was found that the drug sample of Levodopa and selegiline HCl was pure. The calibrations curves of Levodopa and selegiline HCl were prepared by UV method in data obtain were subjected to linear regression.

Levodopa and selegiline HCl loaded CS-NPs were successfully prepared by ionic gelation method. Shape and surface morphology of levodopa and selegiline HCl chitosan nanoparticle was observed using SEM. Shape of levodopa and selegiline HCL loaded chitosan nanoparticle was found to be spherical with smooth surface. The average particle size of prepared nanoparticles was found to be around 63.18nm. The SEM image of prepared nanoparticles is depicted in Figure 1. The shape of nanoparticles is spherical in shape with nanometre size range.

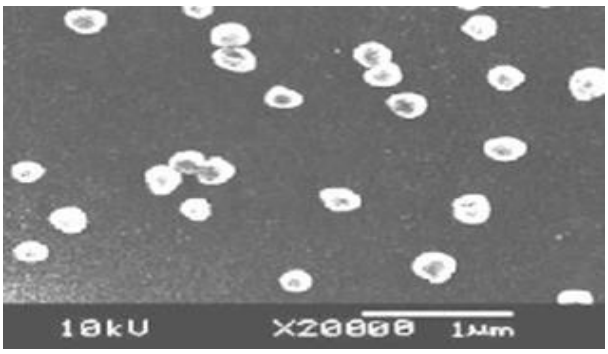

Fig. 1. SEM image of prepared nanoparticles.

The Effects of process variables were observed on final particle size, size distribution and shape of nanoparticles and levodopa and selegiline loading efficiency, which is reported in Tables 1-4 and shown graphically in Figures 2-5. On the basis of and levodopa and selegiline loading efficiency the optimum condition were obtained.

On increasing % chitosan concentration from 0.5% to 2.0%, the entrapment efficiency increased from 62.62±3.07 to 63.78±2.41 while particle size increased from 56.91 to 68.02 nm. Although no significant changes were observed on increasing the % chitosan concentration from 1.5 to 2.0 %. The optimized chitosan concentration was 1.5% observed, with particle size of 64.72 nm and entrapment efficiency 67.42±3.04 %.

Table 1: Optimization of % Chitosan solution with regard to entrapment efficiency & particle size.

Formulation Code	% Chitosan Solution	Conc. of TPP (w/v)	Stirring speed	Stirring time	Entrapment efficiency (%)	Size (nm)
CNP-C1	0.5	0.75	2000	30	62.62±3.07	56.91
CNP-C2	1.0	0.75	2000	30	64.67±2.45	60.21
CNP-C3	**1.5**	**0.75**	**2000**	**30**	**67.42±3.04**	**64.72**
CNP-C4	2.0	0.75	2000	30	63.78±2.41	68.02

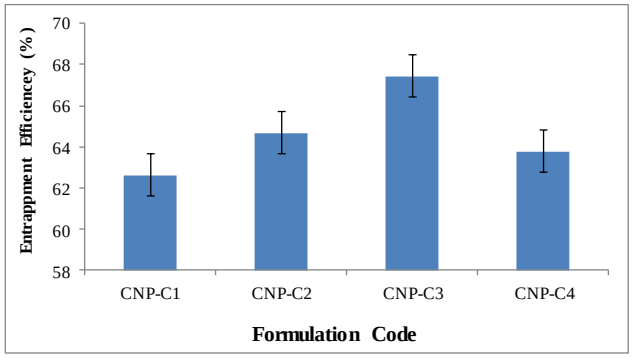

Fig. 2. Effect of % chitosan solution on entrapment efficiency.

Changing the stirring speed with optimized chitosan solution concentration, with regard the particle size and entrapment efficiency was found to be increased the entrapment efficiency of nanoparticle from 56.82±3.37 to 64.94±2.30 % and decreased the particle size of nanoaprticles from 66.29 nm to 57.90 nm respectively, as the stirring speed was increase from 1000 to 2500 rpm. Although entrapment efficiency was decreased from 66.02±2.09 to 64.94±2.30 as on increasing stirring speed from 2000 to 2500 rpm.

Table 2: Optimization of stirring speed with regard to entrapment efficiency & particle size.

Formulation Code	% Chitosan Solution	Conc. of TPP (w/v)	Stirring Speed	Stirring time	Entrapment efficiency (%)	Size (nm)
CNP-S1	1.5	0.75	1000	30	56.82±3.37	66.29
CNP-S2	1.5	0.75	1500	30	63.43±2.44	63.78
CNP-S3	1.5	**0.75**	**2000**	**30**	**66.02±2.09**	**60.34**
CNP-S4	1.5	0.75	2500	30	64.94±2.30	57.90

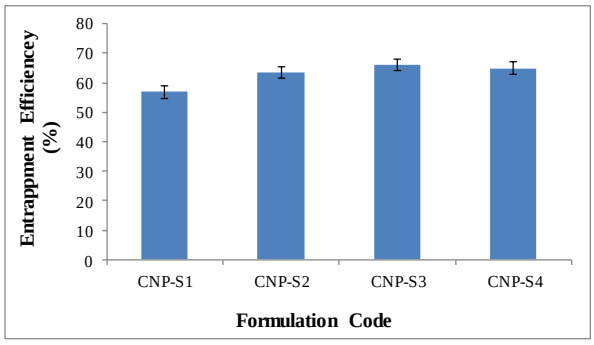

Fig. 3. Effect of stirring speed on entrapment efficiency.

The particle size of prepared chitosan nanoparticles were decreased from 64.19 nm to 56.72 nm, while percent entrapment efficiency increased from 59.52±2.66 % to 60.02±3.08 % respectively, as the TPP concentration was increased from 0.25 to 1.00 %.

Table 3: Optimization of TPP concentration with regard to entrapment efficiency & particle size.

Formulation Code	% Chitosan Solution	Conc. of TPP (w/v)	Stirring speed	Stirring time	Entrapment efficiency (%)	Size (nm)
CNP-P1	1.5	0.25	2000	30	59.52±2.66	64.19
CNP-P2	1.5	0.50	2000	30	62.15±2.27	62.09
CNP-P3	1.5	**0.75**	**2000**	**30**	**60.73±2.83**	**59.01**
CNP-P4	1.5	1.00	2000	30	60.02±3.08	56.72

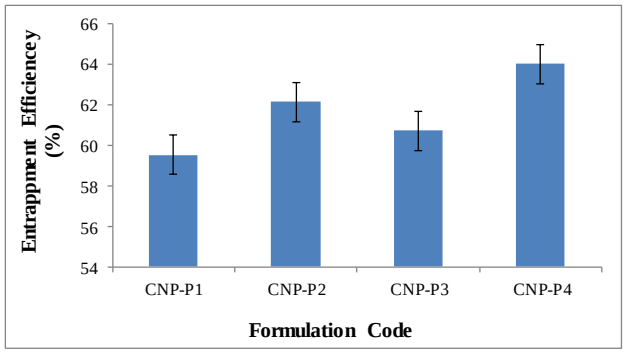

Fig. 4. Effect of TPP con. on entrapment efficiency.

Furthermore, on increasing the stirring time (20-40 min), the entrapment efficiency and particle size both were decreased from 63.12±2.96 to 59.12±3.03 and 66.20 to 61.93 nm.

Optimized condition, entrapment efficiency and particle size for prepared nanoparticles is showed in table 5 (Figure 6).

Table 4: Optimization of stirring time with regard to entrapment efficiency & particle size.

Formulation Code	% Chitosan Solution	Conc. of TPP (w/v)	Stirring speed	Stirring time	Entrapment efficiency (%)	Size (nm)
CNP-T1	1.5	0.25	2000	20	63.12±2.96	66.20
CNP-T2	**1.5**	**0.50**	**2000**	**30**	**62.93±3.38**	**63.89**
CNP-T3	1.5	0.75	2000	40	59.12±3.03	61.93

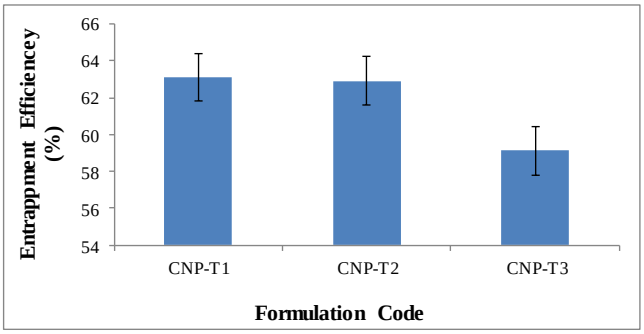

Fig. 5. Effect of stirring time on entrapment efficiency.

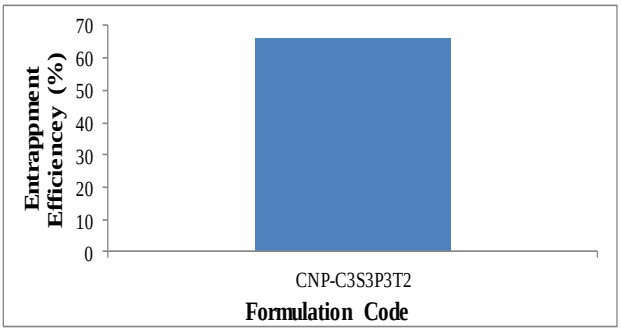

Fig. 6. Optimized formulation with entrapment efficiency.

Table 5: Optimized condition, entrapment efficiency and particle size for prepared nanoparticles.

Formulation Code	% Chitosan Solution	Conc. of TPP (w/v)	Stirring speed	Stirring time	Entrapment efficiency (%)	Size (nm)
CNP-C3S3P3T2	1.5	0.75	2000	30	66.02±2.09	62.18

The drug content in optimized formulation showed in table 6.

Table 6: Drug Content in optimized formulation.

Formulation Code	Drug Content (%)	
	Levodopa	Selegiline HCl
CNP-C3S3P3T2	98.78±0.94	89.84±4.86

The drug content in optimize formulation was found to be within limit and acceptable. The *in vitro* drug release of prepared optimized nanoparticles (CNP-C3S3P3T2) was reported in phosphate buffer of *pH* 5.5 (Table 7). Both drugs showed different *in vitro* drug release rate. However, both drugs were released from formulations within 2 hrs (table 7).

Table 7: *In vitro* % drug release from CNP-C3S3P3T2.

Time (min)	% Drug Release	
	Levodopa	Selegiline HCl
0	0	0
15	10.96±4.67	12.22±0.53
30	28.93±2.10	30.11±1.87
45	46.86±2.09	50.12±1.09
60	61.91±2.88	65.05±1.23
75	74.34±2.24	80.62±2.72
90	93.45±2.29	95.54±2.64
105	97.34±2.57	96.56±2.23
120	96.11±2.01	94.57±1.98

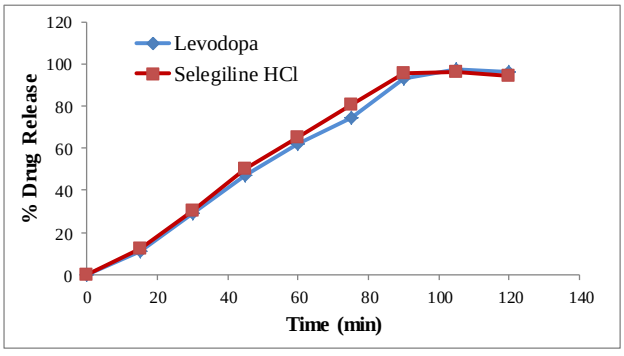

Fig. 7. % Drug Release From nanoparticles in *pH* 5.5.

In vitro drug releases from optimized levodopa and selegiline HCl loaded chitosan nanoparticles were carried out in PBS pH5.5 showed significant release profiles of the levodopa and selegiline HCl from prepared chitosan nanoparticle (Figure 7). The result was show prepared nanoparticles are suitable for brain delivery.

CONCLUSION

In the present research we have reported the dual drug delivery approach for the treatment of PD. Chitosan nanoparticles were prepared successfully by ion gelation technique carrying levodopa-selegiline. The best formulation showed nanosized particle size with good entrapment efficiency. The optimized formulation showed carrying ability of levodopa-selegiline for the treatment of PD.

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None

CONFLICT OF INTEREST

None

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