Simar preet Kaur et al /J. Pharm. Sci. & Res. Vol.3(5), 2011,1227-1232

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Preparation and Characterization of Rivastigmine Loaded Chitosan Nanoparticles

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Abstract

Nanotechnology mediated drug delivery has been reported to enhance the drug efficacy, bioavailability, reduce toxicity and improve patient compliance by targeting the cells and tissues to produce desired pharmacological action. **Aim:** The purpose of the present study was to formulate and evaluate rivastigmine loaded chitosan nanoparticles for sustained release. **Methods:** Rivastigmine is a short acting cholinesterase inhibitors (ChEI). In present study rivastigmine loaded chitosan-tripolyphosphate nanoparticles were prepared by ionic gelation method in five different batches with variable drug/polymer ratios (1:1, 1:2, 1:3, 1:5 and 1:7). **Results:** The prepared nanoparticles were evaluated by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and differential scanning calorimetry (DSC). Further, drug entrapment efficiency and *in vitro* release studies were carried out. Among different ratios studied, 1:3 ratio showed highest drug entrapment efficiency (83.74%). Scanning electron microscopy results revealed that nanoparticles were spherical in shape. Particle size and polydispersity index was analyzed by photon correlation spectroscopy and showed average 258 nm and 0.261 respectively. *In vitro* release studies showed that rivastigmine loaded chitosan nanoparticles were capable of releasing the drug in a sustained manner. **Conclusion:** The experimental results showed the suitability of chitosan nanoparticles as a potential carrier for providing sustained delivery of rivastigmine.

Keywords: Chitosan, Ionic gelation, Nanoparticles, Nanotechnology, Rivastigmine, Sustained release.

INTRODUCTION

Drug delivery to central nervous system is a major menace as multiple cerebral diseases like Alzheimer's, brain tumours, prion diseases are cropping up nowadays. The blood brain barrier (BBB) represents an insurmountable obstacle for a large number of drugs including antibiotics, antineoplastic agents and a variety of central nervous system (CNS) active drugs [1-3]. Currently, nanoparticles are used as drug delivery vehicles to deliver such drugs to brain by infiltrating BBB and these may provide a significant strategy to break this impasse [1, 3]. These drug delivery systems offer numerous advantages over conventional dosage forms, including improved efficacy, reduced toxicity and improved patient compliance. Nanoparticles can also be utilized in the form of carriers in drug delivery [4]. Nanotechnology employs engineered materials or devices with the smallest functional organization on the nanometer scale (1–1000 nm) that are able to interact with biological systems at molecular level. Thus, they may stimulate, respond and interact with target cells and tissues in order to induce desired physiological responses while minimizing undesirable side effects. Furthermore, nanotechnology offers ways to manipulate complex biological systems with greater selectivity and timing than conventional pharmacological approaches [5]. The hydrophilic nanoparticles have received considerable attention to deliver therapeutic agents like peptides, proteins, antigens, oligonucleotides and genes by intravenous, oral and mucosal administration [6]. Nanostructure mediated drug delivery enhances drug bioavailability, improves the timed release of drug molecules, and enables precision drug targeting [7]. They also decrease excessive and unfavourable biodistribution of drug. This leads to delayed drug clearance and retarded drug metabolism, resulting in their prolonged release pattern [8]. It has been observed that the nanoparticles that have reported in literature to cross the epithelium via paracellular transport are greater in number than microspheres [6]. Depending on the method of preparation, nanoparticles, nanospheres, or nanocapsules can be tailored for different properties and release characteristics for the best delivery or encapsulation of the therapeutic agent [9]. The primary advantage of nanoparticle carrier technology is that it can cross BBB entrapping the original characteristics of the therapeutic drug molecule. Furthermore, this system may reduce drug leaching in the brain and decrease peripheral toxicity [1-3, 10-12].

Chitosan is a biocompatible, bioactive, and biodegradable polymer that can be easily engineered. It is widely reported for preparing micro and nanoparticles. Because of its cationic charge, biocompatibility and low toxicity, chitosan has been used as a vehicle system for delivery of genes, proteins (including antibodies) and various categories of drugs [13]. Chitosan was selected for present study because of its recognized mucoadhesive property and ability to enhance the penetration of large molecules across mucosal surface. Chitosan nanoparticles were prepared by the ionotropic gelation process based on the interaction between the negative groups of sodium tripolyphosphate (TPP) and the positively charged amino groups of chitosan. Tripolyphosphate (TPP) was used to prepare chitosan nanoparticles, because it is nontoxic, multivalent and able to form gelate through ionic interaction between positively charged amino groups of chitosan and negatively charged TPP. This process has been well reported for preparation of chitosan nanoparticles for peptides and proteins delivery [6].

Rivastigmine is an anticholinesterase agent having favorable efficacy and safety in patients with Alzheimer type dementia and has been widely used for the treatment of mild to moderate Alzheimer's disease [14, 15]. It inhibits both acetylcholinesterase and butyrylcholinesterase and hence, have additional clinical benefits in patients with dementia related to Alzheimer's disease and recently in Parkinson's disease [16, 17]. But, when administered orally, it has short half-life of 1.5 hours due to hepatic first pass metabolism [17]. Hence, it was chosen as the drug candidate in present work which was designed to overcome the problems of conventional dosage forms and can be used for brain targeting.

MATERIALS AND METHODS

Rivastigmine was provided as a gift sample by Ranbaxy Pvt. Ltd., Gurgaon, India. Chitosan was procured from Indian Sea Foods, Cochin, India. Sodium Tripolyphosphate was obtained from Central Drug House Ltd., New Delhi, India. Dipotassium hydrogen phosphate, potassium dihydrogen orthophosphate, sodium hydroxide pellets (AR) and octanol were obtained from S.D. Fine Chemicals Ltd., Mumbai, India. Methanol (HPLC grade) and Glacial acetic acid were obtained from Ranbaxy Fine Chemicals Ltd., New Delhi, India. Diethyl ether (AR) was purchased from Rankem, New Delhi, India.

Preparation of chitosan nanoparticles

Chitosan nanoparticles were prepared using ionotropic gelation of chitosan with TPP anions. Ionotropic gelation takes place when the positively charged amino groups in chitosan interact with the negatively charged TPP [18]. Chitosan was dissolved in purified water 0.1%, 0.15%, 0.2% and 0.25% w/v. Sodium triploy phosphate (TPP) was also dissolved in purified water at various concentrations to obtain final ratios of chitosan/TPP (8/1, 7/1, 6/1, 5/1 and 4/1 w/w). The nanoparticles were formed spontaneously upon the incorporation of variable amount of the TPP solution into 3 ml of the chitosan solution, with magnetic stirring at room temperature [19].

For the association of rivastigmine to chitosan nanoparticles, rivastigmine was dissolved in purified water and then incorporated into TPP solution. The amount of rivastigmine was added such that various batches were obtained with drug/polymer ratios: 1/1, 1/2, 1/3, 1/5 and 1/7 w/w (Table-1). Nanoparticles were centrifuged at 5000 x g for one hour. Supernatants were discarded and nanoparticles were resuspended in purified water for lyophilization and for *in vitro* characterization in phosphate buffer (pH 7.4) [18]. Further evaluation studies were carried out with lyophilized nanoparticles.

Particle Size and Polydispersity index

Measurement of particle size and polydispersity index was performed by Photon Correlation Spectroscopy (PCS) [20] known as Dynamic Light Scattering using a Zetasizer® 3000 (Malvern Instruments, NIPER, Mohali). All samples were diluted with ultra purified water & measured at 25°C and 90° scattering angle, recorded for 180 s. The mean diameter for each

sample & mean hydrodynamic diameter was generated by cumulative analysis in triplicate.

Surface Charge determination

Nanoparticles were characterized with Zeta potential (ζ) using a Zeta Sizer 4 (Malvern Instruments ltd., Malvern UK) [8]. The measurements were performed using an aqueous dip cell in an automatic mode by placing diluted samples (with ultra-purified water) in the capillary measurement cell and cell position was adjusted.

Morphological Studies

The morphological examination of nanoparticles was performed using transmission electron microscopy (TEM) (Tecnai 20 G2 S TWIN at IIT Roorkee) set at 200 kV by placing an air-dried nanoparticle suspension on copper electron microscopy grids (Formvar filmed) [21].

At structural point of view, the arrangement of components and orientation of molecules within the nanoparticle can determine its behavior and stability. For this purpose scanning electron microscopy (SEM) (Tecnai 20 G2 S TWIN at Punjab University, Chandigarh; set at 200 kV) [22] was employed.

Differential scanning calorimetry (DSC)

DSC studies were performed to understand the behavior of cross-linked chitosan on application of thermal energy. DSC was performed on a DSC-7 (Perkin-Elmer Corp., USA) at a heating rate of 10°C/min in the temperature range of 0-350°C using empty aluminum pan as reference standard [23].

Percentage entrapment efficiency studies

The Entrapment Efficiency (EE%) is also known as Association Efficiency. The drug loaded nanoparticles were centrifuged at a high speed of 3500-4000 rpm for 30 min and the supernatant was assayed for non-bound drug concentration by spectrofluorometer (SL 174, Elico India) [24]. Entrapment efficiency was then calculated as follows:

$$EE\ \% = \frac{\text{Total amount of drug added} - \text{Non-bound drug}}{\text{Total amount of drug added}} \times 100$$

***In vitro* drug release studies**

The drug release profile of nanoparticles was measured *in vitro* [8]. Forty milligrams of lyophilized nanoparticles were dispersed in 100 ml dissolution media consisting of phosphate buffer (pH 7.4) previously equilibrated at 37°C in an incubator shaker at 100 rpm. At definite time interval, 2 ml of the dispersion was withdrawn and replaced with equivalent volume of dissolution medium to maintain the sink conditions. The amount of drug released from the nanoparticles was then analyzed spectrofluorometrically.

Table 1: Composition of Nanoparticles

Batch Code	*Amount of Drug(mg)*	*Amount of Chitosan(mg)*	*Drug-Polymer Ratio*
A	10	10	*1:1*
B	10	20	*1:2*
C	10	30	*1:3*
D	10	50	*1:5*
E	10	70	1:7

A, B, C, D and E represent formulations 1 to 5 respectively.

Table 2: Drug Entrapment Efficiency of Rivastigmine Nanoparticles

Batch (Drug to Polymer ratio)	*Entrapment Efficiency*
A (1:1)	59.63±1.9
B (1:2)	78.65±2.2
C (1:3)	83.74±3.1
D (1:5)	45.19±2.8
E (1:7)	42.2±2.5

Table 3: *In vitro* Release Profile of Rivastigmine nanoparticles

Time (hrs)	*Percent Cumulative Drug Release for Different Batches of Nanoparticles*				
	A	*B*	*C*	*D*	*E*
0	0	0	0	0	0
1	16.46	15.14	14.73	14.92	15.67
2	30.26	28.80	26.46	27.88	28.19
3	40.14	36.29	31.58	33.61	33.26
4	51.19	45.46	37.69	41.11	40.21
5	60.48	52.91	42.65	48.82	49.91
6	68.21	59.48	47.68	56.01	57.24
7	74.63	65.94	54.25	60.49	64.11
8	79.22	73.24	57.91	63.16	69.64
10	88.16	80.21	64.18	67.19	73.93
12	90.31	81.56	67.14	70.38	77.34

Batches A, B, C, D and E represent formulations 1 to 5 respectively.

RESULTS AND DISCUSSION

Nanoparticles were successfully prepared by ionic gelation technique. PCS analysis studies showed the average particle size and polydispersity index of the prepared nanoparticles were 258 nm and 0.261 respectively (Figure-I).

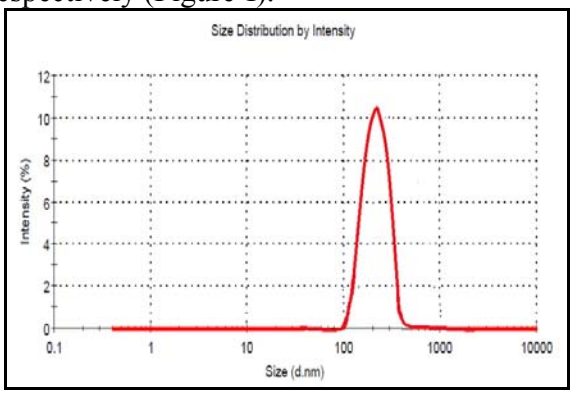

Figure-I: Mean diameter and Polydispersity index of Rivastigmine loaded Chitosan Nanoparticles

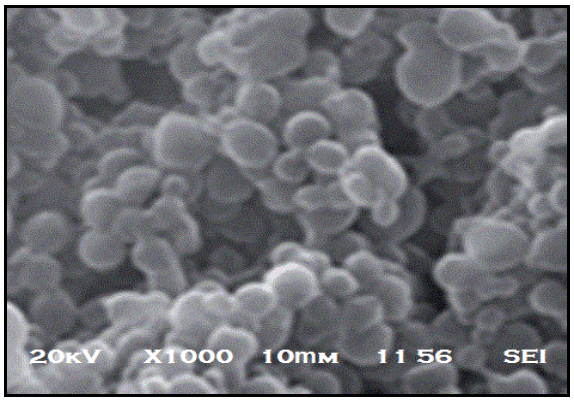

Figure-II (a) SEM image

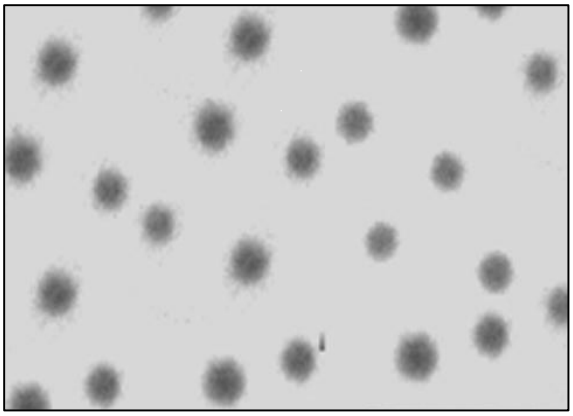

Figure-II (b) TEM image

Figure-2: SEM and TEM images of Rivastigmine loaded Chitosan Nanoparticles.

The SEM photograph (Figure-II a) and TEM photograph (Figure-II b) of rivastigmine nanoparticles indicated that the nanoparticles have a discrete spherical structure. The particle size of rivastigmine chitosan nanoparticles from TEM images accords with that from PCS.

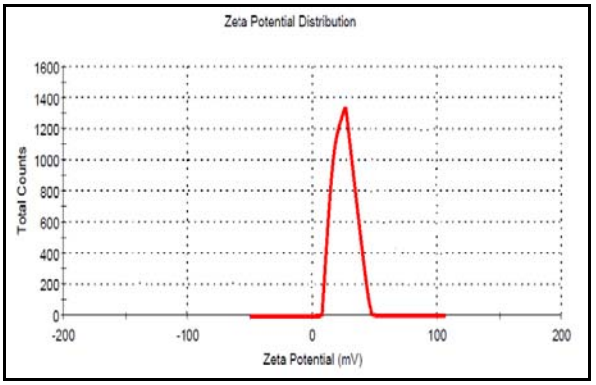

Figure-III: Zeta potential value of Rivastigmine loaded Chitosan Nanoparticles

Further, TEM images showed that rivastigmine chitosan nanoparticles exhibited spherical shape with hairy surface. Zeta potential of the nanoparticles was determined by Malvern zetasizer and it was found to be +35.1mV (Figure-III). DSC is a useful tool to monitor the effect of additives on the thermal behaviour of materials, and used to derive qualitative information about the physicochemical status of drug in nanoparticles [25]. The DSC peaks for rivastigmine sample and nanoparticles were obtained at 122ºC and 124 ºC.

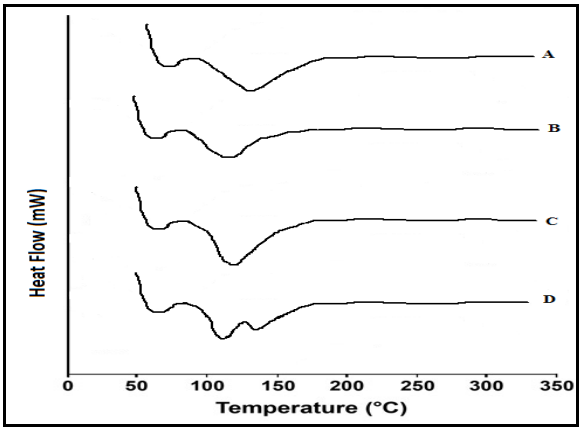

Figure-IV: DSC thermograms of (A) Chitosan (B) Rivastigmine (C) Rivastigmine loaded chitosan nanoparticles (D) Physical mixture of Rivastigmine and Chitosan Nanoparticles.

The physical mixture of drug and nanoparticles (Figure-IV) showed separate characteristic endothermic peaks for drug and nanoparticles. The drug entrapment efficiency of batches A(1:1), B(1:2), C(1:3), D(1:5) and E(1:7) was reported in Table-2 which shows that with increase in proportion of chitosan, entrapment efficiency increases upto drug-polymer ratio 1:3 and above this entrapment efficiency significantly decreases (Figure-V).

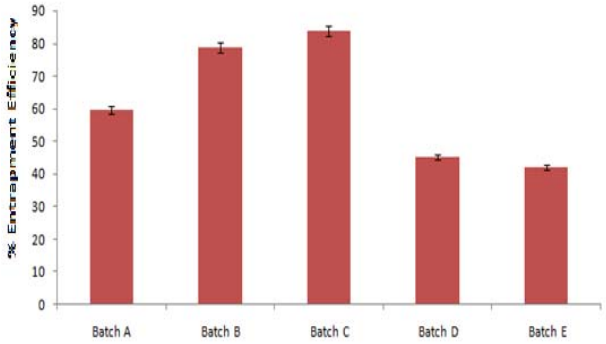

Figure-V: Entrapment efficiency of Rivastigmine loaded Chitosan Nanoparticles. Values represent Mean ± S.D (n=3).

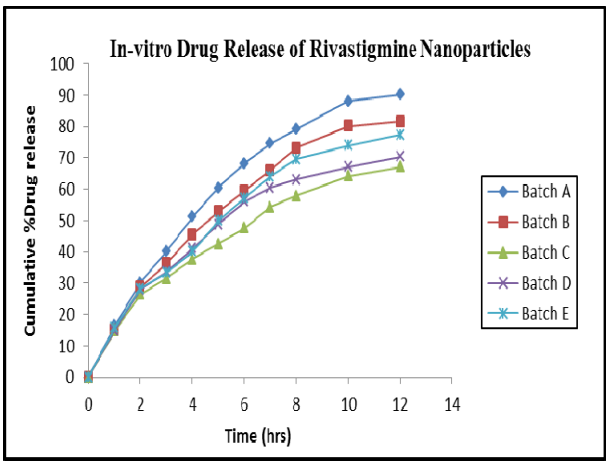

Figure-VI: *In-vitro* release from Rivastigmine Chitosan Nanoparticles

Hence, batch C registered highest entrapment efficiency i.e., 83.74%. The cumulative percentage drug release after 12 hours is reported in Table-3. The cumulative percentage drug release for batches A, B, and E after 12 hours was found more than cumulative release of batches C and D. It was apparent that *in vitro* release of rivastigmine showed a rapid initial burst, followed by a slow drug release. An initial, fast release suggests that some drug was localized on the surface of the nanoparticles. Formulation batch C showed good sustained release as compared to other formulations (Figure-VI).

CONCLUSION

Rivastigmine loaded chitosan nanoparticles were successfully prepared by ionic gelation method. The proportion of chitosan used for formulating these batches of nanoparticles showed significant effect on its efficiency to entrap rivastigmine molecule. Rivastigmine loaded chitosan polymeric nanoparticles with a small size and narrow size distribution were obtained. *In-vitro* release profile showed that rivastigmine loaded chitosan nanoparticles were capable of releasing the drug in a slow sustained manner. The best release pattern was observed in batch C with 1:3 drug polymer ratio. From the present investigation, it may be concluded that rivastigmine loaded chitosan nanoparticles are effective carriers for the design of controlled drug delivery for drugs with short half-life such as rivastigmine and can be further explored for the treatment of Alzheimer's disease and dementia associated with Alzheimer's disease & Parkinson's disease.

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