Colloids and Surfaces A: Physicochem. Eng. Aspects 482 (2015) 34–43

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

journal homepage: www.elsevier.com/locate/colsurfa

Preparation of chitosan–TPP nanoparticles using microengineered membranes – Effect of parameters and encapsulation of tacrine

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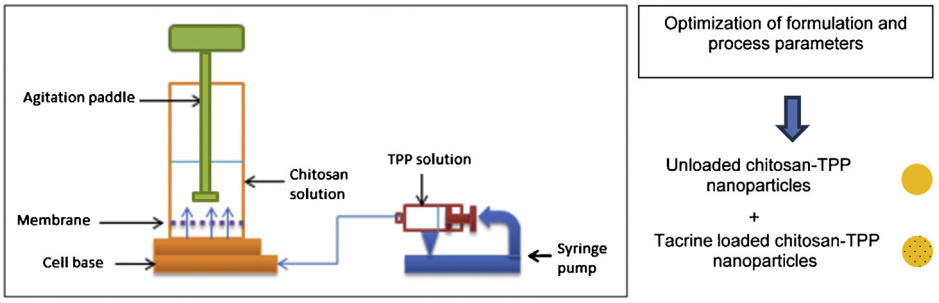
HIGHLIGHTS

- Chitosan nanoparticles were prepared using TPP as a cross-linking agent.
- A membrane was used for micromixing the TTP and chitosan solutions.
- The anti-Alzheimer's drug tacrine was loaded in the chitosan nanoparticles.
- Nanoparticles had a mean size of 90–100 nm and a polydispersity index of 0.22.
- Tacrine encapsulation efficiency was found to equal 66.1%.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 11 March 2015
Received in revised form 10 April 2015
Accepted 12 April 2015
Available online 29 April 2015

Keywords:
Chitosan nanoparticles
Ionic gelation
Membrane emulsification
Membrane contactor
Tacrine
Alzheimer's disease

ABSTRACT

Chitosan nanoparticles were prepared by a novel technique based on ionic gelation using sodium tripolyphosphate (TPP) as cross-linking agent. In this method, the TPP solution flows through the pores of a microengineered membrane into the chitosan solution put in a stirred cell. It is shown that favorable micromixing conditions are created on top of the membrane surface to form chitosan–TPP nanoparticles. The influence of several formulation parameters (chitosan and TPP concentrations, ratio between volumes of the two solutions, pH of the two solutions, chitosan molecular weight) and process parameters (membrane characteristics, injection speed, stirring rate) were investigated. Under optimum conditions, chitosan–TPP nanoparticles had a mean size around 90–100 nm, polydispersity index around 0.22, and zeta potential close to +31 mV. The encapsulation of the anti-Alzheimer's drug tacrine did not change the mean size and polydispersity index of unloaded nanoparticles, whereas the zeta potential was increased to +38 mV due to the positively charge of tacrine. Under optimum conditions, tacrine encapsulation efficiency into nanoparticles was found equal to 66.1%. In addition, chitosan–TPP nanoparticles were shown to be stable at least during 25 days in an acidic medium at 4 or 25 °C. This study demonstrates that ionic gelation using a stirred cell with microengineered membrane is a suitable technique for preparation of chitosan–TPP nanoparticles.

1. Introduction

Many drug delivery systems have been developed from the 1980s to improve drug efficacy and minimize toxic side effects. Nanoparticles are an important research topic in the field of drug delivery, because of their ability to deliver a wide range of active

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http://dx.doi.org/10.1016/j.colsurfa.2015.04.006
0927-7757/

molecules at different levels of the body and for prolonged periods [1–3]. A number of drug delivery systems are based on nanoparticles, for example, those prepared from synthetic hydrophilic polymers. However, many of them are not biodegradable (such as poly (N-isopropyl acrylamine), poly (2-hydroxyethyl methacrylate), polyvinyl alcohol) or have other disadvantages such as local inflammation.

Among other polymers constituting nanoparticles, the natural polymer chitosan, has been used in the preparation of nanoparticles for the vectorization of active ingredients because of many advantages such as biocompatibility, low toxicity and biodegradability by human enzymes [4–7]. Chitosan is a natural polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine. It can be derived by partial deacetylation of chitin from crustacean shells. Various methods have been used to prepare particulate systems of chitosan, including emulsification, cross-linking, coacervation/precipitation, spray-drying, emulsion droplet coalescence method, reverse micellar method, sieving method, and ionic gelation [4]. The ionic gelation method has several advantages such as the use of aqueous solutions, the preparation of small size particles, the control of particle size by the variation of parameters such as chitosan and TPP concentrations, and the possibility of encapsulation of a large range of molecules [4]. In the ionic gelation method, an anionic cross-linking agent, such as sodium tripolyphosphate (TPP), is added to an aqueous solution of chitosan in acetic acid. Positively charged chitosan nanoparticles are formed through the inter- and intra-cross-linking of the amino groups of chitosan with the negatively charged phosphate groups of TPP. The ionic gelation method for the preparation of chitosan–TPP nanoparticles was first reported by Calvo et al. [8]. Since then, the method has been extensively studied, including the effect of several parameters on the particle size distribution and stability of the chitosan–TPP nanoparticles [9]. The particle size distribution depends strongly on the chitosan–TPP mixing procedure and stoichiometry, as well as on chitosan concentration, degree of deacetylation, and molecular weight [10–13]. The nanoparticles size is also sensitive to the ionic strength and pH that the nanoparticles are exposed to [10,14]. A wide variety of drugs, such as bovin serum albumin (BSA) [15], insulin [16], indomethacin [17], and double-stranded small interfering RNA (siRNA) [18] have been encapsulated into chitosan–TPP nanoparticles prepared by the ionic gelation method.

Our new method of preparation uses the ionic gelation method and a membrane contactor for mixing the chitosan and TPP solutions. In a membrane contactor, a microporous membrane is employed as a dispersion media for controlled feeding of one solution into another one to intensify micromixing [19]. The membrane pores act as parallel capillaries for introduction under pressure of one solution into another one. The increasing interest for this technique is due to control of flow, mixing and/or reaction between two phases, and to the fact that the method is easy to scale-up by increasing the membrane surface. The technique has been applied to the preparation of a very large range of colloids including emulsions [20], lipid and polymeric nanoparticles [21,22], albumin nanoparticles [23], nanoemulsions [24], liposomes [25] and micelles [26]. Several membranes have been tested, such as nickel microengineered membranes (Micropores Technologies, UK). These membranes are characterized by a flat smooth surface and low membrane resistance. Their main advantage is the uniformity of the pore size and regularity of the inter-pore distances [20]. A membrane contactor method for chitosan–TPP nanoparticles preparation may provide fine control of size distribution and make easier the extrapolation for an industrial large scale production.

This new method of chitosan–TPP nanoparticles preparation was applied to the encapsulation of tacrine. Tacrine, an acetylcholinesterase inhibitor, was the first molecule proposed for the symptomatic treatment of Alzheimer's disease [3]. Indeed, acetylcholinesterase is an enzyme responsible for the degradation of acetylcholine, a neurotransmitter of the central nervous system involved in memory and learning. Alzheimer is an incurable neurodegenerative disease that causes progressive and irreversible loss of mental function. However, peroral administration of tacrine is associated with low bioavailability, due to an extended first-pass metabolism, short elimination half-life and hepatotoxicity, which are the major reasons for its withdrawal. Hence several alternative routes have been proposed such as transdermal and nasal route for rapid drug delivery to the central nervous system. For example, tacrine hydrochloride nasal delivery has been investigated by means of albumin nanoparticles carrying beta cyclodextrin and two different beta cyclodextrin derivatives [27]. Advantages of intranasal administration include a large surface area for drug absorption, rapid achievement of target drug levels and avoidance of first-pass metabolism. Also, chitosan nanoparticles prepared by spontaneous emulsification [3] and poly(butylcyanoacrylate) nanoparticles [28] have been evaluated after intravenous injection.

The aims of the present study were: (a) to develop a membrane contactor technique for the preparation of chitosan–TPP nanoparticles and optimize the membrane preparation process by studying the influence of various parameters on the characteristics of the prepared nanoparticles; (b) to study the stability of nanoparticles for different storage conditions; (c) to apply the optimized process to the encapsulation of tacrine.

2. Materials and methods

2.1. Materials

2.1.1. Reagents

Three different molecular weight chitosan were obtained from Sigma–Aldrich (France): low molecular weight (LMW) Catalog No. 448869, medium molecular weight (MWM) No. 448877 and high molecular weight (HMW) No. 419419. For all chitosan, the degree of deacetylation given by Sigma–Aldrich was 75–85%. TPP, sodium hydroxide and tacrine (9-amino-1,2,3,4-tetrahydroacridine) were also supplied by Sigma–Aldrich (France). The solvents used (glacial acetic acid and 37% hydrochloric acid) were of analytical grade and were purchased from Carlo Erba Reagents (France). For all experiments, the ultra-pure water used was obtained from a Millipore Synergy® system (Millipore, France).

2.1.2. Experimental device

The chitosan–TPP nanoparticles were prepared using a stirred cell with a flat disk membrane fitted under a paddle blade stirrer. The stirred cell and membranes were provided by Micropore Technologies Ltd. (Hatton, Derbyshire, United Kingdom). The paddle rotation speed was controlled in the range from 200 to 1300 rpm by the applied voltage of a 24 V DC motor (Instek Model 3060, United Kingdom). The membranes used are nickel microengineered membranes, with uniform cylindrical pores in a perfectly ordered hexagonal array with a pore at the center of each hexagonal cell [20]. The membranes with diameters (d_p) of 5, 10 or 20 μm and inter-pore spacing (L) of 80 or 200 μm were used.

2.2. Preparation of chitosan nanoparticles

Chitosan nanoparticles were prepared based on the ionic gelation method reported first by Calvo et al. [8]. This method has been modified using a membrane device through which the anionic aqueous solution of TPP was injected into the aqueous solution of chitosan. A schematic representation of the experimental set-up is shown in Fig. 1.