Journal of Industrial and Engineering Chemistry 62 (2018) 239–249

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Synthesis and characterization of chitosan ascorbate nanoparticles for therapeutic inhibition for cervical cancer and their *in silico* modeling

Vigneshkumar Sekar[a], Karthikeyan Rajendran[a,*], Sugumari Vallinayagam[a], Venkataraman Deepak[b], Surianarayanan Mahadevan[c]

[a] Department of Biotechnology, Mepco Schlenk Engineering College, Sivakasi 626005, Tamil Nadu, India
[b] Department of Biotechnology, Kalasalingam University, Anand Nagar, Krishnankoil 626190, Tamil Nadu, India
[c] Chemical Engineering Department, Central Leather Research Institute (CLRI), Adyar, Chennai 600020, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 22 August 2017
Received in revised form 15 December 2017
Accepted 2 January 2018
Available online 10 January 2018

Keywords:
Chitosan
Ascorbic acid
Chitosan ascorbate nanoparticles
Cytotoxicity
In silico docking

ABSTRACT

This study focuses on synthesis and characterization of chitosan ascorbate nanoparticles (CANs) from a salified carboxylate form of chitosan with ascorbic acid. Evident changes were observed in crystallinity and structure of CANs from their parent polymer. CANs exhibited improved antioxidative properties against DPPH, ABTS, and FRAP. *In vitro* effect of CANs on cervical cancer cells (HeLa) and noncancer human fibroblast cells (WI-38) viability showed portentous toxic effects on cancer cells with reduced viability on increasing CANs concentration. However, no significant effect was observed in the fibroblast cells. Further, *in silico* docking on cervical cancer protein targets also showed efficient binding.

Introduction

Chitosan is a non-toxic natural polysaccharide that is partially or wholly deacetylated from chitin. It is found to be the second most available natural polymer next to cellulose and predominantly extracted from crustacea such as crab and shrimp. Chitin is constituted of 2-acetamido-2-deoxy-β-D-glucose with a β (1→4) linkage falling out as α, β, and γ-chitin polymorph that vary in their intermolecular hydrogen bonding structure [1]. Chitosan is cationic and consists of monomeric glucosamine units with several degrees of N-acetylated residues. The functionalized polymer possess versatile abilities such as biodegradability, biocompatibility, and non-toxicity apart from their anti-oxidative, anti-bacterial, immunoenhancing, anti-inflammatory and anticancer properties [2]. Though advantageous, one issue that hinders its widespread applications is the insolubility in most of the common solvents. To overcome this, chemical modification such as cross-linking, esterification, etherification, and graft polymerization was done on the chitosan chains to the better solubility [3]. These chemically modified chitosan derivatives are converted to functionalized nano and microform so as to engender an intriguing and tractable material for multitude application in biomedical and biopharmaceuticals. In general chitosan and its derivative nanoparticles have wide application in drug delivery, gene delivery, molecular imaging and in some cancer therapy [4–10], which make them as a significant biomaterial of interest. Chitosan derivatized by chemical modification has been shown to improve the functionality of chitosan with specific applications like gene transfer, topical antimicrobial, wound dressing application [11] and detoxification of wastewater [12].

In recent years, intense research activities on the derivatives of chitosan can be seen due to its potential use in cancer drug delivery. Chitosan nanoparticle complexed with sulphobutyl-ether-β-cyclodextrin was used for the delivery of lipophilic drug curcumin for colorectal cancer [13]. Hydrophobically modified glycol chitosan nanoparticles were used as a carrier for water-insoluble camptothecin delivery [14]. Tumor-targeting ability of cisplatin-loaded glycol–chitosan nanoparticles was evaluated in tumor-bearing mice, and its improved antitumor efficacy was observed [15]. Paclitaxel-loaded with O-carboxymethyl chitosan nanoparticles altered using glycyrrhizin elevated internalization levels in liver cancer SMMC-7721 cell to 10.0-fold as compared with the unmodified nanoparticles [16]. Recently, chitosan with surface loaded folate was encapsulated with ursolic acid for

* Corresponding author.
E-mail addresses: Kumarvignesh91phd@mepcoeng.ac.in (V. Sekar), rkarthi@mepcoeng.ac.in (K. Rajendran), Sugumari@mepcoeng.ac.in (S. Vallinayagam), v.deepak@klu.ac.in (V. Deepak), Suri@clri.res.in (S. Mahadevan).

https://doi.org/10.1016/j.jiec.2018.01.001
1226-086X/

enhanced water solubility and improving the acid's targeting and anticancer activity on breast cancer [17]. The nanoparticles exhibited stable targeting that induced significant anticancer efficacy with minimal side effects. Animal experiments performed using 5-fluorouracil loaded chitosan nanoparticle synthesized by ion method exhibited the drug concentration impact on antitumor effect with substantial drug release from the nanoparticles [18]. These studies show the drug delivery ability of the chitosan derivatized nanoparticles in overcoming the implications of conventional methods of drug delivery.

Ascorbic acid is a well-known antioxidant present in cells and plasma of humans [19]. Its beneficial effect and antioxidant properties have been widely studied. The high ascorbic acid dosage intravenously alone has been tested in phase I clinical trials for advanced malignancies. Although the patients well tolerated the levels of ascorbic acid, there was no significance anticancer activity observed [20], which necessitates further research. Ascorbic acid is also desirable for the formation of water-soluble chitosan. Chitosan ascorbate a soluble ketimine formed due to Schiff reactions of chitosan with dehydroascorbic acid. Chitosan salified with ascorbic acid has shown increased permeability and adhesiveness to human epithelial colorectal adenocarcinoma and buccal mucosa [21]. A recent study was reported in the development of sponge-like dressing for the vaginal delivery of hydrophilic high MW drugs like peptides [22]. Chitosan supplemented with ascorbic acid increased the reduction in the apparent fat digestibility for rat fed with high-fat diet [23]. Chitosan ascorbate is also considered useful in wound healing process with potent antibacterial and antioxidative property [24–27].

The properties of chitosan ascorbate have potential to be a compound of interest for many future biomedical applications. To the best of our knowledge, we believe so far there are no reports on the synthesis of nanoparticles for this compound. In view of its unique properties reported earlier with little knowledge about the nano form of this compounds characteristics, the present study aims to investigate the nanoparticle formation, its physiochemical properties, antioxidant efficacy, *in silico* simulation of interaction with possible cervical cancer targets, cytotoxicity and anticancer activity. We hypothesize that this proposed approach would surmount precise toxicity limitations and be a utile choice of material for depot based drug delivery with therapeutic properties.

Materials and methods

Chitosan with a deacetylation degree (DD) of 86.6% and low molecular weight (50–190 kDa), penta-sodium tripolyphosphate (TPP) and L-Ascorbic acid (99%) were purchased from Sigma–Aldrich, India. Rest of the chemical was of analytical grade. All the solutions were prepared in nano pure water.

Synthesis of CANs

CANs were synthesized by ionotropic gelation [28]. 0.1% (w/v) low molecular weight chitosan powder was added to 0.1 M ascorbic acid solution and stirred continuously until a clear solution was obtained. The solution was allowed to stand still for 4 h. This was followed by addition of 0.1% (w/v) TPP and stirring at 700 rpm for 15 min. The solution was then sonicated for 10 min. The obtained solution was then subjected to centrifugation at 12,000 rpm for 30 min at 4 °C. The supernatants were discarded, and pellets obtained were washed three times and filtered using a 0.25 μm membrane filter and resuspended in nano pure water. The obtained solution was freeze-dried without any complex agglomeration by subjecting them to flash freezing in liquid nitrogen and then lyophilized for 6 h. All further analyses were carried out with these lyophilized nanoparticles.

Particle size analysis and morphological characterization

The particle size distribution and polydispersity index (PDI) of the CANs were determined by dynamic light scattering (DLS) in Malvern Zetasizer ZS (Malvern Instruments, Malvern, United Kingdom). A uniform total concentration of 1% was maintained by dispersing an appropriate amount of nanoparticles in nano pure water at 37 °C. The zeta potential values of the nanoparticles were measured by determining electrophoretic mobility with the Malvern ZS equipment. Size and the morphology of synthesized nanomaterials were determined using high-resolution scanning electron microscope (HR-SEM, 15 kV, model 54,160, Hitachi, Japan) and high-resolution transmission electron microscope (LEO920 HR-TEM, Carl Zeiss, Germany).

Moreover, the atomic force microscope (AFM) analysis was performed using scanning probe microscope XE 70 (Park system, Suwan, South Korea). The size of the nanoparticle was measured by AFM in non-contact mode. The CANs 5 mg was dispersed in 20 mL of acetone and coated evenly on a microscopic slide. Then the slide was viewed under scanning probe microscope. The line profile was drawn by using XEI image software. 2D AFM image and 3D AFM image were documented. HR-TEM, AFM images were analyzed with Image J software for size distribution.

Characterization

Lyophilized CANs along with chitosan, ascorbic acid and chitosan ascorbic acid physical mixture were analyzed by Fourier transform infrared spectroscopy (FTIR), solid-state nuclear magnetic resonance (NMR), X-ray diffraction (XRD) and differential scanning calorimetry (DSC) to study its physiochemical characteristics. FTIR spectrum was recorded for all four samples using FTIR 8400S (SHIMADZU, Japan) instrument. Samples were prepared as KBr pellets and were scanned against a blank KBr pellet background at a resolution of 4 cm^{-1} from 4000 to 400 cm^{-1} to analyze the functional modification in CANs. CP/MAS ^{13}C solid-state spectra of the nanoparticles were recorded using 400 MHz Solid-state NMR spectrometer (BRUKER, USA). All sample were analyzed at 25 °C with a frequency of 100 and 79.49 MHz where the powdered samples were kept in a cylindrical rotor and spun at 3 kHz with 2 ms contact time and 5 s repetition time. X-ray diffraction patterns of the CANs were examined to study the nature and atomic structure of the molecule. The diffraction patterns were obtained using an X-ray diffractometer D8 Advance ECO XRD Systems with SSD160 1 D Detector (BRUKER). The voltage was maintained at 40 kV with an intensity of 40 mA. The 2-theta angle was scanned between 3° and 80°, and the counting time was 1.2 s at each angle step (0.05°). Differential scanning calorimetry (DSC) was performed to study the thermal behavior of the nanoparticles using a Pyris™ Diamond DSC (PerkinElmer). An approximate of 2–10 mg of sample was used for the analysis, and it was sealed in aluminum pans. They were scanned from 50 °C to 445 °C at 10 °C/min. The inert atmosphere was sustained by purging nitrogen at a rate of 360 cm^3/minute.

Solubility test

The solubility of CANs synthesized in different concentration of ascorbic acid was dissolved into 10 mL of three different solvents such as 1% acetic acid (pH 3.4), 1% sodium hydrogen carbonate (pH 8.0) and nanopure water (pH 7) [29]. CANs were stirred continuously at 30 °C for 24 h and then centrifuged at 8000 rpm