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Pectin-zinc-chitosan-polyethylene glycol colloidal nano-suspension as a food grade carrier for colon targeted delivery of resveratrol

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ABSTRACT

The aim of the present study was to develop chitosan-zinc-pectinate-polyethylene glycol (PEG) nanoparticles (NPs) for colon-targeted delivery of resveratrol. The effects of pectin:$ZnCl_2$:chitosan (PZnC) % w/v, pH and ionic strength of media, and addition of PEG on the colloidal stability and release behavior of resveratrol from NPs were examined by Zeta potential, particle size analyzer, scanning electron microscopy (SEM), and Fourier transform-infrared (FTIR) methods. The particle size and Zeta potential of PZnC NPs in the ratio of 10:1:3% w/v were 399 ± 18 nm and +25 ± 1 mV, respectively. The addition of PEG to PZnC as a solvent for resveratrol (10% w/v) noticeably decreased the size of NPs to approximately 83 ± 4 nm. More than 63% of the resveratrol was encapsulated into the developed NPs; furthermore, a low amount of resveratrol was released during one month, using simulated juice model (pH = 4) as investigated by High Performance Liquid Chromatography (HPLC) analysis of resveratrol.The remaining resveratrol in NPs (~49%) was released in simulated colon fluid in the presence of pectinase. These NPs can be introduced as a novel platform for successful colon delivery of resveratrol in fruit juice matrix.

1. Introduction

The incidence of chronic intestinal diseases such as ulcerative colitis and cancer is recurrently increasing in the world [1]. *Trans*-resveratrol (3,5,4′-trihydroxystillbene), which is known to have low water solubility and oral bioavailability, has been proven to be capable of retarding or preventing the stages of carcinogenesis in various *in vitro* and *in vivo* experiments [2–5]. With respect to chemo-preventive effect of resveratrol in colon cancer, there is a growing interest in designing multipurpose nanodevices for the enrichment of natural food products such as grape juice [6]. Nano-particulated colon-specific delivery systems can be manipulated to protect resveratrol from oxidation and isomerization, thereby preventing rapid absorption and metabolism [6]. Natural biopolymers such as pectin and chitosan have recently been utilized in nanoparticle-based structures [7–10]. The main criterion for selecting biopolymer for designing delivery systems is the presence of functional groups in the structure of biopolymers that can be conjugated with a bioactive component and aggregated to form a cross-linked dense network to encapsulate a bioactive component [11,12]. The high specific surface area of pectin nanoparticles (NPs), resistance to the enzymes present in the stomach and intestine as well as complete degradation by the colonic bacterial enzymes have made the pectin-based delivery systems the most suitable choice for colon-specific delivery purposes [13,14]. The main disadvantage of pectin-based delivery systems is solubility and swelling properties of pectin in aqueous media, leading to the release of the bioactive component during transit via the upper gastrointestinal tract before entering the colon [14,15]. Chitosan, as a linear polysaccharide with cationic properties, is a suitable choice for amendment of pectin limitation through the formation of polyelectrolyte complexes [16–21]. Chitosan can act as a coating for pectin, provided that the critical factors such as charge-to-charge

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stoichiometry, charge density, pH, ionic strength, polymers concentration and molecular weight are set in appropriate ranges [22–24]. Polyethylene glycol (PEG) is an FDA-approved, non-ionic, hydrophilic polymer that has recently been employed in the formulation of NPs due to the stability improvement of the system during the storage time by its preventing effect on the aggregation of nanoparticles via the steric stabilization [25].

The purpose of the present study was to characterize physicochemical properties, colloidal stability, and release behavior of resveratrol-PEG-loaded pectin: $ZnCl_2$: chitosan (PZnC) system, using particle size and Zeta measurement, Fourier transform infrared spectrometry (FTIR), scanning electron microscopy (SEM), and HPLC. Thus, in this study, we developed a composite of pectin and chitosan in order to design a food grade colloidal nanosuspension for colon delivery systems.

2. Materials and methods

Low molecular-weight chitosan (poly D-glucosamine) with a high degree of deacetylation >75%, high-methoxylated Pectin (HMP) (P9135, from citrus peel, galacturonic acid ≥71%) and PEG (average molecular weights of 4 kDa) were purchased from Sigma company (USA). Sodium chloride, zinc chloride, phosphate and acetate buffer saline, ethanol, methanol, glacial acetic acid, and sodium hydroxide were obtained from Merck Chemical Company (Germany). *Trans*-resveratrol (3,4′,5-trihydroxystillbene, >99% pure, M = 228 g mol^{-1}) and pectinase from *Aspergillus niger* were purchased from Sigma Aldrich (USA).

2.1. Preparation of NPs

The pectin stock solution (1% w/v) was prepared by placing pectin in sterile bi-distilled water and agitating it overnight until complete hydration; thereafter, it was centrifuged at 7000 rpm for 120 min and filtered with 0.45 μm syringe filter (cellulose acetate).The degree of esterification (DE) of pectin was determined, using titration according to the method applied by Bochek et al. [26]. Pectin solution (1.25 mg/mL) was prepared in the presence of different concentrations of NaCl (0, 0.01, 0.03, and 0.05 M). Zinc chloride ($ZnCl_2$) solution (0.018 M = 2.5 mg/mL) was added dropwise to pectin solution at a ratio of 1:10 and stirred for 2 h. Chitosan solutions were prepared by placing 10 mg/mL of chitosan in 3 mg/mL of acetic acid solution for complete dissolution.The solutions were agitated overnight at 40 °C. Pectin: $ZnCl_2$ solution was added dropwise to chitosan solution at a ratio of 10:1, 5:1, 3:1, and 1:1, and the final concentrations of pectin: chitosan were 1.25:0.125, 1.25:0.25, 1.25:0.41, and 1.25:1.25 mg/mL, respectively. The pH was measured at room temperature (25 °C) by pHmeter (Metrohm, Germany). The pH of the solution was found to be ~3.8 ± 0.2. All experiments were performed at room temperature, and the solution was stored in the refrigerator.

2.2. Preparation of resveratrol-Loaded NPs

Resveratrol was dissolved in PEG solution (50 w/v%) and added to the pectin solution.The other stage was repeated as described in Section 2.1. The final concentrations of pectin, $ZnCl_2$, resveratrol, and chitosan in all formulations were kept constant (1.25 mg/mL pectin, 0.125 mg/mL $ZnCl_2$, 0.524 mg/mL resveratrol, and 0.41 mg/mL chitosan). Physically- loaded resveratrol powder at the same concentration was considered as the blank sample. Resveratrol-Loaded NP_S were stored in refrigerator at 4 °C for approximately one month.

2.3. Encapsulation efficiency

Encapsulation efficiency (EE) of resveratrol in PZnC NPs was assessed, using Amicon® centrifugal filter (Millipore, MW cut off 100 kDa) [27]. The dispersion of resveratrol-loaded NPs was placed on the upper compartment of Amicon® tubes and centrifuged at 4000 rpm for 30 min to separate free resveratrol from the lower compartment of Amicon®. A volume of 500 μL of the filtrated solution was removed and mixed with 500 μL of ethanol; in addition, 50 μL of this mixture was injected into HPLC-UV to determine resveratrol concentration. HPLC-UV analysis was carried out on a Knauer (Germany) system equipped with a UV–visible detector (K-2600, Knauer, Germany), a pump (K-1001, Knauer, Germany), and a Knauer injector consisting of a 20 μL loop. The separation was performed on an analytical C_{18} column (5 μm particle diameter, 4.6 mm i.d × 25 cm) (Knauer, Germany) at room temperature. The mobile phase of the methanol/phosphate buffer solution (10 mM) at a ratio of 63/37 (V/V) and the final pH adjustment of 6.8 were utilized in the isocratic mode at a flow rate of 1 mL/min. The peak for t-resveratrol was detected at 306 nm by UV detector, and the calibration curve was provided with resveratrol solution (1–75 μM in ethanol 50% v/v). The EE was calculated, using the following formula [28]:

$$EE\ (\%) = \frac{\text{Total resveratrol - free resveratrol}}{\text{Total resveratrol}} \times 100$$

2.4. In vitro *release*

The *in vitro* cumulative release of resveratrol from the PZnC NPs was evaluated by dialysis bag diffusion technique (cellulose acetate 12 KDa MWCO, Sigma, USA) [29,30]. The behavior of NPs was studied in mimicking transit gastric condition (pH 1.2 for 2 h for stomach) and intestinal condition (pH 6.8 for 3 h for small intestine) to reach colon (pH 7.4 for 2 h) in the presence and absence of pectinase for large intestine [31]. To mimic stomach condition without enzymes under sink condition, 1 mL of zinc pectinate-chitosan containing resveratrol was added to the dialysis bag and suspended in 20 mL of HCl (0.1 M)-ethanol w/v (at a ratio of 80:20). Thereafter, the dialysis bag was suspended in 20 mL of phosphate buffer-ethanol (at a ratio of 80:20) to mimic the small intestine condition. For the simulated colon condition, the buffer was altered (pH 7.4) in the presence and absence of 0.6 mg/mL of pectinase enzyme under stirrer in incubator (50 rpm, 37 °C). Resveratrol content was assayed at the end of the simulated duration. Release behavior in mimicking grape juice pH was assayed by incubating 1 mL of PZnC containing resveratrol inside the dialysis bag and was suspended in 20 mL of acetate buffer. The pH was adjusted at pH 3.5 and 4.5 by 0.1 M acetic acid during one month at 4 °C. Moreover, the sampling process was performed in sequence, and the same volume of fresh incubation medium was replaced after every sampling.

2.5. Fourier transform infrared spectrometry (FT-IR) analysis

FT-IR spectra of pectin and chitosan powder and freeze-dried samples in KBr pellets were recorded on a Bruker-Tensor27 Spectrometer. Interferograms were accumulated over the spectral range of 4000–500 cm^{-1} with nominal resolution of 2 cm^{-1} and 100 scans. The freeze-drying process was performed at the temperature of −30° C and the pressure of 0.07–0.1 mbar for 48 h in a Christ Alpha1-4 (Germany) freeze drier.

2.6. Scanning electron microscopy

The morphology and structure of NPs were visualized, using scanning electron microscope (SEM). Freeze-dried samples were