International Journal of Biological Macromolecules 88 (2016) 171–178

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Encapsulation and release studies of strawberry polyphenols in biodegradable chitosan nanoformulation

Rama Pulicharla[a], Caroline Marques[b], Ratul Kumar Das[a], Tarek Rouissi[a], Satinder Kaur Brar[a,*]

[a] INRS-ETE, Université du Québec, 490, Rue de la Couronne, Québec G1K 9A9, Canada
[b] UEPG, State University of Ponta Grossa, 4748, General Carlos Cavalcante Avenue, Ponta Grossa, PR 84030-900, Brazil

ARTICLE INFO

Article history:
Received 8 February 2016
Received in revised form 18 March 2016
Accepted 18 March 2016
Available online 19 March 2016

Keywords:
Strawberry fruits
Polyphenols
Chitosan
Nanoformulation
Controlled release

ABSTRACT

Polyphenols (negative groups) of strawberry extract interacts with positively protonated amino groups of chitosan which helps in maximum encapsulation. This approach can improve the bioavailability and sustained release of phytochemicals having lower bioavailability. The optimum mass ratio of chitosan-tripolyphosphate and polyphenols (PPs) loading was investigated to be 3:1 and 0.5 mg/ml of strawberry extract, respectively. Prepared nanoformulation were characterized by UV–vis spectroscopy, Fourier transform infrared spectroscopy and scanning electron microscopy. The formed particles size ranged between 300 and 600 nm and polydispersity index (PDI) of ≈ 0.5. The optimized formulation showed encapsulation efficiency of 58.09% at 36.47% of polyphenols loading. Initial burst and continuous release of PPs was observed at pH 7.4 of *in vitro* release studies. PPs release profile at this pH was found to be non-Fickian analomous diffusion and the release was followed first order kinetics. And at pH 1.4, diffusion-controlled Fickian release of PPs was observed.

1. Introduction

Polyphenols (PPs) are largely found in fruits, vegetables, cereals, and beverages derived from plants such as coffee, tea and wine. Based on the studies so far, PPs definitely have ecological roles [1]. These are abundant plant secondary metabolites involved in plant defense; having inherent propensity to donate hydrogen and/or electrons to act as free radical scavengers. Thus, PPs protect the plants from pathogens and ultraviolet radiation. PPs show significant diversity in their structure which influences their mechanism of action and the bioavailability [2]. Further, most of the PPs functions are still unclear. Epidemiological studies on animals support the fact that PPs have inverse relation with cardiovascular or cancer diseases. Even though our diet contains high concentration of polyphenols, it is not necessary that all PPs would be absorbed within the body; as they have lower solubility and absorption, higher metabolism, or rapid elimination kinetics. And the health effects of PPs are highly variable which depend on their intake and bioavailability [1,3]. Strawberry fruits were ranked 7th among the fruits and 33rd among the foods containing rich dietary sources of polyphenols [4]. Ellagitannins and ellagic acid are the main PPs in strawberry fruits showing positive health effects, such as antioxidant, anti-carcinogenic, anti-inflammatory and anti-neurogenerative effects [5,6]. Studies on the bioavailability of these PPs in animals proved that intestinal microbiota played an important role to enhance the absorption in the form of derivatives of urolithins [7]. This rate limiting step of absorption of PPs can be overcome by encapsulating them in a biodegradable polymer to enhance the permeability across the intestine.

Chitosan is a biocompatible, biodegradable and naturally occurring biopolymer. It is a deacetylated form of chitin comprising glucosamine and N-acetyl glucosamine units. This biopolymer shows diverse biological properties, including mucoadhesion [8], antibacterial, immunostimulating [9], wound healing properties and antitumor effects [10]. Chitosan shell has been investigated as potential nano/submicron carrier system for controlled/sustainable release of drug. As they are smaller, they are capable of crossing the biological barrier. Being versatile in nature, chitosan can encapsulate both lipophilic and hydrophilic compounds and is widely used in biomedical and pharmaceutical applications. In recent study of Khan et al. [11], the encapsulated green tea polyphenols in chitosan nanoformulation have shown increased bioavailability and controlled release to inhibit the cancer cell growth.

* Corresponding author.
E-mail address: satinder.brar@ete.inrs.ca (S.K. Brar).

http://dx.doi.org/10.1016/j.ijbiomac.2016.03.036
0141-8130/

Encapsulating technique has been successfully applied as a promising delivery system for many plant-derived PP [11]. However, potential systemic usages of PPs have been plagued by poor bioavailability. Food industries are now targeting to improve the stability, bioavailability and slow release of bioactive phytocompounds. In this context, nanoencapsulation provide smart tolls for prolonged effect of PPs at specific targeted sites. Strawberries are a good source of antioxidants such as ascorbic acid, anthocyanins, tanins, and flavonols with highest radical absorbance capacity [12]. Marketability of strawberries has limited due to its short life. This limitation can be overcome by incorporating the polymer encapsulation process to strawberry extract for extended shelf life. For such encapsulation chitosan can serve as a carrier material for PPs due to its permeation enhancing properties [13].

The present work relates to the preparation and characterization of PPs loaded chitosan particles by ionic gelation and their sustained release. PPs were extracted from strawberry fruits and PPs loaded chitosan nanoformulation was developed to overcome the pharmacokinetic challenges of PPs. Optimization of PPs loading, characterization of the particles and *in vitro* PPs release behavior was also studied.

2. Materials and methods

2.1. Materials

Fresh strawberries were purchased from local market (Farmer's market, Quebec, Canada). High molecular weight chitosan (600–800KDa), sodium tripolyphosphate (TPP), acetone (99.7%) and acetic acid (99.7%) were purchased from Fisher scientific (Ontario, Canada). Phosphate buffer saline (PBS) and other reagents used were of analytical grade.

2.2. Methods

2.2.1. Extraction of polyphenols

Fresh strawberry fruits were washed and cut into small pieces. They were soaked overnight in 70% of acetone and acetone and water (70:30) were used as PPs extracting solvent. The soaked material was ground, filtered and the filtrates were dried at room temperature for 3 days. The dried filtrate was frozen at $-20 \pm 1\,^{\circ}C$ for 12 h and later lyophilized (Dura Frezz Dryer, Kinetics). The resulting powdered strawberry fruit extract was stored in a desiccator at room temperature [14,15].

2.2.2. Determination of polyphenols

Total polyphenols were determined by Folin Ciocalteu (F-C) method using gallic acid as standard [16,17]. About 10 mg of gallic acid was dissolved in 10 ml of 10% methanol (10 mg/ml) and further diluted to plot a calibration curve ranging from 10 to 100 μg/ml. About 0.5 ml of diluted extract or standard samples were mixed with 2 ml of Folin-Ciocalteu reagent (1 ml in 10 ml of water) and allowed to stand at room temperature for 2 min. Further, to this solution, 2 ml of sodium carbonate solution (7.5% w/v) was added and the mixture was then incubated at room temperature for 1 h. Absorbance was measured at 765 nm using UV–vis spectrophotometer (Varian Carey 50) against water. The total concentration of polyphenols was expressed as gallic acid equivalents (GAE) in g/100 g material. (Correlation coefficient: $R^2 = 0.9992$).

2.2.3. Preparation of PPs loaded nanoparticle formulation

About 0.1% w/v of chitosan dissolved in 0.1% acetic acid was used throughout the studies. The nanoparticles were synthesized by initial drop wise addition of PPs to the high-molecular-weight chitosan solution under magnetic stirring. After 5 min of PPs addition, drop wise TPP (1% w/v TPP in milliQ water) was added to the chitosan-PPs solution under magnetic stirring (700 rpm, 3 h), pH 4.0 at room temperature. In the first step, chitosan-TPP mass ratio has been optimized from 1:1 to 5:1 at constant PPs concentration of 0.5 mg/ml. Where, in the second step, optimized ratio of chitosan-TPP was used to enhance PPs loading by increasing the concentration of PPs from 0.25 to 2 mg/L. Formed nanoparticles were separated by centrifugation at 47,815 *g* for 30 min and washed twice in milliQ water and freeze dried and further lyophilized.

2.2.4. Characterization of chitosan nanoparticles

Particle size, size distribution and zeta potential of dispersed solution was measured by dynamic light scattering technique using a Zetasizer (Malvern Instruments, US). The binding of chitosan functional groups to PPs of strawberry extract was analyzed by FTIR spectroscopy in transmission mode (Cary 670 FTIR Spectrometer). FTIR measurements identify the possible characteristic functional groups of molecules responsible for stabilization of nanoparticles. Measurements were carried out by forming thin and transparent KBr pellets of prepared NPs. Spectra was operated at a resolution of 4 cm^{-1} in the range of 4000–400 cm^{-1} with 100 scans.

Morphology and size of prepared nanoparticles were studied by scanning electron microscopy (SEM) images, by using Carl Zeiss EVO® 50 instrument at the required magnification. The SEM samples were prepared by placing very small drops of the NPs diluted solution on the aluminum foil and dried at room temperature. And further, this dried aluminum foil was mounted on the SEM stub and then coated with gold metal using a sputter coater. For previously mentioned experiments prior to analysis, lyophilized nanoparticles were dispersed in water by sonication for 5 min at room temperature using a sonication bath (Fisher, FB 15069).

2.2.5. Evaluation of PPs loading efficiency

The encapsulation efficiency (EE) and loading capacity (LC) of PPs was determined by separating chitosan nanoparticles by pelletization using ultracentrifugation at 47,815 *g* for 30 min. The amount of free PPs in the supernatant was determined by UV spectrophotometer at an absorption maximum of 765 nm. EE and LC have been calculated by correlating the PPs content in supernatant with the standard calibration curve.

EE is the ratio of amount of PPs present in the nanoparticles to the amount of drug used in the preparation and is given by Eq. (1):

$$EE = \frac{Total\ PPs - Free\ PPs}{Total\ PPs} \times 100 \quad (1)$$

LC was also calculated with respect to the yield of nanoparticles gained after centrifugation using Eq. (2):

$$LC = \frac{Total\ amount\ of\ drug\ entrapped\ in\ the\ pellet}{Nanoparticles\ weight} \times 100 \quad (2)$$

2.2.6. In vitro PPs release studies

In vitro drug release profile of nanoparticles was carried out for optimized PPs loaded chitosan-TPP nanoparticles using direct dispersion method as explained by Jain et al. [18]. *In vitro* release studies were performed at three different pH (1.4, 7.4 and 10.4) to determine the effect of pH on drug release. A known quantity of PPs loaded nanoparticles were dispersed in 30 ml of hydrochloric acid and PBS at various pH and incubated in a water bath shaker at $37\,^{\circ}C \pm 0.5\,^{\circ}C$, 120 rpm. At pre-determined time intervals (15 min, 30 min, 2, 4, 6, 8, 10, 24 and 48 h), 1 ml of aliquots were withdrawn followed by replacement of the withdrawn volume by fresh buffer to operate release studies under sink conditions. The withdrawn aliquots were centrifuged at 17 213*g* for 10 min and the released PPs into the supernatant was monitored by UV spectrophotome-