Co encapsulated resveratrol and quercetin loaded peg biology essay

Science, Biology

ABSTRACT

Chitosan modified with polyethylene glycol (PEG) nanoparticles prepared by ionic gelation method as merely modify for improving delivery of polyphenols. Resveratrol and quercetin as a model compound because it has been suggested that most potent plant derived polyphenols. But these two drugs have poor solubility, intestinal degradation by bacteria and quick first pass metabolism. Drugs loaded PEG modified chitosan nanoparticles might improve the above said problems and improve the bioavailability. Encapsulation of the drugs confirmed by differential scanning calorimetry, X-ray powder diffractometry, Fourier transformed infrared spectroscopy, Ultraviolet-Visible spectrum. Morphology of the formulation was characterized by Tanning electron microscopy. In-vitro release of nanoparticles shows an initial burst release followed by a sustained release. PEG modified approach having long circulating efficiency that is confirmed by lower protein absorption with bovine serum albumin. Antioxidant activity of combined resveratrol and quercetin nanoparticles is higher rather than individual, which is confirmed using in vitro methods (free radical scavenging and reducing ability). Key words: Resveratrol; Quercetin; Chitosan; PEG; ionic gelation;

Introduction

Polyphenols are " bioactive compounds" that gained a lot of importance due to its variety of biological effects relevance to numerous area of health care. Because of their disease-preventing property and therapeutic usefulness in multiple biological effects, it focused on various bio-field of research. Among

them resveratrol (RES) and quercetin (QUR) are the most attracting and well beneficial molecules [1-5]. RES (3, 5, 4′-trihydroxy-trans-stilbene) present in many plants such as grapes, berries, nuts and red wine etc,. It has powerful antioxidant properties that persuade the anti-aging properties [6-8], anti-inflammatory [9-11], cardio protective [12-15], neuroprotective [16-20] and antitumor activities [21-24]. At the same point of view QUR (3, 5, 7, 3 and 4 - pentahydroxyflavanone) also one of the most prevalent flavonoids present in health food or herbal products with numerous health benefits [25]. It has been shown to have a variety of biological activities and pharmacological actions, such as dilating coronary arteries [26], decreasing blood lipid [27], anti-inflammation [28], anti-aging [29-30] and anti-tumour activities [31-32]. The synergism between QUR and RES may have potential clinical implications in the various studies [33-35]. Recent focuses on co-encapsulation of the molecules are not much attention. RES is very potent drug, it is focused various area of research and co-treat with QUR; it reduces the bioavailability issues and might double the effect of RESChitosan nanoparticles (NPs) can be prepared by the ionic gelation method using TPP as a cross linking agent [36-37]. Ionic gelation method is advantageous over other methods attributed to its mild conditions achieved without applying harmful organic solvent, heat or vigorous agitation that are destruct the sensitive compounds [38]. Delivery of polyphenols with chitosan NPs for improves their biological and pharmacokinetic property due to its mucous adhesive, non-toxic, biocompatible and biodegradable properties. Chitosan matrix to protect the active compounds from the processing condition and the benefits of encapsulating active agents in a chitosan matrix include their

protection from the surrounding medium or their release pattern [39]. Although some blends of polyethylene glycol (PEG) with chitosan NPs have been prepared by incorporation of PEG in the chitosan TPP linkage [40-41]. Both chitosan and PEG are biocompatible and nontoxic; these polymers have considerable importance in many fields, such as drug delivery, biotechnology and biomaterials [42]. PEG is an amphiphilic in nature and it has approved by FDA for human intravenous, oral and dermal applications. Addition of PEG in the therapeutic product has been improving immunological profile by reducing the ability of the compound to raise antibodies in humans [43]. Based on the findings, the current study was carried out to determine the drug loading, in vitro drug release, drug polymer interaction and antioxidant studies of the combination of these two compounds in nanoparticles (NPs) would cause an enhanced synergistic research in the various area compared to either compound itself.

Experimental

Materials

Resveratrol gift sample from Safetabe life sciences (Pondicherry), Quercetin dihydrate, 2, 2-diphenyl-1-picrylhydrazyl hydrate and bovine serum albumin was purchased from Sigma (Sigma-Aldrich, St. Louis), Chitosan from Cognis (Cognis, Mumbai, India), PEG 4000, PEG 6000, PEG 8000, other reagents and solvents were analytical grade. The solutions were prepared using double distilled water.

Methods

Preparation of RES and QUR-loaded PEG modified chitosan NPs.

Based on the Calvo et al ionic gelation method chitosan NPs were prepared by the slightly modification with TPP anions. Chitosan (1 mg/ml) was dissolved in 20 ml of acetic aqueous solution. Chitosan NPs are formed by drop wise addition of tripolyphosphate (TPP, 1mg/ml) aqueous solution into chitosan solution under magnetic stirring at room temperature. PEG-modified chitosan NPs were formed spontaneously upon incorporation various concentration of PEG (2. 5, 5, 7. 5 mg/ml) with various molecular weight (PEG 4000, PEG 6000 and PEG 8000) into TPP solution. Then the TPP containing PEG solution are added drop by drop into the acetic aqueous solution of chitosan, it form a PEG modified chitosan NPs. For the association of RES and QUR into the PEG modified NPs, dissolve (1mg/ml) of RES and QUR in acetic aqueous solution are added into chitosan solution. PEG containing TPP solution is added into chitosan solution containing RES and QUR under magnetic stirring at room temperature. The opalescent suspension was formed under the same above mentioned conditions. They were separated by centrifugation [44-45] and the empty NPs are prepared without drugs. Formulation conditions are given in Table 1.

S. No

Formulation code

Chitosan : TPP in mg

PEG in mg

QUR: RES in mg

1FQR20: 20

10: 102FQR1PEG 4000 501001503FQR24FQR35FQR4PEG 6000

501001506FQR57FQR68FQR7PEG 8000 501001509FQR810FQR9

Table 1. Formulation conditions of PEG modified chitosan NPs contain drugs.

Characterisation

Particle size (PS) and polydispersity index

The mean PS and polydispersity index of the drug loaded all formulation and empty NPs were determined by zetasizer (Malvern Instruments Ltd). The samples were diluted 10-times with distilled water for analysis. The results are showed as mean ± standard distribution. Polydispersity index of the formulation or SPAN factor (range of size distribution) was calculated by using following equation, Polydispersity index or SPAN = D0. 9, D0. 5 and D0. 1 are the particle diameters determined respectively at the 90th, 50th, and 10th percentile of undersized particles.

Drug loading (DL) and encapsulation efficiency (EE)

The RES and QUR content of chitosan-PEG NPs was determined for all the formulations were accurately weighed and then dissolved with acetic aqueous solution then the solution was diluted with freshly prepared PBS. The absorbance was measured at 320 and 370nm by UV–Vis spectrophotometry [46] and equivalent concentration was determined using the calibration curve prepared using the same proportion of solvents. The percentage (%) DL and % EE of the blend were calculated using the following formula.% DL =% Theoretical loading =% EE =

In vitro drug release

Drug loaded formulations prepared using chitosan and PEG polymers were tested for in vitro release of RES and QUR with phosphate buffered saline (PBS, pH 7. 4). Weighed 10mg of NPs in 2 ml PBS dispersed in a dialysis bag (12-14 kDa cut-off, Himedia Labs, India). This dialysis bag was placed in a 40ml PBS containing beaker under magnetic stirring in 600rpm at room temperature. At the specific time interval 2ml of samples were withdrawn and replace the same amount of fresh PBS. The aliquots were analysed for the concentration of RES and QUR released by spectrophotometry at 320 and 370 nm respectively.

Transmission electron microscopy (TEM)

The morphology of the RES and QUR loaded chitosan NPs and PEG modified chitosan NPs was examined by a TEM (Philips EM430 Transmission electron microscopy, USA). Before analyzing TEM, NPs were fixed on copper grids with 0. 5% (w/v) phosphotungstic acid staining.

Fourier-transformed infrared spectroscopy (FTIR)

FTIR spectra of chitosan, PEG, RES, QUR and loaded formulation were recorded from 4000 to 400 cm−1 on a FTIR spectrometer (Affinity 1, Shimadzu, Japan) using potassium bromide pellets with 32 scans and resolution of 4 cm−1.

Powder X-Ray Diffraction (PXRD)

PXRD of chitosan, PEG, RES, QUR and formulation were obtained with JEOL, JDX 8030 using Ni filtered Cu K-alpha a radiation (40 kV, 20mA) to determine the crystallinity of the drug, before formulation and after formulation. All these experimental works were done at room temperature.

Differential scanning calorimetry (DSC)

The thermal properties of the pure polymers, drugs and formulated NPs were characterized by DSC analysis (TA Instruments) with refrigerated cooling. The purge gas was purified nitrogen at a pressure of 20 psi. For pure polymers, drugs and weighed NPs were sealed in crimped aluminium pans with lids using an empty pan as a reference and were heated at a rate of 10°C/min from 0 to 300°C.

Protein adsorption studies

Determine protein adsorption of NPs, chitosan and PEG modified NPs were incubated with 1 ml (400µg/ml) bovine serum albumin (BSA) and stirred vigorously with a magnetic stirrer for 2 h at 37°C. Centrifuge the protein containing NPs to discard the unabsorbed BSA and dilute the particles. Then

analyse the surface charges of NPs before and after incubated with protein using Zetasizer [47].

In vitro anti oxidant study

2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) free radical scavenging activity

DPPH (0. 3Mm) was incubated with 2. 0mL of original RES, QUR and NPs at various concentrations at 37°C. The absorbance was measured, after 30 min of incubation at 517nm using a UV-Vis spectrophotometer. The percentage inhibition of the experimental samples was evaluated by comparing the absorbance values of control and test samples. It was calculated using the formula: Scavenging effect (%) =

Measurement of the reductive ability

Formulated NPs in 3. 5mL phosphate buffer (0. 2 M, pH 6. 6) were mixed with 1wt % K3 [Fe (CN) 6] solution and subsequently incubated at 50°C for 20 min thereafter centrifuged at 1560 g for 10 min. Separate the supernatant, mixed with equal volume of distilled water and aqueous FeCl3 (0. 1% w/v) solution and then the absorbance was measured at 700nm using UV-Vis spectrophotometer. Increasing absorption directly proportional to the reducing the ability of the samples [48].