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A comparison of eugenol and menthol on encapsulation characteristics with water-soluble quaternized β-cyclodextrin grafted chitosan

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ABSTRACT

Two guest molecules (eugenol and (−)-menthol) were investigated on inclusion complex formation with water-soluble quaternized β-CD grafted with chitosan (QCD-g-CS). The inclusion complexes were prepared at varying mole ratios between eugenol or (−)-menthol and β-CD (substituted on QCD-g-CS) by a conventional shaking method and obtained as solid powder by freeze-drying process. The results showed that encapsulation efficiency %EE decreased with increasing of initial eugenol or (−)-menthol loading whereas %loading increased with increasing of initial eugenol or (−)-menthol loading. The results indicated that inclusion complex formation between eugenol and QCD-g-CS was more favorable than that of (−)-menthol. To clarify this mechanism, molecular dynamics simulations were performed to explore their binding energy, solvation energy and total free energy of those complexes. It was found that the total free energy (ΔG) of eugenol and (−)-menthol against QCD-g-CS (mole ratio of 1) in water-explicit system were −2108.91 kJ/mol and −344.45 kJ/mol, respectively. Moreover, molecular dynamic simulation of eugenol absorbed on surface QCD-g-CS (−205.73 kJ/mol) was shown to have a higher negative value than that of (−)-menthol on QCD-gCS (3182.31 kJ/mol). Furthermore, the release characteristics of the encapsulated powder were also investigated in simulated saliva pH 6.8 at 32 °C. The results suggested that (−)-menthol had higher release rate from the complexes than eugenol. In all cases, the release characteristics for those guest molecules could be characterized by the limited-diffusion kinetics.

1. Introduction

Cyclodextrins (CDs) are well-known macrocyclic oligosaccharides that produced by enzymatically degradation of starch. CDs are most commonly built of 6, 7 and 8 glucopyranose unit calling α-CD, β-CD and γ-CD, respectively, in which their conformations resemble hydrophobic cavity and hydrophilic outer surface [4,22]. In recent years, CDs have been widely used as a promising approach to improve solubility of several non-polar molecules [14,18,26,32,34]. The inclusion complexes can enhance their solubility, dissolution rate, bioavailability, stability as well as controlled release [5,21,33]. Moreover, in some cases β-CD can form self-assembly at very low concentrations (18.5 mg/L) [13,24], which is not occurred in case of α-CD and γ-CD, resulting in higher encapsulation efficiency.

Chitosan (CS) obtained by alkaline deacetylation of chitin is one of natural popular biopolymers due to its non-toxic, biocompatible, biodegradable and mucoadhesivity [10,27,30]. It is a linear polysaccharide of randomly distributed glucosamine and *N*-acetylglucosamine units linked by β-(1–4) glycosidic bonds [17,40]. Chitosan has been extensively used for drug delivery [30], gene delivery [3,30] as well as for cancer treatment [41]. Both beneficial properties of β-CD and CS lead to continuously growing interest in grafting of β-CD onto CS backbone [31,44]. We previously reported the synthesis of water-soluble quaternized β-CD grafted with CS

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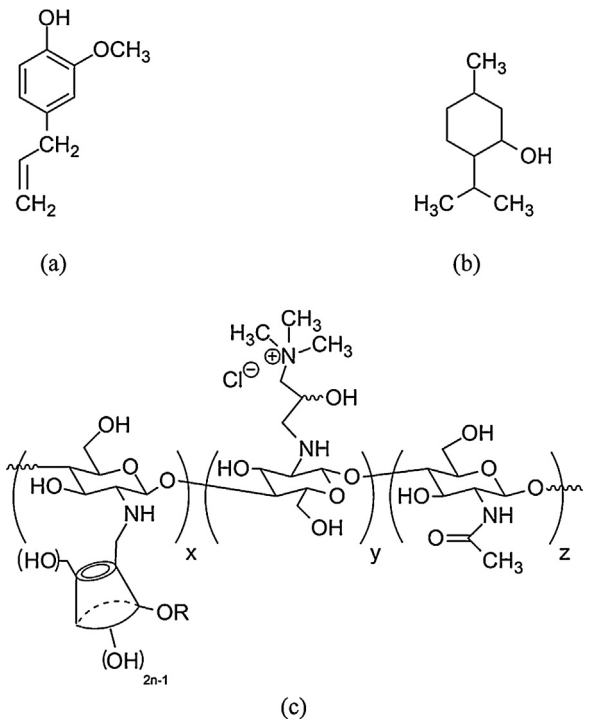

Fig. 1. Chemical structures of (a) eugenol, (b) (−)-menthol and (c) QCD-g-CS (n = 7, R = Tosyl or H, x = 11, y = 80, Z = 9).

(QCD-g-CS) [12,37–39]) to improve inclusion complex entrapment, sustained release as well as to maintain mucoadhesive properties.

In this study, eugenol and (−)-menthol were investigated as two hydrophobic compounds. Eugenol (4-allyl-2-methoxyphenol) (Fig. 1a) is found as a major compound in clove oil, nutmeg, cinnamon and basil and has been widely used as preservatives in food, active pharmaceutical ingredient as well as cosmetics. Several biological properties have been reported including antibacterial activity [9], antioxidant activity [28], anti-inflammatory and local anesthetic [6,23]. (−)-Menthol (L-menthol) (Fig. 1b), monocyclic terpene alcohol, is found as a major constituent in several members of the mint families. (−)-Menthol also exhibited various biological properties such as analgesic, antimicrobial, antifungal and anti-inflammatory activities [15,16]. However, the use of these compounds is currently limited due to their poor water solubility. Thus, the inclusion complex of two guest molecules (eugenol and (−)-menthol) with QCD-g-CS at 11% of degree of substitution (DS) of β-CD moieties on CS chain was investigated in this study (Fig. 1c). The physicochemical characteristics of the formed complex were explored in terms of encapsulation efficiency (%EE) and loading capacity (%loading), crystallinity using differential scanning calorimetry (DSC), chemical composition using infrared spectroscopy (FT-IR) and the release characteristics. To clarify the mechanism of interaction of the complex, molecular dynamics simulations were also evaluated.

2. Materials and methods

2.1. Materials and chemicals

Eugenol, (−)-menthol, *p*-toluenesulfonyl chloride, and glycidyltrimehylammonium chloride (GTMAC) were from Sigma–Aldrich Co. Ltd. (St. Louis, USA). Chitosan (CS) (90% DDA) practical grade from shrimp biowaste with molecular weight of 22,000 Da was obtained from Bio21 Co. Ltd. (Chonburi, Thailand). β-CD was from Wacker Chemical AG (München, Germany). Acetic acid, methanol and *N,N*-dimethylformamide (DMF) were from Carlo Erba (Rodano, Italy). Sodium hydroxide (NaOH), disodium hydrogen phosphate (Na_2HPO_4), Monopotassium phosphate (KH_2PO_4) and Sodium chloride (NaCl) were from Fisher Scientific (Loughborough, UK). Dialysis membranes with molecular weight cut-off of 1,000 Da and 3,500 Da were from Membrane Filtration Products, Inc. (Segiun, TX, USA). The deionized (DI) water was produced from a MilliQ Plus (Millipore, Schwalbach, Germany).

2.2. Synthesis of quaternized chitosan containing β-CD moiety

The synthesis of quaternized β-CD grafted chitosan (QCD-g-CS) was prepared according to the procedure described previously by Gonil et al. [12]. Briefly, O-*p*-toluenesulfonyl-β-CD was preferential synthesized tosylation of either the primary or secondary sugar hydroxyl groups with *p*-toluenesulfonyl chloride occurring under basic condition at 0–5 °C for 5 h. O-*p*-toluenesulfonyl-β-CD was substituted on chitosan (CD-g-CS) backbone under acidic condition at 100 °C for 24 h producing the degree of substitution of 11 ± 2%. Quaternized β-CD grafted CS was carried out by quanternization with GTMAC under acidic condition at 52 °C for 6 h producing the degree of quanternization around 80 ± 5%. The quaternized β-CD grafted chitosan was purified by using dialysis bag (molecular weight cut-off of 3500 Da) and the powders were performed by freeze-drying technique.

2.3. Preparation of inclusion complex

The inclusion complexes were prepared by freeze-drying technique. The QCD-g-CS was dissolved in deionized water (2%, w/v) at room temperature until appearing the clear solution. Eugenol and (−)-menthol were separately added into the QCD-g-CS solution according to the following mole ratios of β-CD (β-CD grafted with chitosan backbone) and eugenol or (−)-menthol; 1:1, 1:5, 1:10 and 1:20. In addition, (−)-menthol crystals were dissolved in ethanol at the concentration of 1.5 g/mL before adding in the QCD-g-CS solution. The inclusion complexes were prepared by a conventional shaking method, in which the inclusion complex was shaken at 25 °C and 40 °C at 250 rpm for 4 h for eugenol and (−)-menthol, respectively. These mixtures were placed into round bottom flask in order to pre-freeze and consequently dry by freeze dryer (Christ Gamma 2-16 LSC, UK). The complexes were stored in a sealed bottle at 4 °C until further analysis.

2.4. Determination of entrapment efficiency (%EE) and loading efficiency (%loading)

The amount of eugenol and (−)-menthol content in an inclusion complex were determined by solvent extraction method with methanol. The freeze-dried complexes of 50 mg were dissolved with methanol 12 ml in a sealed glass tube. The mixtures were vigorously shaken at 60 °C, 250 rpm for 8 h. The supernatant was separated by centrifuge (Kubota, Japan) at 8000 rpm, 25 °C for 15 min. The solvent layer was undertaken under the gas chromatography–mass spectrometry (GC–MS) (Agilent technologies, California, USA). GC–MS conditions were as followed; the silica capillary columns 60 m long, 0.25 mm ID with DB-5ms stationary phase, film thickness 0.25 μm were used. Helium was used as a carrier gas, programmed temperature of the capillary column from 70 °C at 10 °C/min to 250 °C at a constant pressure (100 kPa). The temperature of the split injector was 250 °C and the split ratio was 50:1. The transfer line temperature was 280 °C. The ion source temp was kept at 230 °C. The ionization occurred with a kinetic energy of the impacting electrons of 70 eV. All measurements were carried out in triplicate and the data were expressed as mean average. The percentage of entrapment efficiency (%EE) and loading