Supramolecular Encapsulation of Pulegone from Oriental Herb, *Schizonepeta tenuifolia* Briquet by β- and γ-Cyclodextrins

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Pulegone is a naturally occurring monoterpene ketone obtained from the essential oils of a variety of plants such as Nepeta cataria (catnip), Mentha piperita, and pennyroyal.[1] It was reported that the content of pulegone in *Schizonepeta tenuifolia* Briquet, the dried aerial part of Jingjie, is more than 0.5% and as high as 4.1% in its spike.[2] Pulegone has bio-functional activities including antinociceptive, antibacterial, fumigant and acaricidal activity.[3] As pulegone was characterized as Generally Recognized As Safe (GRAS) status by the USA FDA.[4] pulegone has been widely used in flavoring agents, in perfumery, and in aromatherapy. Pulegone is also known as one of the terpenses, which has been extensively used to enhance the transdermal permeability of several drug molecules such as 5-fluorouracil, propranolol hydrochloride, indomethacin, ketoprofen, and tamoxifen.[5]

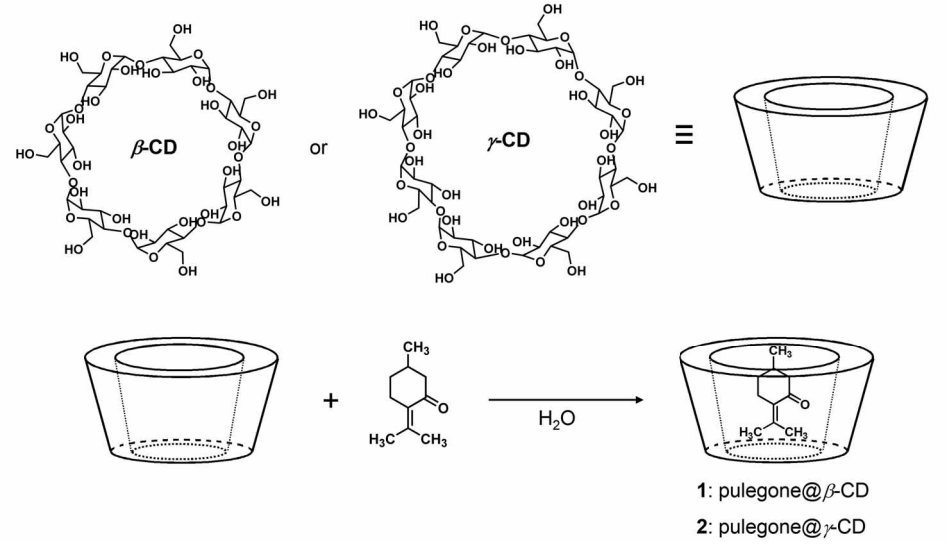
However, this useful pulegone is practically insoluble in water,[6] which has been regarded as a major drawback in many applications. To increase the solubility of pulegone in water, hydrophilic group can be synthetically attached to pulegone through organic chemical reactions. But these procedures need high cost and time consuming, and also may cause to change its physical and chemical properties, which results in unfavorable altering the efficacy and stability of itself. It is thus important to solubilize pulegone in water without any changes of physicochemical properties for its practical use. One promising method to increase solubility in water could be the encapsulation of pulegone by a water-soluble macrocyclic host such as cyclodextrins.

Cyclodextrins are cyclic oligosaccharides with bucket-shape, consisting of six (α-cyclodextrin), seven (β-cyclodextrin), eight (γ-cyclodextrin), or more glucopyranose units linked by α-(1.4)-glycosidic bonds.[7] Most importantly, cyclodextrins provide a micro heterogeneous environment.[8] Namely, cyclodextrins have hydrophilic exterior due to the decoration with hydroxyl groups on edges of the rings, and an apolar cavity providing a hydrophobic matrix. As a result of this structural feature, cyclodextrins are able to encapsulate a variety of hydrophobic guest molecules in aqueous environment.[9] Such supramolecular encapsulations by cyclodextrins have been widely used in many industrial products, technologies and analytical methods.[10] Furthermore, the negligible cytotoxic effects and the ability to permeate biological membranes of cyclodextrins afford important advantages in applications such as food and flavors, pharmaceuticals, cosmetics, environment protection, bioconversion, packing and the textile industry.[11]

For the purpose of further biochemical applications, we have investigated supramolecular interactions between pulegone molecules and water-soluble host molecules such as β- and γ-cyclodextrins to solubilize pulegone in water. The cavities of β- and γ-cyclodextrins are large enough for pulegone to accommodate in the cavity while that of α-cyclodextrin is too small. In addition, β-cyclodextrin is the most readily accessible, the lowest-priced and generally the most useful among cyclodextrin homologues.[9a] We here report the investigation on the supramolecular encapsulation of pulegone by β- and γ-cyclodextrins.[12]

We extracted and purified pulegone from *Schizonepeta tenuifolia* Briquet for the future studies on phytochemicals.[13] The purity of yielded pulegone exceeds over 99%, which was estimated by the ^{1}H NMR spectrometry, gas chromatography (GC), and GC-Mass techniques. Scheme 1 illustrates the inclusion of pulegone in the cavity of cyclodextrins (CDs) with their chemical structures. The encapsulation of pulegone by CDs was performed by direct mixing pulegone with aqueous solution of CDs at room temperature. The inclusion complexes **1** (pulegon@β-CD) and **2** (pulegone-@γ-CD) were isolated after simple work-up processes. The inclusion complexes were characterized by various spectroscopic methods as well as elemental and thermogravimetric analyses.

The formation of supramolecular complex between pulegone and CDs was supported by IR spectroscopy which is a useful tool to prove the presence of both host and guest components in an inclusion complex. The IR spectra of the inclusion complexes (**1** and **2**), CDs, and pulegone are shown in Figure 1. All changes of the stretching frequency for pulegone can be not clearly observed upon supramolecular complexation with β or γ-CDs because large parts of the characteristic bands of pulegone were overlapped with those of β or γ-CDs. But we could evidently observed the C=O stretching vibration band originated from the complexed pulegone at 1678 and 1687 cm^{-1} for **1** and **2**, respectively. The intensities of the bands were noticeably reduced



Scheme 1. Schematic illustration of the encapsulation of pulegone by cyclodextrins.

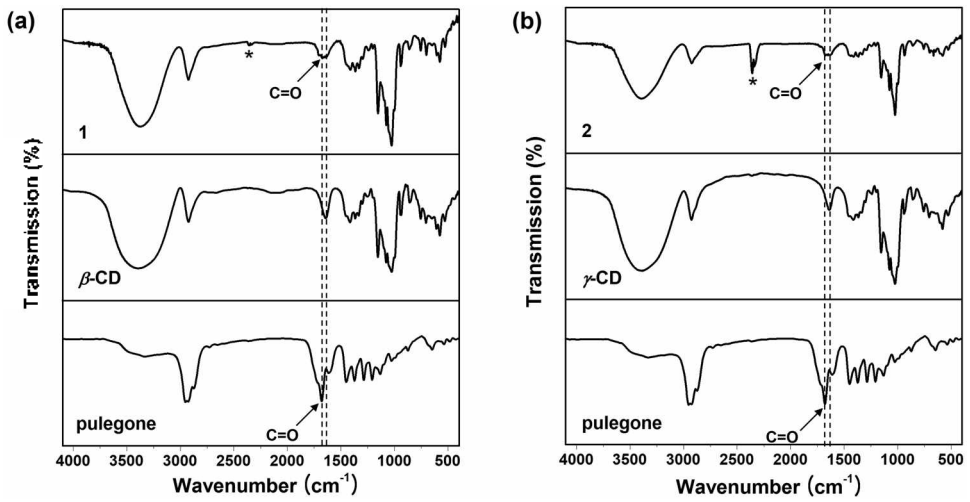


Figure 1. Comparison of IR spectra for inclusion complexes, CDs and pulegone. (a) **1** (top), β-CD (middle) and pulegone (bottom); (b) **2** (top), γ-CD (middle) and pulegone (bottom). Asterisk (*) represents the bands for the vibration of CO_2 molecules in ambient atmosphere during the measurements.

owing to the complexation. An additional key observation to support the inclusion of pulegone in CDs is the sharpening with the decreased intensity of the peak between 3500 and 3000 cm^{-1} in the spectrum of the inclusion complexes. The broad bands between 3500 and 3000 cm^{-1} correspond to stretching vibration of hydrogen bonded O-H groups. Thus, the decreased intensities of OH vibrational modes in the region of 3500-3000 cm^{-1} in the spectrum of the inclusion complexes **1** and **2** are resulted from the release of included water molecules in CDs upon the inclusion of larger guest molecules. It has been well understood that the main driving force for the inclusion of a larger hydrophobic guest molecule in cyclodextrins is the release of enthalphy- and entropy-rich water molecules from the cavity.[7,14]

In order to examine the thermal stability and stoichiometry for the inclusion complexes in solid states, we performed the thermogravimetric analysis (TGA) of CDs and the inclusion complexes **1** and **2** (Figure 2). The thermal analysis of CDs and their inclusion complexes have been well documented, and the data obtained in this work are in good agreement with those reported in the literature.[15] The TGA curves for β- and γ-CDs indicate that loss of water contents occurred up to 120 °C and β- and γ-CDs were decomposed above 290 °C (dashed lines in Figure 2a and 2b). However, the TGA curves for the inclusion complexes **1** and **2** exhibited significantly different behaviors in the course of thermal decompositions. It was found that the inclusion complexes showed at the beginning a small weight loss due to the loss of water molecules, followed by a larger weight loss corresponding to the decomposition of the pulegone and CDs.[16] From these TGA data of the inclusion complexes **1** and **2**, the ratio between pulegone and CDs was estimated to be 1:1. Our TGA investigation thus supports that pulegon molecules are supramolecularly encapsulated in β- and γ-CDs with 1:1

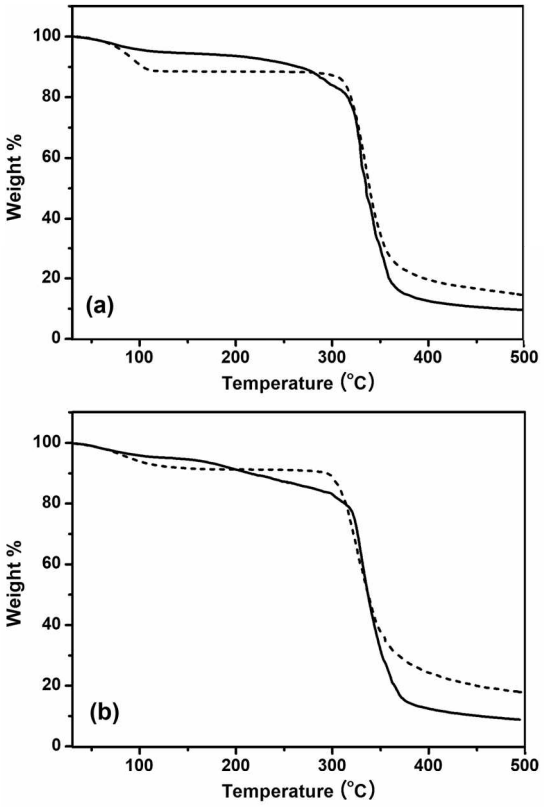


Figure 2. Thermogravimetric analysis diagrams of (a) **1** (solid line) and β-CD (dashed line); (b) **2** (solid line) and γ-CD (dashed line).

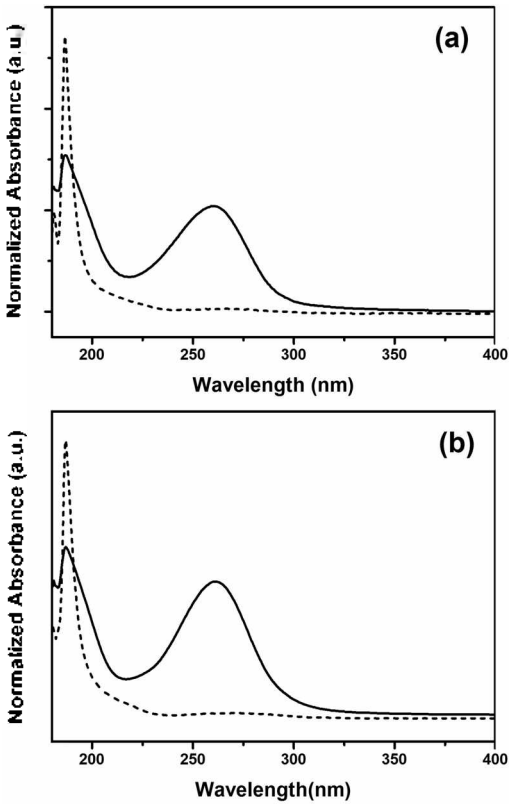


Figure 3. Normalized UV-visible absorption spectra in water of (a) **1** (solid line) and β-CD (dashed line); (b) **2** (solid line) and γ-CD (dashed line).

complexation.

We also studied supramolecular encapsulation of pulegone by CDs in aqueous solution state using UV-vis absorption and ^{1}H NMR spectroscopy. Free β- and γ-CDs exhibited only absorption bands at ~190 nm in their aqueous solutions due to the absence of proper chromophores (dotted lines in Figure 3). However, new absorption band appeared at around 260 nm in the UV-vis absorption spectra obtained from the aqueous solutions of inclusion complexes **1** and **2**, as shown in Figure 3 (solid lines). This absorption is attributed by the new incorporated chromophore (C=C-C=O) of pulegone in the inclusion complexes **1** and **2**. To further substantiate the supramolecular encapsulation of pulegone by CDs in aqueous solution, the inclusion complexes **1** and **2** were also characterized by ^{1}H NMR spectroscopy. In the ^{1}H NMR spectra of **1** and **2** in D_2O, not only the resonances in the range 2.7-0.8 ppm attributed by the protons of pulegone but also the characteristic resonances of CDs were evidently observed (Figure 4). From the estimation of the area ratios, pulegone molecule is encapsulated in β- and γ-CD with 1:1 stoichiometry for **1** and **2**, respectively. This stoichiometric estimation is also in good agreement with the TGA data. Therefore, the UV-vis and ^{1}H NMR spectral data confidentially corroborate that water-insoluble pulegone molecules are readily dissolved in water through the supramolecular encapsulation by water-soluble host molecules, CDs.

In summary, we have investigated supramolecular encapsulation of pulegone by cyclodextrins with the aim of

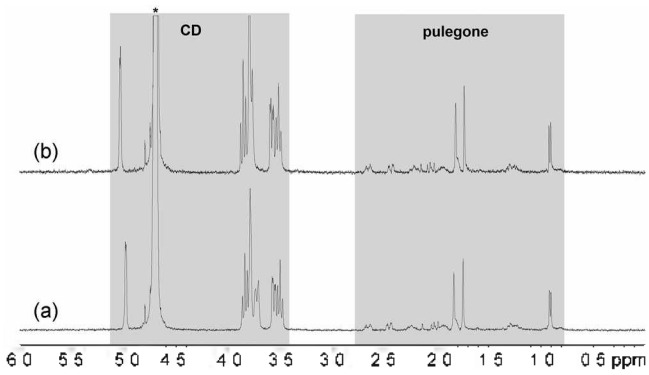


Figure 4. ^{1}H NMR spectra in D_2O solution (1 mM) of (a) **1** and (b) **2**. The resonances in each shaded region correspond to the protons of cyclodextrins (β-CD in **1** and γ-CD in **2**) and pulegone, respectively. Asterisk (*) represents the peak owing to residual undeuterated solvent molecules.

solubilization of water-insouble phytochemicals for biological applications. Our investigations reveal that pulegone molecules are readily included in β- and γ-cyclodextrins with 1:1 stoichiometry, and are substantially stabilized in aqueous solution as well as solid state through the supramolecular encapsulation.

Experimental Section

Measurements. UV-vis and IR spectra were recorded on a

Hewlett-Packard 8453 diode array and a Jasco FTIR-460 Plus spectrophotometers, respectively. ^{1}H NMR spectra were obtained on a Bruker AVANCE III 400 Micro Bay spectrometer. Matrix-assisted laser desorption ionization (MALDI-TOF) mass spectra were recorded on an Applied Biosystems Voyager DE-STR spectrometer. Elemental analyses were performed on a ThermoQuest EA 1110 analyzer. TGA data were obtained on a Perkin-Elmer Pyris 1 TGA instrument with a heating rate of 10 °C min^{-1} under a N_2 atmosphere.

Materials. β- and γ-cyclodextrins were purchased from Aldrich. Although pulegone can be available commercially, we used our purified samples from *Schizonepeta tenuifolia* Briquet for the future studies on phytochemicals.[13] A typical procedure is as follows: A mixture of phytochemicals including pulegone was extracted from grinded *Schizonepeta tenuifolia* Briquet (220 g) with chloroform (4.5 L) by heating at 55 °C for 12 h. The solvent of the extract was removed under reduced pressure. Pulegone was separated from the crude extract by column chromatography on silica gel using gradient eluents with hexane and chloroform (10:0 to 5:5, v/v). The purity of yielded pulegone exceeds over 99%, which was estimated by the ^{1}H NMR spectrometry, gas chromatography (GC), and GC-Mass techniques.

Encapsulation of pulegone by cyclodextrins. To an aqueous solution of β- or γ-cyclodextrins (10^{-3} M, 10 mL), pulegone (10^{-1} mmol) was directly added. The solution was stirred for 1 h at room temperature and the solvent was removed under reduced pressure. The residue was thoroughly washed with diethyl ether to remove uncomplexed pulegone, and then dried in vacuo at room temperature. For **1**: Yield 95%. ^{1}H NMR (400 MHz, D_2O): δ 5.00 (d, J = 3.6 Hz, 7H, β-CD), 3.87-3.80 (m, 21H, β-CD), 3.72-3.70 (m, 7H, β-CD), 3.60-3.50 (m, 14H, β-CD), 2.70-2.66 (m, 1H, pulegone), 2.49-2.44 (m, 1H, pulegone), 2.25 (t, 1H, pulegone), 2.16-2.02 (m, 1H, pulegone), 1.98-1.94 (m, 1H, pulegone), 1.85 (s, 3H, pulegone), 1.76 (s, 3H, pulegone), 1.34-1.31 (m, 2H, pulegone), 0.92 (d, J = 1.6 Hz, 3H, pulegone). IR (KBr, cm^{-1}): ν 3374 (O-H), 2930, 1678 (C=O), 1636, 1421, 1370, 1335, 1293, 1164, 1087, 1036, 950, 873, 762, 710, 590. UV-vis (H_2O, nm): λ_{max}(log ε) 188 (2.21), 260 (1.71). MS (MALDI-TOF): m/z 1325.74 ([M+K]$^+$ requires 1325.45). Anal. Calcd. for $C_{52}H_{86}O_{36}$: C, 48.52; H, 6.73. Found: C, 48.83; H, 6.83. For **2**: Yield 92%. ^{1}H NMR (400 MHz, D_2O): δ 5.04 (d, J = 3.6 Hz, 7H, β-CD), 3.88-3.76 (m, 28H, γ-CD), 3.61-3.58 (m, 7H, γ-CD), 3.55-3.50 (m, 7H, γ-CD), 2.69-2.65 (m, 1H, pulegone), 2.47-2.42 (m, 1H, pulegone), 2.16 (t, 1H, pulegone), 2.10-2.03 (m, 1H, pulegone), 1.99-1.95 (m, 1H, pulegone), 1.83 (s, 3H, pulegone), 1.75 (s, 3H, pulegone), 1.30-1.29 (m, 2H, pulegone), 0.93 (d, J = 1.6 Hz, 3H, pulegone). IR (KBr, cm^{-1}): ν 3401 (O-H), 2930, 1687 (C=O), 1645, 1456, 1402, 1365, 1329, 1164, 1079, 1036, 993, 941, 865, 770, 702, 667, 590, 530. UV-vis (H_2O, nm): λ_{max}(log ε) 187 (2.55), 262 (1.78). Anal. Calcd. for $C_{58}H_{96}O_{41}$: C, 48.06; H, 6.68. Found: C, 48.12; H, 6.38.

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