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Effect of concentration and degree of saturation on co-precipitation of catechin and poly(L-lactide) by the RESOLV process

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ABSTRACT

Rapid expansion of subcritical solutions into liquid solvents (RESOLV) was able to consistently produce catechin (CTC)-loaded poly(L-lactide) (PLLA) nanoparticles. The effects of CTC concentration and processing conditions – pre-expansion temperature and pressure, and degree of saturation – on the size and morphology of CTC-loaded PLLA nanoparticles were investigated, as well as the loading capacity (LC), entrapment efficiency (EE), and release of CTC. RESOLV experiments were carried out with 0.1 wt% CTC + 0.2 wt% PLLA and 0.2 wt% CTC + 0.2 wt% PLLA solutions in mixtures of EtOH and CO_2 (3:2, wt/wt) with pre-expansion temperature and pressure of 60–100 °C and 265–325 bar, respectively. The obtained CTC-loaded PLLA nanoparticles were spherical, with average sizes, LC, and EE ranges of ~30–40 nm, 2.4–7.3%, and 4.7–22.0%, respectively. CTC concentration, pre-expansion temperature, and degree of saturation had significant effects on LC and EE of CTC, without affecting the size of composite nanoparticles. The LC and EE of CTC increased with increasing pre-expansion temperature and degree of saturation, and with decreasing CTC concentration. The release profiles of CTC from the nanoparticles exhibited an initial stage of burst release (first 8 h) followed by a sustained release (~1–9 days). Furthermore, the fraction of CTC being released from the nanoparticles decreased with increasing the degree of saturation of subcritical solutions prior to rapid expansion and with increasing the LC of CTC.

1. Introduction

Catechins, polyphenolic flavonoids predominantly found in pine bark and green tea, recently have been of great interest for use in cosmetic, pharmaceutical and food applications because of numerous health-promoting properties, including antioxidant [1–5], antimicrobial [6,7] and anticarcinogenic activities [8,9]. In addition, catechins have been approved for use as a natural antioxidant in oils and animal feed supplements, and as an antimicrobial agent in foodstuffs and health functional ingredients [10]. In spite of these advantages, the application of catechins has so far been limited because their chemical structures are sensitive to oxygen, light and pH, rendering them unstable and with a short half-life, particularly when they are present in the solutions [11–15].

Encapsulation is an alternative technique to improve the stability of catechins. Several previous studies have reported success in increasing catechin stability by encapsulation in a variety of biodegradable and biocompatible polymers (e.g. chitosan, calcium pectinate, and kafirin) [16–19]. Recently, incorporation of an active compound in polymeric nanoparticles with a controllable and narrow size distribution has attracted great attention in the pharmaceutical, cosmetic and food industries in order to increase the stability and control the release of active components [20,21].

Currently available techniques which have so far been investigated for encapsulating catechins include phase inversion, ionotropic gelation, liposomes, emulsification, spray-drying, and freeze-drying [6,18,19,22–28]. Only phase inversion, ionotropic gelation, and liposome techniques were capable of producing catechin-loaded nanoparticles with a reported size range of ~20–400 nm and encapsulation efficiency of ~25–85% [24–28]. However, these conventional techniques still have several limitations, including: generation of particles with broad size distributions; lack of control over particle size; long processing time; use of excessive organic solvents that need to be removed from the final product; and requiring large quantities of surfactants [29]. To overcome these limitations, supercritical fluid technology has been investigated to produce fine polymeric particles loaded with active compounds.

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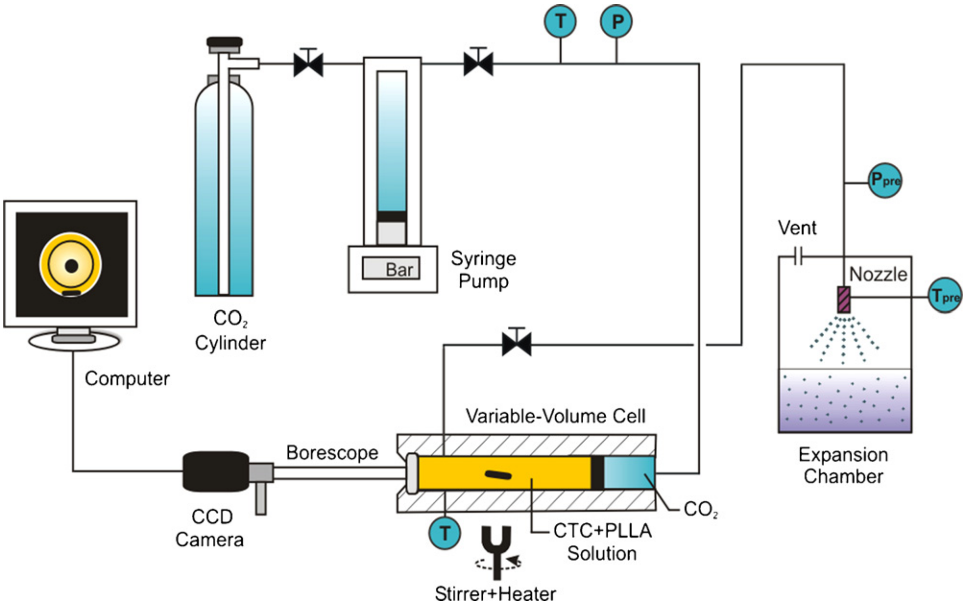

Fig. 1. Schematic of the combined phase-behavior and rapid expansion apparatus.

Rapid expansion of supercritical solutions (RESS) into air and into liquid solvents (RESOLV) are promising techniques for producing nano- and submicron-sized particles. In these processes, a homogeneous solution containing a solute and a supercritical solvent is rapidly expanded (~10^{-6} s) across a nozzle into an expansion medium. Along the expansion path, the solution density decreases dramatically, causing solute precipitation and then the formation of small particles through nucleation and particle growth processes [30–32]. Due to very high supersaturation during rapid expansion, the process favors the formation of small particles with narrow size distributions [32,33]. Additionally, rapid expansion processes can also be used for producing composite particles by dissolving an active substance and a polymer in a supercritical solvent and then expanding the obtained solution in order to simultaneously co-precipitate both components in the form of nano- to micron-sized particles [33–38]. Matsuyama et al. [36] applied RESS to incorporate drugs (e.g. *p*-acetamidophenol and acetylsalicylic acid) into polymeric particles such as poly(ethylene glycol) and poly(L-lactic acid) with an average size range of ~6–55 μm. Türk et al. [37] reported that RESS was able to produce smaller composite microparticles consisting of phytosterol and poly(L-lactic acid) with a particle size range of 1–5 μm. Compared to RESS, RESOLV has more potential in producing nanosized composite particles. Our previous work has shown that RESOLV was capable of fabricating retinyl palmitate-loaded poly(L-lactide) (PLLA) nanoparticles with an average size range of 40–110 nm and loading capacity of 0.9–6.2% [33]. Wen et al. [39] also reported the capacity of RESOLV to incorporate hinesol into liposomes with a size range of ~30–530 nm and entrapment efficiency of 88%. Most rapid expansion studies have focused only on RESS and RESOLV of solutions in the supercritical state, and have been limited to CO_2-soluble compounds. However, our group has demonstrated that RESOLV of solutions in the subcritical state was able to produce asiatic acid-loaded PLLA nanoparticles with size, loading capacity, and entrapment efficiency ranges of ~30–100 nm, 7.6–20.7%, and 38–62%, respectively [40]. However, it remains a challenge to produce composite nanoparticles consisting of compounds with low solubility in supercritical CO_2 via rapid expansion of subcritical solutions which contain a large concentration of an organic solvent compared to the amount of CO_2.

In this work, catechin-loaded PLLA nanoparticles were prepared via rapid expansion of subcritical solutions. PLLA was employed as the matrix because it is biocompatible and approved by the U.S. Food and Drug Administration (FDA) [41,42]. The effects of CTC concentration and processing variables – i.e. pre-expansion temperature and pressure (T_{pre}, P_{pre}), and degree of saturation (S) – on the morphology and size of CTC-loaded PLLA nanoparticles were investigated, as well as the loading capacity, entrapment efficiency, and release of CTC.

2. Experimental

2.1. Materials

(+)-Catechin (CTC) with 96% purity was obtained from Sigma–Aldrich (Germany). Poly(L-lactide) (PLLA, P2294-LLA) with a number-average molecular weight of 4500 g/mol and a polydispersity index of 1.32 was purchased from Polymer Source (Canada). Ethanol with 99.9% purity (analytical grade) was obtained from Merck (Germany). Carbon dioxide (CO_2) with a high purity grade (≥99.98%) was purchased from Chattakorn Lab Center (Thailand). Tetrahydrofuran (THF) with 99.9% purity was supplied by Labscan (Thailand), and distilled water was obtained from Burdick & Jackson (B&J) (USA).

2.2. Phase-behavior measurements

The phase behaviors of CTC and PLLA in subcritical mixtures of EtOH and CO_2 were measured prior to carrying out rapid expansion experiments, because the phase state of a solute–solvent mixture before rapid expansion has been found to affect the size and morphology of products obtained from rapid expansion of sub- and supercritical solutions [33,40,43,44]. Our preliminary work has shown that both CTC and PLLA are practically insoluble in neat CO_2 at pressures up to ~345 bar (the pressure limit of our high-pressure pump) and temperatures ranging from ~30 to 80 °C. Hence, EtOH was mixed with CO_2, with a weight ratio of EtOH to CO_2 of 3:2, in order to sufficiently enhance the solubility of both CTC and PLLA. A schematic of the apparatus used for phase-behavior measurements is shown in Fig. 1. Note that the same apparatus was used for both phase-behavior and rapid expansion experiments. The details of