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# Effect of rapid expansion of subcritical solutions processing conditions on loading capacity of tetrahydrocurcumin encapsulated in poly(L-lactide) particles

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ABSTRACT

Encapsulation of tetrahydrocurcumin (THC) in poly(L-lactide) (PLLA) was achieved by rapid expansion of subcritical solutions of THC and PLLA into water. A pressurized mixture consisting of ethanol and carbon dioxide (3:2 wt/wt) was used as a solvent for THC and PLLA with concentrations up to 2 wt%. The influences of pre-expansion conditions (temperature and pressure) and THC:PLLA weight ratio on the size and morphology of THC-loaded PLLA particles, as well as the loading capacity of THC, were systematically investigated. All the obtained particles were spherical in shape with average size and THC loading capacity ranges of ~80–110 nm and ~13–25%, respectively. The loading capacity increased with (i) increasing pre-expansion temperature and reducing pre-expansion pressure and (ii) increasing THC:PLLA weight ratio at high pre-expansion temperature and low pre-expansion pressure. The antioxidant activity of THC remained unchanged after rapid expansion process, and encapsulation in PLLA prolonged the release of THC.



## 1. Introduction

Currently available conventional encapsulation techniques of bioactive compounds present several disadvantages, including use of substantial amounts of surfactants, risk of thermal and chemical degradation, and difficulty in controlling particle size [1]. Encapsulation processes using supercritical fluid technology have become an attractive alternative to overcome those limitations. Rapid expansion of supercritical solutions is a promising process for producing composite particles by co-precipitation of active substances and polymers [2–5]. In this process, active and polymeric substances are dissolved in a supercritical solvent, and the solution is then rapidly expanded through a micro-nozzle into air (RESS) or a receiving liquid (RESOLV) under atmospheric conditions. During the expansion, the solution density and solvent power decrease substantially, leading to a drastic increase in supersaturation of the solution and then co-precipitation of the solutes, resulting in generation of ultrafine composite particles through nucleation and particle growth processes [6–8].

Previous works reported that RESS produced composite microparticles consisting of bioactive compounds (e.g., *p*-acetamidophenol, acetylsalicylic acid, phytosterol, and protein) and biopolymers (e.g., poly(ethylene glycol) and poly(L-lactic acid)) with sizes ranging from ~1 to 60 μm [2–4]. However, particles obtained from RESOLV process are usually smaller and more uniform with sizes in nano- to sub-micron range [5,8,9]. Moreover, most previous RESOLV studies focused on preparation of nanoparticles from single compounds. To the best of authors knowledge, only a few have focused on the simultaneous co-precipitation of binary components. RESOLV operating parameters, including solute concentration, degree of saturation, and pre-expansion conditions–i.e., pre-expansion temperature and pressure ($T_{pre}$ and $P_{pre}$)–have been found to affect the loading capacity of active substances. In our previous work, the RESOLV process was carried out to co-precipitate retinyl palmitate with poly(L-lactide) [5]. Retinyl

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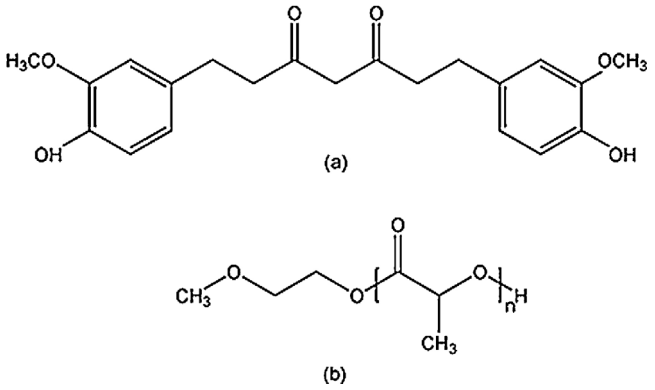


**Fig. 1.** Chemical structures of (a) tetrahydrocurcumin (THC) and (b) poly(L-lactide) (PLLA).

palmitate-loaded poly(L-lactide) nanoparticles with an average size range of ~40–110 nm and loading capacity of 0.9–6.2% were produced through rapid expansion of supercritical carbon dioxide solutions. The particle size slightly increased with degree of saturation, and the loading capacity of retinyl palmitate increased with pre-expansion temperature and retinyl palmitate concentration. The same size range of poly(L-lactide) nanoparticles loaded with asiatic acid was obtained from rapid expansion of subcritical mixtures of carbon dioxide and ethanol (1:1 wt/wt); however, the loading capacity of asiatic acid (7.6–20.7%) [9] was higher than that of retinyl palmitate. Additionally, the loading capacity decreased with increasing pre-expansion temperature and polymer matrix concentration. For catechin-loaded poly(L-lactide) nanoparticles produced by rapid expansion of subcritical mixtures of carbon dioxide and ethanol (2:3 wt/wt), approximately the same ranges of particle size (10–140 nm) and loading capacity (2.4–7.3%) were obtained [10]. Furthermore, the catechin loading capacity increased with pre-expansion temperature and degree of saturation, but decreased with increasing catechin concentration. The results suggest that rapid expansion of sub- and supercritical solutions is a promising process for co-precipitation of binary solutes. However, these previous rapid expansion experiments were limited to dilute solutions in which the concentration of individual solutes in high pressure solutions was no more than 0.4 wt%. In order to achieve a more economically practical process, the feasibility of co-precipitation with higher solute concentrations was investigated in this work.

Tetrahydrocurcumin (THC) and poly(L-lactide) (PLLA) were chosen as model compounds. THC (Fig. 1a) is one of the hydrogenated metabolites of curcumin (*Curcuma longa* Linn.) and possesses potent antioxidant and pharmacological activity [11–13]. Several researchers have demonstrated that THC exhibited greater antioxidant activity than curcumin and vitamin E [12,13]. Moreover, the strong antioxidant activity and lack of yellow color render THC more favorable for food and cosmetic applications [14,15]. However, the sensitivity to oxygen and poor aqueous solubility of THC still limit its applications [13,16]. Therefore, encapsulation of THC in polymeric nanoparticles is a promising alternative to overcome such limitations. To date, only a few studies have reported on encapsulation of THC in polymer matrices [16,17]. Sethacheewakul et al. [16] and Siraksa et al. [17] incorporated THC into mm-sized gelatin capsules and alginate beads, respectively, using extrusion/spheronization and ionotropic gelation techniques in order to develop novel drug delivery systems. Thus far, there have been no reports on encapsulation of THC in polymer nanoparticles. In the present work, PLLA (Fig. 1b) was employed as a polymer matrix for encapsulating THC because it is a biodegradable, biocompatible polymer approved by the U.S. Food and Drug Administration (FDA) [18].

The main objectives of this work were to examine the effects of rapid expansion processing conditions ($T_{pre}$ and $P_{pre}$) and THC:PLLA weight ratio on the product size and morphology as well as THC loading capacity. Moreover, the stability of THC during the rapid expansion process and the release kinetics of THC from encapsulated products were also investigated. Prior to co-precipitation experiments, phase-behavior measurements of THC and PLLA in compressed mixtures were carried out to systematically select pre-expansion conditions.

## 2. Experimental

### 2.1. Materials

Tetrahydrocurcumin (≥99% purity) was provided by Sabinsa Corporation (USA). Poly(L-lactide) (PLLA, P3937-LA) with a number-average molecular weight of 4700 g/mol and a polydispersity index of 1.09 was purchased from Polymer Source (Canada). Ethanol (EtOH, 99.9% purity), tetrahydrofuran (99.9% purity), and distilled water were obtained from Merck (Germany), RCI Labscan (Thailand), and Burdick & Jackson (USA), respectively. Potassium ferricyanide (≥99% purity) was acquired from Ajax Finechem (Australia), and ferric chloride (≥99% purity) was obtained from Loba Chemie (India). Trichloroacetic acid was purchased from Fisher Scientific (UK), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was acquired from Sigma-Aldrich (Germany). Carbon dioxide ($CO_2$) with a high purity grade (≥99.98% purity) was purchased from Chattakorn Lab Center (Thailand). All chemicals were used as received without further purification.

### 2.2. Phase behavior of THC and PLLA in subcritical EtOH + $CO_2$ mixtures

Fig. 2 illustrates a schematic diagram of the experimental apparatus; the main part is a variable-volume view cell used for measuring the phase behavior of THC and PLLA in subcritical mixtures (EtOH + $CO_2$). A detailed description of the experimental procedure can be found elsewhere [5]. The phase behavior of THC and PLLA in subcritical EtOH + $CO_2$ mixtures were investigated prior to performing the rapid expansion experiments, because the size, morphology, and composition of products obtained have been found to depend on the phase state of the solute/solvent mixture and on the operating conditions [5,9,10,19–22]. Because both THC and PLLA are sparingly soluble in pure $CO_2$, a mixture of EtOH and $CO_2$ in a weight ratio of 3:2 was used as the solvent mixture in order to dissolve THC and PLLA at concentrations up to 2 wt%. For each run, the variable-volume view cell was loaded with known amounts of solute (0.11–0.22 g of THC or PLLA), EtOH (6.59 g), and $CO_2$ (4.39 g). Then the view cell was pressurized to ~340 bar and heated to ~55 °C under continuous mixing using a horseshoe magnet (200 rpm) located below the view cell (Fig. 2) until the solute was completely dissolved and a single-phase solution was obtained. To determine the cloud-point (i.e., liquid–solid) pressures, the homogeneous solution in the cell was maintained in the single-phase region at a controlled temperature (±0.1 °C) for at least 30 min; then the system pressure was slowly decreased until the solution became cloudy. The cloud-point pressure was defined as the first point at which the clear liquid solution started to phase-separate and turn slightly cloudy. In the case of 1 wt% THC solution, the phase transition occurred at a temperature below room temperature. Thus, the cloud point was measured as the solution temperature was gradually reduced (≤0.1 °C/min) by supplying cold nitrogen gas to the isothermal bath under constant