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Encapsulation of propolis flavonoids in a water soluble polymer using pressurized carbon dioxide anti-solvent crystallization

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ABSTRACT

This study investigated the pressurized carbon dioxide anti-solvent co-precipitation process (abbr. PAS) on encapsulation of propolis with water soluble polyethylene glycol (PEG). The extent to which recovery of propolis flavonoids, total yield, amount of propolis (i.e. drug content) affects the precipitation was examined using a two-factor central composite schemed experimental design method. Analysis results indicated that the drug content and total yield of the precipitates are conversely related to each other in the PAS process. Additionally, micro-sized amorphous particulates of propolis encapsulated on the surface of PEG were generated, as evidenced by chromatography and X-ray diffraction analyses. Total yield of the PAS co-precipitation process reached 88%, and recovery of propolis flavonoids achieved 97%. Experimental results also indicated that the concentration ratio of propolis to PEG in the feed more significantly affects the drug content than that of the pressure factor. Moreover, nearly spherical and aggregated micro-sized co-precipitated particulates are more soluble in an aqueous solution than those of ethanol extracts.

1. Introduction

A propolis is a collection of bees from plants or bark of the tree buds with the mixing of resin [1]. Over 300 materials are found in propolis [2,3] while approximately 80–100 compounds are found in different propolis [4]. The major constituents of propolis are often categorized as derivatives of cinnamic acid, *p*-coumaric acid, isoamylene, artepillin C, and some other organic matrixes [5]. The compositions of propolis are usually complex, and flavonoids are the major constitutes of propolis [6]. Most compounds of flavonoids are caffeic acid phenethyl ester, quercetin, wogonin, pinocembrin, naringin, galangin and chrysin [7]. Flavonoids have been recently considered as having beneficial bioactive effects on human organs [8,9]. In ancient medical therapy, these bioactive constituents of propolis were used as a folk medicine with antiseptic and antibacterial properties [4]. Moreover, the acetone extract of propolis enhances antibacterial activities [10]. The ethanol extract of propolis significantly affects rats during anti-arthritic treatment [11]. Caffeic acid phenethyl ester has been adopted to adjust the macrophage in the inflammation path [12]. Cinnamic acid and chrysin of propolis in the solvent extract have been used to inhibit tumors, induced apoptosis [13,14], and reduce the side effects of cancer drug cyclophosphamide [15]. Recent studies have recommended the use of propolis in an alcoholic solution as a healthy supplement to improve the immune system as well as protect the liver [16] and the brain [17] from the attack of free radicals. HPLC analysis of flavonoids plays an important role in quantifying the amount of flavonoids in the extract.

While water soluble and non-toxic polyethylene glycol (PEG) does not react with human immunoreactive [18]. Commonly found in the food industry as an adhesive or an emulsifier to become a food additive [19]. PEG is also widely used in biology and medicine as a drug carrier [1,20]. The PEG drug carrier shows numerous benefits in the control release rate of drugs [21,22]. In addition to enhancing the dissolution rate of drugs, the PEG modified triamterene in an in vivo test also protects the drugs degradation [23,24].

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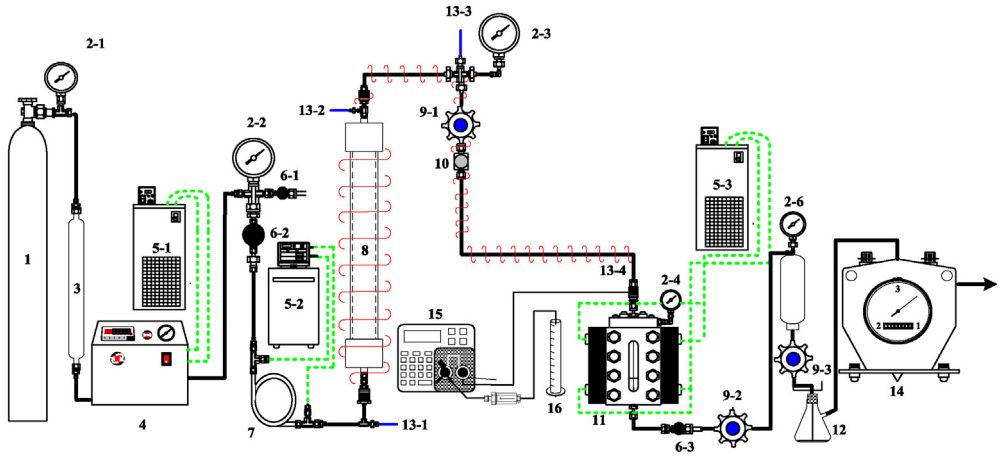

1.	CO_2 cylinder	9-1~9-3.	Back pressure regulator
2-1~2-4.	Pressure gauge	10.	Metering valve
3.	Gas dryer	11.	Precipitator
4.	High-pressure pump	12.	Separator
5-1~5-3.	Cool circulator	13-1~13-4.	Thermocouple
6-1~6-4.	Needle valve	14.	Wet gas meter
7.	Heat exchanger	15.	HPLC pump
8.	storage tank	16.	Feeding cylinder

Fig. 1. Schematic flow diagram of pressurized anti-solvent precipitation equipments.

Frequently used in some industries, encapsulation or co-precipitation can be divided into physical or chemical processes. Physical processes include spray-drying, spray-cooling, spray-chilling, air suspension coating, extrusion, centrifugal extrusion, lyophilized, annular jet and rotational suspension separation. Meanwhile, chemical processes include acervation, co-crystallization, liposome entrapment, interfacial polymerization, and molecular inclusion [25,26].

Supercritical anti-solvent (SAS) co-precipitation has been extensively used to precipitate drugs and natural components with a high yield [27–29]. However, when the solutes (i.e. propolis) have a lower solubility in the supercritical CO_2, SAS might have a low yield of precipitates. Hence, in this study the PAS (pressurized anti-solvent) process is adopted for the encapsulation of propolis. PSA generally lead to the precipitation of a solute with a high yield. Furthermore, precipitation can be controlled by adjusting both the pressure and temperature [30].

Different solvents may extract different compounds, explaining the popular use of ethanol extracts used in biological assays and commercial products [31]. In this study, flavonoids are extracted from Brazalian propolis using ethanol. An attempt is also made to increase water solubility of the hydrophobic propolis extracts by studying the encapsulation of propolis with PEG via a pressurized carbon dioxide anti-solvent process in order to generate micro-sized water soluble particulates.

2. Materials and methods

2.1. Materials and reagents

Extra-green Brazalian propolis was kindly donated by Bio-Joint Natural Co., Ltd., Taiwan; it was stored at 4 °C before extraction. Analytical grade solvents were used for the extractions, column chromatographs and PAS processes, including 99.9% ethanol (Mallinckrodt, USA), dimethyl sulfoxide (Sigma–Aldrich, USA), ethyl acetate (Mallinckrodt, USA), tetrahydrofuran (Mallinckrodt, USA), 99.5% acetone (Mallinckrodt, USA), and 99% diethyl ether (J.T. Baker, USA). Ultra pure water (>18 M) was obtained by using the Ultrapure-TM water purification system (Louton Co., Ltd., Taipei, Taiwan). The water was then filtered through a 0.45 μm PTFE (Advantec) membrane filter before use. 99.95% CO_2 (Toyo gas, Taiwan) was used for the PAS process. The molecular weight of 95% polyethylene glycol is 35,000 Da (Sigma–Aldrich, USA). The standards used for UV/vis quantification of propolis flavonoids are aluminum nitrate nonahydrate (Fluka, CH), sodium hydroxide (Sigma–Aldrich, USA), sodium nitrite (Sigma–Aldrich, USA), quercetin (Sigma–Aldrich, USA). The purity of all standards is above 95% (the HPLC grade).

2.2. Ultrasonic extractions and soxhlet extractions

In ultrasonic extractions, 2 g of propolis powder was extracted by using 200 ml of a solvent (i.e. the SSR value was 100) under 303 K with a frequency of 40 kHz and a power of 300 W for 1 h extraction. In Soxhlet extraction, 10 g of propolis powder was extracted by 7920 ml of solvent (i.e. the SSR value was 792). Following extraction, extracts were concentrated with a vacuum rotor and freeze-dried for 12 h. The dried extracts were analyzed by a gravimetric method and an UV chromatographic method.

2.3. Solubility test of PEG and propolis in solvents

Precise weight of both the powdered PEG and the powdered propolis are ranged from 0.25 to 1 g. A certain amount of solids were put into several tubes, followed by an addition of 1 ml of various solvents, and vibration of the solutions at 318 K for 30 min by an ultrasonic vibrator, individually. After this treatment, the mixed super-saturation solutions were filtered with 0.45 μm nylon filters. Additionally, samples were formed by taking 100 μl of the filtered solutions and evaporating the solvent. Finally, the solubility of PEG and propolis in the solvent was determined using a gravimetric method, individually.