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Simultaneous recovery of ionic liquid and bioactive alkaloids with same tropane nucleus through an unusual co-crystal after extraction

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ABSTRACT

It was found for the first time that natural tropane alkaloids from *Physochlaina infundibularis* Kuang and tropine-based ionic liquid ($[C_3tr][PF_6]$, as component of solvent) could form a unique co-crystal through simple cooling after extraction process, which had the same parent nucleus in structure. A series of instruments and techniques including microscope, infrared spectroscopy (IR), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), ultraviolet spectrum (UV) together with high-performance liquid chromatography (HPLC) were used to analyze the structure, composition and formation process of the co-crystal comprehensively. The molecular ratio of $[C_3tr][PF_6]$ to alkaloids was determined as 6:1, and the optimal crystal formation conditions were explored; finally, 0.119 g co-crystals could be obtained from the aqueous solution of $[C_3tr][PF_6]$ and alkaloids after 1.000 g of dried herbal material was extracted. The formation mechanism of this new co-crystal was also studied in detail. The results prove structural similarity between solute and solvent molecules can not only improve extraction selectivity, but also induce the formation of their co-crystal and make their recovery convenient.

1. Introduction

In recent years, co-crystallization has been applied to many chemical and industrial fields as a strategy to improve the solid state properties of compounds and production efficiency [1,2]. Co-crystals are special crystal forms where two or more neutral molecules present in the unit cell [3] and can be prepared by a variety of approaches including solution crystallization, solid state grinding, thermal methods, freeze-drying, and slurrying, etc. An important consideration with co-crystallization is that not every pair of molecules has the propensity to form a co-crystal. In fact, molecules which will co-crystallize with a given compound can sometimes be an arduous process [4]. Co-crystal is essentially a supramolecular self-assembly system [5], which is the equilibrium results of thermodynamics, kinetics and molecular recognition. During its formation process, the interaction between molecules and spatial effects affect the formation of the networks, which directly affect the composition of crystals, so the interactions between different molecules are mainly including hydrogen bonding, π-π stacking, Van der Waals force and halogen bond. Most of the co-crystals formed under the action of hydrogen bond, such as N—H···O, O—H···O, N···N and O—H···N. For example, in the works of Basavoju [6], the co-crystal of indomethacin and saccharin formed by the hydrogen bonding of N—H···O, which generated with the carboxyl in the structure of indomethacin and amido in saccharin. It is found that some weak hydrogen bonds can also cause the co-crystal formation, including C—H···O and C—H···N, N, N′-two oxidation-2, 2′-two pyridine formed in the co-crystal of fumaric acid, itaconic acid, succinic acid and oxalic acid through the hydrogen bond of C—H···O [7]. At the same time, halogen bond is the active force to generate co-crystal, which usually forms through halogen atoms and heteroatom with lone electron pairs [8]. Moreover, it has been found that multiple forces rather than the single force are always required to stabilize the structure of co-crystal.

Co-crystal has some obvious change by the comparison of raw material in the of properties of color, melting point, solubility, physic chemical stability, crystallinity, mechanical properties and so on. For example, caffeine and theophylline can form co-crystal with malonic acid, maleic acid or glutaric acid. Compared with active ingredient, the co-crystal with oxalic acid enhanced hydration stability and could not be converted to hydrate for 7 weeks even at exposure to 98% relative humidity [9]. In addition, co-crystal can increase dissolution rate and solubility of active compounds. The co-crystal of saccharin and carbamazepine showed chemical stability of good solubility properties and good suspension stability in the study of Hickey [10]. At the same time, it was found that the dissolution rate slowed down after the formation of co-crystal [11].

In the last decade, ionic liquids (ILs) are widely used in many chemical fields because of their special physicochemical properties and intermolecular interactions, especially in the field of extraction and separation of natural products from industrial crops. It has been reported that a series of ionic liquids are used as the environment-friendly solvents for liquid-liquid extraction, microwave/ultrasonic wave-

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assisted extraction or liquid-phase micro extraction with high efficiency [12–14]. After the extraction is completed, the effective recovery of ionic liquids and target compounds will be an unavoidable problem, and related treatment is usually time/energy-consuming or composed of complex operation. Back extraction is considered as the most common method for separation of ILs and nonvolatile or thermal sensitive products; however, long-time concentration and high consumption of extraction solvents are needed. Adsorption is another useful selection, and the most widely used sorbents in fixed bed or other modes are activated carbon and ion-exchange resins. Furthermore, aqueous two-phase system and commercial membrane are also appropriate for the efficient separation of ILs and target products [15]. How to obtain them with satisfied efficiency and purity after extraction through newer and easier ways is worthy to explore. In our recent study, it was found that the solubility of propyl-substituted tropine-based IL with hexafluorophosphate anion (abbreviated as [C_3tr][PF_6]) showed temperature sensitivity and its 0.05 mol/L aqueous solution had the ideal selective extraction ability for tropane alkaloids in *Radix Physochlainae* for their structural similarity, which also resulted in a unusual phenomenon that both [C_3tr][PF_6] and tropane alkaloids could crystallize from the system under low temperature at the end of extraction. In this way, IL and alkaloids could be easily recovered without any concentration, which offer a new way in the post-treatment process after extraction of target bioactive constituents.

On the basis of above backgrounds, the unique co-crystal of tropane alkaloids and [C_3tr][PF_6] was found for the first time, which was used to develop the post-treatment methods after the former are extracted by the IL with similar structure. Here it is necessary to study and investigate the formation conditions, composition and mechanism of co-crystal, and the study of [C_3tr][PF_6] and tropane alkaloids separation is also expected. Analytical methods were used to investigate the composition of the co-crystal formed with tropane alkaloids and [C_3tr][PF_6], such as SEM, IR, UV, XPS, TG and XRD. The physical/chemical properties and formation conditions of co-crystal were also studied to explore its nature. At the same time, the mechanism of the formation of co-crystal was studied through IR, XRD, TG, NMR and molecular simulation.

2. Experimental

2.1. Reagents and materials

All chemicals involved in this study were at least of analytical grade and purchased from Kelong chemical reagents factory (Chengdu, China). Chromatographic methanol used for high performance liquid chromatography (HPLC) was purchased from Chengdu Chemical reagents factory and filtered with microporous membrane (0.45 μm) before use. Experimental deionized water was obtained from ultra-pure water purification system (0.4 mm filter) manufactured by Millipore Co. Ltd. (Bedford, USA). [C_3tr][PF_6] was synthesized and purified according to the previous method [16], and its purity was found above 99.0% after analysis by HPLC method coupled with evaporative light-scattering detector. All samples were stored in closed desiccators until use. Herbal raw materials originating in Shannxi province were obtained from local pharmacy and identified as the dried roots of *Physochlaina infundibularis* Kuang by Prof. Fangyan Li in the Department of Pharmaceutical & Biological Engineering. They were milled and dried and then the sample powders passed through a stainless steel sieve with the particle size of 60 mesh.

2.2. Apparatus

JSM-7001F scanning electron microscope (abbreviated as SEM, JEOL, Tokyo, Japan) and N-117 M biological microscope (Boyu Instrument Co. Ltd., Shenzhen, China) were used to observe the morphology of the crystal. Nicolet 6700 Fourier transform infrared spectrometer (abbreviated as FT-IR, Thermo Scientific, Madison, USA), TU-1810 ultraviolet spectrophotometer (Purkinje General Instrument Co. Ltd., Beijing, China), STA 449 F3 simultaneous thermal analyzer (NETZSCH, Selb, Germany), EMPYREAN X-ray diffractometer (abbreviated as XRPD, PANalytical B.V., Almelo, Netherlands) and XSAM800 X-ray photoelectron spectroscopy (abbreviated as XPS, Kratos, Manchester, UK) were used to characterize and determine the composition of the co-crystal. Extraction experiments were carried out using HH-S constant temperature water bath with an uncertainty of ±0.05 K (Wanke Instrumental Co. Ltd., Jintan, China) and KQ-2200DA ultrasonic extractor (Shumei Co. Ltd., Kunshan, China). The process of crystal precipitation was controlled in DC2006 low-temperature thermostatic bath (Hengping Instrumental Co. Ltd., Shanghai, China). HPLC analysis was performed with EC2006 HPLC (Elite, Dalian, China) consisting of UV1201 UV–Vis detector and ELSD2000 evaporative light-scattering detector (Alltech, Lexington, USA). Refractive index of [C_3tr][PF_6] and co-crystal was determined by WAY-2S Abbe refractometer (Shenguang Instrumental Co. Ltd., Shanghai, China). DDS-12A conductivity meter (Kangyi Scientific Instrument Co. Ltd., Shanghai, China) was used to measure conductivity and polarity of mother liquor after crystallization. Bruker AV II-600 MHz and 400 MHz (Bruker Biospin Co. Ltd., Switzerland) were used to measure the one dimensional and two dimensional NMR of related samples.

2.3. Quantitative analysis of target alkaloids

Based on the reported method for five representative alkaloids [17], the HPLC analysis was carried out on Welch C_{18} chromatographic column (4.6 × 250 mm, 5 μm) at column temperature of 25 °C; the mobile phase was composed of 10 mmol/L ammonium acetate solution (A) together with methanol (B) and the flow rate was 1.0 mL/min. Detection wavelength was set at 210 nm and injection volume was 10 μL. The gradient procedure was operated as: 0–10 min, A:B = 7:3 (V/V); 10–30 min, A:B = 3:7 (V/V); after 30 min, A:B = 1:4 (V/V). The concentrations of five standard alkaloids had good linear relationship with peak area in the investigated range of content, and corresponding standard curves of anisodamine, hyoscyamine, scopolamine, aposcopolamine and scopoline were determined as $y_1 = 12{,}261.40 \times_1 - 368.19$ (R^2 = 0.9998, linear range: 0.50–3.50 μg), $y_2 = 3560.60 \times_2 - 619.55$ (R^2 = 0.9997, linear range: 0.24–1.28 μg), $y_3 = 1628.80 \times_3 - 135.65$ (R^2 = 0.9999, linear range: 0.17–1.73 μg), $y_4 = 2188.80 \times_4 - 266.52$ (R^2 = 0.9997, linear range: 0.15–0.40 μg), $y_5 = 110.59 \times_5 - 1.84$ (R^2 = 0.9998, linear range: 0.20–1.20 μg) successively, where *x* was their amount (μg) and *y* was the UV absorbance value of their corresponding peak area, respectively. As the result, the total mass fraction of five alkaloids was calculated as 0.165% in the herbal material.

2.4. Preparation procedure of co-crystal

According to the experimental optimization, 1.00 g powders (60 mesh) of *Radix Physochlainae* were weighed accurately and placed in 50 mL Erlenmeyer flask, and then they were mixed with 35 mL of 0.05 mol/L [C_3tr][PF_6] aqueous solution. The target constituents were extracted at 75 °C in constant temperature water bath for 55 min [18]. At the end of extraction, the solution was filtered and the filtrate was precipitated to obtain the co-crystal on 5 °C for 3 h, meanwhile the filtrate was concentrated and then diluted to 10 ± 0.010 mL with chromatographic methanol in volumetric flask. Finally, the sample solution was filtered with a microporous membrane of 0.45 μm before HPLC analysis. The yield of target alkaloids (mg/g) in co-crystal was determined by the developed HPLC method and calculated through the following equation:

$$\text{Yield of tropane-based alkaloids} = (0.165\% \cdot m_0 - m_1)/m_0 \times 100\% \quad (1)$$