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Desorption of artemisinin extracts of CIM-Arogya by supercritical carbon dioxide

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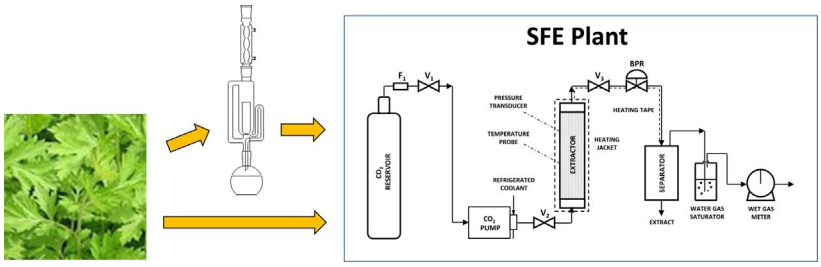
GRAPHICAL ABSTRACT



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ABSTRACT

Artemisinin is a drug for chloroquine resistant malaria and cerebral malaria treatments. In the recent past, there was an acute shortage of this drug and hence World Health Organization made a strategy to fulfil the Artemisinin demand.

In this study, artemisinin was extracted by supercritical Carbon Dioxide ($SFCO_2$) from CIM-Arogya, a variety of Artemisia annua, in temperature and pressure ranges of 313.1-333.1 K and 15–25 MPa. Artemisinin global yield isotherms were determined obtaining a maximum yield of 3.65 wt%. Artemisinin extracts were also obtained by hexane Soxhlet extraction: then, the crude extracts were purified using $SFCO_2$, after adsorption on silica gel. Different desorption runs were performed with a 6 ml/min CO_2 flow rate, in temperature and pressure ranges of 313.1–333.1 K and 15–25 MPa. At different time intervals, extracts were collected and analysed: their yields varied from 2.75% to 4.34% function of the experimental conditions. Desorption trials were also correlated with different models.

1. Introduction

Isolated from the aerial parts of Artemisia annua (Family: Asteraceae), artemisinin, a sesquiterpene lactone, and its derivatives are powerful medicines known for their ability to swiftly reduce the number of Plasmodium parasites in the blood of patients with malaria. This drug has been developed from the Chinese traditional medicine and is known as Qinghaosu.

The World Health Organization as the first-line treatment for uncomplicated Plasmodium falciparum malaria recommends Artemisinin-based combination therapies (ACTs). Expanding access to ACTs in malaria-endemic countries has been integral to the remarkable recent

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success in reducing the global malaria burden. In 2015, 311 million ACT treatment courses were procured by endemic countries – up from 187 million in 2010 [1].

Further optimization of artemisinin-based therapy for malaria is ongoing. A number of semisynthetic routes to prepare artemisinin analogues such as artemether and artesunate with changes to the d-lactone portion have been developed with the goal of improving the pharmacokinetic properties. New combination therapies in which one of the components is an artemisinin-derived antimalarial either are in clinical development or recently approved therapies [2].

In addition to natural artemisinin and dihydroartemisinin, the semisynthetic artemisinin derivatives artemether, arteether, and artesunate have been increasingly used for about 20 years. These drugs are metabolized to dihydroartemisinin, the main bioactive compound. The artemisinins act faster than any other antimalarial drugs, with an approximate parasite- and fever-clearance time of 32 h, in contrast to 2–3 days needed with conventional antimalarial drugs to resolve the symptoms. To shorten the treatment duration and to prevent the development of resistance, the artemisinins are progressively associated with other antimalarial drugs with longer half-lives.

Although the total synthesis of artemisinin has been realised, its product is not yet competitive in price with the natural one, because of its high abundance in the plant (0.6–1.2%). Due to the use of artemisinin as such and its chemical modification to various semisynthetic drugs, there is a high demand of this compound in pharmaceutical industry. Presently, liquid solvent extraction with hexane, petroleum ether, and toluene is the most applied technique. However, these procedures exhaust a large amount of potentially hazardous solvents to the environment, and its recovery yield is low. Therefore, alternative extraction technique with better selectivity and efficiency are highly desirable.

Artemisinin solubility in supercritical carbon dioxide ($SFCO_2$), in terms of mole fraction, was found to range from 10^{-4} to 10^{-3}, which is higher than typical solubilities of many biological molecules [3–5].

Quispe-Condori et al. [6], in 2005, report the global yield isotherms and the kinetic of extraction from Artemisia annua leaves using $SFCO_2$. They obtained the maximum yield of 5.7% at 323.1 K and 30 MPa, with artemisinin purity in the extracts around 12 wt%. Lin et al. [7], at 323.1 K and 173.4 MPa, achieve higher purity values of 73 wt%, with a total yield of 0.5%.

Some papers report supercritical carbon dioxide artemisinin extractions, from Artemisia annua L., by adding co-solvents: 3% of methanol at 150 bar and 40 °C by Kohler et al. [8,9], 16.25% of *n*-hexane at 70–208 bar and 40–60 °C by Lin et al. [7] and 16.25% of ethanol at 173–311 bar and 40–60 °C by Tzeng et al. [10]. The co-solvent modified $SFCO_2$ extraction produces more pure artemisinin than classical Soxhlet solvent extraction.

Martinez-Correa et al. [11], in 2017, introduce a process with two-step extractions of bioactive compounds present in Artemisia annua L. They use $SFCO_2$ in the first step and ethanol or water in the second one (on the solid residue of the supercritical extraction). The conclusion is that in the supercritical extraction most of the artemisinin is extracted and therefore a second extraction step it is not necessary. Recently, an interesting article reports the supercritical fractional extraction of Artemisia annua L., producing extracts enriched in Artemisinin [12]. The work demonstrates the efficiency of the fractional cooling separation that allows complete elimination of waxes that confer solid or semi-solid consistency to SFE extracts.

From the comparison between the different extraction techniques [7–9,11] it seems that the solvent extraction with hexane still gives the better results in terms of total yields.

A combination of extraction with traditional organic solvents and supercritical carbon dioxide desorption is proposed, in some cases, for the obtainment of high purity extracts from natural sources. Guyer et al. [13] recover onion flavour from onion juice by adsorption and $SFCO_2$ desorption from polymeric adsorbent. Braida et al. [14] obtain greater antioxidants concentration from labiatae family herbs. In their work, crude oleoresins, obtained from dried leaves of rosemary using organic solvents, are used as starting material. Supercritical extraction of oleoresin followed by an adsorption step with various adsorbents is carried out. After adsorption, retained compounds are recovered in a desorption step under the same supercritical condition but using ethanol as co-solvent.

The first part of this work concerns the direct artemisinin $SFCO_2$ extraction from CIM-Arogya, a variety of Artemisia annua. In the second part a simplified solvent extraction-$SFCO_2$ desorption approach is applied: after a Soxhlet plant extraction, with hexane, the crude extracts are directly coated with silica gel and the solvent removed under vacuum. Then the supercritical carbon dioxide is used only in the desorption step. Several desorption conditions are studied and correlated with different models. All the obtained extracts are analysed by HPLC technique.

2. Materials and methods

2.1. Materials

The Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow India, developed a variety of Artemisia annua, named CIM-AROGYA. This variety is characterized by the high content of artemisinin ranging from 1 to 1.2%. The raw material was dried at 40 °C, in a tray drier with air circulation (Memmert Universal Oven UF30, Germany), and comminuted. The resulting material packed in a plastic bottle placed in a freezer.

The particle size distribution was determined using a vibratory sieve shaker (Giuliani, Turin, Italy): different mesh size of sieves (35, 40, 50, 60, 80 and 100) were taken and arranged in a tower shape.

Particles from 35 to 60 meshes were selected for the extraction experiments.

The real density of the particles was measured by helium gas pycnometry (Ultrapycnometer-1000, Quantachrome Instruments – USA) and is of 1030.0 kg/m^3.

Carbon dioxide with a purity of 99.98% was purchased by Società Italiana Acetilene e Derivati (SIAD, Italy). Hexane pro analysis grade and Silica gel 60–120 mesh (Merck) were used in the organic extraction and in the desorption process, respectively.

In HPLC analysis the used methanol and absolute ethanol were purchased by Sigma-Aldrich.

2.2. Experimental procedures

2.2.1. Supercritical fluid extraction

The supercritical fluid extraction of the plant material was performed in the LAB SFE 100 ml, from Separex, that is composed of a CO_2 heater, an extractor with extraction cells of different volumes, a temperature and pressure control system, and an extract collector [15].

The extraction unit is designed for operation up to 50 MPa and 423.1 K. Liquefied CO_2 from the reservoir is cooled (Haake K cooling bath) and pumped by a dual-piston pump (Lewa EKM210V1). Pressure is controlled with a heated back-pressure regulator (Tescom 26-1762-24-043).

Ten grams of dry material, with an artemisinin content of 1.2 wt%, were charged in the extraction cell. The same procedure was repeated for all the experiments.

Flow rate of carbon dioxide was maintained constant in all the runs at 25 g/min.

The influence of extraction temperature and pressure was investigated in the range 313.1–333.1 K and 15–25 MPa. Samples were collected at different time intervals (from 10 to 90 min).

2.2.2. Supercritical fluid desorption

The supercritical fluid desorption assays were executed on raw CIM-