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Conceptual process synthesis for isolation and purification of natural products from plants - A case study of artemisinin from *Artemisia annua*

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Abstract

A systematic method based on conceptual process synthesis for recovery of natural products from their biological sources is presented. The proposed methodology divides the task into two major subtasks namely, isolation of target compound from a chemically complex solid matrix of biological source and purification of the target compound from the crude extract. This methodology consists of three major separation techniques to achieve the task and provides a platform to generate different process alternatives by employing different combinations of separation units and with different set of key process variables. Decision making about the different combinations of separation units as well as key process variables is based upon the process information collected at every step with the help of process analytical techniques (PAT) and heuristics. The optimal combination of different separation units and set of key process variables is determined in an iterative approach. In the present work, this methodology is applied to isolate and purify artemisinin, an anti-malarial drug from the plant *Artemisia annua*.

Keywords: conceptual process synthesis, natural products, separation and purification, process flow sheet

1. Introduction

Natural products form a vast resource of compounds with unlimited chemical and functional diversity and also serve as a major resource for drug discovery. However, the isolation and purification of target compounds from their biological sources represents one of the most challenging tasks due to the presence of many other compounds in the biomass and also due to lack of basic process information. Associating compounds having similar chemical and physical properties to that of the target compound significantly influence the performance of a stand alone separation technique. In addition, the current chemical process industries face challenges like quicker development of new products and processes, making existing processes more efficient by reducing capital and operating costs, and improving the safety and environmental performance. To cater to these demands, a systematic approach in designing a commercial separation process consisting of multiple separation techniques for isolation and purification of natural products, which can evaluate different possible process alternatives within short span of time, is necessary.

The approach of conceptual process design provides such a systematic framework to rapidly evaluate different process alternatives and thus enable major decisions making at very low cost in a speedup way. It identifies the steps within a process that are the

most critical operations to determine the process economy and product quality. That will also provide insights into how costs can be reduced and what affects final product quality and thus guiding future research and development efforts. A detailed separation process design for recovery of natural products can be very time consuming and laborious, while a rapid conceptual process design can screen different process alternatives within short period of time with less resources used (Douglas J. M, 1988). A detailed description of manufacturing process design for phytochemicals along with heuristics is given by Harjo et al., while the effect of different process variables on the efficiency of different stand alone separation processes is described by Ndocko et al. However, there is scarcity of information on determination of synergistic effects between various separation processes used in combination for recovery of natural products.

The objective of this work is to perform a real case study to recover the target compound artemisinin from *A. annua*, which aims at providing a generalized model to evaluate different separation process alternatives for recovery of natural products. The methodology is expected to fit in the majority of natural product recovery problems. In the present work, the methodology is developed and formulated through evaluating different process alternatives for recovery of artemisinin from dried leaves of *A. annua*. The focus is on the interaction and synergistic effect between the chromatography and crystallization separation operations.

2. The methodology

The basic structure of the process model for isolation and purification of natural products is shown in Fig. 1. The task is divided into two subtasks of isolating the target compound from its source and then purification of the target compound from complex

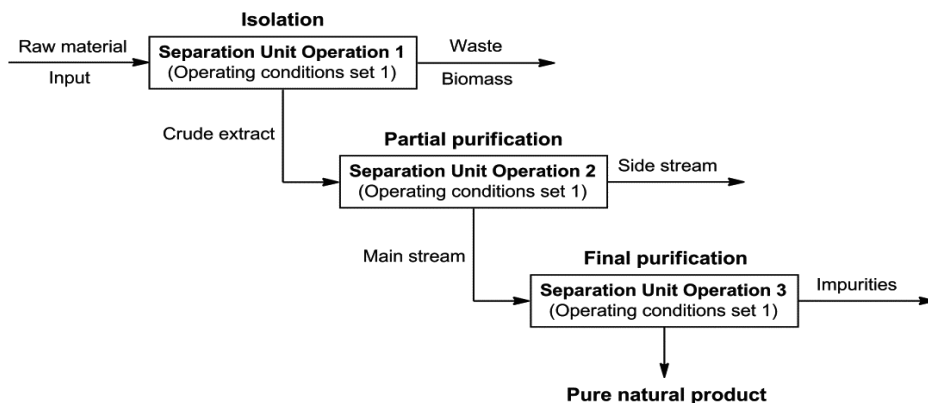


Figure 1. Basic process structure for isolation and purification of natural products.

crude extract. This methodology also emphasizes the use of process analytical technology (PAT) to analyze the process streams at various stages of the process in order to collect information about critical process parameters, which affects critical quality attributes as well as the overall design of the process.

2.1. Isolation

The process begins with the task of isolating target compound from the biomass designated by separation unit operation 1 (Fig. 1). Separation unit operation 1 can be represented by one of the numerous industrial applied separation techniques. A

comprehensive review of the separation techniques used to isolate natural products is given by Sarkar et al. The decision to pick up the best suitable one and selection of optimal operating conditions is product specific and depends upon many factors like amount of target compound present in the biomass, distribution of target compound in the source, final product specifications and also the physico-chemical properties of the target compound.

2.2. Purification

Taking into consideration the complex nature of the crude extract obtained from the previous step, the use of combined separation techniques is suggested for the purification of the target compound. In practice, high purity natural products can be obtained by highly efficient stand alone separation techniques like preparative high performance liquid chromatography (HPLC), but the cost of such process is always high. The present methodology suggests the use of preparative separation methods with less operating cost compared to, e.g. HPLC, to only partial purification of target compound combined with final purification by using cost effective techniques like crystallization. In this way, feasible process alternatives can be generated by employing different separation techniques with different arrangements in a sequence to achieve the tasks of isolation and purification of the target compound.

3. Case study of artemisinin from *Artemisia annua*

The proposed methodology is applied to generate process flow sheet alternatives to isolate and purify an anti-malarial drug, artemisinin from dried leaves of *A. annua*. In the present work, one such process flow sheet is evaluated in regard to the synergistic effect between chromatography and crystallization separation techniques. The basic process flow structure is shown in Fig. 2. Three major separation unit operations are used: solid-liquid extraction to isolate artemisinin into the crude extract, flash column chromatography (CC) to fractionate the crude extract, and crystallization to recover and purify artemisinin from the fractions of the crude extract.

3.1. Solid-Liquid Extraction

Artemisinin is extracted from the dried leaves of *A. annua* with dichloromethane (DCM) using maceration technique. DCM is chosen as the extraction solvent mainly due to the high solubility of artemisinin in DCM combined with its low boiling point that facilitates easy recovery of the solvent. The extraction procedure included immersion of 150 g of dried leaves containing 2.08% w/w artemisinin into 1 L of DCM followed by filtration after 5 hrs. The procedure is repeated one more time with 1 L of DCM. The combined DCM extract (2 L) is evaporated to obtain 12.5 g of crude extract. The concentration of artemisinin in the crude extract is determined by analytical HPLC–Charged Aerosol Detection (CAD). The yield of the extraction process in terms of artemisinin recovery using this extraction method is 90%.

3.2. Flash Column Chromatography

The crude extract obtained in the previous step is partially purified using flash CC to obtain artemisinin rich fractions with less number of other components. 15 g of crude extract is separated on a 7 cm diameter column filled with normal phase silica. Adsorbent (silica gel) to solute (crude extract) ratio of 20:1 is used. Gradient type of elution is used to run the column under the applied pressure. The eluent was selected based on the results of solvent screening by thin-layer chromatography (TLC). Column conditioned with 100% *n*-hexane and the gradient starts with 100% *n*-hexane followed by 10% stepwise gradient from 100 % *n*-hexane to 100% ethyl acetate.

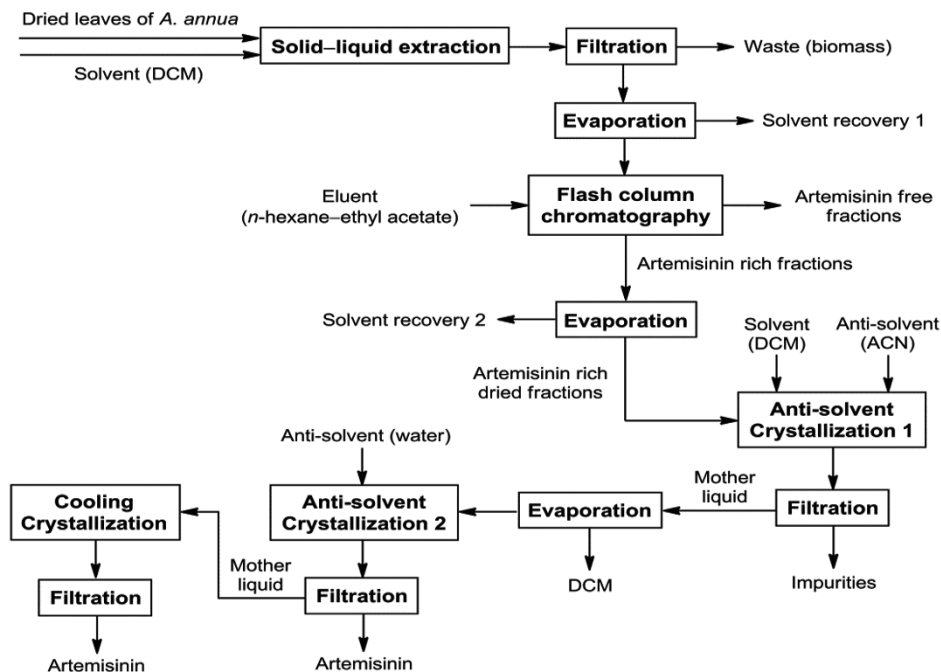


Figure 2. Process flow sheet for isolation and purification of artemisinin from dried leaves of *A. annua*. (DCM: dichloromethane, ACN: acetonitrile)

Each gradient of 900 mL is used and the size of fractions collected is 100 mL. Collected fractions were analyzed by TLC to identify the artemisinin rich fractions and these were then analyzed by HPLC-CAD for quantification of artemisinin. The results of this analysis are shown in Fig. 3.

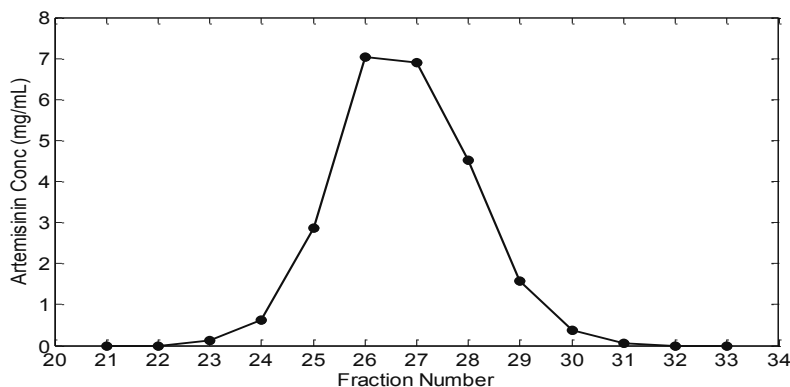


Figure 3. Concentration profile of artemisinin in fractions obtained by flash CC.

3.3. Crystallization

The artemisinin containing fractions obtained in the previous step are purified further by using a three-step crystallization process as shown in Fig. 2. The two-step anti-solvent crystallization process has been reported in our previous work by Qu et al. The artemisinin rich fractions 25-28 are processed individually, whereas fractions 23-24 and

29-31 are combined together to form two fractions. However, no artemisinin is crystallized out from these combined fractions because of the low concentration of artemisinin. Yield and purity of artemisinin crystals obtained from each fraction are shown in Table 1. Artemisinin is crystallized from fractions 25-28 with varying purity and yield. One remarkable observation from Table 1 is that the yield of artemisinin from fraction 25 is much higher than that from fractions 26, 27, and 28; regardless of the higher artemisinin concentration in the fractions 26-28. This fraction wise variation in purity and yield of artemisinin may be attributed to the associating compounds present in the fractions, which might be affecting the solubility of artemisinin and furthermore some of which may have crystallization behavior similar to artemisinin during the anti-solvent crystallization. FT-Raman spectroscopy measurement confirmed that thermodynamically stable orthorhombic polymorph of artemisinin has been obtained from all fractions.

Table 1: Purity and yield of artemisinin obtained from fractions during crystallization process.

| Fraction No. | Artemisinin purity | | Yield (%) |
|--------------|-----------------------------------|-----------------------------|-----------|
| | Anti-solvent Crystallization2 (%) | Cooling Crystallization (%) | |
| 25 | 91.37 | 100 | 87.4 |
| 26 | 84.85 | 92.35 | 45.8 |
| 27 | 100 | 100 | 64.0 |
| 28 | 66 | 100 | 28.8 |

4. Conclusion

The systematic method for synthesis of conceptual processes for isolation and purification of natural products from plants is investigated. A case study for recovery of artemisinin from dried leaves of *A. annua* is performed. Three major separation techniques of maceration extraction, flash CC and crystallization are selected to formulate the process flow sheet. Lab scale experiments are studied to confirm and understand the effectiveness of the separation methods. To do so, PAT is used in each step to get the necessary information and data of the process. The results showed that the formulated process flow sheet is feasible to isolate and purify the target compound artemisinin from *A. annua*. One outstanding observation is that the associating compounds in the fractions have significant effects to the purity and yield of the target compound artemisinin during crystallization steps. This indicated that seeking synergy between chromatography and crystallization to obtain optimal hybrid separation process is the key direction for commercial manufacturing process of artemisinin from *A. annua*. Effort in this direction is underway.

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