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Systematic synthesis of production process for the isolation and separation of bio-products from plants

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Natural products can be a tremendous primary source for modern drug discovery. However, the isolation and purification of the target product from plants represents one of the most challenging tasks, which require the knowledge both at the molecular level and at the process level. On the one hand, there might be hundreds of compounds extracted from plants together with the target product; on the other hand, many of the compounds contained in the crude extracts may have similar chemical structures as the target compound. Therefore, it is a quite complex and challenging task to develop and synthesize the production process to produce the final target compound from the raw materials. One needs to identify the necessary unit operations and interconnect and integrate them in a systematic way to synthesize the whole production process. At the same time, one needs to optimize the production process at both the unit level for the unit operations and the system level for the whole process system to pursue the multi-objective optimised solution.

In this work, a systematic synthesis method was proposed to generate a production process for the isolation and separation of bio-products from plants. The synthesis method of such processes consists of the following four steps:

1. Identification of the separation task and determination of the basic structure of the process.
2. Collection of the physical properties of the target compound and decide the preliminary operation parameters of the process.
3. Identification of the performance-limiting components for the crystallization process.
4. Optimization of the synergistic effect of chromatography and crystallization processes.

The proposed synthesis method was applied to the design and development of the separation process for the isolation and purification of artemisinin which is the most effective antimalarial drug from the medicinal herb *Artemisia annua*. Extraction of the aerial parts of *A. annua* by dichloromethane (DCM) yielded a complex mixture of natural products of which many have chemical structures very similar to artemisinin, such as artemisinic acid, arteannuin B, artemisitene, deoxyartemisinin and dihydroartemisinin, and hence similar chemical and chromatographic characteristics as artemisinin. Therefore, the isolation of artemisinin in high purity is very challenging. Instead of using a single separation method, a hybrid chromatography-crystallization separation process is proposed in the present work. Chromatography is capable of fractionating a very complex mixture containing hundreds of compounds. Crystallization is appropriate for the isolation of a product with high purity from a multi-components mixture. The hybrid between chromatography and crystallization can have a synergistic effect, which may lead to an improved recovery and productivity.

After the basic structure of the separation process has been decided, the physical properties of the target compound artemisinin were collected in step 2. The solubility of pure artemisinin in various organic solvents was measured at room temperature. The two structurally similar solvents, DCM and chloroform, provided remarkably high solubility of artemisinin, which was about 4 times higher than the ideal solubility of artemisinin (5.61 mol %). The solubility of artemisinin in acetone, ethyl acetate and acetonitrile is close to the ideal

solubility of artemisinin. The alcohols (ethanol, methanol, 1-butanol, 1-propanol) and hexane provided low solubility for artemisinin probably due to their different polarity compared to the solute. The solubility of artemisinin in binary solvent mixtures of hexane–ethanol, ethanol–water, ethyl acetate–acetonitrile, and acetonitrile–water was investigated. It has been observed that the solubility profiles exhibit one maximum in the solvent mixtures with relatively low polarity, and the solvent solubility parameter corresponding to this maximum solubility is between 19–21 $MPa^{1/2}$ regardless of the nature of the component solvents. For the mixtures of ethanol–water and acetonitrile–water, the solubility of artemisinin remarkably decreased with increasing water concentration, which suggested the feasibility of performing an anti-solvent crystallization of artemisinin by using water as the anti-solvent. Artemisinin is capable of forming two different polymorphs, the orthorhombic form is considered as the thermodynamically stable form at room temperature, and the triclinic form is the metastable one. The anti-solvent crystallization of artemisinin was performed from acetonitrile solution by using water as the anti-solvent. It was observed that the orthorhombic form always crystallized out regardless of the feeding rate of the anti-solvent. Based on the solubility of artemisinin in different solvents and solvents mixtures, and the crystallization behaviour of the polymorphs of artemisinin, it was decided that DCM was used as the solvent for the plant extraction and as the eluent for column chromatography (CC). After separation by CC, a two-step anti-solvent crystallization process was used to produce artemisinin crystals.

The eluate from CC was analyzed with thin layer chromatography (TLC), and the fractions, which were rich in artemisinin and containing the same components, were combined and the composition of the combined fractions was analyzed by high performance liquid chromatography (HPLC). It was observed that all the combined fractions are complex and contain several other compounds besides artemisinin. A two-step crystallization process was utilized to isolate artemisinin. The first step crystallization was performed by feeding acetonitrile to the DCM solution, which caused the precipitation of a white powder that was identified as 1-nonadecanol by ^{1}H and ^{13}C NMR spectroscopy. The second step anti-solvent crystallization was induced by feeding water to the acetonitrile solution. Artemisinin crystals were produced from this step, and were further purified by re-crystallization. The purity of these products was determined by HPLC (Fig. 1).

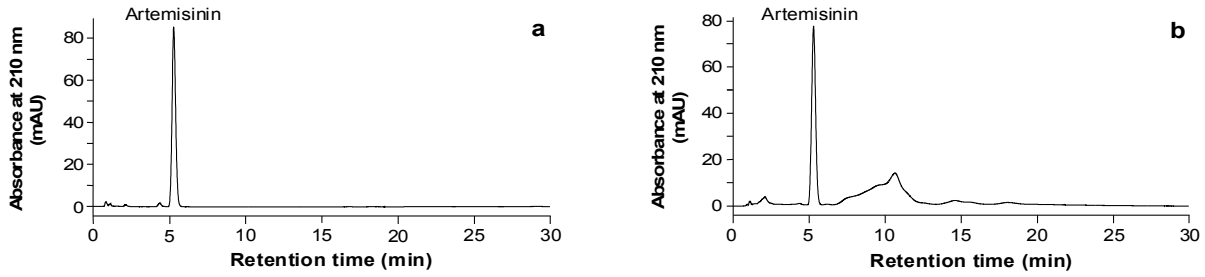

Fig. 1 HPLC chromatogram of (**a**) artemisinin from the supplier (purity 99%), (**b**) artemisinin purified by two times re-crystallization (purity 90%).

It has been observed that most of the compounds co-existing with artemisinin in the CC fractions can be removed by the re-crystallization. However, the compound with a retention time of 10.5 min was difficult to remove and it turned out to be the main impurity in the re-crystallized crystals. This observation possibly indicated that this compound has similar solubility characteristics as artemisinin in acetonitrile–water mixtures and the complete separation of this main impurity need to be done in the CC separation process, possibly by optimizing the operation conditions of CC, such as using an appropriate gradient elution. Research to this direction is underway.