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Concentrical coils counter-current chromatography for natural products isolation: *Salvia miltiorrhiza* Bunge as example☆

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ABSTRACT

Countercurrent chromatography (CCC) is an efficient separation technique without the solid support matrix, largely depending on the partition of two-immiscible liquid phases in the separation column. Since the helical coil planet centrifuge was invented in early 1970s by Yoichiro Ito, a series of coils columns, including spiral coils and conical coils columns have been developed for CCC separation. In this work, we introduced a new simple and efficient concentrical coils column for CCC separation, which was prepared by winding the whole polytetrafluoroethylene (PTFE) tube into the circular grooves from the rotation axis in the same direction. Once the PTFE tube filled in all space of one round of the circular groove, it was jumped into the nearby outer circular groove through the gap and until the whole groove was filled. The three same concentrical coils distributed on three disc-shaped holders were connected by the same PTFE tube to form concentrical coils separation column. The separation capacity was further investigated using ten tanshinones of the extracts of a Traditional Chinese Medicine *Salvia miltiorrhiza* Bunge as a model natural product. All results indicated that the concentrical coils column could hold satisfactory retention of the stationary phase, higher theoretical plate number and better resolution for CCC separation of more than ten tanshinones. It may be an alternative CCC column for non-targeted and targeted isolation of bioactive natural products.

1. Instruction

Counter-current chromatography (CCC) employs highly efficient fractionation by a hybrid technique of liquid–liquid distribution and liquid chromatography to rapidly separate and purify compounds [1]. Since the method does not employ a solid support matrix, CCC has several distinctive advantages over the traditional liquid–solid separation methods [2]. For example, it eliminates some complications, such as adsorptive sample loss, deactivation, the tailing of solute peaks and contamination. In addition, CCC is easy to couple with other online separation techniques [3]. Therefore, there is an increasing interest in application of CCC to analyze and prepare various natural and synthetic products [2,4–6], especially some high polar and unstable compounds.

Since the pioneer work of Yoichiro Ito was done in the early 1970s [7,8], many types of CCC instruments and methods have been developed, such as types I, J, cross-axis and non-synchronized CCC apparatus [9,10]. Usually, CCC column was wound with helical coils and distributed on the cylinder holder in type I and type J flow-through CPC, multi-layer CPC, eccentric multi-layer CPC, high-speed CCC, cross-axis CPC, low-speed CCC, and even high-performance CCC developed recently [11,12]. Helical coils could provide the stable axial driving force and attain the unilateral hydrodynamic equilibrium in the coiled tube.

After that, spiral coils or disks assembly [13,14] were found to improve the retention of the stationary phase and enhance the partition efficiency for high-speed [13–16] and low-speed CCC [17] in the isolation of peptides and proteins. More recently, we built conical coils to form centrifugal force gradients both in axial and radial directions for CCC [18,19]. It not only put CCC column in a two-dimensional centrifugal field, but also provided a potential centrifugal force gradient both in axial and radial directions. Compared with helical and spiral coils CCC, the extra centrifugal gradient in conical coils allowed the mobile phase move faster and enabled CCC have much higher retention of stationary phase, higher theoretical plate number and better resolution. However, these coil columns were not full symmetrical and it is still hardly to fabricate and keep machine balance.

In this work, we introduced a simple concentrical coils column for CCC separation. All concentrical coils were wound in the cir-

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cular grooves and hold on the disc-type column holder for better balance. Further, we selected the extract of the rhizome of *Salvia miltiorrhiza* Bunge as a model natural product, which is a famous Traditional Chinese Medicine and has potent anti-bacterial, anti-oxidant, and anti-neoplastic activities [20]. As results, more than ten tanshinones could be well isolated and purified by the concentrical coil CCC.

2. Experimental

2.1. CCC apparatus

Similar to the previous upright three-channel type-J CCC device [21], concentrical coils CCC apparatus was fabricated as shown in Fig. 1A and Fig. 1B. It held three identical disc-shaped holders (diameter, 160 mm) replacing the previous cylindrical column holders, and their revolution radius was 10 cm. On each disc-shaped holder, there were five well-distributed circular grooves (3.3 mm width and 5 mm depth) ranging from an inner diameter of 90.4 mm to an outer diameter of 145 mm (Fig. 1C and D). Each concentrical coiled separation column was prepared by a long polytetrafluoroethylene (PTFE, Great Wall Fluorine Plastic Mechanical and Electrical Market, Hangzhou, China) tube (1.8 mm i.d. and 0.6 mm wall thickness), which was first fitted into the innermost circular groove and wound in the left-handed direction from the rotation axis. Once the PTFE tube filled the space of one round of the circular groove, it was drawn into the nearby outer circular groove through the gap and wound in the same direction (Fig. 1F). When PTFE filled the space of the outermost groove, it was drawn into the nearby inner groove through gap and wound in the same direction (Fig. 1G). Then, the PTFE tube was repeatedly wound in the same mode until the whole disc groove was filled to form a concentrical coils column (capacity, 80 mL; β, 0.5–0.75). After the concentrical coils were well connected with the axis connectors and all coiled PTFE tubes were well fixated by stuffing bulking agent, the disc-type column holder was covered with a cover (Fig. 1E) to form a balanced column with concentrical coils (Fig. 1H). Three same concentrical coils distributed on three disc-shape holders were connected by the same PTFE tube making a total 260 mL of column volume. The volume of

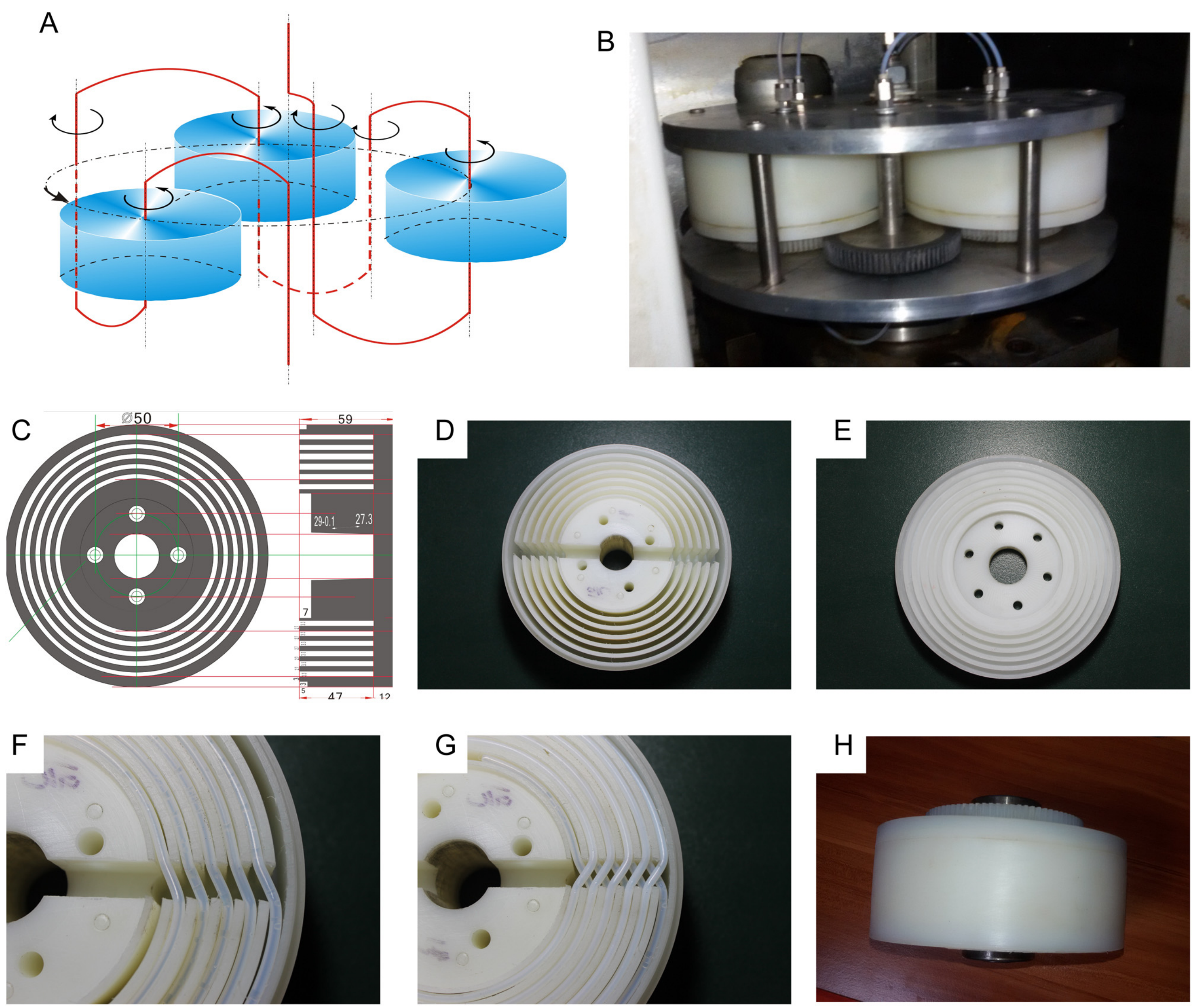

Fig. 1. The principles of (A) instrument and (C) column design and (B, D-H) instrumental photos of concentric CCC, (A) Principles of instrument, (B) The photograph of fabricated concentric coils CCC apparatus. (C) The diagrammatic figure of cross section and the vertical section of disc-shaped holders. (D) The picture of real disc-shaped holder with five well-distributed circular grooves. (E) The cover of disc-type column. (F) – (G), the ways of polytetrafluoroethylene (PTFE) tube wound in the circular groove in the left-handed direction from the rotation axis. (H) The disc-type column holder with concentric coils.