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Review

Dual counter-current chromatography —its applications in natural products research

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ABSTRACT

Dual counter-current chromatography is a newly developed separation method which allows the performance of classic counter-current distribution in a highly efficient manner. The principles of CCC and its capabilities, as illustrated by several applications in natural products isolations, are reviewed.

CONTENTS

1. INTRODUCTION

The development in the 1980s of modern counter-current chromatography (CCC) based upon the fundamental principles of liquid–liquid partition has caused a resurgence of interest in the separation sciences. The advantage of applying continuous liquid–liquid extraction, a process for separating of a multicomponent mixture according to differential solubility of each component in two immiscible solvents have long been recognized. In spite of the limitations of the traditional counter-current distribution methods which prevailed in the 1950s and 1960s [1], liquid–liquid partition was used succesfully to fractionate commercial insulin into two subfractions differing by only one amide group in a molecular weight of 6000 [1].

In recent years, significant improvements have been made to enhance the performance and efficiency of liquid–liquid partitioning [2–8]. The high-speed CCC tech-

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nique utilizes a particular combination of coil orientation and planetary motion to produce a unique hydrodynamic, unilateral phase distribution of two immiscible solvents in a coiled column. These hydrodynamic properties can effectively be applied to perform a variety of liquid–liquid partition chromatographies including high-speed CCC (HSCCC) [2], foam CCC [8,9] and dual CCC (DuCCC) [10,11]. The name of DuCCC is redundant, however, it is useful to distinguish between HSCCC which ought to be called high-speed epicyclic partition chromatography, because only one solvent phase is mobile and the other solvent phase is stationary [2]. Strictly speaking, DuCCC should have two solvents counter-crossing each other from opposite directions and there is no stationary phase involved.

DuCCC has several advantages which are common to many types of liquid–liquid partition chromatography, such as no limitation of the two phase solvent system which can be employed and no sample losses due to irreversible adsorption or decomposition on the solid support. In addition, DuCCC is extremely powerful in separation of crude natural products which usually consist of multicomponents with a wide range of polarities. In a standard operation, the crude sample is fed through the middel portion of the column. The extreme polar and non-polar components are readily eluted from the opposite ends of the column followed by components with decreasing orders of polarity in one phase solvent and increasing order of polarity in the other phase solvent. A component with a partition coefficient equal to 1 will remain inside the coiled column. Essentially, the DuCCC permits a highly efficient performance of classic counter-current distribution.

Below, the principles, instrumentation of DuCCC, and its capabilities in natural products isolation are reviewed.

2. PRINCIPLES AND MECHANISM

The fundamental principle of separation for modern DuCCC is identical to classic countercurrent distribution. It is based on the differential partitions of a multicomponent mixture between two counter-crossing and immiscible solvents. In general, the crude sample is applied to the middle of the coiled column and the extreme polar and non-polar components are readily eluted by two immiscible solvents to

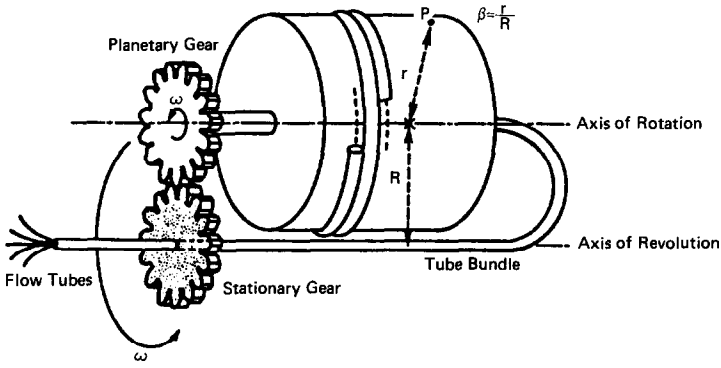

Fig. 1. Epicyclic rotation of DuCCC column holder.

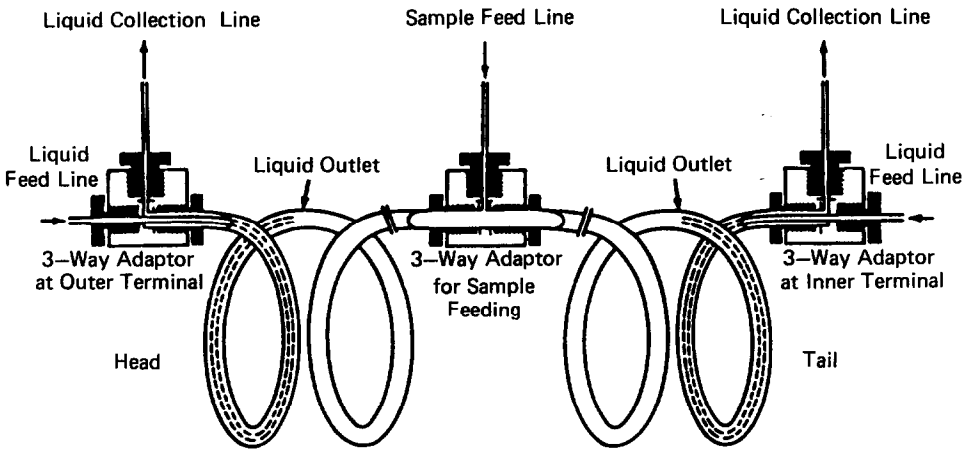

Fig. 2. Column design for DuCCC.

opposite outlets of the column and the component(s) with partition coefficient(s) of 1 will remain in the column. However, in comparison to classic countercurrent distribution, modern DuCCC allows the entire operation to be carried out in a continuous and highly efficient manner. Modern DuCCC is based on the ingenious design of Ito [8] and is illustrated in Figs. 1 and 2. In Fig. 1, a cylindrical coil holder is equipped with a planetary gear which is coupled to an identical stationary sun gear (shaded) placed around the central axis of the centrifuge. This gear arrangement produces an epicyclic motion; the holder rotates about its own axis relative to the rotating frame and simultaneously revolves around the central axis of the centrifuge at the same angular velocity as indicated by the pair of arrows. This epicyclic rotation of the holder is necessary to unwind the twist of the five flow tubes caused by the reovlution, eliminating the use of rotary seals to connect each flow tube. This unique design enables the performance of DuCCC using five flow channels connected directly to the column, as shown in Fig. 2. When a column with a particular coil orientation is subjected to a epicyclic rotation, it produces a unique hydrodynamic phenomenon in the coiled column, in which one phase entirely occupies the head side and the other phase occupies the tail side of the coil column. This unilateral phase distribution enables the performance of DuCCC in an efficient manner. A theoretical calculation of the hydrodynamic forces resulting from such an epicyclic rotation is very complicated and has not yet been elucidated.

3. METHODS AND APPARATUS

The DuCCC experiments are performed with a table top model high-speed planet centrifuge equipped with a multilayer coiled column connected to five flow channels. The multilayer coiled column is prepared from 2.6 mm I.D. PTFE tubing by winding it coaxially onto the holder to a total volume capacity of 400 ml. The multilayer coiled column is subjected to an epicyclic rotation at 500–800 rpm. The fractions are collected simultaneously from both ends of the column and analyzed by

thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC) [8,12].

4. APPLICATIONS

In the past decade, the rapid development of sophisticated spectrosocpic techniques, including various 2D-NMR methods, automated instrumentation and routine availability of X-ray crystallography has greatly simplified structural elucidation in natural products investigations. Consequently, the challenge to today's chemists has shifted to one's capability of isolating the bioactive components from crude extracts of either plants or animals. The extract of crude natural products usually is comprised of hundreds of components over a wide range of polarities. In isolating these natural products, it is essential to preserve the biological activity while performing chromatographic purifications. DuCCC represents one of the mild methods of isolation.

DuCCC has several advantages over HSCCC or centrifugal partition chromatography (CPC) [13] in dealing with crude natural products. One distinct feature of DuCCC is the capability of performing normal-phase and reversed-phase elutions simultaneously. This provides a highly efficient method which cannot otherwise be achieved easily in the separation of crude natural products. In many instances, fractions eluted from DuCCC are pure enough for recrystallization or structural study. For example, Fig. 3 shows an analytical HPLC trace of the crude ethanol extract of *Schisandra rubriflora*. Because the major bioactive lignan, schisanhenol 6 is closely eluted with its acetate 5, it has been a major problem to isolate the pure lignan 6. The fractions collected from DuCCC after injection of a crude ethanol extract of *S. rubriflora* (125 mg) were analyzed by TLC (Fig. 4). Fig. 5 shows the reversed-phase HPLC

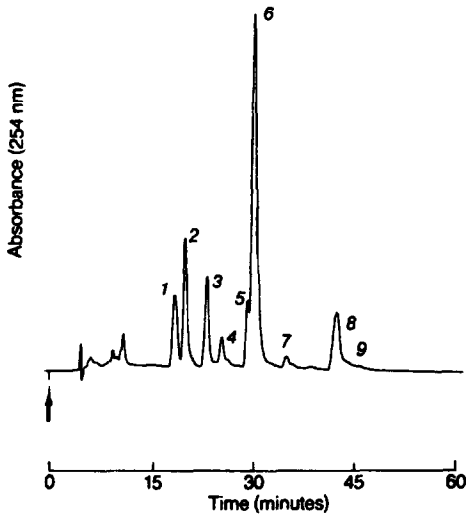

Fig. 3. Analytical HPLC trace of crude ethanol extract from *Schisandra rubriflora*. Column: Zorbax ODS 250 × 4.6 mm I.D.; detection: UV, 254 nm; mobile phase: methanol–water (75:25); sample: ethanolic extract of the kernels of *Schisandra rubriflora* Rhed et Wils. Peaks: 1 = wuweizisu C; 2 = (−)-rubschisandrin; 3 = rubschisantherin; 4 = deoxyschisandrin; 5 = schisanhenol acetate; 6 = schisanhenol; 7 = schisanhanol B; 8 = gomisin O; 9 = pregomisin.

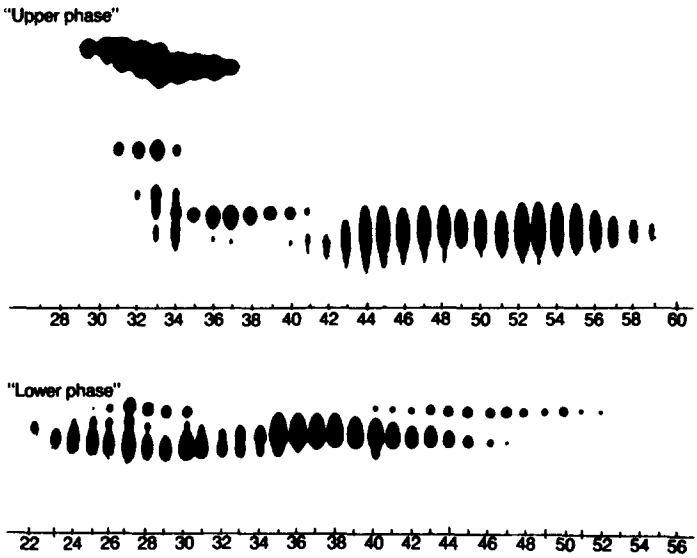

Fig. 4. DuCCC of crude ethanol extract from *Schisandra rubriflora*. Solvent system: hexane–ethyl acetate–methanol–water (10:5:5:1); flow-rate: 2 ml/min; sample crude extract of *Schisandra rubriflora* Rhed et Wils, 125 mg. Detection: TLC, acetone–dichloromethane (5:95). Numbers in figure are fraction Nos.

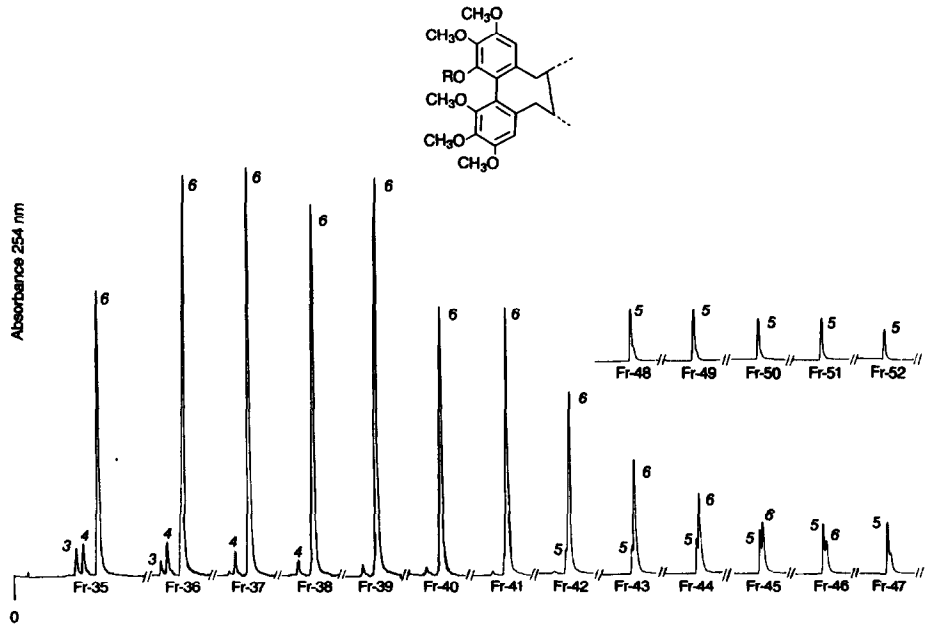

Fig. 5. HPLC traces of the fractions from DuCCC. Column: Zorbax ODS, 254 × 4.6 mm I.D.; detector: UV, 254 nm; mobile phase: methanol–water (75:25); sample: fractions from true counter-current chromatography. 5, R = CH_3CO; 6, R = H. Fr = fraction.

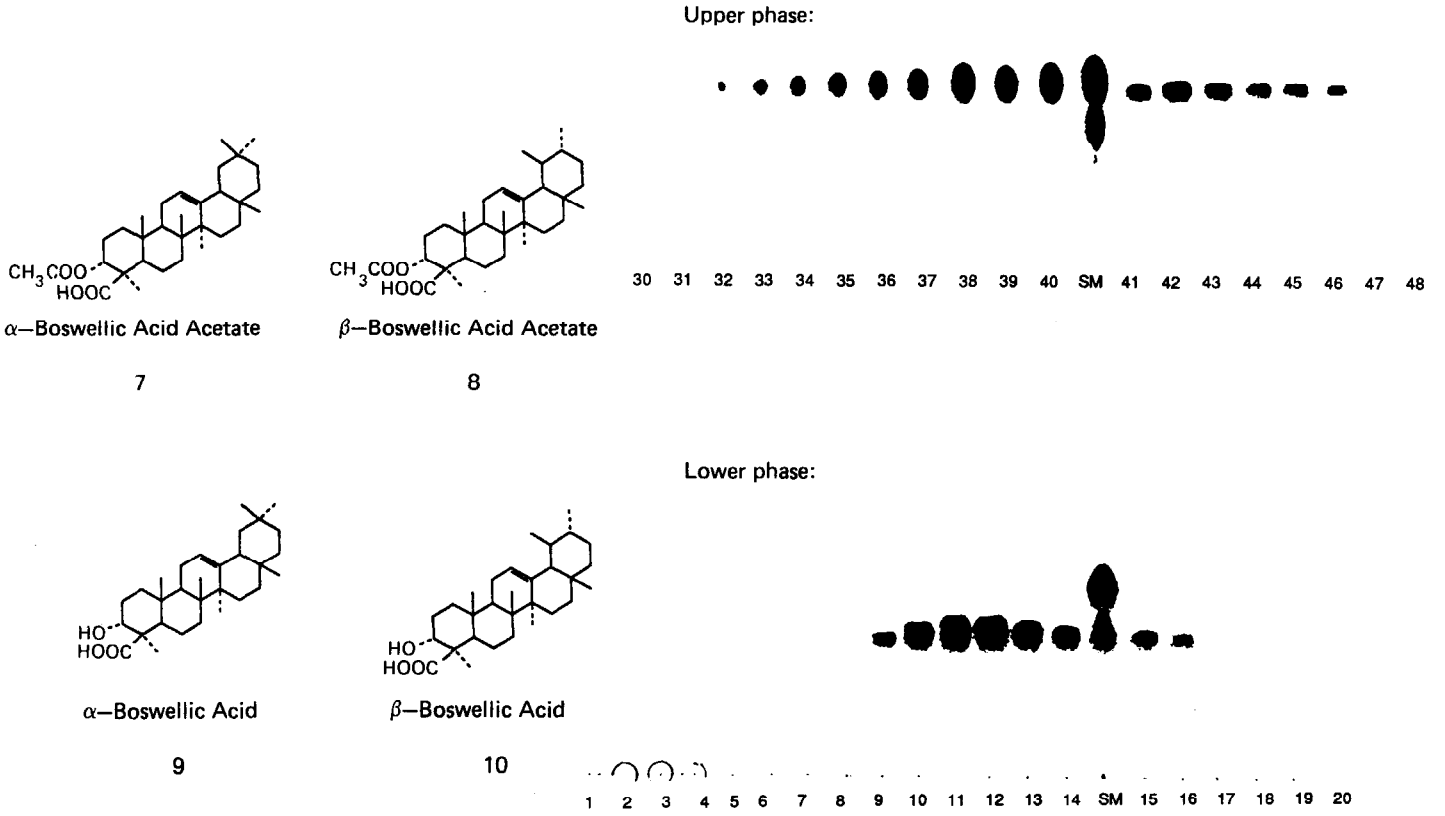

Fig. 6. DuCCC of triterpenoic acids. Sample: mixture of *Boswellic carterii* extract. (400 mg); solvent system: hexane–ethanol–water (6:5:1); flow-rate: upper phase, 2.0 ml/min; lower phase, 2.0 ml/min; rotational speed: 800 rpm; detection: TLC. SM = Starting mixture. Numbers in DuCCC are fraction Nos.

analyses of DuCCC fractions eluted from the lower phase. The solvent system employed for DuCCC was hexane–ethyl acetate–methanol–water (10:5:5:1). The upper phase, being less polar than the lower phase, results in a sequence of elution similar to normal-phase chromatography, while the lower phase provides a sequence of elution resembling reversed-phase chromatography. The bioactive components, schisanhenol acetate 5 and schisanhenol 6, were eluted in the lower phase. Reversed-phase HPLC analyses of fractions 36 to 40 accounted for 32 mg of almost pure schisanhenol 6. A total of 4 mg of schisanhenol acetate 5 was also obtained from fractions 50 to 57. As evidenced by this experiment, DuCCC offers an excellent method for semi-preparative isolation of bioactive components from very crude natural products [11].

The isolation of the topoisomerases inhibitor, boswellic acid acetate from its triterpenoic acid mixture has also been acomplished by DuCCC [12]. As shown in Fig. 6, when an isomeric mixture of triterpenoic acids (400 mg) was subjected to DuCCC, using a hexane–ethanol–water (6:5:1:) as the solvent system, provided 215 mg of the boswellic acid acetes (7 + 8, α + β isomer) and 135 mg of the corresponding boswellic acid (9 + 10, α + β isomer). Some highly polar impurities were eluted immediately in the solvent front, from fraction 1 to 4. The isomeric boswellic acids (7 + 8) were eluted in the lower phase solvent and the less polar acetates (9 + 10) were eluted simultaneously in the upper phase solvent. Although the α and β isomers were only partially resolved by DuCCC, this experiment demonstrates that DuCCC is a highly efficient system for preparative purification.

5. CONCLUSION

The capability and efficiency of DuCCC in performing classic counter-current distribution has been demonstrated in the isolation of bioactive lignans and triterpenoic acids from crude natural products. Besides the resolution and sample loading capacity offered by DuCCC, the unique feature of elution the non-polar components in the upper phase solvent (assuming upper phase is less polar than the lower phase) and concomitant elution of the polar components in the lower phase results in an efficient and convenient preparative method for purification of crude natural products.

The capability of DuCCC has not yet been fully explored. For instance, a particular solvent system can be sclected to give the desire bioactive component a partition coefficient of n1. This will allow the "stripping" of the crude extract with DuCCC to remove the impurities or inactive components. Consequently, the bioactive component will be concentration inside the column for subsequent collection. This strategy can also be applied to extract and concentrate certin metabolites in the biological fluids such as urine or plasma. A large amount of sample can also be processed by DuCCC because there is no saturation of the stationary phase.

DuCCC needs more studies in order to explore its capability in isolation of not only natural products but also biological macromolecules.

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