Recent trends in counter-current chromatography

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Counter-current chromatography (CCC) has been widely used as a preparative chromatography technique since Ito first developed it in the late 1960s. This technique uses a support-free liquid stationary phase, which is retained by gravity or centrifugal force. The analytes can access the whole stationary phase, leading to effective separation.

In the past few decades, high-speed CCC has been a versatile preparative-scale separation technique, especially in isolation of natural products.

The aim of this review is to describe different aspects of recent interesting applications in CCC, including instrumentation, solvent selection and elution methods.

Keywords: Counter-current chromatography (CCC); Elution mode; Elution-extrusion counter-current chromatography (EECCC); High-speed counter-current chromatography (HSCCC); Instrumentation; Natural product; Preparative chromatography; Separation; Solvent selection; Stationary phase

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1. Introduction

As an integral part of analytical chemistry, liquid chromatography (LC) has been widely used as a separation tool. Counter-current chromatography (CCC) is a branch of LC, which relies on the partition of the solutes between the two immiscible solvent phases. The original model of this technique came from counter-current distribution (CCD), which was invented by Craig in the 1940s [1,2], when CCD was widely applied to separation of natural products. However, time-consuming, complex instrument construction and the large amount of solvent consumption restrained further development of the technique.

On the basis of CCD, an improved separation technology, called CCC, was developed by Ito in the 1970s [3]. The separation procedure and instrument construction of CCC were much simpler than those of CCD. Since the 1970s, more people have chosen CCC as a preparative or semi-preparative separation tool to deal with comprehensive mixtures (e.g., natural products) [4,5]. Today, a SciFinder survey with the search term ''counter-current chromatography'' lists more than eight times as many articles in 2011 as there were in 2000, which reflects the development of the CCC technique.

As the task of separation processes is to decrease the complexity of mixtures, there is always a need for a convenient high-throughput technique with minimal sample loss, high efficiency, high resolution, and ease of sample recovery without contamination. Compared with the conventional chromatographies [e.g., thin-layer chromatography (TLC), silica-gel column chromatography and high-performance LC (HPLC) which utilizes a solid stationary phase and a liquid mobile phase], both stationary and mobile phases are liquids in CCC. CCC therefore benefits from great advantages compared to traditional solid-LC. It can eliminate the complications resulting from the solid matrix (e.g., irreversible adsorption sample loss and deactivation). Besides, the retention volumes of the analytes can be calculated using a simple formula. The target effluents can be fixed in advance by the results of this calculation. Extracted material is always easily dissolved in an optional solvent system, which is able to cope with crude extracts without any pre-conditioning. The operating costs are reduced by simplifying processing steps, decreasing solvent consumption, and dispensing with expensive solid-support materials. Though the theoretical plate numbers of CCC are lower than those of HPLC, the unique

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features of liquid stationary phase and ease of scale-up make CCC complementary to HPLC. Due to the above advantages, CCC has been considered a powerful preparative method to separate peptides [6–11], pharmaceutical drugs [12,13], chiral compounds [14–17], and natural products [18–25].

In the past 40 years, there have been a number of publications including monographs [26–31], encyclopedia [32–34] and review articles [4,35], together with a great number of research articles on CCC in scientific journals. The purpose of this review is to let people know more about the powerful technique of CCC. This review covers recent progress in CCC in instrumentation, solvent selection, and modes of elution.

2. Instrumentation

The improvement in analytical instruments represents the development of the corresponding analytical technique to some extent. To develop better instruments has been a continuing trend in analytical chemistry, as the demand of analytical-chemistry assignments has become more critical. As the main part of CCC, the centrifuge plays an important role in CCC separation. Commercial CCC instruments can be divided into two systems:

(1) hydrostatic centrifugal partition chromatography apparatus (commonly known as CPC) using a constant-gravity field produced by a single-axis rotation mechanism and two rotary-seal joints for inlet and outlet of the mobile phase; and,
(2) hydrodynamic coil planet centrifuge using a variable-gravity field produced by two-axis rotation mechanism (like planetary motion) and a rotary seal-free arrangement for the column.

CPC instruments are generally more effective at retaining the solvent system with little density difference between the two phases (e.g., aqueous-aqueous systems). Polar compounds (e.g., proteins and polypeptides) generally separate better using CPC than hydrodynamic centrifuge. The hydrodynamic centrifuge usually has higher retention of the stationary phase, resulting in better peak resolution. Besides, the continuous flow paths allow for unprepared crude samples or even raw samples in suspension. CCC users can choose different types of instrument for different purposes.

In recent years, there have been some breakthroughs in development and improvement of CCC instruments, including integration of different separation techniques into one system, new rotary seal-free centrifuges and new column designs. More and more scientists have focused on biological sample analysis, and novel instrument designs were developed for this purpose in recent years.

Several designs of the CCC system sought to improve the separation efficiency at the analytical scale. The retention of the stationary phase is the critical parameter in CCC separation. The higher the retention of the stationary phase, the better is the peak resolution obtained. The most direct way to increase the retention of the stationary phase is to change the CCC-column construction. The hydrostatic CCC used to use a coiled column mounted around the periphery of the centrifuge bowl. The retention of the stationary phase is about 30% of the total column volume. In recent years, various configurations of the helical column were designed to study the influences on separation efficiency and chromatographic behaviors. First, a triangular helical column was designed to reduce the dead volume. Stationary-phase retention was increased to over 40% compared with about 30% in a typical system [36]. Then, a novel column design, a zigzag toroidal column, was developed to decrease the dead space and increase the peak resolution further [37]. The result indicated that the retention of stationary phase and peak resolution is considerably improved by changing the shape of the tube. In the subsequent study, geometric designs of tubing (plain, mid-clamping, flattened and flat, twisted) were evaluated for their performance in CCC. The plain and flattened tubing both had good performance in the zigzag toroidal column [38]. To reduce the dead space further, a configuration of the saw-tooth column was constructed with short radial segments and long slanted segments [39]. A figure-8 column was designed to improve the peak resolution and stationary-phase retention. The results indicated that the small figure size yielded better peak resolution at a higher flow rate [40]. All the above designs increased the retention of the stationary phase in different approaches. Besides, all these instruments could be applied in analytical separation. This promises that more efficient CCC instruments will be designed in the next 10 years.

In 1981, based on the coil planet centrifuge, high-speed CCC (HSCCC) was developed to shorten separation time from days to hours with high partition efficiency up to a few thousand theoretical plates. In the past, there was a limitation for HSCCC, which produced low resolution for the separation of polar compounds (e.g., peptides and proteins). The polar solvent system developed for separation usually cannot be well retained in the traditional HSCCC instrument. In order to cope with this problem, the spiral disk [41,11,42,43] assembly and the spiral tube assembly [6,44] were developed to enhance the retention of the stationary phase, and successfully applied to separation of dipeptides [6]. The partition efficiency was further improved by modifying the tubing [7], and it is effective, especially for protein separation using the polymer-phase system. It is a realistic improvement for separation of peptides and proteins by HSCCC.

Another interesting design in CCC was a novel non-synchronous centrifuge. The instrument was successfully constructed recently (see Fig. 1), and was more