



An overview of recent progress in elution mode of counter current chromatography



Xin-Yi Huang^{a,b}, Svetlana Ignatova^b, Peter Hewitson^b, Duo-Long Di^{a,*}

^a Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Science, Lanzhou 73000, China

^b Brunel Institute for Bioengineering, Brunel University, Uxbridge UB8 3PH, UK

ARTICLE INFO

Keywords:

Countercurrent chromatography
Elution mode
Gradient elution
Dual-mode elution
Multiple dual-mode elution
Recycling elution
Extrusion elution
Cocurrent elution
pH-zone-refining CCC

ABSTRACT

Countercurrent chromatography (CCC) is a developing chromatographic technique that achieves separation based on the distribution of the target in an immiscible biphasic or multiphasic solvent system. In the past decades, this technique has advanced greatly in application and theory. This overview is mainly focused on the development of elution modes, which have been easily achieved with the classical CCC apparatus in recent years. It includes gradient elution, dual-mode elution, multiple dual-mode elution, recycling elution, extrusion elution, cocurrent elution and pH zone refining. The basic principles of each elution mode are described in detail and then summarized. The comparison and the scope of application of these elution modes are also discussed.

© 2016 Elsevier B.V. All rights reserved.

Contents

1. Introduction	214
2. Advance in elution mode	215
2.1. Gradient elution	215
2.2. Dual-mode elution and multiple dual-mode elution	215
2.3. Recycling elution	218
2.4. Extrusion elution	218
2.5. Cocurrent elution	219
2.6. pH-zone-refining CCC	221
3. Summary	222
4. Conclusion	223
Acknowledgment	223
References	223

1. Introduction

Countercurrent chromatography (CCC) is a chromatographic separation and preparation technology that is based on the liquid–liquid partition coefficient of the solute, as no adsorptive matrix is used to retain the stationary phase [1]. The liquid stationary phase is retained in the column by a centrifugal force field, whereas the immiscible mobile phase passes through. Due to its free solid stationary

phase and continuous liquid–liquid partition design, CCC has many distinctive advantages over conventional chromatography techniques. CCC prevents the sample loss caused by irreversible adsorption and solute degeneration caused by surface chemistry. It can be directly applied to crude extracts, exhibiting sustained high efficiency, high recovery and low solvent consumption, as well as the ability to produce a large amount of the compound. In addition, CCC can be easily coupled with other online separation techniques [2–4]. Therefore, CCC has increasingly been applied in several fields. In addition, CCC displays unique advantages and great application potential in the separation of some special compounds, such as high polar compounds and unstable compounds. At present, it is used as a separation and purification technique across the globe.

It should be noted that CCC has been used in a broad sense in this study. It includes two main different types: hydrostatic

Abbreviations: CCC, countercurrent chromatography; CPC, centrifugal partition chromatography; HSCCC, high-speed countercurrent chromatography; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography.

* Corresponding author. Tel.: +86 931 4968248; Fax: +86 931 8277088.

E-mail address: didl@licp.cas.cn (D.-L. Di).

centrifugal partition chromatography (CPC), which is based on a constant centrifugal force field, and hydrodynamic coil planet centrifuge, which is based on a variable centrifugal force field and is called high-speed CCC (HSCCC). CCC is a powerful and effective preparative technique due to its high capacity and low cost of solvent. In a CCC separation, the selection of solvent system has been considered the foremost and crucial factor because both the column and the eluent of the solid-support chromatography can be chosen in a single step simultaneously [5]. Many different and effective CCC solvent systems have been proposed, studied and successfully applied, and a number of different approaches have been established for selecting a suitable CCC solvent system [6–13]. It must also be noted that the elution mode is an important factor contributing to the success of the separation. It can improve the separation efficiency and greatly reduce the separation time using various elution modes. In recent years, studies on the elution mode in CCC have contributed significantly, with several novel elution modes being developed and several related articles published. However, only a few reviews have presented this topic in brief [14,15]. Therefore, we reviewed the progress of the elution mode in CCC and briefly summarize the recent progress in research into the applications of different elution modes in CCC.

2. Advance in elution mode

The separation of a complex sample is one of the development tendencies in the CCC technique. In contrast to other chromatographic techniques such as liquid chromatography and electrophoresis, CCC has a lower number of theoretical plates. Consequently, it is inefficient in the separation of complex samples. In the conventional isocratic mode, it is a simple and effective method of isolating and purifying few major compounds from complex mixtures. However, in practice, separation of more different compounds with a broad range of hydrophobicity is difficult. Fortunately, as the CCC technique is an all-liquid method without a solid phase, it also displays greater flexibility in the choice of elution mode. Therefore, different elution modes have been developed and applied in the case of real complex samples in CCC methodology.

2.1. Gradient elution

Gradient elution has been frequently applied in high-performance liquid chromatography (HPLC) analysis, through the changes in the eluting medium or operating conditions. This method is increasingly used in CCC as well. Depending on the various forms of change, typically, two kinds exist: the stepwise gradient elution mode and the linear gradient elution mode. In the stepwise elution mode, the elution condition is changed stepwise at one or several occasions. It can be treated as a composition of multiple steps of isocratic elution, and several solutes may be eluted in each step. The stepwise elution mode is frequently used to separate complex samples in CCC. It generally includes stepwise changes in the mobile phase composition [16–18], flow rate [19,20], pH value [21,22] and salting-out concentration [23]. In the linear gradient elution mode, the elution condition is changed continuously toward the condition that is favourable for separation. During the whole gradient elution, the elution condition is sustained variation. This is usually achieved by altering the mobile phase composition [24] and pH value [25,26]. In practice, the stepwise mode is a more common method than the linear mode, probably because the gradient elution in liquid-liquid chromatography is vastly different from liquid-solid chromatography and the stepwise mode is relatively easy to implement.

In the gradient elution of CCC, the majority of the process involved a change in the mobile phase composition or flow rate. When the target solutes have a wide range of polarities and the conventional isocratic solvent system fails to adequately separate all target

solutes, the most effective method of improving the separation is to change the mobile phase composition [27,28]. With the rapid change in the polarity of the mobile phase, the solutes in the solvent system can be eluted faster. Similarly, the stepwise increase in flow rate is used in separation to reduce the time required [29,30]. The procedure is started at a low flow rate; subsequently, the solutes with small partition coefficient (K) values are eluted first. Then, an increase in the flow rate leads to faster elution of the remaining target solutes with higher K values. Recently, certain gradient elution modes of pH value [31,32] and salting-out concentration [23] were applied in CCC separation. Adjustment of the pH value or salt concentration of the solvent system can improve efficiency and achieve the best separation with good manipulability and flexibility.

Gradient elution is a useful approach for separating solutes with vastly different polarities. Its advantages broaden the range of CCC application, in addition to reducing the separation time considerably. However, its applications in CCC are not as easy and simple as in HPLC. The major limitation of gradient elution in CCC is that any change in the operating condition may induce a change in the composition of stationary phase and loss of stationary phase retention (S_r), especially in the change of mobile phase composition [18]. Thus, in gradient elution, the stationary phase must remain relatively stable in composition when the composition of the mobile phase is rapidly changed during the separation. Not all liquid systems can be used to perform gradient elution in CCC experiments. It is generally assumed that during a gradient run the change in stationary phase composition should be <20% to prevent instability of the stationary phase [33]. In practical applications, ternary solvent systems such as hexane/methanol/water, chloroform/methanol/water, hexane/1-butanol/water and ethyl acetate/1-butanol/water system and quaternary solvent systems such as heptane or hexane/ethyl acetate/methanol/water have been proven as useful and appropriate solvent systems for gradient elution due to their stability and a board range of polarity. Some studies suggested the use of phase diagrams to build gradients and predict the stability of the stationary phase, as well as to calculate the composition of the initial and final phases for gradient elution [34,35]. Other studies on such benefits have also been conducted. In the literature [36], a three-stepwise gradient elution combined with a descending stepwise flow rate gradient was introduced by Du's group. In this experiment, the lower phase of the solvent system composed of n-hexane/1-butanol/0.05M NaOH (5/1/6, v/v) was used as the stationary phase and the upper phase was used as the initial mobile phase. To prevent the loss of the stationary phase, the flow rates were significantly reduced from the initial 5.0 mL/min to 3.0 mL/min (step 1), 2.0 mL/min (step 2) and 1.5 mL/min (step 3), along with the stepwise rise of the 1-butanol content in the mobile phase from an initial ratio of 5:1 to 1:1 (step 1), 1:2 (step 2) and 1:4 (step 3) consisting of n-hexane/1-butanol. Further, the retention of the stationary phase during the gradient steps decreased to 67%, 65%, and 64%, respectively. It is well known that reducing the flow rate helps improve the retention of the stationary phase in CCC. In this example, the authors reduced the flow rate while also changing the mobile phase to minimize the adverse effects on the stationary phase and provide satisfactory separation for the target compounds. Four ursane triterpenoids (asiatic acid, madecassic acid, asiaticoside and madecassoside) have been successfully separated by the gradient elution method in a single-step CCC separation.

2.2. Dual-mode elution and multiple dual-mode elution

The dual-mode elution is a unique elution method in CCC. CCC instruments can run in either normal-phase or reverse-phase modes and can freely switch between both modes during their operation. The process that uses both the normal and reverse modes (in CPC, they are also called ascending and descending modes) to elute

Download English Version:

<https://daneshyari.com/en/article/1247698>

Download Persian Version:

<https://daneshyari.com/article/1247698>

[Daneshyari.com](https://daneshyari.com)