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Counter-Current Motion in Counter-Current Chromatography

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Abstract

After the CCC2012 meeting, I have received an e-mail regarding the terminology of “**Countercurrent Chromatography**”. It stated that the term “**Countercurrent**” is a misnomer, because its stationary phase is motionless in the column and that the method should be renamed as liquid-liquid separations or centrifugal separations. However, it was found that these names are already used for various other techniques as found via Google search. The term “**Countercurrent Chromatography**” was originally made after two preparative methods of **Countercurrent** distribution and liquid **Chromatography**, both having no **countercurrent** motion in the column. However, it is surprising to find that this F1 hybrid method “**Countercurrent Chromatography**” can clearly exhibit **countercurrent** motion within the separation column in both hydrodynamic and hydrostatic equilibrium systems. This justifies that “**Countercurrent Chromatography**” is a proper term for this chromatographic method.

Keywords

Countercurrent chromatography; liquid-liquid separations; centrifugal separations; terminology; countercurrent; stationary phase

I. Introduction

After CCC2012 meeting, I have received an e-mail from Prof. Berthod stating “The technique denomination: countercurrent chromatography (CCC) is confusing by itself and needs some explanations. In normal CCC use, there is no countercurrent circulation of any fluid. The stationary phase is liquid but motionless, most often maintained steady by centrifugal fields. The liquid mobile phase is flown through the liquid stationary phase as in any other chromatographic techniques. The technique naming was coined in the early 1970s by its inventor, Yoichiro Ito, as a reference to the Craig’s countercurrent distribution method [1]. Unfortunately, the countercurrent term is a misnomer. The term chromatography is correct but it frightens process chemists who do not like the cost and complexity supposedly attached to any preparative chromatographic techniques. Consequently, it is proposed to call the purification methods using liquid-liquid exchanges between a mobile and a stationary phase: Liquid-Liquid Separations (LLS)”. Since I named countercurrent chromatography, I am compelled to address my comments on this issue. In order to justify the new name for CCC suggested by the above letter, I first, consulted through Google search which will

furnish the common usage of terms in various fields including science. The results are shown in Table 1.

In this table Google search items are each evaluated by the number of matches, the number of CCC listed in the first 10 matches and its major contents. The first search on liquid-liquid separation, that was suggested as a new term in the above letter, yielded over 10 million matches, but it does not contain CCC in the first 10 hits. Besides, this name is already given to a totally different technique for separating two liquid phases from emulsified liquid, and the method is extensively used in oil industries and dairy factories. Over one hundred of instruments have been developed for this purpose. Second, the centrifugal separation, that was suggested as an alternative new name for CCC, gave 15 million matches including no CCC in the first 10 matches. It contained various other centrifugal techniques. Next, liquid-liquid chromatography or liquid partition chromatography showed 15–1 million matches but without CCC in the first 10 matches. It contained mostly liquid chromatography with solid support. This clearly indicates that “liquid-liquid chromatography uses always solid support” is the common understanding. When the term “without solid support” was added to these terms, the name CCC starts to appear in the first 10 matches. And the search on countercurrent chromatography contained CCC in all of the first 10 matches. From the results of these Google searches, it is certain that changing the CCC name to liquid-liquid separations or centrifugal separations would produce tremendous confusion in the scientific community.

As mentioned in the above letter, the term “countercurrent chromatography” was coined in our Science publication in 1970 [2]. At that time there were two major preparative separation techniques, namely, countercurrent distribution method (CCD) [1] and liquid chromatography. Then, we developed a new hybrid method which could share their merits with these two methods while eliminating their problems: CCC is more efficient than CCD while it provides higher yields by avoiding irreversible adsorption of samples onto the solid support used in liquid chromatography. Since neither CCD nor liquid chromatography has a capability of countercurrent, this F1 hybrid is not expected to have countercurrent motion of two phases. However, it is surprising to find that CCC literally exhibits countercurrent motion of the two phases in the separation column in both hydrodynamic and hydrostatic equilibrium systems.

2. Seal-free flow-through centrifuge systems

Figure 1 shows a series of rotary-seal-free centrifuge systems developed for performing CCC [3]. In all these centrifuge systems a bundle of flow tubes from the cylindrical column holder supported at the upper end of the centrifuge axis is free from twisting so that the solvent can be continuously eluted through the rotating column without the conventional rotary seal device which may become a potential source of complications such as leakage and contamination. These systems are divided into three categories according to the motion of the column holder, i.e., synchronous (left column), non-planetary (middle column) and non-synchronous (right column). Among those the synchronous systems (left column) are mostly used for performing countercurrent chromatography. Therefore, two typical synchronous systems, type-I (left, top) and type-J (left, bottom) synchronous systems are

chosen to illustrate countercurrent motion of the two liquid phases. In the type-I system (left, top), the column holder synchronously counter-rotates about its own axis while revolving around the centrifugal axis of the centrifuge whereas in the type-J system the column synchronously rotates in the same direction to the revolution and this planetary motion is used for high-speed CCC. As illustrated in Fig. 3, these two types of planetary motions produce countercurrent motion of the two phases in the coiled column but with totally different hydrodynamic distribution patterns.

3. Counter-current motion of two phases enclosed in a coiled tube under two typical hydrodynamic CCC systems

Figure 2 shows the motion of the two immiscible phases enclosed in the coiled tube subjected to the above two different planetary motions. In this figure an end-closed coiled tube at the top is filled with two immiscible solvent phases half-and-half and subjected to these two different types of planetary motion. On the left diagram, the coiled tube is filled with the black phase on the head side and the white phase on the tail side (or *vice versa*), and subjected to the type-I planetary motion. Stroboscopic observation revealed that two phases undergo countercurrent movement through the coiled tube and they are finally distributed evenly in each helical turn as shown in the bottom coil. This hydrodynamic equilibrium pattern is identical to that observed in the original coil planet centrifuge published in Nature in 1966 [4] in which the countercurrent chromatographic separation of 4 different dyes was demonstrated by injecting the sample mixture at the middle portion of the coil prior to the centrifugation [5]. The result is shown in Figure 3 in which each coil was made by winding a single piece of long narrow polyethylene tubing around a glass rod. The coil at the top shows separation of 4 dyes according to their partition coefficients while the rest of the coils show distribution of individual dyes to demonstrate the reproducibility of the method. This CCC method also has been successfully applied for separation various other samples such as proteins and blood cells using the suitable two-phase solvent systems.

Next, the right diagram in Figure 2 shows the hydrodynamic distribution of the two phases in the enclosed coil subjected to the type-J planetary motion. The coil is first filled with black lower phase on the head side and the white upper phase on the tail side and it is subjected to the type-J planetary motion. Surprisingly the two phases undergo countercurrent movement to separate completely the upper phase on the head side and the lower phase on the tail side.

The mechanism of hydrodynamic distribution in the coil produced in the these two planetary motions is not well understand, but it is extensively discussed in my earlier paper [6]

4. Counter-current motion of the two phases in the type-J hydrodynamic CCC system

The above bilateral hydrodynamic equilibrium of the two phases is efficiently utilized for performing high-speed CCC as shown in Figure 4 where all coiled columns were each drawn as a straight tube for simplicity. The column at the top shows the bilateral hydrodynamic equilibrium described above. This equilibrium condition indicates that the

white phase, if introduced at the tail end, would move toward the head, and similarly the black phase introduced at the head end would move toward the tail. This hydrodynamic motion is utilized to perform countercurrent chromatography using the separation column equipped with a pair of flow tubes at each end and the sample feed tube at the middle portion of the column (Figure 4, middle). In this dual CCC system two phases literally undergo countercurrent motion through the column. In the standard CCC separation system shown in the bottom, however, we take out one flow tube from each end and the sample feed tube so that one of the phases is always forced to stay in the column. We call this phase “stationary phase” according to the terminology used in liquid chromatography. However, the term “stationary phase” in CCC is a misnomer. The stationary phase in liquid chromatography is confined in the column and literally stationary in the column. Although the stationary phase in CCC is similarly confined in the column, it is not stationary in the column. Instead it actively repeats countercurrent motion against the mobile phase in the rotating column as shown in Figure 5.

Figure 5A diagrammatically shows two phase motion and distribution in the spiral column subjected to the type-J planetary motion under stroboscopic illumination [6]. The two phases show two distinct hydrodynamic zones, the mixing zone near the center of revolution and the settling zone in the opposite side. Now, I would like to have your attention to the lateral loops where two phases undergo countercurrent motion. Figure 5B similarly shows the two phase hydrodynamic motion in a single spiral loop. As mentioned earlier, the two phases are mixed in the tube near the center of revolution, and separated again into the two phases on the opposite side. As shown by curved arrows, two phases undergo countercurrent motion through the lateral loops where the upper phase moves downward and the lower phase moves upward under a strong centrifugal force field. This indicates that although the stationary phase is confined in the column, it is not stationary in the column and instead actively repeats countercurrent movement against the mobile phase. So far I illustrated the countercurrent motion of the two phases in the hydrodynamic CCC system.

5. Counter-current motion of two phases in the hydrostatic system

Figure 6 shows a portion of the single column of droplet CCC, a typical hydrostatic CCC system [7]. The upper phase introduced into the straight vertical column previously filled with the lower stationary phase forms a series of droplets at regular intervals. In this droplet CCC, the rising droplets of the upper phase steadily produce countercurrent movement of the lower phase downward through the column as indicated by curved arrows in the figure.

6. Conclusion

As presented above, the stationary phase of CCC is not stationary in the column, but locally repeats active countercurrent motion against the mobile phase in both hydrodynamic and hydrostatic CCC systems. I believe that CCC is the only chromatographic system which exhibits countercurrent movement of two phases in the column among all the existing chromatographic systems. I think that this justifies the term countercurrent chromatography.

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Figure 1.

A series of rotary-seal-free centrifuge systems developed for performing CCC

Countercurrent Motions in Hydrodynamic CCC Systems

Figure 2.
Countercurrent motion of the two solvent phases enclosed in a coiled tube by two typical planetary motions

Figure 3.
Separation of 4 dyes in an end-closed coil by countercurrent chromatography.

Mechanism of High-Speed CCC

Figure 4.
Mechanism of high-speed countercurrent chromatography

Countercurrent motion in the hydrodynamic CCC system

Figure 5.

The hydrodynamic motion of two phases in a spiral tube subjected to the type-J planetary motion A: stroboscopic observation; B: countercurrent movement of the two phases in a spiral loop under the type-J planetary motion

Countercurrent motion in the hydrostatic CCC system

Droplet CCC Column

Figure 6.

Countercurrent motion of the two phases in a droplet CCC column

Table 1

Google search terms	No. of matches	Occurrences of CCC in first 10 matches	Most frequently matched topic(s)
Liquid-liquid separation	10,500,000	0	separation of two phases
Centrifugal separation	15,000,000	0	centrifugal technologies
Liquid chromatography	10,100,000	0	LC with solid support
Liquid-liquid chromatography	15,600,000	0	LC with solid support
Liquid partition chromatography	970,000	0	LC with solid support
Liquid-liquid chromatography without solid support	5,400,000	3	CCC, GC & LC
Liquid-liquid partition chromatography without solid support	244,000	6	mostly CCC
Countercurrent distribution chromatography	170,000	5	CCC & CCD
Countercurrent chromatography	199,000	10	all CCC