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Visualisation of J-type counter-current chromatography: A route to understand hydrodynamic phase distribution and retention

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ABSTRACT

This paper has addressed decade sought-after questions on phase bilateral distribution and stationary phase retention in any J-type high-speed counter-current chromatographic (CCC) centrifuge. Using a 2-D spiral column operated on such a CCC device and an aqueous two-phase system, this work systematically observed the phase interaction during transitional period and at dynamic equilibration under stroboscopic illumination. The experimental results thus obtained were used to examine the effects of the liquid–solid friction force, tangential centrifugal force, and physical properties of the two-phase system on hydrodynamic phase behaviour. We identified that (a) density difference between lower and upper phases is the critical factor to cause unusual phase bilateral distribution in the 2-D spiral column and (b) interfacial tension (manifested primarily as phase settling time) of any two-phase system is the critical factor in explaining inability to retain stationary phase in 3-D helical column and, for certain flow modes, in the 2-D spiral column. This work thus has extended or modified the well-established rule-of-thumb for operating J-type CCC devices and our conclusions can accommodate virtually all the anomalies concerning both hydrophobic and hydrophilic phase systems. To this end, this work has not only documented valuable experimental evidences for directly observing phase behaviour in a CCC column, but also finally resolved fundamentally vital issues on bilateral phase distribution orientation and stationary phase retention in 2-D spiral and 3-D helical CCC columns. Revised recommendations to end users of this technology could thus be derived out of the essence of the present work presumably following further experimental validation and a consensus in the CCC R&D and manufacturing circle.

1. Introduction

Any form of counter-current chromatography (CCC) is featured by a liquid stationary phase, through which the mobile phase flows, and thus chromatographic separation of dissolved components is achieved through numerous partition steps between the two liquid phases along a CCC column. There is an established portfolio of seal-free flow-through CCC centrifuge schemes, in which J-type has been the most popular due primarily to its construction simplicity, operation robustness and scalability [1,2].

The invention of high-speed CCC (HSCCC) by Ito [3] was the most significant milestone for impacting on a steadily increasing range of applications and for driving commercialisation of CCC apparatus. HSCCC is characterised by both fast mobile phase flow and high stationary phase retention. By all means, the liquid "stationary" phase can in certain situations be made to flow either simultaneously or intermittently with the "mobile" phase in CCC. Compared to HSCCC, the scope of (gravity-driven) slow speed CCC has been limited to rather specific applications.

On a macro scale, a chromatographic process usually entails continuous and thus gradual concentration profile development for each component along the column longitudinal path [4]. Shortly ensuing the invention of HSCCC, Ito used a 2-D model [5] to show that the separation process under s dynamic J-type centrifuge scheme is governed by a discrete pattern of phase mixing and settling cycle over each rotation. Indeed, it was this understanding that led to some to adapt Craig's counter-current distribution model for discrete processes to HSCCC which possesses genuine chromatographic profiles [6]. Amongst other restrictions, such adaptation can become possible only when thorough phase mixing and settling over each rotation has been achieved. As a guidance and clarification, throughout this paper readers are referred to Fig. 1 for 3 column geometries to be discussed and compared.

Abbreviations: ATPS, aqueous two-phase system; CCC, counter-current chromatography; H, head; HSCCC, high-speed counter-current chromatography; LP, lower phase; S, supplementary material; T, tail; UP, upper phase.

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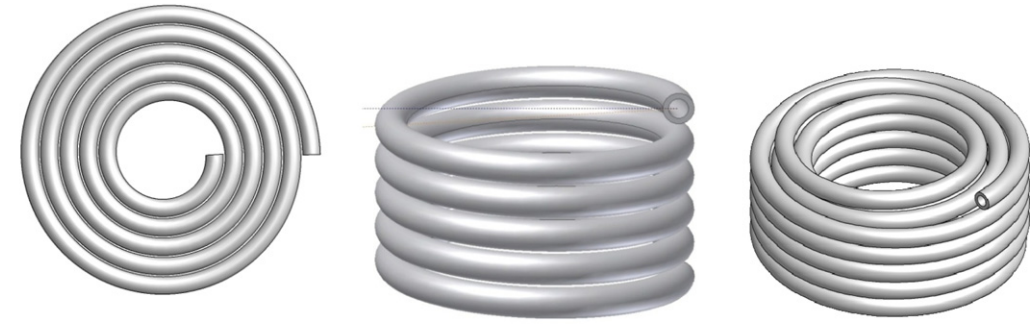

(A) A 2-D spiral column (i.e. single-layer spiral column)

(B) A 3-D helical column (i.e. single-layer cylindrical helix)

(C) A multi-layer 3-D helical column (i.e. multilayer cylindrical helix)

Fig. 1. Illustration for the three column (coil) geometries discussed and compared in this work.

In the period 1984–1986, Conway and Ito [7], Conway et al. [8,9] and Sutherland and Heywood-Waddington [10] reported stroboscopic observation results on 2-D spiral columns (Fig. 1A) in a few conferences, with abstracts being restrictively available. In Conway's CCC monograph of 1990 were published 4 original black-and-white photos taken under stroboscopic illumination for chloroform-acetic acid-water (2:2:1) two-phase solvent system [11]. For illustrating Conway's findings, Sutherland et al. disclosed 4 original photos for an unknown two-phase system in 2000 [12]. Overall, photographic results for phase behaviour of HSCCC have been sparse in the public domain. Speculatively, the less satisfactory quality of photos taken in the past and hence consideration for their suitability in printed version may well have contributed to this situation.

In 2007, Guan et al. reported digital images taken under stroboscopic illumination to show the anticipated mixing and settling pattern for a PEG-phosphate aqueous two-phase system (ATPS) in a 2-D spiral column undergoing J-type CCC planetary motion [13]. This work was conducted mindful that the 3-D helical column on J-type CCC has a great difficulty in retaining satisfactorily any chosen stationary phase out of polar two-phase systems (typically ATPSs) [14]. In line with existing experimental results, the 2-D spiral model published in 2007 [15] confirmed theoretically that certain flow modes for a 2-D spiral column have the potential in achieving sound stationary phase retention for polar two-phase solvent systems. However, at that time neither experimentally nor theoretically were we able to differentiate the intensity of phase mixing between the 3-D helical and the 2-D spiral columns.

The most compelling feature of HSCCC is the use of a centrifuge to establish hydrodynamic and hydrostatic forces for retaining a liquid stationary phase. Our understanding on the physical working of HSCCC has been improved considerably in recent years [15,16], and we are now able to explain why 3-D helical columns have difficulties in retaining stationary phase for more polar two-phase systems like ATPS and this knowledge advancement inevitably sheds light on application scope that the solvent selection approach per se could achieve (e.g. ref. [17]).

Against such a backdrop, the objective of the present work was to observe, using stroboscopic illumination, the dynamics of an ATPS for a 2-D spiral column under all the 8 flow modes. It was further endeavoured to address the following aspects, (a) to observe the effect of J-type planetary motion for hydrodynamic phase distribution in the 2-D spiral column, (b) to observe the hydrodynamic phase behaviour during a transitional period, which leads to dynamic balance between stationary phase retention and mobile phase flow, (c) based on existing experimental results, to make systematic observations for the mixing and settling pattern, and (d) finally to amend and generalise the presently existing rule-of-thumb for determining mobile phase flow orientation based on head and tail locations. Most of colour digital images taken will be left as Supplementary materials at Elsevier Publisher website in JPEG format and thus further uses of these original experimental results are possible.

2. Materials and methods

2.1. The ATPS

The ATPS was composed of 18% (w/w) polyethylene glycol (PEG) 1000 (Sigma–Aldrich P3515) and 18% (w/w) K_2HPO_4 (Sigma–Aldrich P3786) in deionised water. This ATPS was prepared by dissolving 180 g of PEG 1000 and 180 g of anhydrous dibasic potassium phosphate in 640 g of distilled water aided by a magnetic stirrer at 30 °C. The lower phase is rich in phosphate and the upper phase is rich in PEG. The ATPS was equilibrated to 25 °C, mixed and allowed to phase separation.

The phase system has a volume ratio of the upper phase to the lower phase close to one. For this ATPS, the settling time is 51 s, density difference of the two phases 130 kg/m^3, interfacial tension 2.76 mN m^{-1}, upper phase viscosity 18.3 mPa s, and lower phase viscosity 2.14 mPa s [13,18]. The upper PEG phase was coloured blue using Cibacron blue 3G-A (Sigma C9534) alone or green with a combination of Cibacron blue 3G-A and crocin (Fluka 17304). Because of the unilateral partitioning of most dyes in this type of ATPS, the lower phase appeared to be colourless under daylight and greyish in the colourful digital images taken under the stroboscopic lighting described below.

2.2. The J-Type CCC system

The experimental set-up highlighting key hardware parts is illustrated in Fig. 2. A custom-made J-Type CCC device was made by modifying an MSE benchtop centrifuge. An electrical heater kept the space inside the centrifuge casing at 25 °C, with the temperature sensor being located on the internal side of the metal casing. The centrifuge was turned 90° on its axis so that the originally vertical rotor shaft became horizontal and the top of the original centrifuge became the vertical front face of the present J-Type CCC centrifuge. This front was covered by a 15-mm thick transparent Perspex panel to separate the rotor from its surrounding. A solid metal base was