Journal of Chromatography A, 1218 (2011) 6072–6078

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Intermittent counter-current extraction—Effect of the key operating parameters on selectivity and throughput

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ARTICLE INFO

Article history:
Available online 8 April 2011

Keywords:
Intermittent counter-current extraction
ICcE
True moving bed
TMB
Flying leads

ABSTRACT

Intermittent counter-current extraction (ICcE) has proved itself as a method for splitting compounds into streams and/or concentrating compounds in the column. In this paper a model mixture sample based on a modified GUESSmix (containing salicin, caffeine, aspirin, coumarin, salicylic acid, carvone, ionone and biphenyl) was separated into two eluant streams across a range of HEMWat phase system polarities from the polar system 11 through to non-polar system 23. ICcE could provide throughput of over 1 kg/day with this model sample, at the preparative scale, Changing the time cycle to adjust where the sample mixture is split into two streams was demonstrated. It is established that for the continuous running of ICcE, on a conventional twin bobbin counter-current chromatograph instrument, it is necessary to adjust the dead volumes of the flying leads to maintain similar phase retention in each column so the instrument does not become hydrodynamically and mechanically unbalanced due to the difference in densities between the upper and lower phases.

1. Introduction

The unique nature if the liquid stationary phase in counter-current chromatography (CCC) allows great flexibility in operating methods [1–3]. The pharmaceutical industry requires high throughput continuous processes to reduce solvent usage and minimise separation times, but these processes need to be robust. Continuous counter-current extraction (CCCE) where phases are flowed truly counter-current to each other and the sample is loaded continuously to the centre of the column has been explored [4,5] but requires a bespoke column. Intermittent counter-current extraction (ICcE) is a relatively new method for hydrodynamic CCC [6]. The importance of this operational method is that a conventional twin-bobbin CCC instrument may be used by operating the columns in series alternating between normal phase and reversed phase operation with the sample injected continuously between the columns.

Historically, the overarching concept of separating a sample by “the introduction of the mixture at the centre of a perfectly operating continuous column (of test tubes)” with the separated products eluting from opposite ends of the column was first introduced by Craig and Craig [7]. The concept of flowing initially in normal phase mode, then switching to reversed phase mode, with the sample injected at the beginning of the column, to improve the efficiency of a separation was first described by Zhang et al. [8]. This is described as dual-mode [1] and in a further development by Delannay et al. [9] a semi-continuous method for separating two components using a hydrostatic centrifugal partition chromatography (CPC), is termed multiple dual-mode (MDM). By loading a bolus of sample at the beginning of the column then running the system through an ascending and descending cycle before loading another bolus and repeating the cycle, the column length is effectively increased to give a better resolution, using only a single column. A similar principle but using a pair of CCC columns with injection between the columns and elution from one end was described as a “simulated dual-flow approach” by Dubant et al. [10]. A patent by Couillard et al. [11] describes a novel practical method for separating a crude sample into two component groups using a CPC device. The sample was loaded continuously between two columns consisting of serially connected chambers and the centrifuge was run alternately in ascending (less dense phase mobile) and then descending (more dense phase mobile) modes so splitting the sample into two groups which eluted from opposite ends of the system. An extension of dual-mode, as described above, is to repeatedly flow in normal and reversed phase. This is theoretically modelled by Kostanyan and Voshkin [12], as is controlled-cycle counter-current chromatography [13] where the flow of mobile phase is switched between the heavy and lighter phases, with a delay between the switching periods to allow for full equilibration. The current authors with Ye published the first results for separations with ICcE [6], where a sample was injected between a pair of CCC columns and the phases were alternately switched between normal and reversed phase on both a model mixture sample based on the GUESSmix proposed by Friesen and Pauli [14] and a representative Chinese

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doi:10.1016/j.chroma.2011.03.072

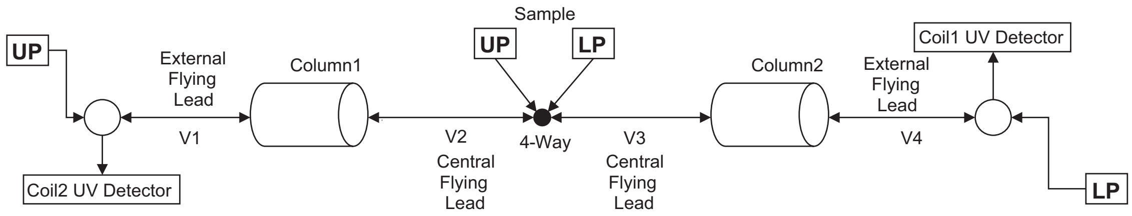

Fig. 1. ICcE method setup for a standard Midi instrument, V1 to V4 are the dead volumes of the four flying leads connecting the columns and the ancillary equipment.

herbal medicine, the herb *Tripterygium wilfordii* Hook. f. The authors [15] then demonstrated the scale up of the ICcE method from prep to pilot scale. Yang et al. [16] has proposed a theoretical model for the prediction of peak elution with ICcE for both hydrostatic and hydrodynamic systems, with separation of DNP-amino acids to confirm the predictions. Peng and Ye [17] recently compared ICcE and batch CCC for the separation of honokiol and magnolol, the main bioactive isomers in the traditional Chinese medicine “Houpu”, showing approximately a 4× improvement in throughput.

To allow continuous running of the ICcE method it is important that the phase retention within the column is maintained over a reasonable continuous running period and that within the two columns the phase retention is similar so that the instrument does not become hydrodynamically and mechanically unbalanced.

This paper assesses the robustness of the phase retention within the CCC columns when using the ICcE method across a range of HEMWat phase systems polarities from the polar system 11 through to non-polar system 23 demonstrating the need for the correct flying lead dead volumes to maintain similar phase retention between the columns with extended operation.

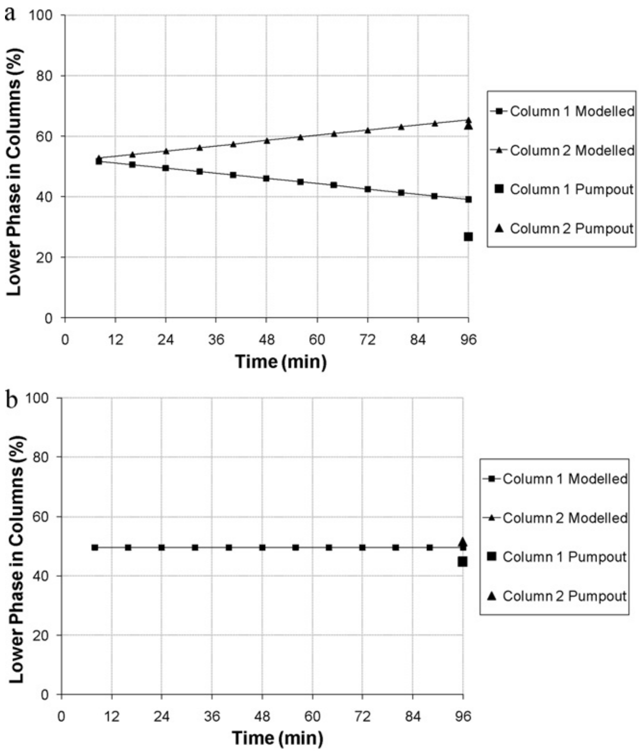

Fig. 2. (a) Un-balanced columns with unmatched volume flying leads, V1 = V4 ≠ (V2 + V3) and (b) balanced columns with matched volume flying leads, V1 = (V2 + V3) = V4, predicted and actual lower phase in columns after 96 min ICcE run; HEMWat system 23; upper phase and lower phase flow rate 40 ml/min; flow switched every 4 min; rotational speed: 1400 rpm; temperature: 30 °C.

Further, this study establishes the effectiveness of ICcE to successfully separate compounds of a sample into two eluant streams across a range of HEMWat phase system polarities. The specific compounds used for this study were a model sample mixture based on a modified GUESSmix proposed by Friesen and Pauli [14], containing salicin, caffeine, aspirin, coumarin, salicylic acid, carvone, ionone and biphenyl. Over the course of the study the sample mixture was successfully split by polar (HEMWat 11), intermediate (HEMWat 17) and nonpolar (HEMWat 23) phase systems at different points dependant on the polarity of the compounds. The feasibility to achieve a throughput of over 1 kg of crude processed per day with a preparative scale Midi instrument was investigated by doubling the eluant and sample loading flows which gave a throughput equivalent to 1.2 kg/day. Finally, the change of the splitting point by adjusting the flow switching times of the phases was experimentally demonstrated with ICcE for the first time.

2. Experimental

2.1. Reagents

Solvents used for ICcE were of analytical grade and for HPLC detection were HPLC grade from Fisher Chemicals (Loughborough, UK). Carvone, salicylic acid, coumarin and caffeine were also purchased from Fisher Chemicals. Biphenyl, ionone, aspirin and salicin were purchased from Sigma–Aldrich (Gillingham, UK). HPLC water was purified from a Purite Select Fusion pure water system (Thame, UK).

2.2. Apparatus

A high performance Midi CCC instrument (Dynamic Extractions, Slough, UK) fitted with 4 mm I.D. preparative columns made of polyfluoroalkoxy tubing (PFA) with volumes of 459.5 ml and 453.0 ml was used to perform the intermittent counter-current extraction. The ICcE set up consisted of two preparative Knauer K-1800 HPLC pumps (Berlin, Germany) and two Knauer K-6 valves to

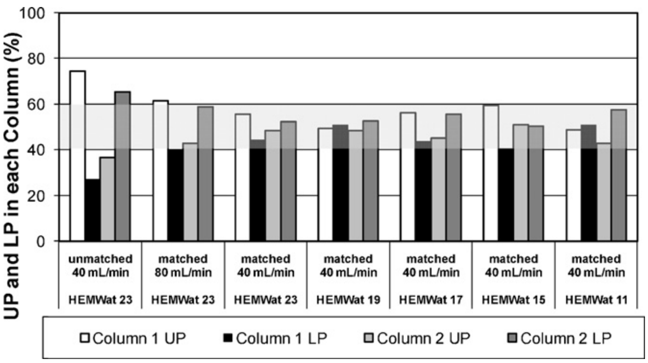

Fig. 3. Percentage phase in columns after 96 min ICcE run across a range of HEMWat systems; unmatched volume flying leads, V1 = V4 ≠ (V2 + V3); matched volume flying leads, V1 = (V2 + V3) = V4; upper phase and lower phase flow rates as indicated; flow switched every 4 min; rotational speed: 1400 rpm; temperature: 30 °C.