Hydrodynamics studies of the behaviour of traditional and two-phase ionic liquid solvent systems in countercurrent chromatography (CCC)

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HIGHLIGHTS

- No single-phase flooding is observed at low beta values (<0.5) in spiral coil for the conditions studied.
- Ionic liquid produces previously unseen, highly non-uniform phase distribution.
- Local velocity of each phase changes significantly along the coil.
- Effectiveness of wave mixing depends on beta value, mobile phase pumping direction and flow rate.

ABSTRACT

The hydrodynamics and retention of 2D countercurrent chromatographic systems were investigated for the first time for ionic liquid based biphasic solvents using high-speed imaging. The results were compared against 3D chromatographic systems and different solvent systems for a wide range of beta values (0.27–0.83). The studies revealed details of the phase distribution, the mixing and settling areas within the column, as well as the interfacial characteristics that can enhance mass transfer (such as waves). There was no single-phase flooding in the beta range 0.27–0.5 contrary to previous limited visualisation studies, which suggested that for the biphasic solvents they utilized, single-phase flooding at low beta values (less than 0.5) occurs. Previously unseen, stable, six segment phase distributions, involving alternate high and low stationary phase retention were observed for the ionic liquid biphasic solvent system but not for the organic-aqueous and aqueous-aqueous biphasic solvent systems also studied. This unusual behaviour and the resulting considerable variations in interfacial height and thus local velocity of each phase along the coil, are discussed in the text. The ionic liquid biphasic solvent also demonstrated novel behaviour in other aspects. In the mixing zone around the proximal key node, interfacial waves were observed; however, it was found that their amplitude and frequency depended on the chosen elution mode as well as the mobile phase flow rate and beta value. While there was some agreement in the performance of both column geometries, it was found that for the serial 2D column there was always some retention of the stationary phase over the entire range of flow rates tested, unlike the case for the 3D column.

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1. Introduction

Current approaches for liquid-liquid extractions in many industrial applications often involve the use of organic solvents, which have low flash points and are inherently unsafe. The long residence times, and the large volumes in the mixer-settler or pulsed column extraction units that are most commonly used, further increase the high hazard ratings for the process. A promising intensified separation technology is high-performance countercurrent chromatography (CCC). It is a form of liquid-liquid extraction that achieves separation by repeated partitioning of solutes between two immiscible liquid phases, as they interact in a continuous length of coiled tubing under centrifugal and Archimedean forces. The tubing is wrapped around a cylindrical drum (called a bobbin) to form either a 2D spiral column geometry or more typically it is wound to form a 3D helical configuration with one or several layers. Within the CCC column, one of the liquid phases is held stationary...
combination of hydrodynamic and hydrostatic forces generated as a result of rotating the column in planetary motion, while the other mobile phase is continuously pumped through the coil and serves to transport the solutes through the system.

Since the 1970s and the pioneering work of Ito and his colleagues (Ito and Bowman, 1970a,b), many different versions of CCC instruments have been developed. In J-type synchronous centrifuges, the bobbin is mounted on a planetary axis, which is driven by a central axis so that the column rotates about its own axis while it revolves around the central axis at the same velocity in the same direction. The double rotation of the column during its planetary motion produces a variable centrifugal force field. This force field creates a unique mixing pattern in which a series of sequential mixing and settling zones is generated simultaneously along the length of the column. These alternating mixing and settling steps are essential to the chromatographic process as they promote solute transfer between the phases, and, therefore, separation of species with different partition coefficients. As described by Conway (1990) and Ito (1992), the mixing zones are coincident with the lower accelerations experienced at the proximal key node (region of the coil closest to the centre of revolution around the central axis) and primarily take the form of wave mixing. During mixing, the interfacial area increases and depending on the physical properties of the solvent system, each phase can also form droplets in the other phase to enhance mass transfer. On the other hand, the settling zones are coincident with the high accelerations at the distal key node (region of the coil furthest from the centre of revolution around the central axis) and are characterised by a smooth interfacial area. In the settling zone, no significant mass transfer occurs and the phases travel individually as separate layers. There is one mixing and one settling zone per coil loop per revolution. Due to the high rotational speed of a typical modern analytical instrument, a large number of mixing and settling stages can therefore be generated in a single instrument. For example, a sample injected into the mobile phase can experience 800 repetitive mixing and settling steps in a minute when the rotor is spun at 800 rpm, thus promoting highly efficient separations.

For over four decades the CCC technique has been successfully applied to the separation and purification of a wide range of natural products and synthetic compounds (Ito, 2005, 2010; Pan and Lu, 2007; Yin et al., 2010). However, it may be argued that the complex hydrodynamic phenomena inside the rotating coils have not been adequately studied and described. For example, there are still many questions regarding the local movement of each phase during the column rotation, as well as the mechanism and physical forces involved in retaining the stationary phase within the column. For high resolution and efficient separations, high retention levels are necessary where values of up to 95% can be achieved in J-type machines using standard organic-aqueous two-phase systems. To retain the stationary phase, it is generally agreed that CCC utilizes the Archimedean screw effect. This is an internal pumping effect that redistributes the upper (lighter) and lower (heavier) phases to opposite ends of the rotating column when no externally applied experimental conditions (Ito, 1992). The Archimedean effect occurs because the internal surface of the rotating coil exerts a drag force (i.e. solid-liquid friction force) on all the column contents toward the tail end of the column as both phases are being screwed toward the head (Guan et al., 2007a, 2012; Guan and Heuvel, 2011). Guan et al. (2007a) hypothesized that this drag force applies more to the lower phase than the upper one, and as a result, in most cases all of the upper phase tends to preferentially occupy the head whilst the lower phase is displaced to the tail. For CCC separations with a mobile phase flow, the direction of the interfacial liquid-liquid shear force (based on the pumping direction of the mobile phase) should therefore be arranged to counteract this solid-liquid friction force, so that the two phases are in effect being pumped in opposite directions and the stationary phase can be retained in the column to a reasonable extent. The situation is further complicated because the hydrostatic centrifugal forces generated due to the column rotation can be separated orthogonally into normal and tangential force components; while the former force plays a role in the mixing of the two phases, the latter acts along the same axis as the drag forces and can influence the unilateral phase distribution. It is therefore a delicate interplay of all three hydrodynamic forces that determines whether reasonable stationary phase retention can be achieved for a given solvent system to enable separations in the CCC device.

In the last decade, the use of ionic liquids either as solvents or additives in liquid-liquid extractions has expanded considerably because of their unique properties. Ionic liquids are organic salts that are liquid below 100 °C. Some of their most salient features include: negligible vapour pressure at ambient temperature and pressure, high chemical/thermal stability, and very good solvating capacity for a wide range of compounds. Moreover, through careful selection of the constituent ion, their properties can be fine-tuned allowing them to be optimised for a particular application. The combination of the two technologies, ionic liquids and CCC, therefore represents an exciting approach to intensified liquid-liquid separations. However, the use of ionic liquids in CCC is not a trivial task due to their relatively high viscosities, which can introduce significant problems for the majority of traditional CCC machines currently on the market that are mostly low pressure. As a result, to date, their application in CCC has been very limited. Initial work using diluted ionic liquids in CCC was conducted by Berthod and colleagues (Berthod and Carda-Broch, 2003, 2004; Berthod et al., 2008, 2009), who concluded that CCC was not suitable at the time, as the instrumentation available was not able to cope with the high backpressures generated by the high viscosities of many ionic liquids. To overcome the pressure limitations previously encountered using the CCC technique, AECS in collaboration with QUILL Research Centre have reported on the design and construction of a modified high backpressure CCC instrument, which has proven successful in using ionic liquids as a major solvent component for a wide range of separations (Brown et al., 2017a,b). In addition to viscosity, the physical properties of ionic liquid based solvent systems such as interfacial tension and phase density difference can differ significantly from those of standard solvent systems. To determine the optimal flow rate and rotational speed for a given separation, Brown et al. (2017a,b) measured the degree of stationary phase retention achieved with increasing mobile phase flow rate and rotational speed in four 3D multilayer helical coils of varying capacity. However, the phase distribution and thus hydrodynamics of the ionic liquid solvent systems are not known as these studies were carried out using metal coils. Thus, in this paper, to gain understanding of the hydrodynamics that govern ionic liquid based extractions in CCC, we reveal for the first time, the dynamic flow characteristics of a two-phase ionic liquid solvent system in these machines.

In the literature, the phase distribution in 3D helical and 2D spiral columns has been extensively studied using a variety of organic-aqueous systems in order to predict the direction that a given mobile phase should be pumped for maximum stationary phase retention inside the column (Ito, 1984a,b, 1992; Sutherland et al., 2000). In his work, Ito performed retention studies in 3D and 2D columns with both the lower and upper phases as
mobile, whereas Sutherland et al. performed head and tail studies in sealed 2D spiral coils (no mobile phase) using dyed liquid phases. Based on the empirical observations from these studies, two different hypotheses were proposed. Ito (1992) hypothesized that for hydrophobic binary solvent systems with one phase being water, the lower phase always moves toward the tail side of the column and the upper phase to the head side. On the other hand, for hydrophilic systems where the non-aqueous phase is polar, the lower phase moves toward the head while for intermediate systems it can go in either direction. This means that for optimum results, the pumping direction of the mobile phase should be selected based on the physical properties of the solvent system. However, Sutherland’s research suggested that for all types of solvent systems in 2D columns, the lower phase always accumulated at the tail and the upper phase at the head, when the spiral coil was orientated with the head at the centre, and the tail at the periphery of the column (Sutherland et al., 2000). Therefore, for a mobile lower phase it should be introduced through the head toward the tail, whilst a mobile upper phase should be pumped in the opposite direction. To explain these findings, Sutherland et al. (2000) proposed that with this arrangement, the Archimedean screw effect and centrifugal forces combine to produce the same hydrodynamic behaviour for each phase regardless of whether the phase system was hydrophobic, hydrophilic or intermediate. As a result, the main recommendation was to always use this column orientation for CCC separations.

The dynamic motion and phase distribution of the two phases in rotating 2D spiral coils when there is an externally pumped mobile phase flow, have also been directly observed under stroboscopic illumination (Conway and Ito, 1984; Conway, 1985, 1990; Conway et al., 1986; Guan et al., 2007b, 2012; Sutherland and Heywood-Waddington, 1985). However, nearly all these photographic results were presented at a few conferences and are not in the public domain. Exceptions are the 4 black-and-white images published by Conway (1990), which show the wave motion in the mixing zone for chloroform-acetic acid-water (2:2:1) two-phase solvent system. Sutherland et al. (2000) also presented images showing waves in the mixing zone; however, no details were given for the solvent system used. In later studies, Guan et al. (2007b and 2012) reported an extensive collection of coloured digital images taken of a polyethylene glycol (PEG)-phosphate aqueous two-phase system (ATPS) which show the phase distribution before and at equilibrium as well as the mixing and settling patterns in the coil. These images showed wavelike interfacial structures in the mixing zone, with no evidence of droplet generation.

Against this background, the first aim of this study was to establish the effect of operating conditions such as elution mode, mobile phase flow rate and rotational speed on the retention of the stationary phase for ionic liquid solvent systems in CCC. The results were compared against those from a 3D CCC column and from traditional organic-aqueous and aqueous-aqueous biphasic solvent systems. The second aim was to view directly with the aid of a high-speed camera and dyes, details of the phase distribution and mixing cycle resulting from the dynamic interaction of all utilized biphasic solvent systems in the column to offer insight into the operation of the CCC apparatus for ionic liquid based chromatographic extractions.

2. Materials and methods

2.1. The solvent systems

The ionic liquid (IL) solvent system used consisted of a 1:1:4 (v/v/v) ratio of the IL trihexyl(tetradecyl)phosphonium chloride ([P66614]Cl), ethyl acetate (EtOAc) and water. It was prepared at room temperature by mixing 150 cm³ of [P66614]Cl (Sigma-Aldrich) with 150 cm³ of ethyl acetate (Sigma-Aldrich) and 600 cm³ of deionised water. The IL was used as supplied, without further purification. The system was then equilibrated at room temperature and the phases were allowed to separate in a separating funnel. It was found that the upper phase was rich in the IL, while the aqueous phase formed the lower phase. This solvent system was successfully used by Brown et al. (2017a,b) for the separation of transition metal salts (copper (II), nickel (II), and cobalt (II) chloride). For these separations, a small percentage of ethyl acetate was added primarily because it is a water immiscible solvent that reduces the viscosity of the pure IL [P66614]Cl which is 2161 mPa s and therefore too viscous to pump. Furthermore, this co-solvent is less dense than water compared to others and helps to keep the stationary ionic liquid phase less dense than the mobile aqueous phase. This is important for the separation of these metals because the density of the ionic stationary phase can increase with increasing concentration of dense metal salt samples. This can lead to phase inversion and ultimately column bleed which should be avoided. Moreover, in comparison to other potential co-solvents, ethyl acetate is favoured because it is biodegradable to ethanol and acetic acid, cheap, readily available and possesses very low toxicity.

To aid with visualisation, the upper IL-rich phase was coloured dark blue using bromophenol blue (Sigma-Aldrich), while the lower aqueous phase was colourless. For comparison, a simple 1:1 (v/v) hexane-water system and a PEG-ATPS system were also investigated. The hexane-water system was selected because it is a known standard organic-aqueous system, characterised by high interfacial tension, low viscosity and therefore fast settling times. Experiments have shown that these hydrophobic systems can yield a high level of stationary phase retention in both the 2D spiral and 3D multilayer helical columns (Ito, 1992, 2010; Sutherland et al., 2000). For this study, the lower aqueous phase was dyed dark blue using water-based pen ink, whilst the upper hexane phase was colourless.

The work involving the PEG-ATPS system was conducted due to the similarity in physical properties with the IL solvent system. It was undertaken mindful that it has been previously shown that in the 3D helical J-type CCC column there are low retention levels of any chosen stationary phase of the ATPS system (Al-Marzouqi et al., 2005; Guan et al., 2009, 2012; Ito, 2010). These aqueous polymer systems have high viscosity, low interfacial tension and hence require a long settling time of over 1 min. The ATPS used contained 18% (w/w) PEG 1000 (Sigma-Aldrich) and 18% (w/w) anhydrous dibasic potassium phosphate (K₂HPO₄) (Sigma-Aldrich) in deionised water. It was prepared by dissolving 180 g of PEG 1000 and 180 g of (K₂HPO₄) in 640 g of deionised water using a magnetic stirrer at approx. 30°C. The ATPS was then equilibrated at room temperature and allowed to phase separate in a funnel. The lower phase was rich in phosphate and the upper phase was rich in PEG.

Table 1 summarizes the relevant physical properties of all the systems used in this work. The density of each phase was determined gravimetrically, while the viscosity was measured using an ARES rheometer (TA Instruments). The interfacial tension was measured using the Du Noüy ring method in a Krüss K100 tensiometer. The settling time was determined by introducing 5 mL each of the upper and lower phases into a capped tube and inverting it 5 times to measure the time it takes to separate and form two clear layers (Ito, 2010). All measurements were made at room temperature.

2.2. Experimental set up

The viscosity of ILs is often higher than that of common molecular solvents and can pose significant problems for traditional CCC
devices, which are mostly low pressure. To overcome these challenges, a custom-designed J-type centrifuge – Quattro IL-Prep™ ILLC machine (AECS-QuikPrep Ltd) was utilized in this research. To allow visualisation of the flow distribution and mixing patterns within the rotating CCC column, the ILLC machine was fitted with a bespoke 2D spiral rotor set which consists of four 2D spiral coils that are connected in series (Fig. 1). Each coil, made from transparent PFA tubing (2 mm ID, 3 mm OD), expands clockwise and is characterised by a \( R \) value (\( \beta = r / R \)) range of 0.27–0.83, where \( r \) is the distance from the holder axis to the coil, and \( R \) is the distance between the holder axis and the central axis of the centrifuge (Fig. 1). The volume of each coil is 25 mL, which gives a total column volume of 100 mL. The visualisation side of the instrument is covered by a 10 mm thick transparent polycarbonate plate to separate the rotor from the surroundings. In the device, the rotor and coils are enclosed in a temperature controlled housing that was maintained at 30 °C for all experiments. The operating rotational speed can be varied in the range between 0 and 865 rpm. For comparison studies with the 2D column geometry, the ILLC was also equipped with an interchangeable, 3D multilayer helical coil that was made from the same tubing (material and dimensions) as the 2D spiral coils. Unlike spirally wound coils, with a 3D column the first wind is along the longitudinal plane of the bobbin before it rises to the next level of winding to create another layer (see Fig. 1 in Guan et al., 2012). For the 3D column examined in the present study, two layers of tubing were used giving \( \beta \) values in the range of 0.8–0.88 and a total column volume of 135 mL. Other \( \beta \) values in the range of 0.27–0.8 have yet to be studied, but will be discussed in future publications. To deliver independently the stationary and mobile phases to the ILLC device, two SSI HPLC pumps (0–40 mL/min) were used. The coils are connected to the pumps using poly[1,1,2,2-tetrafluoroethylene]/(PTFE) flying leads with 0.8 mm ID and 1.68 mm OD. The maximum working pressure for the system is 100 psi.

The Archimedean screw effect and hence the type of helix (right-handed or left-handed) as well as the rotation direction of the centrifuge have been used traditionally to determine the location of the CCC head and tail terminals. According to Ito (1992), the Archimedean effect drives the fluids in the column toward the head of the coil and a change from clockwise to anticlockwise rotation, changes the direction of the screwing effect and therefore inverts the location of the head and tail ends. Unlike helical coils which have 4 elution modes [upper mobile phase (head → tail), upper mobile phase (tail → head), lower mobile phase (head → tail), lower mobile phase (tail → head)], spiral coils can be characterised by 8 possible elution modes due to the combination of three operational parameters: rotation direction (clockwise or anticlockwise), selection of the stationary phase (upper or lower phase) and the flow direction for the mobile phase (centre → periphery or periphery → centre). Following the terminology originated by Ito et al. (2003), these 8 elution modes can be termed as L-I-H, L-O-T, U-I-H, U-O-T, L-O-H, L-I-T, U-O-H and U-I-T. In this terminology system, the first letter specifies the mobile phase (either the upper, U, or the lower, L, phase), the second letter specifies the inlet terminal for the mobile phase (either the inside, I, or the outside, O, of the coil) and the third letter specifies the head and tail orientation (either the head, H, or the tail, T, is located at the entrance terminal of the mobile phase). For instance, L-I-H refers to the case where the lower phase is used as the mobile phase and flows from the inside terminal of the coil, which is the head during coil rotation (clockwise rotation for clockwise-wound coils).

### 2.3. Stationary phase retention measurement

To obtain the stationary phase retention curves, the column geometry under study was initially filled with the chosen stationary phase. Once completely filled, the column was rotated in either the clockwise or anticlockwise direction at the selected speed. The mobile phase was then pumped through the column either from the head or the tail terminal at various increasing flow rates (0.5–20 mL/min). For each mobile phase flow rate investigated, the eluted stationary phase was collected in a graduated measuring cylinder until hydrodynamic equilibrium was achieved, that is when no more stationary phase co-eluted with the mobile phase. The volume of the eluted stationary phase was used to calculate the volume remaining in the column, taking into account the dead volume in the system. Several experiments were performed in triplicate and the maximum variation in the measured phase retention was found to be ±2%.

### 2.4. Observation of phase distribution

To observe the distribution of the phases as well as the mixing and settling patterns inside the rotating column, high-speed imaging was employed. With the column located at an axial position of

![Fig. 1. An external view of the ILLC instrument fitted with a custom-designed 2D rotor set.](image-url)
3. Results and discussion

3.1. Stationary phase retention

Fig. 2 shows the phase retention behaviour of the IL solvent system ([P_{66614}]Cl-EtOAc-water (1:1:4, v/v/v)) in the 3D multilayer helical column operated at a rotational speed of 800 rpm for the case when the lower mobile phase is pumped in the head to tail direction. As can be seen, satisfactory phase retention is achieved. Consistent with the prevailing head-and-tail rule of thumb for operating 3D CCC devices using traditional solvents (Ito, 2005), it is found that this elution mode also gives the best performance for the IL solvent system. Furthermore, as the viscosity of the ionic upper phase is relatively high, it is best to avoid selecting this phase as the mobile phase since no stationary phase is retained in the column under these conditions. In Fig. 2 the present experimental results are compared with those reported by Brown et al. (2017a,b) for the same IL solvent system in a 3D multilayer helical column of 2 mm stainless steel bore tubing. The CCC instrument used by Brown et al. (2017a,b) was also a Quatto ILPrep™ with similar design parameters to the present study, although with a much wider beta value range. As can be seen from Fig. 2, a good agreement is found.

In the literature, there remains some question regarding the extent to which the 3D helical and the 2D spiral column geometries installed on the same J-type CCC device give rise to similar performance (Guan et al., 2007a,b, 2012; Guan and Heuvel, 2011). To examine this, the IL solvent system retention levels in the 3D multilayer helical coil (β: 0.8–0.88) are compared with those obtained in the 2D spiral column (β: 0.27–0.83) for the LIH elution mode in Fig. 2. It is interesting to observe that for low to intermediate flow velocities < ~8 mL/min, there is a close agreement between the retention results obtained for the 2D LIH (i.e. head to tail) and the 3D (head to tail) modes. In contrast, when the mobile phase velocity is increased to above 8 mL/min, the 2D spiral column performs better. Guan and Heuvel (2011) examined theoretically the difference in performance between the 2D and 3D CCC configurations and suggested that due to the difference in column geometry, the 3D column has a three-dimensional centrifugal force system that intensifies the phase mixing and causes more of the stationary phase to leave the system together with the mobile one. However, it is possible that the difference in beta values of the two column configurations also plays a role in the observed difference in performance since this will affect the resulting centrifugal force field generated in each column. In this work, the 2D column is characterised by a much wider range of low beta values so that the phase mixing may actually be better in comparison to the 3D column also examined. These two effects are contradicting and in the future the phase distribution in both types of configuration will be investigated in more detail.

The performance of the 2D spiral column for all the 8 possible elution modes using the IL solvent system is shown in Fig. 3. In general at low mobile phase flow rates there is initially a steep decline in retention before it stabilizes at higher flow rates. In this plateau region, it appears that there is very little difference in the retention levels attained for the different elution modes. However, unlike the 3D column results, for the 2D column there is always some retention of the stationary phase over the entire range of flow rates tested. It is found that the LIH flow mode has the highest stationary phase retention, whilst the LOH is associated with lower yet robust levels of stationary phase retention. This is consistent with the prevailing rule of thumb for operating 2D CCC devices (Sutherland et al., 2000), as in both cases the lower phase is being pumped in the opposite direction to which the upper phase is internally pumped (tail to head) by the device. To explain the observed difference in performance between these two elution modes, it has been suggested by Sunderland et al. (2000) that for the case where the head is located at the centre (LIH), this configuration results in the Archimedean screw and centrifugal forces acting in the same direction so that higher retention is achieved. On the other hand, for anticlockwise rotation, with the head now located at the periphery (LOH), the Archimedean and centrifugal forces act in the opposite direction so that lower retention is achieved.

In terms of choice of mobile phase for this system, when the more viscous upper IL-rich phase is used as the mobile phase (UOT, UOH, UIH and UIT modes), the measured backpressures generated in the CCC very quickly approached the working maximum for the setup. As a result, it was only possible to investigate a smaller range of flow rates. In addition, it is found that similar to the 3D column performance, the rate of loss of the stationary phase is...
lower interfacial tension and higher viscosities which leads to a faster flow rate. Both the IL and PEG-ATPS solvent systems have lower stationary phase retention compared to the hexane-water system (Ito, 2010). In Fig. 5, the phase retention results for the IL solvent system are compared to those obtained using a standard organic-aqueous system (hexane – water, 1:1 v/v) and the PEG-ATPS for the same elution mode (LIH) in the 2D spiral column. For the 3D column, a similar comparison is also made in Fig. 5 between the IL and PEG-ATPS solvent systems for the same elution mode. From these results, it is clear that there is a close agreement between the IL and PEG-ATPS data in both the 2D spiral and 3D helical columns, which is likely due to the similarities in the physical properties, in particular, interfacial tension and liquid viscosities (Table 1). Furthermore, it can be observed that the IL and PEG-ATPS systems display significantly lower stationary phase retention than the hexane-water system over the range of mobile phase flow rates tested. Both the IL and PEG-ATPS solvent systems have lower interfacial tension and viscosities which leads to a much faster settling time, in comparison to the hexane-water system. As a result, the upper and lower phases of these viscous systems have difficulty separating in the CCC column so that more of the stationary phase is dragged out of the column reducing the observed retention at equilibrium.

3.2. Visualisation studies

3.2.1. Phase distribution of conventional aqueous-organic two-phase system

To further compare the hydrodynamics of the IL solvent system with the well-known phase behaviour of aqueous-organic two-phase systems, examples of the high-speed images obtained for the hexane-water system inside the rotating 2D spiral column are presented in Fig. 6. The results shown are for the LIH elution mode, that is when the column was originally filled with the dyed aqueous phase and the colourless upper organic phase. These images were taken at a constant mobile phase flow rate of 20 mL/min, which corresponded to a measured stationary phase retention of 67% at hydrodynamic equilibrium (Fig. 5). Fig. 6(a) reveals that a non-uniform layered pattern is formed. The denser aqueous phase occupies mainly the outer portions of the coil while the lighter organic phase mostly occupies the inner portions. Observations also indicate that the phases do not distribute equally throughout the coil and an interfacial height gradient exists. Variations of the interfacial height with both angular position and beta value can be discerned, implying that the local velocity of each phase not only varies circumferentially over each loop of the coil but also with distance from the bobbin centre axis. Interestingly, in contrast to previous visualisation studies of 2D spiral centrifuges which showed that at beta values below 0.5 only one phase occupied the column (Conway, 1990; Sutherland et al., 2000), the current results show that even at the lowest beta value (0.27), both phases are present within the coil.

In agreement with the current understanding of the phase distribution of aqueous-organic two-phase systems in CCC (Conway, 1990), our observations showed that around the distal key node (labelled 1 in Fig. 6a), the phases are completely separated into distinct layers. In contrast, around the proximal key node (labelled 2 in Fig. 6a) the two phases are mixed and it is found that one of the liquid layers penetrates the other. Fig. 6(b) reveals details of the dynamic mixing patterns of the solvent system (0.27 < β < 0.36) that were obtained using the high-speed camera equipped with a monozoom lens. Due to the vigorous mixing and low viscosity of
this system, the physical structure of the interface appears discontinuous. The water phase is broken up into ligaments of irregular shape that penetrate the hexane phase, whilst the hexane phase also forms small droplets of varying size which are entrained in the aqueous phase. For high partition efficiency, droplets are important because they have a high specific interfacial area which enhances mass transfer between the two phases. From examination of the high-speed video recordings it was found that the hexane phase dispersed more easily in the aqueous phase, whilst the formation of water droplets was only occasionally observed for the lower range of aqueous mobile phase flow rates tested. The visual observations also revealed that the mixing cycle for the aqueous-organic system is not a stationary process and the appearance of the interface changed with each revolution.

3.2.2. Phase distribution of IL solvent two-phase system

The images presented in Fig. 7 illustrate the influence of the choice of elution mode on the motion and phase distribution of the IL solvent system in the rotating 2D column once dynamic equilibrium was established at a lower mobile phase flow rate of 2 mL/min. The upper ionic liquid phase appears black, whilst the lower aqueous phase appears white. The corresponding images taken at the end of the rotation period are given in S1 (Supplementary material). From Fig. 7, it is interesting to observe that in comparison to standard organic-aqueous systems, the IL solvent system produced a very unique, segmented phase distribution in the 2D spiral column. These images reveal the formation of six distinct sections on the first 2D spiral coil, which were seen to be repeated on the remaining three spiral coils located at the back. The six sections consist of three sections where the column is predominantly filled with the dyed ionic liquid phase alternating with three other sections where the colourless aqueous phase fraction is significantly greater. Each triangular section is widest at the column periphery (highest beta value). To the best of our knowledge, this kind of alternating flow distribution pattern has never been previously observed for any type of solvent system inside a CCC column. Contrary to expectations, the IL solvent system phase distribution results are not consistent with those reported by Guan et al. (2012) for a PEG-ATPS with similar physical properties to the IL solvent system. The imaging results presented by Guan et al. (2012) showed that the PEG and the aqueous phases are more uniformly distributed in the column.

Comparing the standard organic-aqueous, hexane-water system presented earlier (Fig. 6), to Fig. 7, it should be noted that both the IL and the aqueous phases are present in each coil layer with the denser aqueous phase directed in the outward portions of the coil whilst the less dense IL-rich phase occupies the inner portions over the entire range of beta values. The bilateral spreading of the two phases throughout the entire column during rotation is confirmed from the static images obtained following each dynamic elution mode (see S1 in Supplementary material). As discussed previously, the elution modes where the upper IL-rich phase is used as the mobile phase result in poor retention of the stationary phase. This is supported by the images in S2 and S3 (Supplementary material) which show that for these elution modes the column is predominantly filled with the dyed IL-rich phase both during and after the column rotation respectively.

Historically several publications have studied extensively the force field that acts on a point mass fixed on a coil column undergoing planetary motion (Ito, 1984a; Ito, 1984b, 1992; Conway, 1990). Ito (1992) proposed a mechanism for the distribution of two liquid phases in a rotating coil based on the simultaneous action of two force components, a radial and a tangential one, on the point mass. The tangential force component (Eq. (1)) generates the Archimedean screw effect which drives both solvent phases toward the head of the column; whilst the radial component (Eq. (2)) interferes with the relative motion of the two solvent phases to establish the phase distribution throughout the coil. Since, however, both the mobile and stationary phases are not fixed to this point and are free to move, the actual acceleration of the phases will differ to that of the coiled tubing.

\[ F_t = R\omega^2 \sin \theta \]  
\[ F_r = R\omega^2 (\cos \theta + 4\beta) \]

Fig. 8 presents the variation of the total acceleration that is exerted on a point mass on the tube wall within one loop of the coil at beta values of 0.27 and 0.83 (minimum and maximum beta
values of the current CCC unit). The 0° and 360° angular position represent the distal key node whilst the 180° represents the proximal key node. Superimposed on these subplots are the approximate angular locations of the six distinct regions identified in the dynamic phase distribution patterns for the LIH, LOH, LIT, LOT elution modes (Fig. 7). The six segmented zones consist of three regions where the column is predominately filled with the ionic liquid phase, which alternate with very sharp boundaries, with three other regions where the aqueous phase fraction is significantly greater. It can be seen from Fig. 8 that there is good agreement between the phase location results for the elution modes where the liquid-liquid interfacial shear force is acting in the same direction (i.e. either in the centre to periphery direction or alternatively the periphery to centre direction) and this is regardless of the rotation direction of the CCC centrifuge. For example, it is observed that there is a good agreement between the locations of the regions for the LIH and LIT elution modes, which is when the lower phase is pumped from the centre to the periphery terminal and the centrifuge is rotated in the clockwise and anticlockwise direction respectively. Likewise, a relatively good agreement can also be seen between the results for the LOT and LOH elution modes, which is when the lower phase is pumped from the periphery to the centre, and the column is rotated in the clockwise and anticlockwise directions respectively. A change in rotation direction, changes the direction of the Archimedean screw effect. The current results suggest that the phase distribution effect caused by the Archimedean effect can mostly be overcome by the liquid-liquid shear force. Thus, ultimately these results provide evidence of the influence of the hydrodynamic liquid-liquid drag force on the resulting IL solvent system phase distribution in the 2D spiral column, which is not taken into account using Eqs. (1) and (2).

Fig. 7. High-speed visualisation of the phase distribution for the IL solvent system, 1:1:4 (v/v/v) \( [P\text{emix}]^\text{Cl}-\text{EtOAc}-\text{H}_2\text{O} \), inside the 2D spiral column during rotation for LIH, LOH, LIT, LOT elution modes. The upper ionic liquid phase (black) is the stationary phase. The lower aqueous phase (white) is the mobile phase. Mobile phase flow rate: 2 mL/min. Operational rotational speed: 800 rpm.
3.2.3. Phase mixing and settling in IL solvent two-phase system

3.2.3.1. Effect of elution mode on interfacial waves. Fig. 9 shows the variation of the radial acceleration (Eq. (2)) within one loop of the coil at beta values of 0.27 and 0.83 (beta value range of the present work CCC unit). Examination of these curves shows that the minimum occurs at the proximal key node (180° position) which is where mixing is more likely to occur, whilst the maximum values occur at the distal key nodes (0° and 360°) where the phases are separated. The effect of elution mode on the dynamic physical structure of the interface between the IL and aqueous phases in the mixing zone (0.27 < β < 0.36) is illustrated in the close-up images presented in Fig. 10, at a constant mobile phase flow rate of 2 mL/min at the same angular position (180°). Examination of the video recordings suggests that after hydrodynamic equilibrium of the system was established, the appearance of the interface at a given angular position did not change with time. It is interesting to observe that in contrast to the aqueous-organic systems discussed previously (Fig. 6) these images indicate a capillary wave motion at the interface in the mixing zone. Due to the higher viscosity of the IL solvent system, the physical structure of the interface also appears to be continuous, whilst generation of droplets of either phase was not observed. Comparison of the interfacial
characteristics for the various elution modes suggests significant differences in the wave mixing particularly depending on the pumping direction of the mobile phase. For the cases where the lower aqueous mobile phase is pumped in the head to tail direction (i.e., LIH and LOH), and good stationary phase retention was achieved, Fig. 10 indicates that there is no significant wave activity on the interface and only very small amplitude, low frequency waves are generated. On the other hand, when the pumping direction of the mobile phase is reversed to the tail to head direction (i.e., LIT and LOT) where the resulting stationary phase retention was much lower, well-defined waves are observed, with increased frequency and amplitude. Previously, the mixing observed at the proximal key node has been attributed to Kevin-Helmholtz (KH) instability on the interface (Sutherland et al., 1986; Wood and Sutherland, 2001). According to the KH instability criterion, the interface will be unstable, and waves will grow when the relative velocity of the two phases exceeds a critical value (Craik, 1992). Therefore, referring to the observed difference in mixing shown in Fig. 10 it may be possible that for the elution modes when the lower phase is pumped in the tail to head direction (LIT and LOT), the relative velocity of the phases exceeds the KH threshold velocity for the system, so that a large amplitude interfacial wave motion is generated. Conversely, it may be that pumping the mobile phase in the opposite head to tail direction (LIH and LOH) leads to a reduction in the relative velocity below the threshold velocity so that the interface is more stable and appears mostly flat.

Fig. 9. Variation for 2D CCC of radial acceleration exerted on a point on the tube vs. angular position in a single coil loop at different beta values. R: 100 mm, rotational speed: 800 rpm, $\beta$: 0.27 (solid), 0.83 (dotted). Proximal key node at 180° position, distal key node at positions $0^\circ$ and $360^\circ$.

Fig. 10. High-speed close-up visualisation of the interface in the mixing zone ($0.27 < \beta < 0.36$) around the proximal key node for the IL solvent system, 1:1:4 (v/v/v) [P66614]Cl-EtOAc-H$_2$O, inside the 2D spiral column for the elution modes where the lower aqueous phase is the mobile phase. Mobile phase flow rate: 2 mL/min. Operational rotational speed: 800 rpm.
3.2.3.2. Effect of beta value on interfacial waves. Fig. 11 shows the appearance of the interface in the mixing zone at different beta values for the LIT elution mode. Consistent with the suggestion by Wood et al. (2005) and Wood (2010) on the effect of beta value on mixing in coil-planet centrifuges, these images provide definitive evidence that there is enhanced wave mixing at lower beta values in 2D spiral columns. Close examination of these images shows that as the beta value is increased in the proximal region, and by definition the “G” force is increased, the interfacial waves are gradually dampened and there is almost a complete lack of wave activity present at the highest beta value. The difference in the mixing waves with beta value could be attributed to the change in direction of the tangential velocity \( \left( \frac{R}{2} \cos \theta + \frac{2}{R} \right) \) of the column just either side of the proximal key node (180° position) for a beta value less than 0.5, as can be seen in Fig. 12 (Wood et al., 2005; Wood, 2010). It is possible that this change in direction of the tangential velocity, causes the tangential velocities of the two phases to also change and increase their relative velocity. On the other hand, for beta values greater than 0.5, Fig. 12 shows that the tangential velocity of the column always remains positive. In general, the tangential velocity of the column increases with increasing beta value.

3.2.3.3. Effect of mobile phase flow rate on interfacial waves. The dynamic high-speed images presented in Fig. 13 illustrate the

Fig. 11. High-speed close-up visualisation of the interface in the mixing zone at different beta values (0.27 < \( \beta \) < 0.80) around the proximal key node for the IL solvent system, 1:1:4 (v/v/v) [Pb(N(Tol))4]:EtOAc:H2O, inside the 2D spiral column for LIT elution mode. Mobile phase flow rate: 2 mL/min. Operational rotational speed: 800 rpm.

Fig. 12. Variation of tangential velocity vs. angular position in a single coil loop at different beta values for the 2D spiral column. R: 100 mm, rotational speed: 800 rpm. Proximal key node at 180° position, distal key node at positions 0° and 360°.
effect of increasing aqueous mobile phase flow rate on the inter-
face shape in the proximal mixing zone (0.27 < \( b \) < 0.36) for the
LIT and LIH elution modes respectively. These results suggest that
with increasing mobile phase flow rate, the lower aqueous film
thickness increases in the column as the IL-rich phase retention
simultaneously decreases. In addition, as the lower mobile phase
flow rate increases, the interfacial waves gradually dampen and
eventually for flow rates above 6 mL/min a flat interface is seen
(Fig. 13). It is possible that the increase in mobile phase flow rate
leads to a reduction in the relative phase velocity below the KH
threshold velocity.

3.2.3.4. Settling pattern observations. Visual observations have also
been carried out in the distal settling area of the column. Fig. 14
presents the close-up imaging result obtained for the LIT elution
mode at a mobile phase flow rate of 2 mL/min in the region of
the distal key node. In a similar manner to standard aqueous-
organic systems, the IL and aqueous phases are separated in this
region. The interface is flat and, as expected, the less dense IL-
rich phase occupies the inner portions of the coils whilst the denser
aqueous phase occupies the outer portions. It was also seen from
examination of the video recordings that during the settling cycle
a small portion of IL-rich phase forms a thin film that stretches
over the entire tube circumference forming an annulus before
completely joining with most of the settled phase. The presence
of a thin film of one phase surrounding the other phase could be
important for chromatographic resolution as it increases the con-
tact area between the two phases.

3.2.4. Film thickness and local velocity measurements

The effect of beta value on the measured average film thickness
of the IL-rich phase at the proximal and distal key nodes for the LIH
and LIT elution modes is presented in Fig. 15. To obtain these
results, each image was discretized, and a uniform orthogonal grid
was generated (20 x 20 cells). For each channel, about 8 points
were selected at the interface where the height was measured
and the results were averaged. The maximum variation in the mea-
sured values was found to be ±13%. The average value was then
divided by the tubing inner diameter (2 mm) to give the average
dimensionless film thickness in each channel. It is found that the mixing wave amplitude decreases with increasing beta value in the proximal region (Fig. 11), whereas the average IL film thickness in the column increases. A similar increasing trend of IL film thickness with beta value can also be seen in the distal region. Fig. 15 also illustrates that for a given beta value, there is a significant decrease in the average IL film thickness between the proximal and distal key nodes, with the IL film thickness in the proximal zone approximately 50% higher than in the distal zone. The recorded IL film thickness is higher for LIH mode compared to the LIT mode, which agrees with the higher stationary phase retention levels associated with the LIH elution mode.

The variation of the average local velocities of the mobile phase with beta value in the proximal and distal regions are presented in Fig. 16. The assumption of constant stationary phase retention means that the stationary phase retention has zero linear velocity for all positions within the coil and the mobile phase local velocity is constant. These results suggest that due to the variation of the interfacial height within one loop of a coil (i.e. moving from the proximal to distal key nodes at the same beta value) there is in fact significant variation in the mobile phase velocity. Fig. 17 summarizes the clear inverse relationship between the mobile phase flow rate and the measured average IL film thickness values for the LIH and LIT modes in the proximal region. These results were extracted from the images presented in Fig. 13.

4. Conclusions

Ionic liquid-liquid based countercurrent chromatography represents a new approach to liquid-liquid extractions. Using high-speed imaging, this paper describes the first detailed study of the retention and hydrodynamic behaviour of ionic liquid biphasic solvent systems in 2D countercurrent chromatographic systems for a wide range of beta values (0.27–0.83). The results were compared against studies of different solvent systems and with a 3D column using the same ionic liquid biphasic solvent system. For the different solvent systems studied, the results revealed that contrary to previous limited visualisation studies, there was no single-phase flooding in the coil at low beta values (0.27–0.5). The results also showed that 6 segments with very sharp boundaries form in the column with alternating high and low stationary phase retention. These patterns were observed for the first time with the ionic liquid biphasic solvent system but not with the traditional organic-aqueous and aqueous-aqueous biphasic solvent systems also studied. Despite the widely held assumption of constant stationary phase retention that is used in CCC theory to predict separations, this unique phase distribution and the resulting large variations in interfacial height observed for the ionic liquid system, suggest significant changes to the local velocity of each phase along the coil. This phenomenon certainly adds to the complexity of the hydrodynamics governing the extracting process and further examination is recommended.

The ionic liquid biphasic solvent system also demonstrated novel behaviour in other aspects. In the mixing zone around the proximal key node, interfacial waves which would enhance mass transfer were observed. However, it was found that their amplitude, frequency and hence effectiveness depended on certain operational/design parameters such as pumping direction of the mobile phase, mobile phase flow rate and beta value. For the cases where the lower aqueous mobile phase was pumped in the head to tail direction (i.e. LIH and LOH), and good stationary phase retention was achieved, the results showed that there was no significant wave activity on the interface and only very small amplitude, low frequency waves were generated. On the other hand, when the pumping direction of the mobile phase was reversed to the tail to head direction (i.e. LIT and LOT) and the resulting retention was
much lower, larger amplitude, higher frequency waves manifested on the interface. As the mobile phase flow rate increased for these cases, it was found that the mixing waves gradually dampened and eventually for flow rates above a critical value, the interface became flat. It was also confirmed that low beta values in the proximal region favoured mixing for the ionic liquid system.

The effect of operating conditions such as elution mode, mobile phase flow rate and rotational speed on the retention of the stationary phase have also been determined. For optimum retention and hence high resolution separations involving the IL solvent system in the 2D coil configuration, it is recommended that the maximum rotational speed should be applied and the more viscous IL phase should be used as the stationary phase. Compared against the 3D column there was some agreement in the performance. However, it was found that for the serial 2D column there was always some retention of the stationary phase over the entire range of flow rates tested, unlike the case for the 3D column. Overall, it can be concluded that the results presented in this paper have successfully provided much needed insight into the operation of the CCC apparatus for ionic liquid based chromatographic extractions. These results, obtained for a specific case of IL solvent system, may be instructive for other two-phase solvent systems in the J-type counter-current chromatography. Sep. Purif. Technol. 65, 79–85. https://doi.org/10.1016/j.seppur.2008.07.016.

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