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Rational improvement of centrifugal partition chromatographic settings for the production of 5-*n*-alkylresorcinols from wheat bran lipid extract
I. Flooding conditions—optimizing the injection step

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Abstract

A dedicated CPC prototype permits direct flow pattern visualization in the partition cells of a CPC column. It was used to understand ''flooding'', a frequent phenomenon associated with large injections. A general strategy was developed to optimize the injection step in the framework of a particular preparative separation: the purification of 5-*n*-alkylresorcinols from a wheat bran lipid extract on a several hundred milligram scale. The construction of the ''mobile phase/stationary phase/sample'' pseudo ternary diagram characterizes the effect of the injected solution on mobile and stationary phases. The position of the binodal curve maximum indicates if the biphasic system is ''robust'' towards a large injection or not, and can be used for optimum mass load determination.

Keywords: Centrifugal partition chromatography; Injection methods; Visualization; Instrumentation; Alkylresorcinols; Lipids

1. Introduction

Counter-current chromatography (CCC) originated from the pioneering studies of Ito (see Ref. [1]) and refers to a support-free chromatographic method based on the partitioning of solutes between two immiscible liquid phases. According to the nomenclature of the inventor, instruments can be classified into two families, i.e. hydrodynamic or HDES, and hydrostatic or HSES. HDES instruments, mainly developed by Y. Ito and co-workers, are usually named CCC instruments [1], while HSES instruments, mainly developed by Nunogaki (Sanki Laboratories, Japan), are usually named CPC instruments, or centrifugal partition chromatographs [2]. A typical CPC column has a single rotation axis and two rotary seals, and consists of a series of cells connected by ducts in cascade [2]. Based on the

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density difference between the two phases, stationary phase retention is obtained by means of a centrifugal acceleration field. The mobile phase is pumped from cell to cell and flows through the stationary phase in the centrifugal direction when it is the more dense phase (this operating mode is called the descending mode) or in the centripetal direction when it is the less dense one (the ascending mode). Mass transfer occurs in each cell while the two phases are in contact. As a result of the absence of any solid support for stationary phase retention in the column, CCC and CPC instruments can be distinguished from other chromatographic devices by their large capacity. This feature makes them especially promising on a preparative or production scale. In fact, the volume of stationary phase usually available in CCC or CPC is commonly greater than 50% of the column volume, a property that delays saturation of the column in the case of injections of large sample amounts.

The flow-pattern, mass transfer and solute residence time distribution in CPC is fundamentally different from that of CCC, and has been extensively investigated in our laboratory [3,4].

At a given purity level, productivity enhancement of a chromatographic process requires treatment of the largest sample quantity in the shortest amount of time. The amount of injected sample can be raised by increasing the sample solution concentration and/or volume, whereas the separation time can be lowered when flow-rate, selectivity and stationary phase retention are increased. The increase of the sample solution volume has quite well-known effects in liquid chromatography. A large injection volume produces band broadening (broader peaks with more fronting and tailing [5]) that is especially important for the less retained solutes [6]. The increase of the sample mass load can have more dramatic effects as a total carryover of the stationary phase volume, a phenomenon called ‘‘flooding’’ [7–9]. This problem is sometimes reported in the framework of lipid purification using non-aqueous solvent systems. As it is often encountered at the development stage of the separation, only the parameters of the successful operation are communicated and the phenomenon remains unclear. This work makes some hypothesis formulation and experiments on how solutes can alter CPC conditions and lead to ‘‘flooding’’.

Our interest in injection optimization arose from the need of a preparative CPC separation procedure of 5-*n*-alkylresorcinols from wheat bran [10]. These compounds are extracted as a mixture of homologs (17, 19, 21, 23 or 25 carbons in the linear saturated hydrocarbon side chain). The assessment of the biological properties of each homolog requires several hundred milligrams of pure product. The first separation trial involved the injection of 250 mg of alkylresorcinol extract using the non-aqueous heptane–methanol biphasic system on a CPC instrument from Kromaton Technologies. Purified compounds were obtained at the milligram scale [11]. Transposition of this experiment to a five-fold larger injected sample led to flooding.

The sample is usually dissolved in the liquid phases previously chosen for the separation. There are three possibilities for sample dissolution: (i) in the mobile phase, (ii) in the stationary phase or (iii) in a mixture of both. According to the usual CPC working conditions (stationary phase retention larger than 50%), if the solubility of the sample in the stationary phase is higher than the solubility in the mobile phase, a saturated solution in the mobile phase can be injected without any risk of stationary phase overload [6]. However, when sample mass load is increased, other disturbing factors appear in addition to the possible saturation of the column. The physical properties of the injected solution with such a large amount of sample are substantially different from those of the stationary and mobile phases. Thus, the injected solution should be considered as a third phase that is not in equilibrium with the initial ones.

A special CPC prototype was available at our laboratory, developed for direct visualization of flow patterns in a CPC cell [3,12–14]. This prototype, named ‘‘visual-CPC’’, has been useful in understanding what occurs in the CPC cells during an injection.

We will discuss the influence of a highly concentrated injected solution on the elution mechanism in CPC. Elution in CPC is based on two main rules: (i) operating phases are not miscible, and (ii) operating phase densities are different and the elution mode is chosen according to this difference. Flooding may be due to a failure in one or both of these rules.

First, the unbalanced state between the injected matter and the operating phases is investigated by

using a pseudo ternary diagram mobile phase–stationary phase–sample. This diagram gives information on mass load optimization and explains why high mass and large volume injections have similar effects on chromatographic efficiency, but it does not fully explain flooding appearance.

Then, phase densities are accurately determined. Results show that elution becomes possible after dilution of the injected volume. There is a concentration limit value for which, at the end of the dilution step, the whole stationary phase volume has been pushed out of the column.

Lastly, results on the effect of mass transfer direction on liquid–liquid hydrodynamics will be used for a judicious phase choice for sample preparation.

This work is the first part of a complete approach on adjustment of CPC settings for production of *n*-alkylresorcinols (ARs). It will be followed by a second article on the optimization of the water percentage in the *n*-heptane–methanol system for the separation on a 200 ml CPC column, and a third one on the linear scale-up to a 1 l CPC column.

2. Experimental

2.1. The ‘‘visual-CPC’’

The visual-CPC was a prototype designed to visualize what occurs in each cell of a CPC column. The cells of the prototype used for experiments were engraved in a 5-mm-thick steel ring. The ring was clamped between a steel plate and a glass ring of optical quality (Ediver, Rebais, France). A 0.05 mm thick PTFE sheet was tightened on each side of the engraved ring to ensure a leakage-free rotor. There were 66 cells, nearly parallelepipedic (10×4×5 mm) connected by 0.8 mm width ducts. The rotor was connected through 1/16 in. PEEK tubing (UpChurch Scientific, Oak Harbor, USA) and two rotary seal joints (Tecmeca, Epernay, France) (1 in.=2.54 cm). The visual-CPC rotor was driven by an asynchronous motor (Leroy Somer, Angoulême, France) allowing rotational speeds up to 2500 r.p.m. The visual-CPC column volume is 16.1 ml. According to the number of cells of this instrument, it is inefficient for chromatographic separation and it will only be used for the analysis of operating parameters. Separation with a ‘‘complete’’ commercial instrument will be described in the next article.

2.2. Video instrumentation

A slightly modified VideoStrobe system was used (Sysmat Indutrie, St Thibault des Vignes, France), consisting of a TMC-9700 progressive scan charge-coupled device (CCD) color camera (Pulnix, Sunnyvale, CA, USA) with an asynchronous shutter, two Phylec stroboscopic units (Sysmat) and a VLS7T optical speed sensor (Compact, Bolton, UK) which triggered both stroboscopes and camera. The camera was equipped with an 18–108 mm F 2.5 TV zoom lens.

2.3. Chromatographic equipment

A P4000 quaternary gradient pump equipped with inert assemblies was used (ThermoQuest, San Jose, USA), which delivered constant flow-rates from 0.1 to 30 ml min^{-1}. A 6-port injection valve and a 4-port switching valve, both in PEEK material (UpChurch) were used for injection and selection of either ascending (upper phase mobile) or descending (lower phase mobile) mode.

More details on the visual-CPC, the video instrumentation and chromatographic equipment can be found in Refs. [3,13].

2.4. Sample preparation

The studied crude lipid extract from wheat bran was a kind gift from A.R.D. (Pomacle, France). This oil (525 g) was first fractionated in light petroleum (b.p. 60–80 °C)–methanol (13 l:7 l) and, after decantation, the two phases were separately evaporated under reduced pressure. The petroleum ether extract (80 g) was partitioned in the original triphasic system heptane–methyl tert.-butylether–acetonitrile–water (1.5:1:2:1.5, v/v), using 1 l of each phase. The upper one, enriched in 5-*n*-alkylresorcinols (ARs), was evaporated under reduced pressure to furnish 74.3 g of the desired extract. All solvents were purchased from Carlo Erba (Rodano, Italy).

2.5. The two-phase system and the sample solution

The selected non-aqueous two-phase system is heptane–methanol. *n*-Heptane and methanol are purchased from Carlo-Erba. The physical properties of each phase are summarized in Table 1. Heptane is partially miscible with methanol at room temperature (miscibility around 30%, w/w at 20 °C and 1 atm). Due to this miscibility, the two phases have close density and viscosity values, and the interfacial tension is low (it was measured by the spinning drop method at 23 °C [15]). The viscosity was calculated by pressure drop measurement in a capillary tube, previously calibrated with pure solvents.

In this system, it appears that the ARs are distributed between the two phases (heptane and methanol mutually saturated) with some preference for the methanol-rich phase. In order to have sufficient selectivity, the separation was performed in the ascending mode (the normal mode) in which the mobile phase is the heptane-rich one.

2.6. Operating conditions

All experiments are done at 22 °C (±0.5 °C). The rotational speed is fixed at 900 r.p.m. (giving a centrifugal acceleration of 92.8 g) and the flow-rate is fixed at 15 ml min^{-1}. The elution is done in the ascending mode: the methanol-rich phase is stationary and the heptane-rich one is mobile. The selected injection procedure is the sandwich injection. This name stems from the fact that the sample solution is introduced behind the solvent front. It is useful when the injection method is a syringe or any low-pressure device, as there is no pressure at the column head before the hydrodynamic equilibrium. A noisy baseline is obtained when the mobile phase front appears as a consequence of the absence of preliminary equilibrium between the phases in the column. This noisy baseline may be present for a long time if the system used is quite unstable, and can prevent the monitoring of the readily eluted sample constituents [16].

An injection loop of 1.61 ml is used (10% of the column volume, consisting of a 2 m× 1/16 in. O.D. PEEK tubing).

3. Results and discussion

3.1. Flooding conditions

In CPC, the chromatographic process is based on the interfacial mass transfer and occurs in each cell when the two liquid phases are in contact. Initially, cells are entirely filled with stationary phase. When the mobile phase is introduced in the entrance zone of the cells, an interface is formed, and until the mobile phase has reached the outlet of the cell, part of the stationary liquid phase initially present has been moved out of the cell. The resulting ratio depends on the mobile phase velocity powered by the centrifugal acceleration and on the flow-rate. At the outlet of each cell, the phases need to separate (mobile phase coalescence).When only mobile phase leaves the cell, an equilibrium, called the hydrodynamic equilibrium, is reached. When flooding occurs, it follows the solute injection, the hydrodynamic equilibrium is never reached, and the stationary phase is completely carried over. The effects of the injected solution on the phase behavior in the column may have the following origins:

(a) Solutes have surfactant properties.

(b) The injected solution is not in equilibrium with the phases present in the column, such that the phases are no longer immiscible.

(c) The interface between the two phases is

Table 1
Physical properties of the heptane–methanol two-phase system at 22 °C (except σ, at 23 °C)

ρ Density UP ($kg\ m^{-3}$)	ρ Density LP ($kg\ m^{-3}$)	$\Delta\rho$ Density difference ($kg\ m^{-3}$)	σ Interfacial tension ($10^{-3}\ N\ m^{-1}$)	μ Viscosity UP ($10^{-3}\ Pa\ s^{-1}$)	μ Viscosity LP ($10^{-3}\ Pa\ s^{-1}$)
684	756	72	1.16	0.47	0.65

UP, upper phase (heptane-rich one); LP, lower phase (methanol-rich one).

strongly affected by an important interfacial mass transfer.

According to the specificity of the application, effects may not result from one but rather a combination of these origins.

The tensioactive property of a solute does not strongly depend on its concentration. Surfactants affect the interface even if they are present in trace amounts. Surfactants are classified according to the time they need to migrate towards the interface and to orientate themselves in the interfacial media. In a process like CPC, the lifespan of the interface in the cells is of the order of tenths of a second and the surfactant should lack time to act and disturb the hydrodynamic. A confirmation was obtained at our laboratory for DEHPA (di-2-ethylhexylphosphoric acid) in heptane–water two-phase system. No significant effects on phase behavior were observed whatever the DEHPA concentration [17].

In the ideal case of a diluted sample solution, its physical properties are close to those of the initial operating phase and its injection does not alter the liquid–liquid equilibrium in the column. When the concentration of the sample increases, one has to expect that the corresponding solution will have physical properties so distant from that of both mobile and stationary phases that the injected volume will behave like a third phase, partially miscible with the two operating phases.

For the previously given operating conditions, at the hydrodynamic equilibrium, the flow pattern is a spray and the stationary phase retention is about 60%. The sample solution is the lipid fraction dissolved at 300 g l^{-1} in a volume of stationary phase. The sandwich injection of this sample leads to a significant change in the column. In the cells where the sample comes into contact with the blank mobile phase, a darkening of the phases is observed, making it difficult to determine if this is due to the presence of undistinguishable droplets (a kind of an emulsion) or to a complete mixing and a dissolution of the phases. The propagation of the phenomenon from cell to cell along the column can be followed and the hydrodynamic equilibrium is never reached. The resulting carryover of the stationary phase is complete and no separation is obviously conceivable. This behavior is clearly due to the presence of the solutes even if their concentration value is far from the solubility limit one [this limit value was not finely determined, but the extract remains soluble at 1000 g l^{-1} in both phases (in the heptane rich phase, sonication was needed to reach this concentration)].

If the flooding appearance in this experiment is related to the presence of a surfactant in the extract or to an important interfacial mass transfer, flooding would also occur for an extract concentration still high but three times lower, i.e. 100 g l^{-1}. This injection was done in the same operating conditions. The phase ratio in the column was slightly modified, involving some stationary phase volume loss (from 63 to 47%), but no flooding appeared. Flooding seems to be related to a nonequilibrium state in the column between the operating phases and the injected one. The observed darkening in the column may be due to a dilution event rather than to a micro-emulsion formation.

3.2. An unbalanced state in the column—introducing a pseudo ternary diagram

Thus, in order to determine the influence of the unbalanced state between the injected phase (a highly concentrated sample dissolved in a volume of stationary phase) and the operating phases themselves, a pseudo ternary diagram—mobile phase, stationary phase, extract (w/w/w)—is introduced. As is often encountered in liquid–liquid extraction, a solubility isotherm (or a binodal curve) of the crude extract in the heptane–methanol two-phase system is built. Pre-defined ratios (w/w) of stationary phase on mobile phase are successively added to 400 mg of pure extract until the appearance of the conjugated phases. The mass quantities of stationary phase and mobile phase added to the 400 mg of extract for nine selected ratios are summarized in Table 2, giving the coordinates of the points on the solubility isotherm. The ternary diagram is presented in an orthogonal form in Fig. 1.

It clearly appears that, in the framework of this application, if the extract mass concentration exceeds 9%, the injected solution can not be in equilibrium with the two-phase system. In other words, a phase with an extract mass concentration larger than 9% will first need to be saturated with either the mobile or stationary phase to be considered as nonmiscible with them. Using this diagram, the optimum in mass

Table 2
Masses of both mobile and stationary phases added to 0.4 g of the considered lipid extract when biphasic state appears, and the associated coordinates of points on the solubility isotherm of the extract in the heptane–methanol system at 23 °C

MP (g)	0.758	1.278	1.548	2.010	2.642	3.498	6.838
SP (g)	6.841	3.833	2.576	1.996	1.582	1.167	0.761
MP (w/w/w)	9.5%	23.2%	34.2%	45.6%	57.1%	69.1%	85.5%
S(w/w/w)	5.0%	7.3%	8.8%	9.1%	8.7%	7.9%	5.0%

MP, mobile phase; SP, stationary phase; S, sample.

load can be determined; it is located on the extremum of the binodal curve (point E on Fig. 1). Its mass composition is about 9% of extract, 45.5% of mobile phase and 45.5% of stationary phase. The optimal quantity of equilibrated extract solution is obtained when it is diluted in a mixture of equal volumes of stationary and mobile phases. This is consistent with the already observed good distribution or partitioning of the extract compounds between the two phases.

Points F_1 and F_2, that correspond to the early injected solutions at 100 g l^{-1} and 300 g l^{-1} in the stationary phase, are placed in the diagram. These points are not on the isotherm, but above it, confirming that the two injected solutions were not in equilibrium with the operating phases in the column. The diagram indicates the mobile phase amounts needed in order to achieve phase immiscibility with the mobile phase, and calculation of sample saturation is possible (Table 3).

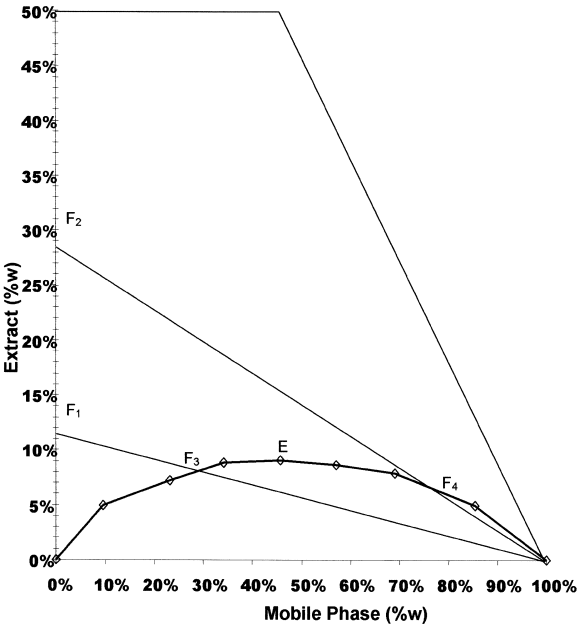

Fig. 1. Pseudo ternary diagram of the system extract-mobile phase-stationary phase (in %, w/w) for the considered lipid extract and the heptane–methanol two-phase system at 23 °C.

The first injection F_1 needs 0.45 g of mobile phase to become biphasic, giving a solution F_3 rather rich in stationary phase (64.5% w/w), whereas the injection F_2 needs 3.71 g of mobile phase and gives a solution F_4 rather rich in mobile phase (75% w/w). Despite this difference, F_3 and F_4 are two solutions of identical densities (about 0.7 in both cases) and similar mass concentration of extract (about 8% w/w). When the injected sample concentration increases, the mobile phase volume needed to get immiscible phases in the column and thus to start the elution or the chromatographic step also increases. When the chromatographic step begins, the high mass injected volume has been strongly diluted, explaining that a large mass load and a large volume injection have the same peak broadening effect. Here, when the sample mass load is multiplied by 2.4, the resulting sample volume is multiplied by 2.9.

Table 3
Properties of the injected solutions and mobile phase quantities needed to get biphasic state appearance

	Sample 1 100 g/l^{-1}	Sample 2 300 g/l^{-1}
Extract mass concentration (F_1, F_2)	11.7%	28.4%
Loop volume (ml)	1.6	1.6
Injected mass (g)	1.2	1.2
MP mass added (g) to reach the biphasic appearance	0.45	3.71
Corresponding MP volume (ml)[a]	0.7	5.4
Resulting sample volume (ml)	2.4	7.0

[a] With respect to the mobile phase density.

As the solubility isotherm is quite symmetrical, injections of extract in mobile phase solutions are expected to have similar effects. The mobile phase–stationary phase–sample ternary diagram is then a useful tool for the optimization of both injection step and separation; it gives information on optimal conditions, gives criteria for the choice of the phases and helps for result interpretation. In Fig. 2 a ''weak system'' and a ''robust system'' are presented based on the fact that in the second case, the extract mass load possibilities are much higher than in our example with the ARs.

The unbalanced state between the phases in the CPC column explains why a large mass load has the same broadening effects as a large volume injection in CPC, because in both cases the injected matter at equilibrium occupies a large volume ratio in the column. Unfortunately, it does not fully explain the appearance of flooding.

3.3. Phase hydrodynamics and phase density

Miscibility was the main notion previously discussed, but another parameter, phase density, can be strongly modified by highly concentrated solutes, thus disturbing the elution process in CPC based on density difference and centrifugal acceleration. Phases densities are reported in Table 4.

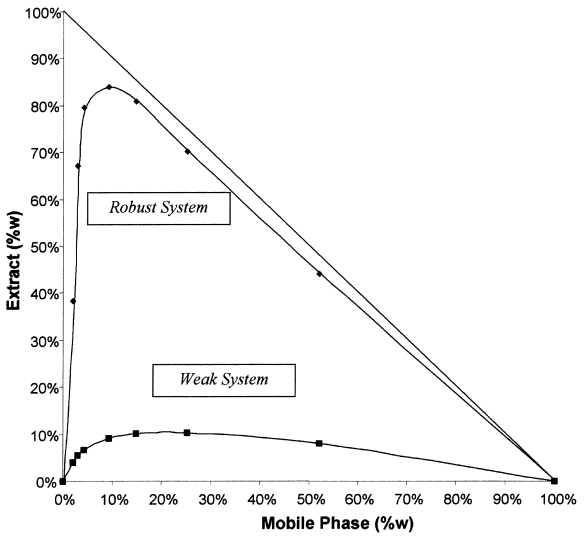

Fig. 2. Characteristic shapes of the solubility isotherm curves for a weak or a robust two-phase system with respect to the extract of interest.

Table 4
Characteristics of the interesting points in the ternary diagram at 23 °C

% (w/w)	Extract	MP	SP	Density
MP	0.0	1	0.0	0.684
SP	0.0	0	1.0	0.756
F_1	11.7	0	88.3	0.770
F_2	28.4	0	71.6	0.769
F_3	8.5	27	64.5	0.700
F_4	7.5	75	17.5	0.702

Densities were accurately measured via the weighing of gauged volumes, with a reproducibility of about 1.5%. Before any contact with the mobile phase, the injected solutions (F_1 and F_2) have densities about 0.77. They are denser than both mobile and stationary phases. During the dilution of the phases F_1 and F_2, the density of the solutions diminishes until a common value of about 0.7 for F_3 and F_4 is reached. This density value is intermediate between that of the mobile and the stationary phase. Conceptually, in the case of a sandwich injection, the column is initially filled with the stationary phase and the sample solution F_1 or F_2 is in the column inlet (Fig. 3a). In the ascending mode, the mobile phase is the less dense one. It can rise up through the sample solution but that one, being heavier than the stationary phase, pushes it instead of percolating through it. During the sample solution dilution with the mobile phase, supported by the fact that the mobile phase can flow through it, its volume increases and its density decreases (Fig. 3b). The configuration changes when the density at the borderline between the sample solution and the stationary phase reaches the stationary phase density value (Fig. 3c). This concentration of the sample can be defined as C_c, the critical concentration. For further calculation, density is assumed to be a linear function of the sample concentration in the close bounds of our study, i.e. between 0.700 and 0.770, and the mixing of the sample solution with the mobile phase in each cell is modeled by a perfectly agitated reactor (Fig. 4). In order to verify such a mixing behavior in CPC cells, some monophasic tracer injections are needed. Such injections have already been done in order to verify the column volume by use of the

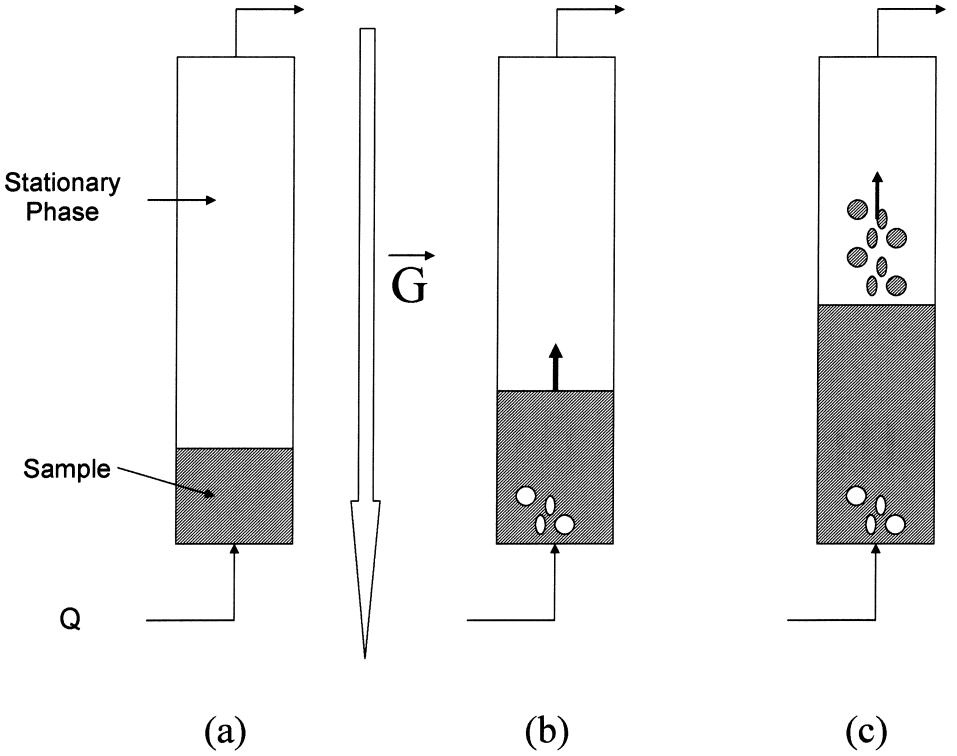

Fig. 3. Scheme of the CPC column, at its initial state (a) with the position of the sample solution and the stationary phase for a sandwich injection in the ascending mode, when elution of the mobile phase is started (b)—the mobile phase can percolate through the sample solution, but this one, heavier than the stationary phase pushes it—and when the sample solution, diluted by the mobile phase, becomes lighter than the stationary phase, elution starts (c).

main residence time of a tracer [8,18]. As the standard deviation of the solute time distribution is also available, the number of perfectly agitated reactors giving the same blending can be determined. According to an acceptable experimental error of 15% on the standard deviation value (cf. reproducibility and noisy baseline), the number of cells and the calculated number of perfectly agitated reactors are equal. Then, the time evolution of the extract concentration in each cell can be estimated and the time necessary to reach the stationary phase density value corresponding to C_c can be calculated.

3.3.1. Mixing plate model for dilution

The initial number of cells, J_0, used to model the dilution of the injected solution is equal to the ratio of the injected volume and the cell volume rounded to the higher integer. In our example, the injected volume is equal to one tenth of that of the column which includes a number $N=66$ cells; thus $J_0=7$.

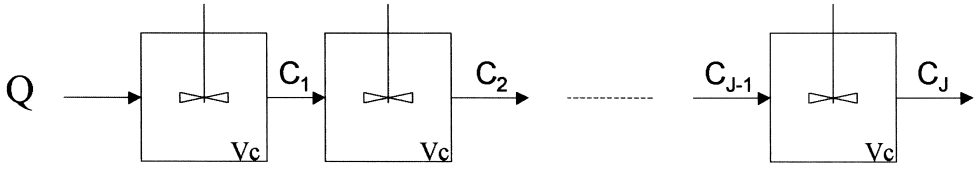

Fig. 4. The CPC column as it is seen in the mixing plate model for the dilution of the sample solution. V_{cell} is the cell volume, C_i is the extract mass concentration at the cell i outlet and Q the mobile phase flow-rate.

Table 5
Simulation results — position of the front J_c where the sample solution density reaches the stationary phase density value ($\rho = 0.756$)

	Sample 1	Sample 2
C_0	11.7%	28.4%
Critical mass concentration C_c for $\rho = 0.756$	11.1%	9.2%
Mass concentration at the biphasic state appearance (F_1 and F_2)	8.5%	7.5%
J_c	15	66

If Q is the mobile phase flow-rate, $C(t)$ the extract mass concentration and V_{cell} the cell volume, the solute concentration in the first cell C_1 is:

$$Q\,C_1(t) + V_{cell}\frac{dC_1(t)}{dt} = 0$$

and in the following cells n where the sample solution is present:

$$Q\,C_{n\text{-}1}(t) = Q\,C_0(t) + V_{cell}\frac{dC_n(t)}{dt}$$

Remark: since the injected solution is diluted and its volume increases, the number of cells where injected matter is present, J, is then implemented with the elapsed time every (V_{cell}/Q).

The differential equation resolution is done with a simple program in Visual Basic for Excel. For specified values of initial and critical concentrations (C_0 and C_c), Q and V_{cell}, it gives the position of the front between the sample solution and the stationary phase J_c (or critical J), when the concentration at this front reaches the critical value at which the sample density becomes lower than the stationary phase density. Results are summarized in Table 5 and calculated elution curves for various cells along the column are presented in Fig. 5.

For F_1 injection, the critical concentration is obtained at the outlet of 15th cell whereas for F_2 injection, when the critical condition is reached, the column is emptied of its stationary phase volume and flooding is then predicted. In Fig. 6, where the % volume of the column needed to get critical concentration C_c (J_c/N) is plotted against the initial % volume corresponding to the injection (J_0/N), it appears that J_c increases very quickly with the injection volume and that flooding is predictable for injection volumes comprised between 4 and 5 times the cell volume (i.e. about 7% of the column volume).

From the above demonstration, the density of the sample solution seems to be a fundamental parameter during the injection step of a CPC purification. The

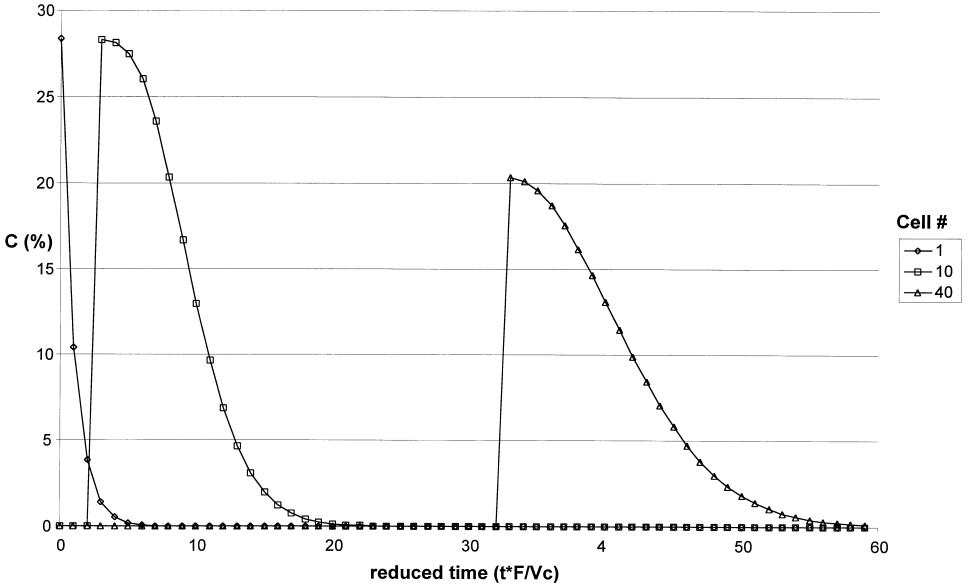

Fig. 5. Extract mass concentration calculated curves in various cells of the CPC column (cell numbers 1, 10 and 40).

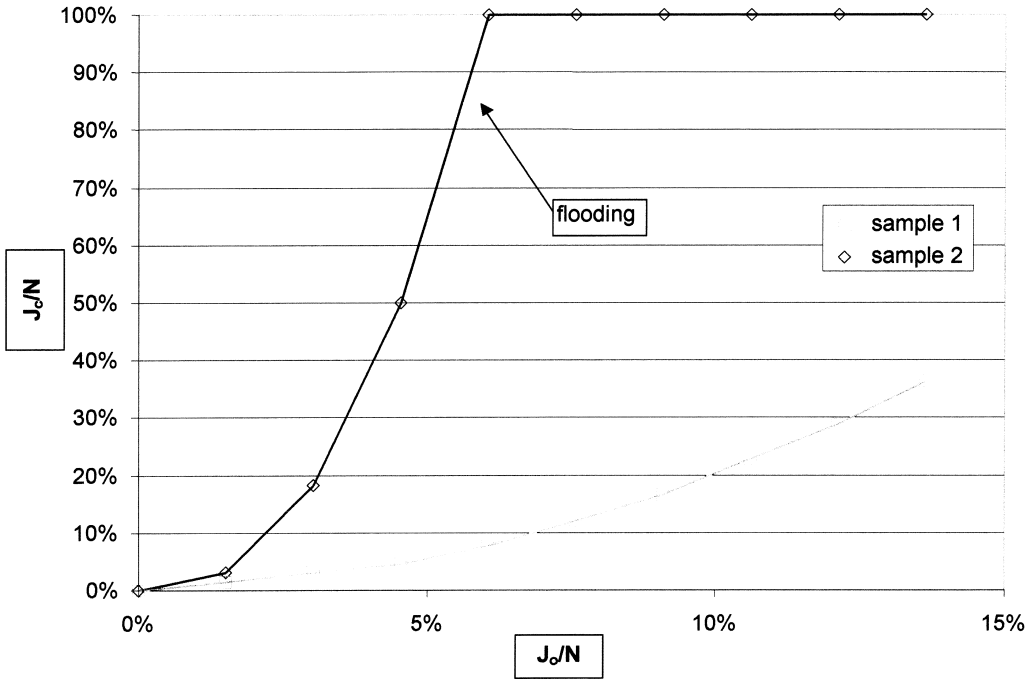

Fig. 6. Curves giving the calculated % volume of the column needed to get the critical concentration C_c, (J_c/N), as a function of the initial % volume corresponding to the injection (J_0/N) for the two considered sample concentrations (100 g l^{-1} for sample 1 and 300 g l^{-1} for sample 2). Experimental results were obtained at $J_0/N=10\%$.

presence of solutes in large quantities in the injected sample strongly alters the physical properties of the liquid phase used for make-up (mobile, stationary or both), mainly its density. Generally denser than both the upper and lower operating phases, the injected volume needs to be diluted until it reaches the critical concentration allowing it to percolate through the lower phase in the ascending mode.

For injections in the descending mode (the stationary phase is then the less dense phase), a similar approach can be done. In that case, the sample solution is still the densest one, so it can percolate through the stationary phase but it cannot be percolated by the mobile phase, which is lighter. This results in dilution of the injected solution at its forefront by the stationary phase instead of its back by the mobile phase, and in this case the critical concentration then corresponds to the sample concentration at which the sample solution density will reach that of the mobile phase (i.e. the lower phase too). As it is shown in Table 4, saturated solutions F_3 and F_4 have physical properties between those of the blank phases. The injection of such phases may prevent ''flooding''.

3.4. Influence of mass transfer direction

Additional experiments are proposed in order to better describe the contribution of the unbalanced state and the physical properties of the injected solution. 1.6 ml of F_3 was directly injected (see Fig. 1) using the same conditions as for the F_1 injection (at 100 g l^{-1}, out of the flooding conditions). In this case there is no unbalanced state effect, as sample saturation is previously obtained, and only physical properties will have an influence. The stationary phase retention is less altered, varying from 63% to 53% (instead of 63% to 47%). As the loss of stationary phase retention is divided by more than 2 in our example, the saturation of the sample solution with the operating phases simultaneously prevents the appearance of flooding, and has a positive effect on the phase ratios in the column. This can be explained as follows:

(i) The F_3 sample solution density is intermediate between that of the mobile and the stationary phase. The elution of the mobile phase through the sample solution, in the same conditions of flow-rate and centrifugal acceleration, is thus more difficult than through the blank stationary phase. Dynamic equilibrium is then altered in the first cells where the solutes are initially present.

(ii) The flow pattern is modified by the interfacial mass transfer from the stationary phase to the mobile phase. The mass transfer from the continuous phase to the dispersed one (i.e. from the stationary phase to the mobile phase in CPC) is known to be unfavorable to dispersion and coalescence. It tends to stabilize the interface [19]. Mass transfer from the dispersed phase to the continuous one has an opposite effect. The phenomenon is proportional to the solute concentration and is thus more important in the first cells of the column.

In fact, if 1.6 ml of F_4 are injected using the same conditions as for F_3 (extract mass concentration about 8%, in a rather mobile phase rich solution — symmetrically from F_3 on the solubility isotherm), we observe in the first cells that the flow pattern is much more dispersed than in the absence of solutes, but the resulting stationary phase retention is not perceptibly modified (around 63%). The mass transfer orientation is the only parameter that changes between these two experiments (as the binodal curve is symmetrical and the sample solutions prepared in the mobile and the stationary phase has the same density). As mass transfer towards the stationary phase supports the contact between the two phases and the stationary phase retention, injections of sample in mobile phase solutions should be preferred.

4. Conclusion

According to our experiments, a large scale injection in CPC should follow the steps of Table 6.

We have demonstrated here that in the case of large solute amount injections, even if we are far from the solubility limits of the extract, the injected solution behaves like a third phase. The latter can be mutually soluble with the mobile and stationary phases, and its density can be higher than the two others. The flooding conditions in the ascending mode are easily modeled and predicted according to the stationary phase retention principle in CPC based on density difference and centrifugal acceleration; needed information is the unsaturation degree of the injected solution and its density. In order to optimize the injection conditions, it is preferable to avoid non-equilibrium states in the column in order to use the whole column for the separation step, and not for dilution. A pseudo ternary diagram (mobile phase–stationary phase–extract) is introduced. It characterizes the ‘‘injected third phase’’, and immediately gives the optimum mass load. This study on the unbalanced state between the phases also shows that a large mass load has an almost equal effect as a large volume one on the phase behavior in the

Table 6
Logical approach for a large scale injection in centrifugal partition chromatography

Step	Action	Objectives and remarks
1	Build the ternary diagram upper phase, lower phase, sample using weight units	Define the biphasic zone where points corresponding to the composition of the injected volumes must be. Allow the determination of the maximum concentration of sample to be injected
2	Evaluate the density of the injected volume issued from step 1	If higher than that of the operating lower phase, it needs further dilution before injection
3	Prefer the injected phase to be similar to the operating mobile phase	This will result in a much better mass transfer between the injected phase and the stationary phase, starting from the beginning of the CPC column

column, thus clarifying the reason why they have similar effects on chromatographic efficiency. Solute effects on the interface can be judiciously used, as the mass transfer orientation between the two phases in the column has a significant influence on the interface stability. Mass transfer from the mobile phase to the stationary one increases the mobile phase dispersion and supports its coalescence at the cell outlet. As this phenomenon is proportional to the solute concentration, mobile phase injection should be preferred, especially for preparative applications. Notions that are used here are not properly related to the nature of the solutes, except the position of the binodal curve; in our example, the best conditions are a sample solution at 9% mass in the mobile phase-rich conjugated phase, injected in descending mode. This methodology can be easily transposed for other applications, starting with the realization of a ternary diagram.

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