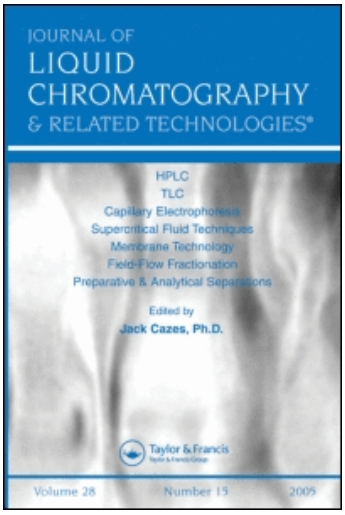

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Modelling CCC Using an Eluting Countercurrent Distribution Model

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Modelling CCC Using an Eluting Countercurrent Distribution Model

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ABSTRACT

As countercurrent chromatography (CCC) is becoming an established method in chromatography for scaling from analytical CCC in the laboratory to full process scale in the industrial manufacture of products, it is becoming increasingly important to model the process and to be able to predict coil/column scale-up parameters for a given process. This paper offers a method of modelling CCC on the basis of an eluting countercurrent distribution (CCD) model. The model confirms that peak width in CCC varies in proportion to the square root of the length of the column, establishes a formula predicting peak width in terms of retention factor and retention time, and provides a method for determining the efficiency of a given CCC instrument. This allows, for the first time, the mixing efficiency of different CCC approaches and/or devices to be compared and perhaps, more importantly, predictions to be made that are outside the current operating parameters of existing CCC instrumentation. This will

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greatly assist in the design of new equipment, particularly in scale-up, and will also help users optimize the results from their CCC instruments.

Key Words: CCC; CCD; Liquid–liquid chromatography; Modelling; Distribution ratio; Retention; High resolution; Scale-up; Distribution constant.

INTRODUCTION

Countercurrent chromatography (CCC)[1–3] is a form of liquid–liquid chromatography, which takes place along a continuous length of tubing. The tubing is initially filled with the phase intended to be the "stationary" phase and then the mobile phase is pumped into the tube at a given flow rate. Winding the tubing on a drum or bobbin, which is rotated in planetary motion on a coil planet centrifuge, sets up zones of mixing and settling between the two solvent phases at a rate dictated by the speed of rotation of the centrifuge. One thousand revolution per minute, for example, will result in a sample injected with the mobile phase experiencing 1000 mixing and settling steps per minute. The hydrodynamic behavior of the phases in the tubing is such that the lighter phase wants to move toward the head end of the coil and the heavier phase wants to move toward the tail end of the coil, where the head end is defined as the end to which a ball or bead would screw under Archimedean screw action.[4] The "stationary" phase is held in equilibrium and is retained in the tubing against quite high flows of the mobile phase.[5–7] The word "stationary" is purposely put in "quotes," as it is now known that within each coil unit the stationary phase undergoes a back and forth "swish-swash" motion, which enhances the mixing between the two phases.[8] The retention of the stationary phase has been found to have a linear relationship with the square root of the mobile phase flow,[9] and that the square of the mobile phase linear velocity is linearly related with mobile phase flow.[10] The stationary phase has been found to remain in equilibrium when these relationships are linear and become unstable, and slowly elute when they are not—this latter phenomenon being known as "stripping."

Countercurrent chromatography can be considered as a form of column chromatography without a solid support or, alternatively, as a continuous form of countercurrent distribution (CCD). In the 1950s Craig's CCD apparatus (Fig. 1) was extensively used for the purification of natural products[11] based on their distribution ratio or partition coefficient, as it used to be known, where the distribution ratio is the ratio of sample concentrations in the two immiscible phases. It is a fine example of a separation system that is completely mathematically predictable, once the solute distribution ratio and

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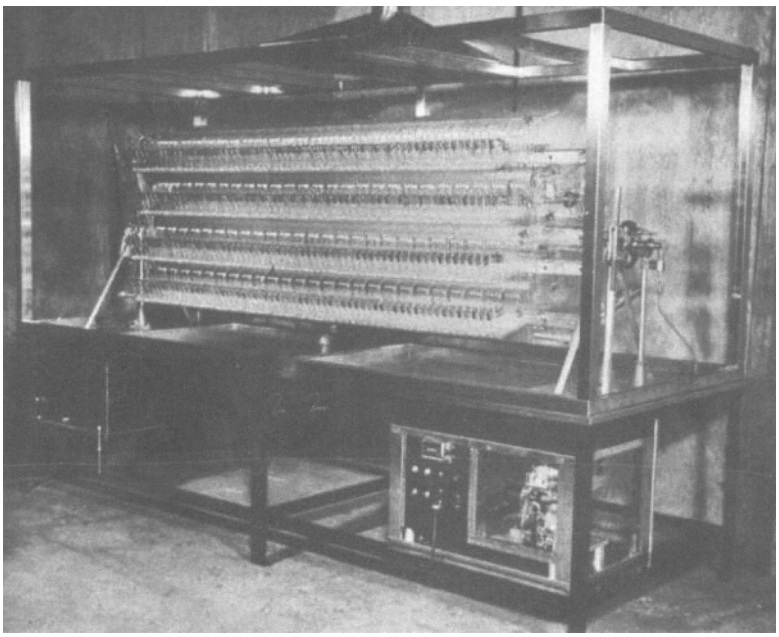

Figure 1. Craig 100 transfer CCD apparatus.

the ratio of mobile to stationary phase volumes is known. This paper builds on CCD theory by extending beyond a given number of transfers allowing the transfer of mobile phase to continue into a fraction collector (elution CCD), thus rendering it similar to standard liquid chromatography with comparable chromatograms.

This paper describes the theory behind this elution-CCD model, its validation against known CCC theory, and its use to predict CCC chromatograms. It then describes a simple spreadsheet process for predicting partitioning behavior that can be used in conjunction with or instead of the model, using data supplied by Bousquet et al.[5] as an example. The importance in CCC of not using the normal chromatography term "number of theoretical plates" as an efficiency term is emphasized, as it does not relate very well to resolution "efficiency" due to the very large volume of the stationary phase. Finally, it makes recommendations to future authors on the procedures they should use and the information they should give when writing CCC applications papers. In this way, it will be possible in the future, to build up a useful database on the efficiency of different CCC instruments for a given set of operating parameters and phase systems.

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THEORY

The theory of CCD was established by Martin and Synge in the 1940s[12] and re-used, in some depth, by Mandava and Ruth[13] in Mandava and Ito's book on CCC. In CCD the retention factor (k') is defined as the mass in the upper transferred phase divided by the mass in the lower stationary phase as follows:

$$k'_{\mathrm{CCD}} = \frac{m_u}{m_l} = \frac{C_u V_u}{C_l V_l} = \frac{D_{\mathrm{CCD}} V_m}{V_s} = \frac{D_{\mathrm{CCD}}(1 - S_f)}{S_f} \tag{1}$$

The distribution of a sample, with retention factor (k'), after τ mixing, settling and transfer steps, can be described as follows:

$$(x + y)^{\tau} = x^{\tau} + {}_{\tau}C_1 x^{\tau-1} y + {}_{\tau}C_2 x^{\tau-2} y^2 + \cdots + y^{\tau} \tag{2}$$

where

$${}_{\tau}C_r = \frac{\tau!}{(\tau - r)! r!} \quad x = \frac{1}{(1 + k')} \quad \text{and} \quad y = \frac{k'}{(1 + k')}$$

In CCC the separation is not restricted to the chain of "τ" test tubes. Elution takes place in such a way that the upper phase volume is passed on to a fraction collector with fraction 1 being the "$\tau + 1$" transfer. The quantities in each eluted fraction from the CCD chain can be described as follows:

$$[\mathrm{Fraction}]_{(i-\tau)} = \sum_{i=\tau}^{n_{\mathrm{tot}}} \frac{i! x^{(i-\tau)} y^{\tau}}{(i - \tau)! \tau!} \tag{3}$$

where τ is the number of transfers to elution (i.e., the size of the CCD chain), n_{tot} is the total number of transfers and $(i - \tau)$ is the fraction number at the ith transfer.

A computer CCD model (described below) was developed based on Eqs. (1–3) but defining retention factor (k') and distribution ratio (D) from the perspective of CCC:

$$k'_{\mathrm{CCC}} = \frac{m_s}{m_m} = \frac{C_s V_s}{C_m V_m} = \frac{D V_s}{V_m} = \frac{D S_f}{(1 - S_f)} \tag{4}$$

where $k'_{\mathrm{CCC}} = 1/k'_{\mathrm{CCD}}$

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DESCRIPTION OF MODEL

The model is built around an array based iterative solution of Eqs. (1–3) above. The programming language used was Delphi 3, which is similar to Pascal, but is Microsoft Windows oriented.

An array of positions, representing test tubes is set up in one dimension, with mixing settling and transfer activities set up in the other. This is illustrated schematically in Fig. 2 for two transfers in a three test tube chain. Initially each tube is filled (step 0) with a given proportion of each phase (50 : 50 in the example), defined by the retention of stationary phase (S_f). Next, a sample

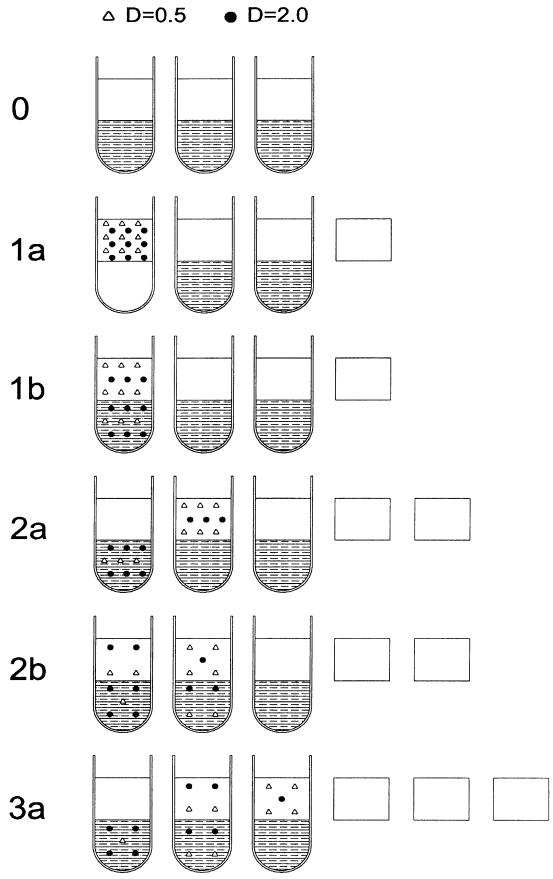

Figure 2. Schematic diagram of CCD elution model for two solutes with distribution ratios of 0.5 and 2 undergoing two mixing and settling/transfersteps.

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component containing equal amounts of two solutes of distribution ratio, D_1 and D_2, dissolved in mobile phase, is transferred to the first tube successively displacing the mobile phase components of the other test tubes in the chain until a mobile phase volume is eluted from the chain into, for example, a fraction collector (step 1a). The whole chain is then mixed and the phases allowed to settle (step 1b) assuming 100% mixing and ideal mass transfer/distribution between the two phases, as defined in Eq. (4). After this, the mobile phase parts of each tube are again transferred to the next tube (step 2a) and so on. Even in this two transfer example, a clear fractionation between the two solutes can be seen in the first and third test tubes, where there is a 4 : 1 enrichment of the $K_D = 2$ and $K_D = 0.5$ solutes, respectively.

The model assumes a starting sample mass of 100% of each solute and calculates the mass distribution as transfers progress from $i = 0$ to n_{tot}.

Model Display

A typical elution profile in "run mode" is shown in Fig. 3a. The input parameters for the model are set in the windows provided in the top of the display. From left to right these are: the distribution ratios of a mixture of samples 1 and 2 (0.2 and 1.0 respectively, but shown in the old partition coefficient notation of k_1 and k_2); the number of transfers to elution of the solvent front (τ—set at 200, maximum 10,000); the total number of transfers (n_{tot}—set at 1600, maximum 100,000); the number of sample insertion steps into the first tube (set at 1, but must not exceed the number of run steps); the cycles per mobile phase transfer (normally set to 1); the percentage retention of stationary phase ($S_f = 85\%$, range 0–100); and the number of animation steps.

The top of the display contains pull down menus. "File" contains an export function which allows the export of data to an excel spreadsheet. "Calculate" gives the option of "run" and "time" modes. Selecting "run" will initiate the programme and the display will show the distribution of the two samples as the programme steps through, in this example, from $i = 0$ to 1600 as shown in Fig. 2. In practice, samples are generally collected in a fraction collector or optical density is monitored with a spectrophotometer and readings displayed on a chart recorder in "time" mode, which reverses the time order. This arrangement can be displayed by selecting "time mode" in the "calculate" menu, which will display the chromatogram as in Fig. 3b with the y axis at $t = 0$, representing the solvent front or the point in time when a sample with a distribution ratio of $D = 0$ elutes. The "options" menu allows the animation to be turned on or off and allows auto-scaling to be enabled or not. The centre of the display contains a graph of sample mass in percent compared to a starting mass of 100% against transfer number. The lower part of the display contains the analysis of the chromatogram described below.

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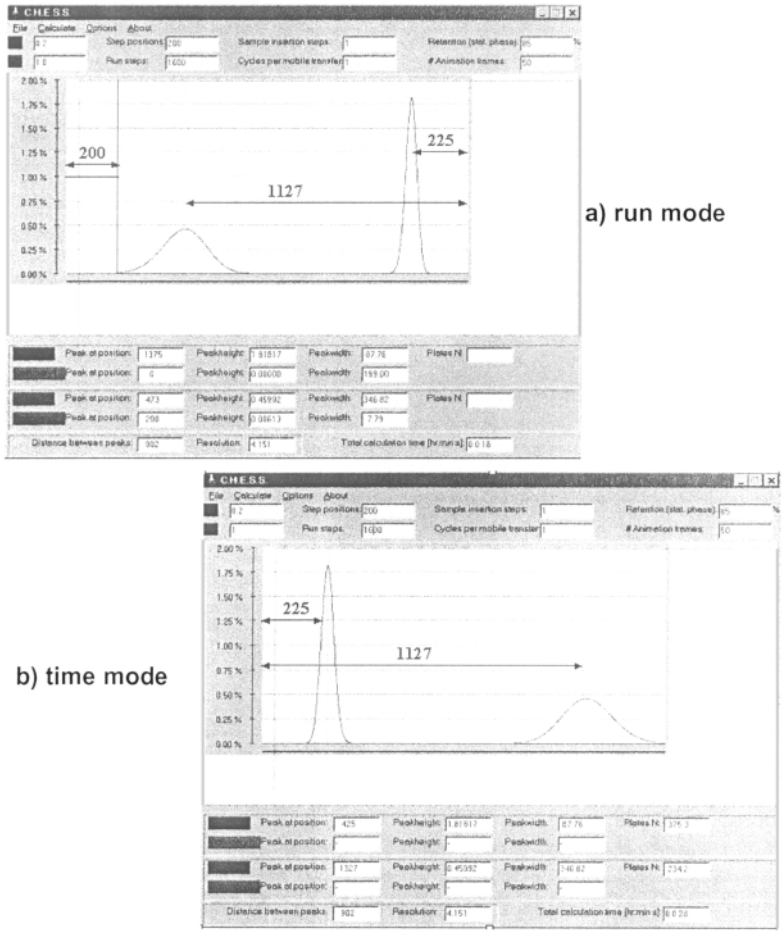

Figure 3. A model chromatogram for solutes with partition coefficients 0.2 and 1.0 and a stationary phase retention $S_f = 85\%$ for a $\tau = 200$ step transfer chain with 1600 transfers in all (i.e., 1400 elution steps) presented (a) in Run Mode and (b) in Time Mode. Note run mode if from the point of sample injection, while time mode is from the solvent front although peak positions are always expressed in transfers from sample injection.

Model Output Analysis

The first column of output data in the bottom left hand corner of the display gives the peak positions (in number of transfers from sample injection) of the mobile and stationary phase proportions of the two sample components,

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as well as, the peak separation between them. It should be noted, that the model can be used as a CCD model by equating the total number of run steps with the number of step positions (i.e., $n_{\text{tot}} = \tau$). The model will display two traces during the first "i" transfers or steps (where $0 < i < \tau$)—one for the upper phase and one for the lower phase. The total quantity in each test tube would have to be the sum of the upper and lower components. Once eluted, the sample is only in the mobile phase and the stationary phase box will remain blank unless there is sample residue still waiting to be eluted.

The second column contains each respective peak height expressed as a percentage of the 100% starting mass and the resolution (Rs) between the two peaks [see Eq. (7) below]. The third column contains the respective peak widths based on tangents drawn at the points of inflection and projected onto the $y = 0$ axis to give a baseline width. This is equivalent, for a Gaussian distribution, to taking the peak width at 0.6 of the peak height[14] and multiplying by two. The right hand column gives the number of theoretical plates (N_D) for each solute where:

$$N_D = 16\left(\frac{t_D}{w_D}\right)^2 \tag{5}$$

where peak retention time is taken from the point of sample injection. Finally, the total computing run time is given in the bottom right hand column. If run times are long, it is possible to speed them up by running without animation.

Model Validation

In CCC[15,16] the position of a solute peak can be predicted if the distribution ratio K_D and the percentage volume retention of stationary phase (S_f) are known. In Fig. 3 the samples have distribution ratios of 0.2 and 1.0. The $K_D = 1$ peak, being equally soluble in both phases, will elute in the system volume, and the $K_D = 0.2$ peak can be considered as the test sample. The distance between the elution point of the peak and the solvent front (Y) divided by the distance between the $K_D = 1$ peak and the solvent front (Z) will be equal to the distribution ratio, as shown in Fig. 4.

$$K_D = \frac{V_D - V_m}{V_s} = \frac{Y}{Z} \tag{6}$$

The eluting CCD model can be validated if Eq. (6) is shown to be true. It can be seen from Fig. 3b, that the ratio $Y/Z = 225/1127 = 0.19964$ compared to the set value of 0.2. Note, that the values used for $Y$and Z are calculated from the solvent front. The step values for peak positions calculated from sample

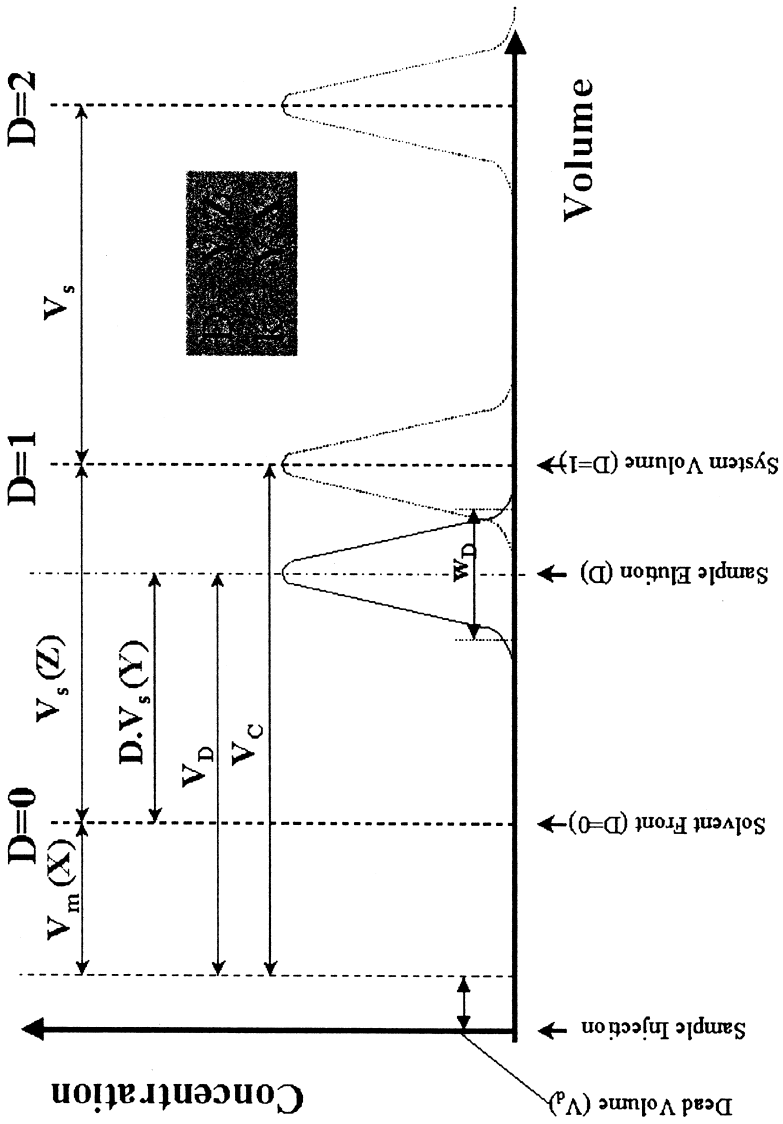

Figure 4. Schematic diagram of CCC elution profile for a solute with distribution ratio (D).

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injection (as listed in the display boxes) will, therefore, have to have the number of transfers in the CCD chain (200) subtracted. These values can also be calculated in "Run Mode," as shown in Fig. 3a, but with the knowledge that the solvent front is the extreme right hand side (i.e., $n_{\text{tot}} = 1600$ transfers).

Another validation check from CCC theory is the proportion of stationary phase retained in the coil [$S_f = V_s/V_c = V_s/(V_s + V_m)$], which can be calculated from the chromatogram, shown in Fig. 4, as the ratio $Z/(X+Z)$ for the elution of the $K_D = 1$ peak. From Fig. 3b, this $1127/(200 + 1127)$ where $X = 200$ is the number of transfers from sample injection to the elution of the solvent front. This works out as 0.84928 (84.9%) compared to the set value of 85% of the total system volume. Rounding errors can accumulate with a large number of iterations, but accuracies in the order of $\pm 0.1\%$ were considered acceptable, as most experimental errors were in the order of ± 1–5%. It was found in the course of developing the programme, that more exact solutions could be obtained using "extended floating point" variables for calculating array concentrations, but at the expense of extending computing time beyond what was considered acceptable. The current accuracy of 0.1% is, therefore, a suitable compromise.

It is well known in chromatography, that resolution can be increased by increasing the length of the column and this has been confirmed by Du et al.[17] for CCC. Doubling the column length increases the resolution by a factor $\sqrt{2}$ (i.e., $\text{Rs} \propto \sqrt{L}$). The resolution between two samples is commonly expressed[18] as:

$$\text{Rs} = \frac{2(t_2 - t_1)}{w_1 + w_2} \tag{7}$$

where t_1 and t_2 are the retention times of two solutes and w_1 and w_2 their respective baseline widths. This was confirmed using the model illustrated in Fig. 3. Running it for 50, 100, 200, and 400 transfers gave respective resolutions of 2.059, 2.930, 4.151, and 5.880. Plotting resolution (Rs) against the square root of the number of transfers (t) gave a linear relationship with a correlation coefficient $R^2 = 1$.

Finally it was confirmed that the sum of the samples contained in all the fractions equalled the starting mass.

USING THE MODEL TO PREDICT CCC CHROMATOGRAMS

The demonstration (above) that resolution (Rs) increases in proportion to the square root of coil volume or length of the column ($\text{Rs} \approx \sqrt{L}$) and that the

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time for peak elution increases in proportion to the length of the column ($t_D \approx L$), leads to the conclusion from Eq. (7) that the width of the peaks also increases with the square root of the length of the column and/or the square root of the time for peak elution ($w_D \approx \sqrt{L} \approx \sqrt{t_D}$). This is verified in Fig. 5, where the model has been used to plot the peak width (w_D) against the number of transfers to peak elution (n_D) for distribution ratios of $K_D = 0.5$, 1.0, 2.0, and 5.0 and a stationary phase volume ratio of $S_f = 75\%$, where the units are numbers of steps or transfers. It can be seen, that there is a good correlation ($R^2 = 1$) between the peak width (w_D) and $\sqrt{n_D}$ and that the peak width increases with distribution ratio. In fact, it is found that these curves all fall on one line (Fig. 6) if peak width is plotted against $\sqrt{(n_D k')}$, where k' is the retention factor and the following relationship is established:

$$w_D = 4\sqrt{n_D k'} \tag{8}$$

where all terms are non-dimensional quantities.

It was also confirmed that this relationship did not just hold for $S_f = 75\%$, but was valid for all values of stationary phase retention (S_f). Equation (8) can be extended to be applied in CCC providing a scaling factor (χ) can be introduced which defines the number of transfers per unit time, and keeps the relationship dimensionally correct.

$$w_D = \chi w_{Dt}$$
$$n_D = \chi t_{Dt}$$
$$w_{Dt} = 4\sqrt{\frac{t_{Dt} k'}{\chi}} \tag{9}$$

where t_{Dt} is the time of elution of a peak with distribution ratio (D) and retention factor (k') and χ is a scaling constant with units of steps or transfers/unit time. For CCD where the unit of time is a step, then $\chi = 1$.

Rearranging Eq. (9) to make χ the subject gives:

$$\chi = 16\frac{t_{Dt}k'}{{w_{Dt}}^2} = 16\frac{t_{Dt}k'}{{w_{Dt}}^2}\frac{(t_{Dt} - t_{mt})}{t_{mt}} \tag{10}$$

Substituting for w_{Dt} from Eq. (5) gives χ in terms of the number of theoretical plates, which is a term more familiar to solid phase chromatographers:

$$\chi = \frac{N_D k'}{t_{Dt}} \tag{11}$$

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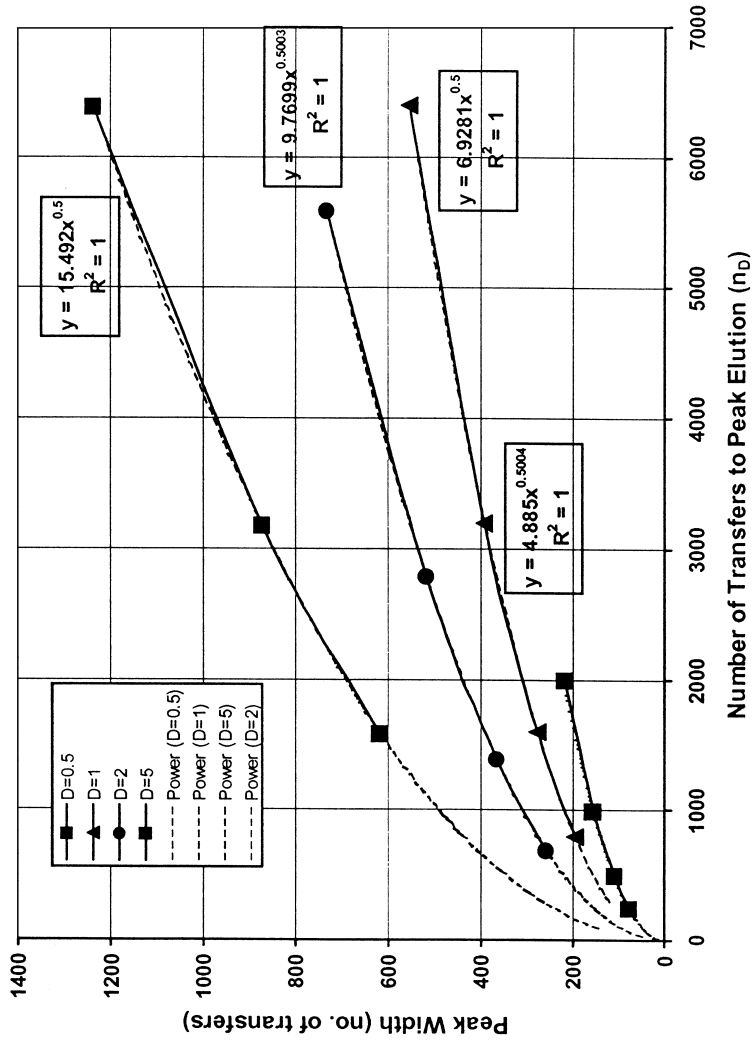

Figure 5. Variation of peak width with number of transfers to peak elution for different distribution ratios (D) and a stationary phase retention $S_f = 75\%$.

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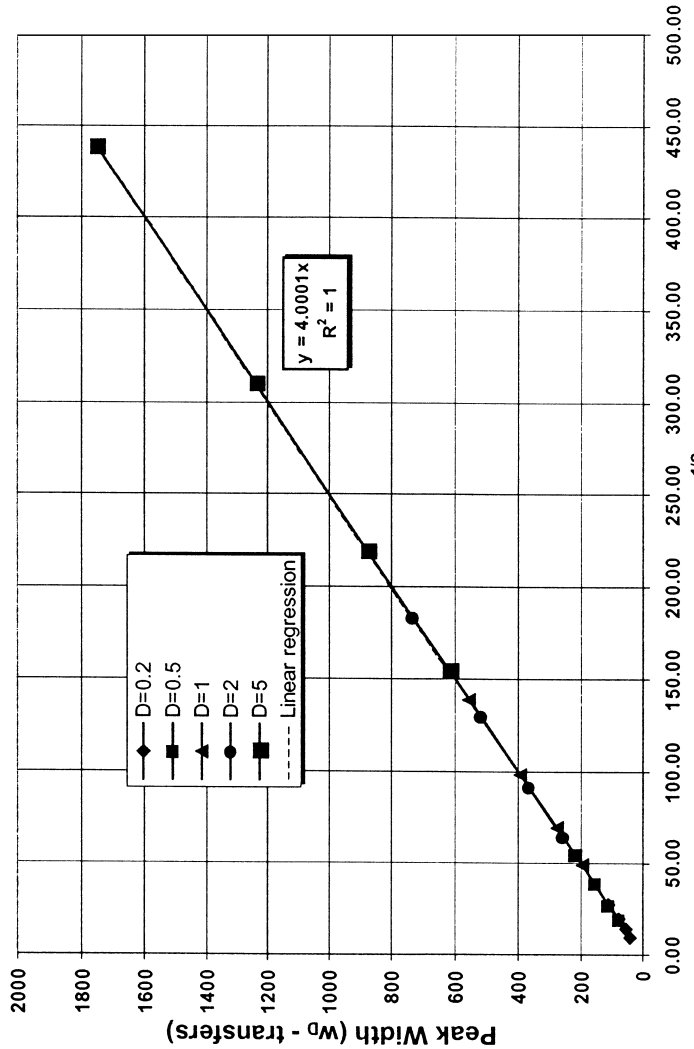

Figure 6. Variation of peak width (w_D) with $(n_Dk')^{1/2}$ for different distribution ratios (D) demonstrating the relationship $w_D = 4(n_Dk')^{1/2}$.

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If peak elution times were being measured in minutes, for example, then χ would have units of steps/min. If multiplied by the elution time of the solvent front ($t_m = V_m/F$), this would give the equivalent number of CCD transfers to elution (τ) for a given CCC chromatogram:

$$\tau = \frac{\chi V_m}{F} \tag{12}$$

Furthermore, as the number of mixing and settling steps in a CCC instrument is defined by the speed of rotation ($\omega -$ rev/min), then a percentage efficiency (E) can be defined as:

$$E_{\text{mix}} = \frac{\chi 100}{\omega} \tag{13}$$

Substituting for χ from Eq. (10), the efficiency can be expressed in terms of peak width and peak elution time. In this way, if the time of elution of the solvent front or $D = 0$ peak is known, then the efficiency of the process can be obtained from a single chromatogram as follows:

$$E_{\text{mix}} = 16 \frac{t_{Dt} k'}{{w_{Dt}}^2} \frac{(t_{Dt} - t_{mt})}{t_{mt}} \frac{100}{\omega} \tag{14}$$

If t_m is not known and the retention of the stationary phase (S_f) is, then a substitution can be made for t_m as follows:

$$t_m = \frac{V_m}{F} = \frac{V_c(1 - S_f)}{F} \tag{15}$$

substituting for t_m in Eq. (14) gives:

$$E_{\text{mix}} = 16 \frac{t_{Dt}}{{w_{Dt}}^2} \left[\frac{F t_{Dt}}{(1 - S_f) V_c} - 1 \right] \frac{100}{\omega} \tag{16}$$

which can be simplified with substitutions from Eqs. (5) and (15) to give:

$$E_{\text{mix}} = N_D \left(\frac{1}{t_{D=0}} - \frac{1}{t_{Dt}} \right) \frac{100}{\omega} \tag{17}$$

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Using Retention Data to Predict Peak Elution Times

It will be assumed in the following theory, that the volume of the inlet outlet tubing is small in comparison with the coil volume and can be ignored. If this is not the case, then it will have to be compensated for.[19] The solvent front will elute in the time it takes the mobile phase to pass through the volume of mobile phase in the coil, as in Fig. 4 and Eq. (15). The $K_D = 1$ peak has a special significance in CCC. The sample sees no difference between the mobile and stationary phase and, therefore, elutes in the time it takes for the mobile phase to pass through the whole system volume.

$$t_{D=1} = \frac{V_c}{F} \tag{18}$$

The volume position of the $K_D = 1$ peak in CCC will always remain the same whichever phase system or flow is used, whereas, the $K_D = 0$ position will move toward the $K_D = 1$ elution point as the retention decreases.

The elution time for a solute with distribution ratio (K_D), with reference to Fig. 4, is as follows:

$$t_{Dt} = \frac{V_m + K_D V_s}{F} = t_{mt} + K_D t_{st} \tag{19}$$

As mentioned in the model validation section, the distribution ratio of a given peak can easily be worked out by dividing the elution volume measured from the solvent front by the stationary phase retention volume, as shown in Fig. 4 and Eq. (6).

The retention factor k' (k-prime), which is the ratio of sample mass in the respective phases regardless of volume ratio [see Eq. (4)], can also be obtained from the elution profile as follows:

$$k' = \frac{K_D V_s}{V_m} = \frac{D t_{st}}{t_{mt}} = \frac{Y}{X} \tag{20}$$

Application of the Model in Practice

The example below utilises a sample set of results from the CCC paper by Bousquet et al. published in 1991.[5] This was one of the first papers to explore how phase retention and sample resolution were affected by flow. They used a PC Inc coil planet centrifuge with $R = 100\,\text{mm}$, $\omega = 710\,\text{rpm}$, $V_c = 143\,\text{mL}$, $L = 61.4\,\text{m}$, and $d_c = 1.6\,\text{mm}$. In practice, the flow range used was 0.4–9 mL/min, but the model allows elution predictions outside this range.

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The procedure for developing a model for a given set of running conditions is to first establish the relationship between stationary phase retention and the square root of mobile phase flow after Du et al.[9] as set out below;

$$S_f = (100 - \mathrm{B}F^{0.5}) \tag{21}$$

Figure 7 shows Bousquet's results taken from his Figs. 7 and 13 plotted in this way. Note, they are linear up to about 4 mL/min, after which some non-linearity starts to occur. A linear regression of the first three points (on the linear part of the curve) gives a negative value for the gradient B of 10.209 [units – percentage retention per $(\mathrm{mL/min})^{0.5}$]. This slope is used in association with Eq. (21) even though Bousquet's intercept is 97.5%. This is because it is now known that failure of these plots to intercept at $S_f = 100\%$ means that the "extra coil volume" has either been over- or under-estimated.[19] Knowing the volume of the stationary phase, allows the mobile phase volume to be calculated. This will establish the point of elution of the $K_D = 0$ peak [Eq. (15)].

Next, a chromatogram should be obtained using a solute of known distribution ratio and the intended solvent phase system. Measure t_P and w_P from the chromatogram and calculate N_D from Eq. (5) and k' from Eq. (4) or (20) as appropriate. It will then be possible to calculate χ, the number of CCD steps per minute from Eq. (10) or (11). This was found to be 32.2 CCD steps/minute.

Once χ is known, a spread sheet like the one in Table 1 can be prepared to give resolution (Rs) and any other separation parameters, such as total run time, that are required.

Figure 8 shows theoretical and measured resolutions vs. retention for Bousquet's results. Here, the scaling factor ($\chi = 32.2$ transfers/min) was calculated from an average of 12 different values obtained from the four eluted peaks for the three different flows. This was done, in each case, by measuring the time of elution (t_{Dt}), the base width of the peak (w_{Dt}), calculating the distribution ratio (K_D) from Eq. (6) or (19), and the number of CCD steps per minute from Eq. (10) or (11). All other predictions were made from Eqs. (13 and 19), assuming distribution ratios remain the same and that CCC follows Du's flow relationship of Eq. (18). Note, that the predicted results lay at least 3% to the right of the measured ones. This comes from assuming that the Du flow relationship (18) passes through 100%. In Bousquet's result, it passed through 97.4%, which suggests his extra coil volume was underestimated and that his actual retentions were, in fact, 2.6% higher.

Figure 9 shows the theoretical and measured resolutions vs. mobile phase flow for the same results, together with a prediction of the resolution flow profile that would be obtained by doubling the column capacity or length of tubing. Note, that the two measured results at flows up to 4 mL/min lie close

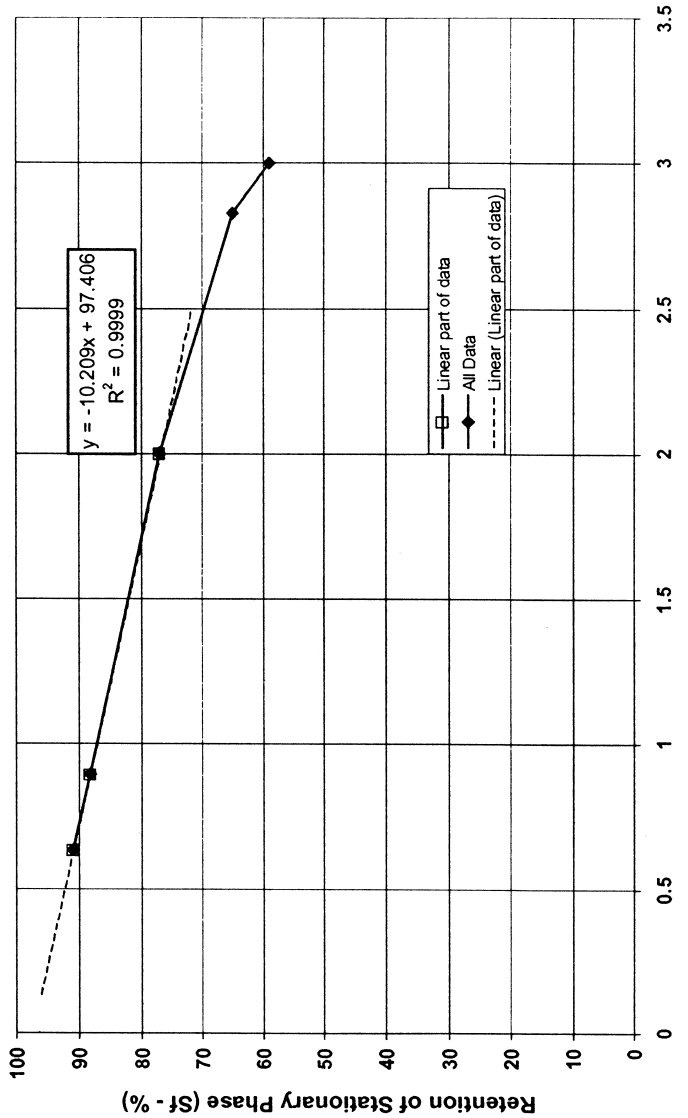

Figure 7. Variation of stationary phase retention (S_f) vs. the square root of mobile phase flow from Bousquet's results.[5]

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Table 1. Spreadsheet for deriving stationary phase volume retention and target sample resolution from the Du Plot gradient and the number of CCD steps per minute

Vc	143 ml											
B=	10.209 %			Sf=100-BF$^{1/2}$								
D_1=	0.233			χ =	32.2 steps/min							
D_2=	0.4			Ts=	0.031056 mins/step							
F	S_f	V_m	V_s	t_0	t_{D1}	t_{D2}	Δt	w_{D1}	w_{D2}	N_{D1}	N_{D2}	Rs
(ml/min)	(%)	(ml)	(ml)	(min)	(min)	(min)	(min)	(min)	(min)			
0.25	94.90	7.30	135.70	29.20	155.67	246.32	90.65	18.30	30.17	1157.22	1066.60	3.74
0.5	92.78	10.32	132.68	20.65	82.47	126.79	44.31	11.08	18.00	886.79	794.11	3.05
1	89.79	14.60	128.40	14.60	44.52	65.96	21.44	6.73	10.74	699.47	603.70	2.45
2	85.56	20.65	122.35	10.32	24.58	34.79	10.22	4.11	6.40	573.12	472.62	1.94
4	79.58	29.20	113.80	7.30	13.93	18.68	4.75	2.51	3.80	493.86	385.80	1.51
8	71.12	41.29	101.71	5.16	8.12	10.25	2.12	1.52	2.24	455.79	334.89	1.13
16	59.16	58.40	84.60	3.65	4.88	5.76	0.88	0.90	1.29	465.65	320.31	0.81
32	42.25	82.58	60.42	2.58	3.02	3.34	0.32	0.51	0.70	570.61	367.07	0.52

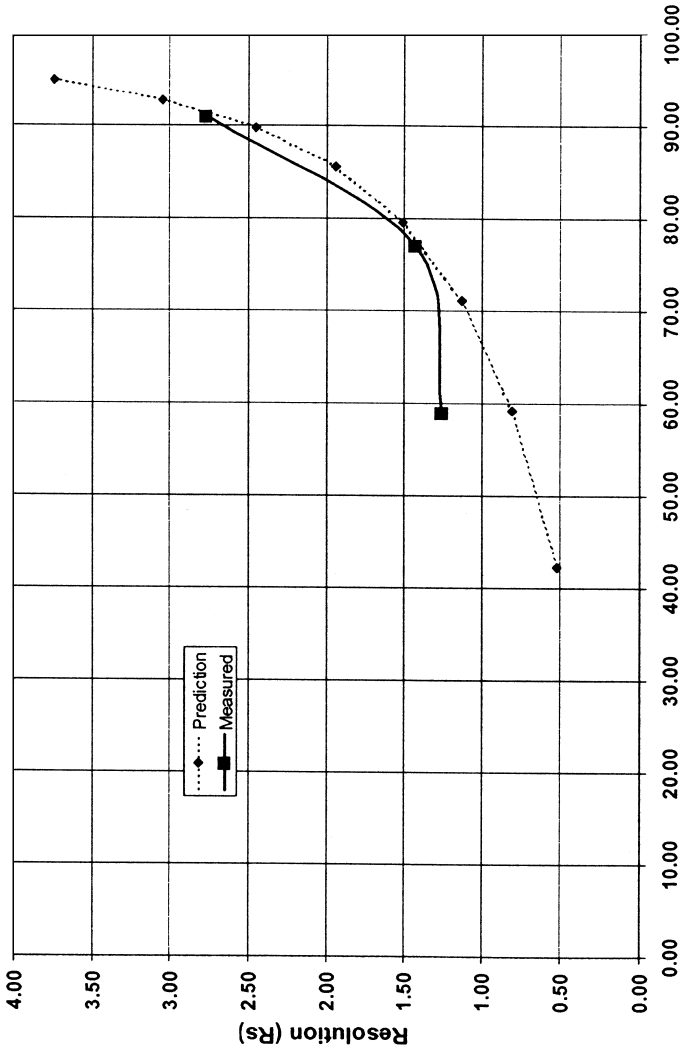

Figure 8. Theoretical and measured resolutions vs. S_f retention from Bousquet's results.[5]

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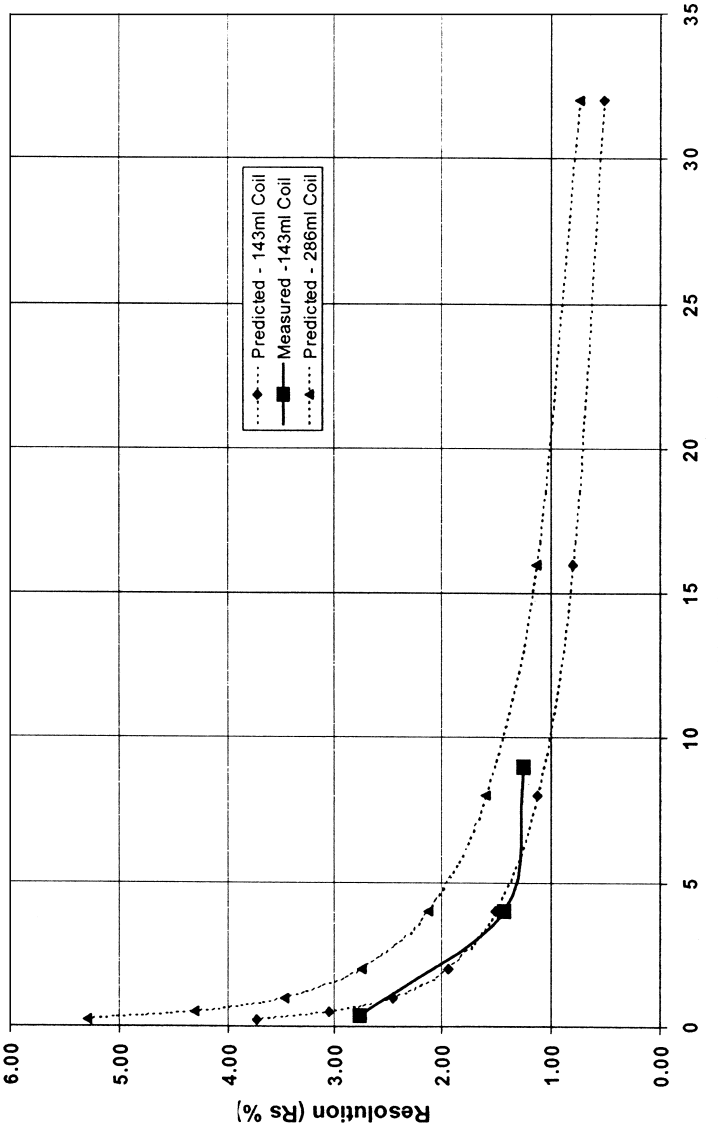

Figure 9. Theoretical and measured resolutions vs. mobile phase flow from Bousquet's results.[5]

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to the curve predicted from the new theory. The 9 mL/min flow rate gave better resolution than expected, which shows that the mixing efficiency increased at this higher mobile phase flow rate.

A closer analysis of all the eluted peaks in Bousquet's Fig. 7 shows, in Fig. 10a, that mixing efficiency does, in fact, increase steadily with flow for the

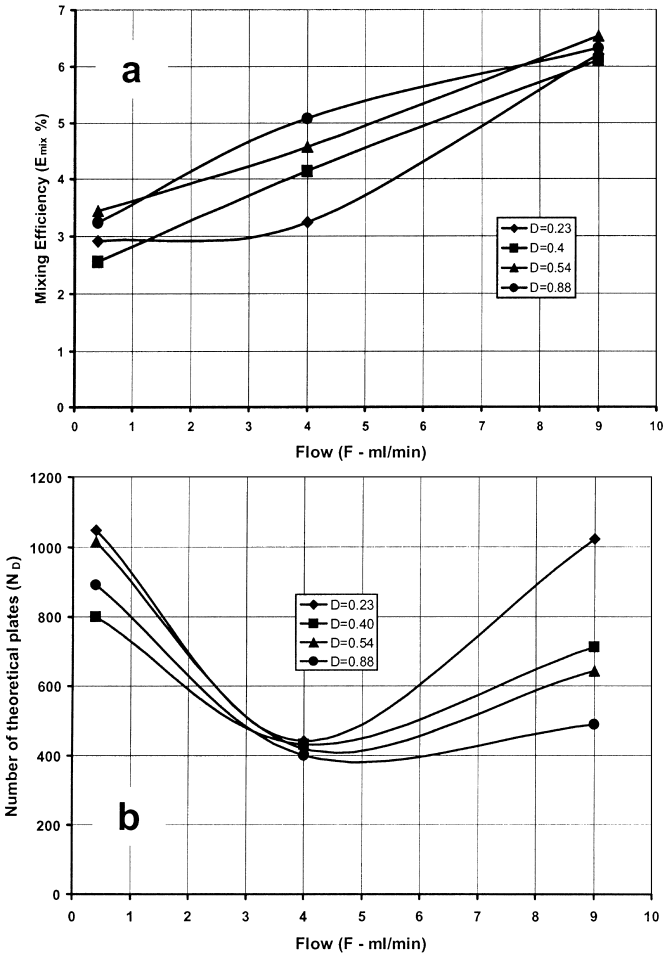

Figure 10. Variation of (a) mixing efficiency, (b) number of theoretical plates with flow from Bousquet's results.[5] Variation of (c) resolution and (d) resolution per unit run time with flow from Bousquet's results.[5]

(*continued*)

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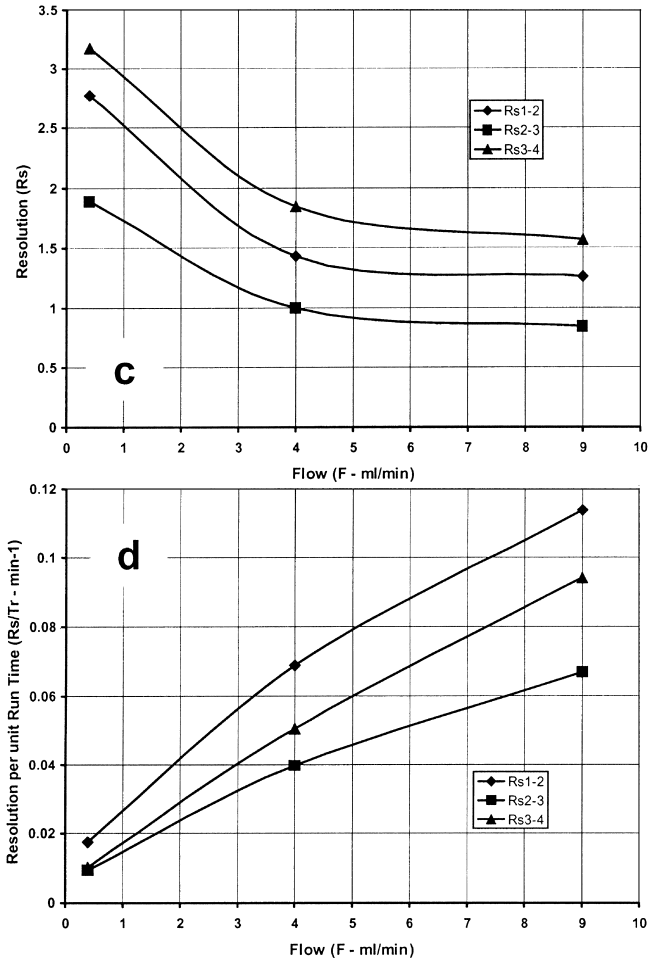

Figure 10. Continued.

phase system used. Figure 10b shows how the number of theoretical plates changes with flow (solid-phase chromatographers sometimes refer to this as efficiency). In fact, efficiency is a relative term as it depends what "efficiency" is related to. To the analytical chemist, the most efficient separation may be the one that gives the best resolution—this would be the lowest flow, as shown in Fig. 10c, regardless of how long the separation takes. On the other hand, the

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process engineer would be much more interested in throughput and his most efficient run would be the one that gives the best resolution per unit time. This would be the highest flow in Fig. 10d.

CONCLUSIONS

The mixing efficiency introduced in this paper mimics resolution per unit time, and if used wisely, could aid the analytical chromatographer also, giving them just as high a resolution if longer coils were used to compensate for reduced resolution at the higher flows. It appears though, that the number of theoretical plates (N_D) in CCC bears little relationship with either resolution or resolution per unit time and should, therefore, be avoided or used with extreme caution for CCC when the retention volumes of stationary phase are so high.[5]

In the future, it would be desirable for the CCC community to agree to a standard test system for comparing different CCC devices—in this way all operators would be able to gain some satisfaction that their system is operating satisfactorily, before proceeding with the separation of their choice.

NOMENCLATURE

Symbols Used

β	The ratio r/R
χ	Number of CCD steps/unit time in CCC
τ	No of transfers or test tube steps to the elution of the solvent front
ω	Rotational speed (rev/min)
A	Cross-sectional area (m^2 or cm^2)
B	Negative slope of S_f vs. v_F plot ($min^{0.5}$ $mL^{-0.5}$)
C	Concentration of solute (mol/l)
D	Distribution ratio (most often noted K_D)
E	Efficiency ($=100\,\chi/\omega$) (plate)
F	Mobile phase flow rate (mL min^{-1})
g	Earth's gravitational field ($10\,ms^{-2}$)
i	Numeric describing the ith position in the test tube chain
K_D	Distribution ratio (also noted D)
k'	Retention factor
L	Total length of tubing in coil system (m)
m	Mass of solute in a given phase volume (g)
n_D	Number of transfers until elution of a peak with distribution ratio (D)

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n_{tot}	Total number of transfers
N_D	Number of theoretical plates for a peak with distribution ratio K_D (plate)
r	Distance from the planetary axis to a given point on the planetary rotor (bobbin, m or cm)
R	Distance from centre of main rotor to the planetary axis (m or cm)
R	Correlation coefficient
Rs	Resolution between two peaks
S_f	Retention of stationary phase (percentage of machine volume)
t	Time (s or min)
t_D	Time until elution of a peak with distribution ratio K_D (s or min)
w	Peak baseline width (time unit, s or min)
w_D	Baseline width of a peak with distribution ratio K_D (time unit)
x	$1/(1+k')$
X	Scalar measurement on chromatogram ($=V_m$ or t_m and corres. unit)
y	$k'/(1+k')$
Y	Scalar measurement on chromatogram ($=DV_s$ or Dt_s and corres. unit)
Z	Scalar measurement on chromatogram ($=V_s$ or t_s and corresponding unit)
V	Generic volume term (mL)

Subscripts

c	Coil or whole system
CCC	With reference to countercurrent chromatography
CCD	With reference to countercurrent distribution
D	With reference to peak with distribution ratio D or K_D
l	Lower phase
m	Mobile phase
mix	Mixing
s	Stationary phase
t	Measured in time units
tot	Total
u	Upper phase

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