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Counter-current chromatography: Simple process and confusing terminology

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ABSTRACT

The origin of counter-current chromatography is briefly stated, followed by a description of the mechanism of elution of solutes, which illustrates the elegance and simplicity of the technique. The CCC retention equation can be mentally derived from three facts; that a substance with a distribution coefficient of 0 elutes at the mobile phase solvent front (one mobile phase volume); and one with a distribution coefficient of 1 elutes at the column volume of mobile phase; and solutes with higher distribution coefficients elute at additional multiples of the stationary phase volume. The pattern corresponds to the classical solute retention equation for chromatography, $V_R = V_M + K_C V_S$, K_C not being limited to integer values. This allows the entire pattern of solute retention to be visualized on the chromatogram. The high volume fraction of stationary phase in CCC greatly enhances resolution. A survey of the names, symbols and definitions of several widely used chromatography and liquid–liquid distribution parameters in the IUPAC Gold Book and in a recent summary in LC–GC by Majors and Carr revealed numerous conflicts in both names and definitions. These will retard accurate dissemination of CCC research unless the discordance is resolved. It is proposed that the chromatography retention parameter, K_C, be called the *distribution coefficient* and that a new biphasic distribution parameter, $K_{\Delta(A)}$, be defined for CCC and be called the *species partition ratio*. The definition of V_M should be clarified. V_H is suggested to represent the holdup volume and V_X is suggested for the extra-column volume. H_V and H_L are suggested to represent the volume and length of a theoretical plate in CCC. Definitions of the phase ratio, β, conflict and should be clarified.

1. Introduction

The discovery of counter-current chromatography (CCC) was a serendipitous outgrowth of a search by Dr. Yoichiro Ito for means to improve the separation of lymphocytes by a centrifugal sedimentation process using a helical coil-planet centrifuge [1–3]. First referred to as liquid–liquid partition [2], the name counter-current chromatography was introduced in 1970 [4–6] with the introduction of helix, droplet, locular and gyration locular chromatography, all of which maintained a fixed stationary phase and a flowing mobile phase, as in conventional liquid–liquid or partition chromatography [7], but without requiring the solid matrix to support the stationary phase. Only the original demonstration and a few later separations, using either a specially designed multilayer column [2,8], or a machined spiral disk [9] for a method called dual CCC employed simultaneous counterflow of the phases. The name counter-current chromatography was derived by analogy to Craig's counter-current distribution (CCD) system, which also did not provide true counter-current flow [10,11].

2. The simplicity of CCC

2.1. The retention equation

The mechanics and kinematics of CCC apparatus will not be discussed, but an attempt will be made to show its conceptual identity of CCC with conventional chromatography and to compose a picture of the elegant and simple and visually perceptible separation process it provides. This simplicity is best conveyed using a mechanical or computer-simulated moving diagram, but in print, parts of a stationary diagram must be imagined to move.

The separation process is illustrated in stepwise fashion in Fig. 1, in which, after equilibration, 60% of the column remains filled with stationary phase. The volumes of eluate are shown in correct proportion. The relative elution volumes for the sample components are correctly illustrated, but the peak widths are not drawn precisely and will be discussed later. The sample contains four solutes with distribution coefficients, K_C, of 0, 1, 2 and 3 where

$$K_C = \frac{C_S}{C_M} \quad (1)$$

and C represents the total concentration of solute (all forms; neutral, ionized, dimers, ion pairs, etc.), in the stationary phase, C_S,

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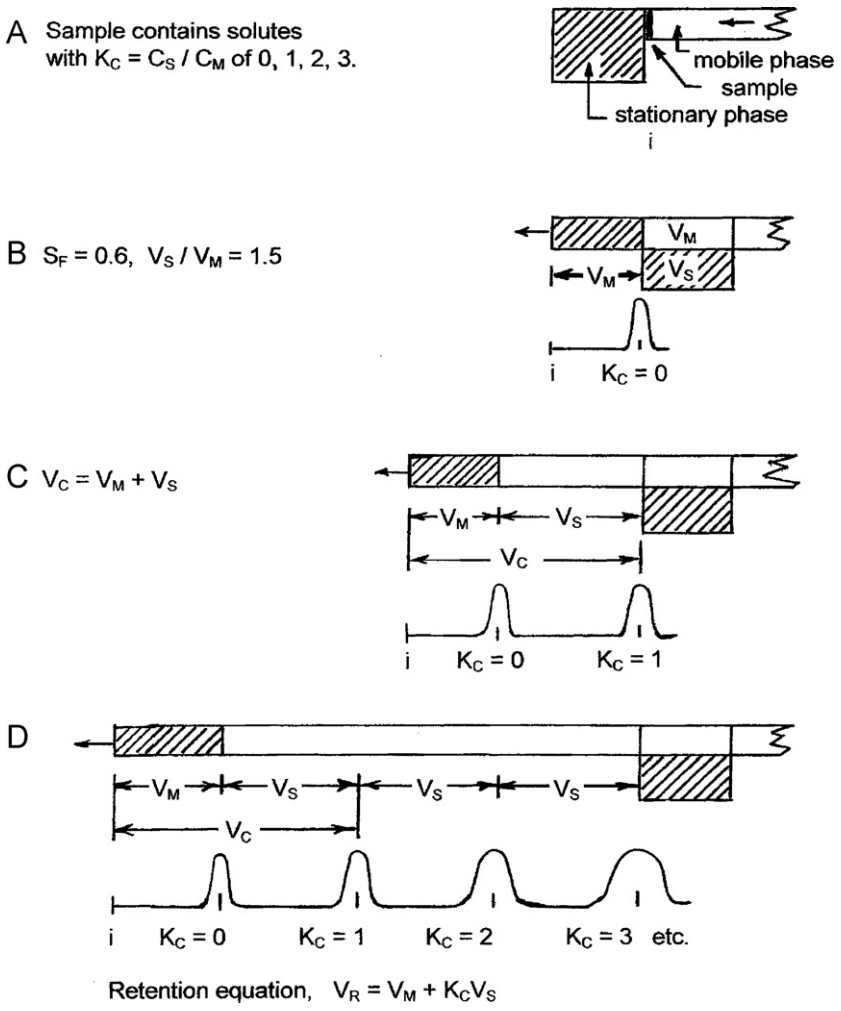

Fig. 1. The simplicity of CCC.

divided by the total concentration of solute in the mobile phase, C_M.

The column (or coil) is first filled with stationary phase (shown shaded) prior to the start of column rotation and often the sample is placed at the front of the mobile phase stream as illustrated in panel 1A. Then column rotation is begun and shortly thereafter, mobile phase flow is begun. (In practice, using an injection loop, the sample can also be injected any time after mobile phase flow has begun.) The mobile phase is shown as an unshaded stream being pumped in a leftward direction, indicated by the arrow, about to enter the column.

Panel 1B shows that after one mobile phase volume, V_M, has entered the column, one mobile phase volume of stationary phase has been displaced (eluted) and the peak maximum of the solute with $K_C = 0$ is eluting. Measurement of this volume of displaced (eluted) stationary phase is one way to measure V_M. Since the column volume, V_C, is known (1 unit in the illustration), the stationary phase volume, $V_S = V_C - V_M$ and the phase volume ratio, V_S/V_M, can be calculated. The phase volume ratio in CCC is often indicated by β_V, but, at this time, there is confusion about which phase is indicated in the numerator.

After an additional stationary phase volume of mobile phase enters the column, panel 1C, the solute with $K_C = 1$ elutes, its retention volume, V_R, being one column volume, V_C. Regardless of column size and operating parameters, such as flow rate, $K_C = 0$ will always emerge at one mobile phase volume and $K_C = 1$ will always emerge at one column volume.

As seen in panel 1D, subsequent solutes with unit K_C values above one will elute with each passage of another stationary phase volume of mobile phase. Examination of the chromatogram in panel 1D allows one to intuitively write the general chromatographic retention equation

$$V_R = V_M + K_C V_S \tag{2}$$

This equation is applicable to chromatography in general, but in packed column chromatography the stationary phase volume is not usually known.

Given a real counter-current chromatogram, it is a simple matter to find the $K_C = 0$ point either by collecting the initially expelled stationary phase or by injecting a non-retained solute or by using a contaminant peak as an estimate. V_C will be known and V_S can then be calculated. The retention equation is linear, so laying off these reference points on the chromatogram allows even a visual estimation of the K_C values of the peaks. Conversely, if K_C values of unknowns are measured by non-chromatographic means, the expected retention times for the counter-current chromatographic system can be estimated.

Because separation in CCC is visually apparent, this author has found that it is easier to introduce CCC in teaching analytical chemistry and then convert the CCC retention equation, using the relationship between K_C and the retention factor, k,

$$k = \frac{V_S C_S}{V_M C_M} = \frac{V_S K_C}{V_M} = \beta_V K_C \tag{3}$$

Table 1
Corresponding equations for liquid–solid chromatography (LSC) and countercurrent chromatography (CCC).

	LSC	CCC
Retention equations	(a)$t_R = t_M + kt_M$	(b)$t_R = t_M + \beta_V K_C t_M$
	(c)$V_R = V_M + kV_M$	(c)$V_R = V_M + kV_M$
	(d)$V_R = V_M + K_C V_S$	(d)$V_R = V_M + K_C V_S$
Retention parameters	(e)$k = \frac{V_R - V_M}{V_M}$	(f)$K_C = \frac{V_R - V_M}{V_S}$
Conversion factors	$k = \frac{t_S}{t_M} = \frac{Q_S}{Q_M} = \frac{V_S C_S}{V_M C_M} = \beta_V K_C$	

All equations apply to both LSC and CCC, but the value of V_S is known only in partition chromatography (LLC) and CCC. Symbols: Q = quantity of solute in the phase volume; t_M is the time required for one V_M of mobile phase to pass through the column, and also represents the time spent by the solute in the mobile phase; t_S is the time the solute spends in the stationary phase. The definition of the phase volume ratio, β_V, is presently unsettled, but is expressed here as V_S/V_M. Equations are indicated by letters to avoid confusion with those in the text which are numbered.

Table 2
R_S as a function of S_F for $N = 400$.

R_S as a function of S_F for N = 400

$K_{C(1)}$	$K_{C(2)}$	α	S_F	0.2	0.4	0.6	0.8
			$(1\text{-}S_F)/S_F$	4	1.5	0.67	0.25
0.01	1	100	R_S	1.10	2.48	4.24	6.60
1	2	2	R_S	0.91	1.67	2.31	2.86
2	3	1.5	R_S	0.77	1.25	1.58	1.82
3	4	1.33	R_S	0.67	1.00	1.20	1.33

$\alpha = K_{C(2)} / K_{C(1)}$, $N = 16(V_R / W_b)^2 = 400$
Values of S_F are shown at the top of each column. Values of the resulting phase volume ratio are shown in row 2 and the R_S values are shown in rows 3 to 6. Minimum R_S value for baseline resolution is 1.5.

to obtain the retention equation for conventional liquid–solid chromatography (LSC)

$$V_R = V_M + kV_M \tag{4}$$

These and other corresponding equations, all valid for both LSC and CCC, are summarized in Table 1. The retention equation (a) and (c) in Table 1 (Eq. (c) appears as Eq. (4) in the text), are commonly used in LSC instead of Eq. (d) because V_S is not known in LSC. On the other hand, Eq. (d) (Eq. (2) in the text) is the predominant equation in CCC because V_S is known; K_C is perceptible from the chromatogram and independent determination of K_C allows prediction of retention volumes and run times. The quantities k and K_C can be generically referred to as retention parameters since they (along with β_V in CCC) determine the slope of the retention equations (a), (b) and (d). They are related by the phase volume ratio β_V as $k = \beta_V K_C$ (Table 1). Note that β_V employed here represents the ratio V_S/V_M. This will be further discussed at the end of this article.

2.2. The role of stationary phase fraction retained in the column, S_F

If no stationary phase is present in the column, no solutes are retained and all emerge at one column volume as illustrated in panel A of Fig. 2. Panel B illustrates the separation when the stationary phase fraction retained in the column, $S_F = V_S/V_C$, is 0.2. The solutes then emerge in a pattern corresponding to that in panel D of Fig. 1 where K_C of 0 elutes at V_M followed by the other solutes at intervals of V_S. Many solvent systems provide S_F values in the range of 0.4–0.8. In contrast, classical partition chromatography only retains volumes of stationary phase well under 20% of column volume. Panels C–E extend the illustration to S_F values of 0.4, 0.6 and 0.8. The solute with a K_C of 1 always emerges at V_C, while those with K_C values of 0 and 2 diverge symmetrically from 1, each separated from K_C of 1 by 1 stationary phase volume, V_S. As V_S increases, V_M decreases allowing K_C of 0 to emerge earlier, always one stationary phase volume before K_C of 1. The solute with K_C of 3 emerges one stationary phase volume later than $K_C = 2$.

In general, Fig. 2 illustrates what is expected from Eq. (2), when applied to the difference in the retention volumes for two solutes with different K_C values. The retention volume, and consequently the distance between the peaks of adjacent solutes, increases in proportion to the stationary phase volume, V_S as indicated by $\Delta V_R = \Delta K_C V_S$. Dividing by V_C allows this to be expressed in terms of the stationary phase fraction, S_F as $\Delta V_R/V_C = \Delta K_C S_F$. This is a significant effect and, if the efficiency, N, remains constant (or decreases only slightly), as S_F is increased, one expects an increase resolution, R_S. Efficiency is related to the ratio of the retention volume (or time) and the base width, W_b, of the peak. But, the base width is also influenced by factors unrelated to S_F.

2.3. Resolution, R_S

Resolution in CCC is determined by three independent terms in the resolution, Eq. (5), [12,13]. These are: the separation factor term, $\alpha - 1$; the efficiency term, $N^{0.5}$; and the remaining combination term, often called the distribution coefficient term, but actually containing $K_{C(1)}$, α and S_F. The parameter α is the ratio of distribution coefficients, $K_{C(2)}/K_{C(1)}$ where $K_{C(2)}$ is greater than $K_{C(1)}$, so both distribution coefficients are accounted for.

$$R_S = 0.25(\alpha - 1)\sqrt{N}\left[\frac{K_{C(1)}}{K_{C(1)}[(\alpha+1)/2] + [(1-S_F)/S_F]}\right] \tag{5}$$

If the distribution coefficients (and therefore α) and N remain constant, the effect of S_F on resolution can be evaluated.

The expected resolution, R_S, for which peak separation is illustrated in Fig. 2, is summarized in Table 2 for a column of 400 theoretical plates, N. Another hypothetical solute with K_C of 4 is included. A K_C value of 0.01 is used for the $K_C = 0$ solute to avoid dividing by zero. A minimum resolution of 1.5 is required for baseline resolution. The staircase line in Table 2 divides the baseline-resolved solutes, above, from those below with resolutions less than 1.5. None are resolved with S_F of 0.2 and resolution improves with increasing S_F, leaving only the 4/3 pair partially resolved with S_F loadings of 0.6 and 0.8.

The 4-fold increase in S_F, from 0.2 to 0.8, produces a 16-fold decrease, 4 to 0.25, in the ratio $(1 - S_F)/S_F$. This provides a very large increase in resolution, especially for solutes with K_C values of 1 or lower. Because of the role played in CCC by the high fraction of stationary phase in the column, much higher resolution is obtained than might be expected from the number of theoretical plates in the column.

This dramatic increase in R_S as S_F is increased is well illustrated in Fig. 1, p. 350 of ref. [21] for 10 solutes as S_F increases from 0.3 to 0.9.

The expected resolution for columns with other N values can be calculated by multiplying the $R_{S(N=400)}$ values in Table 2 by the ratio $(N_{\text{New column}}/400)^{0.5}$. For instance, with a column of 800 theoretical plates: all pairs will be baseline resolved when S_F is 0.6

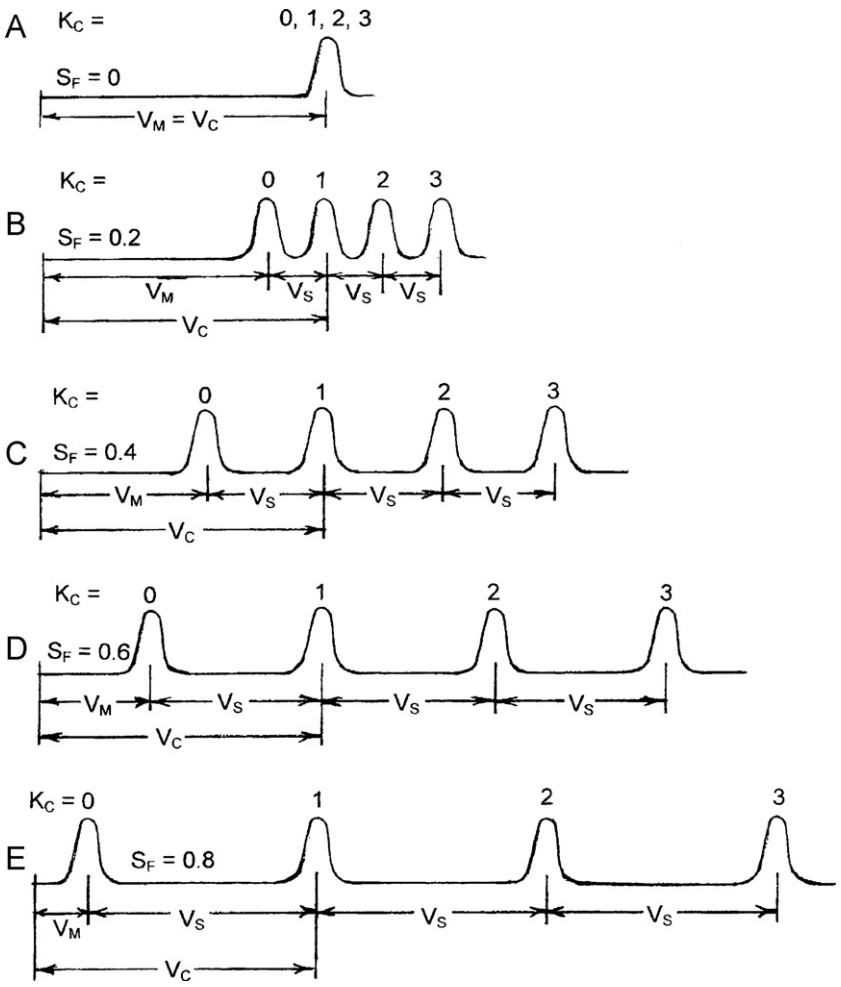

Fig. 2. The effect of S_F on peak separation.

or higher; with S_F of 0.4, only the 4/3 pair with R_S of 1.41 will be slightly less than baseline resolved; and with S_F of 0.2, only the 1/0.01 pair will be resolved. Two useful general rules are: (1) when all other factors remain constant, to double resolution N must be increased four-fold. (2) If the efficiency, N, is reduced by half, resolution is reduced only by the factor $(2)^{0.5} = 0.707$, or to roughly three-quarters of its former value. All of the parameters in Eq. (5) are important in achieving resolution and while increasing S_F does often provide a significant increase in R_S, other factors in the apparatus or experimental procedure may frustrate this achievement.

3. The terminology of CCC

3.1. The problem

The title of the oral presentation of this work, which referred to the nomenclature of CCC, is updated here to the terminology of CCC since the IUPAC now reserves the term *nomenclature* for the rules for naming organic compounds. While the counter-current chromatogram can be said to be simple, elegant and visually apparent, the lexicon of its terminology lacks clarity. The state of present day CCC terminology is disarranged, confounded and confusing. Several principal terms in mainstream chromatography and shared by CCC also lack precision. This inhibits the accurate presentation and mutual assimilation of research by both groups. It exhorts the chromatography community to harmonize the deficiencies in terminology.

Two recent authoritative compilations of chromatography terminology were used to summarize the terminology and definitions applicable to counter-current chromatography. These are the International Union of Pure and Applied Chemistry, IUPAC Gold Book, 2nd ed. on-line at http://goldbook.iupac.org/ [14] and the Summary of Liquid-Phase Separation Terms by Majors and Carr in LC–GC in 2001 [15] also available on-line (dated February 1, 2008) at http://chromatographyonline.com and is referred to in this article as the M & C summary. The on-line version is titled Glossary of HPLC/LC separation terms and can be accessed directly with the following link:

http://chromatographyonline.findanalytichem.com/lcgc/article/articleDetail.jsp?id=494774&pageID=1&sk=&date=.

The definitions are the same in the on-line article and the earlier print version.

The compilation in the Gold Book emerged from three editions of the so called "Orange Book", derived from the color of its binding, which were published at about 10-year intervals (1978, 1987 and 1997) [16–18] and two papers published respectively by Ettre [19] and by Rice et al. [20] in J. Pure and Appl. Chem. The Ettre paper summarized recommendations for the field of chromatography and the Rice et al. paper summarized the recommendations for the fields of liquid–liquid distribution, LLD, including extraction.

Table 3
Inconsistencies in IUPAC terminology for retention and equilibrium parameters.

Symbol	Name	Gold Book page index	Field of Use
Definition Definition continued or comment			
k	Retention factor	R05359	CHR
$k = C_S V_S / C_M V_M = (C_S / C_M) K_C$, $V_R = V_M + kV_M$ C is total concentration, all forms of the solute in stationary, S, or mobile, M, phase.			
K_C	Distribution constant	D01814	CHR
$K_C = C_S / C_M$, $V_R = V_M + K_C V_S$ C is total concentration, all forms of solute Symbol K_C is not given on page D01814 but is found in the cited source, the Ettre paper [19]. D01814 also states this term is also called the distribution coefficient, but that distribution constant is closer to general usage			
$K_{D(A)}$ or $(K_D)_A$	Partition ratio	P04440	LLD
$K_{D(A)} = [A]_{org} / [A]_{aq}$ A is concentration of a single species, one definite form of solute Footnote 1 in P04440 suggests distribution constant as a synonym.			
$(K_D^\circ)_A$	Partition constant	P04438	LLD
$(K_D^\circ)_A = \frac{a_{A(org)}}{a_{A(aq)}}$ a is the activity of species A, single species, in the extract (organic) and the other phase			
D	Distribution ratio	D01817	LLD
$D = C_{extract} / C_{other\ phase}$ C is total concentration, all forms of solute			
No symbol Not a synonym for distribution ratio	Distribution coefficient	D01812	–
No symbol Synonymous with partition ratio P04440	Distribution constant	D01813	LLD
No symbol [obsolete] not recommended, not a synonym for partition constant, partition ratio or distribution ratio	Partition coefficient	P04437	–

3.2. Retention and equilibrium parameters

Table 3 summarizes the relevant IUPAC-recommended terms for retention parameters used in chromatography, CHR, along with equilibrium parameters used mainly for liquid–liquid distribution, LLD, studies. The M & C summary lists only chromatography terms and includes names that are believed to reflect current usage, as well as those recommended by IUPAC. These will be commented on in conjunction with the terms in Table 3 and later with respect to some other terms. A specific Gold Book page index number is listed in Table 3 for the reference to each term. If inserted in the URL for the Gold Book the page will be obtained directly, as shown here for the retention factor, *k*: http://goldbook.iupac.org/R05359.html. If/R05359-plain.html is used, a page more suitable for printing will be obtained.

Since at least the 1960s, the symbol K was alternately referred to as either the *partition coefficient* or the *distribution coefficient*. Historically, k was written as k' and was called the *capacity factor*. Ettre, in 1993, [19] recommended dropping the prime and changing the name to the *retention factor*. To avoid confusion between the printed symbols k and K, the IUPAC [19] recommended to add the subscript c (meaning concentration) to K and to rename K_C the *distribution constant*. In retrospect this was an unfortunate choice because K_C is not a constant; it varies with pH, solute concentration, dimerization and other factors and this parameter had always previously been termed a coefficient. Continuation of the historical term, *distribution coefficient* would have been, and still is, a better choice. The term *partition coefficient* was felt to be confusing because it had been applied to various other symbols (such as P) with various definitions. But in chromatography, K and K_C have always shared the same definition as C_S/C_M. The terms k, K and K_C are the only terms being discussed here that have been defined by the IUPAC for chromatography.

The symbol K_D is a source of considerable confusion. The symbol K_D was defined for chromatography in section 9.4.8.37 of the 2nd edition of the Orange Book (1987) [17] as the ratio of the concentration of a component, in a single definite form, in the stationary phase to its concentration in the mobile phase and it was called the *distribution constant*. The M & C summary does not explicitly define K_D, but lists it as a retention parameter along with K_C in their definition of the *partition coefficient*, K. The summary calls K_D the *distribution coefficient*. The summary also employs it in the retention equation, $V_R = V_M + K_D V_S$, when defining the retention volume. This author has not located a source for the definition of K_D as a retention parameter (all species of solute) in chromatography, but presumably one exists.

A recently published IUPAC technical report [21] employed K_D as the symbol for the CCC retention parameter and it was alternately called the *partition ratio* (if referring to all forms) and the *partition coefficient* (if referring to a single specific form). This use of K_D directly conflicts with its former definition cited above [17]. The report also equates V_M and the holdup volume, which, especially in CCC, often includes appreciable extra-column volume.

The Orange Book definition of K_D as a chromatography parameter cited above [17] does not appear in the Gold Book. However, the symbol $K_{D(A)}$ (or alternatively $(K_D)_A$) is defined (see Table 3) for LLD, not chromatography, as the ratio of the concentration of a single form of the solute in the organic phase to its concentration in the aqueous phase. The A in the symbol indicates that the formula (or an abbreviation) of the species is to be indicated in parentheses following the subscript D, although in practice, the species is sometimes placed on the same line as K, or as a superscript to K. In the Orange Book, this term is called the *partition ratio*, although this parameter is often regarded as a constant, in contrast to K_C. The choice may have been made because another term, $(K_D^\circ)_A$, defined as an activity ratio for a single species, had already been called the *partition constant* (see Table 3). But, it would have been better to call them both *partition ratio* and qualify $(K_D^\circ)_A$ as the *thermodynamic partition constant*. Another problem that arises with $K_{D(A)}$ is that in general discussions the (A) is often dropped and K_D is used as

the stand-alone symbol, which then becomes confused with other definitions discussed above.

Based on their former definitions, none of the terms K_D, $K_{D(A)}$ or $(K_D)_A$ is credible as a chromatography retention parameter, which must include *all forms* of the solute and should be defined in the format C_S/C_M characteristic of chromatography. The symbol K_C (Table 3 and Eqs. (1) and (2)), presently called the *distribution constant* is the appropriate retention parameter for chromatography. However, it is not a constant as the IUPAC name implies and would be better referred to by the well established historical name, the *distribution coefficient*.

The symbol *D* is called the *distribution ratio* and is defined for extraction as the total concentration of solute in the extract (usually organic phase) divided by the concentration in the other phase (usually water). This presents no problem when employed in LLD, but solute diffusion is an important variable in CCC and the symbol *D* is almost universally used for the diffusion coefficient.

The final three entries in Table 3 allow certain synonyms or recommend they not be used. A noteworthy observation on the assigned names is that *distribution coefficient* is not assigned or recommended for any parameter; see the proposal below.

A summary of a recently presented poster illustrates the frustration of coping with existing chromatography terminology. The poster described a meticulous study of benzoic acid equilibria in the CCC systems heptane/water and heptane/1-butanol/water [22]. *D* was chosen as the retention parameter because its IUPAC name, *distribution ratio*, better reflected the variability of its chromatographic behavior with pH and concentration; whereas the IUPAC-recommended parameter, K_C, is named *distribution constant*, which misrepresents its observed behavior. The retention parameter, *D*, was observed to vary from 0 to 25 as the aqueous mobile phase pH decreased from 7 to 1.

The IUPAC defines *D* for LLD as $C_{extract}/C_{other\ phase}$, which happens to correspond in reversed phase CCC to C_S/C_M, where C includes all forms of the solute. *D* was correctly calculated from the C_S/C_M ratio expressed in terms of the known dimerization constant, K_2, the acidity of the aqueous phase, $[H^+]$, and the constant, K_D (which represents the concentration ratio $[HB]_{org}/[HB]_{aq}$ of the single species, protonated benzoate, [HB], between the organic and aqueous phases). The name *partition constant* was assigned to K_D to reflect the fact that biphasic distributions of single species do not vary with pH or concentration and are considered constant. (The IUPAC would use the symbol $K_{D(A)}$, defined for LLD, to be written in this case $K_{D(HB)}$ and recommends the name *partition ratio*.) Dimerization is significant in the heptane/water system, but is suppressed when 1-butanol is added. Adding 1-butanol also greatly increases partitioning into the organic phase.

Here is an excellent experimental study, technically flawless, which is very difficult to discuss precisely because of the existing confusion in terminology. Some chromatographers will find this presentation difficult to follow because few use *D* as the retention parameter and some even use K_D as the retention parameter [15], whereas the IUPAC [14,19] recommends K_C. The symbol names amplify the confusion.

With the emergence of preparative CCC, not only are liquid–liquid equilibrium studies of solutes desired, but CCC methodology provides the means to conduct these studies in much greater detail than previously. Facilitation of such studies requires a unique and unambiguous term defined specifically for CCC to serve as an equilibrium parameter representing the partitioning of a single species of the solute (such as benzoate ion, B^-, or the neutral benzoic acid molecule, HB) between the two phases. This should be defined for chromatography as C_S/C_M to be compatible with the retention parameter K_C which applies to the overall distribution of all species of the solute.

3.3. Two proposals

It will be difficult to harmonize the names for the chromatography retention parameters, *k*, *K*, K_C (and K_D?) and those for the biphasic equilibrium parameters, K_D, $K_{D(A)}$, $(K_D^\circ)_A$ and *D*, defined for use in LLD. The alliterative similarity of the names makes remembering their assignment difficult; only *k* is unique.

3.3.1. Proposal no. 1, K_C

In CCC and applicable to all liquid–liquid chromatography, the symbol for the retention parameter should be K_C and, for CCC, it should be called the *distribution coefficient*. $K_C = C_S/C_M$ where *C* is the total concentration of all forms of the solute in the stationary and mobile phases respectively. K_C serves as the retention parameter in the equation $V_R = V_M + K_C V_S$. In effect, this proposal is to continue use of the name *distribution coefficient* in CCC as a synonym for the IUPAC recommended name *distribution constant* for K_C as defined by the IUPAC.

The symbol K, modified by the IUPAC in the 1990s to K_C, has the longest history of any symbol in chromatography with an unchanging definition as $K_C = C_S/C_M$, where *C* is all forms. The concern here is only about the name. The IUPAC calls it the *distribution constant*, when it is really not a constant. M & C, who are in close touch with contemporary usage, call it both the *distribution constant* and the *distribution coefficient*. The name distribution coefficient has as long a history as the symbol K which was alternately called either the *partition coefficient* or the *distribution coefficient*. J. Calvin Giddings lists both names in his 1965 book, Dynamics of Chromatography [23] and his 1991 book Unified Separation Science [24], though he uses *distribution coefficient* in the text. Snyder and Kirkland use distribution coefficient in their 1979 book Introduction to Modern Liquid Chromatography [25]. At the present time the IUPAC does not assign the name *distribution coefficient* to any symbol.

3.3.2. Proposal no. 2, $K_{\Delta(A)}$

It is proposed that in CCC, and applicable to all liquid–liquid chromatography, that the symbol for the biphasic equilibration parameter for a single species should be $K_{\Delta(A)}$. The subscript is upper case Greek delta and it should be called the *species partition ratio*. $K_{\Delta\,(A)} = [A]_S/[A]_M$ where $[A]$ is the concentration of a single specific form of the solute in the stationary (S) and mobile (M) phases respectively. In use, the formula for the specific solute species is to be inserted either in parentheses following Δ or as a superscript to the symbol. It is recommended that when the symbol is used in stand-alone fashion, the additional subscript (A) be included. However, at the present time, no confusion will result from using K_Δ alone. Some chromatographers consider the biphasic equilibrium parameter for single species to be constant. However, others [20, p. 2376] prefer to apply the name *constant* only to thermodynamic constants. The term *ratio* does not preclude the parameter being constant. Adding the qualifying term *species* will clarify its application and avoid it being used as a retention parameter. Upper case Δ was chosen as the subscript to continue the sense (distribution) of the existing symbols and to follow the IUPAC practice of using upper case subscripts on symbols referring to aspects of the solvent system.

At the present time, there is no biphasic single species parameter defined for use in CCC or LLC and there is confusion when the corresponding parameters, $K_{D(A)}$ and K_D defined for liquid–liquid distribution (LLD) are used.

Fig. 3 shows the relationship between K_C and the proposed term $K_{\Delta(A)}$ by illustrating the variability in K_C and retention volume seen in reversed phase CCC and in bonded phase chromatography for weak acids, HB, and weak bases, B, as the result of changing the mobile phase pH. For this purpose, $K_{\Delta(A)}$ is defined for chromatography as $[A]_S/[A]_M$, where A is a single form of the solute. For a

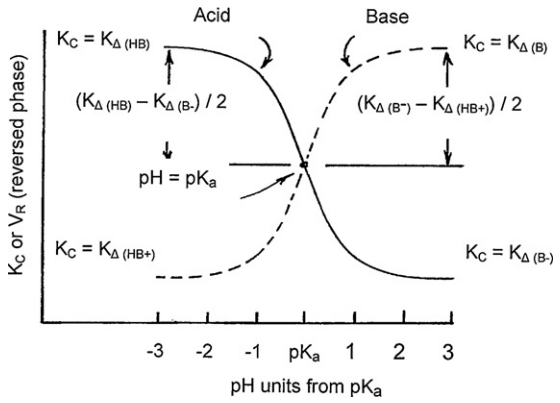

Fig. 3. Retention of acids and bases in reversed phase CCC.

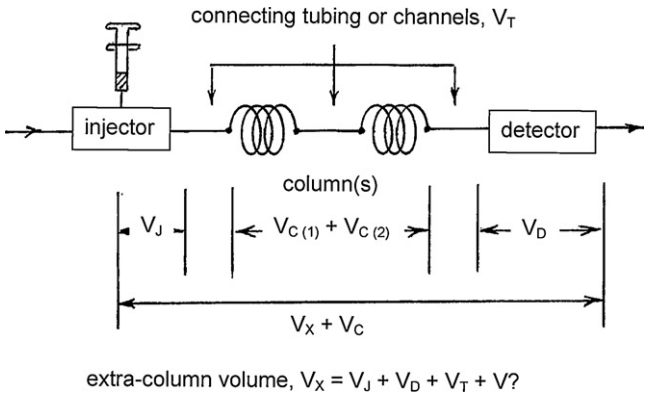

Fig. 5. Various volumes in a CCC apparatus.

weak acid at 3 pH units below the pK$_a$, virtually all the acid will be in the form of the protonated acid which may penetrate the organic phase. At this point, K_C is equal to $K_{\Delta(HB)}$. As pH increases, HB concentration decreases while that of B^- increases until at the pK$_a$, [HB] = [B^-]. HB concentration continues to decrease with further increase in pH until, at a pH 3 units above the pK$_a$, essentially all the acid is in the form of B^- and $K_C = K_{\Delta(B^-)}$. If B^- is sufficiently polar K_C may be 0, or near 0, at high pH. The opposite behavior is observed for weak bases.

The acid could be any monoprotic weak acid, such as benzoic acid and the base could be any monofunctional weak base, such as aniline. The diagram shows the expected sigmoid shape of the respective curves (barring adsorption on a matrix, dimerization or interaction with substances in the mobile aqueous phase). It is not intended to imply that both curves are the same height, with K_C for the acid equal to K_C for the base. The curves are independent, since they represent different substances, the asymptotes of which will likely be at different heights. The ionized species, being polar, will likely elute near zero. No vertical scale is shown.

Fig. 4 illustrates a more complete biphasic equilibrium for a weak acid including dimerization of the acid in the organic phase (dimerization constant K_2) and dissociation of the acid in the aqueous phase (dissociation constant K_a) and the resulting K_C expression. Again the proposed equilibrium parameter $K_{\Delta(A)}$ is shown. This would represent a reversed phase CCC mode.

3.4. Mobile phase volume and symbols V_M, V_H, and V_X

Both the IUPAC Gold Book and the M & C summary imply that V_M is the mobile phase within the column; for instance when it is used in the retention equation. But, both lists define V_M as being equivalent to the holdup volume, which both designate by the symbol V_M. The holdup volume is determined by measuring the retention volume of an unretained solute, for which K_C is 0 (Gold Book index H02833). The holdup volume will include the extra-column volume, which because of the long inlet and outlet flow lines (flying leads) is often not negligible in CCC, especially those systems with small column volumes [26]. Extra-column volume should not be referred to as dead volume, which the IUPAC reserves for those volumes in the system not swept by flowing mobile phase. Perhaps it is an error, but the M & C summary includes the term dead volume and defines it in the same way as holdup volume and again assigns it the symbol, V_M. It is necessary therefore in CCC to clarify the definition of V_M and to assign new symbols for holdup volume and extra-column volume.

Several relevant volumes in a CCC system are shown in Fig. 5. The extra-column volume includes the volume V_J from the point of injection to the column entrance and the volume V_D from the column exit to the point of detection as well as any additional tubing or channels, V_T, and any other volumes not specified in figure, V?. The sum of these volumes constitutes the extra-column volume for which the symbol V_X is suggested. For CCC, the symbol V_M should be defined as the volume of mobile phase within the column after correction for extra-column volume, V_X. Authors should indicate whether V_M in their paper represents the corrected mobile phase volume or the uncorrected mobile phase volume.

Fig. 6 illustrates the effect of extra-column volume on the CCC chromatogram. The figure shows a correction for extra-column volume which introduces a delay, V_X, in the elution of all peaks. If the position of $K_C = 0$ is determined by using only an unretained marker, and no correction for V_X is made, the position for $K = 1$, based on the known column volume, would actually lie much earlier, giving an incorrectly low estimate of V_S. In the example shown, this would lead to an erroneously high estimate of K_C for the solute peak shown to the right of $K_C = 1$.

V_H is a good symbol to represent holdup volume because the meaning is self-evident. One objection might be that V_H should be reserved for the volume of a theoretical plate. However the IUPAC assigns the symbol upper case H for the height equivalent to a the-

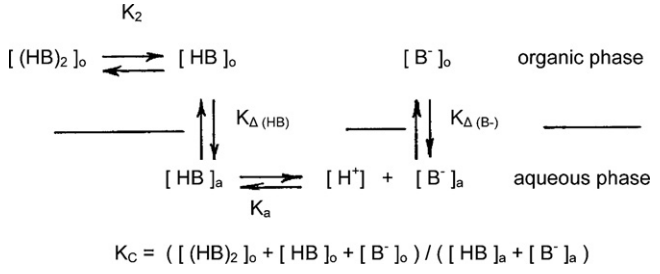

Fig. 4. Biphasic weak acid equilibrium and expression for K_C for reversed phase CCC.

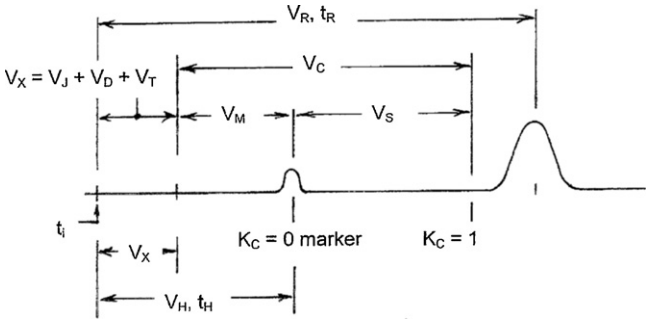

Fig. 6. The effect of extra-column volume, V_X, on the CCC chromatogram.

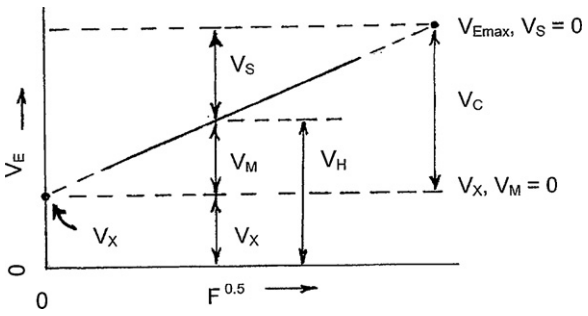

Fig. 7. CCC volumes illustrated on Wood et al. plot for measuring extra-column volume.

oretical plate and since the size can be stated in either volume or length, the symbol could be modified to H_V or H_L to accommodate either choice.

To summarize: the suggestions for these related volumes are; holdup volume, V_H; mobile phase volume within the column, V_M; extra-column volume, V_X; theoretical plate volume, H_V and theoretical plate length, H_L.

A procedure for measuring V_X, described by Wood et al. [27], can be used to illustrate the relationship between V_M, V_S, V_X and V_H.

When an initially empty CCC is filled with stationary phase, a volume, V_C, fills the column (coil) and an additional volume, V_X, occupies any extra-column volume in the system. This is the maximum volume of stationary phase that the system can contain and will be represented by $V_{Emax} = V_C + V_X$ and called the maximum expulsion volume. On starting mobile phase flow, the extra-column volume, V_X, of stationary phase, along with an increment of the stationary phase in the column, is expelled from the system. At this point the cumulative expelled volume, V_E, is equal to $V_X + V_M$, where this V_M is the volume of mobile phase now in equilibrium with the stationary phase remaining in the column at the current flow rate, F, (exactly what is illustrated in Fig. 3, where V_X is neglected). As F is increased incrementally, more stationary phase is expelled from the column, each time reaching an equilibrium when $V_E = V_X + V_M$, with V_E increasing as accumulation of more V_M in the column displaces more stationary phase from the column.

Du et al. [28] had shown that S_F remaining in the column is related to the mobile phase flow rate, F, as

$$S_F = A - BF^{0.5} \tag{6}$$

where A and B are constants. Wood et al. transformed Eq. (6) to Eq. (7)

$$V_E = \frac{BV_C F^{0.5}}{100} + V_X \tag{7}$$

and used this as the basis of a procedure to measure the extra-column volume (see Fig. 7).

The cumulative expelled volume, V_E, of stationary phase is collected and plotted, on the vertical axis, against the flow rate. When the plot is extrapolated to 0 flow rate, the intercept is the extra-column volume, V_X. The maximum expulsion volume, V_{Emax}, can then be calculated from $V_X + V_C$ and the plot extrapolated to that level as well. Vertical distances from V_X to the plot at a particular flow rate represent V_M in the column at that flow rate, while distances from the plot to the maximum expulsion volume, V_{Emax}, represent V_S. The distances from the horizontal baseline to the plotted line represent what chromatographers call the holdup volume, $V_H = V_M + V_X$. In addition to providing a means of measuring V_X, this procedure presents a very practical illustration of the relationship of the four volumes, which are illustrated in Fig. 7.

3.5. The phase ratio, β_V

The phase ratio should be indicated by β_V to avoid confusion with the coil radius ratio $\beta_r = r/R$, where r is the radius of a planetary helical coil or of a loop in a multilayer planetary coil (measured from the planetary axis) and R is the orbital radius of the planetary axis.

However, the definition of β_V is uncertain at the present time. M & C define β_V as V_S/V_M in agreement with the definition in the Orange Book, 2nd ed. [17] in section 9.4.8.6. However, in the Ettre paper [19] section 3.2.17 and in the Gold Book (index P04531) it is defined as the reciprocal value, V_M/V_S. The phase ratio symbol is convenient and serves to show the similarity of some equations, but it is useless until a definition is agreed upon. The V_S/V_M configuration agrees with traditional chromatography formulations as stationary phase/mobile phase.

4. Summary

The process of CCC can be inferred from three facts: (1) a non-retained solute, $K_C = 0$, elutes with a retention volume, V_R, equal to one mobile phase volume; (2) a solute distributed with equal concentration in each phase, $K_C = 1$, elutes later, with passage of an additional volume of mobile phase equal to a stationary phase volume, V_S, a retention volume of one column volume, V_C; and (3) subsequent solutes with K_C values of 2, 3, 4, etc. elute at successive mobile phase volumes, each equal to a stationary phase volume. The solute with $K_C = 1$ always has a retention volume of V_C. These facts readily allow the deduction of the standard chromatographic solute retention equation, $V_R = V_M + K_C V_S$. A high stationary phase fraction, $S_F = V_S/V_C$ is characteristic of CCC and leads to high values of solute resolution.

The discussion of terminology is limited to addressing the inconsistencies and inadequacies of terminology for some mathematical terms used in chromatography and liquid–liquid distribution, particularly as they relate to CCC. Terms defined for these related fields are similar but are not interchangeable without redefinition. The meanings of several terms summarized by authoritative bodies have become so confusing that their symbol, definition or name must be clarified or in some cases abandoned. Some new terms must be introduced to facilitate development of emerging methodologies in CCC. These should be as unique as possible and their field of use must be indicated. The terms for closely related fields need not be interchangeable, but they should not conflict and the assigned name should not misrepresent the properties of the parameter it represents.

It is suggested that K_C be employed to represent the solute retention parameter and that it be referred to as the *distribution coefficient*, instead of the IUPAC-assigned name *distribution constant*, since the retention parameter is not a constant. It is also proposed that a new term for the biphasic equilibrium parameter for a single form of the solute be defined for use in CCC as well as other forms of chromatography. It should be defined as $K_{\Delta(A)} = [A]_S/[A]_M$, where $[A]$ is the concentration of a single specific form in the stationary and mobile phases respectively. In use, the species formula would replace A in the symbol and the term $K_{\Delta(A)}$ should be called the *species partition ratio*. At the present time, there is no such term defined for the field of chromatography. It should be useful for both CCC and reversed phase (bonded or LLC) liquid chromatography.

The definition of the mobile phase volume, V_M, particularly in CCC, but also in mainstream chromatography, should be clarified so as not to include the extra-column volume. To facilitate this, the symbols V_H and V_X are suggested for the holdup volume and the extra column volume respectively. The terms H_V and H_L are suggested for the volume and length of a theoretical plate in CCC.

The phase ratio should be represented by β_V in CCC but its definition must be clarified in consultation with representatives in mainstream chromatography. The M & C glossary [15] defines it as V_S/V_M, in agreement with the Orange Book, 2nd edition [17]. However, the Gold Book defines it as V_M/V_S. This is a convenient term, but unless authoritative bodies can agree, it should be avoided or defined each time it is used.

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