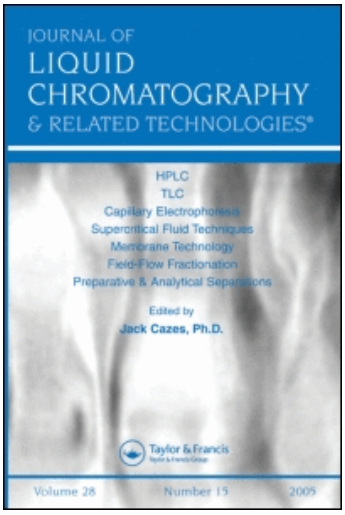

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Estimation of Separation Factor for Analogues in CCC Using UNIFAC

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ABSTRACT

Separation factor is an important index for judging the eluotropic sequence of solutes in countercurrent chromatography (CCC). This paper has proven that the contribution of the common basic agglomerate can be eliminated when calculating the separation factor for analogues and introduced a computational equation based on UNIFAC. According to the equation, the estimation of separation factors needs only a few different groups of analogues. Eleven kinds of analogues, such as phytosterol, chlorinated s-triazines, flavonoids, theaflavins, anthocyanins, sporaviridins, etc., have been tested with the method. Though the solvent systems used are different from each other, the estimated eluotropic

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sequences are all consisted well with the literatures except for two chlorinated s-triazines.

Key Words: Eluotropic sequence; Separation factor; Analogue; Countercurrent chromatography; UNIFAC.

INTRODUCTION

Recent studies have shown that countercurrent chromatography (CCC) is a powerful method for the preparative separation of many chemicals, such as natural products, bio-products, drugs, etc. The key work when using CCC is to select an appropriate solvent system that must be a biphasic liquid system and gives higher separation factors for the solutes being separated. It is difficult work and takes much time for the researchers who try to find the appropriate solvent system experimentally. Fortunately, our recent studies show that solution theories such as UNIFAC,[1] a group contribution model for activity coefficient, can help us to accomplish this target without experiments. By using UNIFAC, one can judge whether a solvent system divides into two liquid phases or not, and estimate the partition coefficient of a solute between the two liquid phases. However, there are many cases where UNIFAC parameters are not available, or the solute molecules are too complex to be split simply into UNIFAC groups. In these cases, the partition coefficient cannot be calculated with conventional procedure.

Separation factor, defined as the ratio of the partition coefficients of two solutes, is an important index for judging the eluotropic sequence of solutes in CCC. The present paper will establish a strategy to estimate the separation factor for analogues with common structural agglomerate, by which one can evaluate the solvent system selected and preview the eluotropic sequence of the analogues in CCC.

THEORY

It is known that many natural products, bio-products, drugs, etc., are analogues with certain common and complex basic agglomerates. In general, analogues with large molecular weights consist of common agglomerate and some different branches. The volume and the surface of the common agglomerate are very large, even the contribution of the branches can be neglected when calculating the molecular volume or molecular surface area. Therefore, it is assumed that both the molecular volumes and the molecular surface areas are all the same for the homologues.

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The partition of an organic solute between two liquid phases can be estimated by using UNIFAC if the parameters of the structural groups are all known. However, in many cases, it is hard to split the basic agglomerate of the homologues into detailed groups. Thus, we treat the agglomerate as one group and split the branches according to UNIFAC.

If two liquid phases, I and II, are equilibrated and analogues, 1 and 2, are distributed between them, according to the principle of equality of chemical potential, we have

$$\gamma_1{}^{\mathrm{I}} x_1{}^{\mathrm{I}} = \gamma_1{}^{\mathrm{II}} x_1{}^{\mathrm{II}} \tag{1}$$

$$\gamma_2{}^{\mathrm{I}} x_2{}^{\mathrm{I}} = \gamma_2{}^{\mathrm{II}} x_2{}^{\mathrm{II}} \tag{2}$$

The partition coefficients K_1 and K_2 are then represented respectively as

$$K_1 = \frac{x_1{}^{\mathrm{I}}}{x_1{}^{\mathrm{II}}} = \frac{\gamma_1{}^{\mathrm{II}}}{\gamma_1{}^{\mathrm{I}}} \tag{3}$$

$$K_2 = \frac{x_2{}^{\mathrm{I}}}{x_2{}^{\mathrm{II}}} = \frac{\gamma_2{}^{\mathrm{II}}}{\gamma_2{}^{\mathrm{I}}} \tag{4}$$

The separation factor for the two compounds is defined as

$$S_{12} = \frac{K_1}{K_2} = \frac{\gamma_1{}^{\mathrm{II}}\gamma_2{}^{\mathrm{I}}}{\gamma_1{}^{\mathrm{I}}\gamma_2{}^{\mathrm{II}}} \tag{5}$$

In CCC, the concentrations of the solutes are generally very low. Therefore, for common applications, we discuss the separation factor at infinite dilution ($S_{12}{}^{\infty}$),

$$S_{12}{}^{\infty} = \frac{\gamma_1{}^{\infty\mathrm{II}}\gamma_2{}^{\infty\mathrm{I}}}{\gamma_1{}^{\infty\mathrm{I}}\gamma_2{}^{\infty\mathrm{II}}} \tag{6}$$

According to UNIFAC, the activity coefficients at infinite dilution are calculated by

$$\ln \gamma_i{}^{\infty\mathrm{I}} \gamma_i{}^{\mathrm{C}(\infty\mathrm{I})} + \ln \gamma_i{}^{\mathrm{R}(\infty\mathrm{I})} \tag{7}$$

$$\ln \gamma_i{}^{\infty\mathrm{II}} \gamma_i{}^{\mathrm{C}(\infty\mathrm{II})} + \ln \gamma_i{}^{\mathrm{R}(\infty\mathrm{II})} \tag{8}$$

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Due to almost the same molecular volume and surface area of the analogues, we have

$$\gamma_1^{C(\infty I)} \approx \gamma_2^{C(\infty I)} \tag{9}$$

$$\gamma_1^{C(\infty II)} \approx \gamma_2^{C(\infty II)} \tag{10}$$

and

$$\ln S_{12}^{\infty} = \ln \frac{\gamma_1^{\infty II}\gamma_2^{\infty I}}{\gamma_1^{\infty I}\gamma_2^{\infty II}} = (\ln \gamma_1^{R(\infty II)} - \ln \gamma_1^{R(\infty I)}) - (\ln \gamma_2^{R(\infty II)} - \ln \gamma_2^{R(\infty I)}) \tag{11}$$

According to UNIFAC,

$$\ln \gamma_i^{R} = \sum_k v_k^{(i)}(\ln \Gamma_k - \ln \Gamma_k^{(i)}) \tag{12}$$

making $x_1^{I} \to 0$, $x_2^{I} \to 0$, and $x_1^{II} \to 0$, $x_2^{II} \to 0$, we have

$$\ln \gamma_i^{R(\infty I)} = \sum_k v_k^{(i)}(\ln \Gamma_k^{\infty I} - \ln \Gamma_k^{(i)}) \tag{13}$$

$$\ln \gamma_i^{R(\infty II)} = \sum_k v_k^{(i)}(\ln \Gamma_k^{\infty II} - \ln \Gamma_k^{(i)}) \tag{14}$$

hence

$$\ln S_{12}^{\infty} = \sum_k (v_k^{(1)} - v_k^{(2)})(\ln \Gamma_k^{\infty II} - \ln \Gamma_k^{\infty I}) \tag{15}$$

Because an analogue has only one common agglomerate (B), $v_B^{(1)} = v_B^{(2)} = 1$, the above equation becomes

$$\ln S_{12}^{\infty} = \sum_{k \neq B} (v_k^{(1)} - v_k^{(2)})(\ln \Gamma_k^{\infty II} - \ln \Gamma_k^{\infty I}) \tag{16}$$

According to UNIFAC,

$$\ln \Gamma_k = Q_k \left[1 - \ln\left(\sum_m \theta_m \Psi_{m,k} \right) - \sum_m \frac{\theta_m \Psi_{k,m}}{\sum_n \theta_n \Psi_{n,m}} \right] \tag{17}$$

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where

$$\Psi_{n,m} = \exp\left(-\frac{a_{n,m} + b_{n,m}T + c_{n,m}T^2}{T}\right) \tag{18}$$

For liquid phase I, making ${x_1}^{\mathrm{I}} \to 0$, ${x_2}^{\mathrm{I}} \to 0$, we have

$$\theta_m \to {\theta_m}^{\mathrm{I}} \tag{19}$$

and

$$\ln {\Gamma_k}^{\infty \mathrm{I}} = Q_k\left[1 - \ln\left(\sum_m {\theta_m}^{\mathrm{I}}\Psi_{m,k}\right) - \sum_m \frac{{\theta_m}^{\mathrm{I}}\Psi_{k,m}}{\sum_n {\theta_n}^{\mathrm{I}}\Psi_{n,m}}\right] \tag{20}$$

Similarly, for liquid phase II, making ${x_1}^{\mathrm{II}} \to 0$, ${x_2}^{\mathrm{II}} \to 0$, we have

$$\theta_m \to {\theta_m}^{\mathrm{II}} \tag{21}$$

and

$$\ln {\Gamma_k}^{\infty \mathrm{II}} = Q_k\left[1 - \ln\left(\sum_m {\theta_m}^{\mathrm{II}}\Psi_{m,k}\right) - \sum_m \frac{{\theta_m}^{\mathrm{II}}\Psi_{k,m}}{\sum_n {\theta_n}^{\mathrm{II}}\Psi_{n,m}}\right] \tag{22}$$

Thus

$$\begin{aligned}
\ln {S_{12}}^{\infty} &= \sum_{k \neq \mathrm{B}} ({v_k}^{(1)} - {v_k}^{(2)})Q_k\left[\ln\left(\sum_m {\theta_m}^{\mathrm{I}}\Psi_{m,k}\right)\right. \\
&\quad \left. - \ln\left(\sum_m {\theta_m}^{\mathrm{II}}\Psi_{m,k}\right) + \sum_m \frac{{\theta_m}^{\mathrm{I}}\Psi_{k,m}}{\sum_n {\theta_n}^{\mathrm{I}}\Psi_{n,m}} - \sum_m \frac{{\theta_m}^{\mathrm{II}}\Psi_{k,m}}{\sum_n {\theta_n}^{\mathrm{II}}\Psi_{n,m}}\right] \\
&= \sum_{k \neq \mathrm{B}} ({v_k}^{(1)} - {v_k}^{(2)})Q_k\left[\ln \frac{\sum_m {\theta_m}^{\mathrm{I}}\Psi_{m,k}}{\sum_m {\theta_m}^{\mathrm{II}}\Psi_{m,k}}\right. \\
&\quad \left. + \sum_m \left(\frac{{\theta_m}^{\mathrm{I}}\Psi_{k,m}}{\sum_n {\theta_n}^{\mathrm{I}}\Psi_{n,m}} - \frac{{\theta_m}^{\mathrm{II}}\Psi_{k,m}}{\sum_n {\theta_n}^{\mathrm{II}}\Psi_{n,m}}\right)\right]
\end{aligned} \tag{23}$$

The above equation shows that S^{∞} has no relation to the common agglomerate. This indicates that only the different groups of the analogues are required for the estimation of separation factor.

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Sometimes, analogues may have two or more common agglomerates which bond together by different hydrocarbon chains or other groups. In these cases, the common agglomerates can be considered as one and Eq. (23) is still available.

The first step in estimation of separation factor is to calculate the equilibrium compositions of the two liquid phases of the solvent system using UNIFAC (in this work, we use the modified UNIFAC).[1,2] Then, the separation factor is calculated using Eq. (23) with all groups of the solvents and only different groups of the solutes.

APPLICATION AND DISCUSSION

Phytosterol is a kind of analogue with common agglomerates (sterol base). The structures of three familiar phytosterols: campesterol, β-sitosterol, and stigmasterol are very similar to each other, as shown in Fig. 1. By using the very few different groups, the separation factors for these sterols were estimated with the proposed method. The results were consistent with our experiments,[3] as shown in Table 1.

Many studies have been carried out on the separation of analogues by CCC. Examples of such analogues are shown in Figs. 2–11. All of them differ in a few substituted groups. The separation factors for each kind of analogue by various solvent systems are calculated with Eq. (23), using only the different groups as listed in Table 2. Because the eluotropic sequence agrees with the separation factor, we can estimate the eluotropic sequences of these analogues from the separation factors calculated.

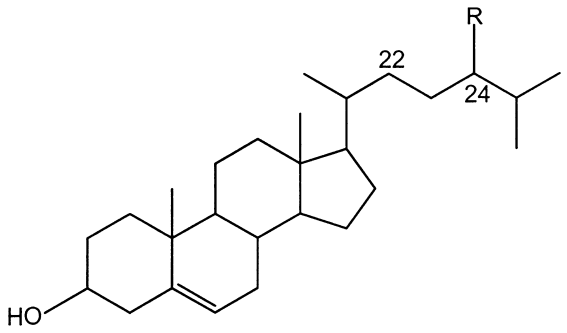

Figure 1. The structures of the steroids campesterol (R═CH_3), β-sitosterol (R═C_2H_5), and stigmasterol (R═C_2H_5, Δ^{22}).

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Table 1. The separation factors for campesterol, β-sitosterol, and stigmasterol in five solvent systems.[a]

		$S_{21} = K_2/K_1$		$S_{31} = K_3/K_1$	
No.	Solvent system (volume ratio)	Exp.	Cal.	Exp.	Cal.
1	Heptane/methanol (1 : 1)	1.06	1.42	1.01	1.19
2	Heptane/acetonitrile/ethyl acetate (5 : 5 : 1)	1.35	1.33	1.03	0.91
3	Hexane/methanol (1 : 1)	1.04	1.32	1.00	1.14
4	Hexane/acetonitrile/ dichloromethane (5 : 5 : 1)	1.10	1.29	1.02	0.91
5	Hexane/acetonitrile/ dichloromethane (5 : 5 : 2)	1.12	1.18	1.02	0.94

[a]Partition coefficients (K_1, K_2, K_3) are defined as the ratio of the mole concentration of a given sterol in the nonpolar phase vs. that in polar phase. (1: campesterol, 2: β-sitosterol, 3 : stigmasterol).

It should be noted that the different groups indicated in the figures cannot be used directly in the calculation in many cases. Generally, they need further detailed treatment according to UNIFAC and the bonding point may be taken into account. Therefore, the groups finally used in this work are not always the same as that shown in the figures.

Up to now, both the groups and the parameters of UNIFAC cannot cover all the molecules in the world. Sometimes, we cannot find suitable groups for a compound from UNIFAC tables. For example, we could not find suitable UNIFAC groups for chlorinated s-triazines, because there are conjugated bonds between the nitrogen atoms of the amino groups and the carbon atoms of the ring (Fig. 2). Due to this reason, the estimated eluotropic sequence of propazine and trietazine (the first two compounds) is reversed compared with the experimental results.

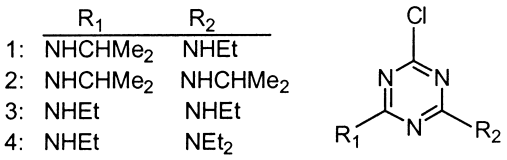

Figure 2. Structures of chlorinated s-triazines. 1: atrazine, 2: propazine, 3: simazine, 4: trietazine.

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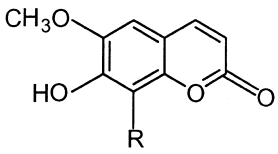

Figure 3. Structures of (1) isofraxidin (R═OCH_3) and (2) scopoletin (R═H).

In some analogues, no difference in groups can be found, but the bonding positions are different, 7,8-dihydroxyflavone and chrysin are one of the examples (Fig. 5). Theoretically, UNIFAC model cannot distinguish such isomers. Therefore, the calculated separation factors for 7,8-dihydroxyflavone and chrysin are unity, and we cannot get their difference in eluotropic sequences with the present method.

Theaflavins are compounds with a somewhat large common agglomerate, differing in two groups (Fig. 9). Here, the different group (G) is relatively large, and there is no difference between theaflavin-3-gallate and theaflavin-3′-gallate according to UNIFAC. The separation factors estimated are $S_{AB} = S_{CD} = 25.9$ and $S_{BC} = 1$, implying the eluotropic sequence is A–(B, C)–D, when they are separated by CCC with hexane/ethyl acetate/methanol/water (1 : 3 : 1 : 6)

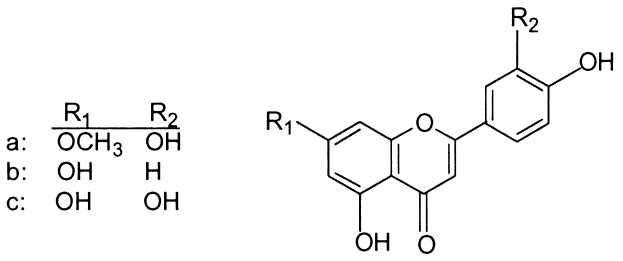

Figure 4. Structures of 3′-hydroxygenkwanin (a), apigenin (b), and luteolin (c).

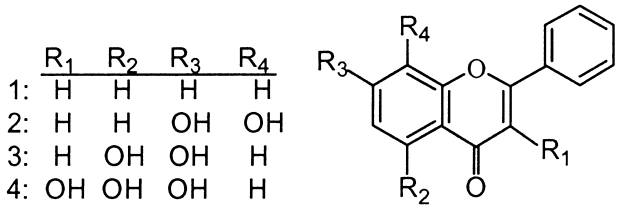

Figure 5. Structures of flavonoids, 1: flavone, 2: 7,8-dihydroxyflavone, 3: chrysin, 4: galangin.

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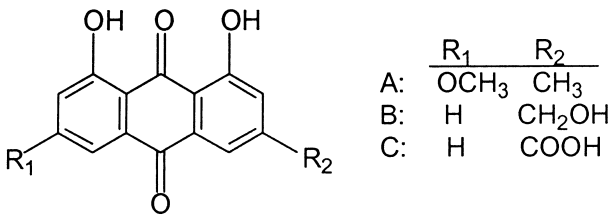

Figure 6. Structures of physcion (A), aloe-emodin (B), and rhein (C).

system and the lower phase mobile. Other workers reported the same eluotropic sequence.[11] Theaflavin-3-gallate and theaflavin-3′-gallate could not be separated with this solvent system.

The most complex analogue that was tested is anthocyanin, differing in three different groups (Fig. 10). All five analogs were successfully separated by CCC with t-butyl methyl ether/*n*-butanol/acetonitrile/water (2 : 2 : 1 : 5) system (acidified with 0.01% TFA) and the aqueous phase mobile. The eluotropic sequence was 1–2–3–4–5. Ignoring the acidification with 0.01% TFA, we calculated the separation factors with the present method. Their

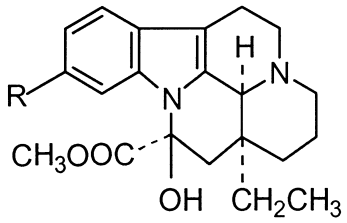

Figure 7. Structures of (1) vincamine (R=H) and (2) vincine (R=OCH_3).

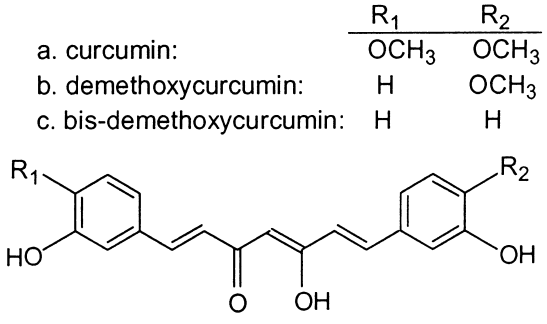

Figure 8. Structures of curcuminoids.

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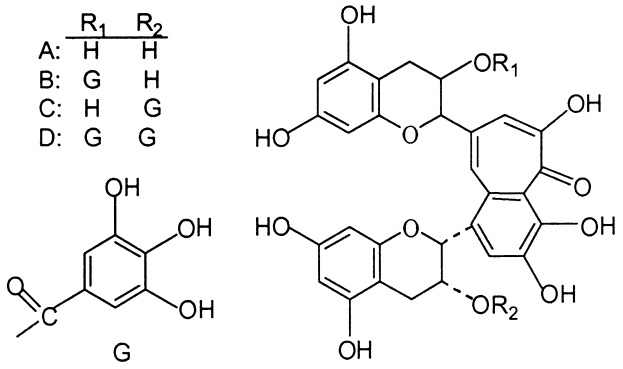

Figure 9. Structures of theaflavins. A: theaflavin, B: theaflavin-3-gallate, C: theaflavin-3′-gallate, D: theaflavin-3,3′-digallate.

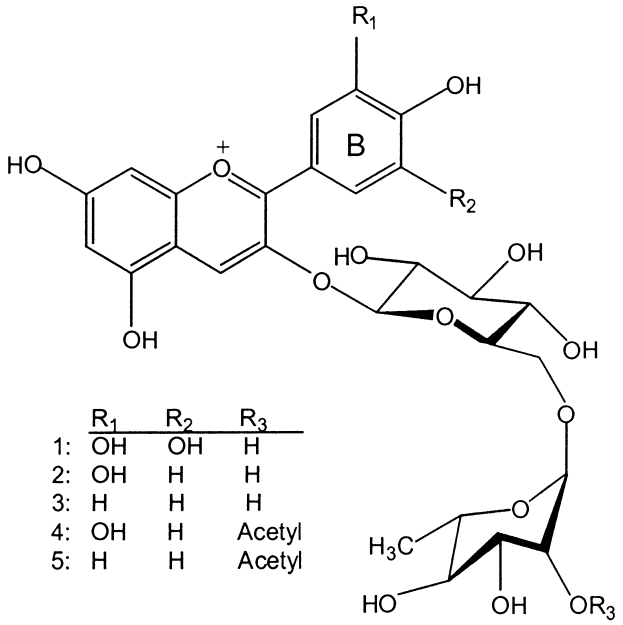

Figure 10. Structures of five anthocyanins. 1–3: The 3-rutinosides of delphindin, cyaniding and pelargonidin. 4–5: The 3-(2‴-acetyl-rutinosides) of cyaniding and pelargonidin.

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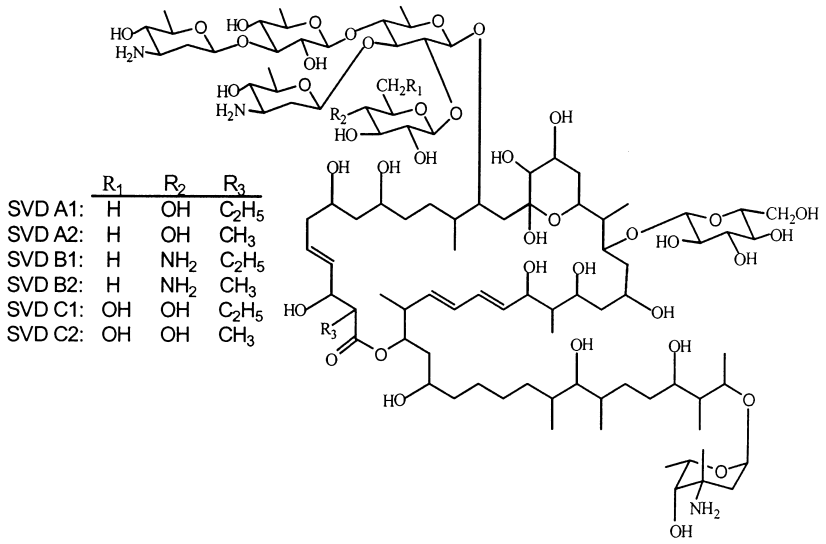

Figure 11. Structures of sporaviridins.

values obtained are $S_{12} = 4.623$, $S_{23} = 4.623$, $S_{34} = 3.487$, $S_{45} = 4.623$, from which we can obtain the same eluotropic sequence as reported by Torskangerpoll et al.[12]

Other complex analogues are six sporaviridins, also differing in three groups (Fig. 11). Harada et al.[13] measured the partition coefficients of these sporaviridins in several solvent systems and found that they were eluted by CCC in the order of their partition coefficients. For example, the eluotropic sequence was C_2–C_1–B_2–A_2–B_1–A_1 in the case of chloroform/ethanol/water (5 : 3 : 4) system and upper (aqueous) phase mobile. In our study, the separation factors calculated with the three different groups R_1, R_2, and R_3, are $S_{C2,C1} = 2.37$, $S_{C1,B2} = 7.12$, $S_{B2,A2} = 1.34$, $S_{A2,B1} = 1.77$, $S_{B1,A1} = 1.34$ for the same solvent system. The result indicates that the eluotropic sequence estimated is consistent with the experiment.

It was found that all the estimated eluotropic sequences were coherent with the experimental ones, except for chlorinated s-triazines (as already explained above).

CONCLUSION

It has been proven that the contribution of the common basic agglomerate can be eliminated when calculating the separation factor for analogues in

Table 2. The grouping according to UNIFAC for the different groups in the analogues tested.

			Figure 1		
Name of solute	CH_2	CH=CH			
Campesterol	1	0			
Sitosterol	2	0			
Stigmasterol	0	1			
			Figure 2		
Name of solute	CH_3	CH_2	CH_2NH	CHNH	CH_2N
Atrazine	3	0	1	1	0
Propazine	4	0	0	2	0
Simazine	2	0	2	0	0
Trietazine	3	1	1	0	1
			Figure 3		
Name of solute	ACH	AC	OCH_3		
Isofraxidin	0	1	1		
Scopoletin	1	0	0		
			Figure 4		
Name of solute	ACH	AC	ACOH	OCH_3	
3′-Hydroxygenkwanin	0	1	1	1	
Apigenin	1	0	1	0	
Luteolin	0	0	2	0	

Figure 5

Name of solute	ACH	ACOH
Flavone	4	0
7,8-Dihydroxyflavone	2	2
Chrysin	2	2
Galangin	1	3

Figure 6

Name of solute	ACH	AC	$ACCH_3$	$ACCH_2$	OCH_3	COOH	OH(s)
Physcion	0	1	1	0	1	0	0
Aloe-emodin	1	0	0	1	0	0	1
Rhein	1	1	0	0	0	1	0

Figure 7

Name of solute	ACH	AC	OCH_3
Vincamine	1	0	0
Vincine	0	1	1

Figure 8

Name of solute	ACH	AC	OCH_3
Curcumin	0	2	2
Demethoxycurcumin	1	1	1
Bis-Demethoxycurcumin	2	0	0

(*continued*)

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Table 2. Continued.

			Figure 9			
Name of solute	OH(s)	ACH	AC	COO	ACOH	
Theaflavin	2	0	0	0	0	
Theaflavin-3-gallate	1	2	1	1	3	
Theaflavin-3′-gallate	1	2	1	1	3	
Theaflavin-3,3′-digallate	0	4	2	2	6	
			Figure 10			
Name of solute	ACH	ACOH	CH_3COO	OH(s)		
1	0	2	0	1		
2	1	1	0	1		
3	2	0	0	1		
4	1	1	1	0		
5	2	0	1	0		
			Figure 11			
Name of solute	CH_3	CH_2	CH	OH(p)	OH(s)	$CHNH_2$
A1	2	1	2	0	1	0
A2	2	0	2	0	1	0
B1	2	1	1	0	0	1
B2	2	0	1	0	0	1
C1	1	1	2	1	1	0
C2	1	0	2	1	1	0

CCC. The introduced equation for the calculation of separation factor for analogues is useful and powerful, especially when the common basic agglomerate is too complex to split into UNIFAC groups. The theoretical predictions of eluotropic sequences were consistent with the experimental results for almost all 11 kinds of analogues tested. The introduced method widens the application field of UNIFAC and is convenient to CCC researchers in saving time and solvents.

Supporting Information Available: We have elaborated a computer program by which one can conduct all the calculation easily. At the same time, we have registered it as a formal software that will be put on sale in the year 2003 with a price of $2000.

NOTATION

$a_{n,m}$	UNIFAC group interaction parameter between groups n and m (K)
$b_{n,m}$	UNIFAC group interaction parameter between groups n and m
$c_{n,m}$	UNIFAC group interaction parameter between groups n and m (K^{-1})
x	mole fraction
K	partition coefficient
Q	relative van der Waals surface area of a group
S	selectivity coefficient
T	absolute temperature (K)
γ	activity coefficient of a molecule
Γ	activity coefficient of a group
$v_k^{(i)}$	number of structural groups of type k in molecule i
θ_m	surface fraction of group m in the liquid phase
$\Psi_{n,m}$	UNIFAC group interaction parameter between groups n and m

Superscript

I	liquid phase I
II	liquid phase II
∞	at infinite dilution
(i)	in the molecule of component i

Subscript

1	component 1
2	component 2
B	basic agglomerate of a homologue

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i	component i
k	group k
m	group m
n	group n

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