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THEORY FOR MOBILE PHASE EFFECTS IN SEPARATIONS OF ISOMERS BY LIQUID-SOLID CHROMATOGRAPHY

APPLICATION TO THE RELATIVE RETENTION OF CERTAIN DIASTEREOMERS ON SILICA

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SUMMARY

Literature data (Palamareva *et al.*) on the separation of diastereomeric hydroxy-diarylpropionates on silica have been interpreted in terms of a recent theory of solvent selectivity in liquid-solid chromatography. The data of Palamareva *et al.* show that separation factors for these compounds depend markedly on the composition of the mobile phase used for separation. These latter data can be quantitatively rationalized in terms of the relative localization of adsorbed mobile phase molecules. The present study suggests that non-localizing mobile phases will usually provide maximum resolution of isomeric solute pairs.

INTRODUCTION

A recent report¹ has shown that solvent-solute localization plays a major role in determining the relative separation of adjacent bands in liquid-solid chromatography (LSC). Furthermore, the theoretical model presented in ref. 1 allows the quantitative prediction of changes in resolution or separation factor, α , as a function of the composition of the mobile phase. In other studies, Palamareva and co-workers²⁻⁷ have reported extensively on the separation of diastereomeric tetrasubstituted ethane derivatives, and have shown that the retention order *threo* > *erythro* generally predominates. This has been explained in terms of a simple model of the configuration of the two isomers in LSC systems**. The *erythro* isomer generally exhibits greater steric hindrance of polar groups within the adsorbate molecule.

Occasional exceptions to the above rule for diastereomers are observed; most of these can be explained in terms of the exceptional structures of the molecules in question. The latest publication by Palamareva *et al.*⁷, dealing with 3-hydroxy-2,3-diarylpropionates as solutes, reports data on the variation of α values for these

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** By "configuration" is meant the position and orientation of the adsorbate molecule with respect to the silica surface.

diastereomers as a function of change in the mobile phase composition. These data are interesting for several reasons: (1) solvent selectivity can affect values of α for a diastereomeric pair, and this could conceivably explain some of the anomalous retention orders observed (*i.e.*, *erythro* > *threo*); (2) isomer separations are of particular interest in LSC systems, and the enhancement of such separations by the judicious choice of mobile phase composition is also of interest; (3) the data of Palamareva *et al.*⁷ offer yet another test of the theory put forth in ref. 1, and the more general model^{8,9} upon which that theory is based.

In this paper I will show that the explanation advanced in ref. 1 to explain the most important solvent-selectivity effects in LSC systems is capable of quantitatively predicting the solvent effects for diastereomeric separation reported in ref. 7. I will also explore some practical implications of this correlation.

THEORY

Diastereomer separations of ref. 7

Palamareva and co-workers have reported data for the separation on silica by thin-layer chromatography (TLC) of compounds of the structure Ar-CH(X)-CH(Y)-Ar', where Ar and Ar' refer to various substituted phenyl (aromatic) groups, X is variously -OH, -OCOCH₃ or -NH(C₆H₅), and Y is -COOR. The group R is variously methyl, isopropyl, *n*-butyl, isobutyl or *tert.*-butyl. The *threo* isomer is generally more strongly adsorbed, and this is explained in terms of the configurations of the adsorbed molecules (Fig. 1a). Here it is assumed that both polar groups X and Y interact with the adsorbent surface for each diastereomer. It is then seen that steric hindrance to adsorption (by the group Ar') is greater for the *erythro* isomer, which explains the preferential adsorption of the *threo* isomer.

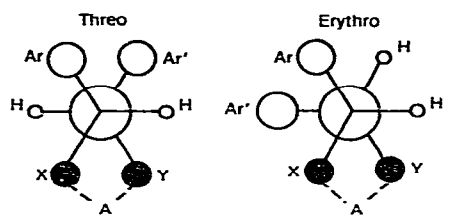
As the alkyl group R increases in bulk, in going from methyl to isopropyl to *n*- and isobutyl and finally to *tert.*-butyl, there will be increasing steric interference between groups X and Y, and eventually the configuration of the adsorbed molecule will be as in Fig. 1b. Assuming that the group X is more strongly adsorbed *vs.* Y, the group Y will be desorbed from the surface for each isomer. It can be argued that the *threo* conformation in Fig. 1b is more crowded than the *erythro*, and therefore the latter isomer will be more strongly retained; *i.e.*, a reversal in predicted retention for compounds with more bulky groups R.

Experimental data reported in ref. 7 can be explained in terms of the above model. That is, the one exception to the retention order *threo* > *erythro* occurs for a diastereomer pair where R is the bulky *tert.*-butyl group. However, two other diastereomer pairs with R = *tert.*-butyl do not show this inversion of retention order. While these latter examples could be rationalized in terms of the structures of the compounds involved, it appears questionable that retention order can be predicted with confidence for diastereomer pairs of similar structure. Therefore it would be helpful to have additional experimental criteria when making predictions of structure (*threo vs. erythro*) on the basis of retention data.

Solvent-solute localization

Previous papers^{1,10-12} have developed a general theory for the effect of mobile

(a)

Adsorption Model—Small Group Y

(b)

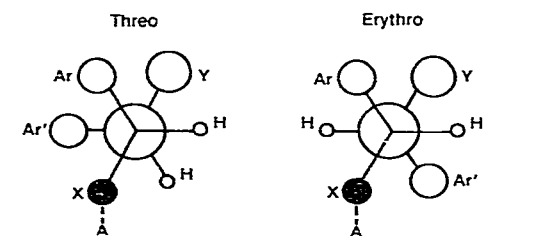
Adsorption Model—Bulky Group Y

Fig. 1. Models for adsorption of *threo* and *erythro* isomers on silica. (a) Polar groups X and Y do not sterically interact; (b) group Y is more bulky and would sterically interact in the configuration of (a).

phase composition on the separation of adjacent bands; *e.g.*, a diastereomeric pair. For a given solute

$$\log(k_1/k_2) = \alpha' A_s (\varepsilon_2 - \varepsilon_1) \quad (1)$$

where k_1 and k_2 are capacity factor values (k') for the solute with mobile phases 1 and 2, α' is an adsorbent activity factor, A_s is the molecular cross-sectional area of the solute, and ε_1 and ε_2 are the solvent strength values (ε^0) for mobile phases 1 and 2.

For binary-solvent mobile phases A–B as used in ref. 7, the ε^0 value of the solvent A–B can be calculated¹¹:

$$\varepsilon^0 = \varepsilon_A + [\log(N_A/\theta_A)]/\alpha' n_b \quad (2)$$

ε_A refers to the ε^0 value of solvent A, N_A is the mole fraction of A in the mixture A–B, and θ_A is the mole fraction of adsorbed A in the stationary phase. θ_A is in turn given by:

$$\theta_A = N_A/[N_A + N_B \cdot 10^{2' n_b (\varepsilon_B - \varepsilon_A)}] \quad (3)$$

Here, N_B is the mole fraction of B in the mixture A-B, and ε_B refers to the ε^0 value of pure B. For polar solvents B with $\varepsilon^0 > 0.4$, ε_B varies with the value of θ_B , where $\theta_B = (1 - \theta_A)$; see also ref. 12.

For the separation of any pair of solutes X and Y, there will generally exist an optimum value of ε^0 for the mobile phase, such that k' values for X and Y (k_x, k_y) fall within an optimum range $2 \leq k' \leq 5$. Separation can be further improved, with ε^0 for the mobile phase held constant, by varying the solvent B in the mobile phase mixture A-B. This leads to change in the separation factor, $\alpha = k_x/k_y$. In ref. 1 it was shown that variation in α with change in mobile phase composition (ε^0 constant) can be expressed as

$$\log \alpha = A + B m \quad (4)$$

where A and B are constants for a given adsorbent and pair of solutes; m is a function of mobile phase composition:

$$m = m^0 f(\theta_B) \quad (5)$$

The parameter m is a measure of the extent to which the mobile phase solvent B localizes onto adsorption sites. The quantity m^0 is the value of m for pure solvent B, and $f(\theta_B)$ is a continuous, single-valued function of the mole fraction, θ_B , of B in the stationary phase. In practice, we can maximize α and separation by varying mobile phase composition so as to vary m .

Eqn. 4 is a consequence of the competition of polar solutes and solvents for localization onto adsorption sites. Increasing localization of the mobile phase (larger values of m) leads to greater competition for localized adsorption, and results in a relative decrease in retention of the more strongly localizing solute molecule. Thus, if X is more strongly localized than Y (because of a more polar group k in the molecule X, or a less sterically hindered group k), the value of the constant A in eqn. 4 will be positive, B will be negative, and α will decrease with increase in m for the mobile phase.

Eqns. 4 and 5 should apply also to solute pairs that are isomeric. In this case, the value of A_x for each solute X and Y will generally be similar, so that eqn. 4 should then apply over a wider range of ε^0 values for a given solute pair. The relative adsorption or retention of two isomers will often be determined by the more favorable adsorption of one or more substituent groups within the molecule. This is the case in Fig. 1a, where group X is more favorably situated for adsorption in the *threo* isomer than in the *erythro* isomer (because of steric hindrance by Ar'). This stronger adsorption of a solute group (X in Fig. 1a) will mean generally greater localization of X in that isomer. Since increasing mobile phase localization (larger m) will preferentially reduce the adsorption of more strongly adsorbing groups X, the differences in retention of the two isomers would then be predicted to be a maximum for smaller values of m . That is, for isomeric solute pairs, A in eqn. 4 should be positive and B should be negative. Furthermore, solvent-solute localization works to reduce the increased retention of group X in the *threo* isomer vs. the *erythro*, so with complete delocalization of X (via competitive B-solvent localization) the retention of the two isomers will approach a similar value (at larger m). The maximum value of m (com-

plete delocalization of X) is about 1.2 for silica as adsorbent, which means that $(\log \alpha)_{m=1.2}$ and the quantity $(A + 1.2B)$ (see eqn. 4) should each equal about zero. That is, for a mobile phase with a maximum value of m , isomer separation will be minimal. Some exceptions to this rule can be expected, as where a change in configuration of the adsorbed isomers occurs when m (or other variable) is varied.

INTERPRETATION OF THE DATA OF REF. 7 IN TERMS OF SOLVENT-SOLUTE LOCALIZATION

Mobile phase properties

Retention data for fifteen diastereomeric pairs of compounds and four mobile phases were reported in Table II of ref. 7. Six compounds of similar structure (Nos. 5–10, with X = –OH and Y = –COOC₄H₉) were selected by me to evaluate the solvent strength, ϵ^0 , and θ_B values for these four mobile phases, as summarized in Table I. Experimental values of ϵ^0 were determined from eqn. 1, assuming $\alpha' = 0.57$ (constant for most wide-pore silicas), estimating a value of A_s (Table 8-4 of ref. 13) equal to 31, and using methylene chloride ($\epsilon^0 = 0.30$) as mobile phase 2. Values of ϵ^0 for the remaining three mobile phases of ref. 7 were averaged for the six solutes chosen (Nos. 5–10). These ϵ^0 values are compared in Table I with values calculated from eqns. 2 and 3. The latter calculation also furnishes the values of θ_B shown in Table I.

TABLE I
PROPERTIES OF MOBILE PHASES USED IN REF. 7

Mobile phase	ϵ_{4B}		θ_B (<i>calc.</i>)	m^{0***}	m	
	<i>Calc.</i> [*]	<i>Exptl.</i> ^{**}			<i>Calc.</i> [‡]	<i>Exptl.</i> ^{§§}
1 Methylene chloride	0.30	(0.30) ^{§§§}	1.00	0.10	0.10	0.08
2 Diethyl ether– methylene chloride (5:95)	0.31	0.36	0.09	0.43	0.11	0.12
3 Acetone– heptane (17:83)	0.36	0.27	0.90	0.87	0.84	0.79
4 Diethyl ether–heptane (33:67)	0.32	0.29	0.91	0.43	0.42	0.45

* Calculated as described in refs. 10 and 12, using eqns. 2 and 3 with following parameter values (ϵ_B^0 , ϵ_B^0/n_b) for each solvent and $\alpha' = 0.57$: methylene chloride (0.30, 0.30, 4.1); diethyl ether (mobile phase 2) (0.49, 0.43, 4.5); diethyl ether (mobile phase 4) (0.78, 0.43, 4.5); acetone (1.00, 0.53, 4.2).

** Calculated from R_F values of ref. 7 as described in text, using eqn. 1; $A_s = 31$, $\alpha' = 0.57$ (solute 5–10 of ref. 7).

*** Values from eqn. 6 and data of ref. 1; m^0 for diethyl ether and acetone on alumina is 0.62 and 1.02, respectively.

‡ Calculated from eqn. 5, with $f(\theta_B)$ given in ref. 1 as a function of θ_B .

§ Calculated as described in Appendix I of ref. 1 (eqn. 8): $m = \left(\sum_{15} \log \alpha - C \right) / D = \left(\sum_{15} \log \alpha - 4.14 \right) / 3.94$

§§§ Experimental value of ϵ^0 for reference mobile phase 2 assumed equal to calculated value.

Values of m for each mobile phase could be calculated, using eqn. 5 with values of θ_B from Table I, and $f(\theta_B)$ taken from ref. 1. The required values of m^0 for the various B-solvents of Table I were generally not available. However, limited data

suggest a correlation between m^0 values for various solvents on silica vs. alumina:

$$m^0(\text{silica}) = -0.25 + 1.1 m^0(\text{alumina}) \quad (6)$$

Since m^0 values for the B-solvents of Table I are available for alumina as adsorbent¹, eqn. 6 allowed the calculation of the m^0 values (silica) shown in Table I.

Verification of eqns. 2, 4 and 5 for data of ref. 7

R_F values reported in ref. 7 for various solute–mobile phase combinations were converted to values of k' via:

$$k' = (1 - R_F)/R_F \quad (7)$$

In the case of mobile phases 3 and 4 of Table I, values of R_F in ref. 7 correspond to a double development of the TLC plate. Reported values of R_F for this case (R_2) can be related to the usual (single development) R_F value as (ref. 13, p. 32):

$$R_F = 1 - (1 - R_2)^{\frac{1}{2}} \quad (7a)$$

Comparison of calculated vs. experimental values of ϵ_{AB} in Table I shows fair agreement (± 0.06 units). Similar comparisons for column high-performance liquid chromatographic (HPLC) data¹² show four-fold better agreement (± 0.016 units). This may be the result of complicating factors in the TLC separations of ref. 7: solvent demixing for the diethyl ether–heptane and acetone–heptane mobile phases, and large solvent–solute delocalization effects for these polar solutes.

The calculated values of m given in Table I can be compared with experimental values derived from the data of ref. 7. Thus, we can sum the $\log \alpha$ values for the fifteen diastereomeric solute pairs from ref. 7, for each of these four mobile phases. From eqn. 4 (see also ref. 1, Appendix I) we then have:

$$\Sigma \log \alpha = \Sigma A + (\Sigma B) m = C + D m \quad (8)$$

Best values of C (4.14) and D (-3.94) were estimated for the solutes of ref. 7, to yield m values in agreement with calculated values (Table I). Finally, comparison of the experimental and calculated m values in Table I shows agreement within ± 0.03 units (1 S.D.), for $0.10 \leq m \leq 0.84$. This confirms the application of eqn. 4 for these solutes of ref. 7, and also verifies eqn. 6 for the B-solvents diethyl ether and acetone. A summary of calculated (eqn. 6) vs. experimental m^0 values (silica) for all solvents studied as mobile phases for both alumina and silica is given below:

<i>B-Solvent</i>	m^0		
	<i>Alumina</i>	<i>Silica</i> <i>(exptl.)</i>	<i>Silica</i> <i>(eqn. 6)</i>
Chloroform	0.34	0.10	0.12
Methylene chloride	0.29	0.10	0.07
Diethyl ether	0.62	0.46	0.43
Acetone	1.02	0.81	0.87
Acetonitrile	1.31	1.19	1.19

Further verification of eqn. 4 for predicting the solvent dependence of α values for these diastereomeric solutes is given in Fig. 2, where $\log \alpha$ for some individual diastereomer pairs is plotted vs. m . Linear plots as predicted by eqn. 4 are generally observed. Table II summarizes data from similar plots for all fifteen diastereomer pairs from ref. 7. These latter data are organized according to the size of the group R attached to the ester group Y of the solute pair.

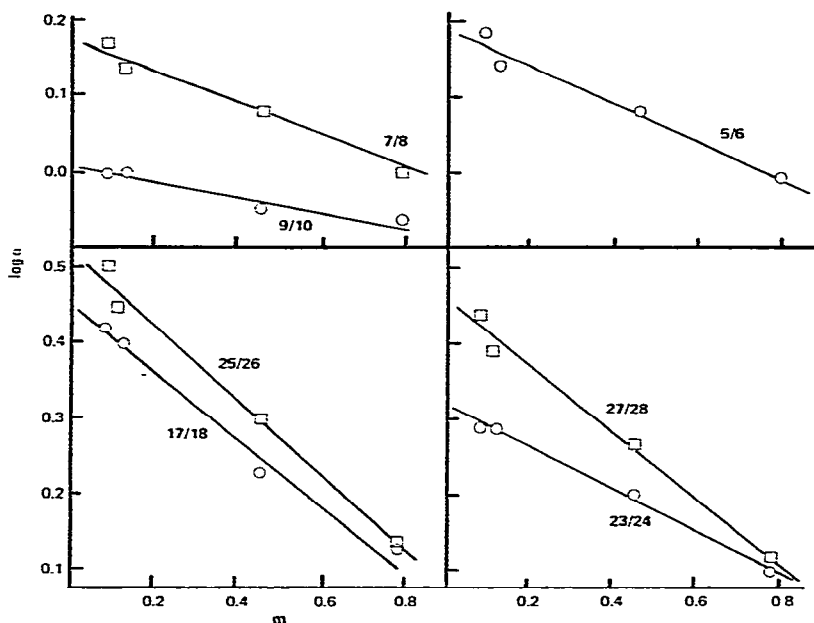


Fig. 2. Correlation of solvent selectivity for diastereomer pairs of ref. 7 in terms of eqn. 4. Numbers on lines refer to solute numbers from Table II of ref. 7.

The overall standard deviation of the correlation of the data of ref. 7 with eqn. 4 is ± 0.027 log units. Two values of R_F are comprised in each measurement of α , so the implied agreement of values of $\log k'$ with eqn. 4 is $\pm 0.027/\sqrt{2} = \pm 0.02$ (1 S.D.). This in turn suggests agreement of R_F values with eqn. 4 within $\pm 0.01 R_F$ units or better (eqn. 7). Thus, eqn. 4 describes the experimental data of ref. 7 within the probable experimental error of these data.

Further analysis of the data of ref. 7

The discussion in the Theoretical section suggests for isomeric pairs as in Table II that the coefficient A should be positive and the coefficient B negative (eqn. 4). This is seen for values of A and B in Table II to be true in every case. This relationship has important practical consequences.

First, the maximum value of α is determined (eqn. 4) by the range in values of m that can be obtained by varying mobile phase composition. The range in m^0 values (alumina as adsorbent) for slightly polar to polar B-solvents is about 0.2–1.3¹, so the corresponding range in m^0 values for silica (eqn. 6) is roughly 0.0–1.2. From eqn. 5

TABLE II
SOLVENT-SOLUTE LOCALIZATION OF DIASTEREOMER PAIRS

Fit of eqn. 4 to data of ref. 7 as in Fig. 2.

Solute pair*	Fit to eqn. 4**			$(A + 1.2B)$	Av. value of A	$\log k_{im}^{***}$
	A	B	S.D.			
<i>R = tert.-butyl</i>						
9/10	0.01	-0.11	± 0.01	-0.12	0.06 \pm 0.09	0.25
13,14	0.16	-0.14	± 0.02	-0.01		-0.21
29/30	0.00	-0.07	± 0.05	-0.09		-0.87
<i>R = isopropyl, n-butyl or isobutyl</i>						
3-4	0.20	-0.23	± 0.03	-0.07	0.18 \pm 0.03	0.39
5-6	0.19	-0.25	± 0.02	-0.11		0.33
7-8	0.17	-0.21	± 0.01	-0.08		0.31
11-12	0.04	-0.05	± 0.02	-0.02		-0.14
<i>R = methyl</i>						
1,2	0.40	-0.37	± 0.01	-0.04	0.40 \pm 0.08	0.58
15,16	0.32	-0.28	± 0.03	-0.02		0.35
17-18	0.45	-0.44	± 0.02	-0.08		0.45
19/20	0.44	-0.46	± 0.06	-0.07		0.58
21,22	0.32	-0.26	± 0.03	0.01		0.79
23,24	0.31	-0.27	± 0.01	-0.01		0.39
25,26	0.53	-0.51	± 0.01	-0.07		0.53
27,28	0.46	-0.43	± 0.01	-0.06		0.83

* Numbers refer to designations in Table II of ref. 7.

** A and B refer to least-squares fit to eqn. 4; S.D. refers to standard deviation of fit.

*** k_{im} is the k' value of the *threo* isomer, with methylene chloride as mobile phase.

this also corresponds to the maximum range in values of m for silica. The form of eqn. 4 is such that the largest separation factor α will result either for minimum or maximum values of m , which then means (for $0 \leq m \leq 1.2$) that this maximum value of $\log \alpha$ must equal either A (for $m = 0$) or $|A + 1.2B|$ (for $m = 1.2$). Values of A and $(A + 1.2B)$ for each diastereomer pair or ref. 7 are listed in Table II. With the exception of the diastereomer pairs 9/10 and 29/30, A is always larger than $|A + 1.2B|$, which means that maximum separation occurs for minimum m ($m = 0.0$) in thirteen cases out of fifteen. That is, mobile phases of low m are generally favored for the separation of these isomers, as predicted by theory. However, it should also be noted in Table I that exceptions to this rule occur for solute pairs that are generally the most difficult to separate (smallest values of α).

Second, consider the experimental conditions that favor the preferential retention of the *threo* isomer. Such conditions also allow the more accurate prediction of diastereomer configuration on the basis of observed k' or R_F values. Values of A correspond to $[\log k'(\textit{threo}) - \log k'(\textit{erythro})]$, for the case of $m = 0$. It is seen that A is positive for each of the fifteen diastereomer pairs in Table II, which means that when $m = 0$ the *threo* isomer was more strongly retained in every case (except for solute pairs 9/10 and 29/30), where retention of the two isomers is equal). While most of the compounds of Table II will not elute with convenient k' values when mobile

phases of very small m are used (ϵ^0 values are then too small), extrapolated values of $\log \alpha$ vs. m for several mobile phases of differing m allow evaluation of A , which is equivalent to a value of $\log \alpha$ for $m = 0$. Therefore, for maximum confidence in assigning diastereomer conformation—or the structures of other isomers—either a low- m -value mobile phase should be used, or an extrapolated value of A should be obtained as in Fig. 2.

Third, consider how the role of the R group in the compounds of ref. 7 affects preferential retention of the *threo* isomer. We have argued above that the value of A is the best measure of the effect of solute structure on relative retention. Average values of A are given in Table II for each group of compounds where R is the same or of similar size. Thus, the average value of A for compounds where R is *tert.*-butyl is 0.06 ± 0.09 (i.e., zero within statistical significance). Similarly, for R groups of intermediate size (iso-C₃, iso-C₄, *n*-C₄), the average value of A is 0.18 ± 0.03 . For R = methyl, A is 0.40 ± 0.08 . Thus, the effect of the size of the group R on relative retention is consistent throughout this series of compounds, within the probable error in values of A . There is no need as in ref. 7 to invoke special effects to explain minor changes in α and occasional reversals of retention order within the three groups of Table II.

Palamareva *et al.*¹⁴ have more recently reported retention data for diastereomers of similar structure as in ref. 7, where the group R is the equally bulky menthyl radical. Here, the *erythro* isomer is consistently more strongly adsorbed. This accords with these authors proposal that a change in retention configuration (Fig. 1b vs. 1a) occurs after the R group reaches a certain size. My interpretation of the roughly zero values of A for the diastereomers with R = *tert.*-butyl is that for these compounds the retention configuration cannot be specified on the basis of the data of ref. 7, whereas the configuration of Fig. 1a applies for compounds with R smaller than *tert.*-butyl, and the configuration of Fig. 1b applies for R larger than *tert.*-butyl.

Fourth, according to the discussion of ref. 1, the parameter B of eqn. 4 is expected to be small or zero for isomer pairs in which solute localization does not occur, and to increase in magnitude as solute localization increases. Since solute localization is a function of the adsorption energy of the polar group(s) in the solute molecule, solute localization should tend to increase with increase in the adsorption energy and retention of the solute molecule. This increase in B with increasing k' value for a series of related compounds will be most obvious when the solutes of interest are structurally related—as in the case of the present X,Y-diarylethane derivatives. The retention of the diastereomers of ref. 7 can be approximated by $\log k'$ of the *threo* isomer for a given mobile phase, preferably one with small m value. I have chosen methylene chloride here, and define the resulting k' value for each diastereomer pair as k_{tm} ; values of $\log k_{tm}$ are given in Table II. Finally, the theory of solvent-solute localization¹ predicts that values of B should be near-zero for small values of $\log k_{tm}$ (where solute localization is minimal), and decrease for larger k_{tm} values (as solute localization becomes increasingly more important). This hypothesis is tested for average values of B and different ranges in $\log k_{tm}$:

$\log k_{tm}$	B (av.)
<0	-0.09 ± 0.05
0-0.5	-0.26 ± 0.10
>0.5	-0.41 ± 0.10

The predicted trend in values of B vs. solute retention is observed. It appears that localization of diastereomer solutes is minimal when $k_{tm} < 1.0$, corresponding to $R_F > 0.5$ for the *threo* isomer with methylene chloride as mobile phase.

Last, the present treatment and its application to the data of ref. 7 have been somewhat simplified. Thus, hydrogen-bonding between these hydroxylic compounds and the basic B-solvents used in ref. 7 would be expected to contribute further to the relative retention of these diastereomers¹³. However, there is a tendency for the cancellation of such effects among isomers of the same compound, except where the extent of hydrogen-bonding is obviously different among the various isomers (e.g., ref. 15). Furthermore, solvent-solute localization is usually of primary importance in determining solvent selectivity, and its effects will generally determine relative retention, even in the presence of solvent-solute hydrogen-bonding.

The use of A_s or n values in the interpretation of retention mechanism

The value of A_s for a solute molecule is a function of its configuration in the adsorbed state (ref. 13, p. 54; refs. 8, 16, 17). For example, the cross-sectional area will generally be smaller for a solute molecule adsorbed in a vertical as opposed to flat configuration. Likewise, for the case of solutes with polar substituents X and Y (as in Fig. 1) adsorbed onto silica, A_s is largely determined by the number of these groups which can interact with the adsorbent surface. In the latter case (see ref. 8) we have to a good approximation

$$\log k' = \log k_B - n \log X_B \quad (9)$$

for the variation of k' as a function of the mole fraction X_B of a polar solvent B in a binary mixture with a non-polar solvent A. Here, k_B refers to k' for pure B as mobile phase, and n is the ratio of A_s values for solute vs. solvent molecules. The parameter n is also *approximately* equal to the number of *strongly* polar groups (X, Y, etc.) in the solute molecule which can interact with the adsorbent surface (see ref. 8).

Palamareva *et al.*⁷ have attempted to define the configuration of these adsorbed diastereomers by determining the n values (eqn. 9) for diethyl ether-heptane mobile phases. They observe that n decreases with increasing bulkiness of the R group, suggesting that the configuration of these isomers is shifting from two-point to one-point attachment (Fig. 1b vs. 1a), as is reasonable from steric considerations. This conclusion would be more convincing, however, if the actual n -values observed were to cluster around values of either 1.0 or 2.0. In most cases, the n values reported in ref. 7 have values intermediate between 1.0 and 2.0. My analysis of the data of Table II and the n values reported in ref. 7 suggests a continuous decrease in the relative retention of these compounds as R becomes more bulky. This in turn means a decrease in the adsorption energy of the ester group, which should result in a decrease in its contribution to A_s and n ; on silica the apparent A_s value of a polar solute molecule is larger than the cross-sectional area, in proportion to the adsorption energy or polarity of individual groups within the solute molecule. Therefore, the concomitant reduction in n with increase in the size of R can be interpreted in terms of this effect, with no need to assume a change in the configuration of adsorbed solute molecules. *These observations also suggest that using A_s or n values to decide adsorbate configuration (especially on silica) must be done cautiously.* The basic problem is that

as the polarity of solute groups decreases (and/or R increases in bulk), the contribution of the remainder of the solute molecule to A_s becomes relatively more important. That is, eqn. 9 with n equal to the number of polar solute groups (that are adsorbed) only applies to a limiting case: retention on silica from a binary solvent A-B where B is quite polar, where the solute groups X, Y, ... are also quite polar, and where $\theta_B \approx 1.0$.

CONCLUSIONS

The data of Palamareva *et al.*⁷ provide a useful example of the effects of solute structure and mobile phase composition on the separation of certain isomeric compounds. Elaboration of the theory of ref. 1, as in the present paper, provides a reasonable interpretation of these data, and allows the quantitative prediction of the effects of mobile phase composition on the relative retention and separation of these diastereomers.

The present study further suggests that isomer separations in general will be favored by the use of mobile phases that exhibit little or no localization of the B-solvent. Predictions of isomer structure (*e.g.*, *threo* vs. *erythro*) on the basis of relative retention is likewise favored by the use of non-localizing mobile phases and/or the extrapolation of relative retention data to $m = 0$ (no localization of the mobile phase).

GLOSSARY

A, B	constants in eqn. 4 for a particular pair of solutes and given LSC system
A_s	solute molecular cross-sectional area
C, D	constants in eqn. 8 for a given set of solute pairs and various LSC systems (different mobile phases)
$f(\theta_B)$	a function of θ_B defined in ref. 10; eqn. 5
k'	solute capacity factor
k_1, k_2	values of k' for given solute in mobile phases 1 and 2
k_x, k_y	values of k' for solutes X and Y, in a given LSC system
k_{tm}	value of k' for a <i>threo</i> isomer with methylene chloride as mobile phase
$(\log \alpha)_{\bar{m}=1.2}$	value of $\log \alpha$ calculated from eqn. 4 for $m = 1.2$
m	mobile phase localization function; eqn. 4
m^0	value of m for pure solvent ($\theta_B = 1.0$); eqn. 5
n	constant in eqn. 9 for given solute and mobile phase A-B where X_B is varied
n_b	molecular cross-sectional area of mobile phase molecule B (for mixture A-B, where B is more polar than A)
N_A, N_B	mole fractions of A and B in mobile phase A-B
R_F	TLC solute migration parameter; eqn. 7
R_2	observed value of R_F for a TLC system where mobile phase development has been carried out twice; eqn. 7a
α	separation factor, equal to k_x/k_y
α'	adsorbent activity parameter, equal to 0.57 for wide-pore silica

ε^0	solvent strength parameter
$\varepsilon_1, \varepsilon_2$	values of ε^0 for mobile phases 1 and 2; eqn. 1
$\varepsilon_A, \varepsilon_B$	values of ε^0 for pure solvents A and B
$\varepsilon'_B, \varepsilon''_B$	values of ε_B for θ_B equal 0.0 and 1.0, respectively; see ref. 10
θ_A, θ_B	mole fractions of solvents A and B in adsorbed phase

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