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A DENSITOMETRIC STUDY OF CO-ELUTION IN THIN-LAYER CHROMATOGRAPHY, AND ITS PHYSICOCHEMICAL MODELING

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SUMMARY

In our earlier efforts to scrutinize lateral interactions among test analyte molecules, and their impact on the shape of the respective concentration profiles of thin-layer chromatographic bands, we focused our attention on single analytes able to self-associate by hydrogen bonding of their functional groups (and primarily on alcohols and carboxylic acids). Distinct demonstration of lateral interactions is possible only when the analyte is applied to a stationary phase in an amount within the non-linear region of its adsorption isotherm. One must, moreover, ensure the chromatographic conditions are relatively mild, i.e. a low-activity adsorbent used as stationary phase and a low-polarity mobile phase. Such conditions guarantee that lateral interactions among the analyte molecules are to a large extent unaffected and therefore observable by densitometry.

In the TLC study reported in this paper we focused on co-elution of a binary mixture of test analytes. For the purpose of our experiment we chose two such mixtures: (i) a carboxylic acid (2-phenylbutyric acid, which is, simultaneously, a Lewis acid and a Lewis base (AB)) and a ketone (benzophenone, which is a Lewis base (B) only); and (ii) an aliphatic alcohol (5-phenylpentanol, which is, simultaneously, a Lewis acid and Lewis base (AB)) and the same ketone (i.e. benzophenone). Because of their functionality, these two pairs of compounds can form mixed associative structures by hydrogen bonding. It is worthy of note that the retention (i.e. R_F values) of each of the two analytes from a given pair is perceptibly different if each is chromatographed as a single species. Using mild chromatographic conditions and working in the non-linear region of the adsorption isotherm we managed to (i) demonstrate co-elution of the two analytes from each

pair in the form of a single chromatographic band; (ii) suggest (optional or complementary) three different physicochemical explanations of their densitometrically measured concentration profiles; and (iii) perform semi-quantitative simulations of these profiles.

Most of the results discussed were obtained by planar chromatography (TLC) but some experiments were repeated with a column-chromatographic technique, HPLC, using fully analogous working conditions (i.e. low-activity carbohydrate stationary phase and low-polarity hydrocarbon mobile phase). Some of the results obtained by HPLC were similar to those from planar chromatography whereas others were dissimilar.

INTRODUCTION

Practical applications of thin-layer chromatography (TLC) in both its analytical and preparative mode tend to be conducted under optimum conditions, which are usually selected by trial-and-error (although sometimes with the aid of the simplex method or other chemometric methods) [1–4]. Unsuccessful attempts are usually discarded without too much reflection, and more specifically without reflecting on the origins of the failure, understood as a failure to achieve full separation of the components of a given mixture of compounds. Occasionally an excuse for an unwanted chromatographic result is provided in physicochemical terms, and sporadically on a molecular level also, even if this explanation is a pure guess only.

Thorough explanation of lack of separation success under experimental conditions which are relatively close to acceptable (if not optimum) is often difficult, because the drawbacks are usually not very strongly pronounced and hence quite difficult to assess.

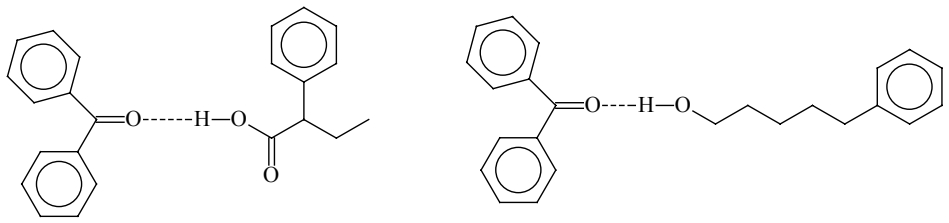
Within the framework of our long-term studies devoted to tracing lateral interactions in liquid chromatographic systems and to demonstrating their measurable impact on the overall chromatographic process, we have assumed a strategy of employing very specific stationary and mobile phases which ensure at least partial preservation of lateral interactions [5–10], and consequently their sufficiently distinct demonstration also. Such systems inevitably embrace low-activity adsorbents and low-polarity (preferably monocomponent) mobile phases. Our favourite systems usually comprise cellulose as stationary phase and a single hydrocarbon (e.g., *n*-hexane, *n*-octane, decalin, etc.) as mobile phase.

In our earlier work [5–10] we focused our major attention on the impact of lateral interactions among molecules of a single analyte (which can also be described as self-association by hydrogen bonding) on the concentration profile of the chromatographic band. These studies focused mostly on selected alcohols, and mono- and dicarboxylic acids. Usually the shape of the concentration profile of a band obtained in a non-linear region of the isotherm could be explained and, ultimately, even simulated with aid of the anti-Langmuir-type adsorption isotherm.

In the work discussed in this paper thorough attention was paid to elution of two binary mixtures of test solutes (one comprising 2-phenylbutyric acid and benzophenone, the other 5-phenylpentanol and benzophenone) under the customary, i.e. deliberately mild, working conditions (microcrystalline cellulose was used as adsorbent and either decalin or *n*-octane as monocomponent mobile phase). One of the test solutes in each of the two binary mixtures (2-phenylbutyric acid or 5-phenylpentanol) can be viewed as having the double properties of Lewis acidity and Lewis basicity (AB) whereas the other component of both mixtures (benzophenone) is a typical Lewis base (B) only. Thus 2-phenylbutyric acid and 5-phenylpentanol both tend to self-associate by hydrogen bonding and to form mixed associates either with a Lewis base or a Lewis acid. In contrast, benzophenone cannot self-associate by hydrogen bonding, but it can participate in mixed associates with a Lewis acid (in our work with either the carboxylic acid or the aliphatic alcohol).

In our study we managed to demonstrate lack of success in the separation of binary mixtures either of 2-phenylbutyric acid and benzophenone or of 5-phenylpentanol and benzophenone under these chromatographic conditions, because of preservation of the hydrogen bonds between the two different species (as shown in Fig. 1). In the other words, we witnessed the phenomenon of co-elution. Although for both mixtures the retention of the two compounds is measurably different when developed separately (i.e. for most of the examples studied $\Delta R_F \geq 0.10$), in a mixture they remain inseparable. Moreover, the retention (i.e. R_F) of the chromatographic band of the co-eluting mixture tended to be lower than that of either of the two individual test solutes developed separately (this was always so for the acid–ketone mixture and sporadically so for the alcohol–ketone mixture) or at least lower than that of the individual test solute migrating faster (almost always for the alcohol–ketone mixture). This striking effect was interpreted in three different (optional or complementary) ways in semi-quantitative physicochemical terms, ultimately resulting in simulation of

the concentration profiles of the chromatographic bands of the co-eluting mixture of analytes.



(a) (b)

Fig. 1

Schematic representation of mixed associative complexes of (a) molecules of 2-phenylbutyric acid and benzophenone, and (b) molecules of 5-phenylpentanol and benzophenone, kept together by hydrogen bonding

EXPERIMENTAL

Binary Mixtures of 2-Phenylbutyric Acid and Benzophenone (TLC)

This part of the chromatographic experiment was performed with three test solutions: (i) monocomponent solutions of 2-phenylbutyric acid in carbon tetrachloride (concentrations 0.75, 1.00, and 1.25 mol L^{-1}); (ii) a monocomponent solution of benzophenone in carbon tetrachloride (concentration 0.10 mol L^{-1}); and (iii) binary solutions of 2-phenylbutyric acid and benzophenone in carbon tetrachloride (concentrations of the acid 0.75, 1.00, and 1.25 mol L^{-1} and concentration of the ketone always 0.10 mol L^{-1}). The stationary phase was microcrystalline cellulose (pre-coated glass TLC plates from Merck, Darmstadt, Germany; #1.05730) and the mobile phase was decalin. The volumes applied to the plates (irrespective of concentration) were 1 μL. Development was performed in the ascending mode in a Stahl-type open-space chromatographic chamber. The mobile-phase front migration distance was 15 cm.

All thin-layer chromatograms were developed in the two ways which differed in the manner of application of the samples to the adsorbent layer. The first procedure was the classical one, with the carbon tetrachloride being removed (with a hairdryer) from the origin before chromatography. The second procedure was development of the chromatograms without evaporation of the solvent from the origin. By comparing the results obtained we intended to demonstrate the impact of dissolving single analytes

and analyte mixtures on the relative velocity of their migration (expressed as R_F values).

Binary Mixtures of 5-Phenylpentanol and Benzophenone (TLC)

These chromatographic experiments were performed on three types of test solution: (i) monocomponent solutions of 5-phenylpentanol in carbon tetrachloride (concentrations 1.00, 1.50, and 2.00 mol L^{-1}); (ii) a monocomponent solution of benzophenone in carbon tetrachloride (concentration 0.10 mol L^{-1}); and (iii) binary mixtures of 5-phenylpentanol and benzophenone in carbon tetrachloride (concentrations of the alcohol 1.00, 1.50, and 2.00 mol L^{-1} and concentration of ketone always 0.10 mol L^{-1}). The stationary phase was again microcrystalline cellulose (as indicated above) and the mobile phase was *n*-octane. Again, the volumes applied to the plates (irrespective of concentration) were 1 μL, development was performed in ascending mode in a Stahl-type chamber, and the mobile-phase front migration distance was 15 cm.

All thin-layer chromatograms were again developed with and without drying the samples applied to the adsorbent, again to demonstrate the impact of dissolving single analytes and analyte mixtures on the relative velocity of their migration (expressed as R_F values).

Binary Mixtures of 5-Phenylpentanol and Benzophenone (HPLC)

For purposes of comparison we performed a brief experiment with high-performance liquid chromatography (HPLC) analogous to one originally performed by TLC. Again the experiment was performed with three test solutions: (i) monocomponent solutions of 5-phenylpentanol in *n*-octane (concentrations 0.40 and 2.00 mol L^{-1}); (ii) monocomponent solutions of benzophenone in *n*-octane (concentrations 0.02 and 0.10 mol L^{-1}); and (iii) solutions of binary mixtures of 5-phenylpentanol and benzophenone in *n*-octane: (a) concentration of alcohol 2.00 mol L^{-1}, that of the ketone 0.10 mol L^{-1}; (b) concentration of alcohol 0.40 mol L^{-1}, that of the ketone 0.02 mol L^{-1}).

Densitometric Evaluation of the Chromatographic Concentration Profiles (TLC)

All the thin-layer chromatograms obtained in this study were evaluated by means of densitometry. Densitograms were acquired with the Desaga (Heidelberg, Germany) model CD 60 densitometer equipped with Windows-compatible ProQuant software. Concentration profiles were re-

corded in UV light (in reflectance mode) at 260 nm; the dimensions of the rectangular light beam were 0.02 mm × 0.4 mm. The densitograms obtained were relatively smooth and therefore needed no extra smoothing.

High-Performance Liquid Chromatography (HPLC)

HPLC was performed with a Merck–Hitachi model L-7100 La Chrom pump, a Merck–Hitachi model L-7455 La Chrom diode-array detector (DAD), a Merck–Hitachi model D-7000 La Chrom interface, a Merck–Hitachi model L-7350 column oven, a Merck model L-7612 solvent degasser, a 20-μL injection loop, and a 150 mm × 4.6 mm, average particle diameter 5 μm, Chiralcel® OB-H cellulose tribenzoate column (Daicel Chemical Industries, Chiral Technologies Europe, Illkirch-Cedex, France). Cellulose tribenzoate is somewhat less active than microcrystalline cellulose, because the three hydroxyl groups in its structural unit are esterified with benzoic acid (Fig. 2). The mobile phase (*n*-octane) flow rate was 1 mL min^{-1}, the absorbance was measured at 270 nm, and the column temperature was 20°C. Elution was performed in isocratic mode.

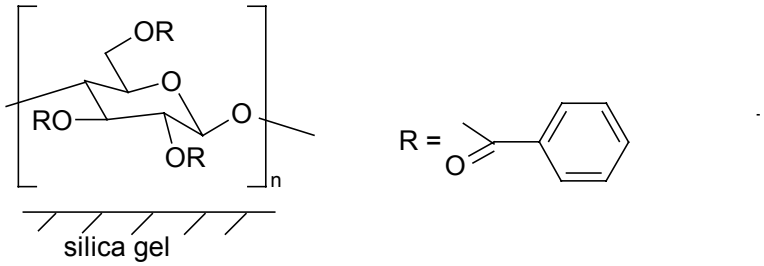


Fig. 2

Schematic diagram of the cellulose tribenzoate structural unit

RESULTS AND DISCUSSION

The numerical values of the R_F coefficients of the test analytes developed with and without drying of the starting spots, observed for the individual species and for binary mixtures, are given in Tables I and II. It is worthy of note that all the R_F values presented in this paper were calculated taking into the account the maxima of the bands' concentration profiles as their central points and, consequently, measuring the distance between the origin and the maximum of a given band ($R_{F(max)}$). Alternatively, all the

R_F values were determined from the centre of gravity of the distribution of analyte mass in the band ($R_{F(int)}$) [5,6]. Carefully selected densitograms, which are representative of all of the experimental results obtained, are shown in Figs 3–6.

Table I

Numerical values of the $R_{F(max)}$ and $R_{F(int)}$ coefficients for 2-phenylbutyric acid and benzophenone chromatographed as individual species and as a binary mixture (the test samples were developed with and without drying the starting spots). The stationary phase was microcrystalline cellulose and the mobile phase was decalin. The sample volume applied was 1 μL

Sample	Concn (mol L^{-1})	With drying		Without drying	
		$R_{F(max)}$	$R_{F(int)}$	$R_{F(max)}$	$R_{F(int)}$
Benzophenone	0.10	0.95	0.94	0.95	0.94
2-Phenylbutyric acid	0.75	0.82	0.88	0.87	0.86
	1.00	0.81	0.83	0.85	0.85
	1.25	0.81	0.82	0.84	0.85
Binary mixture (2-phenylbutyric acid/benzophenone)	0.75/0.10	0.81	0.87	0.86	0.85
	1.00/0.10	0.79	0.82	0.84	0.85
	1.25/0.10	0.79	0.81	0.84	0.85

Table II

Numerical values of the $R_{F(max)}$ and $R_{F(int)}$ coefficients for 5-phenylpentanol and benzophenone chromatographed as individual species and as a binary mixture (the test samples were developed with and without drying the starting spots). The stationary phase was microcrystalline cellulose and the mobile phase was *n*-octane. The sample volume applied was 1 μL

Sample	Concn (mol L^{-1})	With drying		Without drying	
		$R_{F(max)}$	$R_{F(int)}$	$R_{F(max)}$	$R_{F(int)}$
Benzophenone	0.10	0.97	0.94	0.99	0.96
5-Phenylpentanol	1.00	0.86	0.88	0.89	0.89
	1.50	0.86	0.85	0.88	0.88
	2.00	0.75	0.82	0.78	0.81
Binary mixture (5-phenylpentanol/benzophenone)	1.00/0.10	0.88	0.87	0.92	0.94
	1.50/0.10	0.81	0.79	0.87	0.88
	2.00/0.10	0.81	0.78	0.83	0.85

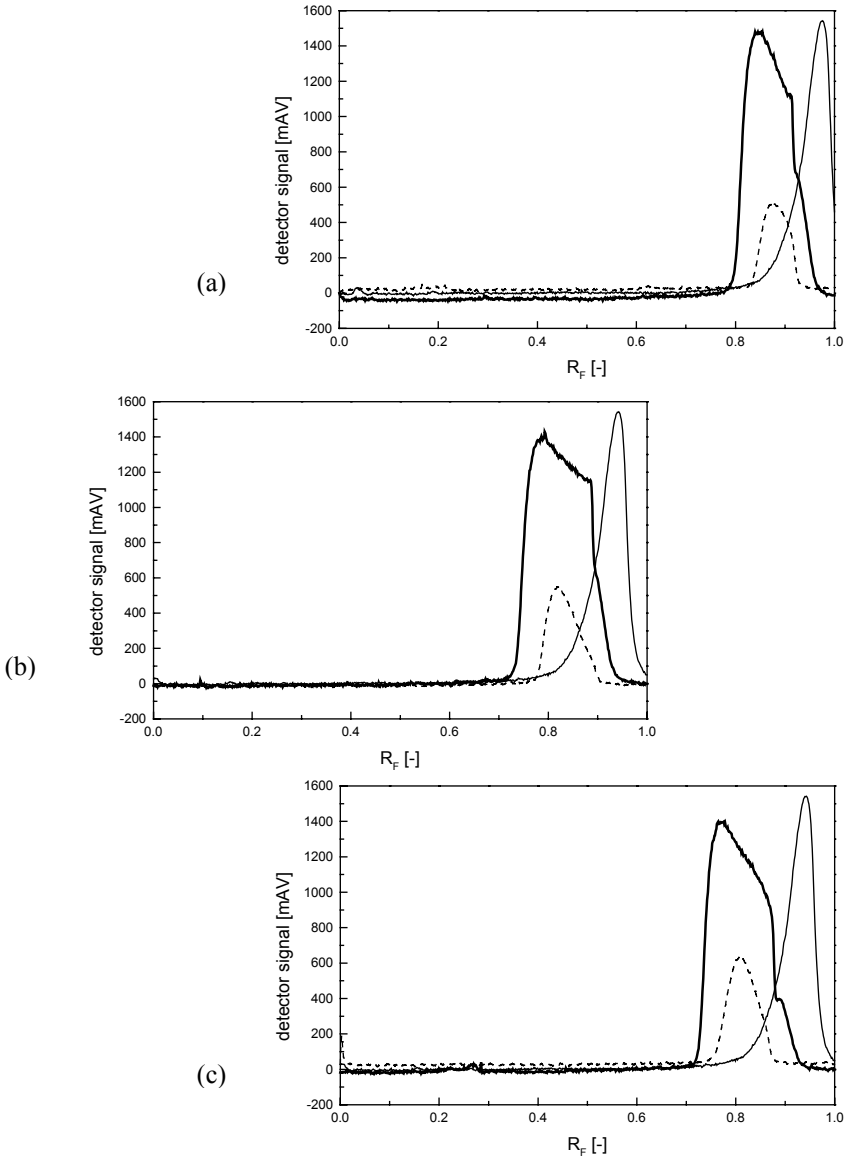


Fig. 3

Comparison of the concentration profiles of 2-phenylbutyric acid (dashed line) and benzophenone (thin solid line) developed as single analytes and as a binary mixture (bold solid line), with drying of the sample after application to the plate, showing their dependence on the concentration of 2-phenylbutyric acid in the sample: (a) 0.75 mol L^{-1}; (b) 1.00 mol L^{-1}; and (c) 1.25 mol L^{-1} (the concentration of benzophenone was always 0.10 mol L^{-1}). Microcrystalline cellulose was used as stationary phase and decalin as mobile phase. The sample volume applied to the plate was always 1 µL. The mean velocity of decalin migration was 0.74 mm min^{-1}

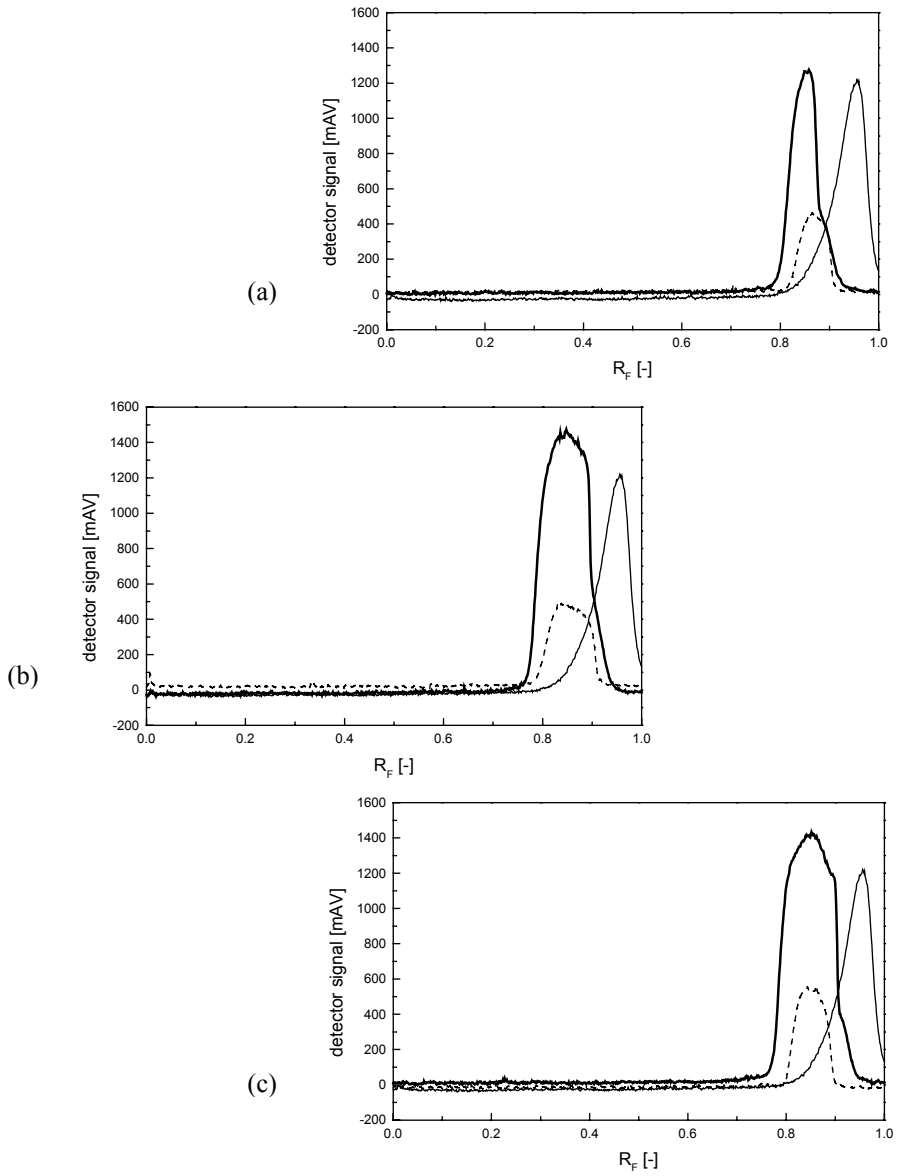


Fig. 4

Comparison of the concentration profiles of 2-phenylbutyric acid (dashed line) and benzophenone (thin solid line) developed as single analytes and as a binary mixture (bold solid line), without drying of the sample after application to the plate, showing their dependence on the concentration of 2-phenylbutyric acid in the sample: (a) 0.75 mol L^{-1}; (b) 1.00 mol L^{-1}; and (c) 1.25 mol L^{-1} (the concentration of benzophenone was always 0.10 mol L^{-1}). Microcrystalline cellulose was used as stationary phase and decalin as mobile phase. The sample volume applied to the plate was always 1 µL. The mean velocity of decalin migration was 0.74 mm min^{-1}

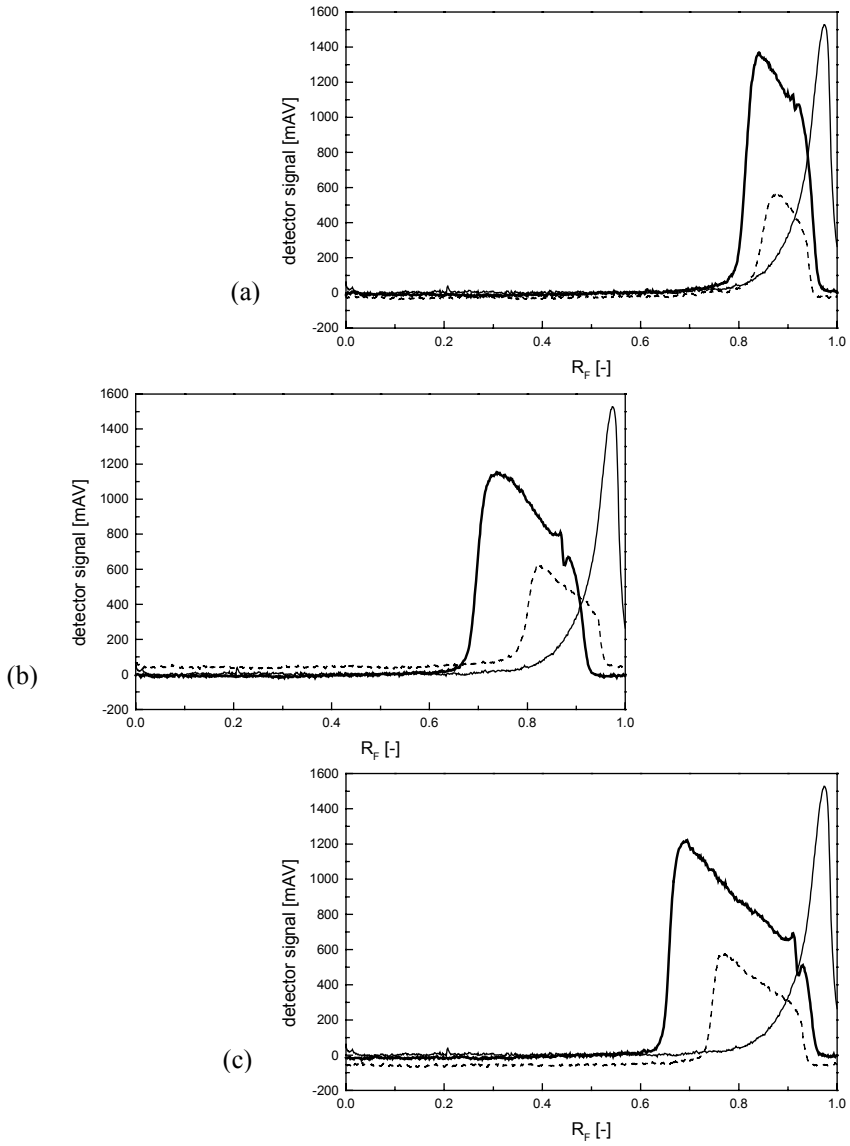


Fig. 5

Comparison of the concentration profiles of 5-phenylpentanol (dashed line) and benzophenone (thin solid line) developed as single analytes and as a binary mixture (bold solid line), with drying of the sample after application to the plate, showing their dependence on the concentration of 5-phenylpentanol in the sample: (a) 1.00 mol L^{-1}; (b) 1.50 mol L^{-1}; and (c) 2.00 mol L^{-1} (the concentration of benzophenone was always 0.10 mol L^{-1}). Microcrystalline cellulose was used as stationary phase and *n*-octane as mobile phase. The sample volume applied to the plate was always 1 µL. The mean velocity of *n*-octane migration was 2.06 mm min^{-1}

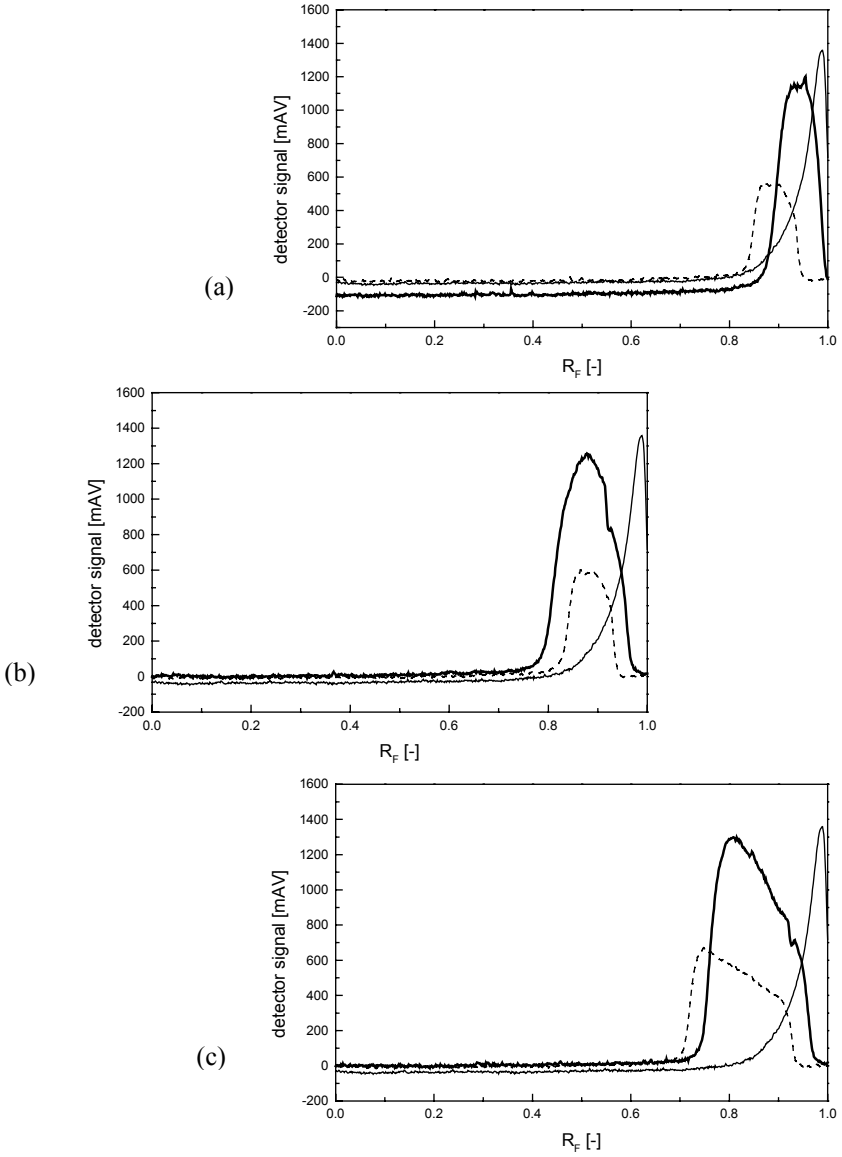


Fig. 6

Comparison of the concentration profiles of 5-phenylpentanol (dashed line) and benzophenone (thin solid line) developed as single analytes and as a binary mixture (bold solid line), without drying of the sample after application to the plate, showing their dependence on the concentration of 5-phenylpentanol in the sample: (a) 1.00 mol L^{-1}; (b) 1.50 mol L^{-1}; and (c) 2.00 mol L^{-1} (the concentration of benzophenone was always 0.10 mol L^{-1}). Microcrystalline cellulose was used as stationary phase and *n*-octane as mobile phase. The sample volume applied to the plate was always 1 µL. The mean velocity of *n*-octane migration was 2.06 mm min^{-1}

Analogous experimental results obtained by means of HPLC are presented as chromatograms of the investigated test analytes (injected as single species and in binary mixtures) in Fig. 7.

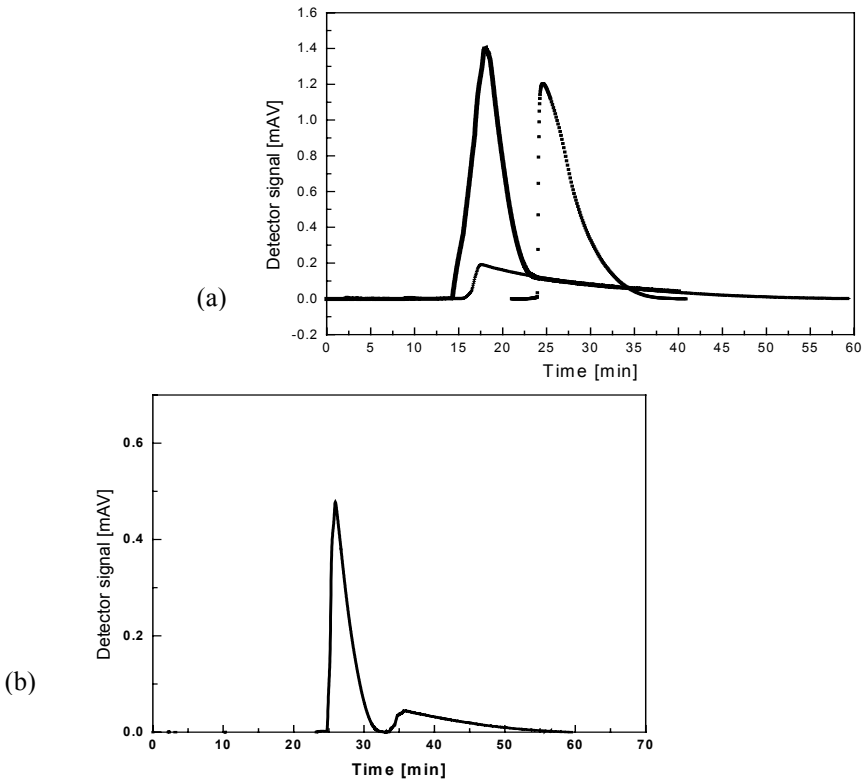


Fig. 7

(a) Comparison of HPLC chromatograms obtained from 5-phenylpentanol (×) and benzophenone (▪) developed as single analytes and as a binary mixture (solid line). The concentrations of the single components were 2.00 mol L^{-1} for 5-phenylpentanol and 0.10 mol L^{-1} for benzophenone; the same concentrations were used in the unresolved binary mixture. (b) The concentrations of the single components were 0.40 mol L^{-1} for 5-phenylpentanol and 0.02 mol L^{-1} for benzophenone (solid line). For the single analytes at the lower concentrations full separation was achieved. All HPLC chromatograms were obtained on Chiralcel® OB-H (i.e. cellulose tribenzoate) as stationary phase with *n*-octane as mobile phase; the sample volume was 20 μL. The detector signal was obtained at 270 nm.

The Impact of Drying the Starting Spots (TLC)

The impact of drying the starting spots before beginning TLC is readily apparent from the results given in Tables I and II – R_F values are sub-

stantially lower for dried spots than for the wet. This regularity is equally valid for individual analytes and for their binary mixtures. The only exception is benzophenone developed as an individual analyte; for this compound the R_F values are the same (0.95 with decalin as mobile phase) irrespective of sampling mode, most probably because the ketone – even with the starting spot dried – migrated very close to the mobile phase front (and the wet spot could hardly migrate any further). In other words, there is practically no doubt that the time-consuming process of dissolving the analytes at the starting point usually contributes measurably to the overall retardation of their migration.

Co-elution of 2-Phenylbutyric Acid and Benzophenone (TLC)

It is apparent from the results presented in Table I and in Figs 3 and 4 that the amounts of 2-phenylbutyric acid and benzophenone developed as individual analytes were within the non-linear region of the adsorption isotherm. This is readily deduced from the asymmetric concentration profiles of the acid and ketone. With pure acid (which tends to participate in lateral interactions), we observe concentration profiles giving proof of an anti-Langmuir-type adsorption isotherm, typical of such cases. For pure benzophenone (which does not tend to participate in lateral interactions), we observe concentration profiles indicative of a Langmuir-type adsorption isotherm, which for this compound is to be expected. The numerical values of the retardation factor (R_F) of the acid and the ketone are quite different, with the difference falling within the range $\Delta R_F \approx 0.08–0.14$, irrespective of whether the analyte samples were developed with or without drying. Despite such evident differences between ΔR_F values the mixture of the two analytes cannot be separated under the chromatographic conditions used and the two analytes co-elute. As can be deduced from Figs 3a–3c and Figs 4a–4c, the shape of the concentration profile of a co-eluting mixture of the two analytes (kept together by mixed hydrogen-bonding interactions) depends on the amount of 2-phenylbutyric acid present. The concentration profile of a co-eluting binary mixture is least symmetric for the binary mixture containing the largest amount of acid – a small and poorly resolved chromatographic band clearly precedes the predominant band. It can only be speculated that because of the absolute quantitative predominance of 2-phenylbutyric acid over benzophenone (ca. 10:1 ratio) the small and poorly resolved chromatographic band obtained from the binary mixture represents the pure, self-associated acid.

Co-elution of 5-Phenylpentanol and Benzophenone (TLC)

From the results shown in Table II and in Figs 5 and 6 it can easily be deduced from the asymmetric concentration profiles that the amounts of 5-phenylpentanol and benzophenone developed as individual analytes were also within the non-linear range of the adsorption isotherm. For pure alcohol (which tends to participate in lateral interactions) we observe concentration profiles indicative of the anti-Langmuir-type adsorption isotherm, typical of such cases. This phenomenon is most pronounced for the two highest concentrations (i.e. 1.50 and 2.00 mol L^{-1}) of 5-phenylpentanol. For pure benzophenone (which does not tend to participate in lateral interactions) we again observe concentration profiles indicative of the Langmuir-type adsorption isotherm, which again is to be expected. The R_F values of the pure alcohol and the pure ketone are also markedly different (with the difference occasionally as high as $\Delta R_F = 0.22$) , irrespective of whether the analyte samples were developed with or without drying. Despite the distinct difference between their R_F values the two analytes cannot be separated under these chromatographic conditions, and they simply co-elute. As can be deduced from the plots in Figs 5 and 6, the shapes of the co-eluting concentration profiles give perceptible evidence of the two types of co-eluting moiety, one most probably the mixed associate of 5-phenylpentanol and benzophenone, kept together by mixed hydrogen-bonding, and the other corresponding to the pure alcohol. Intermolecular interactions between molecules of alcohol and ketone engage the entire capacity of the alcohol to participate in hydrogen-bonding, thus perceptibly hindering its interactions with the active sites of the adsorbent (which is not true for the acid, the carboxyl group of which can participate in two intermolecular hydrogen-bonds which involve, separately, the carboxyl –OH and >C=O groups). Because of this important structural difference between the alcohol and the acid, the mixed associative moiety formed between alcohol and ketone occasionally migrates “more quickly” (higher R_F value) than the pure alcohol.

Physicochemical Modelling of Co-elution of 2-Phenylbutyric Acid and Benzophenone

In our TLC experiment it was clearly shown that the two-component peak profiles had remarkably lower R_F values than any of the co-eluting compounds chromatographed separately, and that the entire envelopes of the two-component peaks were shifted to the left compared with benzophenone. Remembering that in our experiment the molecular ratio of ketone

to acid was ca. 0.1, it seems justifiable to assume that all the ketone molecules were in some way bonded to acid molecules. In these circumstances the three co-elution scenarios seem possible: (i) ketone molecules are adsorbed by previously adsorbed acid (or vice versa), forming two layers; (ii) in the mobile phase ketone and acid form a quasi-molecule, kept firmly together by hydrogen bonding (Fig. 1), which can be adsorbed by cellulose; and/or (iii) adsorbed ketone and acid interact by hydrogen bonding (lateral interactions). In scenario (ii) it is relatively easy to explain the single and binary peak profiles observed on the densitograms. The ketone and acid molecules coupled together form a larger unit, which interacts more strongly with active sites of the adsorbent and migrates more slowly along the solid bed of the stationary phase. Intuitively, scenarios (i) and (iii) can also explain the observed densitograms.

After deeper reflection, scenario (ii) seems rather improbable (see next section devoted to results obtained by use of HPLC), so in this study we are going to scrutinize scenarios (i) and (iii) in greater detail. Because quantitative investigation of the isotherm model and the retention process in experimental TLC data is impossible, we will restrict our considerations to giving an acceptable qualitative explanation of the observed peak profiles only, although for obvious reasons a detailed explanation of these profiles is not possible. For example, the peak of the acid has a characteristic long tail, which suggests that the adsorbent surface is heterogeneous. Of course, it is not a surprise, because cellulose has a complicated surface structure; in the following discussion, however, we will ignore surface heterogeneity.

Isotherm Representative of Scenario (i)

It was assumed that the ketone can be adsorbed by the previously adsorbed acid molecule (or, vice versa, acid can be adsorbed by the previously adsorbed ketone or another acid molecule). Further, assuming that the adsorption–desorption process is instantaneous and that a maximum of two layers only can be formed, it is easy to obtain the isotherm model (eqs 1 and 2) by following the method described elsewhere [11]:

$$q_1 = q_s \frac{K_1 C_1 \cdot (1 + 2K_{11} C_1 + K_{12} C_2) + C_1 C_2 K_2 K_{21}}{D} \tag{1}$$

$$q_2 = q_s \frac{K_2 C_2 \cdot (1 + 2K_{22} C_2 + K_{21} C_1) + K_1 K_{12} C_1 C_2}{D} \tag{2}$$

where $D = 1 + K_1C_1 + K_2C_2 + K_1K_{11}C_1^2 + K_2K_{22}C_2^2 + (K_2K_{21} + K_1K_{12})C_1C_2$, q_s is the maximum capacity of the adsorbent, C_i is the concentration of component in the mobile phase, q_i is the concentration of the adsorbed component, K_i is the equilibrium constant of adsorption of the *i*th component on the adsorbent surface, K_{ii} is the equilibrium constant for adsorption of the *i*th component on the same previously adsorbed *i*th component, and K_{ij} is the equilibrium constant for adsorption of the *i*th component on the *j*th component. It was also assumed that $K_{ij} = K_{ji}$. Index $i = 1$ refers to 2-phenylbutyric acid and $i = 2$ denotes benzophenone. Because benzophenone cannot self-associate, $K_{22} = 0$.

Isotherm Representative of Scenario (iii)

Lateral interactions between the adsorbed ketone and acid molecules can be depicted by using, e.g., the Fowler–Guggenheim [12] isotherm model:

$$q_1 = \frac{q_s a_1 C_1 \exp(\chi_1\Theta_1 + \chi_{12}\Theta_2)}{1 + a_1C_1 \exp(\chi_1\Theta_1 + \chi_{12}\Theta_2) + a_2C_2 \exp(\chi_{21}\Theta_1 + \chi_2\Theta_2)} \qquad (3)$$

$$q_2 = \frac{q_s a_2 C_2 \exp(\chi_{21}\Theta_1 + \chi_2\Theta_2)}{1 + a_1C_1 \exp(\chi_1\Theta_1 + \chi_{12}\Theta_2) + a_2C_2 \exp(\chi_{21}\Theta_1 + \chi_2\Theta_2)} \qquad (4)$$

where a_i is the isotherm parameter, Θ_i is the fractional coverage of the *i*th component, and $\Theta_i = q_i/q_s$. Terms χ_1 and χ_2 relate to the energy of lateral interactions between the molecules of the corresponding components. Terms χ_{12} and χ_{21} take into account cross-interaction between separated components. As in the previous example, index $i = 1$ denotes 2-phenylbutyric acid and $i = 2$ refers to benzophenone. Again, because benzophenone cannot self-associate, $\chi_2 = 0$. It was also assumed that $\chi_{12} = \chi_{21}$.

The surface heterogeneity was ignored in the isotherm models represented by eqs (1) and (2) and by eqs (3) and (4). To simulate the empirical concentration profiles, an appropriate mass-transfer model must be used. We chose a simple model [13] given by the equation:

$$\frac{\partial c_i}{\partial t} + F\frac{\partial q_i}{\partial t} + u\frac{\partial c_i}{\partial x} = D_{a,x}\frac{\partial^2 c_i}{\partial x^2} + D_{a,y}\frac{\partial^2 c_i}{\partial y^2} \qquad (5)$$

where t is the time, x and y are, respectively, the longitudinal and perpendicular coordinates of the plate, u is the linear flow rate, F is the phase ratio, and $D_{a,x}$ and $D_{a,y}$ are the apparent dispersion coefficients in directions x and y. Because of the qualitative nature of the experimental data, interpretation of dispersion in the y direction, perpendicular to the direction of development of the chromatogram, was ignored.

Finally, eq. (6)

$$\frac{\partial c_i}{\partial t} + F\frac{\partial q_i}{\partial t} + u\frac{\partial c_i}{\partial x} = D_a\frac{\partial^2 c_i}{\partial x^2} \qquad (6)$$

in combination either with the isotherm represented by eqs (1) and (2) or with that represented by eqs (3) and (4) was solved for the values summarized in Table III.

Table III

Terms of the models used to simulate densitometric peak profiles

Magnitude	Value
Analyte migration distance, L	15 cm
Phase ratio, F (assumed value)	0.25
Linear flow rate, u	0.074 cm min^{-1}
Apparent dispersion coefficient, D_a (assumed to be equal to molecular diffusivity)	0.00033 cm^2 min^{-1}
Maximum adsorbent capacity, q_s	1 mol L^{-1}
Terms of the isotherm represented by eqs (1) and (2)	
Equilibrium constant, K_1	1.2 L mol^{-1}
Equilibrium constant, K_2	1.2 L mol^{-1}
Equilibrium constant, K_{11}	2 L mol^{-1}
Equilibrium constant, K_{22}	0 L mol^{-1}
Equilibrium constant, $K_{12} = K_{21}$	1.9 L mol^{-1}
Terms of the isotherm represented by eqs (3) and (4)	
a_1 (assumed value)	1 L mol^{-1}
a_2 (assumed value)	1 L mol^{-1}
χ_1 (assumed value)	2
$\chi_{12} = \chi_{21}$ (assumed value)	2
χ_2 (assumed value)	0

To solve eq. (6), the initial and the boundary conditions had to be established. We assumed that the initial concentration and the concentration gradient for $x = L$ were equal to zero. For $x = 0$, the concentration was as-

sumed to be equal to the initial concentration in the spot at time $t = 4$ min. This time is equal to the ratio of the initial spot diameter (ca. 3 mm) to the mobile phase velocity. The values assumed in Table III were chosen so as to obtain the best qualitative agreement between the shapes of the experimental and theoretical peak profiles. The models given by eqs. (1), (2), and (6) or by eqs. (3), (4), and (6) were solved by the method discussed elsewhere [13].

The results of calculations for the isotherm models represented by eqs (1) and (2) and by eqs (3) and (4) are presented in Figs 8 and 9, respectively. The theoretically obtained peak profiles were calculated from the ratio of the concentration to the detector signal, using the previously obtained calibration plot. For the simulated two-component peak profiles the signal from the mixture was calculated by summation of the two one-component signals.

As is apparent from the results obtained, relatively good qualitative agreement was obtained between the experimental peak profiles and the theoretical profiles simulated for the acid–ketone and alcohol–ketone co-elution experiments, thus confirming the possibility of the adsorption mechanisms assumed in models (i) or (iii).

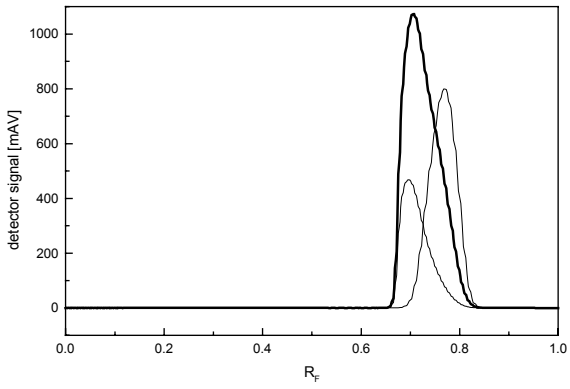


Fig. 8

Calculated signal profiles for the single components (thin lines: the first peak represents 2-phenylbutyric acid and the second peak represents benzophenone) and the mixture (thick line). The isotherm model used is that given by eqs (1) and (2)

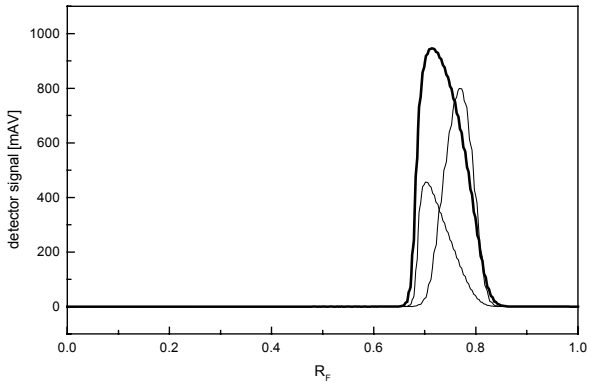


Fig. 9

Calculated signal profiles for the single components (thin lines: the first peak represents 2-phenylbutyric acid and the second peak represents benzophenone) and the mixture (thick solid line). The isotherm model used is that given by eqs (3) and (4)

Elution of 5-Phenylpentanol and Benzophenone (HPLC)

These models do not make use of scenario (ii). This scenario does not seem possible because of results obtained from HPLC and presented in Fig. 7.

As is apparent from Fig 7b, for relatively low concentrations of the mixture components (concentration of 5-phenylpentanol 0.40 mol L^{-1} and that of benzophenone 0.02 mol L^{-1}) both species could be separated (i.e. no co-elution was observed); co-elution occurred only for higher concentrations of the alcohol and ketone, as shown in Fig. 7a. This is sufficient proof that for *n*-octane as mobile phase (the same mobile phase was used for both TLC and HPLC) the acid and ketone cannot form a quasi-molecule held together by hydrogen bonding. It also seems impossible that the hypothetical alcohol–ketone quasi-molecule could split (i.e. be disrupted) on the Chiralcel® OB-H adsorbent surface yet be kept together on the more polar crystalline cellulose.

The general shapes of the band profiles obtained from TLC differ from that from HPLC (compare the band profiles from the densitograms with those shown in Fig. 7a). The main difference is that the TLC band profiles obtained for the binary mixtures migrate more slowly than the profiles obtained for the single analytes (especially for higher concentrations of the alcohol) and in HPLC the binary band profiles always migrate more quickly than the peaks of the single components. The HPLC results can easily be explained, by bearing in mind that the analytes compete for

active sites; at this stage of our study, however, we cannot provide any sensible explanation of why the binary band profiles obtained in TLC migrate more slowly than those of the analytes developed as single species.

For the single species the TLC and HPLC band profiles of the ketone are very similar. For the alcohol, however, the HPLC peak profiles are indicative of a Langmuir-like isotherm whereas the TLC band profiles are indicative of an anti-Langmuir isotherm (the band profiles in TLC are mirror reflections of the band profiles in HPLC). This discrepancy evidently results from the different surface properties of microcrystalline cellulose on TLC plates and on the Chiralcel® adsorbent (i.e. cellulose tribenzoate) in the column.

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