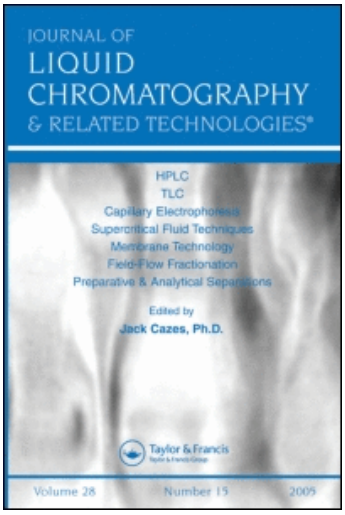

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THE ROLE OF LATERAL ANALYTE–ANALYTE INTERACTIONS IN THE PROCESS OF TLC BAND FORMATION. II. DICARBOXYLIC ACIDS AS THE TEST ANALYTES

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ABSTRACT

A second attempt has been made to investigate intermolecular hydrogen-bonding interactions among adsorbed analyte molecules (so-called lateral interactions), and their impact on the retention process. In this work, three compounds from a homologous series of dicarboxylic acids were selected as the test analytes. To this effect, a novel model was investigated.

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The quantitative validity of this novel model was checked experimentally with three dicarboxylic acids (succinic, adipic, and suberic) as test solutes. TLC was performed with cellulose powder and 1,4-dioxane, respectively, as stationary and mobile phases. The results obtained, fully confirmed the practical utility of our approach.

The traditional definition of the R_F coefficient was re-examined and its irrelevance to analytes participating in lateral interactions was demonstrated, as was the use of densitograms (rather than flat overall pictures of TLC chromatograms).

Key Words: TLC; Retardation factor; Succinic acid; Suberic acid; Adipic acid; Lateral interactions

INTRODUCTION

The aim of this study was:

- to provide new and reliable experimental proof from TLC that lateral analyte–analyte interactions have a measurable impact on chromatographic band shape and, thus, considerably affect the entire chromatographic process (earlier work on this topic, with selected higher fatty acids as the test analytes, has been presented elsewhere);[1]
- to verify a novel physicochemical model able to furnish the theoretical foundations of the aforementioned and experimentally proven phenomenon of lateral analyte–analyte interactions;
- to re-examine[1] the traditional definition of analyte R_F coefficient, and the technique used to measure it, when the concentration profile of the chromatographic band is taken into consideration (instead of measuring the respective distances on the freshly visualized chromatogram).

THEORY

We explored a novel approach (based on the model of gas adsorption by Wang and Hwang)[2] devised to describe analyte behavior on the stationary phase surface, which takes into the account lateral interactions among adsorbed molecules of the analyte. It is a fairly sophisticated approach to this phenomenon, assuming intermolecular interactions by hydrogen-bonding and making a clear distinction between initial formation of the adsorbed analyte monolayer and subsequent, stepwise accumulation of further adsorbed layers on this initial

monolayer. Detailed assumptions about the isotherm for adsorption of the analyte on the stationary phase surface are given in the forthcoming section.

Model

The following adsorption processes are regarded as possible:

1. Analyte molecules are adsorbed on the active sites of the adsorbent. The kinetic rates of adsorption and desorption are denoted by k_1 and k_{-1}, respectively, and the ratio of the amount of the adsorbed compound to the saturation capacity (q_s) of the stationary phase is denoted by Θ_1.
2. Analyte molecules are adsorbed on the previously adsorbed monolayer. The kinetic rates of dimerization and dissociation (i.e., the reverse process) are denoted by k_{p1} and k_{-p1}, respectively. The ratio of the active sites occupied by the dimer to the saturation capacity of the stationary phase is denoted by Θ_2.
3. Analyte molecules are adsorbed on the previously adsorbed binary layer. The kinetic rates of trimerization and dissociation are denoted by k_{p2} and k_{-p2}, respectively (typically, the values of k_{p2} and k_{-p2} can differ from those of k_{p1} and k_{-p1}, respectively, because association between the analyte molecules can be more complex than the example presented in Fig. 1). The ratio of the active sites occupied by the trimer to the saturation capacity of stationary phase is denoted by Θ_3.
4. This chain process of stepwise building of consecutive adsorption layers can be continued *ad infinitum* (theoretically at least).

Taking these assumptions into account, kinetic equations can be formulated: Let Θ_0 denote the ratio of the free active sites to the saturation capacity:

$$\Theta_0 = 1 - \Theta_1 - \Theta_2 - \Theta_3 - \cdots - \Theta_n \tag{1}$$

The reaction mechanism for multi-layer adsorption can be described as follows: for the free active sites

$$\frac{d\Theta_0}{dt} = k_{-1}\Theta_1 - k_1 C_1 \Theta_0 \tag{2}$$

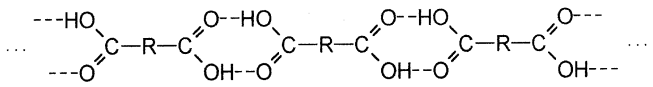

Figure 1. Schematic representation of the self-association of dicarboxylic acids as a result of intermolecular hydrogen bonding.

for the first layer

$$\frac{d\Theta_1}{dt} = k_1 C_1 \Theta_0 - k_{-1}\Theta_1 - k_{p1} C_1 \Theta_1 + k_{-p1}\Theta_2 \tag{3}$$

for the second layer

$$\frac{d\Theta_2}{dt} = k_{p2} C_1 \Theta_1 - k_{-p2}\Theta_2 - k_{p3} C_1 \Theta_2 + k_{-p3}\Theta_3 \tag{4}$$

for the nth layer

$$\frac{d\Theta_n}{dt} = k_{pn} C_1 \Theta_{n-1} - k_{-pn}\Theta_n \tag{5}$$

Assuming pseudo-steady-state conditions, the left-hand sides of Eqs (2)–(5) are equal to zero and, hence, the following algebraic equations are fulfilled:

$$KC(1 - \Theta_1 - \Theta_2 - \Theta_3 - \cdots) - \Theta_1 = 0 \tag{6}$$

$$K_{p1} C\Theta_1 - \Theta_2 = 0 \tag{7}$$

$$K_{p2} C\Theta_2 - \Theta_3 = 0, \text{ etc.} \tag{8}$$

where C is concentration of the analyte in the mobile phase, and $K = k_1/k_{-1}$, $K_{p1} = k_{p1}/k_{-p1}$, etc.

The total amount of the adsorbed analyte can be calculated from the equation:

$$q = q_s(\Theta_1 + 2\Theta_2 + 3\Theta_3 + \cdots) \tag{9}$$

or, after substitution of Eqs (6), (7), (8), etc., into Eq. (4) and assuming that $K_{p1} = K_{p2} = \cdots = K_{pn} = K_p$, we obtain:

$$q = q_s \frac{KC(1 + 2K_pC + 3(K_pC)^2 + 4(K_pC)^3 + \cdots)}{1 + KC + KCK_pC + KC(K_pC)^2 + KC(K_pC)^3 + \cdots} \tag{10}$$

For an infinite number of adsorbed layers, Eq. (10) takes the simpler form:

$$q = q_s \frac{KC}{(1 - K_pC) * (1 - K_pC + KC)} \tag{11}$$

It seems, however, that Eq. (11) has hardly any practical sense for liquid adsorption.

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EXPERIMENTAL

Working Conditions

The stationary phase was cellulose (precoated cellulose TLC plates manufactured by E. Merck, Darmstadt, Germany; cat. # 1.05730); the mobile phase was 1,4-dioxane; the analyzed acids are in Table 1.

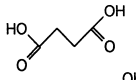
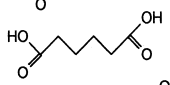
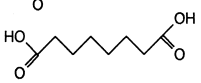
Visualization of the chromatograms was achieved by spraying with a solution of bromocresol green in ethanol, prepared in accordance with a procedure described elsewhere.[3]

Densitograms were obtained by use of a Shimadzu (Columbia, MD USA) model CS9301 PC scanning densitometer, by use of a rectangular cross-section light beam (wavelength 625 nm; rectangle dimensions 0.05 mm × 0.5 mm). The detector used in this densitometer can detect analytes active in the visible or ultraviolet range only, i.e., absorption or fluorescence of the analyte is quantified. In our experiment, the analytes were optically inactive, and it was, therefore, necessary to visualize them. The visualizing procedure, however, spoils the quality of the densitogram, basically because of noise originating as a result of uneven distribution of the visualizing agent (the bromocresol green dye) on the stationary phase surface. Thus, interpretation of the densitograms obtained was possible only after preliminary removal of the noise; this was achieved by approximation, by use of a smoothing spline fit.

The Ability of Test Analysts to Self-Associate by Hydrogen-Bonding

Dicarboxylic acids can form the tape-like associative multimers by hydrogen-bonding (a maximum of four hydrogen bonds can be formed by each molecule of acid). This is because of the presence of the two negatively polarized

Table 1. The Test Solutes Used

Compound	Structure	Description
Succinic acid		C_4
Adipic acid		C_6
Suberic acid		C_8

oxygen atoms from the carbonyl groups and the two positively polarized hydrogen atoms from the carboxyl groups.

Determination of the Numerical Values of Analyte R_F Coefficients from the Densitograms

The traditional (and so far only) method of determination of the numerical values of analyte R_F coefficients quasi-automatically assumes the preconditions:

(a) circular chromatographic band shape; and
(b) Gaussian distribution of the mass of the analyte in this band.

On the basis of these assumptions, the position of a band on the chromatogram is defined by measuring the distance between the origin and the geometrical center of the band. Despite the considerable imprecision of this definition for asymmetric (i.e., tailing) and non-Gaussian bands, two features of the definition are very important:

(i) The traditional definition regards the center of a chromatographic band as the point at which the local concentration of the analyte is highest.
(ii) The traditional definition also regards the center of the chromatographic band as the center of gravity of the mass distribution of the analyte in the band.

For ideal, circular bands with Gaussian analyte concentration profiles, the band centers described by assumptions (i) and (ii) are, in fact, identical.

For densitograms obtained from non-circular (i.e., tailing) bands with non-Gaussian concentration profiles, it can be stated that:

- The numerical value of the R_F coefficient for a given chromatographic band can be determined for the maximum value of the concentration profile of the band (which is the point at which the local concentration of the analyte is the highest). In our study, the R_F coefficient determined according to this definition was denoted $R_{F(max)}$.
- Alternatively, the numerical value of the R_F coefficient can be determined from the center of gravity of the distribution of analyte mass in the band. In our particular experiment (i.e., involving non-symmetrical chromatographic bands), this value cannot be identical with that obtained from the maximum of the analyte concentration profile. In our study, the R_F coefficient determined in this manner was denoted $R_{F(int)}$.

To determine the center of gravity of analyte mass distribution in the chromatographic band we established the baseline, removed the noise from the

densitogram, subtracted the baseline signal, defined the beginning ($i = 0$) and end ($i = k$) of the chromatographic band, and, finally, calculated the position of its center of gravity by use of the relationship:

$$d_{sr} = \frac{1}{S}\sum_{i=1}^{k} I\left(\frac{d_i + d_{i-1}}{2}\right)\frac{d_i + d_{i-1}}{2}(d_i - d_{i-1}) \tag{12}$$

where S denotes the chromatographic band surface, and $I(d_i)$ is the detector signal at a distance d_i.

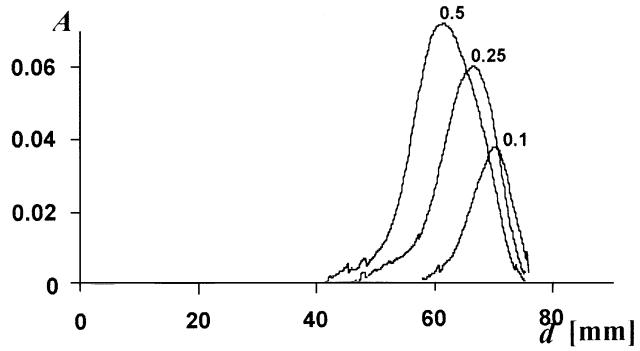
RESULTS AND DISCUSSION

The first part of this section will be devoted to discussion of the results obtained in the context of the model (Eq. (10)) of the adsorption isotherm that anticipates lateral interactions among the analyte molecules. Figures 2a and 2b show the processed densitograms (after noise removal and subtraction of the baseline) obtained by scanning the chromatograms of succinic (C_4) and suberic acids (C_8), spotted on to the chromatographic plates as solutions in ethanol (2 μL). The initial spot diameter was always constant at 2 mm. The results obtained for adipic acid (C_6) were similar to those in Fig. 2 and, therefore, to keep this paper concise, are not included.

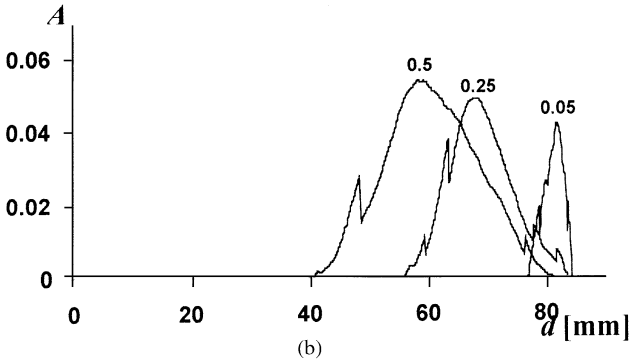
It is clearly apparent that the densitogram peak areas obtained for different concentrations of the same analyte (Figs 2a and 2b) are not proportional to the respective initial sample concentrations. This phenomenon undoubtedly results from the mode of visualization employed; the scanned signal depends (at least to some extent) on the amount of visualizing agent applied to the chromatographic plate. We can, on the other hand (and owing to even application of the visualizing dye to the entire chromatographic plate surface), expect that the qualitative picture of concentration distribution in the analyte band profiles is correct.

Figure 3 shows the dependence of R_F on analyte concentration. R_F values determined from the band maxima and from the band centers of gravity for different concentrations of the different analytes are compared in Table 2. From these data it is apparent that:

- As the concentration of solute in the solution applied to the plate is increased, the numerical value of the R_F coefficient decreases; this was confirmed unequivocally by the respective experimental results obtained for the three different dicarboxylic acids by use of the two independent approaches to measurement.
- Numerical values of the R_F coefficient, irrespective of the technique used for their determination, are similar to each other, although not completely identical. Numerical values of the R_F coefficient obtained by use of the center of gravity are usually (but not always) somewhat



(a)



(b)

Figure 2. Densitograms obtained for (a) succinic acid (C_4) and (b) suberic acid (C_8). Sample concentrations were: (a) 0.1, 0.25, and 0.5 mol L^{-1} and (b) 0.05, 0.25, and 0.5 mol L^{-1}.

lower than the values obtained from the band maxima. The reason for this difference is the band shape (non-symmetrical distribution of the detector signal). The difference between the R_F values obtained by use of the two different approaches evidently depends on the quantity of the test solute applied to the chromatographic plate. As the amount of analyte applied to the stationary phase is increased, the shapes of the resulting chromatographic bands become increasingly non-symmetrical. For very small amounts of analyte, differences between the numerical values of $R_{F(max)}$ and $R_{F(int)}$ should eventually disappear.

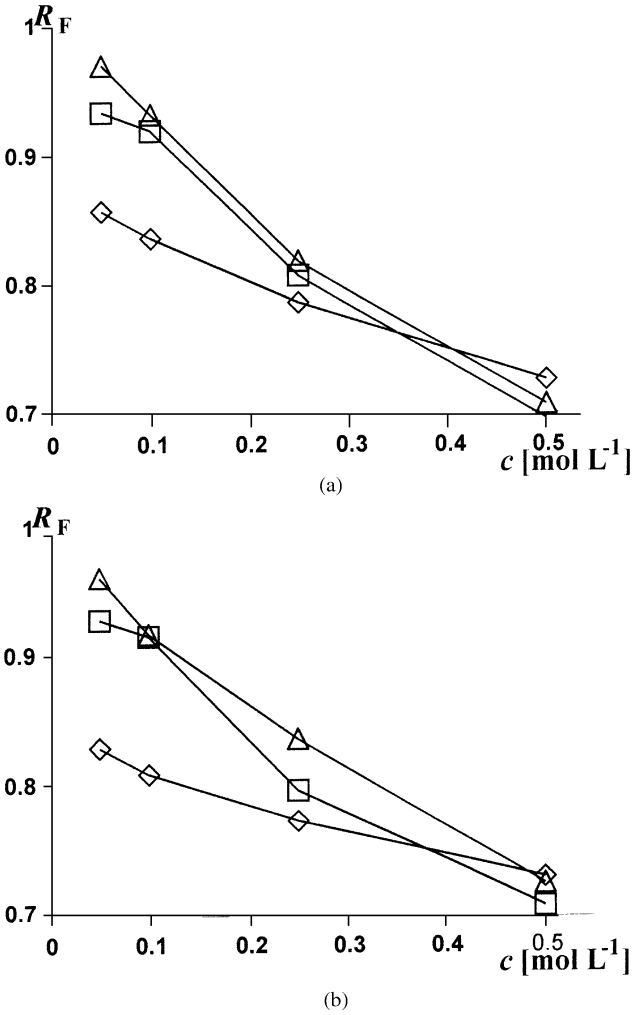


Figure 3. Dependence of the numerical values of the R_F coefficient on the concentration of the analyte for the C_4 (◇), C_6 (□), and C_8 (△) dicarboxylic acids, determined from the band maximum (a) and the band weight center (b).

- The differences, however insignificant, between the numerical values of the R_F coefficients determined in two different ways from the densitometric concentration profiles indisputably have two causes, which result directly from the technique used for data processing. (i) For analytes with strongly pronounced lateral interactions, the traditional

Table 2. Numerical Values of the R_F Coefficients Determined from the Maximum (max) and from the Center of Gravity (int) of the Chromatographic Bands

Test Analyte	C_4		C_6		C_8	
Conc. of Acid in Sample ($mol\,L^{-1}$)	$R_{F(max)}$	$R_{F(int)}$	$R_{F(max)}$	$R_{F(int)}$	$R_{F(max)}$	$R_{F(int)}$
0.05	0.86	0.83	0.93	0.93	0.97	0.96
0.10	0.84	0.81	0.92	0.92	0.93	0.92
0.25	0.79	0.77	0.81	0.80	0.82	0.84
0.50	0.73	0.73	0.70	0.71	0.71	0.73

method of determination of the geometrical center of the chromatographic band as an important reference (without taking its concentration profile into the account) seems an entirely unfounded simplification. Now the real problem emerges: which point should be regarded as the center of a given chromatographic band? By analogy with column-chromatographic techniques (i.e., high performance liquid chromatography and gas chromatography), the maximum in the concentration profile of a band can correctly be regarded as its reliable reference point. Alternatively, and in the spirit of the traditional concept of the geometrical center of the chromatographic band, the center of gravity of a band can be assumed to be its central point. It is rather obvious that the two approaches are not equivalent. (ii) Determination of the maximum of the concentration profile of a band and of its center of gravity are both founded on fairly arbitrary determination of the baseline of the densitogram. The arbitrariness of this determination, however, affects the accuracy of determination of the maximum of the concentration profile of the band, or of its center of gravity, to somewhat different extents.

Qualitative Comparison of Densitograms with Simulated Concentration Distributions

We have evaluated the possibility of qualitative modeling of the experimentally observed peak profiles. To do so, we applied the differential equation of the mass balance given by Eq. (13):[1]

$$\frac{\partial C}{\partial t} + w\frac{\partial C}{\partial x} + F\frac{\partial q}{\partial t} = D_x\frac{\partial^2 C}{\partial x^2} + D_y\frac{\partial^2 C}{\partial y^2} \tag{13}$$

where w is the average mobile-phase flow rate; C and q are, respectively, the concentrations [mol dm^{-3}] of the analyte in the mobile phase and on the adsorbent surface; D_x and D_y are, respectively, the effective diffusion coefficients lengthwise (x) and in the direction perpendicular to the plate (y); and F is the so-called phase ratio (i.e., the ratio of the volume of stationary phase to that of the mobile phase), which in our experiments was assumed, somewhat arbitrarily, to be 0.25.

It seems noteworthy that the model expressed by Eq. (13) is a two-dimensional representation of the widely applied equilibrium-dispersive model.[4]

The simulations presented below were obtained assuming three-layer adsorption as a maximum, so the isotherm model can be expressed as:

$$q = q_s \frac{KC(1 + 2K_pC + 3(K_pC)^2)}{1 + KC + KCK_pC + KC(K_pC)^2} \tag{14}$$

Equation (13), with the isotherm expressed by Eq. (14), was solved using the initial conditions:

$$\text{if } (x < 0.2) \text{ and } (y > 0.4) \text{ and } (y < 0.6), \text{ then } C = C^0; \text{ otherwise } C = 0$$

and the boundary conditions:

$$\left.\frac{\partial C}{\partial x}\right|_{x=0,x=10} = \left.\frac{\partial C}{\partial y}\right|_{y=0,y=1} \tag{15}$$

Constants in the adsorption isotherm equation and the effective diffusion coefficients were chosen to obtain shapes of the lengthwise cross-sections of the chromatographic bands, similar to those from the experimental chromatograms. It was assumed, that:

$$D_x = 2.5 \times 10^{-3} \text{ [cm}^2 \text{ min}^{-1}\text{]},$$
$$D_y = 1 \times 10^{-4} \text{ [cm}^2 \text{ min}^{-1}\text{]},$$
$$q_s = 1.5 \text{ [mol dm}^{-3}\text{]},$$
$$K = 0.5 \text{ [dm}^3 \text{ mol}^{-1}\text{]},$$
$$K_p = 5 \text{ [dm}^6 \text{ mol}^{-2}\text{]}.$$

The velocity of the mobile phase, $w = 0.13$ cm min^{-1}, was established as velocity of the analyte migration, averaged from all the experiments.

It was assumed that, at the start of development of the chromatograms the analyte was spotted on to the adsorbent surface as a rectangular band of dimensions 2 mm × 2 mm.

The orthogonal collocation method on finite elements (OCFE) was used to solve the set of equations given by Eq. (13). The OCFE method used in this study has been described elsewhere.[5–7] The set of ordinal differential equations

obtained after OCFE discretization was solved by means of the Adams–Moulton method implemented in the VODE procedure,[8] using relative and absolute errors equal to 10^{-6}. The VODE procedure automatically chooses an appropriate time increment to fulfill the assumed error conditions. In each calculation, the number of internal collocation points in subdomains (elements) was equal to three in direction *X* and to six in direction *Y*. The number of subdomains was chosen such that there were no visible oscillations in simulations of the band profiles; the number was 80 in direction *X* and 5 in direction *Y*.

The simulated spot profiles and the lengthwise cross-sections of the chromatographic bands are depicted in Figs. 4 and 5. It is apparent from Fig. 5, that the adsorption fronts are much less steep than the desorption fronts, and that adsorption fronts simulated for the different initial concentrations of the spots overlap.

Similar behavior can be observed when analyzing the typical experimental densitograms presented in Figs. 2a and 2b. In all of these densitograms, the adsorption fronts for the different concentrations of the acid C_4 coincide with each other. The concentration changes in the adsorption fronts are typically slightly steeper than in the desorption fronts of the spots. For the C_8 acid, the desorption front is generally steeper than the adsorption front.

These results suggest that adsorption of the analyzed acids must be connected with additional lateral interactions, probably more complex than those assumed in the isotherm model used. The coincidence of the adsorption fronts can be explained only if interactions between the adsorbed molecules are assumed. Also, the adsorption front is less steep than the desorption front when attraction between the adsorbed analyte molecules cannot be ignored.

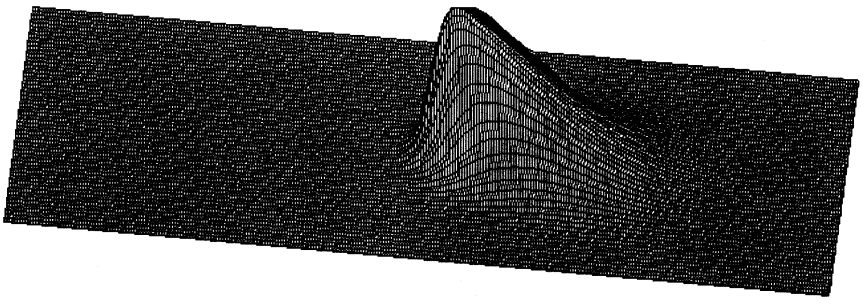


Figure 4. Simulated chromatogram according to the model expressed by Eq. (13) implemented with the isotherm given by Eq. (14). The concentration of the solution used for spotting was $0.25\,\mathrm{mol\,L^{-1}}$.

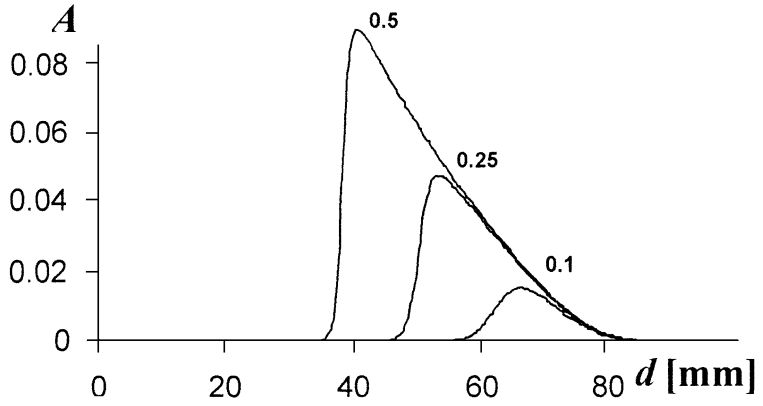


Figure 5. The lengthwise cross-section of the simulated chromatogram according to the model expressed by Eq. (13) with the isotherm given by Eq. (14). The concentrations of the solutions used for spotting were 0.5, 0.25, and 0.1 mol L^{-1}.

CONCLUSIONS

- Our observations justify the assumption that lateral interactions play an important role in the adsorption of dicarboxylic acids on cellulose-coated TLC plates.
- We remain uncertain which approach is better for determination of the numerical values of analyte R_F coefficients, especially for analytes that tend to participate in lateral interactions. Maybe this matter warrants thorough discussion by the entire community of TLC practitioners, followed by a broad consensus.

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