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Experimental evidence of a delta-shock in nonlinear chromatography

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ABSTRACT

We report about a new type of composition front in nonlinear chromatography that is called delta-shock, which has to be added to the family of classical transitions, i.e. simple waves, shocks and semi-shocks. Recently, the occurrence of delta-shocks in the case of mixed competitive-cooperative isotherms of the following type

$$n_i = \frac{H_i c_i}{1 - K_1 c_1 + K_2 c_2} \quad (i = 1, 2),$$

(with $H_2 > H_1$, where components 1 and 2 have anti-Langmuir and Langmuir adsorption behavior, respectively) was predicted theoretically and their behavior was analyzed in the frame of the equilibrium theory of chromatography. The delta-shock can be viewed as a growing traveling spike superimposed to the discontinuity separating the initial and the feed state, which propagates along the column at constant speed and constant rate of growth. In this work we complement these findings from an experimental point of view. The binary system consisting of phenetole (component 1) and 4-tert-butylphenol (component 2) in methanol–water (about 2:1, v/v) on a Zorbax 300StableBond-C18 column from Agilent has been shown, through a series of overloaded pulse experiments and of frontal analysis experiments with the pure compounds, to be subject to the competitive-cooperative isotherm of the type above, up to rather large concentrations. This system does exhibit a delta-shock when the operating conditions are chosen according to theory, namely when phenetole initially saturating the column is displaced by 4-tert-butylphenol, both at high concentrations (the minimum concentrations exhibiting a fully developed delta-shock in this series of experiments were $c_1 = 20$ g/L and $c_2 = 75$ g/L). The propagation of the delta-shock matches the theoretical predictions in terms of both the effect of concentration and the effect of column length. This is the first experimental observation ever of a delta-shock in chromatography. It is noteworthy that the proof of the occurrence of the delta-shock reported here has been obtained in both laboratories cooperating in this project.

1. Introduction

In chromatography, a typical situation is where a mixture of chemical species that initially saturates the chromatographic column is displaced by flushing the column with a new mixture of the same species with a different composition. The species are dissolved in a mobile phase, i.e. a solvent or a mixture of solvents of fixed relative concentrations. They have different affinities for the stationary phase, hence different retention times when injected individually into the column. One such process is the adsorption of a mixture on an empty column, i.e. filled with the mobile phase only, and the ensuing saturation of the column with the mixture itself. Another one is the regeneration of a column initially saturated with a mixture at a given composition by flushing the column with pure mobile phase. When a given amount of a mixture is fed to an empty column for a finite time, in a so called pulse injection, two successive events like those described above take place, first adsorption, then regeneration.

The application of a new feed mixture to a column saturated with an initial feed mixture causes a dynamic response that leads to the displacement of the initial mixture (or state) by the new one (state). As this happens, the initial and new composition states are connected by composition fronts that travel through the column from the inlet to the outlet. For instance, for a mixture of two retained compounds, there are up to two composition fronts, which are separated by one composition plateau, i.e. by an intermediate

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composition state. Under linear chromatographic conditions, such fronts have velocities that are independent of the compositions of the initial and new feed states, and shapes that are controlled by the column efficiency. Under nonlinear, overloaded chromatographic conditions, the composition fronts propagate at speeds that depend on the compositions upstream and downstream the front and on the adsorption isotherm. They can belong to significantly different types and their shape is only partly affected by the column efficiency.

The theory of nonlinear chromatography is well established [1,2]. It allows classifying composition fronts in three different types. Simple waves are those where the transition from one composition state to the adjacent one occupies a larger and larger portion of the column as the front travels; they occur when for instance a strongly retained species is eluted by the mobile phase. Shocks propagate as constant patterns, without changing shape whatever the column length; this happens during the adsorption of a retained component on an initially empty column. Finally, semi-shocks are combinations of these two types; they typically occur during the adsorption or desorption of species subject to an isotherm with an inflection point and cannot take place in the case of a simple Langmuir isotherm. It should be emphasized that within the equilibrium theory of nonlinear chromatography a shock is a mathematical discontinuity. Its existence is a consequence of the fact that the upstream composition state would propagate faster than the downstream state. The propagation rate of the shock is such that mass conservation is fulfilled through the traveling discontinuity, which has neither volume nor capacity for the species present in the mixture under consideration.

Recently, it was shown through theoretical considerations and calculations that there may be new types of composition fronts in nonlinear binary chromatography. These would take place under particular conditions and consist of a continuous non simple wave transition and a delta-shock [3,4]. Focusing on the delta-shock, which constitutes a brand new dynamic phenomenon in nonlinear binary chromatography, this paper demonstrates the first observation of such a delta-shock and it proves that its experimental properties are consistent with the theory.

2. Theoretical background on the delta-shock

Recently, the classical solution of the equilibrium theory of chromatography originally developed for Langmuir isotherms [2] has been extended to the binary generalized Langmuir isotherms where, during their adsorption, the two species can either compete or co-operate [5]. Also the extension of the so-called "triangle theory" for the design of Simulated Moving Bed chromatographic processes was possible [6,7]. Among these new isotherms, the mixed isotherm called M2, where the more retained component follows a Langmuir adsorption behavior whereas the less retained one follows an anti-Langmuir behavior, is in several ways unusual among the four generalized Langmuir isotherms. It is given by the following equation:

$$n_i = \frac{H_i c_i}{1 - K_1 c_1 + K_2 c_2} \quad (i = 1, 2), \tag{1}$$

where c_i and n_i are fluid and adsorbed phase concentrations, respectively (same units); the subscript 1 indicates the less retained component with anti-Langmuir behavior and the subscript 2 stands for the more retained component with Langmuir behavior. The coefficients in the numerators are the Henry's constants of adsorption, H_i (dimensionless), where $H_2 > H_1$; those in the denominator are the equilibrium constants K_i, with $i = 1, 2$ (with units corresponding to the reciprocal of a concentration). It is worth noting that this isotherm can describe real systems, at least in the range of compositions where the denominator is positive, even though it is non-consistent from a thermodynamical point of view [5].

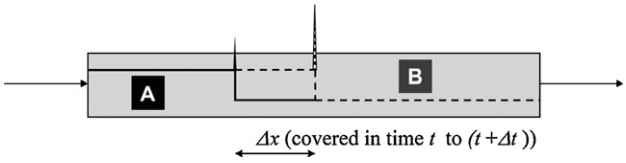

Fig. 1. Propagation of a delta-shock between states A and B. The solid and the dashed lines represent the concentration profiles of either species at time t and at time $t + \Delta t$, respectively.

Through a mathematical analysis based on the equilibrium theory of chromatography, it was demonstrated that such isotherm exhibits non-classical behavior [3,4]. More specifically, a new type of composition front was discovered, the delta-shock, which should be added to the classical fronts, i.e. simple wave, shock, and semi-shock transitions, that are exhibited by the systems considered so far and have been briefly described in the introduction [1].

In this section we summarize the key theoretical findings on the delta-shock that were reported earlier. As illustrated in Fig. 1, a delta-shock separates two composition states, the feed and the initial states mentioned in the introduction, and consists of a shock discontinuity superimposed to a spike, both traveling at the same constant propagation speed along the column. From a mathematical point of view the spike has a zero volume like a shock, but, in contrast, it has a finite capacity. In fact, like a Dirac-delta injection, it contains a finite amount of the compounds in the mixture under consideration in an infinitely small volume; as a consequence, the spike's height is infinite. However, the compounds accumulate in the spike as the delta-shock propagates hence the amount of each compound in the spike, i.e. its strength, increases. These qualitative characteristics are confirmed by detailed simulations using the equilibrium dispersive model of the chromatographic column in which shocks become shock layers, the spike's strength is finite and growing along the column, and the spike's height is also finite and growing [3,4].

From a physical point of view, the delta-shock phenomenon originates in the synergistic-competitive behavior of the two species as described by the adsorption isotherm (1). This means that the adsorption of the less retained species 1 enhances the adsorption of the more retained species 2, whereas the adsorption of 2 hinders that of 1. The delta-shock occurs for instance when species 1, initially present in the column, is displaced by species 2, provided that the concentrations of both species are sufficiently large. In Fig. 2 such a process is illustrated for a model isotherm through concentration profiles calculated at the column outlet for increasing values of the concentrations of the two species. Therefore, the occurrence of a delta-shock requires that the synergistic-competitive behavior described by Eq. (1) takes place up to high concentrations. This behavior has some times been reported at low concentrations but it seems to be normally shut off by saturation effects as the concentrations increase (see for instance [8]). Species 1, being anti-Langmuir, is displaced through a shock the velocity of which decreases when its initial concentration increases, as shown in Fig. 2 for the following initial concentrations: $c_1 = 2.0$, 4.0, and 4.5 g/L (note that in these chromatograms the shocks are rather broad because the column efficiency is finite and the concentration changes are relatively small). In contrast, the adsorption front of species 2 (Langmuir isotherm) is a shock that propagates at a velocity which increases with increasing feed concentration (see Fig. 2 again, for $c_2 = 7.5$, 15.0, and 16.875 g/L, where the calculated shocks are steeper than the rear fronts of species 1). As the two concentrations increase, a level is reached, namely $c_1 = 5$ g/L and $c_2 = 18.75$ g/L, for which the two shocks collide and

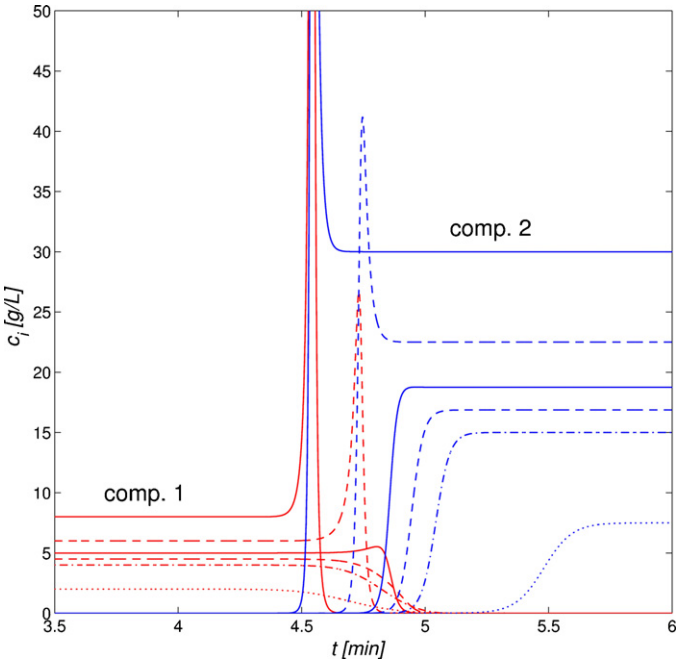

Fig. 2. Frontal analysis simulations where species 2 fed at $t = 0$ displaces species 1 initially saturating the column. The initial concentration of species 1 (red lines in the figure) and the feed concentration of species 2 (blue lines) can be read directly from the figure. Lines corresponding to the same simulation are identified because they are of the same type. There are three simulations without interaction between the fronts of species 1 and 2, where concentration profiles are plotted as dotted lines, dash-dotted lines and dashed lines (in order of increasing concentration). There are three simulations where fronts interact leading to delta-shocks of increasing strength, where concentrations profiles are plotted as solid lines, dashed lines and again solid lines for the highest concentration level. Simulations have been carried out using an equilibrium dispersive model and the adsorption isotherm (1) with $H_1 = 2.381$, $H_2 = 3.342$, $K_1 = 0.0155$ L/g, and $K_2 = 0.0162$ L/g. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

as a result, no classical shock can exist between the initial and the feed compositions that would allow for mass conservation [3,4]. Thus, matter accumulates at the discontinuity, in an amount that increases as the front travels along the column, thus resulting in the traveling spike of increasing size, or strength, that is schematically illustrated in Fig. 1. The larger the concentrations of species 1 and 2, the stronger the interactions between the desorption front of species 1 and the adsorption front of species 2, and the larger the size of the delta-shock at the column outlet, as shown in the calculated chromatograms in Fig. 2 for concentrations up to $c_1 = 8.0$ g/L and $c_2 = 30.0$ g/L.

From a mathematical point of view, a classical shock transition corresponds to a step function, i.e. a Heavyside function. A delta-shock can be represented as the superimposition of a Heavyside function and a Dirac-delta function. Actually, the latter is a generalized distribution, which is zero almost everywhere but has an integral over the real axis that equals one; in other words it is zero everywhere except at its origin where it is undefined or infinitely large and its integral is one. The Dirac-delta is a mathematical object that has a finite mass concentrated in a zero volume. Delta-shocks have been observed previously for a few other systems of nonlinear hyperbolic equations (see for instance [9] and a recent review [10]). Their mathematical treatment is very difficult and a general theory is still missing. Thus, every new mathematical model exhibiting a delta-shock solution must be dealt with in new, original ways.

In the case of the delta-shock considered here, it was possible to reach several general conclusions. First, exact criteria for the occurrence of a delta-shock were derived [3,4]. In the case of the pure compound 2 fed at concentration c_2 and displacing the pure compound 1 initially present in the column at concentration c_1, a delta-shock occurs if and only if:

$$\frac{n_1}{c_1} = \frac{H_1}{1 - K_1 c_1} > \frac{H_2}{1 + K_2 c_2} = \frac{n_2}{c_2}, \tag{2}$$

where the adsorbed phase concentrations n_1 and n_2 are calculated using Eq. (1) for the initial state (state B in Fig. 1) and for the feed state (state A in Fig. 1), respectively.

Second, the speed of propagation of the delta-shock was calculated. In the case of the pure compound 2 displacing the pure compound 1, the delta-shock will exit the column after a time following the injection of species 2 that is given by [4]:

$$t_R^{ds} = \frac{V}{Q}\left(\varepsilon + (1-\varepsilon)\frac{H_1 K_2 n_2 + H_2 K_1 n_1}{H_1 K_2 c_2 + H_2 K_1 c_1}\right), \tag{3}$$

where all the concentrations in this equation have the same meaning as above, V and ε are the volume and the total porosity of the column, respectively, and Q is the volumetric flow rate.

Finally, it was also possible to determine explicitly the mass of component i present in the traveling spike when it reaches the column outlet [4]:

$$h_i^{ds} = \frac{V H_i K_{3-i}(1-\varepsilon)(c_2 n_1 - c_1 n_2)}{H_2 K_1(\varepsilon c_1 + (1-\varepsilon) n_1) + H_1 K_2(\varepsilon c_2 + (1-\varepsilon) n_2)} \quad (i = 1, 2), \tag{4}$$

which is always positive thanks to inequality (2). It is easy to demonstrate that as c_1 and c_2 increase, while their ratio remains constant, the delta-shock hold up of species i, i.e. h_i^{ds}, increases as well.

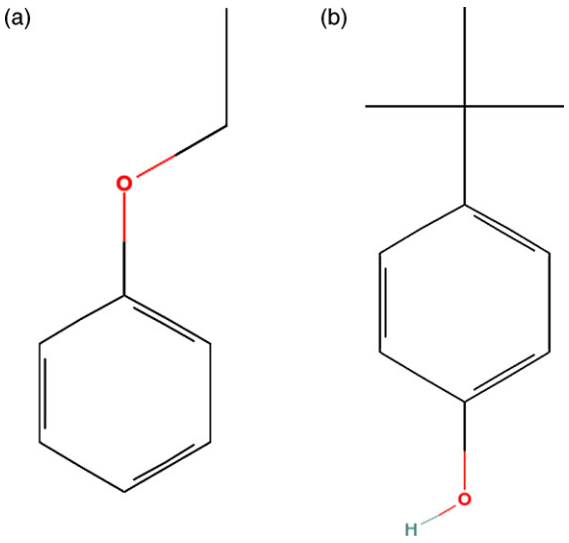

Fig. 3. Chemical structure of phenetole (a, $C_8H_{10}O$) and of 4-tert-butylphenol (b, $C_{10}H_{14}O$).

3. Experimental

3.1. Materials and methods

Over the last few years in our laboratories several systems were tested unsuccessfully for a behavior corresponding to the one described by the mixed M2 isotherm in a concentration range that would be wide enough to satisfy the delta-shock prerequisite conditions. Finally, it was observed that phenetole (ethoxybenzene or ethyl-phenyl-ether) and 4-tert-butylphenol (see Fig. 3 for their chemical structures) exhibit an anti-Langmuir and a Langmuir, respectively, adsorption behavior in a methanol–water (2:1, v/v) solution, at room temperature on a C18 column. Since the for-

mer is less retained than the latter, they qualified to be components 1 and 2 in an adsorption isotherm like that of Eq. (1), which could lead to a delta-shock.

Before carrying out chromatographic experiments, the solubilities of the two compounds were determined at 294 K in a methanol–water (67:37, v/v) solution. The solubility of phenetole is slightly larger than 20 g/L while that of 4-tert-butylphenol is around 700 g/L. Both values were measured with a precision better than 10%, sufficient for the needs of this study. Achieving a higher precision was difficult due to the nature of the two compounds. We also verified that the solubility of the two compounds in the presence of the other one exceeds 80 g/L for 4-tert-butylphenol and 35 g/L for phenetole in this same eluent.

In order to be able to rule out any possible artifact interfering with this new chromatographic phenomenon, we decided to repeat the experiments several times and to do so independently in the two laboratories involved in this research, following similar protocols. In particular all the experiments reported in Section 5 have been repeated from two to five times in different days. In all cases the chromatograms are reproducible not only in the general behavior but also as far as their details are concerned. This is true only if fresh solutions are prepared before each experiment. We have in fact observed that phenetole degrades when dissolved in the mobile phase, and that a solution of it in the mobile phase cannot be kept and used longer than for a few hours. In this section we describe the materials and methods applied in the two laboratories.

3.1.1. Zurich laboratory

The experiments were carried out in a modular HPLC setup from Agilent (HP1100, Palo Alto, CA, USA), equipped with a four-solvent delivery system. The setup has an online vacuum degasser, a diode-array detector to monitor simultaneously a broad range of wavelengths (with a detection UV-cell of 13 μL volume), and a column temperature controller. The extra-column volume from the mixer to the column inlet is 0.90 mL, a volume that was accounted for in all data reported. In all experiments, the temperature was kept at 294 ± 1 K, thanks to the lab air-conditioning system. The flow rate was kept constant and equal to $Q = 1$ mL/min. The mobile phase had a composition of 63% methanol/37% water (v/v), and was prepared manually by measuring the appropriate volumes (740 mL water + 1260 mL methanol = 2 L mobile phase), and by premixing these volumes with a magnetic stirrer bar for approximately 20 min. For the sake of consistency in future experiments, the solvent masses (730.4 g water and 986.9 g methanol) were also measured. The chromatographic columns used were Zorbax 300StableBond-C18 from Agilent of three different lengths (50 mm × 4.6 mm, 150 mm × 4.6 mm, and 250 mm × 4.6 mm). The eluent was prepared from de-ionized water, purified with a "Synergy" water purification system of MILLIPORE, and from methanol bought from Fisher Scientific. The chemicals phenetole (98% pure, Fluka) and 4-tert-butylphenol (99% pure, Fluka) were used as delivered without further purification.

During some breakthrough experiments, fractions were collected using an auto fraction collector (GILSON FC203B). During the interacting mixture experiments, they were collected from 3 to 19 min, at 0.5 min interval for each fraction. For the pure component experiments, they were collected from 3 to 15 min at 0.5 min interval for phenetole, and from 2.5 to 14 min at 0.5 min interval for 4-tert-butylphenol. These fractions were analyzed with the same HPLC system, injecting 5 μL of each fraction, using the auto-sampler of the HPLC module.

3.1.2. Tennessee laboratory

The chromatograph used for these experiments is an HP1090 series II from Hewlett-Packard (now Agilent) equipped with a three-solvent delivery system (paths A, B, and C). The detection UV-cell has a 1.7 μL volume. The extra-column volume from the exit of the low pressure mixer to the column inlet is 0.90 mL. In all experiments, the temperature was fixed by the lab air-conditioner at 295 ± 1 K. The flow rate was kept constant at 1 mL/min. The mobile phase composition was: 67% methanol/33% water (v/v). This eluent mixture was prepared manually by measuring the appropriate volumes in two graduated cylinders (1000 mL for methanol and 500 mL for water), premixed with a magnetic stirrer bar, degassed in a sonicator during 1 min, and finally filtered before use on a surfactant-free cellulose acetate filter membrane, 0.2 μm pore size (Suwannee, GA, USA). All the chemicals were purchased from Fisher Scientific (Fair Lawn, NJ, USA). The purity of phenetole and 4-tert-butylphenol are 99% and 98%, respectively.

The chromatographic column used for the delta-shock experiments is a Zorbax 300StableBond-C18 column (150 mm × 4.6 mm; Agilent, Little Falls, DE, USA). Zorbax 300StableBond-C18 is made by chemically bonding diisobutyl n-octadecyl silane instead of the conventional dimethyl n-octadecyl silane to specially prepared, ultra-high-purity, 5 μm Zorbax particles (300 Å average meso-pore size). The manufacturer indicates that this is a "densely covered", "sterically protected" stationary phase.

Two more columns have been tested, both from Agilent. The first is a Zorbax 80StableBond-C18 column that has the same surface chemistry as the Zorbax 300StableBond-C18 but with 5 μm Zorbax particles of only 80 Å average meso-pore size. The second is a Zorbax Extend-C18 column, which has a different surface chemistry as it incorporates a bidentate organosilane (attached to the silica surface by two distinct silanol groups) with a double end-capping process. The bonded bidentate ligand is propylene-bridged bidentate-C18 silane. The 5 μm Zorbax particles have also in this case a 80 Å average meso-pore size.

During the frontal analysis experiment in which the two components interact, fractions were collected starting just before the elution of the front shock of 4-tert-butylphenol ($t_{start} = 8.08$ min) during 90 s. Each fraction was collected during 9 s and consisted of 11 droplets (e.g. a total volume of about 150 μL); 5 μL of each fraction was injected into the HPLC system SB300 and its elution recorded simultaneously at 282 and 287 nm. The UV signals of the individual overloaded band profiles of phenetole and 4-tert-butylphenol were transformed into concentration profiles from concentration–absorbance calibration curves obtained previously at 282 nm (with phenetole) and 287 nm (with 4-tert-butylphenol). The reference standard injections were those of known concentrations of 15 g/L solutions of phenetole and 70 g/L of 4-tert-butylphenol.

3.2. Overloaded pulse experiments

Overloaded pulse experiments were carried out at ETH Zurich using a methanol–water (65:35, v/v) mobile phase, i.e. slightly different from the mobile phase used for the frontal analysis experiments. Both pure components and the binary mixture were used. In the latter case the mixture composition was $c_1 = 3.5$ g/L and $c_2 = 9.0$ g/L, and the experiments were carried out by injecting increasing amounts of such a mixture, namely 5, 10, 20, 30 and 40 μL.

3.3. Frontal analysis experiments

Frontal analysis experiments were carried out following two different protocols, namely one where only one species was used, and another where both species were injected sequentially in the same experiment. Before each frontal analysis experiment, the column was rinsed and equilibrated with the eluent mixture for 30 and 60 min in Zurich and Tennessee, respectively. In the case of pure

phenetole, it was injected from $t = 0$ to 5 min, followed by the eluent for $t > 5$ min. Sample concentrations injected were 20, 18.3, and 16.2 g/L in Zurich, and 15 g/L in Tennessee. The same procedure was followed for pure 4-tert-butylphenol experiments, whose concentrations were 75, 67.5, and 60 g/L in Zurich, and 70 g/L in Tennessee.

The experiments leading to the occurrence of a delta-shock were divided in four successive steps. After an initial equilibration time, which might be reported in the experimental plots or not, the phenetole sample was injected, followed by 4-tert-butylphenol, and then finally by the mobile phase solution.

4. Preliminary results: proof of concept

In this section we report about a first series of experiments carried out in both laboratories on the same type of chromatographic column, the 15 cm Zorbax 300StableBond-C18 column provided by Agilent. First, overloaded pulse experiments, then frontal analysis experiments with pure compounds, and finally with the two species are presented and discussed. In the last case the plots of the frontal analysis experiments include 5 min injection of phenetole, then 5 min injection of 4-tert-butylphenol, followed by the mobile phase, but they do not include any equilibration period. The time when the interaction between the two species starts coincides with the start of the injection of 4-tert-butylphenol, i.e. $t = 5$ min, in the plots in this section.

4.1. Overloaded pulse experiments

In order to characterize the retention behavior of the species considered in this work, a series of overloaded pulse injections of the pure components was carried out (results not shown here). The results indicated that pure phenetole and 4-tert-butylphenol have anti-Langmuir and Langmuir adsorption behavior, respectively. Characteristic features of the chromatograms are a triangular shape with a sharp desorption front for phenetole and a sharp adsorption front for 4-tert-butylphenol while the retention times of the triangle apices increase and decrease with increasing amounts injected for phenetole and 4-tert-butylphenol, respectively.

Then a series of five binary overloaded pulse injections were carried out in Zurich, as described in Section 3.2 and illustrated in Fig. 4. It was observed that, beside a few impurities eluting earlier, the peaks of the two main components exhibit the same anti-Langmuir and Langmuir behavior as they exhibit when injected alone. As expected, the peaks get closer as the amount injected increases (see Fig. 4 of [7] that shows the same effect as found in the calculations), and the resolution becomes worse and worse. In the experiment with the largest amount injected, the two peaks have merged into one, but remarkably the positions of the leading front of phenetole and of the tail front of 4-tert-butylphenol are consistent with those observed in the other four experiments and with those predicted by the calculations carried out for a system following the mixed M2 generalized Langmuir isotherm [7].

4.2. Frontal analysis experiments with the pure compounds

A series of frontal analysis experiments with the pure components, reaching the concentration levels required for the delta-shock experiments, have been carried out, to be used as reference for the experiments resulting in a delta-shock. These experiments consisted in the adsorption of the chosen species at the concentration of interest, followed by its desorption after 5 min (see the detailed description in the previous Section 3.3).

Some of these experiments were carried out on the Zurich set-up, using a methanol–water (63:37, v/v) mobile phase. The results of two of these are illustrated in Fig. 5, one experiment

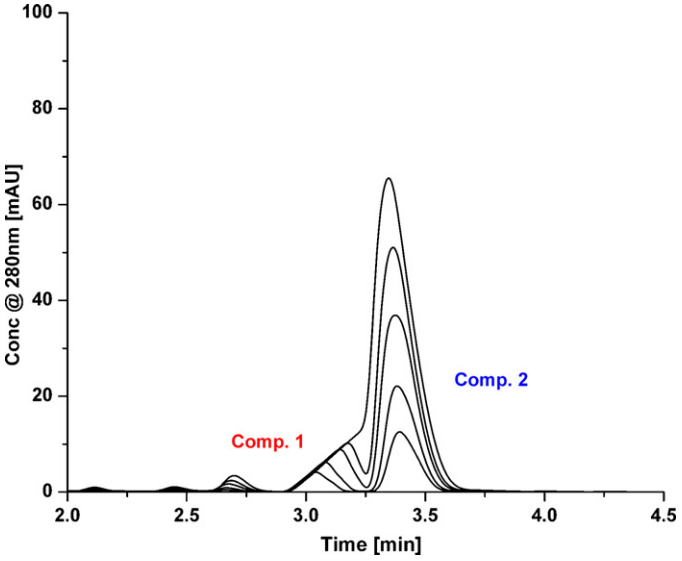

Fig. 4. Overloaded binary pulse experiments carried out in the Zurich laboratory, where the concentrations in the injected pulse are $c_1 = 3.5$ g/L and $c_2 = 9.0$ g/L, and the injected volumes are 5, 10, 20, 30 and 40 μL. UV absorbance is measured at 280 nm.

involving phenetole, component 1 (open red circles), and the other 4-tert-butylphenol, component 2 (open blue circles). Both sample solutions were injected for 5 min. However, for the sake of comparison with the profiles discussed in the next section and shown in the same figure, the elution profiles of 4-tert-butylphenol were plotted with a 5 min shift, so as they appear as if they had been injected between $t = 5$ and $t = 10$ min. The symbols give the concentrations measured for the samples collected during the experiments and analyzed off-line. The dashed lines in Fig. 5 serve only to guide the eye, and highlight the fact that, at these concentrations and under these conditions, the adsorption–desorption profiles of the two compounds overlap for more than 4 min. This shows that the requirements for the occurrence of a delta-shock will be met when the two components are injected sequentially, as shown below.

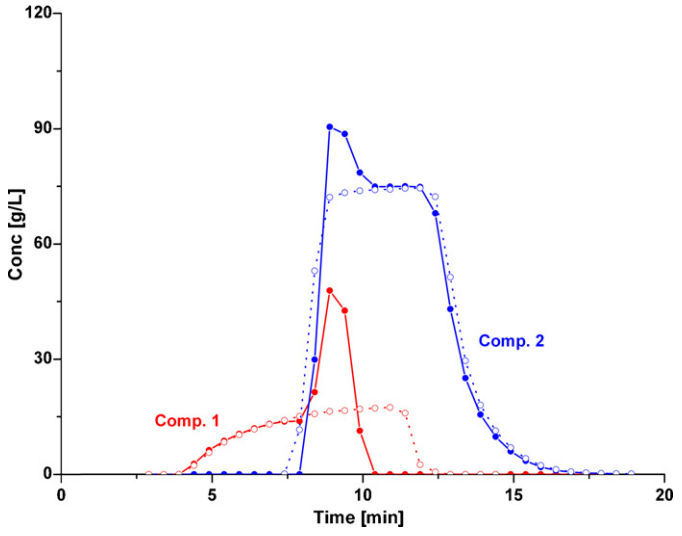

Fig. 5. Frontal analysis experiments carried out in the Zurich laboratory, with feed concentrations of $c_1 = 20.0$ g/L and $c_2 = 75.0$ g/L. Open symbols and dashed lines correspond to single component experiments. Closed symbols and solid lines correspond to the experiment where the two components are made interact. Red symbols and lines are phenetole; blue ones are 4-tert-butylphenol. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

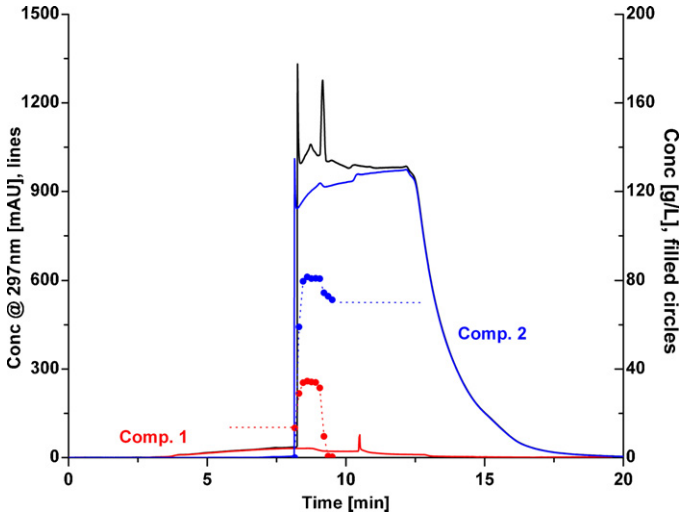

Fig. 6. Frontal analysis experiments carried out in the Tennessee laboratory, with feed concentrations of $c_1 = 15.0$ g/L and $c_2 = 70.0$ g/L. Colored solid lines correspond to single component experiments. Closed symbols, dashed lines and the black solid line correspond to the experiment where the two components are made interact. The solid lines are the UV absorbance profiles at 297 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Two experiments of the same type, one for each species, were carried out in the Tennessee laboratory under the conditions described above. They are illustrated in Fig. 6. The profiles of UV absorbance at 297 nm during elution of the two species are plotted (solid lines). This figure highlights the high sensitivity of the UV signal, and confirms the fact that the elution front of phenetole elutes after the adsorption front of 4-tert-butylphenol, as in the experiments illustrated in Fig. 5.

4.3. Frontal analysis experiments with interaction between phenetole and 4-tert-butylphenol

The closed symbols in Fig. 5 show the concentration of phenetole and of 4-tert-butylphenol in the fractions collected at the corresponding time during the experiment carried out by first injecting phenetole and then displacing it with 4-tert-butylphenol, the concentration of the former being 20 g/L and that of the latter 75 g/L. The solid lines connecting the closed symbols serve only to guide the eye.

The difference between the solid symbols and the open ones obtained in the corresponding pure component experiments demonstrates that there is indeed interaction between the two species. In other words, if there were no interaction, the closed and open symbols would overlap. To be more specific, on the one hand the elution (rear) front of component 1 is eluted earlier in this experiment than when it is pure; its band is in fact completely eluted after about $t = 10$ min whereas its elution takes $t = 12$ min when injected alone. This influence of component 2 on the elution of component 1 demonstrates the competition of 2 with 1. On the other hand, the adsorption front of component 2 is slightly but clearly (several seconds) retarded with respect to the front of the pure component 2. This influence of component 1 on the adsorption of component 2 indicates cooperation. Both effects are consistent with the binary adsorption isotherm (1), i.e. with the mixed generalized Langmuir isotherm called M2. It is worth pointing out that while the competition of species 2 towards species 1 is obvious, the cooperation effect of species 1 on species 2 is very small, as reflected by the rather small retardation of the adsorption front of species 2. This small effect illustrates well the great difficulties in finding a proper cooperative–competitive system for which the delta-shock could take place.

The outcome of the interaction described above is the clear formation of a peak eluting between $t = 8$ and 10 min, in which both species are present and enriched with respect to their initial concentrations. The maximum phenetole concentration is about twice that in the corresponding feed, whereas the maximum 4-tert-butylphenol concentration is almost 20% larger than in the feed. The amounts of components 1 and 2 eluted in this peak are roughly 25 and 4% of the whole, respectively. This difference is consistent with the observations made earlier that the elution of species 1 is more accelerated than the breakthrough of species 2 is delayed compared to their elution when these species are injected separately. This result is fully consistent with the theory [4].

The occurrence of the phenomenon observed in Fig. 5 (and in other similar experiments carried out in Zurich and not reported here) was confirmed in an independent experiment carried out in the Tennessee laboratory, and illustrated in Fig. 6. The filled symbols in this figure indicate the concentration of the two species in the samples collected, but in this case only during the elution of the delta-shock peak. The dashed lines serve to guide the eye and indicate the levels of the feed concentrations, namely $c_1 = 15$ g/L and $c_2 = 70$ g/L. As in the Zurich experiments, the delta-shock elutes in the time period during the breakthrough of 4-tert-butylphenol and the elution of the rear front of phenetole in the experiments in which these compounds were injected separately (see the profiles of the UV signal given by black lines in the same Fig. 6). Moreover, the two species are significantly more concentrated in the peak itself as expected, and the amount of phenetole eluting in the peak is larger than that of 4-tert-butylphenol, exactly as in the case of the Zurich experiment shown in Fig. 5.

4.4. Discussion

We believe that the peaks reported and discussed above represent the experimental manifestation of a delta-shock, i.e. a new phenomenon in nonlinear chromatography that had never been observed earlier, or that had neither been recognized nor understood as such if ever observed.

Nevertheless, let us compare the band profiles recorded in the experiments involving interaction between phenetole and 4-tert-butylphenol made at ETH Zurich and discussed in the previous section with the profiles obtained as numerical solution of the equilibrium dispersive model using a large number of theoretical plates (typically one thousand) and the binary isotherm in Eq. (1) (see Fig. 2). There are obvious differences between the experimental and the calculated profiles that need to be discussed, even though the simulations do not refer to the same conditions and are made using a model isotherm, i.e. not necessarily the one corresponding to the experimental system. In particular the experimental peak is much broader than the calculated one, i.e. about 2 min instead than about two-tenths of a minute.

In the calculated profiles, the delta-shock is a traveling spike that exhibits a self-sharpening behavior on both sides. Therefore its shape and breakthrough position change with the column efficiency, i.e. with the intensity of the dispersive effects when the number of theoretical plates becomes unrealistically small (see Fig. 12 in [4]). Although it would in principle be possible to tune the number of theoretical plates used in the simulations in such a way to better describe the rather broad experimental peak of Fig. 5, this would not lead to a physically meaningful number of theoretical plates and would lead to inconsistencies in the description of other experiments, e.g. the overloaded peak profiles in Fig. 4.

The model predicts that very high concentrations are reached in the column when the delta-shock occurs [4]. In principle this mech-

anism might drive the system beyond the solubility limit, leading to supersaturation, and might trigger the nucleation of crystals of the solutes. This might lead to complex, unpredictable band broadening. However, we have neither evidence that this happens in our experiments nor any proof that it does not. It is certain, however, that both species are significantly enriched within the spike with respect to their initial concentrations.

Another problem exhibited by this first series of experiments was the rather irregular and poorly reproducible UV signal, as observed in Fig. 6 and in the other experiments of the series not shown here.

We have carefully verified our experimental protocols, as well as the chemistry of the system, and we have concluded that phenetole slowly reacts and degrades when dissolved in the mobile phase, thus forming degradation products. The corresponding impurity is difficult to detect because it co-elutes with phenetole in the analytical column. It represents however a major disturbance in the experiments leading to a delta-shock, also because its concentration varies in the different experiments with the different aging of the feed solution. Based on this observation, all the experiments carried out in the second experimental campaign, which is presented and discussed in the next section, have been carried out using always a fresh phenetole solution, i.e. a solution that had not been prepared more than a few hours before its use, certainly not the day before. As it will be discussed, such measure has been decisive in improving quality and reproducibility of the experimental results.

5. Main results: delta-shock phenomenology

In this section we present a rather comprehensive set of results about the delta-shock observed with the system consisting of phenetole and 4-tert-butylphenol in methanol–water 63%/27% (v/v) on three Zorbax 300StableBond-C18 columns of different lengths (5, 15 and 25 cm long, with the same internal diameter of 0.46 cm) at 294 ± 1 K. All experiments presented here led to interaction between the two species and were carried out at a flow rate of 1 mL/min. All of them were repeated from two to five times, always obtaining identical results.

In the 5 and 15 cm columns, the frontal analysis experiments were carried out as follows, and are plotted accordingly; after 5 min equilibration (included in the figures that follow), phenetole was injected for 15 min, followed by 4-tert-butylphenol, also injected for 15 min, followed finally by the mobile phase solution. In the case of the 25 cm column, the injection times of the two species were chosen to be 20 min. The time when the interaction between the two species starts coincides with the start of the injection of 4-tert-butylphenol, i.e. $t = 20$ min in the first two columns and 5 min later in the longest column. For the sake of clarity and in order to align the start time of the interaction, the equilibration period is not included in the plots referring to the 25 cm column. It is worth noting that it is therefore possible to compare the positions in the short columns of neither the adsorption fronts of phenetole nor the elution fronts of 4-tert-butylphenol with their positions in the 25 cm column.

The analysis of the effect of concentration and of column length will be based on UV absorbance at 305 nm, i.e. a wavelength that results to be most suitable for this study. Concentrations will be indicated as percentage values of the reference concentration levels, namely those applied in the experiments illustrated in Fig. 5, i.e. $c_1 = 20$ g/L and $c_2 = 75$ g/L, which are identified as 100% in the following. We have not attempted to calibrate the UV signal, as this was not necessary for the purpose of this work. We will also consider the whole UV absorption spectra in order to establish the evidence of the occurrence of the delta-shock.

5.1. Effect of concentration

In this section two sets of experiments are presented and discussed. The first set, illustrated in Fig. 7a, refers to experiments in the short 5 cm column at concentrations ranging from 75% to 120%, whereas the second set, shown in Fig. 7b, consists of experiments carried out in the long 25 cm column in a low concentration range, namely from 2% to 100%. Note that the UV absorbance profiles shown in these and in the next figures account for the concentrations of both species; in other words they represent a weighted average of the concentrations of the two species, where the unknowns weights follow from the different absorbance of the two species. It is also worth noting that UV absorbance is not linear in the species concentrations in the very large range of compositions explored here, as it is evident in Fig. 7b. The UV signal becomes completely saturated at an absorbance of about 3300 mAu, as it is evident in Fig. 7a.

All experiments in Fig. 7a exhibit a rather evident peak that separates the two plateaus corresponding to the inlet concentrations of species 1 and 2 and reaches enrichments well above these. The peak's size increases with increasing concentration, as expected based on the observations made about Eq. (4) and on the theory [4]. Looking at the inset, it can be observed that a fully developed peak such as those corresponding to the two highest concentrations in Fig. 2 is attained at concentrations of 100% or higher. However, at concentrations of 105% or higher the UV signal is saturated and the delta-shock appears truncated at about 3300 mAu. The delta-shock width, if not the height due to UV absorbance saturation, increases with increasing concentration; all delta-shocks are eluted in no more than half a minute. At concentrations below 100%, when zooming in, the peaks appear much less well defined (see inset), and one is reluctant to assimilate them to what observed at 100%. They bear however some resemblance to the very weak delta-shock shown in Fig. 2 ($c_1 = 5$ g/L and $c_2 = 18.75$ g/L).

UV saturation makes it impossible to measure the concentration in the delta-shock's spike. Nevertheless, this can at least be estimated by considering just 4-tert-butylphenol, i.e. the component exhibiting the higher absorbance and achieving the higher concentration levels, and noting that at 100% concentration UV absorbance is about 600 mAu. This implies that at more than 3000 mAu, i.e. where the spike has its peak, its concentration is at least five times larger, i.e. 750 g/L.

The range of concentrations covered in the experiments shown in Fig. 7b extends to very low values and allows for a thorough comparison with Fig. 2. At the lowest concentration of 2% there is baseline separation, as in the case of the lowest concentration in Fig. 2: the elution front of phenetole has not yet collided with the breakthrough front of 4-tert-butylphenol. At 10% concentration, there is neither baseline separation nor enrichment with respect to the feed concentrations; this is similar to the second and third lowest concentrations in Fig. 2. At concentrations from 50% to 95% peaks similar to those below 100% concentration in Fig. 7a are observed. At 100% concentration a fully developed delta-shock is finally observed (not entirely shown here).

Thus summarizing, we can divide the concentration range in three intervals. Up to at least 10%, phenetole and 4-tert-butylphenol do not interact yet and their elution takes place as if they were alone in the column. From at least 50% to 95%, an enriched peak above the feed concentration plateaus can be observed; it seems reasonable to explain this peak as a manifestation of the interaction that leads theoretically to a delta-shock, but under these conditions and in the presence of dispersion effects the delta-shock is too weak to generate a very sharp peak. At concentrations of 100% or more (we have checked up to 120%) a fully developed delta-shock is obtained, which is characterized by a very sharp peak and follows the theoretically expected behavior as concentration increases.

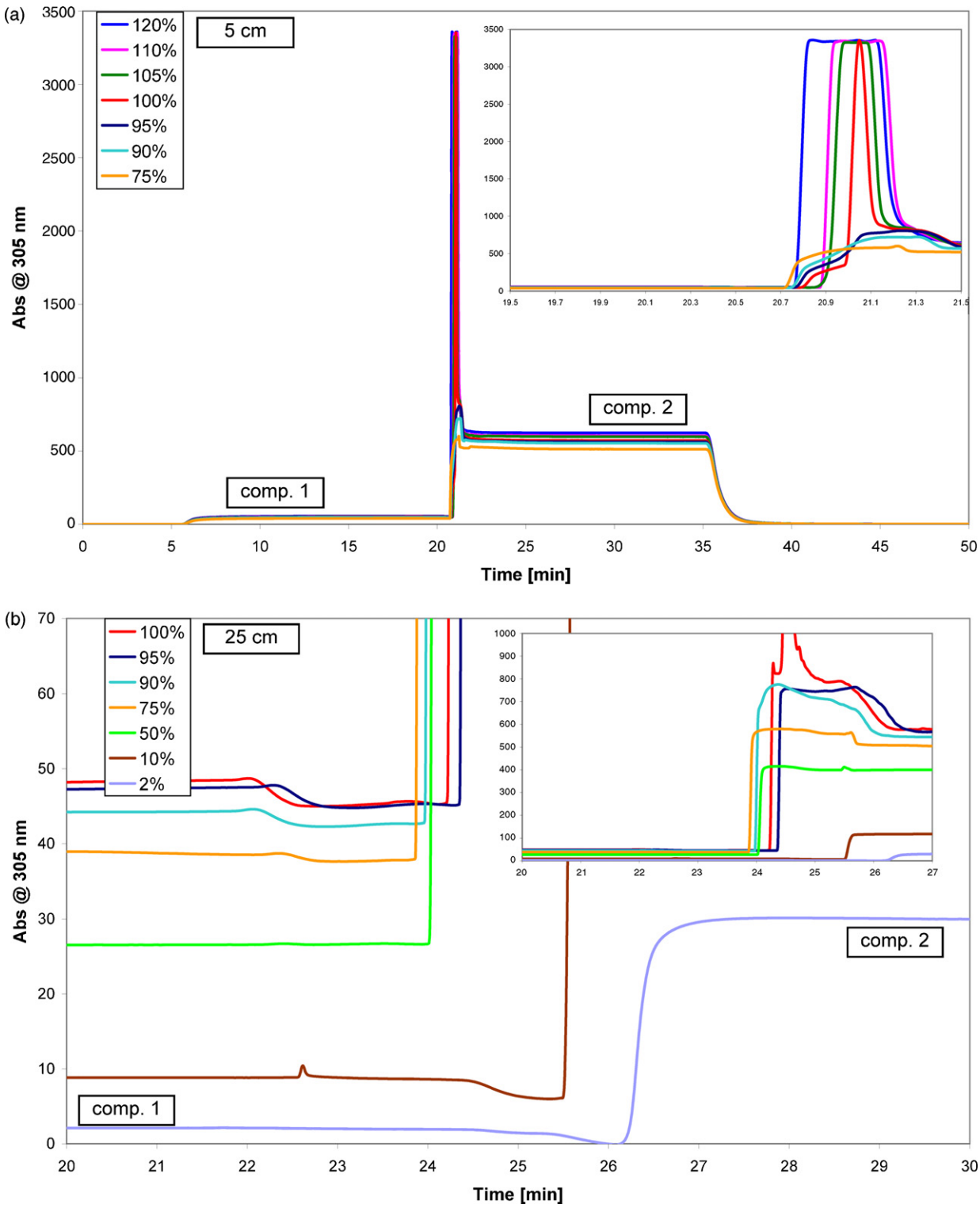

Fig. 7. Effect of feed concentration on the interaction between phenetole (comp. 1) and 4-tert-butylphenol (comp. 2) in frontal analysis experiments (Zurich laboratory). (a) 5 cm column, high concentration range; (b) 25 cm column, low concentration range.

Two final remarks are worth making. The first remark refers to the shape of the peak in the experiment at 100% concentration (see Fig. 7a, inset), which is in this case clearly different from that exhibited by the peaks obtained at higher concentration. Both before and after the main sharp peak, the UV profile reaches two plateaus, which are above the feed concentrations of the two species; they elute for a time, namely between 0.2 and 0.3 min, which is comparable to the elution time of the main peak itself, i.e. about 0.2 min. We do not have an explanation for this effect, which is common to all three columns, but is not so evident or not at all exhibited at higher concentration.

The second remark refers to the two sharp fronts exhibited by all delta-shocks' spikes. It is well known that sharp fronts in nonlinear chromatography exhibit a constant pattern behavior, which is called shock layer, when they separate two constant states and propagate through long enough columns [11–13]. Although there

is an important difference, namely that while the states on either side of the spike are well defined the spike's height keeps increasing as the spike propagates, we think that the relationship between the delta-shock and the shock layer should be explored more than what was possible in this work.

5.2. Evidence of delta-shock

Looking at UV absorbance at one wavelength only is somehow arbitrary and somewhat limited in this case. The analysis of the whole spectrum provides much richer information and allows clarifying the difference between the behavior observed at concentrations smaller and larger than 100%.

In Fig. 8 the time-resolved UV spectra, in the wavelength range from 290 to 325 nm, are shown for four different experiments carried out in the three different columns, namely the 5 cm column in Fig. 8a and b, and the 15 and 25 cm columns in Fig. 8c and d, respectively, and at two different concentrations, i.e. 90% in Fig. 8a and 120% in Fig. 8b–d.

In all cases and at all wavelengths, one can see the two plateaus corresponding to the feed concentration of component 1, on the left hand side, and of component 2, on the right hand side. As expected the absorbance intensity varies a lot between the short wavelengths, corresponding to very high absorbance, and the long ones, where absorbance is very low. It is worth mentioning that the UV spectra of the two compounds are given by sections of the plots in Fig. 8 at constant time, namely with reference to Fig. 8a at $t = 15$ min for phenetole and at $t = 30$ min for 4-tert-butylphenol.

Let us consider the two experiments in the short column shown in Fig. 8a and b (the same experiments are also shown in Fig. 7a). The difference is striking. At 90% concentration the two plateaus are separated just by a ripple, which is present at all wavelengths but reaches absorbance intensities that are only slightly above those of the concentration plateaus. On the contrary at 120% concentration the two plateaus are separated by a high ridge that at all wavelengths saturates the UV signal. This is consistent with the theory of the delta-shock that demonstrates that in the traveling spike the concentrations of the two species are virtually unbound, which should indeed imply that at all wavelengths the UV signal reaches its upper bound.

We have observed this behavior, i.e. the high ridge, in all experiments carried out in all three columns at concentrations of 100% or higher, as shown for two specific cases in Fig. 8c and d. On the contrary, in all columns at concentrations between 75% and 95% only the ripple has been observed, i.e. even in the 25 cm column where one would expect that the traveling spike has had the time to develop fully. We argue that the high ridge shown in Fig. 8b–d and observed in all the experiments at concentrations of 100% or more is the undisputable fingerprint of a delta-shown. For the sake of simplicity, in all the experiments presented in the next section we will show conventional chromatograms reporting the UV absorbance at 305 nm, but in all cases the time resolved UV spectra (not shown here) exhibit the typical fingerprint of a delta-shock.

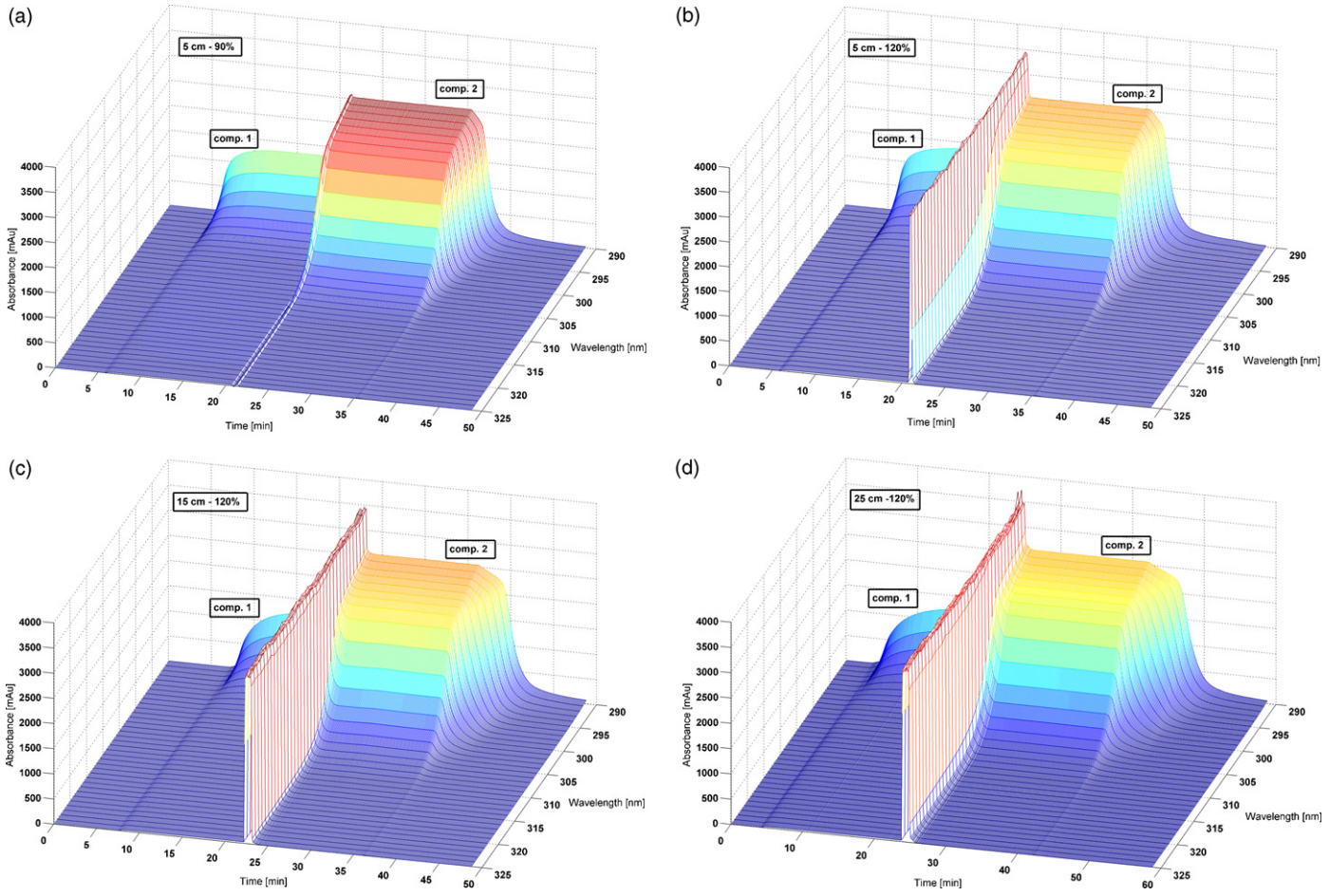

Fig. 8. Time resolved UV spectra of frontal analysis experiments (Zurich laboratory) exhibiting or not the characteristic fingerprint of a delta-shock. (a) 5 cm column, 90% concentration; (b) 5 cm column, 120% concentration; (c) 15 cm column, 120% concentration; (d) 25 cm column, 120% concentration. Comp. 1 is phenetole; comp. 2 is 4-tert-butylphenol.

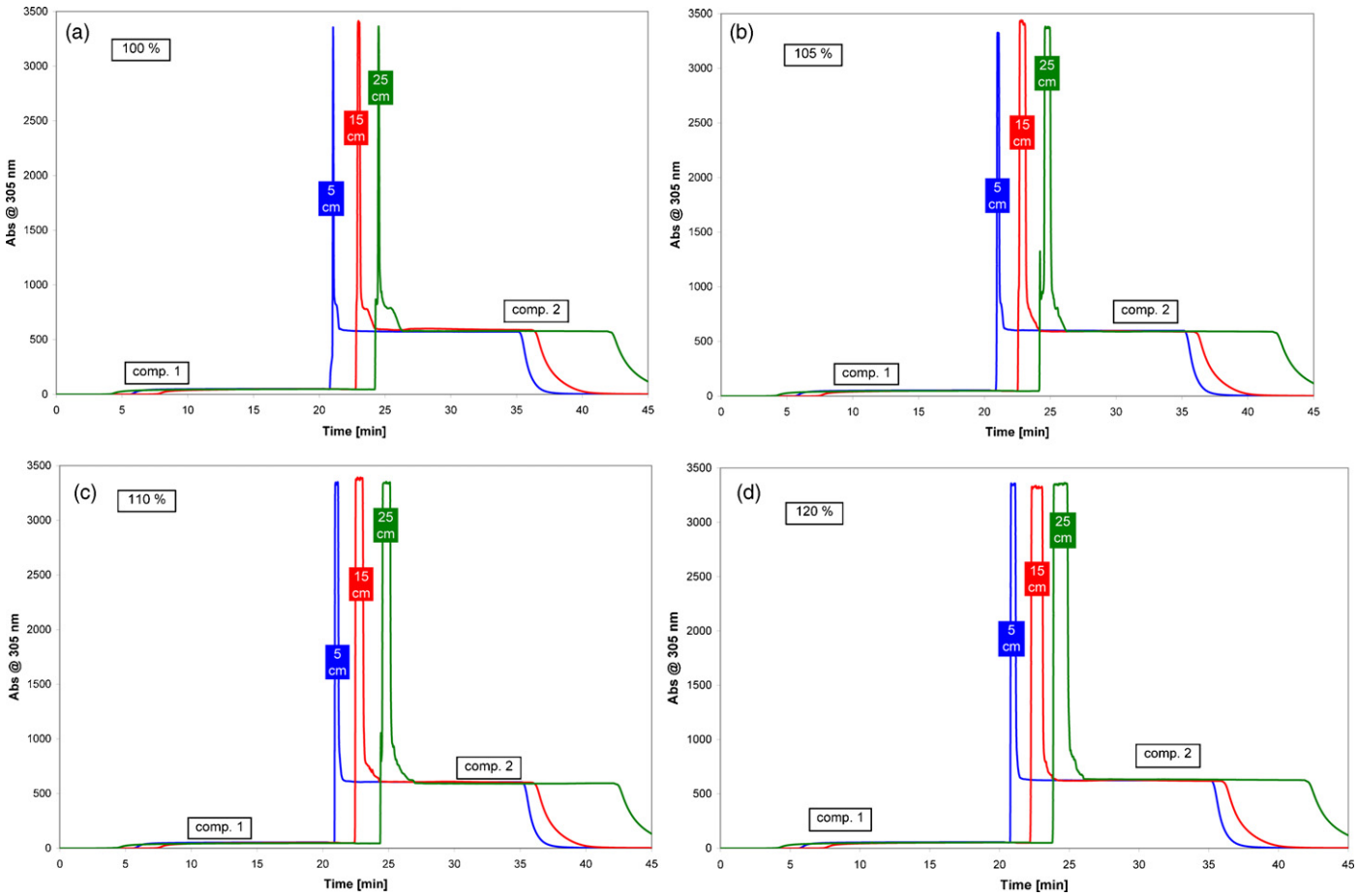

Fig. 9. Effect of column length and feed concentration on the delta-shock (experiments in the Zurich laboratory). (a) 100% concentration; (b) 105% concentration; (c) 110% concentration; (d) 120% concentration. Blue lines are for the 5 cm column, red ones for the 15 cm column, and green ones for the 25 cm column. Comp. 1 is phenetole; comp. 2 is 4-tert-butylphenol. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

5.3. *Effect of column length*

In the previous section, we have demonstrated that delta-shocks occur under the same conditions in all three columns of different lengths used in this study. This proves that such a phenomenon is generic for phenetole and 4-tert-butylphenol in methanol–water on the packing material used in Zorbax 300StableBond-C18 columns.

Using columns of different lengths allows also for an experimental check of the scaling rules expressed by Eqs. (3) and (4). The former equation states that at constant flow rate and for the same feed concentrations the elution time, or retention time, of the delta-shock scales linearly with the column volume. The latter demonstrates that under the same conditions also the size of the spike scales linearly with the column volume. The experiments aimed at verifying these properties are illustrated in Fig. 9; each of its four parts refers to a different concentration, namely 100%, 105%, 110% and 120%, and shows the corresponding chromatograms obtained in the three different columns, as indicated. Due to the different procedure applied to the 25 cm column, the corresponding chromatograms are shifted backwards by 5 min, so as in all these plots the start time of the interaction between the two species that generates the delta-shock is the same for all experiments and equal to 20 min.

In this context the saturation of the UV signal that occurs in most of these experiments is a problem, because it makes it difficult or impossible to obtain precise measurements of the retention time and of the spike's size. Therefore, we use the breakthrough time of the spike, which is rather well defined by the spike's steep front, as an approximation of the retention time, and the width of the spike at 1700 mAu, i.e. at half of the signal saturation level, as an estimate of its size.

The progression of the retention times and of the spikes' sizes with column length according to theory is rather obvious in Fig. 9d, where the experiments at 120% concentration are shown. There is some tailing in the rear front of the delta-shock, particularly in the two longer columns, for which we do not have a justification yet. A similar qualitative behavior is observed also in the case of the concentrations 105% and 110% (see Fig. 9b and c), though in these cases comparing the spikes' sizes for the two longer columns is really difficult. It should also be noticed that the absorbance profiles in the 25 cm column exhibit in both cases a tiny but evident peak before the main peak; also this could not be clarified yet. Finally, the experiments at 100% concentration shown in Fig. 9a are the least consistent with the theory in terms of delta-shock's shape, size and retention time (the retention time in the 15 cm column is clearly inconsistent with those in the 5 cm and in the 25 cm columns). We have already discussed the shape of the 100% peak in the 5 cm column with reference to Fig. 7a; the shape of the 100% peaks in the other two columns exhibits the same qualitative features, which are anyhow unclear at this point in time.

6. Discussion and conclusions

The first and foremost objective of this work, its main result and its novelty are the demonstration of the experimental occurrence of the delta-shock. This has been indisputably achieved for the binary system phenetole and 4-tert-butylphenol in methanol–water

(about 2:1, v/v) on a Zorbax 300StableBond-C18 column from Agilent. The most striking evidence is provided by the time resolved UV spectra shown in Fig. 8, whose characteristic high ridge separating the plateaus of the components can be considered as the fingerprint of a delta-shock.

These results demonstrate that the delta-shock predicted by the theory of nonlinear chromatography is a real physical phenomenon. We are convinced that this result is general and that delta-shocks should be observed in all binary systems exhibiting an adsorption behavior accounted for by an isotherm equation like Eq. (1), in a sufficiently wide range of concentrations, or by a similar competitive-cooperative adsorption isotherm.

In this context two more columns were tested with the same system. The delta-shock was observed also with a Zorbax 80StableBond-C18 column, which differs from the previous one only by its smaller pore size. However, neither the delta-shock nor its associated cooperative–competitive behavior were observed on a Zorbax Extend-C18 column, which has a completely different surface chemistry. These results suggest that the cooperative–competitive character of the system is related to the surface energy of the adsorbent used in the column and not to the differences in pore size distribution. Unfortunately, not many systems exhibit an adsorption isotherm of type M2 such as Eq. (1) and this seems to limit the extent to which the delta-shock phenomenon can be exploited in chromatography research and applications.

We have shown that many features of the experimental delta-shock are fully consistent with the theory, particularly the effect of feed concentration and that of column length. However, we have also highlighted experimental observations that so far lack an explanation. This is not at all surprising considering the underlying complexity of a system exhibiting the delta-shock behavior, and the fact that this is the first study ever where such a phenomenon has been observed. We believe that it will be worth studying delta-shocks in nonlinear chromatography even further in order to address all the open issues above. This promises to be stimulating certainly from a fundamental point of view and possibly also from an applicative perspective.

Notation

c_i	fluid phase concentration of component i
h_i^{ds}	hold up of component i in the traveling spike at the column outlet
H_i	Henry's constant of component i
K_i	adsorption equilibrium constant of component i
n_i	adsorbed phase concentration of component i
Q	volumetric flow rate
t	time
t_R^{ds}	elution time of the delta-shock
V	column volume
ε	overall void fraction

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