A closer study of overloaded elution bands and their perturbation peaks in ion-pair chromatography

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Abstract

There is strong renewed interest in ion-pair chromatography (IPC) because of its great importance for separating new-generation biosimilar pharmaceuticals such as oligonucleotides. Due to the complexity of the IPC process, its mathematical modeling is challenging, especially in preparative mode. In a recent study, Leško et al. (2021) developed a mathematical model for predicting, with good accuracy, overloaded concentration profiles for sodium benzenesulfonate, describing how the overloaded solute concentration profiles change from Langmuirian to complicated U-shaped, and then back again to Langmuirian profiles, with increasing concentration of the ion-pair reagent in the mobile phase. This study identifies and explains the underlying mechanism generating these complex peak shapes and band-shape transformations; this was only possible by visualizing and modeling the underlying equilibrium perturbations that occur upon injection in preparative IPC. In the 2021 study, the model was derived based on the concentration profiles obtained using a conventional UV detector principle, so the concentration gradients and perturbation zones of the mobile-phase components were not visualized. In this study, the necessary mechanistic information was obtained via complementary experiments combining two detection principles, i.e., refractive index detection and UV detection, with modeling efforts. The models correctly described the invisible equilibrium perturbations and how these formed internal gradients of the mobile-phase components. The models also explained the complex overloaded solute-band deformations reported in the recent study. In addition, a rule of thumb was developed for predicting experimental conditions that could result in deformed solute elution profiles and/or for avoiding these deformations. The latter is crucial for the practical chromatographer, since such U-shaped solute-band profiles are undesirable in preparative separation due to the broader elution zones, resulting in lower productivity than that of normal band shapes.

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1. Introduction

In ion-pair chromatography (IPC), a lipophilic component called the ion-pair reagent (IPR) is added to the mobile phase for the separation of polar or charged solutes such as inorganic and organic ions, proteins, peptides, oligonucleotides, and basic drugs in reserved-phase liquid chromatography systems [1–4]. Interest in IPC has been increasing greatly in recent years, as efficient separations of oligonucleotide and peptide pharmaceuticals require IPR in the mobile phase [5–7].

IPC has been under development for almost 40 years, but there is still no single universal mechanism that fully explains this kind of chromatography [1–4,8,9]. Generally, two main mechanisms are considered: (i) ion-pair formation occurs in the mobile phase and thereafter the complex binds to the stationary phase; and (ii) ion-pair formation occurs between the solute and already adsorbed IPR on the stationary phase. To describe these mechanisms, stoichiometric, electrostatic, and combined stoichiometric-electrostatic models have been proposed [2–4,10,11]. Moreover, most studies of IPC concern the analytical mode, with only a few covering overloaded elution profiles and their modeling [12]. This modeling is a challenge because the nonlinear adsorption of the ion-pair solutes must also be considered in the models, and doing so is very difficult. Nevertheless, due to the observed growth in the use of IPC, and the common band deformation occurring in it [13–16], such models as well as a workable modeling process are highly desirable. Work on nonlinear IPC and the development of tools for
predicting overloaded concentration profiles are needed and crucial for the future.

Recently, we developed a multilayer electrostatic modified adsorption model describing the adsorption process in IPC separations, particularly for modeling and predicting overloaded concentration profiles in IPC [17]. The model was proposed and validated based on concentration profiles obtained for sodium benzenesulfonate salt (SBS) recorded using a UV detector. The agreement between experimental and simulated profiles was very good in the 0–10 mM range of the IPR concentration in the mobile phase. Using the model, one can follow the peak evolution from Langmuirian to U-shaped, and then back to Langmuirian again, without changing anything in the equations or model parameters. Hereafter, we refer to these complex band shapes as “unusual,” meaning that their shapes are unusual, not that they occur infrequently.

The model in the previous study was derived and validated using only concentration profiles recorded with a UV detector [17]. To explain the complicated band-shape changes, i.e., the change of the band shape from Langmuirian to U-shaped, and then back to Langmuirian again, we must also investigate the complicated perturbations of the equilibria that generate internal gradients of the IPR and other mobile-phase components. Nevertheless, these perturbation gradients are not detected using conventional UV detection, as in the former study; with this detection principle, we could only visualize how the solute-band shapes appeared and not the underlying events causing these deformations. For a complete understanding of the effects, it is also necessary to visualize the perturbations of the mobile-phase components (e.g., IPR and salt) generated by the injection of the solute and to visualize how their zones travel along the column and form internal gradients. In this study, we combined two detection principles, i.e., refractive index (RI) and UV detection, with modeling efforts to visualize the perturbations of the mobile-phase components in order to provide the necessary complementary information, so that the models can be validated and used in the fundamental investigation of IPC separations.

Additives are often added to the eluent to improve the separation. One common additive is an amine added to the eluent [16] to improve the peak shape or reduce the retention of amine solutes; in this case, the additive operates by competing with the solutes for the limited adsorption sites on the stationary-phase surface. In this study, we are considering IPR, which also adsorbs to the surface but instead increases the retention.

Let us now consider a separation system: when the mobile phase contains an adsorbing additive, a certain amount of additive will be adsorbed to the stationary phase at equilibrium. Injection of a sample with surplus of the additive perturbs the equilibrium of the additive, generating a peak called the primary perturbation peak. The primary perturbation peak migrates along the column at a characteristic speed depending on the strength of the additive adsorption to the stationary phase and the amount of additive in the mobile phase. Primary perturbation peaks will also be generated if solute is introduced into the column, because the solute will perturb the equilibrium of the additive [18–21]. In preparative mode, a large amount of solute is generally injected and the resulting high-concentration elution bands depend essentially on the adsorption strength of the solute in the separation system. The same preparative injection is done when the mobile phase contains an additive, generating a large primary perturbation peak that may deform the solute elution profile [20,21]. For additives that compete with the solute for the available surface on the stationary phase, the prerequisites for the deformation are that the additive should be retained more strongly than or as strongly as the solute is in an eluent not containing the additive and that the solute should elute near the primary perturbation peak [20]. These deformations are especially serious for large solute loads due to the creation of large perturbation peaks. However, the above rule of thumb is not validated for IPC, because during its theoretical development no ion-pair mechanism was assumed [20]. Moreover, the perturbation peaks are usually difficult to detect using UV detectors due to the lack of chromophores. In a recent study, we observed deformed peaks; here, as mentioned, we will visualize the perturbation peaks using an RI detector and systematically investigate how they affect the solute elution profile.

This study experimentally and theoretically investigates and explains the complex band deformations reported in earlier publications [17]. For this purpose, additional experiments were conducted using an RI detector, which enabled registration of the perturbation peaks. We also aim to develop a rule of thumb that can predict under what experimental conditions we can expect peak deformation in IPC.

2. Theory

To correctly predict the overloaded elution band, a dynamic column model connected with an appropriate description of the adsorption/desorption process must be used. In a recent study, we proposed a mathematical model for predicting overloaded concentration profiles in IPC [17]; this model will be used here. Below, we briefly describe the model; for more details, see Leško et al. [17].

The assumptions used in formulating the equations are the following. The lipophilic ions of the IPR adsorb to the stationary phase and thus dynamically create charged active sites. These active sites on the first layer of IPR serve as active exchange sites for the solute ions. Apart from the solute ions, anions coming from the salt added to the mobile phase can also adsorb to the first layer. The model also assumes that the next layer of solute can form on the already adsorbed solute layer. In other words, we have up to three layers on the stationary phase: a first layer of IPR; a second layer on the IPR layer of solute or of anions coming from the salt added to the mobile phase; and a third layer of solute on the already established IPR–solute complex. Moreover, based on electrostatic theory, it was assumed that the affinity of IPR to accumulate on the stationary phase decreases due to the repulsion of positively charged IPR ions from the positively charged first layer of IPR. Analogously, the model assumes that the adsorption affinity of solute and of ions from the salts increases, due to electrostatic attraction, with the increasing concentration of IPR on the stationary phase.

The ratio of the free adsorption sites to the saturation capacity Θ0 can be expressed by the following equation:

\[ \Theta_0 = 1 - \Theta_H - \Theta_{HE} - \Theta_{HE_2} - \Theta_{IX} \]

where \( \Theta_H \), \( \Theta_{HE} \), \( \Theta_{HE_2} \), and \( \Theta_{IX} \) are the ratios of the sites occupied by: \( H \), the first layer of \( E \) on the \( H \) layer, two layers of \( E \) on the \( H \) layer, and \( X \) on the \( H \) layer to the saturation capacity, respectively. The electrostatic contribution is handled by modifying the equilibrium constant \( T_e \) as follows:

\[ T_H = K_{HE} \exp (-S_1 \Theta_H) \]

\[ T_{HE} = K_{HE} \exp (S_2 \Theta_H) \]

\[ T_{IX} = K_{IX} \exp (S_3 \Theta_H) \]

and

\[ T_{HE_2} = K_{HE_2} \]

The constant \( K \) is the equilibrium constant of the adsorption process for each considered case. To model the repulsion/atraction of the ions, constants \( S_1, S_2 \), and \( S_3 \) are introduced.
The kinetic adsorption process of the fractional coverage for each layer is described using the Langmuir or the electrostatic modified Langmuir adsorption isotherm; see Eqs. (2)–(6) below. We start by introducing $C_{H}$, $C_{E}$, and $C_{X}$, i.e., the mobile-phase concentrations of $H$, $E$, and $X$, respectively. We also define the rate constant of the adsorption process as $k_{s}$. Note that the equilibrium constants could be calculated as $K_{i} = k_{j}/k_{i}$. 

For $\Theta_{i}$, only the IPR adsorb:

$$\frac{d\Theta_{0}}{dt} = -(k_{h}C_{H}\Theta_{0} - k_{j2}\Theta_{H}) = -k_{h} \left( C_{H}\Theta_{0} - \frac{\Theta_{H}}{T_{H}} \right). \quad (2)$$

For $\Theta_{HE}$, we must consider that $H$, $E$, and $X$ adsorb:

$$\frac{d\Theta_{HE}}{dt} = (k_{HE}C_{H}\Theta_{E} - k_{HE2}\Theta_{HE}) = k_{HE} \left( C_{H}\Theta_{E} - \frac{\Theta_{HE}}{T_{HE}} \right). \quad (3)$$

For $\Theta_{HE2}$, the adsorption of $E$ needs to be considered,

$$\frac{d\Theta_{HE2}}{dt} = (k_{HE}C_{E}\Theta_{HE} - k_{HE3}\Theta_{HE2}) = k_{HE} \left( C_{E}\Theta_{HE} - \frac{\Theta_{HE2}}{T_{HE2}} \right), \quad (4)$$

and for $\Theta_{HEX}$, we need to consider the adsorption of $X$,

$$\frac{d\Theta_{HEX}}{dt} = k_{HE} \left( C_{X}\Theta_{HE} - \frac{\Theta_{HEX}}{T_{HEX}} \right). \quad (5)$$

The concentration changes of the adsorbed compounds ($q_{i}$) are:

$$\frac{dq_{H}}{dt} = q_{0} \frac{d}{dt} \left( \Theta_{H} + \Theta_{HE} + \Theta_{HE2} + \Theta_{HEX} \right), \quad (6)$$

$$\frac{dq_{E}}{dt} = q_{0} \frac{d}{dt} \left( \Theta_{HE} + 2\Theta_{HE2} \right), \quad (7)$$

and

$$\frac{dq_{X}}{dt} = q_{0} \frac{d}{dt} \Theta_{HEX}. \quad (8)$$

Using steady-state approximation for Eqs. (2)–(9) and Eq. (1a), we obtain:

$$\Theta_{0} = \left( 1 + T_{H}C_{H} + T_{HE}C_{HE} + T_{HE2}C_{HE2} + T_{HEX}C_{HEX} + T_{H}T_{HE}C_{HE} + T_{H}T_{HE2}C_{HE2} + T_{H}T_{HEX}C_{HEX} \right)^{-1}. \quad (9)$$

The rest of the fractional surface coverage can be expressed as:

$$\Theta_{H} = C_{H}T_{H}\Theta_{0}, \quad (10)$$

$$\Theta_{HE} = C_{E}T_{HE}\Theta_{H}, \quad (11)$$

$$\Theta_{HE2} = C_{E}T_{HE2}\Theta_{HE}, \quad (12)$$

and

$$\Theta_{HEX} = C_{X}T_{HEX}\Theta_{HE}. \quad (13)$$

The kinetic adsorption model was solved using the transport dispersive model [25]:

$$\frac{\partial C_{i}}{\partial t} + u \frac{\partial C_{i}}{\partial x} + \frac{1}{\varepsilon_{t}} \frac{\partial q_{i}}{\partial t} = D_{t} \frac{\partial^{2} C_{i}}{\partial x^{2}}, \quad (14)$$

where $u$ (m s$^{-1}$) is the superficial velocity, $\varepsilon_{t}$ is the total porosity, and $D_{t}$ (m$^{2}$ s$^{-1}$) is the axial dispersion coefficient. Initial conditions for solving this transport dispersive model are found in the previous study [17]. The above-formulated model is nonlinear and can only be solved using numerical methods. We used the method of orthogonal collocation on finite elements (OCFE) [22–24]. To solve the sets of ordinal differential equations resulting from the discretization of spatial derivatives with the OCFE method, the CVODE solver, available in the SUNDIALS package [25], was used.

3. Experimental

The experiments were similar to those described in detail in a previous study [17]. Below we present only the basic information about the chromatographic system and the additional experiments conducted, as well as deviations from the previous study.

3.1. Chemicals and column

The mobile phase consisted of gradient-grade MeOH purchased from Fisher Scientific (Loughborough, UK) and deionized water with a resistivity of 18.2 MΩ cm delivered from a Milli-Q Plus 185 water purification system (Merck Millipore, Darmstadt, Germany). Constant ionic strength was maintained using sodium chloride. The IPR used in this study was tetrabutylammonium bromide (TBuABr), while the solutes were SBS, 2,6-naphthalenedisulfonic acid disodium salts (S26NS), and p-toluensulfonic acid monohydrate (SPS). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The column was a 100 × 2.1-mm XBridge C18 from Waters Corporation (Milford, MA, USA) with an average particle diameter of 5.0 μm; the pore size is 147 Å and the carbon load is 18%, according to the vendor.

3.2. Instrumentation and procedure

The experiments were performed on an Acquity U-Class Bio System (Waters) equipped with a PDA detector with a 500-nL flow cell. Additionally, an RID-10A RI detector from Shimadzu (Kyoto, Japan), located behind the PDA detector, was used throughout this study. The extra volume from the PDA detector to the RI detector was 110 μL, which was determined chromatographically. The mobile phase was 25/75 (v/v) MeOH/water. Several different concentrations of IPR with constant ionic strength in the eluent were considered. For overloaded elution profiles, 30 μL of 1 g L$^{-1}$ SBS, S26NS, or SPTS were injected. We also injected a very low concentration (2 μL of 1 mg L$^{-1}$) of SBS, S26NS, or SPTS. Under these conditions the peaks were almost Gaussian (estimated by ocular inspection); in the following we will regard these operational conditions as “analytical.” For the IPR perturbation peak, 2 μL of a sample containing a small excess of IPR relative to the eluent was injected. The experiments were conducted at a mobile flow rate of 0.25 mL min$^{-1}$ and the temperature of the column oven and heat exchanger before the column inlet was 25 °C. The reference cell in the RI detector was filled with the mobile phase for at least 30 min. Signals from both detectors were recorded simultaneously.

4. Results and discussion

This section is divided into two parts. Section 4.1 presents the comparison of the simulated elution bands of SBS with the experimental elution bands recorded with UV and RI detectors. We also conduct a detailed analysis of the perturbation peaks and their impact on the solute concentration profiles. In this section, we propose a rule of thumb that can be used to predict conditions under which deformed solute profiles can appear. Section 4.2 discusses the propagation of the elution bands along the column.
4.1. Effects of perturbation peaks on solute bands

As discussed in the “Theory” section, the anions from the sodium chloride added to the eluent and the cations from IPR all adsorb to the stationary phase. Introducing a sample to a column equilibrated with an eluent containing adsorbing compounds will result in perturbation peaks. Even if the injected sample contains the same concentrations of IPR and salt as does the eluent, perturbation peaks are still induced. This is because of the high concentration of solute introduced to the column, which disturbs the equilibrium obtained between the adsorbed IPR and salt in the stationary and mobile phases. The perturbation peaks are generally not observed because they represent disturbances of eluent compounds that are normally not detected. To detect the elution of IPR, an RI detector was used; everything affecting the reflective index, such as changes in salt, IPR, and solute concentrations, was recorded.

The number of negative or positive peaks induced by the injection of high-concentration sample solution depends on the number of compounds involved in the chromatographic system [20,26]. Generally, for each additive, there is one perturbation peak per component, migrating with the peak of this component, and one primary perturbation peak, migrating at a velocity characteristic of the additive. In our case, we consider a three-component system comprising the solute compound and the IPR and salt added to the eluent. Therefore, we should expect: (i) for salt, one primary peak eluted at a velocity following from the salt adsorption, accompanied with two more peaks for the IPR and solute; and (ii) for the IPR, one primary peak eluted at a velocity following from the strength of IPR adsorption, accompanied with two additional IPR peaks for the solute and salt. Fig. 1 shows the simulated and experimental elution bands from a 30-μL injection sample containing 1 g L⁻¹ SBS recorded using RI (blue line) and UV (gray line) detectors for different concentrations of IPR in the mobile phase: a) 0.5, b) 1, c) 1.5, d) 2, e) 3, and f) 5 mM IPR in the eluent. Moreover, each subplot is complemented by simulated elution bands of SBS (black dotted line), IPR (red dotted line), and X (green dotted line) (see Section 2 for more details about the mathematical modeling). Note that X is the excess of salt (anions) relative to the IPR. In Fig. 1, we can see that the elution profile of SBS (solid gray) goes from Langmuirian shaped, with a small amount of IPR in the eluent (0.5 mM, Fig. 1a), through U-shaped, with moderate amounts of IPR (1–2.5 mM, Figs. 1b–d), and back to Langmuirian shaped, with a greater amount of IPR in the eluent (5 mM, Fig. 1f).

In the chromatograms recorded using the RI detector, we cannot see all the mentioned perturbation peaks and solute peaks because some of them are hidden by the main peaks; however, the main salt and IPR elution bands are visible. Inspecting the predicted elution profiles for each component (i.e., X, SBS, and IPR), we clearly see that the model-predicted elution profiles and recorded RI signals agree well from a qualitative perspective. From a modeling perspective, the most important factors describing the retention are the IPR and the solute. During the model building in the previous study, we noted that salt was added to fine tune the model [17]. However, the primary factors describing the adsorption process and, thereby, the retention are the solute and the IPR. This discussion will therefore mainly focus on the IPR and the solute.

Briefly, the first-eluted peak band for the simulated X is the primary perturbation band for X. At lower IPR concentrations, X will have a positive perturbation band that co-elutes with the IPR primary perturbation band, and at higher IPR concentrations, X will have a negative perturbation band (see Fig. 1a and f). From a modeling perspective, X adsorbs much more weakly than does SBS to the already established IPR layer on the stationary phase; as well, the amount of adsorbed X increases with the amount of IPR in the mobile phase. At lower IPR concentrations in the eluent, X and the solute do not compete for the established layer of IPR on the column; when the IPR concentration is higher, this results in a positive X band that co-elutes with the primary perturbation band from the IPR. At higher IPR concentrations in the eluent, SBS co-elutes with the positive IPR perturbation band, resulting in a deficiency of X due to competition on the established IPR layer on the stationary phase. Interpreting the elution profiles in Figs. 1b–e, is harder, as they show a transition between the two cases discussed above.

From the presented concentration profiles of SBS (see Fig. 1) and the observed shape of the solute elution profile, three experimental areas have been defined, i.e., Zones I, II, and III, for different IPR concentration ranges. These three zones are marked in Fig. 2a, showing the retention times of small analytical solute peaks and perturbations of IPR, respectively, versus the actual bulk concentration of IPR; the Zone I–III areas coincide with the concentration ranges of IPR, with Langmuirian and U-shaped profiles prevailing according to the rule of thumb (see below). Fig. 2a shows the retention time of a 2-μL injection of 1 mg L⁻¹ SBS and the retention time of the perturbation peak of IPR from a 2-μL injection of a small excess of IPR relative to the eluent for different IPR concentrations in the mobile phase. Figs. 2b–d present concentration–elution profiles of SBS observed in the three zones.

In Zone I (see Fig. 2a), the amount of IPR in the eluent is small and the retention time of the perturbation is longer than that of the solute profile. In this zone, normal Langmuirian-shaped solute elution profiles are observed (see Fig. 2b). Fig. 1a compares the simulated and recorded elution bands for an IPR concentration of 0.5 mM in the eluent. In this case, three positive elution bands are observed in the signal recorded using the RI detector. The first peak is the signal from the salt, the second is from the solute, and the third, not visible with the UV signal, is the positive band from the IPR. In this range, the IPR perturbation peak only slightly affects the characteristics of the solute elution profile. Observe that the solute injection generates three perturbation bands in the IPR concentration: the early-eluting band from the salt and two later-eluting bands from the solute and the IPR. These bands can have excess IPR relative to the eluent, here called positive bands, or a deficit of IPR relative to the eluent, here called negative bands. In Zone I, all these IPR elution bands are well separated from one another and the solute profile elutes in a negative IPR perturbation peak.

In Zone II (see Fig. 2a), the amount of IPR has increased so that the IPR perturbation peak and the solute profile elute very close to each other. For SBS, it starts above 0.5 mM and ends with about 4 mM IPR in the eluent. In this zone, we can observe deformed elution bands such as U-shaped profiles (see Fig. 2c). This phenomenon is clearly visible in the chromatograms recorded using the RI detector (see Figs. 1b–e). When the negative IPR elution band is eluted close to the positive elution band of the IPR, then the solute elution profile is distributed between these two bands. The portion of solute propagating through the column in a negative IPR elution band will be less retained (the solute’s linear velocity will increase in this band), whereas the portion of solute propagating through the column in a positive IPR elution band will be more retained (the solute’s linear velocity will decrease in this band). As a result, the unusual U-shape of the solute profiles is observed (see Fig. 1b–e). Note that in some cases the negative IPR elution band in the RI chromatograms is not visible (see Fig. 1b). Only a small valley in front of the right shoulder of the U-shaped profile is recorded. In this case, this is because the signal from the negative zone of the IPR and the positive band of the solute compensate for each other.

As mentioned above, small-load experiments were also carried out (2 μL of 1 mg L⁻¹). The black arrows in Fig. 1 indicate the sim-
Fig. 1. Comparison of elution profiles from 30-μL injections of 1 g L⁻¹ of SBS (black dotted line), X (green dotted line), and IPR (red dotted line) simulated using a multilayer model, and corresponding elution profiles recorded using UV (gray solid line) and RI (blue solid line) detectors for: a) 0.5, b) 1, c) 1.5, d) 2, e) 3, and f) 5 mM IPR in the eluent. The signal response was normalized for visualization reasons. The arrows indicate the simulated retention time of the analytical peaks (2 μL of 1 mg L⁻¹).
lected retention time of SBS under analytical conditions. In Zones I and III, the analytical peak elutes later than the apex of the SBS profiles. However, this is not the case in Zone II. Here, the analytical peak's retention is between the two profiles, making the SBS elution band U-shaped. It should be noted that for a Langmuir isotherm, the analytical peak elutes at the end of the triangular overloaded concentration profile. In our case, the analytical peak elutes inside the triangular elution band (see Figs. 1a and f). The position of the analytical peaks is connected to multilayer adsorption. This is especially visible in Fig. 1e, where the experimental profile of SBS (gray line) is a classic example of multilayer adsorption [17,26].

The calculated retention times of the analytical peaks indicated by the arrows in Figs. 1a–f agree with the experimental retention times shown in Fig. 2a, with an error not exceeding 7.3%.

In Zone III (see Fig. 2a), the amount of IPR has increased even more, so that the IPR perturbation peak elutes before the solute profile elutes. In this case, the negative and positive IPR bands are well separated (see Fig. 1f). As a result, the SBS will not propagate in two different velocity zones generated by the perturbation peaks from the IPR. Therefore, no peak deformation is observed (see Fig. 2d).

Supplementary Material Fig. S1 shows the retention time of 2-μL injections of 1 mg L⁻¹ S26NS and SPTS and the retention of the perturbation peak of IPR from a 2-μL injection of a small excess of IPR relative to the eluent for different IPR concentrations in the mobile phase. Figs. S2 and S3 compare overloaded elution profiles of S26NS (Fig. S2) and SPTS (Fig. S3) recorded using UV (gray line) and RI (blue line) detectors. It is worth comparing Zone II for the different considered compounds. The smallest IPR concentration range in which the U-shaped profiles are observed is for SPTS. This compound is characterized by a great increase in retention with increasing IPR concentration. Thus, the range of IPR concentrations in which the negative perturbation peak of the IPR can influence the SPTS elution band is small. For the other considered compound, S26NS, Zone II is similar in width to that for SBS because the increase in the solute retention with increasing IPR concentration in the mobile phase is similar for both compounds.

From all these observations, we can formulate a rule of thumb. The prerequisite for the occurrence of distorted solute elution profiles is that the IPR is more strongly retained than the solute in an eluent without IPR. In this case, deflected peaks may arise given large sample loads for eluents containing IPR depending on the relative retention of the primary perturbation peak of the IPR and the solute peak. Three different zones can be identified:

- Zone I—at low bulk levels of IPR in the eluent, the primary perturbation peak of IPR elutes much later than the retention of solute; under these conditions Langmuiran profiles of the solute are observed (cf. Fig. 2b).
- Zone II—at moderate bulk levels of IPR in the eluent, the primary perturbation peak of IPR and the retention time of solute are similar; here deformed profiles are observed (cf. Fig. 2c).
Zone III—at high levels of IPR in the eluent, the primary perturbation peak of IPR elutes much earlier than the retention of the solute; here the solute profiles are Langmuirian again (cf. Fig. 2d).

To summarize, all simulated profiles agree well with the corresponding experimental profiles UV and RI signals, respectively (Fig. 1). This is interesting and perhaps even remarkable, because the original mathematical model was elaborated on and validated based only on the concentration profile obtained using the UV detector, without consideration of the negative and positive elution bands from the IPR or the salt.

4.2. Propagation of the elution bands in the column

Mathematical modeling is not only of value for process optimization, but can also be used to understand the chromatographic process. This is of great importance when considering complicated chromatographic processes that are hard to intuitively understand. Using valid models, we can gain insight into how elution bands develop along the columns, and how they influence each other during propagation through the column.

As discussed in Sections 2 and 4.1, the concentration bands along the column that most merit consideration are the solute bands and IPR bands. To illustrate the influence of the perturbation peak on the solute elution band along the column, these two bands are plotted at three different distances from the column inlet, i.e., 2, 5, and 8 cm, and with four different concentrations of IPR in the eluent, i.e., a) 0.5, b) 2, c) 3, and d) 5 mM (see Fig. 3).

Fig. 3a presents the simulated elution band inside the column for 0.5 mM IPR in the mobile phase and represents an elution in Zone I (see Fig. 2a); for elution at the outlet of the column, see Fig. 1a. As can be seen in Fig. 3a, the positive and negative IPR perturbation peaks are well separated, even 2 cm from the inlet. In addition, the SBS concentration profile is well separated from the primary IPR perturbation peak (i.e., the positive zone) 2 cm into the column. Because the solute propagates a considerable distance into a zone where the positive and negative IPR perturbation peaks are well separated, the IPR system will only slightly influence the SBS peak shape and the resulting elution profile will be Langmuirian.

Fig. 3d presents the simulated elution band inside the column for 5 mM IPR in the mobile phase and represents an elution in Zone III (see Fig. 2a); for elution at the outlet of the column, see Fig. 1f. As in the 0.5 mM IPR case, because the solute propagates a considerable distance into a zone where the positive and negative IPR perturbation peaks are well separated, the IPR system will only slightly influence the SBS peak shape and the resulting elution profile will be Langmuirian. Comparing IPR elution bands in Zone I (Fig. 3a) and Zone III (Fig. 3d), we can observe that at low IPR concentration in the eluent, the SBS elution band propagates together with the negative IPR elution band, whereas at high IPR concentration in the eluent, the solute elution band will propagate together with the positive IPR elution band.

Fig. 3b shows the same simulations of the propagation of the solute and perturbation peaks, but this time the concentration of IPR in the mobile phase is 2 mM, representing an elution in Zone II (see Fig. 2a); for elution at the outlet of the column, see Fig. 1d. According to the rule of thumb formulated in Section 4.1, we expect to observe deformed elution profiles, such as U-shaped profiles. In this case, the solute is eluting in both the negative and positive IPR elution bands, which migrate all along the column. Two cm into the column, the U-shape is already established and the distance between the two-peak maximum of the U-shape increases as the elution band propagates along the column. This is because the solute in the early zone of the U-shape has a much higher linear velocity than does the solute distributed to the later zone of the U-shape profile.

Fig. 3c shows the propagation of the solute and perturbation peaks for IPR, but this time the IPR concentration in the mobile
phase is 3 mM, representing an elution in Zone II (see Fig. 2a); for elution at the outlet of the column, see Fig. 1e. As in the elution with 2 mM IPR in the eluent, we expect to observe deformed elution profiles. With 3 mM IPR in the eluent (Fig. 3c), the solute is eluting in both the negative and positive IPR elution bands, which migrate all along the column. Two cm into the column, the U-shape is already established. However, in this case, a rather small proportion of the solute is distributed to the early negative IPR band versus in the 2 mM IPR case (compare Figs. 2b and c). Moreover, in this case we can observe the U-shaped profile disappearing during the propagation of the elution band along the column. This is because of the greater distance between the negative and positive perturbation bands of IPR versus in the case with 2 mM IPR in the eluent. The elution profile is still deformed, but a clear U-shape is only present near the column inlet. Inspecting a corresponding experimental elution profile (Fig. 1e, gray line), we can observe the deformed elution profile of SBS. However, in this case the peak recalls its origins in a type-IV adsorption isotherm [26]. Type-IV isotherms occur in gas due to capillary condensation in the pores of the solid support [27]. However, in this case, it is mainly due to the complicated effect of the IPR perturbation peak.

5. Conclusion

In this study we considered nonlinear IPC. Attention was focused on overloaded concentration profiles and their shape, which varied depending on the concentration of IPR in the mobile phase. It was observed that the overloaded concentration profiles of some sodium salts of sulfonic acid change in shape from Langmuirian to deformed profiles such as U-shaped profiles, and then back again to the Langmuirian shape with the increasing concentration of TBuABr in the mobile phase. In a recent paper, we developed a mathematical model that, with good accuracy, could predict the overloaded concentration profiles of SBS. However, that model was based only on UV detector signals (only for SBS) without consideration of the negative and positive elution bands from the IPR or the salt.

Here, we experimentally visualized the unusual band profiles with profiles recorded using an RI detector. The chromatograms recorded using the RI detector confirmed that the previous model could also correctly describe the salt- and IPR-concentration elution zones.

A rule of thumb was developed for identifying experimental conditions that could result in deformed solute elution profiles. The prerequisite for the occurrence of distorted solute elution profiles is that the IPR is more strongly retained than the solute in an eluent without IPR. In this case, three different experimental zones were identified, i.e., Zones I, II, and III, depending on the relative retention between the IPR primary perturbation peak and the solute peak (cf. Fig. 2):

- Zone I—experimental conditions under which the solute peak elutes earlier than the IPR primary perturbation peak lead to Langmuirian elution profiles.
- Zone II—experimental conditions under which the solute peak and the IPR perturbation peak have similar retentions lead to deformed elution profiles.
- Zone III—experimental conditions under which the solute peak elutes later than the IPR primary perturbation peak lead to Langmuirian elution profiles.

In Zone II, simulations showed that the solute elution band was split between the negative and positive IPR perturbation peak bands, so the eluted overloaded profiles were U-shaped. The early-eluting part of the solute band was eluted together with the negative elution band of the IPR, and the later-eluting part of the solute elution band was eluted together with the positive IPR elution band. This is because the solute retention increases with the IPR concentration in the mobile phase. The portion of the solute propagating together with the negative elution band of the IPR is less retained and the portion propagating with the positive elution band of the IPR is more retained. This induces the U-shape. Finally, using simulations, we also investigated how the deformed peaks develop along the column. Here, we showed that the deformation was already present near the inlet of the column and that, along the column, the two-peak maximum of the U-shaped profile becomes more separated or that one of the apexes disappears.

This study is useful in several ways. For the practical chromatographer, this study provides a rule of thumb for predicting experimental conditions under which the unusual band shapes can be avoided, without needing to do any numerical modeling. This is very beneficial because the more broadly deformed band shapes (under Zone II conditions) lead to lower productivity than is the case with Langmuirian band profiles. From a more scientific perspective, the present results give an improved fundamental understanding of the preparative IPC separation process. The engineering chromatographer can perform numerical process optimization, accounting for the deformations in cases of preparative IPC separations of complicated therapeutic biomolecules where the band deformations (under Zone II conditions) are impossible to avoid by applying the rule of thumb. For this purpose, however, the model should be expanded to cover a broader range of solutes as well as, for example, temperature effects, for rapid and accurate numerical process optimization of the unusual band shapes in preparative IPC.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Marek Leško: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization. Krzysztof Kaczmarski: Conceptualization, Methodology, Software, Formal analysis, Data curation, Writing – review & editing, Supervision. Torgny Forsnstedt: Conceptualization, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition. Jörgen Samuelsson: Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing.

Data Availability

Data will be made available on request.

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Supplementary materials

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References


