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Peak interactions under concave isotherm conditions in preparative ion chromatography

CHARLES A. LUCY*, THI-LUONG LUONG and STEVE ELCHUK

General Chemistry Branch, AECL Research, Chalk River Laboratories, Chalk River, Ontario K0J 1J0 (Canada)

ABSTRACT

There have been recent improvements in the understanding of the behaviour occurring when chromatographic columns are severely overloaded and neighbouring peaks start to interact. However, these studies have concentrated exclusively on convex non-linear isotherms (e.g. Langmuir). In many preparative separations concave isotherms are operative and result in fronting peaks.

The separation of lanthanides on an ion-exchange column, with α -hydroxyisobutyric acid (HIBA) as eluent, is used as a model system for the study of peak interactions under concave isotherm conditions. Experimental evidence suggests that the concave isotherm results from the HIBA in the mobile phase being unable to complex all of the lanthanide injected. Two types of peak interaction have been identified: the retainment effect, where a more retained peak shifts to greater retention times due to the overloading of an earlier peak, and the pull-back effect, where a peak is smeared into a more retained peak due to the overloading of the later peak.

INTRODUCTION

Ion chromatography is increasingly being looked upon as a means of separating commercial quantities of a variety of products. One of its advantages for such preparative separations is the wide variety of modes of separation which have become available: ion-exclusion chromatography has been used for the large-scale separation of sugars from molasses [1], and ion-pair chromatography has been used to purify tripeptides [2]. However, despite preparative ion-exchange chromatography dating back to the Manhattan project [3], there is still little understanding of the peak interaction behaviour which often affects the quality of the separation.

It has been recognized that productivity and throughput in preparative chromatography can best be achieved by overloading the column. Under these conditions, the distribution isotherm of the sample between the stationary and mobile phases will be non-linear. The non-linearity may be either convex, as for the Langmuir isotherm, in which case the peak will tail when the column is overloaded, or concave for which fronting peaks are observed. Theoretical [4–6] and experimental [7–10] studies have been made of the interactions between adjacent peaks under convex isotherm conditions. These studies have identified “displacement” and “tag-along” effects which affect the productivity and purities obtained from a preparative system.

On the other hand, the interaction between adjacent peaks under concave isotherm conditions has received essentially no attention, despite this behaviour having been observed in almost every branch of chromatography [8,11–13]. In this work, the separation of binary solutions of lanthanides is used as a model system for the study of peak interactions in preparative chromatography under concave isotherm conditions.

BACKGROUND

Convex isotherm

A convex isotherm results when the sample adsorption capacity of the stationary phase becomes limited. Under these conditions the solute molecules in the band centre will remain in the mobile phase longer and thus migrate faster than those molecules at the edges of the peak where the solute concentration is lower. This results in a steepening of the peak front and a broadening of the tail.

Analytical chromatography operates under linear isotherm conditions and thus the overlap of adjacent peaks does not alter the original peak shape. This will not be true under non-linear convex isotherm conditions, since the overlapping of peaks will exacerbate the already limited capacity of the stationary phase. Theoretical studies using the numerical “semi-ideal” model have identified two types of interaction operating between adjacent bands [4,5,14]. The first is “sample self-displacement”, where the more strongly retained component of a pair of solutes has a stronger interaction with the stationary phase. Consequently, when the bands overlap the less retained component is displaced by the more retained component. In severe cases this leads to an earlier and sharper elution of the first component^a. This displacement behaviour has been observed experimentally in a number of preparative chromatographic separations [8–10].

The second type of interaction predicted is the “tag-along” effect. For adjacent peaks the strength of the solute interaction with the stationary phase is similar. Therefore, while the more retained solute has a slightly stronger interaction with the stationary phase, it can still be displaced by the less retained solute if the localized concentration of the latter is large enough. The “tag-along” effect appears then as a smearing forward of the more retained solute into the peak of the less retained solute. This behaviour is enhanced by increases in the relative concentration of the less retained solute and results in decreases in the separation between the two bands. Experimental verification of the tag-along effect was recently obtained by Katti and Guiochon [10].

Concave isotherm

Concave isotherms are observed less frequently than convex isotherms discussed above. A concave isotherm results when the sample capacity in the mobile phase becomes limited. Under these conditions the resultant peak shape is a fronting peak, with a broad gradual upward slope culminating in a steep tail (Fig. 1). Profiles of peaks fronting due to a concave isotherm have been generated using the fundamental models

^a Similar behaviour has been modelled using a Craig counter-current simulation of preparative chromatography assuming Langmuir isotherms [6].

of Houghton [12,15] and Haarhoff and Van der Linde [16], and by the numerical semi-ideal model (using an S-shaped isotherm) [17]. However, no theoretical or experimental studies of the interaction of adjacent peaks under concave isotherm conditions have been made. Thus, no theoretical or experimental guidelines exist regarding the behaviour to be expected.

In this work, the separation of lanthanides by ion-exchange chromatography with α -hydroxyisobutyric acid (HIBA) is used as a model system for the study of peak interactions under concave isotherm conditions. The fronting behaviour of the lanthanides under preparative isocratic elution conditions was first noted by Campbell and Buxton [13]. They ascribed this behaviour to the formation of a precipitate which they observed on their glass columns. In addition, however, the lanthanide separations are particularly well suited to the study of peak interactions under concave isotherm conditions because of their narrow, sharply-defined spectral peaks. The sharp absorption bands result from f-f transitions within the 4f shell, which is well shielded from distortions from the surroundings of the ion, and so the transitions take on a free atom like character. Many of these bands are characteristic to a particular lanthanide [18], and UV-visible spectroscopy can be used to selectively monitor the behaviour of an individual lanthanide in the presence of another. While it is possible to perform such a study using pairs of organic solutes having differing absorption spectra, these solutes would have dissimilar adsorption isotherms and so little general understanding of the processes involved would be gained.

EXPERIMENTAL

The high-performance liquid chromatography (HPLC) equipment consisted of a Shimadzu LC-610 solvent delivery system, a Rheodyne 7125 sample injection valve fitted with a 100- μ l loop, a Shimadzu 6AV variable-wavelength UV-visible detector and an IBM-XT computer containing a LAB MASTER data acquisition board. All data handling was performed with Lotus 1-2-3. The column was a 150 \times 4.6 mm I.D. stainless-steel jacket packed with 30-45 μ m Dowex AG 50W-X8 sized by elutriation [19]. The column was slurry packed at 1500 p.s.i. from 1.0 M HIBA pH 4.6 solution. The injector and column were thermostatted at 30.0°C using a Haake water bath.

The HIBA (Aldrich, Milwaukee, WI, U.S.A.) was purified with a 200 \times 3 cm AG 50W-X8 cation-exchange column and filtered with a 0.8 μ m/0.4 cartridge filter (Nalge Co.). The eluents and solutions were prepared from quartz-distilled and deionized water (Milli-Q system). All eluents were filtered through 0.45- μ m Millipore filters before use. The eluent was 0.30 M HIBA, pH 4.6, and was pumped at a flow-rate of 1.0 ml/min. Neodymium and praseodymium standards were prepared in distilled water from their nitrate salts (Aldrich). Calibration curves for Nd at 755 nm and Pr at 444 nm were linear (correlation coefficients of 0.9997 and 0.9994, respectively) over the absorbance ranges used in this study, with zero intercepts (within the 95% confidence interval).

RESULTS AND DISCUSSION

Individual peak profiles

The changes in peak profiles with increasing amounts of neodymium and

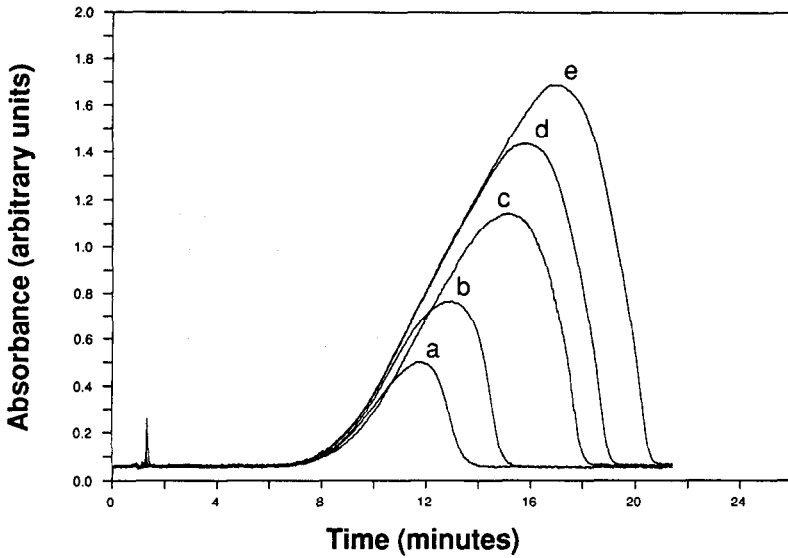


Fig. 1. Peak profiles for neodymium under concentration overload conditions. Nd loadings: a = 1 mg; b = 2 mg; c = 4 mg; d = 6 mg; e = 8 mg. Nd monitored at 575 nm.

praseodymium injected onto the Dowex AG 50W-X8 cation-exchange column are shown in Figs. 1 and 2. These figures illustrate the effect of column overload on the individual peak shapes. In both cases the profiles are asymmetrical with a gradual rise at the front of the peak followed by a sharp drop. The degree of the asymmetry

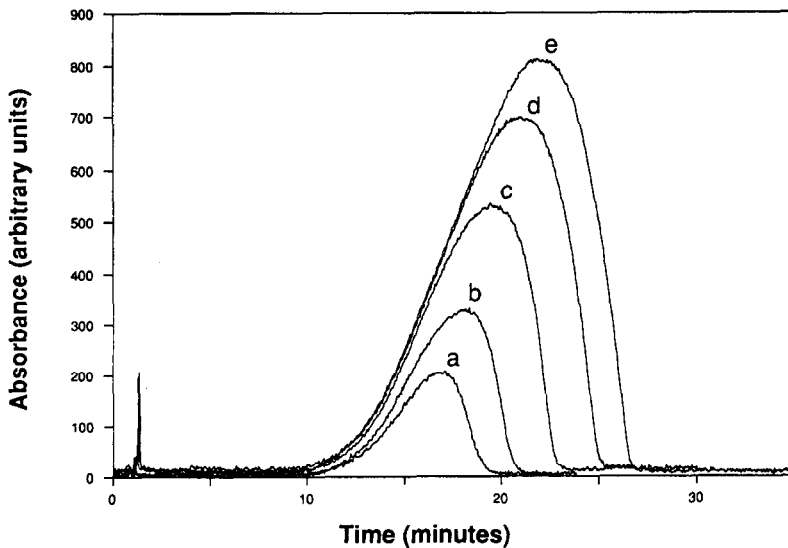


Fig. 2. Peak profiles for praseodymium under concentration overload conditions. Pr loadings: a = 1 mg; b = 2 mg; c = 4 mg; d = 6 mg; e = 8 mg. Pr monitored at 444 nm.

increases with the degree of loading, with a resultant increased retention time for the peak maximum. This indicates that the migration rate of the solute decreases with increasing solute concentration in the mobile phase.

However, the fronting behaviour and increased retention time for the peak maximum are not sufficient, by themselves, to assure that the isotherm is concave. Indeed, similar peak shapes have been observed in both theoretical and experimental studies of adsorption chromatography with binary eluents containing a strongly sorbed additive [20,21]. In the adsorption chromatography case, the peak profiles result from competition between the molecules of the strong solvent component and those of the sample for the adsorbent surface (*i.e.*, competitive Langmuir isotherms). When the mobile phase additive is more strongly retained than the sample, the resultant peak profiles display a diffuse front and a sharp tail [20], and increases in the sample loading result in increased peak asymmetry and movement of the peak front to earlier retention times. This last characteristic is unlike the behaviour resulting from a concave isotherm, where the peak front does not change retention time when the loading is changed. In Figs. 1 and 2 it can be seen that the peak front does not move when the loading of either neodymium or praseodymium is increased. Thus, both lanthanides follow true concave isotherms.

Fig. 3 shows a plot of the lanthanide concentration measured at the peak maximum *versus* the retention time for the peak maximum. Theoretical relationships derived from the Houghton model of non-linear chromatography predict that such a plot fundamentally follows an orthogonal hyperbola [12]. However, over a small concentration range such a plot would appear rectilinear as observed in Fig. 3 for both neodymium ($r = 0.989$) and praseodymium ($r = 0.998$). Thus for both lanthanides studied, no discontinuities exist in the isotherm behaviour, unlike other concave isotherm systems studied previously [12].

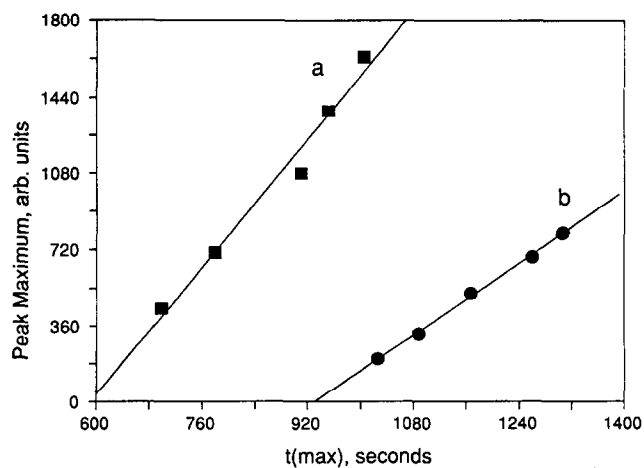


Fig. 3. Variation of retention time of peak maximum [$t(\max)$] with concentration measured at peak maximum for (a) neodymium and (b) praseodymium. Lines are the best-fit linear regression of the points.

Binary mixture: effect of overload on trailing peak

Fig. 4a shows a composite of the retention behaviour of neodymium and praseodymium when each is injected alone. The concentrations are such that neodymium is severely overloaded (*i.e.*, exhibiting extreme fronting) while praseodymium is only slightly overloaded. When the two lanthanides are injected together, the praseodymium is pushed backwards to longer retention times (Fig. 4b). This behaviour is analogous to the "displacement" effect observed in convex (Langmuir)

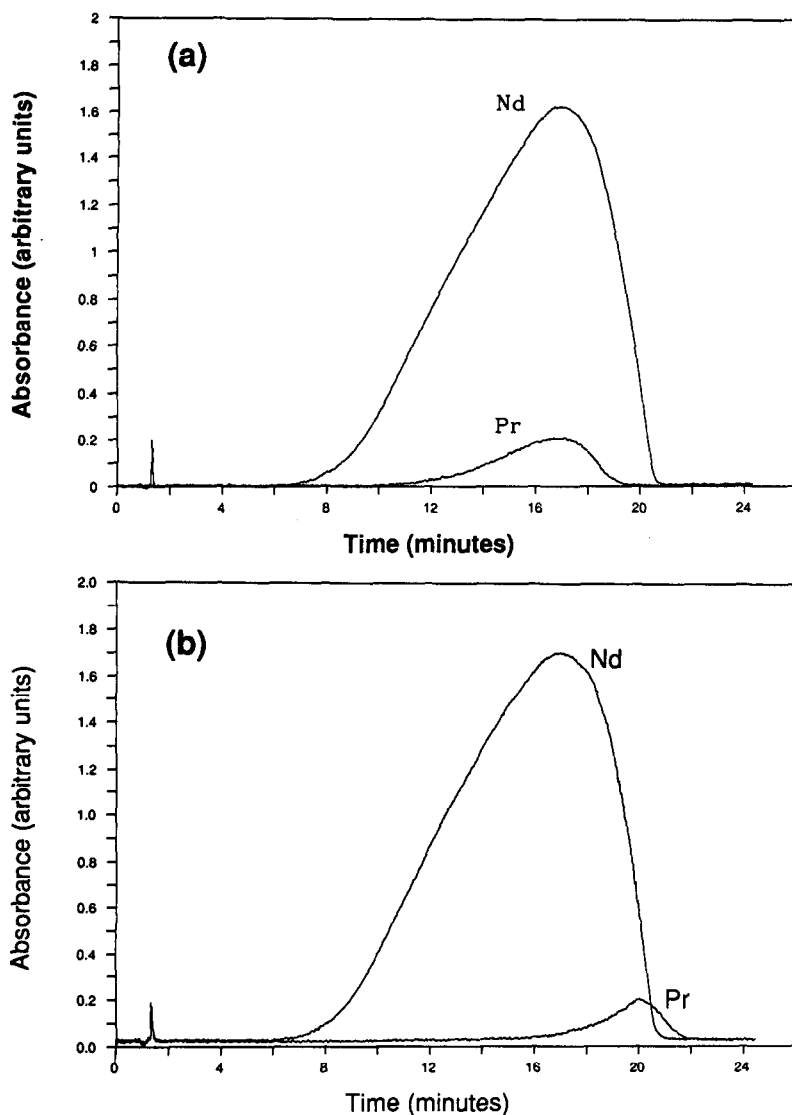


Fig. 4. Effect of neodymium loading on later-eluting praseodymium: (a) composite of retention behaviour of praseodymium (1.0 mg monitored at 444 nm) and neodymium (8.0 mg monitored at 575 nm) injected separately; (b) retention behaviour of 0.5 mg praseodymium and 8.0 mg neodymium when injected together.

isotherm conditions [8–10], and in keeping with the terminology of Golshan-Shirazi and Guiochon [22], it will be referred to as the *retainment effect*.

The behaviour of the praseodymium peak when increasing loadings of neodymium are co-injected with the praseodymium is shown in Fig. 5. As the neodymium loading is increased, the praseodymium peak shifts to longer retention times and becomes sharper. A plot of the retention time for the praseodymium peak maximum *versus* the neodymium loading was linear [$r = 0.994$, intercept of 15.6 min (95% confidence interval is 0.2 min)]. This behaviour is similar to that predicted and observed for the displacement effect in convex isotherm systems [4,5,8–10].

The cause of the concave isotherm can be determined based on the behaviour observed in Figs. 4 and 5. Assuming that the cause of the concave isotherm is precipitation of the lanthanide–HIBA complex, as had been postulated by Campbell and Buxton [13], one would expect praseodymium to be unaffected by the neodymium precipitation, since the praseodymium concentration is still below its solubility limit. However, since the retainment effect was observed this expectation must be modified. The extra retention of praseodymium could be due to praseodymium co-precipitating with the neodymium–HIBA complex. However, upon redissolving, such a precipitate would liberate both neodymium and praseodymium at the same rate, which would result in comparable peak shapes and peak maximum shifts for both neodymium and praseodymium. This is not what is observed in Figs. 4 and 5. Therefore, the concave isotherm behaviour exhibited by the lanthanides does not result from the formation of a precipitate.

An alternative hypothesis is that the concave isotherm behaviour results from a shortage of HIBA in the mobile phase. At the highest loadings studied, 0.55 M Nd is injected into an eluent stream containing only 0.30 M HIBA. Using this assumption, the retainment effect can be explained in an analogous fashion to the displacement

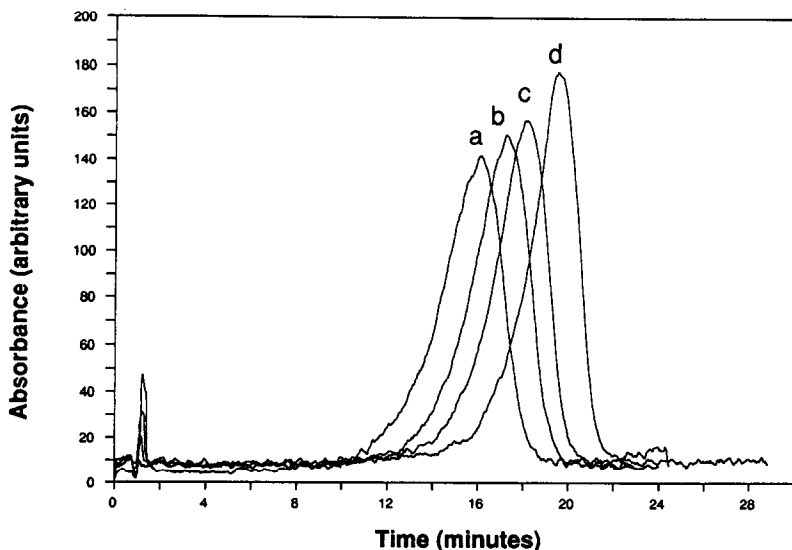


Fig. 5. Behaviour of later-eluting praseodymium with increasing loadings of neodymium. Nd loading: a = 1.0 mg; b = 3.0 mg; c = 5.0 mg; d = 7.0 mg. Pr loading: 0.5 mg. Detector wavelength: 444 nm.

effect observed when overloading a convex isotherm. The less retained of the pair of solutes (Nd) forms a stronger complex with the eluent, HIBA [23]. Consequently, when the peaks overlap the Nd will be preferentially complexed by the HIBA, leaving the more retained solute (Pr) stranded on the stationary phase and resulting in increased retention for the more retained solute (Pr). In cases of greater relative concentration of the less retained solute to the more retained (*i.e.*, Nd *vs.* Pr), the situation will be exacerbated such that the degree of the retainment effect will increase, and the more retained peak (Pr) will become sharper. This is the behaviour observed in Fig. 5. Therefore it is concluded that the concave isotherm results from the limited capacity of the mobile phase, brought on by the limited concentration of HIBA in the eluent.

Binary mixture: effect of overload on leading peak

Fig. 6 shows the neodymium peak profiles observed for the injection of 0.5 mg neodymium along with various larger loadings of praseodymium. The neodymium peak shape broadens and undergoes a transition from a fronting to a tailing peak as the praseodymium concentration is increased. Some increase in the retention time is also observed. This behaviour is analogous to that in the convex isotherm situation. In this case, the results mirror the “tag-along effect” [10,14], and have been termed the *pull-back effect* [22].

This pull-back effect can be explained by assuming that limited complexation capacity in the mobile phase is the cause of the concave isotherm. The less retained component, neodymium, forms a more stable complex with the eluent, HIBA, than does praseodymium. While this difference in complex stability causes the retainment effect, this difference is small. Thus, increasing the concentration of praseodymium will result in shifting the equilibrium away from the neodymium–HIBA complex,

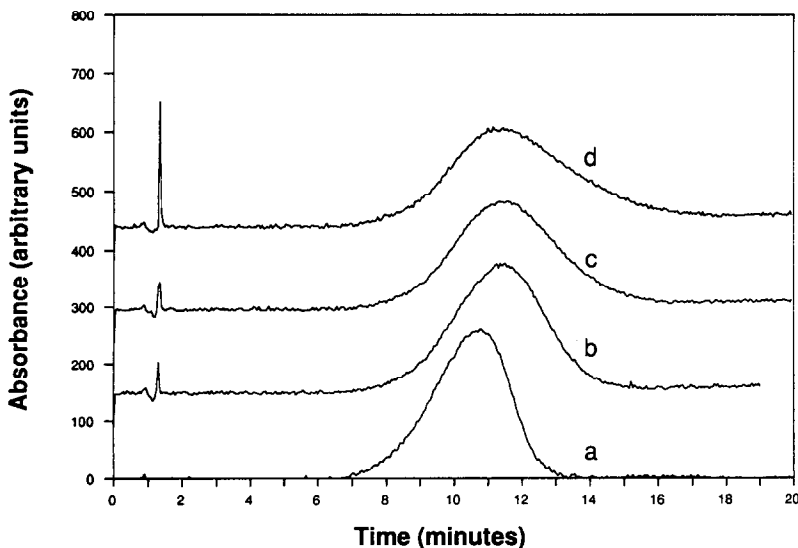


Fig. 6. Behaviour of earlier-eluting neodymium with increased loading of praseodymium. Nd loading: 0.5 mg. Pr loading: a = 1.0 mg; b = 3.0 mg; c = 5.0 mg; d = 7.0 mg. Detector wavelength: 575 nm.

leading to greater retention for neodymium. Concurrently, the neodymium peak broadens due to the praseodymium concentration gradient (*i.e.*, the praseodymium peak front) which is spread across the width of the neodymium peak. The praseodymium, present in high concentrations, complexes the available HIBA with the result that the “free” HIBA concentration across the neodymium peak also varies. This non-uniform eluent concentration across the neodymium peak leads to varied migration rates and thus peak broadening.

CONCLUSIONS

Fronting peaks are observed when ion-exchange columns are concentration overloaded by a lanthanide while using HIBA as eluent. This is typical of preparative chromatography under concave non-linear isotherm conditions. The peak profiles display consistent behaviour over the full concentration range studied, indicating that no secondary phenomenon are occurring which would complicate the observed behaviour. The concave isotherm could result from either precipitation of the lanthanides on the column or limited complexation capacity of the mobile phase. The peak interaction behaviour indicates that the latter mechanism is most probable.

The experimental studies have identified two types of peak interactions which occur when peaks overlap under concave isotherm conditions. These are the *retainment* and *pull-back* effects, which result from the two solutes competing for the limited complexation capacity of the mobile phase. The retainment effect appears as a delay and sharpening of the more retained peak when the less retained peak is severely overloaded. The intensity of the effect is proportional to the concentration of the less retained solute injected. The pull-back effect is the smearing of the less retained peak when the more retained peak is severely fronting. The severity of the effect is related to the concentration of the more retained solute injected.

The peak interaction behaviour observed under concave isotherm conditions is analogous to that observed in convex isotherm system, allowing for the mobile phase having the limited capacity rather than the stationary phase. Thus it is possible to take the wealth of understanding available for convex isotherm systems [4–10] and use it as a guide for the behaviour expected in preparative chromatographic separations which follow a concave isotherm.

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