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Linearization of Column Responses by Correlation Chromatography. Separation Improvement and Reduction of Undesired Adsorption Effects.

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Key Words

Gas chromatography
Correlation chromatography
Simulation model
Adsorption effects
Separation improvement

Summary

Experiments and simulations prove that correlation chromatography can greatly reduce the disadvantage of a non-linear response of the chromatographic column. A factor that has been accepted as being an important source of error in correlation or multiplex chromatography, has been shown not to be. Separations affected, improve dramatically when correlation chromatography is used, and a substantial amount of correlation noise only arises when there is a large difference in separation between a conventional chromatogram and a correlogram.

A model has been developed for simulating these non-linearities. It is shown that, especially for correlation chromatography, the simulation results match the practical measurements very well.

Introduction

A non-linear response of the chromatographic column, caused by e.g. adsorption, is well known but in general unwanted in chromatography. When non-linearities in the equilibration process of a chromatographic column are not negligible, they can give rise to deformation of the peak shape and a shift in retention time. The asymmetry of the peak shape (fronting or tailing) will be greater for the affected component and because of this asymmetry, the quality of the separation worsens. Quantification of an asymmetrical peak is more difficult and sometimes less accurate. Commercial integra-

tors often have difficulties finding the „peak-stop“, and a longer integration time will increase the integration error [1].

Non-linearities show up when the distribution constant K_i (Eq. (1)), being the ratio of the concentration in the stationary and the mobile phase of component i , is not constant at all concentrations.

$$K_{i, \text{ideal}} = \frac{c_i^s}{c_i^m} = \text{constant} \quad (1)$$

where: c_i^s = concentration of component i in the stationary phase,

c_i^m = concentration of component i in the mobile phase.

When a component is present at a high concentration, a deviation from the ideal linear distribution function can be caused by column overloading when c_i^s/c_i^m will be less than $K_{i, \text{ideal}}$ (Figure 1). At a low concentration undesired adsorption can cause a deviation from the ideal situation and c_i^s/c_i^m will be greater than $K_{i, \text{ideal}}$. In between, the distribution function is constant and

Figure 1

C^s as a function of C^m . A double Langmuir function (Eq. (8)) describes the relation between C^s and C^m , with $A_1 = 2 \cdot 10^{-2}$, $A_2 = 10^8$, $K_1 = 1$ and $K_2 = 50$.

normal peak shapes appear, there is no overloading and the undesired adsorption can be neglected. Similar effects occur in the separation of proteins (because of denaturation and adsorption) [2], and in capillary electrophoresis (sample overloading and adsorption) [3, 4].

The best way to deal with these non-linearities is to avoid or to prevent them. A lot of effort has been made to reduce these effects. To reduce undesired adsorption, special column materials have been developed, the inside surface of the column is treated, and the stationary phase support material (for instance silica), is chemically modified or shielded. A column has to provide a concentration working range that has to be linear for several decades, in order to get a peak shape which is independent of the concentration range of the analyzed samples.

Another way to deal with the problem of non-linearity, e.g. in case of undesired adsorption, is to add a polar modifier to the mobile phase. This is often successfully used in liquid chromatography, but adding a modifier to the mobile phase in gas chromatography is more troublesome. Phillips et al. [5] illustrated for correlation chromatography (CC) the effect of a non-linear response of the column. Unfortunately, no comprehensive research has been published since. CC can be compared, to a certain extent, with adding a modifier with the components themselves acting as the modifier. The large amount of injections in CC, ideally sample is injected for half the time, creates a situation in which the column is continuously fed with components. In conventional chromatography most of the time the component concentration is very low and close to zero at any point in the column. In CC on average there is a constant and relatively high component concentration at all times throughout the column. Where in conventional chromatography the differences in the concentration of a component throughout the column is a large number of decades, this is not the case in CC. Therefore, the drawbacks of a non-linear distribution function can be expected to be relatively smaller; the asymmetry of the peak shape, due to a non-linear distribution function, is reduced. In CC a component, present at a relative low concentration level at a certain point in the column, cannot be assigned to a peak front or a peak tail only, in contrast to conventional chromatography. This results in an extra reduction of the asymmetry (caused by a non-linear response) of the peak shape. This phenomenon will be explained later in more detail.

In CC the sample is introduced to the column a large number of times according to a pseudo random binary sequence (PRBS). Deconvolution of the detector signal with e.g. a cross-correlation procedure will give a so called correlogram [6]. Usually a correlogram can be compared to a single injection chromatogram with a reduced noise level. The calculation of the correlogram is a linear process; non-linearities will give rise to errors appearing as noise and ghost peaks in the correlogram.

These errors can be defined as correlation noise, as they do not originate from detector noise. It is to be expected that correlation noise will appear when non-linearities are still present in CC, and also that the level of this correlation noise will be dependent on the presence of non-linearities.

In this paper a non-linear model is described capable of simulating column overloading at high concentrations and undesired adsorption at low concentrations, as an example of possible non-linearities. In the experimental sections a mixture of three components is analysed on a GC column, one of the components shows a strong non-linear behaviour due to undesired adsorption.

Simulation Model

To simulate the non-linear response of a chromatographic column, a model, called "Non-linear Mixers in Series" (NMS), is developed being a modification of the model with a gamma density function (GDF) impulse response [7]. The GDF was a.o. developed for fitting chromatographic peaks. The peak shape $f(t)$ of a GDF as a function of time, can be described mathematically by the impulse response of a series of n perfect mixers (first order systems), each having the same time constant.

$$f(t) = I \left(\frac{t - t_0}{\tau} \right)^{n-1} \cdot e^{-\left(\frac{t - t_0}{\tau} \right)} \quad (2)$$

where: I = constant representing the "intensity" of the peak,

t_0 = time of input into the series of mixers,

τ = time constant of all mixers,

n = number of mixers in series, $n \geq 1$.

It has to be noted that n is not necessarily an integer, in which case the basic model of perfect mixers is of course no longer realistic. Figure 2 shows the peak shape for several values of n , keeping τ and t_0 constant.

Figure 2

Gamma density functions, not normalised, with constant τ and t_0 . $n = 1, 2, 5, 10, 20$ and 40 from left to right.

Some important peak parameters are determined for this model [7]:

$$t_R = t_0 + (n - 1)\tau \quad (3)$$

where: t_R = retention time (top of the peak).

$$\sigma = \sqrt{n} \cdot \tau \quad (4)$$

where: σ = standard deviation, or the second central moment.

$$a_3 = \frac{2}{\sqrt{n}} \quad (5)$$

where: a_3 = skewness, or the third dimensionless moment.

The asymmetry of the peak, related to the skewness in Eq. (5), depends on n . For a large n the peak shape becomes more and more symmetrical, finally approaches a Gaussian shape. Eqs. (3) and (4) show a dependence of both σ and t_R on τ and n . When τ is enlarged keeping n constant, both σ and t_R will enlarge. This can be used to introduce non-linearities when τ is made dependent on c_i^s/c_i^m . The value of n is not used to introduce non-linearities, because not only t_R and τ are dependent on n , but also the asymmetry, giving a more complex situation. The concentration dependent $\tau, \tau(c_i^s/c_i^m)$, should not change in the linear part of the distribution function; the peak shape is constant.

$$\tau \left(\frac{c_i^s}{c_i^m} \right) = \tau(K_{i, \text{ideal}}) = \text{constant} \quad (6)$$

However, outside the linear part when overloading or undesired adsorption effects are simulated, $\tau(c_i^s/c_i^m)$ should change.

The individual mixers in the mixers in series model are not equal when τ is made dependent on the concentration. This makes Eq. (2) no longer valid outside the linear part of the distribution function. The GDF can be rearranged to a convolution of separate first order processes. For integers of n the NMS as a function of time is shown in Eq. (7), making each mixer i dependent on c_i^s/c_i^m .

$$f(t) = I \cdot e^{-\left| \frac{t}{\tau_1(c_1^s/c_1^m)} \right|} * e^{-\left| \frac{t}{\tau_2(c_2^s/c_2^m)} \right|} * \dots * e^{-\left| \frac{t}{\tau_n(c_n^s/c_n^m)} \right|} \quad (7)$$

where: $*$ = convolution operation.

For simulating column overloading and undesired adsorption processes, a double Langmuir equation was chosen as a model describing C^s as a function of C^m :

$$C^s = \frac{K_1 \cdot C^m}{1 + A_1 \cdot C^m} + \frac{K_2 \cdot C^m}{1 + A_2 \cdot C^m} \quad (8)$$

where: K_1, K_2 are the (ideal) distribution coefficients of the „wanted“ chromatographic process and the “unwanted” adsorption respectively,

A_1, A_2 are corresponding constants.

The first term in Eq. (8) describes the column overloading and the linear chromatographic distribution process. The second term describes the undesired adsorp-

tion process. Four areas can be distinguished when $\tau = c^s/c^m$ and $K_1/A_1 \gg K_2/A_2$.

$$\begin{aligned} 1) \quad A_1 \cdot C^m \text{ not } \ll 1 &\Rightarrow C^s = \frac{K_1 \cdot C^m}{1 + A_1 \cdot C^m} \\ \tau = \frac{C^s}{C^m} &= \frac{K_1}{1 + A_1 \cdot C^m} \end{aligned} \quad (9)$$

Overloading reduces τ , the retention time and the peak width will be reduced. The component is moving faster through the column at high concentrations.

$$\begin{aligned} 2) \quad A_1 \cdot C^m \ll 1 \text{ and } \frac{K_2}{A_2} \ll C^s &\Rightarrow K_1 \cdot C^m \\ \tau = \frac{C^s}{C^m} &= K_1 \end{aligned} \quad (10)$$

Within the linear distribution range τ is constant, the peak shape will be independent of the concentration. Eq. (2) can also be used here.

$$\begin{aligned} 3) \quad A_1 \cdot C^m \ll 1 &\Rightarrow C^s = K_1 \cdot C^m + \frac{K_2 \cdot C^m}{1 + A_2 \cdot C^m} \\ \tau = \frac{C^s}{C^m} &= K_1 + \frac{K_2}{1 + A_2 \cdot C^m} \end{aligned} \quad (11)$$

If the second Langmuir term is no longer negligible than undesired adsorption influences τ , making it larger. The retention time and the peak width will increase, the component is retarded by an extra amount.

$$\begin{aligned} 4) \quad A_2 \cdot C^m \ll 1 &\Rightarrow C^s = K_1 \cdot C^m + K_2 \cdot C^m \\ \tau = \frac{C^s}{C^m} &= K_1 + K_2 \end{aligned} \quad (12)$$

At extremely low concentrations τ is constant again, the distribution function is linear. The retention time and the peak width are at the upper limit.

In this paper the second- and third area (Eqs. (10) and (11)) are of main interest.

Experimental

Experiments were performed on a GC, adapted and tested for CC in our laboratory [8]. Figure 3 outlines the configuration used for performing the experiments and Table I lists the equipment. As injection device a pair of pneumatic needle valves, MOVPT-1/100 from Scientific Glass Engineering, were used, controlled by the computer with an optocoupler as a circuit breaker. The valves were connected to the column by a 0.8 mm

T-piece from Valco. Headspace compartments used for sample and reference were 300 cm³ home made glass vessels, connected to the needle valves with 1.6 mm OD steel capillary. The injection device was placed in a temperature controlled rebuilt GC oven, type PV 4000 from Philips. The chromatograms and correlograms were produced with an open tubular GC column, 30 m × 0.32mm ID, coated with 0.2 µm SP2331 purchased from Supelco. Nitrogen was used as carrier gas. Table II lists the chromatographic conditions.

Table I List of equipment.

Apparatus	Type
GC injector	Packard Becker Model 421 with FID home-made
i-v converter	Atlas MAT DC60CH
data filter	Frequency Devices series 900 low-pass filter
CC-computer	Tulip dc386 with DAS-16 DAC/ADC controlled by Assyst
data-handling computer	HP-9000/350

Table II Chromatographic conditions.

Gas	Pressure (10 ⁵ kPa)	Temperature control	Temperature (°C)
N ₂	0.6	injector	97
H ₂	1.8	column	95
air	1.5	detector	180

Table III Resolution calculated for 1-propanol and 2-propanol from single injection experiments with variable injection times of Samples I and II.

Injection time (s)	Resolution	
	Sample I	Sample II
0.2	36	
0.4	35	
0.8	29	
1.6	19	20
3.2	10	11
6.4	5.5	6

Figure 3

Headspace configuration used for the single injection- and correlation chromatography experiments.

Two test samples were prepared: samples I and II, containing respectively 1.5 ml/l and 0.15 ml/l of 1-propanol, 2-propanol and 2-aminopropane each, in demineralized water. All chemicals were p.a. quality purchased from Merck. The headspace of each sample was analyzed at room temperature (22 °C) with the headspace of demineralized water as a reference. The CC experiments were carried out with a PRBS of 511 elements, and a clock period (the time length of one element) of 0.4 s. The data sampling period was also 0.4 s. A filter frequency of 1 Hz was chosen to avoid aliasing. To produce a correlogram at least two PRBSs were injected; the collected data during the last injection sequence were used to calculate the correlogram. For single injection experiments, data sampling period and filter frequency were chosen equal to the CC experiments.

The resolution (the ratio of the distance of two peaks and the σ of the first peak) was calculated by the results of a peak fitting procedure. The t_R and σ were estimated with non-linear regression and a Fraser-Suzuki function [9] to describe the peak shapes.

Results and Discussion

Single injection chromatograms of Samples I and II are shown in Figure 4. The injection times were 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 seconds for Sample I (Figure 4a), and 1.6, 3.2 and 6.4 s for Sample II (Figure 4b). The chromatographic behaviour of 1-propanol, and 2-propanol (with boiling points of 97 and 82 °C respectively) is normal. The peak shapes are symmetrical and a small shift in retention time appearing at longer injection times can be attributed to the enlarged injections (data collection starts at the same time as the start of the injection). When the injection time increases the peaks broaden and the resolution, listed in Table III, decreases rapidly.

2-Aminopropane (boiling point 32 °C) adsorbs very strongly and shows an extremely tailing peak. A larger amount of injected sample causes a sharpening of the peak shape and a decrease in retention time. Injecting more sample can be used here to counter the influence of adsorption, however, at longer injection times there is a unacceptable broadening of the other component peaks. This is clearly illustrated in Figure 4b (Sample II).

Figure 4

Single injection chromatograms; (a) from left to right, with injection times of 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 seconds of Sample I; (b) idem, with injection times of 1.6, 3.2 and 6.4 seconds of Sample II.

The injection time has to be at least 1.6 seconds to get a 2-aminopropane peak, which is still badly tailing. Due to this long injection time, the resolution for 1-propanol and 2-propanol is considerably decreased. Quantification of 2-aminopropane is difficult, and also quantification of other components can be a problem when they overlap with the amine.

To prove that adsorption of 2-aminopropane is caused by the column and not by the injector or other parts outside the column, Figure 5 shows a series of single injections at different column temperatures; other instrumental parameters were kept constant. Adsorption decreases very clearly at higher temperatures.

Correlograms, calculated by correlating the detector signal of the PRBS response with the PRBS itself, are shown in Figure 6 for both samples. Normal correlograms (Figures 6a1, 6b1), inverse-bit correlograms (Figures 6a2, 6b2), and the inverse-bit subtracted from the normal correlograms (Figures 6a3, 6b3) are shown. Inverse-bit correlograms are obtained by inverting the injection-bits of the PRBS. A '1' stands for 'not injecting' instead of 'injecting' in case of a normal

Figure 5

Single injection chromatograms of Sample I at different column temperatures. Temperatures are, from left to right, 95, 115, 135, 175, and 205 °C.

correlogram and a '0' stands for 'injecting' instead of 'not injecting' [8]. The peak shapes of 1-propanol and 2-propanol in the correlograms are similar to the peak shapes in the chromatogram when the results of equal injection times are compared (Figure 4a, 0.4s).

The resolution for both propanol peaks in the correlogram, was calculated as 36 and 35 for Samples I and II respectively, which is almost equal to a resolution of 35 in the corresponding chromatogram of Sample I. The correlograms show a considerably better peak shape for 2-aminopropane, especially for Sample II (Figure 6b) where it is impossible to get a corresponding single injection chromatogram (0.4 s injection) with the same resolution for the propanol peaks and a 2-aminopropane peak present at the same time. But also for Sample I (Figure 6a) a substantial improvement is attained. The correlograms only show a slightly tailing peak for 2-aminopropane and the peak width is comparable with the propanol peaks. A resolution of 20 was calculated for 2-aminopropane and 2-propanol with Sample I.

The improved peak shape and the counteracted adsorption can be attributed to the CC process. Due to the semi-continuous injection in CC there is a constant supply of sample. In CC at every point in the column there is an average concentration which is constant in time, and which fluctuates pseudo randomly around this average. In conventional chromatography every component passes the column only once. Before and after the levels are very low. Compared to CC, conventional chromatography uses a much larger part of the (non-linear) distribution function, and the peak shape will be more affected by non-linearities. In the case of adsorption, the improvement of the symmetry in CC can also be explained by comparing both processes. In conventional chromatography the component present at low concentrations at the front of the peak is retarded more than at higher concentrations at

Figure 6
Correlograms of Sample I (a1-a3) and Sample II (b1-b3). Normal correlograms (a1, b1), inverse-bit correlograms (a2, b2) and the inverse-bit subtracted from the normal correlogram (a3, b3) are shown.

the peak maximum, causing a more steep peak front. Because of the falling concentration at the rear of the peak, the component is retarded more and more, causing tailing. In CC the component present at a relatively low concentration at a certain point in the column cannot be assigned only to the start or the end of a peak. A point in the column with a relatively low component concentration on average contains an equal amount of information both from peak starts and peak endings. Since the component present at lower concen-

trations is retarded more, both start and ending will be influenced equally, causing a symmetrical broadening of the peak shape in the correlogram but not necessarily a tailing of the peak. Every point in the correlogram includes a contribution out of the total concentration range. A statistical averaging effect is obtained. Considering the more symmetrical peak shape it looks as if CC "linearizes" the distribution function. This linearizing effect is comparable to the dither effect known from electronics.

It also can be noted very clearly that noise is present in the correlograms. This noise is known as correlation noise, as it does not originate from detector noise since the noise level of the single injection chromatograms is low. The correlation noise most probably originates from the non-linear chromatographic behaviour of 2-aminopropane, because ghost peaks of both propanol peaks cannot be identified while ghost peaks of 2-aminopropane are present. The experimental results show that a considerable amount of this correlation noise (including ghost peaks) can be eliminated by subtracting the inverse-bit correlogram from the normal correlogram (Figures 6a3, 6b3). The eliminated part of the correlation noise therefore can be seen as though it comes from non-complementary injection errors [8]. Obviously, for these non-linear processes the theory of origins and properties of ghost peaks, described by Mulder et al. [8] is, at least partly, valid. This can be illustrated by the three largest ghost peaks in the correlograms of Sample I (Figures 6a1-a3). They show up at 54, 82 and 149 s and can be determined respectively as first-order non-complementary (λ_3), second-order complementary (λ_7) and second-order non-complementary (λ_5) ghost peaks, where λ_3 , λ_5 and λ_7 describe the nature of the ghost peak [8]. The non-complementary ghost peaks (λ_3 and λ_5) have the same sign in both the normal and the inverse-bit correlogram, they are eliminated with subtraction. The complementary ghost peak (λ_7) has an opposite sign and can not be eliminated by subtraction. The correlation noise, excluding the ghost peaks, is also partly non-complementary and partly complementary. Non-complementary correlation noise is eliminated by subtraction. Figure 6 illustrates this very clearly for both samples.

Simulation Set-up

The NMS model, described earlier, was used to simulate the experiments. The simulations were performed with the double Langmuir function of Eq. (8), describing the concentration dependent distribution between stationary and mobile phase, and the NMS of Eq. (7) describing the peak shape. The NMS only describes the average total concentration. Besides Eq. (7) a mass balance is needed:

$$C_t = C^m \cdot V_{fr} + C^s \cdot (1 - V_{fr}) \quad (13)$$

where: C_t = average total concentration,

V_{fr} = volume fraction of the mobile phase.

Figure 7 shows the simulated process which is the discrete-time equivalent of the continuous-time system of Eq. (7). The following lines of Fortran sub-code describe the heart of the simulations.

```

do j = 1, n
  do i = 1, npts - 1
    if (x(i) > 0) then
      call langmuir (K1, A1, K2, A2, Vfr, x(i), Cs, Cm)
      τ = Cs/Cm
      x(i+1) = x(i+1) + x(i) * τ / (1 + τ)
      x(i) = x(i) * 1 / (1 + τ)
    endif
  enddo
enddo

```

where: n = number of mixers,
 npts = number of datapoints,
 x [] = data array of C_t ,
 langmuir = subroutine for calculating C^s and C^m based on Eq. (8) and Eq. (13).

C^m (and C^s) was derived as a function of C_t (or $x[i]$) using Eq. (8) and (13). A third order equation was formed. Two imaginary and one real solution were derived, with help of the program Mathematica from Wolfram Research. The real solution was introduced in the subroutine Langmuir to calculate C^m and C^s out of the other parameters.

A simulated injection period was represented by 8 datapoints, both for the single injection and for the PRBS injections. A single injection chromatogram was simulated by assigning a concentration level to $x[1]$ until $x[8]$ and assigning all other values of $x[i]$ to be zero at the start of the described process (Eq. (14)). A CC experiment was simulated by filling up the $x[]$ array sequentially with 2 PRBSs of 511 elements. Every PRBS element contains 8 datapoints, all being 1 or 0 according to the PRBS. All datapoints were multiplied by a concentration factor before the start of the process. After processing the datapoints the second sequence was correlated with an inverse PRBS function [10] in order to get a correlogram. Random noise was not added, noise present after the correlation procedure therefore only depends on non-linearities (or imprecise computer algorithms).

Figure 7

Schematic diagram of the simulated non-linear mixers in series model. Where $\tau(c)$ and $1+\tau(c)$ multiply the signal, $\oplus$ add up signals and Δ is a time delay.

Simulation Results and Discussion

To produce data similar to the experiments, the values of the parameters of the Langmuir function (Eq. (8)) were selected in such a way that the adsorption behaviour of the simulated single injection chromatograms was roughly the same as in the experiments. The values of the parameters were: $K_1 = 1$, $A_1 = 2 \cdot 10^{-2}$, $K_2 = 50$ and $A_2 = 10^8$. The volume fraction, V_{fr} , was chosen to be 0.5. To produce a symmetrical peak shape, n was chosen to be 40. Figure 1 presents the relation between C_s and C_m for these parameters, calculated by the subroutine "Langmuir". Figure 8 shows the result of simulated single injections at several concentration levels. The level of the first simulated peak was selected in a concentration range which was not disturbed by non-linearities. The others started at a concentration level of $12 \cdot 10^{-6}$ and successively down with a factor of 2 until $19 \cdot 10^{-8}$. The peak areas were normalised to compare the different concentration levels in Figure 8. The result of the simulated single injections match quite well with practical measurements (Figure 4), a comparable adsorption behaviour can be observed. The distortion of the peak caused by the simulated adsorption is similar, which makes the NMS model useful in describing a column with a non-linear distribution function.

Figure 8

Simulated single injection chromatograms. The first (furthest left) peak was not affected by non-linearities. The other peaks start with a simulated injection level of $12 \cdot 10^{-6}$ and successively down with a factor of 2 until $19 \cdot 10^{-8}$ for the last simulation. The peak areas were normalised.

Figure 9
Simulated correlograms. Injection levels of $3 \cdot 10^{-7}$ (a1-a3) and $75 \cdot 10^{-9}$ (b1-b3) were simulated. Normal correlograms (a1, b1), inverse-bit correlograms (a2, b2) and the inverse-bit subtracted from the normal correlogram (a3, b3) are shown. The correlograms were shifted circularly in time to match the practical experiments.

The results of the simulated correlation experiments are presented in Figure 9. Two areas were chosen in the non-linear part of the distribution function: $3 \cdot 10^{-7}$ (Figure 9a) and $75 \cdot 10^{-9}$ (Figure 9b). Normal correlograms (Figures 9a1, 9b1), inverse-bit correlograms (Figures 9a2, 9b2), and the inverse-bit subtracted from the normal correlograms (Figures 9a3, 9b3) are shown. For direct comparison between simulations and prac-

tice, the simulated correlograms were shifted circularly in time until the retention times of the simulations matched those of the practical experiments.

The similarity of the simulations (Figure 9) and the experimental results (Figure 6) is astonishing. Not only are the peaks similar in shape, but also the correlation noise, including ghost peaks, match to a large extent. The ghost peaks appear and disappear with subtraction in exactly the same way as in the practical experiments. Obviously, the NMS model together with the double Langmuir function describe the non-linear processes in CC very well.

Conclusion

Up to now some papers [11–14] in the field of correlation chromatography, multiplex chromatography or multiple input chromatography have attributed an important part of the correlation noise to a non-linear response of the column. Simulations and practical experiments show that correlation noise only contributes substantially when there are severe non-linearities. In common practice non-linearities can be present, but in most cases they will not be as extreme as the examples presented in this paper. When there is not much difference between a single injection chromatogram and a CC correlogram with respect to separation, it can be expected that correlation noise caused by non-linearities is a negligible factor when a high noise level is present in the correlogram. CC can even be used as a tool to enhance the separation when non-linearities are causing problems in chromatography.

The NMS model presented here worked beyond expectations. It can be of further use in the theoretical development of CC, and perhaps also in conventional chromatography. Undesired adsorption, overloading, and other non-linearities can be simulated, if wanted in combination with CC. The double Langmuir function is mathematically a very simple model. It can be questioned here whether it is the most suitable function in combination with the NMS model. With a polynomial or another suitable function, it might be possible to describe non-linear processes in a more accurate way. Starting from a number of single injection chromatograms or correlograms, the coefficients of the polynomial can be estimated by an iterative optimization routine.

It may be concluded that applying CC to reduce the disadvantages of a non-linear response of the column, opens a new field in CC.

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