CHEMICAL REVIEWS

Review

pubs.acs.org/CR

Artifacts in Liquid-Phase Separations—System, Solvent, and Impurity Peaks

Boguslaw Buszewski,*[,†] Szymon Bocian,[†] and Attila Felinger[‡]

[†]Department of Environmental Chemistry & Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Gagarin 7, 87-100 Torun, Poland

[‡]Department of Analytical and Environmental Chemistry, Faculty of Science, University of Pécs, Ifjúság útja 6, H-7624 Pécs, Hungary

CONTENTS

1. INTRODUCTION

The adsorption and partition of analytes and solvents on the stationary phase—which control the retention during the chromatographic separation—are very complex phenomena.[1] The chromatographic elution in liquid−liquid and liquid−solid systems brings about many effects, the most important of which are the hydrophobic effect, preferential solvation, and displacement effects.[2−4]

To increase the selectivity of a chromatographic system, a binary or multicomponent mobile phase is used.[5] During an analysis, apart from the signals of sample components, some additional signals are also observed on a chromatogram.[6−8] They are caused by the perturbation of the equilibrium between mobile and stationary phases, which takes place during the injection of a sample with a composition different than that of the mobile phase.[9] The sample injection causes the perturbation of the equilibrium in the column. As a result, a number of additional peaks are generated. However, some system peaks are unrecorded, depending on the type of detection.[10] The detectable system peaks (using a selective detector, e.g., UV detector) that appear in chromatograms are called solvent peaks. In the literature, these signals are described variously as solvent peaks, system peaks, ghost peaks, and vacancy peaks.[11−16] System peaks have been observed in a number of types of liquid chromatography.[6,7,17−23]

In reversed-phase liquid chromatography (LC), the hydrophobic effect, preferential solvation, and displacement effects caused by complex retention mechanisms result from the individual properties of the molecules present in the chromatographic system and from the specific interactions between them.[24] Approximately 90% of analytical separations have been carried out in reversed-phase LC with chemically bonded stationary phases.[25] Under these conditions, hydrophobic, nonpolar packing materials and a polar mobile phase are used.[16] A detailed description of a system functioning as a mobile phase−stationary phase is more difficult due to a very large number of parameters that determine the properties of the phases and the interactions between all the components.

The solvent peaks cannot be assigned to any of the separated solutes,[16] and they prevalently appear at the beginning of the chromatogram. The retention volume of the solvent peak is usually more or less equal to the dead volume of the column.[6] The presence of the system peak (solvent peak) can complicate the qualitative and quantitative measurement of individual peaks in a chromatogram, if the separated substances exhibit small retention factors, as seen in Figure 1,[5,8] or when those compounds coelute. Furthermore, it is also difficult to determine the correct dead volume of the column. However, the retention of solvent peaks was proposed as one of the methods used to characterize the dead volume of the column.[23,26−31]

2. SOLVENT PEAKS IN LIQUID CHROMATOGRAPHY

2.1. First Theories about Solvent Peaks

A number of authors have considered the reasons for the presence of solvent peaks. Šlais and Kreči[6] claim that solvent peaks are caused by the local concentration change of the eluent components after the injection of a sample or pure eluent either more or less concentrated than the equilibrium concentration between the phases. McCormick and Karger[20,32] and Solms et al.[18] propose that solvent peaks are caused by displacing the adsorbed organic modifier after an injection. Melander et al.[21] suggest that, in solvent peak creation, the solvation effect of a stationary phase by eluent components is the most important factor. Knox and Kaliszan[30] introduced a

Received: May 25, 2011
Published: February 6, 2012

   dx.doi.org/10.1021/cr200182j | Chem. Rev. 2012, 112, 2629−2641

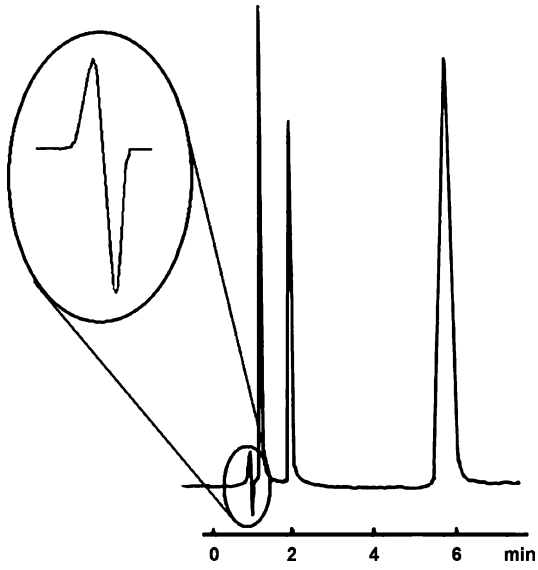

Figure 1. Chromatogram of reversed-phase liquid chromatography (RP LC) separation of aromatic hydrocarbons showing the position of the solvent peak.

relationship between the extent of the disturbance and the retention volume of the solvent peak:

$$V_r = V_M \frac{dy_A}{dx_A} \quad (1)$$

where V_r is the retention volume of solvent peak, V_M is the dead volume of the column, dx_A is the change of the volume fraction of component A in the binary eluent (A + B), and dy_A is the change of the volume fraction of component A in the stationary phase.

Riedo and Kováts[17] showed that, for a $(n + 1)$-component eluent, when one of the components is injected, n solvent peaks are obtained and their retention volumes are constant and independent of the injected component if the perturbation caused by the injection is small enough. Berek and co-workers suggested a strict relationship between the presence of solvent peaks and changes in the solvation processes of the stationary phase by the solvent molecules.[19,33,34] Erkelens et al.[35] noticed that the injection of pure water can form a solvent peak by dilution of the mobile phase. In the measurements carried out by Buszewski et al., the retention volume of solvent peaks was variable on the octadecyl stationary bonded phase and varied together with the change in the mobile phase composition. Still, on an alkylamide stationary bonded phase containing polar groups, the retention volume was stable.[16] These results confirmed the role of the stationary phase solvation in the solvent peaks formation.

All the above-mentioned theories connect the presence of the solvent peaks with interactions between a stationary phase and components of a mobile phase. The primary effects are solvent adsorption on the stationary phase, the hydrophobic effect, the displacement effect, preferential solvation, and local change of the mobile phase concentration. These effects take place when the sample is injected onto the column.[26]

2.2. Detection of Solvent Peaks

Solvent peaks can be observed when nonselective detectors are used (e.g., a refractometer or a microadsorption detector). It is also possible to observe solvent peaks on a chromatogram recorded by a specific detector (e.g., a UV, an electrochemical, or a polarographic detector).[6,26]

In RP high-performance LC (HPLC), the most popular mobile phases are mixtures of methanol (MeOH) or acetonitrile (ACN) with water. These organic modifiers are commercially available and transparent to the UV radiation. Surprisingly, one can also detect solvent peaks at λ = 254 nm. At this wavelength the organic modifiers are fully transparent. In this case, the detection of solvent peaks must be connected with effects not yet discussed in this review.[26] The solvent peaks may be connected with disturbance of the light absorption in the detector. This effect is caused by a simple construction of UV detector cells and its miniature size. This explains why modern UV detectors use a focused light beam or specially shaped cell.[36] In reality, the intensity of radiation transmitted is lower than the intensity of incident radiation. Light scattering in some liquid is proportional to the increase in the square of the refractive index, as well as to the wavelength raised to the negative fourth power. Light scattering is described by the Rayleigh equation:

$$R = \frac{\pi^2 V (n^2 - n_0^2)^2}{2\lambda^4 r^2 N}(1 + \cos^2 \Theta) \quad (2)$$

where R is the Rayleigh scattering, V = the volume of scatter center, n = the refractive index, n_0 = the refractive index of the pure solvent, Θ = the scatter angle, λ = the wavelength, r = the length between the scatter center and the measurement point, and N = the amount of molecules.

The change in the refractive index depends on the contraction effect between the mobile phase components.[5] As seen in Figure 2, the refractive indices of methanol–water and

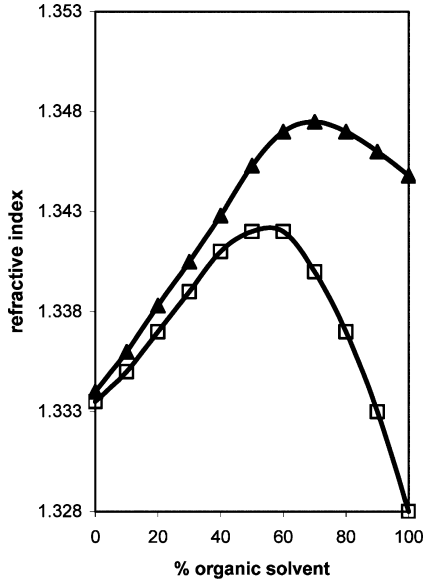

Figure 2. Refractive index of a mixture of methanol–water (□) and acetonitrile–water (▲) vs concentration of organic solvent in the mixture.

acetonitrile–water mixtures change with their compositions. In the both cases, the mixtures exhibit refractive indices higher than each of their pure components. For a methanol–water mixture, the maximum value of n_{20}^D = 1.342 is observed when the methanol content is ca. 50%.[37] A similar situation is observed for acetonitrile–water mixtures. The maximum value

dx.doi.org/10.1021/cr200182j | Chem. Rev. 2012, 112, 2629–2641

of n_{20}^{D} = 1.347 is obtained when the mixture contains 70% acetonitrile. In this case, the refractive index of the eluent in the detector cell varies with the liquid flow (of varied compositions). The amount of scattered radiation varies and the total absorbance changes. The change in absorbance may be detected as a solvent peak.[5]

Changes in the refractive index of an organic modifier mixture with water are easily detectable with the use of a refractive index detector. This detection method makes solvent peaks the most observable. In a spectrophotometer detector, its simple construction and small volume of the detecting cell makes light scattering larger. A change in the refractive index of solvent passing through the UV detector cell may cause deflection of a part of the radiation beam from the optical path. It affects the detector signal, even though there may be a small difference in the absorbance of pure liquids. The change of the absorbance may also be caused by wetting of the cell wall by a mobile phase component percolating through the detector.[5]

The mobile phases used in RP HPLC exhibit strong specific interactions between molecules. Mixtures of water and polar nonelectrolytes show extremes in thermodynamic, optical, and other properties when their compositions change. Light scattering may be caused by volume contraction. When water is mixed with methanol to the concentration of 47.73% methanol, the contraction equals about 1 mL/mol.[38] This effect is brought about by the creation of hydrogen bonding between water and alcohol molecules. This observation is very important from a separation processes modeling point of view.

The presence of a solvent peak, its shape, and its size depend on many factors in the chromatographic system.[26] The most important parameters are the type of the stationary phase as well as the qualitative and quantitative composition of the mobile phase. The wavelength of the detection and impurities in the mobile phase also affect the solvent peak detection.

The solvent peaks can be related to the local change in the mobile phase concentration; thus, the retention volume of a solvent peak is equal to the retention volume of an organic modifier under the same chromatographic conditions. In binary, hydroorganic mobile phases with a UV detection system, solvent peaks may be positive or negative, depending on the mobile phase composition. Positive peaks occur when the concentration of an organic modifier in the eluent increases. Negative peaks (vacancy peaks) occur when the concentration of water increases (and organic modifier concentration decreases).

2.3. Mobile Phase Composition

The retention volume of the solvent peaks changes with the equilibrium concentration of the mobile phase. The shape of the curve depends on the type of organic modifier and stationary phase.[16] As seen in Figure 3, the retention of ACN changes in a parabolic manner over a range of eluent compositions. In general, the retention volume of ACN is lower than the retention volume of methanol under the same conditions. A minimum of the retention of all the modifiers is observed between 50% and 70% organic solvent concentration in the eluent.[39−41] Changes in the solvent peak retention together with the mobile phase composition may be caused by a number of factors. The general trend of changes in the retention together with the increase in water concentration is connected with the mobile phase elution strength. Elution strength of a hydroorganic mixture increases with the increase of the organic solvent concentration.[20] During their passage

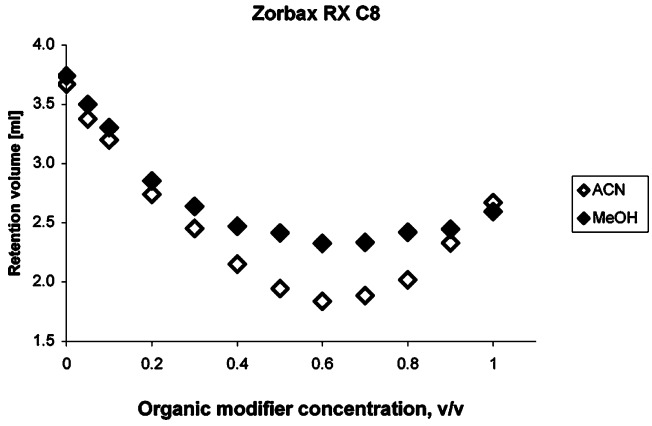

Figure 3. Relationship between the retention volume of solvent peaks of MeOH and ACN and the mobile phase concentration on the Zorbax RX C8 stationary phase.

through a chromatographic column, the solute molecules constantly and reversibly transfer from the mobile phase to the stationary phase. On the other hand, the mobile phase composition has a large influence on the solvation and conformation of chemically bonded ligands.[16,41,42] These phenomena may change the mass transfer kinetics between the mobile phase and the solvated stationary phase if the structure of the stationary phase is changing from a "brushlike" to a collapsed state structure.[26]

The retention volume and the shape of the system peaks do not depend on separated sample components. They only depend on the mobile phase composition. When a pure solvent is used as a mobile phase and the same solvent is injected, no peak occurs in a chromatogram.[5,16,26]

2.4. Impurities in the Mobile Phase

The eluents used in liquid chromatography may contain some impurities. These impurities can also absorb UV radiation that passes through the binary mobile phase. However, the UV absorption by impurities cannot lead to the occurrence of solvent peaks. It is impossible to eliminate the solvent peaks by using "HPLC-grade" solvents, but at least in this case, their height decreases to ~40% of the original value in comparison to solvents without special purification.[5] Impurities in the mobile phase cannot be a reason for the presence of solvent peaks; however, nonretained impurities can cause the solvent peaks to be more visible in a UV detector. Even when using a gradient-grade or LC-MS-grade solvent for an HPLC analysis, the solvent peaks are always present in chromatograms. This suggests that the presence of these peaks is connected with the processes that take place in the column during chromatographic elution.

Solvent peaks are not caused by the presence of air dissolved in the eluent. When the mobile phase is degassed under vacuum, ultrasonically, or by boiling under reflux, the solvent peaks do not disappear.[5] However, some extra peaks that appear to be identical to analyte peaks may be caused by the injection of air into the system. Obviously, these signals cannot be named "solvent peaks". Air injected onto the column will be immediately compressed and dissolved into the mobile phase due to the pressure of the system. The gas-enriched zone of the mobile phase will diffuse in the same way as a chemical analyte band when it progresses down the column. The mobile phase saturated with air has a higher absorbance than the degassed

dx.doi.org/10.1021/cr200182j | Chem. Rev. 2012, 112, 2629−2641

one.[43] In this case, a gas-saturated mobile phase peak that looks very much like an analyte peak may be observed.[44]

2.5. Wavelength of UV Detection

The height and the area of the solvent peaks depend on the wavelength of a detection. The height of the solvent peaks decreases when the detection wavelength increases above the "cut-off" limit for an organic modifier. It is very low or almost negligible at wavelengths above 320 nm. At a wavelength of 254 nm, the most clear solvent peaks are observed for a 70/30% (v/v) mixture of methanol–water.[5] As seen in Figure 4, the shapes

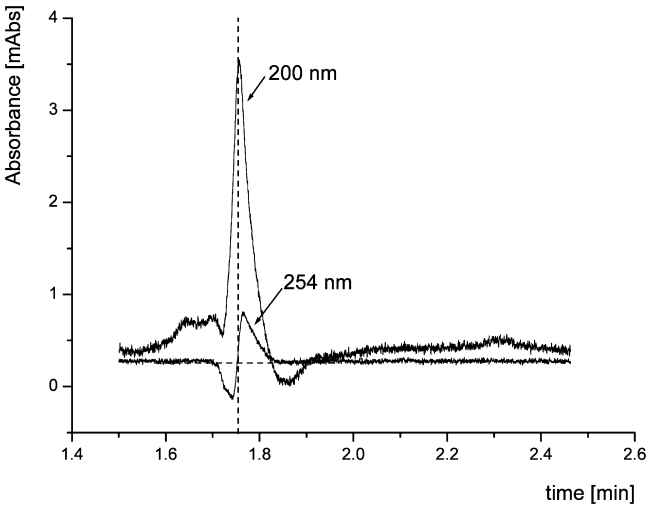

Figure 4. Solvent peaks recorded at λ = 200 nm and λ = 254 nm. Conditions: stationary phase, Waters Symmetry C18; mobile phase, 70% methanol in water.

of the solvent peaks depend on the wavelength of the detection. The peak obtained at λ = 254 nm shows both positive and negative absorbance parts. The second one, recorded at λ = 200 nm, has only positive absorbance. The maximum of the peak obtained at λ = 200 nm has the same retention volume as the crossing point of the signal with the baseline on the chromatogram obtained at λ = 254 nm. However, at λ = 200 nm, when the concentration of an organic modifier is high in the mobile phase, a negative peak occurs, and it disappears when the concentration of water increases.

The shape of the solvent peak recorded at λ = 200 nm depends on the mobile phase concentration. In general, highly symmetrical peaks may be measured from 100% to ~70% of an organic modifier in the eluent. Obviously, this parameter depends on the type of the stationary phase. When the concentration of the organic modifier decreases, the peaks become asymmetrical. It is noteworthy that, for some stationary phases, such as C30 or C4, and for low concentrated eluents, the peaks are quite symmetrical. When pure water is used as a mobile phase, solvent peaks have very significant tailing.[45]

2.6. Solvent Peaks in the Measurement of Column Dead Volume

The accurate measurement of the column dead volume is necessary for the determination of fundamental retention parameters, such as the retention factor (k) and the selectivity (α).[16,46] In the literature several definitions have been suggested for the dead volume.[30] The kinetic dead volume (V_0) is the volume of the mobile phase measured by the elution volume of a nonretained marker. The thermodynamic dead volume (V_M) is measured by the excesses of adsorption of all the eluent components in the bulk phase.[30] The determination of the dead volume in RP HPLC is difficult because the separation is carried out with a hydroorganic mobile phase, in which specific and nonspecific interactions between the individual mobile phase components occur. These interactions also influence the physicochemical properties of the stationary phase.[23,24,26,47–49]

Many methods for the determination of the column dead volume are known. These methods are based on gravimetric measurements,[50] linearization of a logarithmic dependence of reduced retention time, and the number of carbon atoms for a homologous series.[51–53] The column dead volume can be determined by injecting a nonretained substance, e.g., inorganic salts (sodium nitrate, sodium chloride)[54,55] or other organic species, for instance, uracil,[56] acetone,[4] and dimethylformamide,[57] among others. Reliable results are obtained when deuterium-labeled substances are used (CH_3OD and most commonly D_2O).[17,20,26,30,49,58–60] It should be mentioned that both size exclusion and ionic exclusion effects may alter the elution volumes of nonretained markers in HPLC systems. Additionally, significant differences can be found between the column dead volumes measured with different markers, as was shown by Jandera et al.[61]

In RP HPLC, thiourea is very often used as a dead volume marker because this substance exhibits a negligible retention on the stationary phase.[62] Comparing the retention of the solvent peaks with thiourea, one can find that the retention volume of the solvent peak is rather close to the dead volume of the column. In this case, it is suggested in literature to use the solvent peak as a marker of the column dead volume.[5,26] In a methanol–water mobile phase, the retention volume of the solvent peak does not depend on the separated mixture (sample) but depends slightly on the composition of the mobile phase.[26] In our opinion, this change may be caused by the change of the bonded ligands' conformation, which is affected by the solvation process. The retention volume of the solvent peak changes in a nonlinear manner over the range of mobile phase compositions. Buszewski et al.[16] observed that the retention volume of the solvent peak depends also on the type of stationary phase used. For the chemically bonded alkyl phase, the retention volume of the solvent peak under MeOH–water and ACN–water conditions changes in a parabolic manner over a range of eluent compositions. However, when an alkylamide stationary phase is used, the retention time is nearly

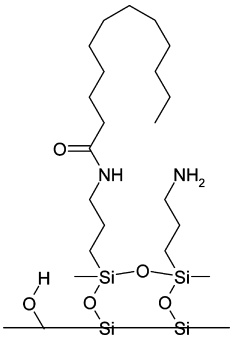

Figure 5. Structure of alkylamide stationary bonded phase.

constant—it does not depend on the composition of the mobile phase. This phenomenon may be caused by the structure of the alkylamide phase, which is more complex than

dx.doi.org/10.1021/cr200182j | Chem. Rev. 2012, 112, 2629–2641

the alkyl phase. In the structure of the alkylamide phase, there are organic chains and three types of polar groups. Thus, water and an organic modifier can penetrate the stationary phase. The solvated alkylamide phase forms a more energetically stable structure.[16] This type of stationary phase is better solvated by water molecules than the alkyl phases.[63]

3. SOLVENT PEAKS OF DEUTERATED SOLVENTS OR SOLUTES

The solvent peaks obtained after the injection of water or methanol are slightly different from their isotopically labeled derivatives. These differences are clearly visible by noting the peak shape.[26] In addition, the dependence of the retention volume on the mobile phase composition differs for deuterated compounds.[20]

In Table 1, the retention volumes of MeOH and MeOD measured on five columns are compared. These volumes are nearly the same for each compound in the given column. Small differences are visible between the retention volumes of H_2O and D_2O. The retention volumes of all the markers decrease with the increase in coverage density of the bonded ligands.[26] As seen in Figure 6, the shapes of MeOH, MeOD, and water solvent peaks are similar. However, when D_2O is injected onto the column, one additional positive peak is observed (V_{R0} in the table).[20,26]

Table 1. V_{RS} and V_{R0} Values for Different Markers Using 70/30% vol. Methanol–Water Composition As the Mobile Phase on C18 Columns with Different Coverage Density of Bonded Ligands (Reproduced with Permission from Ref 26; Copyright 1993 Taylor & Francis Ltd.)

		V_{RS} (mL)				V_{R0} (mL)
phase code	coverage density (μmol/m^2)	MeOH	MeOD	H_2O	D_2O	D_2O
# 1	0.72	1.20	1.18	1.23	1.20	0.57
# 2	1.61	1.16	1.16	1.22	1.13	0.56
# 3	2.60	1.09	1.09	1.15	1.06	0.59
# 4	3.46	0.93	0.95	1.00	0.97	0.56
# 5	4.24	0.84	0.86	0.94	0.99	0.58

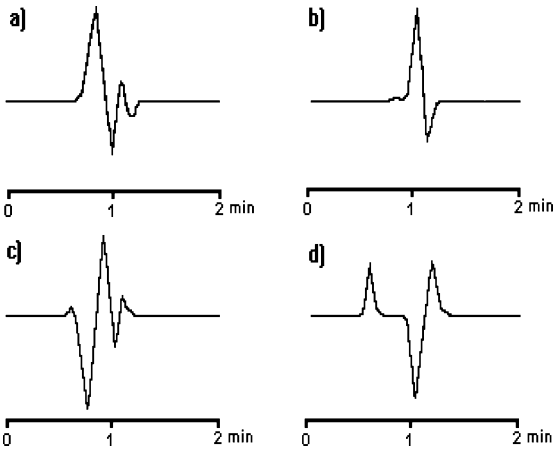

Figure 6. Shapes of solvent peaks obtained after injection: (a) MeOH, (b) MeOD, (c) H_2O, and (d) D_2O. Eluent: methanol–water 70/30% v/v. Reproduced with permission from ref 26. Copyright 1993 Taylor & Francis Ltd.

Experiments by Krstulovic et al.[60] demonstrate that the column dead volume measured with D_2O seems most appropriate. However, the retention volume of deuterated water or methanol depends on the mobile phase composition.[60] The retention volumes of these compounds exhibit a minimum between 40 and 50% organic modifier content in the mobile phase, similar to nondeuterated compounds.[20,21,41,45,54,64,65] The retention volume of the solvent peaks depends on the properties of the stationary phase, e.g., coverage density,[49] which is an effect of the column dead volume.[27,66]

As shown in Figure 7, the retention volume of solvents changes with the coverage density of the packing surface with alkyl ligands. When the coverage density increases, the retention volume of all the solvent peaks decreases.[26] The retention volume of the first peak of D_2O is nearly constant. Most probably, D_2O does not interact with alkyl chains. However, it may interact with residual silanols, but this interaction is rather weak.[20] In this case, D_2O seems to be a nonretained solute and its retention volume may be applied to determine the kinetic and thermodynamic dead volume of the column.[26,58,59]

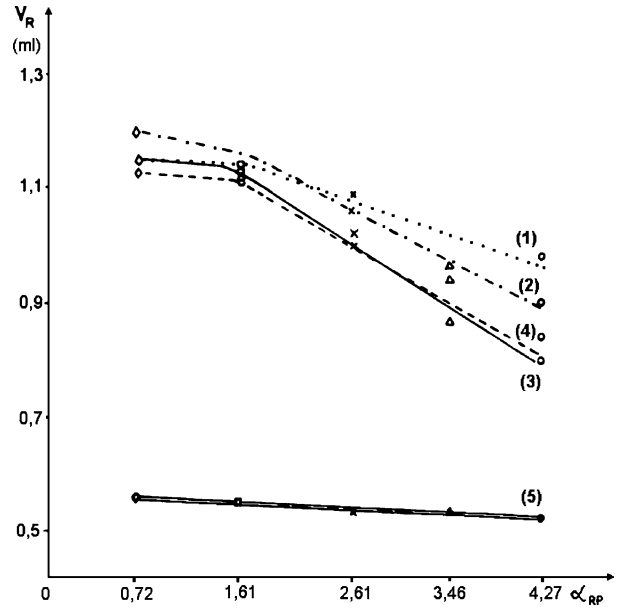

Figure 7. Dependence of the retention volume of solvent peaks on the coverage density for different markers: (1) D_2O, (2) H_2O, (3) MeOH, (4) MeOD, and (5) the first peak of D_2O. Reprinted with permission from ref 26. Copyright 1993 Taylor & Francis Ltd.

4. SYSTEM PEAKS IN GRADIENT ELUTION HPLC

Reversed-phase gradient HPLC is an essential and extremely powerful technique in liquid chromatography. However, gradient HPLC can sometimes be plagued with seemingly random and uncontrollable system peak problems.[67] These problems were discussed in detail by Jandera and Churacek[68] and Snyder and Dolan.[69]

The potential sources of the system peaks present in gradient elution are mobile phase impurities. Other reasons could involve physical or mechanical aspects of mobile phase delivery, sample introduction, and effects of the interaction between the

dx.doi.org/10.1021/cr200182j | Chem. Rev. 2012, 112, 2629–2641

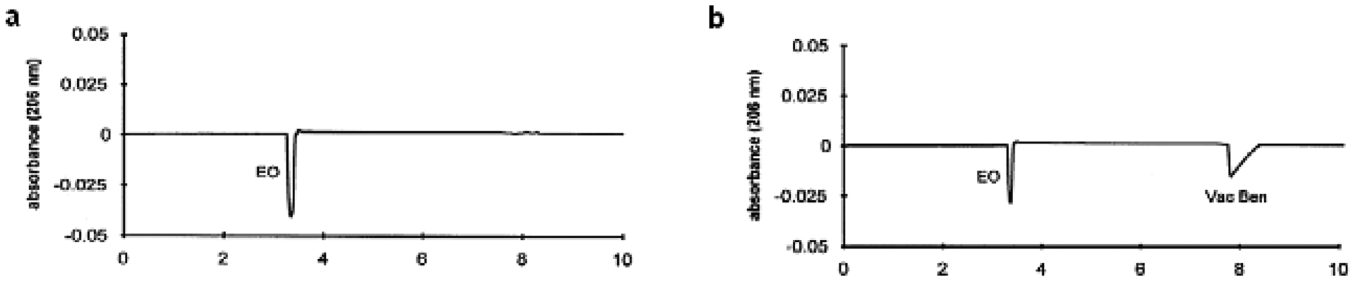

Figure 8. Effect of sample concentration on the electroosmotic flow peak (EO) and benzoic acid vacancy (Vac Ben) in reverse capillary electrophoresis (CE) analysis. BGE = 10 mM monochloroacetic acid (ClAc), 10 mM tris(hydroxymethyl)aminomethane (Tris), and 10 mM benzoic acid (Ben). (a) Injection of water; (b) injection of 10 mM ClAc−Tris (1:2, v/v). Reprinted with permission from ref 74. Copyright 1997 Elsevier.

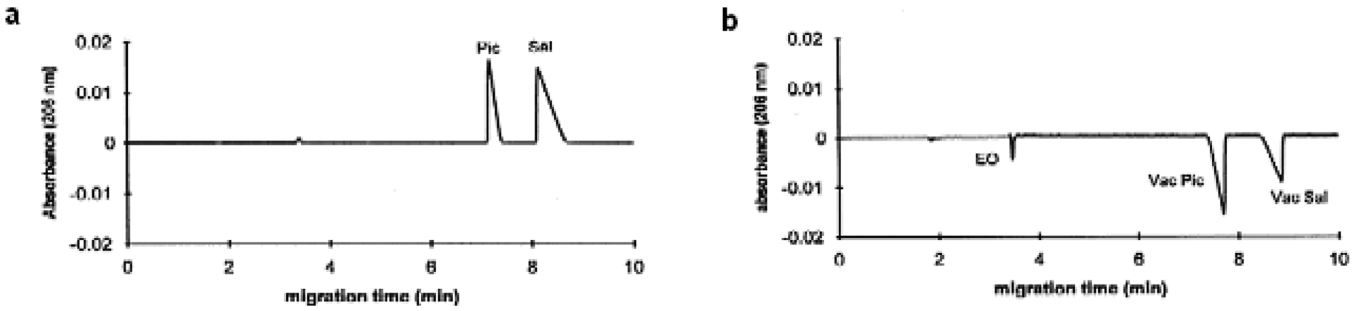

Figure 9. Direct CE analysis of picric and salicylic acids in direct 2-morpholinoethanesulfonic acid (MES) BGE. Injection of 0.8 mM (each) of picric acid and salicylic acid (a) and reverse CE analysis of picric acid and salicylic acid vacancies (b). BGE = reverse MES injection of 5 mM MES−Tris (1:2, v/v) buffer. EO stands for electroosmotic flow. Reprinted with permission from ref 74. Copyright 1997 Elsevier.

mobile and the stationary phase. A chromatogram may contain system peaks from a variety of these sources. This makes the system peak problem quite complex.[67] However, it should be emphasized that these signals cannot be named "solvent peaks".

During gradient elution, the band compression mechanism may focus organic impurities present in the mobile phase. Impurities that show some retention at low-eluent strength may be focused into peaks as the gradient progresses.[70] When a solvent linear gradient is applied, the concentration of a stronger organic solvent is always higher at the tail of the band than at the front of the band. Hence, the component molecules at the front of the band are more strongly retained by the stationary phase than the molecules at the back of the band. If the focusing is sufficient, system peaks appear to be identical to injected analyte peaks.[67,70] During elution, the impurities from the organic solvent would be concentrated on the reversed-phase HPLC column and then eluted by a trace enrichment and focusing mechanism.[71] In the early part of the gradient, when the solvent mixture has relatively low overall eluotropic strength, impurities from the organic solvent may still be retained and eluted in the same manner. Indeed, some impurities may be strongly retained with 80% organic solvent and eluted as system peaks at 90−100% organic solvent.[72,73]

5. SYSTEM PEAKS IN CAPILLARY ELECTROPHORESIS

Studies of system peaks (eigen peaks) in capillary electrophoresis as well as in HPLC systems were carried out by many authors.[74−79] These zones move through the medium but consist only of disturbances in the concentrations of the background electrolyte (BGE). They contain no analytes but an electropherogram often contains additional peaks.[77−81] The number of system zones is the same as the number of constituents in the system. The more complex the electrolyte solution, the higher is the number of system zones. An electrophoresis system with N constituents has N system eigenmobilities, which may generate N system zones. Those moving disturbances that are not associated with any analyte are called system zones.[79,82]

However, the term "system peak" in capillary electrophoresis is poorly defined, and thus it is applied in various ways. Many effects may induce disturbances in the baseline. One of them is the release of the adsorbed ionic species from the capillary wall and its migration through the electrolyte system. If this compound is detected, an irreproducible disturbance is observed in the electropherogram, but this is not a system peak. The term "system peak" may be applied for peaks present in the electropherogram that correlate with zones that do not contain any sample ionic species and contain only the BGE species with different compositions.[78] In this case, more peaks are observed than are expected from the number of ions in a sample.

System peaks can be created by introducing the BGE-like zones with a composition different from the BGE, or can even be caused by the injection of a sample of water.[78] If there is a component of the BGE that is absent in the sample injection, then the injection zone represents a vacancy of such a component, and after applying voltage across the capillary, this vacancy migrates as an individual zone and can be detected. If there are more components missing in the sample and present in the BGE, then the sample represents a vacancy for each individual component that is absent, and these zones migrate individually and can be separated and detected.[74] Every analyte migrating in the capillary is accompanied by a corresponding eigenzone moving at the same position as the analyte. These eigenzones enable indirect detection if the analytes are invisible to the detector.[82] In a system consisting of a BGE with two or more co-ions, zone electrophoresis of a sample provides one normal zone for each sample component that is absent in the

dx.doi.org/10.1021/cr200182j | Chem. Rev. 2012, 112, 2629−2641

BGE and one system zone (vacancy) for each, but one BGE component that is absent in the sample.[75]

System peaks may appear only if the detector is responsive to a component of the background electrolyte, especially in the case of indirect or conductivity detection.[79] In this case, system peaks may or may not be detectable, depending on whether the BGE contains a detector-responsive constituent. However, even when this does not occur due to the system zones being "invisible" to the detector, they may influence the peak shapes of analytes sitting at the same position, analogously to HPLC.[83,84]

The magnitudes of the system peaks created by a sample are proportional to the injected amount of this species in the sample. If a species present in the BGE is also contained in the sample, but at a different concentration, then it can create a migrating vacancy (negative) peak or a positive peak, depending on the ratio of its concentration in the sample to that in the BGE.[74]

The sample injection also creates a zone that is marked by stationary boundaries and does not move, due to the applied electric field. The mobility of this zone is zero or close to zero, and it does not usually cause a problem in practice. If the zone is stationary, it remains in the liquid at the original position of injection. In the literature, this zone is called a stationary zone, injection zone, or water zone.[79] Provided there is no electroosmotic flow (EOF), this zone is stationary and the migrating sample ions are replaced with those of the BGE at a concentration adjusted to the Kohlrausch regulating function (KRF) of the sample.[74]

In the presence of electroosmosis, the above zone is driven by EOF. It can sometimes conveniently serve as a marker for determining the velocity of the EOF, and it is also called the EO zone.[79,82] It is similar to an HPLC system in which the retention volume of solvent peaks may be used to determine the void volume.[26] The problem with the presence of system peaks is much more serious when the mobility of the system zone is the same or very close to the mobility of the separated analytes.[85,86]

Depending on the original KRF of the sample, the EOF peak may be negative or positive. A negative peak observed via an indirect detection of a species corresponds to the species that was present in the sample, whereas a negative peak of a migrating vacancy corresponds to a species that was absent in the sample.[74,82] Some studies provide valuable practical rules for the prediction of system peak positions for simple BGE in electrophoretic systems, which can be described adequately by using the KRF.[87] More sophisticated mathematical tools have also been applied to deal with more complicated situations, e.g., polybasic buffers.[88,89] A linearized mathematical model of electromigration, recently reported in the literature, enables the prediction of the existence of system zones and their mobilities.[82] The model is based on the calculation of the eigenvalues of a certain matrix. The eigenmobilities can be calculated by the freeware program PeakMaster.[90,91] This program allows prediction of the existence and migration times of system peaks with good reliability, as well as the calculation of system eigenmobilities.[92]

Although the presence of system peaks in electropherograms is usually considered as an unwanted phenomenon that accompanies the separation, it offers a simple method of critical micelle concentration (CMC) determination in micellar electrokinetic chromatography (MEKC). The presence of surfactants in the system affects the number of system peaks observed in electropherograms. The positions of some system peaks are strongly dependent on the surfactant concentration (whether the surfactant creates micelles or not).[92]

6. SYSTEM PEAKS IN LINEAR AND NONLINEAR CHROMATOGRAPHY

For a chromatographic system containing $n + p$ constituents (n sample components and p modifiers), $n + p - 1$ system peaks can be observed in the chromatogram, even if the perturbation is very small and even if the modifier is weakly adsorbed. In this case, for an n-component sample injected into the binary mobile phase, $n + 1$ system peaks appear.[17,93,94] All of these signals result from the perturbation caused by the sample injection of the equilibrium of the modifier (organic solvent) between the mobile and stationary phases. A detector selective for the sample components does not detect the system peaks. In the case of a nonselective detection, one system peak that corresponds to the retention of the modifier is observed. The other system peaks are eluted at the same retention volume as the sample components and interfere with them. If the detector is selective for the modifier, all of the $(n + 1)$ system peaks are observed. If the detector responds to the sample components and to the modifier, the detected signal is a combination of those two bands, and it can complicate the interpretation. The position, the sign, and the relative importance of the system peaks connected to the sample components may be calculated using the ideal model of chromatography if the competitive Langmuir model is assumed.[10] Similar results were obtained with a numerical solution of the equilibrium-dispersive model.[94]

The application of the system is the qualitative and quantitative analysis of those analytes that cannot be detected directly. In this case, indirect detection can be used. A modifier is added to the mobile phase, which can be easy detected. This method may be used for analytes that have no UV chromophores using aromatic compounds as modifiers or with fluorescence or eletrochemical detection.[95,96] The indirect detection method is used in ion-pair chromatography to determine the nondetectable cationic and anionic organic components.[97−101] It can also be used in ion-exchange chromatography, where a UV-absorbing counterion is used as a detectable additive.[102−106] In RP HPLC, the indirect detection of uncharged analytes may be carried out using a nonionic additive.[95,107−109]

In ion-pair chromatography, when a large-volume sample is injected, a large modifier system peak occurs because of the vacancy of the modifier. The rear part of this peak profile is an effect of positive gradient of the modifier concentration. If the ionic solutes elute during the passage of this gradient, the peaks are extremely narrow because of the gradient-focusing effect. If a peak is eluted in the front part of the system peak, its profile may be deformed.[110,111]

System peaks can be present also in nonlinear chromatography. This phenomena is discussed by Helfferich and Klein[93] and by Golshan-Shirazi and Guiochon in the case of single-component[112] and multicomponent[113] samples injected into a multicomponent mobile phase. These studies are based on the competitive Langmuir adsorption model. The competition between the strong solvent and the sample components influence the shape of the elution bands. It is possible that a combination of modifiers could enhance the symmetry of the bands.[114] Both the retention time and the component band profile depend on the relative strength of adsorption of the modifier (strong solvent) and that of the components from

dx.doi.org/10.1021/cr200182j | Chem. Rev. 2012, 112, 2629−2641

their solution in a pure weak solvent, as well as on the column efficiency, the modifier concentration, the column saturation capacity, and the sample size.[10]

If the modifier is less well adsorbed than the studied compound, quasi-Langmuirian peak profiles are observed.[114] If the modifier exhibits adsorption stronger than the compound, the anti-Langmuirian peak is observed. The diffusive front parts of the different profiles are not coincidental as should be in the case of a true anti-Langmuir isotherm, because they begins progressively earlier with increasing sample size.[10] If the sample size increases, the amplitude of the band profile increases, and its shape changes progressively. At a low sample size, the band profile may be anti-Langmuirian whereas it can be a smooth rectangle when the sample size becomes larger.[115] This is a result of column overloading.

The band profile depends strongly on the modifier concentration. If the modifier is adsorbed more strongly than the solute, changes in the modifier concentration may intensely affect the compound band profile, e.g., Langmuirian profile at a low modifier concentration and anti-Langmuirian at a high modifier concentration. Band profiles shift from the first to the second type in a narrow concentration range, which corresponds to the reversal of the elution order of the modifier system peak and the peak of the solute.[116]

In the case of a two-component sample, if the modifier is much less well adsorbed than the solute, the positive system peak appears on the chromatogram near the column dead volume. Further, two positive bands of compounds are eluted. Each of these peaks is accompanied by the component system peak that corresponds to the replaced modifier from the stationary phase.[114] While increasing the modifier concentration, the component bands become more symmetrical and less well retained. Thus, strong solvents are used to elute compounds, which exhibit high adsorption energies, faster.[10] If the modifier is adsorbed more strongly than the components, the two component bands are highly unsymmetrical. These two components are poorly resolved and eluted immediately after the modifier system peak.[114] When the concentration of the modifier increases as a result of competition between the modifier and compounds, a progressive change in the direction of the band asymmetry is observed.[113,114]

7. SOLVATION IN HPLC

7.1. Adsorption and Partition

In liquid chromatography, four types of separation mechanisms may be distinguished: adsorption, partition, ion-exchange, and size exclusion. The basic property that is applied to separate substances in RP HPLC is the difference in solute adsorption on the stationary bonded phase. The organic solvent is distributed between the mobile and stationary phase as well as the solute.[20,117] The organic modifier is enriched in the stationary phase.[1,65,118] This process is more complicated due to a number of interactions between the multicomponent mobile phase and the variety of adsorption centers in the structure of the stationary phase.

Adsorption is a phenomenon that takes place when some substance accumulates on the surface of the solid phase. This process is a consequence of surface energy activity.[1] In a bulk material, all the bonding requirements of the constituent atoms are fulfilled. Atoms on the surface experience a bond deficiency. Thus, it is then energetically favorable for these atoms to interact with whatever happens to be available.[1] In RP HPLC, the liquid phase close to the adsorbent surface is enriched with the organic modifier because of the interactions of the mobile phase components with the adsorbent surface. This effect has a great influence on the interaction of the solute with the adsorbent surface and, accordingly, on the solute retention.[40]

In RP LC systems, the mobile phase is in contact with the hydrophobic surface of the stationary phase. The composition of the mobile phase at the surface is different from its bulk composition. Under these conditions, an alternative mechanism of distribution can take place: partition. Partition is a volume process that is favored when the stationary phase is thick enough to accumulate the solute molecules in its volume. The partitioning mechanism in reversed-phase chromatography systems differs significantly from that which takes place in a system of two immiscible liquids.[1] We are aware that the presented differentiation between adsorption and partition in RP LC is very simplified. The discussion of the retention mechanism that was started a number of years ago is ongoing. An excellent treatment of this topic was presented by Riedo and Kováts.[17]

For the separation of a polar compound, other chromatographic techniques have recently been developed: hydrophilic interaction liquid chromatography (HILIC) and aqueous normal phase (ANP) chromatography.[119,120] In the HILIC mode the residual silanols and different polar groups in the organic ligand structure provide the retention of polar compounds. These groups are polar adsorption centers, and they are preferentially solvated by water molecules, whereas acetonitrile (which is the most common organic modifier in this mode of LC) solvates organic ligands. ANP phases can provide the retention of both hydrophilic and hydrophobic solutes.[120] If, in HILIC mode, the concentration of water in the mobile phase is rather small (usually <15%), it is possible to apply a much higher concentration of water in the eluent when the ANP mode of LC is used. The polarity of the used stationary phase and the concentration of water in the mobile phase strongly influence solvation processes in the chromatographic systems. Similar results might be obtained on RP phases with very low surface coverage density where the amount of the residual silanols is significant.[121]

It is commonly believed that solvent adsorption in RPLC is controlled by the distribution of solvent molecules between the bulk mobile phase and the stationary phase. Interactions of ligands bonded on the stationary phase surface with a solute are primarily influenced by the hydrophobic effect.[122] The concentration of the mobile phase at the surface of the stationary phase differs from its bulk concentration.[20] The residual silanols also play a very important role in the adsorption of a solute.[42] Indeed, the retention of the solute is controlled by partitioning between the layer of the bonded nonpolar groups onto the stationary phase, or by adsorption, or by a combination of the two.[123] The exemplary excess of the extracted solvent from the mobile phase into the stationary phase on the series of octadecyl bonded phases is shown in Figure 10.

The partition coefficients can be transformed into free energy of transfer involving enthalpic and entropic components:[124]

$$\Delta G = \Delta H - T\Delta S \tag{3}$$

The relationship between the Gibbs free energy (ΔG), retention factor (k), equilibrium constant (K), and thermody-

dx.doi.org/10.1021/cr200182j | Chem. Rev. 2012, 112, 2629–2641

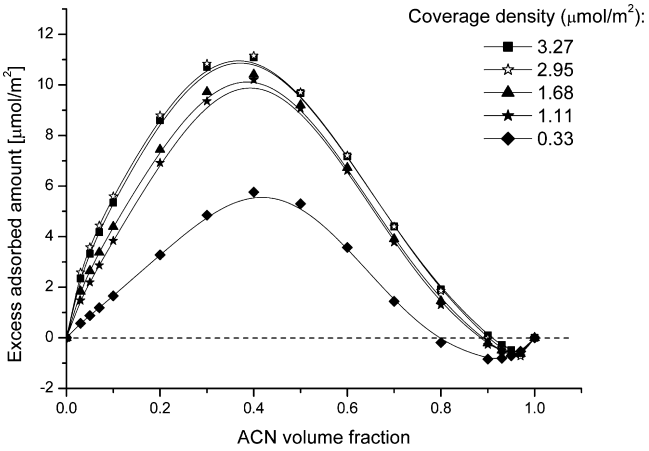

Figure 10. Excess isotherms of acetonitrile from binary mobile phase on the octadecyl stationary bonded phases with different coverage densities. Reproduced with permission from ref ref 66. Copyright 2009 American Chemical Society.

namic temperature, T (in Kelvin),[125] is described according to the following expression (vaṅt Hoff equation).[126]

$$\ln k = \ln K + \ln \frac{V_S}{V_M} = \frac{-\Delta G^\circ}{RT} + \ln \frac{V_S}{V_M} = \frac{\Delta S^\circ}{R} + \ln \frac{V_S}{V_M} - \frac{\Delta H^\circ}{RT} \quad (4)$$

where ΔH is enthalpy, ΔS is entropy, and V_S and V_M are the volumes of the stationary phase and the mobile phase, respectively.

7.2. Model of Adsorption

In a mixture of water and an organic modifier, the organic modifier is less polar, so it has a higher affinity toward an alkyl chain than water in RP LC systems. The organic modifier is preferentially adsorbed on the surface of the alkyl chains, whereas water is preferentially adsorbed on the silanol groups. This is shown in Figure 11. In this case, the organic modifier, acetonitrile, solvates the tip of the organic chains better, and the concentration of the adsorbed acetonitrile molecules decreases near the silica surface.[127] When some portion of the eluent adsorbs on the stationary phase, its concentration in the column is changed. An equilibrium is established between the adsorbed molecules and the migrating molecules. A small change in the eluent composition or a sample injection can disturb this equilibrium.

In RP HPLC, when a binary mobile phase is used (for instance, MeOH−water or ACN−water) and one of the mobile phase components or a mixture with a different concentration is injected, solvent peaks appear.[16,26] The same situation is observed when an injected sample is dissolved in one of the components of the mobile phase.

When the mobile phase flows through the column, an equilibrium between the mobile and the stationary phase is established. The organic solvent and water molecules are preferentially adsorbed on the stationary phase. These stationary phases are always heterogeneous. There are bonded organic ligands and the residual silanol groups on the silica surface. In this case, two different adsorption sites are in the

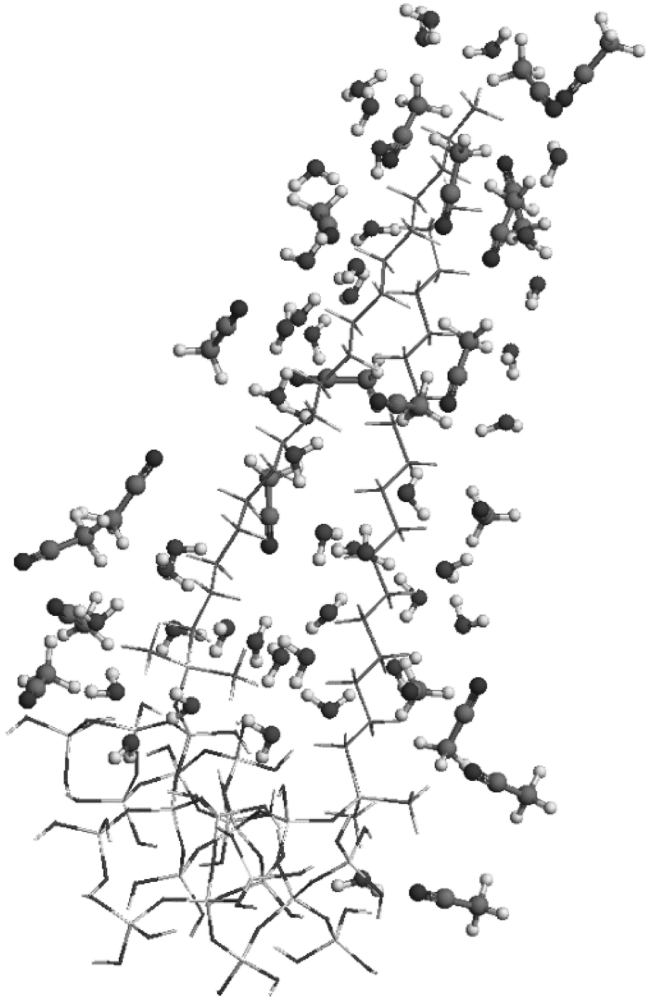

Figure 11. Hypothetical model of an equilibrium mobile phase—a stationary phase in the column with a C18 chemically bonded phase. The mobile phase consists of water and acetonitrile molecules. Reprinted with permission from ref 127. Copyright 2010 Wiley.

stationary phase: hydrophobic organic ligands and polar silanols.[128] When the mobile phase contains MeOH and water, the molecules of MeOH adsorb on the organic ligands and the water molecules adsorb on the silanol groups.

When a pure substance (methanol or water) is pumped through the column, an equilibrium between mobile phase and stationary phase is established. In this case, a signal of the UV detector at 254 nm is observed as a line. If a pure mobile phase is injected onto the column, there is no change in the equilibrium. The signal observed by the detector is still a line (Figure 12, parts a and f′). A different situation is observed when pure methanol is pumped through the column and pure water is injected (Figure 12a′) or pure water is pumped and methanol is injected (Figure 12f). In this case, negative peaks are observed. The injection of one solvent disturbs the equilibrium distribution of solvents between the binary mobile phase and the stationary phase in the column. The system regains the original equilibrium by extracting the second solvent from the stationary phase, leaving the solvent-deficient region in the mobile phase.[20] When a mixture of MeOH−water is used and a sample of pure MeOH is injected (Figure 12, parts b, c, d, and e), two peaks appear in the chromatogram. One of them is positive and the other is negative. An analogous situation

dx.doi.org/10.1021/cr200182j | Chem. Rev. 2012, 112, 2629−2641

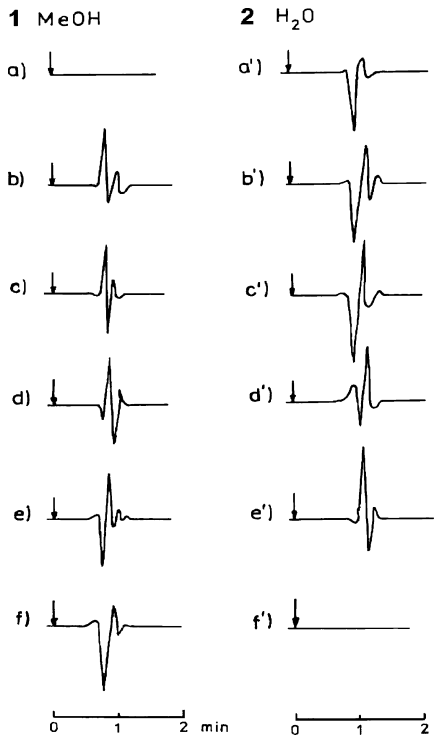

Figure 12. Solvent peaks observed in methanol–water system. Conditions: eluent = methanol/water (%$_{vol}$): (a) 100/0, (b) 70/30, (c) 50/50, (d) 47/53, (e) 30/70, (f) 0/100; (1) injection of methanol, (2) injection of water.

occurs when a sample of water is injected. One positive and one negative peak are observed (Figure 12, parts b′, c′, d′, and e′).

8. CONCLUSIONS

The presence of solvent peaks in reversed-phase chromatograms is a very interesting phenomenon. Unfortunately, the mechanism of solvent peak generation is rather complex, so it is repeatedly misunderstood in the broad analytical community. The most probable theory is that the presence of the solvent peak is connected with competitive adsorption of the binary mobile phase component on the stationary phase. The changes in their retention volume in different mobile phase compositions correspond to a dynamic stationary phase structure caused by preferential solvation of bonded ligands. Thus, the measurement of solvent peak retention may be useful for the column dead volume determination. The solvent distribution between the mobile and the stationary phase is determined by the same rules as solute distribution, so the understanding of solvent retention in the column may provide useful information about the solute retention mechanism during chromatographic elution.

The measurement of solvent distribution between the mobile and the stationary phase may be performed by the determination of excess adsorption isotherms from binary mixtures. The minor perturbation method is based on the differences in solvent peak retention over the whole range of a mobile phase composition. Organic solvent adsorption depends on the type of bonded ligands and their coverage density, and it is a competitive process for water adsorption on the residual silanols. In this case, the solvent excess isotherm may be used for a chemically bonded phase characterization.

The selectivity of the chromatographic separation depends on the specific and nonspecific interactions between a solute and the stationary phase and solute–mobile phase components. The mobile phase composition and the type of an organic modifier are the most important parameters of a chromatographic system. They influence the separation selectivity because the physicochemical properties of the stationary phase are determined to a large extent by the qualitative and quantitative composition of the adsorbed mobile phase components in the structure of bonded ligands.

AUTHOR INFORMATION

Corresponding Author

*Prof. dr hab. Bogusław Buszewski. E-mail: bbusz@chem.umk.pl. Phone: +48 56 611 43 08. Fax: +48 56 611 48 37.

Biographies

Prof. Bogusław Buszewski graduated from the Faculty of Mathematics-Physics-Chemistry of the Maria Curie Sklodowska University in Lublin, Poland, with a master degree. In 1986 he received a Ph.D. degree from the Faculty of Chemical Technology, Slovak Technical University, in Bratislava, Czechoslovakia. In 1992 he received the Doctor of Sciences (habilitation) degree. In 1994 he has received a professorship at the Nicolaus Copernicus University in Toruń, Poland, and in 1999 received the title of professor of chemistry. After M.Sc. graduation he became an assistant in the Department of Physical Chemistry, M. Curie Skłodowska University, in Lublin. He then worked in the Department of Chemical Physics and Physic-Chemical Separation Methods until 1994. From 1994 he began to organize his research group at the Faculty of Chemistry, Nicolaus Copernicus University, in Toruń and became the Head of the Department of Environmental Chemistry & Ecoanalytics, and later became Chair of Environmental Chemistry & Bioanalytics, a position he currently holds. Since 2010 he has also been the head of Center for Education & Research in Separation Methods & Bioanalytics *BioSep*. Prof. Buszewski has been a Humboldt Fellow at Tübingen University (Germany) and a visiting professor at several universities in the U.S.A., Japan, China, U.K., South Africa, Austria, The Netherlands, Taiwan, and many other European countries. He serves as a member of the Committee of Analytical Chemistry of the Polish Academy of Sciences and as a member of the editorial boards of 25 national and international journals and has authored or coauthored 15 books, numerous patents (most of which have been already implemented), lectures, and more than 290 scientific papers (h index = ca. 30). He is one of the most citied chemists in Poland (over 3000 citations). He has received awards from numerous national and international organizations (including Doctor Honoris Causa) and has supervised

24 Ph.D. students. Prof. Buszewski is also the chairman of the *Central European Group for Separation Sciences* (CEGSS), the president-elect of the Polish Chemical Society (2010–2012), the president of the *Societas Humboldtiana Polonorum*, and a member of the steering committee of *Division of Environmental Chemistry* of the *European Association for Chemical and Molecular Sciences* (EuCheMS). In October 2007 he was awarded the prestigious award Master (Mistrz) by the Foundation for Polish Science (FNP) and in 2009 was awarded the Polish Prime Minister award. In 2009 Prof. Buszewski was elected to the board of the *European Society for Separation Science (EuSSS)* (tenure 2009–2014) of which he is now president. His main scientific interests include environmental analysis, chromatography and related techniques (HPLC, SPE, GC, CZE, adsorption, and sample preparation), spectroscopy, utilization of waste and sludge, and chemometrics.

Szymon Bocian is Assistant, Chair of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, at Nicolaus Copernicus University. Szymon Bocian received his M.S. from the Nicolaus Copernicus University for work in the field of solvent peaks in liquid chromatography. After finishing his master degree studies, he continued his training at the university. In 2011 he received his Ph.D. from the Nicolaus Copernicus University for work in the field of retention mechanism in liquid chromatography under the direction of Prof. D. S. Bogusław Buszewski. After finishing his doctoral studies, he continued his training as a research assistant. His research is focused on the synthesis and characterization of the stationary phases for liquid chromatography. He is the author of 21 publications.

Attila Felinger is a Professor of Analytical Chemistry at the University of Pécs (Hungary), in addition to serving as President of the Hungarian Society for Separation Sciences. He graduated with a degree in chemical engineering from the University of Veszprém (Hungary) and obtained his Ph.D. in chemistry in 1988 from the same university; he also holds a D.Sc. degree from the Hungarian Academy of Sciences. His research interests focus on the fundamentals of chromatography including nonlinear, preparative, and analytical separations, as well as the statistical analysis of measurements by analytical chromatography. Prof. Felinger has published some 100 scientific papers and two books, and serves on the Editorial Board of *Journal of Chromatography A* and *LC-GC Europe*.

ACKNOWLEDGMENTS

Financial support from Polish Ministry of Science and Higher Education, Grant No. IP2010 003470 for 2010–2011 period, and Foundation for Polish Sciences "Start" subsidy are gratefully acknowledged.

REFERENCES

(1) Jaroniec, M. *J. Chromatogr., A* **1993**, *656*, 37.
(2) Horvath, C.; Melander, W.; Molnar, I. *J. Chromatogr.* **1976**, *125*, 129.
(3) Tanaka, N.; Goodell, H.; Karger, B. L. *J. Chromatogr.* **1978**, *158*, 233.
(4) Karger, B. L.; Gant, J. R.; Martkopf, A.; Weiner, P. H. *J. Chromatogr.* **1976**, *128*, 65.
(5) Buszewski, B.; Bleha, T.; Berek, D. *J. High Res. Chromatogr.* **1985**, *8*, 860.
(6) Šlais, K.; Kreči, M. *J. Chromatogr.* **1974**, *91*, 161.
(7) Mizrotsky, N.; Kristol, L.; Grushka, E. *J. Chromatogr., A* **1995**, *691*, 21.
(8) Srbek, J.; Coufal, P.; Bosakova, Z.; Tesarova, E. *J. Sep. Sci.* **2005**, *28*, 1263.
(9) Levin, S.; Abu-Lafi, S.; Golshan-Shirazi, S.; Guiochon, G. *J. Chromatogr., A* **1994**, *679*, 213.
(10) Guiochon, G.; Felinger, A.; Shirazi, S. G.; Katti, A. *Fundamentals of Preparative and Nonlinear Chromatography*; Academic Press: Boston, MA, 2006.
(11) Middleditch, B. S.; Zlatkis, A. *J. Chromatogr. Sci.* **1987**, *25*, 547.
(12) Grushka, E. *Anal. Chem.* **1986**, *58*, 1602.
(13) Fritz, J. S.; Gjerde, D. T.; Becker, R. M. *Anal. Chem.* **1980**, *52*, 1519.
(14) Dolan, J. W. *LC-GC* **1988**, *6*, 116.
(15) Melander, W. R.; Erard, J. F.; Horvath, C. *J. Chromatogr.* **1983**, *282*, 229.
(16) Buszewski, B.; Gilpin, R. K.; Jaroniec, M. *Chem. Anal. (Warsaw)* **1994**, *39*, 653.
(17) Riedo, F.; Kováts, E. *J. Chromatogr.* **1982**, *239*, 1.
(18) Solms, D. J.; Smuts, T. W.; Pretorius, V. *J. Chromatogr. Sci.* **1971**, *9*, 600.
(19) Berek, D.; Bleha, T.; Pevna, Z. *J. Chromatogr. Sci.* **1976**, *14*, 560.
(20) McCormick, R. M.; Karger, B. L. *Anal. Chem.* **1980**, *52*, 2249.
(21) Melander, W. R.; Erard, J. F.; Horvath, C. *J. Chromatogr.* **1983**, *282*, 211.
(22) Hackzell, L.; Rydberg, T.; Schill, G. *J. Chromatogr.* **1983**, *282*, 179.
(23) Smith, R. J.; Nieass, C. S.; Wainwright, M. S. *J. Liq. Chromatogr.* **1986**, *9*, 1387.
(24) Weber, S. G.; Tramposch, W. G. *Anal. Chem.* **1983**, *55*, 1771.
(25) Gadzała-Kopciuch, R.; Kluska, M.; Welniak, M.; Buszewski, B. *Mater. Chem. Phys.* **2005**, *89*, 228.
(26) Buszewski, B.; Kulpa, M. *J. Liq. Chromatogr.* **1993**, *16*, 75.
(27) Gritti, F.; Kazakevich, Y.; Guiochon, G. *J. Chromatogr., A* **2007**, *1161*, 157.
(28) Slaats, E. H.; Markowski, W.; Fekete, J.; Pope, H. *J. Chromatogr.* **1981**, *207*, 299.
(29) Eletkov, A.; Kazakievich, V. *J. Chromatogr.* **1987**, *395*, 473.
(30) Knox, J. H.; Kaliszan, R. *J. Chromatogr.* **1985**, *349*, 211.
(31) Sander, L. C.; Wise, S. A. *Crit. Rev. Anal. Chem.* **1987**, *18*, 299.
(32) McCormick, R. M.; Karger, B. L. *J. Chromatogr.* **1980**, *199*, 259.
(33) Bleha, T.; Berek, D. *Chromatographia* **1981**, *14*, 163.
(34) Macko, T.; Berek, D. *J. Chromatogr.* **1985**, *592*, 109.

(35) Erkelens, C.; Billiet, H. A. H.; Galan, L. d.; Leer, E. W. B. d. *J. Chromatogr.* **1987**, *404*, 67.
(36) *Trade literature*; Waters Assoc. Inc.: Milford, MA.
(37) Vit, J.; Popl, M.; Fahnrich, J. *J. Chromatogr.* **1983**, *281*, 293.
(38) Benson, G. C.; Kiyohara, O. *J. Solution Chem.* **1980**, *9*, 791.
(39) Gritti, F.; Guiochon, G. *Anal. Chem.* **2005**, *77*, 4257.
(40) Kazakevich, Y. V.; McNair, H. M. *J. Chromatogr. Sci.* **1995**, *33*, 321.
(41) Rustamov, I.; Farcas, T.; Ahmed, F.; Chan, F.; LoBrutto, R.; McNair, H. M.; Kazakevich, Y. V. *J. Chromatogr., A* **2001**, *913*, 49.
(42) Buszewski, B.; Jaroniec, M.; Gilpin, R. K. *J. Chromatogr., A* **1994**, *668*, 293.
(43) McCown, S. M.; Southern, D.; Morrison, B. E.; Garteiz, D. *J. Chromatogr.* **1986**, *352*, 465.
(44) Egi, Y.; Ueyanagi, A. *LC-GC* **1998**, *16*, 112.
(45) Buszewski, B.; Bocian, S.; Felinger, A. *J. Chromatogr., A* **2008**, *1191*, 72.
(46) Wang, M.; Mallette, J.; Parcher, J. F. *J. Chromatogr., A* **2008**, *1190*, 1.
(47) Snyder, L. R.; Kirkland, J. J. *Introduction to Modern Liquid Chromatography*, 2nd ed.; Wiley & Sons Inc.: New York, 1997.
(48) Isaaq, H.; Jaroniec, M. *J. Liq. Chromatogr.* **1989**, *12*, 2067.
(49) Engelhardt, H.; Muller, H.; Dreyer, B. *Chromatographia* **1984**, *19*, 240.
(50) Slaats, E. H.; Kraak, J. C.; Brugman, W. I. T.; Poppe, H. *J. Chromatogr.* **1978**, *149*, 255.
(51) Berendsen, G. E.; Pikaart, K. A.; de Galan, L. *J. Liq. Chromatogr.* **1980**, *3*, 1437.
(52) Colin, H.; Ward, N.; Guiochon, G. *J. Chromatogr.* **1978**, *149*, 169.
(53) Laub, R. I.; Madden, S. J. *J. Liq. Chromatogr.* **1985**, *8*, 173.
(54) Berendsen, G. E.; Schoenmakers, P. J.; Galan, L. D.; Vigh, G.; Varga-Puchony, Z.; Inczedy, J. *J. Liq. Chromatogr.* **1990**, *3*, 1669.
(55) Jinno, K. *Chromatographia* **1983**, *17*, 367.
(56) Wells, M. J. M.; Clark, C. R. *Anal. Chem.* **1981**, *53*, 1341.
(57) Unger, S. H.; Feuerman, T. F. *J. Chromatogr.* **1979**, *176*, 426.
(58) Fukuhara; Nakamura, A.; Tsuboi, M. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 2169.
(59) Engelhardt, H. *High Performance Liquid Chromatography*; Springer-Verlag: New York, 1979.
(60) Krstulovic, A. M.; Colin, H.; Guiochon, G. *Anal. Chem.* **1982**, *54*, 2438.
(61) Jandera, P.; Bocian, S.; Molíková, M.; Buszewski, B. *J. Chromatogr., A* **2009**, *1216*, 237.
(62) Fini, O.; Brusa, F.; Chiesa, L. *J. Chromatogr.* **1981**, *210*, 326.
(63) Bocian, S.; Vajda, P.; Felinger, A.; Buszewski, B. *J. Chromatogr., A* **2008**, *1204*, 35.
(64) Billiet, H. A. H.; Dalen, J. P. J. v.; Schoenmakers, P. J.; Galan, L. d. *Anal. Chem.* **1983**, *55*, 847.
(65) Scott, R. P.; Kucera, P. *J. Chromatogr.* **1979**, *175*, 51.
(66) Bocian, S.; Vajda, P.; Felinger, A.; Buszewski, B. *Anal. Chem.* **2009**, *81*, 6334.
(67) Williams, S. *J. Chromatogr., A* **2004**, *1052*, 1.
(68) Jandera, P.; Churacek, J. *Gradient Elution in Column Liquid Chromatography: Theory and Practice*; Elsevier: Amsterdam, The Netherlands, 1985.
(69) Snyder, L. R.; Dolan, J. W. *High-performance gradient elution*; John Wiley & Sons: Hoboken, NJ, 2007.
(70) Cartoni, G. P.; Coccioli, F. *J. Chromatogr.* **1986**, *360*, 225.
(71) Dolan, J. W. *LC-GC* **1990**, *8*, 516.
(72) Bristol, D. W. *J. Chromatogr.* **1980**, *188*, 193.
(73) Otsuki, A. *J. Chromatogr.* **1977**, *133*, 402.
(74) Desiderio, C.; Fanali, S.; Gebauer, P.; Bocek, P. *J. Chromatogr., A* **1997**, *772*, 81.
(75) Gebauer, P.; Bocek, P. *J. Chromatogr., A* **1997**, *772*, 73.
(76) Gebauer, P.; Pantuckova, P.; Bocek, P. *J. Chromatogr., A* **2000**, *894*, 89.
(77) Sellmeyer, H.; Poppe, H. *J. Chromatogr., A* **2002**, *960*, 175.
(78) Beckers, J. L.; Everaerts, F. M. *J. Chromatogr., A* **1997**, *787*, 235.
(79) Gaś, B.; Hruska, V.; Dittmann, M.; Bek, F.; Witt, K. *J. Sep.Sci.* **2007**, *30*, 1435.
(80) Poppe, H. *Anal. Chem.* **1992**, *64*, 1908.
(81) Bruin, G. J. M.; Asten, A. C. v.; Xu, X.; Poppe, H. *J. Chromatogr., A* **1992**, *608*, 97.
(82) Stedry, M.; Jaros, M.; Hruska, V.; Gas, B. *Electrophoresis* **2004**, *25*, 3071.
(83) Gebauer, P.; Borecka, P.; Bocek, P. *Anal. Chem.* **1998**, *70*, 3397.
(84) Beckers, J. L.; Gebauer, P.; Bocek, P. *J. Chromatogr., A* **2001**, *916*, 41.
(85) Beckers, J. L. *J. Chromatogr., A* **1994**, *662*, 153.
(86) Beckers, J. L. *J. Chromatogr., A* **1994**, *679*, 153.
(87) Kohlrausch, F. *Ann. Phys.* **1897**, *62*, 209.
(88) Beckers, J.; Bocek, P. *Electrophoresis* **1999**, *20*, 518.
(89) Bocek, P.; Gebauer, P.; Beckers, J. L. *Electrophoresis* **2001**, *22*, 1106.
(90) Jaros, M.; Hruska, V.; Stedry, M.; Zusková, I.; Gas, B. *Electrophoresis* **2004**, *25*, 3080.
(91) Hruska, V.; Stedry, M.; Vcelaková, K.; Lokajová, J.; Tesarová, E.; Jaros, M.; Gas, B. *Electrophoresis* **2006**, *27*, 4610.
(92) Lokajová, J.; Hruska, V.; Tesarová, E.; Gas, B. *Electrophoresis* **2008**, *29*, 1189.
(93) Helfferich, F.; Klein, G. *Multicomponent Chromatography*; M. Dekker: New York, 1970.
(94) Golshan-Shirazi, S.; Guiochon, G. *J. Phys. Chem.* **1990**, *94*, 495.
(95) Hackzell, L.; Schill, G. *Chromatographia* **1982**, *15*, 437.
(96) Ye, J.; Baldwin, R. P.; Rarichandran, K. *Anal. Chem.* **1986**, *58*, 2337.
(97) Parris, N. *Anal. Biochem.* **1979**, *100*, 260.
(98) Bidlingmeyer, B. A.; Warren, F. V. *Anal. Chem.* **1982**, *54*, 2351.
(99) Sokołowski, A. *Chromatographia* **1986**, *22*, 177.
(100) Crommen, J.; Herne, P. *J. Pharm. Biomed. Anal.* **1984**, *2*, 241.
(101) Levin, S.; Grushka, E. *Anal. Chem.* **1986**, *58*, 1602.
(102) Jenke, D. R. *Anal. Chem.* **1984**, *56*, 2468.
(103) Jackson, P. E.; Haddad, P. R. *J. Chromatogr.* **1985**, *346*, 125.
(104) Larson, J. R.; Pfeiffer, C. D. *Anal. Chem.* **1983**, *55*, 393.
(105) Foley, R. C. L.; Haddad, P. R. *J. Chromatogr.* **1986**, *366*, 13.
(106) McAleese, D. L. *Anal. Chem.* **1987**, *59*, 541.
(107) Crommen, J.; Schill, G.; Herne, P. *Chromatographia* **1988**, *25*, 397.
(108) Herne, P.; Renson, M.; Crommen, J. *Chromatographia* **1984**, *19*, 274.
(109) Perkin, J. E. *J. Chromatogr.* **1984**, *303*, 436.
(110) Nilsson, L. B.; Westerlund, D. *Anal. Chem.* **1985**, *57*, 1835.
(111) Fornstedt, T.; Westerlund, D.; Sokołowski, A. *J. Liq. Chromatogr.* **1988**, *11*, 2645.
(112) Golshan-Shirazi, S.; Guiochon, G. *J. Chromatogr.* **1989**, *484*, 125.
(113) Golshan-Shirazi, S.; Guiochon, G. *Anal. Chem.* **1989**, *61*, 2380.
(114) Golshan-Shirazi, S.; Guiochon, G. *J. Chromatogr.* **1988**, *461*, 19.
(115) Golshan-Shirazi, S.; Guiochon, G. *J. Chromatogr.* **1988**, *461*, 1.
(116) Fornstedt, T.; Guiochon, G. *Anal. Chem.* **1994**, *66*, 2686.
(117) Kazakevich, Y. V. *J. Chromatogr., A* **2006**, *1126*, 232.
(118) Scott, R. P.; Kucera, P. *J. Chromatogr.* **1977**, *142*, 213.
(119) Dejaegher, B.; Heyden, Y. V. *J. Sep. Sci.* **2010**, *33*, 698.
(120) Pesek, J. J.; Matyska, M. T. *LCGC North Am.* **2007**, *25*, 480.
(121) Bocian, S.; Buszewski, B. *J. Sep. Sci.* **2010**, *33*, 3033.
(122) Gadzała-Kopciuch, R.; Buszewski, B. *J. Sep. Sci.* **2003**, *26*, 1273.
(123) Gritti, F.; Kazakevich, Y. V.; Guiochon, G. *J. Chromatogr., A* **2007**, *1169*, 111.
(124) Buszewski, B.; Gadzała-Kopciuch, R.; Michel, M. *Accredit. Qual. Assur.* **2011**, *16*, 237.
(125) Vanhoenacker, G.; Sandra, P. *J. Chromatogr., A* **2005**, *1082*, 193.
(126) Melander, W. R.; Horvath, C. In *High-Performance Liquid Chromatography, Advances and Perspectives*; Horvath, C., Ed.; Academic Press: New York, 1980; Vol. 2.
(127) Buszewski, B.; Bocian, S.; Nowaczyk, A. *J. Sep. Sci.* **2010**, *33*, 2060.

(128) Buszewski, B.; Bocian, S.; Rychlicki, G.; Vajda, P.; Felinger, A. *J. Colloid Interface Sci.* **2010**, *349*, 620.