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System peaks and their impact in liquid chromatography

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ABSTRACT

A sample injected into a chromatographic system can generate extra peaks, called "system peaks", which in general are undetectable. However, for small analytical injections, solute zones eluting with a system zone will often give strongly deformed solute peaks. But, if a solute zone is eluted in a particular region of the system zone it will instead be strongly compressed and well-shaped. For overloaded solute injections, another type of complex band deformation may take place due to large system peaks. This review will present results related to system peak distortions of both small analytical peaks and large preparative ones. Guidelines will be given on how to avoid unwanted distortions and how to utilize the distortions for increased detectability in analytical chromatography, or enhanced production rate in preparative chromatography. The works reviewed here were mainly made by Georges Guiochon, and some of his close colleagues, and is dedicated to his memory.

Contents

1. Introduction

In all modes of chromatography the mobile phase is more or less complex containing several different components and some of these might, to a certain extent, be adsorbed to the stationary phase. In reversed phase chromatography the main solvent is water, the additives are buffer components and one have large portions of polar organic solvents, such as acetonitrile or methanol. The polar organic solvents are weakly adsorbed and modulate solute retentions mainly by solubilising the solutes better into the mobile phase, such additives are therefore often called modifiers [1]. In normal-phase chromatography, the main solvent is hexane or heptane and the additives are small portions of polar organic solvents, here mainly acting by competing with the solutes about the stationary phase. A chromatographic system which has the potential to generate system peaks requires additives that are adsorbed more or less strongly to the stationary phase [1].

The injection of a sample into a chromatographic system with mobile phase additives may results in more peaks than there are components in the sample [2–5]. These extra peaks are called system peaks by the analytical chemistry community [6,7] and perturbation peaks by the chemical engineering community [8]. A prerequisite for the detection of these extra peaks at the column outlet is that at least one of the mobile phase components is detectable and that there is a mutual interaction between the mobile phase components and/or analytes [4,9]. In general, injecting a sample containing N undetectable and retained components into a system containing one detectable component (the probe) will result in $N + 1$ detectable mobile phase component peaks [4].

The very first utilisation of system peaks was for indirect detection, i.e., to detect sample components that are more or less undetectable, and this technique is still frequently used in

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ion-chromatography. Here a "probe" is included in the mobile phase, i.e., a highly detectable additive component which also has an affinity for the stationary phase and interacts with the sample components lacking detectable properties by the used detector. Early important studies in this field were done by Schill and co-workers [4] in the 1980's; they found that the signal strength for the indirectly detected component peaks depends strongly on how close they are to the probe system peak [4]. In the early 1990's Guiochon contributed significantly to this topic by presenting a more complete theory than published before for indirect detection [10]. This theoretical study was successfully confirmed experimentally a few years later by Levin et al. [11]. One drawback with the more complete theory presented 1992 was that it still depended on a particular type of adsorption isotherm, and a more general theory was later presented by Forssén and Fornstedt [12].

An adsorption isotherm relates the adsorbed amount of component to its concentration in the mobile phase, at constant temperature, and the retention times of the system peaks are related to the tangential slope of the adsorption isotherms at the actual plateau additive concentration. Therefore, analytical-size system peaks can also be utilised to determine adsorption isotherms [13]. This is an important application since competitive adsorption isotherms is essential in order to perform reliable computer simulation of preparative chromatography [13]. Here pioneer contributions were made by Helfferich [14] and Morgenstein [15,16] from the chemical engineering community where system peaks are known as "perturbation peaks". The authors of this review have followed up these studies by extensive analytical validation of the method for both the single component case [17] and the competitive case [18] and found that the method was even preferable to the classical frontal analysis method for multicomponent eluents [18]. Although, this aspect of system peaks was not a main topic for Georges Guiochon he was very fascinated about it and was probably the one that best explained the method for a broader audience in his book [13]. Moreover Gritti and Guichon proposed a very useful strategy for determination of large adsorption isotherm data sets where they combined the more reliable system peak method with the faster inverse method [19]. The former method was used for key experiments and the latter one mostly for confirmative purpose.

Extensive research has also been focused on how large system zones, generated by large disturbances, affects the solute peaks [20–25]. When large disturbances are induced, large system peaks will develop that contain sharply increasing, or decreasing, gradients of the mobile phase components in the front and tail of the system peak. When small sample analyte peaks are eluted in such internal gradients they are usually distorted, making it impossible to use them for quantitative analysis. However, in some cases it is possible to achieve strong analyte peak compression effects that improve the efficiency and detection limits considerably. The studies by Georges Guiochon and us in this field have concentrated on understanding these distortions in order to give guidelines on how to eliminate them. In this review we will cover both analytical peak distortion and compression, i.e., how to avoid the unwanted "bad" effects of large system peaks and how to utilise the desired effects. By the latter we mean increased detectability due to peak compression effects in analytical chromatography [26] and higher production rates in preparative chromatography by tuning of the overloaded peaks shapes [27].

When large sample sizes are injected, e.g. in preparative separations, this will by necessity generate large equilibria disturbances of the mobile phase additive concentrations. This will in turn usually cause deformed sample components peak shapes. This phenomenon is very complex and has been studied, both theoretically and experimentally, by Guiochon et al. [1,28–32] on achiral straight phase and reversed phase model systems. One example of these phenomena is when an additive adsorbs stronger than the solute component and the additive has such a high bulk concentration that its system peak elutes before the solute peak, then the solute peak will appear as anti-Langmuirian, with a diffuse front and a sharp rear, although it has a Langmuir adsorption isotherm [33] . In the most recent publication by Guiochon in this field, high-concentration binary and individual band profiles for the solutes benzyl alcohol and 2-phenylethanol were reported in a system consisting of a C18 column, a binary mobile phase (MeOH:H_2O, 1:1 v/v) and 2-methylbenzyl alcohol as additive [34]. This was the first time overloaded binary solute bands were due to the large system peak, i.e. a three-component problem: two solutes + one additive. It was suggested that under certain circumstances, the use of a properly chosen additive could significantly increase band separation and hence the production rate, the recovery yield and/or the purity of the fractions. It was therefore concluded that for the design of preparative separation systems *"more attention should be paid to nonlinear system peaks and their potential usefulness. . ."*

Interestingly, some years after Guiochon's observation it was found that these remarkable band shape effects take place especially often in modern chiral separation systems. One such system was the separation of 2-phenylbutyric acid enantiomers on the chiral stationary phase Kromasil CHI-TBB using hexane/methyl *tert*-butyl ether (90/10 v/v) as eluent with 0.1 % formic acid as additive [35]. At moderately high concentration injections of the racemic solute the first eluted S-enantiomer exhibited peak tailing, with a deformed rear portion, while the R-enantiomer exhibited peak fronting. When increasing the solute concentration 10 times, the R-enantiomer peak was deformed to an almost rectangular zone, the same kind of deformation that was observed earlier by Guiochon [1,28–32]. Here the system peaks are generated by the high solute concentration only, not as an effect of sample solvent composition deviation from the eluent, as is the case in analytical peak deformations, see above. Another example is the separation of several β-blocker enantiomers on a Teicoplanin stationary phase (Chirobiotic T) using a mixture of acetonitrile and methanol in the presence of triethylamine/acetic acid as mobile phase. Here, it could be demonstrated that it is possible to tune the peak shapes of the two enantiomers by varying the organic solvent composition [36]. This is a three-component problem with two enantiomers and one additive (triethylamine) that also adsorbs to the stationary phase. A most advantageous situation seems to occur when the first eluted peak is transformed to an anti-Langmuirian shape while the second has a normal Langmuirian shape. In this situation the two peaks tail in opposite directions, with their sharp sides pointing closely to each other, and it should then be possible to obtain baseline resolution at higher loads than when the peaks tail in the same direction. Later systematic computer simulations studies showed further surprising results which will be reviewed here [27,33].

2. Generation of system peaks

In the first example we have standard C_{18} reversed phase system with an eluent containing 1 mM of the strongly UV-absorbing tricyclic antidepressant protriptyline as additive in a phosphate buffer. Fig. 1 shows the resulting chromatogram for the protriptyline signal after injecting 100 µL of either (a) pure buffer or (b) eluent without protriptyline. In both situations the sample lacks protriptyline, but protriptyline diffuses into the injection zone from the bulk eluent and from the mobile phase ahead of the injection zone. The buffer injection in Fig. 1a results in a positive system peak because of an increased adsorption of protriptyline onto the stationary phase in the injection zone. The mobile phase deficiency of protriptyline in this zone is eluted with the non-retained buffer while the stationary phase excess of protriptyline in the injection zone is eluted as a positive system peak. In Fig. 1a the buffer zone is indirectly detected as a negative peak in the front. The retained system peak is