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Peak deformations in preparative supercritical fluid chromatography due to co-solvent adsorption

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ABSTRACT

In supercritical fluid chromatography (SFC) the mobile phase comprises of carbon dioxide (CO_2) as main solvent and smaller amounts of an organic polar solvent (often an alcohol) as co-solvent. The co-solvent is considered to function by changing the overall polarity of the eluent, *i.e.* by acting as a "modifier". However, recent studies indicate that the co-solvent methanol can also adsorb to some common SFC stationary phases. Hence, the co-solvent should also be able to function as an "adsorbing additive", *i.e.* an eluent component that competes with the injected solutes about the stationary phase surface. In this study it was found by fitting different mechanistic models to systematic experimental data, that the co-solvent methanol can have both functions: at low co-solvent fractions, methanol acts as an additive whereas at larger fractions it acts as a modifier. Moreover, it was found that when the co-solvent adsorbs more strongly to the stationary phase than the solute, "bizarre" deformations of the preparative band shapes can occur. This is illustrated by a solute that converts from a normal "Langmuirian" band shape to an "anti-Langmuirian" shape when changing from neat carbon dioxide (CO_2) to an eluent containing co-solvent. This peak shape transition is dependent on both (i) the relative retention of the solute and co-solvent to the stationary phase in eluent containing neat CO_2 and on (ii) the relative retention of the additive perturbation peak and the solute peak in eluent containing also co-solvent.

1. Introduction

Peak deformations appear for several reasons in liquid chromatography (LC), the more complex chromatographic system, especially its mobile phase, the greater the risk of peak deformations. It is often possible to identify the underlying reasons for deformations in LC. Many types of peak deformations are due to sample diluent and eluent contrast in viscosity [1–5], solvent-strength [6,7] and pH [8,9]. To avoid this sample diluent should be made as similar to the eluent as possible. If there is a difference in viscosity between the sample and the eluent, the sample band in the column may suffer from hydrodynamic instability [10]. The instability can give rise to a "fingering" structure as the low viscous solution fingers into a high viscous solution thereby deforming the peak [1–5].

In supercritical fluid chromatography (SFC), peak distortions are harder to avoid than in LC because of the compressible eluent used in SFC. Since samples cannot be dissolved in the mobile phase (often CO_2 and an alcohol), they are often dissolved in the co-solvent. This can cause distortions mainly due to solvent-strength mismatch between the sample and the eluent, which has been recently investigated [11–13]. In one of these fundamental investigations, it was calculated that although the contrast between the solvent strength of the sample diluent and the eluent is the main reason for the peak distortions in SFC a secondary effect causing additional broadening can be viscous fingering effects [12].

Another type of peak distortion, only studied so far in LC and recently reviewed [14], can occur when components from the mobile phase, other than the main solvent are adsorbed onto the stationary phase. Usually the mobile phase consists of a main solvent mixed with a co-solvent, commonly called "modifier" as the purpose of the co-solvent is to modify the elution strength and hence the retention of the solutes. In addition, also other components so called "additives" can be dissolved in the mobile

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phase, which also may influence solute retentions, by e.g. competing for stationary phase adsorption sites, affecting the solvation layer or interacting with the solute molecules with ion paring mechanism [15–17]. However, it is generally accepted that a modifier also adsorbs to the stationary phase [18,19], although that is often of minor importance compared with its polarity modulating function. When large sample volumes are injected in preparative separations, a large equilibria disturbance of the mobile phase is generated, which may result in strongly distorted solute elution band shapes. This phenomenon has been thoroughly studied in LC, both theoretically and experimentally [20–23], and may take place when the mobile phase contain at least one additive that adsorbs more strongly to the stationary phase than the solute component. Although very interesting, these effects seldom occur in preparative LC because the additives used are usually weakly adsorbed compared to the solute. Peak distortions due to this phenomenon have to our knowledge never before been reported and explained in SFC. However, recent investigations shows that the co-solvent methanol adsorbs very strongly to silica and diol stationary phases [24–26]. This gives reason to suspect that, in common SFC systems, there might be another important putative reason for serious peak deformations, but not yet investigated and explained.

Most mobile phases of practical interest in SFC contain some amounts of polar organic solvents dissolved in carbon dioxide as the main and weak solvent. In this study, as well as in earlier similar SFC studies using polar stationary phases [24,26] it has been assumed that the adsorption of the main solvent CO_2 can be regarded as negligible in the presence of the polar organic solvent methanol. This assumption is motivated from the fact that methanol is much more polar than the nonpolar (planar) CO_2 and from the few studies done so far on CO_2 adsorption to polar SFC stationary phases [27,28]. In the study by Strubinger et. al., the adsorption of CO_2 was investigated in the presence of 2% methanol showing that under the density conditions used in our actual study, the adsorption of CO_2 has drastically declined from its maximum [27].

This study has a two-folded synergistic aim. First (I), we want to investigate if the above mentioned peak deformation effect due to solvent adsorption also take place in SFC. This will be done by studying the retention and peak shape of several polar uncharged solutes on a diol column using methanol as co-solvent. By using neutral and stable solutes other possible sources for deformations will be minimized. Second (II), we aim to investigate if the co-solvent used in SFC acts as a modifier or as an additive. For this purpose, two fundamentally different mechanistic models were investigated. The synergy between the two aims is that with the knowledge of how the co-solvent acts (Aim II) we can investigate if its adsorption can result in the same strong deformations shown previously both theoretically and in LC (Aim I).

2. Theory

2.1. Adsorption isotherms

An adsorption isotherm describes the equilibrium of a solute between the stationary and mobile phases at a specific and constant temperature. Generally, we consider two principally different adsorption isotherms: excess adsorption isotherms and absolute adsorption isotherms. Excess adsorption isotherms are often used to describe solvent adsorption to a surface whereas absolute adsorption isotherms are used to describe the solutes adsorption [29]. An excess adsorption isotherm describes the difference between the amount of component actually present in the system, and that amount which would be present in a reference system, without adsorption, for a particular bulk concentration in the adjoining phase at the surface [30]. An absolute adsorption isotherm describes the surface or stationary phase concentration (q) of the compound.

There are several adsorption isotherm models that could be used to describe the adsorption of solutes to a surface [29]. In this study, the bi-Langmuir model is used:

$$q = q_{s,I} \frac{K_I C}{1 + K_I C} + q_{s,II} \frac{K_{II} C}{1 + K_{II} C}, \quad (1)$$

where K is the association equilibrium constant, q_s is the monolayer saturation capacity, C is the mobile phase concentration, and q is the stationary phase concentration. The indices I and II represent adsorption sites I and II, respectively. If several components in the mobile phase compete for the available surface, a multi-component mode of the adsorption isotherm is required. This will be the case, if the mobile phase contains an additive; in the model the additive must be treated as one component and the solute as another. The multicomponent version of the bi-Langmuir adsorption isotherm can be expressed as:

$$q_i = q_{s,I,i} \frac{K_{I,i} C_i}{1 + \sum_{j=1} K_{I,j} C_j} + q_{s,II,i} \frac{K_{II,i} C_i}{1 + \sum_{j=1} K_{II,j} C_j}, \quad (2)$$

where the index i stands for the i:th compound in the mixture.

The adsorption isotherm can be determined using many methods [29,31–33]. One of the fastest methods for determine the adsorption isotherm is the elution by characteristic point (ECP) method [29,34], in which the raw adsorption isotherm data is determined from the diffuse part of an overloaded elution profile [29]. In the present study the slope version of the ECP method is instead used [35,36]. For a type I adsorption isotherm (convex, e.g. Bi-Langmuir), the raw slope data are determined using [36]:

$$\frac{dq}{dC}(C) = \frac{V_R(C) - V_0 - V_{inj}}{V_a}, \quad (3)$$

where $V_R(C)$ is the retention volume corresponding to the mobile phase concentration C, V_0 is the holdup volume, V_a is the volume of the stationary phase, and V_{inj} is the injection volume.

The tracer pulse method and the perturbation peak method are two other accurate methods for adsorption isotherm acquisition [36–40]. These methods are performed by injecting a small excess of molecules into a column already equilibrated with an eluent containing the same molecules, a single peak will appear in the chromatogram, i.e., the perturbation peak. The detected perturbation peak will contain the displaced plateau molecules whereas the injected molecules will elute later in the so-called tracer peak [38,41]. In the later eluting zone, the tracer peak cannot be detected using a standard detector principle because it has the same elution time as the deficiency zone created by the propagation of the perturbation peak. To visualize the tracer peaks, the injected molecules need to be labeled in some way; in this study, stable isotopes were used. The retention volumes of the perturbation (Eq. (4a)) and tracer (Eq. (4b)) peaks, respectively, are expressed by using the following adsorption isotherms:

$$V_R = V_0 \left(1 + F \frac{dq(C_0)}{dC_0}\right), \quad (4a)$$

$$V_R = V_0 \left(1 + F \frac{q(C_0)}{C_0}\right), \quad (4b)$$

where F is the phase ratio (i.e., ratio of the volumes of stationary and mobile phases in the column), C_0 is the concentration of the established concentration plateau, and V_R is the retention of the perturbation or the tracer peak.