



Band deformation in non-isocratic liquid chromatography

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ABSTRACT

Peak deformation is a plague in both analytical and preparative chromatographic separations. The phenomenon may be particularly pronounced in non-isocratic separations, which involve solvent or temperature gradient. Solvent gradient can be exploited for column loading as well as elution of sample components. In the former case the solvent environment of the sample is different than the mobile phase, whereas in the latter one the mobile phase composition is varied during chromatographic elution. Temperature gradient can be used to alter adsorption behavior of eluities in temperature-sensitive chromatographic systems. It may also arise from column thermal heterogeneity caused by viscous friction of the mobile phase or improper thermostating of the column.

In this review we described typical sources of peak tailing in non-isocratic chromatography including solvent and temperature gradients, and elucidated underlying mechanisms of this phenomenon. The analysis was supported by theoretical predictions based on equilibrium theory, dynamic simulations, and experimental illustrations.

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1. Introduction

Peak deformation is a common cause of qualitative and quantitative problems in both analytical and preparative chromatography. It makes interpretation of chromatographic analysis or separation difficult in regard to the retention factor, resolution factor, and column performance. It also deteriorates productivity and yield of separations in industrial applications.

The departure from the Gaussian peak shape can manifest itself in many different ways such as in the form of peak fronting, tailing or splitting. It may be related to thermodynamic, kinetic, and hydrodynamic effects, which accompany chromatographic processes.

The thermodynamic effects are associated with isotherm non-linearity, which arises from column overload, i.e., it occurs when the concentration of the solute loaded into the column is high enough to reach non-linear range of the isotherm. Column overload is typical for preparative and industrial applications, where chromatographic columns are usually operated under strong isotherm non-linearity. Nevertheless, the amount of solute that triggers column overload depends on the isotherm shape. In case of strongly favorable isotherms, which arise from strong interactions between

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the adsorbate molecules and the chromatographic matrix, even a small-volume injection of a low-concentration sample may cause column overload and peak asymmetry. For concave isotherms the solute peak may be distorted with a sharpened adsorption front (shock front), and a sloping desorption front (spreading wave). This type of asymmetry is called peak tailing. The opposite holds true for convex isotherms, i.e., a dispersed adsorption front and a sharpened desorption front can be observed. The latter phenomenon is called peak fronting.

When adsorption isotherms have inflection points in their course, semi-shocks can be formed, i.e., both desorption and adsorption fronts can split into parts, each comprising a sharpened and a dispersion front [1,2].

The peak shapes get more complex for multicomponent mixtures eluted under overload conditions. When all mixture components exhibit a concave isotherm behavior, the displacement effect takes place, which stems from competitive adsorption of elute molecules on the adsorption sites. The molecules of a less adsorbed compound are excluded by others that are more strongly adsorbed. As a result, the migration of the less adsorbed compound is accelerated, hence, it elutes from the column faster in the mixture than if this compound were alone. In case of convex isotherms, the so called retainment effect can be observed, which arises from cooperative (synergistic) adsorption of the mixture components. In this case adsorption of a strongly adsorbed compound is enhanced in the presence of other compounds, which delays its migration through the column [3–6]. When the chromatographed species are characterized by two opposite types of adsorption behavior, convex or concave, mixed synergistic-competitive adsorption takes place, which can involve formation of the so called delta-shock. The delta shock is a traveling spike comprising a certain amount of sample component compressed in a very small volume that exhibits a self-sharpening behavior on both sides [7,8].

A mathematic description of the formation of sharpened fronts and waves can be provided by equilibrium theory (ET), which accounts for thermodynamic factors driving retention, while it neglects all types of non-ideal behavior in the column that may accompany mass and heat transfer [9].

Another thermodynamic source of peak tailing is heterogeneity of the surface of the stationary phase due to the presence of different types of adsorption sites. This is a common problem in separations with RP adsorbents, where low-energy adsorption sites with a high saturation capacity, and high-energy adsorption sites with a low saturation capacity can be distinguished [10,11]. A similar problem is reported for chiral phases that contain low-energy non-selective sites and highly selective sites, which give strong interactions with at least one of the two enantiomers [12]. Peak tailing occurs because a part of the adsorbate population overloads high-energy sites.

Nevertheless, peak distortion is also a common problem under linear isotherm conditions. It may arise from: (a) non-ideality of the system hydrodynamics due to: dispersion in dwell and extra-column volumes [13–16]; heterogeneous particle-size distribution of the packing material [17]; radial distributions of the mobile phase flow velocity and the solute concentration caused by heterogeneity of the structure of packed beds in radial direction [18]; (b) slow mass transport kinetics arising from slow diffusion of elute molecules in external film and in pores [19], or slow rates of mass transport associated with high-energy interactions on heterogeneous adsorbent surfaces [13]; (c) thermal heterogeneity of the column due to heat dissipation, which is particularly relevant for separations on ultra-HPLC columns packed with submicron porous particles and operated under high inlet pressure up to 1000 bar or more [20]. The launch of ultra-HPLC columns allowed achievement of high resolution by diminishing the contribution of mass transfer resistances to band broadening. On the other hand, large

pressure drops trigger excessive heat generation inside the column [21], while small peak retention volumes involve problems with increased influence of dwell and extra-column volumes [14].

Nevertheless, in general, the contribution of extra-column sources of peak tailing has become a less important with progress in the construction of modern instruments. Also, the packing techniques are continuously improved to reduce heterogeneity of the structure of packed beds. To reduce the contribution of energetic heterogeneity of the adsorbent surface to peak tailing, novel support materials with reduced content of highly polar functional groups have been developed [22,23]. Moreover, significant reduction of peak tailing on energetically heterogeneous surfaces could be achieved by modifying the mobile phase to include a tail-suppressing compound, proper selection of the mobile phase pH, and elevating temperature [22].

Most of the above sources of peak tailing and hints for their troubleshooting have been thoroughly discussed in literature for many years. Nevertheless, there remains room for further investigation and elucidations of that phenomenon. This particularly holds true for non-isocratic chromatography, which includes: solvent gradient, where the mobile phase composition is altered during chromatographic elution, or the solvent used for the sample loading is different than the mobile phase; column's internal gradient, which is axial pH gradient formed along the column in response to step changes in buffer composition; temperature gradient, where temperature of the mobile phase is varied to modulate the retention pattern of eluities.

Because of the complexity of thermodynamic, kinetic, and hydrodynamic effects underlying this process, selection of optimal conditions for the separation is often impossible without understanding the band migration phenomenon.

Therefore, in this review article, various sources of peak deformation in non-isocratic chromatography are discussed. ET is exploited to facilitate comprehension of mechanism lying behind that phenomenon. The theoretical discussion is supported by graphical illustrations and experimental evidences.

2. Band deformation in gradient elution

2.1. Peak compression and peak splitting in solvent gradient

Solvent gradient is routinely used for laboratory-scale purifications as well as large-scale applications.

In this elution mode, the mobile phase composition is varied from low to high elution strength. It is realized by an abrupt (step gradient) or a continuous (linear gradient) change in the concentration of a strong solvent (eluent modifier), or, if ionic species are separated, in the content of inorganic salts or pH in the elution buffer.

The advantage of solvent gradient is the possibility to separate multicomponent mixtures that differ markedly in the retention behavior, acceleration of the speed of separation, and improvement of the sensitivity of detection due to compression of band profiles. The peak compression has a thermodynamic background; it arises from changes of adsorption properties of eluities during gradient propagation [24,25].

However, when the gradient shape is not properly chosen, instead of the expected peak compression, peak splitting occurs. In practice, it can be recognized when the solute elutes in two or more parts that belong to the same substance. That phenomenon can be easily explained by graphical representations of ET.

The band propagation of the elute along the column can be illustrated by characteristic lines plotted in a coordinate system, in which the elute position is determined by the time (t) and axial coordinate (x).

The slope of the characteristic line, dt/dx , is directly related to the isotherm course, as follows [9]:

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