

"Forced modulation of operating conditions in chromatographic separation: potential and pitfalls"

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Efficiency of chromatographic separations can be improved by modulating the operating variables, such as mobile phase composition and temperature, as well as column loading conditions. The potential of the modulating methods and troubleshooting related issues will be discussed.

Chromatographic processes in both laboratory and large-scale applications are often performed in gradient mode, whereby operating conditions are modulated during the course of separation to improve its effectiveness. In liquid chromatography the composition of mobile phase (solvent gradient) and temperature (temperature gradient) can be used as operating variables.

In solvent gradient mode the mobile phase composition is varied from low to high elution strength. It is realized by an abrupt or a continuous change in the concentration of the so-called modifier, i.e., solvent that is added to alter adsorption properties of feed components, or by modulation of pH or the content of inorganic salts in the mobile phase. The advantage of solvent gradients is the possibility of separating multicomponent mixtures of components differing markedly in the retention behavior, accelerating the separation speed and compressing band profiles. However, when the added gradient shape is wrongly chosen peak deformation occurs. Moreover, when the modifier is retained by the stationary phase over the gradient concentration range, the shape of the imposed gradient may also be deformed, which disturbs the retention behavior of eluting compounds and deteriorates the column performance. Such deformations may occur in the course of gradients of organic solvents as well as inorganic salts or pH.

Gradient mode can be also employed for loading the column, where different solvents are used for preparing the feed solution (strong solvent) and for the mobile phase (weak solvent). Strong solvents are employed to increase the column load with feed components exhibiting poor solubility in the mobile phase. Because the elution strength of the feed solvent and the mobile phase usually differs markedly, the presence of the feed solvent may alter the adsorption behavior of solutes and cause deformations of their bands.

Another process variable that can be modulated in the course of chromatographic elution is temperature. Temperature gradients modulate retention of compounds to be separated on temperature-sensitive chromatographic media. It can be used to replace solvent gradient and avoid problems with handling multicomponent mobile phases, or both gradients can be combined to improve separation efficiency. Uncontrolled temperature changes may however cause non-uniform distribution of temperature in radial and axial direction and departure of the temperature gradient and the corresponding peak shape from the desired form.

Because of the complexity of thermodynamic, kinetic, and hydrodynamic effects underlying gradient elution, selection of optimal conditions for the separation is often impossible without understanding the band migration phenomenon. As efficient tools for understanding band migration phenomena and process design equilibrium theory and detailed models of the column dynamics are available, which will be discussed and exemplified in the lecture.