Editorial

Displacement Chromatography

by Huba Kalász

Displacement is a universal concept to indicate that one competitor is strong enough to replace the other competitor from its recent position or situation. The displacement mode of chromatography is a normal method of development in separation techniques.

The displacement phenomenon has been recognized among the main separation procedures since the begining chromatography: Tswett, in his first paper already indicated the role of displacement in the separation process, "…the more strongly adsorbed pigments displace the more weakly adsorbed ones" (1). In the early 1940s, Tiselius (2) clearly defined the three types of chromatography: elution, frontal analysis, and displacement development.

Displacement chromatography had wide usage in classical liquid column chromatography, in the preparative scale separation of rare metals, peptides, and proteins. On the basis of these results, Horváth et al. (3,4) initiated the use of high-performance displacement liquid chromatography (D-HPLC) for the separation of various compounds of industrial and biological importance.

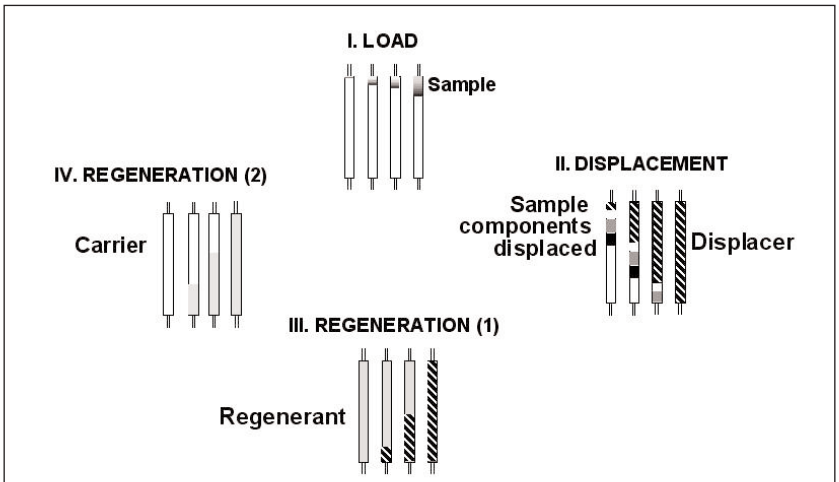

Figure 1. Column displacement chromatography.

Displacement chromatography works with the same instrumentation (hardware) generally used for analytical separation (the only usual exception is a large-volume sample loop) (3). The key difference is in the quality and order of mobile phases. Elution-type HPLC employs one (isocratic elution) or several similar eluents (stepwise gradient), or mixtures of two eluents with changing concentration (gradient elution) as the mobile phase. On the other hand, displacement chromatography employs three basically different mobile phases, such as the carrier, displacer, and regenerant(s). The carrier serves to facilitate the load; the carrier has to be able to dissolve the sample, and the sample components have to be definitely retarded on the stationary phase. The function of the displacer is to displace the sample components from the stationary phase; during this step the individual components also displace each other. By the end of the displacement step, the displacer occupies all binding sites of the stationary phase. Finally, the regenerant removes the displacer from the stationary phase and it prepares the column for the separation of the next sample. The consecutive steps of displacement chromatography are shown in Figure 1.

The quintessential procedure of D-HPLC is that the displacer is bound to the binding sites of the stationary phase very strongly, dislocating from there the earlier bound sample components. The front of the displacer pushes the sample components, and their concentration is determined by their isotherms and the actual concentration of the displacer. This means that the concentration in the fully developed displacement train does not depend on the sample concentration, but on the (generally Langmuirian)

isotherms of the individual components. This means that while in elution-type development, the sample components are diluted, and they are concentrated in displacement chromatography. The elements of a displacement train cannot be characterized by their migration parameters (3,5), the retention factor (such as k' in HPLC), or the RF value [in thin-layer chromatography (TLC)].

The three major steps of D-HPLC can be preferentially optimized using TLC. The planar arrangement of the stationary phase makes the modeling of individual steps of displacement chromatography possible either separately or as a series, and permits to follow visually the development of the displacement train together with the displacing procedure that separates the bands. The stationary phase of TLC is an open complex, and several samples (from 5 to 20 spots) can be simultaneously subjected to displacement chromatography. The silica-gel stationary phase is first used to scout the carrier, the displacer, and the content of the displacer. The results of displacement TLC (D-TLC) can be confirmed by D-HPLC.

The displacement type of development generates bands, which displace each other. The separation cannot be calculated by the usual ways, as these bands are ab ovo touching each other (3). There are certain methods to characterize the results of separation. Horváth et al. (3) suggested the calculation of the yield of the pure fractions. Separation and yield of the highly concentrated, closely located bands can be improved in several ways: (a) the use of multiple carriers generates several displacement fronts; (b) each of them displaces one of the various components to be purified (6); (c) the use of spacers separates the components of the displacement train from each other, and the spacers are generally easily removable (6); and (d) sample displacement chromatography is a valuable combination of frontal and displacement development, and it has been mainly used for the purification of proteins (7).

The primary use of D-HPLC is for preparative scale purification. Horváth's group separated proteins, peptides, nucleic acids, antibiotics, etc. Application of D-HPLC in biotechnology was one of their most remarkable contributions. Extremely large volumes of fermentation broth could be purified with an HPLC system generally used for analytical-scale separation. The increase of the sample volume was over six orders of magnitude. D-HPLC had also been used for the purification of peptides and proteins by the groups of Pinto (8), Cramer (9), and Hodges (10). As shown by Frenz et al. (11), some peptide fragments present in trace amounts could be concentrated by displacement chromatography.

Monitoring the individual components of the displacement train is not always as simple as it is seems. The generally used UV detectors are not able to detect the highly concentrated bands, such as solutions having a concentration of several tens of milligrams/milliliter (5). For this reason, the fractions are often collected and analyzed off-line.

D-TLC had been suggested by Horváth (6) primarily to scout the optimal composition of mobile phases for D-HPLC. However, the method can also be equally well applied in the metabolism research. D-TLC is especially favorable when the metabolites of a radiolabelled drug are sought. D-TLC has outperformed the original expectations, and it is also applied to find and identify new metabolites and natural products and novel rules of displacement chromatography. D-TLC was also utilized to verify that the displacing process of chromatography works with a real counteraction, and the sample components are counteracting by pushing the displacer front backwards. It is hard to observe the slight retardation of the displacement front in D-HPLC on the effect of the displaced bands, but it is easy to recognize the deformation of the displacer front on a TLC plate.

Displacement chromatography is the proper method for both preparative and analytical work when separation is operating in the nonlinear region of the isotherms. D-HPLC can be chosen for preparative scale purification, whereas D-TLC is useful for analytical purposes. As mentioned, D-TLC is very useful in the identification of metabolites (6). Therefore, it is an excellent alternative to HPLC or gas chromatography–mass spectrometry. D-TLC definitely reduces the the possibility of any misjudgment (through an oversight) when investigating the absence of metabolites of radiolabelled drugs. The bands of radiolabelled and unlabelled components comigrate. Therefore, the absence or presence of radioactivity in the displaced section of the tentative (unlabelled) standard compound clarifies the role of the metabolism in the origin of any compound.

The use of two-dimensional TLC yields easy and sure results for either the identification process or any other analytical task (11). Elution chromatography in the first dimension can be combined with displacement mode of development in the second dimension. The two-dimensional technique of TLC is simple and relatively inexpensive. For the evaluation of radiolabelled metabolites, the detection on x-ray film is rather more practical than the digital autoradiography.

The separation of isotopes (12) proved the strength of separation. However, the most important goal has remained the preparative scale separation of biomolecules using HPLC (13) and the analysis with D-TLC (11). A low-molecular-size displacer is generally preferred (14).

What is the reason that a relatively low number of publications are released on displacement chromatography? The answer is in the conflict of industrial interests. The benefit of displacement chromatography can be the scale-up of purification with three to six orders of magnitudes without any significant investment in the hardware of the separation system. Identification can be done without the slightest doubt of reliability. These advantages trigger the restraint from the fast publication of displacement chromatography dealing in industrially significant projects.

Another reason for the limited use of D-HPLC is the lack of information available about its strengths. The basic books on chromatography consider HPLC to be an excellent method for analytical separations, and this is very much the case. However, the further possibilities of HPLC are generally neglected, and this happens in the cases of D-HPLC, determination of physicochemical constants, and many more.

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