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CHAPTER 1

Principles and theory of chromatography

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1.1 Basic terms

It is useful to begin the chapter on the theory of chromatographic separation methods with a definition of chromatography. However, several such definitions can be formulated according to various classification aspects. For the sake of accuracy a phenomenological definition, a molecular kinetic definition and various working definitions can be introduced. According to the first definition chromatography is understood as a *phenomenon* of differential migration of solute compounds in a system of two phases, of which one is stationary and the other mobile. According to the molecular kinetic definition, chromatography is taken as a continuous *process* of convective upsetting and diffusional reestablishment of equilibrium between the concentrations of the solute compound in the stationary and in the mobile phase of the chromatographic system. This process results in a differential migration of the solute compounds. According to the working definitions chromatography is a certain method (specifically a separation and analytical method and various methods of physicochemical measurements). From the point of view of the theory of chromatography we are particularly interested in the chromatographic process.

Whereas the realization of a chromatographic experiment is often surprisingly simple — a number of important chromatographic processes proceed spontaneously — the mechanism of the chromatographic process is relatively complex. A prerequisite of the proper understanding of the mechanism of chromatography is the concept of dynamic equilibrium between the concentrations of a solute in a system of two coexisting phases; more accurately, equilibrium between the concentrations of the solute should be understood as a result of the identity of its chemical potentials in the individual phases of the system. Even when assuming that such a system is stationary and in equilibrium, molecules of the solute permanently pass from one phase to the other, remaining for a certain time in one or other phase after each transition. As the process is random at this level, the individual time intervals of the occurrence of the solute molecules in a given phase are also random and,

hence, very different. The mean time intervals of the occurrence of all solute molecules in each phase during a certain time are, however, constant under given conditions, and their ratio represents a basic factor of chromatographic retention. Thus, the ratio at which a given amount of the solute at equilibrium is distributed between the phases of the system is not determined by a static presence of the solute molecules in these phases but rather by the probability of their occurrence in the phases of the system. When, under these conditions, one phase moves with respect to the other, the solute molecules move together with the moving phase during their occurrence in that particular phase, but remain stagnant when in the stationary phase. Due to the statistical fluctuation some molecules of a given solute migrate a shorter or longer distance during a certain time interval than that corresponding to the mean time intervals of the occurrence of the molecules of this solute in the phases. This results, together with the longitudinal diffusion, in a spreading of the migrating zone of the solute. However, due to its statistical nature, this spreading increases only as the square root of the mean migration distance, so that, in the case of differential migration of zones of different solutes, the zones can be separated. This assumption of the mechanism of the chromatographic process will be formulated quantitatively in subsequent paragraphs of this chapter.

1.2 Classification of chromatographic systems and procedures

1.2.1 State of the aggregation of the coexisting phases

The traditional definition of the phases in a chromatographic system is often rather problematic. Whereas the term mobile phase is usually clear, specification of the chromatographic stationary phase is not always unambiguous. For instance, the whole content of the chromatographic column is sometimes considered as the stationary phase, but sometimes only those components of the packing that are functioning as sorbents of the solute compound are termed in this way. In the former case, the concept of chromatographic stationary phase apparently differs from the classical physical concept of the phase. Whereas in the physical conception the phase is a homogeneous part of the system, the chromatographic stationary phase may contain even more physical phases. In the latter case, the inert support of the sorbent is not considered to be the stationary phase, in spite of the fact that it represents a rather substantial physical phase of the system. However, when an active adsorbent plays the role of the sorbent support, it must then be considered as the chromatographic stationary phase. A problem then arises, viz. what part of the used adsorbent is really active with respect to the solute compound in the given system. Naturally, in a given chromatographic packing, chromatographic stationary phases cannot be unambiguously identified with physical phases. The above indeterminacies should be considered when classifying chromatographic systems according to the state of the aggregation of the phases; a summary of typical chromatographic systems according to this classification is presented in Table 1.1.

TABLE 1.1
Chromatographic systems

Stationary phase	Mobile phase	
	Liquid	Gas
Solid compound	LSC	GSC
Solid compound + liquid	LSLC	GSLC
Liquid	LLC	GLC

LSC, liquid-solid chromatography; GSC, gas-solid chromatography; LSLC, liquid-solid-liquid chromatography; GSLC, gas-solid-liquid chromatography; LLC, liquid-liquid chromatography; GLC, gas-liquid chromatography.

1.2.2 Physical arrangement of the system and the accomplishment of the chromatographic experiment

According to the physical arrangement chromatographic systems can be divided into planar and column ones. The planar arrangements are represented by systems of paper and thin layer chromatography. When further dividing the planar systems according to their physical arrangement we come to systems in the equilibration chamber and to the so-called sandwich systems. According to development procedures (flow of the mobile phase in the planar bed) the systems can be further classified as ascendent, horizontal, descendent and, occasionally, centrifugal; in orthogonal beds the development may proceed in one or more directions. When, during the development of the chromatogram, the composition of the mobile phase remains constant the development is termed isocratic, on the other hand, when the composition of the mobile phase varies, we speak of gradient development.

A more exact classification of column systems according to the physical arrangement leads to various types of packed and capillary columns. In column chromatography the use of several columns that can be suitably switched over, so that chromatographic fractions eluted from one column can be further chromatographed on other columns, is somewhat analogous to two-dimensional development in planar beds. In column chromatography the separation may proceed isocratically or with a programmed gradient of composition of the mobile phase, isothermally or with programmed changes of column temperature, and isobarically or with programmed changes of mobile phase pressure at the column inlet. The programming of the composition of the mobile phase is important practically only in liquid chromatography, whereas temperature and pressure programming is used primarily in gas chromatography.

In planar chromatographic systems the solute compounds are usually not eluted from the chromatographic bed but rather detected directly in it, whereas in modern column chromatography the solute compounds are gradually eluted with the mobile phase and detected in the effluent at the column outlet.

1.2.3 Development of the chromatogram

1.2.3.1 Frontal chromatography

A continuous supply of the analyzed material, or of its mixture with a non-sorbed mobile phase, into the column or into the planar bed results first in frontal chromatography and then in the saturation of the sorbent with all the components of the supplied material. After the development of the chromatogram, and during continuing supply of the mixture, the front of the least sorbed component is washed out first, followed by a mixture of the first component and the more strongly sorbed component etc., and, finally, after all the components of the mixture break through, a mixture identical in composition to that of the mixture supplied flows out of the column. By interrupting the supply of the analyzed mixture to the previously saturated column, and connecting the supply of the mobile phase alone, the opposite (desorption) frontal chromatogram arises. Initially, the mixture of all the components flows out of the column. After the least sorbed component has been eluted the mixture deprived of this component flows out of the column. After the further, more strongly sorbed component is eluted the mixture deprived of the first and second components flows out of the column. Finally, the most strongly sorbed component is washed out and only the supplied mobile phase leaves the column. Both versions of development of the frontal chromatogram are schematically and in an idealized form illustrated in Fig. 1.1.

1.2.3.2 Elution chromatography

Elution chromatography is simpler, and, with respect to the separation of an analyzed mixture, more effective. With this alternative a dose of the analyzed

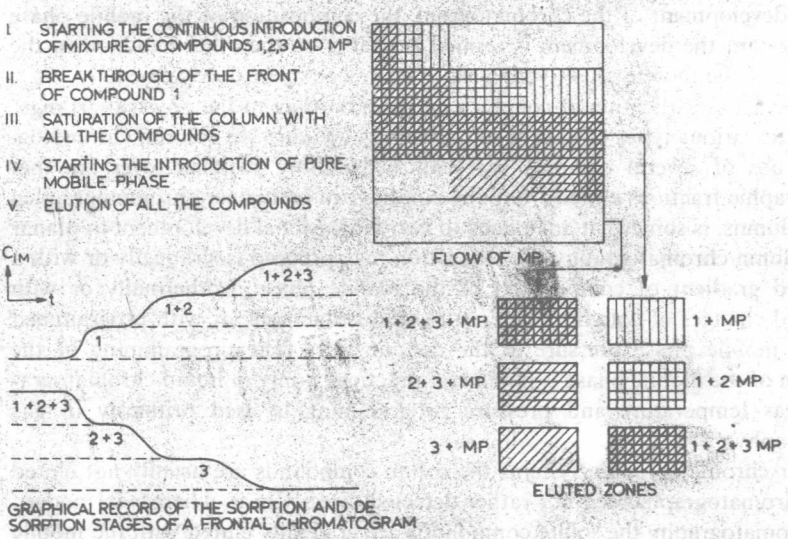

Fig. 1.1.

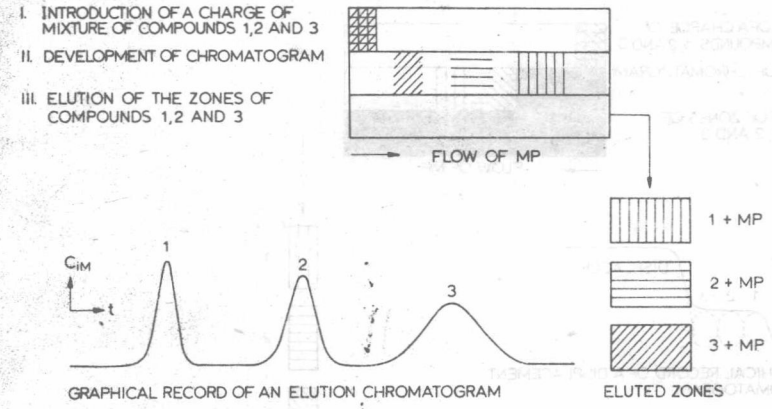

Fig. 1.2.

material is supplied to the column inlet or to the planar bed and is then washed with a non-sorbed mobile phase through the column. The development and differential migration of elution zones of individual components of the mixture thus take place. When the supply of the mobile phase continues the individual zones are gradually washed out of the column; the zone of the most weakly sorbed component is washed out first, followed by the zone of a more strongly sorbed component etc., and, finally, after the elution of the zone of the most strongly sorbed component, only the supplied mobile phase flows out of the column. A schematic illustration of the elution chromatography is presented in Fig. 1.2.

1.2.3.3 Displacement chromatography

When the stationary phase functions as an adsorbent and a compound that is adsorbed more strongly than any other component of the analyzed mixture serves as the mobile phase, the procedure otherwise similar to that used with elution chromatography is termed displacement chromatography. With this alternative the most weakly adsorbed component is displaced by the more strongly adsorbed component, this latter is then displaced by the more strongly adsorbed component, etc., resulting in a situation when the most strongly adsorbed component of the analyzed mixture is displaced by the supplied displacement agent. After the chromatogram has been developed, the zones of all the components migrate closely next to each other and, when the supply of the displacement agent continues, they leave the column in the order of increasing adsorption ability. In the case of elution chromatography (and in frontal chromatography when the mixture of the analyzed material is supplied together with the mobile phase) the eluted fractions are in fact mixtures of the solute compounds with the mobile phase, whereas in the case of displacement chromatography the individual zones are more or less the solute compounds alone. A scheme of displacement development is illustrated in Fig. 1.3.

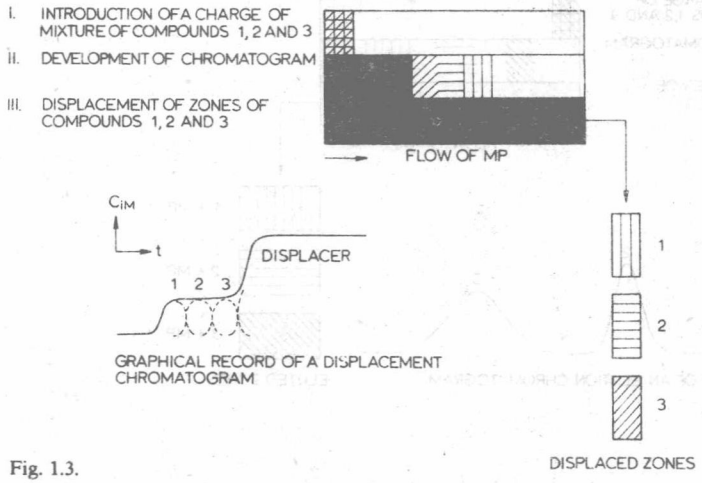

Fig. 1.3.

1.2.4 Mechanism of the distribution of the solute compound between the phases of the system

The mechanisms of sorption and/or the interaction of the solute with the mobile phase can be summarized as follows: *a*, physical dissolution in the phase; *b*, physical adsorption on the surface of the phase; *c*, chemical reaction in the bulk phase or on its surface (acido-basic equilibrium, formation of coordination complexes or chelates, association of ionic pairs, exchange of ions, precipitation); *d*, steric exclusion (molecular sieving effect, gel permeation); *e*, bioaffinity association.

The cases presented in paragraphs 1.2.1–1.2.4 can be mutually combined. The number of all possible combinations naturally exceeds the number of the real combinations, however, the number of real chromatographic systems and procedures is still very large. From the practical point of view, the alternatives of elution chromatography are most important. Therefore, with the exception of general problems, only elution chromatography will be discussed in this chapter.

1.3 Development of chromatography — a review

The oldest intentional chromatographic experiments were performed as frontal chromatography in a liquid-solid system and date from the beginning of the 19th century [1]. Elution chromatography (liquid-solid) was discovered at the beginning of the 20th century [2], but developed rapidly only after the discovery and theoretical explanation of liquid-liquid elution chromatography [3] in the forties and particularly after the discovery of elution gas chromatography [4–6] in the fifties. The pioneers in chromatography are noted in Table 1.2. A detailed description of the development of chromatography can be found in reviews by Ettre [7,8] and Zechmeister [9].