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PREFACE

The aim of this book is to be of help to those involved in the process of developing chromatographic methods. I have tried to write a text that is comprehensible and useful for both chromatographers with some experience, and for novices to the field with a background in science.

The fundamentals of chromatography are not covered in detail; the reader is referred to one of the introductory textbooks or courses on the subject.

Method development in chromatography today requires skills, knowledge and, above all, experience. Therefore, it is a particularly difficult field to enter for newcomers. I feel that an organized approach to method development, as presented in this book, may shift the emphasis from experience to knowledge. In this way, it may help newcomers to understand the process of method development. Also it may open the way for those already involved in method development to go beyond their personal experience and to apply different chromatographic techniques and optimization procedures.

The approach followed should be equally beneficial for chromatographers who do not develop their own methods but wish to improve (optimize) existing ones.

Procedures for developing and optimizing chromatographic separations have attracted increasing attention not only from researchers, but also from instrument manufacturers. Already, several of the procedures described are commercially available. The approach followed does not include describing existing methods. One reason for not doing this is that the elements that constitute a complete optimization package can be discussed and understood separately. Therefore, an existing method may be good in one respect, but poor in another. A second reason is that whereas complete optimization packages may be expected to change a great deal in the next few years, I expect this to be much less true for the underlying principles; so I would like to think that the material presented here will still be of value in the years to come.

This book is intended to be a critical assessment of procedures for method development and selectivity optimization. It is not intended to be a survey of available information, therefore references to the literature are included only when they are relevant to the text. Consequently, a number of references have been omitted. No doubt, some may also have been overlooked.

I am very grateful to a number of people who have reviewed the manuscript of this book (or parts of it) at various stages during the preparation. Together, they are responsible for an immense number of corrections, improvements and clarifications.

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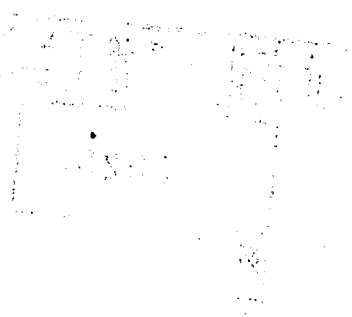
Eindhoven, February 1986
Peter Schoenmakers



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CONTENTS

CHAPTER 1

INTRODUCTION

In this chapter the concepts of chromatography, as far as they are relevant to the context of this book, will be outlined.

The chromatographic system, the column, and the basic fundamentals of chromatographic separations will be briefly discussed.

The extent of separation can be quantified in terms of the resolution obtained between two consecutive chromatographic peaks. This resolution can be expressed in terms of three elemental characteristics of chromatographic separation: retention, selectivity and efficiency. The influence of each of these three factors on resolution will be discussed.

1.1 CHROMATOGRAPHY

Chromatography can be defined as the separation of molecules by differential migration*, i.e. separation is achieved on the basis of different speeds of transportation for different molecules.

In this book column chromatography will be discussed almost exclusively, although occasional reference will be made to thin layer chromatography (TLC), the fundamentals of which are not different from those of column chromatography.

Furthermore, this treatment is limited to those forms of chromatography which involve two phases (a stationary and a mobile phase) and in which the necessary differences in speed of migration are caused by differences in chemical interactions between the molecules of the different sample components ("solutes") and the two chromatographic phases, as well as between the solute molecules themselves. Interaction chromatography is sometimes used as a term to describe such systems.

Separations that are achieved on the basis of the size of the molecules (e.g. size exclusion chromatography) are not dealt with in this book. Such separations are not selective, and hence there is no selectivity to be optimized.

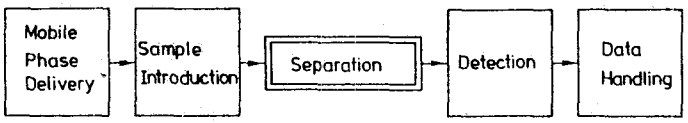

Figure 1.1: Schematic representation of a chromatograph.

A schematic representation of a chromatograph is given in figure 1.1. This figure applies to all kinds of column chromatography, but the various boxes will have different contents for different chromatographic techniques, notably for gas chromatograhy (GC) and for liquid chromatography (LC) (for definitions see section 2.1).

* In this broad definition some techniques which are not usually considered as chromatography are included, for example field flow fractionation (FFF) techniques and electrophoresis. However, isotachophoresis is not included.

For GC the mobile phase delivery box could consist of a gas cylinder, a reducing valve and a flow controller. For LC a high pressure pump will be required. In this book the instrumentation required for chromatography will not be discussed. Only where the equipment used is relevant to the cause of optimization of selectivity will it feature in the present text (e.g. sections 5.6 and 7.4).

The rest of this book will focus on the thick box in the centre of figure 1.1, identified as separation.

1.2 SEPARATION – THE COLUMN

The chromatograph is built around the column, in which the actual separation takes place. The column accommodates the two chromatographic phases: the stationary phase, which remains in the column, and the mobile phase, which is transported through it. Separation is achieved because different sample components (solutes) show different distributions over the two phases. A solute, having such a high affinity towards the stationary phase that it resides in this phase exclusively, will stay in the column indefinitely. A solute, that does not enter the stationary phase at all, will be transported through the column at the same speed at which the mobile phase is transported. In chromatographic terms, the latter is called an "unretained" solute.

If a column is packed with porous particles, then an unretained solute is assumed to be swept through the entire volume of the column that is occupied by the mobile phase, either outside the particles or in the pores. A solute that does not enter any of the pores is called a (completely) "excluded" solute. Throughout the remainder of this book we will assume that the solutes will not be (partially or completely) excluded from the pores.

1.2.1 Retention times and capacity factors

The above discussion can be quantified as follows. A solute i distributes itself over the two phases, resulting in a total quantity $q_{i,m}$ to be present in the mobile phase (m), and a quantity $q_{i,s}$ in the stationary phase (s). The solute molecules which find themselves in the mobile phase will be transported through the column at the same speed (u) as the molecules of the mobile phase. However, this is only a fraction of all the solute molecules, so the average speed for all solute molecules will be only a fraction of u given by

$$v_i = \frac{q_{i,m}}{q_{i,m} + q_{i,s}} u , \tag{1.1}$$

where v_i is the migration speed, the average speed at which the solute band travels through the column. The time $t_{R,i}$ needed for the solute band to elute from the column is determined by the column length and the average migration speed:

$$t_{R,i} = L / v_i . \tag{1.2}$$

$t_{R,i}$ is called the retention time of the solute. Similarly, the time which a mobile phase molecule will spend in the column is

$$t_0 = L / u . \tag{1.3}$$

t_0 (frequently also denoted by t_m) is known under different names: the hold-up time, mobile phase time, or unretained time.
The combination of eqns.(1.1), (1.2) and (1.3) yields

$$t_{R,i} = (1 + q_{i,s}/q_{i,m})\, t_0 \,. \tag{1.4}$$

By definition, the capacity factor (k_i) of the solute i is

$$k_i = q_{i,s} / q_{i,m} \tag{1.5}$$

and hence

$$t_{R,i} = (1 + k_i)\, t_0 \,. \tag{1.6}$$

Eqn.(1.6) is the fundamental equation for retention in chromatography. Throughout this book, extensive use will be made of the capacity factor as a convenient means to describe retention. A major advantage of the use of k for this purpose is the fact that it is a dimensionless quantity. It follows from eqn.(1.6) that

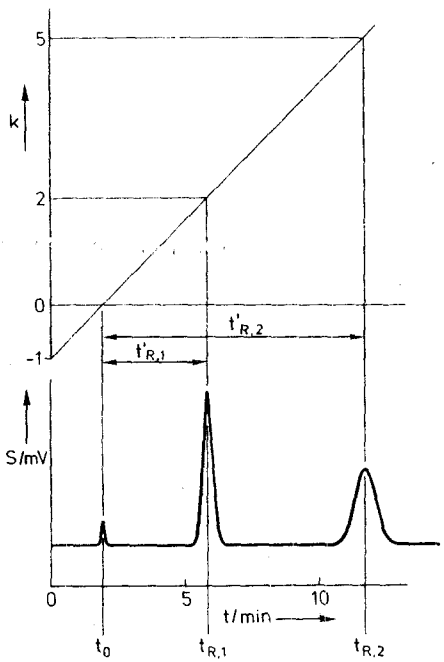

Figure 1.2: Schematic chromatogram illustrating the meaning of various retention parameters

$$k_i = (t_{R,i} - t_0)/t_0 = t'_{R,i}/t_0 \qquad (1.7)$$

where $t'_{R,i}$ is the net retention time of the solute, i.e. the (average) time which a solute molecule spends in the stationary phase.

Eqn.(1.7) also shows that the capacity factor k can easily be determined from the chromatogram. This is illustrated in figure 1.2. If a signal at $t = t_0$ is obtained in the chromatogram, then the quantities t_0, t_R, and t'_R can all be measured directly. The capacity factor can either be calculated from eqn.(1.7), or determined from a calibration line as shown in figure 1.2. Two points can be used to construct the line, for instance $k = 0$ at the occurance of the unretained peak and (a fictive point) where $k = -1$ at the time of injection ($t = 0$). The capacity factor of any peak in the chromatogram can be determined very easily in this way. However, to avoid inaccuracies if high k values occur, the calibration line may be constructed by using eqn.(1.7) once for a point at a high value of k.

1.2.2 Distribution coefficients

The quantity q of the solute i in one of the phases is the product of the average concentration ($\bar{c}$) of i in that phase (where the average is taken along the length of the column) and the volume of that phase. Hence, for the capacity factor (eqn.1.5) we find

$$k_i = \frac{q_{i,s}}{q_{i,m}} = \frac{\bar{c}_{i,s}}{\bar{c}_{i,m}} \cdot \frac{V_s}{V_m} . \qquad (1.8)$$

The ratio $\bar{c}_{i,s}/\bar{c}_{i,m}$ is a constant if the distribution isotherm*, i.e. a plot of $c_{i,s}$ vs. $c_{i,m}$, is linear. This is usually the case at high dilutions. Preferably, all (analytical) chromatography is performed in this linear region. The distribution coefficient in terms of concentrations (K_c) may be defined as

$$K_{c,i} = c_{i,s}/c_{i,m} . \qquad (1.9)$$

Since K_c may be independent of the solute concentration, but will always be a function of the temperature (and pressure), the term distribution coefficient is to be preferred to the alternatives: distribution constant and equilibrium constant. If the distribution isotherm is linear, K_c will also equal the ratio of average concentrations in eqn.(1.8), and hence

$$k_i = K_{c,i} \cdot V_s/V_m . \qquad (1.10)$$

Eqn.(1.10) relates retention in chromatography (k) to a thermodynamic parameter (K_c). The so-called phase ratio V_s/V_m is a characteristic of the column**.

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* If the stationary phase is a solid surface, then the term adsorption isotherm is more commonly used.

** However, in some kinds of chromatography (e.g. reversed phase liquid chromatography, see section 3.2) the phase ratio may vary with variations in the mobile phase composition.

1.2.3 Selectivity

It was stated at the beginning of this section that solutes are separated in a chromatographic column on the basis of differences in their speed of migration through the column. We can define the relative retention (α_{ji}) of two peaks as

$$\alpha_{ji} = t'_{R,j} / t'_{R,i} . \tag{1.11}$$

In this equation i represents the first eluting peak of a peak pair and j the last eluting peak. Hence, by definition α is always larger than unity. Sometimes α is called the separation factor, which is somewhat unfortunate terminology because separation is influenced by other factors than just α (see section 1.3)*. α is the chromatographic parameter that is most directly related to the selectivity of the phase system. In this book, therefore, the word selectivity will often be associated with α. Using eqns.(1.7) and (1.10) we can write two other equations for α_{ji}:

$$\alpha_{ji} = k_j / k_i \tag{1.12}$$

and

$$\alpha_{ji} = K_{c,j} / K_{c,i} . \tag{1.13}$$

Eqn.(1.12) is very useful in practice, because it expresses α directly in terms of the capacity factors. We will make frequent use of this equation throughout this book.

Eqn.(1.13) relates α to the distribution coefficients. Since no phase ratio term appears in eqn.(1.13), it is clear that the selectivity (α) of the chromatographic system is determined only by thermodynamic factors.

The relative retention will be affected only by those factors which affect the distribution coefficients, i.e.

- the solute
- the mobile and the stationary phase (together constituting the phase system)
- the temperature
- the pressure.

The effect of the pressure on α and on k is usually negligible. Only in some particular cases (e.g. in supercritical fluid chromatography, SFC; see section 3.4) will it be a relevant parameter.

1.2.4 The phase ratio

The phase ratio V_s / V_m occurs in eqn.(1.10) as one of the factors that determine retention (k) in chromatography. We can influence the phase ratio by varying one or more of several parameters:

* In chapter 4 we will define a separation factor S which provides a more realistic measure of the contribution of chromatographic retention to separation.

– *The type of column*

In particular, we can choose between open (capillary) columns and packed columns*. A wall coated open tubular (WCOT) column has a much smaller phase ratio than a packed column, due to the small surface area of the wall.

– *The column diameter*

If open columns are used, then the phase ratio will vary with the column diameter (provided that the film thickness is kept constant). The cross-sectional area of the column (and hence the mobile phase volume) is proportional to the square of the column diameter, while the wall area is proportional to the diameter itself. Hence, the phase ratio is inversely proportional to the column diameter.

– *The surface area*

The area available for the stationary phase will directly affect the phase ratio. If a solid material is used as the stationary phase in a packed column, if a liquid phase is deposited on a solid adsorbent with a constant film thickness, or if chemically bonded phases are employed, the phase ratio (through V_s) will be directly proportional to the available surface area. The surface area of an adsorbent is usually given per unit weight (i.e. the specific surface area in m^2/g). However, it should be noted that the relevant quantity is the surface area per unit volume (m^2/ml) in the packed column.

– *The column porosity*

This is the fraction of the column volume that remains available for the mobile phase after packing. There are two contributions to the total column porosity. One part of the volume available to the mobile phase is in between the particles (interparticle space). For uniform, spherical particles this is about 40% of the column volume. The second contribution is due to the very porous structure of materials with large specific surface areas. This makes a significant part of the intraparticle volume available to the mobile phase (usually 20 to 30% of the column volume).

– *The film thickness of a liquid stationary phase*

Clearly, with all other factors constant, V_s will increase linearly with the film thickness (this is also true for the phase ratio V_s/V_m, as long as $V_s \ll V_m$). For solid adsorbents this effect does not occur. For chemically bonded phases the (mono-)layer thickness is not as well defined as the film thickness of a bulk liquid, and neither is the description of variation in the layer thickness as straightforward as it is for liquids (see section 3.2.2).

In general, the effective volume of a stationary phase (V_s) can be increased in a predictable manner by increasing the surface area, but only for liquids can the same be said for increasing the film thickness.

Obviously, there are many ways to influence the capacity factors. However, the effects described above are predictable (see section 4.2.3) and in a sense trivial. It is worth noticing at this point that certain parameters do not at all affect the capacity factor and therefore do not at all affect chromatographic selectivity. These parameters include column length, flow rate and the diameter of packed columns. This renders these parameters irrelevant to the selectivity optimization process. In some cases they may be considered as parameters

* For gas chromatography (and for supercritical fluid chromatography) there is a real choice. Open columns may theoretically be used in liquid chromatography as well, but their diameter should then be so small that they do not yet form a realistic alternative to packed columns in practice.