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retention and selectivity in liquid chromatography

prediction, standardisation and phase comparisons

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Roger M. Smith

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Contents

Preface

This book grew out of a long standing interest in the ways in which retention and the selectivity of separation in liquid chromatography are dependent on the structure of the analyte and on changes in the mobile and stationary phases. These relationships are at the heart of an understanding of the operation of liquid chromatography and of the ways in which the chromatographer can manipulate the conditions of a separation to achieve the analysis of a complex sample.

The factors involved in these processes are complex and even 90 years after the pioneering work of Tswett are still not fully understood. Any progress is linked to the development of an understanding of the physical chemical process of solvation and the physicochemical nature of the stationary and mobile phases. Chromatography is also a valuable practical analytical method and much can be learnt by studying relative interactions and by comparing the behaviour of analytes with different chemical structures under different separation conditions. To achieve this objective, techniques for recording relative retentions are needed so that results can be reproduced in different laboratories or by different operators.

However, liquid chromatography has a notoriously poor transferability, the same high versatility which enables separations to be precisely optimized also means that small changes between systems can alter the separations. This book addresses some of the ways in which these problems have been overcome to enable retention predictions, identifications and the characterization of the properties of mobile and stationary phases, to be carried out. The work owes much to studies in gas chromatography, in particular the work of Kováts in providing a retention index scale and of Rohrschneider and McReynolds on the comparison of stationary phases.

A theme which leads through the different chapters is the value of relative measurements. Most obviously in the descriptions of the different retention index scales in liquid chromatography and their application to the identification of a wide range of analytes. The indices also form the basis of one of the studies on retention prediction, the other relating retention to the contribution of analytes to partition coefficients. Related methods have been used to compare analytes and their interaction properties. The final group of chapters investigates methods for the comparison of mobile and stationary phases not just by using a simple solvent strength parameter but by examining the comparative interaction of the phases to different types of analytes either in terms of their shapes or physical properties.

Bringing these chapters together enables the different approaches to be compared and illustrates the values of each. Hopefully, this will stimulate further research or different approaches for this is by no means the full description of the mechanism of retention. Much more still needs to be done, in particular to understand how complex molecules behave. In this case, the chromatographic behaviour of the analyte under different conditions may itself provide valuable information about the physical properties of the analyte.

I would like to thank many of the contributors for useful and interesting discussion of their work and the stimulation it has provided for our own studies. I would also thank my research and project students at Loughborough University of Technology, who have contributed to our own studies in this field. In the same way that their individual contributions have together built our overall study, so I hope that the chapters of this book will contribute to an overall greater understanding of the retention process in liquid chromatography.

Roger M. Smith
May 1994

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CHAPTER 1

Retention prediction based on molecular structure

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1.1 INTRODUCTION

The retention of a particular analyte in a reversed-phase liquid chromatographic system is dependent on many factors, the structure of the analyte, the nature and chemistry of the stationary phase, the composition of the mobile phase and the temperature. Some of these factors are reasonably well understood, at least on an empirical level, and chromatographers can manipulate eluent composition and even temperature to alter retentions in a predictable manner.

However, the effect of the chemical structure of the analyte on retention is probably the least well described parameter. Most chromatographers recognise the broad influence of polarity and size and their effect on hydrophobicity but not the detailed impact of the addition of a methoxyl, carboxamide or other functional group. Nor in most cases is it possible to predict the composition of the eluent required to result in a predetermined retention (capacity) factor (k). Instead the experimental conditions to achieve a particular retention are usually selected by analogy with related compounds or from experience of analysing a wide range of samples. Most analytical methods in liquid chromatography are then refined on a trial and error basis.

However, in recent years two methods to aid the chromatographer in refining a separation have become available. The first requires no knowledge of the structure of the analyte. A computer programme, often an expert system, uses the retention factors of the components of a mixture from a gradient or isoeluotropic set of separations to propose an eluent mixture, which is predicted to provide optimal resolution or overall run times. These techniques include systems such as Drylab, PESOS, ICOS, and DIAMOND, which are based on prediction and mapping methods, and chemometric techniques, including iteration and Simplex optimization methods. These methods have been well reviewed in recent years [1–3]. Future developments are likely to see the expert systems being sup-

References pp. 45–46

plemented by neural networks, which should enable them to "learn" about the properties of a particular column and instrument, before making their predictions [75].

In most assays the structure of the analyte is known and the second approach has been to predict the retention from the molecular structure. This can be carried out directly by the summation of the retention properties of the structural components or by deriving a physical property, such as the octanol–water distribution coefficient (log P), which can then be related to retention by comparison with analytes of known value [3]. As the structures of any impurities or metabolites in a sample are often known, it should also be possible to predict the optimum conditions for their resolution from the main components. This approach has the potential for true prediction as it can propose initial chromatographic conditions, designed from the start to achieve a particular separation. Two different but closely related aspects of this approach form the subject of this and the following chapters.

The recent literature also includes numerous papers on retention prediction which related retentions under one set of conditions with those using a different proportion of modifier or temperature. For example, changes in retention with mobile phase composition have been recently discussed by Valko *et al.* [4]. A second closely related area has been the selection of robust methods that although they may not be optimized to give the maximum resolution, nevertheless provide methods which are less susceptible to small changes in eluent composition, temperature and or different columns [5,6]. In real life situations this may be an important consideration if the method is to form part of an official method or is required for long-term studies of the stability or quality of a product. Again computer assistance has been provided for the selection of testing conditions and the evaluation of the results.

1.2 STRUCTURE AND RETENTION

The concept that the retention of an analyte in gas or liquid partition chromatography can be expressed as the summation of factors related to its skeleton and individual functional groups was originally proposed by Martin [7]. He suggested that the retention of a analyte can be expressed by the summation of contributions from each of the structural components, alkyl-chains, aromatic rings and functional groups. These substituent values are related to their effects on other equilibria and are recognised as examples of a linear free-energy relationship. The early work in this area on gas-liquid chromatography and thin-layer chromatography have been reviewed by Kaliszan [8,9].

These concepts have led to a wide range of studies, which have examined the effect of the different substituents on the retention of an analyte in liquid chromatography. These quantitative structure–retention relationships (QSRR) studies have encompassed physical properties, topological indices, and additive functions and have been reviewed in detail [8–12]. Similar concepts have long been used for the prediction and calculation of octanol–water partition coefficients (log P) in quantitative structure-activity relationship (QSAR) studies which are important in relating biological activity to structural features. Hansch and Leo [13] have shown that the log P can be calculated by the summation of a value for a parent compound with contributions for each substituent (π constants) and a

similar approach based on fragmental (f) constants has been used by Rekker [14]. There is often a good correlation between the octanol-water partition coefficients and chromatographic retention and numerous studies have used HPLC techniques to measure effective log P values [10,12,15]. The technique works well if a group of analytes are structurally related but compounds of different structural types may show a poorer correlation.

However, comparatively relatively little use has been made of the π or f constants to calculate log P values for retention prediction. In a series of studies, Jinno and Kawasaki [16–18] predicted the retention factors of alkylbenzenes and substituted aromatic compounds. More recently, the relationship has been used by Valko and co-workers [19] as the basis of a retention prediction system (see Chapter 2). This work has formed the basis of a computer program, which also incorporates the ability to handle partially ionized analytes. Some of the advantages and limitations of this method have recently been evaluated by Fekete *et al.* [20].

An alternative approach for the prediction of retentions in liquid chromatography is to use the summation of retention increments, which have been determined by comparison of substituted and unsubstituted analytes. These can be expressed either as functional group contributions (Section 1.2.1) or retention index increments (Sections 1.3–1.5). This approach has also been examined in other branches of chromatography. Peng *et al.* [21] have examined the prediction of the retention indices of analytes, based on their molecular structure on an apolar column in gas chromatography. The number of atoms, the aromatic increment and the group retention functions (GRF) were all important. They used a combination of the number of carbon atoms, carbon atom equivalents, and group retention factors for substituents and functional groups. They took into account the effects of rings, *iso* and *neo*-carbons and found that predicted and experimental values were within ±3%. In a second paper, they examined these effects for separations on polar columns [22]. A similar approach has also been reported by Evans and co-workers [23] based on the molecular weights and selectivity indices of the analytes.

1.2.1 Chromatographic functional group contributions

A frequently applied approach, to relate retention to changes in structure, has been the functional group contribution (τ) to the logarithm of the retention factor. The values of τ are determined by comparison of the retention of substituted analytes with the corresponding unsubstituted analyte (Eq. 1.1).

$$\tau_x = \log k_{R-X} - \log k_{R-H} \tag{1.1}$$

The measurement and application of group contributions have been comprehensively reviewed by Smith [24]. The magnitude of the contributions for individual functional groups differ with the eluent composition and their magnitude usually decreases significantly with increased organic modifier. The contributions also differ with different organic modifiers in the mobile phase. However, these parameters have not been widely used for retention prediction because few studies have examined the relationship between mobile phase composition and the magnitude of the contribution. The contributions were

frequently deliberately extrapolated to 100% water as the eluent to give composition independent values (τ_0), which were then compared with other physical parameters.

Probably the most widely investigated functional group change in retention with structure is the methylene group contribution. Numerous studies of homologous series [24] have shown that there is a systematic change in the logarithm of the retention factor with the carbon number. This change is usually similar for all homologous series, irrespective of the other groups present. For example, Figge and co-workers [25] reported a constant change for a series of homologous analytes, *n*-alkanes, *n*-alkenes, *n*-alkylbenzenes, fatty acid methyl esters, alkan-3-ones, 2-*n*-alkyl-pyridines, 1-*n*-alkanols. This relationship also forms the basis of most retention index series and is discussed further in Chapter 3.

A difficulty with many of the retention studies, such as the functional group contributions, which are based on retention factors (k), is that the increments are very dependent of the experimental conditions, such as temperature and the eluent composition. Frequently these have not been closely controlled and the resulting retention values are often unique to that individual system of mobile phase and column. Many of these problems of reproducibility and transferability between systems can be overcome by using relative retention measurements, such as retention indices (see Chapter 3). A retention index scale effectively compares the increment for a functional group with the corresponding methylene increment in the same system. Both should be similarly affected by the small changes in the strength of the eluent and by temperature, so that retention index based group contributions should be almost independent of the eluent composition and of the make of stationary phase. Unless there are changes in the relative interactions between the methylene or other functional groups and the stationary phase, the retention increments should be largely independent of the brand of stationary phase and carbon loading of the columns, even though these differences can significantly effect retention factors (k).

1.2.2 Related prediction studies

In an extensive series of studies, Jandera has examined the description of the interaction of an analyte in terms of lipophilicity (n_{ce}) and polarity interaction indices (q). The values of these terms for a number of functional groups have been determined by comparison with the retentions of the homologous *n*-alkylbenzenes. This work has been recently reviewed [26] and is described in more detail in Chapters 7 and 8.

Galusko proposed that it should be possible to predict the retention of a compound based on the summation of the effects of the bond dipoles and partial molar volumes of the substituents [27]. This system has now been developed into ChromDream, a computer-based prediction system [28]. Their model is based on a two-layer continuum model of reversed-phase chromatography and the differences in molecular solvation energies in the two phases. The retention of an analyte is described by Eq. (1.2), in which V_i are the increments of the partial molar volume fragments in water and $G_{e.s.j,H_2O}$ are the increments of energy of interaction of bond dipoles with water. a, b and c are the parameters of the reversed-phase system and depend on the column and phase ratio and are characteristic of the stationary layer and mobile phase.

$$\ln k_x = a(\sum_i V_i)^{2/3} + b(\sum_j \Delta G_{\text{e.s.}j,\text{H}_2\text{O}}) + c \quad (1.2)$$

The values of a, b and c for a particular separation system have to be determined by using reference compounds. The parameters a and b are related to differences in the surface tension and dielectric permeability of the sorbent surface layer and mobile phase, respectively, and can be related to differences between stationary phases. The programme gave accurate predictions over a range of eluent composition for a wide range of aromatic analytes. However, the model does not take stereochemical and intramolecular interactions into account and discrepancies were found for *ortho*-disubstituted analytes.

A common approach for retention correlation has been to relate a range of physical parameters related to structure, such as shape and connectivity parameters, to retention factors using multivariant analysis [9,10]. The resulting regression equations can then be used for retention prediction. However, although the correlations are frequently excellent, the addition of new model compounds to the data set will often markedly change the coefficients and even cause the significant terms to change. Thus although the regressions can "predict" the retention of analytes that are included the original model data set, they frequently fail to predict accurate retentions for new compounds. A conceptual problem is that there is often no clear connection between the properties that are used as terms in the regression and structural or physical properties, which are generally accepted as being significant in liquid chromatography. The correlation may be valid but only because the parameters are also indirectly related to a parameter of relevance. This purely chemometric approach has been reviewed [9,11] but the real prediction power appears to be limited.

1.3 FUNCTIONAL GROUP EFFECTS ON RETENTION INDICES

Retention indices, which are determined by logarithmic interpolation between the retention factors of a series of homologous standards, provide a reproducible mode of retention measurement (Chapter 3). They can form the basis of reliable and transferable retention comparisons. In comparison to functional group contributions, they are more independent of the eluent composition and stationary phase. Functional group or substituent increments ($I_{\text{S, X}}$) can be determined as the differences between the retention index of a parent compound ($I_{\text{R-H}}$) and those of substituted derivatives ($I_{\text{R-X}}$) (Eq. 1.3).

$$I_{\text{S, X}} = I_{\text{R-X}} - I_{\text{R-H}} \quad (1.3)$$

As the functional group index increments are not related to the absolute retention times but to the methylene group contribution ($I_{\text{S,CH}_2} = 100$) by definition) they should be largely independent of eluent composition. If interactions occur between multiple substituents, the differences between the simple summation of the contributions from the individual groups and the experimental retention index value, are defined as the interaction index I_{I}.

Because of the range of polarities of analytes in HPLC, a number of different retention index scales have become established ([29], Chapter 3). The most frequently employed scales have often also been used for retention prediction. As each scale is based on the methylene increment, the results should be equivalent but small differences might be ex-

pected from changes in separation conditions. The *n*-alkanes and *n*-alkylbenzenes are both highly retained and have been used for non-polar analytes (Section 1.3.1). The alkyl aryl ketones (Section 1.4) cover a wide range of polar and non-polar analytes and have formed the basis of a major prediction study (Section 1.4). The more rapidly eluted alkan-2-ones have been used primarily for the prediction of drugs and their metabolites (Section 1.3.2). Although the 1-nitroalkanes have a similar polarity, no prediction studies have been carried out with these standards. Some prediction work has used a retention index scale based on standards with increasing numbers of aromatic rings (Section 1.5).

1.3.1 Retention prediction based on *n*-alkanes and *n*-alkylbenzenes

Morishita *et al.* [30] determined the retention index increments for four aryl substituents using the *n*-alkane scale (Table 1.1a). The values of the increments reflected the expected hydrophobicities, although the methyl group increment ($\delta I = 89.3$) was lower then the nominal value for a methylene group ($\delta I = 100$) and may have reflected hyperconjugation with the ring. The relative magnitudes of the increments corresponds to those obtained in later studies on the alkyl aryl ketone scale [31].

They also studied the interactions between two substituents on the aromatic ring, by measuring the difference between the experimental indices and the calculated values obtained by the summation of substituent increments (Table 1.1b). The increments for inter-

TABLE 1.1

SUBSTITUENT AND INTERACTION INCREMENTS OF RETENTION INDICES BASED ON THE *n*-ALKANE SCALE

(a) Substituent increment $\delta I_X = I_{Ar-X} - I_{Ar-H}$. For comparison, substituent index $I_{S,Ar-X}$ values are given from the regression equations based on alkyl aryl ketone retention index scale [31]

Functional group	Substituent increment (δI_X)	$I_{S,Ar-X}$ [31]
CH_3	89.3	104
NO_2	–61.9	–87
OH	–195.7	–290
NH_2	–213.6	–302

(b) Interaction increments between substituents on an aromatic ring. Calculated as the differences between experimental retention index and summed index values for substituents and parent

Groups		Interaction increment ($\delta I_{I,X-Y}$)		
X	Y	2-Y	3-Y	4-Y
CH_3	CH_3	–12.7	3.7	5.0
CH_3	OH	–11.1	–24.5	–24.1
CH_3	NH_2	–24.7	–24.3	–23.5
CH_3	NO_2	–27.4	–2.5	–19.2
NH_2	NO_2	140.8	84.5	48.8

Based on Morishita and co-workers [30]. Conditions: column, Partisil ODS-3; eluent, methanol–water (70:30).

actions between methyl groups were small ($\delta I = -13$ to 5) but between amino and nitro groups much larger changes were observed. The *ortho*-nitro substituent ($\delta I = 141$) on aniline had a major effect and increased the retention by considerably more than the *meta*- or *para*-substituents ($\delta I = 85$ and 49, respectively) suggesting that hydrogen-bonding was occurring between the *ortho*-groups. Using the index increments for the substituents and interactions, they calculated the predicted retention indices for a number of trisubstituted aromatic compounds and found a good correspondence with experimental values. The deviations were between –14 and +10 units; for example, 3,5-dimethylaniline $I_{calc} = 163$ and $I_{obs} = 166$; 2-methyl-4-nitroaniline, $I_{calc} = 79$ and $I_{obs} = 80$.

A similar approach was subsequently used in a study of sulphur compounds by Möckel [32]. He compared the retention of a series of homologous thiols and alcohols with the corresponding alkanes and determined the retention index increments for the replacement of a methylene group by a hydroxyl (OH) group (–510 to –519) or thiol (SH) groups (–180 to –206) (the values increased slightly with chain length) using methanol–water (70:30). Slightly confusingly, the retention indices of the parent compounds in this paper were based on the number of non-hydrogen skeletal atoms (C plus S) so that the replacement increment for the thiol group in heptylthiol ($I = 599$) was calculated as 599 – 800 [$(C_7 + S) \times 100 = -201$)]. These replacement values correspond to substituent increments for hydroxyl of $I_{OH} = -410$ to –419 units and for thiol of $I_{SH} = -80$ to –106 units. Thioethers (R–S–R) had retentions similar to the monothiols. The increments for the hydroxyl group were similar to those found later for the aliphatic hydroxyl ($I_{S,\ R\text{-}OH} = -362$ in methanol-buffer (50:50), see Table 1.7) using the alkyl aryl ketone scale.

The retentions of the alkylpolysulphides (R–S_n–R) were also examined [32]. When a methylene group in tetradecane ($I = 1400$) was replaced by a sulphur atom to give hexylheptyl sulphide ($I = 1060$) the retention decreased markedly. A second replacement by a sulphur atom gave dihexyl disulphide with a similar retention but as the proportion of sulphur atoms in the chain was increased further, the polarity decreased to eventually give dipropyl octasulphide ($I = 1259$) and dimethyl dodecasulphide ($I = >1400$). These changes were explained as the initial formation of a local polar centre and then an increase in retention as the non-polar polysulphide replaced the methylene groups.

In subsequent studies, Möckel and co-workers [33] determined the coefficients, which related the retention indices of homologous analytes to the number of carbon atoms (n_C) (Eq. 1.4).

$$I_K = A + Bn_C \tag{1.4}$$

They used these values to demonstrate that for most homologues the change (B) in the retention index on the *n*-alkane scale on the addition of a methylene group was close to 100 units. However, smaller values were found for Ph–$(CH_2)_n$–Ph (88.99 units) and R–S_9–R (78.84 units). The A term indicated the effect of the functional group. A comparison of the saturated and unsaturated hydrocarbons suggested that the addition of an olefinic group increased retention by 61.85 units but an acetylene group decreased retention markedly ($A = -265.96$). These increments were translated into "chromatographic free energy" changes. They also found a linear relationship for each series between retention index and calculated total surface area. In a subsequent study [34] they reported the cor-