CHAPTER 2

CONCEPTS OF THE RETENTION INDEX AND PARAMETERS FOR THE CORRELATION

After the pioneering work by Martin in 1949 (6), chromatography has been classically defined as the process by which substances in a mixture physically separate by distributing themselves between 2 phases, (i) a "stationary phase", which can be a solid or liquid supported on a solid; and (ii) a "mobile phase", either liquid or gas, which flows continuously around the stationary phase. The four possible combinations of mobile and stationary phases lead to four types of chromatography: liquid-solid, liquid-liquid, gas-solid and gas-liquid. Gas chromatography (GC), which consists of the combination of gas-solid and gas-liquid, is a very suitable method to separate mixtures of compounds that can be easily volatilized. The physical separation of the different compounds results primarily from differences in their affinity for the stationary phase.

2.1 Chromatographic Interactions

The distribution of a solute between the mobile phase and the stationary phase during the chromatographic separation process results from the interactions between the solute molecules and the molecules of each phase. In gas chromatrography the interactions in the gas phase are relatively less important compared to those in the stationary phase.The interaction forces between solutes and different phases are of the following four types:

1. Orientation or Keesom Forces. These are forces resulting from the interactions between two permanent dipoles. For example, the hydrogen-bond.

2. Induced Dipole or Debye Forces.These are forces resulting from the interactions between a permanent dipole in one molecule and the induced dipole in a neighbouring molecule. These forces are usually very small.

Both polar interactions can be found between a solute and a polar stationary phase such as polyethylene glycol.

3. Dispersion, London or Non-polar Forces. These are forces arising from synchronised variations in the instantaneous dipoles of the two interaction species.These interactions can be found between all molecules, but are the only source of attraction between non-polar substances. In gas chromatography this interaction can be found between a solute and a non-polar stationary phase such as squalane.

4. Specific Interaction Forces. These are interactions resulting from specific chemical bonding or complex formation between solute and stationary phase.

These four types of interactions determine the solubility of the solutes and therefore the separation can be achieved. Their combined effects determine the sorption and desorption on the stationary phase, thereby slowing their motion in varying amounts. This is expressed by the distribution or partition coefficient, K :

$$K = \frac{[x]_S}{[x]_m} \qquad (2.1)$$

with $[x]_S$ and $[x]_m$ being respectively the concentrations of compound x in the solvent or stationary phase and in the mobile phase. A large value of K indicates that the component favors the stationary phase and moves slowly through the column because only a small

fraction will be in the carrier gas at any given time. For a small value of K the component favors the mobile phase and moves quickly through the column. Thus separation between products is possible if their distribution coefficients are dissimilar.

The time the solute needs to pass the column from injector to detector is the retention time, t_R (Figure 2.1) and is the characteristic for the solute and the liquid phase at a given temperature and flow rate. Several compounds can have identical or close retention times, but each compound has only one retention time. This retention time is normally not influenced by the presence of other compounds.

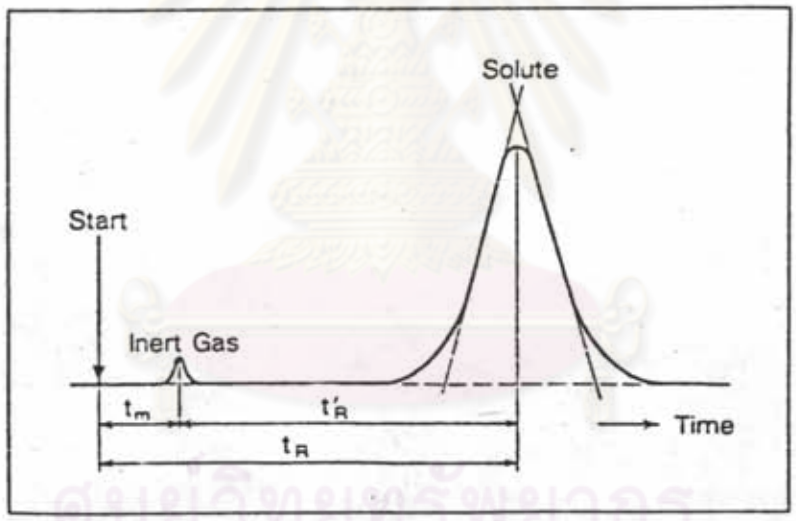

Figure 2.1 Relationship between absolute and adjusted retention time.

If the nature of the interaction between the solute molecules and the phase can be determined, the behavior of a particular solute in a given chromatographic system, such as the retention time can be predicted. Many articles that deal with the theory of solute distributions between two phases at the molecular level have been published, but they never provide an explicit equation that relates the distribution coefficient to phase

composition. The most common approach for studying the behavior of the solutes in the chromatographic system is to use the linear relationship between structure and/or physico-chemical properties of solutes and retention parameters, such as retention index (see hereafter). Many kinds of compounds have been studied by this approach. Only a few of them will be cited here:

- Bermejo et al. (7) studied the relationship between parameters related to electronic polarizability, like molar refraction, refractive index, Van der Waals volume and molar volume, and the retention indices of alkylbenzenes on squalane and PEG-20M at 100 °C.

- Morishita et al. (8) studied the relationship between the retention index and the molecular structure of chlorinated alkanes. The retention index can be predicted by considering the increment of retention index through chlorine atoms and the correction values for the hindrance effect through alkyl chains.

- Bermejo and Guillen (8) related the retention indices of saturated alcohols to their topological and physico-chemical parameters (like molar refraction, Van der Waals volume and connectivity index) on stationary phases of different polarity.

- Papp and co-workers (10) studied the correlations between molar refractions and retention indices of heterocyclic nitrogen-containing compounds

- Engewald et al. (11) studied the correlations between molecular structure and retention indices of alkylphenols in gas-liquid and gas-solid chromatography

- Saura-Calixto et al. (12) found the correlations between the Kovats retention indices of esters and molecular parameters of

orbitals, such as total energy, binding energy and charge density values of the molecules

More examples of studies on the relationship of retention indices and physico-chemical parameters can be found in the article entitled "Thirtieth Annivesary of the Retention Index According to Kovats in Gas-liquid Chromatography" (13). Retention parameters will be described in the next section.

2.2 Determination of Chromatographic Retention Index

After the introduction of gas chromatography in the early 1950s, it became apparent that a uniform system of data presentation was necessary to give the accuracy required for the comparison of data derived by different techniques and laboratories. In order to solve this problem, Kovats (5) proposed a system using the n-alkanes as a series of standards. He introduced his widely known formula (figure 2.2) :-

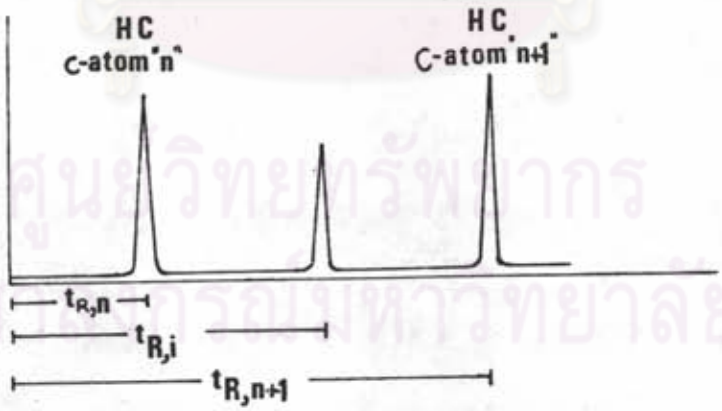

Fig. 2.2 Chromatogram for Kovats retention index calculation

$$I_i(T) = 100n + 100 \frac{[\log t'_{R,i} - \log t'_{R,n}]}{[\log t'_{R,n+1} - \log t'_{R,n}]} \qquad (2.2)$$

where $I_i(T)$ = Kovats retention index of a substance i chromatographed on a given column (stationary phase) at a definite temperature

$t'_{R,n}$ = adjusted retention time of a homologue standard with carbon number n which elutes before the substance i

$t'_{R,n+1}$ = adjusted retention time of a standard with carbon number n+1 which elutes after the substance i

$t'_{R,i}$ = adjusted retention time of a substance i

In gas chromatography the property directly measured is the real retention time, t_R (Figure 2.1). This value is a sum of two factors : the "dead time, t_M" which is dependent on the system flow rate and the "adjusted retention time, t'_R" which is independent of equipment used and could be related to solute structure. Therefore

$$t_R = t_M + t'_R \qquad (2.3)$$

According to equation (2.3), the adjusted retention time used in the Kovats retention index can be calculated by substracting the column dead time from the measured retention time. Thus, the dead time, t_M, must be known from a given column. James and Martin (14) designated the air peak which is unretained in the column as the dead time. But in the case of flame-ionization detectors,which are insensitive to air or to inert gases, McReynolds (15) has suggested methane as t_M marker.

Various mathematical methods of determination of adjusted retention time rely on the accepted linear relationship between ln $t'_{R,n}$ and retention index, I. Under isothermal conditions Kovats indices can be calculated by the following relationship :

$$\ln t'_{R,n} = bI + c \qquad (2.4)$$

$$\text{where} \quad I = 100n \qquad (2.5)$$

b and c are constants and the Kovats retention indices for n-alkanes are defined as 100 times the carbon number for each liquid phase at all temperatures.

The work of Kovats produced seven relationships concerning retention index and chemical structure. The first four propositions concerned the measurement of the retention index on a single phase while the remaining three propositions concerned the measurement of the retention index of a particular solute on different stationary phases. Those seven propositions (16) are

1. Within a homologue series the retention index of a higher homologue increases by 100 for each methylene unit introduced.

2. The differences in boiling points and retention indices of two isomers on a non-polar stationary phase such as pure or mixtures of n-alkanes are given by

$$dI = 5dT_B \qquad (2.6)$$

where dI and dT_B are the differences in the retention indices and the boiling points of the two isomers, respectively.

3. The retention index of asymmetrically substituted compounds could be calculated from the retention index values of the symmetrical counterparts.

4. Similar substitution in compounds of similar structures resulted in the same retention index increase.

5. The retention index of non-polar compounds (alkanes) remain almost constant for any type of stationary phase.

6. The retention index of any compound determined on various non-polar stationary phases are identical or very close to one another.

7. The difference in the retention index values, I, of a compound determined on a polar and on a non-polar stationary phase

characterizes the structure of the compound. Furthermore, values for retention indices may be calculated for a particular molecule by summation of the individual increments relating to the various adjacent zones within that molecule.

The introduction of the retention indices was then followed by a number of conceptually similar representations together with other retention indices using different calibration series for particular applications. For example :

- The generalized retention index, $I^{x}_{y}(i)$ was developed by Novak and Ruzickova in 1974 (16) and is more general than the Kovats retention index which allowed the retention index to be applied to other homologue series of standards. The generalized retention index can be written as :

$$I^{x}_{y}(i) = 100* \frac{[\log V^{x}_{N(i)} - \log V^{x}_{N(yn)}]}{[\log V^{x}_{N(yn+a)} - \log V^{x}_{N(yn)}]} + 100n + Ky \qquad (2.7)$$

with $V^{x}_{N(i)}$, $V^{x}_{N(yn)}$ and $V^{x}_{N(yn+a)}$ are the net retention values on a stationary phase x of the substance examined and of the reference compounds with N and (N+a) methylene units, respectively. Ky is a constant selected to avoid negative indices, with n-alkanes Ky is equal to zero.

- The retention indices of 221 halogenated aliphatic and alicyclic compounds have been reported on 1-bromoalkane scale by Yasuhara et al.(17).Elemental iodine was determined with an electron-capture detector using retention indices based on n-alkanes and alkyl iodides.

- Raymer et al. (18) determined the retention indices for a

number of ketones using linear programmed-temperature capillary gas chromatography and 2-ketones were used as standards for the index calculation.

2.2.1 Retention indices in programmed-temperature gas chromatography

Analytical applications of chromatography usually deal with the investigation of samples with wide boiling point ranges (most series of compounds are in this case including CFCs). In isothermal gas chromatography, adjusted retention times increase exponentially with carbon number (Figure 2.3). For these cases the isothermal condition is not suitable and temperature programming is preferred (Figure 2.4). Because for low temperature isothermal conditions, the compounds with low boiling points are well resolved and elute at a time that can easily be measured, but the compounds with high boiling points will elute too late and the peaks will be very broad. On the contrary with a high temperature isothermal condition, the high boiling point compounds yield measurable peaks at reasonable times, but the compounds with low boiling points are crowded together at the beginning as sharp, poorly resolved and difficult to measure peaks. In order to facilitate the analysis,the linear programmed-temperature technique was developed (Figure 2.4). By this technique, the temperature of the column will be raised during the analysis with a constant heating rate. When the elution starts at a relatively low temperature, most of the compounds are almost immobilized at the inlet of the column. The components with the lower solubilities in the stationary phase will move normally along the column. When the temperature of the column is raised until it reaches the value at which the vapor pressure is high enough, the compounds will elute. So each compound can elute at its optimum temperature for the heating and flow rate chosen. Under such conditions the programmed-temperature retention index (I_{PTGC} or I_{PT}) can be calculated with the equation of Van den Dool and Kratz (4) using the arithmatic

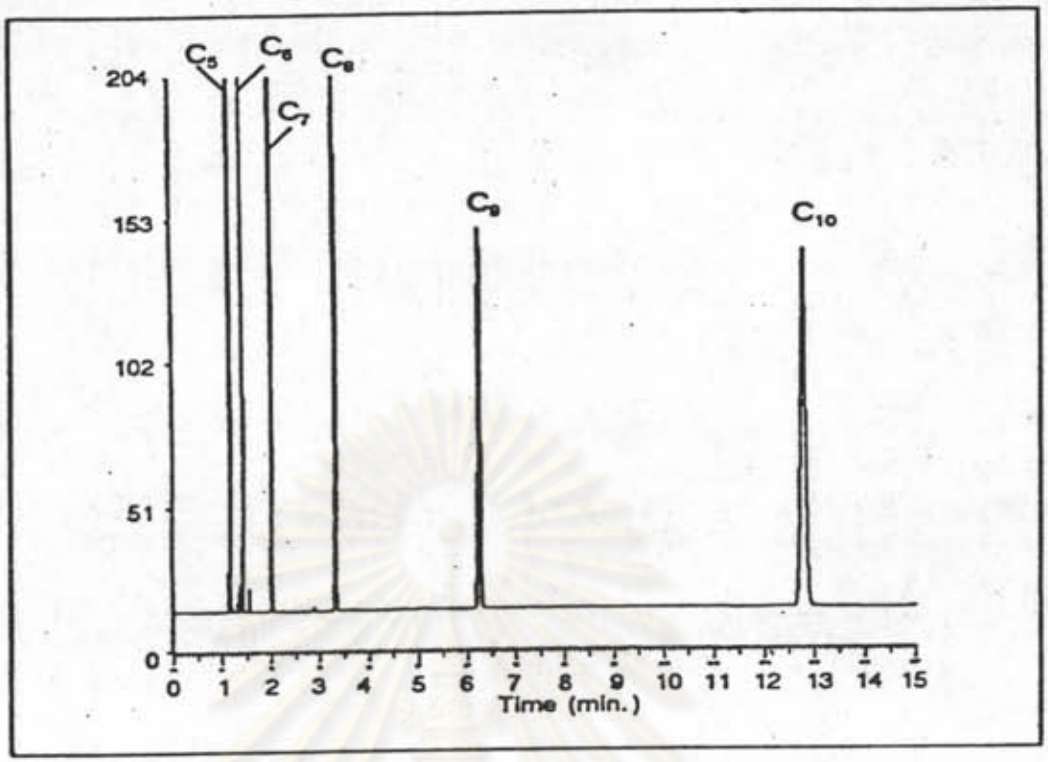

Figure 2.3 Isothermal reference chromatogram.

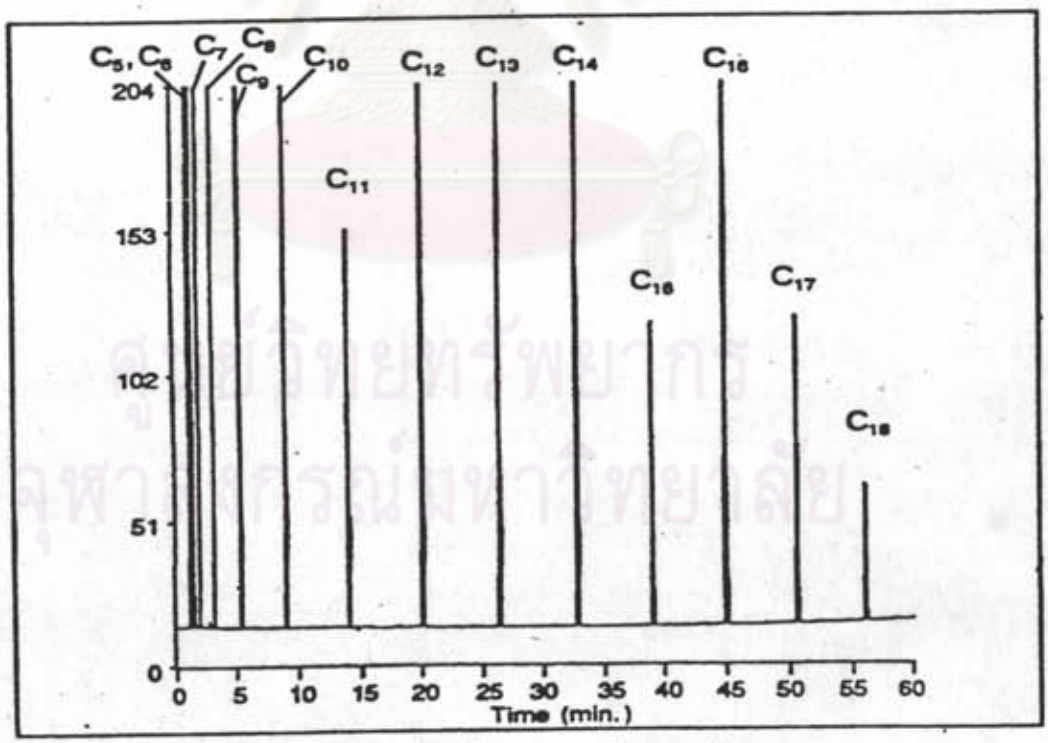

Figure 2.4 Programmed reference chromatogram.

relationship of retention temperature similar to the logarithmic relationship of retention time of Kovats. The equation is given by :

$$I_{PT} = 100n + 100 \frac{[T_{R,i} - T_{R,n}]}{[T_{R,n+1} - T_{R,n}]} \qquad (2.8)$$

and $$T_R = T_0 + rt_R \qquad (2.9)$$

where $T_{R,i}$, $T_{R,n}$, $T_{R,n+1}$ are the retention temperature of the substance i and of reference n-alkanes with carbon number n and n+1, respectively.

T_0 is the initial analysis temperature

r is the heating rate

and t_R is the retention time

This equation is restricted to a simple linear temperature increase without a constant temperature range or without changing the heating rate. The disadvantage of this programmed index is the requirement of an exact specification of operating condition including column dimensions, carrier gas type, flow rate and the temperature profile. The original Kovats isothermal index has the advantage of depending only on the stationary phase and temperature.

2.3 Parameters to Use in the Correlation

In gas chromatography the retention index depends on the chemical nature of the substance examined, the chemical nature of the stationary phase and the column temperature. In a given chromatographic system, the difference in retention indices for the substances depends solely on their nature. Therefore, throughout the history of chromatographic studies, there has been continued interest

in the effort to relate retention index with structural and/or physico-chemical parameters of solutes. Many of the widely used parameters (like boiling point, density, molar refraction, dipole moment, etc.) have continued to be important. But the greatest development concerns the relationship of retention index with topological descriptors (13). For example,

- Sabljic (19) calculated the retention indices of chlorinated alkanes by molecular topology.

- Gorgneyi et al. (20) estimated and predicted the retention indices of selected trans-diazenes correlated with connectivity indices, χ, from the 0^{th} up to the 4^{th} order.

- Bermejo and Guillen(21) found linear relationships between the retention indices of linear, branched, cyclic alkanes and their boiling points, Van der Waals volume, the first-order molecular connectivity and molar refraction.

- Buydens et al. (22,23) concluded that the gas chromatographic behavior of molecules from different chemical families can be satisfactorily described by means of a general topological parameters on non-polar stationary phases. For the more polar phases, a higher order term of the molecular connectivity and an electronic parameter are required. These results were obtained for molecules containing only one functional group.

- Bermejo and Guillen (24) studied the retention indices of aliphatic saturated esters and found only acceptable correlations between retention index and boiling point for low polarity phases. As the polarity increases, the addition of a second parameter (molar refraction, Van der Waals volume or molar volume) becomes necessary.

Based on these articles, the following physico-chemical and structural parameters have been chosen :

2.3.1 Molecular structural parameters

These parameters can be derived from the structural formula of the solutes. For example :

- the number of individual atoms in the structure, like the number of carbon (nC), fluorine (nF), chlorine (nCl) and hydrogen (nH) atoms.

- molecular bulk properties, like molecular weight (MW), structural volume (R) and surface area (Q) parameters, used in UNIQUAC or UNIFAC group-contribution methods, which are based on Van der Waals volume and surface area given by Bondi(25)[1].

- topological descriptors which convert molecular structures into numerical indices by means of the chemical graph theory. The most popular topological descriptor is the connectivity index (χ) in different orders (26)[2].

2.3.2 Physico-chemical property parameters

These parameters are related to the specific properties of a solute that play a role in chromatographic interactions. For example,

- boiling point (BP) of solutes affects directly the chromatographic separation.

1/ see the calculation of these parameters in Appendix A

2/ see the calculation of the connectivity index in Appendix B

- quantum chemical parameters : Many functional groups of compounds do not differ significantly in their "bulk properties" but they differ a lot in their electronic properties, that can better explain the chromatographic separation. The dipole moment (D) is one of these properties which is a measurable and/or calculable theoretical quantity characterizing the polarity of compounds. This can lead to the basic knowledge of polar interaction. For this work, the values of the dipole moment of some CFCs were obtained from the data bank of the Solvay Laboratory Centre.

2.3.3 Parameter describing the polarity of the stationary phase

As described above, the chemical nature of the stationary phase also affects the retention index. Rohrschneider (27) developed a concept for a simple interpretation of the interaction between solutes and the stationary phase. This is based on the interactions of a few selected reference substances (such as benzene, ethanol, methyl ethyl ketone, nitromethane and pyridine) and the specific constants of these examined substances (Rohrschneider's constants). This concept can be applied to the examination of the polarity of the stationary phase. The best known work in this field was carried out by McReynolds (15), based on the work of Rohrschneider. Benzene, n-butanol, 2-pentanone, nitropropane and pyridine are the five standard substances he used to examine the polarity of the stationary phases.The polarity scale is obtained from the sum of the differences in retention indices of these five standard substances tested on the given stationary phase and on squalane :

$$P_{MC} = \sum_{i=1}^{5} [I^{st,ph}(T) - I^{sq}(T)]_i \qquad (2.10)$$

where P_{MC} is the polarity of the examined stationary phase according to McReynolds

$I^{st,ph}(T)$ is the retention index obtained from testing the standard substance i on any stationary phase of a certain temperature.

$I^{sq}(T)$ is the retention index obtained from testing the standard substance i on squalane as stationary phase at the same temperature.

This polarity scale is used to develop one model for the retention index in function of the physico-chemical and/or topological parameters and the polarity of the stationary phase.