Quantitative in silico Analysis of Organic Modifier Effect on Retention in Reversed-Phase Liquid Chromatography

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Retention times in reversed-phase liquid chromatography were quantitatively analyzed in silico using alkanes as standard compounds, much like they have been used for Kovats indices in gas chromatography. The molecular interaction energy was calculated between an analyte and a model hydrophobic phase using a molecular mechanics program. The solvation energy was calculated between an analyte and a model solvent phase. The correlation coefficients between the log k values and the combined molecular interaction and solvation energy values were 0.97 (n = 18) for alkanes, alcohols, alkylbenzenes and polycyclic aromatic hydrocarbons.

Introduction

Modern chromatography began when Martin and Synge developed partition chromatography in 1941 (1). In 1958, Kovats proposed an index to predict the retention time of gas chromatography (2), leading to discussions about the mechanism of retention and studies to validate the prediction of retention time. With the separation of saccharides using a combination of ion-exchange resin and aqueous ethanol without ion-exchange mechanism by Samuelson et al. in 1965 (3), a variety of combination systems have been investigated for liquid chromatographic separation. In particular, the relationship has been studied between the structure and the chromatographic behavior of a variety of mono- and di-substituted benzenes on an anion-exchange resin with anhydrous ethanol used as eluent, and it was found that the retention depends on the type and position of the substituents and that the degree of adsorption is, at least partially, related to electron-withdrawing ability. These results suggested that molecular adsorption on the solid phase occurred by the formation of either hydrogen bonding interactions between the analyte and the anionic groups on the resin, or a charge transfer. These findings were confirmed in studies using a variety of packing materials, ion exchangers, bonded-phase silica gels and organic polymers for the chromatographic separation of organic acids and saccharides in organic solvent mixtures. These results also supported the importance of hydrogen bonding for analyte retention on the solid phase. Similar experiments have been conducted to analyze the chromatographic behavior of phthalate esters in aqueous solvent systems; however, these showed that elution order was related to solubility (hydrophobicity), leading, in 1974 (4), to a liquid chromatography classification scheme based on solubility factors, as proposed by Hanai (5). Furthermore, in 1979, Hanai et al. also demonstrated an optimization method of reversed-phase liquid chromatography using an octanol–water partition coefficient (6).

As a complementary approach to these technological advances, computational chemical analysis is a promising technique with the potential to analyze the mechanisms of molecular interaction between analytes and solid phases, especially given the feasibility of modeling three-dimensional structures of biological macromolecules, such as proteins. This technology can easily be used to study the retention mechanisms in chromatography for a variety of model phases. As a consequence, combining chromatography and computational chemistry offers new possibilities in developing a quantitative description of molecule interactions that are relevant to analytical separations.

When two molecules form a complex, the energy value, calculated in silico using a molecular mechanics program, decreases from the sum of the two molecules’ energy values due to the formation of a stable structure. When the energy value of a pair of molecules is equal to the sum of the two molecules’ energy values, then a complex has not formed. The molecular interaction (MI) energy value is calculated by subtracting the energy value of the complex from the sum of the energy values of the analyte and the model phase. The MI energy values of the final (optimized) structure (MIFS), hydrogen bonding (MIHB), electrostatic interaction (MIES), and van der Waals interaction (MIWV) are given by the following equations:

\[ \text{MIFS} = f_s(\text{analyte}) + f_s(\text{model phase}) - F_S(\text{analyte-model phase complex}) \]
\[ \text{MIHB} = bb(\text{analyte}) + bb(\text{model phase}) - HB(\text{analyte-model phase complex}) \]
\[ \text{MIES} = es(\text{analyte}) + es(\text{model phase}) - ES(\text{analyte-model phase complex}) \]
\[ \text{MIWV} = vw(\text{analyte}) + vw(\text{model phase}) - VW(\text{analyte-model phase complex}) \]

where \( f_s, bb, es \) and \( vw \) are the energy values of the final (optimized) structure, hydrogen bonding, electrostatic force and van der Waals force, respectively; \( F_S, HB, ES \) and \( VW \) are the energy values of the final structure, hydrogen bonding, electrostatic force and van der Waals force of the complex, respectively. The energy units are kcal/mol.

In gas chromatography using an inert gas as the mobile phase, the retention time was determined by a combination of MI and vaporization energy values. The MI (adsorption) was calculated as the MI energy between an analyte and a model...
phase. Vaporization was related to the molecular stability, which was calculated as the optimized energy of a pair of analytes. This approach requires a limited number of standard compounds for the column calibration. These standard compounds were alkanes, benzene, naphthalene and anthracene. The alkanes were used like they are used for Kovats indices. The retention times of various compounds [alkanes, alkenes, alkanols, fatty acid methylesters and polycyclic aromatic hydrocarbons (PAHs)] on both methylsilicone and polyethylene glycol phases were successfully predicted with high precision based on the combination of MI energy and the molecular properties of the analytes. The correlation coefficients were 0.99 for methylsilicone (CPSil5, DB1) and 0.98 for polyethylene glycol phases (CPWAX, DBWAX), respectively (7).

Consistent with the concept that like dissolves like, the retention mechanisms of normal- and reversed-phase liquid chromatography are the same. The retention mechanism depends on the relation between the analyte and the packing material surface. Predicting the retention time in reversed-phase liquid chromatography is relatively simple due to the limited selection of organic modifiers. A direct in silico calculation of MI energy using model phases was developed instead of calculating enthalpy due to the capabilities of the computer. Measured enthalpies are not linearly related to log k values of a variety of compounds. Enthalpies are only related to the log k values of similar compounds (8). In addition, a new pKa prediction method was developed in which Hammett sigma constants were replaced with atomic partial charges. By combining these methods, retention in both reversed-phase (9) and ion-exchange (10) liquid chromatography could be predicted when similar compounds were analyzed because of their similar solvation mechanisms. In normal-phase liquid chromatography, the adsorption strength can be quantitatively analyzed in silico, but the desorption strength cannot be quantitatively analyzed. Although a variety of organic solvents can be used as eluent components, the strength of the solvent does not directly relate to the elution order. The total solubility parameter does not relate to solvent strength in normal-phase liquid chromatography (11, 12), and at present, no solubility prediction method exists. The addition of acids and bases to the eluent modifies the silica gel surface and affects the ionization of the analyte. Therefore, quantitative analysis of retention in normal-phase liquid chromatography, other than for enantiomer separation, remains problematic. However, the retention of triterpenes in normal-phase liquid chromatography using silica gel is not simply related to the direct MI energy values and is affected by the desorption power of the organic solvent. The correlation between the log k and MI energy values has been improved by including the solvent effects obtained from the analyte-solvent complexes (13).

The correlation coefficient between the individual MI energy values and logarithmic capacity ratios indicates the contribution of the primary molecular interaction factor to retention in chromatography. MIHB, MIES and MIVW should represent the primary interaction factors in normal-phase, ion-exchange and reversed-phase liquid chromatography, respectively. Steric hindrance affects the molecular interactions in enantiomeric separation (14).

In reversed-phase liquid chromatography, the retention times (logarithm of capacity ratios) are related to MI energy values of similar compounds because of the lack of a solubility prediction system. However, the precision can be improved when solvent effects are included. Therefore, a similar approach to that used in gas chromatography was studied by using a variety of simple analytes in reversed-phase liquid chromatography, although alkanes and alkanols are not generally used for column calibration because they show poorer detectability and solubility than common analytes. The retention times of the 18 compounds listed in Tables I and II were measured using a variety of hydrophobic columns in aqueous acetonitrile to study the feasibility of this approach. Acetonitrile is the common organic modifier in reversed-phase liquid chromatography, especially for theoretically discussing the relation between the retention times and the log P (octanol–water partition coefficient) values of the analyte. The log k values were related to the MI energy values using a model hydrophobic phase, and the solvent effect was studied using model solvent phases.

### Table I
Molecular Properties of Analytes*

<table>
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<th>Number</th>
<th>Chemicals</th>
<th>log k</th>
<th>fs</th>
<th>hb</th>
<th>es</th>
<th>vw</th>
<th>FS</th>
<th>HB</th>
<th>ES</th>
<th>VW</th>
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<th>HB*</th>
<th>ES*</th>
<th>VW*</th>
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<td>17</td>
<td>Octane</td>
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**Alkyl phase**

<table>
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<tr>
<th>Number</th>
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<th>fs</th>
<th>hb</th>
<th>es</th>
<th>vw</th>
<th>FS</th>
<th>HB</th>
<th>ES</th>
<th>VW</th>
<th>FS*</th>
<th>HB*</th>
<th>ES*</th>
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<tr>
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<td>-8.952</td>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

Note: log k measured on ODS silica gel in 80%aq.ACN at 40°C.

*: results with acetonitrile phase.
**Experimental**

A Dell Latitude C840 computer equipped with a 2 GHz processor and 1.024 MB of memory was used for calculation. The molecular properties of the analytes and model phases and the molecular interactions were calculated by molecular mechanics (MM2) using version 5 of the CAChe program (Fujitsu, Tokyo). Standard parameters, including bond stretch, bond angle, dihedral angle, improper torsion, van der Waals force, hydrogen bond and electrostatic energy (MM2 bond dipoles) were used. The van der Waals force cut-off distance was 9 Å. The energy units were kcal/mol. The Cricket-Graph program from Computer Associates (San Diego, CA) and the Project Reader of the CAChe program were used for data analysis. The retention times of high-grade standard compounds from Wako Pure Chemicals (Tokyo, Japan) were measured at 40°C in reversed-phase liquid chromatography using a detector reflective index from Waters. Water was MilliQ grade and acetonitrile was HPLC grade from Wako Pure Chemicals. The void volume marker was fructose.

**Results and Discussion**

The analytes listed in Tables I and II are flat, so steric hindrance can be neglected when studying their molecular interactions; therefore, a simple and homogeneous model was used, instead of a blush-type bonded phase silica gel model whose blush types and the density were designed to reduce the steric hindrance effects of analytes (15). A model butyl phase with an adsorbed benzene is shown in Figure 1. The calculated MI energy values (summarized in Table I) were correlated with the log k values of these compounds. For example, MIFS = $a \times \log k + b$ the slope $a$ and constant $b$ of all groups should be the same for an ideal system to predict retention time. The following relations were obtained for log k values, measured on the octadecyl bonded silica gel column in 80% aqueous acetonitrile (aqACN) at 40°C:

$$\text{MIFS of alkanes} = 8.191 \times \log k + 4.854, n = 5, r = 0.999$$

$$\text{MIFS of alkanols} = 9.436 \times \log k + 12.808, n = 4, r = 0.997$$

$$\text{MIFS of alkylbenzenes} = 8.774 \times \log k + 7.573, n = 5, r = 0.982$$

$$\text{MIFS of PAHs} = 8.236 \times \log k + 6.978, n = 4, r = 0.985$$

The coefficients of the individual groups were close to 1. The order of the MIFS values was alkanes > alkylbenzenes > PAHs > alkanols. If the relation between alkane log k values and their MIFS values is considered to be the standard for the in silico analysis and the retention is based on
hydrophobic interaction, the retention of other types of compounds should follow the relation of the alkanes. The retention strength was as follows: alkanes > PAHs > alkylbenzenes > alkanes, based on the MI energy values. The van der Waals energy values support the strength of the hydrophobic retentions. If their solvation mechanisms are the same, all compounds should demonstrate the same linear relation between their MI energy values and log \( k \) values measured in liquid chromatography.

The MIFS differences (dMIFS) of alkanols, alkylbenzenes and PAHs from alkanes have to be attributable to differences in their solubilities. The prediction of solubility is impossible at this stage. Acetonitrile molecules form complexes between cyano groups but not methyl groups, as shown in Figure 2. The complex formed (Figure 2B) demonstrated a very low electrostatic (ES) energy drop. Methyl groups were repulsed (Figure 2C) and did not form a complex. The MI energy values of the analyte and solvent complexes were calculated using a model solvent layer, shown in Figure 3, where a benzene interacted with the model phase. The MI energy values are summarized in Table 1. The MIFS values were related to MIES* values between analytes and the model solvent phase. MIES* is MIES values calculated with ACN phase. The relation is given by the following equation:

\[
\text{dMIFS} = 1.498x^2 - 6.598x - 0.954, \quad r = 0.951, \quad x : \text{MIES}^*
\]

The addition of dMIFS values calculated from MIES* to MIFS improved the correlation with the log \( k \) values. The correlation coefficient was 0.973 \((n = 18)\). Although solubility prediction is impossible, the MI energy values with a model solvent phase improved the precision of the relation between the log \( k \) values and MI energy values. The correlation coefficients in 90 and 70% aqueous acetonitrile were 0.967 \((n = 18)\) and 0.971 \((n = 17)\), respectively. These results demonstrated the possibility of a quantitative analysis of retention times in reversed-phase liquid chromatography. In addition, a cyano-group-bonded silicone phase was constructed, and MI energy values were calculated. The sum of MIFS and MIES (cyano-phase) was correlated to log \( k \) values with a correlation coefficient of 0.952 \((n = 18)\). The details are not listed because the ACN phase was considered to be a better model phase than the cyano-group-bonded phase.

Furthermore, the feasibility of the preceding approach was examined for log \( k \) values measured using propyl- and octyl-bonded silica gel columns, an octadecyl-bonded polyvinyl alcohol copolymer gel (ODP) column and a Capcellpack ODS silica gel column. The primary interactions were based on van der Waals force; therefore, the van der Waals energy values were used to determine the MIs between these analytes and the model phases.

The relations between the log \( k \) values and MIW (also MIFS) of alkanes, alkanols, alkylbenzenes and PAHs from alkanes have to be attributable to differences in their solubilities. The prediction of solubility is impossible at this stage. Acetonitrile molecules form complexes between cyano groups but not methyl groups, as shown in Figure 2. The complex formed (Figure 2B) demonstrated a very low electrostatic (ES) energy drop. Methyl groups were repulsed (Figure 2C) and did not form a complex. The MI energy values of the analyte and solvent complexes were calculated using a model solvent layer, shown in Figure 3, where a benzene interacted with the model phase. The MI energy values are summarized in Table 1. The MIFS values were related to MIES* values...
For the octadecyl bonded polyvinylalcohol copolymer column:

\[ \text{MIVW} + \text{dMIVW} = 8.860 \log k + 7.072, \quad r = 0.968, \]
\[ n = 18, \text{in} 90\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 7.683 \log k + 5.284, \quad r = 0.973, \]
\[ n = 18, \text{in} 80\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 7.160 \log k + 3.539, \quad r = 0.972, \]
\[ n = 18, \text{in} 70\% \text{aq.ACN} \]

For the octyl bonded silica gel column:

\[ \text{MIVW} + \text{dMIVW} = 13.987 \log k + 12.277, \quad r = 0.951, \]
\[ n = 18, \text{in} 90\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 11.606 \log k + 8.219, \quad r = 0.963, \]
\[ n = 18, \text{in} 80\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 10.020 \log k + 5.350, \quad r = 0.970, \]
\[ n = 18, \text{in} 70\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 8.719 \log k + 3.239, \quad r = 0.975, \]
\[ n = 17, \text{in} 60\% \text{aq.ACN} \]

For the propyl bonded silica gel column:

\[ \text{MIVW} + \text{dMIVW} = 16.471 \log k + 12.369, \quad r = 0.968, \]
\[ n = 18, \text{in} 80\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 13.373 \log k + 7.753, \quad r = 0.969, \]
\[ n = 18, \text{in} 70\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 10.888 \log k + 5.038, \quad r = 0.975, \]
\[ n = 18, \text{in} 60\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 8.876 \log k + 3.232, \quad r = 0.979, \]
\[ n = 17, \text{in} 50\% \text{aq.ACN} \]

For the octadecyl bonded polyvinylalcohol copolymer column:

\[ \text{MIVW} + \text{dMIVW} = 11.513 \log k + 11.710, \quad r = 0.974, \]
\[ n = 18, \text{in} 90\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 9.404 \log k + 6.951, \quad r = 0.989, \]
\[ n = 18, \text{in} 80\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 8.350 \log k + 4.959, \quad r = 0.989, \]
\[ n = 17, \text{in} 70\% \text{aq.ACN} \]

For the octadecyl Capcellpak column (silica gel matrix):

\[ \text{MIVW} + \text{dMIVW} = 8.237 \log k + 7.999, \quad r = 0.945, \]
\[ n = 18, \text{in} 90\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 7.161 \log k + 6.001, \quad r = 0.952, \]
\[ n = 18, \text{in} 80\% \text{aq.ACN} \]
\[ \text{MIVW} + \text{dMIVW} = 6.736 \log k + 4.463, \quad r = 0.955, \]
\[ n = 17, \text{in} 70\% \text{aq.ACN} \]

These results support that the \textit{in silico} approach for the quantitative analysis of retention times in reversed-phase liquid chromatography is feasible with high precision, if the ideal model phase and solubility prediction methods are available. The liquid chromatography retention time is a combination of adsorption on a packing material surface and solvation in a mobile phase. The precision for gas chromatography is high because vaporization energy can be calculated \textit{in silico} from the analyte properties. The development of a solubility prediction method is required to improve the precision of predicted retention times in liquid chromatography.

**Conclusion**

A new method for the quantitative analysis of retention times measured in reversed-phase liquid chromatography was developed. The feasibility was studied using simple compounds like those used in gas chromatography. Alkanes were used as standard compounds, much like Kovats retention indices. The primary molecular interaction force between the hydrophobic surface and analytes is hydrophobicity, which is calculated as van der Waals energy. The desorption is attributable to solvation of the analytes by an organic modifier. The solvation was analyzed as MI between a model solvent phase and the analytes. The latter interaction was related to an ES energy value from log \( k \) values measured in aq.ACN. The correlation coefficients between the energy values calculated \textit{in silico} and log \( k \) were approximately 0.97. The log \( k \) values in reversed-phase liquid chromatography can be quantitatively calculated if an ideal model phase and a solubility prediction method are used. These results show that this new approach to study the quantitative analysis of chromatographic retention times in reversed-phase liquid chromatography is theoretically possible. The precision should be improved when solubility can be predicted.

**References**

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