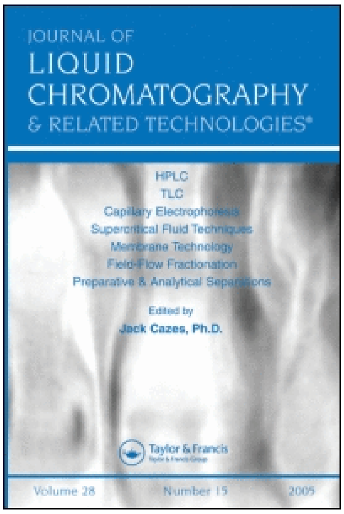

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Quantitative *In Silico* Analysis of the Specificity of a Graphitic Carbon Column

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Abstract: Molecular interaction energy (MI) values calculated by molecular mechanics (MM2) using a model graphitic carbon phase were used to study the specificity of a graphitic carbon column. The analytes were positional isomers of aromatic polar compounds. The log k values were measured using buffered aqueous eluents. The MI values did not correlate with the log k values measured on a graphitic carbon synthesized using silica matrix. The log k values correlated well with the hydrogen bonding energy values calculated using the model silica and carbon phases. The log k values correlated well with MI values calculated using the model silica phase. The graphitic carbon column demonstrated properties of both graphitic carbon and silica gel.

Keywords: Aromatic compounds, Computational chemical analysis, Graphitic carbon, Liquid chromatograph, Molecular interaction

INTRODUCTION

Since the publication of a review about graphitic carbon columns and a computational chemical analysis of the retention mechanism,[1] a number of studies have analyzed the retention mechanism of graphitic carbon phases in normal phase liquid chromatography.[2] Graphitic carbon is mainly used for solid phase extraction, and not for chromatographic columns. Graphitic carbon columns, however, have been used to determine

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trace levels of nitroaromatic explosives and related compounds in water[3] and motor oil[4] using LC-API-MS, to screen underivatized oligosaccharides extracted from Triticum aestivum stems[5] using LC-ECMS, to analyze the effects of a water deficit on carbohydrates in Lupinus albus stems,[6] to retain acrylamide in liquid chromatography-MS/MS,[7] and to fractionate polycyclic aromatic compounds in sediment extracts.[8] In addition, the chromatographic behavior of aromatic compounds measured using different types of graphitic carbon columns in normal phase liquid chromatography has been analyzed using a computational chemical approach with a model graphitic carbon column.[2] The correlation between the calculated molecular interaction energy (MI) values and log *k*s values measured on a graphitic carbon synthesized from 100% organic materials demonstrated the usefulness of this approach ($r = 0.961$, $n = 13$). The correlation coefficient was poor (0.558, $n = 17$), however, for a graphitic carbon synthesized with a silica gel matrix. The latter log *k* values were related well with the MI values calculated using a model silica gel phase ($r = 0.807$, $n = 17$). In addition the log *k* values were better correlated with the contribution of hydrogen bonding energy values ($r = 0.856$, $n = 17$). This is a typical result obtained in normal phase liquid chromatography using silica gel. The results indicate that the silica matrix was not completely washed from the graphitic carbon column, and provide useful clues to determine the retention mechanism of such graphitic carbon columns.

The present study was designed as an explanation to some chromatographers who do not justify the above approach. Liquid chromatographic data measured in reversed phase liquid chromatography[9] were analyzed using a similar previously applied method.[2]

EXPERIMENTAL

Liquid Chromatography

The column used was Hypercarb (ThermoHypersil), and the eluent was aqueous buffer containing acetonitrile.[9] The capacity ratios are indicated in the Figures.

Computational Chemical Analysis

A Dell model Latitude C840 computer equipped with a 2-GHz processor and 1024-MB memory were used. The octanol-water partition coefficients, Vlog P values, were calculated using the program Vlog P 1.0 (Health Design Inc, Rochester, NY). The molecular properties of analytes and model phases and molecular interactions were calculated by molecular mechanics (MM2) using version 5 of the CAChe^{TM} program (Fujitsu, Tokyo, Japan). 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 9Å. The energy unit was kcal/mol (1 kj/mol = 4.18 kcal/mol). The Cricket-Graph^{TM} program from Computer Associates (San Diego, CA) and Project Reader of the CAChe^{TM} program were used for data analysis.

RESULTS AND DISCUSSION

The analytes used and their properties are listed in Table 1. The chromatographic behavior was analyzed to determine whether or not the retention mechanism was based on reversed phase liquid chromatography. These compounds were classified into two groups according to their acidity and basicity. The octanol-water partition coefficients of the analytes, the log P values, were related to log k values measured at pH 2 and 10. There were only 6 compounds. The correlation was better for basic compounds ($r = 0.825$, $n = 6$). Silanol groups existed in their sodium salt form and did not contribute as a hydrogen bonding partner at the high pH, but did affect the retention of the acidic compounds at the low pH. The correlation coefficient was 0.352 ($n = 6$) for acidic compounds at pH 2.

The MI energy values were calculated using the model carbon phase shown in Figure 1, with *p*-anisidine on the model phase. The MI value was obtained by subtracting the energy value of the complex from the sum of energy values of the pair compounds as given in the following equations. The calculated energy values are summarized in Table 1.

$$\begin{aligned}
\mathrm{MIFS} &= \mathrm{fs(analyte)} + \mathrm{fs(model\ phase)} - \mathrm{FS(analyte\text{-}model\ phase\ complex)},\\
\mathrm{MIHB} &= \mathrm{hb(analyte)} + \mathrm{hb(model\ phase)} - \mathrm{HB(analyte\text{-}model\ phase\ complex)},\\
\mathrm{MIES} &= \mathrm{es(analyte)} + \mathrm{es(model\ phase)} - \mathrm{ES(analyte\text{-}model\ phase\ complex)},\\
\mathrm{MIVW} &= \mathrm{vw(analyte)} + \mathrm{vw(model\ phase)} - \mathrm{VW(analyte\text{-}model\ phase\ complex)},
\end{aligned}$$

where fs: energy value of final (optimized) structure; hb: energy value of hydrogen bonding; es: energy value of electrostatic force; and vw: energy value of van der Waals force. FS, HB, ES, and VW: energy value of final

Table 1. Molecular properties of analytes and phases

Chemical	log P	FS	HB	ES	VW
o-Aminobenzoic acid	0.979	−18.0208	−5.74	−11.260	6.452
m-Aminobenzoic acid	1.053	−15.8272	−5.526	−7.258	5.435
p-Aminobenzoic acid	0.995	−15.8508	−5.514	−7.265	5.384
o-Anisic acid	1.621	−15.2144	−4.250	−7.200	6.069
m-Anisic acid	1.708	−16.2962	−4.186	−7.375	5.303
p-Anisic acid	1.670	−16.3114	−4.182	−7.309	5.207
o-Anisidine	1.275	−4.3759	−1.783	0.580	3.729
m-Anisidine	1.391	−4.5510	−1.340	−0.010	3.718
p-Anisidine	1.351	−4.5652	−1.330	−0.008	3.695
o-Phenetidine	1.764	−3.8094	−1.787	0.580	4.229
m-Phenetidine	1.892	−3.9153	−1.340	−0.010	4.289
p-Phenetidine	1.864	−3.9271	−1.330	−0.080	4.270
o-Toluic acid	0.979	−14.6095	−4.184	−6.088	6.476
m-Toluic acid	1.990	−18.4149	−4.180	−7.371	5.029
p-Toluic acid	2.020	−18.4331	−4.179	−7.351	4.971
Silanol phase	–	−842.9634	−34.699	−699.650	−249.575
Carbon phase	–	38.9017	0.000	0.000	65.672
		FS*	HB*	ES*	VW*
o-Aminobenzoic acid		−881.8233	−57.448	−712.530	−247.004
m-Aminobenzoic acid		−887.3857	−61.144	−710.219	−249.389
p-Aminobenzoic acid		−884.0685	−63.337	−709.647	−245.441
o-Anisic acid		−880.2007	−56.102	−708.568	−248.902
m-Anisic acid		−886.5956	−60.135	−709.679	−251.425
p-Anisic acid		−889.3311	−60.788	−710.279	−250.815
o-Anisidine		−866.7141	−49.563	−698.803	−252.456
m-Anisidine		−865.0760	−48.068	−699.812	−251.824
p-Anisidine		−864.4962	−46.271	−700.104	−252.685
o-Phenetidine		−869.4632	−51.005	−699.663	−252.966
m-Phenetidine		−868.0431	−49.567	−699.975	−252.754
p-Phenetidine		−865.4588	−46.325	−700.131	−253.641
o-Toluic acid		−883.2097	−57.534	−706.953	−248.830
m-Toluic acid		−889.9217	−59.044	−710.164	−250.663
p-Toluic acid		−889.6820	−58.758	−710.165	−250.938
		FS*2	HB*2	ES*2	VW*2
o-Aminobenzoic acid		3.9764	−16.312	−11.135	64.200
m-Aminobenzoic acid		6.7896	−13.697	−7.185	61.433
p-Aminobenzoic acid		7.0117	−12.840	−7.190	60.945
o-Anisic acid		6.9083	−10.888	−7.119	60.539
m-Anisic acid		6.0108	−10.550	−7.297	59.597
p-Anisic acid		5.7458	−10.465	−7.230	59.121
o-Anisidine		21.0089	−4.823	0.580	58.778

(*Continued*)

Table 1. Continued

Chemical	log P	FS	HB	ES	VW
m-Anisidine		20.7514	−4.255	−0.010	58.536
p-Anisidine		20.8385	−3.914	−0.008	58.289
o-Phenetidine		20.0854	−4.432	0.580	57.397
m-Phenetidine		19.8381	−4.339	−0.010	57.642
p-Phenetidine		19.9858	−3.961	−0.008	57.405
o-Toluic acid		8.4151	−10.880	−5.990	61.791
m-Toluic acid		4.1319	−10.814	−7.286	59.673
p-Toluic acid		4.2232	−10.543	−7.268	59.497

structure, hydrogen bonding, electrostatic force and van der Waals force of the complexes.

The correlation coefficient between the calculated individual MI and logarithmic capacity ratio indicated the contribution of each individual factor to the retention. MIVW was the main energy value for the interaction in reversed phase liquid chromatography,[10] and MIES was the main energy value for the retention in ion exchange liquid chromatography.[11] Steric hindrance affected the molecular interaction in enantiomeric separation.[12]

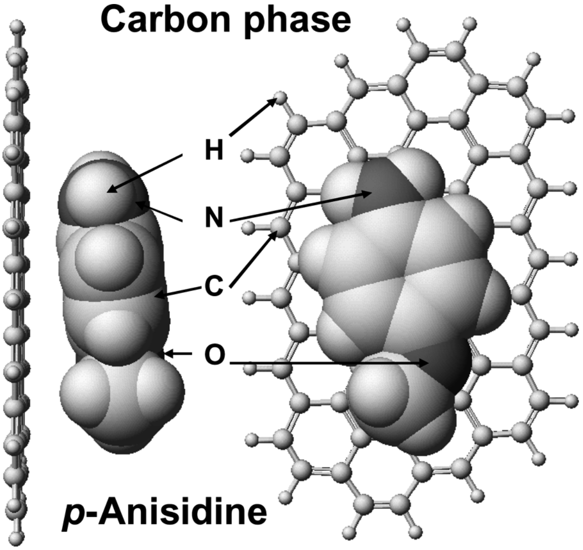

Figure 1. Adsorption of *p*-anisidine on a model carbon phase. Black balls oxygen, dark gray balls nitrogen, large white balls carbon, small white balls hydrogen. Atomic size of the carbon phase is 20%.

MI energy values correlated with reference log *k* values, and the results are given in the following equations.

At pH 10:

$\mathrm{MIFS} = 2.854\,(\log k) + 12.303, \mathrm{r} = 0.902, \mathrm{n} = 6,$

$\mathrm{MIHB} = 0.048\,(\log k) + 2.769, \mathrm{r} = 0.061, \mathrm{n} = 6,$

MIES: No correlation was calculated due to identical MIES values.

$\mathrm{MIVW} = 2.796\,(\log k) + 9.703, \mathrm{r} = 0.822, \mathrm{n} = 6.$

At pH 2:

$\mathrm{MIFS} = 0.328\,(\log k) + 16.078, \mathrm{r} = 0.140, \mathrm{n} = 6,$

$\mathrm{MIHB} = -0.969\,(\log k) + 7.595, \mathrm{r} = 0.844, \mathrm{n} = 6,$

$\mathrm{MIES} = 0.018\,(\log k) - 0.104, \mathrm{r} = 0.372, \mathrm{n} = 6,$

$\mathrm{MIVW} = 1.588\,(\log k) + 9.346, \mathrm{r} = 0.534, \mathrm{n} = 6.$

Why were these compounds retained so strongly on the graphitic carbon column? According to one study,[13] the graphitic carbon was synthesized by washing the silica from the graphitic carbon using potassium hydroxide. Using this method, a high porosity HPLC silica gel was impregnated with a phenol-formaldehyde resin. The resin was carbonized at 2000 to 2800°C in nitrogen or argon, and the silica particles were dissolved using alkali. This process allows for trace amounts of silica and metals in silica and potassium hydroxide in the graphitic carbon, which likely occurred based on the relatively high correlation coefficient of the hydrogen bonding energy values with log *k*.

The probability of silanol affecting the graphitic carbon was studied using a model silanol phase that was used to study retention of silica gels.[4] The MI energy values were calculated using the model silanol phase shown in Figure 2, with *p*-anisidine adsorbed on the model silanol phase. The correlation coefficients are given in the following equations:

At pH 10:

$\mathrm{MIFS} = 7.695\,(\log k) + 14.023, \mathrm{r} = 0.922, \mathrm{n} = 6,$

$\mathrm{MIHB} = 4.718\,(\log k) + 8.993, \mathrm{r} = 0.706, \mathrm{n} = 6,$

$\mathrm{MIES} = 0.482\,(\log \mathrm{k}) - 0.051, \mathrm{r} = 0.408, \mathrm{n} = 6,$

$\mathrm{MIVW} = 1.909\,(\log k) + 5.803, \mathrm{r} = 0.595, \mathrm{n} = 6.$

At pH 2:

$\mathrm{MIFS} = 16.926\,(\log k) + 7.799, \mathrm{r} = 0.929, \mathrm{n} = 6,$

$\mathrm{MIHB} = 10.791\,(\log k) + 7.606, \mathrm{r} = 0.969, \mathrm{n} = 6,$

$\mathrm{MIES} = 4.310\,(\log k) - 2.348, \mathrm{r} = 0.768, \mathrm{n} = 6,$

$\mathrm{MIVW} = 3.319\,(\log k) + 2.435, \mathrm{r} = 0.846, \mathrm{n} = 6.$

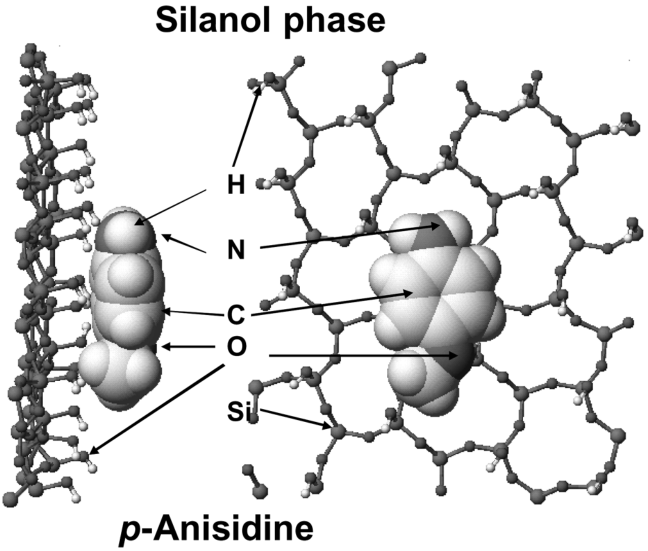

Figure 2. Adsorption of *p*-anisidine on a model silanol phase. Black balls oxygen, dark gray balls nitrogen, large white balls carbon, small white balls hydrogen, small dark balls silicon. Atomic size of the silanol phase is 20%.

There was a strong correlation between log *k* and the hydrogen bonding energy values. The result was typical of normal phase liquid chromatography using silica gels, even when the log *k* values were measured using a graphitic carbon column.

These results support the speculation regarding the silanol activity of one of the graphitic carbon columns, suggesting that the premier matrix silica gel was not completely washed out and silanol activity persisted.

$$\mathrm{MIFS} = 0.183\ \log P + 15.305, \mathrm{r} = 0.057, \mathrm{n} = 15,$$

$$\mathrm{MIHB} = -2.656\ \log P + 9.448, \mathrm{r} = 0.407, \mathrm{n} = 15,$$

$$\mathrm{MIES} = 0.032\ \log P - 0.099, \mathrm{r} = 0.265, \mathrm{n} = 15,$$

$$\mathrm{MIVW} = 2.387\ \log P + 7.380, \mathrm{r} = 0.765, \mathrm{n} = 15.$$

Log *P* values do not relate to MI energy values. They relate weakly with van der Waals energy values, because molecular size is a very important factor for estimating log *P* values.

A semi-empirical molecular statistical theory of adsorption based on an atom-atom approximation for the potential function of the intermolecular adsorbate-adsorbent interaction was studied to obtain Henry's constant based on gas chromatography data using graphitic thermal carbon, even though porous adsorbents are inhomogeneous. A simple quantitative correlation of the thermodynamic characteristics of adsorption was applied in liquid chromatography.[14] Based on several experimental measurements of the isotherms for one of the test solutes followed by non-linear model fitting, the graphite surface was considered to be homogeneous and to have only one type of adsorption site.[15] Hence, an MM2 calculation was performed to obtain Henry's constant using a flat model.[15] The latter method is straightforward for analyzing a variety of chromatographic data, but it is not straightforward for synthesizing model phases other than a graphitic carbon phase. Chromatographic phases are synthesized homogeneously but the steric structure is inhomogeneous. The original computer software is used for the conformational analysis of proteins; therefore, such an approach can be applied to the analysis of chromatographic retention if a prospective model is designed. The MI energy values calculated using MM2 support a set of data measured on a graphitic carbon column synthesized using 100% organic materials, but not the retention data measured on a graphitic carbon column synthesized using a silica matrix. The chromatographic behavior of these graphitic carbon columns differs, as clearly demonstrated by the above computational chemical approach.

CONCLUSION

The chromatographic behavior of aromatic compounds measured using a model graphitic carbon column in reversed phase liquid chromatography was analyzed using a computational chemical approach. For a graphitic carbon column synthesized with a silica gel matrix, the correlation coefficient was low ($r = 0.140$, $n = 6$) at pH 2. The log *k* values correlated well with the hydrogen bonding energy values, ($r = 0.844$, $n = 6$). The log *k*s values were well correlated with the MI energy values calculated using a model silica gel phase, ($r = 0.926$, $n = 6$). This result is typical among those obtained in normal phase liquid chromatography using a silica gel. The log *k* values of basic compounds at pH 10, where silanols should be in their sodium salt form, correlated well with the MI ($r = 0.902$, $n = 6$). The results indicate that the silica matrix was not completely washed from the graphitic carbon column.

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