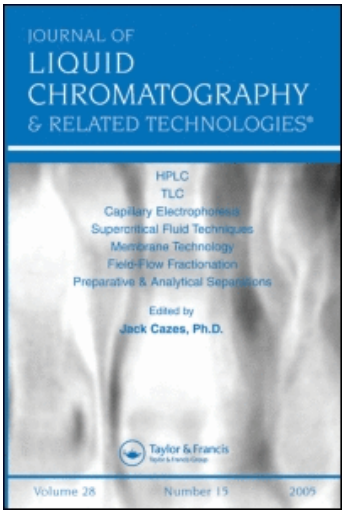

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QUANTITATIVE *IN SILICO* ANALYSIS OF RETENTION IN NORMAL PHASE LIQUID CHROMATOGRAPHY

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□ *The liquid chromatographic retention in normal phase mode was quantitatively analyzed in silico using chromatographic retention of sleeping medicines measured in acidic, basic, and neutral organic solvent mixtures. Hydrogen bonding is the major contribution in the retention that was supported by a high correlation coefficient between log k and hydrogen bonding energy values calculated using a molecular mechanics program.*

Keywords computational chemical analysis, liquid chromatography, molecular interaction, silica gel, thin-layer liquid chromatography

INTRODUCTION

Qualitative analysis of retention in chromatography has been studied from the beginning of chromatographic separation. The factors in chromatographic retention are related with solubility factors.[1] If we can reconstruct solubility factors obtained quantitatively, we may quantitatively analyze the chromatographic retention. On the other hand, computational chemical analysis methods provide the molecular interaction energy as the sum of mainly van der Waals, hydrogen bonding, and electrostatic energy values. The van der Waals energy is related to molecular size, hence, the contact surface area between an analyte and an adsorbent contributes to a part of the molecular interaction energy value. When hydrogen bonding exists between an analyte and an adsorbent, the hydrogen bonding energy value contributes to a part of the molecular interaction energy value. When ion–ion interaction exists, the electrostatic energy value contributes to a part of molecular interaction energy value.

The typical example of the van der Waals energy contribution was observed in the analysis of chromatographic retention using a graphitic

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carbon column. The graphitic carbon column was used in both normal and reversed phase liquid chromatography. The retention of a variety of compounds was quantitatively related with their van der Waals energy change before and after the analyte adsorption on a model graphitic carbon phase.[2,3]

In reversed phase liquid chromatography, an analyte directly contacted with a surface of adsorbent either a bonded phase or an organic polymer. Then, the analyte is replaced by components of the eluent. The strength, retention time difference, is due to the strength of interaction between an analyte and an adsorbent. The quantitative analysis of the retention time in reversed phase liquid chromatography is also analyzed using the same approach used for chromatography using a graphitic carbon column. That is, the direct molecular interaction is related to the retention time.[4] It seems the retention mechanisms in the reversed phase system is different from that of the normal phase system. The difference is the packing materials used. It is based on the concept of "like dissolves like" developed by Prof. Henry Freiser.

The model phase used to study the quantitative analysis of molecular interaction in reversed phase liquid chromatography was modified for the analysis of the ion exchange mechanism. The ionized acidic and basic drugs demonstrated a strong contribution of electrostatic energy to the retention in ion exchange liquid chromatography.[5,6] Furthermore, the combination model of reversed phase and ion exchange allowed to study and to predict drug albumin binding affinity.[7] The approach can be used to predict enantiomer separation, but the bonded phases still have a mixed functional character, the silanol effect cannot be eliminated from present bonded phase silica gels. The feasibility of the approach will be improved in the future. In this presentation, the retention of a variety of compounds on silica gel was analyzed using a model silica gel phase.

Normal phase column liquid chromatography using unmodified silica gel has been used for purification of natural and synthesized compounds. It is a powerful tool for stereoisomer separations. The instability of unmodified silica gel is avoided for use in quantitative analysis in column liquid chromatography. Normal phase separation has, however, been performed using thin layer liquid chromatography. The easy handling permits quick analysis, even if the sensitivity is not so excellent. The relative retention distance is used for the qualitative analysis. The relative retention distance, R_f, can be converted to relative retention time, k, in column liquid chromatography using the following equation: $k = (1 - R_f)/R_f$, if the experimental condition is well controlled.[8] The separation data of sleeping medicines measured using thin layer liquid chromatography[9] was, therefore, used for the quantitative analysis of normal phase liquid chromatography retention.

EXPERIMENTAL

A Dell model Latitude C840 computer equipped with a 2-GHz processor and 1024-MB memory were used. The molecular properties of analytes and model phases and molecular interactions were calculated by molecular mechanics (MM2) using version 5 of the CAChe™ 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 9 Å. The energy unit was kcal/mol (1 kj/mol = 4.18 kcal/mol). The Cricket-Graph™ program from Computer Associates (San Diego, CA) and Project Reader of the CAChe™ program were used for data analysis.

RESULTS AND DISCUSSION

A model silanol phase was constructed with 150 silicones, 288 oxygens, and 48 hydrogens using the CAChe molecular modeling program, and the structure was optimized using the CAChe molecular mechanics (MM2) program. A column liquid chromatogram of polycyclic aromatic hydrocarbons was analyzed to study the feasibility of quantitative *in silico* analysis of retention in normal phase liquid chromatography. The molecular properties of benzene, naphthalene, and anthracene were obtained from the optimized structures using MM2. The optimized (final structure, fs), hydrogen bonding (hb), electrostatic (es), and van der Waals (vw) energy values are summarized in Table 1. The energy values of the complexes are also summarized in Table 1 as final structure (FS), hydrogen bonding (HB), electrostatic (ES), and van der Waals (VW) energy values. The log k values

TABLE 1 Molecular Properties of Polycyclic Aromatic Hydrocarbons

Chemicals	log k	fs	hb	es	vw
Benzene	−0.016	−8.077	0	0	3.006
Naphthalene	0.084	−18.6883	0	0	5.769
Anthracene	0.260	−29.3609	0	0	8.489
Silica phase	–	−842.9634	−34.699	−699.649	−249.575
	FS	HB	ES	VW	
Benzene	−866.2433	−45.423	−699.207	−252.423	
Naphthalene	−882.1955	−47.960	−699.948	−250.884	
Anthracene	−901.7348	−57.069	−698.747	−249.469	

Unit: kcal/mol.

were estimated from a chromatogram obtained on Spherosil XOA 400 (17.5×4 mm i.d.) with n-hexane.[10] The molecular interaction energy values (ΔFS, ΔHB, ΔES, and ΔVW) were obtained by subtraction of the complex energy value from the sum of the analyte and the silanol phase energy values. The interaction energy values were correlated with $\log k$ values. The correlation coefficients between $\log k$ values and ΔFS, ΔHB, ΔES, and ΔVW values were 1.000, 0.985, 0.508, and 0.992, respectively. The $\log k$ values correlated well with hydrogen bonding and van der Waals energy values. The contribution of hydrogen bonding energy is predominant compared to van der Waals energy due to the high slope.

Usually different types of solvent mixtures are used in normal phase liquid chromatography, especially in the thin layer liquid chromatography. Sleeping medicines were separated in thin layer liquid chromatography using different types of eluents (developing solvents). The medicines are usually molecular form in acidic solution, and ionized form in basic solution. The degree of ionization depends on the p*K*a values and eluent pH. The measurement of real pH values in organic solvent mixtures is difficult; therefore, the 100% molecular and ionized forms were used for the discussion. The *k* values were converted from reference R_f values using $k = (1 - R_f)/R_f$. The silica gel was Silica Gel G.[9] The eluents are chloroform and acetone (9:1), benzene and acetic acid (9:1), and dioxane, benzene and aq. ammonium (20:75:5) mixtures. The chemical structures and p*K*a values are obtained from reference 11.

The molecular properties of the medicines were obtained from the optimized structures using MM2. The fs, hb, es, and vw energy values are summarized in Table 2. The energy values of the complexes are also summarized in Table 2 as FS, HB, ES, and VW energy values. Adsorption of barbital on the silanol phase is shown in Figure 1. The ionized forms results are summarized in Table 3.

In the acidic eluent, benzene and acetic acid (9:1) mixture, the following correlations were obtained between log *k*a and molecular interaction energy values.

$$\Delta FS = -0.015\ (\log ka) + 34.659, \quad r = 0.002\ (n = 13),$$
$$\Delta HB = 5.915\ (\log ka) + 22.058, \quad r = 0.641\ (n = 13),$$
$$\Delta ES = -0.846\ (\log ka) + 4.919, \quad r = 0.166\ (n = 13),$$
$$\Delta VW = -0.527\ (\log ka) + 8.359, \quad r = 0.113\ (n = 13).$$

Ethchlorvinal is a neutral molecule, and weakly retained in both neutral and basic eluents, but it retained strongly in acidic eluent. The reason is not clear, therefore, ethchlorvinal was eliminated from the correlation.

TABLE 2 Molecular Properties of Molecular form Sleeping Medicines

Chemicals	log *k*a	fs	hb	es	vw
Allobarbital	0.122	−69.3903	−8.090	−80.052	3.733
Amobarbital	1.140	−58.4183	−7.984	−75.118	5.333
Barbital	0.213	−60.8260	−7.995	−75.123	4.358
Cyclobarbital	−0.087	−56.0490	−8.133	−68.414	6.040
Hexobarbital	0.017	−58.2625	−8.014	−68.372	5.176
Pentobarbital	0.176	−58.1968	−7.957	−75.095	5.995
Phenobarbital	0.250	−72.9552	−8.186	−71.094	5.648
Secobarbital	0.070	−62.9430	−8.312	−77.768	5.054
Thiopental	−0.432	−67.7351	−4.648	−80.264	4.814
Glutethimide	−0.070	−19.1407	−2.753	−37.118	4.086
Ethinamate	−0.269	−17.8171	−5.436	−27.310	4.671
Ethchlorvinal	0.327	−5.0240	−2.831	−5.919	1.782
Methylprylone	−0.631	−10.9322	−2.720	−31.976	4.487
	log *k*n	FS	HB	ES	VW
Allobarbital	−0.087	−948.2816	−67.294	−786.472	−253.606
Amobarbital	−0.176	−934.4776	−64.359	−781.289	−251.590
Barbital	0.000	−940.6903	−68.974	−780.050	−251.964
Cyclobarbital	−0.328	−934.9919	−66.901	−773.484	−251.896
Hexobarbital	−0.525	−935.5317	−63.654	−772.910	−254.266
Pentobarbital	0.000	−937.8826	−66.416	−780.287	−252.078
Phenobarbital	−0.123	−952.8935	−67.179	−775.217	−253.355
Secobarbital	−0.250	−939.9469	−66.109	−781.364	−252.980
Thiopental	−1.195	−941.3991	−57.203	−784.773	−253.607
Glutethimide	−0.602	−899.6517	−57.714	−742.755	−255.640
Ethinamate	−0.631	−895.9510	−61.418	−730.119	−250.332
Ethchlorvinal	−1.284	−875.9328	−57.058	−707.056	−256.154
Methylprylone	0.000	−889.1583	−55.600	−737.374	−254.594

Eluent: *k*a (benzene: acetic acid = 9:1). *k*n (chloroform: acetone = 9:1).

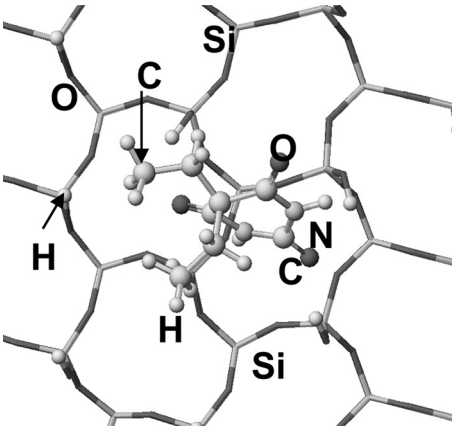

FIGURE 1 Adsorption of barbital on silanol phase, white: hydrogen, light gray: carbon, dark gray: nitrogen or silicone, black: oxygen.

TABLE 3 Molecular Properties of Ionized form Sleeping Medicines

Chemicals	log *k*b	fs	hb	es	vw
Allobarbital	0.035	−46.5193	−4.460	−53.738	3.763
Amobarbital	−0.035	−35.0332	−4.303	−48.209	5.492
Barbital	0.176	−37.5171	−4.307	−48.204	4.442
Cyclobarbital	0.213	−30.7984	−4.368	−40.720	6.350
Hexobarbital	−0.140	−32.7362	−4.331	−40.720	5.591
Pentobarbital	0.017	−34.3887	−4.202	−48.260	6.271
Phenobarbital	0.454	−47.9891	−4.405	−43.830	5.891
Secobarbital	−0.070	−40.2759	−4.406	−50.967	5.299
Thiopental	−0.368	−44.3983	−2.241	−58.241	5.014
Glutethimide	−1.996	6.3333	0.000	−12.266	5.420
Ethinamate	−0.087	−17.8171	−5.436	−27.310	4.671
Ethchlorvinal	−1.276	−5.0240	−2.831	−5.919	1.782
Methylprylone	0.087	−10.9322	−2.720	−31.976	4.487
	p*K*a	FS	HB	ES	VW
Allobarbital	7.77	−919.9919	−59.156	−759.040	−250.696
Amobarbital	7.8	−911.0409	−57.181	−752.930	−255.930
Barbital	7.97	−911.5673	−58.920	−753.044	−252.290
Cyclobarbital	8.60	−908.8343	−63.525	−743.273	−252.699
Hexobarbital	8.2	−909.8929	−59.339	−745.939	−252.496
Pentobarbital	8.0	−908.5543	−58.448	−752.731	−250.514
Phenobarbital	7.4	−925.9632	−64.695	−747.063	−251.303
Secobarbital	7.90/12.60	−916.1206	−60.953	−755.808	−250.336
Thiopental	7.50	−917.0497	−53.620	−761.892	−254.307
Glutethimide	9.2	−863.1612	−44.706	−716.760	−255.538
Ethinamate	–	−897.7109	−63.981	−729.050	−251.009
Ethchlorvinal	–	−863.7277	−47.106	−706.995	−253.996
Methylprylone	12.0	−889.1583	−55.600	−737.374	−254.594

Eluent *k*b (dioxane: benzene: aq. ammonia = 20:75:5).

$$\Delta FS = 2.976\ (\log ka) + 35.343, \quad r = 0.418\ (n = 12),$$
$$\Delta HB = 7.905\ (\log ka) + 22.513, \quad r = 0.833\ (n = 12),$$
$$\Delta ES = 0.561\ (\log ka) + 5.240, \quad r = 0.149\ (n = 12),$$
$$\Delta VW = -0.604\ (\log ka) + 8.342, \quad r = 0.121\ (n = 12).$$

The correlation coefficient was improved, and the contribution of hydrogen bonding energy is clear. When only barbital related compounds were selected, the correlation coefficients were a little improved. The correlation coefficients for ΔFS, ΔHB, ΔES, and ΔVW were 0.431, 0.838, 0.020, and 0.415 (n's = 11), respectively.

In the basic eluent, (dioxane: benzene: aq. ammonia = 20:75:5), the following correlations were obtained. Glutethimide was, however, eliminated

from the calculation; because of the high R_f value (0.99) is close to void volume in column liquid chromatography.

$$\Delta FS = 11.246\ (\log kb) + 32.650, \quad r = 0.873\ (n = 12),$$
$$\Delta HB = 8.589\ (\log kb) + 20.556, \quad r = 0.870\ (n = 12),$$
$$\Delta ES = 1.672\ (\log kb) + 4.374, \quad r = 0.494\ (n = 12),$$
$$\Delta VW = 1.189\ (\log kb) + 7.904, \quad r = 0.247\ (n = 12).$$

In the neutral eluent (chloroform and acetone = 9:1 mixture), a solvent effect appeared. Why methylprylone retained strongly in the eluent is not clear, and the retention was weak in both acidic and basic eluents. The following correlations were obtained after elimination of methylprylone.

$$\Delta FS = 5.195\ (\log kn) + 36.861, \quad r = 0.789\ (n = 12),$$
$$\Delta HB = 4.957\ (\log kn) + 24.445, \quad r = 0.884\ (n = 12),$$
$$\Delta ES = 2.088\ (\log kn) + 5.767, \quad r = 0.617\ (n = 12),$$
$$\Delta VW = -0.474\ (\log kn) + 8.066, \quad r = 0.156\ (n = 12).$$

When only barbitals were selected, the correlation coefficients were a little improved. The correlation coefficients for ΔFS, ΔHB, ΔES, and ΔVW were 0.839, 0.895, 0.293, and 0.434 (n's = 9), respectively. The reproducibility was studied for another set of barbitals R_f values measured in the same silica gel and eluent (chloroform:acetone = 9:1).[12] The correlation coefficients between $\log k$ and DFS, DHB, DES, and DVW were 0.861, 0.935, 0.188, and 0.460, respectively. Hydrogen bonding is predominant for barbital retention on silica gel in normal phase thin layer liquid chromatography.

The above results indicate that hydrogen bonding energy makes the major contribution to retention in normal phase liquid chromatography using silica gels. Van der Waals energy effect is negligible for the similar size compounds. The phenomena are different from the results in reversed phase liquid chromatography. Generally, eluent in normal phase liquid chromatography is relatively hydrophobic compared to that in reversed phase liquid chromatography. Acidic and basic components are, however, usually added in eluent of normal phase liquid chromatography to improve the separation. Another meaning is improving selectivity factor Δ value. Solubility depends on the similarity between an analyte and solvent. The comparison of chromatographic behavior of different types of compounds is very difficult due to unpredictable solubility in normal phase liquid chromatography. Further study is required to predict chromatographic behavior of partially ionized analytes in normal phase liquid chromatography like pH effect in reversed phase liquid chromatography.

CONCLUSION

The retention mechanism in normal phase liquid chromatography can be quantitatively analyzed using a computational chemical method. Hydrogen bonding makes the major contribution to the retention. Further study is, however, required to estimate the retention of partially ionized analytes like that in reversed phase liquid chromatography. The question is how to measure pH in normal phase eluent. Solubility is linearly not related to solvent strength. The retention time prediction of different types of compounds in normal phase liquid chromatography is difficult due to the difficulty of estimating solubility.

REFERENCES

1. Hanai, T. *HPLC A Practical Guide*; RSC: Cambridge, 1999.
2. Hanai, T. Quantitative *in silico* analysis of the selectivity of graphitic carbon synthesized by different methods. Anal. Bioanal. Chem. **2008**, *390*, 369–375.
3. Hanai, T. Quantitative *in silico* analysis of the selectivity of a graphitic carbon column. J. Liq. Chromatogr. &. Rel. Technol. **2009**, *32*, 647–655.
4. Hanai, T. Chromatography *in silico*: Basic concept in reversed-phase liquid chromatography. Anal. and Bioanal. Chem. **2005**, *382*, 708–717.
5. Hanai, T.; Masuda, Y.; Homma, H. Chromatography *in silico*, Retention of basic compounds on a carboxyl ion-exchanger. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28*, 3087–3097.
6. Hanai, T. Chromtography *in silico*: Retention of acidic drugs on a guanidine ion-exchanger. J. Liq. Chromatogr. & Rel. Technol. **2007**, *30*, 1723–1731.
7. Hanai, T.; Miyazaki, R.; Kamijima, E.; Homma, H.; Kinoshita, T. Computational prediction of drug-albumin binding affinity by modeling liquid chromatographic interactions. Internet Electronic Journal of Molecular Design **2003**, *2*, 702–711.
8. Kaizer, R.E. *Einfuhrung in die Hochleistungs-Dunnschicht-Chromatographie*; Institute fur Chromatographie: Bad Durkheim, Germany, 1976.
9. Suzuki, I.; Saitoh, Y.; Toyoda, M. *Hakusoh-Kuromatoguraphi-No-Jissai* (*Practice of thin-layer chromatography*); Hirokawa: Tokyo, 1990.
10. Vermont, J.; Deleuil, M.; De-Vries, A.J.; Gnillemin, C.L. Modern liquid chromatography on Spherosil. Anal. Chem. **1975**, *47*, 1329–1337.
11. Hansch, C.; Sammes, P.G.; Tayler, J.B. *Comprehensive Medicinal Chemistry VI*; Pergamon Press: Oxford, 1990.
12. Macek, K. *Pharmaceutical Application of Thin-Layer and Paper Chromatography*; Elsevier: Amsterdam, 1972; 221.