Computational Chemical Analysis of the Retention of Acidic Drugs on a Pentyl-Bonded Silica Gel in Reversed-Phase Liquid Chromatography

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Abstract

A fast method to obtain a quantitative structure-retention relationship is required in chromatography for the rapid optimization of chromatographic separation conditions. Chromatographic data of acidic drugs are analyzed by a computational chemical method to simulate chromatographic simulation. The direct interaction between a model phase and a drug is calculated as an energy value using the molecular mechanics calculation of CAChe. Computational chemistry using a model adsorbent is a new method for quantitative analysis of retention in reversed-phase liquid chromatography. The correlation coefficient is 0.878 (n = 19) between the retention factors of acidic drugs and interaction energy values of the final structure (ΔFS) between an acidic drug and model pentyl-bonded phase.

Introduction

Optimization of the quantitative structure retention relationship (QSRR) has been required. The octanol–water partition coefficient (log *P*) has been used as a molecular property of analytes (1). Several log *P* calculation methods were evaluated by comparison with reference values (2,3), and a new method—a modified CAlog*P* method—was proposed for the optimization of reversed-phase liquid chromatography (RPLC). The new log *P* values were evaluated with log *k* values of phenolic and nitrogen-containing compounds measured in RPLC (4). However, log *P* is a property of molecular forms of analytes, not ionized forms. This means log *P* is not the final solution to establishing the QSRR in chromatography. QSRR based on the molecular properties of analytes would have limitations when applied under various chromatographic conditions.

A computational chemical analysis was applied to study retention time differences in LC based on the retention mechanisms derived from solubility properties in which hydrophobic interaction is considered as the major driving force in RPLC (1). A model phase was constructed to study the molecular interactions in LC, and the quantitative molecular interactions were proposed (5,6) using the molecular mechanics calculation (MM2) of the CAChe program (7). Simulation of RPLC for simple phenolic compounds was proposed. The correlation between molecular interaction energy values (Δenergy) and retention factors obtained for the molecular forms was used to predict the maximum retention factors, and that for the ionized forms was used to predict the minimum retention factors in given pH eluent (8). This preliminary, successful method was applied to analyze the retention factors of phenolic compounds (9) using a model phase (10). The new model phase was better than the first model phase. Therefore, this new approach was applied using new model phases to QSRR of acidic drugs whose structure is varied compared with homologous phenolic compounds.

Experimental

Drugs used previously to measure albumin–drug binding affinity were obtained from Sigma Chemical Co. (St. Louis, MO) and Wako Pure Chemical Industries (Osaka, Japan). Their properties are summarized in Table I. Sodium dihydrogenphosphate dihydrate and disodium hydrogenphosphate $12H_2O$ were purchased from Wako Pure Chemical Industries. High-performance liquid chromatography grade methanol was obtained from Kanto-Kagaku (Tokyo, Japan). The water used was of Milli-Q grade.

The LC was the same as that used previously (8). The retention factors of acidic drugs were measured by RPLC. A pentyl-bonded silica gel column (50- × 2.1-mm i.d.) was used with various pH eluents. The column temperature was 37°C. The void volume marker was fructose. The eluent was a mixture of 50mM sodium phosphate solution and methanol (1:1). The flow rate was 0.2 mL/min. The measured retention factors are listed in Table I. The computers were the same as those used previously (10). The

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octanol–water partition coefficient (Vlog *P* values) was calculated using TopKat (Fujitsu, Tokyo, Japan).

Results and Discussion

A model butyl-bonded phase that was used previously for a development of new optimization system *in silico* (8) was applied to develop a common optimization system for a variety of compounds. The docking between an acidic drug and the butyl phase was simple. The lowest energy value of a complex was easily obtained. The example of the optimized complex form between benzoic acid and the butyl phase is shown in Figure 1, in which the stick and ball indicate the structure of optimized complex between the model phase and benzoic acid. Butyl groups of the model butyl-bonded phase are highly dense and not pushed down by the analyte that lies on top of the butyl group brush.

The energy values of individual compounds calculated using MM2 are listed in Table I along with the properties (log *P* and p*K*a) of acidic drugs used. The calculated energy values are final (FS), hydrogen bonding (HB), electrostatic (ES), and van der Waals (VW) energy. The energy values of individual complexes for the model butyl phase and an acidic drug are listed in Table II as FS1, HB1, ES1, and VW1.

The interaction energy values between a molecular form compound and the model butyl phase were calculated using MM2 to analyze the retention of molecular form analytes qualitatively [interaction energy values (Δvalue) = energy value of individual molecule + energy value of a model phase – energy value of a complex].

The *r* between ΔFS1 or ΔVW1 calculated using the model butyl phase and measured log *k* values of molecular form acidic drugs listed as log k_2 in Table II was 0.596 (n = 19).

$$\Delta FS1 = 5.235 (\log k_2) + 13.918 \quad \text{Eq. 1}$$

where *r* is 0.596 and *n* is 19.

$$\Delta VW1 = 5.092 (\log k_2) + 12.660 \quad \text{Eq. 2}$$

where *r* is 0.714 and n is 19.

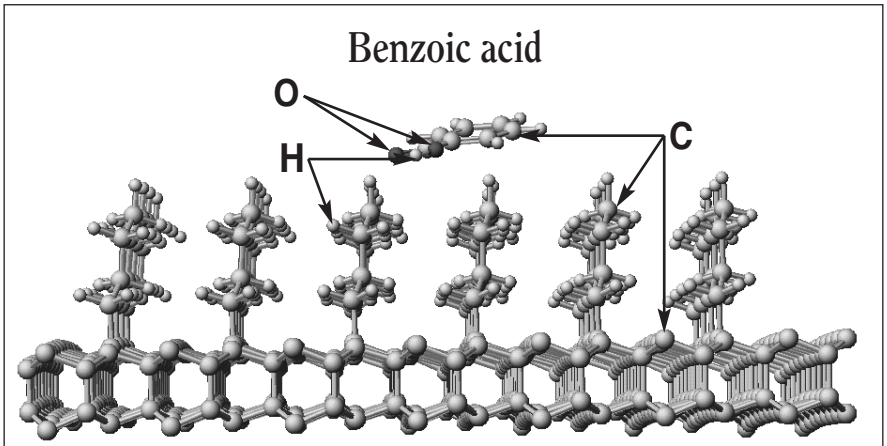

Figure 1. Adsorption of benzoic acid in butyl phase (small white ball, hydrogen; large white ball, carbon; and black ball, oxygen).

Table I. Molecular Properties and Retention Factors of Acidic Drugs

No	Acidic drug	Vlog *P*	p*K*a	log *k*2 (pH 2.00)	log *k*4.5 (pH 4.50)	log *k*6 (pH 6.00)	log *k*7.4 (pH 7.40)	FS	HB	ES	VW
1	*p*-Aminohippuric acid	0.232	3.83	–1.155	–1.854	–1.886	–2.097	–18.7895	–10.241	–9.047	7.603
2	Amoxicillinum	–2.502	9.60	–1.444		–1.796	–1.310	39.4660	–8.568	0.402	5.705
3	Barbituric acid	0.822		–1.131	–1.699	–2.097	–1.921	–59.1784	–8.299	–75.242	–4.501
4	Benzoic acid	1.485	4.20	–0.021	–0.489	–0.759	–0.785	–13.9182	–3.458	–6.671	4.877
5	Furosemide	1.901	3.90	–0.136	–0.479	–0.511	–0.511	9.9626	–5.541	–1.038	6.003
6	*p*-Hydroxybenzoic	1.002	9.46	–0.775	–0.963	–1.538	–1.678	–16.0982	-4.931	–6.668	4.790
7	Ibuprofen	3.550	5.20	1.204	0.910	0.634	0.596	–16.9561	–3.737	–5.043	4.654
8	Indomethacin	3.426	4.50	1.054	0.696	0.594	0.581	–24.0717	–5.284	–12.458	5.883
9	Iopanoic acid	3.873		1.346	1.087	0.692	0.607	–8.2455	–5.634	–4.501	7.048
10	Mefenamic acid	4.971	4.20	1.352	0.935	0.652	0.577	12.5077	–3.951	–11.362	18.894
11	Nalidixic acid	0.966	6.00	0.054	0.008	–0.189	–0.455	–37.4073	–4.051	–40.545	11.771
12	Naproxen	3.047	4.20	0.586	0.262	–0.015	–0.048	–27.7018	–3.755	–5.025	6.778
13	Nicotinic acid	0.477	4.95	–0.796	–1.161	–1.237	–1.174	–18.5217	–4.047	–10.511	3.675
14	Phenylbutazone	3.251	4.40	0.964	0.522	0.346	0.325	18.1704	0.000	–11.325	19.458
15	Probenocid	2.652		0.610	0.088	0.048	0.041	8.8530	–3.455	–3.682	8.863
16	Salicylic acid	1.060	3.00	0.007	– 0.666	– 0.688	–0.706	–15.3507	–5.355	–6.437	5.438
17	Sulfamethoxazole	0.791	5.81	–0.623	– 0.717	– 0.971	–1.301	7.0614	–2.202	2.679	3.090
18	Tolazamide	1.448	5.70	0.407	0.343	0.139	0.078	–3.1534	–2.847	–12.721	8.547
19	Tolbutamide	2.266	5.30	0.372	0.284	0.086	0.032	–29.9856	–2.920	–25.539	4.886
20	Warfarin	2.866	5.10	0.733	0.383	– 0.081	–0.162	–17.5045	–2.808	–5.999	7.411

Table II. Calculated Energy Values of Complexes

No	Acidic drugs	FS1	HB1	ES1	VW1	No	Acidic drugs	FS2	HB2	ES2	VW2
1	*p*-Aminohippuric acid	3344.1074	–10.099	–8.912	416.750	1	*p*-Aminohippuric acid	6560.0353	–10.148	–404.144	–190.940
2	Amoxicillinum	3394.5892	–8.567	0.365	407.435	2	Amoxicillinum	6615.3485	–8.822	–394.697	–195.914
3	Barbituric acid	3302.5911	–8.104	–75.203	414.402	3	Barbituric acid	6522.6549	–8.368	–470.373	–189.924
4	Benzoic acid	3351.5351	–3.457	–6.672	417.054	4	Benzoic acid	6570.5402	–3.508	–401.866	–187.601
5	Furosemide	3368.2982	–5.526	–1.163	410.582	5	Furosemide	6583.5931	–5.955	–396.707	–197.270
6	*p*-Hydroxybenzoic acid	3349.1916	–4.928	–6.669	416.810	6	*p*-Hydroxybenzoic acid	6567.7535	–5.048	–401.922	–188.219
7	Ibuprofen	3342.5102	–3.772	–5.058	412.100	7	Ibuprofen	6562.0379	–3.791	–400.425	–192.483
8	Indomethacin	3332.7620	–5.294	–12.543	409.513	8	Indomethacin	6553.4435	–5.312	–407.812	–193.346
9	Iopanoic acid	3348.7604	–5.636	–4.668	410.919	9	Iopanoic acid	6569.9775	–5.638	–400.012	–193.231
10	Mefenamic acid	3348.8393	–8.633	0.848	411.812	10	Mefenamic acid	6574.6329	–4.919	–406.369	–187.177
11	Nalidixic acid	3320.6021	–4.052	–40.523	416.687	11	Nalidixic acid	6539.7471	–4.064	–435.935	–187.407
12	Naproxen	3331.4418	–3.748	–5.020	412.699	12	Naproxen	6550.4294	–3.762	–400.201	–192.251
13	Nicotinic acid	3347.1021	–4.049	–10.520	416.077	13	Nicotinic acid	6564.9971	–4.121	–405.849	–189.566
14	Phenylbutazone	3371.9024	0.000	–11.299	419.183	14	Phenylbutazone	6591.4219	0.000	–406.505	–184.060
15	Probenocid	3354.0061	–3.442	–3.548	408.495	15	Probenocid	6574.2145	–3.461	–398.621	–193.997
16	Salicylic acid	3350.1086	–5.345	–6.434	417.712	16	Salicylic acid	6568.1128	–5.452	–401.762	–187.487
17	Sulfamethoxazole	3365.6830	–2.274	2.636	408.481	17	Sulfamethoxazole	6582.1412	–2.276	–392.524	–198.790
18	Tolazamide	3352.7883	–3.174	–12.709	411.593	18	Tolazamide	6569.4412	–2.814	–408.588	–197.404
19	Tolbutamide	3326.7263	–2.914	–25.673	408.069	19	Tolbutamide	6543.0870	–2.917	–421.246	–198.103
20	Warfarin	3342.8062	–2.841	–5.915	413.980	20	Warfarin	6562.1148	–2.870	–401.378	–189.830
Butyl-phase		3373.0369	0.000	0.000	419.941	Dimethylpentyl-phase		6594.9954	0.000	–395.235	–181.977

No	Acidic drugs	FS3	HB3	ES3	VW3	No	Acidic drug	FS4	HB4	ES4	VW4
1	*p*-Aminohippuric acid	–651.9113	–10.260	–354.406	–339.926	1	*p*-Aminohippuric acid	–686.4470	–10.405	–412.426	–409.470
2	Amoxicillinum	–604.6880	–9.256	–345.264	–349.126	2	Amoxicillinum	–634.3374	–8.408	–403.125	–420.178
3	Barbituric acid	–685.8185	–9.209	–420.940	–331.687	3	Barbituric acid	–724.4791	–8.012	–478.554	–411.294
4	Benzoic acid	–638.4382	–3.731	–352.083	–331.782	4	Benzoic acid	–683.5664	–3.668	–410.117	–412.996
5	Furosemide	–625.4940	–5.487	–346.596	–344.768	5	Furosemide	–658.6313	–5.662	–403.923	–415.843
6	*p*-Hydroxybenzoic acid	–640.9142	–5.138	–352.038	–332.194	6	*p*-Hydroxybenzoic acid	–681.1793	–5.301	–410.241	–411.627
7	Ibuprofen	–655.7320	–6.630	–350.743	–344.577	7	Ibuprofen	–693.1361	–3.750	–408.423	–419.931
8	Indomethacin	–663.7277	–9.048	–358.140	–342.018	8	Indomethacin	–704.1475	–5.505	–415.957	–423.621
9	Iopanoic acid	–664.4756	–5.660	–350.167	–345.087	9	Iopanoic acid	–686.9940	–5.643	–408.025	–420.321
10	Mefenamic acid	–638.0658	–4.960	–356.660	–335.679	10	Mefenamic acid	–670.4021	–5.178	–414.604	–405.231
11	Nalidixic acid	–670.3637	–4.052	–386.092	–334.377	11	Nalidixic acid	–710.5518	–4.159	–443.681	–411.480
12	Naproxen	–662.6026	–3.773	–350.314	–341.979	12	Naproxen	–701.8769	–3.770	–408.501	–417.874
13	Nicotinic acid	–641.5372	–4.049	–355.938	–331.860	13	Nicotinic acid	–682.1029	–4.188	–413.822	–409.844
14	Phenylbutazone	–622.8831	0.000	–356.835	–336.159	14	Phenylbutazone	–660.0197	0.000	–414.543	–410.521
15	Probenocid	–642.4373	–3.476	–349.401	–345.153	15	Probenocid	–672.2913	–3.450	–407.220	–415.511
16	Salicylic acid	–640.3111	–5.420	–351.835	–331.747	16	Salicylic acid	–684.4083	–5.563	–409.751	–411.829
17	Sulfamethoxazole	–625.8881	–2.205	–342.811	–344.607	17	Sulfamethoxazole	–661.1186	–2.262	–400.829	–415.047
18	Tolazamide	–641.7513	–3.398	–358.438	–344.432	18	Tolazamide	–684.0754	–3.437	–416.628	–420.787
19	Tolbutamide	–668.0298	–3.220	–371.252	–344.875	19	Tolbutamide	–704.1884	–3.450	–430.565	–418.878
20	Warfarin	–663.0003	–2.943	–351.341	–349.078	20	Warfarin	–690.4602	–2.924	–409.217	–414.657
Monomethylpentyl-phase		–608.4140	0.000	–345.403	–321.250	Polydimethylpentyl-phase		–648.6200	0.000	–403.451	–400.524

Table continued...

Table II. Calculated Energy Values of Complexes (continued)

No	Acidic drug	FS4i	HB4i	ES4i	VW4i	No	Acidic drug	FSi	HBi	ESi	VWi
1	*p*-Aminohippuric acid	–	–	–	–	1	*p*-Aminohippuric acid	–	–	–	–
2	Amoxicillinum	–	–	–	–	2	Amoxicillinum	–	–	–	–
3	Barbituric acid	–	–	–	–	3	Barbituric acid	–	–	–	–
4	Benzoic acid	–671.2511	0	–403.332	–412.528	4	Benzoic acid	–2.5511	0	0	4.746
5	Furosemide	–657.0607	–2.056	–402.228	–416.554	5	Furosemide	13.8365	–2.594	–2.736	9.998
6	*p*-Hydroxybenzoic acid	–674.4414	–1.592	–402.960	–413.699	6	*p*-Hydroxybenzoic acid	–4.9589	–0.050	–1.462	4.463
7	Ibuprofen	–679.3902	0	–399.952	–420.940	7	Ibuprofen	3.1510	0	5.220	8.653
8	Indomethacin	–681.6643	–1.743	–407.909	–417.759	8	Indomethacin	–7.2472	–4.273	0	6.009
9	Iopanoic acid	–672.4282	–2.175	–402.479	–417.147	9	Iopanoic acid	2.9680	–2.158	0.411	7.674
10	Mefenamic acid	–660.0112	–1.262	–411.452	–403.430	10	Mefenamic acid	20.2949	–8.420	–0.654	19.891
11	Nalidixic acid	–717.3566	0	–458.411	–411.646	11	Nalidixic acid	–44.4161	0	–55.760	11.793
12	Naproxen	–683.0876	0	–399.852	–412.595	12	Naproxen	–13.5376	3.156	0	6.681
13	Nicotinic acid	–669.4499	0	–410.356	–410.764	13	Nicotinic acid	–7.2772	–7.301	0	3.586
14	Phenylbutazone	–644.7365	0	–401.086	–410.792	14	Phenylbutazone	33.3848	0	2.030	19.586
15	Probenocid	–	–	–	–	15	Probenocid	–	–	–	–
16	Salicylic acid	–670.3145	–1.806	–403.015	–409.905	16	Salicylic acid	–4.1495	–0.150	–1.487	5.234
17	Sulfamethoxazole	–669.4817	–2.061	–405.399	–419.458	17	Sulfamethoxazole	1.4759	1.067	–2.230	3.175
18	Tolazamide	–664.8088	–0.117	–403.589	–418.244	18	Tolazamide	13.5328	–0.089	–0.585	9.727
19	Tolbutamide	–676.9161	–0.125	–407.949	–416.260	19	Tolbutamide	–5.5479	–0.089	–4.856	5.974
20	Warfarin	–697.8105	–3.806	–408.928	–430.282	20	Warfarin	–17.6434	–2.951	–4.952	6.818

In this model system, one side of the analyte was in contact with this model phase, and the steric effect was neglected. The difference of ΔFS1 and ΔVW1 was large for barbituric acid, probenocid, and mefenamic acid.

The new silica gel based pentyl-bonded phase consisted of 682 atoms, 742 bonds, and 5,107 connectors containing 158 silicones, 304 oxygens, 63 carbons, and 157 hydrogens. The monolayer of the polysiliconedioxide phase was locked to avoid deformation of the structure by further optimization because the atomic distance of silica gel does not change under LC conditions. The minimized model bonded phase was constructed for a simple lap-top-computer calculation. The structure of the model bonded phase consists of eight pentyl groups and many oxygens that are kept free to reduce the number of atoms. Figure 2 shows a side view of an optimized structure, and the complex with nicotinic acid is shown in Figure 3, in which the atomic size of nicotinic acid is 1 instead of 0.2 to show the optimized location. Other complexes showed the similar structure. Pentyl groups of the pentyl-bonded polysiliconedioxide phase stand tall before optimization of the molecular interaction with an analyte, then draw close to the analyte after the calculation like a predation of a sea anemone. The FS, HB, ES, and VW energy values of a complex between the pentyl-bonded phase and an acidic drug are listed in Table II as FS2, HB2, ES2, and VW2.

An improvement in the correlation was expected if a low-density phase was used as a model phase because the analyte should be buried in the alkyl chains. The interaction energy values between an acidic drug and the silica gel-based pentyl phase were calculated. The r between ΔFS2 and measured log k values of molecular form acidic drugs listed as log k_2 in Table I improved to 0.773 (n = 19). The correlation (r) was 0.700 (n = 19) from ΔVW2.

$$\Delta FS2 = 9.063 (\log k_2) + 26.133 \quad \text{Eq. 3}$$

where r is 0.773 and n is 19.

$$\Delta VW2 = 6.950 (\log k_2) + 23.963 \quad \text{Eq. 4}$$

where r is 0.700 and n is 19.

The contribution of HB2 and ES2 values was very poor. The HB2 energy value of these model phase is zero. The r for ΔHB2 and ΔES2 was 0.034 and 0.124, respectively. The contribution of ΔVW energy indicated that hydrophobic interaction is the predominant molecular interaction in the retention of these acidic drugs on an alkyl-bonded phase in RPLC. The difference of ΔFS2 and ΔVW2 was large for barbituric acid, probenocid, and mefenamic acid even if the silica gel-based phase diminished the steric effect. The correlation coefficient was still very poor, therefore, further improvement of a model phase was studied.

In the synthesis of the alkyl chain-bonded silica gel, two chloro groups of alkylsiloxaine may bind with two silanol groups of the polysiliconedioxide phase. Therefore, a monomethylpentyl-bonded phase was constructed as a model phase on which there was no free silanol group at the adsorption site. It consisted of 753 atoms, 828 bonds, and 6056 connectors containing 165 silicones, 304 oxygens, 90 carbons, and 210 hydrogens. Fifteen monomethylpentylsilicones bind (bonded) with two oxygens of

the polysiliconedioxide phase within 900 Å^2. The optimized structure of a complex of this model phase and phenylbutazone is shown in Figure 4. In this phase there was not enough space to stick a molecule between brushes. Only one side of molecule contacted with the model phase. This means this type of model phase was not an ideal model even though longer alkyl-chains were used to construct a model phase that required longer calculation time. The FS, ES, HB, and VW energy values of a complex between this monomethylpentyl-bonded phase and an acidic drug are listed in Table II as FS3, ES3, HB3, and VW3. The r between ΔFS and log k_2 in Table I was 0.486 (n = 19). The r was 0.549 from ΔVW3.

$$\Delta FS3 = 3.558(\log k_2) + 17.359 \quad \text{Eq. 5}$$

where r is 0.486 and n is 19.

$$\Delta VW3 = 3.587(\log k_2) + 15.904 \quad \text{Eq. 6}$$

where r is 0.549 and n is 19.

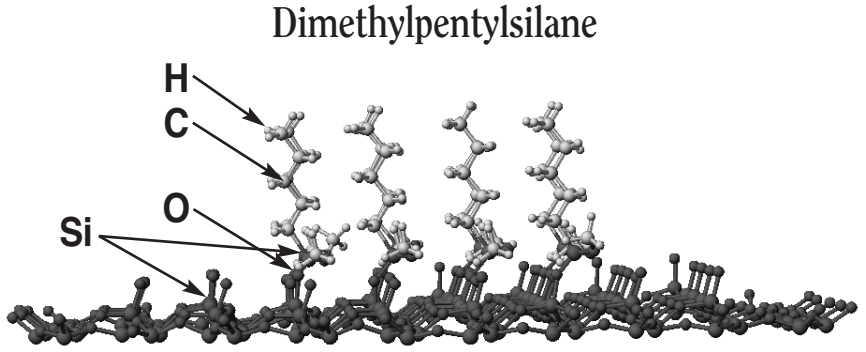

Figure 2. Dimethylpentylsilane-bonded polysiliconedioxide phase (small white ball, hydrogen; large white ball, carbon; small black ball, oxygen; and large black ball, silicone).

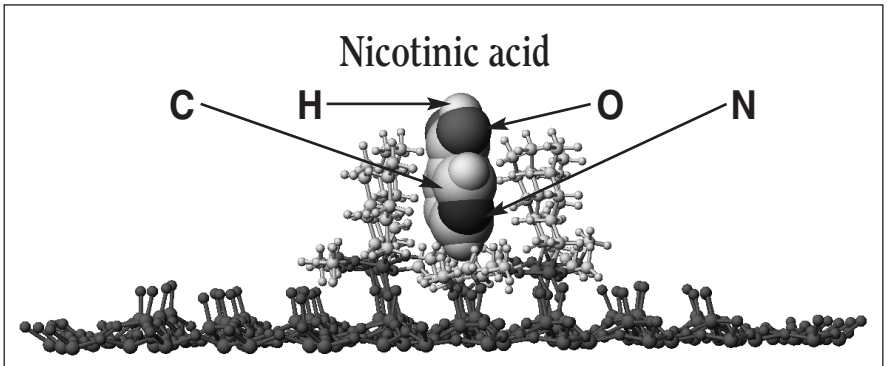

Figure 3. Adsorption of nicotinic acid in dimethylpentylsilane-bonded phase (small white ball, hydrogen; large white ball, carbon; small black ball, oxygen; and large black ball, silicone).

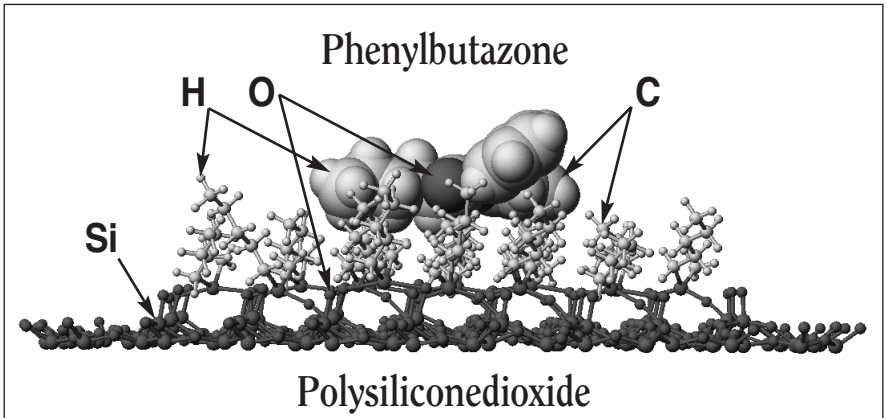

Figure 4. Adsorption of phenylbutazone in monomethylpentylsilane-bonded phase (small white ball, hydrogen; large white ball, carbon; small black ball, oxygen; and large black ball, silicone).

The correlation coefficients were very poor. This type of bonding may not be realistic for an alkyl-bonded silica gel. The difference of ΔFS3 and ΔVW3 was large for barbituric acid, probenocid, and mefenamic acid even in this bonded phase.

Furthermore, a new phase was constructed based on dimethylpentylsilane. It consisted of 991 atoms, 1051 bonds, and 15,193 connectors containing 171 silicones, 328 oxygens, 143 carbons, and 349 hydrogens. Twenty dimethylpentylsilanes and one trimethylsilane were bonded within 900 Å^2 on the polysilicone dioxide phase. The trimethylsilane was considered an end-capped molecule. The optimized structure of a complex of this model phase and iopanoic acid is shown in Figure 5. This upper view of space-filled structure indicates how a molecule is fitted in the pocket. The trimethyl silane is the center of the pocket. The atomic size is 1 instead of 0.2 for the stick and ball model. Dimethylpentyl groups stand close together because of their steric hindrance. Some of them lie in a free space after the optimized molecular interaction

On this new bonded phase, dimethylpentyl groups surrounded one trimethyl group. Silanol groups around the trimethylsilane group are completely covered by alkyl groups. The silanol group may not have contributed. The first circle of dimethylpentyl groups may not be pushed down in the presence of an analyte. The second circle of dimethylpentyl groups should support the first. The interaction energy values between an acidic drug and the new model phase were calculated and are listed as FS4, HB4, ES4, and VW4 in Table II. The r between ΔFS and log k_2 was improved.

$$\Delta FS4 = 6.483(\log k_2) + 23.145 \quad \text{Eq. 7}$$

where r is 0.878 and n is 19.

$$\Delta VW4 = 6.071(\log k_2) + 19.864 \quad \text{Eq. 8}$$

where r is 0.833 and n is 19.

$$\log P = 1.514 (\log k_2) + 1.788 \quad \text{Eq. 9}$$

where r is 0.925 and n is 19.

These results are better than the results for the previous three models, but the difference of ΔFS4 and ΔVW4 was still large for barbituric acid, probenocid, and mefenamic acid at more than 10 kcal/mol. The r between log P and log k_2 was 0.925 (n = 19). This r value was not significantly high compared with the results for phenolic compounds (4). Therefore, log k_2 values measured by LC may not be maximum retention factors. Further development was necessary for simulation chromatography of drugs. The mass of drugs was quite large and the structure was complicated compared with that of phenolic compounds. The retention factors of partially ionized compounds were calculated with the following equation (18) using pKa values:

$$k = \{k_m + k_i(K\text{a}/[H^+])\}/\{1 + (K\text{a}/[H^+])\} \quad \text{Eq. 10}$$

where k_m and k_i are the retention factors of the molecular and ionized analytes, respectively, and Ka is the dissociation constant of analytes. H^+ is the hydrogen ion concentration in eluent. The k_m and k_i were replaced with Δenergy of molecular and ionized forms. The Δenergy of ionized form was calculated from FS4i, HB4i, ES4i, VW4i, FSi, HBi, ESi, and VWi in Table II, in which I means ionized form. The correlation between the retention factors measured and predicted with this new method using molecular interaction energy (ΔFS4) was obtained from equations 11–14. The measured retention factors of acidic drugs are given in Table I.

$$\Delta\text{FS4} = 7.395 (\log k_2) + 22.328 \quad \text{Eq. 11}$$

where r is 0.891 and n is 15 at pH 2.00.

$$\Delta\text{FS4} = 7.603 (\log k_{4.5}) + 24.172 \quad \text{Eq. 12}$$

where r is 0.936 and n is 15 at pH 4.50.

$$\Delta\text{FS4} = 6.954 (\log k_6) + 25.512 \quad \text{Eq. 13}$$

where r is 0.851 and n is 15 at pH 6.00.

$$\Delta\text{FS4} = 6.185 (\log k_{7.4}) + 25.766 \quad \text{Eq. 14}$$

where r is 0.783 and n is 15 at pH 7.40.

The results indicated that the retention time of acidic drugs can be predicted using both energy value changes in the optimized structure calculated with MM2. The addition of pKa values predicted from the atomic partial charge calculated by the molecular orbital package enables the retention factors in a given pH eluent to be predicted.

An octyl-bonded phase was constructed similar to the first pentyl phase without end capping and examined the molecular interactions with these acidic drugs examined. However, the longer alkyl chains did not help to improve the correlation coefficient between Δenergy and log k_2. The addition of one water molecule, besides a polar group of analyte, changed the Δenergy values. However, this MM2 calculation method cannot handle multisolvent molecules.

Molecular interaction in LC can be quantitatively estimated from the energy values calculated by molecular mechanics using analytes and a model phase. The addition of a solvation effect and the construction of a better model phase should improve the precision of qualitative analysis of retention factors in LC.

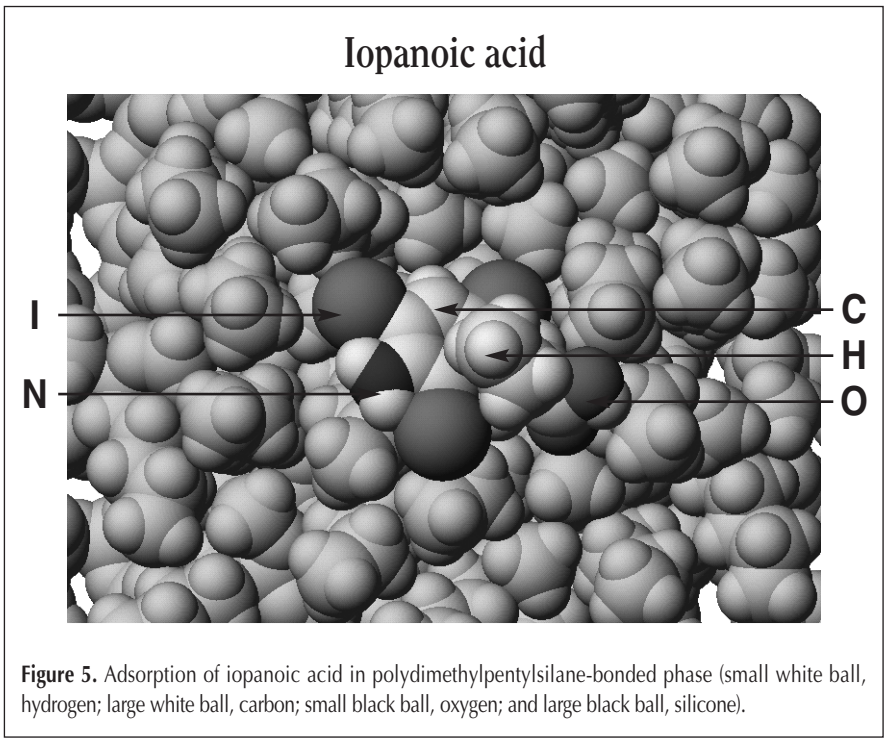

Figure 5. Adsorption of iopanoic acid in polydimethylpentylsilane-bonded phase (small white ball, hydrogen; large white ball, carbon; small black ball, oxygen; and large black ball, silicone).

Conclusion

The retention time of acidic drugs in RPLC was predicted from molecular interaction energy values calculated with MM2. The precision of the retention factors predicted with this new method was equivalent to a former method in which the retention time was predicted from Vlog$P$. Furthermore, the prediction of retention factors of phenolic compounds in RPLC in a given pH eluent was performed using the dissociation constant (pKa). Computational chemical calculation demonstrated a possibility of simulation chromatography of retention of acidic drugs on a pentyl phase. Further computational chemical study with a solvent effect using a better model phase will improve the precision. However, the solvent effect cannot be included in the present calculation system.

References

1. T. Hanai. HPLC *A Practical Guide.* Royal Society of Chemistry, Cambridge, U.K., 1999, pp. 109–32.
2. N. Boder, Z. Gabanyi, and C.-K. Wong. A new method for the estimation of partition coefficient. *J. Am. Chem. Soc.* **111:** 3783–86 (1989).
3. R.F. Rekker. *The Hydrophobic Fragmental Constant.* Elsevier, Amsterdam, the Netherlands, 1977.
4. T. Hanai, K. Koizumi, and T. Kinoshita. Prediction of retention factors of phenolic and nitrogen-containing compounds in reversed-phase liquid chromatography based on log *P* and p*K*a obtained by computational chemical calculation. *J. Liq. Chromatogr. Rel. Technol.* **23:** 363–85 (2000).
5. T. Hanai. Separation of polar compounds using carbon columns. *J. Chromatogr. A* **989:** 183–96 (2003).
6. T. Hanai, H. Hatano, N. Nimura, and T. Kinoshita. Computer-aided analysis of molecular recognition in chromatography. *Analyst* **118:** 1371–74 (1993).
7. CAChe manual. Fujitsu (Sony-Techtronix), Tokyo, Japan, 1994, 2002.
8. T. Hanai, Chi. Mizutani, and H. Homma. Computational chemical simulation of chromatographic retention of phenolic compounds. *J. Liq. Chromatogr. & Rel. Technol.* **26:** 2031–39 (2003).
9. T. Hanai, K. Koizumi, T. Kinoshita, R. Arora, and F. Ahmed. Prediction of p*K*a values of phenolic and nitrogen-containing compounds by computational chemical analysis compared to those measured by liquid chromatography. *J. Chromatogr. A* **762:** 55–61 (1997).
10. T. Hanai. Simulation chromatography of phenolic compounds using a computational chemical method. *J. Chromatogr. A* **1027:** 279–87 (2004).
11. D.J. Pietrzyk and C.-H. Chu. Separation of organic acids on Amberlite-XAD copolymers by reversed-phase high pressure liquid chromatography. *Anal. Chem.* **49:** 860–67 (1977).

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