

Chromatography *in silica*, quantitative analysis of retention mechanisms of benzoic acid derivatives

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Abstract

A quantitative analysis of the retention of benzoic acid derivatives in reversed-phase liquid chromatography was achieved using a molecular mechanics calculation in the CAChe program. Interaction energy values were calculated for both molecular and ionic forms. The predicted retention factors of partially ionized acids obtained using a combination of dissociation constants well correlated with the values measured by reversed-phase liquid chromatography with pH-controlled eluents. The molecular interaction energy value was calculated by subtracting the energy value of the complex from the sum of energy values of a model-phase and an analyte.

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1. Introduction

The retention times of a variety of compounds have been predicted using the octanol–water partition coefficient ($\log P$) and dissociation constant ($\text{p}K_{\text{a}}$) [1–4]. A similar approach was commercialized by the ACD Lab. Toronto, Canada. However, $\log P$ is a property of the molecular form, and the precision of the predicted retention factor of the ionized form is not satisfactory. In addition, the partition coefficient is the sum of various solubility properties, and predictions using a theoretical method with computational chemical calculations are still under development. The accurate prediction of $\log P$ is based on empirically measured data at present. A classic optimization method using $\log P$ demonstrated that the correlation coefficient between $\log k$ values of aromatic acids at low pH, those of the molecular form, and their $V \log P$ and $N \log P$ values was satisfactory, but the slope was far from 1 [3]. The $\log P$ did not hold promise as a molecular parameter for the optimization of ionizable compounds in reversed-phase liquid chromatography.

In reversed-phase liquid chromatography, if the direct interaction between an analyte and the surface of the packing materials or capillary tube surface is considered as the predominant retention force, the retention mechanisms can be quantitatively analyzed and taking the effect of the solvent into accounts should improve the precision of the analysis. Hydrophobic interactions are related to a change in the Van der Waals (VW) energy value, and ion–ion interactions are related to a change in the electrostatic (ES) energy value, which can be calculated using the molecular mechanics function of the CAChe software program. Hydrogen bonding (HB) energy also contributes to ion–ion interactions. If there are water molecules at the site of interaction, the contribution of the hydrogen bonding energy value is decreased, but the contribution of the water molecule is neglected in the present molecular mechanics computer software.

A quantitative analysis of the retention mechanisms for a homologous series of compounds like phenolic compounds was performed with a simple model phase. The precision for drug analysis, however, was not sufficient when using the simple model phase. Drug analysis required a three-dimensional model phase to take into account the contribution of Van der Waals energy, which is related to the

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contact surface area between the analyte and model phase. On the other hand, electrostatic energy values were easily calculated using the simple model phase. The molecular interaction energy together with the dissociation constant makes it possible to predict the retention time of a variety of compounds in reversed-phase liquid chromatography.

Further study has been conducted using a calculation of the direct adsorption energy with model phases for the optimization of aromatic acids in reversed-phase liquid chromatography by a computational chemical method. The direct calculation of molecular interaction energy between a model-bonded phase and a benzoic acid derivative was performed using a molecular mechanics calculation of the CAChe program to develop a quantitative analysis of retention time in reversed-phase liquid chromatography. The dissociation constant, pK_a , was calculated from atomic partial charge using AM1 of MOPAC of CAChe program.

2. Experimental

The computer was a Dell model Latitude C840 equipped with a 2 GHz processor and 1024 MB of memory. The molecular properties of analytes and model phases and molecular interactions were calculated by molecular mechanics (MM2) from Version 5 of the CAChe program from Fujitsu, Tokyo, Japan. The standard parameters used were bond stretch, bond angle, dihedral angle, improper torsion, Van der Waals, hydrogen bond and electrostatic (MM2/MM3 bond dipoles). The Van der Waals cut-off distance was 9 Å. The energy unit was kcal/mole (1 kJ/mol = 4.18 kcal/mol). The molecular design is due to the capacity of the computer used. The optimized energy value was less than 0.00001 kcal/mol. The calculated energy values are listed in Tables 1–3. The Cricket-Graph program from Computer Associates (San Diego, CA, USA) was used for data handling. The chromatographic retention data were from refs. [2,6].

3. Results and discussion

The simplest model-phase in reversed-phase liquid chromatography is a graphitic carbon-phase that is a polycyclic aromatic hydrocarbon. The molecular properties of a model graphitic carbon were analyzed and the adsorption mechanisms of a variety of compounds were studied. A hydrophobic compound retained on the surface by hydrophobic interaction related to Van der Waals energy calculated using molecular mechanics in the CAChe program. No possibility of pi–pi interaction was observed. A polar compound, glucose, was retained at the edge of the model-phase by charge–transfer interaction [5]. The surface of the model-phase is flat, and the calculation of molecular interaction energy between the model-phase and an analyte was simple.

Therefore, a polycyclic aromatic hydrocarbon was first used as a simplest model-phase in reversed-phase liquid

chromatography. The larger phase consisting of 49 aromatic rings was first constructed to calculate molecular interaction energy (Δ value). The adsorption form of benzoic acid on the polycyclic aromatic hydrocarbon is shown in Fig. 1. After subtraction of the complex energy value from the sum of the individual energy value of an analyte and the model-phase (Δ value = energy value of an analyte + energy value of a model phase – energy value of a complex), the retention factors obtained by liquid chromatography were related to their final structure (FS), hydrogen bonding, electrostatic and Van der Waals energy values calculated with MM2 and listed in Table 1. The correlation coefficients between the model-phase and $\log k$ values of benzoic acid derivatives ($\log k_1$) listed in Table 1 were calculated as follows:

$$\Delta FS1 = 3.239 \times (\log k_1) + 13.396, \quad r = 0.724, n = 22, \quad (1)$$

$$\Delta VW1 = 3.765 \times (\log k_1) + 7.492, \quad r = 0.776, n = 22, \quad (2)$$

where, ΔFS and ΔVW were the interaction energy values of the final structure and Van der Waals energies. The $\log k_1$ values were measured in reversed-phase liquid chromatography using an octadecyl-bonded silica gel column in pH 2 eluent [6]. The correlation coefficient indicated that the predominant interaction was hydrophobicity where Van der Waals energy values demonstrated the highest correlation coefficient. The hydrogen bonding and electrostatic energy values did not show the correlation. The correlation between the Δ energy values and $\log k$ measured on an octadecyl-bonded polyvinylalcohol gel [7] was poor. The r -values for ΔFS and ΔVW were 0.405 and 0.505, respectively ($n = 22$). The low r -values indicated that the retention mechanisms on the polymer gel were not the same as those on the octadecyl-bonded silica gel. Some selectivity should be considered when a chemically stable octadecyl-bonded vinylalcohol gel is used for reversed-phase liquid chromatography. No correlation existed between these Δ energy values and $\log k$ values measured on polystyrene gels in reversed-phase liquid chromatography [8]. The slope indicated that the retention of benzoic acid derivatives did not correlate with Van der Waals energy values, even though the $\log k$ values were correlated with $\log P$ values [3]. These results indicated that the retention mechanism on a polystyrene gel is different from that on an alkyl-chain bonded silica gel. The model carbon phase did not explain the retention mechanism even when the polystyrene gel was an aromatic hydrocarbon.

The predominant molecular interaction was hydrophobic, and Van der Waals energy values contributed to the retention when reversed-phase liquid chromatography was performed using an alkyl-chain bonded silica gel for phenolic compounds [9]. The model butyl-bonded phase was therefore used for studying the effect of alkyl groups in the molecular

Table 1
Molecular properties of benzoic acid derivatives

Chemicals	FS	HB	ES	VW	FS1	HB1	ES1	VW1	log k_1	log k_2	log k_{2i}
2,4,6-Trimethylbenzoic acid	-8.6252	-3.442	-4.252	5.538	704.7818	-8.667	-3.990	63.292	1.116	-	-
2,4-Dichlorobenzoic acid	-19.5852	-3.568	-15.694	7.065	694.7570	-9.574	-15.438	65.479	1.178	-	-
2,4-Dimethylbenzoic acid	-11.2572	-3.518	-5.423	6.100	702.9060	-9.229	-5.431	64.577	1.117	-	-0.856
2,4-Dihydroxybenzoic acid	-19.4110	-9.308	-6.717	5.293	691.5560	-27.177	-6.370	72.171	-	-	-
2,5-Dichlorobenzoic acid	-16.3200	-3.561	-12.447	6.979	697.9774	-9.938	-12.195	65.918	1.110	-	-
2,5-Dimethylbenzoic acid	-11.1240	-3.521	-5.296	6.132	703.5891	-9.113	(5.056)	64.787	1.117	-	-
2,5-Dihydroxybenzoic acid	-19.5298	-9.344	-6.797	5.321	692.2385	-24.595	-6.458	69.027	-	-	-
2,6-Dichlorobenzoic acid	-23.4971	-3.452	-22.126	5.983	692.0870	-8.884	-21.545	66.569	0.789	-	-
2,6-Dimethylbenzoic acid	-7.7343	-3.439	-3.949	5.532	707.5040	-8.889	-3.706	65.365	0.815	-	-
2,6-Dihydroxybenzoic acid	-22.7430	-13.031	-7.809	5.950	688.6374	-30.245	-7.762	70.179	-	-	-
2-Bromobenzoic acid	-16.0131	-3.564	-12.649	6.760	699.4113	-9.966	-12.398	67.007	0.774	-	-
2-Chlorobenzoic acid	-17.5343	-3.570	-13.381	6.789	698.3831	-9.817	-13.141	67.100	0.710	-	-
2-Ethylbenzoic acid	-9.1914	-3.545	-5.443	6.198	706.6502	-9.515	-5.060	66.486	-	-	-
2-Methylbenzoic acid	-10.4933	-3.518	-5.251	6.113	705.5074	-9.614	-4.994	66.384	0.824	-	-
2-Methoxybenzoic acid	-11.3306	-3.587	-6.585	5.932	710.4371	-8.392	-6.628	65.162	-	-	-
2-Hydroxybenzoic acid	-17.1254	-7.822	-6.681	5.336	693.8632	-25.636	-6.353	72.260	-	0.912	-0.645
3,4,5-Trihydroxybenzoic acid	-23.1168	-11.337	-6.767	4.883	686.5325	-28.352	-6.519	67.273	-	-0.495	-1.987
3,4-Dichlorobenzoic acid	-9.3448	-3.447	-3.612	6.005	705.1582	-9.532	-3.375	64.671	1.371	-	-
3,4-Dimethylbenzoic acid	-14.3591	-3.462	-6.660	5.364	699.9775	-9.290	-6.421	63.639	1.082	-	-
3,4-Dihydroxybenzoic acid	-18.3220	-6.869	-6.532	4.803	693.6499	-20.933	-6.267	68.525	-	-0.181	-1.510
3,5-Dichlorobenzoic acid	-15.2967	-3.449	-8.568	5.360	699.1785	-9.619	-8.331	64.120	1.442	-	-
3,5-Dimethylbenzoic acid	-15.2490	-3.466	-6.932	4.958	698.6293	-9.421	-6.691	62.901	1.160	-	-
3,5-Dihydroxybenzoic acid	-18.4059	-6.537	-6.743	4.760	693.9818	-21.033	-6.474	69.527	-	-0.242	-1.287
3-Bromobenzoic acid	-13.2092	-3.456	-6.363	5.198	702.3478	-9.809	-6.121	65.233	1.073	-	-
3-Chlorobenzoic acid	-13.3325	-3.453	-6.350	5.111	702.7270	-9.727	-6.110	65.605	0.993	-	-
3-Ethylbenzoic acid	-13.7274	-3.462	-6.736	5.233	701.7864	-9.623	-6.490	64.773	-	-	-
3-Methylbenzoic acid	-14.5031	-3.462	-6.737	4.926	701.2304	-9.596	-6.491	65.010	0.867	-	-
3-Methoxybenzoic acid	-12.4574	-3.470	-6.743	5.165	702.8158	-9.641	-6.496	64.832	-	0.883	-0.369
3-Hydroxybenzoic acid	-16.0948	-4.985	-6.655	4.820	696.4237	-19.737	-6.371	70.077	-	0.246	-0.908
4-Bromobenzoic acid	-13.2872	-3.453	-6.439	5.159	702.2165	-9.553	-6.201	64.926	1.096	1.429	-0.374
4-Chlorobenzoic acid	-13.2874	-3.453	-6.440	5.158	702.6337	-9.465	-6.190	65.270	1.010	1.325	0.163
4-Ethylbenzoic acid	-13.7157	-3.458	-6.719	5.208	701.7422	-9.249	-6.478	64.525	1.157	1.515	0.212
4-Methylbenzoic acid	-14.5173	-3.458	-6.719	4.878	701.3074	-9.331	-6.477	64.677	0.847	1.128	-0.167
4-Methoxybenzoic acid	-10.1574	-3.456	-6.671	6.213	705.1619	-9.330	-6.435	65.564	-	-	-0.187
4-Hydroxy-3-methoxybenzoic acid	-14.4088	-5.174	-6.647	5.166	694.5556	-19.047	-6.451	66.066	-	0.148	-1.084
4-Hydroxybenzoic acid	-15.5198	-5.182	-6.658	4.787	698.8075	-13.986	-6.396	63.782	-	0.262	-1.142
Benzoic acid	-13.9181	-3.459	-6.671	4.876	703.7209	-9.449	-6.438	66.762	0.574	0.765	-0.517
Carbon-phase	731.8482	0.000	0.130	70.778							

FS, HB, ES and VW: energy values of final structure, hydrogen bonding, electrostatic and Van der Waals (kcal/mol) of benzoic acid derivatives; FS1, HB1, ES1 and VW1: energy values of final structure, hydrogen bonding, electrostatic and Van der Waals (kcal/mol) of a complex of model carbon-phase and a benzoic acid derivative; log k_1 : retention factors from ref. [6]; log k_2 (molecular form) and log k_{2i} (ionized form): retention factor from ref. T. Hanai, J. Hubert, J. Chromatogr. 316 (1984) 261–265.

interactions of benzoic acid derivatives in reversed-phase liquid chromatography. The model butyl-bonded phase consisted of 628 carbons and 216 hydrogens and 1197 bonds and 6768 connectors. The molecular weight was 7752. The adsorption form of benzoic acid on the butyl-phase is shown in Fig. 2. After subtraction of the complex energy from the sum of the individual energies of the analytes and the butyl-phase, the retention factors obtained by liquid chromatography were related to their final structure, hydrogen bonding, electrostatic and Van der Waals energy values calculated with MM2 and listed in Table 2.

$$\Delta\text{FS2} = 2.068 \times (\log k_1) + 7.107, \quad r = 0.687, n = 22, \quad (3)$$

$$\Delta\text{VW2} = 1.587 \times (\log k_1) + 7.886, \quad r = 0.521, n = 22, \quad (4)$$

The energy values of flat molecules correlated well with the log k values, but the energy values of compounds having steric hindrance were less than that expected from log k values for phenolic compounds. The correlation coefficient between molecular interaction energy values and the log k of aromatic acids was not excellent due to the steric hindrance of some compounds. Models of several alkyl chain-bonded silica gels were constructed to eliminate steric effect. A model dimethylpentylsilane-bonded silica phase was useful for quantitative analysis of the retention of phenolic compounds [10]. The molecular interaction energy of benzoic acid derivatives was calculated using the model

Table 2
Molecular interaction energy values of benzoic acid derivatives

Chemicals	FS2	HB2	ES2	VW2	FS3	HB3	ES3	VW3
2,4,6-Trimethylbenzoic acid	3354.1217	-3.443	-4.260	415.033	665.7833	-3.480	-355.832	635.528
2,4-Dichlorobenzoic acid	3344.0379	-3.572	-15.695	417.350	655.0733	-3.597	-367.596	636.366
2,4-Dimethylbenzoic acid	3352.6070	-3.518	-5.601	416.364	663.0578	-3.518	-356.985	634.533
2,4-Dihydroxybenzoic acid	3345.7726	-9.284	-6.708	417.203	658.4374	-9.681	-358.449	638.581
2,5-Dichlorobenzoic acid	3346.6643	-3.557	-12.451	416.686	659.0773	-3.547	-364.095	637.426
2,5-Dimethylbenzoic acid	3352.4404	-3.505	-5.280	416.053	663.6032	-3.677	-357.228	635.171
2,5-Dihydroxybenzoic acid	3344.9820	-9.375	-6.794	416.467	659.9836	-9.486	-358.474	640.442
2,6-Dichlorobenzoic acid	3340.8967	-3.488	-22.069	417.507	653.9944	-3.493	-373.662	639.233
2,6-Dimethylbenzoic acid	3355.6645	-3.388	-3.897	415.447	668.7392	-3.466	-355.530	636.880
2,6-Dihydroxybenzoic acid	3350.5446	-13.035	-7.806	425.889	657.4710	-13.060	-359.425	641.281
2-Bromobenzoic acid	3348.0342	-3.567	-12.646	417.569	660.8043	-3.615	-364.393	639.593
2-Chlorobenzoic acid	3346.9728	-3.514	-13.370	416.965	660.0688	-3.695	-365.364	638.950
2-Ethylbenzoic acid	3355.8856	-3.534	-5.273	417.904	668.1971	-3.565	-357.072	639.155
2-Methylbenzoic acid	3354.0768	-3.461	-5.201	416.234	667.1022	-3.534	-356.815	639.154
2-Methoxybenzoic acid	3355.3736	-3.582	-6.687	416.043	667.9472	-3.577	-358.200	640.170
2-Hydroxybenzoic acid	3348.6404	-7.920	-6.623	417.646	663.2607	-7.835	-358.264	641.126
3,4,5-Trihydroxybenzoic acid	3341.5103	-11.358	-6.776	416.315	653.4582	-12.967	-358.548	638.154
3,4-Dichlorobenzoic acid	3353.8374	-3.465	-3.623	416.074	665.3251	-4.367	-355.139	635.672
3,4-Dimethylbenzoic acid	3349.6937	-3.464	-6.662	415.998	660.7502	-3.707	-358.156	635.563
3,4-Dihydroxybenzoic acid	3346.9732	-6.890	-6.545	416.808	659.4534	-7.234	-358.200	638.187
3,5-Dichlorobenzoic acid	3348.1026	-3.450	-8.567	415.519	660.3372	-3.668	-360.664	636.568
3,5-Dimethylbenzoic acid	3347.8388	-3.458	-6.928	414.778	659.5881	-3.653	-358.876	634.681
3,5-Dihydroxybenzoic acid	3346.3498	-6.556	-6.747	416.298	659.0355	-8.377	-358.041	638.253
3-Bromobenzoic acid	3350.2403	-3.462	-6.365	415.520	661.9330	-4.514	-357.892	635.332
3-Chlorobenzoic acid	3350.6834	-3.452	-6.348	415.951	662.9764	-4.364	-357.884	636.237
3-Ethylbenzoic acid	3350.2947	-3.481	-6.749	416.102	663.0791	-3.502	-358.425	637.353
3-Methylbenzoic acid	3349.0077	-3.456	-6.732	415.273	661.7913	-3.986	-358.120	636.001
3-Methoxybenzoic acid	3350.8786	-3.475	-6.747	415.275	664.2649	-3.474	-358.431	636.575
3-Hydroxybenzoic acid	3348.7300	-5.003	-6.659	416.438	662.6316	-5.091	-358.524	638.846
4-Bromobenzoic acid	3350.9572	-3.456	-6.443	416.072	662.1248	-3.507	-358.278	635.897
4-Chlorobenzoic acid	3351.0843	-3.444	-6.422	416.414	662.1190	-3.507	-358.279	635.861
4-Ethylbenzoic acid	3350.0377	-3.460	-6.721	415.770	660.1631	-3.533	-358.514	634.500
4-Methylbenzoic acid	3349.6076	-3.451	-6.714	415.712	661.6807	-3.746	-358.239	636.523
4-Methoxybenzoic acid	3352.7495	-3.444	-6.663	416.004	666.4812	-3.482	-358.429	638.078
4-Hydroxy-3-methoxybenzoic acid	3349.7273	-5.173	-6.736	416.828	662.3273	-5.512	-358.338	637.464
4-Hydroxybenzoic acid	3349.5118	-5.183	-6.657	415.514	662.3578	-5.509	-358.471	638.391
Benzoic acid	3351.6603	-3.475	-6.682	417.184	665.0985	-3.951	-358.075	639.139
Phase	3373.0354	0	0	419.957	693.5732	0	-350.055	649.282

FS2, HB2, ES2 and VW2: energy values of complex with butyl-phase; FS3, HB3, ES3 and VW3: energy values of complex with pentyl-bonded silica phase.

dimethylpentylsilane bonded phase that consisted of 686 atoms, 746 bonds and 5130 connectors. The energy values of complexes are not listed in Table 2. The correlations between molecular interaction energy values and $\log k_1$ values of benzoic acid derivatives are:

$$\Delta FS = 3.972 \times (\log k_1) + 13.000, \quad r = 0.815, n = 22, \quad (5)$$

$$\Delta VW = 4.302 \times (\log k_1) + 12.497, \quad r = 0.771, n = 22. \quad (6)$$

These correlation coefficients were not satisfactory enough to apply to this computational chemical method for the optimization of liquid chromatographic conditions. Notably 3,4-dichloro and 3,5-dichlorobenzoic acids were outliers. The origins of the error were analyzed by making a comparison with the data for phenolic compounds analyzed previously. The comparison of ΔFS values of benzoic acid

derivatives and phenolic compounds measured previously [10] indicated steric hindrance for 2,6-dimethyl, 2,6-dichloro and 2-ethylbenzoic acids, but not for 3,4-dichloro and 3,5-dichlorobenzoic acids. This means that the theoretically calculated energy values should be acceptable, but the retention times of these dichlorobenzoic acids were longer than expected. The reason for this may be the property of the bonded-phase in old-type bonded silica gels and the inertness was not guaranteed. In addition, these dichlorobenzoic acids were not outliers in the chromatography using the octadecyl-bonded polyvinylalcohol gel that did not have any silanol effect [7]. The correlation was recalculated without these dichlorobenzoic acids, and the values were improved. The r for ΔFS and ΔVW was 0.911 and 0.905, respectively ($n = 20$).

$$\Delta FS = 5.455 \times (\log k_1) + 11.660, \quad r = 0.911, n = 20, \quad (7)$$

Table 3
Molecular interaction energy values of benzoic acid derivatives

Chemicals	FSi	HBi	ESi	VWi	FS3i	HB3i	ES3i	VW3i
2,4,6-Trimethylbenzoic acid	5.9723	0	5.574	6.190	681.0784	0	-345.374	636.147
2,4-Dichlorobenzoic acid	-14.7810	0	-15.333	6.665	660.8674	0	-366.549	636.293
2,4-Dimethylbenzoic acid	1.2046	0	2.692	6.089	676.5859	0	-348.248	634.973
2,4-Dihydroxybenzoic acid	-7.1968	-2.813	-2.030	5.953	671.7559	-3.165	-352.926	639.703
2,5-Dichlorobenzoic acid	-11.4074	0	-11.965	6.695	665.6455	0	-363.459	638.731
2,5-Dimethylbenzoic acid	1.3154	0	2.799	6.130	677.4321	0	-348.719	636.905
2,5-Dihydroxybenzoic acid	-7.3230	-2.792	-2.156	5.979	672.9253	-2.935	-353.278	641.287
2,6-Dichlorobenzoic acid	-26.6594	0	-30.310	5.869	650.4113	0	-381.627	637.804
2,6-Dimethylbenzoic acid	6.8717	0	5.884	6.148	684.0866	0	-345.150	637.741
2,6-Dihydroxybenzoic acid	-6.4107	-2.651	-3.513	7.497	674.2711	-2.686	-354.571	643.041
2-Bromobenzoic acid	-10.6595	0	-11.721	6.714	666.8024	0	-362.886	639.259
2-Chlorobenzoic acid	-12.7746	0	-13.032	6.491	666.3541	0	-364.24	639.481
2-Ethylbenzoic acid	3.6903	0	2.980	6.572	681.8481	0	-348.02	639.299
2-Methylbenzoic acid	1.9783	0	2.871	6.105	680.3445	0	-348.181	639.300
2-Methoxybenzoic acid	-0.2372	0	-0.102	5.757	677.5379	0	-351.099	638.103
2-Hydroxybenzoic acid	-5.0058	-1.321	-2.075	6.045	674.6934	-1.334	-353.331	640.498
3,4,5-Trihydroxybenzoic acid	-11.7332	-7.754	-0.208	4.741	666.1980	-9.286	-351.258	638.525
3,4-Dichlorobenzoic acid	2.1915	0	3.213	5.874	677.9470	0	-348.583	636.130
3,4-Dimethylbenzoic acid	-3.0187	0	-0.015	5.231	673.1785	0	-351.224	636.233
3,4-Dihydroxybenzoic acid	-7.0139	-3.339	0	4.668	672.0637	-3.686	-350.903	638.593
3,5-Dichlorobenzoic acid	-3.6542	0	-1.633	5.219	670.9216	0	-354.488	637.136
3,5-Dimethylbenzoic acid	-3.9390	0	-0.315	4.822	672.2625	0	-351.554	635.116
3,5-Dihydroxybenzoic acid	-7.1042	-2.963	-0.247	4.620	671.1521	-4.272	-351.840	638.867
3-Bromobenzoic acid	-1.7129	0	0.429	5.061	674.5229	0	-351.372	635.684
3-Chlorobenzoic acid	-1.8150	0	0.462	4.975	675.5756	0	-351.341	636.763
3-Ethylbenzoic acid	-2.3839	0	-0.095	5.105	675.6559	0	-351.065	637.721
3-Methylbenzoic acid	-3.1647	0	-0.095	4.794	673.9969	0	-351.548	636.300
3-Methoxybenzoic acid	-0.9413	0	0.022	5.027	676.8184	0	-350.937	636.793
3-Hydroxybenzoic acid	-4.8686	-1.466	-0.185	4.686	675.1146	-1.474	-351.345	639.481
4-Bromobenzoic acid	-1.9130	0	0.230	5.025	674.5226	0	-351.058	636.258
4-Chlorobenzoic acid	-1.9144	0	0.230	5.026	674.6309	0	-350.919	636.299
4-Ethylbenzoic acid	-2.3449	0	-0.051	5.078	672.9319	0	-351.077	634.953
4-Methylbenzoic acid	-3.1471	0	-0.051	5.078	674.3795	0	-351.180	637.044
4-Methoxybenzoic acid	1.2012	0	-0.026	6.087	678.5889	0	-351.075	637.998
4-Hydroxy-3-methoxybenzoic acid	-3.0308	-1.690	0.003	5.042	674.9903	-2.036	-350.905	637.845
4-Hydroxybenzoic acid	-4.1908	-1.712	-0.046	4.657	675.1330	-1.984	-350.999	638.814
Benzoic acid	-2.5493	0	0	4.743	677.3723	0	-351.574	639.536
Phase	693.5732	0	-350.055	649.282				

FSi, HBi, ESi, VWi: energy values of final structure, hydrogen bonding, electrostatic and Van der Waals (kcal/mol) of ionized benzoic acid derivatives; F3i, HB3i, ES3i and VW3i: energy values of complex of ionized benzoic acid derivatives and pentyl-bonded silica phase; unit, kcal/mol.

$$\Delta VW = 6.246 \times (\log k_1) + 10.741, \quad r = 0.905, n = 20. \quad (8)$$

$$\Delta FS3 = 7.139 \times (\log k_1) + 11.022, \quad r = 0.948, n = 20, \quad (11)$$

Further study was performed after the eight pentyl groups were unlocked. The adsorption of benzoic acid in the pentyl-bonded phase is shown in Fig. 3. The energy values of complexes are listed in Table 2. The correlation coefficients are:

$$\Delta FS3 = 5.082 \times (\log k_1) + 12.880, \quad r = 0.831, n = 22, \quad (9)$$

$$\Delta VW3 = 7.959 \times (\log k_1) + 10.751, \quad r = 0.916, n = 20. \quad (12)$$

$$\Delta VW3 = 5.413 \times (\log k_1) + 13.052, \quad r = 0.768, n = 22. \quad (10)$$

The correlation coefficients were improved when 3,4- and 3,5-dichlorobenzoic acids were eliminated as outliers.

The correlation coefficients between $\log k_1$ and $N \log P$ [3] or $V \log P$ [3] were 0.638 and 0.680 ($n=20$), respectively. The correlation coefficient of this new system was far higher than that of the old system using $\log P$. Above system was seemed to work fine for developing a quantitative analysis of reversed-phase liquid chromatography of benzoic acid derivatives. This approach was applied to another set of data for aromatic acids used to identify aromatic acids in urine [2]. The retention factors were measured using various pH-controlled eluents. The correlation coefficients for ΔFS of

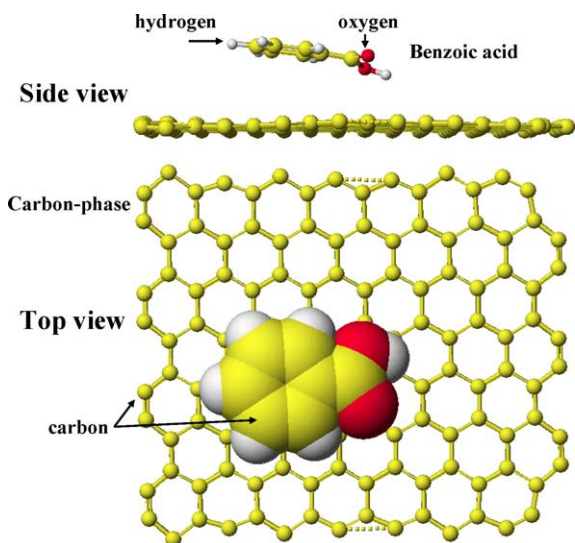


Fig. 1. Adsorption of benzoic acid on a model carbon-phase. Black ball, oxygen; grey ball, carbon; small white ball, hydrogen; atomic size is basically 20% for the phase.

the benzoic acid derivatives is:

$$\Delta\text{FS3} = 4.085 \times (\log k_2) + 9.612, \quad r = 0.947, n = 12, \quad (13)$$

where this ΔFS3 was fixed by subtraction of individual hydrogen bonding energy values. The hydrogen bonding energy value is zero for the model bonded-phase, but the hydrogen bonding energy values of hydroxyl-substituted benzoic acid derivatives were very high. The ΔHB3 values were identical to hydrogen bonding energy values of the original molecules, and are not related to molecular interaction. The adsorption method can calculate the molecular interaction of ionized compounds that the $\log P$ system cannot handle. The energy values of ionized benzoic acid derivatives and their complexes with the phase 3 are listed in Table 3. The correlation coefficient between $\log k$ values of ionized benzoic acid derivatives and ΔFS3i was 0.942.

$$\Delta\text{FS3i} = 4.207 \times (\log k_i) + 17.001, \quad r = 0.942, n = 14. \quad (14)$$

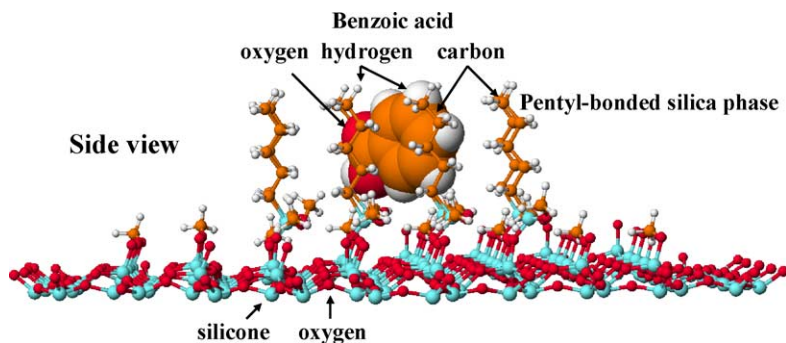


Fig. 3. Adsorption of benzoic acid on a model pentyl-bonded silica phase. Black ball, oxygen; dark grey ball, carbon; grey ball, silicone; small white ball, hydrogen; atomic size is basically 20% for the phase.

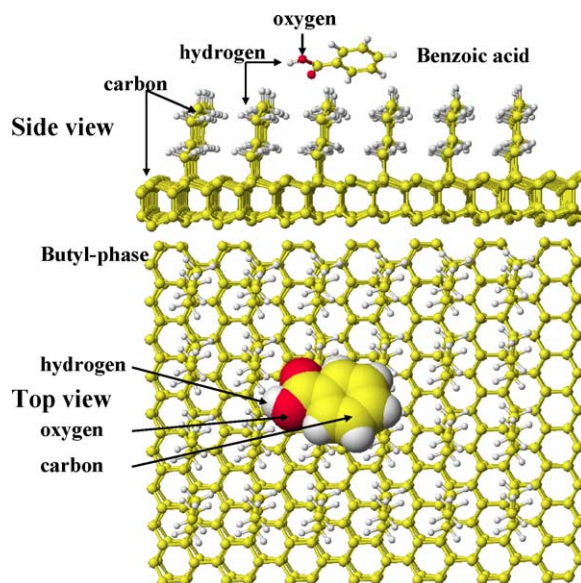


Fig. 2. Adsorption of benzoic acid on a model butyl-phase. Black ball, oxygen; grey ball, carbon; small white ball, hydrogen; atomic size is basically 20% for the phase.

However, the value of *ortho*-hydroxyl benzoic acid was excluded as an outlier. The value of the molecular form can be included, and the correlation coefficient was slightly improved, but the $\log k$ value of ionized form was relatively high.

The retention factor of partially ionized compounds can be predicted using the following equation:

$$k = \frac{\{k_m + k_i([\text{H}^+]/K_a)\}}{\{1 + ([\text{H}^+]/K_a)\}} \quad (15)$$

where k_m is the maximum retention factor of the molecular form of the analytes; k_i is the retention factor of the fully ionized compound; K_a is the dissociation constant; and $[\text{H}^+]$ is the hydrogen ion concentration of the eluent. For evaluation by the above approach, k_m and k_i were replaced with energy values calculated using Eqs. (13) and (14), respectively. The predicted $\text{p}K_a$ values from atom partial charge related to measured $\text{p}K_a$ values in liquid chromatography [3] were used for the calculation.

$$k_{\text{pred.}} = 1.123 \times (k_{\text{mes.}}) + 0.416, \quad r = 0.920, \quad n = 14, \quad \text{at pH } 3.55,$$

$$k_{\text{pred.}} = 1.063 \times (k_{\text{mes.}}) + 0.116, \quad r = 0.936, \quad n = 14, \quad \text{at pH } 4.05,$$

$$k_{\text{pred.}} = 1.001 \times (k_{\text{mes.}}) - 0.069, \quad r = 0.952, \quad n = 14, \quad \text{at pH } 4.56,$$

$$k_{\text{pred.}} = 0.874 \times (k_{\text{mes.}}) - 0.054, \quad r = 0.962, \quad n = 14, \quad \text{at pH } 5.05,$$

$$k_{\text{pred.}} = 1.022 \times (k_{\text{mes.}}) - 0.052, \quad r = 0.962, \quad n = 14, \quad \text{at pH } 5.55.$$

The predicted pK_a values from atom partial charge related to the pK_a values derived from Hammett's equations [3] were used for further evaluation.

$$k_{\text{pred.}} = 1.083 \times (k_{\text{mes.}}) + 0.339, \quad r = 0.918, \quad n = 14, \quad \text{at pH } 3.55,$$

$$k_{\text{pred.}} = 0.988 \times (k_{\text{mes.}}) + 0.024, \quad r = 0.932, \quad n = 14, \quad \text{at pH } 4.05,$$

$$k_{\text{pred.}} = 0.893 \times (k_{\text{mes.}}) - 0.109, \quad r = 0.949, \quad n = 14, \quad \text{at pH } 4.56,$$

$$k_{\text{pred.}} = 0.772 \times (k_{\text{mes.}}) - 0.059, \quad r = 0.960, \quad n = 14, \quad \text{at pH } 5.05,$$

$$k_{\text{pred.}} = 0.931 \times (k_{\text{mes.}}) - 0.046, \quad r = 0.962, \quad n = 14, \quad \text{at pH } 5.55.$$

The measured retention times at low pH were shorter than those expected from Eq. (15) due to an ion-exclusion effect [3]. Those at high pH were too small to obtain precise values. Several approaches have been proposed for the prediction of pK_a values, but it is still difficult to obtain precise values for a variety of compounds at present. However, the above approach using a direct calculation of molecular interaction energy values and pK_a values derived from atom partial charge is better than the old model using $\log P$ and pK_a values. This is, because the correlation coefficient is equivalent and the slope is close to 1. Further development of a model

phase suitable for a variety of compounds and pK_a prediction system based on atom partial charge is necessary to establish as a standard method for determining a quantitative structure retention relationship in liquid chromatography.

4. Conclusion

Computational chemical calculation using a molecular mechanics can replace a quantitative structure retention relationship using octanol–water partition coefficient ($\log P$). The $\log P$ is a property of molecular form compounds but not a property of ionized form compounds. The retention factors of benzoic acid derivatives correlated well with the molecular interaction energy values especially Van der Waals energy values. The correlation coefficient was satisfactory even if the retention factors were predicted with the dissociation constant derived from atom partial charge at a various pH-controlled eluents.

References

- [1] T. Hanai, *Chromatographia* 12 (1979) 77.
- [2] T. Hanai, J. Hubert, J. High Resolut. Chromatogr. Chromatogr. Commun. 7 (1984) 524.
- [3] T. Hanai, H. Homma, J. Liq. Chromatogr. Rel. Technol. 25 (2002) 1661.
- [4] T. Hanai, *HPLC, A Practical Guide*, The Royal Society of Chemistry, Cambridge, 1999.
- [5] T. Hanai, *J. Chromatogr. A* 989 (2003) 183.
- [6] Y. Arai, J. Yamaguchi, T. Hanai, *J. Chromatogr.* 400 (1987) 21.
- [7] Y. Arai, M. Hirukawa, T. Hanai, *Nippon Kagaku Kaishi* 1986 (1986) 969.
- [8] T. Hanai, K.C. Tran, J. Hubert, *J. Chromatogr.* 239 (1982) 385.
- [9] T. Hanai, Chi. Mizutani, H. Homma, *J. Liq. Chromatogr. Rel. Technol.* 26 (2003) 2031.
- [10] T. Hanai, *J. Chromatogr. A* 1027 (2004) 279.