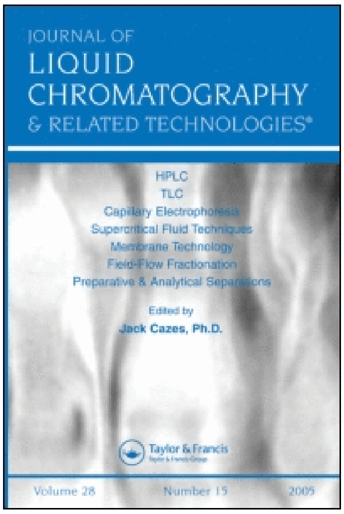

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PREDICTION OF RETENTION FACTORS OF PHENOLIC AND NITROGEN-CONTAINING COMPOUNDS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY BASED ON logP AND pKa OBTAINED BY COMPUTATIONAL CHEMICAL CALCULATION

Toshihiko Hanai[a]; Kuniko Koizumi; Toshio Kinoshita[b]
[a] Institut Pasteur 5F, Kyoto, Japan [b] School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan

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PREDICTION OF RETENTION FACTORS OF PHENOLIC AND NITROGEN-CONTAINING COMPOUNDS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY BASED ON logP AND pKa OBTAINED BY COMPUTATIONAL CHEMICAL CALCULATION

Toshihiko Hanai,[1,]* Kuniko Koizumi,[2] Toshio Kinoshita[3]

[1] Health Research Foundation
Institut Pasteur 5F
Sakyo-ku
Kyoto 606-8225, Japan

[2] Yokogawa Analytical Systems
Mitaka Bldg, Musashino
Tokyo 180-0006, Japan

[3] School of Pharmaceutical Sciences
Kitasato University
Shirokane, Minatoku
Tokyo 108-0072, Japan

ABSTRACT

The new logP values (NlogP) of phenolic and nitrogen-containing compounds based on the modified CAChe™ MOPAC calculation improved the correlation coefficient with measured logP values (MlogP) to 0.950 from 0.662 (n = 87).

The best correlation coefficient was obtained between retention factors of their molecular form and NlogP values among NlogP, CAChe logP, MOPAC-BlogP, Rekker's logP, Hansch's logP, and MlogP. Correlation coefficients were 0.880 (n = 50) for phenolic compounds and 0.900 (n = 48) for nitrogen-containing compounds. The correlation coefficients between predicted and measured maximum retention factors were 0.883 (n = 50) for phenolic compounds and 0.891 (n = 48) for nitrogen-containing compounds.

Addition of pka values predicted by partial charges of atoms calculated using CAChe™ MOPAC to NlogP values could predict their retention factors in eluents at given pH. The correlation coefficient between predicted and measured retention factors of phenolic compounds at pH 8.49 was 0.927 (n = 20), and that of nitrogen-containing compounds at pH 4.0 was 0.833 (n = 45).

INTRODUCTION

The retention time on reversed-phase liquid chromatography can be predicted using the octanol/water partition coefficient, logP, and dissociation constant, pKa.[1-3] Therefore, simple prediction methods are required for logP and pKa values. The logP values are generally used to determine quantitative structure-activity relationships, and can be measured by liquid chromatography. Since Henry et al.,[4] Mirrles et al.,[5] and Miyake and Terada[6] directly measured partition coefficients in liquid chromatography, numerous studies have been performed to measure logP values in liquid chromatography; e.g. for polypeptides and proteins,[7] steroids,[8] heteroaromatic compounds,[9] benzophenones,[10] and barbiturates[11] and N-hydroxyethylamide of aryloxyalkylene and pyridine carboxylic acids.[12] Hanai used logP values to predict retention times in reversed-phase liquid chromatography.[13,14]

The pKa values were measured by both titration and liquid chromatography, or were predicted from partial charges of atoms[15] or Hammett's equations.[16] Hardcastle and Jano reviewed the determination of dissociation constants of polyprotic acids from chromatographic data.[17] The pKa values of non-steroidal anti-inflammatory drugs were estimated,[18] and the effects of components, i.e. buffer and acetonitrile, in the eluent on liquid chromatographic measurements were described.[19]

Hansch et al. first proposed an empirical method for prediction of logP values based on examination of a large database measured by the partition method.[20] Later, Rekker proposed the logP fragmental constants.[21] Hansch pi (ClogP) and Rekker's f (RlogP) constants have been used for prediction of logP values. Moriguchi et al. proposed a method using multiple regression analysis.[22]

The commercial computer program for logP calculation, MaclogP, is based on Hansch's method.[23] Computational chemistry allows the development of such predictive methods based on theoretical calculations. Boder proposed a method known as BlogP™ based on MOPAC calculation.[24] However, this method is not suitable for larger sized molecules, because the molecular size has a negative effect in the equation. A calculation method based on MOPAC calculation called CAChe logP™ was developed by CAChe Scientific.[15] However, the accuracy of the CAChe logP (CAlogP) method was not sufficient due to the poor correlation between CAlogP and logP values obtained experimentally (MlogP) by octanol-water partitioning for phenolic and nitrogen-containing compounds.

The retention factor of ionizable compounds in solution of a given pH can be predicted in reversed-phase liquid chromatography using the following equation:

$$k = (k_m + k_i[Ka]/[H^+])/(1 + [Ka]/[H^+]) \qquad (1)$$

where k_m and k_i are the retention factors of the molecular and ionized form analytes, respectively, and Ka is the dissociation constant of the analyte.[25] $[H^+]$ is the hydrogen ion concentration in the eluent. The k_m, maximum retention factor, can be related to octanol-water partition coefficient (log P) of analytes. The prediction of dissociation constant (pKa) was achieved using partial charges of atoms calculated by computational chemical calculation.[26]

In this study, several logP calculation methods were evaluated by comparison with reference values,[21,24] and a new method was proposed for optimization of reversed-phase liquid chromatography. The new logP values (NlogP) were evaluated with logk values of phenolic and nitrogen-containing compounds measured in reversed-phase liquid chromatography. Furthermore, the retention factors of these compounds in eluents at a given pH were predicted using NlogP values and pKa values were predicted using partial charges of atoms estimated using computational chemical calculations.

EXPERIMENTAL

Sixty-four phenolic and 48 nitrogen-containing compounds were collected for analysis. These compounds can be readily obtained and handled for liquid chromatographic analysis. In addition, 11 alkylbenzenes and 7 alkylphenones were used as calibration standards. Their molecular properties are summarized in Tables 1-3.

The computer used for the calculation was a Macintosh 8100/100 running the CAChe™ program including ProjectLeader™ from Sony-Tektronix (Tokyo), and Cricket-Graph™ from Computer Associates. RlogP values were calculated by a method based on that proposed by Rekker.[21]

Table 1

Molecular Properties and LogP Values of Neutral Compounds*

Chemicals	HOF	SAS	HOF/H_2O	CAlogP	NlogP	MlogP	ClogP	RlogP
Benzene	22.025	59.003	18.563	2.535	2.426	2.13	2.14	2.280
Toluene	14.4.22	65.908	10.869	2.830	2.922	2.73	2.64	2.558
Ethylbenzene	9.124	72.339	5.646	3.137	3.376	3.15	3.17	3.118
Propylbenzene	2.343	79.967	-1.196	3.472	3.917	3.72	3.70	3.648
Butylbenzene	-4.481	87.174	-8.024	3.813	4.430	4.38	4.23	4.178
Pentylbenzene	-11.320	94.788	-14.812	4.171	4.969	4.90	4.76	----
Hexylbenzene	-18.165	102.443	-21.615	4.509	5.512	5.52	5.29	5.238
Heptylbenzene	-25.019	109.758	-28.418	4.839	6.031	----	5.82	5.758
Octylbenzene	-31.860	117.520	-35.034	5.163	6.579	----	6.34	6.297
Nonylbenzene	-38.694	125.148	-41.672	----	7.119	----	----	----
Decylbenzene	-45.547	132.708	49.328	----	7.247	----	----	----
Acetophenone	-15.008	74.803	-24.714	1.950	1.722	1.58	1.58	1.750
Propiophenone	-20.796	81.771	-29.669	2.291	2.207	2.19	2.11	2.280
Butylophenone	-26.883	88.704	-35.497	2.623	2.696	----	2.64	2.810
Pentylophenone	-34.526	96.737	-43.328	2.984	3.269	----	3.17	3.340
Hexylophenone	-41.364	104.473	-50.177	3.333	3.818	----	3.70	3.870
Heptylophenone	-48.201	111.951	-57.071	3.665	4.349	----	4.23	4.400
Octylophenone	-55.028	119.721	-63.902	4.002	4.900	----	4.75	4.930

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* HOF: heat of formation, SAS: surface area, HOF/H_2O: heat of formation at AM1/H_2O geometry, CAlopP: CAChelog P, NlogP: new log P, MlogP: measured log P, ClogP: Hansch's log P, RlogP: Rekker's log P.

The molecules were first optimized by molecular mechanics calculation, optimized again by MOPAC, then their logP values were obtained with the BlogP program.[24] Properties for the calculation were selected according to the manufacturer's manual (CAChe Scientific). CAlogP values were calculated using ProjectLeader™ as part of the CAChe™ program. The properties selected were solvent-accessible surface area at AM1/H_2O geometry (SAS), heat of formation at AM1/H_2O geometry (HOF/H_2O) and heat of formation of MM/AM1 geometry (HOF) of analytes.[15] MlogP is reference value experimentally obtained by the partition method.[23]

The liquid chromatograph was a model HP1090 from Hewlett-Packard (Palo Alto, CA, USA), and the octadecyl bonded silica gel column, Luna C18, 15 cm x 4.6 mm I.D., was from Phenomenex Inc. (Torrance, CA, USA). Chemicals used were from Aldrich and ChemService. HPLC grade acetonitrile was from Fisher Scientific and HPLC grade water was from Merck.

The retention factors of 64 phenolic compounds listed in Tables 2a and 2b were measured by reversed-phase liquid chromatography at 40°C in 70% aqueous acetonitrile containing 20 mM sodium phosphate buffer at pH 4.01, 8.49, and 10.00. The retention factors of 48 nitrogen-containing compounds listed in Table 3a and 3b were measured at 40°C in 60% aqueous acetonitrile containing 20 mM sodium phosphate buffer at pH 1.48, 4.01, and 10.00.

RESULTS AND DISCUSSION

The correlation coefficients between logP values predicted by different systems and the values measured by partition (MlogP) for 112 compounds listed in Tables 2 and 3 including phenolic and nitrogen-containing compounds varied from 0.666 to 0.976 (n ~ 87). The best predicted values were ClogP values with a correlation constant and correlation coefficient of 0.908 and 0.976, respectively. The average predicted logP values were about 91% of measured logP values (MlogP). Further analyses were carried out for individual groups of compounds to improve the calculation system.

The correlation coefficient between logP values predicted for phenolic compounds by different methods varied from 0.753 to 0.960 (n ~ 64), and that for nitrogen-containing compounds varied from 0.595 to 0.986 (n ~ 48). CAlogP and BlogP values were smaller than other logP values. RlogP values were similar to MlogP values but further studies are required to improve the correlation coefficient. ClogP values were correlated well with MlogP values, but the predicted logP values were about 84% of MlogP values for phenolic compounds and 94% of MlogP values for nitrogen containing compounds. The above comparison indicated that ClogP and RlogP values gave the best predictions.

Table 2a

Molecular Properties and Retention Factors of Phenolic Compounds*

Chemical	HOF	SAS	HOF/H_2O	CAlogP	NlogP	Mlogp
Phenol	-22.247	62.809	-30.096	1.386	1.574	1.48
2-Methylphenol	-29.451	68.680	-36.791	1.661	1.994	1.97
3-Methylphenol	-29.828	69.543	-37.714	1.685	2.059	1.97
4-Methylphenol	-29.776	69.560	-37.670	1.686	2.060	1.95
2,3-Dimethylphenol	-35.164	73.728	-42.744	1.878	2.360	2.98
2,4-Dimethylphenol	-36.957	75.343	-44.330	1.957	2.474	2.30
2,5-Dimethylphenol	-37.026	75.443	-44.441	1.960	2.482	2.33
2,6-Dimethylphenol	-36.105	74.543	-42.737	1.944	2.410	2.36
3,4-Dimethylphenol	-36.730	74.718	-44.775	1.906	2.437	2.23
3,5-Dimethylphenol	-37.364	75.852	-45.386	1.960	2.516	2.33
2,3,4-Trimethylphenol	-41.960	78.949	-49.371	2.113	2.738	----
2,3,5-Trimethylphenol	-43.335	80.874	-50.807	2.200	2.874	----
2,3,6-Trimethylphenol	-42.569	80.126	-49.329	2.188	2.814	----
2,4,6-Trimethylphenol	-43.576	81.299	-50.302	2.243	2.897	----
2,3,4,5-Tetramethylphenol	-47.678	84.694	-55.513	2.358	3.152	----
2,3,5,6-Tetramethylphenol	-48.985	85.046	-55.900	2.399	3.173	----
Pentamethylphenol	-52.527	89.476	-59.571	2.598	3.487	----
2-Ethylphenol	-35.157	75.469	-42.053	1.985	2.470	2.47
3-Ethylphenol	-35.109	76.328	-42.916	1.999	2.536	2.50
4-Ethylphenol	-35.092	76.152	-42.871	1.991	2.523	2.43
4-tert.Butylphenol	-40.579	85.620	-48.218	2.438	3.180	3.31
2-Chlorophenol	-28.673	72.260	-35.352	1.860	2.225	2.16
3-Chlorophenol	-29.215	73.450	-37.196	1.876	2.318	2.48
4-Chlorophenol	-29.305	73.378	-37.583	1.863	2.316	2.42
2,3-Dichlorophenol	-33.973	81.532	-40.814	2.288	2.870	2.84

2,4-Dichlorophenol	-35.142	82.971	-41.967	2.355	2.971	3.15	2.96
2,5-Dichlorophenol	-35.098	82.751	-41.808	2.348	2.955	3.20	2.96
2,6-Dichlorophenol	-32.387	82.346	-39.603	2.323	2.921	2.75	2.96
3,4-Dichlorophenol	-34.657	82.538	-42.985	2.288	2.953	3.33	3.17
3,5-Dichlorophenol	-35.386	83.723	-43.328	2.356	3.033	3.62	2.39
2,3,4-Trichlorophenol	-38.901	90.491	-45.917	2.702	3.493	----	3.46
2,3,5-Trichlorophenol	-39.870	91.682	-46.436	2.771	3.573	----	3.58
2,3,6-Trichlorophenol	-37.402	91.244	-44.574	2.740	3.538	3.77	3.25
2,4,5-Trichlorophenol	-40.031	91.750	-46.789	2.767	3.580	3.72	3.58
2,4,6-Trichlorophenol	-38.314	92.619	-45.293	2.810	3.632	3.69	3.37
2,3,5-Trichlorophenol	-39.276	91.443	-47.521	2.709	3.569	4.01	3.79
2,3,4,5-Tetrachlorophenol	-43.281	99.283	-50.004	3.124	4.098	4.21	4.06
2,3,5,6-Tetrachlorophenol	-41.828	100.351	-48.672	3.179	4.164	3.88	3.85
Pentachlorophenol	-44.738	107.547	-51.524	3.521	4.658	5.06	4.32
2-Bromophenol	-16.525	77.032	-23.485	2.133	2.491	2.34	2.35
3-Bromophenol	-17.127	78.257	-25.737	2.139	2.590	2.63	2.63
4-Bromophenol	-17.597	78.290	-26.507	2.129	2.597	2.60	2.63
2,4-Dibromophenol	-10.553	89.988	-18.468	2.761	3.339	3.35	3.31
2,6-Dibromophenol	-7.961	91.511	-16.192	2.836	3.432	3.36	3.03
1,2-Dihydroxybenzene	-62.837	66.587	-76.057	0.820	0.712	0.89	0.88
1,3-Dihydroxybenzene	-66.797	66.576	-78.803	0.842	0.719	0.78	0.81
1,4-Dihydroxybenzene	-65.657	66.701	-77.954	0.843	0.724	0.55	0.81
1-Hydroxynaphthalene	-2.983	81.129	-11.683	2.333	2.720	2.84	2.65
2-Hydroxynaphthalene	-3.807	81.445	-13.094	2.326	2.749	2.70	2.65
2-Hydroxyacetophenone	-55.452	77.726	-70.568	0.736	0.803	----	1.48

(continued)

Table 2a (Continued)

Molecular Properties and Retention Factors of Phenolic Compounds

Chemical	HOF	SAS	HOF/H_2O	CAlogP	NlogP	Mlogp	ClogP
4-Hydroxybenzoic acid butylester	-125.382	107.400	-139.565	1.518	1.114	----	1.07
4-Hydroxybenzoic acid propylester	-118.589	99.777	-132.817	1.170	0.574	----	0.54
2-Nitrophenol	-15.789	73.337	-34.563	0.124	1.665	1.73	1.85
3-Nitrophenol	-17.432	74.455	-34.627	0.219	1.731	2.00	1.85
4-Nitrophenol	-19.557	74.526	-37.283	0.201	1.751	1.91	1.85
2,4-Dinitrophenol	-13.269	84.817	-36.820	0.752	1.782	1.67	1.79
2,5-Dinitrophenol	-10.905	85.081	-33.379	0.808	1.779	1.75	1.79
2,6-Dinitrophenol	-9.411	83.350	-33.963	0.664	1.675	1.37	1.79
3,4-Dinitrophenol	-4.798	84.444	-30.613	1.480	1.754	----	1.79
1-Hydroxy-2,4-dinitronaphthalene	7.751	101.040	-14.393	1.673	2.760	----	2.96
2-Chloro-5-methylphenol	-33.703	78.797	-41.716	2.119	2.696	----	2.65
4-Chloro-2-methylphenol	-35.469	79.258	-43.106	2.146	2.732	2.78	2.98
4-Chloro-3-methylphenol	-36.369	78.550	-44.360	2.095	2.698	3.10	2.98
4-Chloro-3,5-dimethyl-phenyl	-42.941	83.965	-50.926	2.336	3.083	----	3.48

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* HOF: heat of formation, SAS: Surface area, HOF/H_2O: heat of formation at AM1/H_2O geometry, CAlogP: CAChe log P: NlopP: new log P, MlopP: measured logP, ClogP: Hansch's logP.

However, these prediction methods still required further improvement to break the structure into appropriate fragments for which constants are available. Furthermore, RlogP and ClogP calculations are empirical methods, and have disadvantages for new compounds due to the lack of suitable fragment constants. Therefore, a variety of prediction methods for logP values have been developed, and new software is improving the precision.

Generally, increasing molecular size is correlated with increasing hydrophobicity. This phenomenon can be readily observed from the retention time of homologous series compounds including peptides and proteins on liquid chromatography. So, as logP values increase the surface area of molecules increases. The accuracy of prediction of logP values of isomers should be improved by addition of other molecular properties. Therefore, methods for improvement of the CAlogP calculation method were studied. The measured logP values, MlogP, provide less data, and thus ClogP values were chosen as the standard.

The original CAlogP calculation equation was:

$$\text{CAlogP} = -0.33262 + 0.048959 \times (\text{SAS}) - 0.027225 \times (\text{HOF}) + 0.03116 \times (\text{HOF/water}) - 1.4003 \times \sqrt{(\text{N})} - 1.0241 \times \sqrt{(\text{O})} + 1.3851 \times \sqrt{(\text{NO2})} \quad (2)$$

where SAS, HOF, and HOF/water are solvent-accessible surface area, heat of formation in vacuum and heat of formation in water, respectively, as calculated by ProjectLeader™ of CAChe program using MOPAC,[14] and N, O, and NO_2 are numbers of nitrogen, oxygen and nitro groups, respectively.

The correlation coefficients between predicted logP values of standard compounds, alkylbenzenes (n = 9) and alkylphenones (n = 7), calculated by several methods were 1.000.

$$\text{CAlogP (alkylbenzenes)} = 0.632 \times \text{ClogP} + 1.157, \quad r^2 = 1.000, n = 9$$

$$\text{CAlogP (alkylphenones)} = 0.649 \times \text{ClogP} + 0.921, \quad r^2 = 1.000, n = 7$$

The slopes for alkylbenzenes and alkylphenones were considered to be nearly equivalent, and thus modification of the constants of SAS, HOF, and HOF/water was first examined to obtain a slope value of 1.0 between modified CAlogP (NlogP) and ClogP. The relations between logP values and solvent-accessible surface area (SAS) of alkylbenzenes and alkylphenones were excellent, and the correlation coefficients were 1.000.

$$\text{ClogP (alkylbenzenes)} = 0.072(\text{SAS}) - 2.071, \; r^2 = 1.000, \; (n = 9),$$

$$\text{ClogP (alkylphenones)} = 0.071(\text{SAS}) - 3.674, \; r^2 = 1.000, \; (n = 7).$$

Table 2b

Molecular Properties and Retention Factors of Phenolic Compounds*

Chemical	RlogP	BlogP	logk pH401	pre1 logkmax	pKa	k(exp) pH8.49	pre2 pH8.49
Phenol	1.54	1.295	-0.188	-0.225	9.93	0.661	0.575
2-Methylphenol	2.05	1.699	-0.045	-0.109	10.26	0.910	----
3-Methylphenol	2.05	1.706	-0.085	-0.092	10.10	0.830	----
4-Methylphenol	2.05	1.703	-0.085	-0.091	10.10	0.830	----
2,3-Dimethylphenol	2.49	2.163	0.056	-0.009	10.75	1.151	----
2,4-Dimethylphenol	2.49	2.162	0.069	0.023	10.26	1.180	----
2,5-Dimethylphenol	2.49	2.141	0.067	0.025	10.42	1.169	----
2,6-Dimethylphenol	2.49	2.138	0.105	0.005	10.10	1.279	----
3,4-Dimethylphenol	2.49	2.146	0.002	0.012	10.10	1.021	----
3,5-Dimethylphenol	2.49	2.139	0.027	0.034	10.26	1.074	----
2,3,4-Trimethylphenol	3.02	2.562	----	0.095	10.42	----	----
2,3,5-Trimethylphenol	3.02	2.578	0.174	0.133	10.42	1.510	----
2,3,6-Trimethylphenol	3.02	2.568	0.214	0.116	10.42	1.648	----
2,4,6-Trimethylphenol	3.02	2.597	0.229	0.139	10.10	1.710	----
2,3,4,5-Tetramethylphenol	3.55	2.921	----	0.209	11.07	----	-----
2,3,5,6-Tetramethylphenol	3.55	2.922	----	0.215	10.91	----	----
Pentamethylphenol	4.08	3.205	----	0.301	11.56	----	----
2-Ethylphenol	2.58	2.099	0.090	0.022	10.42	1.239	----
3-Ethylphenol	2.58	2.096	0.034	0.040	10.10	1.074	----
4-Ethylphenol	2.58	2.118	0.038	0.036	10.10	1.099	----
4-tert.Butylphenol	3.60	2.887	0.238	0.217	9.93	1.734	----
2-Chlorophenol	2.27	1.743	-0.051	-0.046	8.79	0.700	0.599
3-Chlorophenol	2.27	1.731	-0.016	-0.020	9.28	0.879	0.821
4-Chlorophenol	2.27	1.773	-0.034	-0.021	9.28	0.889	0.820

2,3-Dichlorophenol	2.93	2.203	0.086	0.132	8.31	0.310	0.538
2,4-Dichlorophenol	2.93	2.252	0.108	0.159	7.98	0.460	0.341
2,5-Dichlorophenol	2.93	2.304	0.123	0.155	7.98	0.390	0.337
2,6-Dichlorophenol	2.93	2.278	0.108	0.146	6.35	0.091	0.010
3,4-Dichlorophenol	2.93	2.221	0.125	0.154	8.63	0.920	0.828
3,5-Dichlorophenol	2.93	2.276	0.093	0.176	8.47	0.681	0.733
2,3,4-Trichlorophenol	3.68	2.684	0.252	0.303	7.65	0.140	0.254
2,3,5-Trichlorophenol	3.68	2.740	0.322	0.325	7.49	0.094	0.192
2,3,6-Trichlorophenol	3.68	2.735	0.255	0.315	5.86	0.042	0.005
2,4,5-Trichlorophenol	3.68	2.775	0.303	0.327	7.49	0.110	0.193
2,4,6-Trichlorophenol	3.68	2.802	0.329	0.341	5.70	0.050	0.004
3,4,5-Trichlorophenol	3.68	2.722	----	0.324	7.98	----	----
2,3,4,5-Tetrachlorophenol	4.43	3.170	----	0.469	7.00	----	----
2,3,5,6-Tetrachlorophenol	4.43	3.238	----	0.488	5.54	----	----
Pentachlorophenol	5.17	3.640	0.496	0.623	5.05	0.085	0.002
2-Bromophenol	2.48	2.197	-0.016	0.027	8.63	0.750	0.618
3-Bromophenol	2.48	2.229	0.021	0.055	9.28	0.940	0.976
4-Bromophenol	2.48	2.148	0.007	0.056	9.12	0.971	0.923
2,4-Dibromophenol	3.35	3.001	0.219	0.261	8.14	0.650	0.562
2,6-Dibromophenol	3.35	2.956	0.201	0.286	6.19	0.086	----
1,2-Dihydroxybenzene	1.00	0.878	----	-0.462	6.19	----	----
1,3-Dihydroxybenzene	1.00	0.853	-0.424	-0.460	9.77	0.360	----
1,4-Dihydroxybenzene	1.00	0.878	-0.583	-0.459	9.93	0.280	----
1-Hydroxynaphthalene	2.86	2.618	0.072	0.090	9.93	1.159	----
2-Hydroxynaphthalene	2.86	2.612	0.020	0.098	9.93	1.030	----

(continued)

Table 2b (Continued)

Molecular Properties and Retention Factors of Phenolic Compounds*

Chemical	RlogP	BlogP	logk pH4.01	pre1 logkmax	pKa	k(exp) pH8.49	pre2 pH8.49
2-Hydroxynaphthalene	1.21	1.461	----	-0.437	----	1.330	----
4-Hydroxybenzoic acid butylester	3.20	2.930	----	-0.352	8.63	----	----
5-Hydroxybenzoic acid propylester	2.67	2.488	----	-0.500	8.63	----	----
2-Nitrophenol	1.92	1.644	0.060	-0.200	2.12	0.110	----
3-Nitrophenol	1.27	1.571	-0.158	-0.182	7.98	0.360	----
4-Nitrophenol	1.27	1.723	-0.198	-0.176	7.49	0.040	----
2,4-Dinitrophenol	1.58	1.221	----	-0.168	6.35	----	----
2,5-Dinitrophenol	1.58	1.420	-0.080	-0.169	7.00	0.029	----
2,6-Dinitrophenol	1.58	1.167	-0.234	-0.197	1.31	0.031	----
3,4-Dinitrophenol	0.93	0.849	----	-0.175	5.86	----	----
1-Hydroxy-2,4-dinitronapthalene	2.45	2.173	----	0.101	6.84	----	----
2-Chloro-5-methylphenol	2.71	2.140	0.069	0.084	7.82	1.009	----
4-Chloro-2-methylphenol	2.71	2.176	0.122	0.094	9.77	1.300	----
4-Chloro-3-methylphenol	2.71	2.189	0.202	0.084	9.44	1.169	----
4-Chloro-3,5-dimethyl-phenol	2.34	2.624	0.081	0.190	9.44	1.560	----

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* RlopP: Rekker's logP, BlogP: Border's logP, logk: measured logk, pH 4.01 in 70% aq. AcCN, pre1: predicted max. logk from NlogP in 70% aq. AcCN, pKa: predicted from part. change, k(exp): measured at pH 8.49 in 70% aq. AcCN, pre2: predicted k at pH 8.49 in 70% aq. AcCN.

The slopes were thus considered to be equivalent. Therefore, the results of alkylbenzenes (n = 9) and alkylphenones (n = 7) were used for further studies as standard compounds.

From evaluations using alkylbenzenes (n = 9) , alkylphenones (n = 7) and alkyl, chloro- and bromophenols (n = 44) as standard compounds, the following equation was derived, where NlogP was proposed as new logP value.

$$\text{NlogP} = -1.451 + 0.06687 \times (\text{SAS}) + 0.004180 \times (\text{HOF}) - 0.008687 \times (\text{HOF}/H_2O) - 1.981 \times (\text{O}) - 1.344 \times (\text{OH}) \qquad (3)$$

The individual slopes of NlogP and ClogP values of alkylbenzenes, alkylphenones and these phenols were close to 1.0 in this equation. The constant for carbonyl oxygen, -1.981, was obtained from the difference between alkylbenzenes and alkylphenones. That for oxygen of the hydroxy group, -1.344, was obtained from the differences between alkylbenzenes and these phenols. The NlogP values of phenolic compounds are summarized in Tables 2a and 2b with logP values obtained by other methods and their properties.

The constant -1.8193 (N1) was obtained for primary amines from anilines, and the constant was also used for tertiary amines of pyridines, pyrazines and quinolines. The constants for secondary and tertiary amines were -1.6193 and -1.093 from N-alkylanilines.

The elimination of the nitro group constant was examined, but good results were not achieved. The constants for nitrogen and oxygen of the nitro group were different from the general constants for these atoms. The final equation was :

$$\text{NlogP} = -1.451 + 0.06687 \times (\text{SAS}) + 0.004180 \times (\text{HOF}) - 0.008687 \times (\text{HOF}/H_2O) - 1.8193 \times \sqrt{(\text{N1})} - 1.344 \times (\text{OH}) - 1.6193 \times \sqrt{(\text{N2})} - 1.093 \times \sqrt{(\text{N3})} - 0.937 \times \sqrt{(\text{O})} - 1.981 \times \sqrt{(\text{CO})} - 0.68 \times (NO_2) \qquad (4)$$

where O was divided into two groups, O of ether and carbonyl oxygen.

The NlogP values are summarized in Table 3a and 3b. The correlation constant and correlation coefficient of NlogP values against MlogP values (n = 87) were 0.922 and 0.955, respectively, and these values were improved from 0.670 and 0.666, respectively. The correlation constant and correlation coefficient between NlogP and ClogP values (n = 112) used for improving the CAlogP calculation system were 0.994 and 0.957, respectively, improved from 0.676 and 0.544, respectively. These logP values were correlated with logk values of molecular form phenolic and nitrogen-containing compounds given in Tables 2 and 3.

Table 3a

Molecular Properties and Retention Factors of Nitrogen Containing Compounds*

Chemical	HOF	SAS	HOF/H_2O	CAlogP	NlogP	Mlogp	ClogP
Aniline	20.495	63.994	11.413	1.197	0.995	0.90	0.91
2-Methylaniline	13.183	69.971	4.423	1.471	1.425	1.32	1.36
3-Methylaniline	13.010	70.924	3.945	1.508	1.493	1.40	1.41
4-Methylaniline	12.973	71.236	4.145	1.530	1.511	1.39	1.41
2,4-Dimethylaniline	5.679	76.912	-3.219	1.778	1.924	1.68	1.86
4-Methoxyaniline	-15.364	75.813	-25.947	2.975	1.024	0.95	1.00
2,5-Diethoxyaniline	-65.065	101.191	-79.033	5.304	2.586	----	2.26
2-Chloroaniline	13.111	73.619	4.297	1.648	1.670	1.90	1.91
3-Chloroaniline	13.285	74.738	4.088	1.692	1.747	1.88	1.91
4-Chloroaniline	13.081	75.092	3.674	1.702	1.774	1.88	1.91
2,5-Dichloroaniline	6.278	84.310	-2.446	2.148	2.415	2.92	2.71
3,4-Dichloroaniline	7.537	83.898	-2.103	2.104	2.390	2.69	2.59
4-Bromoaniline	24.648	79.537	14.589	1.944	2.025	2.26	2.06
2-Nitroaniline	20.771	74.728	4.338	1.495	1.096	1.85	1.80
3-Nitroaniline	24.024	75.982	6.200	1.526	1.177	1.37	1.26
4-Nitroaniline	21.559	76.257	2.373	1.487	1.219	1.39	1.26
N-Methylaniline	24.233	71.300	16.709	0.218	1.654	1.66	1.64
N-Ethylaniline	18.675	79.520	11.287	0.603	2.227	2.16	2.17
N-Butylaniline	4.376	94.010	-2.622	1.269	3.257	----	3.23

N-Dimethylaniline	31.685	77.212	25.862	1.989	2.601	2.31	2.34
N-Diethylaniline	21.884	89.487	16.334	2.561	3.463	3.31	3.23
Pyridine	32.039	57.746	24.347	0.980	0.514	0.65	0.64
2-Aminopyridine	32.981	63.325	20.608	-0.290	0.170	0.49	0.32
3-Aminopyridine	30.081	63.070	17.189	-0.329	0.170	0.11	0.32
4-Aminopyridine	29.069	63.212	15.540	-0.346	0.190	0.32	0.32
2-Methylpyridine	25.643	65.051	18.212	1.320	1.029	1.11	1.14
3-Methylpyridine	24.089	64.529	16.473	1.283	1.002	1.20	1.14
4-Methylpyridine	24.164	64.729	16.342	1.287	1.017	1.22	1.14
4-tert.Butylpyridine	13.100	80.817	5.579	2.041	2.140	----	2.47
2,4-Dimethylpyridine	17.812	71.997	10.270	1.627	1.529	----	1.64
2,5-Dimethylpyridine	17.687	72.014	10.389	1.635	1.529	----	1.64
2,6-Dimethylpyridine	19.503	72.383	12.463	1.668	1.543	1.68	1.64
Pyrazine	44.215	56.501	33.553	0.294	-0.352	-0.23	-0.31
2-Methylpyrazine	37.218	63.900	26.956	0.641	0.171	0.23	0.19
2,5-Dimethylpyrazine	30.303	71.217	20.484	0.986	0.687	0.63	0.69
2,6-Dimethylpyrazine	30.470	71.318	20.771	0.995	0.692	0.54	0.69
Quinoline	52.192	77.462	43.481	1.992	1.750	2.03	2.03
2-Methylquinoline	46.030	84.359	37.577	2.313	2.237	2.61	2.53
4-Methylquinoline	45.205	82.768	36.472	2.223	2.137	2.60	2.53
8-Methylquinoline	45.087	82.960	37.526	2.269	2.14	2.02	2.08
8-Hydroxyquinoline	10.673	80.631	-3.323	3.206	1.777	2.59	2.53
1-Aminoindane	16.188	78.380	9.020	1.944	1.960	----	1.51
5-Aminoindane	10.202	79.078	0.777	1.885	2.054	----	1.93

(continued)

Table 3a (Continued)

Molecular Properties and Retention Factors of Nitrogen Containing Compounds*

Chemical	HOF	SAS	HOF/H_2O	CAlogP	NlogP	Mlogp	ClogP
1-Aminonaphthalene	40.297	81.845	29.866	2.106	2.112	2.25	2.09
2-Aminonaphthalene	39.142	83.189	28.335	2.156	2.210	2.28	2.09
1-Aminoantharacene	62.643	100.972	50.511	3.077	3.305	3.69	3.26
1-Aminopyrene	66.697	104.869	53.624	3.254	3.555	----	3.72
5-Aminoindole	54.792	77.518	39.412	0.397	1.046	----	1.93

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* HOF: heat of formation, SAS: surface area, HOF/H_2O: heat of formation at AM1/H_2O geometry, CAlogP: CAChe logP, NlogP: new logP, MlogP: measured logP, ClogP: Hansch's log P.

The correlation coefficient between logP and logk values for phenolic compounds varied from 0.631 to 0.880 (n ~ 50) and that for nitrogen-containing compounds varied from 0.365 to 0.900 (n ~ 48). The following equations were obtained:

MlogP = 4.101 x logk + 2.431, r^2 = 0.859, (n = 42)

ClogP = 3.439 x logk + 2.419, r^2 = 0.869, (n = 50)

RlogP = 3.906 x logk +2.398, r^2 = 0.862, (n = 50)

BlogP =2.700 x logk + 2.018, r^2 = 0.837, (n = 50)

CAlogP = 3.034 x logk + 1.809, r^2 = 0.631, (n = 50)

NlogP = 3.635 x logk + 2.392, r^2 = 0.880, (n = 50)

For nitrogen-containing compounds, the following results were obtained:

MlogP = 1.672 x logk + .0.690, r^2 = 0.845, (n = 37)

ClogP = 1.563 x logk + 0.770, r^2 = 0.840, (n = 48)

RlogP = 1.750 x logk + .0.597, r^2 = 0.831, (n = 40)

BlogP = 1.311 x logk + 0.866, r^2 = 0.774, (n = 48)

CAlogP = 1.184 x logk + 0.928, r^2 = 0.364, (n = 48)

NlogP = 1.644 x logk + 0.635, r^2 = 0.900, (n = 48)

The best correlation coefficients were obtained between NlogP and logk values for phenolic and nitrogen-containing compounds. The predicted retention factors of molecular form phenolic and nitrogen-containing compounds were correlated with the measured values (k_{exp}). For phenolic compounds, the predicted retention factors were correlated with those measured at pH 4.01.

k_{pred} = 1.182 (k at pH 4.01) - 0.199, r^2=0.883, n=50,

For nitrogen containing compounds, the predicted retention factors were correlated with those measured at pH 10.00.

k_{pred} = 1.045 (k at pH 10.00) + 0.167, r^2=0.891, n=48.

Table 3b

Molecular Properties and Retention Factors of Nitrogen Containing Compounds*

Chemical	RlogP	BlopP	logk pH 10.00	pre1 logk max	pKa	k(exp) pH 4.01	pre2 pH 4.01
Aniline	1.03	1.128	0.407	0.219	4.66	2.323	0.297
2-Methylaniline	1.54	1.573	0.611	0.481	4.66	2.314	0.543
3-Methylaniline	1.54	1.576	0.618	0.522	4.91	1.082	0.365
4-Methylaniline	1.54	1.583	0.587	0.533	4.91	0.531	0.374
2,4-Dimethylaniline	1.98	2.026	0.792	0.784	4.79	1.182	0.850
4-Methoxyaniline	1.10	0.630	0.246	0.237	5.39	0.015	0.068
2,5-Diethoxyaniline	2.17	1.523	0.958	1.187	2.85	6.158	14.373
2-Chloroaniline	1.76	1.664	0.858	0.630	2.61	7.188	4.098
3-Chloroaniline	1.76	1.605	0.819	0.677	3.94	5.960	2.539
4-Chloroaniline	1.76	1.600	0.757	0.693	4.06	4.263	2.297
2,5-Dichloroaniline	2.42	2.162	1.232	1.083	1.76	17.216	12.043
3,4-Dichloroaniline	2.42	2.081	1.078	1.068	3.33	12.033	9.635
4-Bromoaniline	1.96	1.981	0.835	0.846	3.58	5.504	5.081
2-Nitroaniline	1.87	1.924	0.716	0.281	0.26	5.438	1.908
3-Nitroaniline	1.22	1.647	0.632	0.330	2.73	4.492	2.029
4-Nitroaniline	1.22	1.708	0.525	0.355	0.55	3.536	2.263
N-Methoxyaniline	1.62	1.498	0.825	0.620	3.70	5.604	2.778
N-Ethylaniline	2.15	1.903	1.067	0.969	0.07	7.974	9.307
N-Butylaniline	3.21	2.810	1.663	1.596	3.94	38.406	21.061

N-Dimethylaniline	2.28	2.103	1.308	1.196	----	12.128	----
N-Diethylaniline	3.34	2.728	1.766	1.721	----	21.868	----
Pyridine	0.70	0.887	0.042	-0.073	5.62	0.866	0.163
2-Aminopyridine	-0.33	0.528	-0.134	-0.283	3.58	0.000	0.377
3-Aminopyridine	-0.33	0.551	-0.327	-0.283	4.18	0.000	0.207
4-Aminopyridine	-0.33	0.510	-0.323	-0.271	2.85	0.000	0.501
2-Methylpyridine	1.06	1.256	0.145	0.240	6.21	0.632	0.065
3-Methylpyridine	1.06	1.257	0.214	0.223	5.39	0.643	0.065
4-Methylpyridine	1.06	1.203	0.196	0.233	6.09	0.516	0.014
4-tert.Butylpyridine	2.65	2.408	0.858	0.916	5.97	3.618	0.087
2,4-Dimethylpyridine	----	1.637	0.340	0.544	6.56	0.207	0.010
2,5-Dimethylpyridine	----	1.693	0.356	0.544	5.86	0.247	0.048
2,6-Dimethylpyridine	----	1.687	0.343	0.553	6.44	0.045	0.013
Pyrazine	----	0.029	-0.284	-0.600	0.64	0.488	0.251
2-Methylpyrazine	----	0.371	-0.177	-0.282	0.37	0.630	0.533
2,5-Dimethylpyrazine	----	0.821	-0.067	0.032	1.71	0.813	1.071
2,6-Dimethylpyrazine	----	0.797	-0.076	0.035	1.77	0.793	1.078
Quinoline	1.98	1.821	0.494	0.679	4.80	0.977	0.653
2-Methylquinoline	2.51	2.158	0.627	0.975	5.39	0.263	0.369
4-Methylquinoline	2.51	2.143	0.657	0.914	5.27	0.421	0.418
8-Methylquinoline	2.51	2.203	0.880	0.916	4.92	2.371	0.884
8-Hydroxyquinoline	----	0.771	0.676	0.695	----	0.780	----
1-Aminoindane	2.27	1.35	0.413	0.806	9.14	0.000	0.000
5-Aminoindane	2.27	2.339	0.859	0.864	4.61	0.676	1.440

(continued)

Table 3b (Continued)

Molecular Properties and Retention Factors of Nitrogen Containing Compounds*

Chemical	RlogP	BlopP	logk pH 10.00	pre1 logk max	pKa	k(exp) pH 4.01	pre2 pH 4.01
1-Aminonaphthalene	2.18	2.448	0.913	0.899	4.42	6.024	2.182
2-Aminonaphthalene	2.18	2.477	0.917	0.958	3.58	6.082	6.585
1-Aminoanthracene	3.35	3.434	1.428	1.625	4.42	22.061	11.610
1-Aminopyrene	4.00	3.747	1.599	1.777	3.58	39.077	43.345
5-Aminoindole	1.36	1.496	0.142	0.250	5.93	0.000	0.021

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*RlogP: Rekker's logP, BlogP: Border's logP, logk: measured logk at pH 10.00 in 60% aq. acetonitrile, pre1: predicted maximum logk from NlogP in 60% aq. acetonitrile, pKa: predicted from partial charge, k(exp): measured at pH 4.01 in 60% aq. acetonitrile, pre2: predicted k at pH 4.01in 60% aq. acetonitrile.

NlogP values can be used for optimization of reversed-phase liquid chromatography of each group of compounds, and replace RlogP values.[1-3] The retention factors of phenolic and nitrogen-containing compounds in solutions of a given pH were predicted using equation (1). The retention factors of alkylphenols at pH 10.0 were not minimum retention factors due to their high pKa values. Therefore, the prediction of retention factors was studied for halogenated phenols. The predicted k_m and pKa values were used for the calculation. However k_i values could not be predicted, and in the calculations these values were either their retention factors measured at pH 10.00 or 1.48 or zero. The values of k_m were derived from the above results, i.e. the relation between NlogP and logk values. The pKa values were predicted from their partial charges of atoms.[26] The predicted retention factors for halogenated phenols at pH 8.49 were correlated with the measured values (k_{exp}).

$k_{pred} = 0.986(k_{exp}) + 0.067$, $r^2 = 0.925$ $(n = 20)$ using k_i at pH 10.00,

$k_{pred} = 0.909(k_{exp}) + 0.020$, $r^2 = 0.927$ $(n = 20)$ using $k_i = 0$.

The retention factors of 48 nitrogen-containing compounds measured at pH 4.01 were correlated with those predicted using the above methods, a combination of logP and pKa values predicted by computational chemical calculations. The relation between predicted and measured retention factors at pH 4.01 was:

$k_{pred} = 0.810(k_{exp}) - 0.224$, $r^2 = 0.836$ $(n = 45)$ using k_i at pH 1.48,

$k_{pred} = 0.797(k_{exp}) - 0.232$, $r^2 = 0.833$ $(n = 45)$ using $k_i = 0$,

$k_{pred} = 0.807(k_{exp}) - 0.095$, $r^2 = 0.763$ $(n = 45)$ using $k_i = 0$ and pKa obtained liquid chromatography.

At present, retention factors of ionized compounds cannot be predicted because logP values are based on the properties of molecular form compounds. However, k_I is close to zero for many compounds, the prediction of retention times can be performed with $k_i = 0$. The pKa values predicted from partial charges are used. However, further precise calculation is necessary to improve the precision of pKa values.

CONCLUSIONS

The NlogP calculation method is a simple approach compared to Hansch's equations (ClogP) that require many fragmental constants based on a large database measured by the partition method. However, the elimination of constants for atoms and groups is necessary as a purely theoretical system for prediction of logP values. The dissociation constant, pKa, can be predicted by either Hammett's equations or partial charges of atoms. It is difficult to fix ortho effects by either methods. The constants for Hammett's equations require a large database. A new method based on partial charges of atoms requires standard values. However, this method is simpler than selecting a specific Hammett's equation. Further development of quantum chemistry and further analyses of the properties obtained by the present computational chemical method are necessary. NlogP and pKa derived from one system can be used for optimization of reversed-phase liquid chromatographic conditions for phenolic and nitrogen-containing compounds.

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