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Hydrophobicity Parameters Determined by Reversed-Phase Liquid Chromatography. XV: Optimal Conditions for Prediction of $\log P_{oct}$ by Using RP-HPLC Procedures

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The use of $\log k$ derived from reversed phase (RP)-HPLC retention times provides a convenient method for estimating $\log P_{oct}$ values (P_{oct}: 1-octanol/water partition coefficient). In order to establish optimal HPLC conditions, the difference between chromatographic (C18 modified column and aqueous methanol eluents) and bulk solvent systems was examined by use of a batch-like equilibration with octane/aqueous methanol. Comparison of values for $\log P_{O/M-W}$ ($\log P$ for the Octane/MeOH–Water partitioning system) and $\log k$ measured for monosubstituted pyrazines at different methanol concentrations, showed closest correlation (*r*, 0.94) with 50% aqueous methanol; and importantly, under these conditions, $\log k$ shows even better correlation (*r*, 0.99) with $\log P_{oct}$. Effects of residual silanols on HPLC retention with C18 stationary phases were examined. The results show that, in the presence of a small quantity of accessible silanols, the use of 50% aqueous methanol (M50) as eluent yields values of $\log k$ directly proportional to $\log P_{oct}$ in accord with our earlier proposal that use of $\log k_{M50}$ provides a convenient means for rapid estimation and prediction of $\log P_{oct}$.

Key words hydrophobicity; partition coefficient; retention factor; hydrogen-bonding

Recently, in connection with the remarkable development of computational technologies for rational drug designs, the necessity has become apparent of rapid measurement or estimation of molecular properties of chemicals having a potential for medical use. In particular, hydrophobicity (as expressed by the logarithm of 1-octanol/water partition coefficient, $\log P_{oct}$) is identified as one of the most important properties that govern potencies; its prediction has attracted much attention from medicinal chemists, agrochemists and many investigators in various fields such as pharmacology, toxicology, environmental chemistry and food chemistry.[1,2] Instead of measuring $\log P_{oct}$ by the standard but time-consuming shake-flask method, reversed-phase chromatography (RP-HPLC) has increasingly been used as a powerful tool to predict $\log P_{oct}$ from the readily accessible retention factor, $\log k$, (given by $k=(t_R-t_0)/t_0$ where t_0 and t_R express elution times of the non-retained substance and sample, respectively).

Extensive studies have been made to establish an optimal RP-HPLC system providing a good linearity between $\log P_{oct}$ and $\log k$.[3—10] Many workers have used an ODS-type column with methanol–water mobile phases to obtain a normalized parameter, $\log k_W$ (k_W: the retention factor extrapolated to 0% organic modifier), as an alternative to $\log P_{oct}$.[3,5,8] In our experience, this $\log k_W$ approach works well for non-polar solutes but tends to provide overestimated $\log P_{oct}$ values for strong H-acceptors.[10—15] From systematic studies of $\log k$ obtained under various HPLC conditions for typical heteroaromatic compounds such as (di)azines, furans and thiophenes, we were led to the conclusion that the use of an eluent containing around 50% methanol, which usually provides a more direct correlation with $\log P_{oct}$, is the most convenient and practical procedure for rapid estimation of $\log P_{oct}$ provided H-donors (amphiprotic compounds) are treated separately.[11—15] Our results so far obtained are illustrated graphically in Fig. 1. This conclusion has been derived from the following results.

First, we have shown that, for each series of monosubstituted (di)azines, $\log P_{oct}$ can be excellently correlated with $\log k$ in different compositions of methanol–water eluents by a general formula Eq. 1 containing two terms correcting for H-bonding effects:[16)]

$$\log k = a\log P_{oct} + \rho\sigma_I + sS_{HA} + \text{const.} \qquad (1)$$

where the σ_I parameter represents the Charton's inductive electronic substituent constant,[17)] and S_{HA} designates the substituent H-acceptor scale that we have recently defined where the value of S_{HA} is set at unity for H (the unsubstituted

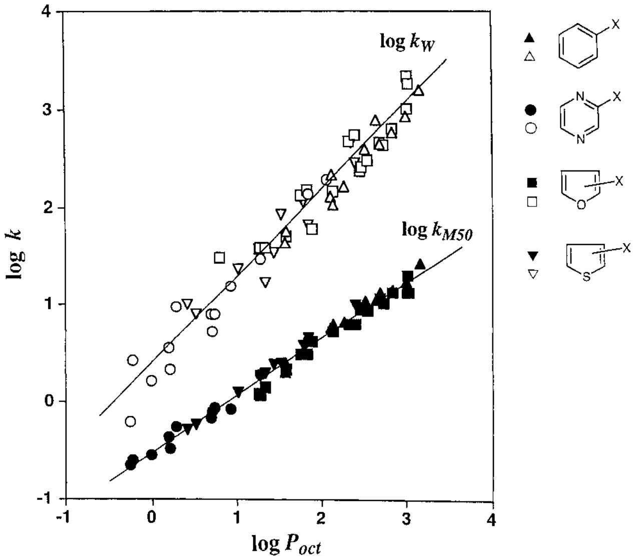

Fig. 1. Plots of $\log k_W$ and $\log k_{M50}$ against $\log P_{oct}$ for Various Monosubstituted (Hetero) Aromatic Compounds, AR-X

Benzenes[11)]: X=H, F, Cl, Br, Me, Et, OMe, OEt, CN, Ac, CO_2Me, CO_2Et. Pyrazines[11)]: X=H, Cl, Me, Et, OMe, OEt, OPr, NMe_2, CN, Ac, CO_2Me, CO_2Et, Ph. Furans[12,13)]: X=H, Cl, Br, Me, Et, OMe, CN, Ac, CO_2Me, CO_2Et, CO_2Pr, $CONMe_2$. Thiophenes[15)]: X=H, Me, Et, OMe, Ac, CO_2Me, CO_2Et, CO_2Pr, $CONMe_2$. Column: CAPCELL PAK C18 SG. Eluents: 0% MeOH ($\log k_W$, extrapolated) and 50% MeOH ($\log k_{M50}$).

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Table 1. log P Values, H-Bonding Parameters and Electronic Constants for Monosubstituted Pyrazines

Compound	Substituent	log $P_{\mathrm{O/M15}}$	log $P_{\mathrm{O/M50}}$	log P_{oct} a)	log P_{O} b)	S_{HA} c)	σ_{I} d)
1	H	−1.10	−1.25	−0.26	−1.04	1.00	0.00
2	Cl	0.18	−0.18	0.70	0.26	0.97	0.47
3	Me	−0.75	−1.05	0.21	−0.64	0.96	−0.01
4	Et	−0.14	−0.56	0.69	0.04	0.91	−0.01
5	OMe	0.23	−0.13	0.73	0.31	1.02	0.30
6	OEt	0.79	0.24	1.28	0.88	1.00	0.28
7	OPr	1.37	0.68	1.84	1.54 e)	0.99	0.28
8	NMe_2	−0.28	−0.78	0.93	−0.09	1.09	0.17
9	CN	−0.92	−1.15	−0.01	−0.93	1.21	0.57
10	Ac	−0.47	−0.75	0.20	−0.48	1.31	0.30
11	CO_2Me	−1.39	−1.50	−0.23	−1.34	1.62	0.32
12	CO_2Et	−0.83	−1.15	0.28	−0.75 e)	1.60	0.30
13	$CONMe_2$	−2.38	−2.43	−0.80	−2.47 e)	1.78	0.28

a) Ref. 11. *b*) Taken from ref. 37 unless otherwise noted. *c*) Ref. 16. *d*) Ref. 17. *e*) This work.

parent compound) and becomes more positive with increasing H-accepting ability of substituents (see Table 1).16) It has generally been observed that for an eluent containing 50% methanol, the regression coefficients of these two parameters, ρ and s, are very small or statistically insignificant, yielding in effect a simple linearity between P_{oct} and log k; the contributions of both of the two H-bonding correction terms increase with decreasing methanol concentration.16)

Secondly, in order to make the chromatographic system approximate more closely to the octanol–water partitioning system, we examined the effects of adding a small quantity of 1-octanol to the eluents for monosubtituted pyrazines.18) It has been found that replacing pure methanol by methanol containing 0.25% octanol by volume in the eluents results in a decrease in the H-bonding effects involved in Eq. 1. However at a concentration of 50% methanol, the effect of octanol was almost negligible (Fig. 4 in ref. 18), suggesting that at this concentration the chromatographic system has properties more similar to the octanol/water partitioning system than to eluents of other compositions.

To elucidate reasons why a better estimation can be made at 50% methanol, properties of the stationary phase should also be studied because the structure of alkyl-bonded phases is much more complicated relative to that of the corresponding liquid alkanes. Recently numerous modern stationary phases have been prepared and the retention mechanism has been studied in detail by using quantitative structure–retention relationships.19—22) Despite these efforts, no universal stationary phase has been found yet to obtain reliable log P_{oct}. Therefore, to get more insight into properties of the stationary phase, the HPLC system was simulated by a batch-like equilibrium between different compositions of aqueous methanol and octane (log $P_{\mathrm{O/M–W}}$), and then the resultant log $P_{\mathrm{O/M–W}}$ values were compared with the corresponding log k. Octane was chosen as a simple model for an alkyl-bonded stationary phase because our previous work23) had shown that log k, for the compounds tested in this study, on C18 column correlated linearly with those on the corresponding C8 column (r>0.999) at each methanol concentration. Measurements were undertaken for the series of monosubstituted pyrazines which we have so far used for various systematic studies.11,16,18) We further examined how the presence of residual silanols affects the relationship between log P_{oct} and log k by comparing the retention behavior on two C18-bonded stationary phases of the same type but with different degrees of residual silanols. Optimal HPLC conditions for predicting log P_{oct} for non-H-bonding and H-accepting solutes will be discussed on the basis of these experimental results.

Experimental

Chemicals Monosubstituted pyrazines, PR-X, with non-H-bonding or H-accepting substituents shown in Table 1 were used. These substituents are similar to those used in the previous study.16)

Measurement of log $P_{\mathrm{O/M–W}}$ Partition coefficients for the octane/aqueous MeOH partitioning system, log $P_{\mathrm{O/M–W}}$, were measured with aqueous methanol containing 15 and 50% MeOH (M15 and M50, respectively) at 25 °C by the conventional shake-flask method used for measurement of log P_{oct}.24)

Measurement of log k We have so far used a CAPCELL PAK C18-SG120 column (Shiseido, 150×4.6 mm I.D.). To examine silanol effects, the corresponding UG120 column was also tested. The packing materials of these columns are high-purity silicone-polymer coated silica gels chemically modified with octadecyl groups and were developed so as to suppress extremely small traces of residual silanols.25,26) Retention factors were measured at 25 °C according to the previous procedure.11,12) A Shimadzu LC9A liquid chromatograph, equipped with an SIL-6A auto injector, an SPD-6AV UV detector (Shimadzu), an ERC-7510 refractive index (Erma, Optical-Works), and a C-R4A Chromatopac (Shimadzu), was used. MeOH-buffer (pH 7.4) solutions containing 5, 15, 30 and 50% MeOH (M5, M15, M30 and M50, respectively) were used as the mobile phase.

Results and Discussion

Relationship between Retention Parameters and Octane/MeOH–H_2O Partition Coefficients As mentioned above, a model of the bulk-solvent partitioning system between octane and aqueous methanol was used as a first approximation to simulate the partition between the stationary (C18) and mobile (aqueous methanol) phases.

The log $P_{\mathrm{O/M–W}}$ values for octane/M15 and octane/M50 partitioning systems, log $P_{\mathrm{O/M15}}$ and log $P_{\mathrm{O/M50}}$, in Table 1 provided excellent correlation with the octane/water partition coefficient, log P_{O}, as shown by Eqs. 2 and 3.

$$\log P_{\mathrm{O/M15}} = 0.944 \log P_{\mathrm{O}} - 0.096 \tag{2}$$

$$n = 13,\ r = 0.998,\ s = 0.059$$

$$\log P_{\mathrm{O/M50}} = 0.780 \log P_{\mathrm{O}} - 0.488 \tag{3}$$

$$n = 13,\ r = 0.992,\ s = 0.103$$

Since the organic phase is the same (octane) in log P_{O} and log $P_{\mathrm{O/M–W}}$, comparison of these equations would afford the

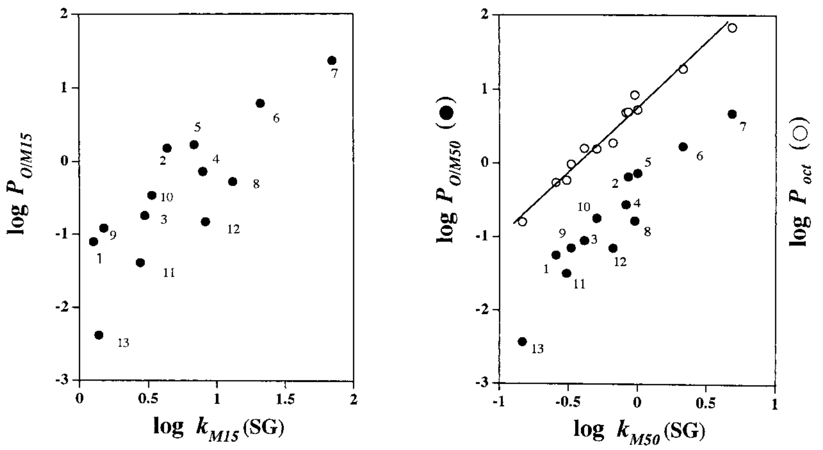

Fig. 2. Comparison between HPLC and Bulk-solvent Partitioning for Monosubstituted Pyrazines

Column: CAPCELL PAK SG C18. The compound numbers are the same as those in Table 1.

information about the difference in solvating features of the polar phases. Finding very high correlations in Eqs. 2 and 3 suggests that the solvation ability of M15 and M50 eluents is proportional to that of water. A lower coefficient of $\log P_O$ for M50 than for the M15 system is thought to indicate that as solutes become more hydrophobic they become solvated more effectively by methanol. Before studying the relationship between $\log k$ and $\log P_{O/M-W}$, it would be important to examine the dependency of $\log P_{O/M-W}$ on methanol concentration in the polar phase (%MeOH). Previously we investigated the relationship between $\log P_{O/M-W}$ and %MeOH for only the unsubstituted and carbomethoxy substituted pyrazines (X=H and CO_2Me), which demonstrated that $\log P_{O/M-W}$ for these substituents decreases linearly with an increase in methanol content from 5 to 70%.[18] Observation of high correlations in Eqs. 2 and 3, then, appears to be indicative of a similar linearity for the other substituents. This reasoning would be also supported by the fact that extrapolation of the coefficients of $\log P_O$ to 0% MeOH yielded a value ($\log P_{O/M0}=1.014$) being close to unity, though derived from only two data points.

In Fig. 2, the values for $\log P_{O/M15}$ and $\log P_{O/M50}$ are plotted against $\log k$(SG) in eluents with the same methanol concentrations. It was shown that the correlation was better at 50% MeOH ($r=0.941$ for M50 and $r=0.846$ for M15); our preparatory experiments had shown that the correlation became poorer with decreasing %MeOH ($r=0.91$ for M30 and $r=0.65$ for M5), suggesting that the chromatographic partitioning system is more directly correlated with the corresponding liquid/liquid partitioning system at 50% MeOH and that the retention mechanism becomes more complicated in water-rich eluents.

If the chromatographic retention mechanism is mainly governed by partition between the stationary and mobile phases and the solvation power of the stationary phase is similar to that of octane, $\log k$ would also be expected to correlate better with the corresponding $\log P_{O/M-W}$ rather than $\log P_{oct}$. However, as shown in Fig. 2, the relationship with $\log P_{O/M-W}$ was non-linear even at 50% MeOH, where strong H-acceptors such as CO_2R and $CONMe_2$ showed significant deviations, and interestingly, $\log k_{M50}$ was found to correlate even better with $\log P_{oct}$ ($r=0.989$) than $\log P_{O/M50}$. This finding is consistent with the conclusion of Carr *et al.* that bulk hexadecane is a good model for the bonded phase for non-polar solutes but not for polar solutes though the solutes and methanol range tested in their work are different from ours.[27] Our results are also similar to those of Tsukahara *et al.* who compared dodecane/MeOH-water (M40, M60, M80 and M100) partitioning coefficients, $P_{D/M-W}$, of typical small organic compounds with the corresponding retention factor, *k*, measured on an ODS column.[28] By using their data for non-amphiprotic benzene derivatives (PhX with X=alkyls, halogens, OEt, Ac, CN, CO_2R and NO_2), we plotted $\log k$ values[28] against $\log P_{oct}$[29] at each methanol concentration, yielding the best correlation at M40, $\log k_{M40}$, ($r=0.982$). It should be noted that here again, this correlation is better than that between $\log k_{M40}$ and $\log P_{D/M40}$ ($r=0.966$); although plot of $\log k_{M40}$ with $\log P_{oct}$ is almost linear, that with $\log P_{D/M40}$ affords two separate lines for non-H-bonders and H-bonders. All these results reveal that, while the $\log k$ parameter is mainly governed by the same factors as those controlling partition between the corresponding bulk solvents, it is also affected by additional interactions specific to the chromatographic system.

The fact that stationary phase modified with octadecyl alkyl chains correlates better with the H-bondable octanol system than the non-H-bondable octane(alkane) system indicates that the stationary phase exerts some H-bonding interactions with the solutes. The stationary phase in RP-HPLC is considered to be a dynamic multicomponent mixture of which formation and character are affected by various factors including the alkyl modifier chain length, organic modifier and mobile phase composition. Many models are proposed to express the analyte distribution between the eluent and the stationary phase.[30—33] Recent investigations have shown that alkyl chains bonded to the silica surface are densely packed or liquid-like[32] and that methanol forms a monomolecular adsorbed layer on a silica surface on the top of collapsed bonded layer.[33] It may therefore be presumed that the dynamic solvated stationary phase exposed to M50 eluent is more liquid-like and behaves like bulk-solvent with hydrogen-bondable sites, showing a close linearity between $\log P_{oct}$ and $\log k_{M50}$; finding an excellent $\log P_{oct}-\log k$ correlation at 50% MeOH seems to suggest that the HPLC phase with M50

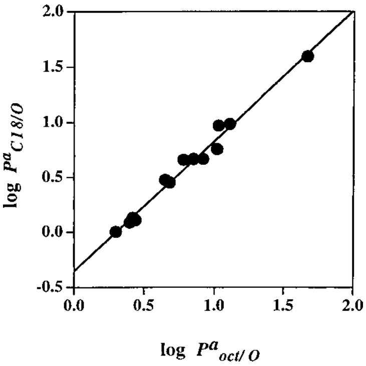

Fig. 3. H-Bonding Properties of Octanol and Stationary Phase (SG)

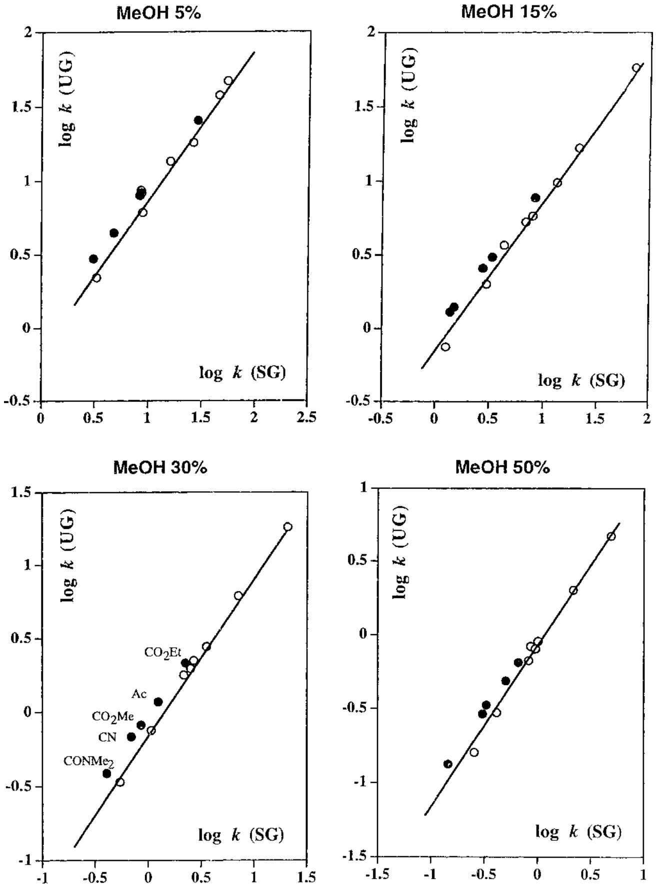

Fig. 4. Comparison of Retention Behavior between SG and UG Columns

Closed circles represent H-acceptor substituents ($S_{HA}>1.2$).

eluent resembles most closely octanol in terms of hydrogen-bonding properties. We attempted to confirm this by comparing the difference in the H-bonding ability of octanol and the stationary phase. To do this, we calculated apparent partitions between octanol and octane, $\log P^a_{oct/O}$, by $\log P^a_{oct/O} = \log P_{oct} - \log P_O$, and also apparent partitions between the C18 stationary phase with M50 eluent and octane, $\log P^a_{C18/O}$, by $\log P^a_{C18/O} = \log k_{M50} - \log P_{O/M50}$, and then the results thus obtained are plotted (Fig. 3), yielding a correlation as shown by $\log P^a_{C18/O} = 1.19 \log P^a_{oct/O} - 0.355$ ($r=0.991$). Not only the extremely high correlation coefficient but also obtaining a slope near 1 would suggest that H-bonding atmosphere of the C18 phase is very similar to that of octanol. Although our model may be too simple to describe the detailed characteristics of the stationary phase, the present results are adequate to establish the advantage of the M50 eluent for prediction of $\log P_{oct}$.

Effect of Silanols on the Relationship between $\log P_{oct}$ and $\log k$ As described above, H-bonding plays an important role in relating $\log P_{oct}$ to $\log k$. In water-rich eluents, the linearity usually fails for various series of heteroaromatic compounds, where strong H-accepting compounds are more retentive than expected.[10—15] One explanation for these phenomena could be that residual silanols which act as H-donors would interact with H-accepting solutes *via* H-bondings. To explore this possibility, we attempted to examine the influence of residual silanols by comparing $\log k$ values for the same compounds measured on the C18-UG column with the previous data obtained on the SG-column. Both columns were designed by the same manufacturer,[25,26] to eliminate residual silanols but the main difference in the characteristics is the amount of silanols; the more recently developed UG-type is considered to be essentially free from accessible residual silanols. Among different ways proposed to assess amount of silanols,[26,34—36] we evaluated qualities of these columns in terms of a separation factor, $\alpha_{C/P}(k_{caffeine}/k_{phenol})$[26,34]; $\alpha_{C/P}=0.359$ and 0.313 for SG and UG, respectively, describing well their characteristic (Smaller value is thought to indicate smaller amount of residual silanols).

The retention factors for these columns are compared in Fig. 4 and the results of analyses by Eq. 1 are summarized in Table 2 where the most reasonable correlation at each eluent composition is accompanied with the 95% confidence intervals of the regression coefficients in parentheses. It is obvious that Eq. 1 works well as a general formula. Extrapolations of the obtained regression coefficients for the $\log P_{oct}$ term to 0% MeOH yield values close to 1 (1.03 for SG and 1.05 for UG), supporting also the validity of Eq. 1.

In Fig. 4, H-acceptors showed enhanced retention relative to non-H-bonders on the UG column, suggesting that stronger interaction of H-acceptor solutes occurs with the UG-stationary phase rather than with SG. Moreover, the correlation between $\log k$ and $\log P_{oct}$ with UG was inferior to that with SG for all eluent compositions studied; even at M50, the plot of $\log k_{M50}$ (UG) with $\log P_{oct}$ showed significant upward deviations from linearity for strong H-acceptors like CO_2R and $CONMe_2$. Inspection of Table 2 indicates that particularly the S_{HA} term makes a greater contribution to these deviations. All these results clearly indicate that H-bonding between residual silanol and H-accepting solute is not responsible for hampering the linearity and that residual silanols are rather favored for establishing a good linearity.

To examine whether such "favored" silanol effects are generally observed, we measured $\log k$ values for a set of typical substituents composed of two strong H-acceptors ($X=CO_2Me$ and CO_2Et) and six non-H bonders[11] ($X=H$, Me, Cl, OMe, OEt and OPr) on various C18-bonded columns with M50 eluent. The resultant $\log k$ values were plotted against $\log P_{oct}$ in Fig. 5; showing two types of plots, A and B. Whereas four stationary phases with greater $\alpha_{C/P}$ factors (0.34—0.53) afforded good linearity (Fig. 5A), seven C18-modified silica with smaller $\alpha_{C/P}$ factors (0.29—0.35) produced significant deviations for strong H-acceptors (Fig. 5B), regardless of the preparation method of packing materials

Table 2. Analyses of log k for Monosubstituted Pyrazines by Eq. 1

Eluent[a]	Coefficients			Const.	n[b]	r[c]	s[d]	F[e]
	log P	σ_I	S_{HA}					
				C18 SG column				
M5	0.572			0.898	12[f]	0.802	0.261	18.0
	0.962	−1.032	1.154	−0.359	12	0.995	0.051	245
	(0.085)	(0.214)	(0.174)	(0.215)				
M15	0.671			0.438	13	0.935	0.187	76.2
	0.907	−0.602	0.862	−0.538	13	0.999	0.032	989
	(0.041)	(0.132)	(0.103)	(0.126)				
M30	0.653			−0.010	13	0.975	0.110	211
	0.784	−0.235	0.485	−0.584	13	0.997	0.040	561
	(0.050)	(0.164)	(0.127)	(0.155)				
M50	0. 565			−0.429	13	0.988	0.064	466
	(0.058)			(0.046)				
				C18 UG column				
M5	0.820			0.654	13	0.874	0.268	35.4
	0.993	−0.766	1.244	−0.613	13	0.998	0.040	688
	(0.051)	(0.165)	(0.128)	(0.157)				
M15	0.659			0.354	13	0.910	0.221	53.0
	0.934	−0.405	1.014	−0.869	13	0.998	0.036	798
	(0.046)	(0.148)	(0.115)	(0.141)				
M30	0.649			−0.083	13	0.954	0.150	111
	0.814		0.613	−0.882	13	0.997	0.042	791
	(0.050)		(0.118)	(0.157)				
M50	0.562			−0.487	13	0.975	0.108	230
	0.617	0.306	0.224	−0.855	13	0.992	0.067	204
	(0.066)	(0.259)	(0.196)	(0.244)				

a) The designation M5, M15, M30 and M50 represents aqueous methanol containing 5, 15, 30 and 50% MeOH, respectively, by volume. *b*) Number of compounds used for correlations. *c*) Correlation coefficients. *d*) Standard deviations. *e*) Values of F-ratio between regression and residual variances. *f*) Compound **7** was excluded because it's log k value was too long to measure.

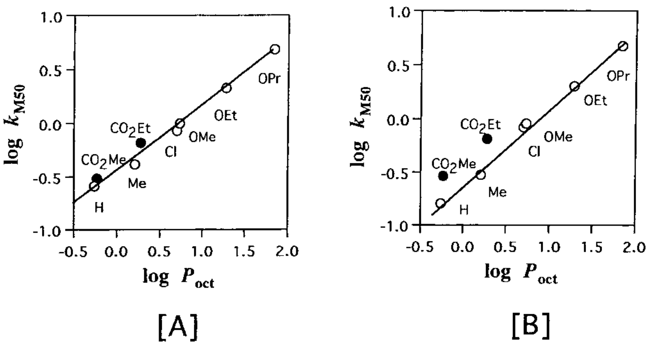

Fig. 5. Effect of Residual Silanols on Relationship between log P_{oct} and log k

Group A ($\alpha_{C/P}$=0.34—0.53): CAPCELL PAK C18 SG 120, CAPCELL PAK C18 AG, Wakosil 2 5C18 HG, J-Sphere ODS M80. Group B ($\alpha_{C/P}$=0.29—0.35): CAPCELL PAK C18 UG 120, CAPCELL PAK C18 UG 80, Cosmosil 5C18-MS, L-column ODS, TSKgel ODS-80TsQA, YMC Pack Pro C18, Develosil ODS UG5.

and manufacturers. Although it is difficult to rationalize these results at the present stage, H-bonding with some H-donors (other than residual silanol) on the dynamic solvated stationary layer may play a significant role in the retention mechanism. It is possible to speculate that methanol absorbed on the stationary phase *via* H-bonding with residual silanol would contribute to make the SG-stationary phase more alcohol-like and thus more octanol-like when combined with the M50 eluent.

In conclusion, on the basis of comparisons of log $P_{O/M-W}$ and log k as well as examination of the effects of silanols on log k, the results demonstrate that log P_{oct} of non-amphiprotic solutes (non-H-donors) can be well predicted by using an HPLC system consisting of the M50 eluent and a C18 stationary phase containing a small amount of accessible silanols. To our knowledge, this work seems to be the first example showing that the presence of a trace of residual silanols plays a significant role in improving the linearity between log P_{oct} and log k. Closer examination of stationary phase properties would be needed to establish a universal procedure to afford the log P_{oct}−log k linearity.

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