Application of a Stepwise Flow Ratiometry without Phase Separation to the Determination of the Chloroform/Water Distribution Coefficients of Volatile Diazines

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The chloroform/water distribution coefficients (K_D) of sixteen diazine compounds were determined by a stepwise flow ratiometry. An aqueous solution of analyte was delivered and merged with chloroform. The flow rate ratio of both the phases was varied stepwise under a constant total (chloroform+aqueous) flow rate. The analyte was extracted to chloroform while both the phases, which were segmented by each other, were passing through an extraction coil. The segmented stream was then led to a UV/Vis detector directly without phase-separation. The absorbance of the chloroform and aqueous phases (A_o and A_a, respectively) was each measured at the maximum absorption wavelength of the analyte. The plots of A^{-1} against R_f, $(AR_f)^{-1}$ against R_f^{-1}, and AR_f against A gave straight lines, where A was A_o, A_a or the sum of them (A_S). The K_D of the analyte was calculated from the slopes and intercepts of the plots. The log K_D values obtained for the analytes (−0.5—1.4) were agreed well with the values measured by a shake-flask method. The present method is simple, rapid (5 min/determination) and applicable to the volatile compounds with reasonable precision (standard deviation of log K_D<0.07).

Key words stepwise flow ratiometry; distribution coefficient; volatile compound; diazine; partition coefficient

Distribution coefficient (K_D), also referred as partition coefficient (P), is an equilibrium constant that is closely relating to the hydrophobicity (lipophilicity) of a substance. It is widely used for the estimation of the activity of drug and pesticide candidates, and of the toxicity of pollutants. Many experimental approaches, such as shake-flask method, reversed phase HPLC, flow injection extraction, micellar electrokinetic chromatography and so forth, have been developed for the determination of K_D; each of them has its intrinsic advantages and disadvantages, as reviewed by Danielsson and Zhang.[1] For example, shake-flask method is accurate in principle but is laborious and time-consuming; HPLC is rapid and simple to operate but requires suitable standards whose K_D values are well established.

Flow ratiometry is a variation of continuous flow analysis, where two independently delivered solutions are merged at various flow ratios (R_f) and the analytical signals are measured at a downstream position.[2] The information of interest is obtained by analyzing the relationship between the R_f and the signals. We reported a system for the K_D determination based on a flow ratiometry.[3,4] The method was applied to the determination of chloroform/water K_D of phenol, benzoic acid and their derivatives. Satisfactory results were obtained for these compounds and the efficiency of the measurement was fairly good (10 min/determination).

In the previous study,[5] the system was further improved by introducing a UV-detection method that required no phase-separation. The phase-separator, which had made the system complicated and limited the efficiency of the measurement, was removed. The absorbance of both the phases was measured almost simultaneously with one detector, which contributed to the ruggedness and reliability of the system. A series of experimental operations can be carried out automatically in a semi-closed flow system. There is no need to determine precisely the initial and final concentration of the analyte so long as the analytical signal of either of the phases at least is proportional to the concentration of the analyte in the corresponding phase.

The features described above are considered to be advantageous to the measurement of volatile compounds, whose concentrations are liable to change during the operations involved in conventional approaches. In the present work, therefore, the improved method with no phase-separation process was further investigated by applying it to volatile compounds. Mono- and di-substituted diazines were selected as the compounds to be analyzed because their K_D values in various solvents systems have been extensively explored in the studies of quantitative structure–activity relationships.[6—11]

Experimental

Apparatus Figure 1 shows the flow system. Chloroform (O) and an aqueous solution of analyte (A) were each delivered and merged with in a Teflon tee-union. The ratio of chloroform/aqueous flow rate (R_f: F_o/F_a) was automatically varied stepwise (each duration time: 1 min) while the total flow rate (F_o+F_a) was kept constant at 1 cm^3 min^{-1}. Both the phases formed small segments and passed through an extraction coil (EC) that was kept at 25 °C with a thermostat (T). The effluent from the coil came into a wide bore Teflon tubing (WT), in which the coalescence between neighboring chloroform segments and that between aqueous segments occurred.[5] Both

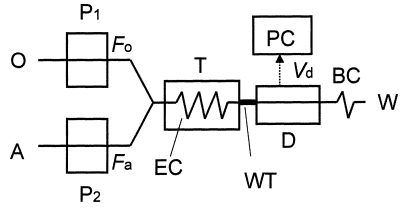

Fig. 1. Flow Diagram

O, chloroform; A, aqueous solution of analyte; P_1 and P_2, Shimadzu LC-10AD$_{VP}$ double-plungers pumps; T, Sanuki R-3000C thermostat; EC, extraction coil (0.5 mm i.d., 3 m long); WT, wide bore tubing (2 mm, i.d., 20 cm long); D, Shimadzu SPD-6AV UV/Vis detector; PC, Toshiba Dynabook Satellite SA70C/5 notebook computer with a Measurement Computing PC-CARD-DAS16/12-AO card; BC, back pressure coil (0.25 mm i.d., 1 m long); W, waste; F_a and F_o, flow rate of aqueous and organic phases, respectively; V_d, detector output voltage (relative absorbance).

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the phases were directly introduced to a handmade optical flow cell[4] set in a commercial UV/Vis detector (D). The relative absorbance was measured at the absorption maximum wavelength of the analyte and acquired in a computer (PC) as a detector output voltage (V_d) at the frequency of 20 Hz.

Materials Sixteen diazines were used in the present study: pyrazine, pyridazine, pyriazineamide (Pyrazine–$CONH_2$) and aminopyrazine (Pyrazine–NH_2) purchased from Nacalai Tesque; methylpyrazine (Pyrazine–Me), 2-chloropyrimidine (Pyrimidine–Cl) and 3-methylpyridazine (Pyridazine–3Me) from Aldrich; 2,6-dimethylpyrazine (Pyrazine–2,6diMe), cyanopyrazine (Pyrazine–CN), acetylpyrazine (Pyrazine–Ac) and 5-methylpyrimizine (Pyrimizine–5Me) from Tokyo Kasei Kogyo; 2-amminopyridazine (Pyridazine–$2NH_2$) from Wako Pure Chemical Industries; 4-methylpyrimizine (Pyrimizine–4Me) from Sigma. Methyl pyrazinecarboxylate (Pyrazine–CO_2Me), *N*,*N*-dimethylpyrazineamide (Pyrazine–$CONMe_2$) and acetylaminopyrazine (Pyrazine–NHCOMe) were synthesized in one of the authors' (C.Y.) laboratory. Chloroform was purchased from Kanto Chemicals. Water is a Milli-Q SP deionized water.

Principles The principle of the K_D determination by flow ratiometry[3,4] and its extension to the method without phase-separation[5] were described before. Briefly, when an aqueous solution of analyte (initial concentration: C_{ai}) is merged with an organic solvent at the flow rate ratio of R_f ($=F_o/F_a$) to reach the distribution equilibrium, the K_D of the analyte is expressed as Eq. 1:

$$K_D=\varepsilon_o^{-1}A_o(C_{ai}-\varepsilon_o^{-1}A_oR_f)^{-1}=(C_{ai}-\varepsilon_a^{-1}A_a)(\varepsilon_a^{-1}A_aR_f)^{-1} \quad (1)$$

where A and ε are the absorbance and the molar absorptivity, respectively, of the analyte in the phase denoted by the subscript (o: organic phase, a: aqueous phase). From Eq. 1, the following three equations are derived for linear plots:

$$A^{-1}=(BC_{ai})^{-1}K_DR_f+(BC_{ai})^{-1} \quad (2)$$

$$(AR_f)^{-1}=(BC_{ai}R_f)^{-1}+(BC_{ai})^{-1}K_D \quad (3)$$

$$AR_f=-K_D^{-1}A+BC_{ai}K_D^{-1} \quad (4)$$

where, A is A_o, A_a or the sum of them (A_S); B is a constant that depends on the species of A, as summarized in Table 1A. The K_D can be calculated from the slopes and intercepts of these linear plots without the information on the values of C_{ai} and B. As for the Eq. 2, for example, A^{-1} is plotted against R_f and K_D is obtained only by dividing the value of the slope by that of intercept. Such the information is summarized in Table 1B.

Table 1. Three Kinds of Linear Plots Based on Eqs. 2—4

(A) A and B

	Plot for A_o	Plot for A_a	Plot for A_S
A	A_o	A_a	A_o+A_a
B	ε_oK_D	ε_a	$\varepsilon_oK_D+\varepsilon_a$

(B) Abscissa, ordinate and K_D

Eq.	Abscissa	Ordinate	K_D
2	R_f	A^{-1}	SI^{-1}
3	R_f^{-1}	$(AR_f)^{-1}$	$S^{-1}I$
4	A	AR_f	$-S^{-1}$

S: slope of linear regression. *I*: intercept at the ordinate.

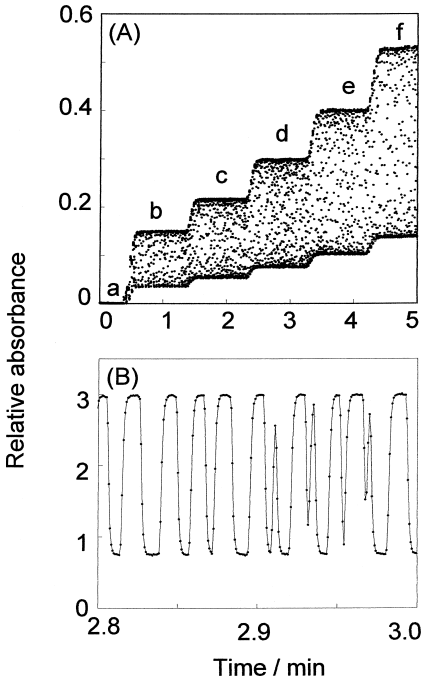

Fig. 2. Typical Example of the Analytical Signals (A) and Its Enlargement in Abscissa in the Range of 2.8—3.0 min (B)

Analyte: pyrazine (*ca.* 1 mmol dm^{-3}). Analytical wavelength: 260 nm. Flow rate ratio (R_f): a, ∞ (chloroform alone); b, 2.333; c, 1.500; d, 1.000; e, 0.667; f, 0.429. The R_f was corrected by taking the mutual solubility of both phases[12] and densities of chloroform[13] and water[14] into account by assuming that there is an additive property of the volumes of both the phases.

Results and Discussion

Selection of the Flow Rate Ratios A set of five R_f was employed for the K_D determination by taking the efficiency of the measurement into account. In the present approach, the K_D is determined not from the ratio of the analyte concentrations in both the phases but from the slopes and intercepts of the linear plots based on Eqs. 2—4. The error in A at each R_f affected, therefore, the calculated value of K_D in a complex way. The K_D is expressed as Eq. 5 for the plot based on Eq. 2 for A_o ($i=1,2,\ldots,5$), for example, when the method of least squares is applied.

$$K_D=\frac{n\sum R_{f,i}A_{o,i}^{-1}-\sum R_{f,i}\sum A_{o,i}^{-1}}{\sum R_{f,i}^2\sum A_{o,i}^{-1}-\sum R_{f,i}\sum R_{f,i}A_{o,i}^{-1}} \quad (5)$$

The extent of the propagation of the error in each A_o ($\partial A_{o,j}$, $j=1,2,\ldots,5$) to that of the final outcome (∂K_D) is, therefore, expressed by the following equation.

$$\frac{\partial K_D}{\partial A_{o,j}}=\frac{\left\{n\sum R_{f,i}^2-\left(\sum R_{f,i}\right)^2\right\}\left\{\sum R_{f,i}A_{o,i}^{-1}-R_{f,j}\sum A_{o,i}^{-1}\right\}}{A_{o,j}^2\left(\sum R_{f,i}^2\sum A_{o,i}^{-1}-\sum R_{f,i}\sum R_{f,i}A_{o,i}^{-1}\right)^2} \quad (6)$$

The higher the $\partial K_D/\partial A_{o,j}$ value becomes, the larger the extent of the propagation is. These equations and those similarly obtained for the other two plots (*i.e.*, the plots based on Eqs. 3 and 4) were used for the selection of R_f values. From the extensive simulations, it was concluded that the R_f values are not so critical for the compounds having the $\log K_D$ below 1.5. On the other hand, for more hydrophobic compounds, even subtle error in A, especially that at high R_f, greatly affects the final result (K_D). Consequently, the R_f set of 2.333, 1.500, 1.000, 0.667 and 0.429 was employed for the diazines except for more lipophilic Pyrazine–2,6diMe and Pyrazine–Ac, to which the set of lower R_f (0.667, 0.429, 0.250, 0.190 and 0.111) was applied.

Flow Signals and Three Linear Plots The raw analytical signals acquired at the frequency of 20 Hz are shown for pyrazine, for example, as a function of time in Figs. 2A. The signals seem scatter in a range of absorbance, whose upper and lower limits increased with a decrease in R_f. However, by

Table 2. The log K_D of Diazines Determinend by a Stepwise Flow Ratiometry

Analyte	λ_{anal} /nm	A	n	log K_D±S.D.				Reference values
				Eq. 2–based plot	Eq. 3–based plot	Eq. 3–based plot	Mean	
Pyrazine	260	A_S	3	$0.53\pm0.00_4$	$0.53\pm0.00_8$	$0.53\pm0.00_7$	0.53	0.59[9]
Pyrazine–Me	264	A_o	5	0.99±0.01	0.96±0.01	0.97±0.01	0.97	1.04[9]
Pyrazine–2,6diMe	271	A_o	8	1.39±0.03	1.35±0.01	1.36±0.01	1.37	1.54[9]
Pyrazine–CN	268	A_o	2	$1.07\pm0.00_1$	$1.06\pm0.00_0$	$1.06\pm0.00_0$	1.07	1.03[9]
Pyrazien–CO_2Me	268	A_o	5	1.41±0.06	1.41±0.03	1.41±0.03	1.41	1.36[9]
Pyrazine–$CONMe_2$	268	A_S	5	0.95±0.04	0.95±0.03	0.95±0.03	0.95	0.71[10]
Pyrazine–$CONH_2$	268	A_S	4	−0.34±0.01	−0.34±0.01	−0.34±0.01	−0.34	−0.34[11]
Pyrazine–Ac	268	A_o	6	1.26±0.02	1.24±0.04	1.24±0.03	1.24	1.42[9]
Pyrazine–NHCOMe	290	A_S	4	$0.07\pm0.00_3$	$0.07\pm0.00_3$	$0.07\pm0.00_3$	0.07	0.05[11]
Pyrazine–NH_2	317	A_S	4	−0.49±0.05	−0.49±0.06	−0.49±0.05	−0.49	−0.56[11]
Pyrimidine–2Cl	250	A_o	7	1.08±0.05	1.08±0.03	1.08±0.03	1.08	1.16[9]
Pyrimidine–4Me	250	A_S	3	$0.71\pm0.00_5$	$0.70\pm0.00_7$	$0.70\pm0.00_6$	0.70	0.74[9]
Pyrimidine–5Me	250	A_S	4	1.01±0.06	0.97±0.02	0.97±0.03	0.98	0.95[9]
Pyrimidine–$2NH_2$	290	A_S	4	−0.26±0.02	−0.26±0.02	−0.26±0.02	−0.26	−0.31[11]
Pyridazine	250	A_S	4	−0.11±0.01	−0.12±0.01	−0.11±0.01	−0.11	−0.15[11]
Pyridazine–3Me	250	A_S	4	0.35±0.04	0.35±0.03	0.35±0.03	0.35	0.29[11]

The log K_D for each plot was the log($\Sigma K_D/n$); standard deviation of log K_D=0.43429 s_{KD}/K_D, where s_{KD} is the standard deviation of K_D.

enlarging the abscissa (time) scale as shown in Fig. 2B, it can clearly be seen that the signals did not scatter randomly but oscillating almost regularly between upper and lower plateaus. The upper and lower plateaus correspond to the absorbance of chloroform and aqueous phases, respectively, because the width of the former plateaus was decreased whereas that of the latter increased with the decrease of R_f (chloroform/aqueous flow rate ratio). The signals between the both plateaus are transition signals corresponding to the interfacial regions between the organic and aqueous phases.

Figures 3A—C show the linear plots based on Eqs. 2—4, respectively, for pyrazine. The linearity of the plots was sufficiently. The coefficients of determination (r^2) of the plots are ranged from 0.9977 to 1.0000. Similar results were obtained for the other diazines. The K_D values obtained from these three plots were 3.390, 3.322 and 3.342 from A_o, 3.305, 3.425 and 3.399 from A_a, and 3.372, 3.343 and 3.353 from A_S, respectively.

Applications to Diverse Diazines Table 2 is the summary of the results obtained for the diazines. The log K_D values determined by a shake flask method[9,11,15] are also listed, as reference. The log K_D obtained from A_S ($=A_o+A_a$) were listed for less lipophilic compounds (log $K_D<1$), because the addition of A_o and A_a is considered to give more reliable results.[5] For more lipophilic compounds, A_o was used instead of A_S, because A_a was too low to be precisely measured.

The log K_D of the present study (Y) agreed well with the reference values (X) listed in the rightmost column in Table 2; the relationship between them is $Y=0.9373X+0.0435$ ($r^2=0.9822$); $Y=0.9281X+0.0390$ ($r^2=0.9802$) and $Y=0.9287X+0.0041$ ($r^2=0.9806$) for the plots based on Eqs. 2, 3 and 4, respectively. The weight of each data for the linear regression depends on the type of plot. This effect seems, however, not so significant because similar values were obtained irrespective of the plot type.

In conclusion, the present approach could realize the simple and rapid (5 min/sample) determination of K_D of diazines with a semi-closed flow system. The method has distinct advantages over conventional methods in the respect that it needs neither standard materials for K_D calibration nor information on the initial and final concentration of analyte: the information needed is only the flow rate (R_f) and the absorbance (A). This approach would be applicable to other volatile compounds whose K_D values are difficult to measure by conventional methods.

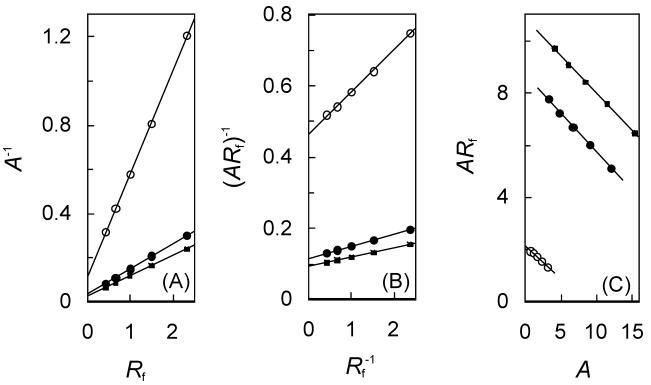

Fig. 3. Typical Example of Three Kinds of Linear Plots Based on Eqs. 2 (A), 3 (B) and 4 (C)

The data shown in Fig. 2 are used for these plots. ●, A_o; ○, A_a; ■, A_S ($=A_o+A_a$). The absorbance was relative absorbance in arbitrary unit.

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