Measurement of Diffusion Coefficients of Parabens and Steroids in Water and 1-Octanol

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Diffusion coefficients (*D*) of parabens and steroids in water and 1-octanol were determined by using the chromatographic broadening method at 37 °C, and the relationships between the *D* values and the physicochemical properties of the drugs were discussed. The *D* values in 1-octanol were lower than those in water because of the higher viscosity of 1-octanol. The *D* values depend on not only the molecular weight (*MW*), but also the lipophilicity of the drugs in water and on the ability for hydrogen-bonding in 1-octanol. When the lipophilic index (*LI*), calculated from the retention time using in a reverse-phase column, was used as a parameter of drug lipophilicity, the following equation was obtained for *D* in water (D_w); $\log D_w = -0.215 \cdot \log MW - 0.077 \cdot \log LI - 4.367$. When the hydrogen bond index (*HI*), the logarithm of the ratio of the partition coefficient of the drugs in 1-octanol and cyclohexane, was used as an index of hydrogen-bonding, the following equation was obtained for *D* in 1-octanol (D_o); $\log D_o = -0.690 \cdot \log MW - 0.074 \cdot \log HI - 4.085$.

Key words diffusion coefficient; steroid; paraben; lipophilicity; hydrogen bond

In modern drug discovery, *in silico* prediction of the properties of drug candidates is useful for high-throughput screening[1—3] and for quantitative predictions, well-designed *in silico* models are needed.[4] Diffusion phenomena are important in all processes when a drug is administered as a preparation: from the initial releasing step to the final elimination from the body.[5] The prediction of diffusivity of drug candidates is required to establish an *in silico* model for the evaluation of bioavailability. Since the diffusivity of a drug depends on the molecular volume of the drug and the viscosity of the solvent, most models for predicting diffusivity involve parameters such as the size of the molecule and the viscosity of the solvent.[6,7] Verification of these models is needed to improve any *in silico* model used for the evaluation of bioavailability.[6] Many different methods for the measurement of the diffusion coefficient (*D*) have been used to understand diffusion phenomena in various media.[8—13] The chromatographic broadening method (CBM) is one such which has been used for the determination of *D* in water and organic solvents.[14—17] In our pervious study, the *D* values of parabens were measured by CBM using an HPLC apparatus, and their dependence on concentration and temperature was examined.[18]

In this study, the *D* of parabens and steroids, model compounds, in water and 1-octanol were determined by using CBM at 37 °C, and the relationships between the *D* values and the physicochemical properties of the drugs are discussed. Although the molecular volume is the principal parameter for diffusion, it is worthwhile examining the contributions of other parameters such as drug lipophilicity and drug–solvent interactions.

Experimental

Chemicals Parabens, 11α-hydroxymethyltestosterone, 11α-hydroxyprogesterone, Δ^4-androstene-3,17-dione, $\Delta^{9(11)}$-methyltestosterone, and 16,17-epoxyprogesterone were purchased from Tokyo Kasei Kogyo. 1-Octanol, androsta-1,4-diene-3,17-dione, testosterone, methyltestosterone, 17α-hydroxyprogesterone, hydrocortisone, deoxycorticosterone acetate, prednisone, prednisolone acetate, spironolactone, triamcinolone acetonide, and betamethasone valerate were obtained from Wako Pure Chemical (Osaka). Beclomethasone and fluocinolone acetonide were purchased from Sigma (St. Louis, MO, U.S.A.).

Determination of Lipophilic Index The lipophilic index (*LI*) values were determined by HPLC. The HPLC system consisted of a pump (LC-10AD), Rheodyne 7125 injector with a 20 μl quantitative sample loop, column (LiChrospher 100 RP-18e, Kanto Chemical, Tokyo), detector (SPD-6A), and integrator (CR-5A). A mixture of methanol (95—30%) and distilled water (5—70%) was employed as the mobile phase. The flow rate of the mobile phase was 1.0 ml/min and the detector was operated at 255 nm. Each drug was dissolved in methanol (20 μg/ml) and the solution was injected to the HPLC system. The elution time of a solvent (R_0) and the retention time of a drug (R) were determined at different mobile phase compositions. The $\log k'$ value defined by Eq. 1 was plotted against the methanol concentration in the mobile phase and the $\log k'$ value extrapolated to 0% methanol was obtained as the *LI* of the drug ($\log k_0'$).[19]

$$\log k' = \log[(R - R_0)/R_0] \quad (1)$$

Examination of Hydrogen Bond Index The drug partition coefficients in 1-octanol/water ($P_{octanol}$) and cyclohexane/water ($P_{cyclohexane}$) were determined at room temperature. The hydrogen bond index (*HI*) was defined by Eq. 2.[20]

$$HI = \log(P_{octanol}/P_{cyclohexane}) \quad (2)$$

Measurement of Viscosity The viscosity of water and 1-octanol was determined using Rotovisco RV100 equipment and measuring system CV100 (HAAKE, Germany) at 37 °C.

Determination of Diffusion Coefficient The diffusion coefficients (*D*) of parabens and steroids were determined by CBM as described previously.[18] An HPLC system (LC-10A, Shimadzu) equipped with a 10 m, 0.8 mm i.d., stainless-steel tube (Supelco Inc., PA, U.S.A.) was used for the studies. The HPLC system consisted of a pump (LC-10AD), oven (CTO-10AC), detector (SPD-6A), and integrator (CR-5A). A Rheodyne 7125 injector with a 10 μl quantitative sample loop was used for the sample injection. The stainless-steel tubing was placed in the oven and connected directly to the injector and detector, and kept at 37 °C. The solvent flow was 0.1 ml/min and the detector was operated at 255 nm. Each drug was dissolved in distilled water (20 μg/ml) or 1-octanol (100 μg/ml) and the solution was injected to the system. The values of the residence time (t_R) and the eluted peak width at half height ($W_{1/2}$) were obtained by the integrator.

The *D* values were determined from Eq. 3, where *r is* the radius of the capillary tube (0.426 mm) determined from calibration runs.[17,18]

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Table 1. Diffusion Coefficients (D) of Parabens and Steroids in Water and 1-Octanol at 37 °C

	MW	LI[a)]	$\log P_{octanal}$	$\log P_{cyclohexane}$	HI[b)]	D_w[c)]±S.D. (cm/s)×10^6	D_o[d)]±S.D. (cm/s)×10^6
Methyl paraben	152.14	1.61	0.914	−1.939	2.85	10.88±0.14	1.46±0.01
Ethyl paraben	166.17	2.08	1.590	−1.493	3.08	9.80±0.07	1.41±0.01
n-Propyl paraben	180.20	2.63	2.876	−0.478	3.35	9.06±0.05	1.36±0.01
n-Butyl paraben	194.23	3.11	3.428	0.301	3.13	7.04±0.03	1.32±0.01
Androsta-1,4-diene-3,17-dione	284.40	2.85	1.769	0.410	1.36	8.04±0.06	1.45±0.01
Δ^4-Androstene-3,17-dione	286.41	3.11	2.768	1.509	1.26	7.93±0.07	1.55±0.02
Testosterone	288.43	3.23	2.990	1.135	1.85	7.53±0.06	1.15±0.01
$\Delta^{9(11)}$-Methyltestosterone	300.44	3.32	3.291	1.280	2.01	7.24±0.10	1.15±0.02
Methyltestosterone	302.46	3.46	3.303	1.523	1.78	7.02±0.04	1.14±0.02
11α-Hydroxymethyltestosterone	318.46	2.86	1.611	−0.087	1.70	7.25±0.05	0.895±0.004
16,17-Epoxyprogesterone	328.45	3.57	3.034	1.933	1.10	6.70±0.06	1.53±0.01
11α-Hydroxyprogesterone	330.47	3.12	2.459	1.125	1.33	7.40±0.04	1.11±0.01
17α-Hydroxyprogesterone	330.47	3.43	2.929	1.548	1.38	6.88±0.08	1.12±0.01
Prednisone	358.43	2.50	1.058	−0.688	1.75	7.28±0.05	0.982±0.010
Hydrocortisone	362.47	2.63	2.333	−0.721	3.05	7.05±0.01	0.876±0.004
Deoxycorticosterone acetate	372.50	3.81	3.126	2.134	0.99	5.76±0.13	1.33±0.02
Prednisolone acetate	402.49	3.08	2.550	0.111	2.44	6.72±0.07	0.876±0.004
Beclomethasone	408.90	3.21	2.515	0.330	2.18	6.70±0.06	0.830±0.003
Spironolactone	416.58	3.40	2.619	1.638	0.98	6.53±0.06	1.13±0.02
Triamcinolone acetonide	434.50	3.14	2.477	−0.243	2.72	6.73±0.04	0.906±0.005
Fluocinolone acetonide	452.50	3.25	2.441	1.253	1.19	6.72±0.05	0.914±0.010
Betamethasone valerate	476.59	4.42	3.643	2.733	0.91	4.86±0.07	0.926±0.020

a) Lipophilic index, *b*) hydrogen bond index=$\log P_{octanal}-\log P_{cyclohexane}$, *c*) diffusion coefficient in water (n=11), *d*) diffusion coefficient in 1-octanol (n=11—16).

$$D=\frac{0.231\cdot r^2\cdot t_R}{W_{1/2}^2} \quad (3)$$

The determination of D under each set of conditions was repeated 11 times and the mean value was calculated.

Results and Discussion

The D values of the drugs in water and 1-octanol are shown in Table 1 with the corresponding molecular weight (MW), LI, and HI. The values of MW, LI, and HI were 152—476, 1.61—4.42, and 0.91—3.35, respectively. The D values in 1-octanol were lower than those in water because of the higher viscosity of 1-octanol (4.84 cp at 37 °C) compared with water (0.696 cp at 37 °C). As an example, the D of methylparaben in water (1.088×10^{-5} cm^2/s) was 7.45 times higher than that in 1-octanol (1.46×10^{-6} cm^2/s), and the ratio was comparable with that expected from the ratio of the viscosity (4.84/0.696=6.95). The D values decreased with the MW in both water and 1-octanol. The results of a simple linear regression analysis between MW and D were:

$$\log D_w=-0.434\cdot\log MW-4.059 \quad (4)$$

(squared multiple correlation coefficient adjusted for the degrees of freedom, R^{2*}=0.693)

in water, and

$$\log D_o=-0.435\cdot\log MW-4.861 \quad (5)$$

(R^{2*}=0.442)

in 1-octanol. The slopes were similar in water and 1-octanol. The R^{2*} value in water was higher than that in 1-octanol.

LI, $\log P_{octanol}$, and HI were chosen as additional explanatory variables for multiple linear regression analysis. LI and $\log P_{octanol}$ are parameters related to drug lipophilicity and HI reflects the drug hydrogen-bonding ability. Each parameter was used in combination with $\log MW$. In the case of water, LI and $\log P_{octanol}$ were significant as parameters and the following equations were obtained.

$$\log D_w=-0.215\cdot\log MW-0.077\cdot LI-4.367 \quad (6)$$

(R^{2*}=0.899)

$$\log D_w=-0.374\cdot\log MW-0.040\cdot\log P_{octanol}-4.109 \quad (7)$$

(R^{2*}=0.846)

The regression coefficients for LI and $\log P_{octanol}$ were −0.077 and −0.040, respectively, and the negative values suggest that lipophilic drugs exhibit slightly suppressed diffusion in water. The "ice-bergs" surrounded by the hydrophobic surface of the molecules could be related to this suppression. LI was a better explanatory variable than $\log P_{octanol}$ because of the higher R^{2*} value. LI and $\log MW$ were significant (LI; F_0=41.976, p=0.0001: $\log MW$; F_0=19.176, p=0.0003) as the explanatory variables in Eq. 6. On the other hand, HI was not an explanatory variable for diffusion in water, since the R^{2*} value did not increase when using HI as the additional variable.

In the case of 1-octanol, LI, $\log P_{octanol}$ and HI were significant as parameters and the following equations were obtained.

$$\log D_o=-0.640\cdot\log MW+0.072\cdot LI-4.573 \quad (8)$$

(R^{2*}=0.541)

$$\log D_o=-0.476\cdot\log MW+0.027\cdot\log P_{octanol}-4.828 \quad (9)$$

(R^{2*}=0.463)

$$\log D_o=-690\cdot\log MW-0.074\cdot HI-4.085 \quad (10)$$

(R^{2*}=0.723)

The regression coefficients for LI and $\log P_{octanol}$ were posi-

tive, suggesting suppression of diffusion for hydrophilic drugs. However, the increase in R^{2*} values for both equations was small, and LI and $\log P_{octanol}$ were not good as explanatory variables to predict the D values of drugs in 1-octanol. On the other hand, HI was a suitable explanatory variable to estimate D values. The R^{2*} value of the multiple linear regression equation with $\log MW$ and HI as the explanatory variables was 0.723, and this value was higher than those obtained using other parameters. HI and $\log MW$ were significant (HI; $F_0=21.197$, $p=0.0002$: $\log MW$; $F_0=56.675$, $p<0.0001$) as the explanatory variables in Eq. 10. The regression coefficient for HI was negative, suggesting that the hydrogen bond between the drug and 1-octanol suppresses diffusion. In a hydrophobic environment, the interaction between polar groups of drugs and those of solvents may increase.

In this study, the D values of parabens and steroids in water and 1-octanol were determined and the relationship between these values and the physicochemical properties of the drugs are discussed. Since parabens and steroids have very different basic structures, the rules obtained in this study could be applied to other neutral compounds. Although most drugs are ionized in water, the influence of ionization on drug diffusion could be examined by CBM,[21] and the D values of a wide range of drugs could be obtained by integration of the results of these studies.

In modern drug development, *in silico* evaluation of drug absorption and distribution is important for high-throughput examination of enormous numbers of candidate compounds.[1—3] In order to establish a well-designed model for *in silico* examination of drug absorption and distribution, basic studies like the present one for the determination of essential parameters such as D will become increasingly important.[4]

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