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CORRELATION OF HYDROPHOBIC PARAMETERS OF ORGANIC COMPOUNDS DETERMINED BY CENTRIFUGAL PARTITION CHROMATOGRAPHY WITH PARTITION COEFFICIENTS BETWEEN OCTANOL AND WATER

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SUMMARY

Centrifugal partition chromatography (CPC) of three series of compounds, phenols, benzoic acids and N-phenylanthranilates, was performed with hexane–acetonitrile and octanol–hexane (20:80)–water systems and values of the partition coefficients (P_{cpc}) of these compounds in CPC were determined. In the hexane–acetonitrile system, the values of log P_{cpc} increased linearly with increase in the logarithm of the partition coefficient between octanol and water (P_{oct}), but the relationship depended on the chemical series. However, in the octanol–hexane–water system, a single linear relationship between log P_{cpc} and log P_{oct} was observed for the compounds in all three series, indicating that this chromatographic system reflects the nature of the octanol–water two-phase partition system.

From these results, the elution behavior of compounds in CPC can be predicted in terms of log P_{oct}. Further, CPC is very effective for the simple and accurate determination of log P_{oct}, which is a hydrophobic index of compounds decisive for their biological activity.

INTRODUCTION

Recently, centrifugal partition chromatography (CPC) has become an especially useful technique for the separation of natural products[1–5]. The principle of CPC is the same as that of classical counter-current chromatography (CCC) but, because of the application of a centrifugal force, CPC is much faster than CCC. As CPC continuously partitions sample compounds between the stationary and mobile phases, it can separate compounds according to their partition coefficients between two phases. Hence the separation time of compounds should be predictable if their partition coefficients are known. However, so far CPC has been used mainly for the

separation of natural products[1-5], and the relationship between the separation time and partition coefficient has not yet been studied.

The partition coefficient, P, of an organic compound between an organic solvent and water is a hydrophobic index of the compound. This index has been used extensively in quantitative structure–activity relationship (QSAR) studies[6,7]. In QSAR studies, the logarithm of the biological activity, induced by chemicals, is expressed as a linear combination of the logarithms of their physical and structural properties, such as hydrophobic, electronic and steric properties. Hence the contribution of each physical property can be evaluated quantitatively. Of these properties, hydrophobicity, expressed in terms of the logarithm of the partition coefficient between octanol and water (log P_{oct}), is reported to be decisively important in most instances in determining biological activity[6,7].

As the log P_{oct} values of many compounds have been determined[8], it would be very useful to study the relationship between log P_{oct} values and elution times in CPC for two reasons: (i) for predicting the retention times of compounds in CPC and (ii) for establishing a method for the determination of log P_{oct} values by CPC. The shaking-flask method has been the standard method for determining log P_{oct} values, but it has the disadvantages that it is tedious and requires relatively large amounts of pure samples and octanol. Further, it is unsuitable for the determination of log P_{oct} values below -2 and above 4[9,10].

Therefore, alternative methods for the determination of log P_{oct} have been examined, such as calculations based on the hydrophobicity of substituting groups[11] and structural elements (fragment system)[12,13], and on the retention time in high-performance liquid chromatography (HPLC) or the R_F value in thin-layer chromatography (TLC)[14-17].

Of these methods, HPLC seems to have received most attention recently, because it affords rapid and accurate values of log P_{oct} in ranges beyond those which can be determined by the shaking-flask method and does not require pure test compounds[17]. Although the HPLC method is very useful, it has some limitations, mainly due to the complexity of the chromatographic mechanisms involved, which are not yet fully understood[17].

In view of these facts, CPC is expected to be the most effective, simple, rapid and accurate method for the determination of log P_{oct}. If suitable conditions are established, this method should be useful for predicting elution times in CPC. In this paper, we report the relationships between log P_{oct} values and chromatographic parameters in CPC under various conditions.

EXPERIMENTAL

CPC was performed on three series of compounds, N-phenylanthranilates (kindly supplied by Taisho Pharmaceutical, Tokyo, Japan), phenols and benzoic acids. These compunds were dissolved at concentrations of 0.5–10 mg/ml in the solvent used as the mobile phase, and about 0.2 ml of the solution was subjected to chromatography.

CPC was performed at 25°C in a centrifugal partition chromatograph, Model CPC-LLN (Sanki Engineering, Nagaokakyo, Japan). A diagram of the CPC system is shown in Fig. 1. Twelve cartridges, each consisting of 200 micro-partition channel
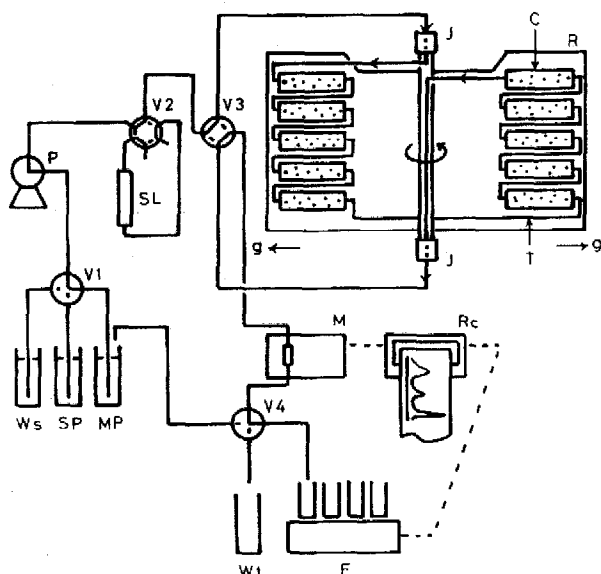
Fig. 1. Diagram of the CPC system. SP = Reservoir for the stationary phase; MP = reservoir for the mobile phase; Ws = reservoir for the washings; Wt = waste tank; V1-V4 = valves; SL = sample solution loop; P = constant-flow pump; J = rotary-seal joint; R = centrifuge rotor; C = micro-partition cells in cartridges; T = connecting tubes; M = flow-cell optical monitor; Rc = recorder; F = fraction collector.

cells (C), built on the centrifuge rotor, R, were filled with the solvent used as the stationary phase via the pump P. The total volume of the 12 cartridges, V_c, was 256.0 ml. Then the solvent used as the mobile phase was introduced continuously at a fixed rate via the pump P under constant rotation.

The volume of the stationary solvent eluted before elution of the solvent front of the mobile phase was measured. This volume corresponds to the volume of the mobile phase V_m. After elution of a sufficient volume of the mobile phase to achieve a steady performance of CPC, the sample solution was applied through the on-line loop injector SL. The sample compound was eluted after repeated partitions between the stationary and mobile phases. The retention volume of the sample compound, V_e, was determined from the chromatogram monitored in a UV spectrophotometer, M, as shown in Fig. 2.
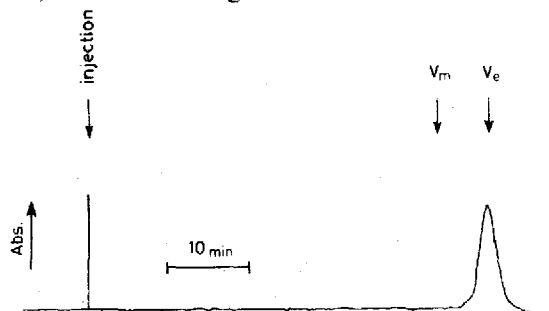

Fig. 2. Chromatogram of flufenamic acid. V_c = 256.0 ml; V_m = 86.5 ml; V_e = 97.6 ml. Chromatography was carried out at a flow-rate of 2.0 ml/min with rotation at 800 rpm.

CPC is based on a multi-partition process between the stationary and mobile phases under centrifugal force. To obtain reproducible chromatograms, a constant flow-rate of the mobile phase and constant rotation of the cartridges were necessary. When these conditions were achieved, the deviations of V_m and V_e values were less than 1%. The flow-rate, V_m and V_e were determined in each run.

Two chromatographic systems were employed, hexane–acetonitrile and octanol–hexane (20:80)–water (0.03 M phosphoric acid solution at pH 2.0). Equal volumes of hexane and acetonitrile or octanol–hexane (20:80) and water were equilibrated by stirring for several hours, then the two phases were separated. The acetonitrile and octanol–hexane phases were used as mobile phases in CPC.

The ratio of the concentration of test compound in the non-polar phase(C_{NP}) to that in the polar phase(C_{PP}) is the partition coefficient in the CPC system (P_{cpc}), expressed by eqn. 1. This value can be obtained experimentally from V_c, V_m and V_e by either eqn. 2 or 3.

$$P_{cpc} = C_{NP}/C_{PP} \tag{1}$$

With the hexane–acetonitrile (HA) system, the polar acetonitrile was used as the mobile phase and the nonpolar hexane as the stationary phase. Thus eqn. 1 could be expressed by

$$\begin{aligned} P_{cpcHA} &= C_s/C_m \\ &= (V_e - V_m)/(V_c - V_m) \end{aligned} \tag{2}$$

where C_m and C_s represent the concentrations of the compound in the mobile and stationary phases, respectively.

With the octanol–hexane–water (OHW) system, the non-polar octanol–hexane mixture was used as the mobile phase and polar water as the stationary phase. Hence eqn. 1 can be expressed by

$$\begin{aligned} P_{cpcOHW} &= C_m/C_s \\ &= (V_c - V_m)/(V_e - V_m) \end{aligned} \tag{3}$$

RESULTS

CPC in the hexane–acetonitrile system

CPC in the hexane–acetonitrile system is efficient for the separation of organic compounds because the viscosities of these solvents are low and they are good solvents for all compounds. Both of these properties are very important for obtaining good resolution in chromatography. In this instance acetonitrile-saturated hexane was used as the stationary phase and hexane-saturated acetonitrile as the mobile phase. We subjected phenols, benzoic acids and N-phenylanthranilates to CPC, and determined their log P_{cpcHA} values according to eqn. 2. As an example, the chromatogram of flufenamic acid (3'-trifluoromethyl-N- phenylanthranilic acid) is shown in Fig. 2. The chromatogram shows good resolution, although the retention volume, V_e, of this compound was the highest of those of the compounds examined in this study. Table I gives the log P_{cpc} values of all the compounds examined.

TABLE I

VALUES OF LOG P_{cpc} IN THE HEXANE–ACETONITRILE SYSTEM AND LOG P_{oct} FOR PHE-NOLS, BENZOIC ACIDS AND N-PHENYLANTHRANILATES

Series	Compound	log P_{cpc}	log P_{oct}[*]	log $P_{oct/cpc}$[**]	Δ[***]
Phenols	4-C$_6$H$_5$	−1.25	3.20	2.88	0.32
	2,4-Cl$_2$	−0.99	3.08	3.61	−0.53
	2,4,6-Cl$_3$	−0.86	3.62	3.97	−0.35
	2,3,4,6-Cl$_4$	−0.76	4.10	4.25	−0.15
	2,3,4,5,6-Cl$_5$	−0.68	5.12	4.47	0.65
	4-CN	−1.71	1.66	1.60	0.06
Benzoic acids	3-Cl	−1.33	2.68	2.62	0.06
	4-Cl	−1.29	2.65	2.70	−0.05
	3-CH$_3$	−1.45	2.37	2.39	−0.02
	4-NO$_2$	−1.76	1.83	1.78	0.05
	4-CN	−1.85	1.56	1.60	−0.04
N-Phenylanthranilates	H	−1.51	4.36	4.51	−0.15
	3'-CF$_3$	−1.18	5.62	5.49	0.13
	2',3'-(CH$_3$)$_2$	−1.16	5.37	5.55	−0.18
	3'-NO$_2$	−1.50	4.57	4.54	0.03
	3'-OH	−1.75	3.49	3.80	−0.31
	3'-OCH$_3$	−1.58	4.56	4.31	0.25
	3'-COCH$_3$	−1.66	4.31	4.07	0.24

[*] Values determined by the shaking-flask method and cited from refs. 8 and 18.
[**] Log P_{oct} determined from log P_{cpc}.
[***] Difference between log P_{oct} and log $P_{oct/cpc}$.

We next examined the relationship between log P_{cpcHA} and log P_{oct}, as shown in Fig. 3. In all instances, log P_{cpcHA} increased linearly with increasing log P_{oct}, but the slope depended on the chemical series. The linear relationships can be expressed by

$$\log P_{oct} = a + b \log P_{cpcHA} \tag{4}$$

where a and b are constants.

For the three series of compounds the following relationships were obtained by least-squares calculation; in these equations, n is the number of compounds, r is the correlation coefficient, s is the standard deviation, $F(p, n - p - 1)$ is the calculated F-value where p is the number of parameters, and $F(p, n - p - 1)_{0.01}$ is the F-value at the 99% level. Values in parentheses under coefficients a and b are the 95% confidence intervals.

For phenols:

$$\log P_{oct} = 6.361 + 2.782 \log P_{cpcHA} \tag{5}$$
$$(1.602) \quad (1.458)$$
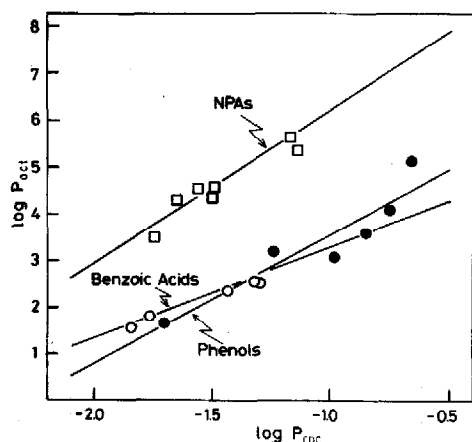$$n = 6, r = 0.926, s = 0.487, F(1,4) = 24, F(1,4)_{0.01} = 21.2$$

Fig. 3. Linear relationships between log P_{cpc} and log P_{oct} for phenols, benzoic acids and N-phenylanthranilates (NPAs) in the hexane–acetonitrile system. The regression lines were drawn according to eqns. 5–7.

For benzoic acids:

$$\log P_{oct} = 5.230 + 1.961 \log P_{cpcHA} \tag{6}$$
$$(0.502) \quad (0.325)$$
$$n = 5, r = 0.995, s = 0.059, F(1,3) = 282, F(1,3)_{0.01} = 34.1$$

For N-phenylanthranilates:

$$\log P_{oct} = 8.991 + 2.965 \log P_{cpcHA} \tag{7}$$
$$(1.591) \quad (1.067)$$
$$n = 7, r = 0.950, s = 0.242, F(1,5) = 46, F(1,5)_{0.01} = 16.3$$

According to the F-test, these equations were statistically significant at the 99% level.

The log P_{oct} values were determined from the values of log P_{cpcHA} according to eqns. 5–7. The values obtained were the logarithms of the partition coefficients between octanol and water, calculated from log P_{cpcHA}. These values are referred to as log $P_{oct/cpc}$, and are listed in Table I, together with log P_{oct} values determined by the conventional shaking-flask method. As can be seen, in most instances, the value of log $P_{oct/cpc}$ agrees well with the corresponding log P_{oct} value.

Of the compounds examined, flufenamic acid has the highest log P_{oct} (5.62)[18]. As shown in Fig. 2, elution of flufenamic acid required about 50 min, but the chromatogram was well resolved and the value of log $P_{oct/cpc}$ agreed very well with the value of log P_{oct} (*cf.*, Table I). Therefore, values of log P_{oct} of up to or more than 6 could be determined easily.

Although good linearities between log P_{cpcHA} and log P_{oct} were demonstrated for the three series of compounds when expressed by eqns. 5–7, the values of a and b in eqn. 4 were different for the three series. Further, the statistical significance of the linear regression equation for all the compounds, regardless of their chemical series, was very low, as shown in eqn. 8.

$$\log P_{oct} = 5.813 + 1.668 \log P_{cpcHA} \qquad (8)$$
$$(2.313) \quad (1.663)$$
$$n = 18, r = 0.468, s = 1.158, F(1,16) = 4, F(1,16)_{0.01} = 8.5$$

CPC in the octanol–hexane–water system

Next, we performed CPC in the octanol–hexane(20:80)–water system. For examination of the chromatographic behaviour in terms of $\log P_{oct}$, it would be appropriate to carry out CPC in an octanol–water system. However, this was not successful because of the high viscosity of octanol, which resulted in an inconsistent flow-rate of the mobile phase. Therefore, as a next step, we used an octanol–hexane(20:80)–water system. The pH of the water used as the stationary phase was adjusted to 2.0 with phosphoric acid in order to minimize the effect of acid dissociation, as the chromatographic behaviour of the ionic molecular form of a compound is different from that of its neutral molecular form[19]. Usually, $\log P_{oct}$ is defined as the partition coefficent of the neutral molecular form.

We carried out the CPC of various phenols, benzoic acids and N-phenylanthranilates in the OHW system and determined their $\log P_{cpc}$ values. The results are given in Table II, and the relationship between $\log P_{cpcOHW}$ and $\log P_{oct}$ is shown in Fig. 4. A single linear relationship was obtained between $\log P_{cpcOHW}$ and $\log P_{oct}$, irrespective of the chemical series. This linear relationship is expressed by

$$\log P_{oct} = -0.168 + 1.732 \log P_{cpcOHW} \qquad (9)$$
$$(0.642) \quad (0.285)$$
$$n = 14, r = 0.967, s = 0.340, F(1,12) = 177, F(1,12)_{0.01} = 9.3$$

TABLE II

VALUES OF PARTITION COEFFICIENTS DETERMINED BY CPC IN THE OCTANOL–HEXANE (20:80)–WATER SYSTEM AND BY THE SHAKING-FLASK METHOD

Series	Compound	log P_{cpc}	log P_{oct}*	log $P_{oct/cpc}$**	Δ***
Phenols	2,4-Cl$_2$	1.95	3.08	3.21	−0.13
	2,4,6-Cl$_3$	2.22	3.62	3.68	−0.06
	2,3,4,5,6-Cl$_5$	3.23	5.12	5.43	−0.31
	4-CN	0.80	1.66	1.22	0.44
Benzoic acids	4-Cl	1.81	2.65	2.97	−0.32
	4-CH$_3$	1.63	2.27	2.66	−0.39
	4-NO$_2$	1.26	1.83	2.02	−0.19
	4-C$_2$H$_5$	1.81	2.89	2.97	−0.08
	4-CH(CH$_3$)$_2$	2.02	3.40	3.33	0.07
	4-C(CH$_3$)$_3$	2.38	3.85	3.96	−0.11
N-Phenylanthranilates	H	2.75	4.36	4.60	−0.24
	3'-CF$_3$	2.93	5.62	4.91	0.71
	3'-Cl	3.23	5.57	5.43	0.14
	3'-OH	1.86	3.49	3.05	0.44

* Values from refs. 8 and 18.
** Log P_{oct} determined from log P_{cpc} according to eqn. 9.
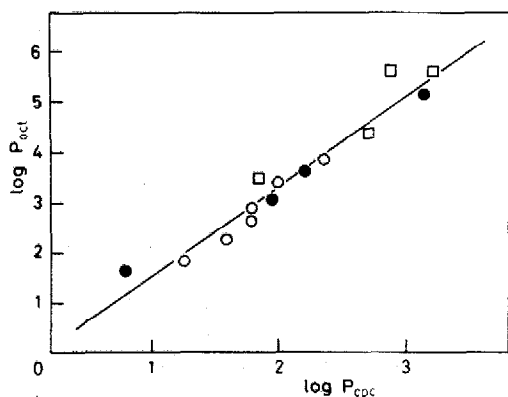*** Difference between log P_{oct} and $P_{oct/cpc}$.

Fig. 4. Linear relationship between log P_{cpc} and log P_{oct} for various compounds in the octanol–hexane–water system. The regression line was drawn according to eqn. 9. ● = Phenols; ○ = benzoic acids; □ = N-phenylanthranilates. The compounds examined are listed in Table II.

Note that according to the *F*-test, eqn. 9 is significant at the 99% level. However, as the value of *a* in eqn. 9 (constant term) is smaller than the value of its 95% confidence interval, in practice the contribution of this constant can be neglected. Hence eqn. 9 can be expressed by

$$\log P_{oct} = 1.730 \log P_{cpcOHW} \tag{10}$$

Table II shows that the values of log $P_{oct/cpc}$ of most compounds agreed very well with the corresponding log P_{oct} values. However, the difference between these values was relatively great for flufenamic acid, probably because of its high log P_{cpcOHW} value. Thus, a value of about 6 may be the highest log $P_{oct/cpc}$ that can be determined with this chromatographic system.

DISCUSSION

In this study, we examined the relationship between log P_{cpc} and log P_{oct} in two systems, hexane–acetonitrile and octanol–hexane–water. For the prediction of the retention time (or retention volume) of organic compounds in CPC, it is useful to know the relationship between log P_{cpc} and log P_{oct}. For the separation of organic compounds by CPC, the hexane–acetonitrile system is more useful than the octanol–hexane–water system, as mentioned under Results. In the hexane–acetonitrile system, linear relationships between log P_{cpcHA} and log P_{oct} were observed, but they were dependent on the chemical series. Thus, the chromatographic behaviour could be evaluated accurately from a plot of log P_{cpcHA} *vs.* log P_{oct} for compounds in the same series as the test compounds, and the elution time could be predicted in terms of P_{oct}.

Values of log P_{cpc} in the octanol–hexane–water system (log P_{cpcOHW}) for various compounds in different chemical series were found to be linearly dependent on their log P_{oct} values. This CPC system is a model of the octanol–water system. Thus, it can be concluded that the chromatographic behaviour in CPC in the octanol–

hexane–water system reflects the nature of the octanol–water system in two-phase partition in the shaking-flask method. Further, the results showed that log P_{oct} can be determined rapidly and accurately by CPC. Because the conventional shaking-flask method for the determination of log P_{oct} has limitations, as described in the Introduction, the CPC method can be used as a more efficient alternative method.

Recently, HPLC has been used to determine log P_{oct}[15–17]. Although the logarithm of the factor, k', increases linearly with increase in log P_{oct}, this relationship is in most instances dependent on the chemical series[17]. As eqn. 9 is independent of the chemical series, CPC should be more effective than HPLC for the determination of log P_{oct} values. Another advantage of CPC is that, because the chromatography is based on liquid–liquid two-phase partition without the use of any solid support, the chromatographic system is simpler than HPLC. Further, in CPC, V_m is determined directly from the elution volume, unlike in HPLC, where the corresponding value (the hold-up volume or dead volume) is determined as the retention volume of an unretained compound. The latter value is, however, dependent on the nature of the unretained compound, and there is not universally accepted method for the accurate determination of this volume[20]. Therefore, in practice, CPC could be a better method than HPLC for this purpose.

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