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USE OF CENTRIFUGAL PARTITION CHROMATOGRAPHY FOR ASSESSING PARTITION COEFFICIENTS IN VARIOUS SOLVENT SYSTEMS

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SUMMARY

Centrifugal partition chromatography (PC) was examined as a technique for measuring the partition coefficient of organic solutes. Two solvent systems of convenient viscosity were used, namely *n*-hexanol–water and cyclohexane–water. The partition coefficients thus determined (expressed as log $P_{CPC/ol}$ and log $P_{CPC/ane}$, respectively) were shown to be well correlated with literature partition coefficients obtained by the shake-flask (SF) method in the *n*-octanol–water and *n*-hexane–water systems, respectively. Hydrogen-bonding parameters, I_H, were calculated as (log $P_{CPC/ol}$ – log $P_{CPC/ane}$) and (log P_{oct} – log P_{hex}); the good correlation between the I_H values derived from the CPC and SF methods indicates that the same partitioning mechanism is operative in both methods.

INTRODUCTION

Lipophilicity is an important molecular property of drugs and other xenobiotics often correlated with their biological activity. A number of experimental models have been developed to simulate partitioning processes in biological systems and to quantify lipophilicity. The shake-flask (SF) technique, using a biphasic liquid system

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consisting of water and an organic solvent, remains the standard method for measuring the lipophilicity of chemical compounds; *n*-octanol is universally accepted as the reference organic solvent[1–3], but other solvents are of considerable value, for example in comparing the hydrogen-bonding ability of series of compounds[4–6]. Thus, lipophilicity is often expressed as the logarithm of the partition coefficient (log P_{oct}), defined as the equilibrium distribution of a single chemical species between the aqueous and the octanol phases. Yet despite its value, the SF method suffers a number of practical limitations and disadvantages due to various perturbing factors such as time consumption, solute stability, solute impurities, solute volatility, formation of microemulsions, concentration and salt effects, etc.[7–9].

Solid–liquid partition chromatography has been explored as an alternative means for measuring lipophilicity. Indeed, the use of chromatographic retention parameters, in particular those obtained from reversed-phase high-performance liquid chromatography (RP-HPLC), are becoming increasingly popular in replacing the *n*-octanol–water partition coefficient in quantitative structure–activity relationship (QSAR) studies[10–13]. Unfortunately, the presence of a solid support with a non-negligible proportion of residual silanol groups on the surface of alkyl-bonded stationary phases dramatically influences the partitioning process of polar basic compounds due to an additional adsorption mechanism[14–17].

Recently, Terada *et al.*[18,19] have used a new chromatographic technique known as centrifugal partition chromatography (CPC) to measure lipophilicity. CPC is a liquid–liquid chromatography technique resembling to some extent droplet countercurrent chromatography (DCCC)[20–22]. As in DCCC, two non-miscible liquids are used as the stationary and mobile phase, respectively. A centrifugal force maintains the liquid stationary phase in a set of interconnected columns (cartridges), while the mobile phase is pumped through the stationary phase in an ascending or descending mode depending on the respective densities of the two phases. General features of the CPC technique such as its efficiency and selectivity have been discussed in detail by Berthod and Armstrong[23,24]. From their studies, Terada *et al.*[18,19] concluded that CPC may be preferable to both the SF and RP-HPLC methods for measuring lipophilicity. Indeed, CPC has advantages over RP-HPLC or other chromatographic methods in that no solid support is present and no adsorption mechanism is involved. Consequently, as in the SF method, the retention mechanism is governed solely by partitioning processes. In addition, CPC can be performed over a very broad pH range since limitations imposed by a solid support such as silica do not apply. Moreover, CPC being a liquid–liquid chromatographic method opens promising possibilities for the precise and accurate assessment of partition coefficients in a large variety of solvent systems.

Exploring some of these possibilities constitutes the major objective of the present study. After optimizing the experimental conditions, the CPC technique was used to measure the partition coefficients of several organic compounds in an amphiprotic (*n*-hexanol) and an aprotic (cyclohexane) organic solvent.

EXPERIMENTAL

Materials

4-Pyridylalkanols were synthesized as previously described[25]. Mono- and di-

substituted benzene derivatives were obtained from Fluka (Buchs, Switzerland); the buffer compound 3-morpholinopropanesulphonate (MPS), *n*-octanol, *n*-hexanol, *n*-hexane and cyclohexane were obtained from Merck (Darmstadt, F.R.G.). All compounds obtained were of analytical grade and used without further purification.

Centrifugal partition chromatography

Measurements were performed at 30°C using a centrifugal partition chromatograph (Model CPC-LLN; Sanki Engineering, Kyoto, Japan) connected to a 2238 Uvicord II detector operating at 254 nm (LKB, Bromma, Sweden) and a 600 chart recorder (W + W Scientific, Basle, Switzerland). Solvent delivery was by a Sanki constant flow pump (Model LBP-V; Sanki Engineering). The chromatograph was fitted with twelve Type 250W cartridges (total volume 250 ml) and samples were injected by means of a six-way valve and 3-ml sample loop. The apparatus was first packed with the stationary phase and then the mobile phase was pumped through. The sample was introduced when elution of the stationary phase was complete and the mobile phase had exited from the cartridges.

Two solvent systems were employed, namely *n*-hexanol–aqueous buffer (0.02 *M* MPS, pH 7.4) and cyclohexane–aqueous buffer (0.02 *M* MPS, pH 7.4). The aqueous and organic phases were mutually saturated. Preliminary studies using an *n*-octanol–aqueous buffer system and an *n*-hexane–aqueous buffer system were not successful. With the *n*-octanol–aqueous buffer system the pump pressure reached its upper limit due to the high viscosity of *n*-octanol. To overcome this problem, *n*-hexanol was chosen as the amphiprotic organic solvent. In the *n*-hexane–aqueous buffer system, inconsistent pump pressures and flow-rates of the mobile phase were observed probably due to the low density and/or low viscosity of *n*-hexane.

In the *n*-hexanol–aqueous buffer system, *n*-hexanol was used as the mobile phase and aqueous buffer as the stationary phase. The flow-rate was 1.8 ml/min and the pump pressure was 55 kg/cm^2 at a rotation rate of 500 rpm. In this solvent system the equilibrium partition coefficient is calculated from the following equation[19]

$$\log P_{\mathrm{CPC/ol}} = \log [V_{\mathrm{S}}/(V_{\mathrm{R}} - V_{\mathrm{M}})] \quad (1)$$

where V_{S} is the stationary phase volume, V_{R} is the retention volume of the solute and V_{M} is the dead volume (mobile phase volume in the cartridges).

In the cyclohexane–aqueous buffer system, water was used as the mobile phase and cyclohexane as the stationary phase. The flow-rate was 2.4 ml/min and the pump pressure was 50 kg/cm^2 at a rotation rate of 700 rpm. The equilibrium partition coefficients are calculated from the reverse of eqn. 1:

$$\log P_{\mathrm{CPC/ane}} = \log [(V_{\mathrm{R}} - V_{\mathrm{M}})/V_{\mathrm{S}}] \quad (2)$$

A systematic determination of the dead volume is very important in CPC due to the continuous decrease of the stationary phase volume. This phenomenon, known as column "bleeding", occurs despite the presaturation of the phases and has been investigated in detail by Berthod and Armstrong[23]. They have proposed the use of a dead volume tracer to take account of the variation in stationary phase volume. In the present study, sodium dichromate and biphenyl were used as the non-retained com-

pounds to assess the dead volumes in the cyclohexane–water system and the *n*-hexanol–water system, respectively.

RESULTS

n-Hexanol–water system

Typical V_R values of the compounds investigated are reported in Table I together with the partition coefficients expressed as log $P_{CPC/ol}$ and calculated according to eqn. 1. Table I also reports literature partition coefficients obtained by the SF method in the *n*-octanol–water system and expressed as log P_{oct}. The excellent reproducibility of log $P_{CPC/ol}$ values should be noted (S.D. $<4\%$). The compounds investigated cover a log P_{oct} range of 2 units. A good linear relationship between log $P_{CPC/ol}$ and log P_{oct} is revealed in eqn. 3 and depicted in Fig. 1

$$\log P_{oct} = 1.25(\pm 0.16) \log P_{CPC/ol} - 0.25(\pm 0.16)$$
$$n = 20,\ r = 0.968,\ s = 0.15,\ F = 269 \qquad (3)$$

where n is the number of compounds, r the correlation coefficient, s the standard deviation of the linear regression and F is the Fischer test of the statistical significance of the equation. The values in parentheses are the 95% confidence limits of the

TABLE I

PARTITION COEFFICIENTS OF VARIOUS ORGANIC COMPOUNDS MEASURED BY CPC IN AN *n*-HEXANOL–WATER SYSTEM, log $P_{CPC/ol}$

Compound	V_R *(ml)*[a]	*log* $P_{CPC/ol}$	*log* P_{oct}[b]
$C_6H_5SO_2NH_2$	159.84	0.44	0.31
$C_6H_5SOCH_3$	138.06	0.60	0.55
$C_6H_5SO_2CH_3$	135.72	0.69	0.49
$C_6H_5CONH_2$	129.60	0.74	0.64
$C_6H_5NHCOCH_3$	118.80	0.99	1.16
$C_6H_5NH_2$	119.34	1.03	0.90
$C_6H_5CH_2OH$	116.64	1.08	1.10
C_6H_5OH	110.16	1.34	1.47
$C_6H_5OCOCH_3$	113.40	1.32	1.49
$C_6H_5NO_2$	110.16	1.45	1.86
$HO\text{–}C_6H_4\text{-}4\text{-}NH_2$	216.45	0.11	0.04
$HO\text{–}C_6H_4\text{-}2\text{-}NH_2$	132.21	0.70	0.52
$H_2N\text{–}C_6H_4\text{-}4\text{-}NO_2$	113.40	1.49	1.39
$H_2N\text{–}C_6H_4\text{-}3\text{-}NO_2$	107.64	1.35	1.37
$H_2N\text{–}C_6H_4\text{-}2\text{-}NO_2$	111.85	1.47	1.83
$4\text{-}C_5H_4N\text{–}CH_2OH$	192.24	0.22	0.06
$4\text{-}C_5H_4N\text{–}(CH_2)_2OH$	179.28	0.30	0.10
$4\text{-}C_5H_4N\text{–}(CH_2)_3OH$	130.57	0.80	0.58
$4\text{-}C_5H_4N\text{–}(CH_2)_4OH$	120.96	1.01	0.90
$4\text{-}C_5H_4N\text{–}(CH_2)_5OH$	112.32	1.47	1.39

[a] V_M values in the range 100–108 ml.

[b] Data taken from ref. 26 for the phenyl derivatives and from ref. 27 for the pyridyl derivatives.

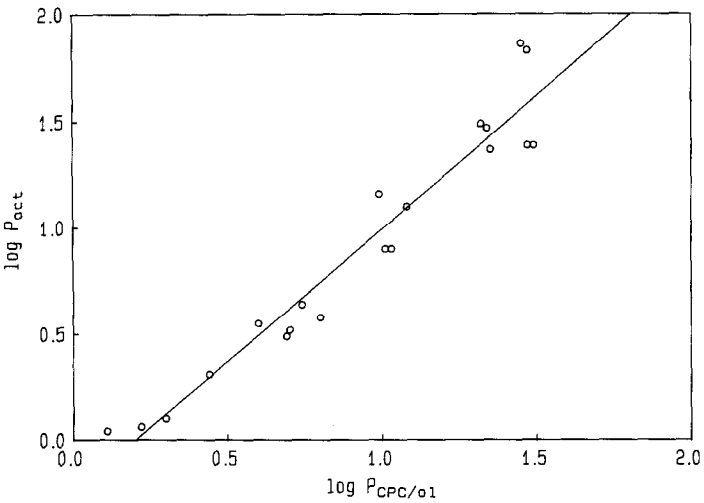

Fig. 1. Relationship between log $P_{CPC/ol}$ and log P_{oct} for the 20 compounds in Table I.

regression coefficients. For the homologous series of 4-pyridylalkanols, the correlation is even better:

$$\log P_{oct} = 1.08(\pm 0.15) \log P_{CPC/ol} - 0.22(\pm 0.13)$$
$$n = 5, r = 0.997, s = 0.05, F = 526 \quad (4)$$

This is presumably due to the good quality and single origin of the log P_{oct} values of these compounds, which were carefully measured under the same conditions in our laboratory[27].

Cyclohexane–water system

In Table II, partition coefficients of a series of mono- and disubstituted benzenes determined by CPC using the cyclohexane–aqueous buffer system were compared with those obtained by the SF method using *n*-hexane–water. The compounds investigated cover a log P_{hex} range of 2.5 units. Although only a limited number of log P_{hex} values are available ($n = 8$), a good linear relationship between log $P_{CPC/ane}$ and log P_{hex} is revealed by eqn. 5 and shown in Fig. 2:

$$\log P_{hex} = 1.05(\pm 0.31) \log P_{CPC/ane} - 0.21(\pm 0.29)$$
$$n = 8, r = 0.959, s = 0.26, F = 69 \quad (5)$$

Hydrogen bonding ability

It is well known that a linear relationship between partition coefficients obtained in different solvent systems is valid only when the organic solvents in the two systems are both either inert (*e.g.*, alkanes), hydrogen-bond acceptors (*e.g.*, ethers), hydrogen-bond donors (chloroform) or amphiprotic (*e.g.*, alkanols)[28,29]. These restrictions have prompted Seiler[4] to define a parameter, I_H, conceived as a measure of the hydrogen-bonding ability of a given solute. This parameter allows the intercon-

TABLE II

PARTITION COEFFICIENTS OF MONO- AND DISUBSTITUTED BENZENES MEASURED BY CPC IN A CYCLOHEXANE–WATER SYSTEM (log $P_{CPC/ane}$), AND HYDROGEN-BONDING PARAMETERS, I_H, CALCULATED ACCORDING TO EQN. 6

Compound	V_R *(ml)*[a]	*log* $P_{CPC/ane}$	$I_{H(CPC)}$	*log* P_{hex}[b]	$I_{H(SF)}$
$C_6H_5SO_2NH_2$	138.82	−2.28	2.72	–	–
$C_6H_5SOCH_3$	144.00	−1.29	1.89	–	–
$C_6H_5SO_2CH_3$	167.04	−0.59	1.28	–	–
$C_6H_5CONH_2$	132.48	−1.92	2.66	−2.35	2.99
$C_6H_5NHCOCH_3$	138.24	−1.31	2.30	−1.80	2.96
$C_6H_5NH_2$	289.44	0.12	0.91	−0.05	0.95
$C_6H_5CH_2OH$	169.97	−0.46	1.54	−0.76	1.86
$C_6H_5CH_2CH_2OH$	252.00	0.01	–	−0.39	1.75
C_6H_5OH	155.52	−0.69	2.03	−0.89	2.36
$HO–C_6H_4$-4-NH_2	132.48	−1.62	1.73	–	–
$HO–C_6H_4$-2-NH_2	141.12	−1.02	1.72	–	–
$H_2N–C_6H_4$-4-NO_2	145.44	−0.92	2.41	−0.62	2.01
$H_2N–C_6H_4$-2-NO_2	446.40	0.42	1.05	0.21	1.62

[a] V_M values in the range 126–139 ml.
[b] Data taken from ref. 26; blanks correspond to unavailable values.

version of partition coefficients from alkane–water systems to alkanol–water systems using the following general equation:

$$I_H = \log P_{alkanol} - \log P_{alkane} \quad (6)$$

The importance and the physicochemical interpretation of this parameter have been discussed at length in several recent papers[5,6,30,31]. According to this concept, we

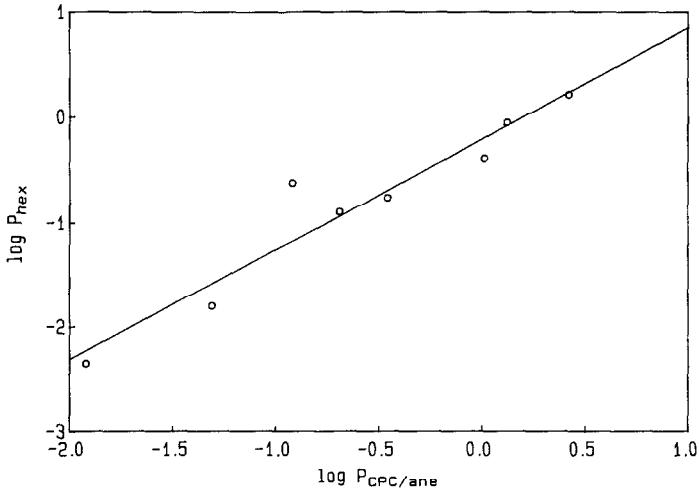

Fig. 2. Relationship between log $P_{CPC/ane}$ and log P_{hex} for eight compounds in Table II.

have examined the relationship between I_H values obtained by the SF and CPC methods. A satisfactory correlation was found:

$$I_{H(SF)} = 0.94(\pm 0.58)\, I_{H(CPC)} + 0.38(\pm 1.15)$$
$$n = 7,\ r = 0.877,\ s = 0.38,\ F = 16.7 \qquad (7)$$

In eqns. 3–5 and 7 the slopes are practically equal to one and the intercepts are close to zero. This leads to the important conclusion that both the SF and CPC methods are governed by the same partitioning mechanism and that the CPC method appears of value for assessing the hydrogen-bonding ability, I_H, of solutes, at least in the lipophilicity range investigated here.

DISCUSSION

In this study, we have examined the use of a CPC technique for the determination of partition coefficients of various compounds in two different solvent systems. The method appears suitable for a study of the partitioning behaviour of compounds in a variety of organic solvents. However, the range of partition coefficients which can be measured by this method was limited due to practical problems such as the long duration of elution and the band broadening characteristic of the more strongly retained compounds. Increasing the speed of rotation of the chromatograph improves the resolution but at same time leads to unacceptably high back pressures. To ensure good precision, the choice of solvent as either the stationary phase or mobile phase is very important. In the *n*-hexanol–aqueous buffer system, *n*-hexanol was chosen as the mobile phase and polar compounds tended to be more strongly retained in the stationary aqueous phase than less polar ones. This condition provided a maximum of selectivity and precision for the more polar compounds. It is clear that the relative error increases when the difference between V_R and V_M decreases. Thus, relatively lipophilic compounds (log $P_{oct} > 2$) cannot be measured accurately under these conditions. Berthod and Armstrong[32] have demonstrated that the retention volume of a given solute must be at least 5 ml higher than the dead volume to determine partition coefficients with less than 10% error. In contrast, polar compounds (log $P_{oct} > -2$) can be accurately measured using *n*-hexanol as the mobile phase. In cyclohexane–aqueous buffer, water was used as the mobile phase, allowing a good selectivity and precision in the lipophilicity range investigated. However, the very long retention time of compounds with log $P_{hex} > 0.5$ limits the usefulness of the conditions chosen. The lower limit which can be measured under these conditions is log $P_{hex} > -2.5$.

Berthod *et al.*[32,33] have also shown that CPC can be used directly to determine octanol–water partition coefficients over a log P_{oct} range of -2.5 to 2.5. For accurate determination of the higher log P_{oct} values, they proposed to use water as the mobile phase and *n*-octanol as the stationary phase. In order to have reasonable retention times while retaining efficiency, they reduced the stationary phase volume using a procedure termed the "underloading mode". Indeed, this method may extend the range of partition coefficients which can be measured by CPC using other amphiprotic organic solvents. However, further studies are needed to examine its utility.

Terada *et al.*[18,19] have compared the *n*-octanol–water partition coefficients of

various organic compounds measured by the SF and CPC methods using two different solvent systems, *n*-hexane–acetonitrile and [*n*-octanol–*n*-hexane (20:80)]–water. A single linear relationship between log k_{CPC} and log P_{oct} was found in the latter but not in the former system, where the relationship was dependent on the chemical structures of the solutes. Terada *et al.*[18,19] thus concluded that the (octanol–hexane)–water solvent system exhibits the same properties as the octanol–water system. Berthod *et al.*[33] have also used the [*n*-octanol–*n*-hexane (40:60)]–water system in order to expand the lipophilicity range which can be measured by the CPC method. The reason for using octanol–hexane mixtures as the organic phase is to reduce the retention time, *i.e.*, to increase the lipophilicity range, and also to decrease the high viscosity of *n*-octanol which causes pressure problems. In these studies[19,33] however, the dramatic decrease in polarity of the organic phase caused by the addition of *n*-hexane was not taken into consideration, particularly at such high proportions of *n*-hexane.

That this neglect may be misleading is shown by the work of Okada *et al.*[34], who investigated the effect of mixed organic solvents on the partition coefficient of procaine and *p*-aminobenzoic acid, demonstrating that partition coefficients in (pentanol–cyclohexane)–water systems gradually increase with increasing proportions of pentanol. They interpreted this behaviour as a polarity-dependent change in the ability of the organic phase to solvate a given solute. As a consequence, the partition behaviour of various solutes, in particular polar compounds, will be quite different in an (octanol–hexane)–water or an octanol–water system. The conclusion to draw from such studies is that (octanol–hexane)–water partition coefficients cannot be used to predict octanol–water partition coefficients in a classical Collander approach[28,29].

It should also be noted that biphasic organic–organic systems such as hexane–acetonitrile or hexane–methanol cannot be used to determine the hydrophobicity of compounds. Indeed, the hydrophobic effect is determined mainly by the hydrogen-bonding network structure of water molecules which cannot be simulated by either acetonitrile or by methanol.

We thus conclude that CPC is a useful tool for measuring partition coefficients in different solvent systems. However, the range of measurable lipophilicities cannot be expanded very far due to the solvent limitations discussed above. Technical improvements of the CPC apparatus, *e.g.*, higher pump pressures, and better stability with low viscosity solvents should alleviate these limitations.

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