CHROM. 21 184

Note

Prediction of alkane–water partition coefficients using a C_{18} derivatized polystyrene–divinylbenzene stationary phase

W. J. LAMBERT* and L. A. WRIGHT[a]

The Upjohn Company, Drug Delivery Research and Development, 301 Henrietta Street, Kalamazoo, MI 49001 (U.S.A.)

(First received October 25th, 1988; revised manuscript received December 12th, 1988)

Octanol–water partition coefficients have been utilized as a reference system in many fields, due primarily to the vast literature base developed by Hansch and co-workers[1,2]. Unfortunately, the hydrogen bonding capability of octanol reduces the intrinsic usefulness of the octanol–water system. Rytting *et al.*[3] and Anderson[4] have suggested that alkanes provide more information on intermolecular forces than octanol due to the lack of hydrogen bonding and dipole–dipole interactions. Furthermore, alkane–water partition coefficients are more relevant in biomembrane transport since the interior region of phospholipid membranes present an alkane barrier to transport[3]. The objective of this report is to disclose a rapid method for determining alkane–water partition coefficients using high-performance liquid chromatography (HPLC) on a new C_{18} bonded support, for future application to transport and structure–activity studies.

Several articles have appeared in the literature which review the use of HPLC retention times to estimate octanol–water partition coefficients[5–7]. Compared to the traditional "shake flask" method, the HPLC method has the advantage of speed, lower expense, less sensitivity to solute degradation, lower requirement for amount of solute, and less sensitivity to impurity interactions. Silica-based columns are generally utilized for the stationary phase, however, polystyrene–divinylbenzene[8,9] and glyceryl-coated glass[10] stationary phases have recently been employed. These columns do not have the mobile phase pH limitations of the silica-based columns[8–11]. Ideally, specific interactions between the stationary phase and the solute of interest should not occur. While silanol groups are typically masked by chemical modification[12], the addition of a lipophilic amine to the mobile phase[13], or by saturation of the stationary phase with octanol[14], outliers due to specific interactions between the free silanol groups and various solutes are commonly seen[6,7,13,14]. Trace metals in silica columns provide an additional site for specific interactions[15]. It has been shown that polystyrene–divinylbenzene stationary phases are also capable of specific interactions[8,9], possibly due to the electron rich pi orbitals which are present[11,16,17].

A C_{18} derivatized polystyrene–divinylbenzene column (Act-I) has been utilized

---

[a] Current address: College of Pharmacy, Florida A&M University, Tallahassee, FL, U.S.A.

in the present study. Benson and Woo[11] have suggested that C_{18} derivatization virtually eliminates solute interaction with the aromatic portion of the stationary phase through steric hinderance. This report shows that this assumption appears to be correct, and alkane–water partition coefficients can be predicted with a high degree of confidence.

EXPERIMENTAL

The chromatographic system utilized in the present study included a Milton Roy/LDC (Riviera Beach, FL, U.S.A.) SM4000 UV detector, a Waters Assoc. (Milford, MA, U.S.A.) WISP 710B autosampler, a Beckman Instruments (Fullerton, CA, U.S.A.) 110A Pump and a Nelson Analytical (Cupertino, CA, U.S.A.) 760 interface with Series 2600 chromatography software. A specially prepared 5-cm Act-I column (normally available as a 15-cm column) was received from Interaction Chemicals (Mountain View, CA, U.S.A.). A methanol–water (60:40) mobile phase was utilized at a flow-rate of 1 ml/min. The pH of the mobile phase was adjusted for acidic and basic compounds. The low ionic strength ($I = 0.01$) buffers of Perrin[18], 0.01 M hydrochloric acid, or 0.01 M sodium hydroxide were used to control the apparent pH (prepared in 60% methanol). The Perrin buffers were chosen to be at least 2 pH units below the pK_a of the acid (or above the pK_a of the base), and two different apparent pH values were utilized for each compound to demonstrate that the maximum retention was achieved. The 30 compounds used as standards in the present study and their sources are listed in Table I. Samples were prepared in the mobile phase at a concentration of approximately 1 mg/ml, as has been previously recommended[7]. An injection volume of 20 μl and a detection wavelength of 230 nm were utilized. Capacity factor (k') was calculated as $k' = (t_R - t_0)/t_0$ where t_R and t_0 are the retention times of the sample (in duplicate) and an unretained solute (methanol), respectively.

Hexane–water partition coefficients for nitroethane and nitrobutane were determined at 25°C (in duplicate) by the traditional "shake flask" method, using a Fisher (Springfield, NJ, U.S.A.) Model 236 shaker bath. The volume ratio of hexane to water was 1, and the initial aqueous concentration of the test solute was 0.5–0.7 mg/ml. The concentration of solute in the aqueous phase was determined using the above HPLC system, except a 25-cm Whatman (Clifton, NJ, U.S.A.) Partisil 10 ODS-3 column and a Brownlee (Santa Clara, CA, U.S.A.) RP-18 Spheri 10 (3 cm × 4.6 mm I.D.) guard column were used in place of the Act-I column. All other partition coefficients (PC) were taken from the literature (see Table I). The alkane–water partition coefficients used were limited to n-pentane through n-decane and cyclo-hexane, and did not include those estimated from HPLC methods or those thought questionable by the authors of the reference. In cases where multiple values were available, the mean was utilized.

RESULTS AND DISCUSSION

In estimating partition coefficients by HPLC, a choice must be made between using a capacity factor determined at a particular mobile phase organic volume fraction, or by linearly extrapolating the capacity factor to 0% organic. The latter

TABLE I

STANDARD COMPOUNDS

Suppliers: a = Aldrich (Milwaukee, WI, U.S.A.); e = Eastman Kodak (Rochester, NY, U.S.A.); f = Fisher Scientific (Fair Lawn, NJ, U.S.A.); k = Fluka Chemie (Buchs, Switzerland); l = Lancaster Synthesis (Windham, NH, U.S.A.); m = Mallinckrodt (Paris, KY, U.S.A.); s = Sigma (St. Louis, MO, U.S.A.). Types (hydrogen acceptor if not specified): 1 = non-hydrogen bonding; 2 = acid–alcohol; 3 = base.

Compound	Supplier	Type	log PC[a]
Acetanilide	a		−1.70
Acetophenone	a		1.16
Aniline	m	3	−0.01
Anisole	s		2.19
Benzaldehyde	a		1.19
Benzamide	l		−2.30
Benzene	f	1	2.30
Benzoic acid	e	2	−1.06
Benzonitrile	f		1.04
Benzophenone	e		3.29
Benzylalcohol	e	2	−0.62
Benzylamine	s	3	−0.21
Biphenyl	k	1	4.10[b]
Chlorobenzene	f		2.95
N,N-Dimethylaniline	s	3	2.32
Ethylbenzene	s	1	3.08[b]
Ethylbenzoate	a		1.40
Methylbenzoate	e		2.08
Nitrobenzene	s		1.52
Nitrobutane	k		1.14[c]
Nitroethane	k		−0.38[c]
Nitromethane	k		−0.93[b]
Phenol	m	2	−0.81
Phenylacetic acid	e	2	−1.23
Phenylacetone	s		0.98
Phenylacetonitrile	e		1.31
Propiophenone	a		2.02
n-Propylbenzene	e	1	4.11[b]
Pyridine	a	3	−0.31
Toluene	f	1	2.86

[a] Ref. 26 if not specified.
[b] Ref. 27.
[c] This study.

method has several drawbacks. First, it is well known, both theoretically and experimentally, that the logarithm of capacity factor is related quadratically to the organic volume fraction[19–21]. Thus, a linear extrapolation can only be performed over a limited (and impractical) range. Second, the quadratic relationship is dependent on the organic solvent used[19,21,22]. Finally, the extrapolation method requires much more time, which defeats a primary advantage of the HPLC method over the shake flask method. Therefore, a 60% methanolic mobile phase was chosen for the present study. This choice was not entirely arbitrary. Methanol is known to produce a less dramatic curvature in plots of log k' versus volume fraction when compared to other

commonly used HPLC solvents[19,21,22]. This is most likely due to the fact that the solubility parameter of methanol is closer to that of water than the other solvents. Furthermore, the physical properties of methanol–water mixtures have been well studied[23,24], which accounts for why methanol–water mixtures are often used for pK_a determination of compounds with low aqueous solubility[25]. A volume fraction of 60% was chosen since it was found to give reasonable retention times for the solutes used in the present study (which is reasonable for many pharmaceutically relevant compounds as well).

A plot of log PC(alkane–water) *versus* log k' and the linear regression line is shown in Fig. 1. The slope, intercept, and correlation coefficient of the regression line are 2.11 (11.7%), −0.998 (30.7%) and 0.953, respectively (95% confidence intervals are in parentheses). This correlation is considered very good due to the wide range of lipophilicities of the solutes (partition coefficients ranging over six orders of magnitude). The deviation from the regression line appears to be most dramatic for compounds with a log PC less than 0. This is most likely due to the error in quantitating k' for solutes with retention times approaching t_0. Therefore, investigators may wish to alter the methanol percentage to match the lipophilicities of the compounds under study.

The compounds in Fig. 1 were classified as non-hydrogen bonding, acid or alcohol, base, or hydrogen bond acceptor (see Table I). The inclusion of various classes of solutes demonstrates the lack of any significant specific interaction between the stationary phase and the solutes. The lack of specific interactions will be treated quantitatively in a future report.

In conclusion, a significant correlation was found between log PC(alkane–water) and log k'. There appears to be no significant specific interaction between the solutes and the polymeric stationary phase, thus, providing a distinct advantage over methods using traditional reversed-phase HPLC columns. Finally, the system can be
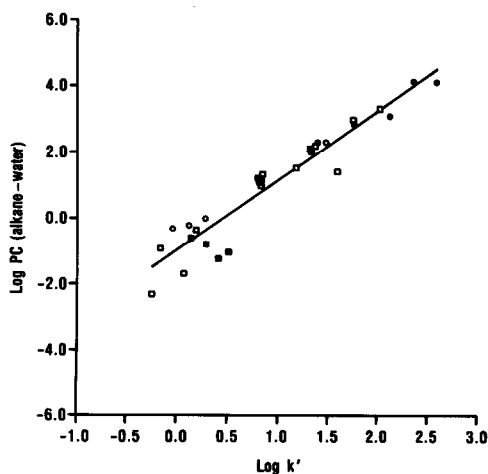
Fig. 1. Relationship between the alkane–water partition coefficient and the HPLC capacity factor [methanol–water (60:40)]. Key: ● = non-hydrogen bonding; ■ = acid–alcohol; ○ = base; □ = hydrogen bonding acceptor.

utilized for the rapid screening of lipophilicity of compounds, including those with acidic and basic functionalities.

REFERENCES

1 A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 71 (1971) 525–616.
2 A. Leo and C. Hansch, *J. Org. Chem.*, 36 (1971) 1539–1544.
3 J. H. Rytting, S. S. Davis and T. Higuchi, *J. Pharm. Sci.*, 61 (1972) 816–818.
4 B. D. Anderson, in S. H. Yalkowsky, A. A. Sinkula and S. C. Valvani (Editors), *Physical and Chemical Properties of Drugs*, Marcel Dekker, New York, 1980, pp. 231–266.
5 R. Kaliszan, *Quantitative Structure–Chromatographic Retention Relationships*, Wiley, New York, 1987, pp. 232–278.
6 R. Kaliszan, *CRC Crit. Rev. Anal. Chem.*, 16 (1986) 323–383.
7 *Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients*, European Chemical Industry Ecology and Technology Centre, Brussels, Belgium, 1983.
8 V. De Biasi, W. J. Lough and M. B. Evans, *J. Chromatogr.*, 353 (1986) 279–284.
9 K. Miyake, F. Kitaura, N. Mizuno and H. Terada, *Chem. Pharm. Bull.*, 35 (1987) 377–388.
10 K. Miyake and H. Terada, *J. Chromatogr.*, 240 (1982) 9–20.
11 J. R. Benson and D. J. Woo, *J. Chromatogr. Sci.*, 22 (1984) 386–399.
12 J. M. McCall, *J. Med. Chem.*, 18 (1975) 549–552.
13 T. L. Hafkenscheid and E. Tomlinson, *Int. J. Pharm.*, 16 (1983) 225–239.
14 M. S. Mirrlees, S. J. Moulton, C. T. Murphy and P. J. Taylor, *J. Med. Chem.*, 19 (1976) 615–619.
15 M. Verzele and C. Dewaele, *J. Chromatogr.*, 217 (1981) 399–404.
16 A. V. Kiselev, *J. Colloid Interface Sci.*, 28 (1968) 430–442.
17 J. Paleos, *J. Colloid Interface Sci.*, 31 (1969) 7–18.
18 D. D. Perrin, *Aust. J. Chem.*, 16 (1963) 572–578.
19 P. J. Schoenmakers, H. A. H. Billiet, R. Tijssen and L. de Galan, *J. Chromatogr.*, 149 (1978) 519–537.
20 P. Jandera, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 435–441.
21 K. A. Dill, *J. Phys. Chem.*, 91 (1987) 1980–1988.
22 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179–195.
23 G. Akerlof, *J. Am. Chem. Soc.*, 54 (1932) 4125–4139.
24 I. T. Oiwa, *J. Phys. Chem.*, 60 (1956) 754–759.
25 L. Z. Benet and J. E. Goyan, *J. Pharm. Sci.*, 56 (1967) 665–680.
26 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.
27 Technical Database Services, 10 Columbus Circle, Suite 2300, New York, NY 10019.