Journal of Chromatography, 538 (1991) 119–123
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 725

Note

Toroidal coil centrifugal partition chromatography, a method for measuring partition coefficients

RUEY-SHIUAN TSAI, NABIL EL TAYAR and BERNARD TESTA*
School of Pharmacy, University of Lausanne, Place du Château, CH-1005 Lausanne (Switzerland)
and
YOICHIRO ITO
Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, Building 10, Room 7N-322, National Institutes of Health, Bethesda, MD 20892 (U.S.A.)

Lipophilicity is a major physico-chemical parameter in quantitative structure–activity relationship (QSAR) studies [1,2]. A number of experimental methods are currently used to simulate partition processes in biological systems and to determine lipophilicity. The traditional shake-flask technique, using a biphasic liquid system, remains the method of reference for measuring partition coefficients (expressed as log P values), but suffers from a number of pratical limitations [3,4].

Solid–liquid partition chromatography has been applied as an alternative means for measuring lipophilicity, and the use of chromatographic retention parameters, in particular those obtained from reversed-phase high-performance liquid chromatography (RP-HPLC) [5], have become popular in replacing solvent–water partition coefficients in QSAR studies. However, the solid supports used in RP-HPLC, particularly octadecylsilane, interact with solutes in modes that in some instances may differ significantly from solute–solvent interactions in the shake-flask method [6,7], rendering difficult comparisons between data and the interpretation of lipophilicity–activity correlations.

Recently, centrifugal partition chromatography (CPC) has been explored as a novel approach for measuring liquid–liquid partition coefficients [8,9]. CPC has been shown to yield highly reproducible log P results, and various technical improvements have transformed it into a fast and efficient method [10,11]. To the best of our knowledge, the only limitation of the CPC method so far is a lipophilicity range that is still too restricted for medicinal use (log P from -3 to $+3$ [12], because the volume of the aqueous stationary phase cannot exceed 95% of the total coil capacity). Any CPC technique that is able, at least potentially, to extend this range is therefore worth investigating. In this paper, we report some preliminary results indicating that the technique known as toroidal coil CPC (TCCPC) can be used to determine partition coefficients, and we discuss its potential for measuring very low and very high log P values.

0021-9673/91/$03.50

The original design of the flow-through toroidal coil centrifuge (Fig. 1A) permits solvent flow in and out through the rotating column without the use of rotating seals, which often cause complications such as leakage, corrosion and contamination [13]. A helical column mounted in the periphery of the column container located on top of the rotor is shown in Fig. 1B. When a two-phase solvent system is introduced into the rotating column, the propagating eluent percolates continuously through the stationary phase retained in each turn of the coil. As a consequence, solutes introduced in either phase are subjected to an efficient partition process. This system has been demonstrated to separate nine dinitrophenylamino acids with efficiencies ranging between 4000 and 1250 theoretical plates [13]. The major difference between a system of this type and other CPC apparatus is that movement of solutes can be observed continuously through the transparent coil, provided that they are coloured; measurement of solute position becomes possible using a stroboscopic light source (see *Methods*).

EXPERIMENTAL

Chemicals

Pure Sudan III, phenol red and pararosaniline were obtained from Sigma (St. Louis, MO, U.S.A.). Crystal violet (94% purity) and *p*-nitroaniline were purchased from National Aniline Division (New York, U.S.A.) and Amend Drug & Chemical (New York, U.S.A.), respectively. 1-Octanol (99.3% purity) was from Aldrich (Milwaukee, WI, U.S.A.). All compounds and reagents were used without further purification.

Methods

The helical column was prepared by winding PTFE tubing (0.55 mm I.D.) (Zeus Industrial Products, Raritan, NJ, U.S.A.) around a nylon tube (110 cm × 4 mm O.D.) to make *ca.* 830 turns with a total capacity of 4.0 ml. The column was then filled with 1-octanol which had been saturated with 0.1 *M* phosphate buffer (pH 7.4). While the rational speed of the column container was adjusted to 1000 rpm, the mobile phase [0.1 M phosphate buffer (pH 7.4) saturated with 1-octanol] was pumped using a Shimadzu Model LC-6A pump at a flow-rate of 0.4 ml/min. When no stationary phase was observed in the eluate, the solute (dissolved in about 20 μl of 1-octanol phase) was injected from a Rheodyne injector.

The movement of the solutes was observed by stroboscopic illumination with a visible light source. To allow measurement of the solute position and propagation speed, the periphery of the column container was scaled into 35 units. Highly lipophilic solutes may take a very long time to elute, rendering almost impossible the direct measurement of their retention times (t_R). In such cases, hower, t_R can be calculated when the solute is still far from the column outlet by measuring the position of the centre of the solute band (X_t) at time t (Fig. 2):

$$t_R = t\,(X_R/X_t) \tag{1}$$

where X_R is the circumference of the support around which the coil is wound. It follows that log P values can be calculated from the predetermined flow-rate (F),

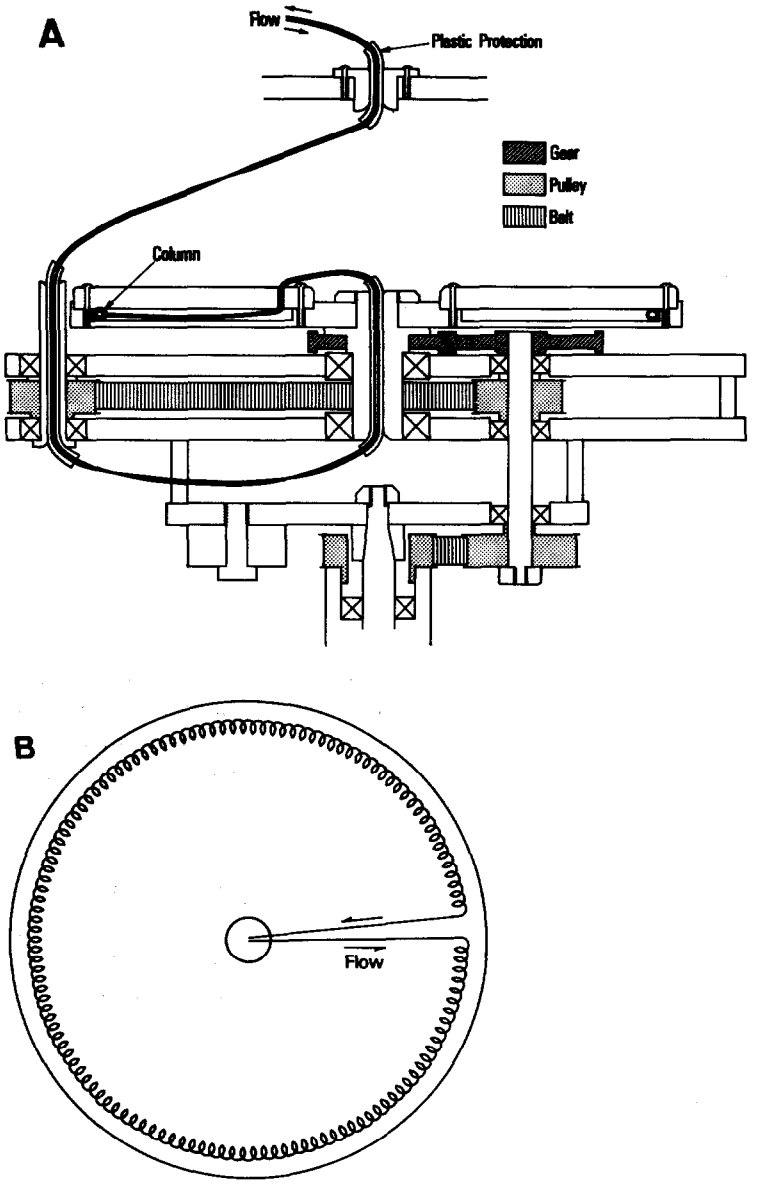

Fig. 1. Design of the rotor in the toroidal coil centrifugal partition chromatograph. (A) Overall view; (B) arrangement of the helical column.

mobile phase volume (V_m) and stationary phase volume (V_s) according to the equation

$$\log P = \log (t_R F - V_m)/V_s \quad (2)$$

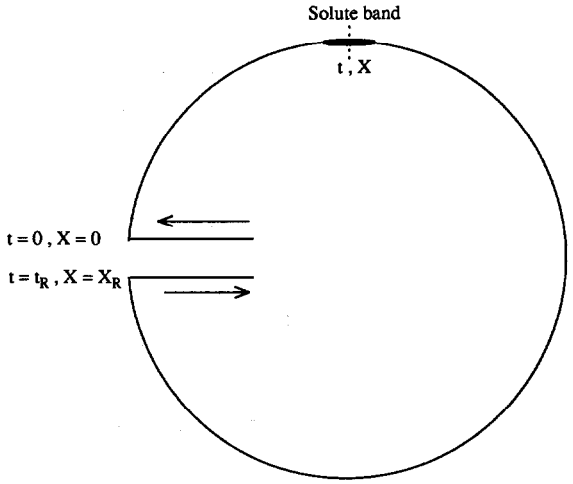

Fig. 2. Propagation of a solute band along the helical column at time $t = 0$ and at time $t = t_R$ (retention time of the solute when it has travelled the full length X_R of the coil).

RESULTS AND DISCUSSION

The time required for the solvent front to elute from the column, as measured by the retention time of potassium chromate, was found to be 5.0 min. The mobile phase volume (V_m) was thus calculated to be 2.0 ml ($V_m = 5.0$ min $\cdot F$) and that of the stationary phase to be 2.0 ml. The retention time of the investigated solutes and their calculated log P_{oct} values are listed in Table I. Note that the measured log P_{oct} value of *p*-nitroaniline (1.30) is close to the literature value of 1.39 [14].

The log P values measured here range from -0.21 to 3.02, but there is no reason why the method should not be applicable to solutes of much higher lipophilicity. Indeed, such compounds would be highly retained, but their X_t distance should become measurable after a sufficient elution time. For highly hydrophilic solutes, one would simply need to use water as the stationary phase and octanol as the eluent.

TABLE I

1-OCTANOL–WATER PARTITION COEFFICIENTS (LOG P_{oct}) MEASURED BY TOROIDAL COIL CENTRIFUGAL PARTITION CHROMATOGRAPHY (TCCPC)

Solute	Retention time, t_R (min)	Log P_{oct} [a]
Pararosaniline	8.1	−0.21
Crystal violet (hexamethylpararosaniline chloride)	21.0	0.51
p-Nitroaniline	105.0	1.30
Sudan III (1-{[4-(phenylazo)phenyl]azo}-2-hydroxynaphthalene)	653.3	2.11
Phenol red (phenolsulphonephthalein)	5250.0	3.02

[a] Measured at pH 7.4, not corrected for ionization.

The most obvious limitation of the method is solute detection, which in the present state of development is restricted to coloured compounds such as those investigated in this feasibility study. However, we believe that the method could be extended to all UV-active solutes by using a stroboscopic UV light source. This would certainly widen considerably the applicability and potential of TCCPC in measuring the lipophilicity of most drugs and many compounds of interest.

ACKNOWLEDGEMENT

B.T. is indebted to the Swiss National Science Foundation for research grant 3.508-0.86.

REFERENCES

1 C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.
2 R. F. Rekker and H. M. de Kort, *Eur. J. Med. Chem.*, 14 (1979) 479.
3 H. van de Waterbeemd and B. Testa, *Adv. Drug Res.*, 16 (1987) 85.
4 J. C. Dearden and G. Bresnen, *Quant. Struct. Act. Relat.*, 7 (1988) 133.
5 T. Braumann, *J. Chromatogr.*, 373 (1986) 191.
6 N. El Tayar, A. Tsantili-Kakoulidou, T. Röthlisberger, B. Testa and J. Gal, *J. Chromatogr.*, 439 (1988) 237.
7 A. Bechalany, T. Röthlisberger, N. El Tayar and B. Testa, *J. Chromatogr.*, 473 (1989) 115.
8 H. Terada, Y. Kosuge, W. Murayama, N. Nakaya, Y. Nunogaki and K.-I. Nunogaki, *J. Chromatogr.*, 400 (1987) 343.
9 A. Berthod and D. W. Armstrong, *J. Liq. Chromatogr.*, 11 (1988) 547.
10 N. El Tayar, A. Marston, A. Bechalany, K. Hostettmann and B. Testa, *J. Chromatogr.*, 469 (1989) 91.
11 P. Vallat, N. El Tayar, B. Testa, I. Slacanin, A. Marston and K. Hostettmann, *J. Chromatogr.*, 504 (1990) 411.
12 N. El Tayar, R. S. Tsai, B. Testa, P. A. Carrupt and A. Leo, *J. Pharm. Sci.*, in press.
13 Y. Ito and R. L. Bowman, *Anal. Biochem.*, 85 (1978) 614.
14 C. Hansch and A. Leo, *The Pomona College Medicinal Chemistry Project*, Pomona College, Claremont, CA, 1983.