

## Note

### Use of droplet counter-current chromatography in log *P* determinations

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Partition coefficients (log *P*) have found a widespread use in the last 20 years and have contributed to our understanding of interactions between bioactive compounds and biological systems. Log *P* has proven to be the most important, by far, of the physicochemical descriptors in quantitative structure–activity studies (QSAR). Traditionally, log *P* has been measured by the so-called “shake-flask” technique<sup>1</sup> where a compound is partitioned between two immiscible liquids such as *n*-octanol and water, which mimic the biological biphasic membranes. Large databases of log *P* values are currently available<sup>2,3</sup>. The method, however, is so tedious, time-consuming and difficult to standardize that several quick alternatives have been developed<sup>4</sup>, mainly based on thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). These methods have been extensively used, but all of them use either a solid stationary phase or a liquid stationary phase fixed on an “inert” support. The packing material in the column tends to complicate the retention process as it may cause adsorption of very polar compounds or produce modifications of the solute at the liquid–solid interface<sup>5</sup>.

To obviate these problems, it would be desirable that new support-free liquid–liquid partition techniques be developed, which could easily be automated and adapted to log *P* determinations. Droplet counter-current chromatography (DCCC) is a recently introduced all-liquid separation technique based on the partitioning of solutes between a steady stream of droplets of mobile phase and a column of surrounding stationary phase<sup>6</sup>. Its application to the separation of various types of natural products has been reviewed by Hostettmann<sup>7,8</sup>.

Our interest lay in testing the validity of this technique for measuring partition coefficients, as the principle of separation was similar to that of the classical “shake-flask” method.

The standard *n*-octanol–water system<sup>1</sup> proved inadequate in DCCC due to the high viscosity of *n*-octanol, which did not allow the formation of suitable droplets

to take place: filling the columns with octanol and pumping water or 5 mM phosphate buffer (pH 7.4) through the columns resulted in flow plugs and displacements of the stationary phase by the mobile phase. The same problem arose when water was used as the stationary phase and *n*-octanol was made to ascend.

A continuous flow of droplets was achieved when mixtures of chloroform (or dichloromethane), methanol and water (or 5 mM phosphate buffer, pH 7.4) in different proportions were used as solvent systems. *p*-Cymene ( $\log P = 4.22$ ) served as an unretained elute to determine  $t_0$ . The capacity factor ( $k'$ ) of a test compound was defined as  $(t_R - t_0)/t_0$ .

The descending mode, *i.e.* use of the heavier phase as the eluent and the lighter one as the stationary liquid phase, yielded the best results for a system composition of chloroform-methanol-water.

## EXPERIMENTAL

An EYELA DCCC-S instrument equipped with an LKB 4701A Uvicord detector (wavelength 254 nm) and an Omniscribe recorder were used for the determinations. Retention times were measured by stop-watch or on the chart paper. For *p*-cymene,  $t_0 = 47.06 \pm 0.59$  s.

A sample chamber was designed that permitted the injection of a few microlitres of the test compound without disturbing the flow (Fig. 1).

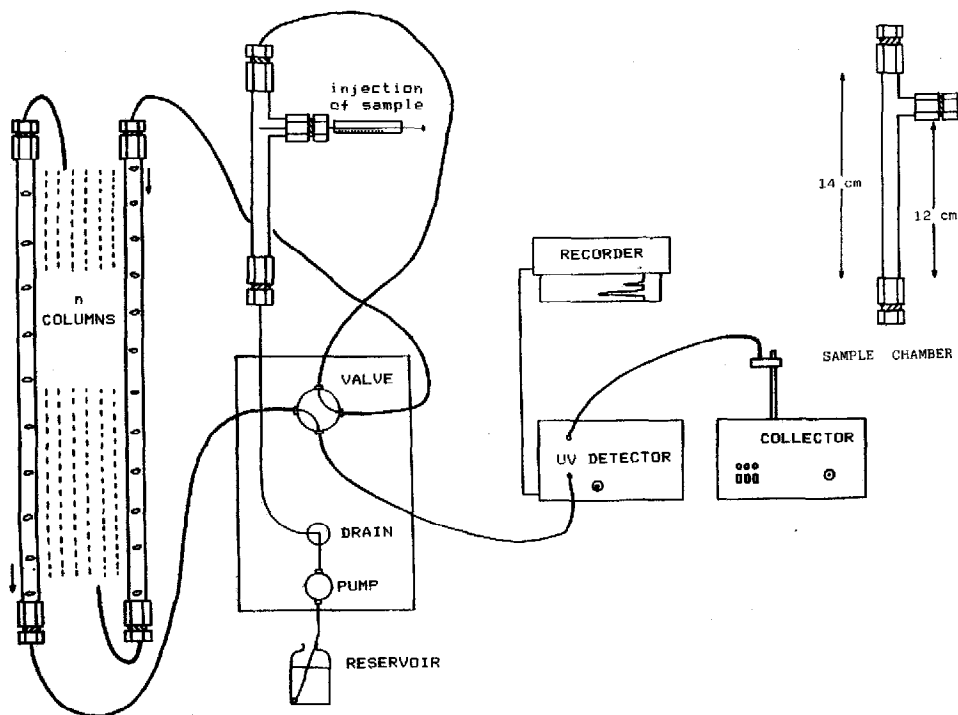


Fig. 1. Schematic representation of the modified DCCC apparatus used in  $\log P$  determinations.

TABLE I

LOG  $P$  LITERATURE VALUES AND EXPERIMENTAL LOG  $k'$  VALUES OF THE COMPOUNDS USED IN THE CORRELATION STUDIES

$\log P_{o-w}$  = log partition coefficient determined in octanol-water (ref. 2);  $\log P_{CLF-w}$  = log partition coefficient determined in chloroform-water (ref. 2);  $\log k'_w$  = capacity factor for the system chloroform-methanol-water;  $\log k'_b$  = capacity factor for the system chloroform-methanol-buffer (pH 7.4).

No.	$R_1$	$R_2$	$\log P_{o-w}$	$\log P_{CLF-w}$	$\log k'_w$	$\log k'_b$
1	-CH <sub>2</sub> CH <sub>2</sub> OH	-H	1.36		+0.08*	
2	-CHO	-H	1.48		-0.40 ± 0.02	-0.36 ± 0.02
3	-CN	-H	1.56		-0.45 ± 0.03	-0.36 ± 0.03
4	-CH <sub>2</sub> CN	-H	1.56	2.71	-0.45 ± 0.03	
5	-COCH <sub>3</sub>	-H	1.73	2.79	-0.43 ± 0.03	-0.54 ± 0.02
6	-NO <sub>2</sub>	-H	1.85	2.93	-0.51 ± 0.02	-0.54 ± 0.02
7	-CH=CHCOCH <sub>3</sub>	-H	2.07		-0.69 ± 0.05	
8	-H	-H	2.13	2.80	-0.51 ± 0.04	-0.66 ± 0.02
9	-CH <sub>2</sub> Cl	-H	2.30		-0.74 ± 0.04	
10	-CH <sub>3</sub>	-H	2.69	3.41	-0.86 ± 0.05	-1.01 ± 0.07
11	-CH <sub>3</sub>	-2CH <sub>3</sub>	3.12		-1.01 ± 0.04	-1.12 ± 0.04
12	-NHC <sub>6</sub> H <sub>5</sub>	-H	3.34		-1.01 ± 0.02	-1.13 ± 0.04
13	-CH(CH <sub>3</sub> ) <sub>2</sub>	-H	3.66		-1.12 ± 0.02	

\* See text.

The number of columns (400 × 4 mm I.D.) employed was usually 25 in order not to unnecessarily prolong the time of analysis. The best droplets were formed when the flow adjuster was fixed at 50 and the pump switch at the High position.

Measurements were performed at room temperature, and from 15 to 25°C no significant changes in the values of  $k'$  were observed.

## RESULTS AND DISCUSSION

Results of  $\log k'$  determinations are presented in Table I, where values for the chloroform-methanol-water ( $\log k'_w$ ) and the chloroform-methanol-buffer ( $\log k'_b$ ) systems are shown separately. Both series were plotted against the  $\log P_{o-w}$  values described in the literature<sup>2</sup> (Fig. 2) and correlation equations were derived using a weighted regression analysis program<sup>9</sup> (eqns. 1 and 2):

$$\log k'_w = 0.09 (\pm 0.03) - 0.33 (\pm 0.01) \log P_{o-w} \quad (1)$$

$$n = 12^* \quad r = 0.991$$

$$\log k'_b = 0.26 (\pm 0.06) - 0.43 (\pm 0.03) \log P_{o-w} \quad (2)$$

$$n = 8 \quad r = 0.987$$

\* 2-Phenylethanol ( $\log k'_w = 0.084$ ) showed a standard error of the mean in its determination that was larger than acceptable, as longer retention times tended to produce flattened peaks in which  $t_R$  measurements were inaccurate. As we later observed, this effect took place with compounds for which  $\log k' > 0$ , i.e. rather polar substances, and is one of the limitations of the instrument.

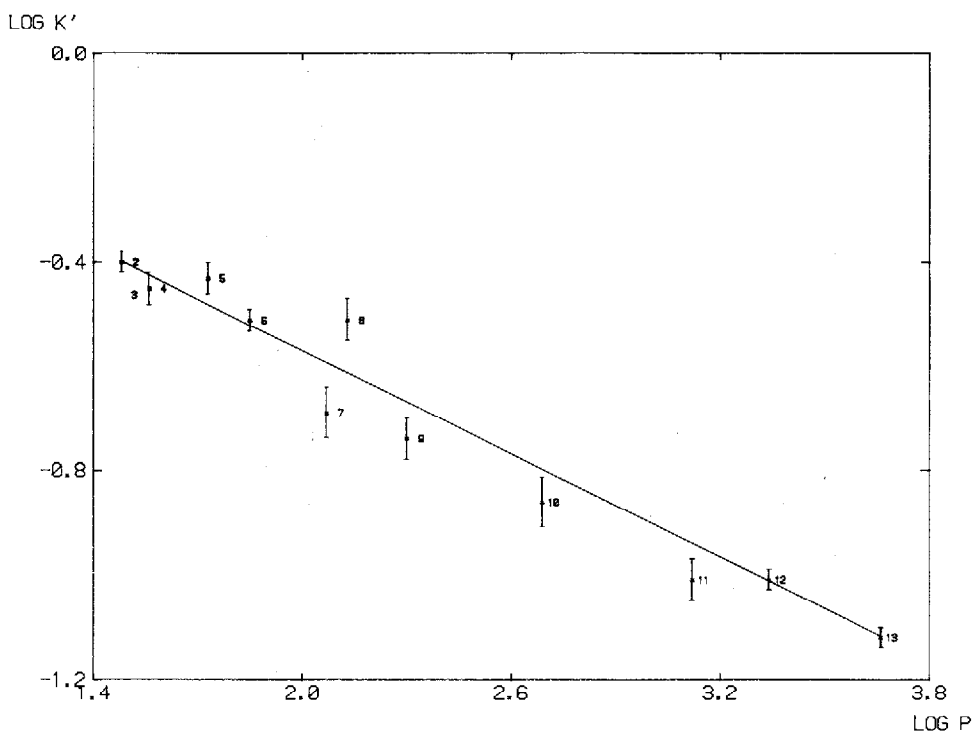


Fig. 2. DCCC  $\log k'$  values vs.  $\log P$  (ref. 2).

As it can be seen, substitution of pH 7.4 buffer for water did not result in a significant improvement in terms of  $r$  values, which on the other hand are as good as those reported for other chromatographic techniques<sup>10,11</sup>.

Trying to improve the quality of the regression for eqns. 1 and 2,  $\log P_{\text{CLF-w}}$  (chloroform-water partition coefficients) were used instead of  $\log P_{\text{o-w}}$ . This parameter is scarcely reported in the literature<sup>2</sup> and only five compounds could be compared (*cf.* Table I). The results were poorer (0.92 and 0.93, *vs.* 0.95 and 0.96) for the same set of compounds ( $n = 5$ ). Thus, even though generalization should be taken with care, it might be accepted that the DCCC system employed behaves more like an octanol-water partitioning system than like a chloroform-water one.

In conclusion, although determinations have been performed with a standard preparative apparatus, results are encouraging enough to support the view that further developments in analytical DCCC and the use of octanol-water as the partitioning system under appropriate conditions could provide more precise measurements and yield still better correlates between  $\log k'$  and  $\log P_{\text{o-w}}$  values.

This new technique could turn out to be an interesting alternative to the presently used HPLC-based methods. Lack of interactions with a solid packing material would provide results very similar to those obtained by the original "shake-flask" method.

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