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Note

Preparation of a column with octanol-like properties for high-performance liquid chromatography

Direct measurements of partition coefficients in an octanol–water system

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The hydrophobic character of chemicals, which is expressed in terms of the partition coefficient, P_{oct}, between *n*-octanol and water, is one of the most important parameters in the analysis of quantitative structure–activity relationships (QSAR)[1,2]. However, there is no convenient method available to determine the P_{oct} value accurately; the flask-shaking method usually adopted is time-consuming, involves troublesome quantitative measurements and requires high purity of the chemicals and of *n*-octanol.

There have recently been attempts using high-performance liquid chromatography (HPLC) to determine the hydrophobicities (P_{HPLC}) of chemicals. If this method could be perfected, it would be very useful for avoiding the disadvantages of the flask-shaking method. In most previous studies on HPLC, ODS[3,4] or persilanized ODS[5] has been used as the stationary phase. However, it would be preferable to use an octanol-coated column to obtain hydrophobicities directly reflecting P_{oct}. Mirrlees *et al.*[6] prepared a column by coating *n*-octanol on Kieselguhr that had previously been fractionated and silanized, and eluting excess *n*-octanol with the mobile phase (*in situ* coating method). Using this column they obtained good correlations between the P_{HPLC} values of miscellaneous compounds and their P_{oct} values. However, their method of preparing the column seems rather tedious. Moreover, Henry *et al.*[7] did not obtain good correlations between P_{HPLC} and P_{oct} values for sulphonamides, using Corasil II coated with 1% octanol as the stationary phase.

Thus in order to establish a practical method of using octanol in HPLC, it is necessary to find suitable experimental conditions, including a simple method for preparation of the column. This paper reports a convenient method for preparing an octanol-coated column that has high stability and gives good results.

HPLC PROCEDURE

All chemicals were obtained from commercial sources and were used without further purification.

HPLC was performed on a JASCO high-performance micro liquid chromatograph, Model Familic-100, because this apparatus is designed so that the length of

the column can be easily varied. The supporting medium was packed in a PTFE tube, 0.5 mm I.D., connected to a UV-detector, JASCO UVIDEC-100. Retention times were measured from the chromatographic peaks, recorded on a Hitachi QPD-54 recorder.

If the adsorption of solutes on solid supports does not affect the retention time and the properties of the eluent are the same in the pores of the support and in the mobile phase, the retention time, t_R, should be linearly dependent on the partition coefficient of the solute between the stationary and mobile phases, P_{HPLC}, as shown in eqn. 1 (ref. 8):

$$t_R = t_0 + t_0 q P_{HPLC} \quad (1)$$

where t_0 is the retention time of the non-retained compound and q is the volume ratio of the stationary and mobile phases (V_s/V_m). Then the capacity factor, k', is expressed by

$$\log k' = \log[(t_R - t_0)/t_0] = \log P_{HPLC} + \log q \quad (2)$$

When adsorption chromatography of methanol, benzyl alcohol and nitrobenzene was performed using Corasil I, the t_R values were almost the same: t_R(methanol):t_R(benzyl alcohol):t_R(nitrobenzene) = 1.0:1.0:1.2; however, on silica gel (WC-01, JASCO) the t_R values were very different: t_R(methanol):t_R(benzyl alcohol):t_R(nitrobenzene) = 1.0:1.3:3.5. Thus, we used Corasil I as the solid support.

To prepare the column, Corasil I was heated at 110° overnight and then, while still hot, mixed with *n*-octanol. The slurry was packed in a PTFE tube, and excess *n*-octanol removed by elution with the mobile phase (HCl–KCl buffer, pH 2.0, saturated with *n*-octanol) until a stable baseline was obtained on the recorder. This took about 10 min at a flow-rate of 16 μl/min. Then 0.1 μl of a solution of the test compound in water or 10% methanol was loaded on the column and eluted with the mobile phase. Methanol ($\log P_{oct} = -0.82$) or benzenesulphonic acid ($\log P_{oct} = -2.25$) was used as a non-retained marker compound. At a flow-rate of 16 μl/min, this column was stable for more than 60 h of use, giving consistent t_R values.

The t_R of *p*-cyanophenol was measured at various flow-rates of the mobile phase and k' was calculated from t_R. Fig. 1 shows the changes of log k' and log t_c with flow-rate ($t_c = t_R - t_0$). Values of log k' were almost constant at flow-rates of up to 20 μl/min, but gradually decreased with increasing flow-rates above 20 μl/min, whereas log t_c decreased continuously with increasing flow-rates. These results indicate that, at flow-rates of less than 20 μl/min, t_c is affected by a small fluctuation of the flow-rate, but k' is not. Thus the hydrophobic property determined by HPLC should be represented by the k' value. In this study, we measured k' values at a flow-rate of 16 μl/min.

The reproducibility of measurements was examined using benzyl alcohol and *p*-cyanophenol. The standard errors of log k' in ten runs with a 14-cm column were 0.003 for benzyl alcohol and 0.005 for *p*-cyanophenol, indicating that log k' can be determined with high reproducibility.

When we examined the *in situ* coating method using Corasil I as a support, as described by Mirrlees *et al.*[6], we did not obtain satisfactory results, *n*-octanol be-

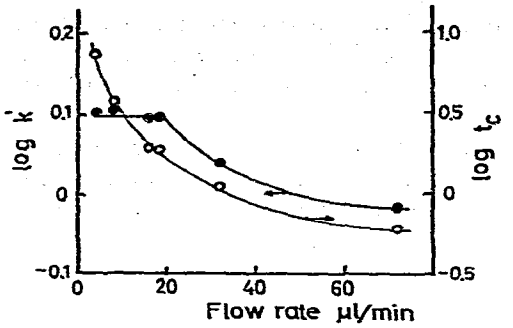

Fig. 1. Effect of the flow-rate of the mobile phase on log k' (●) and log t_c (○) values of *p*-cyanophenol. Mobile phase, 0.2 *N* HCl–KCl buffer, pH 2.0; column length, 14 cm.

coming dislodged and t_R decreasing gradually during the performance of the chromatography.

RELATIONSHIP BETWEEN P_{HPLC} AND P_{oct}

We measured the t_R values of miscellaneous compounds, such as benzene, benzoic acids and phenols, using column lengths of 4 cm and 14 cm; k' was expressed as the mean value of four replicate runs. The linear relationship between log k' and log P_{oct} with 4-cm and 14-cm columns is shown in Fig. 2, and eqns. 3 and 4 were derived.

With a 4-cm column

$$\log k' = 0.960 \log P_{oct} - 1.551 \; (n = 9, r = 0.992, s = 0.023) \quad (3)$$

With a 14-cm column

$$\log k' = 0.965 \log P_{oct} - 1.456 \; (n = 10, r = 0.996, s = 0.024) \quad (4)$$

In eqns. 3 and 4, n is the number of compounds, r is the correlation coefficient and s is the standard error of estimate of log k' on log P_{oct}. With both columns the slope of the straight line is almost unity and a very good correlation is obtained.

Use of a shorter column for compounds with high log P_{oct} values makes it easier to obtain accurate t_R values, because the peak is sharper and the operational time is shorter: for example, with a 14-cm column, the t_R of salicylic acid (log P_{oct} = 2.26) was 23.1 min, but the peak of the more hydrophobic *p*-chlorophenol (log P_{oct} = 2.39) was so broad that t_R could not be determined; with a 4-cm column, however, the t_R of salicylic acid was 11.5 min and the peak of *p*-chlorophenol was sharp enough to obtain a reproducible t_R value of 16.6 min.

The partition coefficients determined in two partitioning systems, P_1 and P_2, can be represented by eqn. 5, if the hydrogen-bonding properties of the two organic solvents are similar[9].

$$\log P_1 = m(\log P_2) + b \quad (5)$$

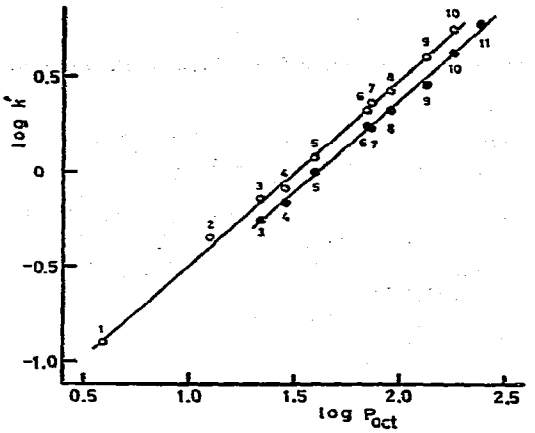

Fig. 2. Plots of log k' against log P_{oct} with different column lengths. 1 = Hydroquinone; 2 = benzyl alcohol; 3 = *p*-methoxyphenol; 4 = phenol; 5 = *p*-cyanophenol; 6 = nitrobenzene; 7 = benzoic acid; 8 = methylparaben; 9 = benzene; 10 = salicylic acid; 11 = *p*-chlorophenol. log P_{oct} values are taken from ref. 9. Mobile phase; 0.2 *N* HCl–KCl buffer, pH 2.0. Column length: ●, 4 cm; ○, 14 cm. Flow-rate, 16 μl/min.

Denoting P_1 as P_{HPLC}, and P_2 as P_{oct}, eqn. 6 is derived by a combination of eqns. 2 and 5.

$$\log k' = m(\log P_{oct}) + \log q + b \qquad (6)$$

The value of the slope, m, in eqn. 6 should be unity, as obtained in this work, if the t_R is governed by the partition between mobile phase and *n*-octanol coated on the support. Thus the value of m is a good measure of whether the stationary phase is octanol-like. Henry *et al.*[7] obtained an m value of 0.72 for sulphonamides with a column of Corasil II coated with 1% octanol, which suggests that there is a direct interaction of silanol groups on the support with solutes in the mobile phase.

Eqn. 5 was reported not to hold for compounds capable of forming hydrogen bonds if the properties of the two organic solvents with respect to hydrogen-bond formation are different[10]. In the case of an ODS column, good linearity between log P_{oct} and log P_{HPLC}, with a slope close to unity was reported for a series of penicillins[4]. However, phenols and monosubstituted benzenes did not give a linear relationship, but two straight lines with different slopes of less than 1 and different intercepts: one for compounds that did not form hydrogen bonds and the other for amphiprotic compounds[11].

It should be noted that all the data-points for compounds with various hydrogen-bonding abilities fall on the same straight line in Fig. 2 with either column length. This also indicates that the properties of the stationary phase used in this study were very similar to those of *n*-octanol, and that the k' value represented P_{oct} well.

It is concluded that P_{oct} values of wide variety of compounds can be determined easily and accurately by the method described in this paper.

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