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Estimating the Hydrophobic Character of Dyestuffs: A Comparison of Partition, Reverse-Phase Thin-Layer Chromatography, and Calculation

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SUMMARY

Several indices of hydrophilic–hydrophobic balance have been compared for 11 water-soluble, monoazo acid dyes. These indices were chromatographic mobilities (R_f and R_m values) in a reverse-phase thin-layer chromatographic system; measured partition coefficients between n-butanol and water; and calculated partition coefficients, for both n-butanol/water and n-octanol/water. The various quantities were strongly correlated, most highly so for R_m values and calculated partition coefficients between n-octanol and water where the linear correlation coefficient was 0·969. On practical grounds the R_f values from reverse-phase thin-layer chromatography were the preferred measure, especially when dealing with a range of dye samples. As calculation of partition coefficients is so easy, this is recommended as a routine procedure, both as a preliminary and as a check on any measurements.

INTRODUCTION

An important characteristic of dyestuffs is their hydrophobic–hydrophilic balance. It has been shown that the hydrophobicity can affect the affinity of dyes for a number of fibre substrates

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including acrylics,[1,2] nylon,[3] and wool.[4] Stapleton and Waters[5] have shown that by varying the hydrophilic–hydrophobic balance in a series of reactive disperse dyes, changes were observed in the distribution of the dye between the fibre components of a 70:30 wool/polyester blend. It was also found that the inclusion of alkyl chains was necessary to increase markedly the hydrophobic character of the dyes. A similar conclusion was reached by Reeves and Harkaway[6] for sulphonated acid dyes. Other interactions influenced by dye hydrophobicity include dye–dye aggregation[7–9] and dye–surfactant complexation.[10,11] The solubility of non-ionic dyes in non-polar materials such as petroleum resins, and varnishes,[12] and also in water[13] will also be influenced by the hydrophobic character of the dyes. Furthermore, toxicity can correlate with hydrophobicity.[14]

In an attempt to assess quantitatively the property of hydrophobicity for dyestuffs, partition coefficients have been used as an index. Thus dyes have been partitioned in *n*-butanol/water,[7,15] water/chloroform[16] and *n*-octanol/water systems.[14] Recently the *n*-octanol/water partition coefficients of dyestuffs have been estimated by calculation.[14] Another experimental procedure for assessing hydrophobicity is reverse-phase thin-layer chromatography (RP TLC). Chromatographic mobility in such systems has been extensively used as a measure of hydrophobic character,[17] though not apparently by dyestuffs or textile workers.

The present paper compares RP TLC with *n*-butanol/water partition measurements, and with calculated partition coefficients as indices of dyestuff hydrophobicity.

MATERIALS AND METHODS

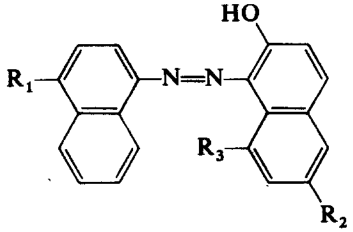
Source of materials

The dyestuffs investigated can be conveniently divided into two groups, (a) and (b), listed in Tables 1 and 2 respectively. The preparation and purification of the dyestuffs have been described elsewhere.[7]

Measurements of partition coefficients

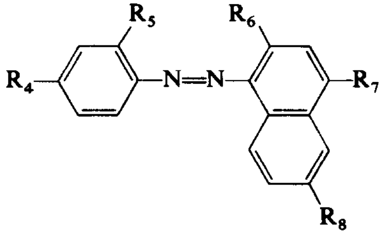
Partition coefficients for the dyestuffs between *n*-butanol and water were determined by the method of Hadfield and Lemin[15] using dye

TABLE 1
Group (a) Dyes of General Formula



Dye number	*C.I. generic name or compound*	R_1	R_2	R_3
I	C.I. Acid Red 88	$—SO_3Na$	—H	—H
II	C.I. Acid Red 44	—H	$—SO_3Na$	$—SO_3Na$
III	1-Naphthylamine-4-sulphonic acid → 2-naphthol-6-sulphonic acid (Schaeffer's Acid)	$—SO_3Na$	$—SO_3Na$	—H
IV	C.I. Acid Red 18	$—SO_3Na$	$—SO_3Na$	$—SO_3Na$

TABLE 2
Group (b) Dyes of General Formula



Dye number	*C.I. generic name or compound*	R_4	R_5	R_6	R_7	R_8
V	*o*-Chloroaniline → Schaeffer's Acid	—H	—Cl	—OH	—H	$—SO_3Na$
VI	C.I. Acid Orange 20	$—SO_3Na$	—H	—H	—OH	—H
VII	C.I. Acid Orange 12	—H	—H	—OH	—H	$—SO_3Na$
VIII	*p*-Toluidine → Schaeffer's Acid	$—CH_3$	—H	—OH	—H	$—SO_3Na$
IX	*p*-Ethylaniline → Schaeffer's Acid	$—C_2H_5$	—H	—OH	—H	$—SO_3Na$
X	*p*-(*n*-Butyl)aniline → Schaeffer's Acid	$—n—C_4H_9$	—H	—OH	—H	$—SO_3Na$
XI	*o*-Ethylaniline → Schaeffer's Acid	—H	$—C_2H_5$	—OH	—H	$—SO_3Na$

concentrations of $1{\cdot}0 \times 10^{-4}$ M. Equilibrium concentrations in the two phases were determined spectrophotometrically using a Unicam SP600 UV spectrophotometer.

Some difficulty was experienced with the most highly sulphonated dye (**IV**) in that it appeared to partition completely in favour of the aqueous layer. Subsequent spectrophotometric measurements indicated the concentration in the *n*-butanol layer to be $0{\cdot}5 \times 10^{-6}$ M. Bearing in mind that the limit of spectrophotometric measurements is about 0·02 absorbance units, the error in the experimental partition coefficient value for this dye could be very large, as can be seen in Figs 1 and 3.

Measurement of chromatographic mobilities

This was achieved in a reverse-phase chromatographic system as follows. Dyes were dissolved in methanol to form approximately 0·1% (w/v) solutions. Disposable 5 μl micropipettes (Camlab) were used to spot dye solutions in duplicate onto a 20 cm × 20 cm Whatman KC_{18} RP TLC plate. These plates consist of an outer layer of octadecylsilane bonded to a layer of silica gel. Solvent was rapidly evaporated from the spots with a hairdryer and elution was carried out in a 6 cm × 23 cm × 18 cm chromotank (Shandon) with a filter paper wick to produce solvent saturation of the atmosphere. The eluting solvent was 70% (v/v) methanol–water with 0·76 M sodium acetate. Chromatographic mobilities, i.e. R_f values, were calculated as the ratio of the distance moved by the dye spot centre from the origin to the distance moved by the solvent front from the origin (8·5 cm).

The RP TLC literature has in the past used the term R_m.[17] This is mathematically more simply related to the partition coefficient than R_f and is thus useful as an index of hydrophobicity. Values of R_m were calculated using the following equation:

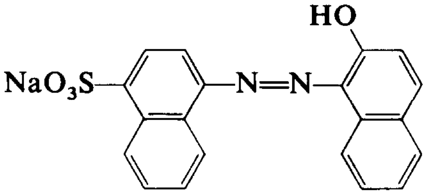
$$R_m = \log(1/R_f - 1)$$

To ascertain the reproducibility of chromatographic mobilities for the dyes, C.I. Acid Orange 20 and C.I. Acid Orange 12 were each spotted ten times onto a 20 cm × 20 cm RP TLC plate. After development under the same conditions as described above, the standard errors of the recorded R_f values were calculated.

Calculation of partition coefficients

Logarithms of the octanol/water partition coefficients (log P_{oct} values) were calculated using the FRAGMENT method developed by Hansch and Leo.[18] A detailed account of this procedure can be found in Chapter 4 of their monograph and here only a brief outline and a worked example will be given.

The contributions to the partition coefficient of a molecular species from the separate fragments out of which the molecule is built up, have been found to be additive. To a first approximation then, the log P_{oct} value for the molecule is obtained by summing the fragmental log P_{oct} contributions (f values).



(I)

Thus dye **I** (C.I. Acid Red 88) can be considered to be built up from:

1 aromatic sulphonate	($f = -4{\cdot}53$)
1 aromatic hydroxyl	($f = -0{\cdot}44$)
1 azo group sandwiched between two aromatic ring systems	($f = +0{\cdot}14$)
12 hydrogen-substituted aromatic carbons	($f = +0{\cdot}36$)
4 other substituted aromatic carbons	($f = +0{\cdot}13$)
4 aromatic carbons which fuse together aromatic rings	($f = +0{\cdot}23$)

We can now write:

$$\begin{aligned}\log P_{oct} &= 1(-4{\cdot}53) + 1(-0{\cdot}44) + 1(0.14) + 12(0{\cdot}36) + 4(0{\cdot}13) + 4(0{\cdot}23)\\ &= 0{\cdot}93\end{aligned}$$

From log P_{oct} values so calculated, log $P_{n\text{-BuOH}}$ values were estimated, using the relationship described by Rekker,[19]

$$\log P_{n\text{-BuOH}} = 0{\cdot}742 \log P_{oct} + 0{\cdot}065$$

RESULTS

The experimental findings and the results of calculations are summarised in Table 3.

The reproducibility of the RP TLC system was found to be good. For dyes VI and VII the standard errors of the mean R_f values were 0·003 and 0·002 respectively.

TABLE 3
Indices of Hydrophobicity, Measured and Calculated

Dye	*Measured values*		*Estimated values*		R_m[c]
	$\log P_{n-BuOH}$	R_f	$\log P_{oct}$	$\log P_{n-BuOH}$	
I	+1·82	0·18	+0·93	+0·76	0·66
II	−0·67	0·67	−3·83	−2·78	−0·31
III	−0·48	0·79	−3·83	−2·78	−0·58
IV	∞[a]	0·98	−8·59	−6·31	−1·69
V	+0·66	0·25	+0·23	+0·24	0·45
VI	+0·93	(0·30), 0·60[b]	−0·25	−0·12	(0·35), −0·18[b]
VII	+0·79	(0·13), 0·38[b]	−0·25	−0·12	(0·83), 0·21[b]
VIII	+0·81	0·25	+0·41	+0·37	0·45
IX	+0·99	0·18	+0·95	+0·77	0·66
X	+1·12	0·08	+2·03	+1·57	1·06
XI	+0·95	0·22	+0·95	+0·77	0·55

[a] Dye concentration in the *n*-butanol phase below detection limit.

[b] For dyes containing more than one component, the R_f and R_m of the minor component is placed in parentheses.

[c] $R_m = \log[1/R_f - 1]$.

DISCUSSION

In attempting to assess the results of Table 3 in terms of the most meaningful indicator of hydrophobicity and the level of agreement between different methods, it must be said that hydrophobicity is an indefinite concept. There is no absolute measure of the property, partition coefficients and chromatographic R_m values being merely plausible empirical indices. Critical evaluation is thus limited to intra- and inter-index comparisons and such comparisons will inevitably be affected by experimental error overlying more fundamental differences.

A comparison of the two experimental indices, partition coefficients and R_m values, is shown in Fig. 1. The two indices were correlated, as shown by the straight line fitted to the plot by least-squares regression, for which the correlation coefficient was 0·694. The data points used to generate this line excluded Dye **IV** (C.I. Acid Red 18) as the experimental log P for this dye was $-\infty$. However, as a limiting minimum value of log P can be assigned, this dye is represented on the plot by a line, though the regression line does not pass through this.

As indicated previously, large errors are expected in the estimation of the $\log P_{n\text{-BuOH}}$ value for dye **IV** since the spectrophotometric limit of detection and the measured absorbance of this dye in *n*-butanol were of the same order ($\sim$0·02 absorbance units). An attempt has been made to estimate the upper and lower limits of concentration in butanol for dye **IV** and hence the limits of $\log P_{n\text{-BuOH}}$. These are shown by lines in Figs 1 and 3. It is probable that a dye of extremely low hydrophobicity such as **IV** is not amenable to partitioning experiments of this sort. The addition of sulphonate groups has a very marked effect on hydrophilic–hydrophobic balance. Earlier work[6] on other, but related, properties of dyes has indicated that best correlation is obtained, not unexpectedly, where structural variations are minimal and regular, as in dyes **VII–X**, where changes are not positional but restricted to the lengthening of an alkyl side chain. In all the intra-index comparisons made here (Figs 1–3) the correlations for dyes **VII–X** are better than for the whole range of dyes where more extreme structural changes are involved.

We can also compare the experimental indices with the calculated. In Fig. 2 the R_m values are plotted against log P_{oct}. In this case the linear correlation coefficient is 0·969, which represents a higher correlation than that found between the two experimental indices. Values of R_f may also be used for correlation analysis and the correlation coefficient for R_f vs. log P_{oct} is $-0{\cdot}962$, which is similar to that for R_m vs. $\log P_{oct}$. A comparison of the measured and calculated butanol–water partition coefficients (Fig. 3) is, however, less clearcut. If the C.I. Acid Red 18 data are excluded, as previously, then again the fitted line (for which the correlation coefficient is 0·801) does *not* run through the line representing Acid Red 18.

An inspection of Figs 1, 2 and 3 indicates that whilst both R_m measurements and calculated partition coefficients show a wide spread of values, the measured partition coefficients are more closely grouped. That is, R_m values and calculated partition coefficients discriminate better

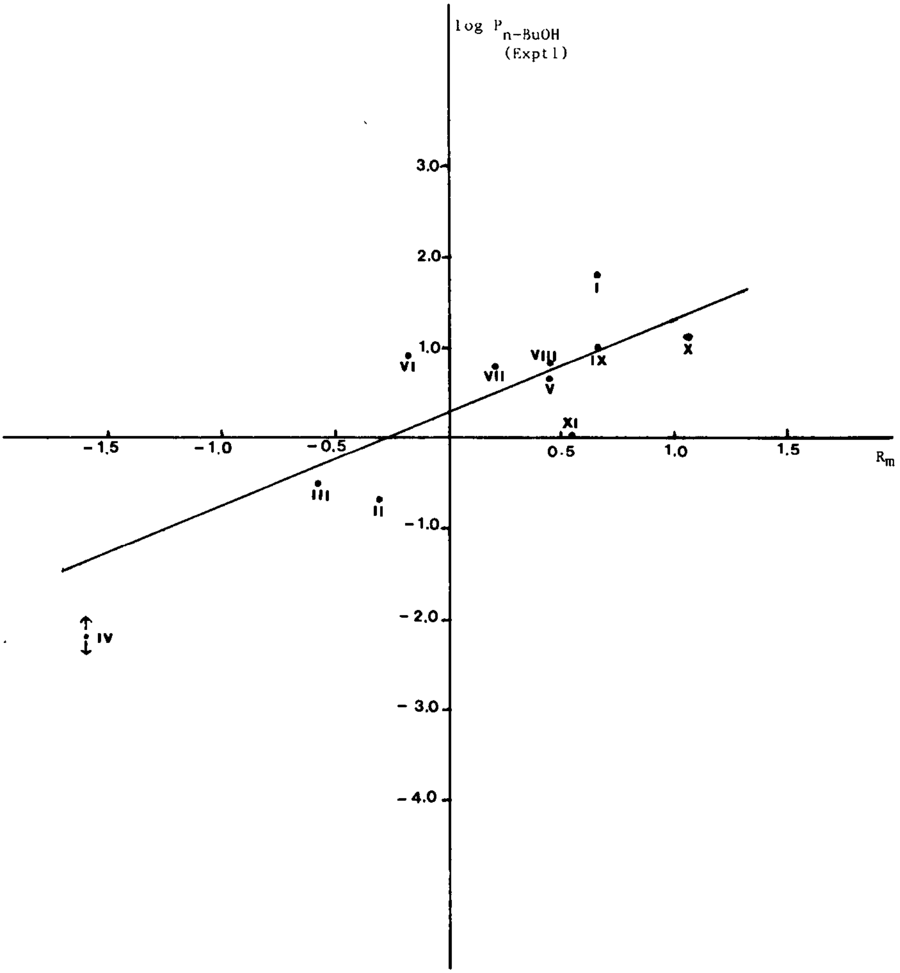


Fig. 1. A comparison of the two experimental indices of hydrophobicity. The line was fitted by least-squares regression, the correlation coefficient being 0·694.

between the different dyes than measured partition coefficients. Moreover, the best inter-index correlation is between R_m and calculated partition coefficients. The following conclusions can thus be drawn. Firstly, as a measured index of dyestuff hydrophobicity R_m values are at least as valid as measured partition coefficients, and, secondly, it is clearly worth calculating partition coefficients, whether or not an index is also to be measured.

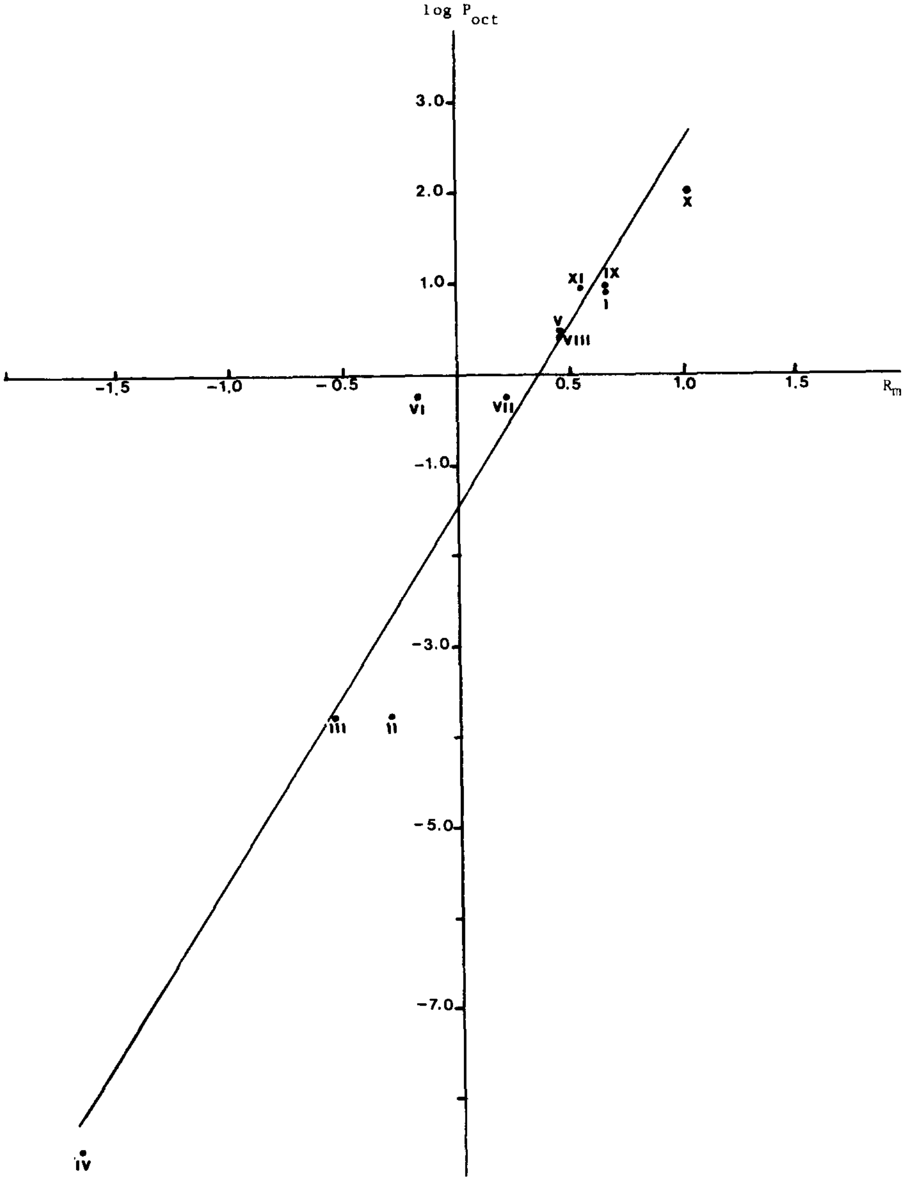


Fig. 2. A comparison of experimentally determined R_m values with calculated $\log P_{oct}$ values. The line was fitted by least-squares regression, the correlation coefficient being 0·969.

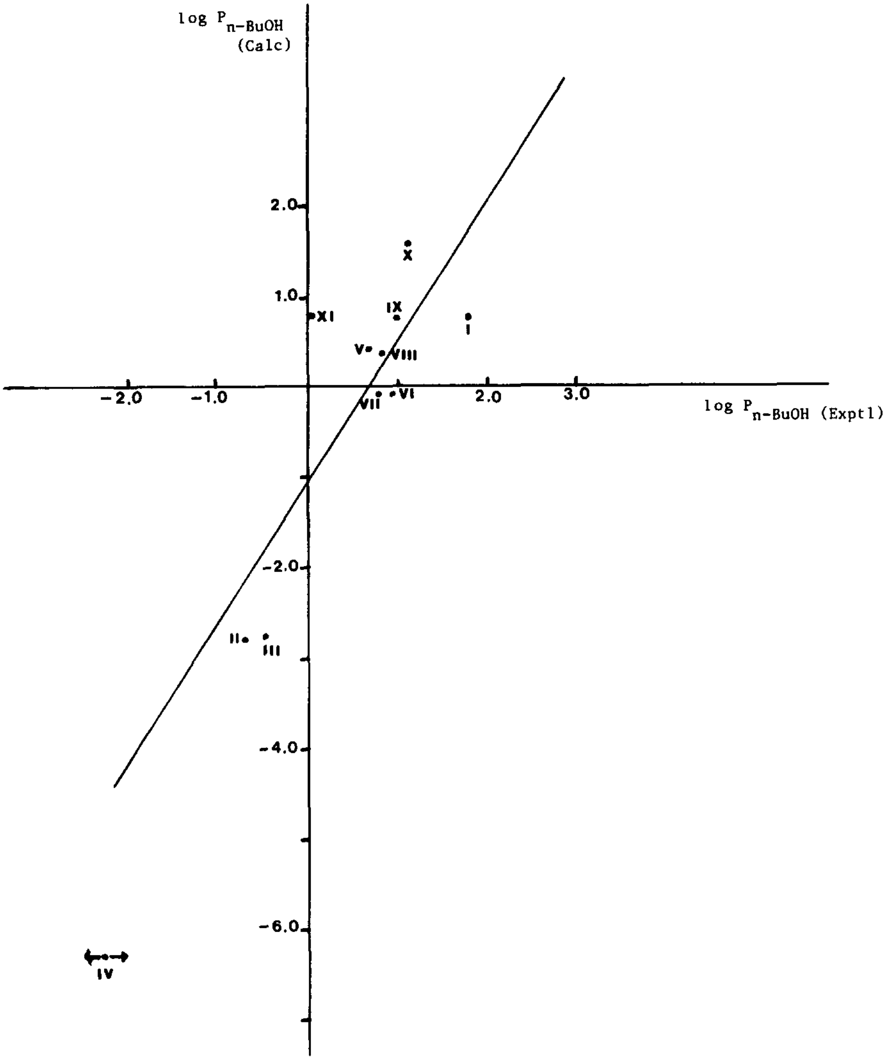


Fig. 3. A comparison of measured and calculated log $P_{n-\text{BuOH}}$ values. The line was fitted by least-squares regression, the correlation coefficient being 0·801.

Some technical considerations

Having established the validity of certain new (to dyestuffs chemistry) indices of hydrophobicity, it is now necessary to compare the indices from a practical and technical viewpoint. Factors to be considered for each method are range of applicability, convenience (i.e. time, skills and materials required), and reproducibility.

Range of applicability

With any given developing solvent RP TLC will only provide R_f values for dyes of a limited range of hydrophobic–hydrophilic character. In the chromatographic system used by us, only dyes in the log P range ca. -9 to $+3$ could be investigated. To mobilise more hydrophobic dyes requires more hydrophobic solvents, and to retard more hydrophilic dyes needs more hydrophilic solvents. Whilst such changes do alter the range of applicability, they do not usually extend it. Measurement of partition coefficients, on the other hand, is not in principle limited by extremes of hydrophobic or hydrophilic character. In practice very hydrophilic or hydrophobic dyes will be so unequally partitioned that they will fall below the limits of detection in one of the phases. In the present study this occurred with dye **IV**. We could have overcome this by using a markedly unequal volume ratio of water and butanol, and then devising a process for concentrating the large volume of very dilute butanolic dye solution to allow spectrophotometric assay. In this case there is no theoretical block but there is the need to compromise between practical convenience and accuracy of measurement. Of course calculation of partition coefficients is not limited in this way.

A related problem concerns the impurities found in commercial dyestuffs, and the extent to which they influence measurement of R_f values and partition coefficients. Such impurities include dyestuffs additional to the major constituent, and colourless materials such as surfactants and electrolyte. Coloured impurities present no great problem with RP TLC, as components of differing hydrophobicities will give rise to spots on the chromatogram of differing R_f values, as can be seen with the dyes **VI** and **VII** in Table 3. Nor will colourless impurities usually affect R_f values, since such compounds will separate from the dyestuffs during the chromatographic process. Measurements of partition coefficients, however, are more susceptible to errors induced by impurities. Not only will partitioning a coloured mixture produce a meaningless coefficient, but salts and surfactants will strongly influence the partition process. The routine response to such impure samples is to use repeated extractions, until the partition coefficient becomes constant. However, unlike the RP TLC case, no information is obtained on the hydrophobicities of the coloured impurities. Once again, this source of error is irrelevant to the calculation of partition coefficients.

Convenience

All three methods involve only standard laboratory skills, thin-layer chromatography and spectrophotometry perhaps being the most complex. The economics of the methods will also be undemanding for most laboratories. RP TLC has no absolute requirements for special equipment, excepting the reverse-phase plates themselves. Partition measurement as carried out by us does require a spectrophotometer for dye assay. It is perhaps the investment in time which shows the largest difference between the methods. Measurements of a single R_f value or partition coefficient take comparable, and fairly short times, with the calculation of a partition coefficient being faster. However, since up to 30 dyes can be run simultaneously on a single thin-layer chromatography plate, RP TLC has a marked advantage when a number of dyes is being studied.

Reproducibility

With partition measurements, repetition will be time-consuming, whereas multiple samples can be run easily in the RP TLC system and reproducibility demonstrated. In the present study, for instance, all the dyes were easily fitted onto a single plate and the standard errors of the mean R_f values for two dyes looked at were small. It may also be noted that inter-laboratory reproducibility, often poor with thin-layer chromatography, is much improved with the use of commercial reverse-phase plates.

Overall conclusions

(i) Chromatographic R_m values, measured *n*-butanol/water partition coefficients, and calculated partition coefficients have proved to be highly correlated indices of hydrophobicity for the dyes studied.

(ii) For investigating single dyes, the two experimental methods were of similar convenience. For multiple dye samples, RP TLC was more convenient.

(iii) Calculation of partition coefficients is very straightforward, and is especially useful for dyes of extremes of hydrophobicity or hydrophilicity. Indeed routine calculations of partition coefficients is recommended as a back-up method even when measurement is also to be carried out.

(iv) The relation between hydrophobicity and structure for a series of dyes is most precise when the structural changes are regular and identifiable, as in the lengthening of an alkyl side chain.

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