# The anomalous chromatographic behaviour of dichlorophen and p-chlorophenol

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The variation of Rf value of p-chlorophenol and di-(5-chloro-2-hydroxyphenyl)
methane when run alone or together on thin layer chromatograms using toluene
and silica gel is attributable to adsorption rather than partition processes. This
conclusion is based on a quantitative study of Rf value variation, adsorption and
partition behaviour. The adsorption isotherms are used to calculate the Rf values.
The latter were of similar magnitude to those obtained directly from the chromato-
grams.

THIN-LAYER chromatography is used to detect p-chlorophenol, an impurity in di-(5-chloro-2-hydroxyphenyl)methane (Dichlorophen) (B.P. 1963, addendum 1964). Mr. C. A. Johnson (personal communication) reported that the Rf values of these substances show anomalous behaviour when toluene and silica gel are used as the solvent and stationary phase respectively. In particular each substance affects the Rf value of the other. Variations in Rf value are explored in terms of the partition coefficients and adsorption isotherms of these two substances.

THEORY

The equation relating the Rf value of a solute to the partition co-efficient (K) and the effective cross-sectional areas of the mobile (a) and stationary (b) phases has been derived by Consden, Gordon & Martin (1944).

$$Rf = \frac{1}{\left(1 + K\frac{b}{a}\right)} \qquad .. \qquad .. \qquad .. \qquad .. \qquad (1)$$

$$\text{where } K = \frac{\text{Concentration of solute in stationary phase}}{\text{Concentration of solute in mobile phase}} \qquad .. \qquad (2)$$

It is generally accepted that the surface of silica gel particles is covered by several layers of chemisorbed and physically adsorbed water molecules, and that such layers are firmly held up to temperatures of 200°. The relevant partition coefficient is that for water and toluene.

From the adsorption aspect, it is convenient to modify equations (1) and (2) so as to apply to unit mass or length of adsorbent or stationary phase used in the chromatography apparatus. If $g_s$ is the amount of solute in the stationary phase associated with one unit of adsorbent, and $g_m$ that in the corresponding amount of mobile phase, then equation 2 becomes

$$K = \frac{g_s}{g_m} \cdot \frac{a}{b} \qquad .. \qquad .. \qquad .. \qquad .. \qquad (3)$$

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and equation 1,

$$Rf = \cfrac{1}{\left(1 + \cfrac{g_s}{g_m}\right)} \qquad \cdots \quad \cdots \qquad \cdots \qquad \cdots \qquad (4)$$

The volume of the mobile phase may be identified as the void volume $(V_v)$ associated with a unit of adsorbent; then $g_m = cV_v$, where c is the solute concentration. Equation 4 may be rewritten

$$Rf = \cfrac{1}{\left(1 + \cfrac{g_s}{cV_v}\right)} \qquad \cdots \qquad \cdots \qquad \cdots \qquad (5)$$

which expresses the dependence of the Rf value on void volume. Any variation in $V_v$ from plate to plate will introduce variations in the apparent Rf value. Although the volume of the stationary phase can be calculated precisely for a chromatographic system in which the support is coated with a known amount of stationary phase, in the toluene and silica gel system this is not possible. The void volume may be identified, however, with the volume occupied by the adsorbate at maximum coverage $(V_m)$, a value which can sometimes be derived from the appropriate isotherm equation. For example if the adsorption is represented by the Langmuir isotherm, then

$$\frac{\rho g_s}{V_m} = \frac{Bc}{1 + Bc} \qquad \cdots \qquad \cdots \qquad \cdots \qquad (6)$$

in which $\rho$ is the density of the adsorbate layer and B the Langmuir constant. Equations (5) and (6) may then be combined to eliminate $g_s$ which shows the relation between the Rf value and the mobile phase concentration of the solute in the region of the chromatogram spot.

$$\frac{1}{Rf} = 1 + \frac{BV_m}{\rho V_v (1 + Bc)} \qquad \cdots \qquad \cdots \qquad \cdots \qquad (7)$$

This concentration is a function of the mean concentration in the spot, since diffusional processes are operating radially. The Rf value is concentration dependent, since the Langmuir equation is non-linear with respect to $g_s$ and c; it is indirectly time dependent because of diffusion. Given the spot area, the total quantity of solute, together with the mass and void volume of the silica gel associated with this area, then in principle the Rf value can be calculated.

The following factors must therefore be determined experimentally if the quantitative aspects of the investigation are to be realized: (a) Variation of Rf values with load and duration of run. (b) Area and total solute concentration of chromatogram spots. (c) Water–toluene partition coefficients. (d) Density and bulk density of silica gel.

## Experimental

Commercially available dichlorophen was crystallized once from dichloroethane and four times from toluene: m.p. 175–176° $E$ (1%, 1 cm) at 304 m$\mu$ in 0·1N sodium hydroxide solution, 276·2. A sample thus

purified contained no *p*-chlorophenol when examined by thin-layer chromatography. Its purity was probably better than 99·9%.

*p*-Chlorophenol was recrystallized from toluene to a constant melting point of 41° (uncorr.): $E$ (1%, 1 cm) at 297 m$\mu$ in 0·1N sodium hydroxide, 188·0. Its purity was probably better than 99·9%.

Merck silica gel G was extracted with toluene to remove substances absorbing in the ultraviolet region. It was then dried for 1 hr at 105°, a treatment corresponding to that employed in the activation of thin-layer silica gel plates.

Toluene was fractionated on a Vigreux column of effective length 43 cm, that fraction distilling at 110·8° was used.

Equal volumes of a 20% w/v solution of ferric chloride and 1% w/v solution of potassium ferricyanide were sprayed on to the chromatograms to detect the spots. The method and materials complied with the requirements of the B.P. (1963), Addendum (1964).

CHROMATOGRAPHIC PROCEDURE

The chromatographic procedure described in the last reference was employed, plates were always used on the day in which they were prepared. The depth of the silica gel was standardized at 250$\mu$ $\pm$ 7$\mu$, solutions of dichlorophen and *p*-chlorophenol of known concentrations in absolute ethanol were applied to the plates using a calibrated Agla microsyringe.

Chromatograms were run at 25° $\pm$ 2° using toluene in glass tanks fitted with grooved glass lids and presaturated with toluene vapour. After running, the plates were removed, allowed to stand for 10 min to remove the toluene, and sprayed with reagent to reveal the spots. Two min later the chromatograms were traced on to semi-transparent paper.

The centre of each spot was assumed to lie at the intersection of the diagonals of a rectangle formed by drawing perpendicular and horizontal tangents at the periphery of the spot. The diameter of the spot was measured with vernier calipers and the spot area calculated. The mean Rf value from five separate determinations on different plates is reported. The coefficient of variation of the Rf values ranged from 2 to 16%. The smaller the Rf value the higher was the coefficient of variation.

ADSORPTION MEASUREMENTS

Solutions of dichlorophen and *p*-chlorophenol of known, but variable, composition in toluene were allowed to equilibrate (24 hr)* at 25° $\pm$ 2° enclosed and in contact with weighed amounts of silica gel. Aliquots of each of the solutions and also identical solutions which had not been in contact with silica gel, were then assayed. The amount adsorbed was calculated from each pair of assays. All experiments were made at least

* Dichlorophen attains equilibrium in 1 hr but more than 4 hr are required for *p*-chlorophenol. It was convenient, however, to allow 24 hr for this process.

in duplicate. This procedure revealed 17% decomposition of p-chlorophenol in 24 hr, and this was allowed for in determining the equilibration concentration.

Solutions of dichlorophen and p-chlorophenol used alone or in admixture were analysed by extracting the toluene four times with 0·1N sodium hydroxide and making up to an appropriate volume. The absorbance at 297 mμ (p-chlorophenol) and 304 mμ (dichlorophen) in 1 cm cells were used for assay. Single component solutions were optically regular at both wavelengths up to 25 μg/ml exhibiting, at the above wavelengths respectively, $E$ (1%, 1 cm) values of 242·0 and 276·2 for dichlorophen; and 188·0 and 168·1 for p-chlorophenol. Control cells were filled with 0·1N sodium hydroxide that had been shaken with toluene. Single component solutions were assayed using the wavelength of greater absorbance. Both wavelengths were used for mixtures.

TABLE 1. ANALYSIS OF KNOWN MIXTURES

| Dichlorophen μg/ml | | | p-Chlorophenol μg/ml | | |
|---|---|---|---|---|---|
| Theory | Found | % error | Theory | Found | % error |
| 5·00 | 5·04 | +0·74 | 15·42 | 15·40 | −0·13 |
| 5·10 | 5·01 | −1·80 | 15·42 | 15·53 | +0·70 |
| 5·00 | 4·82 | −3·60 | 15·00 | 15·45 | +3·00 |
| 10·00 | 10·17 | +1·70 | 10·28 | 10·05 | −2·10 |
| 10·20 | 9·81 | −3·70 | 10·28 | 11·20 | +8·90 |
| 10·00 | 10·15 | +1·50 | 10·00 | 10·04 | +0·90 |
| 15·00 | 15·28 | +1·80 | 5·14 | 4·87 | −5·60 |
| 15·30 | 15·27 | −1·60 | 5·14 | 5·27 | +2·50 |
| 15·00 | 15·29 | +2·10 | 5·00 | 4·66 | −6·70 |

Assays for nine known mixtures are given in Table 1 and, to a first approximation, the error is not random, since the return tends to be low for the lower concentrations of each component. The error is, however, generally small compared with the effects measured.

### BULK, TRUE DENSITY AND VOID VOLUME OF SILICA GEL

The true density ($\rho_1$) of silica gel, determined in a specific gravity bottle with toluene by displacement was 2·21 g/ml. The bulk density ($\rho_2$), determined by the three tap method (Martin, 1960), was 0·56 g/ml. If the weight and mean thickness of silica gel from known areas of thin-layer plates was used, the bulk density obtained was 0·57 g/ml. Void volume ($V_v$) was calculated from the formula: $V_v = 1/\rho_2 - 1/\rho_1$. Values of 1·33 ml/g and 1·30 ml/g were obtained. The void volume of a silica gel layer in a plate is thus significantly lower than that for the dry material.

*Density of* p-*chlorophenol and dichlorophen* were determined in a similar manner and are as follows at 25°, p-chlorophenol 1·31 g/ml, dichlorophen 1·46 g/ml.

### PARTITION COEFFICIENTS

Toluene solutions of each component were shaken with known volumes of water until equilibrium was attained. Toluene solutions were assayed

by extraction with 0·1N sodium hydroxide, aqueous solutions by the addition of sodium hydroxide to give an 0·1N solution. Each was then assayed spectrophotometrically. No dichlorophen was detected in the aqueous layer.

The coefficient for p-chlorophenol is concentration dependent. The following values for the ratio concentration in water/concentration in toluene, were obtained; 0·69, 0·70 and 0·82 at, respectively, concentrations of 2·6, 5·1 and 8·8 $\mu$g/ml in the toluene layer.

RF VALUES

These show an almost linear decrease as the load on the plate is decreased (Table 2). They also decrease with time (Table 3); with dichlorophen the effect is more pronounced as the load is decreased, with p-chlorophenol the effect is greater at the higher loading.

The spot diameter increases with time, though that of dichlorophen at low loads apparently decreases (Table 4). The latter phenomenon is ascribed to the failure of the spray reagent to show the small concentration at the periphery of the spot, a defect which must operate at all loads but which may be masked by the more rapid increase in spot area with time for higher loads.

When the plates are loaded with mixtures of the two components each appears to increase the Rf value of the other (Table 5). In general the increase is greater for dichlorophen.

TABLE 2. VARIATION OF RF VALUE WITH LOAD

| Load (µg) | Dichlorophen | p-Chlorophenol |
|---|---|---|
| 200 | 0·062 | 0·140 |
| 100 | 0·029 | 0·155 |
| 50 | 0·022 | 0·137 |
| 25 | 0·021 | 0·140 |
| 10 | 0·021 | 0·136 |
| 5 | 0·019 | 0·116 |
| 1 | 0·017 | 0·098 |
| 0·25 | 0·017 | 0·098 |
| 0·1 | 0·013 | 0·087 |

The solvent front was allowed to advance 10 cm from the origin.

TABLE 3. VARIATION OF RF VALUE WITH TIME OF RUN

| Time (min) | Load (µg) | | | | | |
|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 10 | 5 | 1 |
| | Dichlorophen Rf values | | | | | |
| 5 | 0·035 | 0·036 | 0·025 | 0·042 | 0·042 | 0·041 |
| 10 | 0·039 | 0·036 | 0·026 | 0·025 | 0·031 | 0·021 |
| 30 | 0·034 | 0·030 | 0·018 | 0·018 | 0·021 | 0·015 |
| 60 | 0·031 | 0·025 | 0·012 | 0·017 | 0·018 | 0·010 |
| | p-Chlorophenol Rf values | | | | | |
| 5 | 0·170 | 0·170 | 0·170 | 0·170 | 0·128 | 0·112 |
| 10 | 0·139 | 0·132 | 0·134 | 0·126 | 0·122 | 0·111 |
| 30 | 0·131 | 0·120 | 0·127 | 0·144 | 0·108 | 0·100 |
| 60 | 0·125 | 0·113 | 0·120 | 0·105 | 0·094 | 0·091 |

The advance of the solvent front for these runs is variable.

TABLE 4. VARIATION OF SPOT DIAMETER WITH TIME AND LOAD

| Time (min) | Load ($\mu$g) | | | | | |
|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 10 | 5 | 1 |
| | Dichlorophen spot diameter (mm) | | | | | |
| 5 | 6·6 | 6·5 | 6·5 | 6·3 | 6·1 | 5·6 |
| 10 | 6·6 | 6·4 | 6·0 | 5·7 | 5·9 | 5·0 |
| 30 | 7·6 | 6·6 | 6·6 | 5·9 | 6·0 | 5·1 |
| 60 | 8·5 | 7·3 | 7·1 | 6·6 | 5·8 | 5·2 |
| | p-Chlorophenol spot diameter (mm) | | | | | |
| 5 | 10·0 | 9·0 | 9·0 | 9·2 | 7·3 | 6·6 |
| 10 | 11·4 | 10·2 | 10·2 | 10·4 | 7·4 | 7·3 |
| 30 | 12·7 | 11·8 | 11·2 | 11·7 | 8·7 | 7·2 |
| 60 | 13·1 | 12·7 | 12·3 | 11·5 | 9·3 | 7·4 |

TABLE 5. RF VALUES OF MIXTURES OF COMPONENTS

| Ratio in mixture Dichlorophen-p-chlorophenol | Rf values | | | |
|---|---|---|---|---|
| | Mixed components | | Components alone at same load | |
| | Dichlorophen | p-Chlorophenol | Dichlorophen | p-Chlorophenol |
| 4 | 0·032 | 0·134 | 0·022 | 0·119 |
| 3 | 0·042 | 0·146 | 0·032 | 0·124 |
| 1 | 0·032 | 0·162 | 0·022 | 0·144 |

TABLE 6. ADSORPTION OF DICHLOROPHEN

| Concentration of solution (C) $\mu$g/ml | Amount adsorbed (X) $\mu$g/g | Amount adsorbed calculated from best fitting Langmuir equation $\mu$g/g |
|---|---|---|
| 0·71 | 190 | 207 |
| 1·79 | 486 | 427 |
| 2·64 | 580 | 574 |
| 3·95 | 768 | 800 |
| 5·88 | 1030 | 1067 |
| 7·60 | 1231 | 1266 |
| 10·32 | 1492 | 1500 |

Langmuir equation $\dfrac{X}{Xm} = \dfrac{BC}{1 + BC}$

B = constant = 0·51.

Xm = maximum X = 2844.

TABLE 7. ADSORPTION OF p-CHLOROPHENOL

| Concentration of solution (C) $\mu$g/ml | Amount adsorbed (X) $\mu$g/g | Amount adsorbed calculated from best fitting Fowler–Guggenheim equation $\mu$g/g |
|---|---|---|
| 4·1 | 31 | 22 |
| 7·6 | 40 | 43 |
| 11·4 | 51 | 71 |
| 13·8 | 123 | 113 |
| 18·2 | 236 | 238 |

Fowler–Guggenheim equation: $\dfrac{C}{C_0} = \dfrac{1}{BC_0} \cdot \dfrac{X/Xm}{1 - X/Xm} \exp\left(\dfrac{WN^1}{MRT}\right) \cdot \dfrac{X}{Xm}$

where B = constant = 1·34; $C_0$ = saturated soln. conc; W = interaction term; M = mol weight; R = Gas const; T = Temp °K; N = Avogadro's Number; Xm* = maximum X = 2718.
* This value has been calculated from the value of Xm for dichlorophen (Table 6) using the densities of dichlorophen and p-chlorophenol on the assumption that two molecules of p-chlorophenol occupy approximately the same volume as one molecule of dichlorophen.

*Adsorption measurements* for dichlorophen and *p*-chlorophenol alone are given in Tables 6 and 7 respectively and must be interpreted bearing in mind the accuracy of the assay procedure. The results for the former are best represented by a Langmuir isotherm equation, whilst those for the latter appear to conform best to a Fowler–Guggenheim isotherm. (Fowler & Guggenheim, 1939.) The same maximum adsorption space is assumed to be available in each case. This conclusion is based on a detailed statistical examination of the results (Scartsiaris, 1966) in which conformity with the Henry, Langmuir, BET, Freundlich and Fowler–Guggenheim isotherm equations was investigated. For each component the amounts adsorbed are given with those calculated from the best fitting isotherm in the adjacent column of Tables 6 and 7. The fit is less satisfactory in the case of *p*-chlorophenol.

TABLE 8. MIXED ADSORPTION

| | Dichlorophen | | | *p*-Chlorophenol | |
|---|---|---|---|---|---|
| Solution conc μg/ml | Amount adsorbed μg/g | Single adsorption calculated μg/g | Solution conc μg/ml | Amount adsorbed μg/g | Single adsorption calculated μg/g |
| 8·53 | 1172 | 1360 | 34·3 | 46·3 | — |
| 19·24 | 1773 | — | 18·75 | 76·2 | — |
| 5·30 | 941 | 987 | 23·6 | 80·1 | — |
| 12·27 | 1362 | — | 12·18 | 17·6 | 79·2 |
| 2·29 | 372 | 520 | 4·86 | 5·86 | 26·5 |
| 12·16 | 1226 | — | 5·62 | 18·2 | 30·8 |
| 3·69 | 746 | 754 | 9·32 | 17·5 | 54·2 |
| 4·08 | 693 | 814 | 8·85 | 23·4 | 50·0 |

Table 8 shows the adsorption when mixtures of the two components are examined. Where possible the anticipated adsorption of each component, had it been alone, has also been calculated from the appropriate isotherms; the results are given in the third and sixth columns of Table 8. Each component depresses the adsorption of the other but *p*-chlorophenol clearly has a disproportionately greater effect on dichlorophen despite the smaller affinity of the former for the surface. The fact that the molecular weight of *p*-chlorophenol is half the molecular weight of dichlorophen is not sufficient to account for this effect.

## Discussion

The Rf values of each component clearly vary with the load and the duration of the run: both these factors involve the concentration of the component in the area of the spot. The relation is given by equation (4) in which the term $g_s/g_m$ occurs. This term is a simple function of the partition coefficient or the slope of the adsorption isotherm. Only *p*-chlorophenol is partitioned between water and toluene and its partition coefficient increases with concentration. Hence by equation (1) the Rf value should decrease with increasing load whereas the reverse has been shown to be the case. Moreover dichlorophen has no measurable water/toluene partition coefficient, so that any interpretation of the observed chromatographic behaviour based on a partition coefficient theory is clearly inadequate.

The slope of the adsorption isotherm for dichlorophen decreases as the concentration increases, giving a predictable increase in Rf value as the load is increased. Diffusional processes tend to decrease the concentration and the system would thus move to a position of the isotherm with a greater slope. Therefore the Rf value will also decrease with increasing duration of run: both these trends have been observed.

For p-chlorophenol the increase in Rf value is less marked but this could be predicted from the generally smaller slope of the isotherm, since this component is less strongly adsorbed.

In mixtures, each component depresses the adsorption of the other and this will effectively decrease the slope of the isotherm for each component; p-chlorophenol has a disproportionately greater effect than dichlorophen. Not only should each increase the Rf value of the other, but also that for dichlorophen should be the more affected. This is in accord with the experimental results.

The Fowler–Guggenheim isotherm equation, to which p-chlorophenol appears to conform, allows for adsorbate - adsorbate interaction. The shape of the experimentally determined isotherm suggests attractive interaction between p-chlorophenol molecules. A repulsive interaction between dichlorophen and p-chlorophenol could explain the phenomenon, in which case the adsorption isotherm for dichlorophen in the presence of p-chlorophenol should exhibit Fowler–Guggenheim characteristics. Initial examination of the results fails to reveal this. Nevertheless an explanation of the anomalous behaviour of the two components based on their adsorption behaviour on silica gel would appear more valid. On thin-layer plates the separation of the components is effected by adsorption rather than partition processes.

The load on the chromatography plate and the spot area (Table 4) together with the bulk density, void volume and thickness of the layer may be used with the adsorption data to calculate values for $g_s/g_m$. These are then inserted in equation (4) to obtain the Rf value. The calculated Rf values of dichlorophen for loads of 1, 5 and 10 $\mu$g are, respectively, 0·004, 0·005 and 0·007 and the corresponding experimentally determined values are 0·017, 0·021 and 0·022. Similarly, the calculated Rf values of p-chlorophenol are 0·192, 0·145 and 0·096 and the corresponding experimentally determined values are 0·108, 0·116 and 0·137. The partition coefficient, K, for p-chlorophenol may be used in equation (1) to calculate the Rf value if the ratio b/a is known. The ratio

$$b/a = \frac{X_m \ (p\text{-chlorophenol})}{V_v \times \text{density of } p\text{-chlorophenol}} = 1\cdot55 \times 10^{-3}.$$

Calculated values based on the partition coefficient thus yield Rf values slightly less than unity. This tends to support the contention that adsorption is the main factor for the system studied. However the values show that whilst the Rf values calculated for p-chlorophenol are of the right order of magnitude, their trend with load is opposite to the experimentally found values. The trend for dichlorophen is in the right direction but the calculated values are a third of those experimentally determined.

# References

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