The effect of molecular structure on the R_F value
A contribution to the theory of paper chromatography

Paper chromatography is perhaps the most widely used research tool. Unfortunately the choice of the solvents is rather intuitional, since no theory is available which connects the R_F with the molecular structure of the solute.

In the present paper a thermodynamical derivation is presented, which defines the R_F in terms of the molar volume and solubility parameter of the material to be chromatographed and the solubility parameters of the solvents used.

Materials and methods

Whatman No. 1 filter paper was used in all experiments. According to our determinations this paper contained about 30% water when it was equilibrated with water-saturated air at room temperature (determined by measuring the loss in weight of the paper after drying at 100°).

The volume of the moving phase was determined by immersing the tip of a 1 × 10 cm paper strip into the appropriate solvent. After the ascending solvent had wetted the strip entirely, it was weighed in a stoppered test tube. The difference between the weight of the wetted and the non-wetted strip, divided by the density of the solvent, gives the volume of the solvent phase.

Results and discussion

According to MARTIN AND SYNGE[1], paper chromatography is a continuous distribution of the solute between a stationary phase and a mobile phase. By assuming adsorption to be negligible he was able to deduce the following equation, which connects the distribution coefficient a^* with R_F:

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* The following symbols are used throughout this paper:

a = distribution coefficient = $\dfrac{\text{conc. in stationary phase}}{\text{conc. in mobile phase}}$

A = cross section
$\Delta\mu$ = free energy of transfer from one phase to the other
T' = melting point in absolute degrees
T = temperature in absolute degrees
V = molar volume
φ = volume fraction
C = $\sqrt{\dfrac{U}{V}}$ = solubility parameter
U = ΔH_{evap} = cohesion energy $\approx$ specific heat of evaporation
M = molecular weight
ϱ = density
X = mole fraction

Subscripts

m = moving phase s = stationary phase
M = solute S = solvent
Mixt. = mixture

$$a = \frac{A_m}{A_s}\left(\frac{1}{R_F} - 1\right) \tag{1}$$

Since the free energy of transfer of one mole of the solute is given by:

$$- \Delta\mu = RT \ln a \tag{2}$$

we obtain by substituting (1) in (2):

$$- \Delta\mu = RT \ln \frac{A_m}{A_s}\left(\frac{1}{R_F} - 1\right) \tag{3}$$

MARTIN postulated that $\Delta\mu$ is the result of the addition of the contributions of the various groups in the molecule of the solute, and obtained the following expression for homologous series, with x as repeating group n times:

$$- \frac{\Delta\mu_x}{RT} = \ln \frac{\left(\frac{1}{R_{F_{n+1}}} - 1\right)}{\left(\frac{1}{R_{F_n}} - 1\right)} \tag{4}$$

The validity of the above expression was confirmed by several authors[2-7].

We wish to present a thermodynamical basis for MARTIN'S postulate. According to KETELAAR[8] the solubility of a solute in a regular solution can be represented by:

$$\ln X_M = - \frac{\Delta\mu}{R}\left(\frac{1}{T} - \frac{1}{T'}\right) - \frac{V_M\,\varphi_s{}^2}{RT}(C_s - C_M)^2 \tag{5}$$

If we apply this equation to the distribution of a given material between two phases, we have in the case of dilute solutions, where $\varphi \approx 1$:

$$- \Delta\mu = RT \ln \frac{X_{Ms}}{X_{Mm}} = V_M\left[(C_m - C_M)^2 - (C_s - C_M)^2\right] + RT \ln \frac{M_m\,\varrho_s}{M_s\,\varrho_m} =$$

$$= V_M\left[C_m{}^2 + 2 C_M(C_s - C_m) - C_s{}^2\right] + RT \ln \frac{M_m\,\varrho_s}{M_s\,\varrho_m} \tag{6}$$

The second part of the right side of the above equation changes the expression of $\Delta\mu$ from mole fraction to concentration.

For homologous series this is approximately equal to:

$$- \Delta\mu_x = A + K V_{xm} \tag{7}$$

which is a mathematical expression of MARTIN'S postulate, as mentioned above.

It is interesting to note that COHN AND EDSALL arrived at the same equation on an empirical basis[9]. Moreover, MASON AND WEGIENKA[10] found an empirical relationship between R_F and molar refraction, which is of course an expression for the molar volume.

Let us consider an application of the above equation.

Table I shows the C_s values of most common solvents. In the case of a mixture we use:

$$C_{s\ \text{mixt.}} = X_{sA} C_A + X_{sB} C_B \ldots \tag{8}$$

C_M can be calculated according to the structure by using Table II, i.e. by dividing the sum of the respective cohesion constants by the calculated molar volume and taking the square root.

TABLE I

THE SOLUBILITY PARAMETER OF VARIOUS SOLVENTS[8]

Solvent	Solubility parameter
n-Pentane	7.05
n-Hexane	7.3
Ether	7.4
Carbon tetrachloride	8.6
Cyclohexanone	8.2
Benzene	9.15
Chloroform	9.3
Chlorobenzene	9.5
Dioxane	9.7
Carbon disulphide	10.0
Pyridine	10.7
Nitrobenzene	11.6
Ethanol	12.7
Methanol	12.7
Water	23.4

TABLE II

THE COHESION ENERGY VOLUME OF VARIOUS CHEMICAL GROUPS[*]

Group	Cohesion energy cal. per mole	Volume c.c. per mole
CH_3	1,780	20.1
$=CH_2$	1,780	22.3
$-CH_2-$	890	13.8
$=CH-$ aliphatic	990	7.3
$=CH-$ aromatic	990	11.0
$\equiv CH$	— 380	7.2
$-O-$	1,630	10.5
$-OH$	7,250	17.0
$=CO$	4,270	12.0
$-CHO$	4,700	19.3
$-COOH$[**]	8,970	30.4
$-COOMe$ aliphatic	5,600	60.0
$-COOMe$ aromatic	5,600	44.1
$-COOEt$ aliphatic	6,230	77.0
$-COOEt$ aromatic	6,230	57.8
$-NH_2$[***]	3,530	14.5
$-Cl$	3,400	22.0
$-F$	1,200	7.0
$-Br$	4,300	28.0
$-I$	5,040	32.0
$-NO_2$	7,200	13.0
$-SH$	4,250	32.0
$-CONH_2$	13,200	27.4
$-CONH-$	10,60	21.0
$=$	—	8.0
$-H$	—	6.5
$\begin{bmatrix} NH_2 \\ H_2C-COOH \end{bmatrix}$ as zwitterion	13,390	45.4

[*] The data in this Table are based on refs. [11-14].
[**] For ionization of carboxyl group deduct 10.0 c.c.
[***] For ionization of amino group deduct 17.0 c.c.
   Varying contractions for hydrogen bonds and ring systems.

TABLE III

THE VOLUME OF MOVING PHASE FOR VARIOUS SOLVENTS
Whatman No. 1 filter paper at room temperature ($\approx 25°$).

| Solvent	Volume, $\mu l/mg$ weight of filter paper
Ligroin	0.98
Chloroform	0.93
Carbon tetrachloride	0.92
Benzene	0.90
Toluene	0.90
Dioxane	0.98
Butanol	0.97
Amyl alcohol	0.92
Ethyl acetate	0.99
Ethanol 96%	1.10
Acetone	1.10
Acetic acid	1.21
Methanol	1.25
Pyridine	1.60
Water	1.66

Moreover, we can assume that the stationary phase always has the same composition, *i.e.* pure water, independent of the composition of the moving phase. This should be true at least for solvents that cause no swelling of the filter paper. The above assumption, together with the calculated constants, allows the calculation of $\Delta \mu$.

In order to calculate R_F it is necessary to have information about the value of $\frac{A_m}{A_s}$. We found experimentally that it is fairly constant for solvents that do not swell the cellulose (Table III). When the moving phase is composed of a mixture of two solvents, one causing and the other not causing swelling, $\frac{A_m}{A_s}$ wil be a function of the mole fraction of the solvent that causes the swelling (Figs. 1 and 2). Since the filter paper used in our experiments contained 30% water, and since the data in Table III and Figs. 1 and 2 are given per mg filter paper, the results should be multiplied by 3.3 in order to obtain $\frac{A_m}{A_s}$.
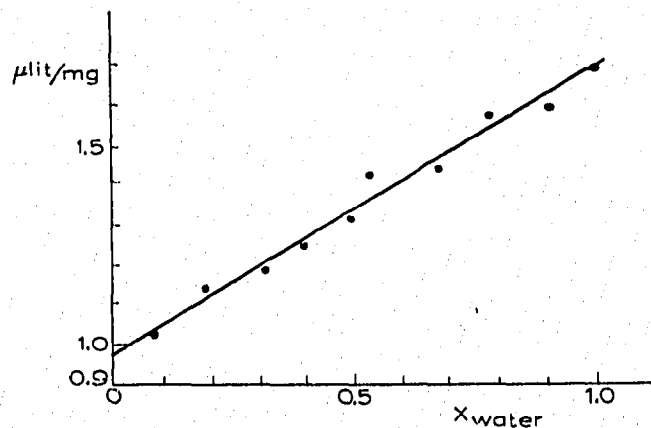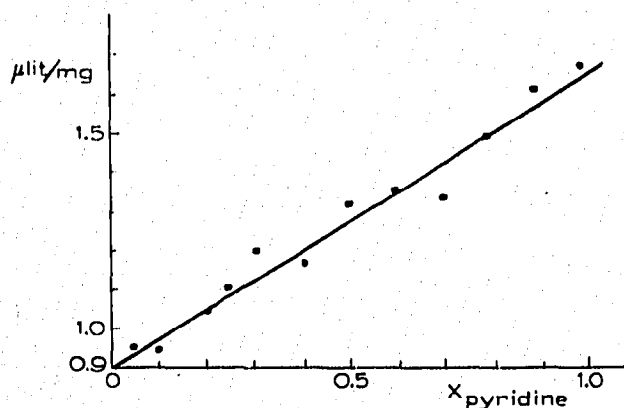
We must mention that there is a serious drawback in the actual calculations. When calculating the value in the square brackets in eqn. 6, a small difference between two large numbers is obtained. An error here can cause a difference of 100% or more in the value of R_F, as a result of the inaccuracy of the constants used. By using more accurate constants, the calculated R_F should be closer to the actual value. Nevertheless we can use the above theory for choosing the appropriate solvent for a given material, or for differentiating between two materials. Moreover, the thermodynamical derivation gives some insight into the structures of related substances through their R_F values and can explain relative R_F values.
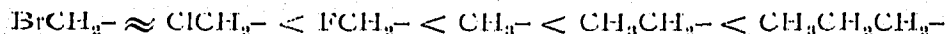
It is well known[15] that the separation of materials differing only by the replacement of a CH_3 group by a CH_2F group, is extremely difficult. Table II also shows that, in view of the identical molar volumes and the small difference in cohesion constants, the calculated R_F should be similar, the more so as the other groups in the molecule further reduce possible differences. Since we know that hydrogen bonding and cyclization reduces the molar volume, we should expect different R_F values for the ammonium salt of $FCH_2CONHOH$ as compared to $CH_3CONHOH$. This was shown to the case by BERGMANN AND SEGAL[15], and was related to the structure:

$$CH_2-CO-N-OH$$
$$|\qquad\quad H\quad |$$
$$|\qquad\quad |\quad\ \ |$$
$$F...H-N-H$$
$$|$$
$$H$$
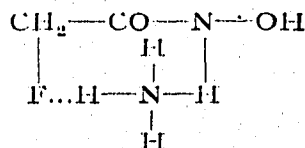
The molecular volume of acid hydroxamates increase in the order:

$$FCH_2- < CH_3- < CH_3CH_2- < ClCH_2- < CH_3CH_2CH_2- < BrCH_2-$$
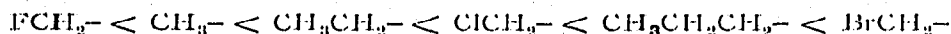
On the other hand the above authors found that the order of the R_F values is:

$$BrCH_2- \approx ClCH_2- < FCH_2- < CH_3- < CH_3CH_2- < CH_3CH_2CH_2-$$

This obvious discrepancy can be clarified as follows:

In eqn. (6) we have two variables, which are dependent on the molecular structure of the moving material, namely: the molar volume (V_M) and the solubility parameter (C_M). In homologous series according to the relative importance of the changes, either factor can dominate and determine the change in the R_F value.

In the above example the equal R_F values for $ClCH_2CONHOH$ and $BrCH_2CO$-NHOH can be explained by the fact that according to Table II the cohesion energy of the $ClCH_2$ group $\left(\frac{4390}{35.8} = 122\right)$ is almost equal to that of CH_2Br $\left(\frac{5290}{41.8} = 126\right)$. On substituting the hydrogen by a halogen the cohesion energy increases since the value for a CH_3 group is only $\frac{1780}{20.1} = 89$. According to eqn. (6), the increase of C_M lowers the absolute value in the square brackets, since in our case $C_s > C_m$. This increases the difference between the two terms of eqn. (6), increasing $-\varDelta\mu$, which causes a lowering of R_F according to eqn. (3). Internal hydrogen bonding decreases V_m causing the same effect. On the other hand in homologous series C_s is approximately constant; only V_M increases which causes the R_F to increase too.

Finally the following consideration makes it possible to measure the interfering effects. When chromatography is carried out with distilled water, eqn. (6) will give $-\Delta\mu = 0$ and from eqn. (3) we obtain

$$\frac{1}{R_F} = \frac{A_s}{A_m} + 1 \tag{9}$$

i.e. R_F is independent of molecular volume and constant (≈ 0.88), which was found experimentally to be true for such widely differing compounds as: xanthine, coffeine, uric acid, tetramethyluric acid, urea, glycine, leucine, acetohydroxamic acid. We postulate that any deviation from this limit is a measure of the effect of adsorption, precipitation, etc.

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Chromatography on paper impregnated with ion exchange resins
IV. The adsorption of metal ions on Dowex-50
from dilute HF solution

In this note we wish to describe a simple method for determining whether a metal ion in a given solution adsorbs on a cation exchanger. For this purpose KRAUS[1] recommends running short column chromatograms. Ion exchange papers yield the same information and offer perhaps some advantages over both columns and equilibrium studies, since the bands can be detected on the paper after development by the use of