

CHROM. 16,098

EFFECT OF SUPPORT CHARACTERISTICS ON THE DETERMINATION OF THE LIPOPHILICITY OF SOME NEUTRAL, ACIDIC AND ALKALINE COMPOUNDS BY REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY

TIBOR CSERHÁTI*, YASSIN M. DARWISH and GYÖRGY MATOLCSY

Plant Protection Institute of the Hungarian Academy of Sciences, Herman Ottó ut 15, 1022 Budapest (Hungary)

(First received January 17th, 1983; revised manuscript received June 15th, 1983)

SUMMARY

The lipophilicity of some neutral, acidic and alkaline compounds was determined by reversed-phase thin-layer chromatography applying silica, aluminium oxide and cellulose in different degrees impregnated with paraffin oil as support. The lipophilicity of all compounds increased on all supports at increasing extent of impregnation; paraffin oil gradually covered the active adsorption sites of supports. The lowest R_M values were determined on cellulose support owing to its lower adsorptive capacity. The alkaline compounds showed higher R_M values on silica and the acidic compounds on aluminium oxide, proving that also the surface pH value of the support influences the lipophilicity determination.

INTRODUCTION

Reversed-phase thin-layer chromatography (RPTLC) has been extensively applied to the determination of relative partition coefficients^{1,2}. Recent research indicates that a support may partially retain its original adsorptive character after impregnation³, and that R_M values are dependent on the impregnation and on the adsorption behaviour of the support^{4,5}.

To further understand this phenomenon we determined the lipophilicity of some neutral, acidic and alkaline compounds by RPTLC using silica, aluminium oxide and cellulose as supports.

MATERIALS AND METHODS

The chemical structures of the sample compounds are shown in Tables I-III. The substituted triazine derivatives were considered to be neutral compounds, the heterocyclic quaternary ammonium salts and phenol derivatives as alkaline and acidic molecules respectively.

To determine the theoretically important ΔR_{MCH_2} value, 2,4-dinitrophenylhydrazones (DNP) derivatives of normal saturated aldehydes containing 1-7, 9, 10 and

12 carbon atoms were prepared according to the method of ref. 6. The aldehydes of greater carbon number were first dissolved in ethanol and then added to the reaction mixture.

Layers of 0.25 mm thickness were prepared on glass plates (20 × 20 cm) from the following supports: I, Kieselgel 60 HF₂₅₄ (Merck); II, MN-Aluminium oxide G (Macherey-Nagel); III, Cellulosepulver MN 300 (Macherey-Nagel). After drying, the plates were impregnated by immersion overnight in solutions of 1, 5 and 10% paraffin oil in *n*-hexane. The triazine derivatives were dissolved in chloroform, the heterocyclic quaternary ammonium salts in methanol, the phenol derivatives in acetone and the DNP derivatives in acetonitrile at a concentration of 2 mg per cm³ solvent; 5 μl of each solution were spotted on the plates. In the case of the heterocyclic quaternary ammonium salts and phenol derivatives, water was used as eluent. Due to their high lipophilicity and low solubility in water the triazine and DNP derivatives cannot be eluted by water. In the eluents chosen, these compounds gave symmetrical spots and their R_F values were readily measurable. As the DNP derivatives differed considerably in lipophilicity, various eluent compositions were necessary to determine their R_M values.

After development, the triazine derivatives were revealed by use of the chloro-toluidine reaction⁷, the heterocyclic quaternary ammonium salts by the Dragendorff reagent⁷ and the other compounds by their visible and UV absorption spectra. For each experiment five independent parallel determination were made.

We have to stress that, owing to the poor reproducibility of R_F values, the comparison of R_M values calculated from R_F measured on different plates should be carried out under strictly controlled conditions; only plates prepared from the same batch, stored under identical conditions, and impregnated and developed in the same TLC chamber can be compared.

RESULTS AND DISCUSSION

The mean R_M values of trisubstituted symmetric triazine derivatives are listed in Table I. The lipophilicity values differ considerably, depending on the quality of the support and on the extent of impregnation, but on each support they increase with increasing extent of impregnation (Fig. 1). The R_M value changes generally non-linearly with the extent of impregnation.

Paraffin oil gradually covers the active adsorptive sites of support. This is a saturation process which can probably be modelled by a Freundlich type isotherm. The order of retention on the supports is silica > aluminium oxide > cellulose.

The lipophilicities measured on the same supports at different extents of impregnation or on different supports at the same extent of impregnation are well correlated, *i.e.*, neither the extent of impregnation nor the support characteristics change the lipophilicity order of substituted symmetric triazine derivatives.

The mean R_M values of heterocyclic quaternary ammonium salts are compiled in Table II. As these compounds showed very elongated spots near to the eluent front on impregnated cellulose layers, determination of the exact R_M value was impossible under these conditions. The lipophilicity values depend more strongly on the support characteristics than is the case with the neutral triazine derivatives, and to a lesser extent on the extent of impregnation. The R_M values on each support

TABLE I

100 · R_M VALUES OF SOME TRISUBSTITUTED SYMMETRIC TRIAZINE DERIVATIVES DETERMINED IN DIFFERENT RPTLC SYSTEMS

Eluent: water-methanol (1:1) except where stated otherwise. Et = Ethyl; i-Pr = isopropyl; t-Bu = *tert.*-butyl.

| | | | Impregnation of silica (%) | | | Impregnation of aluminium oxide (%) | | | Impregnation of cellulose (%)* | | |
|------------------|-------|-------|----------------------------|-----|----|-------------------------------------|-----|-----|--------------------------------|-----|-----|
| R_1 | R_2 | R_3 | 1 | 5 | 10 | 1 | 5 | 10 | 1 | 5 | 10 |
| OCH ₃ | Et | i-Pr | -41 | -10 | 5 | -67 | -47 | -27 | -50 | -45 | -35 |
| Cl | Et | i-Pr | -42 | -6 | 9 | -57 | -27 | -14 | -22 | -13 | 6 |
| SCH ₃ | Et | i-Pr | -27 | 15 | 34 | -46 | -9 | 3 | 23 | 27 | 47 |
| OCH ₃ | i-Pr | i-Pr | -32 | 6 | 24 | -41 | -15 | -6 | 12 | 30 | 50 |
| Cl | i-Pr | i-Pr | -33 | 14 | 37 | -40 | -9 | -2 | 9 | 15 | 26 |
| SCH ₃ | i-Pr | i-Pr | -20 | 36 | 55 | -29 | 11 | 15 | 38 | 56 | 73 |
| OCH ₃ | Et | t-Bu | -34 | 16 | 42 | -38 | -16 | -3 | 4 | 19 | 36 |
| Cl | Et | t-Bu | -30 | 23 | 53 | -24 | 0 | 12 | 33 | 53 | 55 |
| SCH ₃ | Et | t-Bu | -15 | 44 | 66 | -21 | 22 | 41 | 63 | 92 | 132 |

* Eluent: water-methanol (3:1).

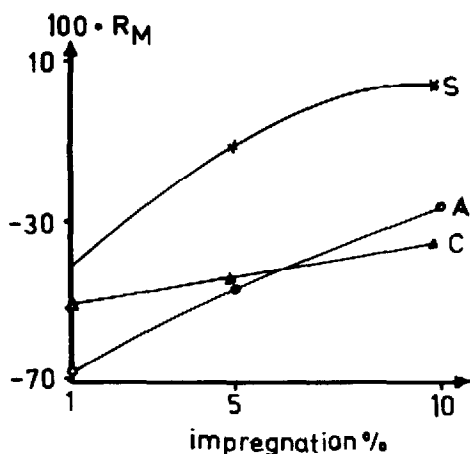


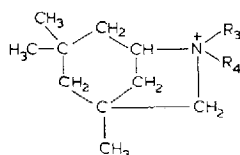
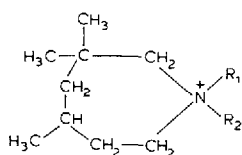
Fig. 1. Effect of support quality and extent of impregnation on the lipophilicity of 2-methoxy-4-ethyl-amino-6-isopropylamino-*s*-triazine. A = Aluminium oxide; S = silica; C = cellulose. Eluent for A and S: water-methanol (1:1), for C: water-methanol (3:1).

increase with increasing extent of impregnation (Fig. 2). As in the case of the triazines, the extent of impregnation has no effect on the lipophilicity order, but the R_M values measured on silica and aluminium oxide supports do not show any correlations. This result indicates that in the case of polar compounds not only the adsorption strength but also the surface pH value of the support exerts a considerable influence on the R_M determination. The order of retention on the supports is the same as for the triazine derivatives.

TABLE II

100 · R_M VALUES OF SOME HETEROCYCLIC QUATERNARY AMMONIUM SALTS DETERMINED IN DIFFERENT RPTLC SYSTEMS

Eluent: water.



| | | Impregnation of silica (%) | | | Impregnation of aluminium oxide (%) | | |
|---|-----------------|----------------------------|-----|-----|-------------------------------------|-----|-----|
| | | 1 | 5 | 10 | 1 | 5 | 10 |
| R_1 | R_2 | | | | | | |
| CH ₃ | CH ₃ | 121 | 139 | 152 | 0 | 46 | 53 |
| CH ₃ | H | 123 | 140 | 147 | -14 | 29 | 31 |
| C ₂ H ₅ | CH ₃ | 114 | 124 | 129 | -19 | 20 | 35 |
| CH ₂ CHCH ₂ | CH ₃ | 121 | 139 | 149 | 50 | 115 | 111 |
| C ₆ H ₅ CH ₂ | CH ₃ | 116 | 130 | 137 | -8 | 36 | 42 |
| (CH ₂) ₄ Br | - | 113 | 123 | 126 | -17 | 25 | 35 |
| R_3 | R_4 | | | | | | |
| CH ₃ | CH ₃ | 125 | 142 | 151 | -5 | 39 | 47 |
| CH ₃ | H | 117 | 127 | 126 | 39 | 88 | 96 |
| C ₂ H ₅ | H | 121 | 135 | 145 | 19 | 65 | 72 |
| CH ₂ CHCH ₂ | H | 97 | 105 | 107 | -16 | 30 | 41 |

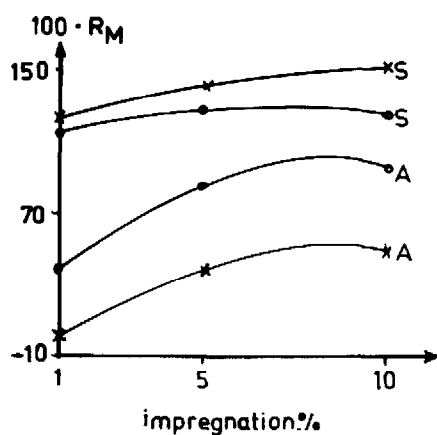
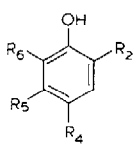


Fig. 2. Effect of support quality and extent of impregnation on the lipophilicity of *N,N*-dimethyl-1,3,3-trimethyl-6-azobicyclo[3.2.1]octane (x) and of *N*-methyl-1,3,3-trimethyl-6-azobicyclo[3.2.1]octane (o). A = Aluminium oxide; S = silica. Eluent: water.

TABLE III

100 · R_M VALUES OF SOME PHENOL DERIVATIVES DETERMINED IN DIFFERENT RPTLC SYSTEMS

Eluent: water.

|  | | | | Impregnation of silica (%) | | | Impregnation of aluminium oxide (%) | | | Impregnation of cellulose (%) | | |
|--|-----------------|-----------------|-----------------|----------------------------|------|-----|-------------------------------------|-----|-----|-------------------------------|-----|-----|
| | | | | 1 | 5 | 10 | 1 | 5 | 10 | 1 | 5 | 10 |
| R_2 | R_4 | R_5 | R_6 | | | | | | | | | |
| | NO ₂ | | | -30 | 27 | 38 | 33 | 70 | 69 | -15 | -11 | -12 |
| NO ₂ | NO ₂ | | | | -92 | -86 | 57 | 100 | 103 | | | |
| NO ₂ | | NO ₂ | | | -48 | -32 | 90 | 153 | 167 | -52 | -53 | -54 |
| NO ₂ | | | NO ₂ | | -67 | -57 | 91 | 153 | 159 | | | |
| NO ₂ | NO ₂ | | NO ₂ | | -53 | -40 | 77 | 125 | 156 | | | |
| | CN | | | -20 | 40 | 57 | 38 | 73 | 89 | -30 | -31 | -27 |
| Br | CN | | | -44 | 14 | 25 | 52 | 87 | 92 | -18 | -19 | -13 |
| NO ₂ | CN | | | | -106 | -94 | -42 | 95 | 100 | | | |
| NO ₂ | CN | | NO ₂ | | -82 | -76 | 64 | 118 | 127 | | | |
| Br | CN | | Br | | -84 | -60 | 64 | 114 | 122 | | | |
| Cl | CN | | NO ₂ | | -106 | -87 | 71 | 123 | 133 | | | |
| I | CN | | NO ₂ | | -85 | -58 | 118 | 177 | 192 | | | |
| Br | CN | | NO ₂ | | -100 | -76 | 85 | 146 | 161 | | | |

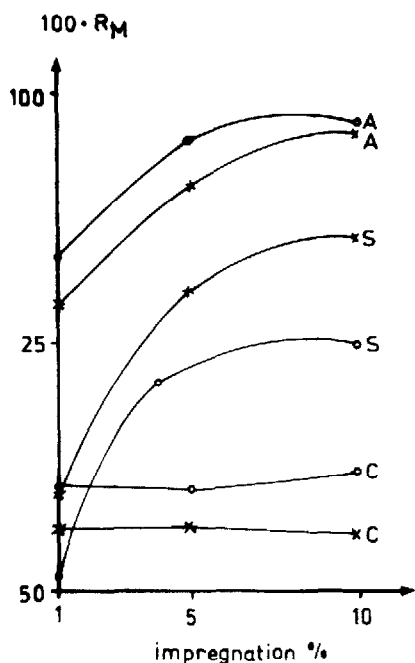


Fig. 3. Effect of support quality and extent of impregnation on the lipophilicity of 4-cyanophenol (x) and of 2-bromo-4-cyanophenol (O). A = Aluminium oxide; S = silica; C = cellulose. Eluent: water.

The mean R_M values of phenol derivatives are summarized in Table II. The blank spaces in Table III indicate that the compound in the given RPTLC system was at the eluent front or so near to it that its R_M value could not be determined accurately. Due to the weak retention of these compounds, only a few R_M values could be measured on impregnated cellulose layers and on silica impregnated with 1% paraffin oil. Similar conclusions to these for the triazines and ammonium salts can be drawn: *viz.*, the support characteristics and extent of impregnation influence the lipophilicity values, which increase with increasing extent of impregnation (Fig. 3). The lipophilicity order does not depend on the extent of impregnation, but the R_M values measured on silica and aluminium oxide supports showed no correlation. Also the retention order was different: aluminium oxide > silica > cellulose. As with the quaternary ammonium salts (alkaline compounds) on silica, the phenol derivatives (acidic compounds) on aluminiumoxide showed higher retention and the lipophilicity orders did not correlate. Therefore the character of the support has a considerable influence on the determination of lipophilicity and also on the lipophilicity order of highly polarizable molecules.

The mean R_M values of DNP derivatives of normal aldehydes are compiled in Table IV. Blank spaces again indicate that the R_M value of the compound would not be determined accurately in the given system (the spot was at the start of near to the front). Spots were often deformed near to the second solvent front, but the R_M values of such spots were never recorded.

TABLE IV

100 · R_M VALUES OF 2,4-DINITROPHENYLHYDRAZONE DERIVATIVES OF SOME NORMAL ALDEHYDES DETERMINED IN DIFFERENT RPTLC SYSTEMS

Eluents: upper part, water-acetonitrile (1:1); lower part, water-acetonitrile (2:1).

| No. of C atoms in aldehyde | Impregnation of silica (%) | | | Impregnation of aluminium oxide (%) | | | Impregnation of cellulose (%) | | |
|-------------------------------|-------------------------------|-----|-----|--|-----|-----|----------------------------------|-----|-----|
| | 1 | 5 | 10 | 1 | 5 | 10 | 1 | 5 | 10 |
| 1 | | | -90 | | | | | | -89 |
| 2 | | | -68 | | | | | | -70 |
| 3 | | -91 | -53 | | | | | | -44 |
| 4 | -96 | -66 | -41 | | -35 | | | | -30 |
| 5 | -88 | -52 | -24 | -83 | -23 | | | | -17 |
| 6 | -72 | -30 | -3 | -74 | -12 | -24 | -66 | -53 | -3 |
| 7 | -43 | 23 | 39 | -46 | 11 | -1 | -33 | -17 | 22 |
| 9 | -20 | 47 | 69 | -37 | 21 | 20 | -18 | 5 | 31 |
| 10 | -3 | 71 | 95 | -30 | 26 | 35 | 3 | 18 | 43 |
| 12 | 20 | 114 | 139 | -5 | 63 | 94 | 43 | 43 | 74 |
| 1 | | 5 | 38 | -92 | -11 | -49 | | -15 | -40 |
| 2 | -17 | 23 | 58 | -68 | -2 | -29 | -53 | 0 | -19 |
| 3 | -7 | 56 | 91 | -41 | 15 | 0 | -36 | 17 | 9 |
| 4 | 14 | 84 | 117 | -17 | 31 | 35 | -13 | 28 | 34 |
| 5 | 34 | 114 | 149 | -4 | 44 | 68 | 10 | 43 | 62 |
| 6 | 49 | 141 | | 9 | 61 | 106 | 35 | 49 | 48 |
| 7 | 84 | | | 26 | 123 | | 85 | 104 | 168 |

All parameters investigated, support type, extent of impregnation and eluent composition, influence the ΔR_{MCH_2} value (Fig. 4). For the DNP derivatives the differences between the supports are lower than for the other derivatives studied (Figs. 5 and 6); however, the ΔR_{MCH_2} values on silica are a little higher and their standard deviation is lower than the corresponding values on aluminium oxide and cellulose.

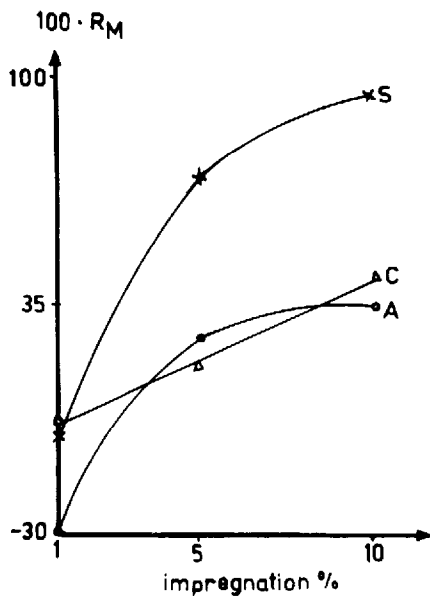
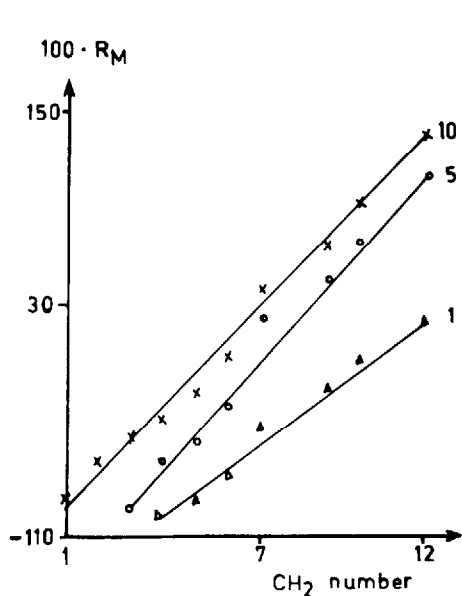


Fig. 4. $100 \cdot R_M$ values of 2,4-dinitrophenylhydrazones of some normal aldehydes on silica. Numbers indicate extent of impregnation (%). Eluent: water acetonitrile (1:1).

Fig. 5. Effect of support quality and extent of impregnation on the lipophilicity of *n*-C₁₂ aldehyde 2,4-dinitrophenylhydrazone. A = Aluminium oxide; S = silica; C = cellulose. Eluent: water acetonitrile (1:1).

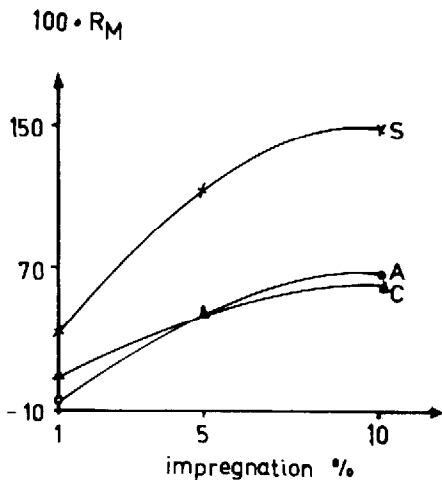


Fig. 6. Effect of support quality and extent of impregnation on the lipophilicity of *n*-C₃ aldehyde 2,4-dinitrophenylhydrazone. A = Aluminium oxide; S = silica; C = cellulose. Eluent: water acetonitrile (2:1).

Summarizing our results, we stress again that in the case of polar compounds the supports have a considerable influence not only on the absolute value of the lipophilicity but also on the order of lipophilicity; therefore different sets of R_M values may point to different optimal molecular structures in quantitative structure-activity studies and these data must be regarded with extreme caution. The situation is not so critical in the case of less polar compounds where the individual R_M values may differ from each other but their order is similar, and probably indicate the same optimal molecular structure.

We are well aware that these series of compounds are too limited to draw firm conclusions and that the problem needs further investigation.

REFERENCES

- 1 G. L. Biagi, A. M. Barbaro, M. F. Gamba and M. C. Guerra, *J. Chromatogr.*, 41 (1969) 371.
- 2 G. L. Biagi, M. C. Guerra, A. M. Barbaro and M. F. Gamba, *J. Med. Chem.*, 13 (1970) 511.
- 3 W. V. van Giesen and L. H. M. Janssen, *J. Chromatogr.*, 237 (1982) 199.
- 4 M. C. Guerra, A. M. Barbaro, G. Cantelliforti, M. T. Foffani, G. L. Biagi and P. A. Borea, *J. Chromatogr.*, 216 (1981) 93.
- 5 L. Ogierman and A. Silowiecki, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 357.
- 6 D. R. Knapp, *Handbook of Analytical Derivatization Reactions*, Wiley, New York, 1979, p. 340.
- 7 E. Stahl, *Dünnschicht-Chromatographie*, Springer, Berlin, 1962, pp. 501, 504.