INVESTIGATIONS OF THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC PARAMETERS

II. STRUCTURAL EFFECTS IN LIQUID–LIQUID PARTITION CHROMATOGRAPHY OF ANILINE DERIVATIVES*

MARIA PRZYBOROWSKA AND EDWARD SOCZEWIŃSKI

Department of Inorganic and Analytical Chemistry, Medical Academy, Lublin (Poland)

(Received February 15th, 1969)

SUMMARY

R_M values of ten methyl derivatives of aniline were determined for systems of the type: weakly polar solvent-formamide and weakly polar solvent-5% formic acid in formamide. The experimental data plotted against the pK_A values of the bases indicate that their chromatographic behaviour in acidic systems is determined by the basicity of the amino group, steric effects and the molecular volume of the solutes; on the other hand, the basicity plays only a minor part in neutral systems. The chromatographic results are compared with analogous data for the system cyclohexane–water and cyclohexane–buffer solution obtained in static experiments.

The results indicate that it is possible to apply the concept of additivity of R_M values to the solutes investigated, provided that steric effects and the basicity of the nitrogen atom are taken into account.

INTRODUCTION

In view of the high sensitivity of nitrogen basicity to constitutional effects[1-3] only sporadic attempts have been made to apply the R_M concept to partition chromatography of nitrogen bases[4]. As ionization and solvation are largely determined by the electronegativity of the nitrogen atom and steric effects, it cannot be expected that ΔR_M values for NH_2, NH or ternary nitrogen will be constant, especially in systems in which the bases are partly ionized. Effects of electronegativity have been observed even for very weak bases such as ethers[5] (*cf.* also ref. 4, p. 410). In earlier work[6], it was demonstrated that the hydration and solvation of the nitrogen atom in related solutes (quinoline bases), were largely dependent on the electronegativity expressed by the pK_A value. Depending on the cohesion of the polar phase, the volume of the solute molecule plays a more or less important role (*e.g.*, ΔR_M (ring-attached CH_2) was *ca.* —0.2 units in aqueous systems and was negligible when the polar phase was 4% formic acid in formamide; the difference of the R_M values of acridine and isoquinoline was —1.2 units in an aqueous system and only —0.2 units in the formamide system).

* For Part I see Ref. 14.

It was also found that the introduction of a methyl group in the 8 position caused a strong increase of R_F in both systems in view of the steric shielding of the nitrogen atom. Steric hindrance was also demonstrated in adsorption of nitrogen bases[7,8], the electronegativity of the nitrogen being an important factor; for related solutes, linear R_M vs. pK_A correlations were also found[9] in adsorption chromatography and this was explained theoretically by assuming that in this case, adsorption by the silica is an acid–base equilibrium in a non-aqueous medium[9,10].

Since attempts, in the case of partition of nitrogen bases, to reduce constitutional effects to the basicity of the nitrogen atom, its steric situation, to the volume of the molecule and the presence of hydrophilic groups gave promising results for liquid–liquid partition systems, further investigations were carried out with the object of accumulating sufficient experimental data for simple organic bases. In the present work we shall discuss chromatographic parameters for aniline and its methyl- and N-methyl derivatives for systems of the type weakly polar solvent–formamide (pure or containing 5 v/v % formic acid to decrease very high R_F values).

EXPERIMENTAL

Whatman No. 4 paper strips, 7 × 23.5 cm, were impregnated with formamide (or 5% formic acid in formamide) by passing them through a 1:4 solution of the non-volatile liquid in acetone, blotting them between two sheets of filter paper and allowing the diluting solvent to evaporate. After 2 min, the impregnation was repeated once again; the impregnated paper strips contained 0.6 g of stationary phase per 1 g of dry paper. The solutes were spotted as 2% solutions in benzene; the chromatograms were developed at 20 ± 1° at a distance of 16 cm in 5 × 7 × 22 cm glass tanks for descending development. The following developing solvents were used: heptane, cyclohexane, benzene, trichloroethylene and chloroform. The spots were detected by coupling with bis-diazotized benzidine (obtained by mixing equal volumes of 10% $NaNO_2$ with a solution of 0.5 g of benzidine and 1.5 ml conc. HCl in 100 ml water).

The pK_A values of the bases refer to their aqueous solutions[1,2], except for N,N-dimethyltoluidines (Fig. 9) for which the pK_A values for 50 % aqueous ethanol were available[11]; pK_A values of three xylidines (2.4, 2.5 and 3.5) were estimated by addition of 0.2 units to the pK_A values determined for 50 % ethanolic solutions (the estimated values are approximate and are marked in the plots by horizontal dashes; the difference in the pK_A values for water and 50 % ethanol amounts to 0.15 for 2,3-xylidine, 0.30 for 2-toluidine and 0.35 for aniline).

RESULTS AND DISCUSSION

The experimental results are presented as R_M vs. pK_A correlations. The solutes are denoted with mnemonic symbols to facilitate the analysis of the plots: A = aniline; T = toluidine; X = xylidine (thus, 4T denotes 4-methylaniline; 26X = 2,6-dimethylaniline; NNDMA = N,N-dimethylaniline etc.). The R_M value is defined in accordance with BATE-SMITH AND WESTALL's proposition, i.e., $R_M = \log (1 - R_F)/R_F$; however, the R_M axis is directed downwards to secure parallel decrease of R_M and R_F values. In parallel on the right-hand side ordinates, R_F values are marked

subordinate to the proportional R_M scales. Diagrams of this type permit direct plotting of R_F values on R_M vs. property relationships.

In Fig. 1 the results for the system cyclohexane–formamide are presented. The analysis of the plot shows that the effect of the pK_A in this system is negligible; on the other hand, the molecular volume and steric hindrance to solvation are decisive effects. The R_F values of aniline and its methyl derivatives follow the sequence below which is in accord with increasing molecular volume: aniline < toluidines < xylidines. The ortho-effect (2T, 23X, 24X, 25X) causes an increase of R_F (decrease of R_M by ca. 0.15 to 0.25 units). In cases where the ortho-effect is absent (A → 3T, 4T → 34X) the ΔR_M(ring-attached CH_2) is ca. —0.25 units and is thus comparable to $\Delta R_M(CH_2)$ for the system ethyl oleate–25% ethanol (see ref. 4, p. 399) where $\Delta R_M(\alpha\text{-}CH_2) = 0.29$.
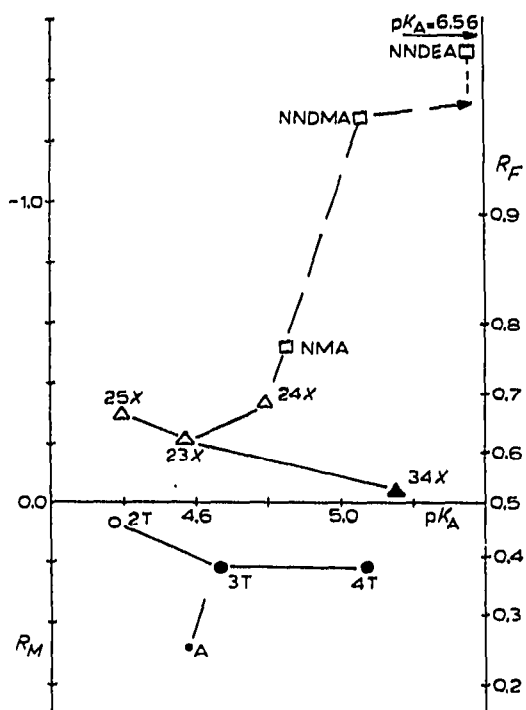

Fig. 1. R_M values of aniline and its derivatives plotted against their pK_A values determined for aqueous solutions. Filled points refer to solutes in which steric shielding of the nitrogen atom is absent. The point of N,N-diethylaniline is beyond the plot ($pK_A = 6.56$) in the direction indicated by the broken arrow. System: cyclohexane–formamide.

A much stronger effect is caused by the introduction of methylene groups into the NH_2 group; thus, ΔR_M(NMA — A) is —1.0 R_M unit and ΔR_M(NNDMA — NMA) is —0.8 R_M units (in the latter case the accuracy is much lower in view of the high R_F value of N,N-dimethylaniline). Substitution of methylene groups by ethylene groups leads to a further increase of R_F; however, the ΔR_M(NNDEA — NNDMA) is difficult to evaluate in view of the high R_F values of the two N,N-dialkylanilines. The strong effects due to substitution of the hydrogen atoms in NH_2 groups are caused by steric effects, i.e., limited accessibility of the nitrogen atom leading to decreased probability of solvation (cf. ret. 9 for analogous effects in adsorption).

Similar conclusions follow from the analysis of Fig. 2 where R_M values obtained

for the system heptane–formamide are plotted. Only slight modifications in the relative positions of the points are observed.

The addition of 5% of formic acid to formamide changes the partition mechanism; the R_M vs. pK_A correlations in Fig. 3 seem to indicate that partition is mainly determined by steric effects and the basicity of the nitrogen atom; on the other hand, the molecular volume effect is insignificant. Thus, the points of aniline, the two toluidines (3T, 4T) and one of the xylidines (34X) in which there are no methyl groups in the 2 position (filled points) lie approximately on a common correlation line of negative slope. On the other hand, the hollow points of *ortho*-toluidine (2T) and the remaining xylidines (23X, 24X and 25X) have much higher R_F values (ΔR_M = ca. —0.6 units, due mostly to the *ortho*-effect). Also in this case the three N-alkylanilines have much higher R_F coefficients.
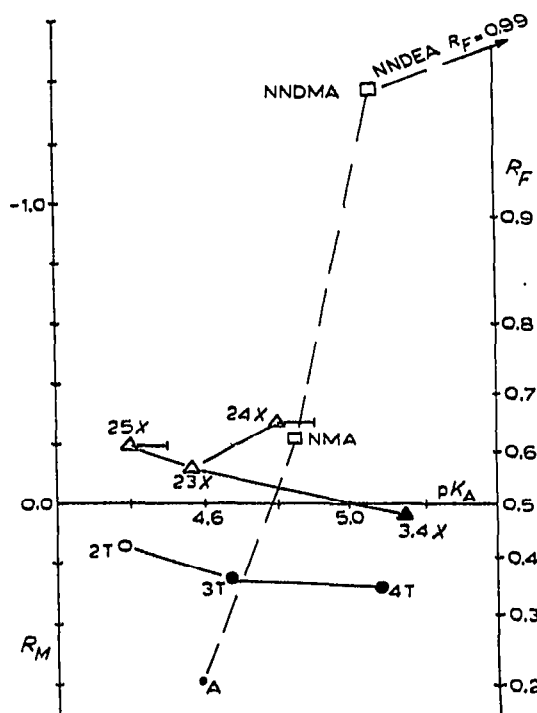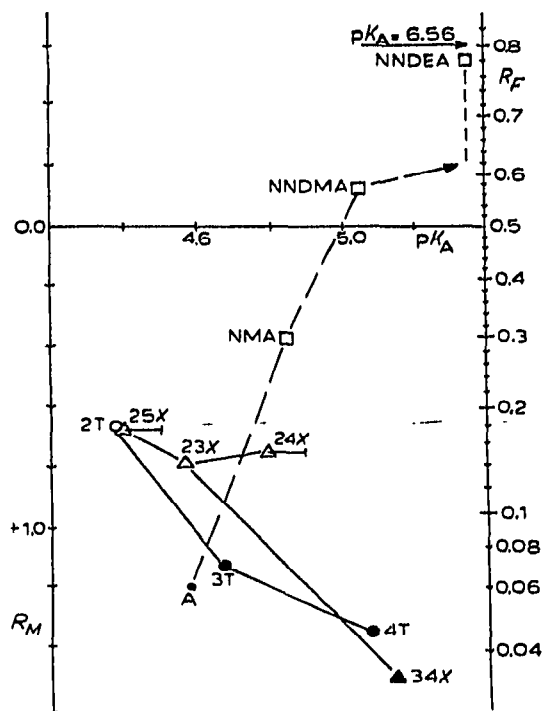

Fig. 2. System: heptane–formamide.

Fig. 3. System: cyclohexane–5% formic acid in formamide.

The results obtained for the system cyclohexane–5% formic acid in formamide indicate that in view of the strong interaction of the anilines with formic acid (presumably due to ionization or partial charge transfer, $-NH_3^+$ $^-OOC-$), the effect of steric shielding of the nitrogen atom by the *ortho*-methyl group is much stronger here than in pure formamide. The weaker molecular volume effects seem to indicate that the presence of 5% formic acid causes a partial disorganization in the structure of the polar phase, so that the formation of "holes" for solute molecules is facilitated.

The point of 2,4-xylidine does not follow the correlation line, which may be due to an inaccurate pK_A value for this compound. Some irregularities in the range of high R_M values ($R_F < 0.08$) may be caused by the low accuracy of R_M values

below $R_F = 0.1$ (compare the right hand side ordinate in Fig. 3, where a single division corresponds to 0.02 R_F units). The results obtained for the system heptane–5% formic acid in formamide (Fig. 4) are somewhat more readable; it can be seen that ortho-toluidine (2T) deviates from the line 3T–4T upwards due to the contribution of the ortho-effect. In addition the line connecting the point of 3,4-xylidine with the remaining xylidines (24X, 23X and 25X) rises more steeply.

For solvents possessing higher solvent power, i.e., benzene, trichloroethylene and chloroform, some individual differences in the relative positions of the points are observed. Thus, in the case of benzene most of the points for the aniline derivatives lie approximately on a common correlation line which seems to indicate a negligible contribution due to molecular volume effects (Fig. 5). The point for N,N-diethylaniline, in this system, lies lower than the point for N,N-dimethylaniline, the slope of the NNDMA–NNDEA line being ca. 0.4, while the "pure" effect of basicity, calculated for the 3T–4T line gives rise to a higher slope (ca. 0.6), which seems to indicate some contribution from the molecular volume effect due to the substitution of two N-methyl groups by two N-ethyl groups; however, the R_M of N,N-dimethylaniline is beyond the range of accurate R_M values so that this conclusion is not certain.

For the system trichloroethylene–5% formic acid in formamide (Fig. 6) the contribution of the molecular volume effect is more pronounced: the point for aniline lies below the correlation line of the three toluidines and the latter—below the points of the xylidines. The slope of the 3T–4T line is in this case higher than in the former system (ca. 0.8).

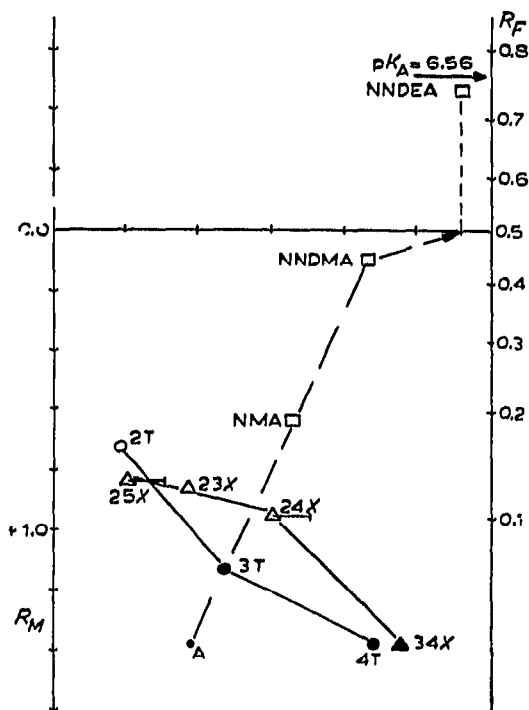The correlation lines for the system chloroform–5% formic acid in formamide

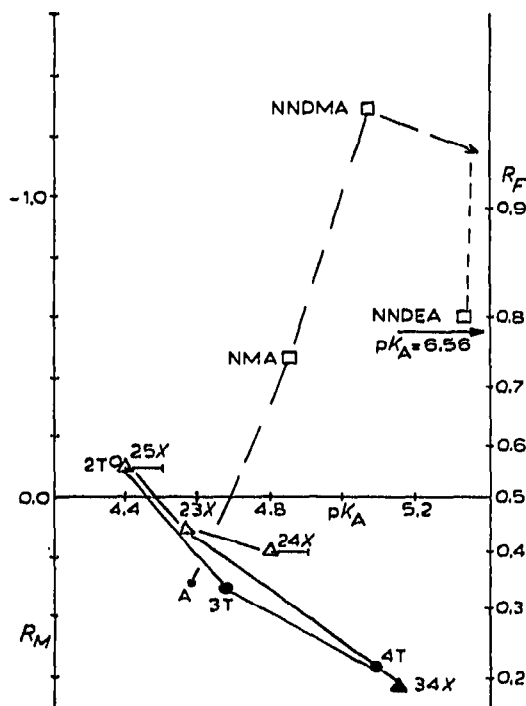Fig. 4. System: heptane–5% formic acid in formamide.

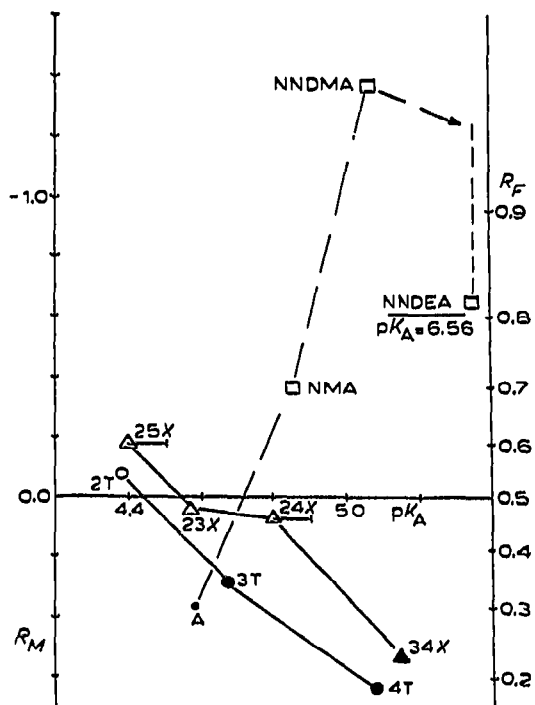Fig. 5. System: benzene–5% formic acid in formamide.

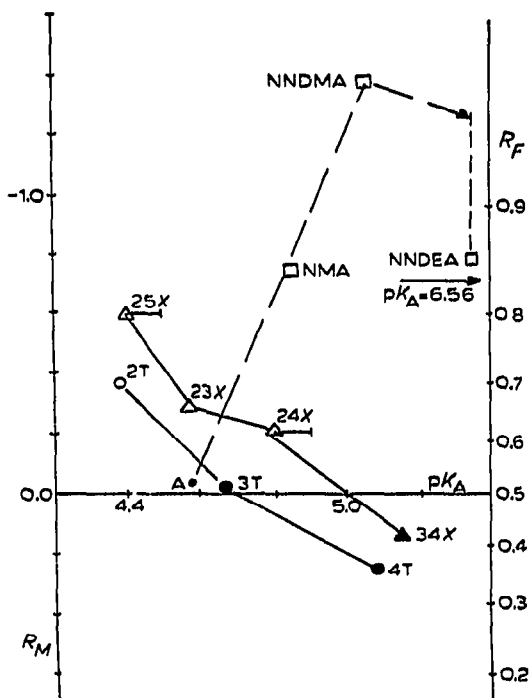Fig. 6. System: trichloroethylene–5% formic acid in formamide.

Fig. 7. System: chloroform–5% formic acid in formamide.

are similar to those in the preceding system; however, they are higher in view of the high solvent power of chloroform in pursuance of its pronounced proton-donor properties.

It is worthwhile discussing analogous results for the system cyclohexane–water and cyclohexane–citrate-phosphate buffer, pH 3.0, obtained by GOLUMBIC AND GOLDBACH[12, 13] in static experiments.

R_M vs. pK_A correlations for the system cyclohexane–water are presented in Fig. 8; R_M is defined here as the negative logarithm of the partition coefficient of the unionized base ($k° = c_{org}/c_w$). The plot resembles Fig. 1, however, the $\Delta R_M(\alpha\text{-}CH_2)$ values are higher and amount to —0.60 units, in accordance with results obtained in the first part of this investigation[14] for longer alkyl chains. The correlation lines of the toluidines (2T, 3T, 4T) and xylidines (24X, 25X, 35X) are almost horizontal which seems to indicate that hydration is largely insensitive to moderate variations of basicity of the amino group[12]. The ortho-effect is insignificant ·for 2-toluidine and furthermore the points of 2,4-xylidine and 2,5-xylidine are almost in line with 3,5-xylidine; however, it is more pronounced for 2,6-xylidine where the amino group is shielded from two sides. The substitution of a hydrogen atom in the amino group by a methyl group decreases the R_M value by ca. 1.2 units. Thus, generally speaking, with respect to volume effects, the aqueous system is much more selective than the analogous formamide system in view of the stronger cohesion of water.

For the system with buffered aqueous phase (pH < pK_A — 1) the following equation was derived[12]:
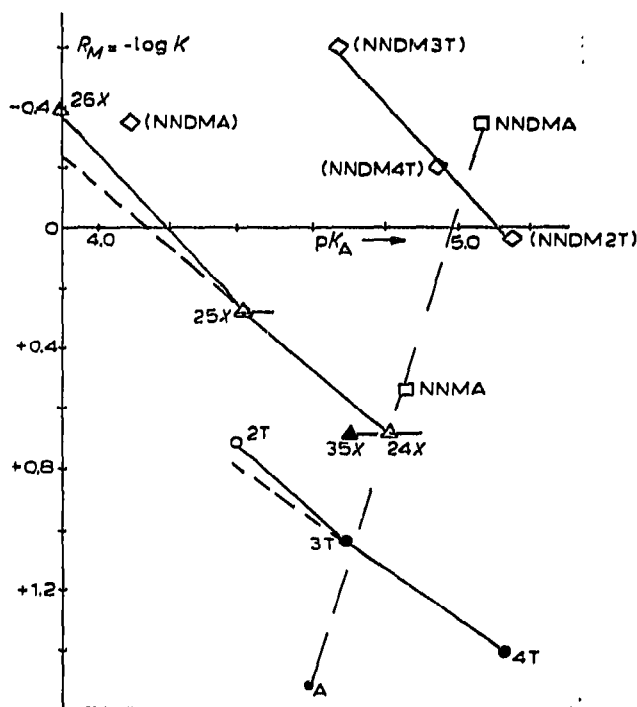
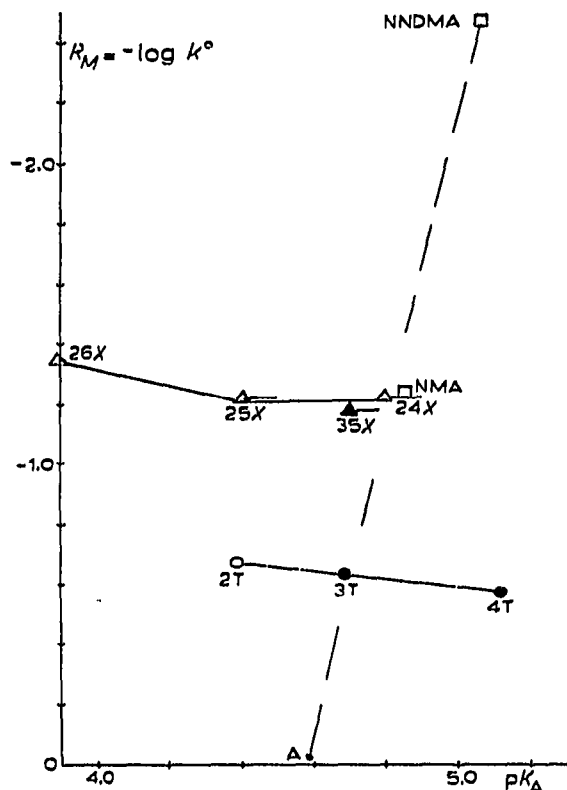$$- R_M = \log K = \log k° - pK_A + pH.$$

Fig. 8. Logarithms of static partition coefficients $k°$ determined for the system cyclohexane–water[12] plotted against the pK_A values of the bases (compare with Fig. 1).

Fig. 9. Logarithms of static extraction coefficients K determined for the system cyclohexane–buffer solution of pH 3.0, plotted against the pK_A values of the bases (compare with Fig. 3). Diamonds refer to pK_A values determined for 50% aqueous ethanol.

Thus, for solutes possessing similar $k°$ values (isomers) linear R_M vs. pK_A correlations of unit slope can be expected. The data for the system cyclohexane–pH 3.0 confirm this assumption; strong volume and steric effects are also observed in this system, especially for N-alkylanilines, the *ortho*-effect being weak, although clearly manifested: thus, the 2T point deviates above the 3T–4T line; the point 35X (no *ortho*-effect) lies below the 24X–25X line and 26X (double *ortho*-effect) deviates upwards. The ΔR_M(*ortho*-effect) in this system can be estimated at *ca.* —0.1 R_M units. The results presented in Fig. 9 are more accurate than those in Fig. 8 since $k°$ values are high in most cases.

In Fig. 9 the points of N,N-dimethylaniline and three isomeric N,N-dimethyl-toluidines are also plotted against their pK_A values determined for 50% ethanol-water solutions (hollow diamonds; symbols in parentheses). In this case, the points of the three isomers also follow a straight correlation line lying above the lower homologue (NNDMA). The pK_A values of these compounds for solutions in pure water would be much higher, *i.e.*, the points would be shifted to the right (as seen from Fig. 9, by *ca.* 1.0 pK_A unit in the case of N,N-dimethylaniline).

The relationships illustrated in Figs. 1–9 show that in the systems investigated it is possible to apply the concept of additivity of R_M values, taking into account the basicity of the nitrogen atom and steric effects. At the worst, at least a semi-quanti-

tative interpretation of chromatographic data is possible. The correlations also explain certain problems of selectivity; thus, the separation factor of the pair aniline–4-toluidine is often low when the polar phase contains formic acid (or for buffered aqueous phase): in these cases the effect of increased molecular volume is counter-balanced by the increased pK_A value of the methyl derivative[6]. Better selectivity is obtained with pure formamide or water. The opposite is true for the pair 3-toluidine–4-toluidine etc.

REFERENCES

1 H. C. Brown, D. H. McDaniel and O. Häfliger in E. A. Braude and F. C. Nachod (Editors), *Determination of Organic Structures by Physical Methods*, Academic Press, New York, 1955.
2 A. Albert and E. Serjeant, *Ionization Constants*, Methuen, London, 1962.
3 A. Albert in A. Katritzky (Editor), *Physical Methods in Heterocyclic Chemistry*, Vol. 1, Academic Press, New York, 1963, Ch. 1.
4 I. E. Bush, *Methods Biochem. Anal.*, 13 (1965) 357.
5 S. Marcinkiewicz, J. Green and D. McHale, *J. Chromatog.*, 10 (1963) 42.
6 E. Soczewiński and W. Maciejewicz, *Separation Sci.*, 2 (1967) 779.
7 L. R. Snyder, *Principles of Adsorption Chromatography*, M. Dekker, New York, 1968.
8 H. J. Petrowitz, G. Pastuska and S. Wagner, *Chemiker Ztg.*, 89 (1965) 7.
9 E. Soczewiński and W. Gołkiewicz, *Chem. Anal.*, 14 (1969) in press.
10 E. Soczewiński, *Anal. Chem.*, 41 (1969) 179.
11 H. C. Brown, D. H. McDaniel and O. Häfliger, in E. A. Braude and F. C. Nachod (Editors), *Determination of Organic Structures by Physical Methods*, Academic Press, New York, 1955, p. 596.
12 C. Golumbic and G. Goldbach, *J. Am. Chem. Soc.*, 73 (1951) 3966.
13 E. Hecker, *Verteilungsverfahren in Laboratorium*, Verlag Chemie, Weinheim, 1955.
14 E. Soczewiński and M. Bieganowska, *J. Chromatog.*, 40 (1969) 431.