

TLC Separation of Nitrophenones on Polar Adsorbents

A. Gupta* and S.A. Nabi**

**Department of Applied Sciences,
College of Science & Engineering, Jhansi (India)*

***Analytical Chemistry Division,
Department of Chemistry, Aligarh Muslim University, Aligarh (India)*

ABSTRACT

A group of nitrophenones, including isomers and closely related compounds, and their reduced derivatives, the corresponding alcohols, have been chromatographed on different polar adsorbents, i.e. silica, alumina and Florisil with mixtures of ethyl acetate or 2-propanol and *n*-heptane as mobile phases. The retention behaviour of these substances and their interactions with different adsorbent sites are discussed, The parameters of linear regression for the dependence of R_M on $\log c$ were analysed and ΔR_{Mf} values for the pairs nitrophenone and corresponding alcohol were estimated. This led to recognition of the effect of reduction on the separation of the investigated compounds and on the selectivity of the separation of both groups of substances on various polar adsorbents; this enabled analysis of similarities of and differences between the chemical character and distribution of the surface-active centres.

KeyWords: TLC, Nitrophenones, Polar Adsorbents.

INTRODUCTION

The aim of derivatization in liquid chromatography is to change the chromatographic properties of solutes. For example, chromatographic compounds having strongly polar substituents such as NH_2 , $=\text{NH}$, COOH or OH groups are strongly adsorbed on active centres. In this case derivatization may reduce solute polarity, with the result that the adsorption energies of the derivatives are much lower than those of the original compounds, and separation is

improved. Pre-column derivatization should be used when it is necessary to change chromatographic properties. Then there are no limitations on retention conditions or reaction rate and excess derivatizing agent does not disturb in chromatographic process. The production of several derivatives or non-quantitative reaction can, however, sometimes lead to problems. Examples of such derivatization are the reaction of amines, amino acids and alcohols with dansyl chloride, of steroids with benzoyl chloride [1, 2], and the formation of chelate complexes or ion pairs.

When chromatographed substances do not absorb UV light, e.g. aliphatic amines, amino acids and alcohols, derivatization is performed to produce substances absorbing UV light or having fluorescence. In such circumstances derivatization should be post-column [3]. With this technique great reaction velocity is required, eluent solvents can affect the reaction, and there is a possibility of simultaneous detection before and after reaction. Post-column derivatization can also lead to problems when the derivatizing agent is UV-absorbing. In TLC post-chromatographic derivatization enables visualization of chromatographic spots.

When chromatographed compounds of closely related structure behave similarly in several chromatographic systems derivatization might be performed to change structural effects (polar group interactions) in the molecule. This might lead to different interactions of the derivatized compounds with surface-active centres of adsorbents, and thus to improved separation.

The aim of this work was to investigate the effect of reduction of nitrophenones on their retention behaviour and on the separation of the chromatographed isomers and other closely related compounds on various polar adsorbent surfaces. Phenones and their reduced derivatives were also interesting groups of model solutes for comparing the properties of polar adsorbents by analysis of their chromatographic behaviour and by the correlation of the retention parameters R_{MI} and R_{MII} in different chromatographic systems.

EXPERIMENTAL

Derivatization

Derivatization was performed by adding solid NaBH_2 to methanolic solutions of the nitrophenones or by applying a methanolic solution of the derivatizing agent directly to the spots of the nitrophenones on the adsorbent layer. Both procedures enable reduction of the nitrophenones to the corresponding alcohol. Compound 9 (see Table I) investigated to monitor the direction of derivatization, was not reduced under these conditions.

Chromatography

TLC was performed on precoated 10×20 cm plates with 0.25 mm layers of silica gel or alumina (E. Merck, Germany) and on 10×10 cm glass plates which were coated in-house with 0.25 mm layers of Florisil for TLC dried in air for approx. 30 min, then at 80°C for 1 h and finally activated at 120°C

for approx. 2 h. Plates were stored in a desiccator over CaCl_2 . The activity of the plates, although not determined, was thus considered comparable.

Binary mixtures of ethyl acetate (AcOEt) or 2-propanol (iPrOH) with *n*-heptane were used as mobile phases. The solvents were analytical grade. Elution was performed in horizontal PTFE DS chambers [4, 5]. To avoid solvent demixing [6] plates were conditioned for approx. 5 min in mobile phase vapours before development.

Spots were visualized under UV illumination (254 nm).

RESULTS AND DISCUSSION

Nitrophenones and their reduced derivatives, the corresponding alcohols (Table I) were chromatographed in normal phase systems using Florisil, silica or alumina as stationary phases and *n*-heptane containing one of the polar modifiers ethyl acetate or 2-propanol as mobile phases.

Ethyl acetate (not hexane or heptane) was chosen as a weak diluent for mobile phase composition because it has more eluting power than hexane or heptane which is based on polarity.

Results are presented as relationships between R_M and $\log c$ (c = concentration of modifier) in Table I. In all cases plots were linear with high regression coefficients ($r > 0.989$). The results obtained confirm the validity of the molecular model of adsorption [7, 8]. The absolute values of the slopes (m) in Table I for the investigated compounds with ethyl acetate as modifier are clearly different - for the nitrophenones $m \approx 1$ whereas for the corresponding alcohols $m \approx 2$. This is indicative of one-point attachment of the nitrophenone molecules to the active centres, and two-point attachment of the alcohols. It seems that changing the C=O group to the HC-OH group results in stronger interactions with adsorbent surface. The values of intercept (a) also indicate stronger retention of alcohols in this chromatographic system (Table I).

Table II lists values of $\Delta R_{Mr} = R_{MRn} - R_{Mn}$ (where the subscripts 'r', 'Rn' and 'n' denote 'reaction', 'reduced nitrophenone' and 'nitrophenone', respectively) [9] for corresponding pairs of nitrophenones and their reduced derivatives. Although reduction leads to similar values for ΔR_{Mr} for most of the nitrophenones, some irregularities are apparent, i.e. a given type of reaction does not always lead to a constant change in R_M . ΔR_{Mr} values depend on the positions of other substituents in the molecule and thus on the possibility of interactions (e.g. hydrogen-bonding interactions) between adjoining groups. For example, differences in R_M values are relatively small (i.e. ΔR_{Mr} values are low) for the pairs 3 and 3R and 4 and 4R because of hydrogen-bonding interactions of the HC-OH group with the *ortho* substituted nitro group in the reduced derivatives (see structures in Table I and values in Table II).

Analysis of the ΔR_{Mr} values presented in Table II also makes it possible to compare the selectivity of separation on different adsorbents. For the two eluent systems investigated values for alumina as adsorbent were in all cases

higher than those for silica. It is also seen that differences in retention parameters are high on Florisil with ethyl acetate - *n*-heptane (2:8) as eluent. Analysis of the retention behaviour of both groups of compounds (nitrophenones and their reduced derivatives) can be performed by constructing R_{MI} - R_{MII} correlation diagrams. It is apparent from these correlation diagrams that the nitrophenones (1-8) have similar retention parameters. They are eluted within a narrow range in most of the systems investigated - in a range of 0.45 R_M units on alumina and a range of 0.30 R_M units on silica with ethyl acetate - *n*-heptane as mobile phase, and in a range of 0.30 R_M units on both silica and alumina with 2-propanol - *n*-heptane as mobile phase; only on Florisil with ethyl acetate - *n*-heptane as mobile phase are the phenones eluted in a range as large as 0.80 R_M units. The reduced derivatives (1R-8R) are eluted in a range of approx. 0.80 R_M units on alumina, approx. 0.70 R_M units on silica and 0.90 R_M units on Florisil with ethyl acetate - *n*-heptane as mobile phase and in a range of 0.50 R_M units on alumina with 2-propanol as mobile phase.

Analysis of correlation diagrams also makes it possible to compare the surface properties of polar adsorbents. Fig. 1 presents plots of correlations between R_{MI} and R_{MII} for ethyl acetate - *n*-heptane (2:8) as mobile phase. Nitrophenones accumulate near one correlation line:

$$R_{M \text{ alumina}} = -0.186 + 1.02R_{M \text{ silica}}; r = 0.944; n = 9 \quad (1)$$

and their reduced derivatives near another:

$$R_{M \text{ alumina}} = -0.03 + 1.28R_{M \text{ silica}}; r = 0.938; n = 8 \quad (2)$$

Because the value of the slope in eq. (1) is approximately unity it seems that the nitrophenones are adsorbed similarly on both adsorbent surfaces, although there are individual differences in selectivity. Thus, for example, the pairs of isomers 2 and 3 or 4 and 3 are better separated on alumina, but pair 2 and 5 is better separated on silica.

Because the value of the slope in eq.(2) is greater than 1 it seems that the reduced derivatives are better separated on alumina; this is in accordance with the differences between the retention parameters of the isomers 2R, 3R, 4R and 5R.

The behaviour of the solutes is similar when 10% 2-propanol in *n*-heptane is used as mobile phase (Fig. 2). The nitrophenones cluster near the line:

$$R_{M \text{ alumina}} = -0.165 + 1.07R_{M \text{ silica}}; r = 0.942; n = 9 \quad (3)$$

The slope of the line (≈ 1) indicates that the nitrophenones are adsorbed similarly on these adsorbents. There are, however, differences in the retention behaviour of the reduced derivatives, which cluster near the line:

$$R_{M \text{ alumina}} = -0.134 + 1.37R_{M \text{ silica}}; r = 0.862; n = 8 \quad (4)$$

It is clear that the compounds in this group are better separated on alumina (slope > 1). For example, isomers 2R, 3R and 4R are eluted together on silica but are better separated on alumina. The low regression coefficient $r = 0.862$ implies that there are differences between the interactions of the alcohols with the various active centres on the surfaces of both adsorbents [10, 11].

Fig. 3 illustrates correlations between $R_{M\text{Florisil}}$ and $R_{M\text{silica}}$ for ethyl acetate - *n*-heptane (2:8) as mobile phase. The compounds again cluster near two lines. For the nitrophenones the line is:

$$R_{M\text{Florisil}} = -1.27 + 2.03R_{M\text{silica}}; r = 0.993; n = 9 \quad (5)$$

and for the reduced derivatives it is:

$$R_{M\text{Florisil}} = -0.14 + 1.06R_{M\text{silica}}; r = 0.952; n = 8 \quad (6)$$

The large slope of eq.(5) (≈ 2) is indicative of much better separation of the nitrophenones on the Florisil surface. This might be as a result of interactions of electron-donor C=O groups with acidic centres (Mg^{2+} ions) on the Florisil surface [12]. The reduced derivatives interact similarly with the active sites on both silica and Florisil - the slope of the line in eq. (6) is approximately 1. There are, however individual differences in separation selectivity.

Fig. 4 illustrates a comparison of the retention behaviour of the solutes on Florisil and alumina. The nitrophenones and their derivatives cluster around one line only:

$$R_{M\text{Florisil}} = -0.586 + 1.22R_{M\text{alumina}}; r = 0.970; n = 17 \quad (6)$$

The high regression coefficient, $r = 0.970$ confirms the similar interactions of the solutes with the active centres of Florisil and alumina. The value of the slope (> 1) seems to imply, however, that separation selectivity is better on Florisil. This similar retention behaviour is because the adsorbents' active centres are similar - Mg^{2+} and Al^{3+} ions [12].

CONCLUSIONS

1. Linear relationships between R_M and $\log c$ are obtained for nitrophenones and their reduced derivatives on Florisil with ethyl acetate - *n*-heptane as mobile phase (high regression coefficients of the slopes; $r > 0.989$).
2. The slopes of the plots of R_M against $\log c$ are approximately unity for nitrophenones and approximately 2 for their reduced derivatives. This implies that the nitrophenones and the alcohols interact by one- and two-point attachment, respectively, with the active centres on the Florisil surface. The reduced derivatives are strongly retained by polar adsorbent surfaces.

3. The reduced derivatives are better separated than nitrophenones by most of chromatographic systems investigated.
4. $R_{MI} - R_{MII}$ correlations are indicative of differences between the chromatographic behaviour of the solutes and, therefore, differences between the properties of the active centres on the surfaces of the polar adsorbents.

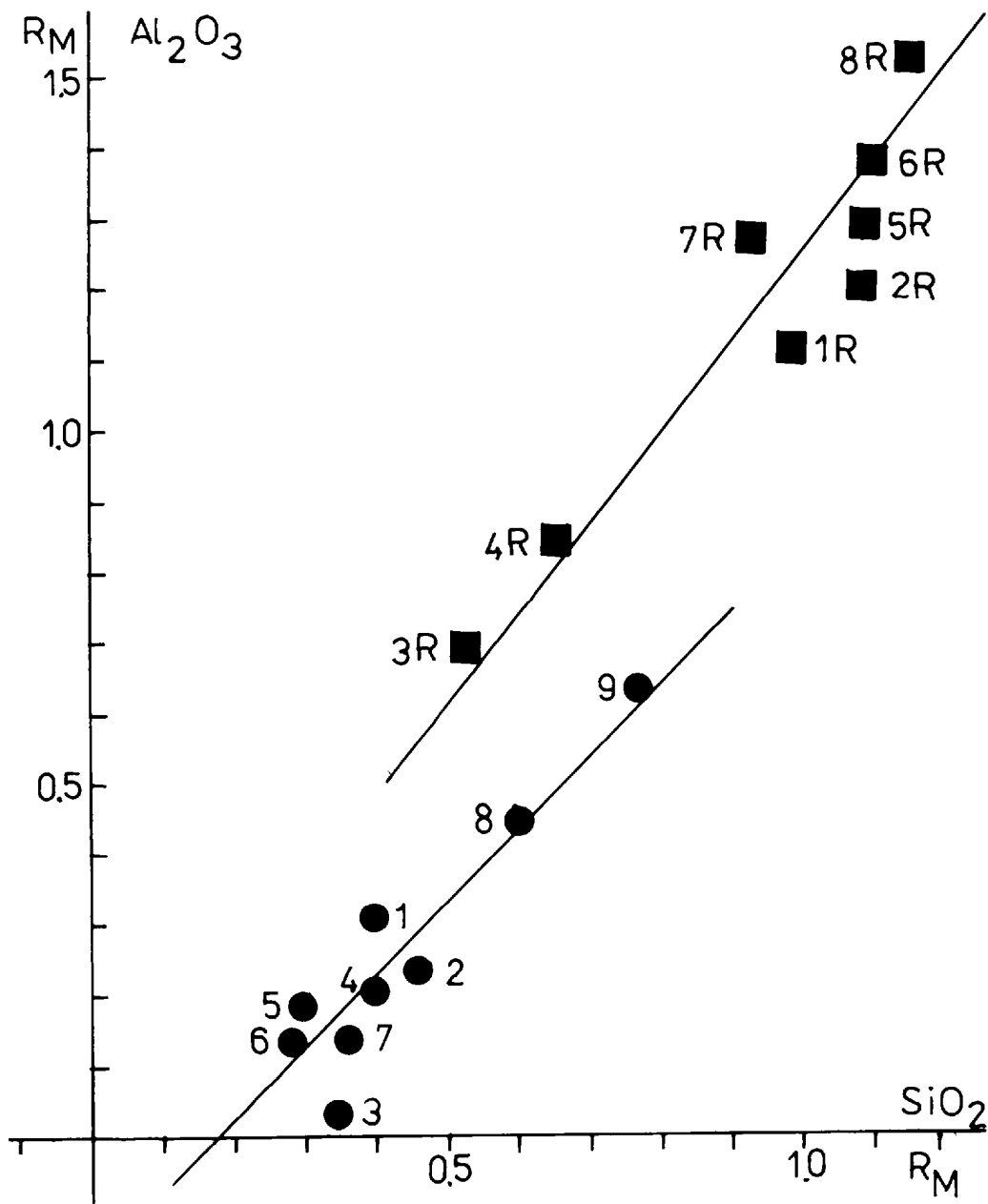


Fig. 1 Correlation of R_M values obtained on alumina and silica with ethyl acetate - *n*-heptane (2:8) as mobile phase. Solute identification as in Table I.

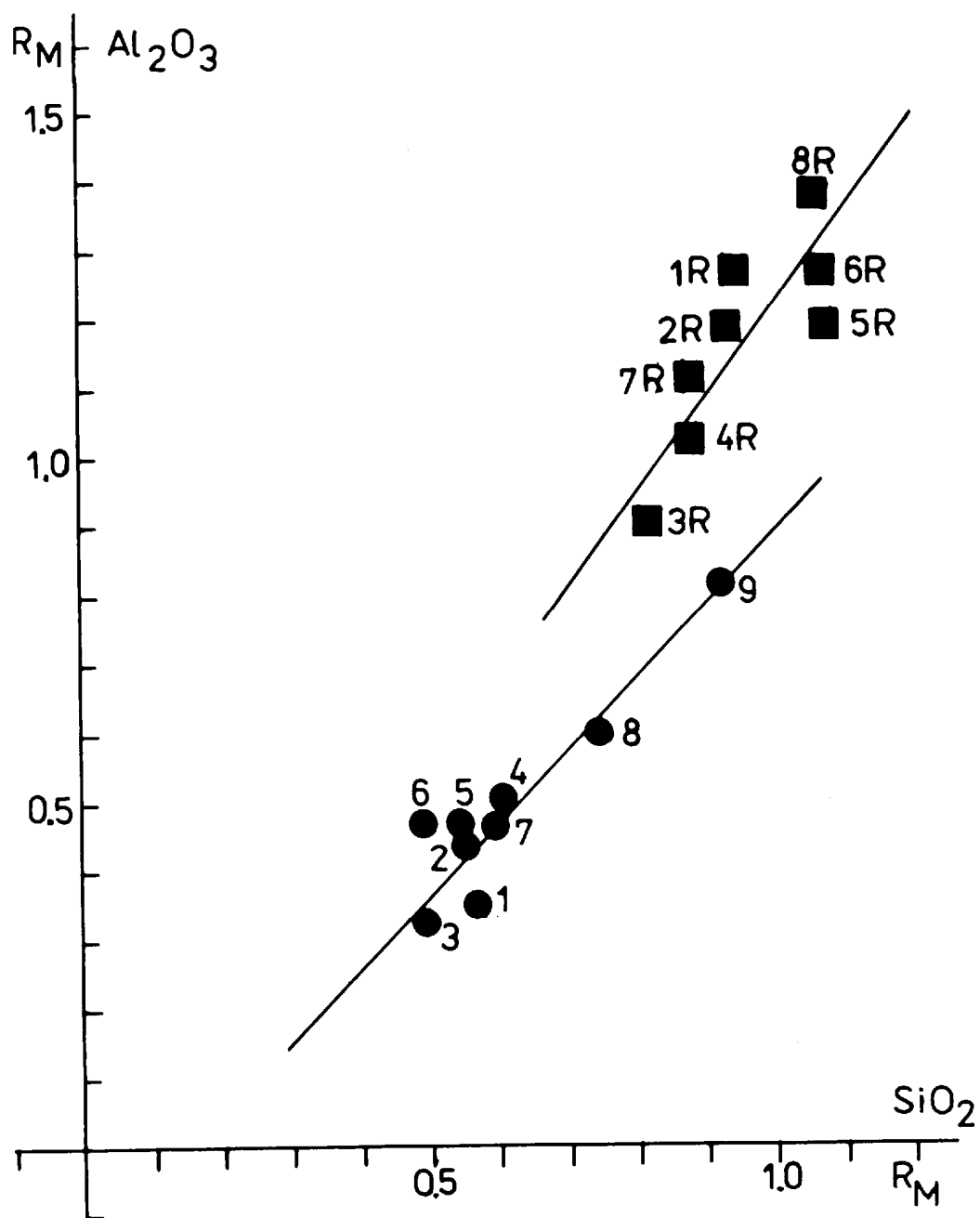


Fig. 2 Correlation of R_M values obtained on alumina and silica with 2-propanol - *n*-heptane (1:9) as mobile phase. Solute identification as in Table I

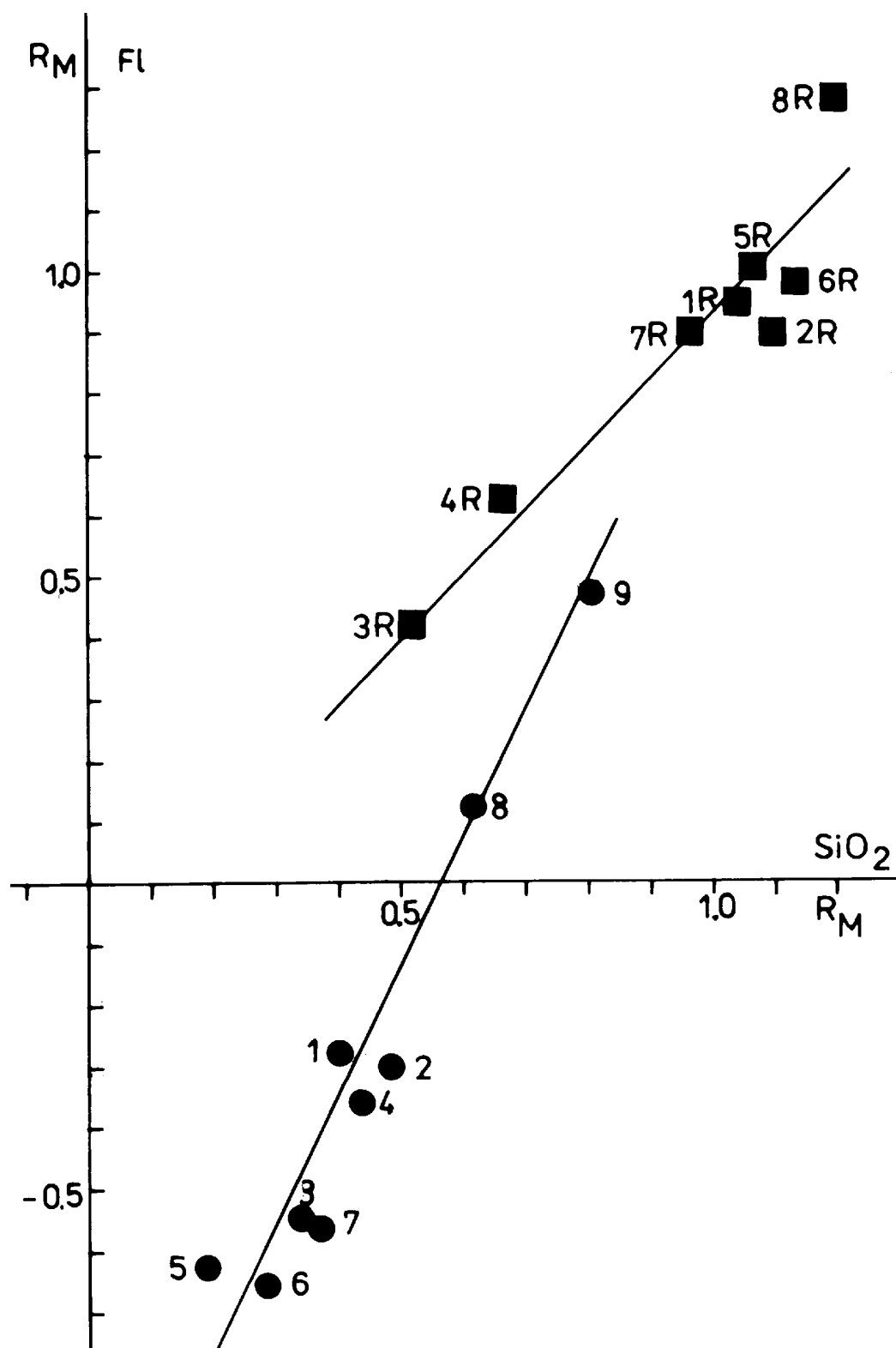


Fig. 3 Correlation of R_M values obtained on Florisil and silica with ethyl acetate - *n*-heptane (2:8) as mobile phase. Solute identification as in Table I

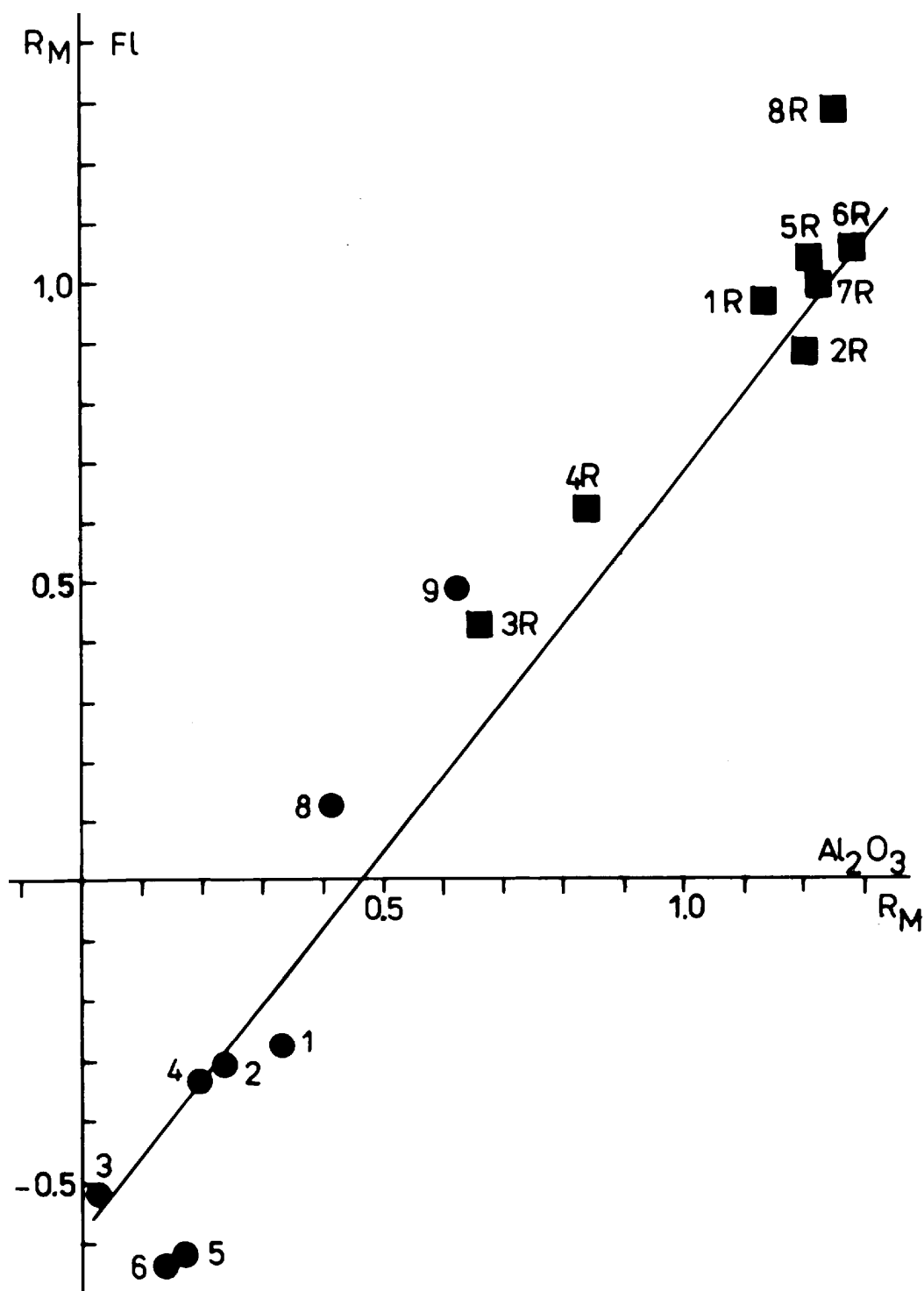


Fig. 4 Correlation of R_M values obtained on Florisil and alumina with ethyl acetate - *n*-heptane (2:8) as mobile phase. Solute identification as in Table I

Table I Parameters of the equation $R_M = a - m \log c$ for the compounds on Florisil with ethyl acetate - *n*-heptane as mobile phase

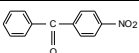
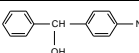
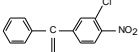
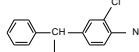
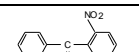
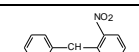
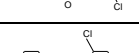
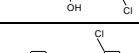
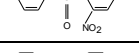
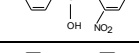
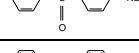
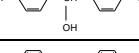
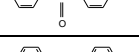
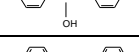
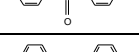
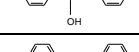
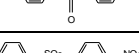
No.	Structure	<i>a</i>	<i>m</i>	<i>r</i>	<i>n</i>	No.	Structure	<i>a</i>	<i>m</i>	<i>r</i>	<i>n</i>
1		0.75	0.95	0.997	5	1R		3.02	2.12	0.997	4
2		0.60	0.88	0.996	5	2R		2.87	2.02	0.995	4
3		0.24	0.88	0.994	5	3R		2.36	1.99	0.997	4
4		0.51	0.87	0.999	5	4R		2.5	1.94	0.998	4
5		0.35	0.94	0.996	5	5R		3.24	2.21	0.995	4
6		0.22	0.88	0.996	4	6R		3.20	2.23	0.989	4
7		0.46	0.89	0.999	4	7R		2.99	2.22	0.996	3
8		1.06	0.95	0.996	5	8R		3.45	2.25	0.989	4
9		1.52	1.09	0.999	4	-	-	-	-	-	-

Table II ΔR_{Mr} ($= R_{MRn} - R_{Mn}$) for pairs of derivatives and the respective nitrophenones for the chromatographic systems investigated

ΔR_{Mr}	Ethyl acetate - heptane (2:8)			Isopropanol - heptane (1: 19)	
	SiO ₂	Al ₂ O ₃	Florisil	SiO ₂	Al ₂ O ₃
$R_{MR1} - R_{M1}$	0.61	0.83	1.12	0.40	0.93
$R_{MR2} - R_{M2}$	0.63	0.96	1.17	0.54	0.78
$R_{MR3} - R_{M3}$	0.17	0.63	0.95	0.31	0.53
$R_{MR4} - R_{M4}$	0.25	0.58	0.92	0.26	0.52
$R_{MR5} - R_{M5}$	0.77	1.12	1.66	0.43	0.76
$R_{MR6} - R_{M6}$	0.85	1.26	1.69	0.58	0.85
$R_{MR7} - R_{M7}$	0.64	1.19	1.18	0.26	0.69
$R_{MR8} - R_{M8}$	0.59	1.09	1.09	0.37	0.81

REFERENCES

- [1] Lawrence J.F and Frei R.W(1976).Chemical Derivatization in Liquid Chromatography' Elsevier, Amsterdam.
- [2] Lammens J and Verzele M(1978). Chromatographia, 11, 376-380.
- [3] Snyder L.R(1976). J. Chromatogr., 125, 287-292.
- [4] Ito Y(2005). J. Chromatogr. A., 1065, 145-150.

- [5] Dzido T.H(1990). *J. Planar Chromatogr.*, 3, 199-205.
- [6] Waksmundzka-Hajnos M and Wawrzynowicz T(1994). *J. Planar Chromatogr.*, 7, 58-64.
- [7] Snyder L.R(1974). *Anal. Chem.*, 46, 1384-1390.
- [8] Soczewiński E(1969). *Anal. Chem.*, 41, 179-184.
- [9] Bush L.E and Glick D. *Methods of Biochemical Analysis*, Wiley-Interscience, New York, 13, 357-360.
- [10] Snyder L.R(1968). *Principles of Adsorption Chromatography*, Marcel Dekker, New York.
- [11] Oęcik J (1982). *Adsorption*, Polish Scientific Publishers, Warszawa, and Ellis Horwood, Chichester.
- [12] Waksmundzka-Hajnos M and Wrońska B, *Chromatographia*, in press.

