R_F VALUES AND STRUCTURE OF THE ADSORBENT IN THIN-LAYER CHROMATOGRAPHY USING PURE SOLVENTS AS THE MOBILE PHASE

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SUMMARY

The object of this paper was to investigate experimentally the reproducibility of R_F values in thin-layer chromatography, and to establish the effect of the adsorbent, the molecular structure of the solute and the solvent on this reproducibility. In order to solve the problem in question, silica gel adsorbents of various micro-porous structure were used in order to show to what extent the specific surface area of the adsorbents affected the R_F values. For this purpose five typical solvents were chosen which belonged to different classes according to the classification introduced by EWELL *et al.* and PIMENTEL AND MCCLELLAN. However, for the purpose of investigating the effect of the kind of molecular structure on the R_F value, substances of different molecular size, composed of one, two, or three aromatic rings, were used. The results obtained are presented as "Chromatographic spectra" and their courses are analysed. It has been concluded that the variations in R_F values, including ΔR_F, depend on the structure of the same type of adsorbent used, but only for some solvents. The specificity of interactions between the solute, solvent and adsorbent is responsible for the changes observed.

In a preceding paper[1] it was shown that the rate of flow of the developing solvent depends, for a given particle size and packing of the adsorbent, on its surface area and structure. This phenomenon would be expected to change the R_F value (the retardation coefficient, defined as the ratio of the rate of migration of the solute to that of the mobile phase). The R_F value also depends on the interaction of the solute with the adsorbent and with the solvent. These effects will influence the reproducibility of R_F values and the ultimate conclusions concerning the separation efficiency and the molecular structure of the solutes to be separated.

On the other hand, it is possible to characterize the properties of the adsorbents using as a criterion the changes in R_F values due to the differences in their properties.

The main purpose of the present work was to investigate experimentally the reproducibility of R_F values in TLC and especially to show the effect of the adsorbent, the molecular structure of the solute, and solvent on reproducibility.

EXPERIMENTAL

Adsorbents of various micro-porous structure were used to investigate the

TABLE I

CHARACTERISTICS OF THE ADSORBENTS

Specific surface areas *(s)* measured using the DUBININ AND TIMOFEEV[7] apparatus by the BET method[8] (adsorption of water vapours); average radii of the capillaries *(r)* calculated from Kelvin's equation; particle size of adsorbents is that used to produce the thin layers; proportions of water are those used to produce a sorbent paste of the required consistence[10].

Symbol of gel	s (m²/g)	r (Å)	Particle size (diameter in mm)	Amount of water (ml/25 g)
A	565	10	0.1	45
B	337	10	0.1	39
C	161	30	0.08	45
D	122	8	0.1	59
E	58	7	0.1	59
G	—	—	0.08	50

effect of the surface area on R_F values. In order to take into account the effect of the solvent, five typical solvents were chosen belonging to different classes in the classification introduced by EWELL *et al.*[2] and PIMENTEL AND MCCLELLAN[3]; the solutes also differed in molecular structure so that their contribution to the chromatographic phenomena could be estimated.

The characteristics of the adsorbents used are given in Table I.

The following solvents were used as the mobile phase:

(1) Water (class AB*)[2,3]
(2) Methanol (class AB)
(3) Acetone (class B)
(4) Chloroform (class A)
(5) Carbon tetrachloride (class N)

In the case of pure developing solvents demixion does not occur, which simplifies the interpretation of the experimental data. Furthermore, model solvents can be chosen of known structure, properties, tendency to association etc. In our case, we have first of all taken into account their ability to form hydrogen bonds.

TABLE II

SOLUTES USED IN THE EXPERIMENT

No.	Solute	Concentration (mole/l)	Classification[2,3]
1	o-Nitrophenol	0.14	AB
2	m-Nitrophenol	0.14	AB
3	p-Nitrophenol	0.14	AB
4	Quinoline	0.10	B
5	Isoquinoline	0.10	B
6	3-Methyl-isoquinoline	0.10	B
7	Fluorenone	0.08	B
8	Carbazole	0.04	AB
9	Acridine orange	0.08	B
10	Acridine	0.08	B

To investigate the effect of the molecular structure of the solute, substances of various molecular size were chosen, composed of one, two or three aromatic rings. A list of the substances is given in Table II; all have flat structures[4].

The method of preparation of the adsorbents and the experimental technique was similar to that in our preceding paper[1], i.e., the adsorbents were spread as 0.3 mm thick layers and dried for 2 h at 135°. The solutes were spotted with calibrated 5 μl micropipettes. The chromatograms were developed by the ascending technique for a distance of 16 cm in glass tanks. The spots were detected by spraying with color reagents or were observed under U.V. light.

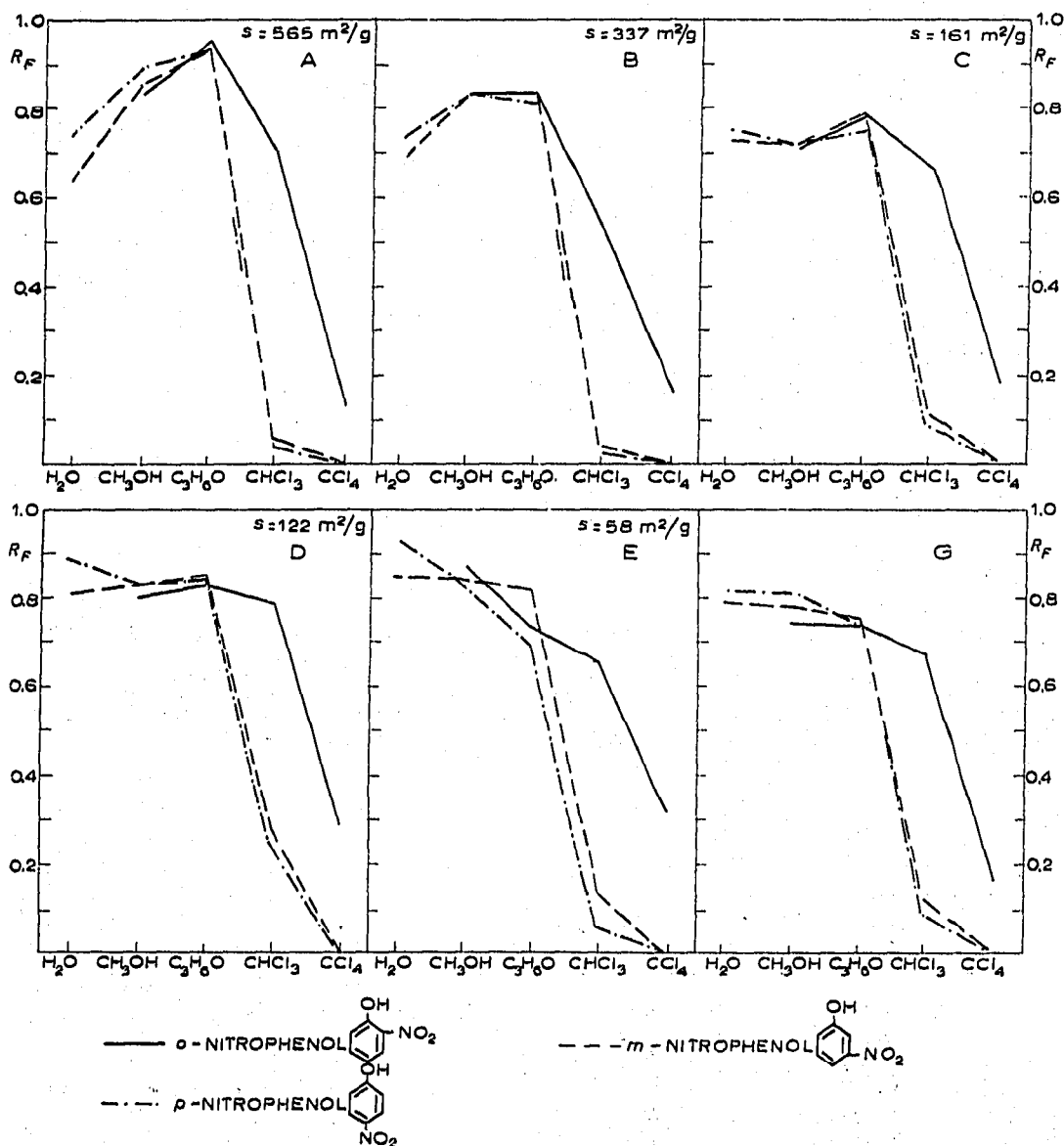The R_F values were determined using a special device[5]. The experiments were

Fig. 1. "Chromatographic spectra" of nitrophenols using various solvents arranged according to the classification of EWELL et al.[2] and PIMENTEL AND MCCLELLAN[3]. At the top of each plot (also in Figs. 2 and 3) the specific surface area (s) of the adsorbent is given, determined by the BET method from the adsorption of water vapours.

carried out in a thermostatted room at 25°. The experimental results are presented as chromatographic spectra[6], the R_F values being plotted against the five solvents arranged on the abscissa according to the classification proposed by EWELL et al.[2] and PIMENTEL AND McCLELLAN[3].

RESULTS AND DISCUSSION

The chromatographic spectra for adsorbents of various micro-porous structures are given in Figs. 1–3.

In Fig. 1 the spectra of o-, m-, and p-nitrophenols are presented. The spectra of nitrophenols are similar for most silica gels. The R_F values of o-nitrophenol are identical with those of its two isomers only for the first two solvents, for the remaining ones
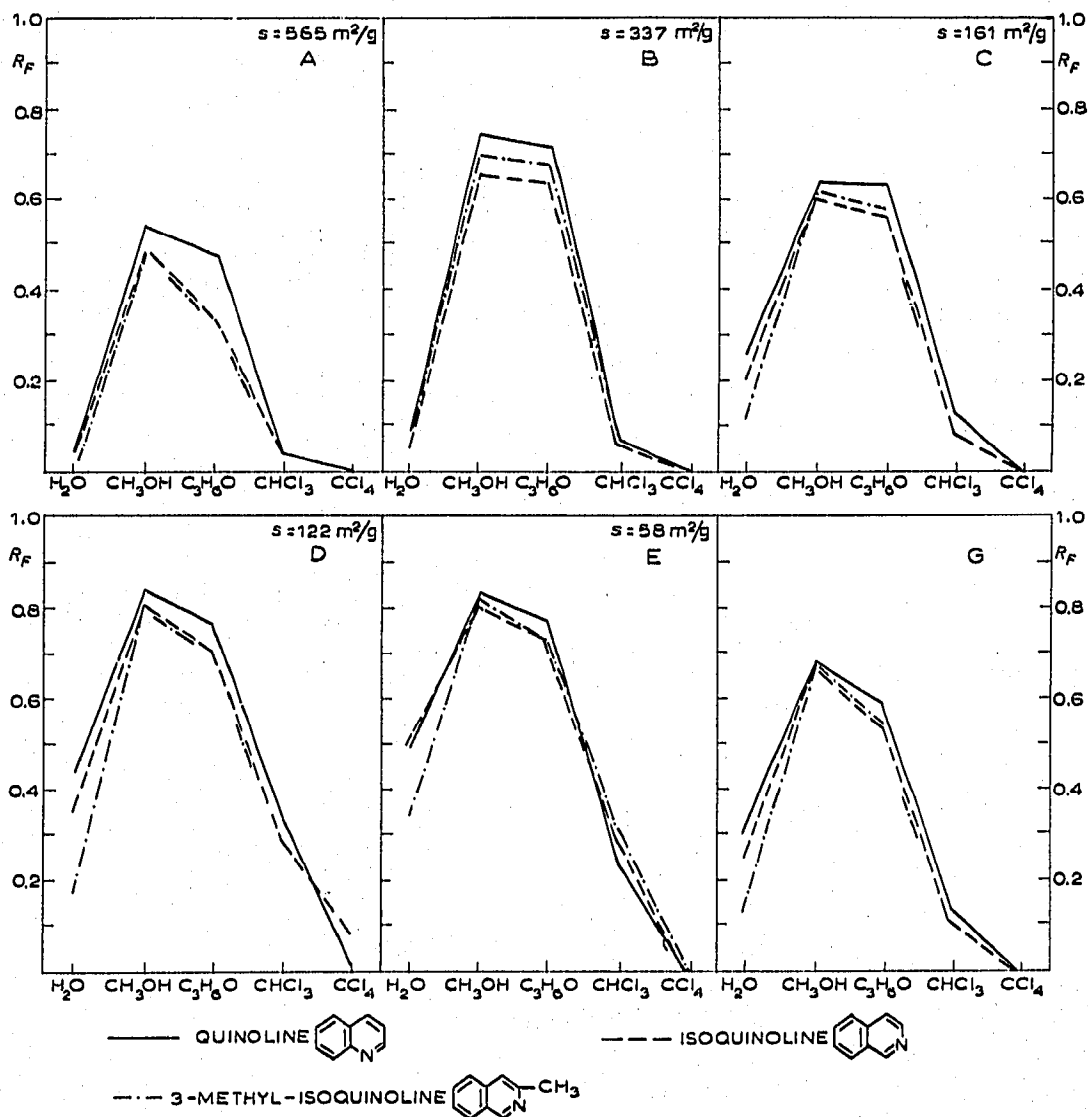

Fig. 2. "Chromatographic spectra" of quinoline, isoquinoline and 3-methyl-isoquinoline using various solvents arranged according to the classification of EWELL et al.[2] and PIMENTEL AND McCLELLAN[3].

they are markedly different. This individual behaviour of *o*-nitrophenol is probably due to the ability of phenols (class AB) to form strong H-bonds with solvents of the same class (water, methanol); *o*-nitrophenol can form an intramolecular H-bond, especially in less polar solvents. Comparison of the chromatographic spectra shows that in the case of water and carbon tetrachloride the R_F values increase with decreasing surface area of the adsorbent; for the remaining solvents this effect is not observed.

The selectivity of the systems, defined as ΔR_F, was greatest for Silica Gel E possessing the lowest surface area; on the other hand, no separation was achieved using Silica Gel G, which was chosen as the reference adsorbent.

In Fig. 2 the spectra of quinoline, isoquinoline and 3-methyl-isoquinoline are
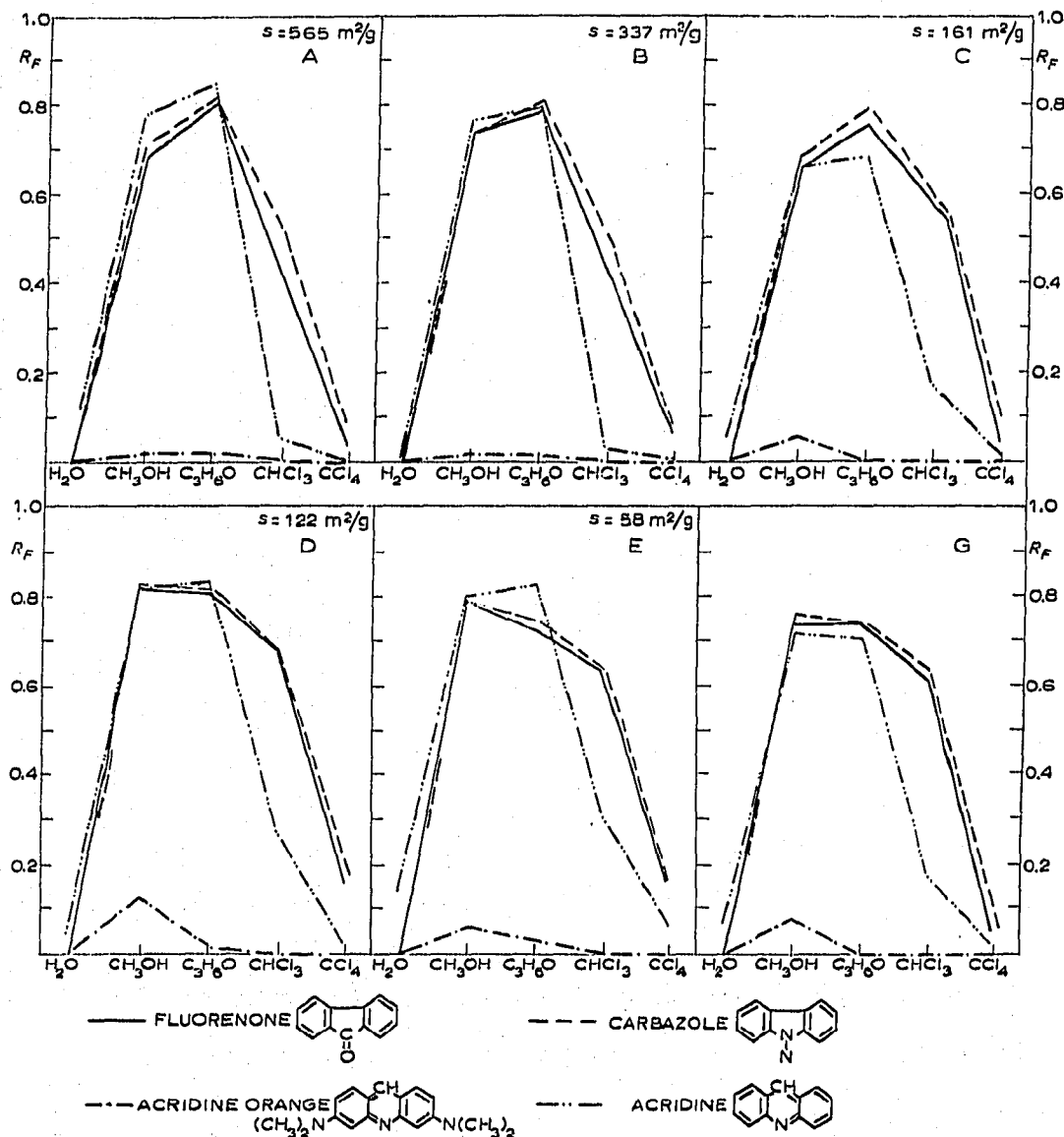

Fig. 3. "Chromatographic spectra" of fluorenone, carbazole, acridine orange and acridine using various solvents arranged according to the classification of EWELL *et al.*[2] and PIMENTEL AND McCLELLAN[3].

presented. As in the case of nitrophenols, when water is used as the developing solvent, the R_F values increase with decreasing surface area of the adsorbent. The solutes belong to class B and thus form weaker H-bonds with water than solutes of class AB.

The spectra of quinoline differ from those of the remaining solutes; this difference cannot be explained by any differences in dipole moments of the substances since the dipole moments of quinoline and isoquinoline are similar. On the other hand, the small differences between the spectra of isoquinoline and 3-methyl-isoquinoline shows that the introduction of the methyl group does not have any significant effect on the adsorbability.

The best separation of quinoline from the isoquinolines is observed with Silica Gel A while with Silica Gel G the separation is poor; it can thus be seen that, in this case, the separation efficiency strongly depends on the structure of the adsorbent.

In the last series of experiments the chromatographic behaviour of fluorenone (B), carbazole (AB), acridine (B) and acridine orange (B) was investigated (Fig. 3). It can be seen that the spectra of carbazole and fluorenone are similar in all cases.

On the other hand, the spectra of acridine and acridine orange are quite different, due to the effect of the dimethylamino groups.

In this case, also, the R_F values increase with decreasing surface area of the adsorbent; however, the effect is only observed for some solvents.

The best separation selectivity is observed with gel A which has the highest specific surface area, in conjunction with chloroform as developing solvent.

To sum up the experimental data, the R_F values, and thus ΔR_F as well, depend on the structure of the adsorbent and this effect is more pronounced for certain solvents, e.g., water and carbon tetrachloride. For other solvents the effect was negligible. The observed variations can be explained by specific interactions between the solute, solvent and adsorbent.

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