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Note

Masking effects in "charge-transfer" thin-layer chromatography

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The effects of masking of silica gel by impregnation with nucleic acid bases during charge-transfer thin-layer chromatography (TLC) has been previously noted[1]. In this note we report some more results of masking effects occurring during charge-transfer TLC of amino acids, nucleic acid bases and organic acceptors with pyrene.

EXPERIMENTAL

The experimental details were similar to those described before[1] except that a thickness of 4 mm silica gel was employed. These plates proved slightly easier to make than 2-mm layers, giving a consistently higher quality as judged by their appearance in both reflected and transmitted light and by the adherence of the silica gel to the glass plates when handled. In addition, our plates were air dried at ambient temperature.

Measurements were made for twelve separate analyses on different plates in order to guarantee that differences in B values of *ca.* 2 were significant. The B value is a measure of the interaction between pyrene and the impregnant as defined by Harvey and Halonen[2]

$$B = \frac{R_F - R'_F}{R_F} \times 100$$

where R_F is the value for pyrene on an unimpregnated plate and R'_F is the value with impregnant.

RESULTS AND DISCUSSION

The results in Table I are for amino acids, nucleic acid bases and the two organic acceptors, tetracyanoethylene (TCNE) and tetrachloroquinone (chloranil).

The behaviours of the three amino acids, tryptophan, glycine and proline (actually an imino acid), are very similar. At low impregnant concentration, the B values are negative and become increasingly negative on increasing the impregnant concentration to *ca.* 0.5% (w/w). At still higher impregnant concentrations the B values increase and finally become positive. It should be emphasized that in no case have we been able to observe movement of the impregnant with the solvent.

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TABLE I

B VALUES FOR CHARGE-TRANSFER TLC OF PYRENE

Impregnant	*% (w/w)*	*B*	*Impregnant*	*% (w/w)*	*B*
Tryptophan	0.025	−1	Adenine	0.025	−9
	0.1	0		0.1	3
	0.5	−7		0.5	3
	2.0	−1		2.0	−8
	5.0	7		5.0	−7
Proline	0.025	−1	ATP	0.025	−3
	0.1	−3		0.1	9
	0.5	−9		2.0	7
	2.0	−7		5.0	1
	5.0	5			
Glycine	0.025	−4	Uric acid	0.025	1
	0.5	−2		0.1	−5
	2.0	4		0.5	−8
				2.0	0
				5.0	1
Isoleucine	0.025	−9	TCNE	0.025	−1
	0.5	−1		0.1	−3
	2.0	−7		0.5	−5
	5.0	−5		5.0	−1
Valine	0.025	−5	Chloranil	0.025	−3
	0.1	0		0.1	−6
	0.5	3		0.5	−5
	2.0	7		5.0	−4
	5.0	−3			

Solvent: chloroform–heptane (1:1); except for chloranil and TCNE, chloroform–hexane (1:1).

In order to explain this effect we need to consider three different interactions, those between the impregnant and pyrene, between silica gel and pyrene and between silica gel and the impregnant. At low impregnant concentrations there is presumably complexing of the active silica gel hydroxyl sites with the appropriate sites of the amino acids, via hydrogen bonding, thus leading to masking of these active sites and hence a weaker interaction between pyrene and silica gel which is not compensated for by the pyrene–impregnant interaction. This therefore leads to negative *B* values, *i.e.*, an apparent increase in the R_F values. Increasing the impregnant concentration leads to increased masking of the active sites and causes a progressive lowering of the *B* values. However, at higher concentrations of impregnant the masking effect is outweighed by the pyrene–impregnant interaction, resulting in a gradual increase in the *B* values.

The two amino acids with large aliphatic side chains, isoleucine and valine, display a somewhat different behaviour. They behave like glycine at the lower impregnant concentrations, showing a decrease and then an increase in *B* values as the impregnant concentration increases. However, at the highest impregnant concentrations they again show a decrease in *B* values. There is no movement of the impregnant in these experiments and we presume that the long aliphatic side chains give rise to

steric hindrance so that at the higher impregnant concentrations the pyrene, impregnant and silica gel interactions are being interfered with.

These results are different from these for the nucleic acid bases which show an increase in *B* value with increasing concentration of impregnant, but then a decrease with above a certain impregnant concentration. This can be explained on the assumption that, at high base concentrations, association and aggregation of these bases occur, effectively lowering the extent of interaction between the bases and pyrene. Many bases are known to undergo such self-association at high concentrations[3]. The behaviour of uric acid is similar to that of amino acids and we assume that at the concentrations used no appreciable self-association occurs.

In Table I are also shown *B* values for pyrene with the two well known electron acceptors TCNE and chloranil as the impregnants. The results are similar to those for the amino acids and again show the effects of masking.

It is clear therefore that the optimum concentration of impregnant for the maximum *B* value in charge-transfer TLC is very system dependent. It is possible to obtain information on the physical nature of the various interactions by use of this technique.

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