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USE OF REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY TO ASSES HYDROPHOBICITY IN STUDIES OF QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS FOR DRUGS*

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SUMMARY

The behaviour of compounds with different polarity on untreated thin layers of silica gel or cellulose, and on layers impregnated with liquid paraffin or 1-octanol, was studied. It was observed that compounds of hydrophilic character such as sulphonic acids and sulphonamides behaved as if the layers did not contain the organic stationary phase, whereas lipophilic compounds followed the reversed-phase partition mechanism. Thus, two pairs of stationary and mobile phases are considered to be present: the partition system organic stationary liquid–mobile phase and the system thin-layer material–mobile phase. These facts should be taken into consideration in studies of quantitative structure–activity relationships to assess hydrophobicity by using R_M values on layers impregnated with less polar liquids instead of partition coefficient determinations.

INTRODUCTION

Recent studies on quantitative structure–activity relationships (QSAR) have shown the importance of the hydrophobic or lipophilic nature of drugs. The hydrophobicity of a drug is usually characterized by the partition coefficient (distribution coefficient) obtained from studies of the distribution of a drug between an immiscible polar and a non-polar solvent. The relationships between the partition coefficient and the chromatographic parameters (R_F or R_M values) enabled the laborious determination of partition coefficients by the simple measurement of R_M values. As hydrophobicity is defined as the tendency of a species to be readily soluble in non-polar solvents (*e.g.*, paraffin oil, silicone oil, 1-octanol) and only sparingly soluble in water (or aqueous buffer solutions, aqueous methanol, aqueous acetone, etc.), chromatographic data obtained in reversed-phase partition chromatographic systems are of value in such QSAR studies. The importance of making the R_M determinations in

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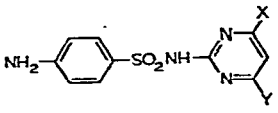
systems where partition either is the sole process taking place or predominates over the others has been emphasized by several workers. An excellent review of this problem has been published[1].

The partition reversed-phase systems introduced into paper chromatography in 1950[2,3] were obtained by impregnating the chromatographic paper with non-polar stationary phases. Partition was shown to be the main separation mechanism in such systems[4]. Later the same technique was introduced into thin-layer chromatography (TLC). Little attention, however, has been devoted to the possible interaction of the chromatographed compounds with the support of the stationary liquid phase, and impregnation of silica gel layers with silicone oil, liquid paraffin or 1-octanol has been widely used in QSAR studies of the type referred to above.



I X=H; Y=H
II X=CH_3; Y=H
III X=CH_3; Y=CH_3

A very good correlation of biological activity of sulphonamides with the data obtained by chromatography on thin layers of silica gel impregnated with 1-octanol was observed by Biagi *et al.*[5]. The R_M values of three homologous sulphonamides, *viz.*, sulphadiazine (I), sulphamerazine (II) and sulphamethazine (III), given by these workers showed that the R_F values of the three compounds decreased in the order III > II > I, although the opposite sequence would be expected in reversed-phase partition systems. Therefore, we investigated the behaviour of these substances on both impregnated and untreated silica gel layers using the same mobile phases and found[6] that the impregnation had no influence on the migration of the spots. This led us to carry out a more thorough study of the chromatographic behaviour of these and similar more polar compounds in order to specify those types of compounds for which the interaction with the support of the non-polar stationary liquid phase is stronger than partition between the non-polar stationary phase and the mobile phases.

EXPERIMENTAL

Chemicals

The compounds chromatographed were standards from our collection or were prepared using procedures described in the literature. Their identity and purity were checked by common methods. All liquids used as stationary phases and components of the mobile phases were of reagent-grade purity.

Thin-layer chromatography

All experiments were carried out on Silufol® (silica gel) and Lucefol® (cellulose) sheets (Kavalier, Votice, Czechoslovakia), both 15 × 15 cm. Impregnation of the layers was effected either by the dipping technique or by capillary ascent; 5% solutions of paraffin oil in *n*-hexane or 1-octanol in ethanol were used for impregnation. The development was carried out in glass tanks. The coloured compounds were observed in visible light, those absorbing UV light using a Camag Universallampe and compounds with primary aromatic amino groups were detected using Ehrlich reagent.

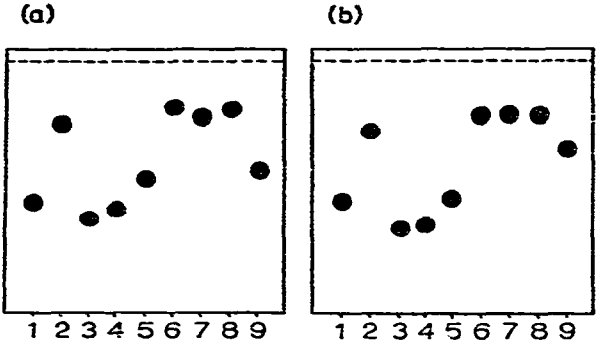


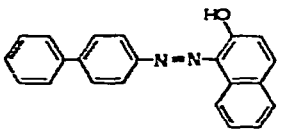
Fig. 1. Chromatograms of sulphonamides on silica gel thin layers. Mobile phase: buffer of pH 7.4. (a) Untreated layer; (b) layer impregnated with 1-octanol. 1 = Sulphamethoxydiazine; 2 = sulphanilamide; 3 = sulphadimidine; 4 = sulphamethoxypyridazine; 5 = sulphathiazole; 6 = sulphacetamide; 7 = sulphaguanidine; 8 = sulphacarbamide; 9 = sulphadiazine.

RESULTS AND DISCUSSION

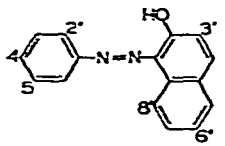
The results of the chromatographic separation of a series of sulphonamides on silica gel, both untreated and impregnated with 1-octanol, are illustrated in Fig. 1. It is evident that the sulphonamides migrated on the impregnated layers as if the latter did not contain the organic stationary phase. The interaction of sulphonamides with silica gel seems to be stronger than their affinity to the non-polar stationary liquid.

The same experiment was carried out with cellulose layers (Fig. 2). Also in this instance the sulphonamides were unaffected by the impregnation. It can be seen from a comparison of Figs. 1a and 2a that the interaction of the chromatographed substances is specific for each layer material. The same results were obtained with buffers of different pH (2.5–0.6), so that the phenomenon cannot be considered to be pH dependent.

Further experiments were carried out with the following series of model dyes:



IV



V —
VI 3′-SO_3H
VII 5-SO_3H
VIII 3′,6′-$(SO_3H)_2$
IX 2,5-$(SO_3H)_2$
X 4,3′,6′-$(SO_3H)_3$
XI 4,3′,8′-$(SO_3H)_3$

Dyes IV and V are lipophilic compounds, IV being more lipophilic because of the presence of an additional phenyl group. In contrast, dyes VI–XI are hydrophilic and their polarity increases with the number of sulpho groups present in their molecules.

The chromatographic mobilities of these dyes on thin layers of silica gel and cellulose are shown in Figs. 3 and 4. 1-Propanol–ammonia (2:1) was used as the

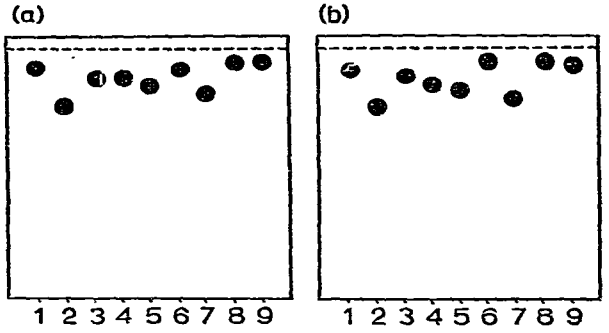


Fig. 2. Chromatograms of sulphonamides on cellulose thin layers. Mobile phase: buffer of pH 7.4. (a) Untreated layer; (b) layer impregnated with 1-octanol. 1–9 as in Fig. 1.

mobile phase. The results on untreated layers are shown in the left-band parts (a) in these figures, and the right-band parts (b) show the results after impregnation with liquid paraffin.

The behaviour of the sulphonated dyes is the same on both untreated and impregnated layers. In contrast, the decrease in the R_F values of dyes IV and V and the

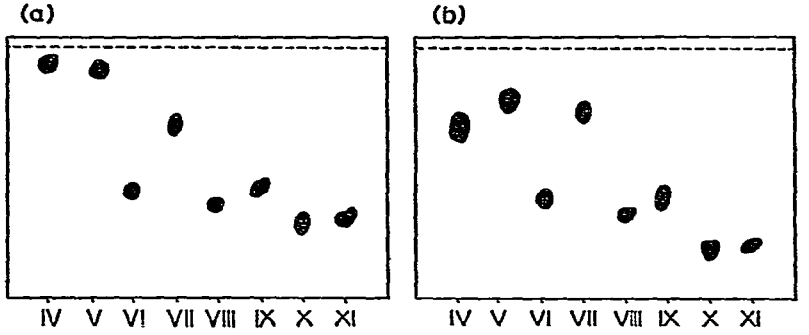


Fig. 3. Chromatograms of azo dyes on silica gel thin layers. Mobile phase: 1-propanol–ammonia (2:1). (a) Untreated layer; (b) layer impregnated with liquid paraffin. IV–XI: see text.

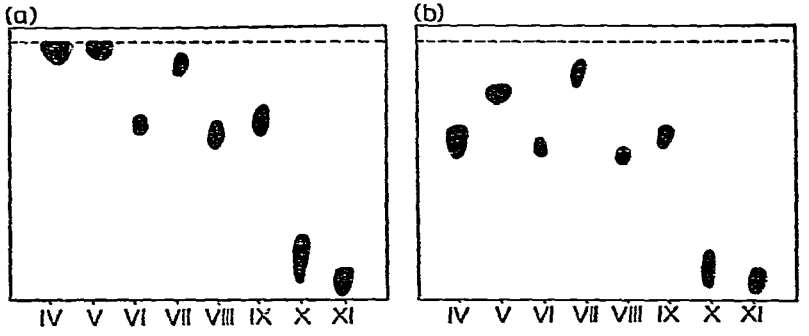


Fig. 4. Chromatograms of azo dyes on cellulose thin layers. Mobile phase: buffer of pH 7.4. (a) Untreated layer; (b) layer impregnated with liquid paraffin. IV–XI: see text.

lower R_F value of the more lipophilic dye IV indicate that these two dyes were separated on the basis of the reversed-phase partition mechanism.

When chromatographed in the system 1-octanol–aqueous buffer (pH 7.4), previously used in QSAR studies on sulphonamides, these dyes migrate in a reversed sequence (Fig. 5b). Dyes containing three sulpho groups have the highest R_F values, whereas the lipophilic compounds IV and V remain at the origin. However, the results obtained on untreated layers of silica gel (Fig. 5a) demonstrate that the reversed sequence cannot be attributed to the non-polar stationary liquid, but rather to the interaction between silica gel and the mobile phase. Such behaviour of sulphonic acids has previously been observed in paper chromatography[7], and was explained by a different separation mechanism involving salting-out.

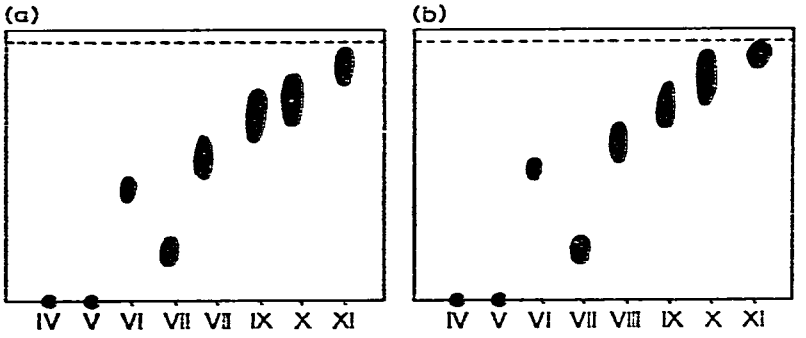
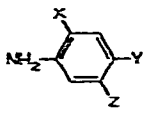


Fig. 5. Chromatograms of azo dyes on silica gel thin layers. Mobile phase: buffer of pH 7.4. (a) Untreated layer; (b) layer impregnated with 1-octanol. IV–XI: see text.

In order to establish which functional groups can cause such problems when using reversed-phase TLC in QSAR studies, a series of model compounds (XII–XX) were chromatographed on both untreated and impregnated silica gel layers. The results obtained are summarized in Fig. 6. The impregnation seems to influence only the behaviour of compounds XIX and XX, which show better formed spots on impregnated layers.



XII X = SO_3H; Y = H; Z = SO_3H
XIII X, Z = H; Y = SO_3H
XIV X, Z = H; Y = SO_2NH_2
XV X, Z = H; Y = $SO_2NHC_4H_9$
XVI X, Z = H; Y = $SO_2N(C_2H_5)_2$
XVII X, Z = H; Y = COOH
XVIII X, Z = H; Y = $CONH_2$
XIX X, Z = H; Y = $COOC_2H_5$
XX X, Z = H; Y = $COOC_5H_{11}$

CONCLUSION

If a thin layer is impregnated with a non-polar organic stationary liquid phase, we cannot simply expect that all compounds chromatographed will undergo partition between the organic stationary phase and the mobile phase. We must take into consideration that after the layer has been impregnated two pairs of stationary and

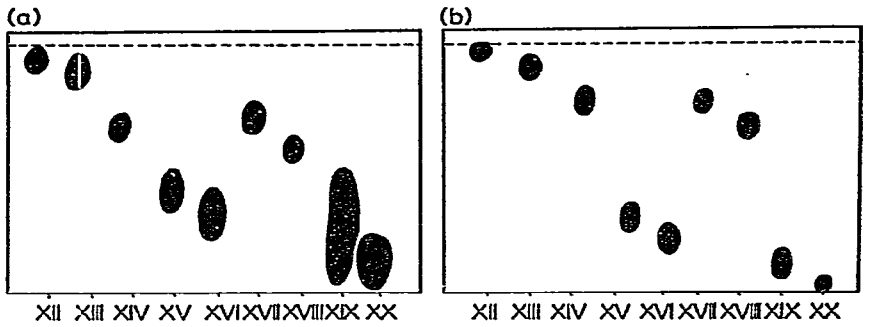


Fig. 6. Chromatograms of model compounds XII–XX (see text) on silica gel thin layers. Mobile phase: buffer of pH 7.4. (a) Untreated layer; (b) layer impregnated with 1-octanol.

mobile phases are present: the partition system organic stationary liquid–mobile phase and the system thin-layer material–mobile phase. The latter can act as an adsorption system in some instances, but can also represent a partition system thin-layer material–water–mobile phase, etc. A similar phenomenon was observed in paper chromatography[8]. Therefore, when thin layers impregnated with a non-polar organic stationary liquid are used to replace partition coefficient determinations, a blank chromatogram with the same mobile phase but on an untreated layer should be run simultaneously to show that the organic stationary liquid has taken part in the separation mechanism.

On the other hand, good correlations between chromatographic mobilities on silica gel layers and biological activity were also obtained in systems without impregnation[9]. This might be due to the fact that the structure–chromatographic behaviour relationships of particular groups of compounds in systems with aqueous mobile phases on untreated silica gel or cellulose happen to be very close to those of a reversed-phase system, or it might also be explained by the fact that phenomena other than partition affect the biological activity of particular compounds.

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