STUDIES IN THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND CHROMATOGRAPHIC BEHAVIOUR*

IX. AN ASSESSMENT OF THE ROLE OF POLYAMIDE SURFACE IN THE THIN-LAYER CHROMATOGRAPHY OF PHENOLS

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INTRODUCTION

Polyamide has been used as a chromatographic substrate by a number of workers[1–17].

It has been suggested that the chromatography of phenolic compounds on polyamide is an adsorption process depending on the reversible formation of hydrogen bonds between the hydrogen atom of the phenolic group and the carbonyl oxygen atom of the amide group[1–4]. Elution from the polyamide is suggested as being a process in which the solute–polyamide bonds are broken by displacement of the solute with an eluent capable of hydrogen bonding with the surface.

When the homologous series of gallic acid esters were chromatographed on polyamide thin layers in the eluent system methanol–acetone–water (60:20:20, v/v) the R_F values were observed to decrease with an increase in the chain length of the esterifying alcohol but they increased with an increase in chain length when the eluent system was light petroleum–benzene–acetic acid–dimethyl formamide (10:10:5:0.25, v/v)[16]. It was proposed[16] that, in the former case, the polyamide was behaving as a non-polar surface. In the latter case, it was behaving as a polar one as a result of the formation of a polyamide–acetic acid complex. The author therefore suggested that the polyamide surface could show a duality of behaviour depending on the nature of the effect of the eluent system on the surface. Hydrogen bonding between the phenolic group and the amido group was considered to play little part in the mechanism. These views were re-iterated in later papers[17,18]. These authors, however, drew their conclusion from their own results only and did not appear to consider whether or not the results obtained by other workers[5–7,12] could be explained in terms of the proposed mechanism.

In this present paper, we report the R_F values of a number of 2-alkyl phenols chromatographed on cellulose impregnated with polyamide (10 %). The R_F values obtained are compared with those obtained by us for the same phenols chromatographed by adsorption chromatography on alumina[19] and by partition chromatography with ethyl oleate as the stationary phase[20]. The results obtained are used to interpret the

* For Parts I, II, IV, V, VI and VIII of this series, see refs. 21, 22, 23, 19, 20 and 24, respectively.

mechanism of the chromatographic process, and in particular, to assess the validity of the supposed duality of behaviour of polyamide surfaces. In assessing the mechanism we have considered not only our results but also those obtained by other workers[5,7,12,16–18].

EXPERIMENTAL

The thin layers were of cellulose impregnated with polyamide.

Polyamide (Nylon 66) was dissolved in formic acid (90%, Hopkin and Williams, Analar grade) to form an approximately 10% solution. After standing for several days, the solution was centrifuged to throw down undissolved material. The resultant clear, pale amber solution contained 0.13 g of polyamide per ml.

Polyamide solution (11 ml) was added to cellulose (13.5 g) and well stirred. Further increments of formic acid (90%) were added, with stirring, to the cellulose, until the combined volume of polyamide solution and formic acid added was 85 ml. This resulted in a homogeneous mixture of polyamide and cellulose of a suitable viscosity for the formation of thin layers. The slurry was applied to glass plates (5 plates 20 × 20 cm) using a Shandon thin-layer applicator, pre-set to give an applied layer of 0.3 mm thickness.

The coated plates remained in the leveller for 1 h. They were then air-dried for 24 h at a constant temperature of 25° ± 0.5°.

Activation of the plates was for 15 min at 80°. The activated plates were cooled in an evacuated desiccator.

Eluent systems

The following eluents were used:

(1) cyclohexane–acetic acid (93:7, v/v);

(2) aqueous acetic acid (10%).

Cyclohexane. This was purified as previously described[21].

Acetic acid. Glacial acetic acid (Hopkin and Williams Analar grade) was cooled until the bulk of it had crystallised, the residue being discarded. The crystallised material was distilled, the fraction boiling at 118°, under 760 mm pressure, being collected and used.

Water. Distilled, de-ionised water was used.

Suitable aliquots of the purified solvents were mixed together to form the eluent systems.

Application of the phenols

The phenols (1 μl of 0.25% w/v solutions in suitable solvents) were applied to the layers using our multiple-spotting device[22]. The chromatograms were then eluted in our double saturation chamber[22], by an ascending technique, at a constant temperature of 25 ± 0.5°. The solvent front travelled a distance of 14.5 ± 0.5 cm in 2 h.

The phenols were detected by spraying the layers with an alkaline solution of potassium permanganate. They appeared as yellow spots on a purple background.

RESULTS

The results, quoted in Table I, are the mean of at least 4 runs on layers carrying

an internal standard, phenol. The values for phenol on each plate did not differ by more than ± 0.01 R_F unit from the predetermined mean values for phenol in each eluent system. The values for individual phenols were also within ± 0.01 R_F units of the mean values quoted.

For convenience, the eluent system, cyclohexane–acetic acid (93:7, v/v) is referred to as System 1, and the eluent system, aqueous acetic acid (10 % v/v) as System 2 in Table I and in the subsequent discussion.

TABLE I

R_F VALUES OF 2-ALKYLPHENOLS IN VARIOUS THIN-LAYER SYSTEMS

Key to solvent/support system:
Ref. 19: Cyclohexane/cellulose paper (Whatman No. 1) impregnated with 2 % alumina.
Ref. 20: Aqueous ethanol (37.5 % v/v)/cellulose impregnated with ethyl oleate.

Phenol	*R_F values*			
	Ref. 19	*System 1*	*Ref. 20*	*System 2*
Phenol	0.10	0.16	0.90	0.62
2-Methyl-	0.24	0.28	0.78	0.38
2-Ethyl-	0.28	0.37	0.61	0.26
2-*n*-Propyl-	0.32	0.43	0.46	0.19
2-*sec.*-Butyl-	0.41	0.50	0.34	0.12
2-*tert.*-Butyl-	0.59	0.55	0.24	0.00

DISCUSSION

Nature of the surface

In this work, the substrate was cellulose impregnated with polyamide by slurrying the cellulose with a solution of polyamide in formic acid. This gave a loading of 10 % polyamide in cellulose, *i.e.* the surface was essentially comparable to the polyamide paper system of MARTIN AND HUSBAND[5]; the eluent systems were also those used by these workers.

The appearance of the plates, and an examination of a thicker layer than those used for the analytical plates, suggests that a polyamide skin is formed over the surface of the cellulose. The robust character of the layers relative to the well known fragility of pure polyamide thin layers substantiates this view. We are of the opinion that the active surface is a microthin layer of polyamide, with the cellulose acting as an inert support for the polyamide.

Chromatographic process

In Table I, a comparison is made between the R_F of the phenols used in this study (Systems 1 and 2) with those obtained from adsorption chromatography[19] and from reversed phase thin-layer partition chromatography[20].

In adsorption chromatography[19,21] we have shown the chromatographic process to occur in two stages:

(i) adsorption of the phenol on to the polar surface (alumina) by the formation of hydrogen bonds between the solute and the substrate;

(ii) desorption of the phenol as a result of the solvation of the hydrophobic part of the molecule by the eluent.

Where the energy of formation of the hydrogen bond predominated, no movement of the phenol occurred. Where the energy of solvation exceeded the energy of hydrogen bond formation, the phenols moved as discrete spots. When neither predominated the phenols streaked.

In System 1, the R_F values increase with an increase in the chain length of the substituent, *i e.* the order of R_F values is the same as that observed both by us[19] and by COPIUS-PEEREBOOM[16], so that there is no doubt that the polyamide surface is acting as a polar surface in this system.

In order to correctly evaluate the mechanism of the process, three alternatives have to be considered:

(i) Is the surface polar by virtue of the formation of phenol/polyamide hydrogen bonds[1–4], and, if so, is the removal of the phenol a result of the solvation of the hydrophobic part of the molecule by the eluent[21]?

(ii) Is the presence of a compound capable of hydrogen bonding with the surface essential?

(iii) Is the polarity of the surface a result of the formation of a polyamide/acetic acid complex[16–18]?

We believe the first of these three alternatives to be the correct one and this belief is substantiated by our results and those of other workers. Thus MARTIN AND HUSBAND[5], WANG[7] and HALMEKOSKI AND HANNIKAINEN[12] all observed that the R_F values of the phenols chromatographed by them increased with an increase in the chain length of the substituent even in eluent systems which did not contain acetic acid or any other acid. Indeed, MARTIN AND HUSBAND[5] reported that their phenols migrated, with streaking, in the simple eluent cyclohexane, WANG[7] also successfully chromatographed phenols in simple hydrocarbon systems. Hence in these cases, the polarity of the polyamide surface could not possibly be attributed to the formation of a polyamide/acetic acid complex[16–18].

In the absence of such a complex, one concludes that the polarity of the polyamide is a result of hydrogen bond formation between the phenol and the polyamide and that the removal of the phenol from the surface is a result of the solvation of the hydrophobic part of the phenolic molecule by the mobile phase. In System 1, the R_F values of the phenols increase with an increase in the chain length of the group in the 2-position, so that steric hindrance of the approach of the phenolic group to the polyamide surface weakens the strength of the phenol/polyamide hydrogen bond. At the same time the size of the hydrophobic part of the molecule is increased, and with it its tendency to dissolve in the non-aqueous eluent system. This view is supported by the fact that the R_F value order is the same as that observed by us when these compounds were chromatographed on an alumina surface with anhydrous cyclohexane as the eluent[19].

The successful chromatography of phenols on polyamide surfaces with simple organic solvents[5,7] suggests that the need for an eluent system containing a compound capable of hydrogen bonding with the surface is unnecessary, although the presence of a hydrogen bond breaker in the eluent system may serve to eliminate tailing of the compounds[5]. Such a hydrogen bond breaker need not, however, function by donating protons to the surface[5,15] but may compete with the surface for the phenolic proton, *i.e.* it too may act as a proton acceptor[5–7].

We now consider the apparent non-polarity of the polyamide surface[16–18].

In the reversed-phase chromatography of phenols[20,23,24] we have shown the chromatographic mechanism to be:

(a) dissolution of the phenol in the stationary phase;

(b) the removal of the phenol from this phase as a result of the solvation of the phenolic group by the aqueous mobile phase.

Table I shows that in System 2 the R_F values of the phenols decrease with an increase in the chain length of the substituent group. In this respect their behaviour is similar to that observed for some of these phenols when they were chromatographed in the system polyamide impregnated paper/aqueous acetic acid[5] and also to that of gallic acid ester chromatographed in the system polyamide/methanol–acetone–water[16]. Thus all three systems would, at first sight, appear to support the suggested non-polar mechanism of COPIUS-PEEREBOOM[16]. However, the mobile phase in System 2, and that used by MARTIN AND HUSBAND[5] both contain acetic acid and it may be expected that in these systems the acetic acid is ionised and hence should form a polyamide complex more readily than when the acetic acid is present in a non-aqueous system. This in turn should maintain the polyamide as a polar surface. The fact that it does not do so is not only added proof that the concept of a polyamide–acetic acid complex is untenable but casts serious doubts on the hypothesis of the non-polarity of the polyamide surface[16]. Thus if the change in the R_F value order cannot be attributed to the effect of the eluent on the surface, it must be attributed to the effect of the eluent on the molecule being chromatographed.

Using the mechanism of solvation of the phenolic group by the aqueous mobile phase, as proposed for the reversed-phase chromatography[20,23,24], we can now equate the results observed with the chromatographic mechanism. As already stated, we believe the application of the phenol to the surface results in the formation of hydrogen bonds with the surface. As the eluent flows over the surface, the acetic acid acts as a hydrogen bond competitor, liberating the phenol which lies flat on the surface. The phenolic group is then solvated by the aqueous mobile phase, and the compound migrates. LE ROSEN[25] has shown that migration of a compound in a chromatographic system takes place only when that compound is in the mobile phase. Thus the mechanism by which the compound is taken into the mobile phase will govern its movement. The values quoted from ref. 19 and for System 2 indicate that solvation of the phenolic group will be impeded by the presence of the alkyl groups in the 2-position. As the chain length of the substituent increases so the degree of steric hindrance to solvation increases, and hence the R_F values fall. At the same time, it can be seen that this is not entirely a molecular weight effect for the R_F values for 2-*sec.*-butylphenol and 2-*tert.*-butylphenol are significantly different in these two systems. Chain branching increases the bulk of the substituent and hence the degree of steric hindrance to solvation, so that the 2-*sec.*-butyl compound has a higher R_F value than the 2-*tert.*-butylphenol.

It can thus be seen that the evidence for the dual nature of the polyamide surface is indeed open to suspicion and that it is more reasonable to consider that the surface in both cases acts as a proton acceptor and that removal of the phenol from the surface is subsequently dependent upon the solvation of the molecule by the mobile phase, the direction of movement of the compounds being governed by the site of solvation. In non-aqueous solvents, this site is the hydrophobic part of the molecule, and so the R_F values increase with an increase in the chain length of the

esterifying group for gallic acid esters, or substituent group for phenols. In aqueous systems, the sovation site will be, for the phenols, the phenolic group. For the gallic acid esters used by COPIUS-PEEREBOOM[17] the increase in the hydrophobic part of the molecule brought about by the esterifying alcohol results in higher R_F values in the non-aqueous eluents and lower ones in the aqueous eluents. Alternatively, it is probable that the carbonyl groups of the esters act as proton acceptors both for the hydrogen atoms of the CONH groups of the surface and for the solvent, and hence represents an additional solvation site. In the case of these compounds it is therefore obvious that solvation of these groups will be reduced by an increase in the chain length of the alkyl part of the esterifying group, and hence the R_F values are reduced, thus accounting for the apparent duality of behaviour of the polyamide surface.

CONCLUSION

We think that hydrogen bonding between a phenolic group and the polyamide surface is the main mechanism of adsorption on to the surface and that removal of the phenol from the surface is a result of the breaking of the hydrogen bond. Once the bond is broken the migration of the phenol with the eluent is a result of solvation of either the hydrophobic part of the molecule for non-aqueous eluents or the phenolic group by aqueous eluents. The previously reported apparent duality of behaviour of polyamide surfaces is considered to be incompatible with the evidence put forward here and the results on which this hypothesis was suggested are explained in terms of the solvation mechanisms described above.

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SUMMARY

The chromatographic behaviour of some 2-substituted alkylphenols on cellulose impregnated with polyamide (10%) as the stationary phase with (a) cyclohexane–acetic acid (93:7, v/v) and (b) aqueous acetic acid (10%, v/v) as the mobile phase has been studied. The results obtained are compared with those obtained from adsorption chromatography, and reversed-phase thin-layer chromatography. The mechanism of the chromatographic process is explained in terms of the breaking of the phenolic/polyamide hydrogen bonds as a result of (i) solvation of the hydrophobic part of the molecule with the non-aqueous solvent (a), or (ii) solvation of the phenolic group with the aqueous solvent (b). The hypothesis of the duality of behaviour of polyamide is discounted.

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