# THE THIN-LAYER CHROMATOGRAPHIC SEPARATION OF NITROPHENOLS ON POLYAMIDE SURFACES

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SUMMARY

The behaviour of 43 substituted nitrophenols on a cellulose–polyamide surface has been compared with their behaviour on precoated polyamide foils from two different sources. In the first of these surfaces, the adsorbent properties of the polyamide surfaces are diluted by the cellulose. The adsorbent strengths of the two precoated polyamide layers are also different.

## INTRODUCTION

In spite of its successful use as a substrate in column chromatography[1], polyamide was initially of limited use as a thin-layer substrate because of the fragility of the layers produced from it.

Several methods have been used to improve the mechanical stability of the layers:

(i) By using starch[2,3] or cellulose[4–6] as a binding agent.

(ii) By coating glass plates with a viscous solution of polyamide in formic acid[7–10].

(iii) By slurrying cellulose with a solution of polyamide in formic acid to give layers containing 10% polyamide[11–14].

More recently, polyamide for thin-layer chromatography has become available as mechanically stable pre-coated foils from two sources, one a Chinese source* and the second, a European source**. The former of these foils is prepared by the method of WANG *et al.*[15]. Details of the method of preparation of the latter are not available. The author and his co-workers have used cellulose–polyamide layers (see (iii) above), with two eluent systems to investigate the chromatographic behaviour of 179 substituted monohydric phenols[11–14]. In the case of substituted nitrophenols[14], those compounds containing a nitro group in a position *ortho* to the phenolic group migrated to the solvent front when cyclohexane–glacial acetic acid (93:7) was used as the eluent. This behaviour prevented an assessment of the effect of the substituent groupings on the chromatographic behaviour of these compounds.

* Chen Chin Trading Company Limited, No. 75, Section 1, Hankow Street, Taipei, Taiwan.
** Macherey-Nagel and Company. Düren, Germany (available in the United Kingdom from Camlab (Glass) Ltd., Cambridge).

For this reason it was decided to re-investigate the separation of the nitrophenols on layers containing a higher percentage of polyamide. Precoated polyamide foils from both the Chinese and European sources were chosen for purpose because this would enable comparisons to be made between the two kinds of foils and also with the cellulose–polyamide layers previously used[14].

## EXPERIMENTAL

### *The chromatographic substrate*

(1) The preparation of the cellulose–polyamide layers has been reported[11–14].

(2) MN Polygram–Polyamide for TLC is available in sheets 20 cm × 20 cm.

A strip of the substrate (1.5 cm) is removed from parallel sides of the foils in order to prevent a capillary pull between the layer and the glass formers used in our saturation chamber technique[16] from distorting the solvent front. The layers were sandwiched between two glass plates and held firmly in position by the glass former and the glass fibre wick.

(3) Polyamide foils (15 cm × 15 cm) prepared by the method of WANG[15] were fastened by their upper edges to glass plates by a strip of polythene adhesive tape. When the plates were formed into a saturation chamber[16], the lower edge of the foil was held firmly in position by the glass fibre wick.

In the discussion which follows these substrates will be referred to as follows:

Cellulose–polyamide = Substrate No. 1
MN Polygram polyamide = Substrate No. 2
WANG polyamide = Substrate No. 3

The foils were activated by the method described for our cellulose–polyamide layers[11–14].

### *Eluent systems*

These are: (a) Aqueous acetic acid (10%), (b) cyclohexane–glacial acetic acid

TABLE I

$R_F$ VALUES OF SUBSTITUTED NITROPHENOLS ON DIFFERENT POLYAMIDE SUBSTRATES USING CYCLOHEXANE–GLACIAL ACETIC ACID (93:7, v/v) AS THE ELUENT

Key to substrate system numbers:
1 = Cellulose impregnated with polyamide[12–15].
2 = MN Polygram polyamide foil.
3 = Polyamide foils prepared by the method of WANG[16].

| *Key* | *Phenol* | *System number* | | |
|---|---|---|---|---|
| | | *1* | *2* | *3* |
| *(a) Phenols containing nitro-groups only* | | | | |
| 1 | 2-Nitro- | 1.00 | 0.59 | 0.79 |
| 2 | 3-Nitro- | 0.07 | 0.00 | 0.00 |
| 3 | 4-Nitro- | 0.03 | 0.00 | 0.00 |
| 4 | 2,4-Dinitro- | 0.33 | 0.12 | 0.15 |
| 5 | 2,5-Dinitro- | 0.51 | 0.17 | 0.19 |
| 6 | 2,6-Dinitro- | 0.26 | 0.10 | 0.13 |
| 7 | 3,5-Dinitro- | 0.02 | 0.00 | 0.00 |
| 8 | 2,4,6-Trinitro- | 0.00 | 0.00 | 0.00 |

*(Continued on p. 120)*

TABLE I *(continued)*

| *Key* | *Phenol* | *System number* | | |
|---|---|---|---|---|
| | | *1* | *2* | *3* |
| *(b) 2-Nitro-; alkyl phenols* | | | | |
| 1 | 2-Nitro- | 1.00 | 0.59 | 0.79 |
| 9 | 2-Nitro-3-methyl- | 0.66 | 0.23 | 0.26 |
| 10 | 2-Nitro-4-methyl- | 1.00 | 0.62 | 0.88 |
| 11 | 2-Nitro-5-methyl- | 1.00 | 0.62 | 1.00 |
| 12 | 2-Nitro-6-methyl- | 1.00 | 0.69 | 1.00 |
| 13 | 2-Nitro-4-*tert.*-butyl-6-methyl- | 1.00 | 0.72 | 1.00 |
| *(c) 2-Nitro-; halogenated phenols* | | | | |
| 1 | 2-Nitro | 1.00 | 0.59 | 0.79 |
| 14 | 2-Nitro-4-chloro- | 1.00 | 0.54 | 0.74 |
| 15 | 2-Nitro-4-bromo- | 1.00 | 0.52 | 0.73 |
| 16 | 2-Nitro-4-chloro-6-bromo- | 1.00 | 0.49 | 0.70 |
| 17 | 2-Nitro-4,6-dibromo- | 1.00 | 0.49 | 0.70 |
| 18 | 2-Nitro-4,6-diiodo- | 1.00 | 0.49 | 0.69 |
| *(d) 2-Nitro-; halogeno-; alkyl phenols* | | | | |
| 19 | 2-Nitro-4-methyl-6-bromo- | 1.00 | 0.58 | 0.76 |
| 20 | 2-Nitro-4-bromo-6-methyl- | 1.00 | 0.68 | 1.00 |
| 21 | 2-Nitro-4-chloro-5-methyl- | 1.00 | 0.56 | 0.82 |
| *(e) 3-, and 4-Nitro-; alkyl phenols* | | | | |
| 2 | 3-Nitro- | 0.07 | 0.00 | 0.00 |
| 22 | 3-Nitro-4-methyl- | 0.35 | 0.04 | 0.04 |
| 3 | 4-Nitro- | 0.03 | 0.00 | 0.00 |
| 23 | 4-Nitro-3-methyl- | 0.00 | 0.02 | 0.02 |
| 24 | 4-Nitro-2-*sec.*-butyl- | 0.13 | 0.04 | 0.06 |
| 25 | 4-Nitro-2-cyclohexyl- | 0.16 | 0.04 | 0.06 |
| *(f) 4-Nitro-; halogenated phenols* | | | | |
| 3 | 4-Nitro- | 0.03 | 0.00 | 0.00 |
| 26 | 4-Nitro-2-chloro- | 0.06 | 0.02 | 0.02 |
| 27 | 4-Nitro-2-chloro-6-bromo- | 0.22 | 0.04 | 0.08 |
| 28 | 4-Nitro-2,6-dibromo- | 0.23 | 0.07 | 0.10 |
| 29 | 4-Nitro-2,6-diiodo- | 0.28 | 0.04 | 0.07 |
| *(g) 4-Nitro-; 2-bromo-; 6-alkyl phenols* | | | | |
| 30 | 4-Nitro-2-bromo-6-methyl- | 0.47 | 0.11 | 0.14 |
| 31 | 4-Nitro-2-bromo-6-ethyl- | 0.61 | 0.19 | 0.26 |
| 32 | 4-Nitro-2-bromo-6-isopropyl- | 0.77 | 0.28 | 0.39 |
| 33 | 4-Nitro-2-bromo-6-*sec.*-butyl- | 0.80 | 0.35 | 0.42 |
| 34 | 4-Nitro-2-bromo-6-*tert.*-butyl- | 0.94 | 0.40 | 0.52 |
| 35 | 4-Nitro-2-bromo-6-cyclohexyl- | 0.78 | 0.31 | 0.45 |
| *(h) Substituted dinitrophenols* | | | | |
| 4 | 2,4-Dinitro- | 0.33 | 0.11 | 0.15 |
| 36 | 2,4-Dinitro-6-chloro- | 0.225 | 0.04 | 0.04 |
| 37 | 2,4-Dinitro-6-bromo- | 0.29 | 0.03 | 0.06 |
| 38 | 2,4-Dinitro-6-iodo- | 0.34 | 0.02 | 0.04 |
| 39 | 2,4-Dinitro-6-methyl- | 0.84 | 0.32 | 0.35 |
| 6 | 2,6-Dinitro- | 0.26 | 0.10 | 0.13 |
| 40 | 2,6-Dinitro-4-methyl- | 0.635 | 0.22 | 0.27 |
| 41 | 2,6-Dinitro-4-*tert.*-butyl- | 0.95 | 0.42 | 0.48 |
| *(i) Nitroso phenols* | | | | |
| 42 | 4-Nitroso-3-methyl- | 0.04 | 0.02 | 0.03 |
| 43 | 4-Nitroso-2,5-dimethyl- | 0.15 | 0.07 | 0.09 |

(93:7, v/v). These will be referred to as eluents (a) and (b) respectively in the following discussion.

The purification and the preparation of the solvents used in the preparation of the eluent systems have already been described[12].

*The application of the phenols and the manner of development of the chromatolayers*

The application of the phenolic solutions (1 $\mu$l of 0.25% w/v solution) using our multiple spotting technique[16] has already been described. Development was by the ascending technique in our double saturation chamber[16]. The phenols were self coloured but in many cases the colour fades in the strongly acidic eluent systems used. The layers were therefore exposed to ammonia fumes and irradiated with ultraviolet light in order to more sharply define the spot perimeters[14,17,18].

## RESULTS

These are given in Table I.

## DISCUSSION

*Nature of the surface*

Substrate No. 1 has already been shown to be extremely robust[11]. Substrate No. 3 is of a comparable nature but the substrate No. 2 is the least robust of the three.

*Reproducibility of results*

The values obtained from the substrate No. 1 were reproducible to $\pm$0.01 $R_F$ units, while those obtained from the foils were reproducible to $\pm$0.02 $R_F$ units.

*The effects of substituents on the chromatographic behaviour of the nitrophenols*

The relationship between the molecular structure of the compounds studied and their chromatographic behaviour on substrate No. 1 in both eluent systems has already been discussed[14]. Lack of migration of the compounds on substrates Nos. 2 and 3 in eluent (a) prevents further discussion of this phenomenon here. In eluent (b), while the $R_F$ values are lower on substrate Nos. 2 and 3 than they are on substrate No. 1, the general pattern of behaviour is similar to that previously reported[14]. This includes the following observations:

(1) Internal hydrogen bonding between the phenolic hydrogen atom and an *ortho* nitro group results in an increase in the $R_F$ values relative to isomeric compounds in which the nitro group is found in some other position in the nucleus.

(2) Polysubstitution with nitro groups results in a reduction of the $R_F$ values.

(3) Substitution with halogen atoms also causes a reduction in $R_F$ values.

(4) Substitution with alkyl groups generally increases the $R_F$ values.

(5) When alkyl groups are substituted in the 2- and or 6- position the $R_F$ values increase with an increase in the size of the alkyl group.

On substrate No. 1 with eluent (a), the substituted 2-nitrophenols were generally found at the solvent front. Retardation of these compounds does, however, occur

on substrate Nos. 2 and 3, so that meaningful $R_F$ values are obtained in these two cases.

Table I(b) shows the buttressing effect of the 3-methyl group in twisting the 2-nitrogroup from the plane of the ring. This weakens the internal hydrogen bond and results in a reduction in the $R_F$ value of 2-nitro-3-methylphenol relative to that of 2-nitrophenol. The results for the remaining compounds in this table show the increases in $R_F$ values which occur as a result of substituting an alkyl group into the nucleus, the effect of the size of the group, and the effect of substitution of an alkyl group in the *ortho* position.

Halogen atom substitution into the nucleus of 2-nitrophenol brings about a decrease in the $R_F$ values of the compounds compared with the value for 2-nitrophenol (Table I(c)). This has been shown to be a result of hydrogen bond formation between the halogen atom and the hydrogen atom of the amido group.

While the presence of a halogen atom in the 2- position has the effect of slightly increasing the $R_F$ values of halogen substituted 4-nitrophenols (Table I(f)), the presence of an *ortho* halogen atom in 2-nitrophenols has the effect of reducing the $R_F$ values. In unsymmetrical 2,6-dihalophenols BAKER AND KAEDING[10] have shown there to be a competiton for the phenolic hydrogen atom between the two *ortho* halogen atoms. If such an equilibrium occurs between the nitro group–phenol hydrogen bond, and the halogen atom–hydrogen bond, then the nitro group will be free to interact with the surface for part of the time and hence cause a reduction in the $R_F$ values. In the case of the 2-nitro-4-methyl-6-bromophenol, this effect will be offset by the increase in the $R_F$ values caused by the presence of the methyl group in the nucleus.

*The adsorptive properties of the layers*

In eluent (a), none of the 43 phenols moved from the point of application on substrate No. 2. On substrate No. 3 some diffusion of the spots from the point of application occurred, but the movement was insufficient to enable $R_F$ values to be determined. This contrasts with the behaviour of these phenols on substrate No. 1 in the same eluent system[14] where the $R_F$ values ranged from 0.00 to 0.615. Thus the order of adsorption for the three layers is: cellulose–polyamide < WANG polyamide < MN Polygram polyamide. The results for the three substrates in eluent (b) confirm this order in every case.

It is therefore apparent that the cellulose present in substrate No. 1 acts as a diluent. Its presence may or may not be advantageous depending upon the nature of the eluent system used and on the structures of the molecules to be separated.

Thus many good separations have been obtained on this substrate in eluent (a)[14], whereas the foils failed to resolve these compounds in this system. In the case of the substituted *ortho* nitrophenols containing a single nitro group (Table I(b), (c), and (d)), the presence of the cellulose substrate No. 1 is decidedly disadvantageous in eluent (b), for these compounds travel with the solvent front in the system. However, resolution of many of this class of phenols becomes possible on the foils in this eluent system. Conversely, better resolution of substituted 3- and 4-mononitrophenols (Table I(e) and (f)) and substituted dinitrophenols (Table I(h)) is obtained in substrate No. 1 than on the foils.

That the cellulose functions largely as a diluent and plays little or no part in

the chromatographic process is seen in so far as the general order of $R_F$ values for the compounds is the same for substrate Nos. 2 and 3 as for substrate No. 1. This is not surprising in view of the fact that we have previously observed[11] that in substrate No. 1 the polyamide is present as a thin skin surrounding the cellulose particles.

The differences in the adsorptive properties of substrates Nos. 2 and 3 may be due either to differences in the methods of manufacture of these foils or they may be due to differences in the thickness of the polyamide layer.

It is interesting to note that the polyamide used in substrate No. 1 is polyhexamethyldiamino-adipate (Nylon 66) whereas that used in substrate No. 3 is an $\varepsilon$-polycaprolactam (Nylon 6).* The comparability of the $R_F$ value order in the three systems therefore suggests that the carbon skeleton of the polyamide plays little or no part in the chromatographic mechanism. Resolution of the compounds is therefore dependent upon the functional groups present in the substrate and the ability of the molecules to be separated to form hydrogen bonds either with the hydrogen atoms of the NH group[13,14] or with the oxygen atoms of the carbonyl groups.

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* *Note added in proof.* Substrate No. 2 is now known to be polyaminodecanoic acid (Nylon 11).

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## DISCUSSION

ZAWTA: Let me point out another possibility of application of polyamide chromatography, namely the separation of structure isomers of free bile acids, which is primarily based on partition chromatography; for details see U. FREIMUTH, B. ZAWTA AND M. BÜCHNER, *J. Chromatog.*, 30 (1967) 607.