THE PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF SIMPLE PHENOLS

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INTRODUCTION

During the past few years a considerable amount of work has been reported in the literature on the paper chromatographic analysis of a wide range of phenols, but very few of the methods described lead to clean separations of the isomers of low molecular weight alkylphenols. The latter materials are of interest to the petroleum industry in connection with the analysis of the low boiling phenolic constituents that can occur in various products *e.g.* gasolines and kerosines.

Attempts to overcome the difficulties caused by the volatility of simple phenols have been made in a variety of ways, including separations on papers impregnated with amides[1,2] or polyamides[3] and on papers held between glass plates[4,5]. However, most workers have used derivatives of phenols *e.g.* the sodium salts of phenylazo-benzenesulphonic acid[6,7], the *p*-nitrophenylazo dyes[8,9] and the 3,5-dinitrobenzoate esters[10]. The separations of low molecular weight phenols reported in the literature were frequently poor, *e.g. m*- and *p*-cresol were rarely resolved[1,11].

A case therefore existed for an improved method of separating mixtures of phenols, by paper chromatography, which would be applicable to a wide variety of samples, particularly petroleum products.

This report describes a method in which phenols are coupled with diazotised aromatic amines to form stable azo dyes. This reaction forms the basis of many well-known quantitative methods for the determination of phenols. In the present method mixtures of these azo dyes are separated into single components on a paper impregnated with formamide. A cyclohexane–benzene mixture containing dipropylene glycol is used as the developing solvent.

The azo dyes are further characterized by the colour reactions they give in alkaline solutions. Examples are given illustrating the successful application of the method in the separation and identification of phenols in a variety of products.

EXPERIMENTAL AND RESULTS

1. *Coupling reagents and the formation of azo dyes*

The choice of amine was governed by several considerations.

(a) The use of the nitro–aci-nitro reaction in alkaline media would be useful in

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characterizing individual phenols, therefore un-nitrated amines such as aniline, toluidines, anisidines, benzidines were not used.

(b) It was intended that the chromatographic papers should not be pretreated with acids or alkalis. It seemed likely that dyes containing sulphonic acid functions, *i.e.* those based on sulphanilic acid, would have a strong affinity for the paper, and hence with developing solvents of low polarity they would have R_F values close to zero; consequently sulphonic acid dyes were not examined.

(c) Since *meta*-nitro dyes would not have quinonoid aci-nitro forms, this investigation was confined to the *ortho*- and *para*-nitro-anilines.

Solutions of the diazotised amines were prepared as follows:

The nitro amine (1.5 g) was dissolved in 1000 ml of N aqueous hydrochloric acid. Just before the coupling with the phenol samples, 25 ml of amine hydrochloride solution were diazotised at 0° with 0.75 ml of sodium nitrite solution (10 % aqueous) giving a solution of p-nitrophenyldiazonium chloride (soln. A); the yellow colour of the amine was completely discharged. A convenient portion of the phenol solution was treated with 1–2 ml of the soln. A, and after 1 min the mixture was made alkaline with sodium carbonate solution (2 ml 20 % w aq.). When phenols were present in the
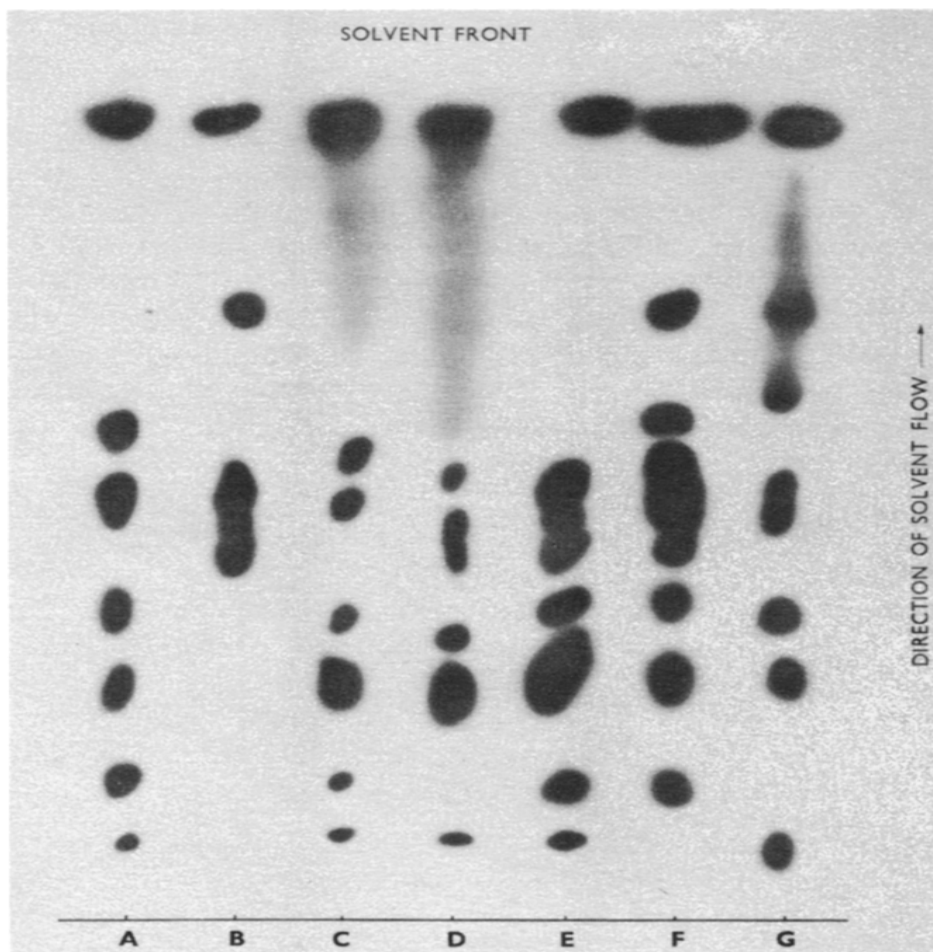

Fig. 1. A = Mixture of phenol, cresols and ethylphenols. B = Mixture of xylenols. C = Phenols from commercial tritolyl phosphate (I). D = Phenols from commercial trixylenyl phosphate. E = Phenols from commercial tritolyl phosphate (II). F = Reference blend of simple alkylphenols. G = Phenols isolated from a Curaçao kerosine.

sample under test a brilliant red/purple dye was formed. The azo dyes, still phenolic, were in solution as their sodium salts. After acidification with excess dilute hydrochloric acid or by adding excess solid carbon dioxide the azo dyes were extracted with ether (2 or 3 extractions with 10 ml ether usually sufficed to remove all the azo dye from the aqueous phase).

The ethereal phase was dried over anhydrous magnesium sulphate. The solvent was distilled off and the azo dyes immediately redissolved in chloroform (ca. 0.2 ml).

This reaction was carried out for all thirteen simple alkylphenols, with both o- and p-nitrophenyldiazonium chlorides. A reagent blank was prepared in the same way, but omitting the phenols. The azo dyes and the blank were examined by paper chromatography (Fig. 1).

2. Development of a suitable solvent system

Most of the systems reported in the literature for the analysis of phenols have required the use of a variety of aqueous solvents. In the case of azo dyes derived from phenols little work has been done, and an example is the use of sodium carbonate treated papers with phenylazobenzenesulphonic acids[6,7].

Since the nitrophenylazobenzene dyes were almost insoluble in water it was decided to use only organic phases. The azo dyes could be partitioned between formamide and hydrocarbon solvents. Therefore in order to achieve separations of the azo dyes, papers impregnated with formamide were used, with hydrocarbon solvents as developing phases. It was found that the best separations were obtained if the hydrocarbon solvent contained a small proportion of dipropylene glycol.

Several conventional systems were also examined, and the nature and behaviour of these systems are listed in Table I. System 9 was finally adopted.

TABLE I

PAPER CHROMATOGRAPHIC SYSTEMS USED FOR THE SEPARATION OF
p-NITROPHENYLAZOBENZENE DYES (DERIVED FROM PHENOLS)

System No.	Mobile phase	Paper* pretreatment	Observation
1	Acetic acid–butanol–water (50:50:10 vol.)	None	Dyes move with solvent front
2	Methyl isobutyl ketone–ethanol (50:50 vol.) saturated with 10 % aq. ammonia	Sodium carbonate	Each dye gave several spots, phenol** retarded
3	Water–methyl ethyl ketone–ethanol (50:50:10 vol.)	Untreated	R_F's 0.5
4	Methyl isobutyl ketone–ethanol, saturated with ammonia	Untreated	R_F's 1.00
5	Sodium hydroxide $N/2$ aq. saturated with n-butanol[9]	Untreated	o-, m-, p**-Cresol not separated
6	Benzene–cyclohexane–dipropylene glycol (30:70:3 vol.)	Untreated	R_F's 1.00
7	Benzene–cyclohexane–dipropylene glycol (30:70:3 vol.)	Sodium carbonate	Spots "comet" badly
8	Benzene–cyclohexane–methanol (50:50:1 vol.)	Formamide	Phenol**, o- and p-cresol separated
9	Benzene–cyclohexane–dipropylene glycol (30:70:3 vol.)	Formamide	Good separation of most phenols**

* Whatman No. 1.
** Refers to azo dyes.

3. Preparation of samples

(i) Phenols uncombined (e.g. in kerosine)

Wherever simple phenols are uncombined e.g. in light petroleum distillates they can be extracted with aqueous sodium hydroxide (2 N)*. To a suitable quantity of the alkaline extract is added 1 ml of diazotised p-nitroaniline solution (soln. A). If a red or yellow colour develops, indicating the formation of an azo dye, the solution is acidified with dilute hydrochloric acid, and extracted with ether. The ethereal solution is dried over anhydrous magnesium sulphate, and then evaporated free of solvent. The solid azo dye is dissolved in 0.1–0.2 ml chloroform.

(ii) Phenols combined (e.g. in triaryl phosphates)

(a) Phosphate esters. When phenols are combined in the form of esters they can be obtained in the free form by saponification e.g. reflux for 30 min to 1 h with alcoholic potassium hydroxide (1 N). The saponification liquid is diluted with water, treated with excess solid carbon dioxide, and extracted with ether, or it can be acidified with dilute sulphuric acid and extracted with ether. In either case the ether extract will contain phenols, from which azo dyes are then prepared as in 3(i).

(b) Phosphate esters and/or mineral oil and/or synthetic ester oil. If other saponifiable material is present with the phosphate esters the saponification liquor (3.ii.a) is diluted with water and extracted with ether or petroleum spirit. This removes alcohols e.g. 2-ethylhexanol, unsaponifiable matter such as hydrocarbon oil, and materials such as antioxidants containing nitrogen e.g. phenothiazine. Excess solid carbon dioxide is added to the alkaline phase, which is then extracted with ether to remove phenols (acids such as sebacic acid remain in the alkaline phase). The alkaline phase can alternatively be acidified with dilute sulphuric acid and steam distilled, in which case the phenols will be in the distillate.

The azo dyes are then prepared as in 3(i).

4. Preparation and running of a chromatogram

A Whatman No. 1 filter paper square 20 cm × 20 cm (Shandon pattern, corner holes) is impregnated with formamide by drawing it once through a 20 % vol. solution of formamide in acetone. Small volumes (1–5 μl) of chloroform solutions of the azo dyes are spotted onto the impregnated paper at appropriate positions along the base line. The paper is developed by the ascending technique with a mixture of cyclohexane–benzene (70:30 vol.) containing 3 % vol. dipropylene glycol. At 21° the solvent takes some 2 1/2 h to travel a distance of 17.0 cm. The atmosphere in the tank need not be saturated with the vapour of the developing solvent. The paper is dried at 40°.

For the most part the chromatographic zones (spots) are self-indicating. Wherever spots overlap e.g., 3,5-, 2,3-, and 2,5-xylenols, they are not easily distinguished from each other. If, however, a chromatogram of the p-nitrophenylazo dyes is exposed to ammonia vapour the spots are changed from pale yellow to intense red or purple. The colours are characteristic, and enable overlapping spots to be identified.

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* Although thiophenols and naphthenic acids are also extracted they do not interfere with the separation and identification of phenols from petroleum distillates. Thiophenols give yellow azo dyes which have R_F values of 1.00, and which do not change colour on spraying with alkali. Naphthenic acids cannot be seen on a chromatogram.

The colour changes given in ammonia vapour by the *o*-nitrophenylazo dyes are only slight, *i.e.* from yellow to orange, or orange red.

On standing for a few minutes in air, *i.e.* after the ammonia has evaporated from the paper the colours return to yellow. In order to form permanent red/purple colours from the azo dyes, the papers are sprayed with sodium carbonate solution (1 % aq.) and dried at 40°. The colours and R_F values of the spots are noted.

DISCUSSION

The structures of the various phenols which have been examined are of two types:

(A) *4-position unsubstituted*   **(B)** *4-position substituted*

phenol	*p*-cresol
o- and *m*-cresol	*p*-ethylphenol
o- and *m*-ethylphenol	2,4- and 3,4-xylenols
2,3-, 2,5-, 2,6- and 3,5-xylenols	*p*-chlorophenol, 4-chloro-2-methylphenol

On the chromatograms of the *p*-nitrophenylazo dyes and of the *o*-nitrophenylazo dyes, the group A phenols have R_F values < 1.00, whereas group B phenols have R_F values 1.00 only. The *para* (or 4) position of the group B phenols is blocked by either methyl, ethyl or chloro groups, whereas the 4 positions in group A phenols are free. It is therefore postulated that the azo dyes are of two types,
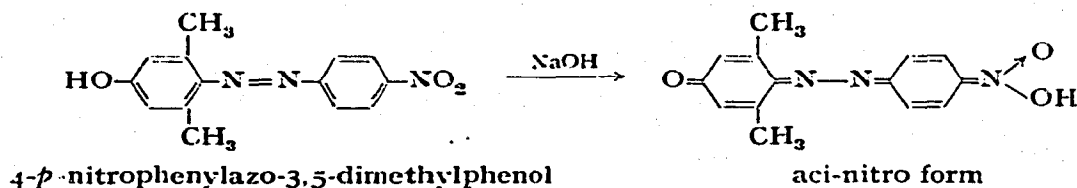
Group A – para coupled
e.g.
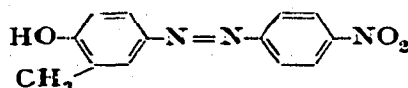
Group B – ortho coupled
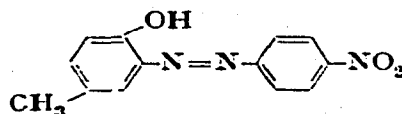
Both types of dye are phenolic, and are soluble in sodium hydroxide solution.

The mechanism whereby the neutral yellow azo dyes assume red/purple colours in alkaline media is most probably due to the formation of quinonoid structures[12].

4-*p*-nitrophenylazo-3,5-dimethylphenol   aci-nitro form

The instability of the quinonoid form is shown by the rapidity with which the purple-red colours formed in ammonia vapour return to yellow in air.

The R_F values of the *p*-nitro- and *o*-nitro-phenylazo dyes are substantially similar (Table II) with the exception of 2,3-, 2,5- and 2,6-xylenols. Hence if the phenols are run as both types of dye, spots which overlap and have R_F values from 0.5 to 0.65 can be more readily characterized.

TABLE II

R_F VALUES AND IDENTIFICATION OF SIMPLE ALKYLPHENOLS (SINGLE SPOTS)

Phenol	*o-Nitrophenylazo dyes*			*p-Nitrophenylazo dyes*		
		Colours of azo dyes			*Colours of azo dyes*	
	R_F value	*Before ammonia treatment*	*After ammonia treatment*	*R_F value*	*Before ammonia treatment*	*After ammonia treatment*
Phenol	0.14	↑	Yellow-orange	0.13	Yellow	Rose
o-Cresol	0.36		Rose	0.35	Yellow	Mauve
m-Cresol	0.29		Orange	0.27	Yellow	Magenta
p-Cresol	1.00		Red-orange	1.00	Orange	Purple
o-Ethylphenol	0.61		Orange-yellow	0.56	Yellow	Mauve
m-Ethylphenol	0.49	Orange-yellow	Orange-yellow	0.47	Yellow	Magenta
p-Ethylphenol	1.00		Red-orange	1.00	Orange	Purple
2,3-Xylenol	0.57		Rose	0.43	Yellow	Lilac
2,4-Xylenol	1.00		Red-orange	1.00	Orange	Purple
2,5-Xylenol	0.64		Rose	0.49	Yellow	Lilac
2,6-Xylenol	0.90		Rose	0.73	Yellow	Pale lilac
3,4-Xylenol	1.00		Red-orange	1.00	Orange	Purple
3,5-Xylenol	0.46		Red-orange	0.41	Yellow	Red-brown
p-Chlorophenol	—			1.00	Yellow	Purple
4-Chloro-2-methyl-phenol	—	↓		1.00	Yellow	Purple

TABLE

R_F VALUES AND COLOUR REACTIONS OF MIXTURES

Phenols	A		B		F	
	R_F	*Colour**	*R_F*	*Colour**	*R_F*	*Colour**
Phenol	0.16	Rose			0.14	Rose
m-Cresol	0.26	Magenta			0.28	Magenta
o-Cresol	0.35	Mauve			0.38	Mauve
m-Ethylphenol	0.48	Magenta			0.55	Magenta
o-Ethylphenol	0.58	Mauve			0.61	Mauve
p-Cresol	1.00	Purple			1.00	Purple
p-Ethylphenol	1.00	Purple			1.00	Purple
3,5-Xylenol			0.43	Red-brown	0.45	Red-brown
2,3-Xylenol			0.48	Lilac	0.51	Lilac
2,5-Xylenol			0.53	Lilac	0.57	Lilac
2,6-Xylenol			0.73	Pale lilac	0.74	Pale lilac
3,4-Xylenol			1.00	Purple	1.00	Purple
2,4-Xylenol			1.00	Purple	1.00	Purple

The R_F values of the azo dye spots are increased by rise in temperature and it is advisable to run chromatograms at $20° \pm 2°$ for best separations. The R_F values of the single spots sometimes differ from those obtained on mixtures.

e.g.:

R_F values of 2,5-xylenol *p*-nitro-phenylazo dye
in mixture of xylenols 0.53 (Fig. 1)

in mixtures of other phenols 0.57 (Fig. 1) ⎫
0.53 (Fig. 1) ⎬ (Table III)
0.56 (Fig. 1) ⎭

Single spot 0.49 (Table II)

However, this phenol can also be identified by the colour which it forms in ammonia vapour.

ACKNOWLEDGEMENTS

III

OF SIMPLE ALKYL PHENOLS (SEE FIG. 1)

R_F	Colour*	R_F	Colour*	R_F	Colour*	R_F	Colour*
	C		**D**		**E**		**G**
0.15	Rose	—	—	0.15	Rose	—	—
0.28	Magenta	0.27	Magenta	0.29	Magenta	0.29	Magenta
0.36	Mauve	0.33	Mauve	0.37	Mauve	0.36	Mauve
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	0.43	Red-brown	0.44	Red-brown	—	—
0.50	Lilac	0.47	Lilac	0.48	Lilac	0.49	Lilac
0.56	Lilac	0.53	Lilac	0.53	Lilac	0.53	Lilac
—	—	—	—	—	—	0.74	Lilac
1.00	Purple	1.00	Purple	1.00	Purple	1.00	Purple
Extended zone		Extended zone				0.64	Lilac
0.69–0.95	Yellow	0.58–0.95	Yellow			0.64–0.74	Yellow
						0.74–0.92 Extended zones	Yellow
0.08	Yellow	0.08	Yellow	0.08	Yellow	0.07	Yellow

SUMMARY

1. With the paper chromatographic system described, mixtures of simple alkyl phenols in the form of their *o*- or *p*-nitrophenylazo dyes can be separated into recognizable components.

2. The yellow azo dye spots undergo the nitro–aci-nitro reaction in alkaline media to give intense red/purple colours.

3. From the chromatographic behaviour of the dyes it is apparent that two types of dye are present:

(i) *ortho* coupled dyes,
(ii) *para* coupled dyes.

4. The method has been used to identify trace amounts of phenols in gasolines and kerosines, and also to ascertain the nature of the "cresyl" groupings in commercial tritolyl phosphate and trixylenyl phosphate. The method has also helped in the examination of triaryl phosphates in mineral and synthetic ester oils.

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