THIN LAYER CHROMATOGRAPHIC STUDIES OF 30 ORGANIC ACIDS ON CALCIUM SULPHATE

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ABSTRACT

Thin layer chromatographic behaviour of 30 organic acids on coatings of calcium sulphate and calcium sulphate containing charcoal, p-dimethylaminobenzaldehyde, flyash, silica gel G etc. has been studied. Farm chemicals: plant growth regulators (benzoic, cinnamic, gallic, β-naphthalene acetic, β-naphthoxy acetic and indole-3-acetic acids) and herbicides (phenoxy acetic acid) have been separated from one another and from several other organic acids.

INTRODUCTION

Thin layer chromatography is a versatile technique for the separation of organic acids. Several new coating materials (1,2) have been discovered and tested for separation. The older material, silica gel-coated glass plates developed in mixed solvent systems have widely been used for the separation of amino acids, benzoic acid, substituted benzoic acids and sorbic acids from fruit beverages, citric acid...
cycle intermediates and lactic acid, esters of p-hydroxy benzoic acid, phosphoric acid etc. Ion-exchange thin layer chromatography has been used for the separation of tryptophan from an aminoacylase-N-acetyltryptophan reaction mixture (1). Most phenyl-thiohydantoin (PTH) and methylthiohydantoin (MTH) amino acid derivatives could also be separated from one another by this method. Thin layers of silica gel (3) containing silver oxide have been used for the separation of substituted benzoic, phthalic, maleic and fumaric acids. Thin layers of silica gel G F254 containing cellulose MN300 F254 (4) have been used for the separation of food preservatives. In our previous publications (5-8) it has been shown that papers impregnated with calcium carbonate/calcium sulphate have a great separation potential for organic acids. Therefore, now an attempt has been made to test the separation potential of calcium sulphate/calcium sulphate containing p-dimethylaminobenzaldehyde, methyl orange, starch, activated charcoal, calcium carbonate, flyash, silica gel G etc. coated glass plates. The results obtained are described in this paper.

EXPERIMENTAL

Apparatus and Materials: A Stahl apparatus with a universal applicator (adjustable thickness of the applied layers from 0-2.0 mm) (made in India), glass plates (20x4 cm), glass jars (25x5 cm) and temperature controlled electric oven were used.
Activated charcoal and acetone (E. Merck, India), ammonium vanadate (Riedel, Germany), benzene (Reechem, India), bromophenol blue and calcium sulphate dihydrate (S.M. Chemicals, India), carbontetrachloride, silica gel G and 1,4-dioxane (Glaxo Laboratories, India), p-dimethylaminobenzaldehyde (BDH, India), starch (NCL, India) of analytical grade and flyash 100-200 mesh (Thermal Power Station, Kasimpur, U.P., India) were used.

Flyash was dried at 100°C in an electric oven before use. The principal ingredients (9,10) of flyash are silica, alumina and iron oxides. Lime and carbon are present in minor proportions. The actual composition of the flyash depends on the variety of coal used and degree of burning.

Aqueous or ethanolic solutions (0.1N) of the test materials were used. In case it was not possible to prepare 0.1N solutions a saturated solution was used.

Preparation of Plates: A slurry of calcium sulphate (I) obtained by mixing calcium sulphate (30 g) with distilled water (D W) (70 ml), was applied on the glass plates with the help of the applicator so that the thickness of calcium sulphate slurry would be 0.75 mm. The plates were first allowed to dry at room temperature and then in a temperature controlled electric oven at 110°C for 1 hr. The plates of silica gel G (II) were also prepared by the same procedure using the slurry of silica gel G made by mixing 48 g of it with 100 ml of distilled water.
The procedure described above was also used to make plates of the following coatings.

<table>
<thead>
<tr>
<th>Coating</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>Calcium sulphate (30 g) + activated charcoal (0.3 g) + D W (70 ml)</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium sulphate (30 g) + activated charcoal (0.6 g) + D V (70 ml)</td>
</tr>
<tr>
<td>V</td>
<td>Calcium sulphate (30 g) + activated charcoal (1.5 g) + D W (70 ml)</td>
</tr>
<tr>
<td>VI</td>
<td>Calcium sulphate (30 g) + calcium carbonate (0.15 g) + D W (70 ml)</td>
</tr>
<tr>
<td>VII</td>
<td>Calcium sulphate (30 g) + calcium carbonate (0.3 g) + D W (70 ml)</td>
</tr>
<tr>
<td>VIII</td>
<td>Calcium sulphate (30 g) + calcium carbonate (0.6 g) + D W (70 ml)</td>
</tr>
<tr>
<td>IX</td>
<td>Calcium sulphate (30 g) + p-dimethylaminobenzaldehyde (p-DAB) (1 ml of 10%) + D W (70 ml)</td>
</tr>
<tr>
<td>X</td>
<td>Calcium sulphate (30 g) + flyash (0.3 g) + D W (70 ml)</td>
</tr>
<tr>
<td>XI</td>
<td>Calcium sulphate (30 g) + flyash (0.6 g) + D W (70 ml)</td>
</tr>
<tr>
<td>XII</td>
<td>Calcium sulphate (30 g) + flyash (1.5 g) + D W (70 ml)</td>
</tr>
<tr>
<td>XIII</td>
<td>Calcium sulphate (15 g) + silica gel G (15 g) + D W (70 ml)</td>
</tr>
<tr>
<td>XIV</td>
<td>Calcium sulphate (30 g) + silica gel G (0.3 g) + D W (70 ml)</td>
</tr>
<tr>
<td>XV</td>
<td>Calcium sulphate (30 g) + silica gel G (1.5 g) + D W (70 ml)</td>
</tr>
</tbody>
</table>
Coating XVI: Calcium sulphate (30 g) + silica gel G (3.0 g) + D W (70 ml).

Coating XVII: Calcium sulphate (30 g) + silica gel G (4.5 g) + D W (70 ml).

Coating XVIII: Calcium sulphate (30 g) + starch (0.6 g) + D W (70 ml).

Coating XIX: Calcium sulphate (30 g) + starch (1.5 g) + D W (70 ml).

Coating XX: Calcium sulphate (30 g) + starch (3.0 g) + D W (70 ml).

Coating XXI: Silica gel G (12 g) + activated charcoal (0.5 g) + D W (25 ml).

Coating XXII: Silica gel G (12 g) + ammonium vanadate (0.5 g) + D W (25 ml).

Coating XXIII: Silica gel G (12 g) + bromophenol blue (0.5 g) + D W (25 ml).

Coating XXIV: Silica gel G (12 g) + calcium nitrate (3.0 g) + D W (25 ml).

Coating XXV: Silica gel G (12 g) + copper sulphate (1 ml of 0.1 M) + D W (25 ml).

Coating XXVI: Silica gel G (12 g) + cresol red (0.5 g) + D W (25 ml).

Coating XXVII: Silica gel G (12 g) + p-DAB (1 ml of 10%) + D W (25 ml).

Coating XXVIII: Silica gel G (12 g) + flyash (0.5 g) + D W (25 ml).

Coating XXIX: Silica gel G (12 g) + resorcinol (1 ml of 10%) + D W (25 ml).
Coating XXX: Silica gel G (12 g) + thymol blue (0.5 g) + DW (25 ml).

In all cases slurry was made as mentioned above except p-DAB and resorcinol. In these cases first the slurry was made and then ethanolic solution of p-DAB or resorcinol was added to it. After thorough mixing the slurry so obtained was coated on the plates.

Spotting of Test Solution: Test solution was spotted on the plate with the help of a fine capillary. The plates were kept at room temperature (30°) for 15 min for the removal of solvent and then developed in a solvent system. For tailing, the front limit (RI) and the rear limit (RT) were measured while for other acids Rf values were taken as usual.

\[
R_f = \frac{\text{Distance travelled by substance (cm)}}{\text{Distance travelled by solvent front (10 cm)}}
\]

Test Solutions and their Detection: The acids on the plates were detected by the reported procedure summarized below:

(1) Alanine, (2) arginine HCl and (3) l-aspartic acid were detected by 1% aqueous ninhydrin solution; (4) acetic, (5) adipic, (6) ascorbic, (7) barbituric, (8) benzoic, (9) cinnamic, (10) citraconic, (11) citric, (12) formic, (13) fumaric, (14) gallic, (15) hippuric, (16) indole-3-acetic, (17) malic, (18) maleic, (19) β-naphthaleneacetic, (20) β-naphthoxyacetic, (21) nicotinic, (22) oxalic, (23) oxaloacetic, (24) phenoxyacetic, (25) quinic, (26) salicylic, (27) sulphamic, (28) tartaric, (29) trans-aconitic
and (30) trichloroacetic acids were detected by 1\% ethanolic alkaline bromophenol blue solution.

RESULTS

Various separations of the acids under study on different coatings are possible, some of them are summarized below. $R_f$ values are given in parentheses that follow the number of the acid, marked in the experimental section.

Coating I: 9(0), 19(0) and 20(0) from 1(1.0), 2(1.0), 4(1.0), 5(0.8), 6(1.0), 7(1.0), 8(1.0), 10(1.0), 11(1.0), 12(1.0), 13(0.85), 14(1.0), 15(0.85), 17(1.0), 18(1.0), 21(1.0), 22(1.0), 23(1.0), 24(1.0), 25(1.0), 27(1.0), 28(1.0), 29(1.0) and 30(1.0).

Coating II: 2(0.6) from 3(0.15), 4(0-1), 5(0-2.5), 6(0-2), 7(0-1.5), 8(0-2), 9(0-1), 10(0-2), 11(0-3.5), 12(0-1), 13(0-3.3), 15(0-0.7), 16(0-2), 17(0-1), 18(0-1), 19(0-0.5), 20(0), 21(0-1.5), 22(0-4.5), 23(0-2.5), 24(0-4), 25(0-0.5), 26(0-1), 27(0-1), 29(0-1) and 30(0-1).

Coating III: 2(1.0), 3(1.0), 6(1.0), 27(1.0), 28(0.9), 30(0.9) from 7(0-6), 8(0-6), 16(0-5), 19(0) and 20(0-7.5).

Coating IV: 22(1.0), 27(1.0) and 30(0.8) from 7(0-3), 8(0-3), 9(0-2), 10(0-3), 15(0-2), 16(0-5), 19(0-1), 20(0-1), 23(0-3) and 26(0-3.5).

Coating V: 3(0-7), 22(1.0) and 27(1.0) from 7(0-2), 3(0-3), 9(0), 10(0-2.5), 11(0-3.5), 13(0-3), 14(0-2.5),
<table>
<thead>
<tr>
<th>Coating</th>
<th>Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>1(1.0) and 2(1.0) from 3(0), 4(0), 7(0-2), 9(0-2), 10(0-2), 12(0), 15(0-3), 16(0-4), 18(0-2), 19(0), 20(0-2), 21(0-4), 22(0-4), 23(0), 24(0-3), 25(0-5), 26(0-5), 29(0-4) and 30(0-2).</td>
</tr>
<tr>
<td>IX</td>
<td>9(0), 19(0) and 20(0) from 1(1.0), 2(0.9), 3(1.0), 4(1.0), 5(1.0), 6(1.0), 7(1.0), 8(1.0), 10(1.0), 11(1.0), 12(1.0), 13(0.8), 14(1.0), 15(1.0), 17(1.0), 18(1.0), 21(1.0), 22(1.0), 23(1.0), 24(0.65), 25(1.0), 27(1.0), 28(1.0), 29(1.0), and 30(1.0).</td>
</tr>
<tr>
<td>X</td>
<td>16(0-5) and 24(0.7) from 6(1.0), 10(1.0), 11(1.0), 14(1.0), 15(1.0), 17(1.0), 18(1.0), 22(1.0), 25(1.0), 27(1.0), 28(1.0), 29(1.0) and 30(1.0).</td>
</tr>
<tr>
<td>XIII</td>
<td>1(0.85), 2(0.4) and 3(0.7) from 4(0), 5(0-2), 6(0-1), 7(0), 8(0-0.5), 9(0), 10(0), 11(0), 12(0), 13(0), 15(0), 16(0), 17(0), 18(0), 19(0), 20(0), 21(0), 22(0), 23(0), 24(0), 25(0), 27(0), 28(0), 29(0) and 30(0).</td>
</tr>
<tr>
<td>XIV</td>
<td>9(0) and 19(0) from 1(1.0), 2(1.0), 3(1.0), 5(0.9), 6(1.0), 7(1.0), 8(0.8), 10(1.0), 11(1.0), 13(0.9), 15(0.8), 17(0.8), 18(5-10), 21(1-10), 22(1.0), 23(1.0), 25(1.0), 27(1.0), 28(1.0), 29(1.0) and 30(1.0).</td>
</tr>
<tr>
<td>XVIII</td>
<td>19(0) from 6(1.0), 11(1.0) and 21(1.0).</td>
</tr>
</tbody>
</table>
| XVI      | 1(0.85) from 4(0-1), 5(0-1), 6(0-1.5), 7(0-1), 3(0-1), 9(0-0.5), 10(0-1), 11(0-1.5), 12(0-1),
13(0-3), 15(0-1), 16(0-2), 17(0-1), 18(0-1), 19(0), 20(0-0.5), 21(0), 22(0), 23(0), 24(0-0.5), 26(0-1), 27(0-1), 28(0-1), 29(0-1) and 30(0-1).

Coating XXIII: 1(0.9) and 2(0.5) from 4(0-1.5), 6(0-1.5), 19(0), 21(0-2), 22(0-1), 25(0-1) and 26(0-2).

Coating XXIV: 1(0.7) and 2(0.6) from 4(0-1), 6(0-1), 19(0), 21(0-2), 22(0-1), 25(0-1) and 26(0-2).

Coating XXV: 1(0.8) and 2(0.45) from 4(0-1), 5(0-1.5), 6(0-1.5), 7(0), 8(0-1), 9(0-1.5), 10(0-1), 11(0-1.5), 12(0-0.5), 13(0-2), 15(0), 16(0-1.5), 17(0-1), 18(0-1.5), 19(0), 20(0), 21(0-1), 22(0-2), 23(0-1.5), 24(0-2), 25(0-1.5), 26(0-2.5), 27(0-2), 28(0-2), 29(0-2.5) and 30(0-2).

Coating XXVI: 1(0.8), 2(0.4) from 4(0-1), 6(0-1), 19(0), 21(0-1), 22(0-1) and 25(0-0.5).

Coating XXVII: 1(0.85), 2(0.4) and 3(1.0) from 4(0-1), 5(0-1.5), 6(0-2), 7(0), 8(0-1), 9(0-0.8), 10(0-0.8), 11(0-1.5), 12(0-0.7), 13(0-2), 15(0), 16(0-1.5), 17(0-1), 18(0-1), 19(0-0.4), 20(0-0.5), 21(0-1), 22(0-1), 23(0-1), 24(0-1), 25(0-0.8), 26(0-1.7), 27(0-1.8), 28(0-1.8), 29(0-1) and 30(0-1.3).

Coating XXVIII: 1(1.0) and 2(0.8) from 4(0), 5(0), 6(0), 7(0), 8(0), 9(0), 10(0), 11(0), 12(0), 13(0), 15(0), 16(0), 17(0), 18(0), 19(0), 20(0), 21(0), 22(0), 23(0), 24(0), 25(0), 26(0), 28(0), 29(0) and 30(0).
Coating XXIX: 1(1.0) and 2(0.65) from 4(0-1), 5(0-1), 6(0), 7(0), 8(0-1), 9(0-1), 10(0-1), 11(0-1), 12(0-1), 13(0-0.5), 15(0), 16(0-1), 17(0-1), 18(0-1), 19(0-1), 20(0), 21(0-1), 22(0-2), 23(0-1.5), 24(0-1), 25(0-1), 26(0-1), 27(0-2), 28(0-2), 29(0-1) and 30(0-1).

Coating XXX: 1(0.9) and 2(0.55) from 5(0-1), 6(0-1), 14(0-1.5) and 21(0-1.5).

Separations achieved are recorded in tables 1, 2, 3 and 4.

DISCUSSION

Organic acids are naturally occurring materials that exist in different parts of the plants and animals. Some of the organic acids (11) are used as herbicides and plant growth regulators and they drain into water generally during monsoon period. Some acids (11) are toxic/mutagenic/carcinogenic to human beings, animals as well as aquatic organisms. Therefore, there is a growing interest in the development of new and inexpensive methods of the analysis of organic acids.

It seems that silica gel G has a very high adsorption capacity for acids. Therefore, most of the acids either have very low $R_f$ values or they tail. Charcoal and flyash show the same behaviour. Flyash is an inexpensive and easily available material. Papers (9,10) describing its utility for the purification of water have been published. Calcium sulphate is a good coating material for the separation
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Acid</th>
<th>Separated from</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cinnamic(0)</td>
<td>Adipic(0.9), alanine(1.0), ascorbic(1.0), barbituric(6-10), citraconic(1.0), citric(1.0), fumaric(0.85), hippuric(1.0), malic(1.0), maleic(1.0), nicotinic(1.0), oxalic(1.0), oxaloacetic(1.0), sulphamic(1.0), tartaric(1.0), and trans-aconitic(1.0) acids.</td>
</tr>
<tr>
<td>2. β-Naphthaleneacetic(0)</td>
<td>Adipic(1.0), alanine(1.0), ascorbic(1.0), barbituric(1.0), citraconic(1.0), citric(1.0), formic(1.0), fumaric(0.85), gallic(1.0), hippuric(1.0), malic(1.0), maleic(1.0), nicotinic(1.0), oxalic(1.0), oxaloacetic(1.0), quinic(1.0), sulphamic(1.0), tartaric(1.0), trans-aconitic(1.0), and trichloroacetic(1.0) acids.</td>
<td></td>
</tr>
<tr>
<td>3. β-Naphthoxyacetic(0)</td>
<td>Adipic(1.0), alanine(1.0), arginine HCl(0.9), ascorbic(1.0), barbituric(0.9), citraconic(1.0), citric(1.0), gallic(0.8), hippuric(0.8), maleic(1.0), nicotinic(0.8), oxalic(1.0), oxaloacetic(1.0), quinic(0.9), sulphamic(1.0), tartaric(1.0), trans-aconitic(1.0) and trichloroacetic(1.0) acids.</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2: Separations Achieved on Plates Coated with Calcium Sulphate in Organic Solvents.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Acid</th>
<th>Separated from</th>
<th>Solvent system</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Benzoic(1.0)</td>
<td>Alanine(0), arginine HCl(0), ascorbic(0), 1-aspartic(0), barbituric(0), citraconic(0), citric(0), formic(0), fumaric(0), gallic(0), hippuric(0), indole-3-acetic(0-5), malic(0), maleic(0), nicotinic(0), oxalic(0), oxaloacetic(0), quinic(0), sulphamic(0), tartaric(0), and trans-aconitic(0) acids.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Cinnamic(1.0)</td>
<td>Acetic(0), adipic(0), ascorbic(0), barbituric(0), citraconic(0-3), citric(0), formic(0), fumaric(0), gallic(0), hippuric(0), malic(0), maleic(0), nicotinic(0), oxalic(0), oxaloacetic(0), quinic(0), sulphamic(0), tartaric(0), and trans-aconitic(0) acids.</td>
<td></td>
</tr>
</tbody>
</table>

continued
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Acid</th>
<th>Separated from</th>
<th>Solvent system</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>$\beta$-Naphthalene-acetic(1.0)</td>
<td>Acetic(0), adipic(0), alanine(0), arginine HCl(0), ascorbic(0), l-aspartic(0), barbituric(0), citraconic(0), citric(0), formic(0), fumaric(0), gallic(0), hippuric(0), indole-3-acetic(0), malic(0), maleic(0), nicotinic(0), oxalic(0), oxaloacetic(0), quinic(0), tartaric(0), and trans-aconitic(0) acids.</td>
<td>Benzene</td>
</tr>
<tr>
<td>4.</td>
<td>Acetic(0)</td>
<td>Adipic(1.0), ascorbic (1.0), barbituric(1.0), benzoic(1.0), cinnamic (1.0), citraconic(1.0), indole-3-acetic(1.0), malic(1.0), $\beta$-naphthoxy-acetic(1.0), and salicylic (1.0) acids.</td>
<td>1,4-Di-o xoano</td>
</tr>
<tr>
<td>5.</td>
<td>Formic(0)</td>
<td>Adipic(1.0), ascorbic (1.0), barbituric(1.0), benzoic(1.0), cinnamic (1.0), citraconic(1.0), indole-3-acetic(1.0), malic(1.0), $\beta$-naphthoxy-acetic(1.0), and salicylic (1.0) acids.</td>
<td>1,4-Di-oxan</td>
</tr>
</tbody>
</table>
TABLE 3: Separations Achieved on Plates Coated with Calcium Sulphate Containing Other Materials in Distilled Water

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Acid Separated from</th>
<th>Material coated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cinnamic(0)</td>
<td>Adipic(0.8), ascorbic(0.75), acetic(0.9), citric(1.0), p-DAB(1 ml of)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>barbituric(0.9), benzoic(0.65), citraconic(0.9), citric(1.0), +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>formic(1.0), fumaric (0.8), gallic(0.9), hippuric(0.8), malic(0.9),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nicotinic(1.0), oxalic(1.0), oxaloacetic(0.9), phenoxycetic(0.65),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>quinic(0.9), sulphamic(1.0), tartaric(0.9), trans-aconitic(1.0) and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trichloroacetic(0.9) acids.</td>
</tr>
<tr>
<td>2.</td>
<td>α-Naphthaleneacetic(0)</td>
<td>Acetic(0.9), adipic(0.8), ascorbic(1.0),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>barbituric(0.9), benzoic(1.0), citraconic(1.0), citric(1.0), +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>formic(1.0), fumaric(0.8), gallic(0.9), hippuric(1.0), malic(0.9),</td>
</tr>
<tr>
<td></td>
<td></td>
<td>maleic(1.0), 10%</td>
</tr>
</tbody>
</table>

continued
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Acid</th>
<th>Separated from</th>
<th>Material coated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nicotinic(1.0), oxalic (1.0), oxaloacetic(1.0), phenoxyacetic(0.9), quinic(1.0), sulphamic (1.0), tartaric(1.0), trans-aconitic(1.0) and trichloroacetic(1.0) acids.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>β-Naphthalene-acetic(0)</td>
<td>Adipic(0.9), ascorbic (0.9), barbituric(0.8), benzoic(0.8), citricnic(0.9), citric(0.7), fumaric(0.8), hippuric p-DAB (0.8) malic(0.7), maleic(0.9), oxalic (0.9), oxaloacetic(0.7), phenoxyacetic(0.7), quinic(0.9), sulphamic (0.9), tartaric(0.7) and trans-aconitic(0.7) acids.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Indole-3-acetic(0)</td>
<td>Ascorbic(0.7), malic (1.0), maleic(1.0), sulphamic(1.0), oxalic + (1.0) and quinic(0.9) 1% flyash acids.</td>
<td></td>
</tr>
</tbody>
</table>
of organic acids because most of the acids move in the form of single spot, three acids remain at the point of application and only two acids, aspartic and salicylic, tail (coating I). The separation potential of calcium sulphate can be further enhanced by mixing it with other materials such as activated charcoal, calcium carbonate, flyash and p-DAB (coatings III-XX). Some important separations achieved are discussed below.

Results recorded in tables 1, 2, 3 and 4 indicate that plant growth regulators (naturally occurring inhibitors) benzoic and cinnamic acids are separated from alanino, barbituric, citric, fumaric, gallic, hipuric, indole-3-acetic and trans-aconitic acids.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Acid</th>
<th>Separated from</th>
<th>Material coated</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.</td>
<td>Oxalic (1.0)</td>
<td>Indole-3-acetic (0-2.5) and phenoxyacetic (0-2.5) acids.</td>
<td>Calcium sulphate + charcoal</td>
</tr>
<tr>
<td>6.</td>
<td>Alanino (0.9)</td>
<td>Arginine HCl (0.6) and l-aspartic (0.7) acids.</td>
<td>Calcium sulphate + silica gel G (1:1)</td>
</tr>
</tbody>
</table>
TABLE 4: Separations Achieved on Plates
Coated with Silica gel 0 (12 g) Containing p-DAB (1 ml of 10%) in Distilled Water

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Acid</th>
<th>Separated from</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alanine(0.85)</td>
<td>Acetic(0), adipic(0), ascorbic(0), l-aspartic(1.0), benzoic(0), cinnamic(0), citraconic(0), citric(0), formic(0), fumaric(0), hippuric(0), indole-3-acetic(0), malic(0), maleic(0), ( \beta )-naphthaleneacetic(0), ( \beta )-naphthoxyacetic(0), nicotinic(0), oxalic(0), oxaloacetic(0), phenoxyacetic(0), quinic(0), salicylic(0), sulfamic(0), tartaric(0), and trans-aconitic(0) acids.</td>
</tr>
<tr>
<td>2.</td>
<td>Arginine HCl (0.6)</td>
<td>Acetic(0), adipic(0), ascorbic(0), l-aspartic(1.0), benzoic(0), cinnamic(0), citraconic(0), citric(0), hippuric(0), indole-3-acetic(0), malic(0), maleic(0), ( \beta )-naphthaleneacetic(0), ( \beta )-naphthoxyacetic(0), nicotinic(0), oxalic(0), oxaloacetic(0), phenoxyacetic(0), quinic(0), salicylic(0), sulfamic(0), tartaric(0), trans-aconitic(0) and trichloroacetic(0) acids.</td>
</tr>
</tbody>
</table>

continued
TABLE 4: continued

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Acid</th>
<th>Separated from</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.</td>
<td>1-Aspartic (1.0)</td>
<td>Benzoic(0), cinnamic(0), citraconic(0), citric(0), formic(0), hippuric(0), indole-3-acetic(0), malic(0), maleic(0), $\beta$-naphthaleneacetic(0), $\beta$-naphthoxyacetic(0), nicotinic(0), oxalic(0), oxaloacetic(0), phenoxyacetic(0), quinic(0), salicylic(0), sulphamic(0), tartaric(0), trans-aconitic(0) and trichloroacetic(0) acids.</td>
</tr>
</tbody>
</table>

etc. on calcium sulphate coating in benzene; plant growth regulators (auxins) $\beta$-naphthaleneacetic and $\beta$-naphthoxyacetic acids are separated from alanine, benzoic, ascorbic, citric, fumaric, gallic, hippuric, sulphamic acids etc. on calcium sulphate containing p-DAB coating in distilled water and indole-3-acetic acid is separated from ascorbic, maleic, sulphamic, oxalic acids on calcium sulphate containing $\beta$-naphthaleneacetic coating in distilled water; herbicide, phenoxyacetic acid is separated from cinnamic, $\beta$-naphthaleneacetic, $\beta$-naphthoxyacetic acids on calcium sulphate containing p-DAB coating in distilled water; aminoacids, arginine HCl, alanine and aspartic acid are separated from benzoic, cinnamic, citric, fumaric, indole-3-acetic, $\beta$-naphthaleneacetic, $\beta$-naphthoxyacetic, phenoxyacetic, salicylic, oxalic acids etc. on silica gel G containing p-DAB coating in distilled water.
These results suggest the possible use of the above coatings in separation and identification of several organic acids.

ACKNOWLEDGEMENT

The authors are thankful to Professor Mohsin Qureshi, Chairman, Chemistry Section, Z. H. College of Engg. & Tech., Aligarh Muslim University, Aligarh for providing research facilities.

REFERENCES


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KEY WORDS: Pressure capillary spot-test, Plant Growth Regulators, Indoleacetic acid

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ABSTRACT

The capillary spot-test, a rapid, sensitive and selective technique, has been developed for the detection and determination of plant growth regulators containing indoleacetic acid. A capillary containing a dilute solution of the growth regulator has been used as detector for the determination of the plant growth regulator. The technique has been applied for the determination of plant growth regulators in wheat shoot.

INTRODUCTION

In the laboratory, p-dimethylaminobenzaldehyde (p DAB) has been used for the detection and determination of auxins, primarily and secondary auxins and esters, often in different procedures such as spot test, capillary spot test and in solution with solid state. It is known that any auxin compound can be obtained quantitatively. In 1925 Klein and Winer reported that free and combined oxalic, succinic, malic, tartaric and citric acids sublime at 10 mm pressure and 110°, 120°, 145°, 170° and 195°
respectively. They separated and determined these acids in plants by fractional sublimation. Hence, this technique seems to be promising for detecting and determining sublimable and volatile pollutants in crops, vegetation and environment. Unfortunately, efforts have not been made firstly to simplify the procedure used by Klein and Werner so that the technique can be used in the unequipped laboratories of the third world persons and secondly to test the potential of the technique for detecting and determining pollutants containing different functional groups as well as those present in a wide variety of samples. Therefore, now an attempt has been made in this direction. In the new procedure a capillary containing reagent on cotton plug is used as detector and a suction pump is used to reduce the pressure as well as a carrier of the test material. Thus the technique is named as pressure capillary spot test (PCST). The results obtained for compounds containing different functional groups are described in this paper.

**EXPERIMENTAL**

**Apparatus and Materials**

Bausch and Lomb Spectronic-20, Vaccustier Pump of 17 cm pressure (Atlantis applications engineering Pvt. Ltd. India), electrically temperature controlled water bath (Tempo, India) and aluminium block were used.

Benzene (Glaxo Laboratories Ltd., India), butanol (BDH, India), p-dimethylaminobenzaldehyde and trichloroacetic acid (Central Drug House, India) were used. All other reagents used were of analytical grade. The detector was made by placing a 1 cm long cotton plug in a glass capillary (3 mm id) and then the plug was impregnated with reagent solution containing p-DAB (1%), trichloroacetic acid (TCA, 1%) in benzene.
General Procedure

Aqueous or ethanolic test solution (0.1 ml of 1\%) was taken in a microbeaker, evaporated to dryness on water bath, one end of the detector was fixed in the mouth of the microbeaker with the help of the rubber cork and the second end was connected with suction pump by a rubber tube and then the beaker was placed in an electrically manually heated aluminium block at 180±2° for 5 min. The colour developed on the plug was noted. The test was also performed at different temperatures by the same procedure.

Semiquantitative Determination of Indole-3-Acetic Acid

Different volumes of standard ethanolic solution of indole-3-acetic acid (10-100 \(\mu\)g) were taken in a microbeaker, evaporated to dryness on water bath and then the general procedure was used to develop the colour at 180±2°. The violet colour obtained on the plug was eluted with butanol, the total volume was made up to 5 ml in a standard volumetric flask with butanol and its absorbance was recorded at 410 nm against the blank containing solution of p-DAB and TCA in benzene and butanol. The absorbance so obtained was used to make a calibration curve. To calculate analytical parameters the following relations were used.

\[
\sigma = \sqrt{\frac{(x_1 - \mu)^2 + (x_2 - \mu)^2 + \ldots}{n - 1}}
\]

\[
\text{C.V.} = \frac{\sigma \times 100}{\mu}
\]

where \(\sigma\) = standard deviation, \(x_1, x_2\) = measured values,
\[ \mu = \text{average value}, \ n = \text{number of sets and C.V. = coefficient of variation.} \]

Determination of Indole-3-Acetic Acids in Wheat Shoots

Wheat seeds were washed with distilled water. They were then sown, embryo up, on moist filter paper in petri-dishes covered with black paper at room temperature (20°), for 3 days. Shoots were cut with the help of razor and indole-3-acetic acids were eluted from 3.35 g shoots with 15 ml of butanol. Indoleacetic acids were detected and determined in 0.2 ml of this solution as above at 180 ± 2°.

RESULTS

The results obtained by PCST for different types of compounds are given in table 1. The results of detection of some compounds at different temperatures are recorded in table 2. The calibration curve is shown in fig. 1. The analytical data of the spectrophotometry are given in table 3. The molar absorptivity is found to be 5576. The percentage of plant growth regulators, indole-3-acetic acids, in wheat shoots is found to be 0.009%.

DISCUSSION

Spot tests play a very important role in preliminary analysis of a test material. Therefore different characteristics of test materials have been utilized in order to bring the specificity, selectivity and sensitivity of the particular test to a maximum. Different spot tests such as solubility test, solution test pyrolysis test, fuming off test, fusion test,
Table 1: Detection of Various Compounds by PEST

<table>
<thead>
<tr>
<th>Compound</th>
<th>sp</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>110 ± 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120 ± 2</td>
</tr>
</tbody>
</table>

**Acids**

<table>
<thead>
<tr>
<th>Compound</th>
<th>sp</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>117.9</td>
<td>NC</td>
</tr>
<tr>
<td>cis-Acetic</td>
<td>130</td>
<td>DR (20)</td>
</tr>
<tr>
<td>l-Alanine</td>
<td>314</td>
<td>NC</td>
</tr>
<tr>
<td>l-Arginine</td>
<td>224</td>
<td>NC</td>
</tr>
<tr>
<td>l-Aspartic</td>
<td>324</td>
<td>NC</td>
</tr>
<tr>
<td>Ascorbic</td>
<td>192</td>
<td>NC</td>
</tr>
<tr>
<td>Barbituric</td>
<td>248</td>
<td>DR (5)</td>
</tr>
<tr>
<td>Benzene</td>
<td>122</td>
<td>NC</td>
</tr>
<tr>
<td>trans-Cinnamic</td>
<td>136</td>
<td>NC</td>
</tr>
<tr>
<td>Citric</td>
<td>153</td>
<td>DR (20)</td>
</tr>
<tr>
<td>Citroconic</td>
<td>94</td>
<td>NC</td>
</tr>
<tr>
<td>Fusaric</td>
<td>300</td>
<td>NC</td>
</tr>
<tr>
<td>Gallic</td>
<td>220</td>
<td>R (50)</td>
</tr>
<tr>
<td>Hippurate</td>
<td>190</td>
<td>NC</td>
</tr>
<tr>
<td>Indigo-3-Acetic</td>
<td>165</td>
<td>DV (0.1)</td>
</tr>
<tr>
<td>α-Keto-lutaric</td>
<td>113.5</td>
<td>DR (20)</td>
</tr>
<tr>
<td>Malic</td>
<td>100</td>
<td>NC</td>
</tr>
<tr>
<td>Salic</td>
<td>155</td>
<td>NC</td>
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<tr>
<td>Malonic</td>
<td>135</td>
<td>NC</td>
</tr>
<tr>
<td>Niacinose</td>
<td>236</td>
<td>DR (5)</td>
</tr>
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</table>

Table 1 (continuation)
(Table 1 continued)

<table>
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<tr>
<th>Compounds</th>
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<th>120 ± 2°</th>
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</thead>
<tbody>
<tr>
<td>B-Naphthalametic</td>
<td>155</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>B-Naphthoxacetic</td>
<td>152</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Benzaldehyde</td>
<td>178*</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>128*</td>
<td>DBH (2)</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Salicylaldehyde</td>
<td>197*</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
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<td>Vanillin</td>
<td>145</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrose</td>
<td>150</td>
<td>BR (1000)</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>146</td>
<td>BR (10)</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>223</td>
<td>BR (10)</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>160</td>
<td>DBH (1)</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>185</td>
<td>BR (1000)</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>256-258</td>
<td></td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Esters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linal acetate</td>
<td>77*</td>
<td>NC</td>
<td>NC</td>
<td></td>
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<tr>
<td>Hydrocarbons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>80*</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>132</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>210*</td>
<td>NG</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>35-60*</td>
<td></td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Toluene</td>
<td>110*</td>
<td>NG</td>
<td>NC</td>
<td></td>
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</tbody>
</table>
### Phenols

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Catechol</td>
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<td>DR (100)</td>
<td>DR (100)</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>173</td>
<td>LBR (5000)</td>
<td>NC</td>
</tr>
<tr>
<td>Ortho-Nitrophenol</td>
<td>217*</td>
<td>BR (50)</td>
<td>NC</td>
</tr>
<tr>
<td>'Ortho'</td>
<td>107</td>
<td>DR (20)</td>
<td>DR (20)</td>
</tr>
<tr>
<td>Oxine</td>
<td>72</td>
<td>LBR (10)</td>
<td>NC</td>
</tr>
<tr>
<td>Phenol</td>
<td>182*</td>
<td>LR (10000)</td>
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</tr>
<tr>
<td>Pyrocatechol</td>
<td>134</td>
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<td>DR (10)</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>111</td>
<td>DR (10)</td>
<td>DR (10)</td>
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### Inorganic compounds

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</thead>
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<tr>
<td>Ammonium acetate</td>
<td>93.5</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Ammonium chloride</td>
<td>340</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Ammonium citrate</td>
<td>184</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Ammonium formate</td>
<td>116</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1339</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Calcium citrate</td>
<td>120</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>1670</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Calcium sulphate</td>
<td>128</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Potassium ferrocyanide</td>
<td>70</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td>240</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Potassium hydrogen carbonate</td>
<td>-14</td>
<td>NC</td>
<td>NC</td>
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</tbody>
</table>

(Table 1 continued)
(Table 1 continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>mp</th>
<th>Colour</th>
<th>180 + 2°C</th>
<th>120 + 2°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium acetate</td>
<td>124</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>150</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Sodium formate</td>
<td>253</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>307</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>271</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Sodium sulphite</td>
<td>Red hot heat</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Sodium sulphide</td>
<td>1180</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Zinc acetate</td>
<td>200</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Zinc sulphate</td>
<td>600</td>
<td>NC</td>
<td>NC</td>
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</table>

**Miscellaneous**

<table>
<thead>
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<th>Compound</th>
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<th>Colour</th>
<th>180 + 2°C</th>
<th>120 + 2°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovistin</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>77</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Indole</td>
<td>52</td>
<td>TV (0.01)</td>
<td>TV (0.5)</td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td>156</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>135</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Grease</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Kerosene</td>
<td>175-325</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Coconut oil</td>
<td>21-25</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Wap</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Detergent</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
<tr>
<td>Synthetic fiber (Kashmilon)</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td></td>
</tr>
</tbody>
</table>
### Abbreviations:
- **BR** = Brown
- **D** = dark
- **L** = light
- **NC** = no colour
- **R** = red
- **V** = violet
- Limit of detection in µg is given in parenthesis.
- Temperatures marked by asterisk mark (*) are the boiling points of the compounds.

### Pressure Capillary Spot Test

<table>
<thead>
<tr>
<th>Compounds</th>
<th>sp</th>
<th>Colour</th>
<th>180 °C</th>
<th>120 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal fiber</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Chlorophyll</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Protein (Bovine serum albumin)</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Resin in acetate form</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Resin in citrate form</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Resin in trichloroacetate form</td>
<td>-</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

Pyrolysis tests have been applied to aromatic compounds containing oxygen. For example, if phenolic compounds are strongly dry heated in a micro-test tube, phenol is split off that can be detected at the mouth of the test tube with a filter paper impregnated with a saturated benzene solution of 2,6-dichloroquinone-4-chloroaniline. In most cases, the quasi dry distillation produces heavy vapours and it usually requires several minutes.

Fusion test has been applied to non-volatile organic compounds such as aromatic hydrocarbons, phenols, aromatic amines, cyclic nitrogen bases, etc. These compounds undergo nitration, oxidation, deamination and oxidative cleavage on heating with concentrated nitric acid. Fusion test with benzoin can be applied for compounds which undergo a pyrohydrogenolysis.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Colour</th>
<th>100 ± 2°</th>
<th>200 ± 2°</th>
<th>400 ± 2°</th>
<th>800 ± 2°</th>
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<tbody>
<tr>
<td><strong>Acids</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Barbituric</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Indoleacetic</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td><strong>Amines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0 (1000)</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Dichloramine</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Toluene</td>
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<td>NC</td>
<td>NC</td>
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<td>NC</td>
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<tr>
<td><strong>Phenols</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Catechol</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Orcinol</td>
<td>BR (100)</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Phenol</td>
<td>BR (100)</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>BR (10)</td>
<td>R (100)</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole</td>
<td>BR (1)</td>
<td>BR (1)</td>
<td>BR (1)</td>
<td>NC</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used are defined in Table 1.

In this test hydrocyanic acid has been applied to compounds with occult acid groups. In this test hydrocyanic acid is produced at 100° and it can be detected with filter paper.
Table 3: Reliability of Colour Development Under the Standard Conditions

<table>
<thead>
<tr>
<th>Amount of Indole-3-Acetic acid taken in µg</th>
<th>Number of observations</th>
<th>mean ± C.V.</th>
<th>Absorbance at 410 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3</td>
<td>0.083 ± 0.015</td>
<td>18.07</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>0.143 ± 0.006</td>
<td>4.19</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>0.187 ± 0.015</td>
<td>0.021</td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>0.417 ± 0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>0.580 ± 0.03</td>
<td>5.17</td>
</tr>
</tbody>
</table>

Abbreviations used are defined in text.
ir'fc RMHORb, CUPTA, AND KHAN

lourteued with cyanide reagent at the mouth of the test tube. The time required is 3 min. in most of the cases. Magic acid can also be detected by heating or sintering it along with area of better biuret at 170 to 200°C. The pyrrole which results in the pyrolysis can be sensitively detected in the gas phase with a filter paper moistened with a benzene solution of TCA and p-DAB. In capillary spot test the characteristics such as nature and intensity of colour, length and direction of movement of the boundary formed are used as signals for detection while the nature and intensity of colour is the only signal available in solution. Hence it is clear that length and direction of movement of coloured boundary are additional signals available in capillary spot test. However, the capillary spot tests can be applied for solids only and it takes relatively more time. In recent years gas-liquid chromatography has revolutionized the area of analysis. It has been successfully used for analysing many complex organic mixtures with high sensitivity and specificity in minimal possible time. The principle of gas liquid chromatography suggests that the detection in the gas phase can be made prompt, sensitive and selective by the use of suction pump and capillary detector. The claim made by Klein and Weiler about the selective sublimation of carboxylic acids at reduced pressure and the results described below prove that the development of PCST is a noteworthy idea.

Table 1 shows that PCST gives colour with cis-aconitic, barbituric, citric, gallic, indole-3-acetic, α-cetoglutaric, nicotinic, and tartaric acids and their lower limit of detection at 180 °C is 20, 5, 20, 50, 0.1, 20, 5 and 20 μg respectively. Hence it is clear that PCST gives colour with
either nitro- or nitrile containing acids such as barbituric, nicotinic and indole-3-acetic or acids such as cis-aconitic, citric, gallic, $\alpha$-ketoglutaric and tartic which gives volatile substances. It has been reported\textsuperscript{11} that in solution spot test p-DAB gives colour with most of the acids given in table 1. The lower limit of detection for $\alpha$-ketoglutaric, citric, barbituric acids is found to be 10, 5 and 5 $\mu$g respectively. In fusion test\textsuperscript{12} p-DAB has also been used for the detection of organic acids. It gives brown colour with cinnamic, gallic and glutamic acids, red colour with cis-aconitic and trans-aconitic acids and yellow colour with several other acids. The lower limit of detection is 300, 50, 5, 100 and 100 $\mu$g for salicylic, citric, barbituric, cis-aconitic and trans-aconitic acids. These results show that PCST is more sensitive, selective and fast than solution test and fusion test. Feigl\textsuperscript{7} has reported that mono-, di- and polyamines can be detected selectively with p-DAB in solution state as monoamines give yellow Schiff base and di and polyamines give orange product\textsuperscript{13}. Tables 1 and 2 show that amines can be detected more selectively by performing PCST at different temperatures. Amongst carbonyl compounds only paraldehyde gives colour and its lower limit of detection is 2 $\mu$g. PCST can also be used for the detection of carbohydrates. It is very sensitive technique for the detection of maltose as its limit of detection is 1 $\mu$g.

Detection of phenols (tables 1 and 2) shows that all the eight phenols produce colour at $180 \pm 2^\circ$, four of them at $130 \pm 2^\circ$, three of them at $80 \pm 2^\circ$ and only one, resorcinol, produces colour at $60 \pm 2^\circ$. Hence it is clear that phenols can be detected selectively by performing PCST at different
temperature and pressure. p-DAB has also been used for the
detection of phenols by capillary-spot tests. However this
test is not as sensitive and selective as PCST. In solution
state as well as in solid state urea gives yellow-red colour
with p-DAB in acidic medium. It does not produce any colour
by PCST. It may be due to break down of urea in anion and
buret at 180 + 2°. Table 2 shows that indole gives colour
at 40 + 2° while none of the compounds listed in table 1 give
colour at this temperature. Thus the traces of indole can be
detected specifically in presence of different types of compounds.

Results recorded in table 3 show that PCST is not only
a device of detection but it can also be used for the quanti­
tative determination. The results obtained for the determination
of indole-3-acetic acid show that molar absorptivity by PCST
(5576) is comparable to that (6307) obtained by a sophisticated
 technique, fluorescence method. p-DAB has been used for the
spectrophotometric determination of various organic compounds with
different procedures in order to enhance its sensitivity and
selectivity. Hence it seems that the color absorptivity (5576)
obtained for reaction of indole-3-acetic acid with p-DAB can be
enhanced by altering the procedure and the sensitivity of the
method can be improved. Further research work in this direction
is in progress. PCST can also be used for the detection as well
as determination of plant growth regulators, indole-3-acetic acid
and its derivatives in wheat shoots. As mp of indole-3-acetic,
5-chloroindole-2-acetic and 5-hydroxy indole-3-acetic acids are
105°, 237° and 161° respectively, the selective detection and
determination of these plant growth regulators may be made.
possible by coupling PCST with sophisticated temperature and pressure control devices.

The results discussed suggest that PCST is a very fast, sensitive and selective technique which may be proved very useful specially in places where sophisticated instruments such as gas-liquid chromatography are not available.

Acknowledgement

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References


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A simple and sensitive redox titration of malathion insecticide

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Key words : Malathion, redox titration, potassium permanganate.

ABSTRACT

A simple, inexpensive, sensitive and safe volumetric method for the determination of malathion in emulsifiable concentrates has been developed. Potassium permanganate in alkaline medium is used as an oxidizing agent. The lower limit of determination has been found to be 0.10 mg of malathion.

INTRODUCTION

Malathion is widely used (Antonine 1984) in the control of stored grain pests, insect vectors of malaria and encephalitis, household pests, animal pests and plant pests. For crop protection malathion (50% E. C.) insecticide is generally used at the dosage rate of 400 to 800 ml per acre. To treat 1 acre of cropped area, the recommended quantity of malathion is mixed in 40 to 50 litre of water for low volume sprays and in 200 to 400 litre for high volume sprays. Thus malathion insecticide is invariably used throughout the world and there is a growing interest in the methods of analysis. The most sensitive and rapid method of determination has been found to be gas chromatography (Hill et al. 1967). In laboratories where sophisticated and costly instruments are not available volumetric methods are used. These methods based upon the hydrolysis of malathion to give O, O-dimethylphosphorodithioate (DPD) and formation of complex/salt of DPD with Ag(I) or Cu(II) or Bi(III). The first category of volumetry is based upon the formation of white insoluble precipitate of DPD with(I) and the detection of end point with the help of dichlorofluorescein (Kolthoff et al. 1957). The second category of volumetry is based upon the determination of amount of Cu(II) or Bi(III) left after complexation with DPD (Lakshminarayana 1966). The third category is based on the oxidation of DPD.
to $O$, O-dimethylphosphoric acid with chloramine T and detection of end point iodimetrically (Meister, 1980). The number of oxidizing equivalents of chloramine T required per mole of malathion have been found to be 16. In all the three categories there is a continuous exposure to malathion. Volumetric methods of the second category are specific but they are laborious, time consuming and expensive due to the involvement of two steps namely extraction of the complex and titration of the metal ion. However, Ag(I) and chloramine T methods are simple and sensitive but they are non-selective. Kolskoff et. al. (1957) & Miles et. al. (1972) have reported that potassium permanganate can be used as a redox titrant in alkaline medium for the determination of various organic compounds such as alcohols, carboxylic acids, carbonyl compounds, phenols, sulphones and thiosulphates. Recently, Antonine (1984), Siquiroff (1976) has claimed that the oxidation is rapid and stoichiometric in buffered solutions and potassium permanganate can be used as a redox titrant for the above compounds. This simple, inexpensive and safe titration has not been applied for the determination of pesticides so far. Therefore an attempt has been made to test the potential of the redox titration for the determination of malathion. The results obtained are discussed in this paper.

**EXPERIMENTAL**

**Apparatus**

A temperature controlled electric oven (Tempo Industrial Corporation, Bombay India), magnetic stirrer with automatic temperature controlled hot plate (Made in India) and Corning glass wares are used.

**Materials**

Potassium hydroxide and sodium hydroxide (Ranbaxy Ltd., India), potassium iodide (E. Merck, India), sodium metal and sulphuric acid (Laboratory grade, BDH, India), Whatman No 1 filter paper (Whatman Ltd., England), soil and wheat grains (Aligarh), sodium thiosulphate (Pfizer Ltd., India) and potassium permanganate (S. M. Chemicals India) are used. Sodium thiosulphate and potassium permanganate solutions are standardized with potassium dichromate and sodium thiosulphate respectively.

**Standardization of Malathion**

Malathion is purified and then standardized by the method of Miguel Siquiroff. The Procedure used is given below.

Fill $300 \times 22$ mm glass column with ca 10 cm 60–100 mesh florisil; then add 1 cm anhydrous $\text{Na}_2\text{SO}_4$. Moisten column with 40 ml Petroleum ether. Add liquid sample equivalent to 0.90–1.00 g active matter to column and elute with 100 ml ethyl ether-petroleum ether (50 + 50) at the rate of 5 ml/min. Evaporate elute, dilute to 50 ml with Methanol in volumetric flask. Take
Redox Titration of Malathion

10 ml of aliquote into 250 ml conical flask with 2 ml of 3 N NaOH and 2 ml of 30% phenol solution in methanol. Shake gently and let stand 20 min. Neutralize with dilute HNO₃ to pH 6.5-8.0. Add 15 drops of indicator solution (0.1% dichlorofluorescein in ethanol), and dilute to ca 100 ml with distilled water.

Titrate with 0.1N AgNO₃ solution, to point at which precipitate formed coagulates and red colour develops on surface. Calculate the concentration of malathion as

\[ \% \text{ Malathion (w/w)} = \left( \frac{V \times N \times 165}{g \text{ sample}} \right) \]

where \( V = \text{ml of AgNO}_3 \) and \( N = \text{normolity of AgNO}_3 \)

Procedure used

Weigh accurately into a 50 ml standard measuring flask directly an amount of emulsifiable concentrates that contain 0.90-1.00 g of pure malathion. Add distilled water up to the mark, mix thoroughly. Transfer 1 ml aliquot of the sample solution into a 250 ml conical flask containing a mixture of 10 ml of 4N sodium hydroxide and 75 ml of 0.2N potassium permanganate solutions. Stopper the flask, shake well and allow to stand for 24 hr. Add 25 ml of 4N sulphuric acid, shake well and allow to stand for 2 hr., and 10 g potassium iodide and titrate it with 0.1 N sodium thiosulphate. Until the solution acquires pale yellow colour. Finally add 1 ml of 1 % aqueous starch solution and titrate dropwise upto the end point (blue to colourless).

To rectify the error due to the photo decomposition of potassium permanganate, take same amount of potassium permanganate and other reagent except malathion into a 250 ml conical flask and titrate with sodium thiosulphate after 24 hr. This procedure was also used for the diluted solutions of malathion (0.01-10.00 mg). The concentration of potassium permanganate was kept always in excess. To standardized diluted solutions of potassium permanganate 0.01N sodium thiosulphate was used. To calculate analytical parameters the following relations were used:

\[ \sigma = \sqrt{\frac{1}{n-1}\sum (x_i - \mu)^2} \]

\[ \text{C. V.} = \frac{\sigma \times 100}{\mu} \]

where \( \sigma = \) standard deviation, \( x_1, x_2 = \) measured values, \( \mu = \) average value, \( n = \) number of sets and \( \text{C. V.} = \) coefficient of variation.

Determination of Malathion in Tube-Well water

Take 50 ml tube-well water containing 10.84 mg malathion and titrate by the above procedure. The maximum error was found to be 8 %. Tube well water alike distilled water consumes no volume of potassium permanganate.
RESULTS AND DISCUSSION

The redox titration under study is a simple and sensitive method of determination of malathion insecticide in aqueous solutions. It is also an inexpensive and safe method because potassium permanganate is used as oxidant in presence of sodium hydroxide in a stoppered conical flask at room temperature, i.e. no

<table>
<thead>
<tr>
<th>Amount of Malathion taken (in mg)</th>
<th>Volume of KMnO₄ consumed (in ml)</th>
<th>Amount of Malathion found (in mg)</th>
<th>% error</th>
<th>μ ± σ</th>
<th>C. V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.68</td>
<td>41.0</td>
<td>22.222</td>
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<td>5.42</td>
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<td>0.00</td>
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<tr>
<td>1.084</td>
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<td>-0.37</td>
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</tbody>
</table>

Abbreviations used are defined in the text.
<table>
<thead>
<tr>
<th>Method</th>
<th>Samples Analysed</th>
<th>Lower Limits of Determination (in mg)</th>
<th>Interference</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentimetric Potentiometric</td>
<td>Emulsifiable concentrates, water dispersible powders and dusting powders</td>
<td>500</td>
<td>O, O-Dimethylphorodithioic acid and other polar impurities</td>
<td>Fast but costly</td>
</tr>
<tr>
<td>Agratimetric Dichlorofluorescein</td>
<td>Emulsifiers, powder formulations and liquid formulations</td>
<td>140 to 180</td>
<td>Emulsifiers formed by mixture of anionic and nonionic surfactants, e.g., toximul</td>
<td>- do -</td>
</tr>
<tr>
<td>Copper (II) Complexometric</td>
<td>Emulsifiers</td>
<td>150 to 180</td>
<td>O, O-Dimethylphosphorodithioic acid, O, O, O, O-tetramethylpyrophosphorotrithioate and bis (dimethox) phosphorothiono disulphide and other pesticides</td>
<td>Costly, time consuming laborious and injurious to health</td>
</tr>
<tr>
<td>Bismuth (III) Complexometric</td>
<td>- do -</td>
<td>200</td>
<td>- do -</td>
<td>- do -</td>
</tr>
<tr>
<td>Redox with Potassium Permanganate</td>
<td>- do -</td>
<td>0.10</td>
<td>Oxidizable materials</td>
<td>Simple inexpensive sensitive and safe but time consuming</td>
</tr>
</tbody>
</table>
exposure to malathion vapours. Results recorded in Table 1 show that the method can be used for determining malathion in wide range of concentration (0.10 to 22.0 mg). Table 2 shows that amongst various volumetric methods the present method is ultra sensitive. The following reaction scheme may be proposed for the redox reaction

\[
\begin{align*}
10\text{CO}_2 + 2\text{H}_2\text{SO}_4 + \text{H}_3\text{PO}_4 + 16\text{H}_2\text{O} \\
\end{align*}
\]

The number of oxidizing equivalents required per mole of malathion are calculated to be 64. The experimental value was found to be 62.6. This method has limited scope as it cannot be used in the presence of redoxable materials. Therefore erroneous results were obtained for the determination of malathion in wheat and soil. The following redox procedures were also studied but unsatisfactory results were obtained.

a) Direct titration of malathion with potassium permanganate in presence of diluted sulphuric acid at 80°C.

b) Hydrolysis of malathion in alkaline medium (Wayne et al., 1972) and then titration with potassium permanganate in acidic medium.

c) Hydrolysis of malathion by sodium metal in alcoholic medium (Wayne et al., 1972) and then titration with potassium permanganate in acidic medium.

The results discussed above suggest the possible use of the method for standardization of malathion in emulsifiable concentrates and determining traces of malathion in ground water and air. It can also be coupled with paper chromatography and thin layer chromatography for determining malathion in wheat, soil, fruits etc.
Redox Titration of Malathion

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