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Review

Chromatographic and related spot tests for the detection of water pollutants

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Abstract

Spot test analyses are used in the preliminary qualitative analysis of pollutants in water. In this review, recently reported spot tests are described. The limit of detection of the spot tests is at the microgram level.

Keywords: Reviews; Water analysis; Environmental analysis; Spot tests; Carbaryl; Aldehydes; Sugars; Pesticides; Nitrites; Carboxylic acids

Contents

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1. Introduction

Generally, a preliminary examination of the test material is required before undertaking sophisticated analysis. Spot tests are simple, sensitive and selective and they have been found to be extremely useful for preliminary on field characterization of sample materials.

The advent of colour reactions made it possible to develop ultra-sensitive and specific spot tests. However, the different characteristics of the test materials have been utilized in order to bring the specificity, selectivity and sensitivity of a particular test to the maximum.

Various spot tests, such as pyrolysis, fuming-off, fusion, sinter, solid-state, paper, fluorescence, enzymatic, ion-exchange, and drop tests were described 30 years ago in Feigl's book [1] for the detection of pure compounds and also the impurities present in trace amounts in purified compounds. Subsequently these tests were made more relevant by utilizing chromatographic properties such as R_F values, sublimation or evaporation at a particular temperature and retention time of the test material. Efforts have also been made to modify the procedures of the already known spot tests [1] in order to utilize such tests in the analysis of pollutants in water. Some versatile tests, such as the column chromatographic, gas chromatographic–pressure capillary spot, paper chromatographic (PC), thin-layer chromatographic (TLC), enzymatic TLC, bubble spot and drop spot tests, have been recently developed and utilized for the detection of pollutants.

The tests which have been used or can be used for detecting pollutants in water are reviewed in this paper.

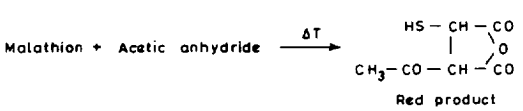

Fig. 1. Tenatative reaction mechanism of malathion with acetic anhydride.

2. Spot tests

2.1. Solution-state spot tests

2.1.1. Detection of malathion using column chromatography

A specific colour spot test [2] was developed for the detection of malathion residues in water. In this test, activated charcoal was used to recover and concentrate malathion from water samples, then 2–3 drops (0.02–0.03 ml) of the malathion solution was hydrolysed by treating it with potassium hydroxide solution to give potassium fumarate, evaporated to near dryness and heated with acetic anhydride. Formation of a red colour indicated the presence of malathion. The limit of detection was 1 μg. The tentative reaction mechanism is shown in Fig. 1.

The spot test can be successfully used for the detection of malathion in acidic, basic and saline water. Soap, detergents, ureas and some other nitrogen compounds which cause water pollution do not interfere in the test. Carbohydrates such as dextrose, maltose and lactose, organic acids such as ascorbic, citric, fumaric, α-ketoglutaric, maleic, malonic and oxaloacetic acids and iron(III) salts give a colour in this test. These are naturally occurring substances which can find their way into water from fruits and vegetables and, as such, they may interfere in the test.

2.1.2. (a) Detection of carbaryl

Diazobenzenesulphonic acid (**II**) obtained by diazotizing sulphanilic acid (**I**) with nitrous acid in the presence of hydrochloric acid, couples instantly with the hydrolysis products (naphthols) of carbaryl and aromatic amines [3] to produce acidic or basic dyestuffs. The limits of detection

Table 1
Detection of carbaryl and related compounds by the Ehrlich diazo test

Compound	Colour produced	Limit of detection (μg)
Phenol	Reddish yellow	0.2
Diethylaniline	Reddish brown	2.0
Resorcinol	Reddish brown	0.8
Dimethylaniline	Reddish brown	15.0
α-Naphthol	Reddish brown	2.0
β-Naphthol	Yellow-brown	5.0
m-Hydroxybenzaldehyde	Brown-yellow	0.1
p-Hydroxybenzaldehyde	Orange-red	50.0
p-Hydroxydiphenyl	Red	5.0
Tyrosine	Red	1.5
Aniline	Greenish yellow	5.0

of α- and β-naphthol were 2 μg (reddish brown) and 5 μg (yellow brown), respectively. The results obtained are given in Table 1. This test is widely used for detecting physiologically important substances, especially derivatives of phenol and imidazole. Nitrogen-containing heterocyclics such as thymine, thiamine (vitamin B_1), primary aliphatic nitro compounds and aldehyde arylhydrazones also gave a positive response.

To perform the test, one drop of a 0.5% solution of sulphanilic acid in 2% hydrochloric acid and the same volume of 0.5% alkali metal nitrite solution were mixed and then one drop of test solution was added to the mixture, followed by one drop of 10% sodium carbonate. The production of colour within 1–2 min was taken as a positive response. A blank test was recommended in this case.

2.1.3. (b) Detection of carbaryl

Nitrous acid containing mercury(II) nitrate reacts with phenols [4,5] to give a red colour or a yellow precipitate which dissolves in nitric acid to form a red solution (Millon's test). The reaction probably depends on the formation of a nitro compound, which then reacts with the phenol. Both aniline and phenol ethers show this reaction, since they produce phenol on boiling with nitrous acid. Di-*o*- and di-*m*-substituted phenols such as picric acid do not react, nor do hydroxy-

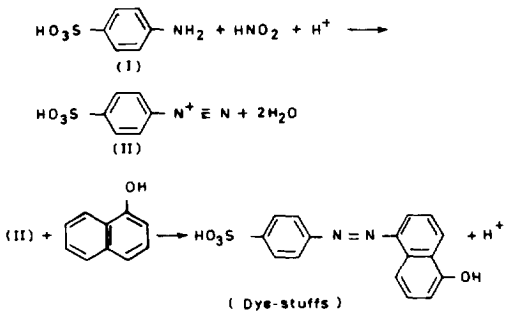

Fig. 2. Tentative reaction mechanism of α-naphthol with diazotized sulphanilic acid.

anthraquinones (Table 2), α- and β-naphthol produce a red colour and their limit of detection is 1 μg.

To perform the test, one drop (0.01 ml) of the aqueous test solution was mixed with a drop of reagent, mercury–fuming nitric acid–water (1:1:2), for a few minutes. If no colour was produced, the mixture was briefly heated to boiling. A red colour was observed if phenols were present.

Table 2
Detection of carbaryl and other compounds by Millon's test

Compound	Colour	Limit of detection (μg)
Phenol	Red	1.0
Resorcinol	Red	0.5
Pyrocatechol	Red	5.0
Hydroquinone	Red	10.0
Orcinol	Red	5.0
Phloroglucinol	Red	5.0
Pyrogallol	Red	5.0
p-Nitrophenol	Red	2.0
Salicylaldehyde	Red	5.0
m-Hydroxybenzeldehyde	Red	10.0
p-Hydroxybenzaldehyde	Red	1.0
Protocatechuic aldehyde	Red	4.0
Vanillin	Red	4.0
Methyl salicylate	Red	1.0
p-Hydroxybenzoic acid	Red	2.0
Methyl *p*-hydroxybenzoate	Red	1.0
α-Naphthol	Red	1.0
β-Naphthol	Red	1.0
o-Hydroxyquinoline	Red	0.5
m-Hydroxycinnamic acid	Red	5.0

2.2. Solid-state capillary spot tests

2.2.1. Detection of carboxylic acid and ureas

A capillary was filled with an equimolar mixture of *p*-dimethylaminobenzaldehyde and N-methylurea (reagent for acid) or *p*-dimethylaminobenzaldehyde and phthalic acid (reagent for ureas) by continuous tapping and the solid test material was added to the open end. Both ends of the capillary were closed with cotton plugs with a further seal of plasticine. Then the capillaries were kept in an oven at a given temperature for a desired period of time. The colour, length and direction of movement of the reagent/test material boundary were recorded after a definite interval of time. These tests utilize the above three properties of the coloured boundary for detection and semi-quantitative determination. Four combinations were tested: (i) undried materials in unsealed capillaries, (ii) undried materials in sealed capillaries, (iii) dried materials in unsealed capillaries and (iv) dried materials in sealed capillaries.

This test [6] was applied to the selective detection of carboxylic acids (tartaric, citric, malic, phthalic, maleic, salicylic and malonic acids), ureas (urea, thiourea, N-methylurea, 1,3-dibutylthiourea, allylthiourea, phenylthiourea and phenylurea) and phenols (catechol, resorcinol, pyrogallol, *m*-nitrophenol and phloroglucinol). The limit of detection is 20 μg.

The colour produced and other characteristics of the test are given in Table 3. The results obtained indicate that the colour, the length and the direction of movement of the boundary can be used for detection. This is an advantage of the solid-state spot test since only the nature and intensity of the colour can be used for analytical purposes in solution-state tests. It appears that the proposed method is selective for the compounds studied and can be made more selective by keeping the capillary longer at a particular temperature and by diluting the test substance.

2.2.2. Detection of aldehydes

The above technique was also used for the detection of some aldehydes and sugars [7,8] using diphenylamine hydrochloride as a chromogenic reagent. The colour and the fluorescence of the product at the junction were de-

Table 3
Solid-state capillary spot test for carboxylic acids

Compound	Colour at junction	Colour of boundary	Direction of movement	Length of boundary in 2 h (mm)	State
Tartaric acid	Orange	Yellow	→	2	–
Citric acid	Orange	Light green	→	5	–
Malic acid	Orange	Light green	→	2	–
Phthalic acid	Green	Green	→	2	–
Maleic acid	Light green	Light green	→	3	–
Salicyclic acid	Green	Light green	→	6	Gap at junction
Malonaic acid	Greenish yellow	Light green	→	2	Gap at junction
Urea	Green	Light green	←	2	–
Thiourea	Greenish yellow	Green	←	2	–
N-Methylurea	Bright yellow	Bright yellow	→	4	–
			←	2	–
1,3-Dibutylthiourea	Yellow	Yellow	←	6	Gap at junction
Allythiourea	Reddish yelllow	Yellow	→	6	–
Phenylthiourea	Yellow	Yellow	→	2	–
Phenylurea	Greenish yellow	Greenish yellow	→	2	–
			←	4	–

termined by a starting from various synthetic mixtures of the solid test material with sodium chloride. Capillaries containing diphenylamine hydrochloride and various concentrations (10–100%) of *o*-nitrobenzaldehyde in starch were kept at 32°C for 4 h and the colour intensity of the product formed at the junction was found to be proportional to the concentration of *o*-nitrobenzaldehyde. The results obtained are recorded in Table 4.

It is clear that the capillary solid-state spot tests are more selective than direct spot tests. Their sensitivity can be enhanced by carrying out the test at elevated temperature and for a long duration. These tests are of greater significance for compounds having high melting points.

2.3. GC–pressure capillary spot test

2.3.1. Detection of phenols

An aqueous or ethanolic test solution was taken in a micro beaker, evaporated to dryness, one end of the detector was fixed in the mouth of the microbeaker with the help of a rubber cork and the second end was connected with a suction pump using rubber tubing. Then the beaker was heated at 180 ± 2°C for 5 min and the pressure of the system was reduced. The change in the colour of the detector plug was noted. The detector was made by placing a cotton plug (1 cm long) in a glass capillary (3 mm I.D.). The plug was impregnated with chromogenic reagent, a 1% solution of *p*-dimethylaminobenzaldehyde and trichloroacetic acid in benzene. The test was used for the detection of pollutants containing amino, carbonyl, carboxylic or phenolic groups. The semi-quantitative determination of the plant growth regulators indoleacetic acids [9] in wheat shoots was successfully achieved. The limit of detection was 0.1 μg. The results obtained are given in Table 5.

Different spot tests, such as vapour-phase solubility, pyrolysis, fuming, sinter and capillary spot tests, have been used for detection. Amongst these tests, vapour-phase detection has been found to be the most sensitive and selective and can be improved further by fitting a device to maintain the required temperature and pres-

Table 4
Solid-state capillary spot test for aromatic aldehydes and sugars

Compound	Solution state		Solid state	
	Colour	Limit of detection (μg)	Colour	Limit of detection (μg)
Aromatic aldehydes				
p-Aminobenzaldehyde	Yellow	2.00	Yellow	2.00
p-Chlorobenzaldehyde	Green	80.00	Light green	60.00
p-Diemthylaminobenzaldehyde	Yellowish green	1.00	Yellowish green	0.04
m-Hydrobenzaldehyde	Greenish yellow	100.00	Light yellow	60.00
p-Hydrobenzaldehyde	Greenish yellow	100.00	Greenish yellow	2.00
o-Nitrobenzaldehyde	Yellow	3000.00	Light yellow	100.00
p-Nitrobenzaldehyde	Yellow	400.00	Yellow	100.00
o-Vanillin	Yellow	1000.0	Yellow	12.00
Sugars				
Arabinose	Blue-violet	7.50	Blue-violet	3.00
Fructose	Blue-black	5.40	Blue-balck	1.80
Galactose	Green-blue	80.00	Light blue	50.00
Glucose	Green-blue	39.60	Light green	19.80
Phamnose	Blue-brown	36.40	Blue-brown	27.00
Sucrose	Green-blue	50.00	Blue	30.00
Trehalose	Light blue	100.00	Blue	50.00

Table 5
Pressure capillary spot test for the detection of nitrogen-containing pollutants in environmental samples

Compound	Colour at 180 ± 2°C	Limit of detection (μg)
Barbituric acid	Dark red	5.00
Indole-3-acetic acid	Dark violet	0.10
Nicotinic acid	Dark red	5.00
Paraldehyde	Dark red	2.00
o-Nitrophenol	Bright red	50.00
Pyrogallol	Dark red	10.00
Resorcinol	Dark red	10.00
Indole	Dark violet	0.01
Glucose	Bright red	10.00
Maltose	Dark bright red	1.00

sure. The results suggest that the pressure capillary spot test is a very fast, sensitive and selective technique which may be very useful especially when techniques such as gas chromatography are not available.

2.3.2. Detection of organophosphates

The test material, an insecticide [10], was placed in a micro test-tube along with 0.25 ml of aqueous ammonium chloride (0.5%) and 5–10 mg of zinc dust. A capillary was fixed in the tube and the second end of the capillary was connected to a Vacuustier pump. The solvent was removed by heating the tube under reduced pressure. Then this capillary was replaced by a capillary detector containing a cotton plug impregnated with chromogenic reagent, *p*-dimethylaminobenzaldehyde (10%) and trichloroacetic acid (20%) in benzene. The contents of the test-tube were heated at elevated temperature under reduced pressure. The colour developed on the plug was recorded. The results obtained are given in Table 6.

The test has been found to be simple, inexpensive, selective and sensitive for the detection of trace levels of organophosphorus insecticides such as malathion, formothion, thiometon, dichlorvos, methyl parathion, dimethoate and phosphamidon in water, soil and the leaves of *Citrus medica* (common lemon plant). The tentative reaction mechanism is given in Fig. 3.

Table 6
Pressure capillary spot test for the detection of trace levels of organophosphorus insecticides in water

Insecticide	Colour	Limit of detection (μg)
Malathion	Dark pink	2
Formothion	Dark orange	5
Thiometon	Dark red	10
Dichlorvos	Dark yellowish red	4
Methyl parathion	Dark bright red	4
Dimethoate	Dark orange	6
Phosphomidon	Dark red	8

2.4. PC–paper spot test

2.4.1. Detection of carbaryl

a paper strip (14 × 3.8 cm) was divided into seven equal divisions (2 × 3.5 cm) and the centre of each division was marked in order to spot the test solution at the centre [11]. The marked strip was impregnated with sodium hydroxide solution

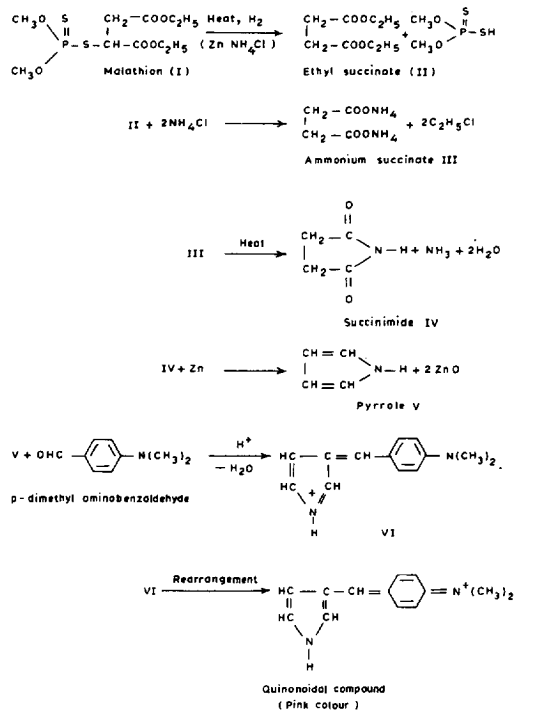

Fig. 3. Tentative reaction mechanism of malathion colour reaction.

for 30 s, the excess solution was drained off, the strip was allowed to dry at room temperature by placing it on a filter-paper sheet and then stored in a polyethene bag. A standard solution of an insecticide, carbaryl (0.01 ml), equivalent to 25, 50, 75, 100 and 125 μg, was spotted at five different marked places on the paper strip. The same volume (0.01 ml) of the unknown solution was spotted at the sixth place on the marked test paper. In this fashion the distance between two spots was kept at 2 cm. The spots were dried by blowing hot air over the strip, then the strip was immersed in a watch-glass full of chromogenic reagent, 2,6-dichloroquinone-6-chloroimine (0.1%) in benzene. Semi-quantitative determination was effected on the basis of comparison of the intensities and natures of the colours of the spots. Colour formation is based on the hydrolysis of carbaryl to give α-naphthol, which reacts with the reagent to produce indophenol, that turns blue with a violet tinge in alkaline medium. The test paper has been successfully used for the detection and semi-quantitative determination of the insecticide carbaryl in water. The results are recorded in Table 7.

Table 7
Paper spot test for the detection of carbaryl and related compounds

Test material	Colour	Limit of detection (μg)
Aldrin	No colour	–
Bavistin	Blue	20.0
BHC	No colour	–
Carbaryl	Blue with violet tinge	1.2
Catechol	Blue with violet tinge	0.5
Cresol red	No colour	–
2,4-D ester	Greenish blue	2.0
2,4-D-Na	Light blue	20.0
Detergent	No colour	–
Dimethoate	No colour	–
Endosulfan	No colour	–
Lemon juice	No colour	–
Malathion	No colour	–
Methyl parathion	No colour	–
Milk	No colour	–
α-Naphthol	Blue with violet tinge	0.06
Phenol	Blue	1.0
Phosphamidon	No colour	–
Pyrogallol	No colour	–
Resorcinol	Reddish violet	–
Soap	No colour	–
Soil extract	No colour	–
Thymol	Blue	0.5

2.5. TLC–thin-layer spot test

2.5.1. Detection of carbaryl

A glass plate was coated [12] with a slurry of silica gel G–water (1:2) to a thickness of 0.25 mm and the plate was activated at 110°C for 1 h. An ethanolic carbaryl solution (0.01 ml, 10 μg) was spotted on the plate and then the plate was developed in hexane–acetone (4:1) to a height of 10 cm. The plate was dried in air and sprayed with 1% copper(II) chloride solution followed by 0.1% ammonium metavanadate reagent. A violet spot of $R_F = 0.45$ conforms the presence of carbaryl. Other insecticides such as malathion, parathion, dimethoate, sumithion, Ekalax, endrin, DDT, gammexane and endosulfan do not produce a colour. However, α-naphthol, a hydrolysis product of carbaryl, gives a spot of the same colour ($R_F = 0.54$). Commercial carbaryl gave two spots of $R_F = 0.45$ and 0.54, demonstrating the presence of α-naphthol in carbaryl. Other carbamate insecticides such as baygon, carbofuran and zineb do not interfere. The limit of detection was 10 μg for carbaryl and 1 μg for α-naphthol. A tentative reaction mechanism is given in Fig. 4.

Another chromogenic reagent, alkaline hexacyanoferrate(III) solution, was also sprayed. This

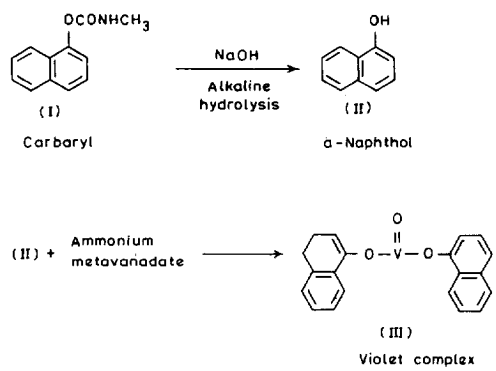

Fig. 4. Tentative mechanism of reaction of carbaryl with ammonium metavanadate.

reagent gave distinct violet spots for carbaryl and α-naphthol ($R_F = 0.45$ and 0.54, respectively). The limit of detection was 1 μg for both carbaryl and α-naphthol. Hence this reagent was claimed to be more sensitive than the former. The latter reagent does not give a colour with baygon and carbofuran but produces a colour with their hydrolysis product, catechol.

2.5.2. TLC separation, detection and determination of carbamate pesticides in water

At present it is difficult to find a single procedure suitable for the analysis of most carbamates. For example, the widely used GLC analysis of intact N-methylcarbamates is hindered by their thermal instability and the lack of sensitivity of the electron-capture detector towards the compounds. The TLC procedure given below [13] has been found to be suitable for the separation of the carbamates both from each other and from their degradation product (phenols). Since the technique does not require sophisticated instrumentation, it can be used successfully for the field detection of carbaryl. The limits of detection and determination are 20 and 70 μg, respectively.

Silica gel G (25 g) in distilled water (68 ml) was coated (0.5 mm) on glass plates. The plates were dried at 110°C for 1 h and pesticide solutions in ethyl acetate were spotted on the plates and developed in different solvents. Spots of carbamates, phenols and *p*-napahthoxyacetic acid were detected by spraying the plates with potassium hydroxide solution followed by *p*-nitrobenzenediazonium tetrafluoroborate solution. Orangish brown spots on a white background were observed for carbaryl, phenol, α-naphthol, propoxur, bavistin, *p*-naphthoxyacetic acid, *o*-nitrophenol and *p*-chlorophenol, but carbofuran appeared as a purple spot. Detection was effected on the basis of colour and R_F value while quantification was performed either by simple calculation of spot areas from their horizontal and vertical dimensions, or by the area–mass, method in which the adsorbent incorporating the spot was scraped from the plate and weighed accurately. A calibration graph was obtained by plotting the amount of carbaryl (μg) against the area of the spot (mm^2) or the mass of silica gel G removed from the plate (mg).

2.6. Enzymatic thin-layer spot test

2.6.1. Detection of fenitrothion

A simple enzymatic method was described [14] for the field TLC detection and determination of fenitrothion as fenitrooxon in water with pig-liver acetone powder as an enzyme source. The limit of detection of fenitrooxon at the nanogram levels and the limit of determination is 5–50 ng. In this method, standard pesticide solutions (1–10 μl) in acetone were spotted on a dried TLC plate (coated with silica gel G) and then exposed to bromine vapour. The plate was exposed to air, developed in acetone–hexane (1:9), dried, sprayed with the pig-liver acetone powder suspension and kept in a moist atmosphere for 10 min. It was then sprayed with α-naphthyl acetate solution in acetone, replaced in the moist atmosphere for 2 min and sprayed with *p*-nitrobenzenediazonium fluoroborate in acetone. A white spot that appeared on an orange-red background confirmed the presence of fenitrothion (as fenitrooxon). Determination was achieved by both the area measurement and area–mass methods. The method can be used for the field analysis of water for fenitrothion residues. The water for the moist atmosphere for enzyme incubation can be boiled on a portable stove and in hot climates the hexane can be evaporated by the heat of the sun. In cold climates the hexane can be evaporated by placing the petri dish on a metal plate previously warmed to about 40–45°C.

2.6.2. Detection of organophosphates

Heyndrickx et al. [15] reviewed cases of parathion poisoning in humans. They determined the distributions of parathion in various organs using fresh horse plasma and α-naphthyl acetate after separating the pesticides by TLC. The detection limit was 0.05 μg for fenthion. The test can be successfully applied to the detection of fenthion in biological fluids. Enzymatic thin-layer spot tests are versatile and sensitive for detecting pesticides in water.

2.7. Ion-exchange spot test

2.7.1. Microgram detection of sulphur, chlorine, iodine and nitrogen in pesticides

Ion-exchange resin beads [3,4] in the nitroprusside form were placed on a spot plate and five or six drops of test solution (sodium fusion extract) were added. The resin beads turn violet and later on become black if the test solution contains sulphur. A test solution free from sulphur was divided into two parts. To one part, 4–5 resin beads in the Ag^+ form were added; if chloride is present, a white precipitate develops on the resin surface which later (after 2–3 min) turns black. To the second part, 4–5 resin beads in the OH^- form and one drop of 30% hydrogen peroxide were added, followed by a few drops of chlorine water. The resins were removed from the solution and treated with 1% starch solution in 10% acetic acid. If the resins turn deep violet, iodine is present. The solution left after removing the resins was evaporated to dryness and the residue was dissolved in 1 drop of distilled water, treated with 4–5 resin beads in the OH^- form, one drop of 0.01% copper sulphate and a few drops of 5% ammonium molybdate solution. The solution was finally treated with concentrated hydrochloric acid. Development of a blue colour on the resin surface shows the presence of nitrogen. The test has been successfully applied for the simultaneous detection of nitrogen, sulphur, chlorine and iodine when they are present in microgram amounts in a given organic mixture, i.e., a pesticide formulation [16]. This test needs an enrichment or preconcentration method to be applicable to water pollutants.

2.7.2. Detection of nitriles

Cation-exchange resins in the hydrogen form are very effective in the hydrolysis of unsubstituted amides and imides [17,18]. The corresponding acid and ammonium salts are formed, and the resin beads in the ammonium form give a red or pink colour with Nessler's reagent. Nitriles are not hydrolysed, hence this test is negative. Nitriles can be hydrolysed either with sodium hydroxide or with dilute sulphuric acid to give ammonium ion, which can be tested with Nessler's reagent. Unsubstituted amides or imides are also hydrolysed with dilute sulphuric acid and thus also give a positive test. Therefore, the following two procedures have been used for the detection of nitrile.

Test I: To one drop of each of the test solutions and water, two drops of concentrated sulphuric acid were added in a semi-micro test-tube. The contents were heated slowly for a few minutes on a low flame. A few resin beads and one drop of water were added and heated again (not boiled). The beads were separated by decantation, washed with 4–5 portions of conductivity water, dried by blotting with filter-paper and placed on a white spot plate. A drop of Nessler's reagent was added. A pink or red colour on the resin beads indicates a positive test.

Test II: A few milligrams of test substance were mixed with two drops of water in a semi-micro test-tube. A few resin beads were added and heated on a low flame (not boiled). The beads were separated, washed, blotted, placed on a white spot plate and tested with a drop of Nessler's reagent. A pink colour indicates a positive test.

Nitriles give a positive response with Test I and a negative response with Test II. For unsubstituted amides and imides, both tests are positive. It is therefore possible to distinguish between unsubstituted amides and nitriles. The results are given in Table 8.

Table 8
Ion-exchange spot test for the detection of nitriles

Nitrile	Amount detected (mg)	Limiting dilution
Acetonitrile	1.05	1:500
Propionitrile	0.137	1:500
Butyronitrile	0.307	1:150
Acrylonitrile	0.215	1:200
Benzonitrile	1.03	1:500
Chloroacetonitrile	0.377	1:150
Cyanoacetic acid	0.425	1:100

2.7.3. Detection of aldehydes

About 5–10 ion exchange beads [19] in the H^+ form were placed in a micro test-tube and one drop (0.05 ml) of the test solution, four drops of a saturated mixture of HCN and NaCN solution and one drop of concentrated sulphuric acid were added. The contents were heated to boiling using a low flame (caution: take precautions against harmful vapour of HCN gas which evolves during heating). The resin beads were washed with demineralized water, blotted dry with a qualitative filter-paper (quantitative filter paper contains ammonium ions) and then a drop of Nessler's reagent was added. A reddish pink colour develops at once on the bead surface if the test solution contains aldehyde. The sensitivity of the test may be improved if freshly regenerated resin in the wet form is used. The results are given in Table 9.

When aqueous solutions of aldehydes are heated with saturated NaCN containing HCN, together with a slight excess of sulphuric acid and beads of cation-exchange resin, cyanohydrins are formed. The resin in the hydrogen form catalyses the hydrolysis of the cyanhydrins to carboxylic acids and ammonium ions. The ammonium ions are retained by the resin and are subsequently detected in the beads with Nessler's reagent. The reaction sequence is an follows:

$$RCHO + CN^- \rightleftharpoons RCH(O^-)CN \quad (1)$$

$$RCH(O^-)CN + H_2O \rightarrow RCH(OH)CN + OH^- \quad (2)$$

Table 9
Ion-exchange spot test for the detection of aldehydes

Aldehyde	Concentration (M)	Volume taken (ml)	Amount detected, (μg)
Crotonaldehyde	0.005	0.05	17.5
Anisaldehyde	0.01	0.05	40.8
Benzaldehyde	0.01	0.03	31.8
Formaldehyde	0.01	0.03	9.0
Salicyaldehyde	0.01	0.03	36.6
Cinnamaldehyde	0.01	0.03	39.6
Propionaldehyde	0.01	0.03	17.44
Isovaleraldehyde	0.01	0.03	25.9
Acetaldehyde	0.01	0.03	13.2
p-Dimethylbenzaldehyde	0.01	0.03	44.7

$$RCH(OH)CN + H_2O + \text{Res-}H^+ \rightarrow RCH(OH)COOH + \text{Res-}NH_4^+ \quad (3)$$

$$\text{Res-}NH_4 + \text{Nessler's reagent (alkaline)} \rightarrow \text{red beads} \quad (4)$$

A number of ketones were tested by this method and gave a negative test. The reason is that in the case of ketones, reaction 2 is generally reversible, whereas in the case of aldehydes and most non-hindered ketones, the formation of cyanhydrins is essentially quantitative if strong acid is added to the mixture containing sodium cyanide.

As expected, a positive test was obtained with nitriles, unsaturated amides and imides. To distinguish among the three functional groups, three tests are recommended, i.e., the ion-exchange amide test [16], the ion-exchange nitrile test [17] and the present ion-exchange aldehyde test. Results of the three tests are summarized in Table 10.

2.8. Bubble spot test

2.8.1. Detection and semi-quantitative determination of trace levels of 2,4-D and related compounds

a sensitive and selective novel technique, the bubble spot test [20], has been developed for the detection of phenoxy herbicides. The test material (one drop) was taken in a micro test-tube (A) and evaporated to dryness, the tube was cooled to room temperature and $ZnSO_4 \cdot 7H_2O$ (35 mg) was added and triturated with a glass rod. The reagent was taken in a U-tube through end B, tube (A) was stoppered and tube B was connected to a suction pump via a rubber tube. About 2 cm of the lower portion of the apparatus

Table 10
Ion-exchange spot test for aldehydes, amides, anitriles and other organic compounds

Compounds	Test I	Test II
Nitriles	Positive	Negative
Unsubstituted amides and imides	Positive	positive
Other organic compounds	Negative	Negative

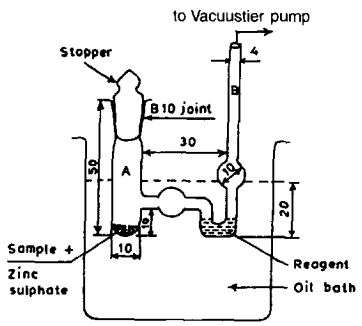

Fig. 5. Apparatus for micro analysis of herbicides in flora, soil and water. All dimensions are in mm.

(Fig. 5) was immersed in an oil-bath at a desired temperature for 2 min and the colour developed was noted. Then the coloured solution was poured into a groove in a tile in order to visualize the colour for a longer time and to use its intensity for semi-quantitative analysis. The technique has been used successfully for the detection of traces of 2,4-D acid, 2,4-D ethyl ester and 2,4-D sodium salt in formulations, leaves, soils and water (tables 11 and 12).

The chemistry of this colour reaction is not known with certainty. It is probable that phenolic chromotropic acid condenses with formaldehyde by oxidation to a *p*-quinoidal compound of violet colour (Fig. 6). This reagent has been reported for the detection of traces (0.14 μg) of formaldehyde in the presence of fructose, saccharose, furfural, arabinose, lactose and glucose. It can also be used for detecting compounds such as phenoxyacetic acid and its halogen derivatives and monochloroacetic acid, which split out formaldehyde when hydrolysed or pyrolysed.

In this reaction, sulphuric acid acts as a dehy-

Table 11
Bubble spot test for the detection of some formaldehyde-producing compounds in different solvents at 180°C

Compound	Solvent used	Colour at 180°C	Limit of detection (μg)
2,4-D acid	Benzene	Light violet	5
	Ethanol	Light violet	5
	Diethyl ether	Light violet	5
	Deep well water	Light violet	5
2,4-D ethyl ester	Benzene	Light violet	4
	Ethanol	Light violet	4
	Diethyl ether	Light violet	4
	Deep well water	Light violet	4
2,4-D sodium	Ethanol	Light violet	4
	Deep well water	Light violet	4
Glycine	Deep well water	No colour	–
Hexamine	Benzene	Light violet	0.5
	Ethanol	Light violet	0.5
Methyl orange	Ethanol	No colour	–
Methyl red	Ethanol	No colour	–

Table 12
Bubble spot test for the detection of 2,4-D acid in soil and vegetation

Soil (mg)	2,4-D (μg)	Colour	Leaves (mg)	2,4-D (μg)	Colour
50	25	Light violet	500	25	No colour
100	50	Violet	500	50	Very light violet
150	75	Dark violet	500	100	Light violet

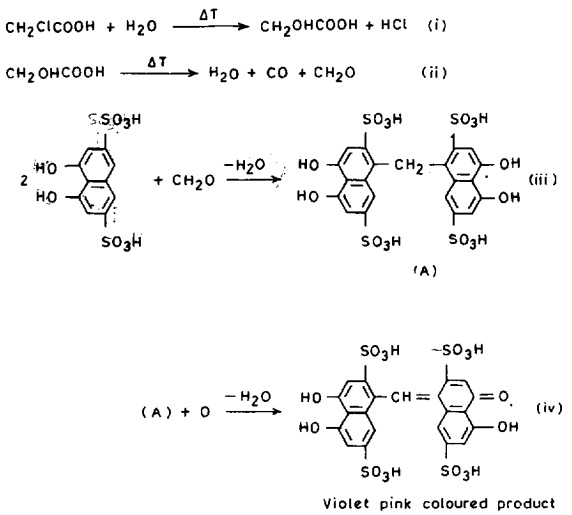

Fig. 6. Tentative mechanism of reaction of chloroacetic acid with chromotropic acid.

drating agent, oxidant and water donor, and cellulose and hydrated sulphates of zinc and manganese, which lose water when heated above 150°C, act in a similar fashion. However, their analytical utility has not been fully explored.

2.9. Drop spot test

2.9.1. Detection of carbaryl in environmental samples

A sensitive, selective and simple drop spot test [21] has been developed for the detection of carbaryl residues in traces present in environmental samples. To perform the test, the test substance was taken in a micro test-tube and a drop detector containing two drops (0.03 ml) of the reagent was fixed in its mouth with the help of a rubber stopper (Fig. 7). The contents of the test-tube were heated at 285°C for 5 min. If the detector containing the reagents, sulphanilic acid–sodium nitrite–sodium hydroxide (1:1:1), 8-hydroxyquinoline in concentrated sulphuric acid and potassium hexacyanoferrate(III), turns pink, brown and violet, respectively, carbaryl is present. The limit of detection was 1 μg. The probable reaction mechanisms are shown in Figs.

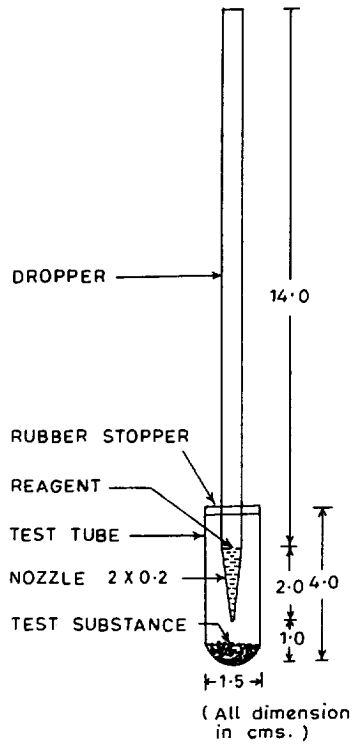

Fig. 7. Drop spot test apparatus.

8–10. The limit of detection of the test is comparable to that of methods such as GC, HPLC and HPTLC. The test can be successfully applied to the detection of carbaryl residues in dust, soil, sediments and grains.

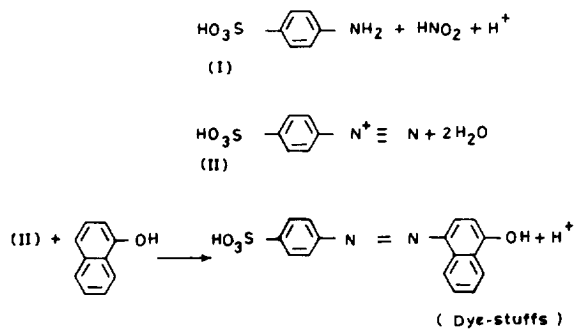

Fig. 8. Colour reaction of α-naphthol with sulphanilic acid in the presence of sodium nitrite.

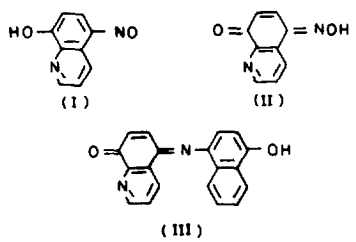

Fig. 9. Colour reaction of α-naphthol with 5-nitroso-8-hydroxyquinoline.

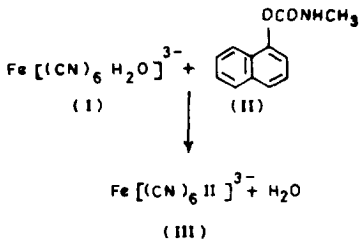

Fig. 10. Colour reaction of α-naphthol with alkaline hexacyanoferrate(III).

3. Conclusion

Spot tests are simple, inexpensive, sensitive and selective and some of them are specific. They can be used for the preliminary characterization of the test material, i.e., qualitative analysis, before costly, sophisticated and highly sensitive instrumental methods are applied. Spot test analysis is extremely useful for the field detection of pollutants in water.

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