VANADIUM PENTOXIDE AS A CHROMOGENIC SPRAY REAGENT FOR THE QUALITATIVE ANALYSIS OF SOME ORGANIC COMPOUNDS ON THIN-LAYER PLATES

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SUMMARY

The use of a very dilute solution of vanadium pentoxide in aqueous sulfuric acid as a sensitive chromogenic spray reagent for the thin-layer chromatographic detection of several classes of organic compounds is described. The limits of detection of these compounds obtained with this spray reagent were compared with those using 25 % aqueous methanol–conc. sulfuric acid (1:1). It was observed that the colors developed by acidic vanadium pentoxide solution were more intense and lasted longer than those with the 50 % sulfuric acid reagent. Further, an attempt was made to correlate the colors developed by this reagent and functional groups of the various compounds under investigation.

INTRODUCTION

Among the various facets of the thin-layer chromatographic (TLC) separation of mixtures of organic compounds into their individual components, the essential aspect that demands considerable attention is the rendering visible of colorless organic materials for their characterization and quantitation. In this endeavour, several transition metal ionic solutions (such as those of antimony, copper, cobalt, iron, cerium, manganese, molybdenum and tungsten) have been used either as chelating agents or as oxidation–reduction plus chelating agents[1].

During our investigations on the degradation of polyols (*e.g.*, carbohydrates and cyclitols), a mixture of sulfuric and nitric acids was used in the presence of catalytic amounts of vanadium pentoxide to yield green (V^{3+}) or blue (V^{4+}) solutions, depending on the nature and amounts of reductants employed. This observation prompted us to investigate the usefulness of pentavalent vanadium (a vanadate anion) as a spray reagent for TLC.

Although V^{5+} has been extensively used in quantitative oxidimetry, a literature survey indicated that the use of V^{5+} as a spray reagent has not been reported except for its concomitant use with ammonium molybdate in the identification of phospholipids[2]. This paper describes the use of V^{5+} in dilute sulfuric acid as a spray reagent for organic compounds with different functional groups, their limits of detection and

the postulation of the mechanism of chromogenicity. Furthermore, the sensitivity of this reagent is compared with that obtained from 50% sulfuric acid after developing the plates.

MATERIALS AND METHODS

All solvents were doubly distilled in all-glass apparatus.

Silica gel SGF_{254} (E. Merck, Darmstadt, G.F.R.) was used for preparing 10×20 cm plates of 0.25 mm thickness according to a standard procedure with a Desaga apparatus. The plates were activated at $110°$ for 1 h and stored in steel cabinets over anhydrous calcium chloride.

All organic compounds used in this experiment were labelled better than 98% pure and were used as obtained from the manufacturers. Analytical standards (*ca.* 4 $\mu g/\mu l$) were prepared by dissolving 7–9 mg of compounds in either water or an appropriate anhydrous solvent in 2-ml volumetric flasks. The solutions were mixed by vibrating with a vortex test-tube mixer with frequent shaking. Exact aliquots of these standards were diluted to 1 ml in a volumetric flask and made homogeneous as described above so as to obtain nine concentrations ranging from 0.1 to 40 μg per 5–8 μl of solution.

Ammonium vanadate (Fisher, Pittsburgh, Pa., U.S.A.; reagent grade) was twice recrystallized from 5% ammonia solution, washed with cold absolute ethanol followed by anhydrous diethyl ether, and vacuum dried at 135–140° for 2 h to obtain anhydrous ammonium metavanadate.

Solution A

A 1.62-g amount of anhydrous ammonium metavanadate was dissolved in 125 ml of concentrated sulfuric acid at 150° to give a homogeneous deep blood-red solution, which, after cooling to room temperature, was added to 125 ml of ice-cold water, which gave a deep orange solution (concentration of $V_2O_5 = 0.5$% in 50% v/v sulfuric acid).

Solution B

A dilute solution, one-tenth of the concentration of solution A, was prepared when required by adding 10 ml of solution A to 100 ml of water. This solution was used as the spray reagent.

Qualitative evaluation of pentavalent vanadium as a spray reagent

Thirty organic compounds (see Table I) with different functional groups, such as acids, alkaloids, amines, carbonyls, phenols, sterols, sugars and vitamins, were chosen for this study. About 80–100 μg of these substances were applied with air drying on several thin-layer plates to obtain spots of 5 mm diameter and sprayed with solution B without development. The steroids, vitamins, sugars and some aromatic amines became visible (see Table I) at room temperature. The plates were then heated in an oven at 50° for 3 min. Some phenolic compounds and organic acids were visible along with changes in color from spots previously recognized at room temperature. Two separate plates, after spraying, were placed in the oven at 110° for 3 and 7 min, respectively, to observe the color changes due to period of heating. It was

observed that most compounds showed colored spots in 3 min at 110° while some of the compounds changed their color or appeared with more intense color than the colors produced at 50°. When the plates were exposed for 7 min or more at 110°, charring was noticed in some of the compounds, especially with steroids and vitamins D$_2$ and D$_3$.

Comparison of detection limits of some organic compounds using pentavalent vanadium and 25% aqueous methanol–conc. sulfuric acid (1:1) as spray reagents

Solutions of five steroids, four fat-soluble vitamins, six phenolics (including antioxidants), two sugars, three aromatic amines and five alkaloids at nine different concentrations (0.1, 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, 25.0* and 40* μg per 5–8 μl) were prepared for this study. Five to eight microliters of every concentration of each solution were applied on two plates (using a 10-μl Hamilton syringe) to obtain spots of approximately 5 mm diameter. In the case of steroids and fat-soluble vitamins, the plates were pre-coated with 10% dodecane in hexane before spotting of the plates. The plates were then developed in acetonitrile–acetic acid (1:1) solvent system.

The phenolic compounds, after spotting on the silica gel TLC plate, were developed in solvent systems of (a) benzene–ethyl acetate–acetone (100:2:1)[3], (b) chloroform–acetic acid (1:1)[4,5], and the sugars in *n*-butanol–acetic acid–water (4:1:5)[6]. The amines and alkaloids, except solanine, were developed in ethyl acetate–methanol–ammonia (85:10:5) according to the method of Fisher *et al.*[7] and solanine was developed in the same solvent system as the sugars[6]. After drying off the solvents, one of the plates was sprayed with solution B and the other with 25% aqueous methanol–sulfuric acid (1:1)[1]. After 5 min at room temperature, the plates were heated to a specific temperature and time as determined by the previous experiment. The colored spots so developed were photographed to ensure minimum errors in detectability with the naked eye.

RESULTS AND DISCUSSION

As vanadium pentoxide in concentrated sulfuric acid was used as an oxidizing agent[8] to degrade biological materials to give either green (V^{3+}) or pale blue (V^{4+}) ionic species, depending on the resulting oxidation state of vanadium, several experiments were carried out to obtain a suitable concentration of vanadic acid in sulfuric acid. By judicious choice, it was found that a solution of 0.05% vanadium pentoxide in 5% aqueous sulfuric acid showed the best chromogenic property with most of the organic compounds under study.

The development of various colors with pentavalent vanadium reagent with organic compounds is shown in Table I. It can be seen that steroids such as cholesterol, β-sitosterol, stigmasterol and campesterol turn deep pink (50–110°) whereas, at room temperature, ergosterol and vitamins D$_2$ and D$_3$ seem to develop an olive-green color which changes to dark brown at 110° with V^{5+}. Similar color change with sterols can be observed with 50% sulfuric acid (ten times more concentrated) at 120° (ref. 1). It is interesting to note that compounds with steroidal skeletons having 3-β-hydroxy and Δ^5-unsaturation definitely show a light pink to deep pink

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* These concentrations were spotted on the plates and developed only when solutions of 10 μg per 5 μl failed to respond to the test.

TABLE I

COLOR OF SPOTS AT THE 80–100 μg LEVEL

Compound	After spray at room temperature	After heating at 50° for 3 min	After heating at 110° for 3 min	After heating at 110° for 7 min
Cholesterol	Pale yellow	—*	Dark pink	Deep purple and charred
β-Sitosterol	Pale yellow	—	Dark pink	Deep purple and charred
Stigmasterol	Pale yellow	—	Pinkish grey	Purplish grey
Campesterol	—	—	Pink to charring	Deep purple and charred
Ergosterol	Pale olive green	Light brown	Charred	Charred
Vitamin D_2	Olive green, turning brown	Deep brown	Charred	Pale brown with charring
Vitamin D_3	Olive green, turning brown	Deep brown	Charred	Pale brown with charring
tert.-Butyl-4-methoxy-phenol (BHA)	Light brown	—	Deep yellow	Deep yellow
2,6-Di-tert.-butyl-hydroxytoluene (BHT)	—	—	Light yellow	Light yellow
Phenol	—	Yellow	Very pale brown	Very pale brown
Resorcinol	—	Yellow	Pale yellow	Orange
Propyl gallate	—	Light yellow	Brownish pink	Orange
Nordihydroguaiaretic acid	Pale brown	Deep yellow	Canary yellow	Canary yellow
Sucrose	Olive green	Light green	Green	Brownish green
D-Glucose	Olive green	Light green	Green	Green to grey
Benzophenone	—	—	Brown	Pale brown
4-Methoxybenzal-dehyde	—	—	Light yellow	Light brown
trans-Cinnamaldehyde	—	—	Greenish yellow	Greenish brown
4-Chlorobenzaldehyde	—	—	Lilac	Pale brown
Glyoxal	—	—	—	—
DL-Tartaric acid	—	Light green	Green to grey	Light green to grey
L-Ascorbic acid	—	Light green	Green to grey	Light green to grey
Aniline	Blue	Turquoise green	Turquoise green	Turquoise green
Benzidine	Light brown	Light brown	Light brown	Deep brown
2-Aminothiophenol	Light pink	Light lilac	Lilac to grey	Lilac to grey
Quinine	—	—	—	—
Strychnine	—	Greenish orange	Pale red	Yellowish red
Solanine	—	—	Deep pink	Deep pink to purple
Cyclohexylamine	—	—	—	—
Triethylamine	—	Greenish yellow	—	—

* A dash indicates no change in color due to change in temperature.

color with V^{5+} at 50–110°. If this inference is correct, we can predict that the same color should be expected with solanine, which is a steroid alkaloid with a 3-β-hydroxy group and Δ^5-unsaturation together with an amino function. As a matter of fact, solanine does turn deep pink with the spray reagent in spite of the amino function present in this alkaloid. Moreover, it is obvious that quinine and cinchonine, alkaloids that contain tertiary amino groups similar to solanine, did not develop any color, indicating the ineffectiveness of the tertiary amino function to produce any color with this reagent.

Furthermore, ergosterol and vitamins D_2 and D_3, containing a 3-β-hydroxy

group and conjugated double bonds, act as powerful reductants towards this spray reagent. These conjugated dienes and trienes react vigorously with V^{5+} to give the green trivalent vanadium below 50°. The charring or deep brown color at higher temperatures may be due to insufficient oxidant and excess of sulfuric acid.

The antioxidants *tert.*-butyl-4-methoxyphenol (BHA), 2,6-di-*tert.*-butyl-4-hydroxytoluene (BHT), propyl gallate and nordihydroguaiaretic acid give yellow spots with the spray reagent at 110°. However, simple phenols, such as phenol and resorcinol, although invisible at room temperature, turn pale brown at 110°. As expected, phenol and resorcinol are less susceptible to ionic oxidation, the phenolic antioxidants are oxidized with comparable ease to their respective quinones to give deep yellow or brown spots.

When sucrose and D-glucose (with polyhydroxyl functions) are sprayed with V^{5+} solution, an olive-green color changing to yellow is obtained. It has been inferred that the oxidant V^{5+} has been reduced to its trivalent state by the polyols. This is exemplified by the oxidation of starch with vanadium pentoxide–sulfuric acid–nitric acid mixture[8]. Similarly, the hydroxy acids DL.-tartaric and L-ascorbic acids reduce pentavalent vanadium to the green-colored V^{3+} ions.

The aromatic carbonyl compounds, such as 4-methoxybenzaldehyde, 4-chlorobenzaldehyde and *trans*-cinnamaldehyde and benzophenone, show light yellow, greenish yellow, pale brown and brown spots, respectively. Apparently, there is no specificity in color changes as regards carbonyl compounds. Similar observations were noted in the case of amines. Aniline, for example, shows a light pink core surrounded by a turquoise green ring, whereas benzidine, with two amino groups, turns brown. It is interesting to note that 2-aminothiophenol shows a light pink to lilac color. Therefore, it is apparent that the color change and the amino functions cannot be correlated to the number or position of the amino groups.

An attempt was made to use this reagent for characterizing different alkaloids. Quinine (quinoline group), strychnine (indole group) and solanine (steroidal alkaloid) were chosen for this study. Quinine does not develop any color, whereas strychnine and solanine produce pale red and deep pink colors, respectively.

In the determination of detection limits of organic compounds, some typical representatives of steroids, fat-soluble vitamins, phenolic compounds, including frequently used antioxidants, sugars, aromatic amines and alkaloids were chosen. As the volume of the solutions taken for spotting was within 10 μl, the diameter of the spots was easily kept within 5 mm.

Table II shows the R_F values of steroids and fat-soluble vitamins together with their change of color and their detection limits with spray reagent B, which are compared with those produced by the 25% aqueous methanol–sulfuric acid (1:1). As it was not our intention in this work to obtain a clear separation of the compounds under study, it is apparent that cholesterol, β-sitosterol, stigmasterol and campesterol had almost the same R_F values, whereas ergosterol had a higher R_F value than the others. With spray reagent B, all the steroids turned lilac at 110°, whereas the same compounds gave a pale red or pink color with methanol–sulfuric acid (1:1). Although the limit of detection seemed to be less than 1.0 μg for the steroids with both reagents, the color imparted by the vanadic acid–sulfuric acid mixture tended to last longer than that produced by the 50% sulfuric acid. In the case of vitamins, vitamin A (R_F = 0.18) could be separated very distinctly from vitamins E (R_F = 0.50) and D₂ and D₃

TABLE II

TLC OF SOME STEROIDS AND FAT-SOLUBLE VITAMINS

Solvent system: acetonitrile–acetic acid (1:1). Heating time and temperature: 5 min at 110°.

| Compound | Spray reagent | | | | |
| | Vanadic acid–sulfuric acid | | | Methanol–sulfuric acid (1:1) | |
	R_F	Limit of detection (µg)	Color	Limit of detection (µg)	Color*
Cholesterol	0.48	0.25	Lilac	0.25	Pale red
β-Sitosterol	0.44	1.0	Lilac	1.0	Pale red
Stigmasterol	0.47	0.5	Lilac	0.25	Pale red
Campesterol	0.47	0.5	Lilac	0.5	Pink
Ergosterol	0.56	0.5	Brown	0.5	Pale red
Vitamin D_2	0.57	0.5	Brown	0.5	Brown
Vitamin D_3	0.57	0.5	Brown	0.5	Brown
Vitamin A	0.18	1.0	Brown	2.5	Brown
Vitamin E	0.50	2.5	Orange	2.5	Yellow

* The color produced by methanol–sulfuric acid (1:1) tends to fade faster than the color produced by the vanadic acid–sulfuric acid.

($R_F = 0.57$). The use of reagent B was advantageous in the detection of vitamin A, whereby about 1.0 µg could be easily detected. However, with 50% sulfuric acid, only amounts higher than 2.5 µg were detected. It was observed that vitamins D_2, D_3 and E were detected at the same levels by both reagents.

TABLE III

TLC OF SOME PHENOLS AND SUGARS

Solvent systems: (a) benzene–ethyl acetate–acetone (100:2:1); (b) chloroform–acetic acid (1:1); (c) n-butanol–acetic acid–water (4:1:5). The plates were heated at 110° for 5–7 min.

| Compound | Solvent system | Spray reagent | | | | |
| | | Vanadic acid–sulfuric acid | | | Methanol–sulfuric acid (1:1) | |
		R_F	Limit of detection (µg)	Color	Limit of detection (µg)	Color
Phenol	a	0.46	0.25	Beige*	1.0	Pink
	b	0.81				
Resorcinol	a	0.1	0.25	Beige*	0.25	Pink
Propyl gallate	b	0.56	1.0	Orange	1.0	Brown
Nordihydroguaiaretic acid	b	0.46	0.5	Brown	1.0	Brown
BHA	a	0.64	2.5	Yellow	1.0	Yellow
BHT	a	0.79	1.0	Pink	>40	No color
Glucose	c	0.32	5.0	Pale brown	20	Pale brown
Sucrose	c	0.22	2.5	Dark brown	2.5	Dark brown

* The plates were heated at 50° for 5 min.

The phenolic compounds were developed in two different solvent systems: (a) benzene–ethyl acetate–acetone (100:2:1)[3] and (b) chloroform–acetic acid (1:1)[4,5]. With system (a), some phenolics such as phenol, resorcinol, BHA and BHT were well separated, as denoted by their R_F values, whereas propyl gallate and nordihydroguaiaretic acid remained at the origin. However, the TLC plates of the latter two phenols were developed (for comparison) in solvent system (b) together with phenol to give a good separation. On spraying with reagent B, it was found that phenol and nordihydroguaiaretic acid could be detected at levels of 0.25 and 0.5 μg, respectively, whereas these two phenolics were detected at the 1.0-μg level with methanol–sulfuric acid (1:1) (Table III). However, BHT showed a light pink color with vanadic acid–sulfuric acid at the 1.0-μg level, and with methanol–sulfuric acid (1:1), the same compound could not be detected even at the 40-μg level. In the case of other phenolics with both spray reagents, the detection limit was about 1.0 μg except in the case of BHA, where the 50% sulfuric acid reagent was found to be slightly superior.

Glucose and sucrose were developed in n-butanol–acetic acid–water (4:1:5)[6] with R_F values of 0.32 and 0.22, respectively (Table III). Although there was no difference in the detection limit of sucrose (2.5 μg for both reagents), glucose was more sensitive to reagent B (5.0 μg) than to methanol–sulfuric acid (1:1) (20.0 μg).

The TLC plates of amines and alkaloids were developed in ethyl acetate–

TABLE IV

TLC OF SOME AMINO COMPOUNDS AND ALKALOIDS

Solvent systems: (a) ethyl acetate–methanol–ammonia (85:10:5); (b) n-butanol–aceticacid–water (4:1:5). RT = room temperature.

Compound	Solvent system	Spray reagent						Methanol–sulfuric acid (1:1)
		Vanadic acid–sulfuric acid				Potassium mercury(II) iodide		
		R_F	Temperature (°C)	Limit of detection (μg)	Color	Limit of detection (μg)	Color	Color
Aniline	a	0.93	RT 110	0.5	Blue Yellow	>40	No color	No color
2,3-Diamino-naphthalene	a	0.87	RT 110	0.25	Beige Beige	0.5	Pale brown (after ~ 12 h)	No color
Benzidine	a	0.90	RT 110	0.1	Pale yellow Deep yellow	0.25	Blue	No color
Cinchonine	a	0.65	110	>40	No color	40	Pale yellow	No color
Quinine	a	0.62	110	>40	No color	40	Pale yellow	No color
Strychnine	a	0.49	110	0.25	Pink	2.5	White spot when wet, not detected when dry	No color
Brucine	a	0.35	RT 110	0.25	Orange Yellow	0.5	White spot when wet, not detected when dry	Yellow
Solanine	b	0.46	RT 110	0.5	Pink Red	2.5	White spot when wet, not detected when dry	Pink

methanol–ammonia (85:10:5)[7], except solanine, which was developed in n-butanol–acetic acid–water (4:1:5). The R_F values of these compounds (Table IV) indicated that these compounds could be easily separated. As methanol–sulfuric acid (1:1) is a poor reagent for these compounds, potassium mercury(II) iodide (Mayer's reagent) was chosen as an alternative for the detection of these compounds at room temperature. With vanadic acid–sulfuric acid reagent, the change of color was recorded at room temperature and at 110°. All of these compounds are more sensitive to spray reagent B than to Mayer's reagent. Aniline, for example, was easily detected at the 0.5-μg level with reagent B, whereas even 40 μg did not show any color with Mayer's reagent nor with methanol–sulfuric acid (1:1). Also, solanine turned pink to red at the 0.5-μg level with vanadic acid–sulfuric acid reagent, while 2.5 μg were required to show any color with Mayer's reagent or with the 50% sulfuric acid reagent. It was noteworthy that both cinchonine and quinine were not detected by spray reagent B even at the 40-μg level.

CONCLUSION

It has been demonstrated that vanadic acid–sulfuric acid mixture is a sensitive spray reagent for some steroids, alkaloids and phenolic compounds, compared with methanol–sulfuric acid (1:1). In the case of amines and certain alkaloids, spray reagent B was shown to be superior to Mayer's reagent.

ACKNOWLEDGEMENTS

REFERENCES

1 E. Stahl (Editor), *Thin-Layer Chromatography*, Springer, New York, 1969, pp. 854–905.
2 H. Jatzkewitz and E. Mehl, *Hoppe-Seyler's Z. Physiol. Chem.*, 320 (1960) 251.
3 D. Simpson and B. R. Currell, *Analyst (London)*, 96 (1971) 520.
4 J. Davidek, J. Janicék and E. Davidkova, *Z. Lebensm.-Unters.-Forsch.*, 131 (1967) 345.
5 J. Davidek, J. Janicék and E. Davidkova, *Z. Anal. Chem.*, 234 (1968) 211.
6 I. S. Shimi and G. M. Iman, *Analyst (London)*, 94 (1969) 65.
7 W. T. Fisher, A. D. Baitshotts and G. S. Grau, *J. Chromatogr. Sci.*, 10 (1972) 309.
8 M. Malaiyandi and J. P. Barrette, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 951.