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VANADIUM PENTOXIDE IN SULPHURIC ACID, A GENERAL CHROMOGENIC SPRAY REAGENT FOR CARBOHYDRATES

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SUMMARY

Vanadium pentoxide in sulphuric acid is a rapid and non-specific chromogenic spray reagent. All carbohydrates and related compounds containing neighbouring functional groups are made visible. A rough guide to the type of compound present may be obtained as the rate of development of the colour is dependent on the type of functional groups present. In addition to its use in thin-layer chromatography on inert supports, the reagent can also be used on paper and cellulose thin-layer chromatograms.

INTRODUCTION

Malaiyandi *et al.*[1] reported the use of vanadium pentoxide as a chromogenic spray reagent for the qualitative analysis of some organic compounds on silica gel thin-layer plates. They used 0.05% solutions of vanadium pentoxide in 5% sulphuric acid. We have studied the oxidation of carbohydrates with 0.1 *M* vanadium pentoxide in 1 *M* sulphuric acid, and we found it to be an excellent chromogenic spray reagent for carbohydrates and related compounds.

Vanadium pentoxide is a one-electron oxidant, the yellow pentavalent vanadium being reduced to the light-blue tetravalent state under the conditions used. All compounds susceptible to attack by this oxidant will therefore be made visible[1–3], including all compounds of carbohydrate origin that contain α,β functions. The method is rapid and convenient because after spraying, the compounds can immediately be made visible by blowing hot air over the plate with a hair dryer. On spraying the plate remains colourless but, as it dries, blue spots and a yellow background appear. Although the blue colour developed is independent of the functional groups in the compounds, the time required to observe the compounds is strongly dependent on the type of groups present. Some compounds initially develop a yellow complex with the reagent, these spots gradually changing to blue. Considerable information concerning a compound can be obtained from the R_F value and from the behaviour with the reagent.

The vanadium pentoxide reagent may be a useful supplement to the already

existing range of chromogenic spray reagents for carbohydrates[4]. Almost all compounds of carbohydrate origin are detected and the reagent may therefore prove useful in detecting compounds in degradation reactions. Malaiyandi *et al.*[1] reported the use of the reagent on silica gel thin-layer plates only, but we have found that it can also be used on paper or cellulose thin-layer chromatograms.

EXPERIMENTAL

Shandon Southern equipment was used to prepare thin-layer plates (20 × 20 cm) of 0.25-mm thickness (wet depth). Plates coated with silica gel G (Type 60) (E. Merck, Darmstadt, G.F.R.) and mixture of silica gel G (two parts) and Kieselguhr G (one part) were dried at 110° for 2 h, stored at the humidity and temperature of the laboratory and used without further activation. Cellulose plates were prepared from cellulose MN 300 (Macherey, Nagel & Co., Düren, G.F.R.), allowed to dry overnight and stored as above. Ascending chromatography was accomplished in closed glass tanks lined with filter-paper to ensure saturation.

Paper chromatography was performed on sheets of Whatman No. 1 paper (20 × 23 cm). After spotting, the papers were rolled into a cylinder, the edge stapled together and the chromatograms developed in cylindrical glass tanks by the ascending method in a saturated atmosphere for 2 h at ambient temperature.

All compounds were used as obtained from the manufacturers.

Solvent systems

We used benzene–ethanol (2:1) as the solvent system for silica gel G and benzene–glacial acetic acid–ethanol (2:2:1) for the mixture of silica gel G and Kieselguhr G (2:1). For cellulose and paper chromatography the solvent system *n*-butanol–glacial acetic acid–water (6:1:2) was used.

Spray reagent (0.1 M vanadium pentoxide in 1 M sulphuric acid[5])

Vanadium pentoxide (18.2 g) was dissolved in 300 ml of 1 *M* sodium carbonate solution by heating. The solution was cooled, 460 ml of 2.5 *M* sulphuric acid were added and the mixture was diluted to 1 l. Excess of carbon dioxide was removed in an ultrasonic bath.

Qualitative evaluation

A stock solution of concentration 10 mg/ml of all compounds was prepared and the following dilutions from the stock solution were made: 0.1, 0.25, 0.5, 0.75, 1.0, 2.5 and 5.0 mg/ml. A 1-μl volume of solution was applied to the plates or paper with Microcap pipettes (Drummond, Broomall, Pa., U.S.A.), giving spots of diameter *ca.* 5 mm.

After development, the chromatograms were dried, sprayed at the rate of about 2 ml per 400 cm^2 (20 × 20 cm plates) unless otherwise stated and left under ambient conditions (no blowing or heating), or were heated in a Termaks drying cabinet fitted with a glass door and fan.

For the sole purpose of locating the compounds on the chromatogram, the following procedure is useful. After removing the chromatogram from the developing tank and drying it briefly with a hair dryer, the chromatogram is sprayed lightly with

the reagent and subjected to further hot air from the hair dryer. In this manner the compounds can be located within 5 min after removing the chromatogram from the developing tank.

The "detection time" was the time from spraying until the compound could be detected on the chromatogram. When in doubt, the spots could be discerned more easily when viewed with light passing through the chromatogram. The spots were photographed at 5-min intervals to ensure minimal errors in detectability with the naked eye.

To compensate for incidental variations in R_F values or detection time, reference compounds with different mobilities and having different detection times were co-chromatographed with the samples. The R_F value of a sample was corrected by the same factor as was necessary for a reference compound of comparable mobility to attain its average (given) R_F value. Similarly, the detection time of a sample was corrected by the same factor as was necessary for a reference compound of comparable detection time to attain its given detection time.

The chromatographic systems used were chosen arbitrarily as it was not our intention to differentiate between all of the compounds under study. Our intention was only to demonstrate the usability of the reagent in different chromatographic systems. The results should therefore be considered as a guide only.

RESULTS AND DISCUSSION

Table I shows the relative times required to detect equal amounts (10 μg) of carbohydrate and related compounds under ambient conditions and the detection limit on silica gel G plates, and the R_F values obtained in four chromatographic systems. The results listed are average values from at least three chromatograms.

The compounds can be classified according to detection time, which can be correlated with the functional groups present in the compounds. The speed of the reaction is dependent on the functional groups in neighbouring positions present in the molecule, and whether these groups are free or bound. If the most reactive function or its neighbour is bound, the rate of development of the colour is substantially retarded. This decrease seems to be proportional to the strength of the bonding.

The reactivity of free functional groups decreases in the order –CO–CO– $\approx$ –CO–COOH $\approx$ –COH–CO–COH– $>$ –COH–CO– $\approx$ –COH–COOH $\gg$ –COH–COH With most of the slow-reacting compounds, a yellow complex becomes visible before the reagent is reduced.

Aldoses and ketoses

Ketoses react substantially faster than aldoses. The triose dihydroxyacetone reacts almost immediately (0.5 min) while glyceraldehyde requires a longer time (3 min). This is probably due to the activation of two neighbouring hydroxyl groups to the carbonyl in the ketose compared with only one hydroxyl group in the aldose.

The carbonyl group in the ketohexoses is masked as a hemiacetal and therefore reacts much more slowly (11 min) than the ketotrioses, but substantially faster than the aldohexoses (25 min). Kumar and Mehrotra[6] have shown that the oxidation of aldoses with vanadium(V) in acidic medium probably proceeds via the free aldehyde form. A higher percentage of the aldopentoses exists in the free form compared with

TABLE I

DETECTION TIMES AND DETECTION LIMITS ON SILICA GEL G PLATES AND R_F VALUES IN FOUR CHROMATOGRAPHIC SYSTEMS

Compound	*Detection time (min)**		*Detection limit (μg)*	*R_F value × 100***			
	Yellow	*Blue*		*A*	*B*	*C*	*D*
Aldoses							
Glyoxal		0.5	0.5	64	78	00	str
Glycolaldehyde		3	0.5	51	48	47	str
Glyceraldehyde		3	0.25	33	60	50	36
Erythrose		5	0.25	22	47	45	34
Arabinose	(15)	18	0.25	15	37	35	19
Xylose	(15)	18	0.25	18	43	36	23
Ribose	(15)	18	0.25	12	37	40	28
Glucose	(20)	25	0.25	11	28	27	20
Galactose	(20)	25	0.25	10	26	25	18
Mannose	(20)	25	0.25	13	32	32	22
Fucose	(20)	25	0.5	18	44	42	30
Rhamnose	(22)	27	0.5	23	50	48	33
2-Deoxyglucose	(20)	28	0.5	22	34	—	—
2-Deoxygalactose	(20)	28	0.5	28	40	—	—
2-Deoxyribose	(20)	28	0.5	20	28	—	—
Ketoses							
Dihydroxyacetone		0.5	0.25	33	56	50	46
Fructose		11	0.25	13	33	30	19
Sorbose		11	0.25	15	36	28	16
Tagatose		11	0.25	13	33	32	18
Substituted monosaccharides							
Methyl-β-D-glucopyranoside	(13)	30	0.5	20	51	—	—
Methyl-β-D-xyloside		50	0.5	30	58	—	—
4,6-O-ethylidene-α-D-glucopyranose		25	0.5	40	68	—	—
Methyl-4,6-O-benzylidene-α-D-glucopyranoside		90	0.75	62	81	—	—
α-D-Glucose pentaacetate		180	1.0	75	90	—	—
Alditols							
1,2-Cyclohexanediol	(11)	25	0.75	58	77	—	—
Ethyleneglycol	(11)	25	0.5	36	64	—	—
Glycerol	(8)	25	0.5	23	50	46	42
Xylitol	2	25	0.5	09	35	27	20
Dulcitol	0.5	25	0.5	06	25	21	15
Mannitol	0.5	25	0.5	06	28	24	15
Sorbitol	0.5	25	0.5	06	22	22	14
Inositol	(8)	25	0.5	03	18	—	—
Acids							
Glycolic acid		5	0.75	str	63	23	60
Glyoxylic acid		0.5	0.75	str	60	58	61
Oxalic acid	0.5	60	1.0	03	04	60	str
Lactic acid		5	0.75	str	70	str	70
Pyruvic acid		0.5	0.5	str	65	75	50
Arabinonic acid		6	0.5	00	18	53	16
Gluconic acid		6	0.5	00	09	22	13
Glucuronic acid		11	0.5	str	22	16	10

TABLE I *(continued)*

Compound	*Detection time (min)* *		*Detection limit (μg)*	R_F *value* × *100***			
	Yellow	*Blue*		*A*	*B*	*C*	*D*
Glucuronic acid lactone		13	0.5	30	25/56	17	30
Galactonic acid lactone		13	0.5	30	15/45	34	25
Galacturonic acid		11	0.5	str	14	31	05
Ascorbic acid		0.5	0.5	str	55	17	35
Oligosaccharides							
Cellobiose	(20)	28	0.5	06	10	—	—
Maltose	(20)	28	0.5	16	11	—	—
α,α-Trehalose	(20)	32	0.5	04	09	—	—
Sucrose		16	0.5	06	14	16	10
Lactose	(20)	27	0.5	03	08	—	—
Melibiose	(15)	21	0.5	03	08	10	05
Melezitose	(15)	20	0.5	03	05	10	05
Raffinose	(15)	18	0.5	02	04	08	03
Gentianose	(15)	18	0.5	02	04	08	03
Isokestose		15	0.5	02	04	11	04
Stachyose	(15)	17	0.5	00	02	03	00

* 10 μg. Values in parentheses: the spot remains faint.

** Chromatographic systems: A = silica gel G with benzene–ethanol (2:1) as solvent system; B = silica gel G–Kieselguhr G (2:1) with benzene–acetic acid–ethanol (2:2:1) as solvent system; C = cellulose with *n*-butanol–acetic acid–water (6:1:2) as solvent system; D = paper with the same solvent system as in C. — = not developed; str = streak.

the aldohexoses[7]. This explains the lower detection time for the aldopentoses compared with that of the aldohexoses. The short-chain (open) aldoses react rapidly.

The aldopentoses and aldohexoses could generally be observed as faint yellow spots a few minutes before they changed to blue. This phenomenon was not observed with the faster reacting ketoses or short-chain aldoses. The aldohexoses were sometimes difficult to detect on paper and cellulose thin-layer chromatograms.

The 2-deoxyaldohexoses react more slowly than the other aldohexoses and were not made visible on paper or cellulose thin-layer chromatograms.

Substituted monosaccharides

Compounds in which the anomer carbon is glycosidically bound react much more slowly than the parent compound, indicating that they must be hydrolyzed before oxidation can take place. Compounds with a high degree of substitution (α-D-glucose pentaacetate) are very resistant towards the oxidant, but they are rendered visible on inert supports.

Alcohols

The reaction of the reagent with the pentitols and hexitols is unique to these compounds, as a distinct yellow complex is rapidly developed. The spot is also clearly visible against the yellow background when the plate is dry. After 8–11 min, glycerol, ethylene glycol, 1,2-hexanediol and inositol also developed a visible yellow complex with the reagent, this spot being much fainter compared with that of the hexitols.

These spots are, however, not specific as several other compounds give comparable yellow complexes. The alditols are slowly oxidized and after 25 min the yellow colour became greyish and gradually changed to blue.

The pentitol and hexitol yellow complexes are clearly visible even when superimposed on other compounds that have been developed, *e.g.*, xylitol on ascorbic acid. On the other hand, if a slow-reacting compound is superimposed on a fast-reacting compound it may be difficult to detect the former.

Acids

The short-chain α-aldo acid glyoxylic acid and the α-keto acid pyruvic acid were made visible within 1 min, the keto acid appearing more rapidly. The α-hydroxy acids glycolic acid, lactic acid, arabinonic acid and gluconic acid required 5–6 min for detection. The acid lactones and uronic acids had a detection time of 11–13 min.

Ascorbic acid, which contains enediol-lactone functions in neighbouring positions, is faintly visible even before spraying. It is easily oxidized and the reagent turns blue almost immediately on spraying.

Oxalic acid behaved in a different manner to other compounds. Within 0.5 min after spraying a faint yellow complex was observed, which was not visible against the yellow background when the plate was dry. However, oxalic acid is slowly oxidized and a blue spot became visible after 1 h. On paper and cellulose thin-layer chromatograms only the yellow spot is visible.

Although the ketohexoses may be difficult to differentiate from the uronic acids by means of the detection time, the rate of migration differs sufficiently in the chromatographic systems used to allow differentiation.

The reagent does not detect monofunctional acids such as formic, acetic or propionic acid. Most acid-specific chromogenic reagents require thorough drying of the chromatogram before spraying, to remove these acids if they are used in the solvent systems. This thorough drying is not necessary when using the vanadium reagent.

Oligosaccharides

The speed of detection is dependent on the monosaccharides present in the molecule. Oligosaccharides containing fructose units react much faster than oligosaccharides containing aldoses alone. Melizitose, which contains a fructose unit between two glucose units, reacts more slowly than compounds that contain fructose as a terminal unit. Isokestose, which has two fructose units, reacts faster than the other trisaccharides containing one terminal fructose unit. All fructose-containing oligosaccharides were non-reducing. The galactose-containing disaccharides react slightly faster than disaccharides containing glucose units only. The non-reducing α,α-trehalose is the most stable of the oligosaccharides investigated and is easily differentiated from the other reducing diglucose disaccharides maltose and cellobiose.

With most oligosaccharides, a faint yellow spot was observable several minutes before it changed to blue. This was difficult to detect on cellulose and paper chromatograms. On silica gel, the yellow spot could not be observed against the yellow background. The oligosaccharides containing aldose units only were not made visible on cellulose and paper chromatograms.

The polysaccharides inulin (polyfructose) and starch (polyglucose) were hydrolyzed, inulin in 0.01 *M* hydrochloric acid for 10 min at 70° and starch with α-amylase. The products were separated in the system silica gel G–Kieselguhr G. The detection times for the oligosaccharides obtained from inulin were much shorter than for the degradation products of starch, as was expected. Inulin itself is also easily oxidized by the reagent, while starch requires severe conditions to reduce vanadium(V).

Dependence of detection time on experimental conditions

The time required to detect a compound may vary considerably, depending on the conditions used. Table II shows the rate of development of vanadium(IV) in the oxidation of 0.1 *M* glucose in 0.1 *M* vanadium pentoxide solution at 60° at different sulphuric acid concentrations. The results show a strong dependence of the rate on the sulphuric acid concentration.

TABLE II

RELATIVE RATE OF DEVELOPMENT OF TETRAVALENT VANADIUM *VERSUS* SULPHURIC ACID CONCENTRATION

0.1 *M* glucose in 0.1 *M* V_2O_5 at 60°. Development of vanadium(IV) measured spectrophotometrically at 680 nm.

(H_2SO_4) concentration (*M*)	1	2.5	4	7.5	10
$\left(\frac{dv}{dt}\ V(IV)\right)_{rel}$	1	3	6	25	80

After spraying the reagent on the chromatograms, the sulphuric acid concentration increases as the water evaporates, the evaporation being accelerated by blowing and heating. The sulphuric acid concentration increases faster with small amounts of reagent sprayed on the chromatogram than with larger amounts. The evaporation can be stopped by covering the chromatographic plate with a glass plate (sandwich).

The time the yellow background takes to develop is proportional to the amount of reagent sprayed on the chromatogram. The yellow background is due to complex formation between vanadium(V) and sulphuric acid. The colour of vanadium(V) solutions changes from light yellow to deep red as the concentration of sulphuric acid is increased[3].

Table III shows the results obtained under different conditions for some selected compounds. The experimental conditions are easiest to reproduce with the sandwich method but this method, however, is not as sensitive or as convenient as the free evaporation method. The yellow background does not appear, nor do the yellow complexes develop. The dependence of the detection time on the amount sprayed is greater for slow-reacting compounds than fast-reacting ones, the variation for ketoses being comparatively small compared with that of the aldoses. On cellulose and paper chromatograms, the detection time for the fast-reacting compounds is comparable with the detection time on silica gel, while the slower-reacting compounds react much more slowly on cellulose and paper.

Table III also shows that the detection time is strongly dependent on the temperature. For slow-reacting compounds, it is more convenient to develop the chro-

TABLE III

DETECTION TIMES FOR SOME SELECTED COMPOUNDS UNDER VARIOUS EXPERIMENTAL CONDITIONS

Experimental conditions				*Detection time (min)***							
Solid phase	*Temperature (°C)*	*Evaporation rate**	*Amount sprayed (ml per 400 cm²)*	*Glyoxal*	*Glycer-aldehyde*	*Fructose*	*Glucuronic acid lactone*	*Arabinose*	*Glucose*	*Xylitol*	*α,α-Trehalose*
Silica gel G	Ambient	Ambient	0.5	0.5	2	6	8	(13) 15	(17) 20	(1) 20	(17) 25
Silica gel G	Ambient	Ambient	2	0.5	3	11	13	(15) 18	(20) 25	(2) 25	(20) 32
Silica gel G	Ambient	Ambient	5	1	11	27	30	(50) 55	(53) 60	(25) 60	(53) 120
Silica gel G	Ambient	Oven	2	0.5	3	5	6	(6) 9	(7) 11	(2) 11	(7) 25
Silica gel G	50	Oven	2	0.5	1.5	2.5	3	(3) 4	(4) 4.5	(1) 5	(4) 8
Silica gel G	95	Hair dryer	5	0.5	1	1	1	1.5	2	(0.5) 2	3
Silica gel G	80	Sandwich	2	1	3	6	7	11	18	14	40
Paper	Ambient	Ambient	2	0.5	6	9	12	30	40	(1) 40	—
Cellulose	Ambient	Ambient	2	0.5	5	7	10	35	45	(0.5) 60	—

* Evaporation rates: sandwich = none; ambient = slow; oven = medium; hair dryer = fast.

** — = not developed. Values in parentheses denote detection times for yellow spots; otherwise blue spots.

matograms at higher temperatures. The order of development of the different compounds was virtually the same in all experiments. For the sole purpose of locating the compounds, the treatment with hot air from a hair dryer on inert chromatograms is rapid. However, compounds that react as slowly as arabinose or even slower will not, on strong heating, become visible on cellulose or paper chromatograms.

Table IV gives the detection time as a function of the amount of sorbose spotted on the chromatogram. On decreasing the amount from 100 to 5 μg, the time required to detect the spot is prolonged by 4 min. With some training, a rough estimate of the amount present on the plate can be made when viewing the size and intensity of the completely developed chromatogram, making a correct classification of the compounds possible.

TABLE IV

DETECTION TIME *VERSUS* CONCENTRATION OF SORBOSE

Concentration ($\mu g \cdot \mu l^{-1}$)	100	50	25	10	5	2.5	1	0.5
Detection time (min)	9	9.5	10	11	13	17	23	30

Tables III and IV show that the detection time must be used with some care and that reference compounds must always be included. With entirely unknown compounds and with unknown amounts of them, the method gives only a rough indication of the type of compound present. Uneven spraying may give erroneous results.

Sensitivity

Table I shows the sensitivity of the reagent with the compounds investigated on silica gel plates. The plates were chromatographed only for a distance of 3 cm so that the spot sizes were comparable. The detection limit was in the range 0.25–0.75 μg for most compounds. The development of yellow complexes could not be detected below 2.5 μg. The limit of detection on cellulose or paper chromatograms is poorer, five times as much of each compound being required.

Stability of spots

On inert supports, the chromatographic plates can be kept for reference for several months. Although the yellow background fades in 2 days if unprotected, the spots still show up as a brighter blue shade against the grey background. The convenient method of transferring the silica gel support to a transparent self-adhesive vinyl sheet and enveloping it with another sheet for reference proved unsuccessful as the reagent was rapidly reduced. Also, the support adhered poorly to the sheet after spraying.

The yellow background disappears if the plate is sprayed lightly with water, and the spots show up as blue against a white background. This may be useful if too much reagent has been used, thus covering faint spots.

The reagent attacks cellulose slowly; after 2 days the spots are invisible on paper and cellulose chromatograms. Slow-reacting compounds are not rendered visible on cellulose or paper.

Interferences

As can be seen from the list of compounds investigated, vanadium pentoxide in sulphuric acid is not a specific reagent. The compounds listed by Malaiyandi *et al.*[1] and Waters and Littler[2,3] will probably react with the reagent also under the conditions used by us.

Compounds with an α-amino function do not reduce pentavalent vanadium; however, other functions in amino acids may react with the reagent. Methionine reacts rapidly, proline faintly while glycine and valine could not be detected on silica gel (10 μg).

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