Technology Section

Pakistan J. Sci. Ind. Res., Vol. 16, No. 5, October 1973

APPLICATIONS OF THE NICHROME WIRE RING CHAMBER IN PAPER CHROMATOGRAPHY

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(Received February 13, 1973; revised August 17, 1973)

Abstract. An application of the nichrome wire ring chamber in combination with paper chromatography is described and it is established that with the use of ring chamber the limit of identification on paper chromatogram is improved, making it possible to run the chromatograms with much smaller samples, which result in better resolution. The utility of the ring chamber for semiquantitative determination of compounds separated on paper chromatograms is also illustrated.

In a previous paper[1] all glass nichrome wire ring chamber was described and methods were developed for microidentification of 46 cations,[1] 20 anions,[2] and semiquantitative determination of 7 amino acids[3] and 10 vitamins[4] from a single drop of solution. In the present paper the utility of the nichrome wire ring chamber in paper chromatography has been described and it is established that the ring chamber can be used successfully for making compounds visible on paper chromatograms even when their concentration in test solution falls below the limit of identification, which are not visible otherwise on chromatograms. The use of the ring chamber for semiquantitative determination of compounds on paper chromatograms has also been illustrated and its advantages over Weisz ring oven[5,6] in paper chromatography have been described.

Experimental

Reagents. All reagents were of analytical grade. Whatman No. 1 filter paper was used throughout. The solution of cations, anions, amino acids and vitamins were prepared by weight. The method for the preparation of spray reagents for these compounds has been described.[1-4]

Apparatus. All glass nichrome wire ring chamber has already been described elsewhere.[1]

Shandon micropipette was used for spotting test solution on paper chromatogram.

Shandon TLC tanks, 27.5×12.5×21 cm, were used for the development of paper chromatograms.

Procedure. Paper chromatograms were developed on 2×10 in strips of Whatman filter paper No. 1, by the ascending technique.[7] The mixture of cations, anions, amino acids or vitamins was applied at a starting line made about 1 in above the lower end of the paper dipped in the developing solvent. On 2-in wide filter paper two separate but similar spots 1 in apart were applied. About 45 min were required for the development of the chromatogram in the usual way.[7] Various solvents used for the development of paper chromatograms are given in Table 1.

After development, the paper chromatogram was folded longitudinally in the middle and was sprayed with appropriate spray reagents. The experimental conditions for the use of spray reagents were similar as described previously. In this way the position of the mixture or compound spotted at one spot is located on the 'guide' paper chromatogram. On the second folded portion of the paper chromatogram (on which same concentration of the mixture was spotted), rings are developed with the nichrome wire ring chamber[1] starting from the place of spotting and ending at the solvent front. Then the paper chromatogram is sprayed with appropriate spray reagents. In this way the substances contained in various spots of the chromatogram are concentrated in circular arcs or rings, whose position depends on the points from where the rings were washed. The experiment is repeated with various concentrations of different compounds and minimum amount of each substance visible on paper chromatogram with and without the use of the ring chamber is determined.

Results and Discussion

The nichrome wire ring chamber[1] is not a paper chromatographic method, but it can be used in combination with paper chromatographic technique for improving the limit of identification of substances separated on paper chromatograms. In Table 2

TABLE 1. SOLVENTS FOR SEPARATION OF DIFFERENT COMPOUNDS ON PAPER CHROMATOGRAMS.

Solvent	Composition (v/v)	For
A	Cyclohexane ether (1:4)	Fat-soluble vitamins
B	Acetone–water–ethanol 96% (1:2:1)	Water-soluble vitamins
C	n-Butanol–glacial acetic acid–water (5:1:4)	Amino acids
D	n-Butanol–pyridine–water (2:1:2)	Anions
E	Acetone–HCl (concd)–water (8:1:1)	Copper, cobalt and nickel

TABLE 2. THE LIMIT OF IDENTIFICATION OF VARIOUS COMPOUNDS ON PAPER CHROMATOGRAMS WITH AND WITHOUT THE USE OF THE RING CHAMBER.

Compound	Spray reagent*	Solvent+ R_f	Minimum amount visible: With ring chamber (μg)	Minimum amount visible: Without ring chamber (μg)
Fat-soluble vitamins		*A*		
A	Antimony pentachloride	1.00	0.25	0.75
	Phosphotungstic acid		0.75	1.00
D_2	,,	0.15	0.20	0.50
D_3	,,	0.13	0.20	0.40
E (α-tocopherol)	2:6-Dichloro-*p*-benzoquinone-4-chlorimine	0.98	0.30	0.60
K_3	L(+)-Cysteine hydrochloride			
	Diethyldithiocarbamate–sodium salt	0.96	0.20	0.30
			1.50	2.00
Water-soluble vitamins		*B*		
B_1	Iodoplatinate	0.86	0.50	0.50
B_2	Stannous chloride	0.65	0.60	0.75
	Cupric chloride–triphenylphosphine complex		0.30	0.45
B_6	Ferric chloride	0.87	0.50	1.00
C	Phosphomolybdic acid	0.85	0.75	1.00
p-Aminobenzoic acid	*p*-Dimethylaminobenzaldehyde	0.98	0.60	0.90
	Ceric sulphate		0.40	0.70
Amino acids		*C*		
L-Arginine	α-Naphthol–urea	0.15	3.00	3.50
L(+)-Cysteine	Phosphomolybdic acid	0.29	1.50	2.00
L-Histidine	Bromine–acetic acid	0.14	1.00	Invisible
DL-Methionine	Sodium nitroprusside	0.43	6.00	Invisible
L-Proline	Isatine	0.24	1.00	1.25
DL-Tryptophan	*p*-Dimethylaminobenzaldehyde	0.44	0.25	0.75
L(—)-Tyrosine	α-Nitroso-β-naphthol	0.41	0.50	1.00
Anions		*D*		
Fluoride	Aluminium chrome–azurol S	0.10	0.04	0.05
Bromide	Fluorescein	0.32	2.50	4.50
Iodide	Palladous chloride	0.54	0.50	1.25
	Thallous sulphate		0.75	1.00
Chlorate	Aniline sulphate	0.40	0.50	0.75
Bromate	Aniline sulphate	0.20	2.50	3.00
Iodate	Indigo carmine–sodium sulphite	0.09	2.00	2.00
	Indigo–sodium sulphite		5.00	5.50
Sulphide	Lead nitrate	0.04	2.00	2.50
Sulphite	Sodium nitroprusside	0.26	0.30	0.35
Thiosulphate	Ammonium molybdate–sulphuric acid	0.06	0.40	0.50
Sulphate	Rhodizonic acid, sodium salt	0.06	0.10	0.15
Nitrite	Sulphanilic acid–α-naphthylamine	0.23	0.10	0.12
Nitrate	Sulphanilic acid–α-naphthylamine	0.38	0.50	0.60
Ferricyanide	Ferrous sulphate	0.21	0.50	0.70
Ferrocyanide	Uranyl acetate	0.08	0.25	0.25
	Ammonium molybdate		0.50	0.60
Arsenite	Uranyl acetate	0.18	8.00	9.25
Oxalate	Potassium chromate	0.11	7.50	8.00
Thiocyanate	Cobalt sulphate	0.56	2.00	2.00
Phosphate	Ammonium molybdate	0.04	0.10	0.20
Borate	Curcumin	0.75	0.03	0.04
Chromate	Fluorescein	0.00	1.00	1.25
Cations		*E*		
Copper	Rubeanic acid	0.62	0.04	0.05
Cobalt	,,	0.23	0.03	0.04
Nickel	,,	0.11	0.01	0.02

*The method of preparation and experimental conditions for use of spray reagents for vitamins,[4] amino acids,[3] anions[2] and cations[1] were similar as described previously. †Solvents (*cf.* Table 1) and *Rf* refer to paper chromatograms developed by ascending technique.

TABLE 3. SOLVENTS FOR DEVELOPMENT OF RINGS ON PAPER CHROMATOGRAMS.

Solvent	Composition (v/v)	For
A	Ethanol (96%)–water (1:1)	Amino acids[3] Water-soluble vitamins[4]
B	Benzene	Fat-soluble vitamins[4] except vitamin K_3
C	Ether	Vitamin K_3
D	Acetone–ammonium hydroxide (1:1)	Anions[2] except borate and phosphate
E	Acetone–acetylacetone–4M HCl (45:2:3)	Borate, phosphate[2]
F	Acetone–HCl (concd)–water (3:1:1)	Cations[1]

some typical results about the limit of identification of some vitamins, amino acids, anions and cations are given which indicate that with the use of ring chamber[1] the limit of identification of these compounds is considerably improved and the compounds otherwise invisible because of low concentration in the test solution can be detected without difficulty. Various solvents used for developing rings with the ring chamber are given in Table 3.

The nichrome wire ring chamber can also be used for semiquantitative determination of substances by comparing the intensities of the ring with that of the standard. If the compound on a paper chromatogram is not distributed in a full circle, then the length of the arc occupying the compound can be found by $\theta = l/r$; where θ is taken in radian, l, length of the arc; and r, radius of the circle. Thus the amount of the substance can be evaluated. For example, if the intensity of an arc of a substance A on a paper chromatogram matches with a circle containing 8 μg of the substance in a circle and length of the arc found by the above equation is x, and circumference of the circle is y (i.e. $2\pi r^2$), then the amount of substance on paper chromatogram which has been concentrated into an arc of a circle corresponds to $8\ x/y$ μg. If the substance distributed in an arc shows different intensities at different points, then the arc is subdivided into different segments according to the intensity and each segment is compared with a circle of standard scale and the amount of substance in each segment is determined separately as above. Of course, the total amount in the arc will be sum of the amounts found in each segment. Some typical results for semiquantitative determination of copper, cobalt and nickel from mixture resolved on paper chromatogram are given in Table 4 which indicate ±5% accuracy.

TABLE 4. SEMIQUANTITATIVE DETERMINATION OF COPPER, COBALT AND NICKEL ON PAPER CHROMATOGRAMS WITH THE RING CHAMBER.

Cation*	Amount present† (μg)	Amount found (μg)	Error (%)
Copper	0.25	0.24	4.00
	0.30	0.31	3.33
	0.35	0.34	2.85
Cobalt	0.20	0.21	5.00
	0.20	0.19	5.00
	0.20	0.19	5.00
Nickel	0.30	0.31	3.33
	0.20	0.19	5.00
	0.30	0.29	3.33

*Copper, cobalt and nickel were separated on Whatman filter paper No. 1 by ascending paper chromatography and 0·5% alcoholic solution of rubeanic acid was used as spray reagent *cf.* Table 2

†Represents actual amount spotted on paper chromatogram.

The nichrome wire ring chamber[1] has many advantages over Weisz ring oven[5] when used in combination with paper chromatography. The ring chamber is made of all glass and no contamination on paper chromatogram from the material of the chamber is possible. It is much easier to prepare rings on a paper chromatogram with the ring chamber[1] than with the Weisz ring oven.[5] The ring chamber is an inexpensive glass apparatus which can be made of different diameters according to requirements.

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