

CHROM. 6454

Note

Ninhydrin-collidine as a sensitive reagent for the detection of the ammonium ion on pherograms

Many of the reagents which have been described for the detection of the ammonium ion, NH_4^+ , after its separation on filter-paper, are not of a high order of sensitivity, only a few giving visible reactions with NH_4^+ in submicrogram quantities. In a comparative study of several reagents, HEISIG AND POLLARD¹ found dimethylaminobenzylidene rhodanine to be the most sensitive, giving a detectable reaction with approximately 0.6 μg of NH_4^+ . But for other reagents—alizarin, lead cobalt nitrite, quinalizarin, and violuric acid—they found ten times this amount of NH_4^+ or more to be necessary.

For the detection of NH_4^+ on paper chromatograms using sodium cobalt-nitrite, STEEL² reported that at least 5 μg of the ion was required, while BLAU³, using Nessler's and other reagents, noted that NH_4^+ was detectable only when present on papers in "high concentrations". The silver nitrate-2,6-dichlorophenol-indophenol reagent of BARNABAS *et al.*⁴ was used by them for the detection of 10 μg of NH_4^+ on paper chromatograms. Dipicrylamine appears to be much less sensitive than any of the above reagents, but a procedure has been described involving its use as a spot-test reagent for NH_4^+ on filter-paper⁵. It is reported to require 200 μg of NH_4^+ for a positive test.

Although ninhydrin is a sensitive reagent for NH_4^+ , its use for locating NH_4^+ on paper chromatograms seems to have been avoided, probably because of its lack of specificity. However, the ninhydrin dip and spray reagents commonly prescribed^{6,7} for the detection of amines and amino acids are of use in locating NH_4^+ on pherograms⁸⁻¹⁰, the lack of specificity of ninhydrin being less of a disadvantage in the paper electrophoresis of NH_4^+ because the characteristically high cationic mobility of this small ion ensures that it separates widely from most other ninhydrin-reactive compounds. The usual ninhydrin reagents give a weak pink reaction with traces of NH_4^+ but it has now been found that the sensitivity of ninhydrin to NH_4^+ is enhanced in the presence of γ -collidine which promotes a strong blue reaction, the limit of detection being about 0.02 μg of the ion per cm^2 of spot area. The sensitivity of the reaction therefore compares favourably with the most sensitive of the tests described by FEIGL¹¹ for NH_4^+ . It is also shown that when the reagent is used in conjunction with paper electrophoresis as a test for NH_4^+ , a high order of specificity may be achieved.

Experimental

Materials. The alkali metal acetates and the amino acids and amines listed in Table I were commercial samples of analytical grade. The amino acids were prepared for use as 0.02-*M* solutions in water and the amines and alkali metal salts as 0.1-*M* solutions. Ammonium acetate (E. Merck, Darmstadt) was dissolved in glass-distilled water to give a 0.1-*M* stock solution which was diluted as required for testing the

TABLE I

RATES OF MIGRATION OF SOME AMINES AND AMINO ACIDS RELATIVE TO NH_4^+ (M_A VALUES)

Compounds were detected with the ninhydrin-collidine reagent¹³ after paper electrophoresis at 21 V/cm for 20 min using 0.05 M potassium acetate in 2M formic acid as electrolyte.

Compound	$M_A \cdot 10^3$	Colour reaction
NH_4^+ ^a	100	Blue
Ethylene diamine	92	Blue
Methylamine	81	Grey
Hydrazine ^b	76	Pale yellow
Hydroxylamine ^b	64	Blue
Ethanolamine	55	Blue-green
Ornithine	41	Blue
Lysine	39	Blue
Arginine	37	Blue
Glycine	29	Blue-brown

^a NH_4^+ migrates at the rate of 27 cm/h/kV of applied potential.

^b Hydrazine is more readily detected with chromium trioxide-permanganate-sulphuric acid¹⁶. Hydroxylamine is the only other compound listed which also reacts with this reagent.

sensitivity of the ninhydrin reagent. The test mixtures were prepared from the ammonium acetate stock solution diluted 100-fold; the other substances (potassium acetate and the amines) were dissolved in separate aliquots of this ammonium acetate solution to give 0.1-M solutions of each.

Electrolytes. The following electrolytes were used: (a) A solution, pH 2.0, containing (0.75 M) formic acid and (1 M) acetic acid¹²; (b) a solution, pH 2.0, containing (2 M) formic acid and (0.05 M) potassium acetate.

Spray reagent. The spray reagent¹³ was prepared by dissolving (0.4 g) of ninhydrin in 95 ml of isopropanol and then adding 5 ml of γ -collidine (2,4,6-trimethylpyridine).

Apparatus and procedure. The enclosed-strip paper electrophoresis apparatus and procedure are described in detail elsewhere⁷. Whatman No. 4 paper was used and test solutions applied in line across the middle of paper strips by means of a platinum loop delivering 1 μ l. Caffeine was used as marker for zero migration without serious error¹⁴ and the rates of migration of amino acids and amines calculated relative to that of NH_4^+ applied to the same paper (M_A values).

Electrophoresis was allowed to proceed for 15–20 min at about 21 V/cm, the temperature of the paper strips being maintained at about 20°. Under these conditions NH_4^+ migrates at the rate of about 27 cm/h/kV of applied potential.

The papers were dried in the oven at 100° and heated for a further 5 min to remove most of the acid electrolyte. Caffeine was located as a dark blue spot under a Hanovia Chromatolite ultraviolet lamp and the papers treated with the ninhydrin-collidine spray reagent. For best results with this reagent, papers should be sprayed (both sides) at the rate of about 5 ml of reagent per 100 cm² of paper area and then heated in the oven at 100° for 4–5 min.

Results and discussion

The ninhydrin-collidine reagent described in the *Experimental* section was for-

mulated by STÜBCHEN-KIRCHNER¹³ as a polychromatic spray reagent for amino acids on paper chromatograms. It was found in the present work to give an easily perceptible blue reaction with as little as 1 nmole NH_4^+ subjected to paper electrophoresis under the above conditions in electrolyte (a) containing formic and acetic acids at pH 2. (An acid electrolyte of relatively low pH is required to retain traces of NH_4^+ on the pherograms during the process of heating to dry them.) Unfortunately, the reagent also reacts with alkali metal ions, as do the common ninhydrin reagents⁹, and Gross¹⁰ has already warned about the possibility of confusing NH_4^+ with K^+ on pherograms, as the two ions have very similar mobilities.

The colour reaction given by K^+ (and other alkali metal ions) with the ninhydrin-collidine reagent is comparatively weak, however, and it was found that small amounts of potassium salts could be incorporated in electrolytes without seriously increasing the intensity of the background colour of the treated pherograms. As expected, K^+ in test solutions subjected to electrophoresis in K^+ -containing electrolytes was not subsequently detected on the pherograms, and for this reason electrolyte (b) containing (0.05 *M*) potassium acetate in 2 *M* formic acid was found to be useful for the unequivocal identification of traces of NH_4^+ present in mixtures containing K^+ in relatively great excess. For example, 1 μl of test solution containing (10^{-3} *M*) NH_4^+ and 0.1 *M* K^+ was subjected to electrophoresis under the standard conditions in electrolyte (b) and NH_4^+ (1 nmole) subsequently detected as a small undistorted blue spot on a paler blue background; K^+ applied separately on the same paper as a 0.1-*M* solution gave no reaction which was detectable against the background.

Some amines and amino acids also react with the ninhydrin-collidine reagent to give blue spots on pherograms but of those tried, none interfered with the separation or identification of NH_4^+ . Of the common amino acids, the most cationic are the basic ones, ornithine, lysine and arginine but, as shown in Table I, their mobilities in electrolyte (b) are less than half that of NH_4^+ . Some of the amines, however, have mobilities approaching that of NH_4^+ and, of these, ethylene diamine is most likely to be confused with it, but tests showed that NH_4^+ in solutions containing each of the amines, including ethylenediamine, in 100-fold molar excess could be separated cleanly under the standard conditions of electrophoresis and 1-3 nmole amounts could easily be detected. It is noteworthy, however, that the clean separation of traces of NH_4^+ from ethylenediamine in electrolyte (a) is difficult, if not impossible to achieve because the latter migrates in this electrolyte as an "inverted comet".

Electrolyte (a) has been recommended for the separation of amino acids¹² and, if required, it is conveniently used for the simultaneous separation and identification of NH_4^+ when K^+ or ethylenediamine are not also present in experimental solutions. Electrolyte (b) is of value for the unequivocal identification of traces of (NH_4)⁺ in the presence of these interfering substances. The ninhydrin-collidine reagent can be used with advantage on pherograms run in either electrolyte, but it is of interest that the ordinary reagent, (0.5%) ninhydrin in acetone⁹, applied to pherograms run in electrolyte (b) gives a reaction with NH_4^+ which compares in intensity with that of the collidine reagent. It appears that the K^+ on these pherograms sensitizes the NH_4^+ -ninhydrin reaction in much the same way as collidine does. This sensitization also works in a converse fashion as can be shown by separating K^+ in an acid electrolyte containing NH_4^+ . The spot of K^+ detected with the ordinary ninhydrin

reagent is much more intense than in the absence of background NH_4^+ . The detection of other alkali metal ions, Na^+ and Li^+ , is facilitated in the same way, and this finding lends support to the suggestion of Gross^{9,10} that the interaction of this group of ions with ninhydrin may be mediated by nitrogenous or other impurities in the paper.

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- 1 G. B. HEISIG AND F. H. POLLARD, *Anal. Chim. Acta*, 16 (1957) 234.
- 2 A. E. STEEL, *Nature (London)*, 173 (1954) 315.
- 3 K. BLAU, *Biochem. J.*, 80 (1961) 193.
- 4 T. BARNABAS, M. G. BADVE AND J. BARNABAS, *Naturwissenschaften*, 41 (1954) 478.
- 5 V. KOUŘÍM, J. KRTIL AND C. KONEČNÝ, *Chem. Listy*, 52 (1958) 262.
- 6 D. GROSS, *Nature (London)*, 184 (1959) 1633.
- 7 J. L. FRAHN AND J. A. MILLS, *Aust. J. Chem.*, 17 (1964) 256.
- 8 R. WEBER, *Helv. Chim. Acta*, 34 (1951) 2036.
- 9 D. GROSS, *J. Chromatogr.*, 10 (1963) 221.
- 10 D. GROSS, *J. Chromatogr.*, 10 (1963) 233.
- 11 F. FEIGL, *Spot Tests in Inorganic Analysis*, Elsevier, Amsterdam, 5th ed., 1958, p. 516.
- 12 D. GROSS, *Nature (London)*, 176 (1955) 72.
- 13 H. STÜBCHEN-KIRCHNER, *Hoppe-Seyler's Z. Physiol. Chem.*, 349 (1968) 1049.
- 14 J. L. FRAHN, *J. Chromatogr.*, 48 (1970) 577.
- 15 J. L. FRAHN AND J. A. MILLS, *Aust. J. Chem.*, 12 (1959) 65.

Received November 6th, 1972