

CHROM. 4773

Isolation of ammonia by use of zirconium phosphate cation exchanger

Most methods currently utilized for the separation of ammonia from interfering compounds are based on the principle of gaseous diffusion of ammonia from an alkaline medium and absorption by an acidic medium¹⁻³.

This communication describes a simple method for separating ammonia from interfering substances. Zirconium phosphate is utilized as an inorganic cation exchanger to adsorb the ammonium ion thus separating it from interfering compounds. Once eluted, the ammonium ion can be oxidized by hypochlorite and reacted with phenol to form a compound whose absorbance obeys Beer's law⁴.

Reagents and chemicals

For the preparation of sodium phenolate 100 g NaOH were dissolved in water and 120 g phenol added; the final volume was brought to 500 ml. Commercial grade clorox was used as the source of sodium hypochlorite. Zirconium phosphate (50-100 mesh) was supplied by Bio-Rad Laboratories.

Other chemicals used were concentrated nitric acid and cesium chloride.

Column apparatus

The zirconium phosphate column (0.8 cm × 0.8 cm) is connected to two reservoirs by a 3-way stopcock (Fig. 1). Flow rates are controlled by a modification of the Mariotte principle⁵. The column is equipped with a removable cap to facilitate loading.

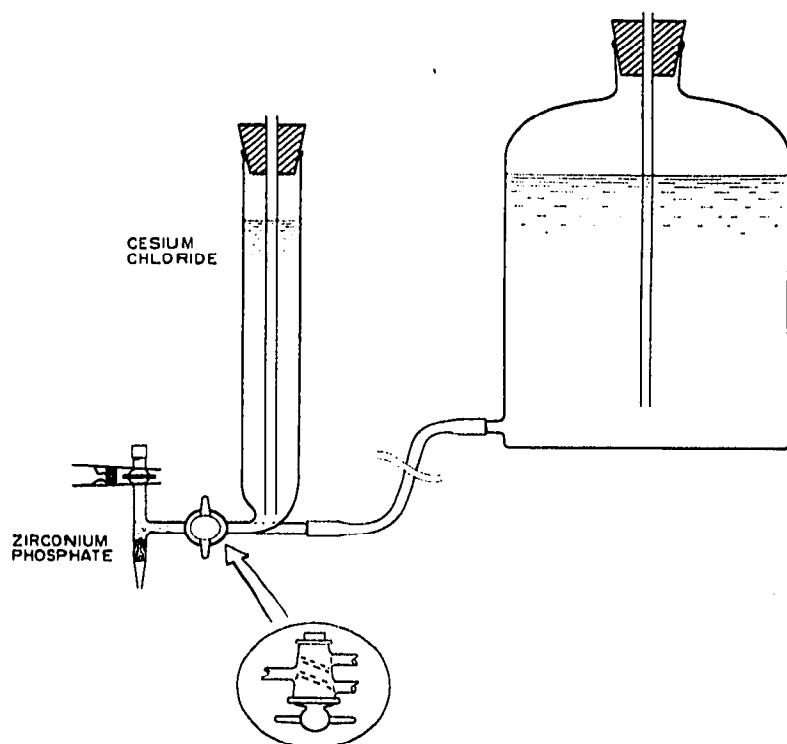


Fig. 1. Zirconium phosphate column for isolation of ammonia.

Method

This procedure contains two fundamental operations. First, ammonia is separated from indigenous interfering substances by column chromatography. Then it is eluted from the column and quantified.

A 1-ml aliquot of sample is adsorbed on the ion-exchange column. The column is rinsed with water (3 ml) at a flow rate of $7.5 \text{ ml min}^{-1} \text{ cm}^{-2}$ to remove contaminants. The adsorbed ammonia is displaced by flowing 1 ml of cesium chloride (1.24 M) at a

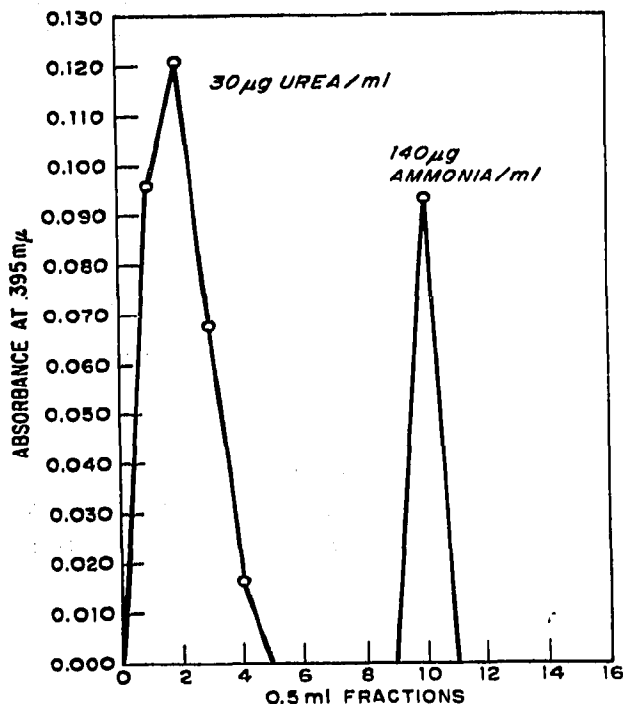


Fig. 2. Elution curve showing the separation of ammonia and urea on the zirconium phosphate column.

rate of $0.05 \text{ ml min}^{-1} \text{ cm}^{-2}$. Subsequent to elution of ammonia the column is regenerated with concentrated nitric acid and the cesium recovered for re-use. After elution of the cesium, residual acid is removed by rinsing with water.

To 1 ml of the eluate from the zirconium phosphate column 0.7 ml phenol reagent and 0.4 ml sodium hypochlorite (5.25%) are added. After 5 min the absorbance is measured at a wavelength of $395 \text{ m}\mu$ or $625 \text{ m}\mu$, depending on the concentration range.

Results and discussion

Ammonium ion can be eluted from zirconium phosphate either by increasing the acidity⁶ or displacing it by the addition of a competing ion with a larger distribution

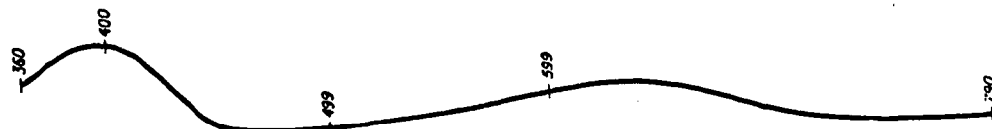


Fig. 3. Absorption spectrum of the phenol-indophenol complex.

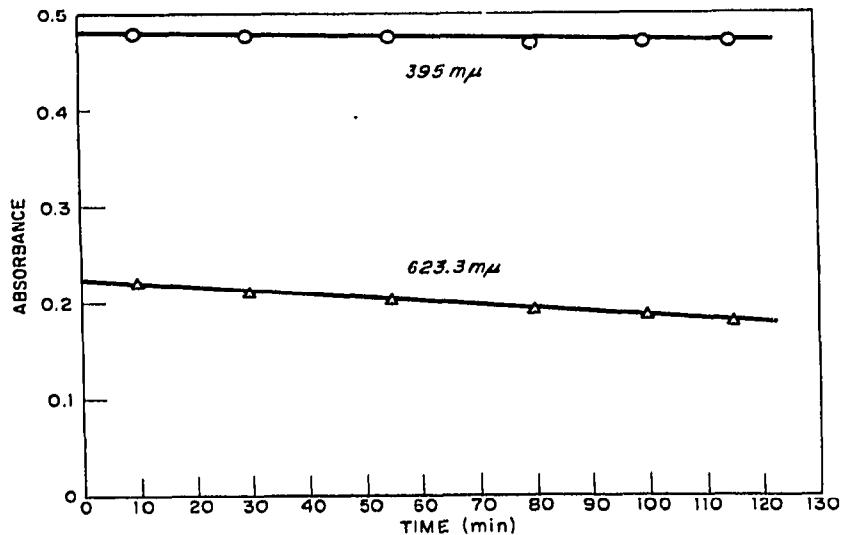


Fig. 4. Stability of the phenol-indophenol compound with time at 395 $m\mu$ and 623.3 $m\mu$.

coefficient. The high distribution coefficient of the ammonium ion necessitated the utilization of concentrated nitric acid for its elution from the ion-exchange column. The concentrated acid, however, interfered with subsequent operations. Therefore, displacement of ammonia with cesium was chosen.

To evaluate the ability of this procedure to separate ammonia adequately from interfering biological substances two probable contaminants were tested. Fig. 2 indicates the ease by which ammonia can be isolated in the presence of urea. Similar separations were obtained in the presence of glutamine.

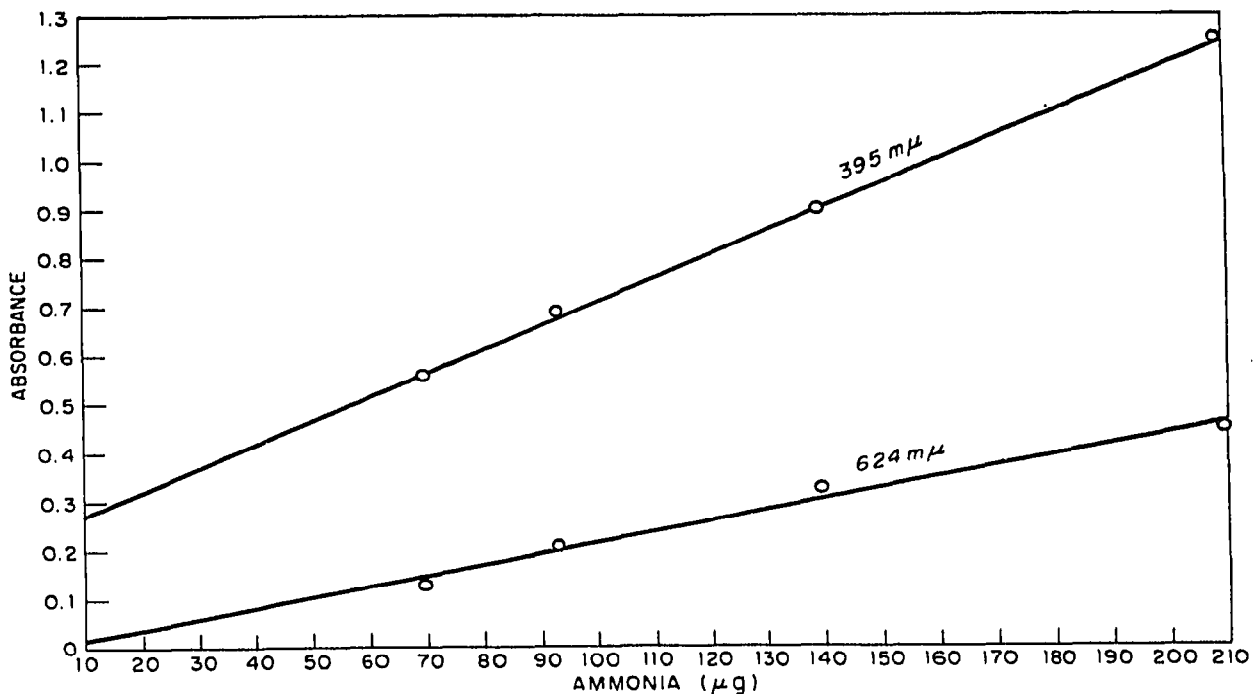


Fig. 5. Calibration curve relating the absorbance at 395 $m\mu$ and 623.3 $m\mu$ with concentration of ammonia.

An absorption spectrum (Fig. 3) of the phenol-indophenol complex was taken and from this spectrum it is apparent that measurement can be made at either of two wavelengths. Experience indicated that the stability of the compound with time was different at the two wavelengths (Fig. 4). Decrease in absorbance occurred at 625 m μ but the absorbance at 395 m μ remained stable. Since absorbance at 395 m μ was stable with time and gave maximum sensitivity, this became the wavelength of choice. Eqns. 1 and 2 are the equations of the line of best fit derived by linear regression from the calibration data (Fig. 5) taken at 624 and 395 m μ , respectively.

$$\mu\text{g NH}_4 = \frac{\text{abs} + 0.003}{0.00222} \quad (1)$$

$$\mu\text{g NH}_4 = \frac{\text{abs} - 0.225}{0.00488} \quad (2)$$

The zirconium phosphate column has possible applications to a wide range of chemical systems. Modifications of the chromatographic parameters would make possible measurement of ammonia in biological extracts and other preparations.

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- 1 E. J. CONWAY AND A. BRYNE, *Biochem. J.*, 27 (1933) 419.
- 2 D. SELIGSON AND H. SELIGSON, *J. Lab. Clin. Med.*, 38 (1951) 324.
- 3 D. SELIGSON AND K. HIRAHARA, *J. Lab. Clin. Med.*, 49 (1957) 962.
- 4 L. T. MANN, *Anal. Chem.*, 35 (1963) 2179.
- 5 F. A. SCHWERTZ, *Anal. Chem.*, 22 (1950) 1214.
- 6 W. J. MAECK, M. E. KUSSY AND J. E. REIN, *Anal. Chem.*, 35 (1963) 2086.

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