J Forensic Sci, Apr. 1977, Vol. 22, No. 2

TECHNICAL NOTE

P. A. F. Pranitis,[1] *B.A. and Abraham Stolman,*[2] *Ph.D.*

Spectrophotometric Determination of Cyanide in Biological Materials

The analytical methods currently used for the determination of cyanide in biological materials are the aeration method with the Prussian blue (ferricyanide) reaction [*1*] and some microdiffusion methods [*2,3*]. The methods are dependent on color reactions, the procedures are either time-consuming or cumbersome, and the results are of questionable accuracy. A method using the ultraviolet light absorption characteristics of a cyanonickelate complex was developed by Scoggins [*4*] for the accurate identification and quantitation of cyanide in aqueous solution. This method was found to be readily adaptable for the determination of cyanide in biofluids and tissues. The resulting cyanide-nickel complex which formed rapidly was found to be stable and could be quantitated readily. This paper presents a method to detect cyanide in biological materials by a spectrophotometric procedure.

Reagents and Procedure

The ammoniacal nickel chloride reagent was produced by diluting 237.7 mg $NiCl_2 \cdot 6H_2O$ and 67.5 ml ammonium hydroxide to 1 litre with distilled water. A 10% solution of lead acetate in distilled water was also used. A 50% solution of sulfuric acid was used to produce hydrocyanic acid (HCN).

The procedure involves the liberation of cyanide from the biological material and then bubbling it through a $NiCl_2$ reaction solution. This is conventionally accomplished by setting up three gas washing bottles in series (Fig. 1) with the appropriate materials and reagents in the respective bottles. Five millilitres of ammoniacal $NiCl_2$ solution are poured into a large test tube (approximately 12.5 by 1.5 cm), which is then inserted into reaction bottle (*3*, Fig. 1). The inlet tubing is placed inside the test tube containing the $NiCl_2$ solution. Twenty millilitres of lead acetate solution are placed in a reaction bottle (scrubber) (*2*, Fig. 1), with the glass tubing submerged in the lead acetate solution to remove sulfides. To the specimen bottle (*1*, Fig. 1) is added 2 ml blood or 2 g homogenized

Presented at the 28th Annual Meeting of the American Academy of Forensic Sciences, Washington, D.C., 17-20 Feb. 1976. Received for publication 28 July 1976; revised manuscript received 12 Oct. 1976; accepted for publication 14 Oct. 1976.

[1]Toxicological chemist, Connecticut State Department of Health, Hartford, Conn. 06101.

[2]Chief toxicologist, Connecticut State Department of Health, Hartford, Conn., and assistant clinical professor, Department of Laboratory Medicine, University of Connecticut School of Medicine, Farmington, Conn.

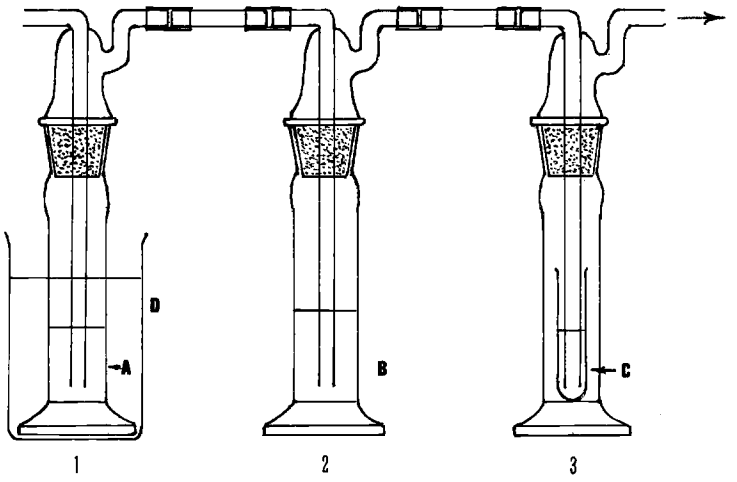

FIG. 1—*Diagram of the three gas washing bottles in sequence;* (1) *specimen bottle;* (2) *scrubber bottle;* (3) *reaction bottle;* (A) *sample;* (B) *lead acetate solution;* (C) *nickel chloride solution; and* (D) *water bath.*

tissue, plus 15 ml water, an antifoam agent,[3] and 2 ml of 50% H_2SO_4. The bottle containing the specimen is then placed in a water bath at 90°C. The water level in the bath is maintained at a higher level than the liquid in the specimen bottle. Suction (sufficient to produce 200 bubbles per min) is then applied for 20 min. At the end of the aeration period the cyanide in the $NiCl_2$ solution is now ready to be determined quantitatively by scanning in the ultraviolet range and recording the optical density at 267 nm (versus a reagent blank of the $NiCl_2$ solution) (Fig. 2).

Discussion

Cyanide ion reacts with the ammoniacal $NiCl_2$ immediately to form the tetracyanonickelate anion complex which can then be determined spectrophotometrically without further treatment or additional preparation. The method is sensitive to detect 0.5 μg cyanide per millilitre of nickel chloride solution. The complex which is formed is very stable at room temperature and need not be analyzed immediately. The presence of sulfides in the sample will turn the nickel chloride solution black and cloudy. This complication is avoided by passing the HCN vapors through the lead acetate solution to remove extraneous materials. Standard solutions of potassium cyanide were analyzed with and without the lead acetate scrubbing solution. As there was no difference in absorption between the two, it was presumed that no lead-cyanide complex was being formed.

A standard curve of cyanide concentration versus absorbance was prepared from a standard solution of potassium cyanide with a concentration of 10 μg cyanide per millilitre by making various dilutions of this solution. The absorbance of the cyanonickelate compound follows Beer's law, and a linear relationship was found over the range 2.5 to 100 μg of cyanide ion per millilitre of $NiCl_2$ solution in agreement with Scoggins' findings [*4*].

Studies are in progress using this method to determine cyanide in biofluids and tissues stored under various conditions and to evaluate the effects of putrefaction on cyanide production.

[3]Silicone defoamer, Dow Corning, Midland, Mich.

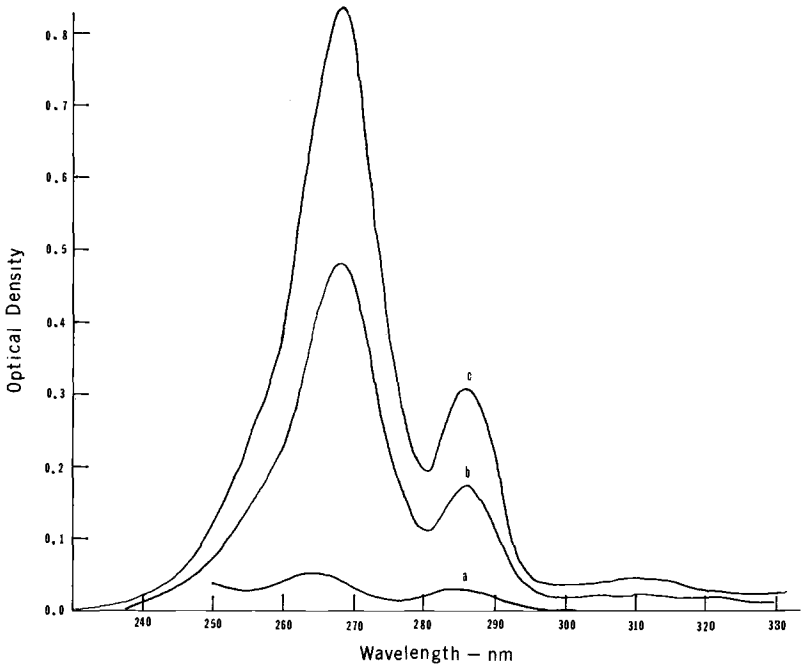

FIG. 2—*Ultraviolet spectrum of tetracyanonickelate complex;* (a) *0.5 μg cyanide per millilitre;* (b) *5.0 μg cyanide per millilitre; and* (c) *8.2 μg cyanide per millilitre.*

References

[*1*] Gettler, A. O. and Goldbaum, L., "Detection and Estimation of Microquantities of Cyanide," *Analytical Chemistry,* Vol. 19, No. 4, 1947, p. 270.

[*2*] Feldstein, M. and Klendshoj, N. C., "The Determination of Volatile Substances by Microdiffusion Analysis," *Journal of Forensic Sciences,* Vol. 2, No. 1, 1957, p. 39.

[*3*] Tompsett, S. L., "A Note on the Determination and Identification of Cyanide in Biologic Materials," *Clinical Chemistry,* Vol. 5, No. 6, 1959, pp. 587–591.

[*4*] Scoggins, M. W., "Ultraviolet Spectrophotometric Determination of Cyanide Ion," *Analytical Chemistry,* Vol. 44, No. 7, 1972, pp. 1294–1296.

Connecticut State Department of Health
P.O. Box 1689
Hartford, Conn. 06101