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The Determination of Sub-microgram Quantities of Cyanide in Biological Materials

In forensic toxicology it is often necessary to determine cyanide levels in biological materials. Reviews on methods for determining cyanide levels include those of Guatelli [*10*] and Bark and Higson [*11*].

Methods commonly used include colorimetric [*1,2,4,9*] and the formation of metal complexes, such as ferric ferri thiocyanate [*3*] or Prussian blue [*3,5,7*].

In Aldridge's method [*1*], claimed to be specific for cyanide [*8*], cyanide is converted to cyanogen bromide, which reacts with pyridine and an aromatic amine (benzidine) to form a dyestuff, the colour intensity being proportional to the amount of cyanide present. Similar methods convert the cyanide to cyanogen chloride, rather than cyanogen bromide, and in place of benzidine use barbituric acid [*4*] or a pyrazolone reagent [*9*] to form a dyestuff. Such micro methods usually remove cyanide from the sampel by diffusion [*2,4,9*] and can detect cyanide down to 0.1 μg.

In Gettler and Goldbaum's method [*5*], cyanide is distilled from the sample as hydrogen cyanide, which is passed through a filter paper impregnated with ferrous sulphate and sodium hydroxide. Upon immersing the paper in dilute hydrochloric acid, a stain of Prussian blue is produced, which may then be compared with standard stains. This method is also specific and the lower limit of detection is 0.1 μg of cyanide.

Although micro-diffusion methods are quantitative and require no supervision, the diffusion process requires several hours to ensure complete removal of hydrogen cyanide from the sample.

Gettler and Goldbaum's method separates cyanide by distillation and, although it requires supervision, is simple and rapid. The comparison of stains, however, renders it only semiquantitative.

The method described here combines the advantages of both methods to give a quantitative cyanide determination which is rapid, simple, and practical. Cyanide is removed from the sample by distillation as hydrogen cyanide, collected in sodium hydroxide, and determined by converting to cyanogen bromide and measuring the absorbance of the complex formed after reacting with pyridine–benzidine.

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Method

Reagents

1. Standard Cyanide Solutions

Stock solution 1—2.5027 g potassium cyanide per litre of solution. Each ml of solution contains 1 mg of cyanide. The solution is diluted as required.

Stock solution 2—5.0054 g potassium cyanide per litre of N NaOH. Each 0.5 ml of solution contains 1 mg of cyanide. The solution is diluted with N NaOH as required.

2. Bromine Water—A saturated aqueous bromine solution is prepared by dissolving 25 ml bromine in 10 ml chloroform, adding 100 ml water and thoroughly shaking [6]. Dilute 1 volume of the aqueous phase with 3 volumes of water before using; prepare the dilution daily.

3. Sodium Arsenite Solution—Dissolve 1 g of arsenious oxide in 5 ml N NaOH and dilute to 50 ml with water.

4. Pyridine–Benzidine Reagent

a) Dissolve 0.18 g of benzidine in 2.5 ml N HCl and dilute to 50 ml with water.
b) Dilute 6 ml pyridine to 10 ml with water and add 1 ml concentrated hydrochloric acid.
c) The reagent is made by adding 2 ml of (a) to 8 ml (b). It does not keep and must be made immediately before use.

5. Saturated Aqueous Lead Acetate Solution

6. 10 percent Trichloroacetic Acid—Dissolve 10 g trichloroacetic acid in water and make up to 100 ml.

Procedure

Nitrogen (or air, if preferred) is passed through the apparatus in Fig. 1 for 20 minutes at the rate of 40 to 50 ml per minute. Tube A contains water and functions as a rough flow meter. Tube B contains 1 ml blood (or an aliquot representing 1 g macerated tissue), 3 ml water, 0.5 ml saturated lead acetate, and 5 ml 10 percent trichloroacetic acid. This tube is immersed in a water bath at 90 C, and the evolved hydrogen cyanide is passed into N NaOH contained in a trap, tube C (a test tube 50 mm long and 10 mm internal diameter). If the sample contains between 0.1 μg and 1.5 μg cyanide, tube C should contain 1.0 ml N NaOH. If however, the sample contains less than 0.1 μg cyanide, it is recommended that tube C contain 0.5 ml N NaOH.

To the N NaOH containing the cyanide, and simultaneously a N NaOH blank, are added 0.1 ml glacial acetic acid and 0.1 ml bromine water. Five minutes later 0.1 ml sodium arsenite, 0.5 ml pyridine-benzidine reagent, and 0.2 ml water are added. Using Pasteur pipettes the contents are transferred to 5 mm pathlength semimicro cells and 5 minutes after the addition of the pyridine-benzidine reagent the absorbance is measured at 525 nm. A Beckman DK–2A Recording spectrophotometer employing standard settings was used in this work.

Where the sample contains less than 0.1 μg cyanide and only 0.5 ml N NaOH in tube C, the quantities of reagents are halved and the colour developed is measured in micro cells with 10 mm pathlength.

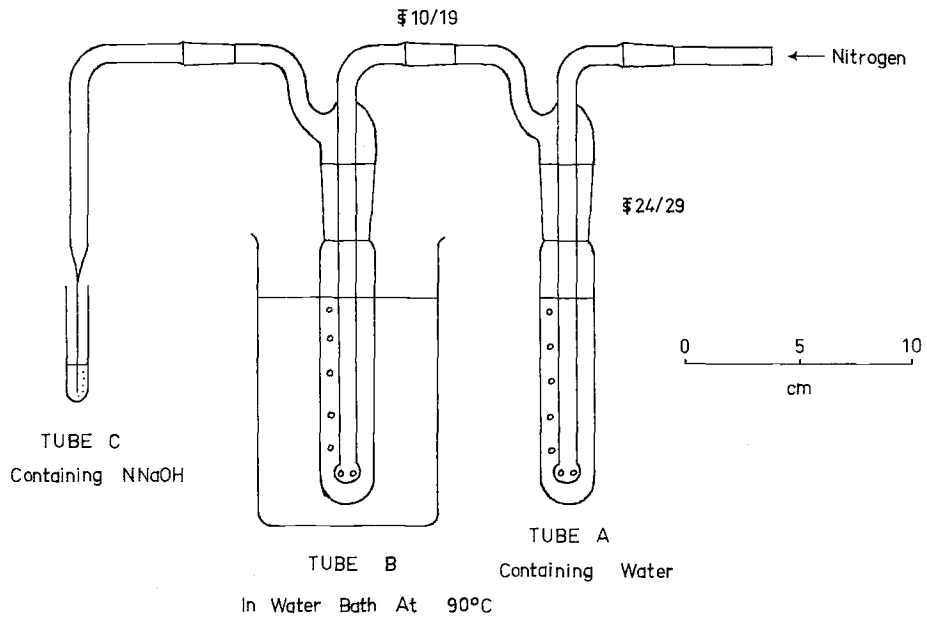


FIG. 1—*Cyanide distillation apparatus (drawn to scale).*

Results

Typical results are shown in Figure 2.

Dilutions of stock solution 1 were prepared so that 1 ml contained from 0.1 μg to 1.5 μg of cyanide. These amounts were added to blood and cyanide determined by distillation, using 1 ml N NaOH in tube C. The procedure was repeated using from 0.025 μg to 0.2 μg of cyanide and 0.5 ml N NaOH in tube C.

Standard curves were prepared by making N NaOH dilutions of stock solution 2 so that 1 ml contained from 0.1 μg to 1.5 μg of cyanide. Cyanide was then determined omitting the distillation step. The procedure was repeated with 0.025 μg to 0.2 μg of cyanide contained in 0.5 ml N NaOH.

The recovery of cyanide by distillation is 95 percent in the upper range and 92 percent in the lower range.

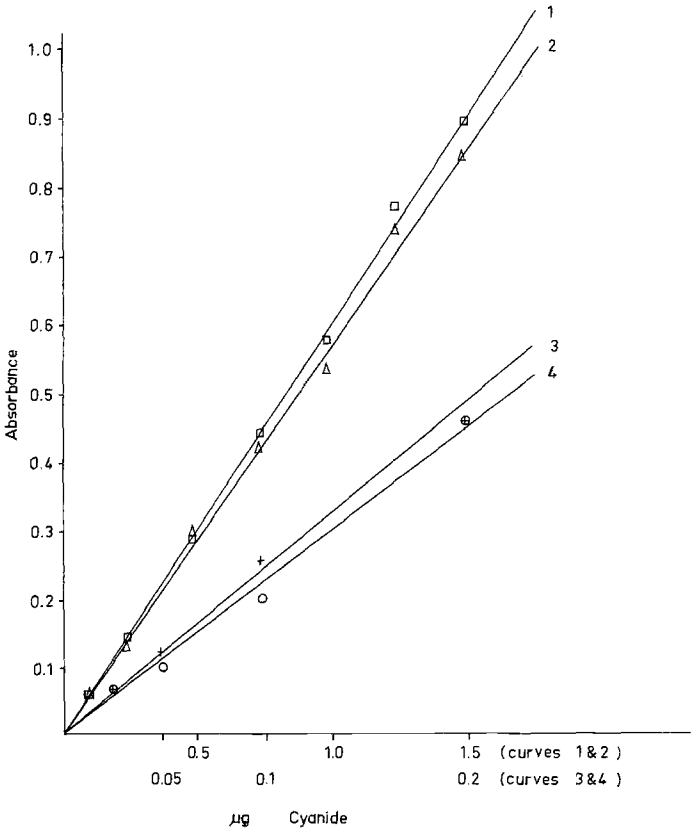
In Fig. 2 each point is the mean of three determinations. Separate determinations of 0.1 μg cyanide added to blood, and those of the 0.025 μg cyanide standard, varied from the mean by 18 percent. With other amounts the variation was not more than 4 percent.

Discussion

Evolution of Cyanide

The nitrogen flow rate should be held constant. Nitrogen flowing at 40 ml per minute for 20 minutes removes all cyanide from the sample. If the rate of flow exceeds 50 ml per minute, some of the solution may be expelled from tube C. Otherwise, its volume remains constant.

Trichloroacetic acid is used to precipitate proteins to prevent foaming, and to acidify the contents, releasing hydrogen cyanide. As HCN is soluble in water below 70 C, it is



1. □ standard cyanide solutions.
2. △ cyanide added to blood.
3. + standard cyanide solutions.
4. ○ cyanide added to blood.

FIG. 2—*Relation of absorbance to cyanide concentration.*

necessary to keep the contents at a higher temperature to ensure that all hydrogen cyanide is removed.

Adding Reagents

When adding the reagents to tube C, it is essential to mix each addition thoroughly before proceeding to the next step. The NaOH solution must be neutralized or made slightly acidic by adding acetic acid before bromine water is added.

If the bromine water is too strong, a precipitate will occur when the pyridine-benzidine reagent is added: the bromine water was therefore standardized to a constant strength that was compatible with the reagent, that is, a four times dilution of saturated bromine water.

Dilution

When determining the larger amounts of cyanide, using 5 mm pathlength semi-micro cells, 0.2 ml of water are added so that all the window in the light path is covered by liquid

in the cell. Although additional volume was not necessary for the microcells, with the scaled down procedure for smaller amounts of cyanide, 0.1 ml water was added so that the reagents were in the same concentrations in both procedures.

Determining Smaller Amounts of Cyanide (0.025 μg–0.2 μg)

In this range of cyanide the final volume was halved from 2 ml to 1 ml and the light pathlength doubled from 5 mm to 10 mm in order to give a fourfold increase in sensitivity. The absorbances of 0.2 μg of cyanide, which can be read from the graphs in Fig. 2, indicate that this was achieved.

Stability of the Developed Colour

Aldridge [*1* (1944)] found that the colour was stable from 6 to 24 minutes. In the method described here the maximum absorbance occurs at 517 nm three minutes after adding the pyridine–benzidine reagent, changes to 525 nm after 5 minutes, and thereafter the absorbance decreases only slowly at this wavelength during the next 35 minutes. The absorbance at 525 nm decreases by 18 percent for 1.5 μg cyanide and 9 percent for 0.05 μg cyanide over this time interval. The absorbance was therefore measured at 525 nm 5 minutes after adding the pyridine–benzidine reagent. If measured at 10 rather than 5 minutes, the error is low by 3 percent for 1.5 μg cyanide and 1.5 percent for 0.05 μg. As indicated in Fig. 2, the Beer–Lambert law was followed over the ranges of cyanide taken.

Absorbance Cells

If preferred, 10 mm pathlength macro cells may be used for measuring absorbances of the larger amounts of cyanide. However, to cover all the cell window in the light path, 3 ml of solution are required; and tube C is too small for efficient mixing of this volume. A suggested procedure would be to use a larger tube with 1.5 ml N NaOH to trap the evolved cyanide, add proportionately more of each reagent for colour development, and thus obtain 3 ml of solution for the absorbance measurement.

Application to Biological Materials

Cyanide levels in biological materials from fatal cyanide poisonings were determined by this method and also by Gettler and Goldbaum's method [*5*]. The results are given in Table 1.

TABLE 1—*Cyanide levels in fatal poisonings.*

Case	Material	Cyanide Detected, mg %	
		Gettler and Goldbaum	This Method
1	Blood	8.0	7.76
	Liver	0.9	1.15
	Kidney	3.0	2.84
2	Blood	5.3	4.94
3	Blood	0.5	0.42
4	Blood	0.3	0.33
	Liver	0.15	0.10
5	Blood	1.0	1.25
6	Blood	1.0	0.85

Summary

A rapid, sensitive method is described for the quantitative determination of cyanide in biological materials. Cyanide, distilled from the sample in a stream of nitrogen, is collected in sodium hydroxide, and the absorption of the pyridine–benzidine complex is measured in a spectrophotometer. The lower limit of detection is 0.02 μg of cyanide.

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