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Assay of Cyanide in Biological Materials Using 2,2-Dihydroxy-1,3-Indanedione

Drochioiu, G. and Mangalagiu, I.
Al. I. Cuza University of Iasi, 11 Carol I, Iasi-6600, Romania

Abstract: The paper describes a novel cyanide assay in biological samples based on its reaction with 2,2-dihydroxy-1,3-indanedione. Being very simple, accurate, fast, selective and sensitive, the method is useful in the determination of as little as 0.025 μg ml^{-1} CN^- in biological samples. The molar absorptivity was found to be 4.8×10^4 l $mole^{-1}$ cm^{-1} for 1.0 μg ml^{-1}. The oxidizers such as oxygen, bromine, chlorine, and even copper (II) and silver (I) ions interfere seriously reacting with the 2*H* indene derivative formed. The coefficient of variation for replicate analyses is 2.4%. The procedure could be applied in forensic studies and to follow the cyanide accumulation in smokers.

Keywords: Cyanide; 2,2-Dihydroxy-; 1,3-Indanedione; Biological Materials; Spectrophotometric Assay

Introduction

Hydrogen cyanide (HCN) is extremely poisonous and acts very rapidly, when absorbed through skin contact and by inhalation, with death occurring within a few minutes from respiratory failure (Brands, 1987). Cyanide reacts with the trivalent iron of cytochrome oxidase (aa_3) in mitochondria, thereby blocking the reduction of oxygen required for cellular respiration, resulting in cytotoxic hypoxia.

Cyanide inhibition of cytochrome oxidase halts electron transport, oxidative phosphorylation, and aerobic glucose metabolism, resulting in a lactic acidemia and high concentration of oxyhemoglobin in the venous return. The lethal dose by mouth in an adult is about 100 mg of potassium cyanide and death because of central respiratory arrest is rarely delayed more than an hour. Early symptoms of toxic reactions to lower levels of exposure to HCN may include weakness, headache, confusion, and occasionally nausea and vomiting, due to the chemoreceptive cells of the carotid and aortic bodies responding to decreased oxygen, and later becomes slow and gasping. Cyanide provokes also the biostructure breakdown, which is maintained intact by oxygen supply (Drochioiu *et al.*, 2001). High concentrations of HCN may cause almost instantaneous collapse, cessation of respiration, and death.

Hydrocyanic acid is extensively used as a fumigant for grain mills, elevators, cargo ships and storage warehouses. It also enters the environment from various technical operations such as blast furnace, electric plating, gas work and coke ovens in steel plant. It is reported to be present in cigarette smoke (Amlathe and Gupta, 1990; Drochioiu *et al.*, 2000). Recent cases of cyanide pollution on Siret River in Romania showed that following the absorption of such cyanogens as acetone cyanohydrin, aliphatic nitriles, nitrile glucosides, or large doses of thiocyanate, prolonged elevated cyanide concentrations in the blood are associated with varying degrees of toxic signs. The origin of blood cyanide concentrations may also be from hydrocyanic acid inhalation from combustion fumes of plastics (Wetherell, 1966 and Sunshine and Finkle, 1964). Normal cyanide content of fresh blood is less than 0.1 μg ml^{-1} CN^-.

Cyanide may be liberated from biological fluids by acidification (Brands, 1987). The evolved HCN is absorbed in alkali and the cyanide thus formed is qualitatively (Guilbauld and Kremer, 1966; Wawschinek *et al.*, 1968) and/or quantitatively (Feldstein and Klendshoj, 1957) determined by measuring the absorbance of chromophores formed by interaction of the cyanide ion with suitable reagents. Also, a sensitive gas-chromatographic method for the determination of cyanide in biological specimens, based on its conversion to cyanogen chloride (Valentour *et al.*, 1974) as well as a fluorometric procedure Felscher and Wulfmeyer, 1998) were described.

Nevertheless, most of these methods (Valentour *et al.*, 1974 and Felscher and Wulfmeyer, 1998) are relatively sophisticated because of the number of steps and their requirement of performance apparatus and a lot of reagents. The others, in spite of their simplicity (Guilbauld and Kramer, 1966), are less sensitive than required for routine analysis.

Therefore, we describe here a novel assay of cyanide in biological materials using 2,2-dihydroxy-1,3-indanedione (Drochioiu, 2002), which is ultra sensitive and highly selective, as well as suitable to follow the change in the blood CN^- content of the smokers, the victims of fire or the victims of poisoning.

Experimental: *Apparatus and reagents*. A Carl Zeiss Spekol spectrophotometer or similar, Conway microdiffusion dishes and usual laboratory glassware and reagents were used. All chemicals were analytical reagent grade. We used silicone lubricant, sodium hydroxide, 0.01 N, cyanide stock solution, 1000 μg ml^{-1} of CN^-, cyanide internal control solutions: 0.2 μg

ml^{-1}, 0.5 µg ml^{-1}, and 1.0 µg ml^{-1} of CN^-, sulfuric acid, 3.6N.

Color reagent. 0.200 g of 2,2-dihydroxy-1,3-indanedione (ninhydrin from Merck) were dissolved in about 40 ml water and mixed with a solution containing 2 g of sodium carbonate. The final solution was then diluted to 100 ml with water.

Procedure A. All glassware to be used was soaked in 3.6 N sulfuric acid for at least ½ hour, rinsed with water, and dried. A volume of 1.5 ml of the samples (whole blood, plasma, serum, cerebrospinal fluid, urine, bile, gastric content, or solid tissue fluidized prior to the analysis by homogenizing 1 part tissue with 2 parts water) was added to each outer reservoir of a series of microdiffusion cells. Simultaneously, a control reference solution (0.2 µg ml^{-1}, 0.5 µg ml^{-1}, and 1.0 µg ml^{-1} of CN^-, depending on the suppositional cyanide concentration of the sample) was treated similarly in another Conway cell. Then, a volume of 1.5 ml of 0.01 N sodium hydroxyde was poured into each inner reservoir of the microdiffusion cells. To each outer reservoir, containing the samples, 1.0 ml of 3.6 N sulfuric acid was added and each cell was covered, sealed immediately by placing a sealant ringed lid over the cell. After at least 2 hour at room temperature, 1 ml of the contents of the inner chambre of each cell was transferred to a test tube to be analyzed.

Procedure B. The same samples were directly analyzed, without being distillated, and the interfering substances studied. These interfering substances were added into the samples. Thus, 1 ml of sample, such as cyanide solution, plasma, serum, cerebrospinal fluid, urine, bile, or gastric content, was analyzed.

Analysis. A volume of 0.5 ml of 28 x 10^{-3} M solution of 2,2-dihydroxy-1,3-indanedione monohydrate (5 mg ml^{-1}) in 2 % sodium carbonate was added to 1 ml of sample solution containing between 0 and 1 µg ml^{-1} CN^-. The development of a purple color indicates cyanide ions are present. The test tube content was stirred vigorously and the absorbance measured at 510 nm in 1 cm glass; cuvettes of a spectrophotometer against a blank of the reagents. The same procedure was followed for the blank, which remained yellow under these conditions. When the absorbance values were too high, then the purple colored solutions were accordingly diluted. A calibration scale was prepared over the range 0-1.0 µg CN^-. The absorbance was measured after 15 min from the beginning of the reaction.

The method using pyridine to form glutaconic aldehyde (Feldstein and Klendshoj, 1957) was used for comparison.

Statistics. The standard deviation (*S*), standard deviation of the mean (s_x) and *t* and *F* parameters, and the coefficient of variation, CV %, were calculated in order to compare the two methods.

Results and Discussion

The color system obeys Beer's law in the range of 0.01 to 1.0 µg ml^{-1}. The molar absorptivity was found to be 4.8 x 10^4 l $mole^{-1}$ cm^{-1} for 1.0 µg ml^{-1}. Six urine samples containing 0.5 µg ml^{-1} cyanide were determined by the proposed procedure as compared with the method using pyridine (Feldstein and Klendshoj, 1957). The results showed an acceptable agreement between the two methods ($F = S_1/S_2 = 1.44 \times 10^{-4}/2.23 \times 10^{-4} = 0.646$; Table 1). Also, 20 urine samples collected from smokers and non-smokers were analyzed by the two methods. A good correlation coefficient was calculated between the two series of values (r = 0.999). The non-smokers presented 0.39 - 1.1 µg ml^{-1} CN^-, while smokers had 1.0 - 4.1 µg ml^{-1} CN^- per ml urine. By adding a mixture of $FeSO_4$ and $CuSO_4$ to eliminate CN^- from urine being analyzed, the purple color did not appeared demonstrating the high selectivity of the proposed method. The method was found to be free from most interferences. Amino acids and ammonia ions as well as thiocyanate, ferro- and ferricyanide did not react with the color reagent under the reaction conditions.

Nevertheless, oxidizers interfere seriously yielding lower values for the absorbance. Even copper (II) and silver (I) ions hinder the formation of the 2*H* indene derivative, which is red in color. Also, chlorine, bromine and potassium permanganate destroyed the colored compound which was formed when added in stoichiometrically proportion with cyanide.

We supposed the formation of 1,2,3-trihydroxy-2*H*-indene-2-carbonitrile and the spectroscopic data (^{1}H-NMR, IR) as well as the properties of the colored solution confirm this structure (Fig. 1).

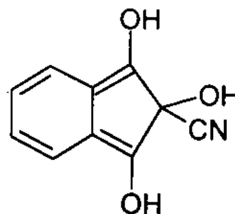

Fig. 1. 1,2,3-Trihydroxy-2*H*-indene-2-carbonitrile

Following the ingestion of ionizable cyanides, signs of acute toxicity are associated with blood concentrations above 0.20 µg ml^{-1} of CN^-. Acute fatalities usually contain more than 1.0 µg ml^{-1} of CN^-. Cyanide in the urine is often below several micrograms per milliliter.

In conclusion, this study describes a novel cyanide assay in biological samples based on its reaction with 2,2-dihydroxy-1,3-indanedione. Being very simple, accurate, fast, selective and sensitive, the method is useful in determination of as little as 0.025 µg ml^{-1} CN^- in biological samples. The coefficient of variation for replicate analyses is 2.4%. The procedure could be applied in forensic studies and to follow the cyanide accumulation in smokers.

Table 1: Evaluation of the Precision of the Proposed Method Compared with the Method Using Pyridine (Feldstein and Klendshoj, 1957) for the Determination of Hydrocyanic Acid in Water

Parameter	Proposed method (μg ml^{-1})	Pyridine method (μg ml^{-1})
C	0.495 ± 0.012	0.497 ± 0.015
s^2	1.44 x 10^{-4}	2.23 x 10^{-4}
s_x	0.0049	0.0061
CV%	2.42	3.01

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