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Technical Note

Cyanide assay based on its novel reaction with resorcinol and picric acid

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ABSTRACT

A highly selective spectrophotometric assay of hydrocyanic acid and cyanide ions based on their novel reaction with picric in the presence of resorcinol is described. Trace concentrations of cyanides in water and hydrocyanic acid collected in dilute sodium hydroxide solution are determined with a solution of 2% sodium carbonate containing 110µg/ml of resorcinol and 229µg/ml of picric acid when a novel indophenol derivative of 2,6-dinitro-5-hydroxy-4-hydroxylamino-1-cyanobenzene is afforded. The formation of the novel dye was proved by elemental and spectral analyses. The mixtures is heated at 100ºC for 30min and, after cooling, the absorbance of the red-brown solution is read at 540nm, where the blank showed a minimum absorption. Detection limit of this method has been evaluated to be below 0.01µg/ml CN^-. The color system obeys Beer's law in the range of 0.1-10µg/ml CN^-, in which a maximum molar absorptivity of 4.53×10^3l/mole.cm has been determined. Cyanide in air, in cigarette smoke, and in waste waters can directly be determined by this selective procedure, without its isolation.

Keywords: Cyanide; Picric acid; Resorcinol; Spectrophotometric assay

1. INTRODUCTION

Hydrogen cyanide is extremely poisonous when absorbed through skin contact and by inhalation and acts very rapidly, with death occurring within a few minutes from respiratory failure (Brands, 1987). High blood cyanide concentrations may also be produced by hydrocyanic acid inhalation from combustion fumes of plastics (Leithe, 1991; Wetherell, 1966; Sunshine and Finkle, 1964). 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, being also present in cigarette smoke (Drochioiu *et al.*, 2000; Amlathe and Gupta, 1990). 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. High concentrations of HCN may cause almost instantaneous collapse, cessation of respiration, and death. 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 O_2-hemoglobin in the venous return. Cyanide proved also to be toxic against the plants and their seeds (Drochioiu *et al.*, 2001).

Cyanide in the environment is frequently determined spectrophotometrically using König reaction (König, 1904; Aldridge, 1945; Epstein, 1947; Pal and Ganguly, 1987; Amlathe and Gupta, 1988). Prussian blue, sodium picrate and guaya resin methods have also been used (Drochioiu *et al.*, 2001; Prodanov, 1964). In addition, spectrophotometric methods such as those using the iron-bathophenanthroline complex (Mariaud and Levillain, 1987) or 2,2-dihydroxy-1,3-indanedione (Drochioiu, 2002a; Drochioiu, 2002b), as

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well as fluorometric (Sano *et al.*, 1987; Felscher and Wulfmeyer, 1998), enzymatic (Stein and Hain, 1995), or potentiometric (Han and Zuo, 2000) procedures have been proposed. Many of these methods are relatively sophisticated because of the number of steps and their requirement of various reagents. Some of them require an intricate equipment, which could be a serious drawback for routine cyanide assay.

Therefore, this paper aims at presenting a novel method for cyanide determination, which proved to be very selective, sensitive, simple, fast and reproducible. The assay is based on the formation of a novel red-brown indophenol dye starting from cyanide and an alkaline solution of resorcinol and sodium picrate. The reaction conditions, the interfering substances and the absorption bands, as well as the other analytical parameters are investigated in order to determine cyanide in air, cigarette smoke, and water.

2. EXPERIMENTAL

2.1. Apparatus and reagents

A Carl Zeiss Spekol spectrophotometer or similar with 1cm matched cells, a laboratory centrifuge, Conway microdiffusion dishes, an absorber with two micro-absorption vessels, connected in series, and a calibrated rotameter to check air flow, as well as usual laboratory glassware and reagents were used. IR spectra were recorded with a SPECORD-71 spectrometer (KBr) and ^{1}H-NMR spectra obtained on a BRUKER-80 AW spectrometer in DMSO and D_2O. All chemicals were analytical reagent grade and all solutions prepared with twice distilled water. Also, sodium and potassium hydroxide, 0.01N, cyanide stock solution, 1000μg/ml of CN^-, and 200μg/ml of CN^-, respectively, cyanide internal control solutions, 5μg/ml, and 10.0μg/ml of CN^-, sulfuric acid, 1.8mole/l were prepared.

Working reagent: 110-mg resorcinol and 229mg of picric acid were dissolved in about 200ml water and mixed with a solution containing 20g of sodium carbonate. The final solution was then diluted to 1000ml with twice distilled water.

Standard cyanide solutions: A 1mg/ml stock standard solution of cyanide was prepared by dissolving of 250.0mg of potassium cyanide in 100ml of 0.01mole/l potassium hydroxide solution. Working standard solutions were prepared by appropriate dilution.

Absorbing solution: A 0.01mole/l sodium hydroxide solution was used.

Materials being analyzed: Natural, industrial and wastewater as well as cigarette smoke were analyzed for cyanide content.

2.2. Procedure

Analysis: A volume of 1ml of working reagent was added to 1ml of sample solution containing between 0 and 10μg/ml CN^-. The mixture was stirred vigorously. The test-tube was kept for 30min in a water-bath at 100°C, then cooled to the room temperature and the absorbance of the red-brown solution was read at 540nm in 1-cm glass cuvettes. The same procedure was followed for a blank, which remained yellow under these conditions. A calibration scale was prepared over the range 0-10μg CN^-. The method using phloroglucinol (Amlathe and Gupta, 1990) was chosen for comparison.

Sample preparation: Controlled amounts of hydrocyanic acid vapor were generated by the dropwise addition of potassium cyanide containing 200μg/ml of cyanide to 5ml of 5M sulfuric acid. The vapor of hydrocyanic acid thus generated were passed through two micro-absorption vessels, connected in series, and containing 0.01M sodium hydroxide (1ml each) as absorbing solution. They were connected to an air sampling train attached to a suction pump. The flow rate was adjusted to 0.250l/min and sampling time was adjusted to 15min after pure air had been passed for 5min to sweep the residue of hydrocyanic acid vapor. Volumes of 1ml HCN dissolved in KOH 0.01M, or cyanide solutions were used in the experiment.

Synthesis of 3-hydroxy-4-(2-hydroxy-4-oxo-cyclohexa-2,5-dienylideneamino)-2,6-dinitro-benzonitrile: In order to establish the mechanism of color formation, we started from the supposition that cyanide ions react with picric acid and resorcinol to afford a new dye. Therefore, potassium cyanide (poison!), resorcinol and picric acid were mixed stoichiometrically (1:1:1 molar ratio) in a solution of sodium carbonate. Thus, a mixture of 340mg of resorcinol, 700mg of picric acid and 200mg KCN was dissolved into 10ml water containing 2g of sodium carbonate. Then, the mixture was heated on a boiling water bath with stirring for 20min. The solution became clear and red-brown in color. Upon adding hydrochloric acid, carbon dioxide evolved (not hydrocyanic acid) and black-brown crystals separated. The resulted precipitate was picked up by filtration. The crude product was recrystallized from alcohol. The structure of the red-brown compound was proved by elemental (C, H, and N) and spectral (IR – KBr, cm^{-1}; ^{1}H-NMR – DMSO or D_2O, δ, ppm) analyses. Additionally, the reaction of cyanide both with picric acid and resorcinol was performed and compared with the above mentioned reaction.

2.3. 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.

3. RESULTS AND DISCUSSION

3.1. Reproducibility and sensitivity

The proposed method is reproducible and the color system was found to obey Beer's law in the range 0.1-10μg/ml of CN^- (Figure 1). The maximum molar absorptivity was found to be $4.53x10^3$ (±75) l/mole. cm at 540nm and $5.19x10^3$ (±82) l/mole.cm at 516nm. Nevertheless, we chose to read the absorbance at 540nm, where the blank of the reagents absorbs lower. Thus, the ratio between the absorbance of the sample (10μg/ml CN^-) and that of the blank was calculated to be 3.65 at 420nm, 8.45 at 516nm and 13.0 at 540nm (Figure 2).

Detection limit of this method was evaluated to be 0.01μg/ml CN^- at 540nm.

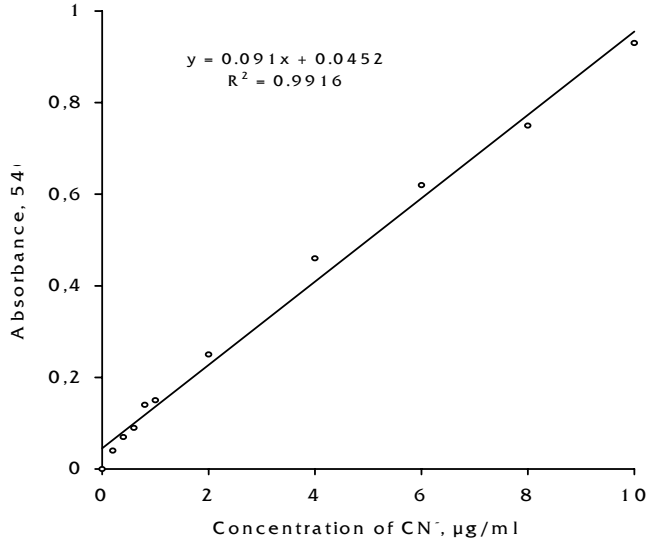

Figure 1. Standard curve for cyanide assay with picric acid and resorcinol

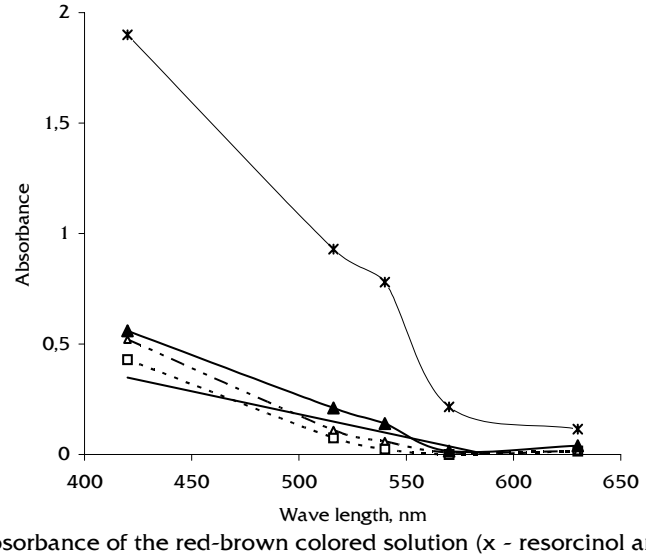

Figure 2. The absorbance of the red-brown colored solution (x - resorcinol and picric acid)

3.2. Effect of various reaction conditions

The effect of reagents, pH, time and temperature on the color reaction was studied after absorption of the hydrocyanic acid in 0.01mole/l sodium hydroxide. Also, the same parameters were investigated with a solution of 10μg/ml of CN^-, prepared in twice distilled water. A minimum of 1ml of working reagent was found to be sufficient for complete reaction of cyanide. The red-brown compound showed a high absorption band in UV, where the blank also absorbed intensively. The absorption decreased drastically from the UV region to IR region. The most suitable region for the spectral measurement proved to be that ranging from 420 to 550nm.

A minimum of 30min was needed for full color development at 100°C. Nevertheless, the test tubes could be kept on a boiling water bath for only 20min to increase the productivity of this procedure. The high sensitivity of this method offers the opportunity to diminish both the reaction time interval and the cyanide concentration without affecting the results.

Upon adding hydrochloric acid the color turns to yellow. Therefore, a high pH of the reaction should be kept. A concentration of 2% Na_2CO_3 proved to be sufficient for the spectral measurement. Higher pH values (by adding potassium hydroxide, for example) decreased the color intensity of the solutions because they created the hydrolytic conditions for the dye.

3.3. Interfering substances

Effect of co-existing species was examined with 10μg/ml of hydrocyanic acid solution. The method was found to be free from most of the interference. Nevertheless, Cu^{2+}, Ag^+ and Hg^{2+} interfered with the color reaction. To eliminate the interference of these ions, we added a solution of hydroxylamine hydrochloride to solution being analyzed. The interference of Ag^+ and Hg^{2+} was thus completely avoided. Contrary, copper ions slowly decreased the values of the absorbance even in the presence of hydroxylamine. Mn^{2+} also diminished the color absorbance and this interference was eliminated using the distillates of the investigated hydrocyanic acid solutions.

Elements, which chelate strongly with CN^-, such as Fe, Co, Ni, and Zn, were avoided by distillation. These elements did not seriously interfere when natural waters were analyzed. Nevertheless, Fe^{2+} and Fe^{3+} ions in water react with CN^- to form non-toxic $[Fe(CN)_6]^{4-}$ and $[Fe(CN)_6]^{3-}$, which cannot react with the working reagent. Still, hexacyanoferate ions could form HCN during the distillation procedure, affecting the results. They could be separated by adding of a copper salt solution to the water sample to form insoluble salts, that are removed by filtration.

3.4. The structure of the novel dye

The new dye, which was isolated in the reaction of cyanide with picric acid and resorcinol (yield: 686mg; 68%), proved to contain 17.19% N (calculated: 16.96% N). Also, the compound melts over 330°C, quite different from the precursors. We supposed the formation of an indophenol structure when treated the red-brown solution with hydrochloric acid and observed the changing in color (Figure 3). The indophenol structure was then proved by spectral analyses. Both position and intensity of the peaks in the IR spectrum were in agreement with the proposed structure. Thus, the strongest absorption peaks were found to be at 1620 and 1670cm^{-1}, which were assigned to >C = O group and C = N group, respectively. and 1640cm^{-1}, aromatic at 1670cm^{-1}, and >C = C< (double bound, 1600cm^{-1}), respectively. The CN group of the novel dye showed a distinct and sharp peak at 2235cm^{-1}. NO_2 groups produced characteristic peaks at 1560cm^{-1} and 1516cm^{-1}, respectively. The aromatic rings were observed at 2950cm^{-1}. Also, the phenol OH group had a characteristic large band from 3300 to 3600cm^{-1}.

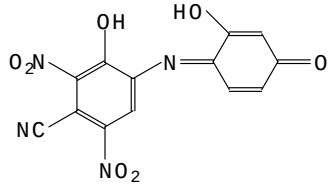

Figure 3. The red-brown dye obtained within the cyanide assay with picric acid and resorcinol

The ^{1}H-NMR spectrum also confirmed the structure of the dye. The large signal at 4.11ppm (from 3.72 to 4.44ppm) was assigned to phenol OH group of the radical provided by resorcinol. The smaller peaks in the aromatic region were assigned both to the other phenol OH group (6.84ppm) and the aromatic H atoms (7.74 – 8.18ppm). We also recorded and compared the IR and ^{1}H-NMR spectra of the precursors and those

of the dyes obtained without using resorcinol. They were quite different from the spectra of 3-hydroxy-4-(2-hydroxy-4-oxo-cyclohexa-2,5-dienylideneamino)-2,6-dinitro-benzonitrile (the ^{1}H-NMR spectrum of isopurpurin – 2,6-dinitro-5-hydroxy-4-hydroxylamino-1,3-dicyanobenzene: 1.91ppm – H_N; 4.03 – 5.06 ppm (H_{NOH}); and 6.84ppm – phenol H_{OH}).

3.5. Application of the method

Water samples: In order to eliminate interference, the sample of water was distilled, hydrocyanic acid vapor was collected in the two collection vessels and the reaction was carried out. Precision of the proposed method was characterized by using a sample of water containing 10μg/ml CN^- (25μg/ml KCN) that was analyzed by the two methods. The result was expressed as the mean of six replicate analyses. The *F* parameter ($F = S_1/S_2$ = 0.162/0.178 = 0.91) showed that the two methods are similar (Table 1). Similar results were obtained using Conway dishes as compared with the standard method (Amlathe and Gupta, 1990; Brands, 1987).

Table 1
Evaluation of the precision of the proposed method compared with the standard method

Parameter	Proposed method/ μg/ml	Standard method/ μg/ml
C	9.94 ± 0.168	9.94 ± 0.196
s^2	0.0262	0.317
s	0.162	0.178
s_x	0.066	0.073
CV%	1.63	1.79

Cigarette smoke: Cigarettes were attached to an air sampling train. The cigarettes were lit and the smoke was drawn through dilute sodium hydroxide solution taken in two collection vessels connected in series to a sampling train attached to a suction pump at the flow rate of 0.25l/min. The solutions were then analyzed both by the present and reported method (Amlathe and Gupta, 1990).

Amounts ranging from 6.5 to 12.5μg of hydrocyanic acid were found in each cigarette by the above method (Table 2). The amount of hydrocyanic acid found in one cigarette was nearly reproducible if the flow rate and the sampling time were the same.

Waste waters: Samples of natural water from drinking sources were taken and artificially contaminated with potassium cyanide to reach a concentration of maximum 10μg/ml CN^-. In addition, some samples of waste waters delivered by Water Cleanliness Station of Iasi were treated similarly. These samples contained also up to 35μg/ml NH_4^+ and heavy metals.

The absorbance at 540nm of the colored solution prepared with natural water was by 5.4% smaller than the absorbance of a 10μg/ml CN^- solution made with distilled water. Moreover, the absorbance of a 10μg/ml CN^- solution in waste water was 11.2% smaller. Nevertheless, calibration curves made with natural and waste water in the range 0 – 10μg/ml CN^- were linear. Therefore, even if the absolute values for absorbance were smaller, the usage of these calibration curves make possible the direct and accurate determination of cyanide in waste waters, without isolation (results not shown).

Table 2
Determination of hydrocyanic acid in air and in cigarette smoke

Sample	No.	Proposed method [a]/ μg/g ($c \pm ts_x$)	Standard method [a]/ μg/g ($c \pm ts_x$)
Air	S_1	2.5 ± 0.26	2.5 ± 0.19
	S_2	5.4 ± 0.37	5.5 ± 0.27
	S_3	7.0 ± 0.46	7.2 ± 0.30
	S_4	10.7 ± 0.38	10.5 ± 0.35
Cigarette smoke	S_1	6.51 ± 0.29	6.39 ± 0.37
	S_2	6.45 ± 0.27	6.50 ± 0.38
	S_3	7.78 ± 0.31	7.77 ± 0.39
	S_4	9.12 ± 0.31	9.35 ± 0.33
	S_5	12.55 ± 0.36	12.38 ± 0.37

[a] Mean of three replicate analyses

Being very simple, accurate, fast, selective and sensitive, the method is useful in determination of as little as 0.01μg/ml CN^- in the environmental samples.

5. CONCLUSION

The proposed method is useful for the determination of hydrocyanic acid in air, waters, and in cigarette smoke, being simple, inexpensive, selective and sensitive. The method can also be used for industrial hygiene work, too. Only common, inexpensive equipment is necessary; no special training is needed except skill in handling simple, chemical glassware and reagents and in reading a colorimeter.

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