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Spectrophotometric determination of cyanide in aqueous samples after its conversion to thiocyanate and complexation to ferric-thiocyanate

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GRAPHICAL ABSTRACT



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1. Introduction

ABSTRACT

A simple spectrophotometric method is developed for the determination of traces of cyanide ion in water samples. Toxic cyanide was reacted with sulfur (S₈) dissolved in acetone and converted to non-toxic thiocyanate which is more stable than cyanide also. This product was reacted to Fe (III) to form red ferric-thiocyanate complex. The complex absorption rate in the first two minutes of its formation is related to the cyanide concentration. By measuring the complex absorbance at 465 nm, the cyanide in the range of 2.0-16.0 µg/mL was determined with a detection limit of 1.7 µg/mL. Relative standard deviation (n = 6) for concentrations of 4.0 and 12.0 µg/mL of cyanide was obtained 4.2 % and 1.5 %, respectively. To avoid interferences from other cations and anions, it is possible to isolate cyanide with a system converting CN⁻ to HCN. This method was successfully applied to determine cyanide in various water samples.

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Cyanides are systemic toxins which are generally called blood agents. The main effect of them is inhibition of cytochrome oxidase and cellular respiration blockage which prevents the transfer of oxygen from the blood to tissues. In this case, cells are unable to use oxygen in spite of the availability of it, so it causes cell death. Also, cyanides are effective inhibitors of metabolic processes, such as glucose metabolism, thyroid hormones operation, and enzyme activity. Indicator symptom for cyanide poisoning is respiration depth increasing that appears quickly, so causes the poison inhalation increases, and if continued, leads to death. The lethal cyanide dosage for humans through oral ingestion, (LD50) is approximately 1.0 mg/Kg. Cyanide is a serious anionic pollutant of water that has a wide use in various industries. About two million tons of cyanides are produced annually in the world, which is mainly used in chemical industries, steel industry, and the mineral industry for precious metals extraction (Halámek et al.

2009; Pitschmann et al. 2011). Hydrogen cyanide is used in nylon preparation and methyl methacrylate plastics. Sodium cyanide and potassium cyanide are used for gold extraction from ore and other cyanide salts are used in brass, bronze, cadmium, gold, silver and zinc electroplating (Gidlow. 2017). Other industrial usages of cyanides include oil refining, chemical and pharmaceutical production (El-Naas et al. 2014). Because of High toxicity and widespread use of cyanide, environmental assay of it is of high importance. Cyanides can be determined using various analytical methods such as electrochemical (Junsomboon et al. 2018; Ghanavati et al. 2014), chromatographic (Bhandari et al. 2012; Lacroix et al. 2011; Randviir et al. 2015; Youso et al. 2010), spectrophotometric (Abbasi et al. 2010; Cacace et al. 2007; Chueachot et al. 2014; Frizzarin et al. 2013) and other instrumental methods (Jermak et al. 2006; Rajamanikandan et al. 2019; Vahid et al. 2019). Most of these methods are not suitable for conventional cyanide measurements. These methods suffer from sever interferences of other compounds, low sensitivity, long reaction times, and toxicity of

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the materials used. Other limiting factors are time consumption and complicated instrumentation of these methods. However, colorimetric and spectrophotometric methods for cyanides measuring in aqueous samples are relatively superior to other methods due to their simplicity and availability of required instruments. Among these methods, there are instructions based on the use of cyanides reaction with benzaldehyde to form cyanohydrin, as an active intermediate (mandelonitrile) or Benzoin, which produce purple nitrophenylhydroxylamine dianion with o-dinitritobenzene reduction. Because of cyanide re-production in this process, the reaction is catalytic; therefore, low detection limit is accessible by this method. When benzaldehyde was replaced with p-nitrobenzaldehyde better results were observed. Other compounds, in particular 2,3,5triphenyltetrazoliumchloride, have been proposed instead of odinitrobenzene, which produces red triphenylformazan, or luminol, that suitable for chemiluminescence determination of cyanides is (Pitschmann et al. 2011). The reagents required in these methods contain a benzene ring that has carcinogenic effect. In pHs < 2 cyanide converts to HCN gas and released from solution, so it is not possible to measure it with methods which require media with high acidity. But it's derivative, thiocyanate is relatively non-toxic and has greater stability at various pH levels (Dzombak et al. 2005). Thiocyanate produces a 1:1 red blood ferric-thiocyanate charge transfer complex with Fe (III). Charge transfer complexes plays an important role in analytical chemistry due to their very high molar extinction coefficients (ϵ_{max} > 10000) (Skoog et al. 2007). So, ferric-thiocyanate complex can be used for detecting and determining of cyanide after its conversion to thiocyanate.

This study presents a simple, fast and sensitive method by nontoxic, inexpensive and available materials for cyanide determination in aqueous samples by spectrophotometric method. The basis of this method is the conversion of cyanide to more stable and non-toxic thiocyanate, by its reaction with the acetone-soluble S_8 . The produced thiocyanate is then complexed by ferric ions to ferric-thiocyanate red blood complex which is then determined by the spectrophotometric method.

2. Experimental

2.1. Reagents and chemicals

All reagents and chemicals used in this research were of analytical grades (Merck). The chemicals used in this study are including potassium cyanide, Potassium thiocyanate, sulfur, acetone, ferric chloride hexa hydrate, ethanol, sodium hydroxide, and sulfuric acid. Double distilled water was used to prepare solutions.

2.2. General procedure

A stock sulfur solution (0.0244 M) was prepared by dissolving 0.0780 g of sulfur in 100 mL acetone by 2-3 hours of continuous stirring. Solutions of 0.02 M KCN, 0.02 M KSCN, and 0.10 M of NaOH were prepared by proper dissolution of solid reagents in 250 mL flasks. 6.0 mM Ferric chloride solution was prepared from dissolution 0.3244 g of ferric chloride hexa hydrate in 5.0 mL redistilled water and diluted to mark with acetone in a 100 mL volumetric flask. Because of the hydrolysis, some ferric ions from this solution precipitate, and after 24 hours sedimentate. At the end of precipitation about half of initial Fe (III) is available. The upper part of the solution was decanted and filtered. This solution is stable for days or even weeks and will be not turbid (Bartlett et al. 1954). To prepare standard solutions, 3.0 mL acetone, 2.0 mL sulfur solution in acetone (0.01 M), 2.0 mL aqueous standard solution containing various cyanide concentrations, and then after 2 minutes, 3.0 mL ferric chloride solution (6.0 mM) were poured in a 10 mL volumetric flask. The blank solution was prepared in the same way, but without addition of cyanide. The mixture then was transferred to a 1.0 cm quartz cell and for maximum sensitivity, sample absorption was measured at λ_{max} of the ferric thiocyanate complex (465 nm) in a spectrophotometer. All spectrophotometric measurements were performed on a JENWAY ultraviolet-visible spectrophotometer (UK) model 6305 with 1.0 cm quartz cells. The absorption spectrum of the ferric-thiocyanate complex was recorded by a photonix Ar 2015 spectrophotometer (Iran).

3. Results and discussion

While cyanide can be converted into thiocyanate by reaction with most compounds containing S-S bonds (Dzombak et al. 2005); there is a high possibility for generation of byproducts that may interfere with

analysis. For example, cyanide can react with thiosulfate according to equation (1) and sulfite ion is produced as a byproduct.

$$CN^{-} + S_2 O_3^{2-} \rightarrow SCN^{-} + SO_3^{2-}$$
 (1)

So, in this study, sulfur was used in its S_8 form which releases no byproducts after reaction, Eq. 2 (Dzombak et al. 2005).

$$S_8 + 8CN^- \rightarrow 8SCN^-$$
 (2)

Moreover, the reaction of cyanide with sulfur is three times faster than its reaction with thiosulfate (Dzombak et al. 2005). Thus, sulfur in the S_8 form has relatively ideal conditions, and with this regard, the reaction of cyanide with dissolved sulfur in acetone was used to convert cyanide to thiocyanate. Thiocyanate was then reacted with Fe (III) to form red blood ferric- thiocyanate complex, equation (3) (Below et al.1958).

$$\mathrm{Fe}^{3+} + \mathrm{SCN}^{-} \xrightarrow{\mathrm{H}_2\mathrm{O}} [\mathrm{Fe}(\mathrm{SCN})(\mathrm{H}_2\mathrm{O})_5]^{2+}$$
(3)

It should be noted that due to slow hydrolysis of the Fe (III) with water component of cyanide solution, red color of ferric-thiocyanate complex changes very slowly and also when exposes to daylight for a long time, but it is stable if stored in the dark. We recommend that all measurements performed in the first 10 minutes of complex formation. Absorption of ferric-thiocyanate complex at 465 nm was used to determine the concentration of cyanide. Absorption spectrum of this complex is presented in Fig. 1.

3.1. Optimization of reaction conditions

Parameters affecting determination of cyanide were studied and optimized. In accordance with general procedure, the ethanol solvent was first used under the same conditions, instead of acetone. In this case, the red color due to the formation of the ferric thiocyanate complex was not observed. However, by adding 3.0 mL aqueous solution of ferric ion (6.0 mM) to 3.0 mL aqueous potassium thiocyanate solution (6.0 mM) the red color of the ferric-thiocyanate complex was immediately formed. And then with drop by drop ethanol addition below 1.0 mL, the red color of ferric thiocyanate complex was disappeared.



Fig. 1. Red blood ferric-thiocyanate complex absorption spectrum (10.0 μg/mL CN⁻).

These results showed that the red color of the ferric-thiocyanate complex disappeared in presence of ethanol, while the color is stable in acetone and the solubility of sulfur in acetone is more than ethanol. Therefore, acetone was chosen as solvent for sulfur dissolution. At high pHs, Fe (III) precipitates in the form of hydroxide, and in acidic solutions, cyanide is released from solution as hydrogen cyanide gas, also it reacts with acetone and produces cyanohydrin (Morrison et al. 1992). Therefore, highly alkaline or acidic media are not suitable for this assay. The suitable pH of the solutions in this study is about 10-11. Adding water to a sulfur solution in acetone through cyanide soluble in water was caused to reduce the solubility of sulfur and colloidizing it in the reaction environment. Also, sulfur concentration should be kept at a level that converts all cyanide into thiocyanate. Therefore, the highest concentration of sulfur concentration was kept in a way that the solution does not become turbid, and the lowest sulfur concentration was kept excess relative to the cyanide content. To determine the volume of water that matches the above conditions, distilled water was added to 5.0 mL of various concentrations of sulfur solution in acetone drop by drop by constant stirring, until the solution became turbid. That's because of formation of colloidal state of sulfur in a solution of acetone and water. 5.0 ml of 5.0 mM sulfur in the acetone was observed to be

turbid after addition of 2.6 mL of water. Thus, maximum water volume from aquatic cyanide samples chosen 2.0 mL to prevent sulfur colloidization. In order to investigate the effect of sulfur concentration, various concentrations of sulfur in the range of 0.5-2.0 mM was tested. Results are shown in Fig. 2. As can be seen, absorbance reaches to its maximum and became constant in the range of 1.0-2.0 mM of sulfur, so in the following experiments sulfur concentration was selected as 2.0 mM. At this concentration, the sulfur is yet excess reagent relative to cyanide with about 3.5 mole ratio, at the maximum cyanide concentration in the dynamic linear range of the method, 16.0 μ g/mL. It should be noted that to avoid sulfur colloidization, concentrations above 2.0 mM were not considered.



Fig. 2. Sulfur concentration effect on absorbance variations of sample solution (Cyanide (0.2 mM) and Ferric (2.4 mM).

Ferric ion concentration was investigated in the range of 0.6-2.4 mM (Fig. 3). The maximum absorbance was observed in 1.8 mM, which was selected as optimum value.



Fig. 3. Ferric concentration effect on the absorbance variations of sample solution (sulfur (2.0 mM), cyanide (0.2 mM)).

In order to investigate the effect of reaction time between sulfur and cyanide, ferric solution (6.0 mM) was added to solution containing sulfur and cyanide and the absorbance was recorded in different times, Fig. 4.





As can be seen, the reaction between sulfur and cyanide, to form thiocyanate is fast and in the first two minutes reaches to its maximum value, and then became constant. So, reaction time between sulfur and cyanide was considered to be 2 min.

3.2. Calibration curve

Fig. 5 shows the dependence of the absorbance on the cyanide concentration. The determination limit found by calculation is1.7 μ g/mL. The following calibration curve was obtained in the 2.0-16.0 μ g/mL cyanide concentration range under optimized conditions that can be used for the analysis of unknown samples in this range. For samples with cyanide concentration greater than 16.0 μ g/mL it is necessary to dilute by water to shift them to this concentration range. The sensitivity of the method expressed by the calibration dependence guideline (molar absorptivity) at 465 nm is 0.35x10⁴ L/mol.cm. The coefficient of determination R²=0.9974. The relative standard deviation for seven repetitive measurements of 4.0 and 12.0 μ g/mL cyanide concentrations was 4.2 % and 1.5 %, respectively.



Fig. 5. Calibration curve in the 2.0-16.0 μg/mL cyanide concentration range (465 nm).

3.3. Interferences

The effect of various cationic and anionic probable interferences on cyanide measurement in a concentration of 10.0 µg/mL was investigated by the proposed method. Permissible limit for all species in Table 1 is obtained based on maximum 1.5% error in cyanide determination. Most cationic interferences are due to complex formation with cyanide ion, and some ions such as ClO_3^{-} , IO_3^{-} , $S_2O_8^{-2}$ interfere due to their oxidizing effect, and for $S_2O_3^{-2}$ is because of S-S bond, that each one of them affect reactions of thiocyanate or ferric-thiocyanate formation.

Table 1.	Effect of	various ior	ns on	cyanide	determin	ation i	n
		ontimized	cond	ditions			

Species	Tolerance limit (w _{ion} /w _{cyanide})		
CH ₃ COO ⁻ , BO ₃ ⁻ , NO ₃ ⁻ , NO ₂ ⁻ , Cl ⁻ , Li ⁺ , Na ⁺ , K ⁺	20/1		
C ₂ O ₄ ²⁻ , S ₂ O ₈ ²⁻ , ClO ₃ ⁻ , IO ₃ ⁻ , SO ₄ ²⁻ , F ⁻ , Br ⁻ , I ⁻	2/1		
SO ₃ ²⁻ , CO ₃ ²⁻ , S ₂ O ₃ ²⁻ , Pb(II), Hg(II), Sn(II), Ni(II), Co(II), Cr(II), Sr(II), Mn(II), Mg(II),	1/5		
Cd(II), $Cl(II)$, $Cd(II)$, $Cd(I$	1/50		

3.4. Interference elimination with cyanide separation

In this method, the cations that form complex with cyanide or other species that interfere in the reaction are regarded as interfering agents. To eliminate them, a separation system as shown in Fig. 6 was applied, which releases all cyanides as hydrogen cyanide gas from the sample matrix. In this system, the cyanide content in the samples is converted into hydrogen cyanide gas in acidic medium of container (1) with the addition of 5.0 mL concentrated H_2SO_4 , and then with a flow of nitrogen gas, transferred to flasks (2) and (3), that each containing

40.0 mL 1.0 mM NaOH as absorbent solution. The temperature of flask (1) was adjusted by a heating mantle at around 90 °C. After two hours N_2 passing, solutions of these two flasks were collected in a 100 ml

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volumetric flask. Moreover, connecting pipes were washed with small volumes of 1.0 mM NaOH solution three times. Then these washing solutions added to collected cyanide solution, and this final solution reached to 100.0 mL with 1.0 mM NaOH solution. To assessing operation of cyanide separation system, at first 100 mL of a 100.0 µg/mL standard cyanide solution was prepared, and divided to two equal 50.0 mL portion. The first portion diluted to 100.0 mL volume with water to prepare 50.0 µg/mL cyanide standard solution. Second portion was used in cyanide separation system illustrated in Fig. 6. With assuming complete separation and absorption, cyanide concentration should be 50.0 µg/mL in this absorbent solution. To ensure complete cyanide recovery, two samples were prepared according to the proposed method at same conditions. At the cyanide addition step, 2.0 ml of standard cyanide solution (50.0 $\mu\text{g/mL})$ was added to the first sample, and 2.0 ml of cyanide absorbent solution (50.0 µg/mL) was added to the second sample and their absorption was measured at 465 nm. The absorbance measured for two samples was very close, which confirms the relatively complete separation of cyanide ions under the given conditions. Results illustrated in Table 2.



Fig. 6. System used for the cyanide separation (flask (1): containing sulfuric acid and cyanide sample, (2) and (3): gas-washing-bottles each containing 40.0 mL 1.0 mM NaOH as absorbent solution).

Table 2. Results of separated cyanide measurement and comparison of results with standard cyanide solution.

	[CN ⁻],	Error, %		
Sample	Real spiked	Proposed	Relative to	
		method	spiked	
Standard	10	9.8	1.5	
Separated	10	9.8	2.0	

It is also necessary to mention that the separation of cyanide by this method is possible even in the presence of species that form a complex with cyanide, so quantitative determination of cyanide in these samples is possible.

3.5. Determination of cyanide in real water samples

In order to validate the proposed method for the cyanide determination in real samples, four samples of water were examined. For real sample analysis, some pretreatment such as removal of all suspended particles by centrifugation and filtration, elimination of chlorine to prevent cyanide reaction and cyanogen chloride production were necessary. The tests showed that the samples were free of cyanide and spiked with a certain amount of cyanide when examined. After separation, cyanide in these samples was collected in the absorbent 1.0 mM NaOH solution and measured by the proposed method and compared with a standard method (Clesceri et al. 1995). The results differences were no more than 11 % and with those given by the standard method confirmed well as shown in Table 3.

Table 3. Determination of cyanide in water (n = 6).			
	CN ⁻ found	l, μg/mL	
Sample	Proposed	Reference	RSD, %* (n=6)
	method	method	
1	3.8	4.2	4.0%
2	5.8	6.1	2.4%
3	7.9	8.2	2.0%
4	11.8	12.1	1.5%

*For the proposed method

4. Conclusions

The results of this study showed that the proposed method can be successfully used to measure trace amounts of cyanide in aqueous samples. Conversion of toxic and unstable cyanide into non-toxic and stable thiocyanate, along with increasing the accuracy and precision, speed, simplicity and sensitivity of this method, and the use of non-toxic, ordinary and inexpensive materials, encouraging users to use this method for measuring cyanide in water samples. The advantages of this method are that each sample analysis takes only 2 minutes. Separation of cyanide with the proposed system reduces the disturbance of cationic and anionic species to a minimum or even zero. Table 4 shows that the materials used in this method are inexpensive and available, with a very low risk of exposure. While other methods mentioned in the Table 4 are time-consuming and used complex and expensive chemicals, which some of them are uncommon, aromatic and carcinogenic. The linear range of this method is relatively large, however, it is necessary to take into account that in comparison with other methods, this range is in higher concentrations and the detection limit of the proposed method is greater than other methods.

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Method-reagents	Determination limit, mg/L	Reference
Janus Green, Ammonium peroxodisulfate, Nitric acid	0.007	Abbasi et al. 2010
Phenolphthalin, EDTA	0.005	Cacace et al. 2007
Ninhydrine, Na ₂ CO ₃ , Tween 20, NaOH	0.006	Chueachot et al. 2014
2,2'-biquinoline 4,4'-dicarboxylic acid, Cu(I)	0.002	Frizzarin et al. 2013
4-hydroxy-3-(2-oxoindoline-3-ylideneamino)-2-thioxo-2H-1,3-	0.016	Hamza et al. 2010
thiazine-6(3H)-one		
p-Nitrobenzaldehyde, teterazolium blue	0.005	Pitschmann et al. 2011
Sulfur, Acetone, Ferric -chloride hexahydrate	1.7	Proposed method in this study

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