

A study on ninhydrin reaction with weak acid dissociable cyanide and its application for toxic cyanide determination

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To cite this article:

Andriana Risk Surleva, Sabina Bancila, Elena Todorova. A Study on Ninhydrin Reaction with Weak Acid Dissociable Cyanide and Its Application for Toxic Cyanide Determination. *Science journal of Analytical Chemistry*. Vol. 2, No. 1, 2014, pp. 1-6.

doi: 10.11648/j.sjac.20140201.11

Abstract: Environmental Protection Agencies impose stringent limits for weak-acid dissociable metal-cyanide complexes (WAD) content in waters. The maximum contaminant level in drinking water is as low as 50 µg/L as CN⁻. Hence, sensitive methods for WAD cyanide determination are strongly required. Recently, ninhydrin reaction with free cyanide has been proven as a very sensitive at 485 nm ($\epsilon = 1.2 \times 10^5$ L/mol.cm), fast (15 min), selective and with non-toxic reagents. In the presence of free cyanide the absorbance measurement was based on the formation of a red hydrindatin resulting from cyanide's catalytic effect on ninhydrin at pH 10.8. This report presents the results from a study on the reaction between ninhydrin and complexed cyanide at ambient conditions. The WAD complexes: Hg(CN)₄²⁻; Ni(CN)₄²⁻; Cu(CN)₄³⁻; Ag(CN)₂⁻; Zn(CN)₄²⁻; Cd(CN)₄²⁻ are discussed. The reaction kinetic and standard curves are presented. Ligand exchange approach was applied without additional separation. Ninhydrin was showed to be selective and sensitive for direct cyanide quantification.

Keywords: Weak Acid Dissociable Cyanide, Ninhydrin, Spectrophotometry, Ligand Exchange

1. Introduction

Water Authorities have divided cyanide compounds into three groups according to their toxicity and environmental fate: (1) free cyanide (HCN, water soluble cyanide salts) – referred as the most toxic cyanide compounds; (2) weak acid dissociable cyanide (WAD) includes free cyanide and metal-cyanide complexes which easily dissociate and release HCN at environmental conditions; (3) total cyanide – all compounds containing CN group. Stringent requirements to WAD cyanide levels in waste and natural waters as well as the sensitivity and reliability of the analytical methods for WAD cyanide determination have been imposed [1-4]. Maximum permissible levels for WAD cyanide in waste waters is as low as 100 µg/L and in portable water – 50 µg/L. Very low levels of free cyanide (5 µg/L) is reported to be lethal for aquatic life.

Recently, free cyanide in trace concentration levels was quantified using ninhydrin as a chromogenic reagent [5, 6]. Ninhydrin and cyanide formed an intensively red colored

adduct in sodium carbonate medium with molar absorptivity of 1.2×10^5 L/mol.cm at 485 nm [7]. The limit of detection and quantification were 8 and 22 µg CN⁻/L, respectively. The reaction was implemented in flow injection and cuvetteless spectrophotometry for free cyanide determination after separation by on-line gas-diffusion or head-space extraction [8-10].

However the determination of weak acid dissociable cyanide has still being a challenge for researchers from two scientific domains. From the toxicological point of view, the cyanide bound in weak-acid dissociable complexes is considered as a very toxic since it is easily liberated at environmental conditions and is available to form HCN – a severe poison, and the Ecological Agencies stipulate for sensitive and reliable methods for WAD cyanide determination. From the analytical point of view, the ninhydrin reaction with cyanide being very sensitive, fast, selective and with non-toxic reagents, is appropriate for implementation for sensitive WAD cyanide determination by spectrophotometry. Recently, the research interest have been directed to WAD quantification and a line of methods

were developed based on different approaches for cyanide liberation and separation: ligand exchange, HCN formation in acidic solutions and gas-diffusion separation [8, 11-14] or head-space extraction [10, 15]; ligand exchange combined standard distillation procedure [10], chromatographic or capillary electrophoresis [15] techniques.

Ninhydrin-cyanide reaction was proved to be strongly interfered by mercury (II), cooper (II) and silver (I), and at less extent by Ni^{2+} , Zn^{2+} and Cd^{2+} [16, 17]. However, a possible implementation of ninhydrin reagent for complex cyanide determination was supposed based on following facts: (1) cyanide bounded in complexes with Hg(II); Cu(I) and Ag(I) is 2-4 times more than the metal ion and respectively the equilibrium concentration of cyanide is higher than metal ion concentration; (2) in cooper-cyanide complex Cu(I) is involved and its reaction with ninhydrin has not been studied; (3) two competing reactions could be supposed metal-ninhydrin and cyanide-ninhydrin and difference in the reaction rates could be explored. This study is aimed at the investigations of the reaction between ninhydrin and WAD cyanide and the possibilities for its application for WAD cyanide determination. The following WAD cyanide complexes: $\text{Hg}(\text{CN})_4^{2-}$; $\text{Ni}(\text{CN})_4^{2-}$; $\text{Cu}(\text{CN})_4^{3-}$; $\text{Ag}(\text{CN})_2^-$; $\text{Zn}(\text{CN})_4^{2-}$; $\text{Cd}(\text{CN})_4^{2-}$ were studied in the range $(0.38 \div 3.8) \times 10^{-6}$ mol/L CN^- . Ligand exchange approach and ninhydrin based detection without additional separation was studied. Cysteine, thiourea and tetraethylenepentamine were chosen as they were reported to give complete recovery in different combinations with sulfur or amine based ligands for on-line gas-diffusion flow-injection determination of WAD cyanide [11, 14]. Moreover, it was described that cysteine did not interfere with ninhydrin-cyanide reaction, but quantitative data were not provided [17]. This report presents the results from a study on the ninhydrin-WAD cyanide reaction in the presence of ligand exchange reagents without separation of liberated cyanide ions.

2. Experimental

2.1. Reagents and Standard Solutions

Stock solution of potassium cyanide was standardized titrimetrically by silver nitrate standard solution. WAD cyanide standard solutions (10^{-2} M) were prepared by adding the stoichiometric quantity of standard KCN solution to $\text{Hg}(\text{CN})_2$, $\text{Zn}(\text{CN})_2$, CuCN and AgCN salts. Standard solutions of $\text{Ni}(\text{CN})_4^{2-}$ and $\text{Cd}(\text{CN})_4^{2-}$ were prepared by dissolving of NiCl_2 or CdSO_4 in stoichiometric quantity of KCN. Working solutions were prepared daily by dilution of appropriate aliquots from stock solution in 10^{-2} M NaOH. Ninhydrin solution was also daily prepared by dissolving 150 mg of ninhydrin in 50 mL of 2% Na_2CO_3 , purged with nitrogen for 15 min. Standard solutions were purged with nitrogen. Cysteine, cystin, thiourea and tetraethylenepentamine hydrochloride (all Fluka reagents) were used. All reagents were of analytical grade.

2.2. Instrumentation and Procedures

Standard solutions of $\text{Hg}(\text{CN})_4^{2-}$, $\text{Zn}(\text{CN})_4^{2-}$, $\text{Cu}(\text{CN})_4^{3-}$, $\text{Ni}(\text{CN})_4^{2-}$, $\text{Cd}(\text{CN})_4^{2-}$ and $\text{Ag}(\text{CN})_2^-$ at concentrations from 1×10^{-6} – 3.5×10^{-6} M (calculated as CN^-) were prepared by adding appropriate volumes of WAD cyanide solutions (7.7×10^{-5} M as CN^-) into 10 mL measuring flasks, a volume of 3.3 mL of ninhydrin reagent (3 mg/mL ninhydrin in 2% Na_2CO_3) was added to each standard solution and the volume was made up to 10 mL by 2% Na_2CO_3 (purged with nitrogen). The mixture was homogenized and left for 15 min for color development. A blank solution was prepared diluting 3.3 mL of ninhydrin reagent up to 10 mL with 2% Na_2CO_3 . UV-Vis absorption spectra at wavelength range from 300 to 700 nm were acquired on a LIBRA S35 PC UV/VIS spectrophotometer (Biochrom, Cambridge, England) in 1-cm quartz cuvettes against blank containing 1 mg/mL ninhydrin in 2% Na_2CO_3 . Kinetics measurements were made measuring the absorbance of WAD cyanide-ninhydrin mixture at 485 nm at every two minutes up to 60 min using the reaction kinetics mode of Acquire Application Software (Biochrom, Cambridge, England). Single wavelength absorbance measurements were made on Specol 11 spectrophotometer in 1-cm quartz cuvettes. In differential spectrophotometry the absorbance was measured against a reference containing: 1.5×10^{-6} M CN^- and 1 mg/mL ninhydrin in 2% Na_2CO_3 .

2.3. Ligand Exchange Procedure

A sample aliquot of 2 mL containing metal-cyanide complex (7.7×10^{-5} M as CN^-) was transferred in a beaker and 2 mL of ligand exchange reagent (1.54×10^{-4} M) were added. The mixture was agitated on magnetic stirrer for 40 min at room temperature. Three aliquots from the obtained solution were taken and transferred in measuring flasks, 3.3 mL of ninhydrin reagent was added and the volume was made up to 10 mL by 2% Na_2CO_3 . The solutions were left for 15 min for color development. The absorbance of each sample was measured at 490 nm against reference containing 1.5×10^{-6} M CN^- .

3. Results and Discussion

3.1. Comparative Study of Ninhydrin-Based Protocols for Free Cyanide Determination

One-step protocol, firstly described by Drochioiu [5], was based on the reaction between cyanide and ninhydrin in 2% sodium carbonate solution at ambient conditions and monitoring the absorbance of the obtained red solution at 485 nm. In sodium carbonate medium cyanide reacted with ninhydrin forming a red colored ninhydrin-cyanide adduct: 2-cyano-1,2,3-trihydroxy-2H indene [16]. Two-step protocol, firstly described by Nagaraja, Kumar, Yathiraja, and Prakash [6], was based on: firstly, formations of a red colored ninhydrin-cyanide adduct in sodium carbonate media, and secondly, formation of blue colored compound

upon addition of sodium hydroxide. The absorbance of the blue solution at 590 nm was monitored. Fig. 1 presents the spectra of ninhydrin-cyanide adduct in sodium carbonate solution before and after addition of sodium hydroxide. As can be seen from the Fig. 1, the addition of NaOH to the red colored ninhydrin-cyanide adduct (monovalent ion) solution caused color transition from red to blue (divalent ion). A bathochromic shift from 485 to 590 nm was observed. The molar absorptivities of red and blue compounds at 485 nm and 590 nm were: 1.4×10^5 L/mol.cm and 8.8×10^4 L/mol.cm, respectively.

The bathochromic shift has been already reported [6, 18, 19]. However, in contrast to the reported results, the absorbance of the blue colored compound at 590 nm was lower than the absorbance of the red colored one at 485 nm. The observed lowering of the absorbance was independent of neither cyanide nor NaOH concentrations. The effect was probably due to the instability of colored compound at high pH in the presence of oxygen. Moreover, at pH > 13 a peak at 352 nm was observed (Fig. 1). As can be seen from the Figure, the blue color of obtained compound vanished with time, quicker in more alkaline solution. The stability of the blue colored compound was lower when compared to the stability of the red colored one – the blue color totally disappeared in 24 hours, while the red solution was found intensively colored. From analytical point of view, a one-step procedure seems to be a better choice due to its simplicity and better stability of the colored adduct.

3.2. Ninhydrin - Free Cyanide Reaction

The mechanism of ninhydrin-cyanide reaction was thoroughly discussed in [16]. Here we confine our study to the low concentration region ($1 - 3 \times 10^{-6}$ M). It was noticed that although the calibration curve was linear an intercept was obtained. We supposed that it is due to the particularity of the two stage ninhydrin-cyanide reaction involving two molecules of KCN in the formation of the red-colored ninhydrin-cyanide adduct and to the existence of a critical concentration of KCN necessary to provoke the reaction. At higher cyanide concentrations, the reaction may follow different mechanism. Thus a modified equation of Beer's law was used $A=a+b \cdot C$, where A is the absorbance, C is the concentration of the cyanide-ninhydrin adduct (mol/L), a and b denote the coefficients of the linear calibration curve. The mean calibration coefficients in the concentration interval $(0.7 \div 2.6) \times 10^{-6}$ M were: slope $(4.32 \pm 0.08) \times 10^5$ and intercept (-0.247 ± 0.073) ($n = 6$; $P = 95\%$). The slope and intercept were independent of the reaction time in the interval 15 - 30 min. The differential spectrophotometric measurements were also studied in the environmentally important concentration interval $(0.7 - 3.85) \times 10^{-6}$ M CN^- . The coefficients of the regression line obtained by measuring the absorbance against reference containing 1.54×10^{-6} M CN^- were: slope $(5.61 \pm 0.05) \times 10^5$ ($n = 3$; $P = 95\%$) and intercept (-0.820 ± 0.050) . As can be seen from the results, at 0.05 confidence interval both slopes were not statistically different and differential spectrophotometry

could be used in studied concentration range.

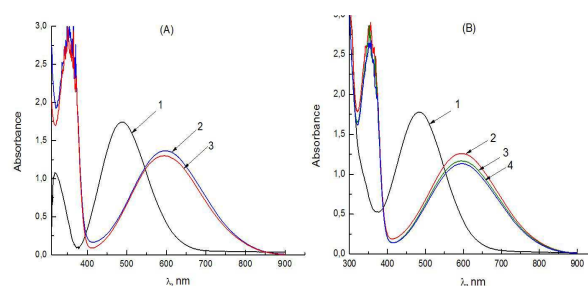


Figure 1 Spectra of ninhydrin-cyanide adduct, $C_{CN} = 0.3 \mu\text{g/mL}$ in 2% Na_2CO_3 ; 1, before and 2, after addition of NaOH: (A) final $C_{\text{NaOH}} = 0.7 \text{ M}$; 2, 30 seconds; 3, 5 min after addition of NaOH and (B) final $C_{\text{NaOH}} = 0.1 \text{ M}$; 2, 1 min; 3, 10 min; 4, 15 min after addition of NaOH.

3.3. Ninhydrin - Complexed Cyanide Reaction

The spectra of ninhydrin-cyanide adduct obtained from complex cyanide are presented in Fig. 2. The wavelength of maximum absorption (490 nm) coincided well with the λ_{max} of CN^- in the case of $\text{Hg}(\text{CN})_4^{2-}$; $\text{Ni}(\text{CN})_4^{2-}$;

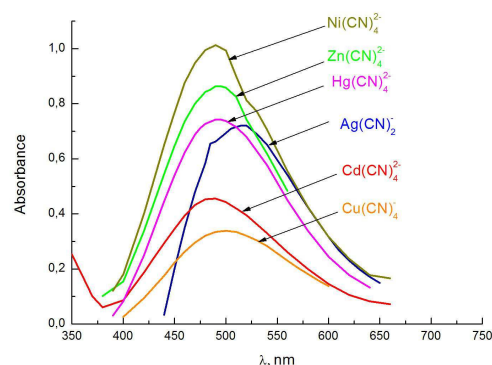


Figure 2 Spectra of WAD cyanide – ninhydrin adduct.

$\text{Cu}(\text{CN})_4^{3-}$; $\text{Zn}(\text{CN})_4^{2-}$; $\text{Cd}(\text{CN})_4^{2-}$. A red shift of 25 nm was observed in the case of $\text{Ag}(\text{CN})_2^-$. The calibration curves were obtained for each WAD complex; the linear range, equations and recoveries are presented in Table 1.

In the case of $\text{Hg}(\text{CN})_4^{2-}$, although linear fit of the data was good enough for quantitative determination, the polynomial fitting resulted in better correlation $r = 0.999$ ($Y = 0.01 + 4 \times 10^4 x + 2.1 \times 10^5 x^2$). Hence, a catalytic mechanism of the reaction between $\text{Hg}(\text{CN})_4^{2-}$ and ninhydrin could be supposed. The calibration data sets (for 15 min color development reaction time) were compared with calibration data obtained using free cyanide and the results showed that (at the 0.05 significance level) the data sets were not statistically different for $\text{Hg}(\text{CN})_4^{2-}$; $\text{Zn}(\text{CN})_4^{2-}$ and $\text{Cd}(\text{CN})_4^{2-}$. The $\text{Cu}(\text{CN})_4^{3-}$ and $\text{Ag}(\text{CN})_2^-$ calibration curves were linear, but with lower absorbance values compared to CN^- calibration curve. In the reaction between ninhydrin and $\text{Fe}(\text{CN})_6^{3-}$ no color compound was obtained even after 60 min reaction time. $\text{Fe}(\text{CN})_6^{3-}$ is regarded as non-toxic cyanide complex and the results showed the selectivity of ninhydrin reagent for WAD cyanide determination.

Table 1 Calibration parameters and recovery study for WAD cyanide.

Me-cyanide complex	Concentration range $\times 10^{-6}$ CN ⁻ /M	Calibration equation	Correlation coefficient	Total cyanide recovery/% ^a	Total cyanide recovery/% ^b
Hg(CN) ₄ ²⁻	0.5-2.0 ^c	- 0.206 + 4.8 × 10 ⁵ C	0.9871 (n = 6)	97.1	100.8
Ni(CN) ₄ ²⁻	0.7-3.1 ^d	- 0.060 + 3.6 × 10 ⁵ C	0.9969 (n = 7)	99.1	102.4
Cd(CN) ₄ ²⁻	1.3-3.6 ^d	- 0.164 + 3.2 × 10 ⁵ C	0.9945 (n = 8)	108.4	95.0
Zn(CN) ₄ ²⁻	0.5-2.1 ^c	- 0.226 + 5.2 × 10 ⁵ C	0.9969 (n = 7)	102.5	111.0
Cu(CN) ₄ ³⁻	0.5-2.1 ^c	- 0.122 + 2.8 × 10 ⁵ C	0.9888 (n = 6)	98.0	55.4
CN ⁻	0.5-2.6 ^d	- 0.155 + 2.9 × 10 ⁵ C	0.9992 (n = 8)	-	103.1

- a. Recovery at total cyanide concentration 1.26×10^{-6} M calculated according WAD cyanide curve.
- b. Recovery at total cyanide concentration 1.26×10^{-6} M calculated according free cyanide curve.
- c. Ninhydrin concentration 5 mg/mL
- d. Ninhydrin concentration 3 mg/mL

The kinetics study of Me(CN)₄ⁿ⁻ – ninhydrin reaction showed that a constant absorbance was obtained after 30 min, which is two times more than ninhydrin-free cyanide reaction (Fig. 3A). Based on these results we supposed that the ninhydrin-Me(CN) reaction was controlled by the rate of

cyanide liberation from the complex. Hg(CN)₄²⁻ and Cu(CN)₄³⁻ seemed to be more inert in the studied conditions than Zn(CN)₄²⁻ and Cd(CN)₄²⁻. To enhance the cyanide liberation cysteine was added as a ligand exchange reagent.

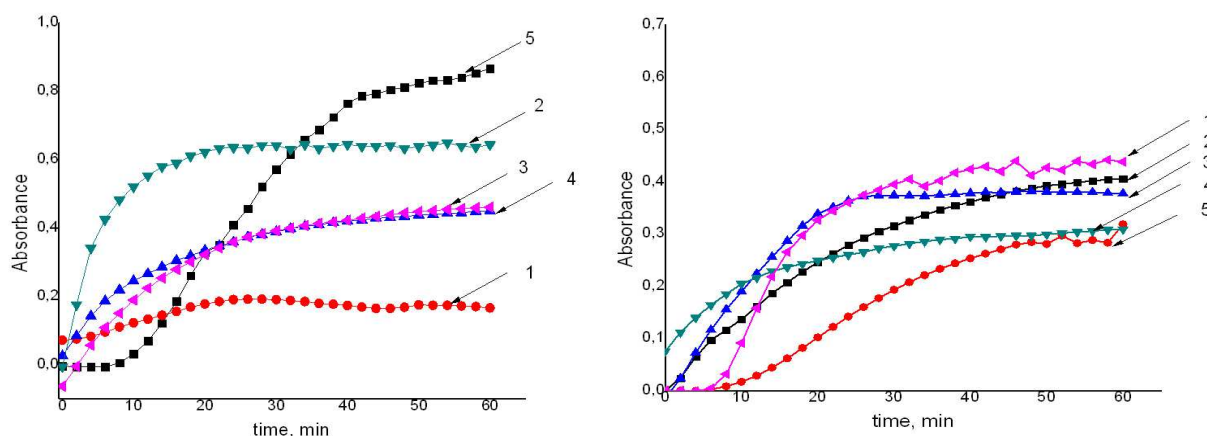


Figure 3 Kinetic curves of WAD cyanide-ninhydrin (A) and WAD cyanide-ninhydrin-cysteine (B) reactions at 485 nm against dist. H₂O: 1, Hg(CN)₄²⁻; 2, Zn(CN)₄²⁻; 3, Cd(CN)₄²⁻; 4, Cu(CN)₄³⁻; 5, Ag(CN)₂⁻

The Me(CN)₄ⁿ⁻ reaction with ninhydrin in the presence of cysteine was followed spectrophotometrically at 485 nm (Fig. 3B). Two reactions might be supposed: (1) a reaction between Hg²⁺ with cysteine and (2) a reaction between liberated CN⁻ and ninhydrin. As can be seen from the Figure, in the presence of cysteine the reaction is slower during the first 6 min and rapidly increased in rate. The slope of the initial part of the curve (b) is higher compared with the curve without cysteine. In both cases the stable absorbance was observed after 30 min. The absorbance intensity increased in the presence of cysteine. Maximum absorbance was reached after 30 min from the beginning of the reaction.

3.4. Ligand Exchange Approach for Complexed Cyanide Liberation

Although the waste water samples contain WAD complexes in different molar ratios and excess of free cyanide, we studied ligand exchange reaction in the most unfavorable case: the studied model samples contained 100% of complexed cyanide and no free cyanide. The kinetic curves of ninhydrin-cyanide reaction in the presence of cysteine, presented on Fig 3B, showed that at least 30 min were necessary to obtain constant absorbance. Hence, we proposed the following procedure for WAD cyanide quantification: cysteine was added to the WAD cyanide sample in molar ratio 1:2 and agitated for 40 min at room temperature (30 °C); the aliquots were taken and transferred in 2% Na₂CO₃ solution, ninhydrin reagent was added and sample was left for 15 min for color development.

3.5. Recovery Study

The efficiency of ninhydrin reagent for direct cyanide measuring after ligand exchange step was evaluated by recovery study. Different ligand exchange reagents were studied: cystine, cysteine, thiourea, tetraethylenepentamine and combined sulphur and amine based ligands. Free cyanide sample was also passed through the ligand exchange step as a control sample for interference. The results are presented on Table 2.

The highest recovery for all WAD complexes was obtained after thiourea ligand exchange and reaction of liberated cyanide with ninhydrin. However, for $\text{Cu}(\text{CN})_4^{3-}$ complex the recovery was still below 50%, better results were obtained at higher molar ratio metal:ligand.

Table 2 Recovery study of total cyanide from WAD complexes by ligand exchange^a

Me-cyanide complex	Recovery/%				
	Cystine	Thiourea	Cysteine	Cysteine+TEPA	Thiourea+TEPA
$\text{Hg}(\text{CN})_4^{2-}$	87.7 ± 0.2	105.6 ± 0.4	74.4 ± 1.3	94.7 ± 0.1	94.3 ± 0.5
$\text{Ni}(\text{CN})_4^{2-}$	50.5 ± 0.8	85.0 ± 0.4 ^a /92.4 ^b	47.8 ± 0.3	77.2 ± 0.5	82.1 ± 0.7
$\text{Cd}(\text{CN})_4^{2-}$	81.6 ± 0.3	83.0 ± 1.1	72.8 ± 0.1	83.7 ± 0.1	88.9 ± 0.2
$\text{Zn}(\text{CN})_4^{2-}$	93.5 ± 0.4	108.9 ± 1.1	-	116.7 ± 0.2	109.0 ± 0.2
$\text{Cu}(\text{CN})_4^{3-}$	48.2 ± 1.6	41.4 ± 0.1 ^a /77.3 ^b	50.6 ± 0.1	68.0 ± 0.5	59.5 ± 0.7
$\text{Ag}(\text{CN})_2^-$	23.9 ± 1.5	74.9 ± 0.3 ^a /72.5 ^b	61.8 ± 1.3	63.2 ± 0.3	47.4 ± 1.2
CN^-	91.2 ± 1.1	98.9 ± 0 ^a /111 ^b	81.6 ± 0.4	91.2 ± 0.3	93.1 ± 0.2

a. Molar ratio Me:thiocarbamide = 0.012. Total cyanide concentration at ligand exchange step: 3.85×10^{-5} M. Cyanide concentration at absorbance measuring step: 1.9×10^{-6} M.

b. Molar ratio Me:thiocarbamide = 0.06; $C_{\text{CN}} = 2.57 \times 10^{-5}$ M.

c. Confidence interval was calculated at $n = 3$ (absorbance was measured in triplicate) and $P = 95\%$.

The free cyanide recovery has showed that ninhydrin is selective towards cyanide in the presence of ligand exchange reagents.

4. Conclusions

The present report has demonstrated the efficiency of ninhydrin as a colorimetric reagent for weak acid cyanide determination. Two protocols for free cyanide determination were compared and the results showed that the measurement of the absorbance of the red colored cyanide-ninhydrin adduct at pH 10.8 maintained by sodium carbonate was more reliable than the procedure involving transformation of red colored adduct into blue one at $\text{pH} > 12$, where less stable pH dependent product was obtained. The results presented here proved the applicability of the reaction between metal-cyanide complexes and ninhydrin for direct WAD cyanide determination. For total WAD cyanide determination the ligand exchange approach was applied. Complexed cyanide was liberated using ligand exchange.

The described protocol was selective towards WAD cyanide in the presence of iron-cyanide complexes regarded as non-toxic cyanide forms. The selectivity of ninhydrin towards liberated cyanide in the presence of ligand exchange reagent is a base for skipping of separation step and simplifying the analytical procedure.

Acknowledgments

The financial support of the University of Chemical Technology and Metallurgy, Sofia, Bulgaria through the Science and Research Program (Contact Nr 11132/2013) and of the European Social Fund through the Human Resources Program (Contract BG051PO001-3.3.06-0014) was gratefully acknowledged.

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