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Quantification of free and metal-complexed cyanide by tetrathionate derivatization

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A sensitive and robust method for detection of free and metal-complexed cyanide in solutions is described. The method does not require a distillation step and is applicable for both low ionic strength and sea-water samples. The method is based on the reaction of cyanide with potassium tetrathionate followed by high-performance liquid chromatography (HPLC) separation and UV detection of formed thiocyanate. The detection limit of the method is 250 nmol L⁻¹ cyanide (6.5 µg L⁻¹ CN⁻) without a pre-concentration step. Storage for three days does not significantly change the results. The sum of free and weak metal-complexed cyanide can be measured by tetrathionate derivatization at a pH of 10. The sum of free, weak metal-complexed cyanide, iron(II) and iron(III)-complexed cyanides may be measured by tetrathionate derivatization at pH 4.4. Derivatization requires heating to 90°C for 20 min at pH = 10 and for 12 h at pH = 4.4. Weighted mean recoveries for free, iron(II), iron(III), nickel(II), silver(I), Cd(II) and Zn(II) complexed cyanide were in the range of 87 to 112% and weighted standard deviations were in the range of 1.7 to 10.0%. The method is not applicable for cyanide complexes of gold and cobalt. We illustrate an application of cyanide quantification using pore-waters from the Delaware Great Marsh.

Keywords: cyanide; iron-cyanide complexes; metallo-cyanide complexes; tetrathionate; liquid chromatography

1. Introduction

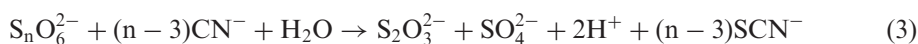
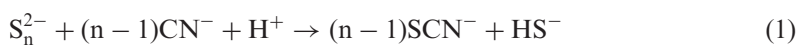
In aqueous systems dissolved cyanide exists in the form of free cyanide (CN⁻), hydrogen cyanide (HCN), weak metal-cyanide complexes (e.g. Cd(CN)₄²⁻, Zn(CN)₄²⁻, Ag(CN)₂⁻, Ni(CN)₄²⁻, etc.) and strong metal-cyanide complexes (e.g. Fe(CN)₆⁴⁻, Fe(CN)₆³⁻, Co(CN)₆³⁻, etc.) [1 and references therein].

Complex matrices interfere with cyanide concentration analysis by colorimetric, titrimetric or electrochemical techniques. The first step of a typical cyanide analysis is manual distillation. Samples are boiled for one to two hours at pH < 2 to release total inorganic cyanide as HCN and at pH 4.5–6.0 to release HCN from free cyanide and weak metal-cyanide complexes [2]. Evolved HCN is trapped in alkaline solution and measured by a variety of different protocols [1 and references therein]. Techniques for analysis of metal-cyanide complexes by liquid chromatography allowed separation and quantification of strong and some weak metal-cyanide complexes with low (µg L⁻¹) detection limit [3–8].

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Two main techniques applied to achieve this goal are ion exchange chromatography and reversed-phase ion-pair partition chromatography. Analysis of the weakest metal-cyanide complexes such as $\text{Cd}(\text{CN})_4^{2-}$ and $\text{Zn}(\text{CN})_4^{2-}$ by chromatographic techniques has not been reported, presumably owing to their low stability. For review of chromatographic techniques for cyanide speciation analysis see [1]. The most sensitive chromatographic technique for quantification of free cyanide (detection limit 8 nmol L^{-1}) utilized derivatization with 2,3-naphthalenedicarboxaldehyde, followed by HPLC with fluorescence detection [9].

Cyanide is known to be reactive toward species, which have sulfur-sulfur bonds. Polysulfides react with cyanide in minutes to hours, depending on pH, at 25°C and in seconds to minutes at 100°C (Equation (1)) [10–13]. Thiosulfate reacts with cyanide three orders of magnitude slower than polysulfides (Equation (2)) [10]. Reaction between thiosulfate and cyanide is catalyzed by Cu(II) [14 and references therein]. Tetrathionate and higher polythionates react rapidly with cyanide (Equation (3)), and trithionate reacts with cyanide much slower (Equation (4)) [13–15 and references therein]. Colloidal and dissolved elemental sulfur is reactive toward cyanide as well (Equation (5)) [12,13].



Tetrathionate was selected as a derivatizing agent from the suite of sulfur compounds that are reactive toward cyanide because it is non-toxic, odorless, is commercially available and rapidly reacts with cyanide in the absence of catalysts. Potassium tetrathionate is stable in solid form if stored at -20°C . Solutions of potassium tetrathionate are stable for days. Recent development of sensitive and robust chromatographic technique for thiocyanate analysis [16] enabled detection of this anion at submicromolar concentration without significant matrix-related problems. Thiocyanate does not undergo protonation even at slightly acidic conditions as its pK_a is -1.1 ± 0.3 [17] and it is stable in aqueous solutions.

This communication describes an easy and robust analytical protocol for quantification of free cyanide and metal-complexed cyanide at concentrations below $1 \mu\text{mol L}^{-1}$ and without the requirement of a distillation step. Cyanide is converted to thiocyanate by reaction with potassium tetrathionate and quantification of thiocyanate is undertaken using liquid chromatography.

2. Experimental

2.1 Materials

All reagents were at least 98% pure. $\text{K}_2\text{S}_4\text{O}_6$ (99%), KSCN (99%), KCN (98%), $\text{K}_4[\text{Fe}(\text{CN})_6] \times 3\text{H}_2\text{O}$ (99.5%), $\text{K}_3[\text{Fe}(\text{CN})_6]$ (99%), $\text{K}[\text{Ag}(\text{CN})_2]$ (purity not specified),

and $\text{K}[\text{Au}(\text{CN})_2]$ (99.98%) used for preparation of standards were purchased from Sigma-Aldrich and used without further purification. $\text{K}_2[\text{Ni}(\text{CN})_4] \times x\text{H}_2\text{O}$ (99.99% metal basis) used for preparation of standards was purchased from Sigma-Aldrich and was dried at 110°C for 24 hours. $\text{K}_3[\text{Co}(\text{CN})_6]$, $\text{K}_2[\text{Cd}(\text{CN})_4]$, $\text{K}_2[\text{Zn}(\text{CN})_4]$ were synthesized from $\text{CoCl}_2 \times 6\text{H}_2\text{O}$ (Sigma-Aldrich, 99%), CdSO_4 (Sigma-Aldrich, 99.99%) and $\text{Zn}(\text{CN})_2$ (Sigma-Aldrich, 98%), respectively, according to [18]. Fresh tetrathionate solution was prepared daily. "Synthetic" sea water was prepared according to [19] with minor differences: No trace constituents (boric acid, silicate and phosphate) were added, pH was adjusted to 8.0.

Delaware Great Marsh sediment cores were taken near the Cannon Laboratory of College of Earth, Ocean, and Environment of University of Delaware in Lewes ($38^\circ48.006\text{N}$, $75^\circ11.684\text{W}$) on April 14, 2010 and transported to the laboratory. Delaware Great Marsh pore-water was extracted with Rhizon samplers inserted through pre-drilled holes in the core liner (type: CSS, Rhizosphere Research Products, NL-Wageningen) with a filter pore diameter of 100 nm [20].

Thiocyanate concentrations were measured by an Agilent Technologies 1200 HPLC system with multiple wavelength UV-visible detector operated at 220 nm. A Nomura Chemical, Japan, Develosil RPAQUEOUS C30 reverse phase column ($150\text{ mm} \times 4.6\text{ mm} \times 5\text{ }\mu\text{m}$) was used for HPLC separation of thiocyanate. The column was prepared according to protocols described by [16] by pumping 5% aqueous solution of PEG-20,000 at 0.3 mL min^{-1} rate for at least three hours.

Agilent vials and caps with septa were used for tetrathionate derivatization and high-performance liquid chromatography (HPLC) analysis.

2.2 Methods

Two methods (Method I and Method II) were developed for analysis of cyanide speciation in aqueous solutions.

Method I was designed to analyse total cyanide (free and metal-complexed). The first step of Method I is acidification of the sample to increase reactivity of iron-cyanide complexes. A 1 mL sample was pipetted into a 2 mL HPLC vial. Acidic buffer (0.1 mL, $\text{pH} = 4.4$, 200 mM boric acid/200 mM sodium chloride) and potassium tetrathionate solution (0.1 mL, 100 mM) were added to the vial; it was closed by screw cap with septa and heated at 90°C for 8–16 h. After cooling, the resulting solution was analysed by HPLC. In addition to the Method I, a variant (Method Ia) was applied to samples with high cyanide concentration. One mL of buffer and 0.1 mL of $\text{K}_2\text{S}_4\text{O}_6$ solution were added to 0.1 mL sample.

In the second method (Method II), a basic buffer (0.1 mL, $\text{pH} = 10.0$, 100 mmol L^{-1} boric acid/100 mmol L^{-1} sodium chloride / 88 mmol L^{-1} sodium hydroxide) was added to a 1 mL sample in order to prevent reaction between tetrathionate and strong metal-cyanide complexes, followed by addition of and potassium tetrathionate solution (0.1 mL, 100 mM). The reaction mixture was kept at 90°C for 20 min, before cooling and analysis by HPLC.

Methods I and II were applied to free cyanide (KCN solution), complexes of iron (II) and iron (III) with cyanide ($\text{K}_4[\text{Fe}(\text{CN})_6]$ and $\text{K}_3[\text{Fe}(\text{CN})_6]$, respectively) as well to other metal-cyanide complexes ($\text{K}_2[\text{Zn}(\text{CN})_4]$, $\text{K}_2[\text{Cd}(\text{CN})_4]$, $\text{K}[\text{Ag}(\text{CN})_2]$, $\text{K}_2[\text{Ni}(\text{CN})_4]$, $\text{K}[\text{Au}(\text{CN})_2]$ and $\text{K}_3[\text{Co}(\text{CN})_6]$. The concentration range of samples analysed by

Methods I and II was 250 n mol L^{-1} – 3 mmol L^{-1} cyanide. Method Ia was applied to KCN, $\text{K}_4[\text{Fe}(\text{CN})_6]$, $\text{K}_3[\text{Fe}(\text{CN})_6]$, $\text{K}_2[\text{Zn}(\text{CN})_4]$, $\text{K}[\text{Ag}(\text{CN})_2]$, and $\text{K}_2[\text{Ni}(\text{CN})_4]$ samples with cyanide concentrations in the range of $50 \text{ } \mu\text{mol L}^{-1}$ – 3 mmol L^{-1} . All experiments were made in triplicates. Details of analytical procedures are summarized in Table 1.

3. Results and discussion

3.1 Final protocol

The reactivity of nine cyanide species toward tetrathionate at various conditions was studied. These nine species included free cyanide, four weak metallo-cyanide complexes ($\text{K}_2[\text{Zn}(\text{CN})_4]$, $\text{K}_2[\text{Cd}(\text{CN})_4]$, $\text{K}[\text{Ag}(\text{CN})_2]$, and $\text{K}_2[\text{Ni}(\text{CN})_4]$) and four strong metallo-cyanide complexes ($\text{K}_4[\text{Fe}(\text{CN})_6]$, $\text{K}_3[\text{Fe}(\text{CN})_6]$, $\text{K}[\text{Au}(\text{CN})_2]$, and $\text{K}_3[\text{Co}(\text{CN})_6]$). Cyano-complexes of Zn(II) and Cd(II), which have response to tetrathionate treatment similar to the one of free cyanides, will be further referred as ‘very weak metallo-cyanide complexes’.

Figure 1 shows principal schemes for analysis of free cyanide and mixtures of metallo-cyanide complexes. Free cyanide should be analyzed by Method II (Table 1) in the

Table 1. Summary of methods used for determination of metal-complexed cyanide.

Method	Sample volume, μL	Buffer volume, μL	$\text{K}_2\text{S}_4\text{O}_6$ solution volume, μL	buffer pH	t , $^\circ\text{C}$	Reaction time
I	1000	100	100	4.4	90	overnight (12 ± 4 h)
Ia	100	1000	100	4.4	90	overnight (12 ± 4 h)
II	1000	100	100	10.0	90	20 min

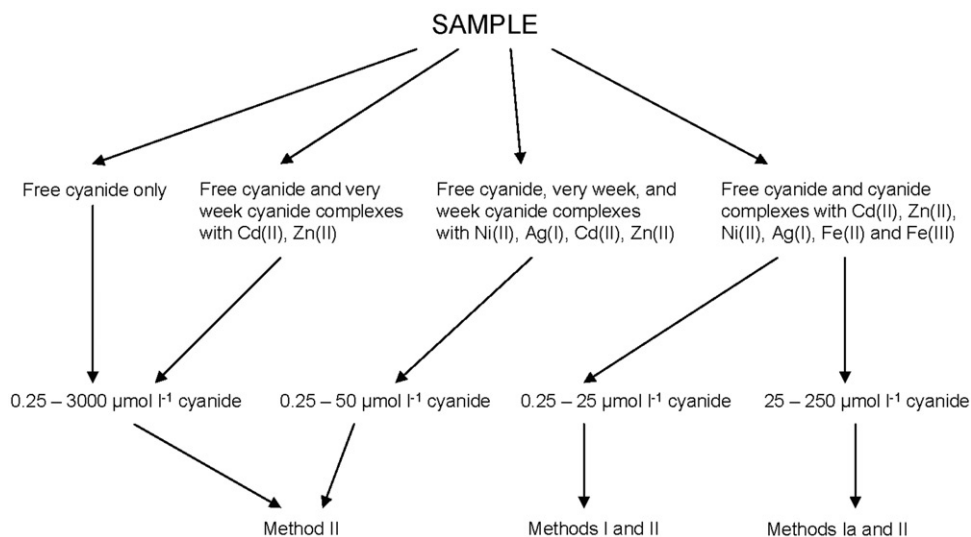


Figure 1. Scheme of analysis of mixtures of free cyanide and various metallo-cyanide complexes.

concentration range of 250 nmol L^{-1} – $3 \text{ mmol L}^{-1} \text{ CN}^{-}$. Very weak and weak cyanide complexes are indistinguishable from free cyanide and can be analysed by the same method in the concentration ranges of 250 nmol L^{-1} – $3 \text{ mmol L}^{-1} \text{ CN}^{-}$ and 250 nmol L^{-1} – $50 \text{ } \mu\text{mol L}^{-1} \text{ CN}^{-}$, respectively. The cyanide content of strong iron-cyanide complexes should be detected by Method I (Table 1) in the concentration range of 250 nmol L^{-1} – $25 \text{ } \mu\text{mol L}^{-1} \text{ CN}^{-}$ and by Method Ia (Table 1) at concentrations up to $250 \text{ } \mu\text{mol L}^{-1} \text{ CN}^{-}$. If free cyanide, very weak and/or weak cyanide complexes, and iron-cyanide complexes present in the sample, Method II should be applied as well, and the concentration of iron-complexed cyanide should be calculated as a difference between results of analyses by the Methods I or Ia and the Method II. The cyanide complex of Co(III) cannot be detected by this protocol. This protocol cannot be applied to system containing cyanide complexes of gold.

The protocol was shown to work for sea water samples and for pore-water samples from a natural salt marsh environment (The Delaware Great Marsh).

3.2 Precision and accuracy

Standard deviation is $\leq 10\%$ for analyses of cyanide content of all metallo-cyanide complexes by all methods in the reported applicability range. Average cyanide recovery by analyses in the reported applicability ranges is 87–112% (Table 2).

3.3 Sample storage

Samples of KCN, $\text{K}_4[\text{Fe}(\text{CN})_6]$, $\text{K}_3[\text{Fe}(\text{CN})_6]$, and $\text{K}_2[\text{Zn}(\text{CN})_4]$, containing $25 \text{ } \mu\text{mol L}^{-1} \text{ CN}^{-}$, were stored after derivatization with potassium tetrathionate at -20°C , 4°C and room temperature ($25 \pm 2^\circ\text{C}$). Samples were analysed immediately after derivatization, after 3 days, and 11–13 days. Analyses of samples stored at room temperature for 3 days were biased relative to results of initial analysis by less than 9%. After 11 days results of most of analysis were reduced by as much as 80% compared with results of initial analysis. Other storage problems arise from disproportionation of residual tetrathionate. Tetrathionate decomposes in aqueous solutions at near neutral pH to lower and higher polythionates [21 and references therein]. Pentathionate elutes before thiocyanate with less than a 2 min difference in retention time [12]. At high pentathionate to thiocyanate ratios precise integration of the thiocyanate peak becomes problematic. Storage at 4°C does not significantly improve recoveries. Storage at -20°C yields low recovery even after 3 days and is therefore not recommended.

3.4 Thiocyanate background

Some aquatic systems contain measurable amounts of thiocyanate. Rong et al. [14] measured 140 – 260 nmol L^{-1} of SCN^{-} in seawater samples from the Japanese coast with different levels of pollution. Kamyshny [12] detected $11 \pm 9 \text{ nmol L}^{-1}$ of SCN^{-} in North Sea water. Up to 274 nmol L^{-1} of SCN^{-} was detected in sulfide rich water layer near the bottom of meromictic Fayetteville Green Lake (NY) (Kamyshny–unpublished results). In anoxic aquatic systems natural processes similar to those utilized in this work may occur. Significant concentrations of sulfur species reactive toward cyanide were reported in anoxic natural aquatic systems: Thiosulfate, tetrathionate, and polysulfides were detected

Table 2. Cyanide concentration ranges ($\mu\text{mol L}^{-1}$) detectable by reported protocols, and recoveries (in parentheses, %).

Method	KCN	$\text{K}_4[\text{Fe}(\text{CN})_6]$	$\text{K}_3[\text{Fe}(\text{CN})_6]$	$\text{K}_2[\text{Cd}(\text{CN})_4]$	$\text{K}_2[\text{Zn}(\text{CN})_4]$	$\text{K}[\text{Ag}(\text{CN})_2]$	$\text{K}_2[\text{Ni}(\text{CN})_4]$	$\text{K}[\text{Au}(\text{CN})_2]$	$\text{K}_3[\text{Co}(\text{CN})_6]$
I	0.25–250 (104.6 \pm 10.0)	0.25–100 (110.4 \pm 9.8)	0.25–25 (94.8 \pm 7.4)	0.25–250 (91.3 \pm 1.9)	0.25–100 (112.1 \pm 7.9)	0.25–250 (96.0 \pm 8.4)	0.25–25 (96.9 \pm 7.6)	N.E.	N.R. (<1.9)
Ia	50–3000 (97.6 \pm 9.6)	50–1000 (94.6 \pm 3.1)	50–1000 (95.0 \pm 5.0)	N.A.	50–250	50–250	50–250	N.E.	N.R. (<1.3)
II	0.25–3000 (102.4 \pm 8.6)	N.R. (<0.7)	N.R. (<2.5)	0.25–3000 (93.1 \pm 4.0)	86.8 \pm 1.7 0.25–3000 (101.3 \pm 1.8)	108.3 \pm 3.2 0.25–100 (103.1 \pm 4.4)	99.7 \pm 6.9 0.25–50 (94.0 \pm 7.6)	N.R. (<8.8)	N.R. (<2.9)

in concentrations of micromoles to hundreds of micromoles per litre in natural aquatic and sedimentary systems [22–27]. These compounds react with cyanide, producing thiocyanate. Measurement of background thiocyanate concentrations is necessary in order to subtract it from the results of analysis for cyanide species.

3.5 Protocol application at the field conditions

Tetrathionate may be added to the sample immediately after collection. However sample should be heated before analysis as the reaction between metallo-complexed cyanide and tetrathionate is too slow to convert CN^- to SCN^- quantitatively at ambient temperature. Another way to apply this method to field samples is to stabilize cyanide by cooling and adjustment of pH to 12 in dark bottle. pH should be adjusted accordingly once the samples are returned to the laboratory. In this case, larger amount of buffers should be used, and dilution of the sample will increase the detection limit of analysis.

3.6 Analytical performance

Calculations – Average recovery of triplicate samples at each cyanide concentration was calculated, and the concentration interval where the calibration is linear (average recovery of 80–120%) was determined. Weighted mean and weighted standard deviation were calculated from results of analysis of triplicate samples at all concentrations in the range of method linear calibration (Table 2). All concentrations are reported as cyanide content of metallo-cyanide complex and not as complex concentration.

Free cyanide – For Method I the linear calibration range for cyanide was 250 nmol L^{-1} – $250 \text{ } \mu\text{mol L}^{-1}$ with recovery of $104.6 \pm 10.0\%$. For Method Ia, the recovery was $97.6 \pm 9.6\%$ in the concentration range $50 \text{ } \mu\text{mol L}^{-1}$ – 3 mmol L^{-1} cyanide. For Method II, the recovery in the cyanide concentration range $250 \text{ } \mu\text{mol L}^{-1}$ – 3 mmol L^{-1} was $102.4 \pm 8.6\%$ (Figure 2a, Table 2). Typical chromatogram for cyanide derivatization is presented in Figure 3a.

Potassium ferrocyanide – For Method I, the linear calibration range for cyanide was 250 nmol L^{-1} – $100 \text{ } \mu\text{mol L}^{-1}$ with recovery of $110.4 \pm 9.8\%$. For Method Ia, the recovery was $94.6 \pm 3.1\%$ in the cyanide concentration range $50 \text{ } \mu\text{mol L}^{-1}$ – 1 mmol L^{-1} CN^- . For Method II, the cyanide recoveries were below 0.7% at all concentrations (Figure 2b, Table 2).

Potassium ferricyanide – For Method I, the linear calibration range for cyanide was 250 nmol L^{-1} – $25 \text{ } \mu\text{mol L}^{-1}$ with recovery of $96.0 \pm 8.4\%$. For Method Ia, the cyanide recovery was $95.0 \pm 5.0\%$ in the concentration range $50 \text{ } \mu\text{mol L}^{-1}$ – 1 mmol L^{-1} . For Method II, the cyanide recoveries are less than 2.5% at all concentrations (Figure 2c, Table 2).

Potassium tetracyanozincate (II) – For Method I, the linear calibration range for cyanide was 250 nmol L^{-1} – $100 \text{ } \mu\text{mol L}^{-1}$ with recovery of $112.1 \pm 7.9\%$. For Method Ia, the cyanide recovery was $86.8 \pm 1.7\%$ in the concentration range $50 \text{ } \mu\text{mol L}^{-1}$ – $250 \text{ } \mu\text{mol L}^{-1}$. For Method II, the cyanide recovery in the concentration range 250 nmol L^{-1} – 3 mmol L^{-1} was $101.3 \pm 1.8\%$ (Figure 2d, Table 2).

Potassium tetracyanocadmiate (II) – For Method I, the linear calibration range for cyanide was 250 nmol L^{-1} – $250 \text{ } \mu\text{mol L}^{-1}$ with recovery of $91.3 \pm 1.9\%$. For Method II,

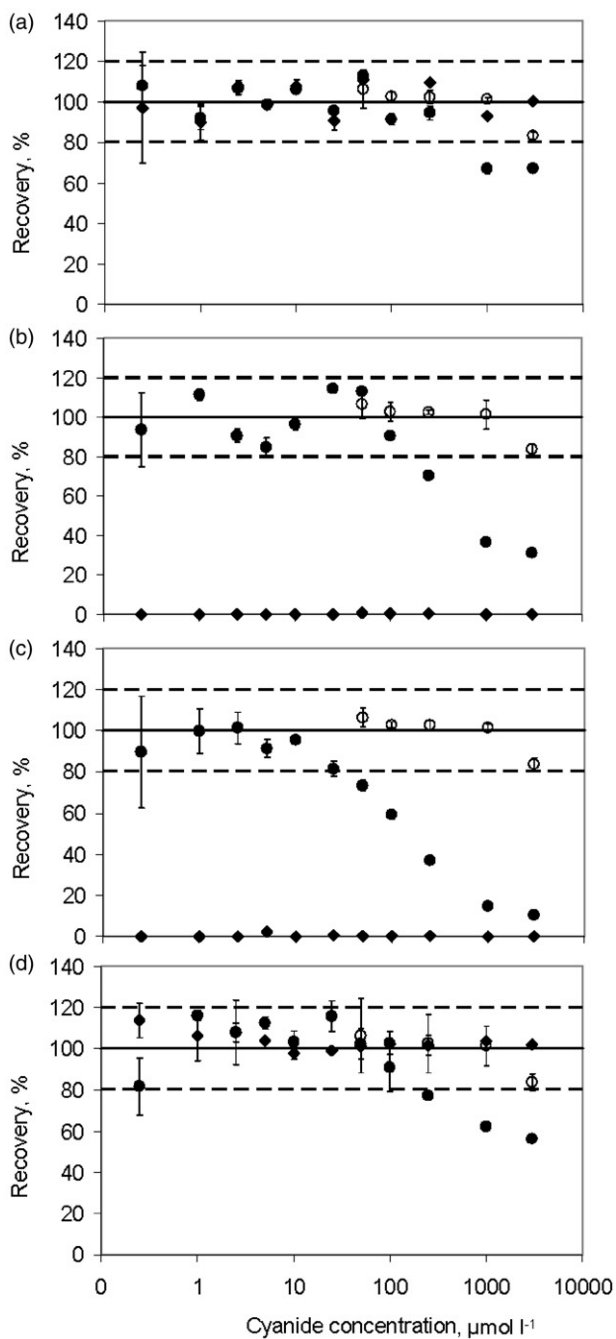


Figure 2. Cyanide recovery by tetrathionate derivatization for KCN (a), $\text{K}_4[\text{Fe}(\text{CN})_6]$ (b), $\text{K}_3[\text{Fe}(\text{CN})_6]$ (c), and $\text{K}_2[\text{Zn}(\text{CN})_4]$ (d) by Method I (closed circles), Method Ia (opened circles), and Method II (closed diamonds).

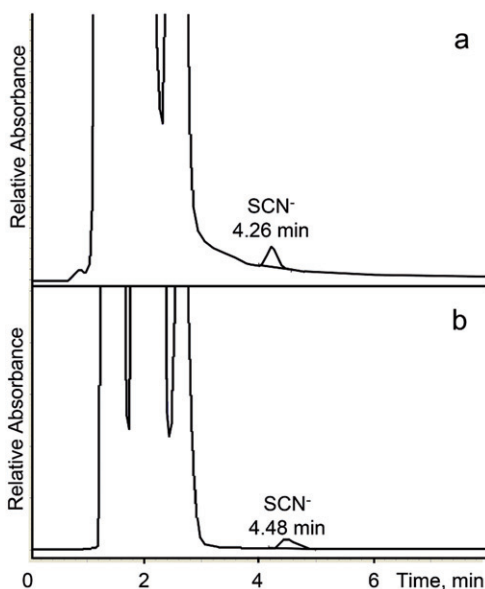


Figure 3. Chromatograms of standard sample containing $10 \mu\text{mol} \mu\text{mol L}^{-1}$ of free cyanide after derivatization according to Method II (a) and of Delaware Great Marsh pore-water sample (25 cm below sediment surface) after derivatization according to Method II (b).

the cyanide recovery in the concentration range 250 nmol L^{-1} – $3 \text{ mmol L}^{-1} \text{ CN}^{-}$ was $93.1 \pm 4.0\%$ (Table 2).

Potassium dicyanoargentate (I) – For Method I, the linear calibration range for cyanide was 250 nmol L^{-1} – $250 \mu\text{mol L}^{-1} \text{ CN}^{-}$ with recovery of $96.0 \pm 8.4\%$. For Method Ia, the recovery was $108.3 \pm 3.2\%$ in the cyanide concentration range 50 – $250 \mu\text{mol L}^{-1}$. For Method II, the cyanide recovery in the concentration range 250 nmol L^{-1} – $100 \mu\text{mol L}^{-1} \text{ CN}^{-}$ was $103.1 \pm 4.4\%$ (Table 2).

Potassium tetracyanonickelate(II) – For Method I, the linear calibration range for cyanide was 250 nmol L^{-1} – $25 \mu\text{mol L}^{-1} \text{ CN}^{-}$ with recovery of $96.9 \pm 7.6\%$. For Method Ia, the recovery was $99.7 \pm 6.9\%$ in the cyanide concentration range 50 – $250 \mu\text{mol L}^{-1}$. For Method II, the cyanide recovery in the concentration range 250 nmol L^{-1} – $50 \mu\text{mol L}^{-1}$ was $94.0 \pm 7.6\%$ (Table 2).

Potassium dicyanoaurate(I) – For Method I, no linear calibration was found at any concentration range. For the Method II cyanide recoveries are less than 8.8% at all concentrations (Table 2).

Potassium tetracyanocobaltate(III) – For the Methods I, Ia and II cyanide recoveries were found to be less than 2.9% at all concentrations (Table 2).

Derivatization in Sea Water Medium – Potassium cyanide, potassium ferrocyanide and potassium ferricyanide solutions with $10 \mu\text{mol L}^{-1}$ cyanide concentration were prepared in synthetic sea water and analyzed by Methods I and II. Results of analyses for all compounds by Method I were in the 84–113% range. Results of analysis by Method II were 93% for free cyanide and 0% for both iron(II) and iron(III) complexed cyanide.

Analysis of natural samples – Cyanide concentrations were determined in the Delaware Great Marsh pore-waters by Methods I and II at 11 cm (non-sulfidic, iron(II) rich zone)

and 25 cm (5.4 mmol L^{-1} sulfide concentration) sediment depths. Background thiocyanate concentrations of 1.54 and $3.86 \text{ } \mu\text{mol L}^{-1}$ were detected at 11 cm and 25 cm depths, respectively. Results of analysis by Method I were 8.58 and $4.13 \text{ } \mu\text{mol L}^{-1}$ cyanide at 11 and 25 cm depths, respectively. Results of analysis by Method II were -0.03 and $3.25 \text{ } \mu\text{mol L}^{-1}$ cyanide at 11 and 25 cm depths, respectively. The negative concentration detected by Method II arises from subtraction of the result of analysis of thiocyanate background concentration from result of analysis. From this data we can calculate that the sum of free and weakly complexed cyanide in the marsh pore-waters is $0.0 \text{ } \mu\text{mol L}^{-1}$ at 11 cm depth and $3.2 \text{ } \mu\text{mol L}^{-1}$ at 25 cm depths. Iron-complexed cyanide concentrations were found to be $8.6 \text{ } \mu\text{mol L}^{-1}$ at 11 cm depth and $0.9 \text{ } \mu\text{mol L}^{-1}$ at 25 cm depth. The fraction of iron-complexed cyanide is 100% and 21% at 11 cm and 25 cm depths, respectively. Typical Delaware Great Marsh pore-water sample derivatization chromatogram is presented in Figure 3b.

3.7 Explanation of observed recoveries

Recovery of cyanide by derivatization with tetrathionate depends on two main factors: the stability of metallo-cyanide complex and stability (or solubility) of metallo-thiocyanate complexes. Tetrathionate does not form stable complexes with transition metals.

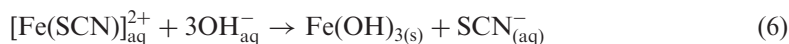
Stronger metallo-cyanide complexes require lower pH for dissociation. The strength of metallo-cyanide complexes used in this work increases in the order $\text{Cd}(\text{CN})_4^{2-} < \text{Zn}(\text{CN})_4^{2-} < \text{Ag}(\text{CN})_2^- < \text{Ni}(\text{CN})_4^{2-} < \text{Au}(\text{CN})_2^- < \text{Fe}(\text{CN})_6^{4-} < \text{Fe}(\text{CN})_6^{3-} < \text{Co}(\text{CN})_6^{3-}$ [1]. Free cyanide and weak metallo-cyanide complexes, $\text{Cd}(\text{CN})_4^{2-}$, $\text{Zn}(\text{CN})_4^{2-}$, $\text{Ag}(\text{CN})_2^-$, $\text{Ni}(\text{CN})_4^{2-}$, react with tetrathionate at pH 10.0. Stronger complexes, $\text{Au}(\text{CN})_2^-$, $\text{Fe}(\text{CN})_6^{4-}$, and $\text{Fe}(\text{CN})_6^{3-}$, react with tetrathionate at pH 4.4. The strongest complex, $\text{Co}(\text{CN})_6^{3-}$, does not react with tetrathionate even at this pH.

The upper limit of detectable cyanide concentrations depends not only on stability of the metallo-cyanide complex, but on stability of respective metallo-thiocyanate complex as well. If thiocyanate produced by the reaction of cyanide and tetrathionate forms a strong complex with cations in solution, the free thiocyanate concentration in sample after derivatization decreases resulting in negative recovery bias.

Silver thiocyanate solubility in water is low ($K_{\text{SP}} = 1.33 \times 10^{-12}$). Precipitation of AgSCN produces negative bias for detection of SCN^- formed in the reaction of dicyanoargentate (I) with tetrathionate. Sodium chloride was added to buffer in order to compete with formation of the thiocyanate complex or silver thiocyanate precipitate formation: $100\text{--}200 \text{ mmol L}^{-1}$ of sodium chloride was added to buffers used in derivatization procedures. As K_{SP} of AgCl (1.77×10^{-10}) is two orders of magnitude lower than that of AgSCN , the final concentration of NaCl in the sample (8.33 mmol L^{-1}) allows us to analyse the silver (I) complexed cyanide at concentrations of up to two orders of magnitude lower ($100 \text{ } \mu\text{mol L}^{-1}$). Solubilities of silver bromide and silver iodide are lower than the solubility of silver thiocyanate. Unfortunately, poor chromatographic separation between millimolar concentrations of these halogen anions and sub-micromolar concentration of thiocyanate restricts their use in buffer preparation. Chloride is a component of mobile phase and it is neither retained on the column nor affects UV detector background.

The strength of water soluble thiocyanate complexes increases in the order $\text{Zn(II)} < \text{Ni(II)} < \text{Cd(II)} < \text{Fe(III)} \ll \text{Au(I)}$ [28]. Metallo-thiocyanate complexes are more stable at low

pH values than metallo-cyanide complexes owing to relatively low pK_a of HSCN [17]. The upper detection limits for Zn(II), Ni(II), Cd(II), and Fe(III) cyanide complexes are in the range of 25–250 $\mu\text{mol L}^{-1}$ cyanide. Upper detection limit for Method II is higher than upper detection limit of method I for all metallo-cyanide complexes, except $\text{Ag}(\text{CN})_2^-$, as at $\text{pH}=10.0$ all metals used in this study (except silver) form (hydr)oxides [29]. Relatively strong blood red thiocyanate complex of Fe(III), $[\text{Fe}(\text{SCN})]^{2+}$, does not form at $\text{pH}>3$ owing to formation of insoluble $\text{Fe}(\text{OH})_3$ (Equation (6)).



Dicyanoaurate (I) can not be detected by the described procedure as gold forms strong complex with thiocyanate.

3.8 Comparison with existing methods of cyanide quantification

Existing analytical protocols for analysis of free and metallo-complexed cyanide may be separated in two main groups.

The first group includes methods that account for free cyanide in a relatively non-complex matrix. The most sensitive method is based on derivatization with a fluorescent reagent followed by HPLC with a fluorescence detector [9]. The detection limit for this technique is as low as 6 nmol L^{-1} . Another group of methods for free cyanide quantification includes titrimetry (detection limit $4 \mu\text{mol L}^{-1}$), colorimetry (detection limit $0.8 \mu\text{mol L}^{-1}$), application of an ion-selective electrode (detection limit $2 \mu\text{mol L}^{-1}$) and amperometry (detection limit 75 nmol L^{-1}) [30]. A significant draw-back of these techniques is the space and time consuming laborious distillation step, which is required for conversion of metallo-complexed cyanide into free cyanide and elimination of complex matrix, such as sea-water salts.

In addition, individual metallo-cyanide complexes can be quantified by ion-interaction chromatography. The detection limit for metallo-cyanide complexes varies in the range of $0.4\text{--}8 \mu\text{mol L}^{-1}$ [6]. These methods [3–8] do not require cyanide distillation or other sample pre-treatment. A combination of ion-interaction chromatography with post-column cyanide derivatization allows detection of free cyanide [31]. The draw-back of these techniques is the necessity of synthesis of all of metallo-cyanide complexes and separate HPLC calibrations for each of them.

The analytical protocol presented in this paper combines the advantages of both approaches: it allows analysis of free cyanide as well as metallo-cyanide complexes, does not require a distillation step, and can be applied to complicated matrices like sea-water. The detection limit of this method ($0.25 \mu\text{mol L}^{-1}$) is lower than detection limit of most of existing techniques, though it is 40 times higher than detection limit for the most sensitive chromatographic technique [9].

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