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Headspace single-drop microextraction and cuvetteless microspectrophotometry for the selective determination of free and total cyanide involving reaction with ninhydrin

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ABSTRACT

Headspace single-drop microextraction has been used for the determination of cyanide with ninhydrin in combination with fibre-optic-based cuvetteless microspectrophotometry which accommodates sample volume of 1 μL placed between the two ends of optical fibres, and has been found to avoid salient drawbacks of batch methods. This method involved hydrocyanic acid formation in a closed vial, and simultaneous extraction and reaction with 2 μL drop of ninhydrin in carbonate medium suspended at the tip of a microsyringe needle held in the headspace of the acidified sample solution. The method was linear in range 0.025–0.5 mg L^{-1} of cyanide. The headspace reaction was free from the interference of substances, e.g., thiocyanate, hydrazine sulphate, hydroxylammonium chloride and ascorbic acid. Sulphide was masked by cadmium sulphate, nitrite by sulphamic acid, sulphite by *N*-ethylmaleimide, and halogens by ascorbic acid. The limit of detection was found to be 4.3 μg L^{-1} of cyanide which was comparable to existing most sensitive methods for cyanide. However, the present method is far more simple. The method was applied to acid-labile and metal cyanides complexes by treatment with sulphide when metal sulphides were precipitated setting cyanide ion free, and to iron(II) and (III) cyanide complexes by their decomposition with mercury(II), the mercury(II) cyanide formed was then determined. These pre-treatment methods avoided cumbersome pre-separation of cyanide by methods such as distillation or gas diffusion. The overall recovery of cyanide in diverse samples was 97% with RSD of 3.9%.

1. Introduction

Cyanide is known for its propensity to bind iron in cytochrome oxidase inhibiting the mitochondrial electron-transport chain and resulting in hypoxia. Consequently, cyanide is extremely toxic and even relatively small amounts of this species are lethal to humans [1]. The World Health Organization has set the maximum contaminant level of 70 μg L^{-1} of cyanide in drinking water [2]. The industrial activities that are responsible for cyanide generation and release into the environment include electroplating, metallurgy, electronic manufacturing, ore leaching, and production of nitriles, nylon and acrylic plastics. Motor vehicle exhaust and fire fumes, therapeutic treatment with sodium nitrosyl-pentacyanoferrate(III) (nitroprusside), pyrolysis of polymers that contain nitrogen, inhalation of tobacco smoke are some other sources of cyanide exposure. Dietary sources such as cassava roots, lima beans and bamboo shoots contain cyanogenic glycosides [3] which produce hydrocyanic acid (hydrogen cyanide) enzymatically after cell rupture. Cyanide has also been identified as a chemical terrorism agent. Besides cyanide and hydrocyanic acid, toxicologically important are also cyanide complexes of zinc(II), cadmium(II), copper(II), nickel(II), mercury(II) and silver(I), labelled as weak acid-dissociable (WAD) complexes, some of which can easily release cyanide in acidic medium, and iron(II) and (III) complexes which are relatively more stable but can be decomposed by sunlight.

Several analytical methods have been reported for the determination of cyanide in diverse sample matrices and relying on a range of experimental protocols and detection techniques. Most of these strategies, however, suffer from some disadvantages of requiring large sample sizes, long analysis times, multi-step procedures with cumbersome sample pre-treatments, high detection limits or use of sophisticated instrumentation which need special operational skill. Many of them also suffer the deleterious interference of other ions and substances which are commonly found in the environmental samples. While free cyanide can be directly determined by a wide variety of methods, such as headspace gas chromatography for hydrocyanic acid [4], sample preparation by distillation of acidified sample and collection of hydrocyanic acid in alkaline solution for analysis is frequently used for metal–cyanide complexes [5]. Iron(II) and (III) complexes which are normally found in petroleum

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refinery effluents have been reported as stubborn examples [6]. Simultaneous clean-up, pre-concentration and derivatization by headspace single-drop microextraction (SDME) [7] or hollow-fibre protected liquid-phase microextraction of hydrocyanic acid [8] and capillary electrophoresis (CE) with UV detection of the derivative tetracyanonickel(II) has been used for cyanide determination in biological samples; the acceptor phase of nickel(II) chloride, ammonium hydroxide and sodium carbonate played a critical role [8], and hydrogen sulphide interfered with the determination due to the precipitation of nickel(II) sulphide. The headspace gas chromatography and atomic emission detection for hydrocyanic acid [9] was unaffected by the presence of hydrogen sulphide, and gave about 15% higher results than the ion-selective electrode (ISE) method ostensibly due to the loss of hydrocyanic acid during the distillation step in ISE method to remove sulphide [10]. Thiocyanate was found to interfere in the determination of cyanide in blood by headspace gas chromatography with nitrogen–phosphorus detection [11]. Ethylenediamine together with dithizone was used for WAD cyanide complexes to liberate cyanide by a ligand displacement mechanism [12], which was determined by headspace formation of tetracyanonickel(II) and its CE [7]. A flow injection method for WAD cyanide complexes allowed skipping of separation step for interference by using a combination of thin-layer electroplated silver chalcogenide ion-selective membranes and electrochemical pre-treatment for release of bound cyanide [13]. Solid-phase reactors packed with cadmium carbonate [14], zinc carbonate [15] or silver carbonate [16] were placed in a single line flow injection-flame atomic absorption spectrometry system, when a metal cyanide complex was released on cyanide injection, and has been used for indirect determination of free cyanide. Many ions, though do not interfere, may deplete the solid reagents.

Separation of cyanide from its WAD complexes by gas diffusion, and flow injection derivatization with o-phthalaldehyde (OPA) and glycine to give a highly fluorescent product, which was measured [17], avoided many of the problems of widely used batch spectrophotometric method based on the Konig reaction [18]. The effect of several species on OPA and glycine reaction was also studied [19], only sulphide interfered when present in large amounts. Determination of free and total cyanide (sum of free and metal-complexed cyanide) was based on focused microwave irradiation of sample to distil hydrocyanic acid, which was collected in alkaline solution and analyzed by flow injection spectrophotometry utilizing the Aldridge reaction [20]. This method was much faster (10–20 min) and convenient than the traditional distillation methods (about 1 h). Hexacyanoferrate(II) and (III) (ferrocyanide and ferricyanide, respectively) required the presence of EDTA during distillation, and produced cyanide recoveries over 90%. Thiocyanate did not interfere, however, elevated levels of nitrate caused liberation of some cyanide due to oxidation of thiocyanate under the prevailing rigorous distillation conditions. Cyanide catalyzes the oxidation of phenolphthalin (colourless) by copper(II) in basic solution giving pink phenolphthalein, which can be measured spectrophotometrically [21]. It was necessary to measure the colour after a pre-determined period of time or to use 'a stabilizing agent,' that reduced copper(II), to quench the reaction. This problem was perhaps best avoided by conducting the reaction in flow injection mode where the reaction mixture was presented before the detector at exactly and reproducibly the same time [22]. Hexacyanoferrate(III), being oxidizing agent, also responded to the same colour reaction in the phenolphthalin method.

Cyanide and thiocyanate formed a coloured ternary complex with copper(II)-2,2′-dipyridyl-2-quinolylhydrazone that was extracted into chloroform in a flow injection manifold [23]; thiocyanate alone was determined after masking cyanide with formaldehyde. The azo dye, 2-methoxy-4-(4′-nitrophenylazo)aniline-*N*,*N*-bis(3′-propanoic acid), complexed with copper(II) giving a colour change from red to yellow; cyanide withdrew copper(II) from the complex and reversed the colour change [24]. Both copper(II) complex methods suffered from the interference of sulphide. Aquacyanocobester, which has a corrin ring with cobalt(III) in its centre and two axial substituents, a cyano group and a water molecule, showed replacement of coordinated water on reaction with cyanide leading to the colour change from orange to violet, which was measured [25,26]. This reagent is particularly free from the interference of thiocyanate and sulphide. Besides these metal complexes, some organic compounds have also been used as chromogenic reagents for cyanide. Coloured species were formed by the nucleophilic addition of cyanide to the imine group of 6-hydroxy-3-(2-oxoindolin-3-ylideneamino)-2-thioxo-2H-1,3-thiazin-4(3H)-one [27] or to the indoline fragment of the oxazines, formed by fusion of benzo-oxazine ring to an indoline moiety [28,29], the colour was measured spectrophotometrically. All these organic reagents are commercially unavailable, and the methods reported using them are applicable only to free cyanide. These methods seem to be unaffected by thiocyanate, which has too slow reaction with the electrophilic cyclic structures, but the interference of sulphide is unknown. Recently reported are thiourea based chemosensors which respond to both cyanide and fluoride [30].

Ninhydrin (2,2-dihydroxy-1,3-indanedione) is commercially available reagent and has been used for cyanide, a method that was adapted from the detection of amino acids [31]. Indeed, cyanide gave a colour reaction with ninhydrin, which was the reason for high blank in amino acids analysis, and formed the basis of cyanide determination by batch spectrophotometry published by two independent research groups [32–34]. In sodium carbonate medium cyanide formed a red colour on reaction with ninhydrin, but it turned to blue in alkaline medium [32]. The method was rapid, simple and sensitive, and formed the basis of automated flow injection [35] and sequential injection spectrophotometry [36]. Though the mechanism of ninhydrin and cyanide reaction was elucidated earlier [37], the subsequent proposals showed disharmony [32,38]. Similarly, selectivity against sulphide was claimed [32,33], there were conflicting reports on the interference of thiocyanate and many metal ions. To attain selectivity, cyanide (as hydrocyanic acid) was removed from sample matrix by gas diffusion and determined using ninhydrin by a stopped flow-sequential injection analysis [39]. The features of diverse analytical methods for cyanide are presented in Table 1.

In the present work, the inherent shortcomings of analytical methods for cyanide involving ninhydrin have been circumvented by using headspace SDME and cuvetteless fibre-optics-based microspectrophotometry. SDME customarily results in high enrichment factor due to a large ratio (V_{aq}/V_o) of the volume of aqueous sample (V_{aq}) to that of organic phase (V_o) [40], and this new technique has of late been used in conjunction with NanoDrop® microspectrophotometer, which is equipped to accommodate sample volumes as small as 1 μL for absorbance measurement with high accuracy and reproducibility [41–47]. In the proposed method hydrocyanic acid that was liberated from free and acid-labile cyanides was extracted into an aqueous drop of ninhydrin held in the headspace, when the red colour developed was measured at 530 nm using NanoDrop® microspectrophotometer. Headspace extraction/reaction avoided the interferences of a number of reducing ions/substances which interfered in batch or flow injection methods, and eliminated the need for gas diffusion to achieve selectivity. Another advancement was determination of cyanide in its salts/complexes with mercury(II), silver(I), nickel(II) and copper(I), which are reported to give poor recovery for cyanide [12], by the addition of sodium sulphide when more stable metal sulphides were formed setting free cyanide ion; the excess sulphide was masked with cadmium(II). Finally, the method was extended

Table 1
Comparison of figures of merit of diverse methods for the determination of cyanide.

Technique[a]	Principle[a]	Sample	Linear range, mg L^{-1}	LOD,[b] μg L^{-1}	Ref.
HS-SDME-CE-UV	Headspace $Ni(CN)_4^{2-}$ formation	Urine, saliva	0.0065–0.52	2.08	[7]
HF-HS-LPME-CE-UV	Headspace $Ni(CN)_4^{2-}$ formation	Urine, saliva	0.0026–0.52	0.26	[8]
HS-SDME-CE-UV	Headspace $Ni(CN)_4^{2-}$ formation	Industrial/river waters	0.013–0.52	3.9	[12]
HS-GC–AED	Headspace collection of HCN	Gases	0.05–1000	50	[9]
FI potentiometry	Use of silver chalcogenide ISM	Water	0.156–13	?	[13]
FI-FAAS	On-line reaction with cadmium carbonate	Electroplating waste	0.2–15	200	[14]
FI-FAAS	On-line reaction with silver carbonate	Electroplating waste	0.04–12	40	[16]
FI-fluorimetry	Reaction with o-phthalaldehyde/glycine	Water	0.001–0.2	0.5	[17]
FI spectrophotometry	Complex formation with Cu(II)/DHQH	Human saliva	0–3	4	[23]
Spectrophotometry	Reaction with azo dye–Cu(II) complex	Water	0.15–2	150	[24]
Spectrophotometry	Reaction with acquacyanocobester	Water	0.04–1.2	20	[25]
Spectrophotometry	Nucleophilic reaction with oxazine	Water	0.026–0.26	26	[29]
Spectrophotometry	Reaction with phenolphthalin–Cu(II)	Water	0.01–3	5	[21]
FI spectrophotometry	Reaction with phenolphthalin–CuS	Water	0.6–4.3	100	[22]
Spectrophotometry	Addition of cyanide to imine group	Water	0.05–2	16	27
FI spectrophotometry	MW irradiation; modified Aldridge reaction	Water	0.05–?	18	[20]
Spectrophotometry	Batch reaction with ninhydrin	Water	0.04–0.24	18	[32]
Spectrophotometry	Batch reaction with ninhydrin	Water	0.01–1	?	[33,34]
FI spectrophotometry	Reaction with ninhydrin	Water	0.01–0.04	1.5	[35]
FI spectrophotometry	Reaction with ninhydrin	Water	2–7	160	[36]
GD-SI spectrophotometry	Reaction with ninhydrin	Water	0.0075–0.2	2.5	[39]
HS-SDME-ND	Headspace reaction with ninhydrin	Water, salts	0.025–0.5	4.3	This work

[a] HS-SDME, headspace single-drop microextraction; CE-UV, capillary electrophoresis with UV detection; HF-HS-LPME, hollow-fibre-headspace-liquid phase microextraction; HS-GC-AED, headspace-gas chromatography-atomic emission detection; FI, flow injection; ISM, ion-selective membrane; FAAS, flame atomic absorption spectrometry; DHQH, 2,2′-dipyridyl-2-quinolylhydrozone; MW, microwave; GD-SI, gas diffusion-sequential injection; and ND, NanoDrop® microspectrophotometry.
[b] LOD, limit of detection; and ?, data not cited in the original paper.

to iron(II) and (III) cyanide complexes involving pre-reaction with mercury(II), when the complexes were decomposed to give mercury(II) cyanide, which was then determined. This pre-reaction allowed the determination of total cyanide, and avoided cumbersome distillation of hydrocyanic acid that is usually adopted to determine metal cyanide complexes.

2. Experimental

2.1. Equipment

All spectrophotometric measurements were made with a NanoDrop® ND 1000 full spectrum (220–750 nm) spectrophotometer (NanoDrop® Technologies, Wilmington, DE, USA). It utilized surface tension of liquid sample to hold it in place between the two ends of optical fibres. This eliminated the need for cuvettes or other sample containment devices and allowed clean up by wiping. For absorbance measurement, a 1 μL sample was placed on to the one end of a fibre optic cable (the receiving fibre) and the second fibre optic cable (source fibre) was then brought into contact with liquid sample causing the liquid to bridge the gap between the two ends of optical fibres. The gap was controlled to both 1 mm and 0.1 mm paths, the former was used for absorbance measurement in the present work. The light source was a pulsed xenon flash lamp, and the spectrophotometer utilized a 2048-element linear silicon CCD array detector to analyze the light after passing through the sample. The instrument was controlled by a software run from a PC. The working of the instrument has been described earlier [41,46].

Four-milliliter vials, sealed by screw cap with PTFE-faced silicon septum (Supelco, New Delhi, India), were used for all HS-SDME measurements. A 10 μL Hamilton microsyringe (Supelco, New Delhi, India) with bevel tip was used for SDME. Thermostated magnetic stirrer and heating plate (Kumars Equipments, Mumbai, India) was used.

2.2. Reagents and samples

All aqueous solutions were prepared in HPLC grade water (Millipore-India, Mumbai, India). The stock cyanide solution, 1000 mg L^{-1}, was prepared by dissolving 250 mg of analytical reagent grade potassium cyanide (Fluka, Switzerland) in 100 mL of 0.01 mol L^{-1} potassium hydroxide. Working solutions were made by sequential dilution of the stock solution with 0.01 mol L^{-1} potassium hydroxide. The ninhydrin (E. Merck, Darmstadt, Germany) reagent solution was prepared daily by dissolving 100 mg of ninhydrin in 1 ml of water and mixing with 1 ml of 10% solution of sodium carbonate. Stock solutions, 1000 mg L^{-1}, of mercury(II) cyanide, potassium hexacyanoferrate(II) and (III), and sodium nitrosylpentacyanoferrate(III) were prepared by dissolving their accurately weighed amounts in water, and were diluted to give less concentrated solutions. All reagents and standards were kept refrigerated when not in use. All safety precautions as mentioned in an earlier paper [39] for handling cyanide salts and its waste solutions must be strictly observed.

River water samples (the Ganga river, Kolkata, West Bengal, India; and the Narmada river, Jabalpur, Madhya Pradesh, India), Jabalpur city tap water, and inorganic salt samples were analyzed. The water samples were collected in amber glass bottles with polypropylene screw caps having PTFE septum. Samples were preserved after treatment with pellets of sodium hydroxide to bring about pH 12, stored at 4 °C and analyzed preferably within 24 h of collection.

2.3. Determination of free and acid-labile cyanide

A 50 μL–1 mL aliquot of sample solution containing 0.025–0.5 mg L^{-1} of cyanide was placed in a 4 mL vial and capped. All subsequent additions were made through the septum in the vial cap by microsyringe. An aliquot of 50 μL of 2 mol L^{-1} sulphuric acid was added, and the total volume was made up to 2 mL by adding HPLC water. The reaction mixture was placed on a hot plate pre-heated to 60 °C and stirred at 500 rpm. For the determination of hydrocyanic acid liberated in the headspace, the needle of a 10 μL Hamilton syringe, containing 2 μL of ninhydrin reagent, was penetrated through the septum of the vial until the tip protruded 1 cm above the meniscus of the solution. The plunger was depressed to form a drop of the reagent at the tip which was then kept exposed in the headspace. After 5 min, the drop was

retracted back into the syringe and, in order to homogenize the drop, the plunger was thrice moved gently to and fro, and the drop left undisturbed inside the syringe for 3 min. The drop was finally transferred to the lower pedestal of the NanoDrop® spectrophotometer for absorbance measurement at 530 nm against the reagent blank.

2.4. Determination of cyanide in the presence of interfering metal ions, oxidizing agents, nitrite and sulphite

A 50 μL–1 mL portion of sample solution containing up to 0.5 mg L^{-1} of cyanide was placed in a 4 mL vial and capped. All subsequent additions were made through the septum in the vial cap by microsyringe. The sample solution was treated with 100 μL of masking agent (prepared by mixing 1 mL each of 50 mmol L^{-1} sulphide, 100 mmol L^{-1} sulphamic acid, 100 mmol L^{-1} *N*-ethylmaleimide, and 100 mmol L^{-1} ascorbic acid), swirled for 2 min, and mixed with 100 μL of 2 mol L^{-1} sulphuric acid. Finally, 100 μL of 20 mmol L^{-1} cadmium sulphate was added and the total volume of mixture was made up to 2 mL by adding HPLC water. The reaction mixture was placed on a hot plate pre-heated to 60 °C, and the liberated hydrocyanic acid was determined as before.

2.5. Determination of cyanide in its iron(II) and (III) complexes, or total cyanide

A 1 mL portion of sample solution containing up to 0.5 mg L^{-1} of total cyanide was placed in a 4 mL vial and capped. All subsequent additions were made through the septum in the vial cap by microsyringe. A portion of 25 μL of 20 mmol L^{-1} mercury(II) chloride was added to the mixture, and the contents were heated on a hot plate at 60 °C for 5 min in the case of hexacyanoferrate(II) and nitrosyl-pentacyanoferrate(III), and at 80 °C for 15 min for hexacyanoferrate(III). Heating at 80 °C for 15 min was done for unknown mixtures. The mixture was cooled to 40–50 °C, treated with 100 μL each of 2 mol L^{-1} sulphuric acid and 10 mmol L^{-1} sulphide, swirled for about 1 min, and then treated with 100 μL of 20 mmol L^{-1} cadmium sulphate. The total volume was made up to 2 mL by adding HPLC water and swirled for 2 min. The reaction mixture was placed on a hot plate pre-heated to 60 °C, and the liberated hydrocyanic acid was determined as before.

2.6. Determination of cyanide formed by the oxidation of semicarbazide

A 50 μL–1 mL portion of sample solution containing up to 1.2 mg L^{-1} of semicarbazide was placed in a 4 mL vial and capped. All subsequent additions were made through the septum in the vial cap by microsyringe. Portions of 100 μL of bromate–bromide mixture (prepared by mixing 3 g of potassium bromide and 2 g of potassium bromate in 100 ml of water) and 200 μL of 2 mol L^{-1} sulphuric acid were added. The mixture was swirled for 2 min, treated with 100 μL of 5% ascorbic acid, diluted to 2 mL by adding HPLC water, and shaken well for 1 min. The reaction mixture was placed on a hot plate pre-heated to 60 °C, and the liberated hydrocyanic acid was determined as before.

2.7. Determination of cyanide formed by the oxidation of thiocyanate

A 50 μL–1 mL portion of sample solution containing up to 1.1 mg L^{-1} of thiocyanate was placed in a 4 mL vial and capped. All subsequent additions were made through the septum in the vial cap by microsyringe. Portions of 100 μL of 1% potassium iodate and 200 μL of 2 mol L^{-1} sulphuric acid were added. The mixture was swirled for 2 min, treated with 100 μL of 5% ascorbic acid, diluted to 2 mL by adding HPLC water, and shaken well for 1 min. The reaction mixture was placed on a hot plate pre-heated to 60 °C, and the liberated hydrocyanic acid was determined as before.

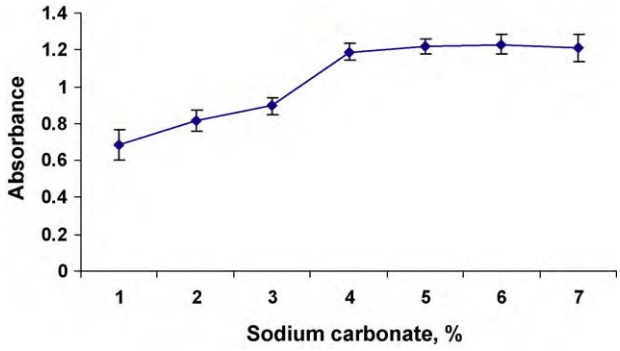

Fig. 1. Effect of concentration of sodium carbonate present in 5% ninhydrin. Reagent drop volume 2 μL, Cyanide 0.5 mg L^{-1}, treated with 50 μL of 2 mol L^{-1} sulphuric acid, heated at 60 °C and stirred at 500 rpm. Equilibration time, 8 min and in-syringe drop equilibration time, 3 min.

3. Results and discussion

3.1. The headspace drop reagent and solvent

For the spectrophotometric detection of cyanide by headspace in-drop reaction, the concentrations of ninhydrin and carbonate in the drop were crucial. To optimize carbonate concentration, the effect of carbonate concentrations in range 1–7% in 5% ninhydrin reagent solution was studied for the analysis of 0.5 mg L^{-1} cyanide. There was an increase in absorbance till 5% of carbonate and thereafter remained practically unaffected (Fig. 1). This effect involved neutralization of collected hydrocyanic acid giving free cyanide which was responsible for colour reaction. The effect of concentration of ninhydrin was studied over the range 1–8% in 5% carbonate solution when an increase in absorbance was observed on increasing the concentration up to 5% and thereafter a sharp decrease in absorbance was observed on further increasing the concentration (Fig. 2). Since cyanide is reported to act as a reducing agent [31], the excess ninhydrin perhaps oxidized the coloured reaction product.

One of the most important criteria of choosing the solvent for headspace SDME while heating is that the solvent drop must not evaporate during the process. Moreover, NanoDrop® spectrophotometer imposes certain restrictions on the solvent that can be used for absorbance measurement. Solvent should be viscous enough so that it does not flow out of the pedestal, and should not be too volatile as to evaporate before measurement is completed. Hence,

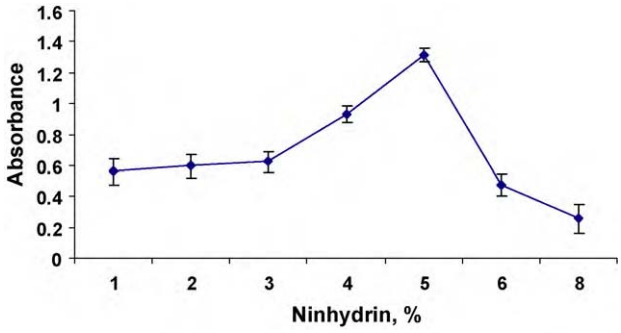

Fig. 2. Effect of concentration of ninhydrin present in 2 μL drop of 5% sodium carbonate. Cyanide 0.5 mg L^{-1}, treated with 50 μL of 2 mol L^{-1} sulphuric acid, heated at 60 °C, and stirred at 500 rpm. Equilibration time, 8 min and in-syringe drop equilibration time, 3 min.

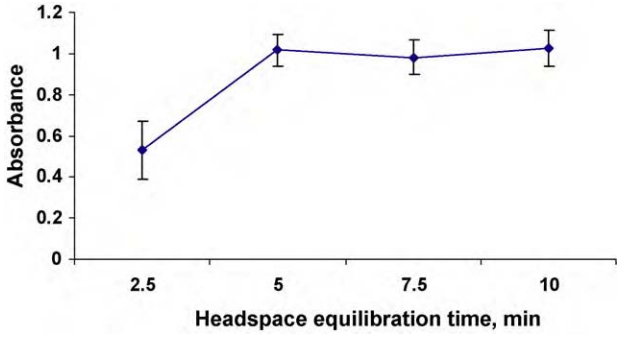

Fig. 3. Effect of headspace equilibration time on the colour intensity of cyanide–ninhydrin reaction product. Cyanide 0.5 mg L^{-1}, treated with 50 μL of 2 mol L^{-1} sulphuric acid, heated at 60 °C, and stirred at 500 rpm. Reagent drop of 2 μL of 5% ninhydrin in 5% sodium carbonate. In-syringe drop equilibration time, 3 min.

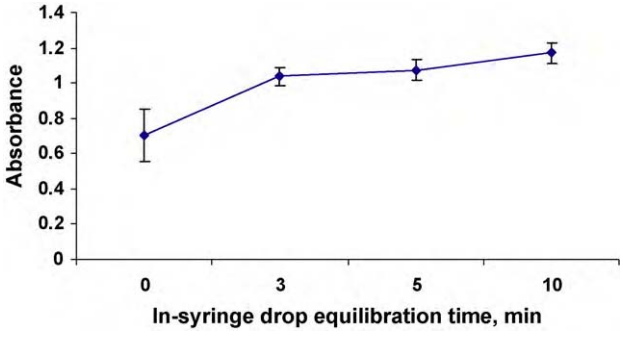

Fig. 4. Effect of in-syringe drop equilibrium time on colour intensity of cyanide–ninhydrin reaction product. Cyanide 0.5 mg L^{-1}, treated with 50 μL of 2 mol L^{-1} sulphuric acid, heated at 60 °C, and stirred at 500 rpm. Reagent drop of 2 μL of 5% ninhydrin in 5% sodium carbonate. Equilibration time, 5 min.

there should be a fair compromise in solvent selection for SDME and cuvetteless spectrophotometry. Water was indeed a good choice in present system as a medium for headspace extraction of cyanide, and for in-drop reaction of cyanide with ninhydrin.

3.2. Optimization of conditions

There were two equilibria in operation involving partitioning of cyanide. First, transfer of hydrocyanic acid from aqueous sample to the headspace and, second, from headspace to aqueous reagent drop. For the favourable position of first equilibrium, acidic medium and high temperature were required to release cyanide into the headspace. It was found 100 μL of 2 mol L^{-1} sulphuric acid was sufficient at higher temperature to release any trace of cyanide into the headspace; 200 μL of sulphuric acid was used when it was also necessary for bromate–bromide or iodate oxidations. In the second equilibrium, the driving force for transfer of hydrocyanic acid from headspace to aqueous reagent was reaction with ninhydrin in carbonate medium giving a red complex within the drop.

While studying the length of equilibration time for the in-drop reaction in the headspace, the absorbance was observed to increase up to 5 min then decreased on further equilibrating. It was traced to the lowering of pH in the drop as more and more hydrocyanic acid was absorbed into the drop. Moving the plunger back and forth after the drop was retracted, the uniform distribution of carbonate re-established the pH in the alkaline range and the colour reappeared. Hence, time required for keeping the drop in headspace as well as in the syringe after retraction was optimized. For the equilibration, the micro-drop was kept exposed in the headspace of sample for 2.5–10 min with a fixed post-equilibration time of 3 min in the syringe. The graph of extraction time versus absorbance of cyanide–ninhydrin complex (Fig. 3) showed that the absorbance levelled off at 5 min, and this time was taken as optimum. For post-equilibration, the retracted drop in the syringe was kept over the period 0–10 min, when a plateau was obtained after 3 min (Fig. 4). Hence, 3 min was optimized for the post-equilibration.

Though, hydrocyanic acid is a volatile gas, it has fair solubility in water. Thus, optimum transfer to the headspace was attempted in acid medium, to suppress ionization, and at high temperatures. For headspace SDME, the effect of sample temperature in range 30–80 °C was investigated. As shown in Fig. 5, the absorbance increased significantly on increasing the temperature and reached a plateau after 60 °C. This effect was due to increase in diffusion coefficient of hydrocyanic acid with temperature. Rapid mass transfer and quick attainment of equilibrium have already been observed in similar systems [48,49]. All subsequent experiments were conducted at 60 °C. The effect of microdrop volume was investigated in the range 1–3 μL. The absorbance increased with increase in the drop volume, but reagent drop bigger than 2 μL was often prone to fall. Since it was necessary to readjust the pH of the reagent drop after colour development, 2 μL drop of reagent was used. Equilibrium between the aqueous and vapour phases can be achieved more rapidly by stirring the aqueous sample. For studying this effect, the aqueous reaction mixture was continuously agitated at 60 °C with stirring rates in range 200–900 rpm. About 20% increase in absorbance was recorded on stirring the mixture at 500 rpm and higher, compared to no stirring.

3.3. Method validation

3.3.1. Calibration range and sensitivity

A rectilinear calibration graph was obtained for 0.025–0.5 mg L^{-1} of cyanide, the correlation coefficient and limit of detection (LOD) were respectively 0.9987 and 4.3 μg L^{-1}. The LOD attained was comparable to methods that are available for most sensitive determination of cyanide (Table 1). The within-day precision of the headspace ninhydrin method was estimated by repeated analyses of cyanide standards. The RSD in six replicate analyses of 0.025, 0.15 and 0.5 mg l^{-1} of cyanide by the present method was 5%, 1.75% and 2.3%, respectively, giving a mean RSD of 3.0% over the calibration range.

The NanoDrop® microspectrophotometer uses a 1 mm path length for absorbance measurement, contrasting 10 mm cuvettes in conventional spectrophotometry, and is liable to yield less sensitivity. However, this limitation was overcome in the present method by headspace pre-concentration of liberated hydrocyanic acid in the reagent drop. A known amount of cyanide (final volume 2 mL) was determined by the reported batch method using ninhydrin [34]

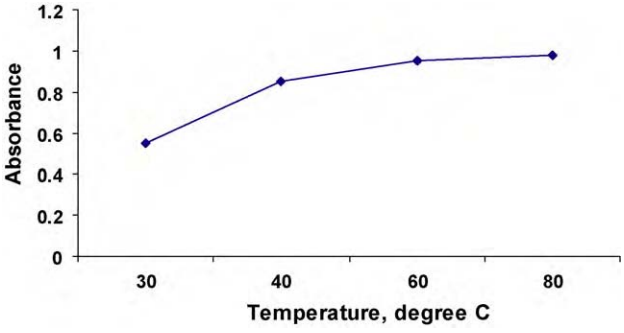

Fig. 5. Effect of temperature on the colour intensity of cyanide–ninhydrin reaction product. Cyanide 0.5 mg L^{-1}, treated with 50 μL of 2 mol L^{-1} sulphuric acid, heated at 30–80 °C, and stirred at 500 rpm. Reagent drop of 2 μL of 5% ninhydrin in 5% sodium carbonate. Equilibration time, 5 min and in-syringe drop equilibration time, 3 min.

and by the present headspace method, the absorbance being measured in both cases by NanoDrop® spectrophotometer. The ratio of absorbances obtained in headspace method to batch method, which was found to be 80, was taken as pre-concentration factor, and it was responsible for high value of molar absorptivity found, 2.7×10^6 L mol^{-1} cm^{-1}.

3.3.2. Selectivity

Sulphide and thiocyanate are often found along with cyanide in samples of diverse origin, and vitiate the results in the determination of cyanide by various methods. Many other anions and cations also interfere principally owing to the medium and the chemistry used for detection [25]. Formation of lead sulphide and distillation of hydrocyanic acid are widely used technique [18], however, the method is cumbersome, and thiocyanate could decompose to cyanide during distillation [20]. Pervaporation [50] and gas diffusion [39] are effective means of separation of interferences, and have been recommended as alternative to distillation method. Headspace analysis of hydrocyanic acid, as used in this work, is further simplification of method to circumvent interferences.

In order to investigate the selectivity of the proposed method, the effect of diverse anions that are usually present with cyanide was studied. The interferences were examined on 0.1 mg L^{-1} cyanide spiked with known amounts of each interferences. Involatile substances such as thiocyanate, hydroxylammonium chloride, hydrazinium sulphate, ascorbic acid and ammonium metavanadate, which all interfere in batch ninhydrin methods [32–34], could be tolerated in the present method up to 1000-fold (w/w) excess to cyanide. Though sulphide was reported not to interfere in ninhydrin methods [32–35], high results were obtained in the present method since on acidification hydrogen sulphide was also released into the headspace and it also produced a similar colour with ninhydrin. The interference of sulphide was avoided by adding cadmium sulphate. Another divergence with reported ninhydrin methods [32–34] was their tolerance to sulphite and nitrite. In the present method both sulphite and nitrite yielded low results when present more than the double amount of cyanide. This was found due to decolourization of cyanide–ninhydrin product by sulphur dioxide and nitric oxide. In the present method sulphite (up to 300-fold excess) was masked by its addition reaction to the double bond of *N*-ethylmaleimide, and nitrite (up to 200-fold excess) was decomposed by sulphamic acid. Since ascorbic acid does not

Table 2
Determination of free cyanide in real samples.

Sample	CN^- found,[a] L^{-1} kg^{-1}	RSD, %	CN^- spiked, L^{-1} kg^{-1}	CN^- recovery,[b] %	RSD, %
Ganga river water	<LOD[c]		300 μg	105	2.8
			50 μg	95	3.5
			50 μg[d]	98	4.5
Narmada river water	<LOD		150 μg	97	3.3
			50 μg[e]	98	2.3
City Tap water	<LOD		200 μg	99	1.5
			50 μg[e]	93	3.8
			50 μg[f]	101	3.2
City Lake water	<LOD		150 μg	94	2.1
			50 μg[g]	95	2.3
Potassium thiocyanate #1	31 μg	3.1	100 μg	102	3.1
			50 μg[h]	97	4.4
Potassium thiocyanate #2	42 μg	2.4	100 μg	105	3.0
			50 μg[f]	96	4.8
Potassium thiocyanate #3	47 μg	2.5	100 μg	95	4.1
			50 μg[d]	104	3.2
$K_4[Fe(CN)_6]$[i] #1	800 mg	4.2	150 mg	108	5.2
			55 mg	98	3.5
$K_4[Fe(CN)_6]$ #2	55 mg	3.5	50 mg	96	3.9
			25 mg	93	4.7
$K_3[Fe(CN)_6]$[j]	71 mg	2.8	50 mg	104	3.3
			25 mg	98	4.5
$Na_2[FeNO(CN)_5]$[k] #1	600 mg	3.9	200 mg	95	6.3
			50 mg	97	5.9
$Na_2[FeNO(CN)_5]$ #2	32 mg	2.7	50 mg	101	5.2
			25 mg	96	6.8

[a] Results are the averages of five determinations.
[b] Results are the averages of five determinations. The recovery has taken into account free cyanide already present in the sample.
[c] <LOD, below the limit of detection.
[d] Sample mixed with sulphide, 200 μg L^{-1} (masked with cadmium sulphate).
[e] Sample mixed with nitrite, 50 μg L^{-1} (masked with sulphamic acid).
[f] Sample mixed with sulphite, 150 μg L^{-1} (masked with *N*-ethylmaleimide).
[g] Sample mixed with thiocyanate, 500 μg L^{-1}.
[h] Sample mixed with sulphite, 400 μg L^{-1} (masked with *N*-ethylmaleimide); and sulphide, 200 μg L^{-1} (masked with cadmium sulphate).
[i] $K_4[Fe(CN)_6]$, Potassium hexacyanoferrate(II).
[j] $K_3[Fe(CN)_6]$, Potassium hexacyanoferrate(III).
[k] $Na_2[FeNO(CN)_5]$, Sodium nitrosyl-pentacyanoferrate(III).

Table 3
Determination of cyanide in metal complexes and as product of redox reactions.

Sample	Taken, μg L^{-1}	CN^- equivalent, μg L^{-1}	CN^- recovery[a], %	RSD, %
Hexacyanoferrate(II)	300	220.7	95	4.1
	150	110.4	98	3.6
	50	36.8	102	5.1
Hexacyanoferrate(III)	200	147.2	87	8.6
	100	73.6	93	5.8
	50	36.8	90	6.2
Nitrosyl-pentacyanoferrate(III)	300	180.5	105	5.0
	200	120.4	96	4.7
	100	60.2	92	3.8
Thiocyanate	500	224.1	94	3.2
	300	134.5	101	2.8
	100	44.8	96	2.4
Semicarbazide	300	104.0	93	2.6
	200	69.3	96	3.0
	150	52.0	104	2.8

[a] Results are the averages of five determinations. The recovery has taken into account any free cyanide already present in the sample.

interfere in the present method, the reported interference of oxidizing agents, e.g., chlorine [34,39], was avoided by the addition of this reducing agent. In the working method, addition of a single masking reagent consisting of sulphide, *N*-ethylmaleimide, sulphamic acid and ascorbic acid (thereafter addition of cadmium sulphate to mask surplus sulphide) has been recommended to avoid interferences in unknown samples.

The interference of nickel(II), cadmium(II), iron(II)/(III) in earlier reported batch and flow methods [32–36] was due to the basic medium of reaction where metal cyanide complexes were formed. These metal ions and cobalt(II), lead(II), calcium(II), magnesium(II) and zinc(II) did not affect the results in present method since the medium was acidic and their cyanide complexes were acid dissociable. Calcium(II), magnesium(II), etc. are likely to precipitate as their carbonates in basic medium but this aspect was not reported in any of batch methods. Silver(I), copper(II) and mercury(II) have been reported to interfere due to their reaction with ninhydrin [38]. In the present work, where the reagent drop was placed in the headspace of sample, the interference was found due to the formation of metal–cyanide complexes. The strategy followed in this work was to add sulphide to form more stable Ag_2S, CuS and HgS [51], thereby liberating cyanide free. The residual sulphide from reaction was masked by adding excess of cadmium sulphate. Under these conditions as large as 500-fold excess of these metal ions could be tolerated in the determination of cyanide.

3.3.3. Applications

Iron(II) and (III) complexes with cyanide, viz., hexacyanoferrate(II) and (III), and nitrosyl-pentacyanoferrate(III) did not response to cyanide reaction in the present method. This was due to high stability of complexes. Since it was possible to determine cyanide in mercury(II) cyanide by the present method involving a pre-reaction with sulphide/cadmium(II), and further because mercury(II) showed ligand exchange with iron–cyanide complexes [52], it was possible to determine cyanide in its iron complexes by reaction with excess of mercury(II) chloride, when mercury(II) cyanide was formed, and determining the latter. Heating for 5 min at 60 °C was enough to complete the reaction of mercury(II) with hexacyanoferrate(II) and nitrosyl-pentacyanoferrate(III), but hexacyanoferrate(III) required 15 min heating at 80 °C; all six, five and six cyanides, respectively, in complexes responded to the test. Thus, the present method could be used to determine free and weak acid-dissociable cyanide, and total cyanide.

The following two redox reactions of semicarbazide with bromine [53], and of thiocyanate with iodate 52[63] were used to determine cyanide formed in respective reactions.

$$NH_2CONHNH_2 + Br_2 \rightarrow HCN + N_2 + 2HBr + H_2O$$

$$5CNS^- + 6IO_3{}^- + 6H^+ + 2H_2O \rightarrow 5HCN + 5HSO_4{}^- + 3I_2$$

Bromine and iodine were reduced with ascorbic acid before the determination of cyanide. Each mol of semicarbazide and thiocyanate produced 1 mol of cyanide, as was confirmed by using the present method. Integrating the present method (when only cyanide is obtained) with oxidation with iodate (when sum of cyanide and thiocyanate is obtained; thiocyanate being obtained by difference), it should be possible to determine mixtures of cyanide and thiocyanate, but no such attempt was made in this work.

The present method was applied to real samples of river waters, lake water and tap water, inorganic compounds for the determination of free cyanide. (Table 2). Standard addition method was used to validate the method. The samples were also spiked with known interferences and free cyanide was determined after masking of interfering materials. In the analysis of spiked samples, an average recovery of 98% (range 93–108%) was obtained with the average RSD of 3.9% (range 1.5–6.8%). Iron(II) and (III) complexes of cyanide were reacted with mercury(II) chloride for ligand transfer, and cyanide was released by reaction with sulphide/cadmium(II) sulphate (Table 3). The average recovery of cyanide in iron complexes was 95% (range 92–105%) with average RSD of 5.2% (range 3.6–8.6%). Thiocyanate and semicarbazide were reacted with iodate and bromine, respectively, to form cyanide which was then determined by the present method (Table 3). The average recovery of cyanide formed as a result of redox reactions was 97% (range 93–104%) with average RSD of 2.8% (range 2.4–3.2%). Thus, the overall recovery of cyanide in diverse samples was 97% with an RSD of 3.9%.

4. Conclusions

The proposed method for the determination of cyanide is simple, sensitive, rapid, eco-friendly and cost effective. The inherent drawbacks of low sensitivity of NanoDrop® spectrophotometry using cuvetteless 1 mm sample path length has been avoided by headspace SDME leading to the 80-fold enrichment of hydrocyanic acid. The method requires low reagent consumption, produces

minimal waste, and is faster (8 min) than previously reported methods which require 15 min [34] and 30 min [32] for reaction with ninhydrin. Comparison of features of merit suggests that the proposed method has better or comparable sensitivity to previously published methods. The methods avoids the use of distillation or gas diffusion and attains selectivity against commonly encountered interferences of involatile substances in cyanide determination by employing reaction in the headspace of the sample. Using a single masking agent, volatile interfering substances can be tolerated in large amounts. These auxiliary reaction chemistries have also permitted to determine cyanide in its stable iron(II)/(III) complexes. The present work is a further example of sample cleanup and simplification of method using headspace SDME and NanoDrop® spectrophotometry as demonstrated previously in the analysis of trimethylamine in fish [44].

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