On-line gas-diffusion separation and fluorimetric detection for the determination of acid dissociable cyanide

Esther Miralles, Dolors Prat*, Ramon Companó and Mercè Granados

Departament de Química Analítica, Universitat de Barcelona, Diagonal 647, E-08028 Barcelona, Spain

A flow injection system with gas diffusion separation and spectrofluorimetric detection is described for the determination of acid dissociable cyanide in waters. Cyanide diffuses through a microporous PTFE membrane from an acidic donor stream into a sodium hydroxide acceptor stream. The cyanide transferred reacts with *o*-phthalaldehyde and glycine to form a highly fluorescent isoindole derivative. Complete recovery of cyanide was found for $Zn(CN)_4{}^{2-}$, $Cu(CN)_3{}^{2-}$, $Cd(CN)_4{}^{2-}$, $Hg(CN)_4{}^{2-}$, $Hg(CN)_2$ and $Ag(CN)_2{}^-$ complexes and low recovery from $Ni(CN)_4{}^{2-}$. No recovery was obtained from the species that are considered as non-free cyanide producing, *viz.*, $Fe(CN)_6{}^{4-}$, $Fe(CN)_6{}^{3-}$ and $Co(CN)_6{}^{3-}$. The sampling frequency was $10\ h^{-1}$ and the detection limit was 0.5 $\mu g\ l^{-1}$. The method tolerates a 40-fold excess of sulfide. The results of cyanide determination in water samples obtained with the proposed method are consistent with those obtained with APHA Method 4500 CN^- I for weak acid dissociable cyanide.

Keywords: Cyanide; flow injection analysis; gas diffusion separation; fluorimetry; water

Cyanide species in the environment originate mainly from industrial wastes. Their high toxicity makes it necessary to develop or improve methods for their determination at very low concentrations. Normally, cyanide testing requirements call for the determination of total cyanide, which includes free cyanide (CN^- and HCN) and metal–cyanide complexes. The US Environmental Protection Agency (EPA) has recently accepted that a distinction should be made between labile cyanides, which are readily bioavailable and extremely toxic, and total cyanides, which include those inert species of low toxicity that do not dissociate to cyanide ions.[1] For this reason the procedure for the measurement of weak acid dissociable (WAD) cyanide is gaining acceptance. This method measures the cyanide released under weakly acidic conditions, which includes free cyanide and relatively unstable cyanide complexes such as those with Zn, Cd and Cu.

Flow injection analysis (FIA) methods with gas diffusion separation have been shown to be efficient in liberating and totally recovering labile cyanide.[1–3] Gas diffusion separations use hydrophobic microporous membranes through which the gas molecules diffuse.[4] The hydrophobicity of the polymer [PTFE or poly(propylene)] prevents the filling of the pores by the reactor liquid phase. The advantage of the membrane is that all interferences related to ionic species are removed because only gaseous molecules diffuse. Hydrogen cyanide (HCN) is thus transferred across the membrane from an acidic donor solution into an alkaline receptor channel, where it is converted to CN^- and determined spectrophotometrically[2,5,6] or amperometrically.[6,7] The method, however, does not separate cyanide from sulfide since H_2S also diffuses across the membrane and must be previously precipitated with Pb^{II}[8,9] or separated by ion

chromatography (IC)[7] if it interferes with the detection system. The additional use of ligand exchange reagents to improve the recoveries of cyanide species of medium stability has also been described in the literature. Dithizone and tetraethylenepentamine (TEP) were added to the sample to recover Hg and Ni cyanide species, respectively,[3] and 1,10-phenanthroline was added to the acidic solution to release cyanide from the metal–cyanide complexes.[2]

Recently, we reported the use of *o*-phthalaldehyde (OPA) as a sensitive fluorogenic reagent for the detection of cyanide in flow systems.[10] The aim of this paper was to apply this fluorimetric detection to determine acid dissociable cyanide in industrial waste water and river water by means of a flow injection system with gas diffusion separation. The method developed is rapid, sensitive, selective, reproducible and easy to automate and can be used as a simple routine method for acid dissociable cyanide monitoring.

Experimental

Apparatus

The flow injection manifold consisted of a Minipuls 3 peristaltic pump (Gilson, Villiers le Bel, France), a Model 5041 injection valve (Rheodyne, Cotati, CA, USA) with a sample loop volume of 170 µl, and a laboratory-made gas diffusion module. Reaction and mixing coils were made from PTFE tubing of 0.5 mm id, and Tygon tubes were used for pumping the solutions.

The gas diffusion unit consisted of two methacrylate blocks, each with a straight groove of 7.8 cm length, 2 mm width and 0.25 mm depth, between which the microporous Teflon membranes were placed. The two blocks were pressed together by six screws.

The on-line detection was carried out with an LS-50 fluorescence spectrometer (Perkin-Elmer, Beaconsfield, Bucks., UK), equipped with a xenon lamp and a Model 176.752 flow cell (25 µl inner volume) (Hellma, Müllheim, Germany), operated at excitation and emission wavelengths of 331 and 379 nm, respectively.

A DR. LANGE LP2W (Neurteck Medioambiente, Zarautz, Spain) spectrophotometer was used for the determination of cyanide by the standard method.

Reagents and solutions

All chemicals were of analytical-reagent grade unless stated otherwise.

Solutions of sodium cyanide (Carlo Erba, Milan, Italy) were prepared at 1000 mg l^{-1} as CN^- in 0.1 M sodium hydroxide. $K_3Fe(CN)_6$ (Merck, Darmstadt, Germany), $K_4Fe(CN)_6\cdot3H_2O$ (Merck), $K_2Ni(CN)_4$ (Aldrich, Milwaukee, WI, USA), $K_3Co(CN)_6$ (Aldrich), $KAg(CN)_2$ (Johnson Matthey Chemicals, Karlsruhe, Germany) and $Hg(CN)_2$ (Merck) were directly weighed and dissolved in 0.01 M NaOH. Solutions of $Zn(CN)_4{}^{2-}$, $Hg(CN)_4{}^{2-}$ and $Cd(CN)_4{}^{2-}$ were prepared by

adding stoichiometric amounts of $Zn(CN)_2$ (Aldrich), $Hg(CN)_2$ (Merck) and $Cd(NO_3)_2$ (Merck, Standard Solution) to an NaCN solution. The cyanide species CuCN (Johnson Matthey Chemicals) was dissolved in a known excess of NaCN in 0.01 M NaOH to form the $Cu(CN)_3^{2-}$ species. All solutions were stored under refrigeration at 4 °C in amber-coloured glass bottles. Working solutions were prepared daily by dilution of the stock solutions in 10^{-3} M sodium hydroxide.

A stock 4×10^{-2} M solution of OPA (Fluka, Buchs, Switzerland) was prepared in ethanol and diluted with borate buffer of pH 8.2 to give a 2×10^{-3} M solution. Glycine (Merck) was used as a 2×10^{-3} M solution in the same buffer prepared by dilution of a stock 2×10^{-2} M aqueous solution. Working solutions were prepared daily. Chloramine-T (Merck), 1% m/v in water, was prepared weekly. Barbituric acid–pyridine solution was prepared by dissolving 15 g of barbituric acid (Merck), 75 ml of pyridine (Carlo Erba) and 15 ml of hydrochloric acid in 250 ml of water. Both solutions were stored under refrigeration.

TEP (Fluka) was prepared as a 0.1% v/v solution in water.

Hydrochloric acid (1 M) and 10^{-2} M sodium hydroxide were used as donor and acceptor solutions, respectively, in the diffusion unit. Zinc acetate, sodium acetate and acetic acid were used in the distillation step of the standard method.

Ultrapure water, MilliQ-plus (Millipore, Molsheim, France), of resistivity 18.2 MΩ cm^{-1} was used throughout.

All glassware used for the experiments was previously soaked in 10% v/v HNO_3 for 24 h and rinsed in ultrapure water.

Procedures

Sample treatment

Water samples were collected in 2.5 l glass bottles, preserved with sodium hydroxide (pH = 11–11.5), filtered if necessary and stored at 4 °C in the dark until analysis.

FIA method

The FIA system was set up as shown in Fig. 1(b). The sample was continuously introduced into the system at a flow rate of 0.85 ml min^{-1}. After acidification by merging with a flow of
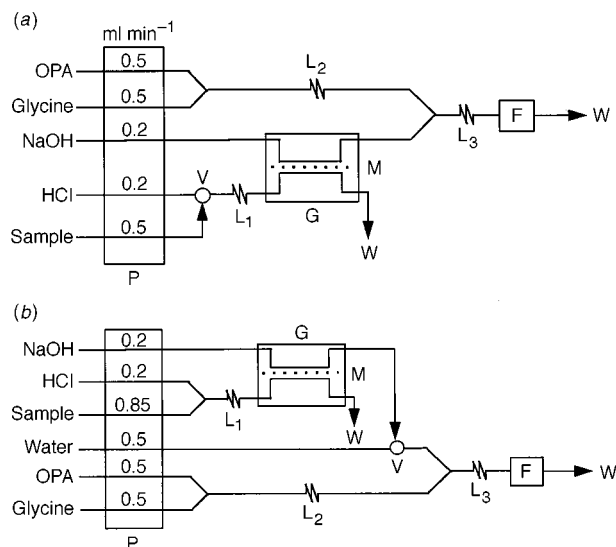
Fig. 1 Diagrams of the flow injection systems. P, Peristaltic pump; G, gas diffusion unit; M, membrane; V, injection valve; L$_1$, mixing coil (a) 60 cm $\times$ 0.5 mm id, (b) 5 m $\times$ 0.5 mm id; L$_2$, reaction coil (2 m $\times$ 0.5 mm id); L$_3$, reaction coil (5 m $\times$ 0.5 mm id); F, fluorimeter; and W, waste.

1 M hydrochloric acid at 0.2 ml min^{-1}, the HCN liberated diffused through the Teflon membrane of the separator and was absorbed by an acceptor solution of 10^{-2} M sodium hydroxide at 0.2 ml min^{-1}, which was continuously filling the injection loop. The diffused cyanide was then injected into an aqueous carrier stream and determined using the OPA–glycine fluorimetric method.[10]

Method 4500 CN^- I

The sample (500 ml) was refluxed for 1 h in a macrocyanide distillation apparatus at pH 4.5, adjusted with acetate buffer solution, and in the presence of zinc acetate as described in the standard method.[8] The distillate was collected into NaOH and analysed colorimetrically with the chloramine-T–pyridine–barbituric acid reagent at 578 nm in a 1 cm cell.

Results and discussion

Optimisation of the experimental parameters affecting cyanide diffusion

Several gas diffusion FIA manifolds have been proposed in the literature in order to separate cyanide from the matrix and interferences. In the conventional FIA manifolds a volume of sample is injected into the donor stream; it diffuses through the membrane and is then collected in a flowing acceptor stream [Fig. 1(a)].[6,11] Other manifolds improve the diffusion efficiency by means of an enrichment step.[2,5] This can be achieved by continuously pumping the sample into the system and either working with a large sample : acceptor flow rate ratio using the manifold shown in Fig. 1(b), or using a stationary acceptor solution in the groove of the diffusion module, which also serves as the injection loop. In the latter, the sample is preconcentrated into a small volume of the stationary acceptor solution.

Some preliminary experiments were performed with two different manifolds (Fig. 1). Working with similar hydrodynamic conditions in both systems, the peaks obtained with manifold 1(b) were six times higher than those obtained with manifold 1(a). In the conventional FIA manifold [Fig. 1(a)], the results showed a marked loss of sensitivity because of low efficiency in the diffusion of HCN through the membrane. These results led us to select and optimise the manifold shown in Fig. 1(b) where, in spite of the low efficiency of diffusion, an enrichment occurs in the slow-flowing acceptor channel.

Two commercial hydrophobic microporous PTFE membranes were tested: Millipore FGLP (0.2 μm pore diameter, 175 μm thickness, porosity 80%) and Teflon tape (average pore size 10 μm, 110 μm thickness). Data on the Millipore FGLP membrane were provided by the supplier, whereas those on the Teflon tape were estimated from observations by electron microscopy. Although the porosity of the Teflon tape was not determined, a comparison of the electron microscopy images of both membranes revealed that the porosity of the Teflon tape was much lower than that of the Millipore FGLP membrane.

Both membranes were tested in cross-current and normal current modes, but no significant differences were observed between the two operational modes, and further experiments were performed with both streams flowing in the same direction.

Since the acceptor channel was flowing continuously, it was expected that the lower the flow rate, the greater would be the preconcentration factor of the HCN in the acceptor chamber. Fig. 2(a) shows the effect of flow rate on the amount of HCN diffusing across the membrane. A flow rate of 0.2 ml min^{-1} for the acceptor stream was chosen since lower flow rates would make the analysis time too long. The effect of the flow rate of the hydrochloric acid channel was also investigated and it was

observed that an increase in the flow rate caused a slight decrease in the peak height because the contact time of the sample solution with the acceptor chamber was shorter. A flow rate of 0.2 ml min^{-1} in the acid channel was selected.

The effect of the flow rate of the sample stream was investigated. It can be observed from Fig. 2(*b*) that the higher the sampling flow rate the higher the peak height obtained, but no significant differences were found at flow rates > 1.5 ml min^{-1}. A flow rate of 0.85 ml min^{-1} was chosen because of the availability of pumping tubes and to minimise sample waste.

When the length of mixing coil L$_1$ was varied no significant differences in the peak height were observed if free cyanide solutions were analysed. However, a 5 m × 0.5 mm id coil was selected because if the cyanide is complexed it is expected to require a longer reaction time to be released from the complex.

The ratio of the signal of a sample injected into the carrier stream without diffusion separation to that of a sample passing through the diffusion cell was about 1 for the Millipore FGLP membrane and 0.65 for the Teflon tape membrane. Therefore, there was no significant loss of sensitivity with respect to a system without diffusion.

The efficiency of the diffusion of HCN across the membrane was estimated from the volume of sample pumped (during the time necessary to fill the injection loop), the volume of the injection loop, the signal obtained with the diffusion step and the signal from direct injection into the carrier stream. The transfer of HCN across the FGLP membrane was about 23% and across the Teflon tape about 14% at a 0.85 ml min^{-1} sample loading flow rate and a 0.2 ml min^{-1} flow rate in the donor and acceptor streams. The efficiency in the transport of HCN through the membrane was relatively low but it does not differ significantly from values reported in the literature.[4] Although the FGLP membrane provided a slightly higher diffusion efficiency than the Teflon tape, the latter was selected for further experiments since it is easier to work with. Because of the thickness of the FGLP membrane it was necessary to tighten the two blocks of the diffusion module considerably to avoid leakage and this might easily cause fissures in the membrane.

The reproducibility of the membrane behaviour was monitored for ten consecutive days. A standard of 50 µg l^{-1} cyanide was injected daily into the diffusion manifold and the signal was compared with that obtained from the injection of the same standard without the diffusion separation. The ratio was 0.62 ± 0.07 . After working with the same membrane for more than 1 month, no memory effects or loss of efficiency were observed. Nevertheless, it is advisable to monitor the efficiency weekly. No significant variations were found when replacing the membrane, which shows that the Teflon tape is fairly homogeneous.

Characteristics of the method

The calibration graph was linear up to the maximum concentration tested, 200 µg l^{-1} (I_f = 0.075 + 0.073 C_{CN^-}, r = 0.9996). The detection limit was 0.5 µg l^{-1} cyanide, calculated as the concentration corresponding to three times the standard deviation of six repeated injections of a blank solution. The relative standard deviation obtained from ten successive injections of a 50 µg l^{-1} cyanide solution was 2.2%.

The gas diffusion technique separates cyanide from most ionic species in the sample but sulfide also diffuses through the membrane and reacts with OPA and glycine to form a fluorescent isoindole derivative. In order to study its interference, solutions of 10, 50 and 100 µg l^{-1} cyanide were spiked with sodium sulfide. Sulfide gave no interference up to about a 40-fold excess.

Carbonate was also studied as a potential interferent because it generates CO_2. Carbonate did not interfere up to about 400 mg l^{-1} when it was added to a solution containing 50 µg l^{-1} cyanide. Above this value, the signal decreased, probably because the large amount of CO_2 produced hinders the diffusion of HCN through the membrane. Nevertheless, the typical levels of carbonates in industrial effluent samples or river water are not expected to cause any problems.

Taking into account that about 5 min were necessary for the system to reach a steady state, the throughput of the method was estimated to be 10 samples h^{-1}.

Dissociation of metal–cyanide complexes

Solutions of different metal–cyanide complexes at 50 µg l^{-1} of cyanide were prepared and analysed by the proposed FIA method. The results (Table 1) show that, with 1 M HCl as donor solution, complete recovery of cyanide was obtained from the labile or relatively unstable complexes of Cu^{II}, Zn^{II}, Cd^{II}, Hg^{II} and Ag^I low recovery from the Ni complex and no recovery from the more inert Fe and Co cyanide complexes.
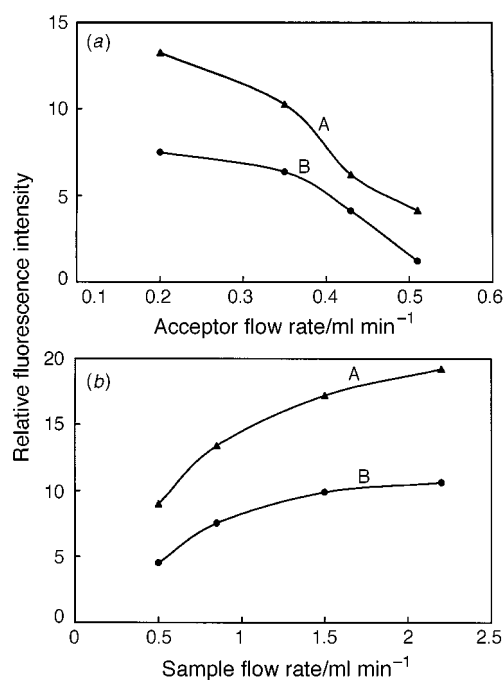
Fig. 2 (*a*) Influence of acceptor flow rate on peak height. (*b*) Influence of sample flow rate on peak height. A, FGLP membrane and B, Teflon tape. Conditions: 100 µg l^{-1} CN$^-$, 2 × 10^{-3} M OPA, 2 × 10^{-3} M glycine, 10^{-2} M NaOH, 1 M HCl.

Table 1 Recovery of cyanide species at different acidifying concentrations

Species (50 µg l^{-1} as CN$^-$)	Recovery (%)	
	0.1 M HCl	1 M HCl
Cu(CN)$_3{}^{2-}$	96	101
Ni(CN)$_4{}^{2-}$	10	20
Zn(CN)$_4{}^{2-}$	99	98
Cd(CN)$_4{}^{2-}$	98	97
Hg(CN)$_2$	60	99
Hg(CN)$_4{}^{2-}$	83	102
Ag(CN)$_2{}^-$	101	100
Fe(CN)$_6{}^{4-}$	0	0
Fe(CN)$_6{}^{3-}$	0	0
Co(CN)$_6{}^{3-}$	0	0

Hence, the FIA method for acid dissociable cyanide excludes the amount of cyanide that is complexed with Ni, with respect to methods involving distillation, but includes complete recovery of cyanide from Hg complexes.[1,8] Table 1 also shows that 0.1 M HCl as donor solution completely released cyanide from Cu^I Zn^{II}, Cd^{II} and Ag^I complexes but not from Hg^{II} complexes. The recovery from the Ni^{II} complex was also lower than that obtained with 1 M HCl, indicating that Hg^{II} and Ni^{II} complexes have medium stability. Hence, the measurement of acid dissociable cyanide is system-dependent. These results are consistent with recoveries reported in the literature using gas diffusion separation with other detection systems.[3,6]

Cyanide can be effectively displaced from the nickel–cyanide complex if TEP is added to the sample before the analysis.[3] Therefore, the use of TEP was also investigated. The recovery of nickel cyanide increased from 20 to 95% if 100 μl of 0.1% v/v TEP were added to a 50 ml sample of $Ni(CN)_4{}^{2-}$ containing 50 and 100 μg l^{-1} of CN^-.

Accuracy

The proposed method was tested by analysing industrial water samples (S1–S3) and comparing the results with those given by the WAD 4500 CN^- I standard method.[8] Samples S1 and S2 came from plating baths and were expected to contain free cyanide plus Cd, Cu and Zn complexes, as well as low concentrations of Ni and Fe cyanide complexes, which originate from contact of the cyanide solutions with the surfaces being coated, but in relatively small proportion with respect to labile species. As these samples contained large amounts of cyanide, they were diluted before analysis by the FIA method. To simulate a polluted river water, sample S3 was prepared by dilution of sample S1 with cyanide-free river water. The results (Table 2) show that the difference between the two methods is not greater than 10%. Thus, the proposed FIA method rapidly provides a value of acid dissociable cyanide at room temperature which is related to the most toxic species, avoiding the tedious (1 h) distillation of the standard method.

The accuracy was also evaluated from recovery studies of spiked cyanide-free natural river water samples. Samples S4

and S5 were spiked with known amounts of NaCN and S6–S8 were spiked with mixtures of labile metal–cyanide complexes at different proportions. The results, given in Table 2, show that the recoveries are usually above 90%. Although the results are satisfactory, recoveries were slightly low, which could be related to sample pre-treatment. Addition of sodium hydroxide to preserve the samples causes precipitation of metal hydroxides which may adsorb some cyanide and hence there may be losses in the filtration step.

Conclusions

A combined gas diffusion–fluorimetric detection method for the determination of labile cyanide species in water samples has been developed. The method gives results in a few minutes whereas the time for a cyanide determination using the standard distillation procedure is at least 2 h. The high sensitivity of the fluorimetric detection and the preconcentration step involved in the gas diffusion unit allow a detection limit of 0.5 μg l^{-1} to be achieved. Other advantages of the fluorimetric detection include less hazardous reagents and less interference from sulfide with respect to the standard method. Although the method has been tested for the analysis of individual samples, it can be applied to near real-time monitoring and continuous surveillance, with the additional advantage of lower errors due to transport and sample storage.

Table 2 Comparison of WAD cyanide methods and recoveries from spiked real samples

Sample	Method 4500 CN^{-1} I	FIA method* Added/μg l^{-1}	FIA method* Found/μg l^{-1}
S1	4.16 mg l^{-1}	—	3.90 ± 0.07 mg l^{-1}
S2	0.62 mg l^{-1}	—	0.56 ± 0.03 mg l^{-1}
S3	81 μg l^{-1}	—	73 ± 4
	43 μg l^{-1}	—	34 ± 2
S4	—	10	8.7 ± 0.4
S5	—	50	45.5 ± 2
S6	—	10	8.8 ± 0.8
S7	—	50	46 ± 3
S8	—	100	96.3 ± 2.1

* S1–S3: mean of three determinations; S4–S8: mean of three separate spiked samples.

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