Indirect fluorescence detection of free cyanide and related compounds by capillary electrophoresis

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Abstract

A capillary electrophoretic method to detect CN^- and some related compounds (CNO^-, SCN^-, and NO_3^-) using a prototype laser-based indirect fluorescence detector has been developed. This method avoids interferences from Cl^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-, and ClO^- and allows the detection of CN^- in less than 4 min and the detection of related compounds in less than 8 min with precise migration times (R.S.D. about 1%). The measured detection limits of CN^- [5×10^{-6} M with DR = 90 (dynamic reserve)] are close to the expected theoretical value [$TR_{CN} = 0.44$ (transfer ratio)]. For the related compounds, the measured detection limits ranged from 10^{-5} to 2×10^{-6} M, depending on noise conditions (DR ⩾ 300). The results showed that the chosen analytical conditions were appropriate and the detection limits could be improved using techniques to further decrease the noise (probably due to mechanical vibrations). Peak-height precision was about 10% R.S.D. and log–log regressions had slopes lower than one. These results seem to be due to instabilities in the indirect signal. The analysis of a quasi-real sample has been achieved, allowing identification of the related compounds present in a CN^-/SCN^- sample oxidized with ClO^- and determination of the CNO^- formed in this alkaline oxidation.

1. Introduction

Free cyanide (CN^- and HCN) is a very toxic contaminant with biocide effects that have to be controlled before reaching the environment. The presence of cyanide in natural waters is mainly due to the uncontrolled discharge of treated wastes from certain industrial processes (electroplating, ore cyanidation). The most extensive treatments of industrial water to eliminate CN^- are based on the alkaline oxidation with ClO^- to produce non-toxic CNO^- and, in a further

oxidation step, HCO_3^- and N_2 [1,2], as well as UV oxidation [3,4], which yields the species CNO^-, NO_3^-, CO_3^{2-}, and HCO_3^-. The species SCN^- could be also present in some cyanide processes [5] or could be formed by the reaction with HS^- [6].

The classical procedures to determine total cyanide in liquid samples consist of acid distillation, collection of the released HCN(gas phase) on NaOH [7,8], and the determination of CN^- by colorimetric (LOD 0.5–20 ppb), electrochemical (LOD 3–50 ppb), or titrimetic methods (LOD 100–1000 ppb) [9–11]. When some metal–cyanide complexes and other species such as

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NO_3^-, CO_3^{2-}, and HCO_3 are present as interference, the distillation procedure will require sample pretreatment. The cited method can detect neither free cyanide (CN^- and HCN) nor CNO and SCN^- in the initial sample and is time-consuming. This prompts the development of new analytical methods for determining individually the amounts of free cyanide and cyanide-related species. These should be fast enough in total analysis time and should be able to detect total cyanide in concentrations lower than that required by law (normally from 10 to 50 ppb) to facilitate monitoring of cyanide in processes and in the environment.

There are several instrumental methods to analyze free cyanide and cyanide species. Atomic absorption spectroscopy (AAS)-based methods could only analyze metal–cyanide complexes with very good detection limits (0.2 to 2 ppb of cyanide) but with long analysis time [12]. GC methods could only analyze derivatized free cyanide also with very good LOD (0.3 to 100 ppb) [9–11]. HPLC-based methods show acceptable analysis time and selectivity. Several modalities and detection methods allow the detection of 20–500 ppb of metal–cyanide complexes [13] and 10–50 ppb of free [14] or derivatized cyanide [15]. As an alternative approach, capillary electrophoresis (CE) methods open the possibility of shorter analysis time, good resolution of sample components, and reasonable detection limits. Several metal–cyanide complexes have been separated in less than 5 min by using CE [16,17]

As CN^- and CNO^- do not absorb in the UV–Vis region [18,19], indirect detection methods have been used. Indirect detection techniques are based on the quantitation of the decrease of the signal due to the displacement of the chromophore ions by the sample ions. This method is used in LC and CE in combination with several detectors (refractive index, conductivity, polarimetry, absorption, and fluorescence) [20]. In the case of CE, indirect fluorescence is one of the most sensitive indirect detection methods and has been widely used for the detection of organic and inorganic anions [21,22]. For these reasons, a study on the de-

termination of CN^- and some related compounds (CNO^-, SCN^-, and NO_3^-) by CZE using a laser-based indirect fluorescence detector will be discussed.

In this technique, the detection limits are directly proportional to the concentration of fluorophore and inversely proportional to the dynamic reserve (DR) and transfer ratio (TR) [21]. TR-values increase when the mobility of the analyte is close to the mobility of the fluorophore to a maximum value given by the ratio between analyte charge and fluorophore charge. Low concentrations of other co-ions (e.g. OH^-) improve the TR. The DR improves with absorption coefficient, quantum yield, and a stable excitation intensity. Increasing the fluorophore concentration and the optical power of the laser increases the signal, but noise is also increased, so it is necessary to operate at low concentrations and low optical power to obtain good DR-values [23].

2. Experimental

2.1. Instrumentation

In both detection modes that were used (indirect absorption and fluorescence) a bare fused-silica, 75 μm I.D., 360 μm O.D., capillary column (Polymicro Technologies, Phoenix, AZ, USA) was used. The total lengths used were 70 and 73 cm and the detection lengths were 44 and 38 cm for the case of indirect absorption detection and indirect fluorescence detection, respectively.

To operate in the absorption detection mode, the column was connected to a commercial electrophoresis instrument (ISCO, Model 3140; Lincoln, NE, USA). Data were collected using commercial software on an IBM-compatible 286 computer.

Fluorescence detection was achieved by replacing the absorption detector by a prototype fluorescence detector as shown in Fig. 1. The excitation source consisted of a 488-nm beam from an argon ion laser (1) (Model 2211-10SL; Cyonics, Uniphase, San Jose, CA, USA) that
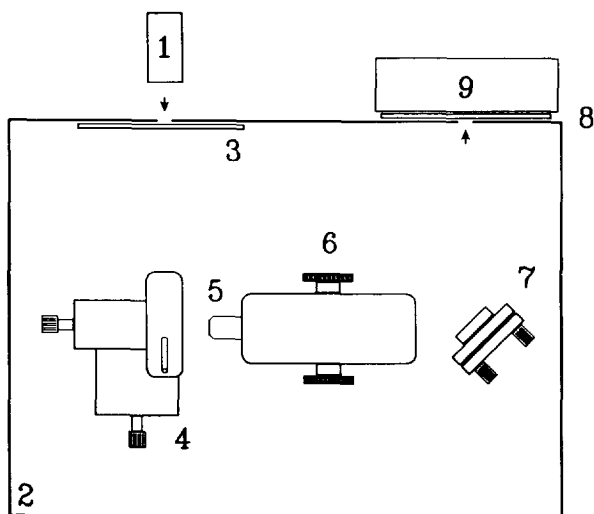
Fig. 1. Laser fluorescence detector setup. The different parts of the setup are explained in the text.

entered to a plexiglass box (2) through a 488-nm interference filter (3). The unfocused laser beam was directed perpendicularly to the capillary window. The capillary was connected to the commercial instrument by using a capillary holder (4). The fluorescence was collected by a 10 × microscope objective (5) placed in an objective holder (6) (Edmund Scientific, Barrington, NJ, USA) and was sent to a silicon photodetector (9) (HC 220-01; Hamamatsu, Bridgewater, NJ, USA) through a 500-nm long-pass cutoff filter (8) using a kinematic mirror mount (7) (Melles Griot, Irvine, CA, USA). Data were collected using an integrator (Datajet, Spectra-Physics, San Jose, CA, USA).

2.2. Operating conditions

The buffer solutions were filtered using a 0.2-μm membrane filter (Alltech, Deerfield, IL, USA) and were degassed for 15 min under vacuum with ultrasonication. After that, the buffer was stabilized at the running voltage for 4–5 h. Because the use of voltages above 25 kV requires increasing the stabilization time, equal or lower applied potentials were used. In all cases, a minimum optical power of 4 to 5 mW was used to avoid dark noise. The sample was

introduced in the system by using vacuum injection.

2.3. Chemicals

Fluorescein disodium salt, 2-hydrate ($C_{20}H_{10}Na_2O_5 \cdot 2H_2O$, $M_r = 412.3$) was purchased from Eastman Kodak (Rochester, NY, USA). Sodium cyanate and sodium hypochlorite were obtained from Jansen Chimica (Gardena, CA, USA). The other cited chemicals were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

2.4. Solutions and reagents

The main problem with free-cyanide samples is that hydrogen cyanide has a pK_a of about 9.2, so the pH has to be kept above this value to have more than 50% of free cyanide as CN^-. Another problem is that CNO^- is an expected species when starting from CN^- in aqueous medium, so the oxidizing properties of the sample matrix and buffer have to be taken into account [24]. Fluorescein solutions between 100 and 2000 μM adjusted to pH between 9.3 and more than 11 with NaOH or KOH were tested. Solutions below 100 μM were avoided because they would probably compromise the buffering capacity. The sodium cyanide standards were prepared every 2 or 3 days in concentrations from 1 to 10 g/l and were stored in the dark. Solutions above 10 ppm of NaCN were prepared each day and solutions lower than that concentration were prepared just before the analysis. Other solutions were prepared also every 2 or 3 days.

3. Results and discussion

3.1. Initial tests

Before using the prototype fluorescence detector, indirect absorption detection of CN^- at 238 nm using fluorescein was performed in order to check the chosen samples and the CE instrument. The best results are shown in Fig. 2(A), where a 1 mM fluorescein solution (pH 11) was
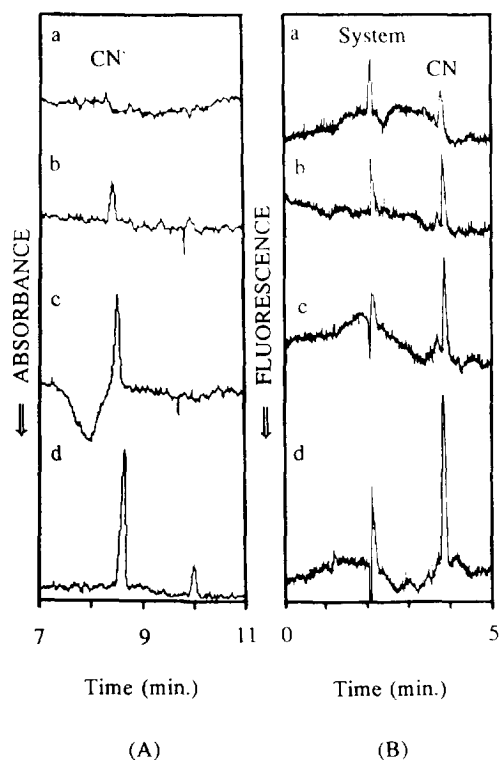
Fig. 2. Comparison of the indirect detection of CN : (a) 10⁻⁵ M; (b) 4 × 10⁻⁵ M; (c) 10⁻⁴ M; and (d) 2 × 10⁻⁴ M. (A) Absorption at 238 nm (1 mM fluorescein at pH 11, 5 kPa·s and 20 kV); (B) fluorescence at 4 mW (0.1 mM fluorescein at pH 10, 25 kPa·s and 25 kV).

the plot of the logarithm of peak height versus the logarithm of concentration (13 measurements) was linear ($r > 0.998$) in the range 2×10^{-10} to 10^{-6} M with a slope of 1.07 ± 0.01.

3.2. Indirect fluorescence detection of CN⁻

After the above tests, solutions with lower concentrations of fluorescein and with different pH were tested in order to optimize the LOD of CN⁻. The buffer solution had to be changed to 0.25 mM fluorescein and pH 9.6 to achieve an LOD similar to that for indirect absorption detection. By decreasing the concentration to 100 μM of fluorescein and pH 9.3 similar results were obtained, but the peaks were deformed. This is consistent with the fact that the chosen pH was close to the pK_a such that the sample was a mixture of CN⁻ and HCN with decreased TR.

After these observations, it was decided to increase the pH to see if there was some interference in the sample displacement by the fluorophore. The best results obtained for the detection of CN⁻ are shown in Fig. 2(B), where a 10^{-4} M fluorescein solution at pH 10 (KOH) was used. In this figure, it can be seen that the CN⁻ peak shape is satisfactory. To the degree that we can optimize each detection mode, the LOD was better for indirect fluorescence compared to indirect absorption, and the analysis time was shorter.

Table 1 shows some analytical results for CN⁻ and related compounds. The detection limits of all the anions studied were calculated from the peak height that is two times the peak-to-peak noise width of the same electropherogram [25]. In the case of CN⁻ the detection limit corresponds to 130 ppb. The dynamic reserve (DR) was calculated from the noise width and the measurement of the background signal obtained after blocking the laser beam and adjusting the output to zero. Depending on conditions, DR-values were between 75 and 300 for 100 μM fluorescein solutions. Operating under similar analytical conditions, the main factors affecting the noise were found to be mechanical vibration of the optics (movements in the order of 75 μm)

used. It can be seen that detection limits were around 1 ppm of NaCN (2×10^{-5} M) and the analysis time was about 9 min.

One important factor to enhance indirect fluorescence detection is to apply high voltages and to operate with high net mobilities for the fluorophore to reduce bleaching. One can thus increase the background signal and reduce analysis time. The high pH-values and voltage used in these experiments produced these two effects [23]. Fluorescein samples were thus studied using direct fluorescence detection in order to evaluate system linearity and the extent of bleaching. The chosen optical power was 22 mW and the injection time and pressure was set to 5 kPa·s. Beyond this setting the peak height did not increase perceptibly, indicating the elimination of bleaching. LOD was around 2×10^{-10} M and

Table 1
Detection performance for various anions

	CN	CNO	SCN	NO₃
Migration time (min ± %R.S.D.)	3.7 ± 1.4[a]	5.7 ± 1.1	6.3 ± 0.9	7.9 ± 1.0
LOD (M)	5×10^{-6}	2×10^{-6}–9×10^{-6}	2×10^{-6}–10^{-5}	2×10^{-6}–10^{-5}
DR	90	≥ 300	≥ 300	≥ 300
TR	0.44	0.07	0.05	0.05

[a] For CN $n = 6$, and for the other anions $n = 10$.

and the stabilization time of the solution in the column. This means that the experimental detection limits could be reduced if we were able to reduce the noise and further increase the DR.

Knowing C_{lim} (calculated as 0.5 times the LOD), DR-values, and fluorophore concentration, one can calculate the value of TR near the LOD. For monovalent anions, the maximum value of TR would be 0.5 because fluorescein at alkaline pH has two negative charges. The observed value of TR for CN⁻ is very close to the theoretical value. This means that the buffer was correctly optimized. Since sample displacement is charge based, one would reduce TR when the concentration of OH⁻ is similar to or larger than that of the chromophore. However, TR increases when the electrophoretic mobility of the analyte is similar to that of the chromophore. The mobility of OH⁻ is very high compared to that of the chromophore, so it is not possible to match the mobilities. Therefore low concentrations of the chromophore at a reasonably high pH were used as a compromise [26].

The LODs achieved are good, although marginal compared to the limit required by law and close to the theoretically expected values. The precision of the CN⁻ peak height was low, even when the samples were prepared just before the analysis. The linear regression of the logarithm of the concentration on the logarithm of the peak height from 10^{-5} to 2×10^{-3} M showed a slope that was different from one. Since for the direct detection of fluorescein with the same setup the slope was one, this deviation has to be due to the indirect technique that was used and not due to the instrument. Actually, TR-values

are dependent on the concentrations of the sample and OH⁻. Therefore this deviation in the slope could be expected.

3.3. Indirect fluorescence detection of related compounds

In this work, the detection of some anions related to CN⁻ (CNO⁻, SCN⁻, NO₃⁻) and possible interfering anions (Cl⁻, SO₄²⁻, and CO₃²⁻/HCO₃⁻) were studied. Mixtures of these anions were analyzed using buffer solutions with fluorescein concentrations and pH in a similar range as used for CN⁻ detection. The separation of CNO⁻, SCN⁻, and NO₃⁻ from Cl⁻ and SO₄²⁻ was achieved, as shown in Fig. 3. In this electropherogram Cl⁻ and SO₄²⁻ were not resolved. This is, however, not important because it is only important to know if these two anions would interfere in the positions of the anions related to CN⁻. In order to know the extent of the interference of these two anions, concentrations of Cl⁻ and SO₄²⁻ eleven times higher than that used in Fig. 3 (28 ppm Cl⁻ and 210 ppm SO₄²⁻), which correspond to concentrations higher than those present in natural waters, were tested. The results showed that detection of CNO⁻ and SCN⁻ was possible even under these conditions.

Compared with other references [27,28], the relative elution order of the anions studied is the same, except for Cl⁻ and SO₄²⁻. The CO₃²⁻/HCO₃⁻ peak was not detected with the positive applied voltage because of their mobilities. All peaks showed tailing which would indicate that the anions have mobilities higher than those of
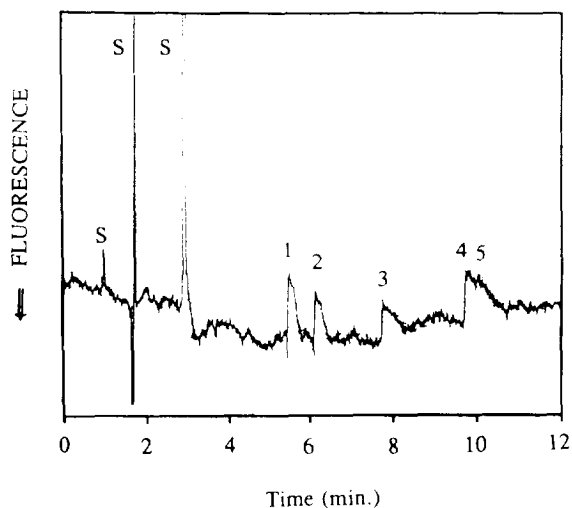
Fig. 3. Electropherogram of the indirect fluorescence analysis at 5 mW [0.1 mM fluorescein at pH 10 (KOH), 25 kPa·s and 25 kV]. Peaks: 1 = CNO 4.6 × 10⁻⁵ M; 2 = SCN 3.9 × 10⁻⁵ M; 3 = NO₃ 3.4 × 10⁻⁵ M; 4 = Cl 7.2 × 10⁻⁵ M; 5 = SO₄ 2.0 × 10⁻⁴ M; and S = system peaks.
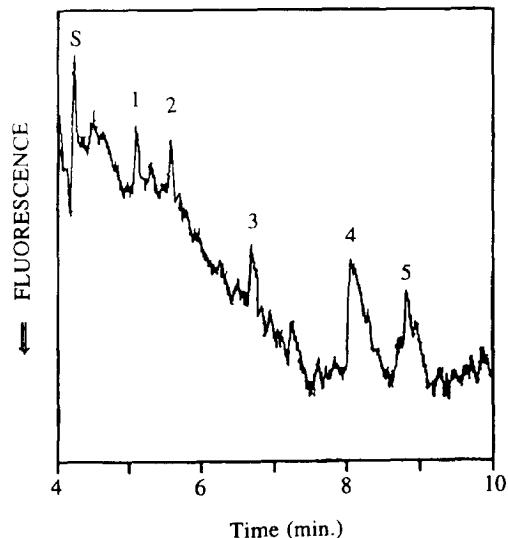

Fig. 4. Electropherogram of the analysis of anions near their LOD under favorable noise conditions [indirect fluorescence at 5 mW, 0.1 mM fluorescein at pH 10 (KOH), 25 kPa·s and 25 kV]. Peaks: 1 = CNO 2.7 × 10⁻⁶ M; 2 = SCN⁻ 2.3 × 10⁻⁶ M; 3 = NO₃ 2.0 × 10⁻⁵ M; 4 = Cl 4.3 × 10⁻⁶ M; 5 = SO₄²⁻ 1.2 × 10⁻⁵ M; and S = system peaks.

the buffer anions [29]. This tailing is probably due to the relatively high concentration of OH⁻.

Table 1 also shows the analytical parameters of CNO⁻, SCN⁻, and NO₃. It can be seen that one of the advantages of this method is that it is possible to analyze all anions of interest in less than 8 min, with good precision in the migration times. Once the fluorophore concentration and pH were chosen, the control of mechanical vibrations and stabilization of the buffer in the column became the limiting factors. By controlling the attachment of the detector to the instrument, the base leveling, vibration deadening, and by using a column stabilization time of 40 h, the results obtained were improved, as shown in Fig. 4, where it can be seen that Cl and SO₄² were clearly separated at low concentrations. The detection limits calculated from this figure are the lower values shown in Table 1. This low noise level lasted for several hours but it was not always possible to reproduce it. So, normal values for the detection limits with a good attachment of the detector and a stabilization time of 4 to 5 h were included in Table 1 as the higher values. Under these conditions DR values of 300 were obtained. Comparing the TR results

between anions (including CN) in Table 1, it can be seen that they are consistent with the fact that TR increases when the mobility of the anion is closer to the mobility of the fluorophore. The calibration curves of the three anions of interest were plotted from concentrations close to the higher LOD to about 10⁻⁴ M of each anion. The precision of the peak heights and the linearity are similar to those for CN⁻. Baseline fluctuations prevented us from evaluating the linearity of the response based on peak areas.

3.4. Study of the alkaline oxidation of CN⁻ and SCN

In order to assess if the analytical method is suitable for the study of the alkaline oxidation of cyanide with ClO⁻, a sample of 100 ml consisting of 1.1 × 10⁻² M of NaCN and 5.5 × 10⁻³ M of KSCN at pH 10 was oxidized using ClO⁻. The oxidation method was based on standard assays [7,8] in which the same ClO⁻ molar concentration (24.8 g active chlorine/l) and the same oxidant-to-sample ratio were used. The sample

to be oxidized was placed in a dark kitasatos to avoid transformation due to sunlight. Through the top part of the kitasatos a pH electrode was inserted through a rubber stopper in order to monitor the alkalinity of the sample. The lateral part of the kitasatos was used to introduce solutions with the help of teflon tubes connected to plastic syringes. The oxidation consisted of the stepwise introduction of ClO^- solution (5 ml each 15 min) for one hour in the stirred sample, adding the necessary volume of 1 M NaOH to keep the pH between 10 and 11. Once this operation was completed, the solution was placed in a volumetric flask and made up to 200 ml by adding NaOH pH 10. In order to analyze the oxidized sample, 100 μl of this solution was diluted to 5 ml with the running buffer.

The electropherogram of this diluted sample is shown in Fig. 5. In this figure we can identify ClO^-, CNO^-, and system peaks by their migration times. The CNO^- anion was also identified by the addition of standard and was found not to be interfered with by ClO^- and other by-products. By using this method, the concentration of

CNO^- was found to be between 1 and 2×10^{-4} M in the oxidized sample, but it was not possible to independently determine if all the CNO^- came from the CN^- plus SCN^- oxidation or only from CN^-.

Acknowledgements

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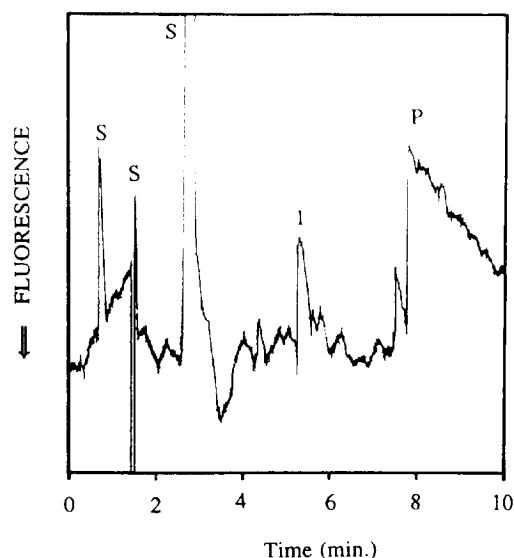[12] J.J. Rosentreter and R.K. Skogerboe, Anal. Chem., 63 (1991) 682–688.

Fig. 5. Electropherogram of an oxidized sample of CN and SCN (see text) diluted 1/100. Indirect fluorescence detection at 5 mW, 0.1 mM fluorescein pH 10 (KOH), 25 kPa·s and 25 kV. Peaks: 1 = CNO ; S = system peaks; and P = by-products (ClO , Cl).

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