An automated colorimetric method for the determination of cyanoguanidine in water

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Abstract

An automated segmented flow analytical procedure for the determination of cyanoguanidine (dicyandiamide or DCD) in surface and ground water was developed. DCD reacts with 1-naphthol and diacetyl to form a red-coloured complex which was measured spectrophotometrically at 540 nm. Interferences of a number of ions were evaluated and the results given. Ethylene diamine tetra-acetic acid (EDTA) was used to eliminate the interference of calcium and magnesium. The method was evaluated in the range 0,1 to 30 mg/ℓ DCD-N. At a sample frequency of 30 samples/h a good precision and accuracy were achieved and the carry-over between samples was less than 2%.

Introduction

Cyanoguanidine, better known as dicyandiamide (DCD), is a widely used chemical. It has been applied as a flocculating agent; either alone, or with others to clear up waste waters (SKW, 1983). It is also employed as a hydrotropic agent for dyeing cellulose polyester fabrics with reactive dyes in the so-called AT-process (Marschner and Hildebrand, 1981).

In agricultural studies, which concentrate on the more effective usage of nitrogenous fertilisers, DCD was found to be a selective and very effective nitrification inhibitor (Yadvinder-Singh and Beauchamp, 1987; Shaviv et al., 1987; Prakasa Rao and Puttanna, 1987; Rodgers et al., 1987; Simpson et al., 1985). Pollution of the underground waters due to the leaching of nitrate should thus be reduced, as the applied nitrogen stays in the soil in the ammonium form, which, as it is positively charged, could adhere to the negatively charged clay particles (Müller, 1986). The accumulation of phytotoxic nitrite and further losses of nitrogen oxides due to chemodenitrification are also prevented (Yadvinder-Singh and Beauchamp, 1987; Rodgers, 1983). Depending on the temperature, water, organic carbon and Fe(III)-hydroxide content of the soil (Amberger, 1989; Reeves and Touchton, 1986), DCD is soluble and stable enough with the nitrogen bases and fluid formulations (Gautney et al., 1985) to inhibit nitrification on average for 1 to 3 months, before hydrolysing through various stages to urea, ammonium and nitrate (Amberger, 1989).

Even though DCD is regarded to be practically non-toxic to higher life forms (SKW, 1983), it may, in excessive quantities, be phytotoxic to plants (Reeves and Touchton, 1986). It has therefore become important to develop an automated procedure for the routine analysis of DCD in runoff, and underground waters, which could also be employed as a quality control measure for fertiliser mixtures containing DCD.

A wide variety of methods has been employed to determine DCD. Harger (1920) quantitatively precipitated DCD as a double compound of silver picrate, named "silver picrate-mono-cyanoguanidine" in the gravimetric determination of DCD. The existence of "silver picrate-di-cyanoguanidine" was also mentioned, and employed by Johnson (1921 a;b) in the volumetric determination of DCD by means of a back titration with thiocyanate. Garby (1925) hydrolysed DCD to guanylurea and precipitated it as nickel guanylurea. All these methods were found to be tedious due to lengthy procedures and also needed a relatively high DCD content for accurate determination.

Dukhovnaya (1986) accurately determined DCD between 0,03 and 0,15 mg/ℓ in water with thin layer chromatography. Milks and Janes (1956) described a paper chromatographic method which dealt with the separation and detection of cyanamide and its derivatives, including DCD, but only gave data on the quantitative determination of urea.

Urbanyi and Walter (1971) used IR-spectroscopy to determine trace quantities of DCD and cyanamide in guanidine sulphate. Hagnauer and Setton (1978) developed a liquid chromatographic method of separation, and identification with Fourier transformed IR spectroscopy in the compositional analysis of epoxy resin formulations. Seiffarth et al. (1966 and 1969) employed IR spectroscopy in the determination of DCD in technical thiourea. However, the lower detection limit of 400 mg/ℓ excluded the application of this method.

Hagnauer and Dunn (1981) described a HPLC procedure for the rapid quantitative analysis of DCD in epoxy resins, whilst Vilsmeier (1984) employed the same technique in the simultaneous determination of DCD, nitrate and nitrite in soil extracts. The DCD concentration ranged between 0,1 and 20 mg/ℓ. The sample frequency of 11 samples/h was found to be inadequate for routine analysis.

Pawlik (1960) determined DCD in soil extracts with UV spectroscopy at 215 nm. This method gives good resolution in the region of 1 mg/ℓ, but is strongly influenced by nitrate and various organic substances. Banyai and Beck (1982) observed a colour reaction between pentacyanoammineferrate (II), oxygen and DCD at pH 12, which seemed suitable for spectrophotometric analysis, but due to a lengthy procedure, was not feasible for routine analysis.

Sakaguchi (1925 a;b; 1950) found that agrinine formed a red-coloured complex on addition of a solution of 1-naphthol, or 8-hydroxyquinoline followed by alkaline hypobromide. He also suggested that the reaction was specific for monoguanidines. Agrinine also reacts with diacetyl, although the Sakaguchi reaction is more sensitive (Eden et al., 1954). Diacetyl was also used in the determination of creatine (Bonas et al., 1963). Vilsmeier (1979; 1982) employed these reactions in the colorimetric determination of DCD in soil extracts. This method was adapted to determine DCD in plants (Müller, 1986)

This paper describes an automated colorimetric procedure, based on the principles of continuous segmented flow analysis, for the determination of DCD in water. The method is based on the

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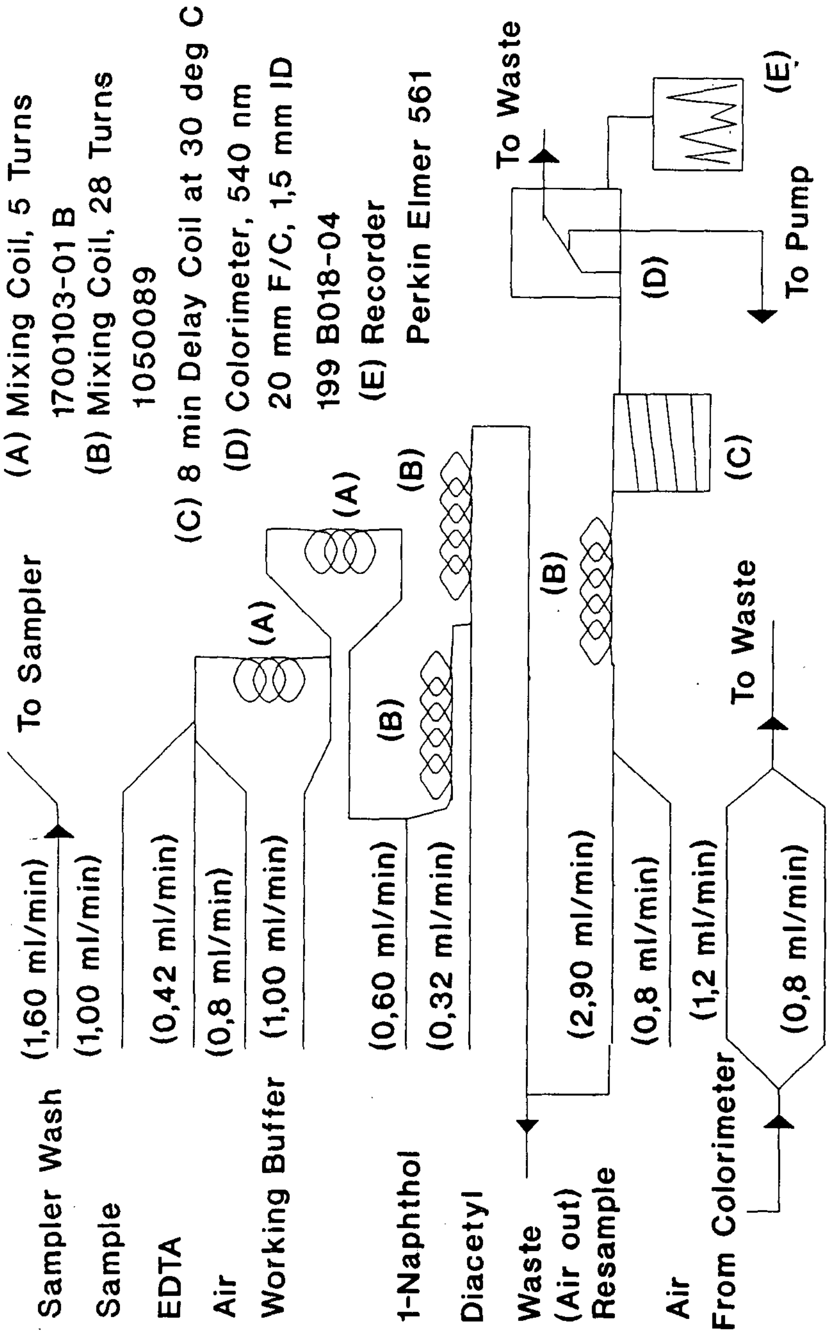


Figure 1
Schematic diagram of the analytical flow system

reaction of diacetyl and 1-naphthol with DCD to form a red complex at high pH (reaction mix pH = 13,30) with adsorption maximum between 535 and 540 nm.

Experimental

The analytical flow system is shown in Fig. 1.

Apparatus

A Technicon Auto-Analyzer Sampler II was used to present samples, at a rate of 30 samples/h, (sample/wash ratio at 1:1) to an Auto-Analyzer Model II peristaltic pump fitted with standard Technicon pump tubes which are resistant to the solvent ethanol (EtOH). The reagents passed through 4 mm OD glass tubes, a series of mixing and delay coils to a Technicon Auto-Analyzer double channel colorimeter with a 20 mm debubble type flow cell (ID = 1,5 mm). Determination of the reaction product was performed at 540 nm and the resulting graph was recorded on a Perkin-Elmer 561 recorder.

Reagents

AR grade chemicals and deionised water were used. All reagents were thoroughly mixed. Filtrations were done through Whatman No. 2V filter paper.

Stock solutions

- Sodium hydroxide (1,72 mol/ℓ): Dissolve 137,57 g NaOH in 1 800 mℓ water. Cool down and dilute to 2 ℓ with water.
- Sodium hydroxide (5,00 mol/ℓ): Dissolve 200 g NaOH in 800 mℓ water. Cool down and dilute to 1 ℓ with water and filter.
- Phosphate buffer (0,5 mol/ℓ): Dissolve 70,98 g sodium phosphate, dibasic, anhydrous in 800 mℓ water. Add 20 g NaOH and dissolve. Dilute to 1 ℓ with water and filter.
- Sodium potassium tartrate (0,71 mol/ℓ): Dissolve 200 g sodium potassium tartrate in 800 mℓ water. Dilute to 1 ℓ with water and filter.
- Standard solution (400 mg/ℓ DCD-N): Dissolve 0,6189 g of DCD (97% pure) in 800 mℓ water. Dilute to 1 ℓ with water. Prepare 30, 25, 20, 15, 10, 8, 6, 4 and 2 mg/ℓ DCD-N standards by suitable dilution of the stock standard solution.

Analytical reagents

- Working buffer: With swirling, add 250 mℓ of the stock sodium potassium tartrate solution to 200 mℓ of stock phosphate buffer solution. Very slowly and swirling vigorously, add 450 mℓ of 5,00 mol/ℓ stock NaOH solution. Dilute to 1 ℓ with water.
- EDTA solution (4,341 x 10^{-2} mol/ℓ): Dissolve 7,81 g EDTA dihydrate (disodium salt) in 400 mℓ water. Dilute to 500 mℓ and filter.
- Diacetyl solution (1,124 x 10^{-2} mol/ℓ): Add 1 mℓ 99% (v/v) pure (d(4°C) = 0,987 g/mℓ) diacetyl with a graduated pipet to 900 mℓ water. Dilute to 1 ℓ and store in a dark bottle.
- 1-Naphthol solution (1,785 x 10^{-1} mol/ℓ): Dissolve 47,20 g sodium carbonate in 390 mℓ of the 1,72 mol/ℓ stock NaOH solution. Cool down and filter into a 500 mℓ stoppered Erlenmeyer flask. Degas this solution, as well as 150 mℓ water and 15 mℓ EtOH (99,8 % v/v pure) for 30 min with nitrogen gas. Rinse a 10 mℓ measuring cylinder throroughly with N_2. With N_2 still bubbling through the alkaline solution, add 12,88g 1-naphthol and dissolve. Add 7,5 mℓ degassed EtOH and dilute to 500 mℓ with degassed water. Degas for another 5 min with N_2 whilst stirring. Note: The 1-naphthol solution has to be prepared daily, and must, at all times, be kept under nitrogen.
- The "sampler wash" solution consists of deionised water.

Method optimisation

Sensitivity

It was clear, from the beginning of the study, that the reaction rate would be the limiting factor in employing the prescribed method at a high degree of sensitivity. According to Vilsmeier (1979) and Bonas et al. (1963), the reaction is only completed in 1 h. Attempts to increase the reaction rate by substituting the air segments in the flow system with oxygen and heating of the reaction mixture to 55°C, were fruitless. Heating led to the total break-up of the reaction product, whilst oxygen caused the baseline to be unstable, possibly due to the oxidation of 1-naphthol to the 1,4-quinone form. Nitrogen segments slowed the reaction rate, but the colour developed, and thus the reaction product was stable for up to 6 months when kept under nitrogen. In relation to the nitrogen segments, air segments showed an increased reaction rate and it is thought that there is enough oxygen in air to have a catalytic effect on the reaction without the oxidation of the 1-naphthol. It was therefore decided to use air segments in the flow system. To operate the prescribed method at optimum sensitivity, the influence of reagent concentration, temperature and sample pH were further investigated.

1-Naphthol and EtOH concentrations of the 1-naphthol solution

The EtOH concentration was varied between 1,253 x 10^{-1} and 5,134 x 10^{-1} mol/ℓ and the sensitivity constantly monitored. The concentration of all the other reagents was kept constant during optimisation. The results are shown in Table 1. Results showed that EtOH had no influence on sensitivity. It acts merely as a stabiliser for the 1-naphthol, but problems may arise at higher concentrations due to the precipitation of sodium carbonate with time, as the latter is insoluble in EtOH. Further tests indicated that the 1-naphthol reagent is optimumly stabilised, and precipitation-free with the EtOH concentration at 2,567 x 10^{-1} mol/ℓ.

The influence of the 1-naphthol concentration on sensitivity was determined as indicated in Table 2. Other than expected, optimum sensitivity is neared at lower 1-naphthol concentrations. However, at 1,504 x 10^{-1} mol/ℓ the precision of the method worsened to

TABLE 1
THE INFLUENCE OF THE EtOH CONCENTRATION ON THE SENSITIVITY OF THE METHOD

EtOH (mol/ℓ) x 10^{-1}	1-Naphthol (mol/ℓ) x 10^{-1}	Relative peak height
1,253	6,015	67,73
2,505	6,016	67,52
3,423	6,015	67,71
5,134	6,015	67,66

TABLE 2
THE INFLUENCE OF THE 1-NAPHTHOL CONCENTRATION ON THE SENSITIVITY OF THE REACTION

1-Naphthol conc. (mol/ℓ) × 10^{-1}	Relative peak height
1,504	79,57
3,008	64,69
4,511	45,99
6,015	34,24
7,519	19,07

TABLE 3
THE INFLUENCE OF THE NaOH CONCENTRATION ON THE SENSITIVITY OF THE REACTION

5,00 mol/ℓ NaOH content (mℓ)	Relative peak height
200	60,10
300	71,08
450	77,12
500	75,15

TABLE 4
THE INFLUENCE OF THE DIACETYL CONCENTRATION ON THE SENSITIVITY OF THE REACTION

Diacetyl conc. (mol/ℓ) × 10^{-3}	Relative peak height
1,798	41,11
3,483	59,86
5,056	73,70
6,966	82,99
11,24	94,34
12,36	96,21

TABLE 5
THE INFLUENCE OF TEMPERATURE ON THE SENSITIVITY OF THE REACTION

Temperature (°C)	Relative peak height
20	65,37
25	68,30
30	70,15
35	67,95
40	65,00

unacceptable levels. A compromise between precision and sensitivity was reached with the 1-naphthol concentration at 1,785 x 10^{-1} mol/ℓ.

NaOH concentration of the working buffer solution

The investigation into the effect of the NaOH and sodium carbonate concentrations on sensitivity were deemed unnecessary as its only purpose was to ensure alkalinity of the reaction mixture. No reference could be found on the exact pH needed for the reaction to divulge optimum sensitivity. However, later studies on the effect of sample pH on sensitivity (discussed under **Sample pH**) furnished poor results. As the reaction mixture's pH dropped from 13 to 12,68, it was believed that the reaction rate was impaired at pH lower than 13. A working buffer, with a high NaOH concentration, was introduced to the system to rectify this problem. The effect of NaOH concentration on sensitivity was evaluated by varying the volume of 5,00 mol/ℓ NaOH added to the rest of the buffer, before diluting to 1 ℓ. The results are given in Table 3. Under optimum conditions the pH of the reaction mixture was 13,30.

Diacetyl concentration

The effect of diacetyl concentration, between 1,798 x 10^{-3} and 1,236 x 10^{-2} mol/ℓ, on sensitivity was studied. Results are shown in Table 4. Even though the sensitivity improved, precision again worsened with the diacetyl concentration at 1,236 x 10^{-2} mol/ℓ. A compromise between sensitivity and precision was reached with the diacetyl content at 1,124 x 10^{-2} mol/ℓ.

Temperature

The 8 min delay coil was placed in an oil bath with a variable thermostat. The temperature was varied between 20 and 40°C and results are given in Table 5. Optimum sensitivity was reached at 30°C.

Sample pH

Weakly buffered water samples, containing varying amounts of sulphuric acid (0,5 mol/ℓ) and sodium hydroxide (1,34 mol/ℓ), with an initial DCD content of 15 mg/ℓ, were prepared to determine the influence of sample pH on sensitivity. The samples were left at room temperature for 3 d before the determination. Results are graphically shown in Fig. 2.

At first it was thought that sample pH, especially at lower pH values, decreased the reaction rate, causing the colour development to be incomplete at the moment of determination. The same tendency was found with some of the elements tested for interference (discussed under **Method evaluation**). However, the pH of reaction mixtures of the various samples divulged a narrow pH-band (between 13,19 and 13,40 pH units) which could not explain the existing disparity between determined and expected values. The only reasonable deduction was that the initial assumption of a decreased reaction rate was wrong. DCD could no longer be determined due to its decomposition. Feigl and Gentil (1959) found that DCD is rapidly reduced through hydrogenation with zinc and dilute hydrochloric acid to form guanidine and monomethylamine. He also proved that the methylamine is not formed directly but that it results from the hydrogenation of hydrogen cyanide, according to the scheme below. This could be used as a spot test determination of DCD and other N-cyano compounds.

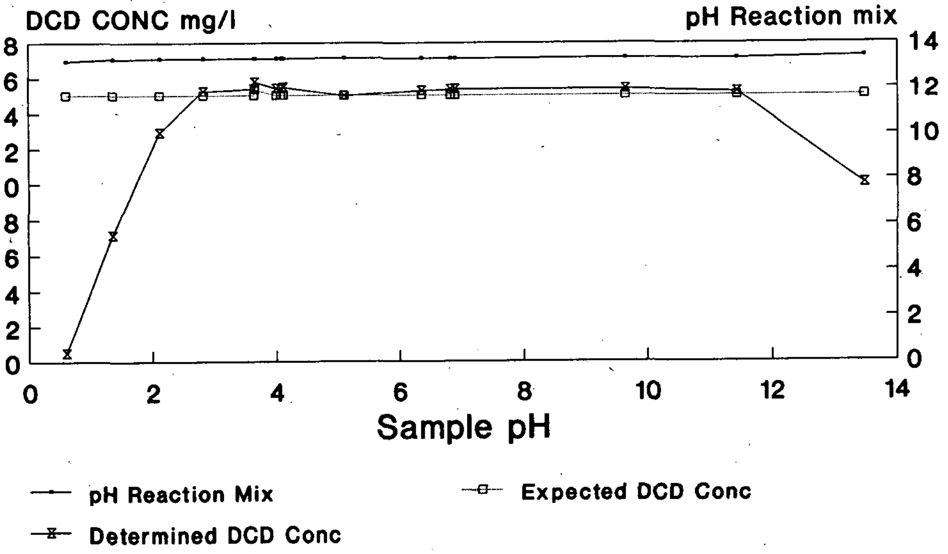
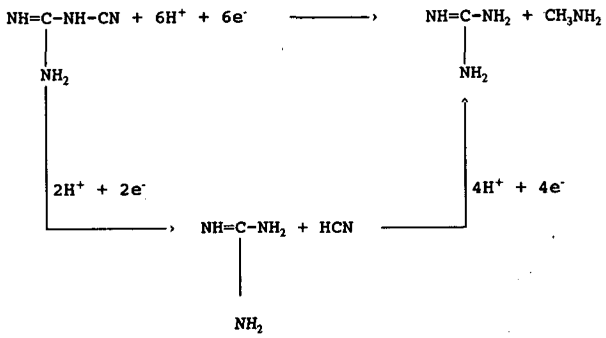
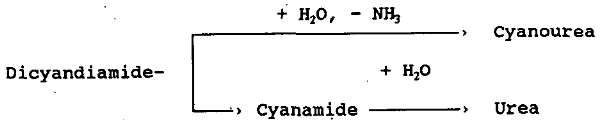


Figure 2
Determining the influence of sample pH on the sensitivity of the reaction



Mushkin and Finkel'shtein (1966) studied the alkaline decomposition of DCD and found that it was hydrolysed through its anion to form cyanourea. They also proved that in strongly alkaline solutions, pH 12,6 to 13,5, DCD decomposed to form cyanamide which in turn was rapidly hydrolysed to form urea. They proposed the following parallel-sequential scheme:



This explained the disparity between expected and determined values at high and low sample pH. The reaction mixture's pH was constant for samples with pH ranging between 1,40 and 11,50 pH units (see Fig. 2), which implied that the working buffer was able to resist the change in sample pH. It can thus be ascertained that sensitivity would not be adversely affected with the sample's pH in the above-mentioned range.

Further tests revealed that conditions for the reaction were now at an optimum. The time delay for the reaction (from when the sampler took the sample until the recorder started to draw the graph) was 17 min. Even 5-min stopped-flow conditions, in other words, stopping the peristaltic pump from pumping the reaction mixture through the flow cell for 5 min, created no difference in peak height. This meant that the reaction was completed within 17 min, a considerable improvement on the reaction time as stated by Vilsmeier (1979) and Bonas et al. (1963), namely 1 h at 20°C and 0°C respectively.

Linearity

The linearity of the calibration curve was studied under optimum conditions. Carry-over between samples at higher sampling frequencies, forced the through-put of samples down to 30 samples/h with a 1:1 sample to wash ratio. This compromise between sampling frequency, sensitivity and precision furnished results with a high degree of linearity and virtually zero carry-over between samples. A typical example of the results obtained is given in Fig. 3. In this case the linear relationship between peak height and DCD concentration is:

$$y = -0{,}35714 + 2{,}96462x;\ r = 0{,}99998$$

with y= peak height and x= DCD concentraiton in mg/ℓ.

Recommended method

The variables as ascertained in the preceding examination can be summarised as follows:

sampling frequency:	30/h
temperature:	30°C
sample pH:	1,40 to 11,50 pH units

The flow diagram of the prescribed analytical flow system is explained in Fig. 1, whilst the concentrations recommended for the different reagents are indicated in the experimental section.

Method evaluation

Accuracy

The accuracy of the method was evaluated by comparing the results with the colorimetric method of Vilsmeier (1982). It has to be noted that most of the samples had to be diluted as the standard method's upper limit of detection is 30 μg DCD-N/2 mℓ. The results as shown in Table 6 revealed an excellent correlation be-

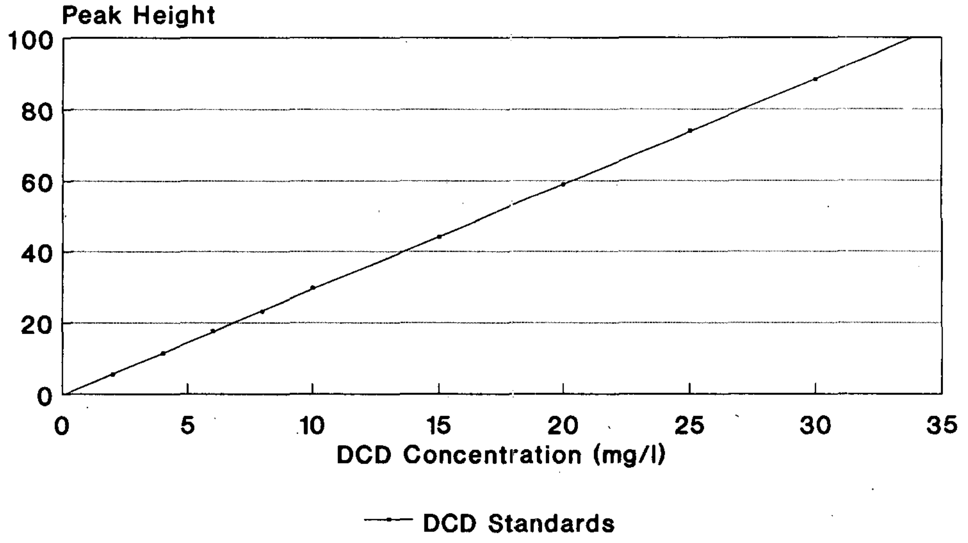


Figure 3
Standard calibration curve indicating the linear relationship between peak height and DCD concentration

tween the two methods. The recovery was determined by analysing a sample to determine its DCD content, after which a known amount of DCD was added and the sample re-analysed. The recoveries of some samples are given in Table 7. The average recovery was 99,16%.

Precision

The precision of the method was determined through 14 repetition analyses of a number of DCD standard solutions as indicated in Table 8. The method provided satisfactory precision as deduced from the calculated standard deviation values.

Detection limit

The detection limit of the method was taken as 3 times the standard deviation of a low DCD concentration (8 mg/ℓ). The detection limit was calculated to be 0,10 mg/ℓ.

Carry-over effect

The transmittance between two consecutive samples was determined by analysing a sample with a high DCD concentration (20 mg/ℓ DCD-N) followed by one with a low DCD concentration (2 mg/ℓ DCD-N). At a sampling frequency of 30 samples/h, a 1:1 sample: wash ratio was adequate to ensure a carry-over of less than 2%.

Interferences

According to the literature (Vilsmeier, 1979), of all the DCD related compounds tested, only guanidine, biguanide and guanidino-acetic acid showed positive interference. Of the 13 amino acids tested only arginine showed positive interference. Except for biguanide the adsorption maximum shifted to lower values. However, biguanide and guanidino-acetic acid are essentially synthetically formed products of cyanamide or DCD, whilst free amino acids contents lie between 0,2 and 0,4 mg/100 g soil, of which only a small percentage is water extractable. This implies that the possibility of these substances interfering under natural conditions is virtually nil. Guanidine on the other hand is the decomposition product of DCD and the possibility of its interference cannot be excluded. However, the decomposition of DCD to guanylurea to guanidine takes place more slowly than that of guanidine to urea, with no consequent danger of guanidine accumulation in the soil, which implies that the possibility of guanidine interfering in the analysis of surface and underground water samples is nil.

The influence of a number of ions, which possibly could interfere with the prescribed method, was evaluated. Ca^{2+} and Mg^{2+} ions were removed from the sample with EDTA, as CA^{2+} formed a precipitate with the carbonate of the 1-naphthol solution. The EDTA was added to the sample before the working buffer as both these ions may precipitate as hydroxides above pH 9,6. Surface water samples, taken near an open-cast coal mine, could pose a problem, at low pH levels (this specific sample's pH was 2,62) due to the possibility of a high iron, manganese and zinc content. These ions also precipitate out of solution at high pH as hydroxides. No interference and good results were obtained when an acidic sample was treated with NaOH to pH ± 11, and the sample, after the precipitation had formed, was filtered into the sample cup with a disposable microfilter. Table 9 indicates the concentrations of the various ions tested at which no interference was experienced.

Discussion

The proposed method was evaluated between 2 and 30 mg/ℓ DCD-N. Good accuracy and precision, with a standard deviation of less than 0,5% were obtained. The calibration curve revealed linearity in this concentration range and the detection limit for this method was calculated at 0,1 mg/ℓ. A frequency of 30 samples/h was maintained using this method. At this frequency the carry-over between samples was negligible and furnished results with a high degree of accuracy and precision. The compromise between sensitivity and precision, as discussed, did not impair sensitivity and provided a vast improvement in the reaction rate. This advantage ensures that this method is by far more accurate, precise and faster than any previously described, which makes it suitable for the routine analysis of water samples.

TABLE 6
COMPARISON OF RESULTS OF A NUMBER OF SURFACE AND UNDERGROUND WATER SAMPLES AS DETERMINED WITH THE AUTOMATED AND STANDARD METHODS

Automated method (mg/ℓ)	Standard method (mg/ℓ)
14,84	15,04
9,89	9,86
0,49	0,56
17,94	18,05
19,89	19,90
23,19	23,46
12,93	12,84
7,14	6,99
3,17	3,24
26,59	26,91
19,79	20,05

TABLE 7
DCD RECOVERY OF A NUMBER OF SAMPLES AS DETERMINED WITH THE AUTOMATED COLORIMETRIC METHOD

DCD concentrations		Recovery (%)
Expected (mg/ℓ)	Determined (mg/ℓ)	
8,57	8,42	98,25
13,33	12,95	97,15
15,64	15,31	97,89
18,00	17,94	99,67
20,00	19,89	99,45
21,67	21,63	99,82
23,48	23,57	100,38
26,11	26,29	100,69

TABLE 8
PRECISION OF THE SEGMENTED FLOW ANALYTICAL METHOD IN TERMS OF THE STANDARD DEVIATION OF 14 REPETITIONS AT 9 DCD CONCENTRATIONS

DCD concentration (mg/ℓ)	Standard deviation (%)
30	0,43
25	0,37
20	0,36
15	0,30
10	0,38
8	0,39
6	0,35
4	0,30
2	0,27

TABLE 9
CONCENTRATION OF IONS WHICH DID NOT SIGNIFICANTLY INTERFERE WITH THE DETERMINATION OF DCD

Ion	Concentration (mg/ℓ)
Fe^{3+}	500
Al^{3+}	500
Zn^{2+}	500
Mn^{2+}	400
Cd^{2+}	500
Ca^{2+}	1 000
Mg^{2+}	125
NH_4^+	40
K^+	1 000
Urea	40
Cl^-	1 000
NO_2^-	10
NO_3^-	40
SO_4^{2-}	1 500

References

AMBERGER, A (1989) Research on dicyandiamide as a nitrification inhibitor and future outlook. *Commun. in Soil Sci. Plant Anal.* **20** (19 & 20) 1933-1955.

BANYAI, I and BECK, MT (1982) Chemical basis of spectrophotometric determination of cyanamide with pentacyanoferrates. *Acta Chimica Academiae Scientiarum Hungarical* **111**(2) 141-148.

BONAS, JE, COHEN, BD and NATELSON, S (1963) Separation and estimation of certain guanidino compounds. Application to human urine. *Microchem. J.* **7** 63-77.

DUKHOVNAYA, IS (1986) Method for the determination of dicyandiamide in water. *Khimia i Tekhnologia Vody* **8**(4) 86-87.

EDEN, E, HARRISON, DD and LINNANE, AS (1954) Studies in carbon tetrachloride poisoning. *Austral. J. Exp. Biol.* **32** 333-340.

FEIGL, F and GENTIL, V (1959) Spot test detection of n-cyano compounds. *Microchimica Acta* **1** 44-46.

GARBY, CD (1925) The determination of dicyandiamide. *Ind. Eng. Chem.* **7** 266-268.

GAUTNEY, J, KIM, YK and BARNARD, AR (1985) Solubilities and stabilities of the nitrogen loss inhibitors dicyandiamide, thiourea and phenylphosphorodiamidate in fluid fertilizers. *Ind. Eng. Chem. Prod. Res. Dev.* **24** 155-161.

HAGNAUER, GL and DUNN, DA (1981) Dicyandiamide analysis and solubility in epoxy resins. *J. Appl. Polym. Sci.* **26** 1837-1846.

HAGNAUER, GL and SETTON, I (1978) Compositional analysis of epoxy resin formulations. *J. Lig. Chromatogr.* **1**(1) 55-73.

HARGER, RN (1920) Dicyandiamide: A rapid, direct method for its determination in cyanamide and mixed fertilizers. *J. Ind. Eng. Chem.* **12** 1107-1111.

JOHNSON, EJ (1921a) A direct method for the determination of dicyandiamide. *J. Soc. Chem. Ind.* **40**(11) 125-126.

JOHNSON, EJ (1921b) The determination of dicyandiamide and of urea in fertilizers. *J. Ind. Eng. Chem.* **13**(6) 533-535.

MARSCHNER, W and HILDEBRAND, D (1981) AT-process- A new method for the continuous dyeing of polyester-cellulosic blended fabrics. *Colourage* Nov. 19, 21-24.

MILKS, JE and JANES, RH (1956) Separation and detection of cyanamide and its derivatives and determination of urea by paper chromatography. *Anal. Chem.* **25**(5) 846-849.

MÜLLER, H (1986) Photometrische Bestimmung von Dicyandiamid in Pflanzen. *Landwirtsch. Forschung* **39**(1-2) 20-23.

MUSHKIN, YI and FINKEL'SHTEIN, AI (1966) Decomposition of dicyandiamide in aqueous alkaline media. *Kinetika Kataliz* **7**(2) 219-223.

PAWLIK, A (1960) Spektralphotometrische Untersuchungen über Dicyandiamid im Boden. *Z. Planzenernaehr. Bodenkd.* **89** 181-187.

PRAKASA RAO, EVS and PUTTANNA, K (1987) Nitrification and ammonia volatilization losses from urea and dicyandiamide-treated urea in sandy loam soil. *Plant and Soil* **97** 201-206.

REEVES, DW and TOUCHTON, JT (1986) Relative phytotoxicity of dicyandiamide and availability of its nitrogen to cotton, corn and grain sorghum. *Soil. Sci. Soc. Am. J.* **50** 1353-1357.

RODGERS, GA (1983) Effect of dicyandiamide on volatilization from urea in soil. *Fert. Res.* **4** 361-367.

RODGERS, GA, PENNY, A, WIDDOWSON, FW and HEWITT, MV (1987) Test of nitrification and of urease inhibitors when applied with either solid or aqueous urea, on grass grown on a light, sandy soil. *J. Agric. Sci. Camb.* **108** 109-117.

SAKAGUCHI, S (1925a) Über die katalytische Wirkung des Blutfarbstoffes auf Natriumhypochlorit nebst einer neuen Farbreaktion des Blutes. *J. Biochem. Tokyo* **5** 13.

SAKAGUCHI, S (1925b) Über die Bindungsweise und quantitative Bestimmung des Arginins im Proteinmoleküle. *J. Biochem. Tokyo* **5** 133.

SAKAGUCHI, S (1950) A new method for the colorimetric determination of arginine. *J. Biochem.* **37**(2) 231-236.

SEIFFARTH, K, WOHLRABE, W and ARDELT, HW (1966) Infrarotspektralphotometrische Bestimmung des Dicyandiamids in technischen Thioharnstoff. *Z. Anal. Chem.* **217** 345-349.

SEIFFARTH, K, WOHLRABE, W and ARDELT, HW (1969) Infrarotspektralphotometrische Bestimmung von Dicyandiamid in technischen Dicyandiamid und Thioharnstofflösungen. *Z. Anal. Chem.* **244** 182-184.

SHAVIV, A, HAGIN, J and NEUMANN, PM (1987) Effects of a nitrification inhibitor on efficiency of nitrogen utilization by wheat and millet. *Commun. in Soil Sci. Plant Anal.* **18**(8) 825-833.

SIMPSON, JR, FRENEY, JR, MUIRHEAD, WA and LEUNING, R (1985) Effects of phenylphosphorodiamidate and dicyandiamide on nitrogen loss from flooded rice. *Soil. Sci. Soc. Am. J.* **49** 1426-1431.

SKW (1983) Produktstudie Dicyandiamid der Süddeutschen Kalkstickstoffwerke Trossberg.

URBANYI, T and WALTER, A (1971) IR determination of trace quantities of dicyandiamide and cyanamide in guanidine sulphate. *J. Pharm. Sci.* **60**(11) 1699-1701.

VILSMEIER, K (1984) Bestimmung von Dicyandiamid, Nitrit und Nitrat in Bodenextrakten mit Hochdruckflüssigkeitschromatographie. *Z. Pflanzenernaehr. Bodenkd.* **147** 264-268.

VILSMEIER, K (1979) Kolorimetrische Bestimmung von Dicyandiamid in Böden. *Z. Pflanzenernaehr. Bodenkd.* **142** 792-798.

VILSMEIER, K (1982) Verbesserte kolorimetrische Bestimmung von Dicyandiamid in Bodenextrakten. *Z. Pflanzenernaehr. Bodenkd.* **145** 503-505.

YADVINDER-SINGH and BEAUCHAMP, EG (1987) Nitrification inhibition with large urea granules, dicyandiamide and low soil temperature. *Soil Sci.* **144**(6) 412-419.