
**Water quality — Determination of
available free cyanide (pH 6) using
flow injection analysis (FIA), gas-
diffusion and amperometric detection**

*Qualité de l'eau — Dosage des cyanures libres disponibles (pH 6)
par analyse avec injection en flux (FIA), diffusion de gaz et détection
ampérométrique*



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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Introduction

Methods using flow analysis automate wet chemical procedures and are particularly suitable for the determination of many analytes in water in large sample series at a high analysis frequency.

Analyses can be performed by flow injection analysis (FIA) using the feature of an automatic dosage of the sample into a flow system (manifold) where the analytes in the sample reacts with the reagent solutions on their way through the manifold. The sample preparation can be integrated in the manifold. The reaction product is measured by a flow detector (e.g. amperometer).

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Water quality — Determination of available free cyanide (pH 6) using flow injection analysis (FIA), gas-diffusion and amperometric detection

WARNING — Persons using this International Standard should be familiar with the normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions including neutralization and proper disposal of waste solutions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this International Standard be carried out by suitably qualified staff.

1 Scope

This International Standard specifies methods for the determination of available free cyanide at pH 6 in various types of water (such as ground, drinking, surface, leachate, waste water, and metallurgical processing waste water) with cyanide concentrations from 5 µg/l to 500 µg/l expressed as cyanide ions in the undiluted sample. The range of application can be changed by varying the operation conditions, e.g. by using a different injection volume ([Figure A.1](#)).

NOTE 1 ISO 2080:2008, 3.105, the concentration of available free cyanide as determined by a specified analytical method.

NOTE 2 The detection limit for this method was determined by interlaboratory testing at the national level using ASTM International D6512 Practice for Interlaboratory Quantitation Estimate.

NOTE 3 Free cyanides according to ISO 14403 and ISO 17690 are not equivalent.

In this method, two suitable mass concentration ranges from 5 µg/l to 50 µg/l and from 50 µg/l to 500 µg/l are described.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

ISO 8466-2, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 2: Calibration strategy for non-linear second-order calibration functions*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1 available free cyanide (pH 6)
sum of HCN, cyanide ions, and cyanide bound in the metal-cyano complexes that are easily dissociated into HCN/CN⁻ at the pH of 6 determined in accordance with this International Standard

4 Interferences

4.1 Interferences by oxidizing agents

Test for the presence of oxidizing agents. Acidify KI starch paper by moistening with acetate buffer (6.7.1). Add a drop of the sample to the test paper as soon as the sample is collected; a blue colour indicates the need for treatment. If oxidizing agents are present, add powdered sodium arsenite (6.9) (0,1 g/l sample) to the sample to avoid degradation of cyanide and mix well. Repeat this test until a drop of treated sample no longer produces a blue colour on the acidified KI starch test paper.

4.2 Interferences by sulfide

Sulfide will diffuse through the gas diffusion membrane and can be detected in the amperometric flow cell. Oxidized products of sulfide can also rapidly convert CN⁻ to SCN⁻ at a high pH. Test for sulfide by moistening lead acetate paper with acetate buffer solution (6.7.1) and then add a drop of sample on the lead acetate paper. If the paper turns black, sulfide is present. Add powdered lead carbonate (6.8) (0,1 g/l of sample). Repeat this test until a drop of treated sample no longer darkens the acidified lead acetate test paper. The supernatant containing cyanide shall be filtered immediately to avoid the rapid loss of cyanide due to the formation of thiocyanate.

Lead acetate test strips might not be sensitive enough to detect low levels of sulfide; therefore, treatment should be performed on samples where sulfide is suspected. Interference can be confirmed by analysing the sample with or without treatment. If the measured cyanide in the untreated sample is significantly higher than in the treated sample, sulfide is likely present and treatment should be performed to remove sulfide.

5 Principle

The sample is introduced into a carrier solution of the flow injection analysis (FIA) system through an injection valve and confluence downstream with a phosphate buffer solution at pH 6 to measure available free cyanide. The released hydrogen cyanide (HCN) gas diffuses through a hydrophobic gas diffusion membrane into an alkaline acceptor stream where the CN⁻ is captured and sent to an amperometric flow cell detector with a silver-working electrode. In the presence of cyanide, silver electrode surface is oxidized at the applied potential ($E_{app} = 0,0$ V vs. the reference electrode). The anodic current measured is proportional to the concentration of cyanide in the standard or sample injected.

Calibrations and sample data are processed with the instrument's data acquisition software.

6 Reagents

WARNING — Cyanide solutions and wastes are toxic. Waste containing these substances shall be removed appropriately.

Use only reagents of recognized analytical grade.

6.1 Water, grade 1, as defined in ISO 3696.

6.2 Sodium hydroxide solution I, acceptor solution, $c(\text{NaOH}) = 0,1$ mol/l.

6.3 Sodium hydroxide solution II, $c(\text{NaOH}) = 1,0$ mol/l.

6.4 Sodium hydroxide solution III, $c(\text{NaOH}) = 0,01 \text{ mol/l}$.

6.5 Potassium cyanide, KCN.

6.5.1 Potassium cyanide solution, KCN, $\rho_{(\text{CN})} = 1\,000 \text{ mg/l}$, (see [Annex B](#)).

Dissolve $2\,500 \text{ mg} \pm 1 \text{ mg}$ of potassium cyanide, KCN, in sodium hydroxide solution III ([6.4](#)) in a $1\,000 \text{ ml}$ graduated flask and make up to volume with sodium hydroxide solution III ([6.4](#)).

This solution is stable for six months at $(5 \pm 3) ^\circ\text{C}$, if stored in the dark or brown bottles.

Alternatively, a potassium tetracyanozincate solution ([6.6.1](#)) can be used.

6.5.2 Cyanide solution I, $\rho_{(\text{CN})} = 10 \text{ mg/l}$.

Pipette $1,00 \text{ ml}$ of the potassium cyanide solution ([6.5.1](#)) in a 100 ml graduated flask and bring to volume with sodium hydroxide solution III ([6.4](#)).

This solution is stable for one week at $(5 \pm 3) ^\circ\text{C}$, if stored in the dark or brown bottles.

6.5.3 Calibration solutions.

Prepare at least five and up to ten calibration solutions with cyanide concentrations, equidistantly distributed over the working range, either by appropriate dilution of the cyanide solution I ([6.5.2](#)).

If, for example, six calibration solutions should be prepared to cover the range of $5 \text{ }\mu\text{g/l}$ to $50 \text{ }\mu\text{g/l}$, proceed as follows.

Pipette 25 ml of the cyanide solution I ([6.5.2](#)) in a 500 ml graduated flask and make up to volume with sodium hydroxide solution III ([6.4](#)). This solution contains $0,5 \text{ mg/l}$ cyanide.

Pipette, in 100 ml graduated flasks, 1 ml , 3 ml , 5 ml , 7 ml , 9 ml , or 10 ml , respectively, of the above mentioned $0,5 \text{ mg/l}$ cyanide solution and make up to volume with sodium hydroxide solution III ([6.4](#)). These solutions contain nominally $5 \text{ }\mu\text{g/l}$, $15 \text{ }\mu\text{g/l}$, $25 \text{ }\mu\text{g/l}$, $35 \text{ }\mu\text{g/l}$, $45 \text{ }\mu\text{g/l}$, and $50 \text{ }\mu\text{g/l}$ of cyanide, respectively. Correct calibration solution concentrations based on the concentration found on titration of the potassium cyanide solution ([6.5.1](#)) following the procedure given in [Annex B](#) by multiplying the nominal value by $\rho_{(\text{CN})}/1\,000$ and round to the nearest $\mu\text{g/l}$, or, for example, if six calibration solutions should be prepared to cover the range of $50 \text{ }\mu\text{g/l}$ to $500 \text{ }\mu\text{g/l}$, proceed as follows.

Pipette 25 ml of the cyanide solution I ([6.5.2](#)) in a 50 ml graduated flask and make up to volume with sodium hydroxide solution III ([6.4](#)). This solution contains 5 mg/l cyanide.

Pipette, in 100 ml graduated flasks, 1 ml , 3 ml , 5 ml , 7 ml , 9 ml , or 10 ml , respectively, of the above mentioned 5 mg/l cyanide solution and make up to volume with sodium hydroxide solution III ([6.4](#)). These solutions contain nominally $50 \text{ }\mu\text{g/l}$, $150 \text{ }\mu\text{g/l}$, $250 \text{ }\mu\text{g/l}$, $350 \text{ }\mu\text{g/l}$, $450 \text{ }\mu\text{g/l}$, and $500 \text{ }\mu\text{g/l}$ of cyanide, respectively. Correct calibration solution concentrations based on the concentration found on titration of the potassium cyanide solution ([6.5.1](#)) following the procedure in [Annex B](#) by multiplying the nominal value by $\rho_{(\text{CN})}/1\,000$ and round to the nearest $\mu\text{g/l}$.

6.5.4 Electrode stabilization solution, approximately 5 mg/l as CN^- .

Pipette $500 \text{ }\mu\text{l}$ of potassium cyanide solution ([6.5.1](#)) into a 100 ml volumetric flask containing $1,0 \text{ ml}$ of sodium hydroxide solution I ([6.2](#)). Dilute to volume with water.

This solution is stable for one week if stored at $(5 \pm 3) ^\circ\text{C}$.

NOTE Lower cyanide concentrations can be used provided the detector signal is near saturation.

6.6 Potassium tetracyanozincate, $K_2Zn(CN)_4$.

6.6.1 Potassium tetracyanozincate solution, $K_2Zn(CN)_4$, $\rho_{(CN)} = 1\,000\text{ mg/l} \pm 2\text{ mg/l}$, commercially available.

This solution is stable for six months at $(5 \pm 3)^\circ\text{C}$, if stored in the dark.

6.7 Reagents for the determination of available free cyanide.

6.7.1 Acetate buffer.

Dissolve 410 g of sodium acetate trihydrate ($NaC_2H_3O_2 \cdot 3H_2O$) in 500 ml of water. Add glacial acetic acid (approximately 500 ml) to yield a pH of 4,5.

6.7.2 Buffer solution A, 2 mol/l sodium phosphate monobasic solution.

Weigh 276 g dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) in a 1 l volumetric flask. Dissolve and dilute to volume with water.

6.7.3 Buffer solution B, 1 mol/l sodium phosphate di-basic solution.

Weigh 142 g disodium hydrogen phosphate (Na_2HPO_4) in a 1 l volumetric flask. Dissolve and dilute to volume with water. If necessary, warm to approximately 40°C on a hot plate and stir to completely dissolve the sodium phosphate dibasic into the water. Allow the solution to cool prior to use.

6.7.4 1 mol/l phosphate buffer pH 6,0 stock solution.

Add 219,25 ml buffer solution A (6.7.2) and 61,5 ml of buffer solution B (6.7.3) to a 500 ml volumetric flask. Dilute to volume with water.

6.7.5 0,2 mol/l phosphate buffer pH 6,0.

In a 1 l volumetric flask, add 200 ml 1 mol/l phosphate buffer solution pH 6,0 stock solution (6.7.4) and dilute to volume with water. The pH should be $6,0 \pm 0,1$. Verify the pH and adjust if necessary with sodium hydroxide solution III (6.4) or dilute sulfuric acid solution (6.7.6).

6.7.6 Dilute sulfuric acid solution, $c(H_2SO_4) = 0,005\text{ mol/l}$.

6.8 Lead carbonate, $PbCO_3$, powder.

6.9 Sodium arsenite, $NaAsO_2$, powder.

7 Apparatus

7.1 Flow injection analysis system.

A suitable example of the system is shown in [Figure A.1](#). Alternative systems are also applicable if the requirements in [Clause 9](#) are achieved.

7.1.1 Autosampler or another device, allowing a reproducible introduction of the sample.

7.1.2 Reagent reservoirs.

7.1.3 Low pulsation pump, with specific chemically inert pump tubes for flow rates as shown in [Figure A.1](#) as an example.

7.1.4 Gas diffusion cell, with hydrophobic semipermeable membrane from, e.g. polypropylene or PTFE, typical thickness 90 µm to 200 µm, pore size 0,1 µm to 1 µm, and minimum area of 150 mm² in contact with acceptor solution. The gas diffusion membrane should be replaced when the baseline becomes noisy or every one to two weeks.

7.1.5 Manifold with highly reproducible dosing of sample and reagents, with appropriate transport systems and connection assemblies made of chemically inert polymers.

7.1.6 Amperometric detector, with flow cell, to include a silver working electrode, an Ag/AgCl reference electrode, and a Pt or stainless steel counter electrode.

7.1.7 Recording unit (e.g. strip chart recorder, integrator, or printer/plotter).

In general, signal peak height is measured. Use the computer hardware and software recommended by the instrument manufacturer to control the apparatus and to collect data from the detector.

7.2 Additional apparatus, materials and measuring device.

7.2.1 Lead acetate test paper, commercially available.

7.2.2 Potassium iodide starch test paper, commercially available.

7.2.3 Syringe membrane filter assembly, with membrane filters having a pore size of 0,45 µm.

7.2.4 pH meter and electrode, capable of measuring $\pm 0,1$ pH units.

8 Sampling and sample preparation

Refer to [Clause 4](#) on Interferences and treat samples prior to adjusting pH.

Immediately after sampling, bring the pH of the water samples to $11 \pm 0,1$ with sodium hydroxide solutions I to III ([6.2](#) to [6.4](#)) such that the quantity of added alkaline yields a negligible dilution of the sample. Alternatively, bring the pH of the water samples to $11 \pm 0,1$ by adding sodium hydroxide pellets (one to two pellets per 500 ml). Avoid excess preservation as it can result in problems with a low recovery and/or poor peak shape of available free cyanide during analysis.

If sodium hydroxide pellets are used, take care not to raise the pH above 11,1.

If the sample appears turbid, remove the particles by filtration at 0,45 µm or by decantation at the laboratory.

Analyse the sample in accordance with [Clause 9](#) as soon as possible after sampling, at the latest within 6 d, but as specified in ISO 5667-3, no longer than 1 d if sulfide is present. Collect and store samples in containers which protect the samples from UV light.

9 Procedure

9.1 Flow system set up

Set up and adjust the flow analysis system according to [Table 1](#).

Table 1 — Adjustment of flow injection analysis system

FIA instrument parameter	Recommended method setting
Pump flow rates	0,5 ml/min to 2,0 ml/min
Cycle period (total)	120 s maximum
Sample load period	At least enough time to completely fill the sample loop prior to injection
Injection valve rinse time between samples	At least enough time to rinse the sample loop and achieve baseline resolution between peaks
Peak evaluation	Peak height or area
Working potential	0,0 V vs. Ag/AgCl

Turn on the power to the apparatus and the auto-sampler (if equipped).

Clamp the pump tube platens in place and start pumping reagents in the flow injection system.

Start the data acquisition system.

Pump reagents for 10 min to 30 min to establish a steady baseline. The analyser is ready for use when the baseline is stable.

Proceed according to [9.2](#) to [9.5](#).

9.2 Reagent blank measurement

Pump reagents through all the tubes and verify that there are no leaks and no air in the sample or reagent tubing. Adjust detector to 0,0 V.

Wait for a steady baseline and ensure that baseline noise is low enough to attain a minimum 2:1 signal to noise ratio for the lowest calibration standard. Use the sodium hydroxide solution III ([6.4](#)) as the reagent blank solution.

9.3 Checking the suitability of the flow injection system

9.3.1 Electrode stabilization

Inject the electrode stabilization solution ([6.5.4](#)) into the apparatus and record the amperometric response (current value) after the cycle period has been completed.

Repeat this procedure until the peak responses are less than 2 % RSD. This process will ensure that the electrode system has stabilized.

After the electrode system has stabilized, aspirate the highest working standard ([6.5.3](#)) into the flow injection apparatus.

Follow the instrument manufacturer's instructions to store the retention time window for cyanide using the data acquisition software.

9.3.2 Recovery rates

If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study shall be performed to demonstrate laboratory capability.

Analyse seven replicates of an independent reference solution containing 25 µg/l available free cyanide as CN⁻ in the low range of 5 µg/l to 50 µg/l and 250 µg/l available free cyanide as CN⁻ in the high range from 50 µg/l to 500 µg/l. The matrix of the solution should be equivalent to the solution samples to be

analysed. Each replicate shall be taken through the complete analytical procedure. The replicates can be interspersed with samples.

Calculate the mean and standard deviation of the seven values. The mean should range from 22 µg/l CN⁻ to 28 µg/l CN⁻, and the standard deviation should be less than 2 µg/l CN⁻, in the low range of 5 µg/l to 50 µg/l. The mean should range from 237 µg/l CN⁻ to 263 µg/l CN⁻, and the standard deviation should be less than 6,3 µg/l CN⁻ in the high range of 50 µg/l to 500 µg/l. Otherwise, the study should be repeated until these criteria are met.

If a concentration other than the recommended concentration is used, apply the F-test and *t*-test in evaluating the acceptability of the mean and standard deviation.

9.4 Calibration

Select the working mode of the flow system and calibrate by sequentially applying the calibration solutions (6.5.3) and the sodium hydroxide solution III (6.4) as the blank. Select calibration solutions most appropriate for the samples to be measured.

Prior to the calibration, establish a steady baseline as given in 9.1 and 9.2.

Determine the detector response values from the calibration solutions.

The test conditions for the calibration and the measurement of samples (9.5) are the same. The magnitude of the measuring signal is proportional to the mass concentration of cyanide. Establish the regression line for the measuring series obtained.

Calibrate the flow system as specified in ISO 8466-1. The following general formula as shown in Formula (1) is appropriate (ISO 8466-1). If the linearity test described in ISO 8466-1 shows that the calibration curve is not linear, calculate the calibration curve as specified in ISO 8466-2.

$$y = b \times \rho_{(\text{CN})} + a \quad (1)$$

where

- y* is the measured value for the calibration solutions in terms of instrument related units (e.g. peak heights in centimetres or counts);
- b* is the slope of the calibration function, expressed in instrument related units/micrograms per litre;
- $\rho_{(\text{CN})}$ are the mass concentrations of the standard solutions, expressed in micrograms per litre, µg/l;
- a* is the ordinate intercept, expressed in instrument related units.

9.5 Sample measurement

Analyse the samples pre-treated according to [Clause 8](#) in the same way as the calibration solutions with the flow injection system.

Check the validity of the calibration function after each sample series, but at least after the measurement of 10 to 20 samples using one calibration solution each for the lower and upper part of the working range.

Make a new calibration, if necessary.

10 Calculations

Determine the mass concentrations of the samples using the measured values, obtained as described in [9.4](#) for the calibration solutions.

Calculate $\rho_{(\text{CN})}$ using Formula (2):

$$\rho_{(\text{CN})} = \frac{y-a}{b} \quad (2)$$

For an explanation of symbols, see [9.4](#).

11 Expression of results

Report the results to two significant figures at most.

EXAMPLE ρ (available free CN) 45 µg/l

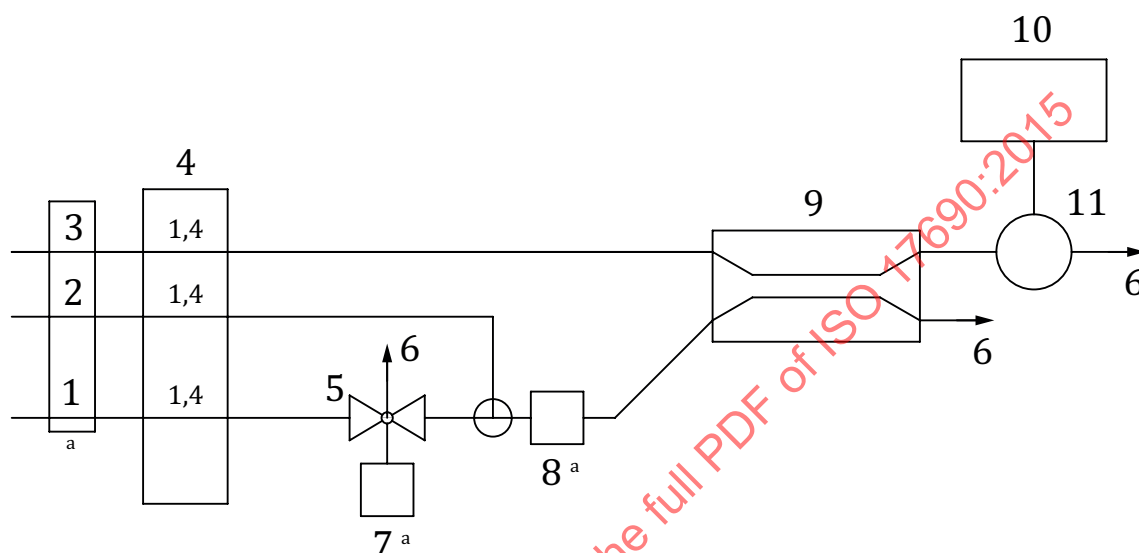
12 Test report

This test report shall contain at least the following information:

- a) the test method used, together with a reference to this International Standard (ISO 17690:2015);
- b) all information necessary for identification of the sample;
- c) any sample pre-treatment such as described in [Clause 4](#) for interferences;
- d) the cyanide concentration, available free cyanide in micrograms per litre (µg/l), in accordance with [Clause 10](#);
- e) any special observations noted during the determination;
- f) documentation framework (e.g. date of the test, operator, etc.);
- g) any deviations from this International Standard which could have affected the result.

Annex A (informative)

Example of a flow injection system



Key

- 1 carrier (water) ([6.1](#))
- 2 reagent buffer (pH 6) ([6.7.5](#))
- 3 acceptor solution (0,1 mol/l NaOH) ([6.2](#))
- 4 peristaltic pump (flow rates in ml/min)
- 5 injection valve (200 µl sample loop)
- 6 waste flows
- 7 sample
- 8 mixing coil (30 cm to 60 cm × 0,5 mm to 0,8 mm i.d.) positioned in optional constant temperature manifold
- 9 gas-diffusion cell
- 10 potentiostat/data collection device running data acquisition software
- 11 amperometric flow cell
- a t = const

Figure A.1

Annex B (normative)

Determination of the real cyanide concentration in the potassium cyanide solution (6.5.1)

B.1 General

If KCN is used to prepare the cyanide calibration solutions (6.5.3), proceed as follows.

B.2 Additional reagents

B.2.1 p-dimethylaminobenzylidene rhodanine.

B.2.2 Indicator solution.

Dissolve 0,02 g of p-dimethylaminobenzylidene rhodanine (B.2.1) in 100 ml of acetone (C₃H₆O). This solution is stable for one week if stored in a refrigerator (5 ± 3) °C.

B.2.3 Silver nitrate solution, $c_{(\text{AgNO}_3)} = 20 \text{ mmol/l}$ or 50 mmol/l .

B.3 Determination of cyanide concentration in potassium cyanide solution (6.5.1)

Pipette into a beaker 10 ml of potassium cyanide solution (6.5.1). Add 0,25 ml of indicator solution (B.2.2). Titrate with the silver nitrate solution (B.2.3) until the colour changes from yellow to yellow-red (consumption V_1).

Calculate the cyanide concentration in the potassium cyanide solution (6.5.1) using Formula (B.1):

$$\rho_{(\text{CN})} = \frac{V_1 c_{(\text{AgNO}_3)} M_{(2\text{CN})}}{V} \quad (\text{B.1})$$

where

$\rho_{(\text{CN})}$ is the cyanide concentration in the potassium cyanide solution (6.5.1) in milligram per litre, mg/l;

V_1 is the quantity of silver nitrate solution (B.2.3) used in millilitres, ml;

$c_{(\text{AgNO}_3)}$ is the concentration of silver nitrate solution in millimoles per litre, mmol/l;

$M_{(2\text{CN})}$ is the molar mass of 2 CN (= 52 g/mol);

V is the volume of the potassium cyanide solution (6.5.1) in millilitres, ml.