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Water quality — Determination of the methylene blue active substances (MBAS) index — Method using continuous flow analysis (CFA)

Qualité de l'eau — Mesurage de l'indice des substances actives au bleu de méthylène (SABM) — Méthode par analyse en flux continu (CFA)

Reference number
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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 16265 was prepared by Technical Committee 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 processing of many analytes in water in large numbers of samples at a high analysis frequency (up to 100 samples per hour).

A differentiation is made between flow injection analysis (FIA) [1], [2] and continuous flow analysis (CFA) [3]. Both methods share the feature of an automatic dosage of the sample into a flow system (manifold) where the analytes in the sample react with the reagent solutions on their way through the manifold. The sample preparation may be integrated in the manifold. The reaction product is measured in a flow detector (e.g. a photometer). The detector produces a signal from which the concentration of the parameter is calculated.

The MBAS (methylene blue active substances) index is an analytical convention (a method-defined parameter) used for water quality control purposes. It measures surfactants and other substances that react with methylene blue under specified conditions.

The user should be aware that particular problems could require the specification of additional conditions.

Water quality — Determination of the methylene blue active substances (MBAS) index — Method using continuous flow analysis (CFA)

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This 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.

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

Trichloromethane and methanol waste solutions should be disposed of properly.

1 Scope

This International Standard specifies a procedure for the determination of the methylene blue active substances (MBAS) index, in the ranges 0,05 mg/l to 0,5 mg/l and 0,5 mg/l to 5,0 mg/l, in various water samples (e.g. ground water, drinking water, surface water, waste water and leachates). Anionic surfactants are the most important substances showing methylene blue activity. This method is therefore useful for estimating the anionic surfactant content [including anionic surfactants with carboxylate groups (e.g. soaps)] of water. Other types of substance may also show methylene blue activity and contribute to the result. On a case-by-case basis, the range of the analysis may be changed and the method used for other concentration ranges provided they cover exactly one decade of concentration units.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the reference document (including any amendments) applies.

ISO 648, *Laboratory glassware — Single-volume pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

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

3 Interferences

The following substances can interfere with the analysis:

- Cationic compounds able to form strong ion-pairs with the active substances in methylene blue.
- Humic acids in concentrations > 20 mg/l.
- Chemicals with a high surface activity (e.g. non methylene blue active surfactants in concentrations > 50 mg/l).

- Chemicals with a strong reductive potential for the oxidation of methylene blue (e.g. S^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, OCl^-). These chemicals shall be removed (e.g. with H_2O_2) prior to analysis.
- High concentrations of inorganic anions (e.g. nitrate, chloride, bromide) may cause positive bias.
- Chemicals which react with any of the reagents used to form a coloured compound soluble in trichloromethane ($CHCl_3$) (excluded are methylene blue active substances).

Filtration of the sample before analysis is advisable for samples containing particles larger than 100 µm in size. Otherwise the particles in the sample may clog the transport tubes. If filtration is necessary, significant losses of anionic surfactants due to adsorbance effects are possible.

The absorbance due to the colour of the sample can be compensated for by a blank analysis which omits the methylene blue from the reagents. The responses of the sample with and without reagent addition are determined. The difference between the two responses is used in Equations (3) and (4).

Samples with an MBAS index concentration > 5 mg/l shall be diluted before analysis.

4 Principle

The sample is mixed, in a continuously flowing stream, with an alkaline methylene blue solution, forming coloured ion-pairs with certain types of organic substance (e.g. anionic surfactants) contained in the sample. The ion-pairs are extracted with trichloromethane. The organic phase is treated with an acidic methylene blue solution and its absorbance determined photometrically at a wavelength of 650 nm ± 10 nm.

The result is expressed in terms of sodium dodecyl sulfate concentration.

5 Reagents

Unless otherwise stated, use only reagents of recognized analytical grade.

Degas reagents if air bubbles appear spontaneously. Unless otherwise specified, degas by passing helium gas at 20 l/h through the reagent for 15 min. Add detergent after degassing. Avoid using reagents showing any turbidity, filtering them if necessary.

5.1 Water, complying with grade 1 as defined in ISO 3696.

5.2 Sodium hydroxide, NaOH.

5.3 Sodium tetraborate decahydrate, $Na_2B_4O_7 \cdot 10H_2O$.

5.4 Sodium dihydrogen phosphate monohydrate, $NaH_2PO_4 \cdot H_2O$.

5.5 Potassium dihydrogen phosphate monohydrate, $KH_2PO_4 \cdot H_2O$.

5.6 Sulfuric acid, H_2SO_4, $\rho(H_2SO_4) = 1{,}84$ g/ml.

5.7 Hydrochloric acid, HCl, $\rho(HCl) = 1{,}18$ g/ml.

5.8 Methylene blue, $C_{16}H_{18}N_3SCl \cdot 2H_2O$.

5.9 Methanol, CH_3OH.

5.10 Ethanol, C_2H_5OH, $w(C_2H_5OH) \approx 96$ %.

5.11 Trichloromethane, $CHCl_3$.

Trichloromethane is stable for 3 months if stored in a dark place.

Before use, degas the trichloromethane for 30 min by purging with a stream of He or by using an ultrasonic bath.

5.12 Sodium dodecyl sulfate (sodium lauryl sulfate), $CH_3(CH_2)_{11}OSO_3Na$.

5.13 Petroleum ether.

5.14 Poly(ethylene glycol) dodecyl ether, $HO(CH_2CH_2O)_nC_{12}H_{25}$, aqueous solution, $w = 30$ %.

5.15 Alkaline borate solution.

Dissolve 15,83 g of sodium tetraborate decahydrate (5.3) and 3,3 g of sodium hydroxide (5.2) in approximately 800 ml of water (5.1) and make up to 1 000 ml with water (5.1) in a volumetric flask (6.2.1).

This solution is stable for one week if stored at room temperature.

5.16 Stock methylene blue solution.

Dissolve 0,35 g of methylene blue in 500 ml of ethanol (5.10) and make up to 1 000 ml with water (5.1) in a volumetric flask (6.2.1).

This solution is stable for 6 months if stored at room temperature.

5.17 Alkaline methylene blue solution.

Add 100 ml of stock methylene blue solution (5.16) to 50 ml of alkaline borate solution (5.15) and mix. Wash this mixture three times with 20 ml of trichloromethane (5.11) until the organic layer is no longer blue, removing the organic layer each time. Extract the aqueous solution thus obtained with 25 ml of petroleum ether (5.13) to remove the trichloromethane remaining in the solution. Filter through a paper filter (pore width 0,45 µm) and make up to 500 ml with water (5.1) in a volumetric flask (6.2.1).

Prepare this solution freshly before use.

5.18 Acidified methylene blue solution.

Add 6,8 ml of sulfuric acid (5.6) to 42,5 ml of stock methylene blue solution (5.16). Dissolve in this mixture 50 g of sodium dihydrogen phosphate monohydrate (5.4). Make up to a volume of 1 000 ml with water (5.1) in a volumetric flask (6.2.1).

This solution is stable for one week if stored at room temperature.

5.19 Trichloromethane extraction solution ($CHCl_3$ in Figure A.1).

Add 1 ml of poly(ethene glycol) dodecyl ether (5.14) to 1 000 ml of trichloromethane (5.11) and mix.

This solution is stable for one week if stored at room temperature.

5.20 Rinsing solution for the sampler.

Dissolve 1,1 g of potassium dihydrogen phosphate monohydrate (5.5) in approximately 800 ml of water (5.1) and make up to 1 000 ml with water (5.1) in a volumetric flask (6.2.1).

This solution is stable for one week if stored at room temperature.

5.21 Methanol solution.

To 800 ml of methanol (5.9) add 100 ml of hydrochloric acid (5.7) and make up to 1 000 ml with methanol (5.9).

This solution is stable for 6 months if stored at room temperature.

5.22 Sodium dodecyl sulfate solutions.

5.22.1 Sodium dodecyl sulfate stock solution, $\rho = 1\ 000$ mg/l.

In a 1 000 ml volumetric flask (6.2.1), dissolve 1 000 mg ± 1 mg of sodium dodecyl sulfate (5.12) in water (5.1) and make up to volume with water (5.1).

This solution is stable for 3 months if stored at room temperature in a glass container.

5.22.2 Sodium dodecyl sulfate solution, $\rho = 100$ mg/l.

In a 100 ml volumetric flask (6.2.1), dilute 10 ml of sodium dodecyl sulfate stock solution (5.22.1) with water (5.1) and make up to volume with water (5.1).

This solution is stable for 1 week if stored at room temperature in a glass container.

5.23 Calibration solutions.

5.23.1 General

Prepare calibration solutions by diluting the sodium dodecyl sulfate solution (5.22.2).

Prepare the calibration solutions in glass containers freshly before use.

At least five calibration standards per working range are recommended.

As an example, if six standards are used, proceed for working ranges I and II as follows:

5.23.2 Range I (0,05 mg/l to 0,5 mg/l)

Pipette 0,5 ml, 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml of sodium dodecyl sulfate solution (5.22.2) into a series of 1 000 ml volumetric flasks (6.2.1). Make up to volume with water (5.1).

The mass concentrations of sodium dodecyl sulfate in the resulting calibration solutions are 0,05 mg/l, 0,1 mg/l, 0,2 mg/l, 0,3 mg/l, 0,4 mg/l and 0,5 mg/l.

In addition, a 0,25 mg/l solution will be required for daily sensitivity testing (see 8.2.2).

5.23.3 Range II (0,5 mg/l to 5 mg/l)

Pipette 0,5 ml, 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml of sodium dodecyl sulfate solution (5.22.2) into a series of 100 ml volumetric flasks (6.2.1). Make up to volume with water (5.1). The mass concentrations of sodium dodecyl sulfate in the resulting calibration solutions are 0,5 mg/l, 1 mg/l, 2 mg/l, 3 mg/l, 4 mg/l and 5 mg/l.

In addition, a 2,5 mg/l solution will be required for daily sensitivity testing (see 8.2.2).

6 Apparatus

Usual laboratory equipment and, in particular, the following:

6.1 Continuous flow analysis (CFA) system.

An example of a suitable system contains the following components (see also Figure A.1). Other systems can be used provided the requirements given in Clause 8 are met.

All tubing which comes into contact with trichloromethane shall be made from a trichloromethane-resistant material.

6.1.1 Reagent containers.

6.1.2 Low-pulse pump, with suitable pump tubes, if required.

6.1.3 Transport tubes, tube connections and T-connections, made of inert material and with minimum dead volumes.

6.1.4 Phase separator system, for aqueous and trichloromethane solutions, e.g. gravimetric or membrane separators.

6.1.5 Photometer with flow cell, wavelength range 650 nm ± 10 nm.

6.1.6 Data registration unit, e.g. strip chart recorder, integrator or printer/plotter, or data acquisition system; in general, peak height signals are evaluated.

6.1.7 Autosampler, if required, having suitable arrangements to avoid memory effects when analysing a series of samples (e.g. intermediate rinsing of the flow system with a mixture of methanol and water).

6.2 Additional apparatus.

6.2.1 Glass volumetric flasks, class A as defined in ISO 1042, of capacities 100 ml, 200 ml and 1 000 ml.

6.2.2 Glass volumetric pipettes, class A as defined in ISO 648, of capacities 0,5 ml to 10 ml.

7 Sampling and sample pretreatment

Store the samples in glass containers at low temperatures (2 °C to 5 °C) for a maximum of 3 days. Longer storage times need to be checked. Some anionic surfactants are metabolized very rapidly due to microbial activity in the sample. This shall be taken into consideration for sample storage.

The addition of a preservative should be considered if the sample is stored for more than 24 h. The addition of 1 % volume fraction of a 40 % formaldehyde solution is suitable for periods of up to 4 days. Do not preserve samples by using mineral acid.

Test samples should be free of suspended matter which can be separated by centrifugation; however, surfactant adsorbed on suspended matter will not be determined.

8 Procedure

8.1 Preparations for measurement

Set up the flow analysis system (CFA) in accordance with 6.1 (see Figure A.1).

Start the system and pump water (5.1) through all the lines.

After 15 min, place the trichloromethane line ($CHCl_3$ in Figure A.1) into methanol solution (5.21) and remove the other lines from the water (5.1).

After 5 min, place the trichloromethane line ($CHCl_3$ in Figure A.1) into the trichloromethane extraction solution (5.19).

Connect up the acidified methylene blue reagent (5.18) (R_2 in Figure A.1) a few minutes later than the alkaline methylene blue reagent (5.17) (R_1 in Figure A.1).

Run the system for 10 min. Wait for a stable baseline and adjust the baseline to zero. The system is ready for analysis when the baseline no longer shows any drift.

Proceed with steps 8.2 to 8.5.

To decontaminate the system, it is recommended that it be rinsed with hypochlorite solution (diluted 1:10) once a week for about 30 min.

8.2 Checks on the measurement system

8.2.1 Checking the reagent blank

Use water (5.1) as the sample and replace the acidified methylene blue reagent (5.18) (R_2 in Figure A.1) with water (5.1).

Wait for a stable baseline and measure the absorbance.

Set up the flow analyser in the original state and measure the change in the absorbance.

If the absorbance per cm increases by more than 0,03 cm^{-1}, the reagents or the water (5.1) entering the system may be contaminated with anionic surfactants, and suitable steps to eliminate the interference shall be undertaken before starting the analysis.

If the photometric detector (6.1.5) does not allow the absorbance to be read, the absorbance can be determined by comparison with an external absorbance-measuring photometer.

8.2.2 Daily sensitivity test and noise test

A calibration solution with a sodium dodecyl sulfate concentration of 0,25 mg/l in working range I (0,05 mg/l to 0,5 mg/l) (see 5.23.2) or of 2,5 mg/l in working range II (0,5 mg/l to 5 mg/l) (see 5.23.3) shall exhibit an absorbance of at least 0,025 cm^{-1}.

A calibration solution with the smallest sodium dodecyl sulfate concentration (0,05 mg/l within working range I, 0,5 mg/l within working range II) (see 5.23.2 and 5.23.3) shall exhibit a signal to noise ratio (S/N) of at least 3/1.

If a recorder is used for the evaluation, the sample probe shall be placed in a calibration solution (see 5.23) with a sodium dodecyl sulfate concentration in the middle of the working range being used. As soon as a positive response due to the colour produced from the calibration solution is visible on the recorder, the response shall be adjusted so that the recorder reading is about 45 % of full-scale deflection.

8.3 Calibration

Set up the required calibration solutions for each working range as specified in 5.23. Calibrate the system for each range separately.

To start the calibration, set the output signal to zero.

Calibrate by sequentially passing the calibration solutions and reagent blanks through the system.

Record the values measured for the calibration solutions used.

The procedure for calibration and for the analysis of samples (see 8.4) is the same.

The following general equation (as given in ISO 8466-1) is used as the equation for the linear calibration function:

$$y = a + b\rho \tag{1}$$

where

y is the value measured, expressed in instrument-related units;

ρ is the mass concentration, in milligrams per litre, of sodium dodecyl sulfate in the calibration solutions;

a is the ordinate intercept, expressed in instrument-related units, of the calibration function;

b is the slope, expressed in instrument-related units × litres per milligram, of the calibration function.

If the curve is not sufficiently linear (as determined by, for example, a test for linearity as described in ISO 8466-1), a second-order equation [Equation (2)] may be used (as described in ISO 8466-2):

$$y = a + b\rho + c\rho^2 \tag{2}$$

where

y is the value measured, expressed in instrument-related units;

ρ is the mass concentration, in milligrams per litre, of sodium dodecyl sulfate in the calibration solutions;

a is the ordinate intercept, expressed in instrument-related units, of the calibration function;

b is a coefficient, expressed in instrument-related units × litres per milligram, of the calibration function;

c is a coefficient, expressed in instrument-related units × (litres per milligram) squared, of the calibration function.

8.4 Measurement

Using the flow analysis system (6.1), analyse the samples in the same way as the calibration solutions.

If the mass concentration of a sample exceeds the selected working range, dilute the sample or use another working range.

Verify the validity of the calibration function for the selected working range before carrying out sample analysis and again after each sample series, at the most after 20 samples, using one calibration solution for the lower part of the working range used and another calibration solution for the upper part of the range. If necessary, recalibrate the system.

9 Calculation of the results

Determine the mass concentration of the MBAS in the solution analysed from the value obtained as described in 8.4 and using the appropriate calibration function as given in Equation (1) or (2) (see 8.3).

Do not extrapolate beyond the working range selected.

Calculate the result using Equation (3) in the case of a linear calibration function or using Equation (4) in the case of a second-order calibration function:

$$\rho = \frac{y - a}{b} \tag{3}$$

$$\rho = \frac{-b}{2c} - \sqrt{\left(\frac{b}{2c}\right)^2 - \frac{a - y}{c}} \tag{4}$$

For the meanings of the symbols used, see Equations (1) and (2). Take into account all dilution steps.

10 Expression of results

Report the results in milligrams per litre to, at the most, two significant figures.

EXAMPLES

MBAS index: 1,7 mg/l

MBAS index: 0,85 mg/l

11 Precision

The precision data given in Table B.1 were obtained by an interlaboratory trial performed in 2008 by DIN.

12 Test report

The test report shall include at least the following information:

a) a reference to this International Standard;

b) full identification of the water sample;

c) the working range used;

d) a description of the sample preparation;

e) the results, calculated in accordance with Clause 10;

f) details of any deviations from the procedure and conditions specified in this International Standard that may have affected the results;

g) the date of the test.