SIAPWS technical documents
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Determination of total iron by the Ferrozine method

Measuring range

2-1000 µg/L	5 cm cuvette
10-5000 µg/L	1 cm cuvette

Principle

Iron oxides are dissolved in hot, diluted thioglycolic acid. Thioglycolic acid rapidly reduces Fe(III) to Fe(II) in acidic solution. Ferrozine forms a stable and colored complex with Fe(II) in the pH range 4-10, and this makes a sensitive determination of iron possible by means of spectrophotometry.

Reactions

Thioglycolic acid dissolves iron oxides:

$Fe_2O_3 + 6HSCH_2COOH \rightarrow 2Fe^{3+} + 6HSCH_2COO^- + 3H_2O$
$Fe_3O_4 + 8HSCH_2COOH \rightarrow 2Fe^{3+} + Fe^{2+} + 8HSCH_2COO^- + 4H_2O$

Ferric ions are reduced by thioglycolic acid in acid environment:

$2Fe^{3+} + 2\ HSCH_2COOH \rightarrow 2Fe^{2+} + HOOCCH_2S\text{-}SCH_2COOH + 2H^+$

The anion of Ferrozine (fz^{2-}) forms a stable complex with Fe(II) between pH 4 and 10:

$Fe^{2+} + 3fz^{2-} \rightarrow [Fe(fz)_3]^{4-}$

Apparatus

Water bath thermostated at 90 °C

Sample bottles with screw cap, e.g. of pyrex glass (Schott Duran Blue/Green Cap, 100 mL)
Rinse sample bottles prior to use by applying a 2 % thioglycolic acid solution for 1 hour at 90 °C using the thermostated water bath.

Dosing burettes for thioglycolic acid (dosing 125 µL volume per sample bottle) and Ferrozine/buffer reagent (dosing 2,5 mL per sample bottle).

Spectrophotometer, preferably with a 5 cm cuvette and sample aspiration

Reagents

Thioglycolic acid, $CH_2(SH)COOH$, p.a.

Ferrozine/buffer reagent – use within 2-3 months
Dissolve 2 g Ferrozin (3-(2-pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine, disodium salt), $C_{20}H_{12}N_4Na_2O_6S_2$, in 300 mL ultrapure water.
Add 250 mL concentrated acetic acid, $C_2H_4O_2$ p.a., and then **slowly** and **under agitation** 210 mL concentrated ammonia, 25 % NH_3, p.a., **Take care, the reaction releases a lot of heat.**

Standards

Buy standard solutions of 1000 mg/L Fe from two different suppliers of chemicals. Use one brand for preparation of calibration standards and the other brand for preparation of control standards.

Prepare calibration standards in ultrapure water containing
0 (blank), 50, 100, 250, 500, and 1000 µg/L (5 cm cuvette)
or
0 (blank), 100, 200, 500, 1000, and 2000 µg/L (1 cm cuvette).
Calibration standards should be prepared fresh for every calibration (the calibration curve is stable for several months).

Prepare control standards in ultra pure water at suitable concentrations according to e.g. specs for the samples or typical measuring range, e.g. at 10 µg/L and 100 µg/L (5 cm cuvette) or 40 µg/L and 200 µg/L (1 cm cuvette). Acidify control standards to pH 2 by means of nitric acid, HNO_3 p.a., and store in HDPE (high density polyethylene bottles).

Procedures

Calibration of spectrophotometer

Add Ferrozine/buffer reagent to the calibration standards, 2,5 mL per 50 mL standard, allow the color to develop for at least 3 min.

Select a wavelength of 562 nm. Nullify the absorbance with ultrapure water in the cuvette. Calibrate the spectrophotometer following the instructions in the manual. If possible, measure the calibration standards twice in the calibration process.

Visually, a plot of absorbance versus concentration should appear perfectly linear, and linear regression should give a correlation indicating linearity ($R^2 > 0,99$).

A positive incept with the Y-axis indicates the combined absorbance from the Ferrozine reagent and the blank level of iron in the ultrapure water. The intercept should be ignored – i.e. the calibration line shifted down to pass through zero – when calculating concentration from absorbance. This is possible with most spectrophotometers. Thus, the relation between measured absorbance and concentration becomes:

$$C^* = K_s * A = 1/b * A$$

where

C^*	is the raw measured concentration
K_s	is the calibration constant
b	is the slope of the calibration line
A	is the measured absorbance

The calibration is normally stable over several month, but should be checked at least when a new batch of Ferrozine is taken into use.

Determination of reagent blank value

Prepare four blank samples using 2,5 mL Ferrozine/buffer reagent to 50 mL of ultrapure water. Allow 3 min for color development.
Prepare four blank samples with double reagent concentration using 5,0 mL of the Ferrozine/buffer reagent to 50 mL of ultrapure water. Allow 3 min for color development.

Measure all blank samples and note the measured concentrations. Calculate the reagent blank concentration as:

$$C_R = C_2 - C_1$$

and the blank concentration of the ultrapure water as:

$$C_B = 2*C_1 - C_2$$

where

C_R	is the concentration corresponding to the reagent absorbance
C_2	is the mean value of the blank samples with double amount of reagent
C_1	is the mean value of the blank samples with normal amount of reagent
C_B	is the blind value of iron in the ultrapure water.

Note, that the determination of reagent blank and blind value is only relevant for spectrophotometers using a 5 cm cuvette. With a 1 cm cuvette both may be neglected, since the reagent blank is usually 1-3 µg/L, and the blind value in ultrapure water is expected to be <10 µg/L.

Measuring samples, blank and control standards

A blind sample and the control standards are treated exactly as the samples in the following procedure.

1. 50 mL samples are taken out in rinsed sample bottles by filling to the mark.

2. 125 µL thioglycolic acid is added to each sample bottle. This conservation must be done as soon as possible after sampling, e.g. at site or after a few minutes transport to the laboratory. When conserved, the samples may be stored for at least 5 days before digestion and analysis.
3. The samples are digested by heating in the water bath at 90 °C in 30 min.
4. The samples are cooled to room temperature.
5. 2,5 mL Ferrozine/buffer reagent is added to each sample bottle. Allow at least 3 min for color development.
6. The spectrophotometer is set at 562 nm and nullified on ultrapure water.
7. The absorbance of the samples is measured.

Calculation

The iron concentration C (µg/L), is calculated from the absorbance by these equations:

$$C^* = K_s * A$$

$$C = C^* - C_R$$

where

C^*	is the concentration including the reagent contribution
K_s	is the calibration constant
A	is the measured absorbance
C_R	is the concentration corresponding to the reagent absorbance

Most spectrophotometers converts to concentration automatically. In this case, the reagent contribution is simply subtracted from the measured values.

A blank concentration in the ultrapure water is subtracted from the result of control samples only, not from real samples.

Safety

Thioglycolic acid is poisonous, smelly and etching. Handle the acid in a fume hood using suitable gloves. Check the local instructions for use of the chemical.

Waste should be collected and disposed of according to local regulations.