Visible Spectrophotometry

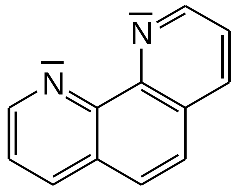
Basic Principles

Skoog 22A-2 (p.500) - p. 504 relations 22-4,5,6,7
22C-5 Beer‘s law rel. 22-8,9
calibration (curve) G-3
method of standard addition G-17
instrumentation 23A(p517) + Fig.23-1 only (b), not (a) and (c)

Analysis of water solutions of iron

Principle

Determination of iron in water samples is based on the formation of the stable orange-coloured complex of iron(II) with three molecules of 1,10-phenanthroline.



1,10-phenanthroline (or *o*-phenanthroline)

To keep the iron in the 2^+ state, a reducing agent, e. g. hydroxylamine hydrochlo-ride, should be added. Since the reaction is pH dependent, sodium acetate is needed for buffering at the optimum pH.

Reagents and Solutions

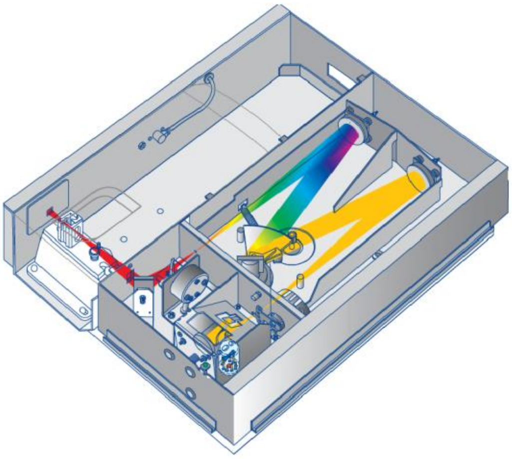
The *o*-phenanthroline solution is in the 2-ml dosing device; the acetate buffer containing hydroxylamine (pH=4.5) is in the 5-ml one.

Preparation of the standard iron(II) solution ($c=5\times10^{-4}$ mol l^{-1}, V=250 ml):

- weight accurately required amount of *bis*-ammonium-iron(II) sulphate hexahydrate $(NH_4)_2Fe(SO_4)_2.6H_2O$ (M=392.13 g mol^{-1});
- transfer the salt into a 250-ml volumetric flask and dissolve it in a small volume of distilled water;
- add 2.5 ml of a sulphuric acid solution using a graduated cylinder;
- dilute the solution up to the mark and mix it.

Samples

Every student will obtain two 50-ml volumetric flasks with sample. Samples in these flasks should be diluted to the mark and mixed thoroughly. The aim of this work is determination of the mass of iron(II) (*m*, mg) content in these samples.

Spectrophotometer Aligent Cary 60 UV-Vis

Instrument

The Agilent Cary 60 is a single beam UV-Vis spectrophotometer, which will be used for the measuring of absorbance when carrying out the working tasks.

Turn on the spectrophotometer Agilent Cary 60 and PC.

Choose the folder ”**photometry, task 5**” on the desktop.

In the folder “methods” you will find the list of the methods for today’s work.

Create the new folder for your data in the folder 2018 on disk *F*

Cuvette (cell)

Fill the cuvette (cell) with at least 1-2 ml of the sample. Before measurement, it is recommended to flush a cell three times with the measuring solution. Dry outer walls of the cell before inserting into the spectrophotometer. Clean optical windows attentively but carefully to avoid scratching of them. *Don’t touch the transparent walls of the cell and never dry cells inside!*

Working instructions

1) Absorption spectrum of the complex

a) Prepare a solution of iron(II): add 2 ml of a standard solution to a 25-ml volumetric flask, then add 5 ml of acetate buffer and 2 ml of the *o*-phenanthroline solution, dilute the mixture to the mark and mix thoroughly.

b) Prepare a solution for “zeroing” and “baseline”: add 5 ml of the acetate buffer and 2 ml of the *o*-phenanthroline solution (0.01 mol l^{-1}) into a 25-ml volumetric flask, dilute the mixture to the mark and mix thoroughly.

c) Setting parameters for recording an absorption spectrum: choose the method “absorption spectrum” and check the parameters in the part SETUP: “start **600** stop **400**”, scan controls “**medium**”, beam mode “**dual beam**”, baseline “**baseline correction**”.

d) Record a baseline spectrum of the “baseline” solution (see part 1 (b)).

e) Record an absorption spectrum of the iron(II) complex solution using command **START**.

f) Write down the wavelength and value of maximal absorption (λ_{max}); use value of the wavelength for all next measurements and value of maximal absorption to calculate the extinction coefficient.

2) *Stability of the complex*

a) Prepare a solution of iron(II) complex by the same way like in 1 (a) and measure absorbance at λ_{max} each 30 second during five minutes.

b) Setting parameters: choose the method “kinetics” and check the parameters in the part SETUP: wavelength – result from previous task 1; sample collect “**stage 1**”; “**cycle 0,5**” (min); “**stop 5**“ (min).

c) Use command **START** for recording.

3) *Calibration curve*

Prepare seven 25-ml flasks and add 5 ml of acetate buffer to each, then add 2 ml of *o*-phenanthroline solution and in successive steps 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml of the standard solution of iron(II) (see table below). Dilute to the mark, mix properly and measure the absorption.

Solution/ Flask	Volume (ml)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
$5 \cdot 10^{-4}$ mol·l^{-1} of Fe^{2+}	0	0.5	1.0	1.5	2.0	2.5	3.0
0.01 mol·l^{-1} of *o*-phenanthroline	2 ml to each flasks						
acetate buffer	5 ml to each flasks						

4) *Determination of iron in the sample*

USING CALIBRATION CURVE

a) Add 1 ml of a sample solution, 5 ml of the acetate buffer and 2 ml of the *o*-phenanthroline solution to three 25-ml volumetric flask. Dilute the mixtures to the mark and mix. Use the same steps for the second sample.

b) Setting parameters for measuring: choose the method “calibration curve” and check the parameters in the part SETUP: wavelength – result from previous task 1; “replicates **3**”; standards: “units **µmol l^{-1}**”; “standards **7**”; calculate the concentration of iron in your calibration solutions and put it in the table; “fit type **linear**”; “samples **6**”.

c) Zero the instrument using command ZERO for “zeroing” solution, see part 1 (b).

d) Use command **START** to record the absorption of the calibration solutions and samples.

e) Write down the absorption and concentration values for your samples.

f) Calculate the iron content (*m*, mg) in your samples using average concentration value. ($M_{(Fe)}$ = 55,847 g mol^{-1}).

5) *Determination of iron in the sample*

<u>by STANDARD ADDITION METHOD</u>

For the determination of iron concentration by this method, use a dilution of your sample having the absorbance value about 0.2.

a) Add a volume *n* (ml) of your sample solution corresponding to absorbance 0.2 to five 25-ml volumetric flasks, then successively add 0, 0.5, 1.0, 1.5, and 2.0 ml of the standard Fe(II) solution. Add 5 ml of the acetate buffer and 2 ml of the *o*-phenanthroline solution to each of the flasks. Dilute the mixtures to the mark and mix.

Solution/ Flask	Volume (ml)				
	1	**2**	**3**	**4**	**5**
sample	*n* ml of sample corresponded to A≈2				
$5\cdot10^{-4}$ mol·l^{-1} of Fe^{2+}	0	0.5	1.0	1.5	2.0
0.01 mol·l^{-1} of *o*-phenanthroline	2 ml to each flasks				
acetate buffer	5 ml to each flasks				

b) Setting parameters for measuring: choose the method “standard addition” and check the parameters in the part SETUP: wavelength – result from previous task 1; “replicates **3**”; standards: “units **μmol l^{-1}**”; “standards **5**”; calculate the concentration of iron in your calibration solutions and put it in the table (write the first five values of concentration from the table for calibration curve); “fit type **linear**”; “samples **0**”.

c) Zero the instrument using command ZERO for “zeroing” solution, see part 1 (b).

d) Use command **START** to measure the absorption of solutions using method of standard addition.

e) Write down the equation describing linear dependence and the correlation coefficient

f) Calculate the iron content (m, mg) in your samples using average concentration value. ($M_{(Fe)}$ = 55,847 g mol^{-1}).

Determination of the molar ratio and formation constant for a complex of iron(II) by the method of continuous variations (Job method)

Spectrophotometry is a valuable tool for elucidating the composition of complex ions in solution and for determination of their formation constants. In the method of continuous variations, cation and ligand solutions with identical analytical concentrations are mixed in such a way that the total volume (and hence the total amounts) of the reactants in each mixture is constant, but the molar ratio of the reactants varies systematically.

Reagents and Solutions

- Standard $5\cdot10^{-4}$ mol·l^{-1} solution of iron(II)
- Standard 5.10^{-4} mol·l^{-1} solution of *o*-phenanthroline (100 ml) will be prepared by diluting of 0.01 mol·l^{-1} solution *o*-phenanthroline.
- Acetate buffer (pH=4.5)

Working instructions

Prepare ten 25-ml flasks. Add the reactants to each flask according to the table below:

Solution/ Flask	Volume (ml)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
$5\cdot10^{-4}$ mol·l^{-1} of Fe^{2+}	0	0.5	1.0	1.5	2.0	2.5	3.0	5.0	8.0	10.0
acetate buffer	5 ml to each flasks									
$5\cdot10^{-4}$ mol·l^{-1} of *o*-phenanthroline	10,0	9,5	9,0	8,5	8,0	7,5	7,0	5,0	2,0	0

Then dilute to the mark, mix thoroughly and measure absorbance of each solution at a suitable wavelength.

Setting parameters for measuring: choose the method “job method” and check the parameters in the part SETUP: wavelength – result from previous task 1.

Zero the instrument using command ZERO for “zeroing” solution, see part 1 (b).

Use command **READ** to measure the absorption of solutions.

Evaluation of a mole ratio and a formation constant

$$\mathbf{M^{n+} + nL^{-} \leftrightarrow ML_n}$$

Prepare the plot of absorbance versus molar fraction of iron ($x_{Fe} = V$(ml) Fe^{2+}/ 10).

Calculate the molar ratio of complex based on A_{max} and x_{Fe}.

$$n = (1 - x_{A_{max}}) / x_{A_{max}}$$

Calculate the formation constant of complex based on the experimental data:

$$\beta = \frac{[ML_n]}{[M] \cdot [L]^n},$$

where

$[ML_n]$ is the molar concentration of formative complex at A_{max}

$$[ML_n] = \frac{A_{max(\lambda)}}{\varepsilon_{(\lambda)} b};$$

b= 1cm (cells)

[M] and [L] are the molar concentration of M and L after reaction corresponded to A_{max}

$$[M] = c(M) - [ML_n]$$

$$[L] = c(L) - n[ML_n]$$

c(M) and c(L) are the molar concentrations of M and L before reaction corresponded to A_{max}

$$c(M) = \frac{c(Fe^{2+})_{st} \cdot V_{Fe^{2+}}}{V_{delute}}$$

$$c(L) = \frac{c(L)_{st} \cdot V_L}{V_{delute}}$$

Problems

1. Explain the relation between the energy and the wavelength.
2. What energetic transitions occur when the molecules are irradiated by ultraviolet or visible light?
3. What is the absorption spectrum?
4. Define visible range of the electromagnetic spectrum in nanometres.
5. Which parts is a single-beam spectrophotometer consisted from?
6. Define transmittance, absorbance and the relation between them.
7. Write Lambert-Beer's law and explain the symbols (use molar concentration).
8. Why it is important to study the time-stability of a complex?
9. What is the calibration curve and explain for which purpose is it used?

10. What is the principle of the standard addition method and what is its advantage?