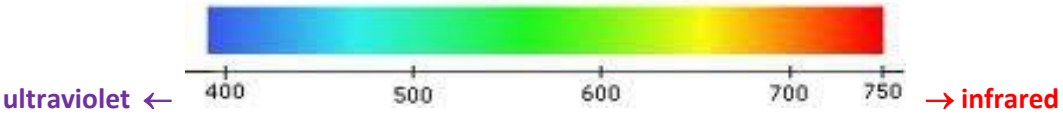
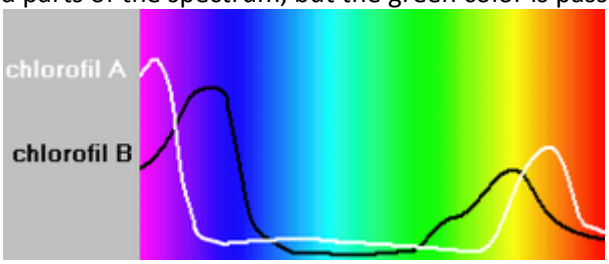
21. Colorimetric determination of Fe(III) using 1,10-phenanthroline or phosphorus using molybdenum blue

Color - it is psychical impression forming in brain when eyes receive electromagnetic radiation in the region of visible light[1].



Spectrum of white light – after diffraction

Colors are seen thanks to cells which are sensible to light and placed in retina, known as *rods* and *cones*. Rods are sensible to the degree of brightness, the cones also to colors. There are three kinds of cones in human eye, they are sensible to different areas of colors – blue, green and red.; these areas overlap. This allows to see all the colors. Our eyes have rather limited resolution to colors, sometimes we are not able to see difference between two areas of different spectral characteristics. In general, sensibility to colors is individual, sometimes also being the result of prolonged contact to colors (painters, printers).
So, human eye has different sensibility to colors; it depends on the number of cones of different kind. Ca. 4% of cones are responsible for detection of blue color, 32% for green and 64% for red. The subtle shades of blue and dark-red are worse detected than those of other colors.
The incredible richness of colors observed in the nature, in particular in plants, comes from specific group of organic compounds, among them are carotenoids (in carrot or tomatoes), betalains (in beetroots) or anthocyanids (petals, fruits, also stems and leafs).
From the physical point of view, colors are created when a part of the electromagnetic radiation forming white light is absorbed, but the remaining radiation is not. In this case one sees the mixture of colors which remain unabsorbed. As example: green color of leafs is caused by the presence of chlorophyll (more precisely, few kinds of this compound, mainly those denoted as A and B). Chlorophylls absorbs simultaneously in blue and red parts of the spectrum, but the green color is passes through.



Spectrum of chlorophylls (in the visible region)

Not only organic compounds are colored. In the qualitative analysis colors of simple inorganic compounds are often exploited in identification of elements and compounds. For example it is used in identification of Fe^{+3} ions using thiocyanates – the respective coordination compound has intensive red color.
For investigation, identification, as well as quantitative analysis one very often uses spectroscopic methods. Spectroscopy concerns the interaction between electromagnetic radiation (including visible light) with the matter. UV-Vis spectroscopy is the type of spectroscopy working in the spectral regions of visible light ("Vis"), near ultraviolet ("UV"), and near infrared ("IR"). The respective wavelengths spread from 200 nm to 1100 nm. The devices used to measure such spectra are known as UV-Vis spectrophotometers.

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[1] **Light** – this term has different meaning in life and in science. In everyday's life it means the visible part of the electromagnetic radiation, received by the retina. Human eyesight is not uniform, the limits of the wavelengths are 380-780 nm maximum, but usually they are lower (in particular for long waves), sometimes this range is limited to 400-700 nm. In sciences the term "**the optical radiation"** is used. It means radiation undergoing the rules of geometrical and wave optics. It is assumed that optical radiation obeys the range of electromagnetic waves with the length between 10 nm to 1 mm. This range is divided into three sub-ranges – infrared (IR), visible (Vis), and ultraviolet (UV).

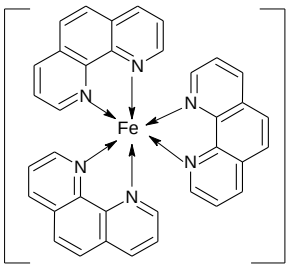
Spectrophotometry belongs to the instrumental analytical techniques, where absorption of electromagnetic radiation is used in analytics. UV-Vis spectrophotometry finds numerous applications in both qualitative and quantitative of substances which absorb light in the appropriate range. The quantitative analyses are based on the absorption laws (the Lambert-Beer law and the law of absorption additivity). These subjects will be presented later.

There is, however, much simpler way to estimate the concentration of colored substance in solution, known as **colorimetry**. Its idea is comparing the intensities of colors of solutions of the analyzed substance with a series of solutions of the same substance prepared in the same conditions, with known, gradually increasing contents. It is obvious that if the content of the colored substance in solution increases, the intensity of the color of the solution also increases. This is the base of the colorimetry. This method is used everywhere one needs fast, cheap (no specialized device is necessary) and easy method although its preciseness is very limited.

The simplest realization of the colorimetric method is preparing a series of standard solution with gradually increasing concentrations, in identical cylinders (rather thick, so called Nessler cylinders are used for this). It is necessary to compare the intensity of the colors of solutions containing known amount of substance with the cylinder with unknown sample concentration. In laboratories there are used also more advanced instruments (colorimeters), which measure color intensity at appropriated wavelength, specific for the compound under test.

<u>Note:</u> students perform only one of the exercises described below, pointed by the teacher.

Colorimetric determination of Fe(II) by 1,10-phenanthroline. Divalent iron ions form red complex with o-phenanthroline AT pH between 2 and 9. The



maximum of absorption of this complex is situated at wavelength λ_{max}=512 nm, its molar absorption coefficient is $\varepsilon = 1{,}11\times10^{-4}\cdot mol^{-1}\cdot dm^3\cdot cm^{-1}$ (specific absorbance 0,2). In the case of shortage of the ligand, yellow complexes 1:1 (Fe^{2+} to phenanthroline) can be formed. Ions of Ti, Al and Bi disturb this analysis because of their hydrolysis in environment of low acidity. Addition of sodium citrate prevents this process. Zn and Cd ions, if in higher amounts, can be masked by EDTA. Copper can be masked by thioglycolic acid. Also trivalent iron Fe^{3+} forms green-blue complex with o-phenanthroline, which after time becomes yellow, thus we will reduce Fe^{3+} ions to Fe^{2+} using hydroxylamine. Optimum condition for this reaction is pH from 6 to 8.

Chemicals (will be prepared by the laboratory technicians)

- Basic solution containing 10 ppm Fe^{3+} : 0.08635 g iron-ammonium sulfate $NH_4Fe(SO_4)_2\cdot12H_2O$ in 1L diluted HCl (5mL concentrated H_2SO_4)
- 0.25% solution of o-phenanthroline in HCl: 0,25 g o- phenanthroline dissolved in 91.5 mL distilled water with addition of 8.5 mL conc. HCl (ρ=1.19 g/mL)
- 10% aqueous solution of sodium citrate: 10 g of sodium citrate dissolved in 90 mL distilled water
- 10% aqueous solution of hydroxylamine hydrochloride: 10 g of hydroxylamine hydrochloride dissolved in 90 mL distilled water (this solution must be prepared fresh)

Equipment: Spectrocolorimeter Hanna, glass cylinders with stand (7 cylinders) and lamp, volume flasks 50 mL (7), bulb and graduated pipettes and/or automatic micropipettes, measuring cylinder á 50 mL.

<u>Procedure</u>

1. Rub a sample of rock in a mortar to fine powder. Try the best method of dissolving it in acids – usually the best is hot 1:1 mixture of concentrated HCl and HNO_3 (use a beaker or mineralizer M9; the latter can be operated only by your teacher).

2. Weigh exactly (using an analytical balance) ca. 2 g of the powdered material and dissolve it in a beaker or in mineralizer (ask the teacher how to do it) in ca. 50 cm^3 of the dissolving mixture. Filter this under vacuum using glass filter G4 and dilute in a volumetric flask to 200 mL.
3. **Preparation of standard solutions and the analyzed solution**. Measure, using pipettes, 0.00, 2.00, 4.00, 6.00, 8.00 and 10.00 mL of the basic solution of Fe^{3+} to 6 volume flasks á 50 mL. Measure 5 mL of the analyzed solution to the seventh flask, and add solid NaOH, pellet by pellet, until the dark precipitate appears. Then add HCl, drop by drop, until solution becomes clear again. Next, add to each flask the following (in the given order, using the measuring cylinder): 3 mL of 10% solution of hydroxylamine hydrochloride, 10 mL of 10% solution of sodium citrate, and 10 mL of 10% solution of o-phenanthroline. Add distilled water to each flask to the final volume 50 mL. The solutions contain now 0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 ppm Fe^{2+} (μg/mL). If the color in the flask with analyzed solution is more intensive than in the most concentrated one in the reference series, repeat the procedure for the analyzed sample but with only 1 mL instead of 5 mL of the basic solution prepared in point 2.
4. **Colorimetric determination of iron using Hanna spectrocolorimeter.** (Before measurements ask your teacher for instructions). Fill one cell with the solution which does not contain iron (0.0 ppm). Pour measured solutions to the second cell in sequence, the analyzed solution is the last. Switch the device on. When the display shows C1, put the cell with reference solution to the apparatus and press the key. Wait until the display shows C2. Change the cells, put that with measured solution and press the key again. Note the result displayed (it is absorbance given in arbitrary units).
5. **Colorimetric analysis using Nessler cylinders.** After the series of measurements described in point 2, pour the solutions to the glass cylinders and place them in the stand. Switch light on and compare the intensity of color in the cylinder with analyzed sample with those with reference solutions. Note, which is the closest to the analyzed one.

Calculations and report

Using Excel or similar datasheet plot the dependence of the measured absorbance on the content of iron, calculate the parameters of the linear function describing this dependence and use them to calculate the content of iron in the original solid sample under analysis (show its data on the plot for illustration). Give the final result, in ppm of iron. Compare the result with that obtained using Nessler cylinders.

Colorimetric determination of phosphorus using molybdenum blue. The content of phosphorus in surface waters determines the degree of their biological usefulness. The more phosphates is present in rivers or lakes because of sewages, the more possible is their eutrophication. Important is also the proper content of phosphates in boilers, while they prevent formation of calcin.

Ammonium molybdate reacts with phosphate ions forming phosphoromolybdic acid which can be reduced to molybdenum blue. Intensity of the blue color of this compound is proportional to the primary content of phosphate ions in solution.

$$PO_4^{-3} \xrightarrow{(NH_4)_2MoO_4} (NH_4)_3P(Mo_3O_{10})_4 \xrightarrow{reductor} (Mo_2O_5 \cdot MoO_3)$$

molybdenum blue

Oxidizing agents present in large amounts avoid formation of this colored complex and should be removed before analysis. H_2S disturbs if its content is above 2 mg/L, heavy metal's ions above 10 mg/L can cause lowering of the results (but vanadium acts reverse). Silicates disturb if above 20 mg/L. Arsenates react as phosphates. Citrates, oxalates and tartrates decrease the sensitivity of the test.

This method is applicable for sea water.

Polyphosphates(V) are added to meat products which undergo thermal processing, to meat jellies and to all types of sausages except dry and semi-dry. Typically, phosphates(V) are added to food together with nitrates(III). Phosphates lead to bonding water by proteins and fats undergo emulgation. The content of phosphates(V) in food, expressed as phosphorus(V) oxide, should not exceed 0.5%.

Preparation of meat or pork-butcher's products for analysis. Homogenize ca. 100 g of meat (butcher's product). Weight in a beaker ca. 0.7-0.9 g (±0.0001 g) of homogenized sample, add to it 5 mL of HCl (1:2) and 1 mL 30% H_2O_2. Put the beaker covered by watch-glass in water-bath at a little less than 100°C. for ca. 20

min. Filter the solution under vacuum using glass filter G4, transfer the filtrate quantitatively to 50 mL volume flask, rinsing the filter with distilled water and fill the flask to the mark with water. The filtrate should be clear.

Chemicals (will be prepared by the laboratory technicians)

- Reference solution containing 10 ppm P/1 L : 43.9mg of anhydrous potassium phosphate dissolved in 1 L of water
- Solution of ammonium molybdate $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in water (40 g/L)
- Solution of antimonyl-potassium tartrate $K(SbO)C_4H_4O_6\cdot 0.5H_2O$ in water (2.7 g/L)
- Solution of H_2SO_4 in water(70 mL of concentrated H_2SO_4 diluted to 500 mL)
- 0.01 M solution of ascorbic acid (1.76 g of ascorbic acid in 100 mL aqueous solution). This solution is unstable and must be prepared shortly before the lesson.
- HCl conc.; 30% H_2O_2

Equipment: Spectrocolorimeter Hanna, 7 glass cylinders with stand and lamp, volume flasks 50 mL (7), volume flask 100 mL (dark glass), bulb and graduated pipettes and/or automatic micropipettes, measuring cylinders á 50 mL, volume flasks á 50 mL, watch-glass, glass rod, funnel for filtering, paper filters (soft).

Procedure

1. **Preparation of the reaction mixture.** To 100 mL volume flask (dark glass) add the following (in the given order, using the measuring cylinder): 50 mL H_2SO_4 solution, 5 mL antimonyl-potassium tartrate solution, 15 mL ammonium molybdate solution and 30 mL ascorbic acid solution. Mix after every component added. If the mixture was opaque, leave it for few minutes. If this does not help, filter it under vacuum using glass filter G4.
2. **Preparation of standard solutions and the analyzed solution**. Measure, using pipettes: 0, 2, 4, 6, 8 and 10 mL of the reference solution to 6 volume flasks á 50 mL. Pour 5 mL of the analyzed extract from meat to 7^{th} flask. Next, add to each flask 8 mL of the reaction mixture prepared in p. 1. Add distilled water to each flask to the final volume 50 mL. The solutions contain now 0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 ppm P (µg/mL).
3. **Colorimetric determination of phosphates using Hanna spectrocolorimeter.** (Before measurements ask your teacher for instructions). Fill one cell with the solution which does not contain phosphates (0.0 ppm). Pour measured solutions to the second cell in sequence, the analyzed solution is the last. Switch the device on. When the display shows C1, put the cell with reference solution to the apparatus and press the key. Wait until the display shows C2. Change the cells, put that with measured solution and press the key again. Note the result displayed (it is absorbance given in arbitrary units).
4. **Colorimetric analysis using Nessler cylinders.** After the series of measurements described in point 3, pour the solutions to the glass cylinders and place them in the stand. Switch light on and compare the intensity of color in the cylinder with analyzed sample with those with reference solutions. Note, which is the closest to the analyzed one.

Calculations and report

Using Excel or similar datasheet plot the dependence of the measured absorbance on the content of phosphates, calculate the parameters of the linear function describing this dependence and use them to calculate the content of phosphates in the sample under analysis (includethe plot for illustration). Give the final result, in ppm of phosphates. Compare the result with that obtained using Nessler cylinders.

Sources:
http://www.chemia.uj.edu.pl/chemanal/dydaktyka/analitycznaI_pliki/spektrometria.pdf
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