XIV. Grand Prix Chimique

Analytical task

Cerimetric Determination of Chemical Oxygen Demand

Spectrophotometric Determination of Levodopa
in Pharmaceutical Preparations

Debrecen, Hungary 2019

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Abbreviations in the Text:

- deionized water = DIW
- mol/dm^3 = mol/L = M = molar
- cm^3 = mL
- relative atomic mass = A_r
- Chemical Oxygen Demand = COD
- levodopa = L-Dopa
- hexacyanoferrate(III) = ferricyanide
- cerium(IV) = ceric
- iron(II) = ferrous
- iron(III) = ferric

Lab Safety

- In the laboratory, safety equipment (lab coat, gloves, safety glasses) must be worn all times. Safety glasses can be found at each workstation. Gloves are available in multiple sizes at the teacher's desk. Please thoroughly study the hazards and safety precautions of the provided chemicals.
- Before using an equipment, always check for damage. Do not use damaged equipment or cracked glassware, but report it to a jury member immediately.
- Eating and drinking in the lab (including chewing gum) is prohibited.
- Always use pipette filler for pipetting, mouth pipetting is strictly prohibited.
- The safety rules must be obeyed.

General Notes:

- Read the task carefully before you start working.
- 30 minutes before the end of the lab session a warning will be issued. Plan your work accordingly.
- If you need to leave the lab, ask a jury member or lab assistant. Do not leave the lab without permission.
- Please write all your answers, calculations and notes in pen. The back of your exam sheet can be used if you run out of space but your final answers must be written in the dedicated area.
- English version of the exam sheet is considered to be standard.
- It is advised to inspect whether the equipment you work with is clean and dry, especially if performed measurement/reaction is sensitive.
- Some equipment and chemical are shared among multiple contestants. Do not take them for yourself and always put them back to their original place after use. Plan ahead to avoid jams.
- Deionized water can be refilled from plastic tanks.
- Cleaning procedure of glassware:
  - wash with tap water and dish soap
  - rinse with deionized water
- The following waste requires special treatment:
  - waste from titration should be collected in containers labelled 'Titration Waste'
  - waste from the spectrophotometric procedure should be collected in containers labelled 'Cyanide Waste'
  - used pipette tips should be collected in crystallizing dishes labelled 'Used Pipette Tips'
- The disposable automatic pipette tips should be used only once (one tip for each solution). If you accidentally touch the tip to anything else, but the solution you transferring, the tip should be disposed of to avoid cross contamination and contamination of the stock solutions.
- A short manual can be found on each spectrophotometer.
- The Excel file you will need for evaluation (named 'Spectro_Evaluation.xlsx') is pre-prepared and can be found on the computer desktop. If you need help with the use of Microsoft Excel ask a jury member.
- You may work on the tasks in any order and multiple measurements can be performed at the same time.
- If you need help with the calculations ask a jury member.

Please obey the rules of fair play. We wish you good luck for the competition.

Available Equipment for each Contestant			
Equipment	**Quantity**	**Equipment**	**Quantity**
Beaker, 50 mL	3	Graduated cylinder, 50 mL	1
Beaker, 100 mL	1	Laminated white paper	1
Beaker, 250 mL	2	Paper towels	-
Beaker, 600 mL	1	Permanent marker	1
Bulb pipette, 5 mL	1	Pipette filler (rubber)	1
Bulb pipette, 10 mL	2	Spatula (plastic)	1
Burette, 25 mL	1	Volumetric flask, 25 mL	7
Burette clamp	1	Volumetric flask, 50 mL	3+1
Burette stand	1	Volumetric flask, 100 mL	1+1
Cuvette, 1 cm (quartz)	2	Wash bottle (for DIW)	1
Erlenmeyer flask, 250 mL	4	Weighing scoop	1
Glass funnel, ⌀45mm	5		

Shared Equipment	
Equipment	**Location**
Analytical balance *(4 pcs.)*	In weighing room
Graduated cylinder, 100 mL *(2 pcs.)*	In fume hood
Hot plate *(2 pcs.)*	Next to computers
Pipette tips	In pipette tip holders, next to spectrophotometers
Pipettor, 100-1000 µL *(4 pcs.)*	On pipette stands, next to spectrophotometers
Stirring rod and beaker for H_2SO_4 *(2 pcs.)*	In fume hood
Ultrasonic bath	On teacher's desk
VIS Spectrophotometer *(4 pcs.)*	In weighing room
Wash bottle (with acetone)	On teacher's desk

Required Chemicals				
Chemical	**Location**	**GHS Hazard and Precautionary Statements**		
		Pictograms	Hazards	Precautions
Ammonium cerium(IV) sulfate 0.1 mol/dm^3	Shared between workstations		H290	P234, P280
Ammonium iron(II) sulfate 0.02 mol/dm^3 in 0.1 M H_2SO_4 *(titrant)*	In fume hood		H290, H315, H318	P280, P305+351+338
Ferroin solution *(indicator)*	Shared between workstations		H302, H412	P280
Iron(III) chloride hexahydrate *(reagent)*	Next to analytical balances		H290, H302, H315, H318	P280, P305+351+338
Levodopa 500 mg/dm^3 *(stock solution)*	Next to spectrophotometers	-	-	-
Potassium hexacyanoferrate(III) 15 mmol/dm^3 *(reagent)*	Next to spectrophotometers	-	EUH 032	-
Sodium oxalate *(primary standard)*	Next to analytical balances		H302, H312	P280
Sulfuric acid 96%	In fume hood		H290, H314	P260, P280, P303+361+353, P304+340+310, P305+351+338

Task 1

Cerimetric Determination of Chemical Oxygen Demand

Introduction:

Chemical Oxygen Demand indicates the amount of oxygen that can be consumed by the oxidation of organic material to carbon dioxide and water in a given solution. It is usually expressed in milligram oxygen consumed per liter of solution (mg/L). A COD test can give information about the amount of organic pollutants in surface water and wastewater and is a useful measure of water quality. Government regulations allows less than 1000 mg/L COD for wastewater in general.
COD can be determined by redox titration, where $Cr_2O_7^{2-}$, MnO_4^- or Ce^{4+} is generally used as an oxidizing agent. The results are evaluated by calculating how much oxidant is consumed and the amount of oxygen that is equivalent to it. However, these oxidizing agents vary in effectiveness of their oxidizing capability of organic substances, thus separate COD_{Cr} , COD_{Mn} and COD_{Ce} values should be considered. Advantages of cerium(IV) include its high redox potential and its low acute and environmental toxicity compared to other reagents.

Basic principle and procedure:

COD is determined with back titration, because the oxidation of organic material is slow, requires high temperatures and an excess amount of oxidant. In this case, after boiling for 20 minutes, the excess cerium(IV) is titrated using ammonium iron(II) sulfate solution as titrant. For the determination of the exact concentration of the titrant, two sodium oxalate standard solutions with different concentrations are needed and each respective aliquot should be treated in the same manner as the sample. During the determination of the exact concentration of the titrant, the following reactions take place, which should be balanced:

$$Ce^{4+} + C_2O_4^{2-} = CO_2 + Ce^{3+}$$

$$Fe^{2+} + Ce^{4+} = Ce^{3+} + Fe^{3+}$$

I. Preparation of solutions

Standard solutions:

Prepare two standard solutions. Weigh by difference accurately between 0.0650-0.0750 g sodium oxalate into a small beaker. Dissolve it in deionized water (an ultrasonic bath can be used to speed up dissolution), then prepare a standard solution by transferring it quantitatively into a 50 mL volumetric flask and filling up to mark. Next, prepare a standard solution into another 50 mL volumetric flask in the same way as before, except now between 0.0950-0.1050 g sodium oxalate should be accurately weighed by difference.

m_1 = _____ g	m_2 = _____ g

Sample:

The sample solution will be at your workstation with your competitor number on it.

Diluted sulfuric acid:

Prepare 350 mL, 4.25 molar sulfuric acid starting from calculated amount of concentrated sulfuric acid. Be careful as the solution heats up considerably. Wait until it cools down before taking it back to your workstation.

Properties of cc. sulfuric acid:
Mass fraction of H_2SO_4: 0.96
Molecular weight of H_2SO_4: 98.08 g/mol
Density: 1.84 g/cm^3

$V_{calculated}$ = _____ cm^3 $V_{measured}$ = _____ cm^3

II. Titration

General procedure:

1. From the solution of interest transfer a 10.00 mL aliquot to an Erlenmeyer flask.
2. Add 5.00 mL of 0.1 M $(NH_4)_4Ce(SO_4)_4$ solution and 25 mL of 4.25 molar sulfuric acid.
3. Cover the Erlenmeyer flask with a small funnel, then place it on a hotplate (do not forget to write your competitor number on it).
4. After 20 minutes, carefully take the flask off the hotplate with the help of a paper handle and let it cool to room temperature.
5. Complete the solution to roughly 50 mL with deionized water.
6. Add a few drops of ferroin indicator to the cooled reaction mixture and titrate with 0.02 mol/dm^3 ammonium iron(II) sulfate solution until a dark red color is reached.
7. Perform as many titrations as you find necessary to get reliable results. Multiple measurements can be performed simultaneously.

Determination of the exact concentration of the ammonium iron(II) sulfate solution:

Follow the general procedure. In step 1, individual aliquots from both sodium oxalate standard solutions should be transferred to separate Erlenmeyer flasks.

COD determination of the sample:

Follow the general procedure. In step 1, the aliquot should be taken from the sample solution.

Titrant consumed for standard solution No. 1 (cm^3)	Titrant consumed for standard solution No. 2 (cm^3)	Titrant consumed for sample (cm^3)

III. Data Evaluation

- Calculate the exact concentration of the $(NH_4)_2Fe(SO_4)_2$ solution.

$A_r(C) = 12.01$ $A_r(O) = 16.00$ $A_r(Na) = 22.99$

$c_{\text{ammonium iron(II) sulfate}}$ = _____ mol/dm^3

- Calculate the chemical oxygen demand of the sample expressed in mg/L unit.

$A_r(O) = 16.00$

COD_{Ce} of the sample: _____ mg/L

- Notes:

Task 2

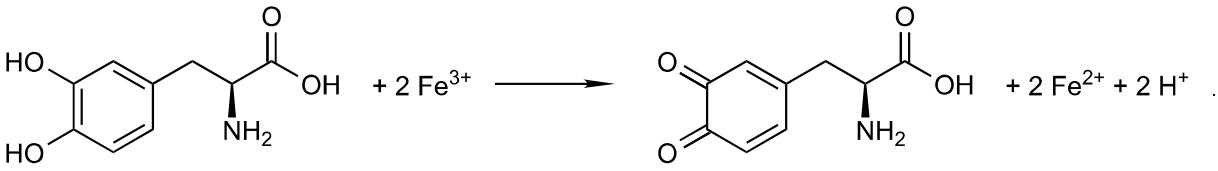
Spectrophotometric Determination of Levodopa in Pharmaceutical Preparations

Introduction:

Levodopa, an amino acid, is naturally part of the human and animal biology. It is the precursor of catecholamine neurotransmitters dopamine, adrenaline and noradrenaline. As a drug it is used in the clinical treatment of Parkinson's disease and Segawa syndrome by increasing the dopamine concentration in the central nervous system. It is often administered together with carbidopa and entacapone. Two Nobel-prize-winning research is related to L-Dopa.

Basic principle and procedure:

L-Dopa is usually determined spectrophotometrically by using a redox reaction. Several substance can -under appropriate conditions- oxidize levodopa, including iron(III), copper(II), periodate and vanadium(V). Then a colorimetric reagent is added to produce a colored complex. The following method was chosen, because it is fast, safe, does not require expensive reagents and most importantly is accurate and reliable.
The mechanism is as follows: first, the levodopa is oxidized by the iron(III) in solution, then the produced iron(II) reacts with potassium ferricyanide to produce colloidal Prussian blue.



$$Fe^{2+} + K_3[Fe^{III}(CN)_6] \longrightarrow 2\,K^+ + KFe^{III}[Fe^{II}(CN)_6]$$

The color develops completely in about 20 minutes, but the produced colloidal particles are not particularly stable, so care should be taken to measure the absorbance as soon as possible. Also, thorough homogenization is necessary. The diluted solution may look mildly opaque, but this does not influence the determination.
The resulting colloidal solution obeys Beer's law when the initial concentration of levodopa is in the range of 0.4-2.0 μg/mL.

I. Measurement

1. A) Preparation of calibration series: Prepare a levodopa working solution in a 100 mL volumetric flask. Next, transfer calculated quantities of this solution to 25 mL volumetric flasks with a pipettor, to obtain the calibration series. Show your calculations to the jury before proceeding.

Aliquot of levodopa stock solution taken: V = _____ cm^3 Final concentrations of the calibration series (from smallest to largest, in μg/mL unit):

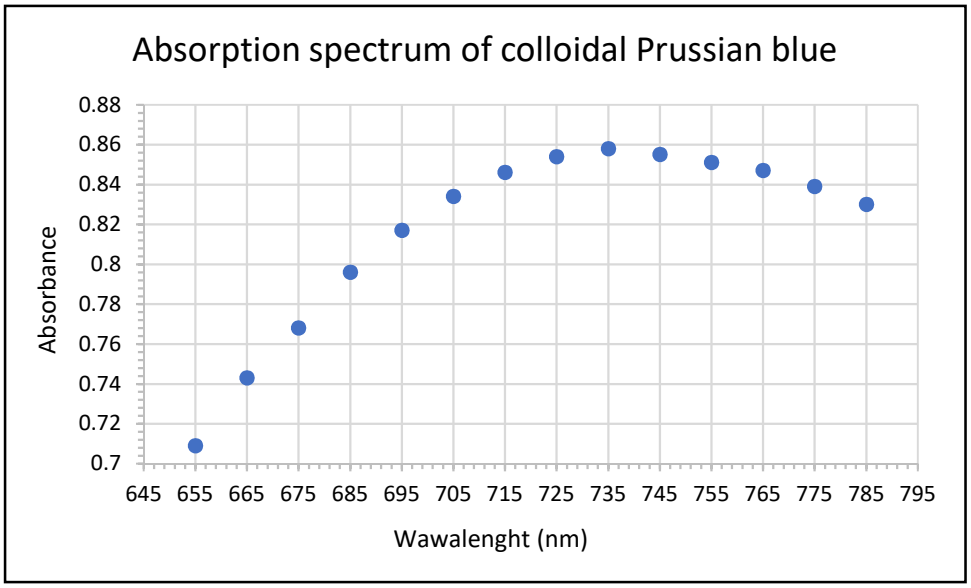
B) Preparation of the sample: 15 tablets of a levodopa containing drug were weighed (m_{total} = 11.9625 g), then ground up in a mortar. 46.50 mg from the fine, homogenous powder was measured, then transferred quantitatively into a 100 mL volumetric flask with the help of deionized water, which you can find at your workstation with your competitor number on it. Fill up the solution to the mark and homogenize it. After transferring a 10.00 mL aliquot of this solution into a 25 mL volumetric flask and treating it as described below, the absorbance of the final solution should be in the concentration range where Beer's law is obeyed.

2. Transfer with a pipettor to each volumetric flask an aliquot containing 18 μmol Fe^{3+}, taken from a stock solution prepared from the calculated amount of $FeCl_3.6H_2O$ in a 50 mL volumetric flask. Show your calculations to the jury before proceeding.

$M_{iron(III)\ chloride\ hexahydrate}$ = 270.30 g/mol $m_{calculated}$ = _____ g $m_{measured}$ = _____ g Aliquot transferred to each flask: V = _____ μL

3. Then add 1000 μL of 15.0 millimolar potassium hexacyanoferrate(III) solution to each flask.
4. Dilute each solution to the mark with deionized water, homogenize them thoroughly, then let them stand for 20 minutes. (The solutions should not stand for more than 25 minutes.)

5. Find the optimal wavelength for the measurements from the absorption spectrum below, then set up the spectrophotometer.



Chosen wavelength: λ = _____ nm

6. Measure the absorbance of calibration series and the sample against a reagent blank, homogenizing the solutions right before the measurement. If some precipitate persists after homogenization, the whole procedure must be started over. All measurements should be made as fast as possible and within 5 minutes.
7. Make as many measurements as you find necessary to get reliable results.

Levodopa concentration (μg/mL) OR Sample number	Absorbance

II. Data Evaluation

- Plot the absorbances of the calibration series against the concentrations of levodopa with the help of Microsoft Excel. A printed copy of the Excel chart must be handed in with this task sheet.
- Find the equation and the coefficient of determination (R^2) of the obtained regression line.

Equation of the obtained regression line: y = _____
Coefficient of determination of the obtained regression line: R^2 = _____

- Calculate the levodopa concentration of the sample (expressed in mg/L) and the average levodopa content of a tablet (expressed in mg).

ρ = _____ mg/L	Number of tablets powdered: 15 Total weight of tablets: 11.96250 g Weight of analyzed portion: 46.50 mg Average levodopa content of a tablet: _____ mg

- Notes: