Exercise 3

Bio-Components characterisation

Faculty of Chemistry, Wroclaw University of Science and Technology

UV-Vis spectrophotometry

1. **Requirements:**
   a. Calculation and re-calculation of concentrations.
   b. Lambert-Beer law
   c. Electromagnetic spectrum

2. **Equipment and reagents:**
   a. UV/VIS spectrophotometer,
   b. polystyrene cuvettes,
   c. 100 mL volumetric flask (2),
   d. pipettes and micropipettes,
   e. hot plate
   f. $KMnO_4$,
   g. 10 mL cylinder (1),
   h. 10 mL volumetric flasks (6),
   i. 125 mL Erlenmeyer flasks or 150 mL beakers (2).

3. **Manual:**

The aim of the exercise is the spectroscopic characterization of of $KMnO_4$ solution, by determining its maximum absorption, calibration curve as well as extinction coefficient.

a. Prepare stock solution (0.008 M): Accurately weigh 126 mg solid $KMnO_4$. Transfer quantitatively to a 100 mL volumetric flask and fill to the mark with water.
b. Prepare standard solutions. Use stock solution for preparation of 10 mL $KMnO_4$ standards: **No.1:** 0.0008 M, **No.2:** 0.0004 M, **No.3:** 0.00016 M, and **No.4:** 0.00008 M.
c. Rinse one of the cuvettes with distilled water and fill it with water. Put the cuvette in the sample compartment. This is the reference solution. Set the wavelength to 400 nm, then set the Absorbance to zero.
d. Rinse a second cuvette once with distilled water and once with standard solution No.1, then fill it with standard solution No.1. Place the cell in the sample compartment, measure the Absorbance at 400 nm.
e. Repeat procedures c and d for the wavelengths 420, 440, 460, 480, 500, 520, 540, 560, 580, 600 nm.

f. Prepare a graph of absorbance A vs. wavelength λ and determine λ_{max} (wavelength with maximum A).
g. Set the wavelength at 525 nm (λ_{max}). Place the cuvette with distilled water in the cell compartment, and again set the Absorbance to zero.
h. Measure and record the Absorbance of each of the four standard solutions, starting with the most dilute standard. After each measurement, rinse the cuvette with the next standard, not with distilled water!
i. Draw a plot having X-axis as concentration (mole/L) and Y-axis as Absorbance at λmax (525 nm)
j. Use Beer's law to calculate ε for $KMnO_4$, given the cell width (path length l) to be 1 cm

Laboratory Data Sheet

Name:_____ Section: _____

1. Stock solution

(1) Mass of $KMnO_4$ = _____ g

(2) Molar mass of $KMnO_4$ = _____ g/mol

(3) Moles $KMnO_4$ = mass/molar mass = _____ mol $KMnO_4$

(4) Molarity of stock solution = mol $KMnO_4$/0.100L = _____ mol/L

2. Preparation of standard solutions

Standard solution 1 (dilute 1.0 mL stock to 10 mL).
$KMnO_4$ molarity = (1.0/10)x(stock solution concentration) = _____ mol/L

Same for concentrations of solutions "2", "3" and "4" with 500, 200, and 100 µL stock. *Remember that 100 µL is 0.1 mL.*

$KMnO_4$ molarity for standard solution 2 = _____ mol/L

$KMnO_4$ molarity for standard solution3= _____ mol/L

$KMnO_4$ molarity for standard solution 4= _____ mol/L

3. The absorption spectrum of $KMnO_4$:
Make a graph of A vs λ at 20 nm wavelength intervals from 400 nm to 600 nm (as measured by the whole laboratory group). Attach the graph with this lab report.

λ ,nm	Asorbance
400	
420	
440	
460	
480	
500	
520	
540	
560	
580	
600	

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4- The Calibration Curve at λ_{max} = 525 nm

Standard	Concentration	Absorbance
1		
2		
3		
4		

Make a graph of A (y-axis) vs M (x-axis). Attach the graph to this report.

5. Calculation of the molar extinction coefficient ε at 525 nm.

Use the A-value of solution 1:

ε = A/(C. *l*), with *l* = 1 cm ε = _____ _____