Lodz University of Technology
Institute of Materials Science and Engineering

Laboratory 4

Colorimetric assay

Instruction for the Laboratory of Biophysics

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I. THEORETICAL INTRODUCTION

The term *absorption* refers to the physical process of absorbing light, which is the basic principal behind ultraviolet-visible spectroscopy. The fundamental law that applies to this technique is the Lambert-Beer law:

$$A = a\,c\,l$$

a – molar extinction coefficient (molar absorptivity)

c – concentration of solution

l – thickness of absorption layer

The law states that there is a linear dependence between the absorption of monochromatic light in a substance and the absorption coefficient of that substance as well as the concentration of the absorbing species and the distance light travels through the material.

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II. EXPERIMENTAL PART

1. Use the stock solution (25 μmol/dm^3) to prepare two sets of dye solution (5 cm^3 each) with the following concentrations:

- 2 μmol/dm^3,
- 3 μmol/dm^3,
- 5 μmol/dm^3,
- 7.5 μmol/dm^3,
- 10 μmol/dm^3,
- 12.5 μmol/dm^3,
- 15 μmol/dm^3,
- 17.5 μmol/dm^3,
- 20 μmol/dm^3,
- 25 μmol/dm^3.

2. Measure absorption for a sample with a concentration of 15 μmol/dm^3 using the PF11 photometer

a) turn on photometer (switch is on the right-hand side)

b) use the M button to choose a method for NANOCOLOR position

c) use the © button to choose method number 013 (Ekstynkcja)

d) place a test-glass tube containing control solution (distilled water) into the measure port, choose colour filter number 1 and reset the photometer by pressing button ZERO

e) place a test-glass tube containing sample solution into the measure port, press button M and record the result.

f) reset photometer (d) and measure the absorption (e) for each of the following filters

filter 1 – 380 nm
filter 2 – 405 nm
filter 3 – 470 nm
filter 4 – 520 nm
filter 5 – 605 nm
filter 6 – 720 nm

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Results put to the Table 1

Filter No.	Wavelength	Absorption
filter 1		
filter 2		
filter 3		
filter 4		
filter 5		
filter 6		

3. Compare the results obtained from 2f and determine which filter gives the highest absorption. Now choose that filter (i.e. highest absorption) and reset the photometer (2d). Next, measure both sets of sample solutions. Results put to the Table 2

Concentrations	Result
2 μmol/dm^3	
3 μmol/dm^3	
5 μmol/dm^3	
7.5 μmol/dm^3	
10 μmol/dm^3	
12.5 μmol/dm^3	
15 μmol/dm^3	
17.5 μmol/dm^3	
20 μmol/dm^3	
25 μmol/dm^3	

4. Measure a sample with an unknown concentration and put results into the Table 2
5. After collecting all data make graphs of absorption dependence and wavelength.
6. Make graphs of dependence and dye concentration. On the basis of your results estimate whether the dye solution complies with the Lambert-Beer law.
Next, calculate the concentration of the unknown dye solution.

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III. THE REPORT MUST CONTAIN

1. A short theoretical introduction.
2. Tables with results and graphical charts of the following
- relation between absorption and wavelength
- relation between absorption and concentration
3. Summary.

VI. ISSUES TO STUDY

1. Definition of transmittance and absorbance.
2. To explain the Lambert-Beer law.
3. Colorymetric methods.

V. LITERATURE

1. Donald L. Pavia, Gary M. Lampman, George S. Kriz Introduction to Spectroscopy. Saunders Golden Sunburst Series
2. Nowicka-Janowska T. *Spektrofotometria UV/VIS w analizie chemicznej* PWN

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