ABSORPTION PHOTOMETRY

MEASURING OF THE ABSORPTION SPECTRUM OF COLOURED SOLUTION

PRINCIPLE OF THE METHOD

The absorption spectrum of coloured substance is the dependence of intensity of electromagnetic radiation passing through the substance (absorbance (A)) on the wavelength. The value of the wavelength (wavelengths), in which the spectral curve reaches a maximum, is characteristic for the substance. Absorbance corresponds with the amount of absorbing substance in the sample.

MATERIALS AND INSTRUMENTS

tubes, cuvette, spectrophotometer SPEKOL 1300

CHEMICALS

Kongo red (solution A), Bromphenol blue (solution B), Coomassie blue (solution C)

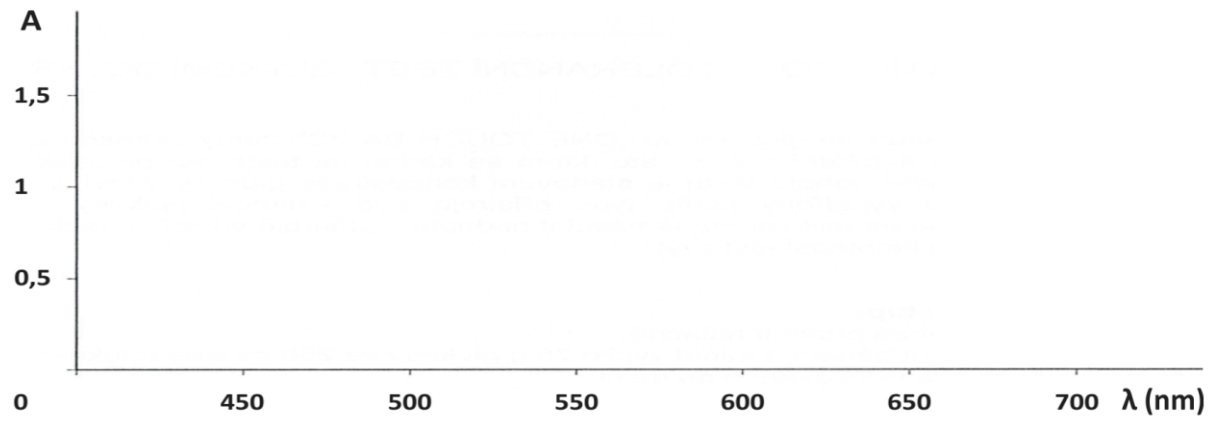
PROCEDURE

1. Turn on the spectrophotometer and leave it for 10 minutes to warm up.
2. Adjust 400 nm wavelenght by arrows.
3. Open the lid of the cuvette space.
4. Place the cuvette containing reference solution (distilled water) into the first position.
5. Place the cuvette containing sample (coloured solution) into the second position.
6. Close the lid of the cuvette space.
7. Insert the rod holder into the first position (pink label).
8. Press the zero button and wait until the display shows the absorbance value of 0.
9. Insert the rod holder into the second position (blue label).
10. Read the value of absorbance.
11. Then change the value of wavelenght by 50 nm and again insert the cuvette with reference solution into the measuring space.
12. Press the zero button, insert the cuvette with test solution into the measuring space and read the value of absorbance.
13. Repeat the procedure and change the value of wavelenght by 50 nm at each step.
14. After each change of the wavelength it is necessary to re-insert the cuvette with reference solution into the measuring space.
15. Continue to the wavelenght of 700 nm.
16. Write the measured values into the table.
17. Draw the graph of the dependence of absorbance on the wavelength and find the wavelength at maximal absorbance.
18. Turn off the spectrophotometer.

MEASURED VALUES

λ (nm)	450	500	550	600	650	700
A						

GRAPHICAL REPRESENTATION



λ_{max} = (nm)

CONCLUSION