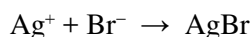


NEPHELOMETRIC DETERMINATION OF COLLOID CONCENTRATION

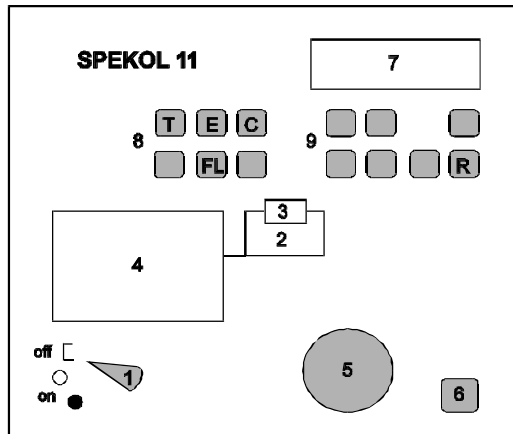
Aim of the practical

The aim of the practical is to become familiar with the phenomenon of light scattering by colloidal solutions. This phenomenon is used for analytical purposes: knowing the turbidity of a solution allows the concentration of the component forming the colloidal solution to be determined. The concentration of the colloidal solution is determined by measuring the intensity of light scattered at a given angle. A "Spekol" spectrophotometer equipped with a nephelometric attachment is used for this purpose; the instrument measures the intensity of light scattered at 90°. In the practical the concentration of a colloidal AgBr solution is determined; it is formed by mixing AgNO₃ solution (solution A) with KBr solution (solution B):



Equipment and solutions:

1. „SPEKOL 11” spectrophotometer.
2. AgNO₃ (solution A).
3. KBr (solution B).



Description of the spectrometers front panel:

1. Light-shutter switch.
2. Cuvette holder.
3. Cuvette.
4. Photocell housing.
5. Wavelength-setting drum.
6. Mains switch.
7. Display window.
8. "Measurement mode" keypad.
9. Data-entry keypad for measurement parameters

pic. 1 Spectrometer front panel

Course of the practical:

ATTENTION! On entering the laboratory, plug in the SPEKOL-11 and press the mains switch (6). After about 20 min the instrument is ready for measurements.

1. Fit a tip from box 3 onto pipette 3 and set the appropriate volume using the upper knob.
2. Using the pipette, transfer **5 ml** of **solution A** into a graduated cylinder. Replace the pipette tip with a new one from box 3.

3. Using the pipette, transfer **5 ml** of **solution B** into the same graduated cylinder that already contains solution A. Mix well.
4. Wait **15 minutes**. During this time the reaction proceeds, producing a colloidal solution of the concentration **$2,5 \cdot 10^{-3}$ mol/dm³**. Then top up the cylinder containing the colloid with distilled water to 20 ml.
5. Measure out the colloid volumes given in Table 2 of the report form into five clean cylinders and top each up with distilled water to a total volume of 25 ml.
6. Top up the remaining colloidal solution (~12.5 ml) with distilled water to 25 ml; this solution — called further **sample C** — will be used to record the turbidity as a function of wavelength.
7. Set the wavelength on the SPEKOL drum (5) to **400 nm**; gently place a cuvette with distilled water in the cuvette holder (2) and insert it into the light path.
8. Press the "FL" key, then "Z — FL MIN", and finally "FAKT" (the display should show 1.0000).
9. Remove the cuvette with water and gently insert the cuvette filled with **sample C** into the light path.
10. Press "FAKT", and after a moment "R" — the turbidity of sample C is measured as a function of wavelength.
11. Perform measurements in the wavelength range from **390 nm** to **560 nm**, changing the wavelength every 10 nm. Take readings approximately 10 seconds after setting up the measurement system. Enter the data into Table 1.
12. Plot the dependence of the turbidity (intensity of scattered light) on the wavelength.
13. From the plot, determine the wavelength λ_{max} at which the turbidity reaches its maximum value.
14. Set the drum (5) to the wavelength λ_{max} read from the plot.
While setting this wavelength, the cuvette with sample C should remain inside the instrument.
15. Measure the turbidity of the samples prepared in step 5 (in order from the **lowest** to the **highest** concentration) at the previously determined wavelength.
16. Pull out the cuvette holder (2) (the display shows "-OFL"), fill the cuvette with the next sample and place it back in the holder (2). Enter the data into Table 2.
17. After each measurement, pour the tested solution back into its cylinder.
18. Using the results, plot the turbidity as a function of sample concentration on plotting paper.
19. Measure the turbidity of a sample of unknown concentration C_x (prepared by the instructor).
20. Using the plot from step 18, determine the concentration of this sample.

Required theoretical knowledge:

1. Colloids. Optical properties of colloids.
2. Rayleigh theory of light scattering.
3. Interaction of light with matter – absorption and scattering
4. Raman scattering.
5. Principle of nephelometric measurement.

Recommended literature:

1. P.R. Bergethon "The Physical Basis of Biochemistry", Springer 1998
2. R. Cotterill "Biophysics An Introduction", Wiley 2003

Wroclaw Medical University Department of Biophysics and Neuroscience	Practical No 14 Nephelometric determination of colloid concentration	
..... Names of students		Faculty: Group No: Date:
Grade:	Tutorial signature	

1. Measurement of the intensity of turbidity by the colloidal solution as a function of the wavelength of the incident light.

Table 1.

Wavelength λ [nm]	Turbidity [%]	Wavelength λ [nm]	Turbidity [%]
390		480	
400		490	
410		500	
420		510	
430		520	
440		530	
450		540	
460		550	
470		560	

2. Plot the turbidity as a function of wavelength
3. From the plot, determine the wavelength at which the scattered-light intensity is highest ($\lambda_{\max}$).

$$\lambda_{\max} =$$

4. Measurement of the dependence of scattered-light intensity on the concentration of the colloidal solution at $\lambda_{\max}$.

Table 2.

Amount of the stock solution in 25 ml of diluted solution	0,5 [cm ³]	1 [cm ³]	1,5 [cm ³]	2 [cm ³]	2,5 [cm ³]	c_x [mol/dm ³]
Solution concentration [mol/dm ³]						
Turbidity [%]						

5. Measurement of scattered-light intensity of the test sample c_x of unknown concentration.

Turbidity = ... [%]	C _x = ... [mol/dm ³]
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