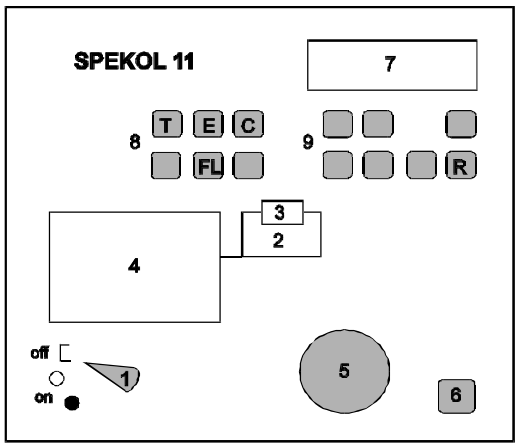
Practical No 2

NEPHELOMETRIC DETERMINATION OF COLLOID CONCENTRATION

CAUTION: SPEKOL-11 is ready for measurements 20 min after switching it on. Therefore it is recommended to turn it on immediately after reading this part of instructions. Press button (6) on the spectrometer front panel (see figure below).

Equipment:

Spectrometer "SPEKOL 11".



Spectrometer front panel

Description of the spectrometers front panel:

1. Diaphragm handle
2. Cuvette holder
3. Cuvette
4. Photoelectric cell box
5. Light wavelength setting knob
6. Power switch
7. Display
8. Buttons setting the type of measurement
9. Buttons setting details of measurement

Course of the practical:

1. In graduated cylinder mix 5 ml of solution A ($5 \cdot 10^{-3}$ mol/dm^3 $AgNO_3$) with 5 ml of solution B ($5 \cdot 10^{-3}$ mol/dm^3 KBr) and wait 15 min for the solution to get opaque. Next add 10 ml of distilled water to the mixture. Due to the reaction $Ag^+ + Br^- \rightarrow AgBr$ the colloid is formed. Concentration of the obtained stock solution is $2.5 \cdot 10^{-3}$ mol/dm^3.

2. In test tubes prepare 5 dilutions of the stock solution ($0.5 \cdot 10^{-4}$ mol/dm^3, $1 \cdot 10^{-4}$ mol/dm^3, $1.5 \cdot 10^{-4}$ mol/dm^3, $2 \cdot 10^{-4}$ mol/dm^3, $2.5 \cdot 10^{-4}$ mol/dm^3). To obtain these dilutions put 0.5 ml, 1 ml, 1.5 ml, 2 ml and 2.5 ml of the stock solution into the subsequent test tubes and add distilled water to obtain finally 25 ml of solution in each tube.
3. Dilute twice the rest of the stock solution. To do it add the the same amount of water as is the volume of stock solution that was left after preparation of the dilutions (if no stock solution was wasted you should have 12.5 ml of the solution left). This solution will be called "solution C" and its concentration is $12.5 \cdot 10^{-4}$ mol/dm^3.
4. Using light wavelength setting knob (5) set the wavelength to 400 nm. Set the light diaphragm switch (1) in the middle position (sign o). Put the cuvette containing distilled water (fill the cuvette up to 3/4 of its height) into the cuvette holder (2) and push holder inside the photoelectric cell box (4). Then subsequently press the buttons FL $\rightarrow$ Z-FL $\rightarrow$ FAKT (the display should show 1.000 after these operations). Replace the cuvette with distilled water by the cuvette containing solution C. Than press subsequently FAKT $\rightarrow$ R. The display (7) now shows now the turbidity of solution C. Changing the light wavelength from 400 nm to 560 nm (increment of 10 nm) measure the dependence of turbidity on the light wavelength T(λ) and put the results into the table. Plot the T(λ) dependence on the plotting paper.
5. Set the light wavelength to the value corresponding to the maximal value of turbidity. Using this light wavelength measure the turbidity of 5 previously prepared dilutions. Put the results into the appropriate table and plot the dependence of turbidity on dilution concentration T(c) on the plotting paper.
6. By mixing random amounts of dilutions 1-5 prepare the solution of unknown concentration (c_x) and measure its turbidity. Using the T(c) plot determine the concentration of this solution and put the value into the table.

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 2** **Nephelometric determination of colloid concentration**
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Grade:	Tutorial signature

1. Measure the dependence of solution turbidity on light wavelength T(λ).

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 T(λ) dependence on the plotting paper. Determine the wavelength for which the turbidity is maximal (λ_{max}).

3. Using λ_{max} measure the dependence of turbidity on solution concentration T(c).

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 [%]						

4. Plot the T(c) dependence on the plotting paper.

5. Measure the turbidity of solution of unknown concentration and using T(c) plot determine c_x.


SPEKOL 11