The Beer-Lambert law

Aims of the experiment

- To define absorbance as a characteristic of a coloured solution
- To recognise the relationship between concentration and absorbance of a solution
- To verify and apply the Beer-Lambert law
- To produce a dilution series of tetramminecopper
- To determine the absorbance coefficient of tetramminecopper

Principles

If a light beam passes through a coloured solution, it is weakened because the transparency to light (or transmission *T*) of the solution is low. Here, transmission *T* is defined as the ratio of the intensity of transmitted light *I* to the intensity of the incident light I_0.

$$T = \frac{I}{I_0}$$

The intensity of the light falls exponentially with the concentration *c* of the solution. It also reduces exponentially on the path *d* through a coloured solution. The following applies:

$$\lg\left(\frac{I}{I_0}\right) = -\varepsilon \cdot c \cdot d = -E$$

The variable ε is referred to as the extinction coefficient or the molar absorption coefficient. It is a substance-specific variable and also depends on the wavelength λ of the light beam. The greater the value of ε, the more light is absorbed by the solution, therefore the more intense the colour of the substance being investigated. The dimensionless variable $\lg\left(\frac{I}{I_0}\right)$ is referred to as absorbance (also absorption or optical density). Absorbance is linked to transmission as follows:

$$\lg\left(\frac{I}{I_0}\right) = \lg T = -E$$

In order to determine absorption, the intensity of the light before (I_0) and after (I) of a sample is measured and the negative logarithm of the ratio of the two intensities is calculated.

The formula

$$E = \varepsilon \cdot c \cdot d$$

is referred to as the Beer-Lambert law. In practice, samples are usually measured in a cuvette with a path length *l* = 1 cm and the absorbance coefficient ε is given in L mol^{-1} cm^{-1}. If the absorbance coefficient of a solution is known, the concentration of the solution can be calculated using the Beer-Lambert law.

Absorbance coefficients are determined using dilution series. Here, several solutions of the substance are produced in different but known concentrations and the absorbance is determined at a defined wavelength. The absorbance values of the various solutions lie on a straight line, the slope of which provides the absorbance coefficient.

In this experiment, the absorbance coefficient of tetramminecopper will be determined. Tetramminecopper, produced from copper sulphate and ammonia, is deep blue.

Fig. 1: Set-up of the experiment.

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$$Cu^{2+} + 4\ NH_3 \rightleftharpoons Cu(NH_3)_4^{2+}$$

In the experiment, the absorbance of solutions of various concentrations is measured with an immersion photometer. For this, the photometer is immersed into the test tubes. In this way, the linearity of the Beer-Lambert law can also be verified.

Risk assessment

Ammonia is corrosive and has a pungent odour. If possible, work under a fume cupboard. Wear protective goggles! Avoid as far as possible skin contact with ammonia and copper sulphate.

Copper (II) sulphate 5-hydrate

Signal word: Caution

Hazard statements

H302 Harmful if swallowed.

H319 Causes serious eye irritation.

H315 Causes skin irritation.

H410 Very toxic to aquatic life with long-lasting effects.

Precautionary statements

P273 Avoid release to the environment.

P302+P352 IF ON SKIN: Wash with soap and water.

P305+P351+P338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

Ammonia solution, 25 %

Signal word: HAZARD

Hazard statements

H314 Causes severe skin burns and eye damage.

H335 May cause respiratory irritation.

H400 Very toxic to aquatic life.

Precautionary statements

P280 Wear protective gloves and protective goggles/face protection.

P273 Avoid release to the environment.

P301+ P330 + P331 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.

P305+P351+P338 IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

P309 + P310 IF exposed or you feel unwell: Immediately call a POISON CENTER or doctor/physician.

Equipment and chemicals

1 Pocket-CASSY 2 Bluetooth 524 018
1 CASSY Lab 2 .... 524 220
1 Immersion photometer S 524 069
1 Holder for immersion photometer S................. 666 2605
1 Volumetric flask Boro 3.3, 100 mL.................. 665 793
1 Volumetric flask Boro 3.3, 50 mL.................... 665 792
6 Test tube, Fiolax, 30 mm diam., from set of 10 664 045
3 Graduated pipette 10 mL................................ 665 997
1 Pipetting ball (Peleus ball) 666 003
1 Test tube holder, wooden, f. 12 tubes 667 054
1 Compact balance 200 g : 0.01 g 667 7977
1 Copper(II) sulphate pentahydrate, 100 g.......... 672 9600
1 Ammonia solution, 25 %, 250 mL.................... 670 3600
1 Water, pure, 1L... 675 3400

Also required:
PC with Windows XP/Vista/7/8
Felt marker pen

Also recommended for wireless measurement:
1 Battery for Pocket-CASSY 2 Bluetooth 524 019
1 Bluetooth dongle 524 0031

Set-up and preparation of the experiment

Preparing the solutions

Preparing a copper sulphate solution (0,05 mol/l): Place 0.62 g of $CuSO_4$ (M = 249.68 g/mol) into a volumetric flask (50 mL) and dissolve in some water. When the substance is completely dissolved, fill the volumetric flask with water up to the calibration mark and mix by inversion. The solution is bluish in colour.

Preparing a ammonia solution (1 mol/l): Pipette 7.5 mL of the 25% ammonia solution into a volumetric flask (100 mL) and fill with water up to 100 mL.

Producing the dilution series of tetramminecopper

For the dilution series, prepare 6 test tubes (TT) in the test tube rack and label with the numbers 1 to 6. Pipette 7.5 mL of ammonia solution (1 mol/l) into each of the test tubes. Pipette various volumes of water and copper sulfate solution into each of the solutions according to the following table. The final volume in every tube is then 15 ml.

TT	c (tetrammine-copper)	V (copper sulphate)	V (water)
1	0 mol/l	--	7.5 mL
2	0.005 mol/l	1.5 mL	6.0 mL
3	0.010 mol/l	3.0 mL	4.5 mL
4	0.015 mol/l	4.5 mL	3.0 mL
5	0.020 mol/l	6.0 mL	1.5 mL
6	0.025 mol/l	7.5 mL	--

Set-up of the experiment.

Connect the Pocket-CASSY to the PC. This is possible via Bluetooth with the battery or via USB cable without battery. Connect the immersion photometer to the Pocket-CASSY.

Performing the experiment

1. Load CASSY Lab settings

The settings are:

Measurement parameter: Transmission 612 nm, absorbance

Measurement recording: manual

2. Calibrating the photometer. For this, use the reference solution with no colour (test tube 1). Immerse the immersion photometer so that the side holes are below the liquid level. Make sure that no air bubbles collect inside the immersion photometer. Choose the measurement parameter Absorbance and click on →0←. The photometer is now calibrated.

3. Prepare CASSY Lab. Now enter the prepared concentrations of the solution into the column c/mmol/L in CASSY Lab. CASSY Lab will then automatically assign the measured absorbance values to these concentrations in order of measurement. Start with the lowest concentration.

Note: The parameter "Concentration" is defined in CASSY Lab in the "Calculator" menu.

4. Measure the coloured solutions one after the other. Start with the solution which has the lowest concentration. This way, it is not necessary to rinse out the immersion photometer between measurements. Once a stable value is displayed after immersion in a solution, record the value by pressing the button on the Pocket-CASSY. Alternatively, save this value manually by clicking on .

Observation

The less copper sulphate the solution contains, the less it is coloured. Therefore, the highest absorbance is measured in the solution with the highest concentration and the lowest absorbance in the solution with the lowest concentration (see Fig. 2).

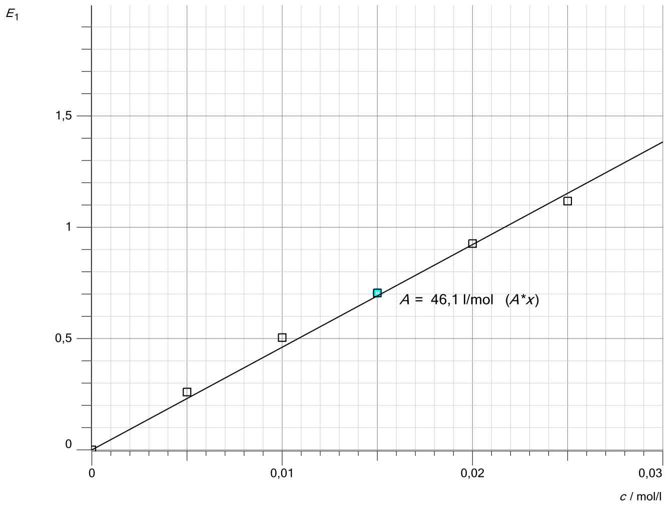

Fig. 2: Plotting the absorbance in dependence on the concentration of the tetramminecopper solution. The slope of the straight line is *A* = 46.1 L/mmol.

Evaluation

Verifying the Beer-Lambert law

The Beer Lambert law states that a linear relationship exists between the absorbance *E* of a solution and the concentration *c* of the coloured substance. The linearity can be confirmed with this experiment (see Fig. 2).

Determination of the absorbance coefficient of tetramminecopper

The absorbance coefficient ε of a substance can be calculated with a dilution series using the Beer-Lambert law. In this experiment, we are dealing with the absorbance coefficient of tetramminecopper. According to the Beer-Lambert law, this is provided by the slope *A* of the straight line when the absorbance *E* is plotted against the concentration *c* of the solution (see Fig. 2).

$$E = \varepsilon \cdot d \cdot c$$
$$y = A \cdot x$$

To simplify the matter, the immersion photometer also contains a path length *d* of 1 cm, as for most other photometers. The absorbance coefficient then corresponds to the slope of the straight line *A.*

$$A = \varepsilon \cdot d \Rightarrow \varepsilon = \frac{A}{d} = \frac{46{,}1\,\frac{\mathrm{l}}{\mathrm{mol}}}{1\ \mathrm{cm}} = 46.1\ \mathrm{l\,mol^{-1}\,cm^{-1}}$$

The absorbance coefficient is dependent on the wavelength used. The complete description of the absorbance coefficient therefore also includes the wavelength at which the measurement was made:

$\varepsilon_{\lambda = 612\ \mathrm{nm}} = 46.1\ \mathrm{L\ mol^{-1}\ cm^{-1}}$

Results

In this experiment it was possible to confirm the Beer-Lambert law. In addition, the absorbance coefficient of tetramminecopper at 612 nm was determined to be ε = 46.1 L $\mathrm{mol^{-1}\ cm^{-1}}$.

Cleaning and disposal

Dispose of the tetramminecopper solution in the waste for inorganic solutions. Store the stock solutions of copper sulphate and ammonia for further experiments. If they are also to be disposed, dispose both solutions in a container for inorganic waste.

Rinse out the immersion photometer with water and store in a dry place.