Related topics

Absorption, spectroscopy, extinction.

Principle and task

The Lambert-Beer law describes the correlation between the absorption behaviour of a substance as well as the concentration and the layer thickness of this substance in the solution.

With the aid of a diode array spectrometers assembled on the optical bench, the concentration dependency of the extinction is investigated at the wavelength of the absorption maximum of a coloured substance.

Equipment

Photodiode array spectrometer	35659.00	1
Softw. diode array spectrometer	35659.01	1
Spectral set f. overhead proj.	35634.00	1
Housing for experiment lamp	08129.01	1
Holder G 6.35 f. 50/100 W halo.lamp	08129.04	1
Halogen lamp, 12 V/50 W	08129.06	1
Single condenser, f 100 mm	08137.01	1
Power supply, univ., anal. disp.	13501.93	1
Optical profile bench l = 60 cm	08283.00	1
Base f. opt. profile-bench, adjust.	08284.00	2
Slide mount f. opt. pr.-bench, h 80 mm	08286.02	4
Table top on rod, 18.5×11 cm	08060.00	1
Plate holder with tension spring	08288.00	1
Swinging arm	08256.00	1
Retort stand, h 750 mm	37694.00	1
Burette clamp, roller mount., 2 pl.	37720.00	1
Microburette 10 ml	36527.01	1
Burette 50 ml, lateral stopcock, S	36513.01	1
Glass cell 60×10×80 mm	29319.00	4
Glass cell 60×20×80 mm	29320.00	1
Volumetric flask 1000 ml, IGJ24/29	36552.00	1
Volumetric flask 100 ml, IGJ12/21	36548.00	1
Volumetric pipette, 50 ml	36581.00	1
Pipettor	36592.00	1
Funnel, glass, top dia. 55 mm	34457.00	2
Pasteur pipettes, 250 pcs	36590.00	1
Rubber caps, 10 pcs	39275.03	1
Analytical balance w. RS 232, 61 g	45024.93	1
Weighing dishes, 85×85× 7 mm, 100	45019.01	1
Wash bottle, plastic, 500 ml	33931.00	1
Spoon, special steel	33398.00	1
Glass rod, boro 3.3, l = 200 mm, d = 5 mm	40485.03	2
Glass beaker, tall, 150 ml	36003.00	2
Copper-II sulphate, cryst. 250 g	30126.25	1
Ammonia solution 25% 1000 ml	30022.70	1
Water, distilled 5 l	31246.81	1

Fig. 1: Experimental set-up: Lambert-Beer law – Diode array spectrometer.
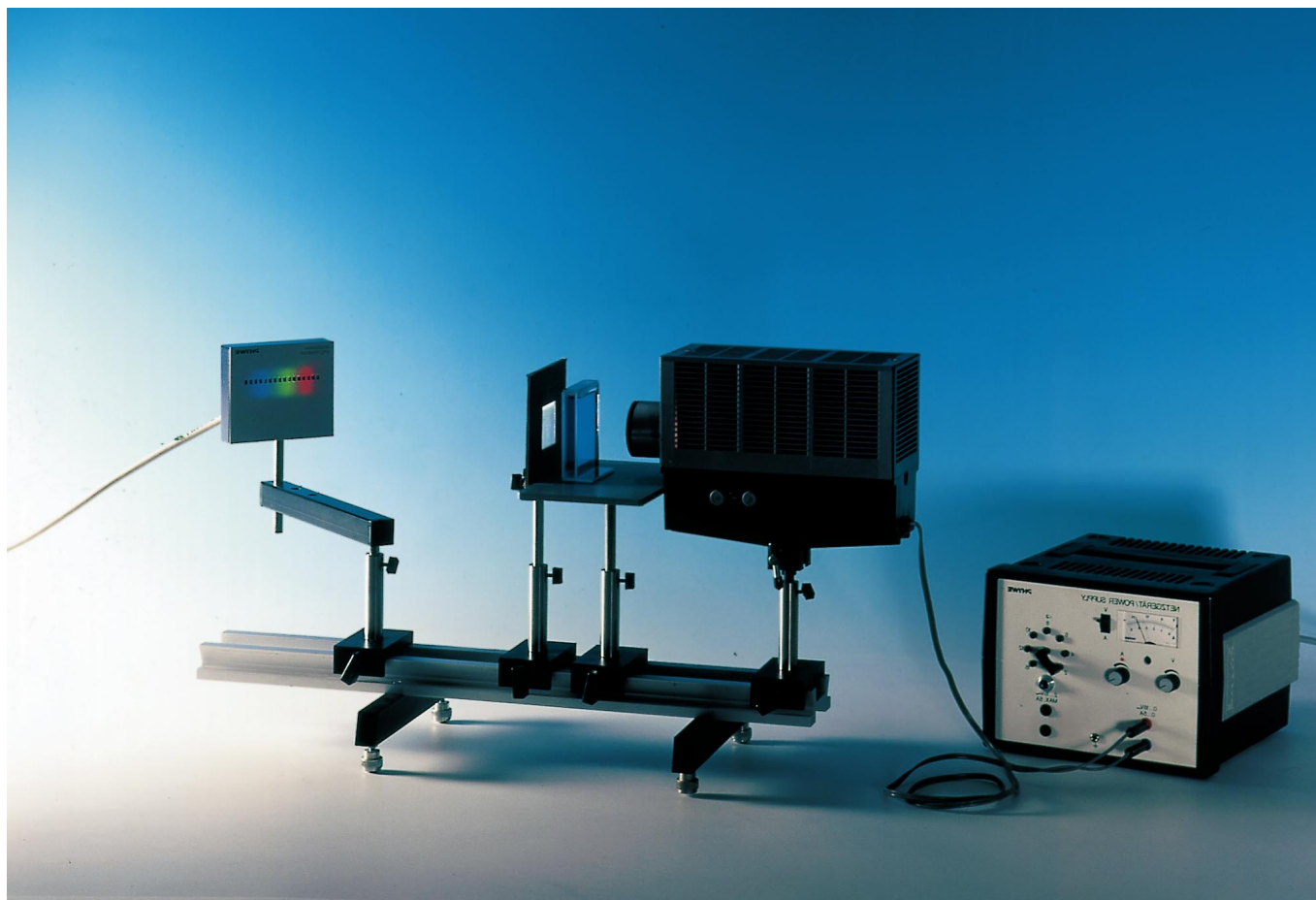
Problems

Record the absorption spectrum of tetrammine copper(II) complex ion and determine its absorption maximum.
Demonstrate the validity of the Lamber-Beer law for the tetrammine copper(II) complex ion using a dilution series. Investigate the layer thickness dependency of the absorption by aligning several cuvettes in a row.

Set-up and procedure

Perform the experimental set-up according to Fig. 1 and the instruction manual for the photodiode array spectrometer.

Prepare the solutions required for the experiment in the following manner:

– 5% NH_3 solution: Pipette 200 ml of 25% ammonia solution into a 1000 ml volumetric flask and fill the volumetric flask up to the calibration mark with distilled water.

– 0.1 molar $[Cu(NH_3)_4]SO_4$ solution: Weigh 2.4969 g of copper(II) sulphate ($CuSO_4 \cdot 5H_2o$) into a 100 ml volumetric flask, dissolve it in 5% ammonia solution and then fill the volumetric flask up to the calibration mark with 5% ammonia solution.

First place a 1 cm cuvette (cell), which is filled with distilled water, into the light path of the spectrometer in such a manner that it is exactly parallel to and in front of the objective aperture of the experimental lamp. Subsequently, perform the intensity adjustment (see instruction manual).

Then measure tetrammine copper(II) solutions having different concentrations. Prepare the solutions directly in the cuvette by mixing the following substance quantities:

$[Cu(NH_3)_4]^{2+}$/ml	1	2	3	4	5	7.5
5% NH_3/ml	39	38	37	36	35	32.5
V_{total}/ml	40	40	40	40	40	40

To achieve this, allow the copper complex solution to flow out of a microburette and the ammonia solution out of a 50 ml burette directly into the measuring cuvette. In each case, replace the water-filled cuvette with the 1 cm cuvette containing the copper complex solution which is to be investigated. After selection of "real time display" in the main menu and depression of the "Enter" key, depress the "F3" key. The spectrum of the copper complex immediately appears on the monitor as a plot of extinction against wavelength (Fig. 2). Subsequent to depression of the "C" key, the cursor is blended in; move it with the arrow keys to the absorption maximum. Depress the "Print Screen" key to obtain a print out of the screen (Fig. 2).

The extinction at the wavelength corresponding to the cursor position is displayed in the upper left-hand margin of the screen. Using the "+" and "–" keys on the right-hand side of the keyboard, the extinction scale can be obtimised.

Record the extinction values for all six mixture ratios in an analogous manner and plot them as a function of the copper concentration (Fig. 3).

Fig. 2: Spectrum of the tetrammine copper(II) complex.
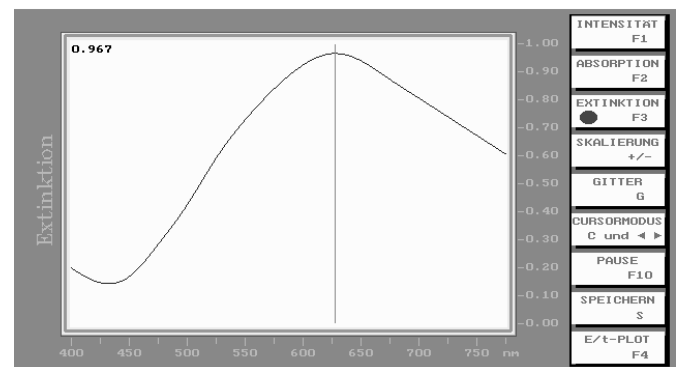
To investigate the dependence of the extinction on the layer thickness (path length), place three 1 cm cells and one 2 cm cuvette, which have been filled with water, in the path of the spectrometers: the cuvette faces should be in direct contact with each other. After completion of this optical adjustment, replace the water in the individual cuvettes successively with the above-described tetrammine copper(II) solution having the lowest concentration (in each case 1 ml of the 0.1 M tetrammine copper(II) sulphate solution in 39 ml of 5% ammonia solution). The respective extinction values at the absorption maximum are then determined as described above. The results are shown in Fig. 4.

Theory and evaluation

If sample solution having a layer thickness d is irradiated with light, the initial intensity I_0 of the radiation is reduced by interaction with the substance. The reduction in intensity dI is proportional to the layer thickness (light path) d and the concentration c.

$$-\frac{dI}{I_0} = k \cdot c \cdot d \qquad (1)$$

where
I = radiation intensity
k = proportionally factor
c = concentration
d = layer thickness

Integration supplies the Lambert-Beer law:

$$I = I_0 \cdot e^{-k \cdot c \cdot d} \qquad (2)$$

or

$$\ln \frac{I}{I_0} = -k \cdot c \cdot d \qquad (3)$$

For more convenient use, the decadic logarithm (lg) is used and additionally defines extinction E as follows:

$$E = \lg \frac{I_0}{I} = \varepsilon \cdot c \cdot d \qquad (4)$$

where
E = extinction
ε = molar (decadic) extinction coefficient
c = concentration
d = layer thickness

Fig. 3: Dependence of the extinction on the copper content (given in ml of stock solution in a total sample volume of 40 ml).
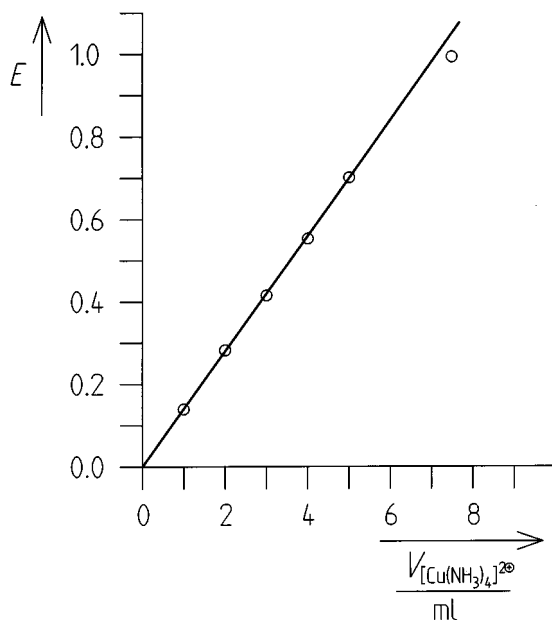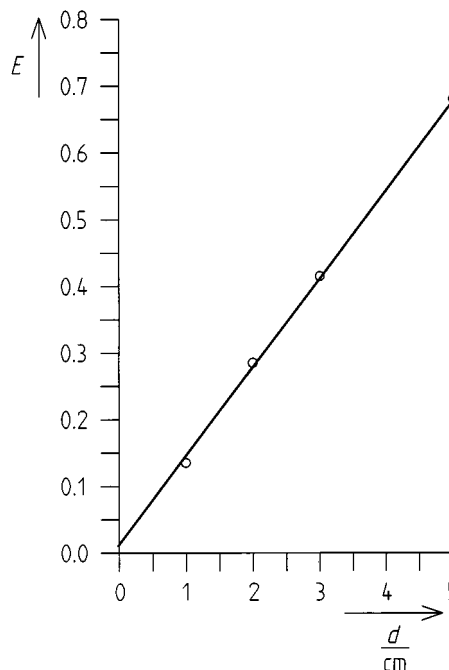

Fig. 4: Dependence of the extinction on the layer thickness (path length).

The expression $\lg I_0/I$ is measure of the absorption behaviour of the substance and is termed extinction. The substance-specific constant ε is a function of the wavelength and has the dimensions $I \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, where the concentration c is given in mol/l:

$$E_\lambda = \varepsilon_\lambda \cdot c \cdot d \qquad (5)$$

As can be seen from equation (5), as a result of the wavelength dependency of ε the extinction is also wavelength-dependent. For a constant wavelength λ and a constant layer thickness d, there is a linear correlation between the substance concentration c and the extinction E.

It also follows from equation (5) that for constant wavelength λ and constant concentration c there is a linear correlation between the extinction E and the layer thickness d.

Theory and evaluation

By plotting the extinction values of the copper complex solution against the concentration of the solution, one obtains a straight line (Fig. 3). This calibration curve definitively shows the linear correlation between the concentration of a substance and its extinction as required by the Lamber-Beer law. This functional correlation is used for quantitative photometric analysis (concentration determination). This linear correlation is only valid for strongly diluted solutions. At higher concentrations deviations from the Lambert-Beer law occur.

Plotting the layer thickness (path length) of an irradiated solution with constant concentration against the extinction also shows, as required, a linear correlation (Fig. 4).