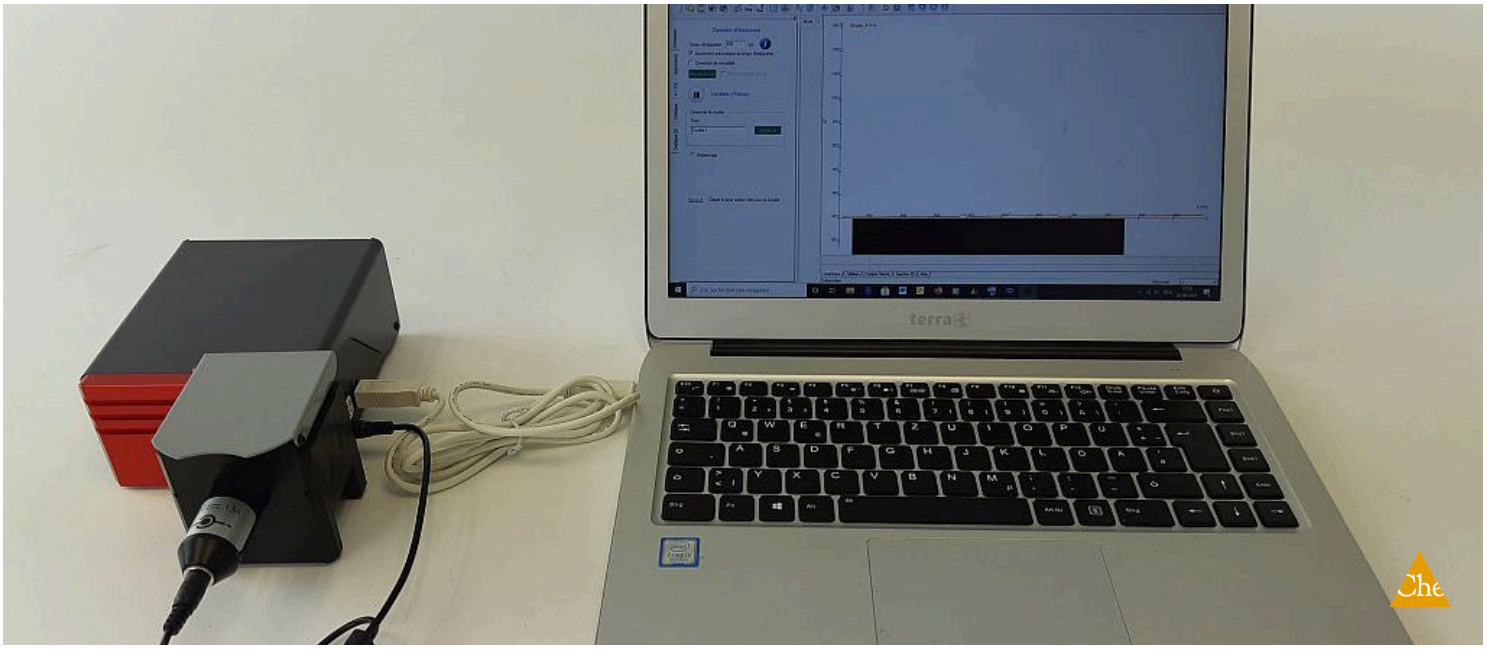
Multicomponent analysis with spectrophotometry (mixed colour photometry)



In dilutions containing substances of different colors, the concentrations of the dyes can be analyzed by spectrometry without prior separation of the substances. The integrated measurement software records the spectra of the pure dye solutions and their mixtures. Calibration curves for each substance make it possible to determine the amount of this substance in the dilution.

Chemistry > Analytical Chemistry > Photometry

Difficulty level	Group size	Preparation time	Execution time
medium	2	10 minutes	10 minutes

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General information

Application

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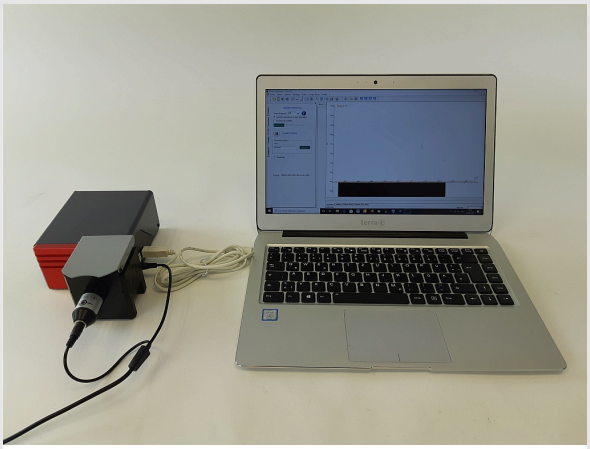

Experiment Setup

Spectroscopy enables the simultaneous determination of several substances in a sample solution, even if their spectra overlap at some wavelengths. A UV spectrometer can be used here for quantitative determination.

If a dye absorbs a certain wavelength (from the continuous light spectrum), the light beam weakens as it passes through a cuvette. The dependence of the absorbance on the concentration is described by Lambert-Beer's law

The sample to be analysed in the cuvette is irradiated with a light beam. The absorbance is displayed graphically as a function of the wavelength. The corresponding software now enables the quantitative evaluation of a measurement.

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Other information (1/2)

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Prior knowledge

A sample of a dye can be analysed in a cuvette with spectrometrometric methods by irradiating with a light beam.

The dependence of the absorbance on the concentration is described by Lambert-Beer's law. The absorbance is displayed graphically as a function of the wavelength. The corresponding software now enables the quantitative evaluation of a measurement.

Scientific principle

In solutions containing different-coloured substances the concentrations of the dyes can be analysed by spectrometry withoutprior separation of the substances.

Using the photometer the spectra of the pure dye solutions and mixtures thereof will be recorded. Calibration curves for eachsubstance enable us to determine the quantity of that substance in the solution

Other information (2/2)

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Learning objective

If several components are present in a solution, the concentrations of the individual substances cannot be determined by measuring a specific wavelength λ. Therefore, the entire UV/VIS spectrum is examined.

To do this, the wavelength λ of the whole UV/VIS radiation is measured continuously. Lambert-Beer's law then applies to each wavelength λ.

Tasks

- Mix two dyes together and fill tem in a cuvette.
- Calibrate the spectrometer
- Put the cuvette into the spectrometer.
- Use the progrannme "A = f(x)" to perform the Multicomponent analysis with spectrophotometry

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Safety instructions (1/2)

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For this experiment the general instructions for safe experimentation in science lessons apply.

For H- and P-phrases please consult the safety data sheet of the respective chemical.

Safety instructions (2/2)

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correct application

Spectrophotometry is very sensitive due to impurities. Turbidities of the solutions can cause an incorrect extinction due to scattering.

Therefore it is important to ensure that the solutions are free of turbidity. Make sure that no gas bubbles are present in the cuvette.

Always insert the cuvettes in the holder in the same orientation with respect to the radiation source. Use the same cuvette for reproducibility of results. Never touch the cells at the optically active surfaces, touch a cuvette only at the matt surface.

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Theory

The Law of Lambert-Beer

$$E = \lambda \cdot d \cdot c$$

with

- E = Absorbance
- $\lambda = wavelenght$
- d = diameter of the cuvette
- c = concentration of the dye in solution

Dyes in nature a mostly mixtures of differnent dyes. If two dye are in one solution, the concentration of one dye can be determined by using the law of Lambert and Beer

If a dye absorbs a certain wavelength (from the continuous light spectrum), the light beam weakens as it passes through a cuvette. The dependence of the absorbance on the concentration is described by Lambert-Beer's law.

The sample to be analysed in the cuvette is irradiated with a light beam. The absorbance (extinction) is displayed graphically as a function of the wavelength. The corresponding software which is used in this experiment enables the quantitative evaluation of a measurement.

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Equipment

Position	Material	Item No.	Quantity
1	Fibre-optics spectrophotometer	35620-00	1
2	Volumetric flask, Borosilicate, 100 ml, IGJ12/21	36548-00	9
3	Graduated pipette 25 ml	36602-00	1
4	Microspoon, steel	33393-00	1
5	Powder funnel upper dia. 100 mm	36893-00	1
6	Patent Blue V (sodium salt), 25 g	48376-04	2
7	Wash bottle, plastic, 500 ml	33931-00	1
8	Fuchsine acid -rubin s-, 25 g	31813-04	2
9	Water, distilled 5 l	31246-81	1
10	Data cable USB, plug type A/B, 1.8 m	14608-00	1
11	Macro-cuvettes, PS, 4ml,100 pcs	35663-10	1

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Setup and procedure

Setup (1/5)

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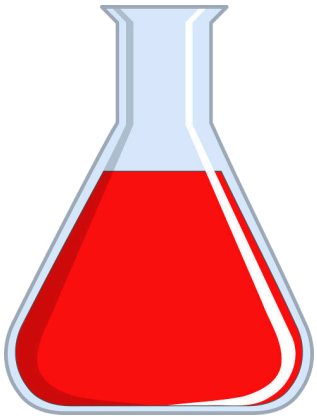

Preparing the solutions

Preparation of the Fuchsine acid solution:

Weigh out 1.6 mg of fuchsine acid into a 100 ml volumetric flasks and top up 100 ml with distilled water to get stocksolution. Pipette the following amounts of fuchsine acid solution into seven 100 ml volumetric flasks: 2.5 ml; 5 ml; 7.5 ml; 10 ml; 15 ml; 20 ml; 25 ml.

Fill each flask up to 100 ml with water.

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Setup (2/5)

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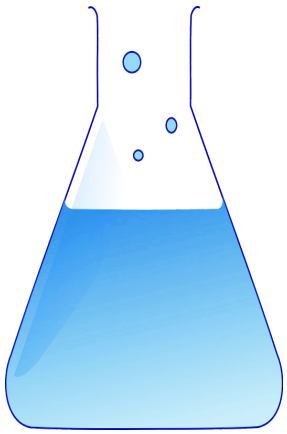

Preparing the solutions

Preparation of the Patent blue V solution:

Weigh out approx. 10.4 mg patent blue V into a 100 ml volumetric flask.Top up to 100 ml with distilled water. Make a Dye mixture of Fuchsine and Patent blue. Pipette 20 ml Patent blue V solution and 15 ml Fuchsine acid solution into a 100 ml volumetric flask and top up to100 ml with water.

These plus the stock solution are the solutions to be examined. We recommend commencing with the least concentratedsolution and rinsing the graduated cell each time with a little of the solution under investigation. As the cells tend to vary slightlyin layer thickness, one cell should be used for one series of measurement

Setup (3/5)

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For this experiment you need a software. The integrated software in the device requires no installation disc. The spectrometer can be used immediately after connecting the spectrometer via USB interface with the PC and installation of the software.

Power is supplied via a separate 12V power supply, which is included in the delivery. For this experiment you need the y-shaped cable to connect both parts of the spectrometer with the power supply.

The important parts of the device is the light chamber (in which you put the cuvette with your sample) and the spectrometer.

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Setup (4/5)

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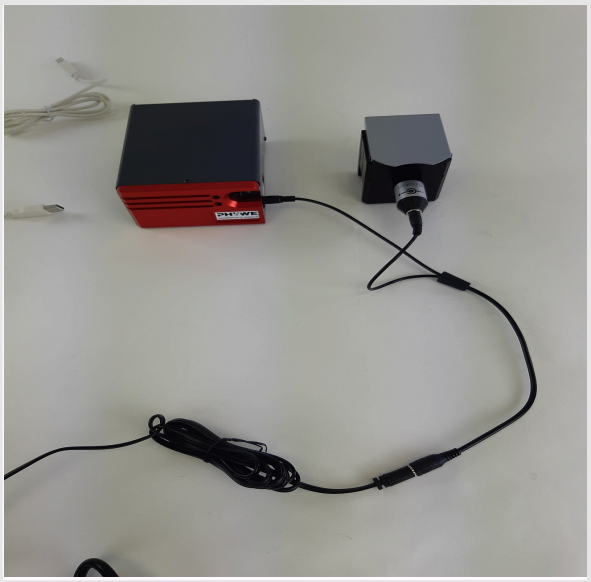

First combine the light source and the spectrometer with the power supply. Take the y-shaped cable and plug it with the power supply (picture left).

Connect the each end of the y-shaped cable with the light chamber (device right in the picture) and the spectrometer (device left in the picture).

Do not plug the power supply into the plug socket, before the light chamber and the spectrometer are not connected (see next side).

Setup (5/5)

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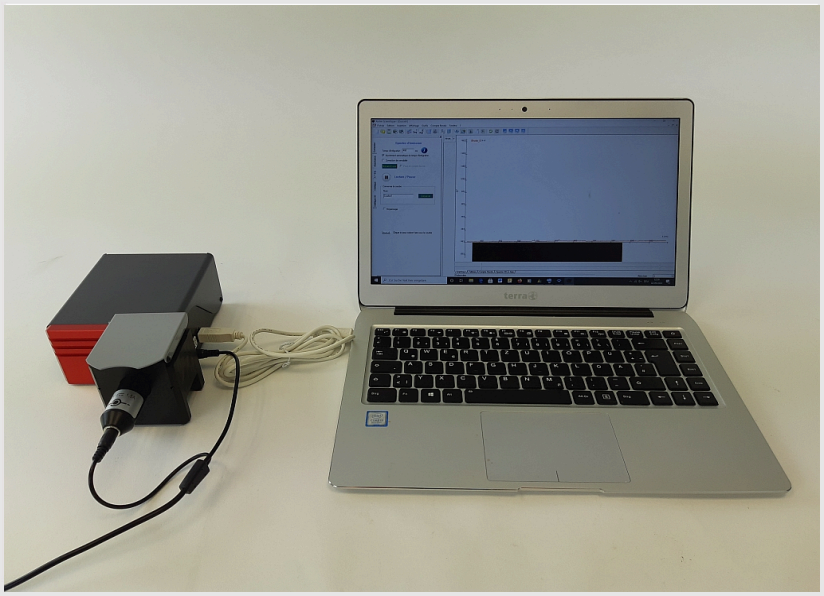
Put both parts (the chamber and the spectrometer) together (like shown in the picture on the right side). The connection work by magnetic way.

Put the light chamber directly in front of the spectrometer. For this experiment you not need a fibre cable to connect light chamber and the spectrometer.

Connect the spectrometer with your PC with the USB-cable, which is included in the delivery.



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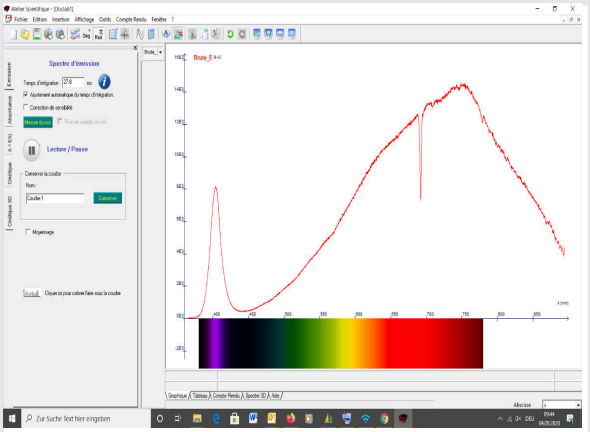
Procedure (1/4)

Before each absorption or transmission measurement, you must perform a calibration of zero (also called "dark measurement") and reference spectrum. The absorption mode allows you to measure the optical density and/or the transmission coefficient of a sample according to the wavelengths.

Each programme perfoms a calibration. For the experiment of Lambert-Beer choose the programme "A = f(x)". For the calibration of the spectrometer yor need a cuvette filled with the solvent (=> water) to perform the reference spectrum. To perform the dark spectrum you need the black metall (included in the scope of delivery), which you put between the light source and the cuvette. The programme will tell you when.



Overview of the spectrometer software

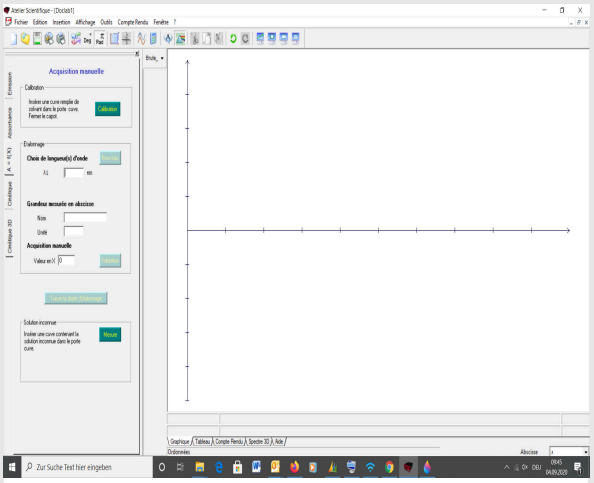
Procedure (2/4)

Note that, as the absolute concentrations of dye in the solutions are very low and the cells display a certain tolerance, the calibration curves are never universally valid.

Therefore, new calibration curves should be drawn up for each new series of measurements.

After choosing the function "A=f(x)" you have to first perform a reference and dark spectrum calibration.

Put a cuvette with water (as solvent) in the light chamber. Start the calibration function (first button in the programme, see picture on the right side)



The function "A = f(x)"

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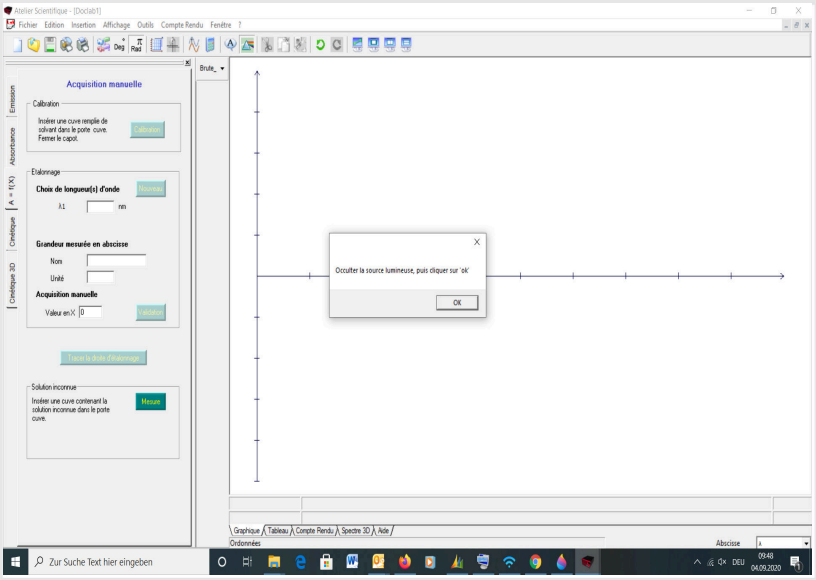
Procedure (3/4)



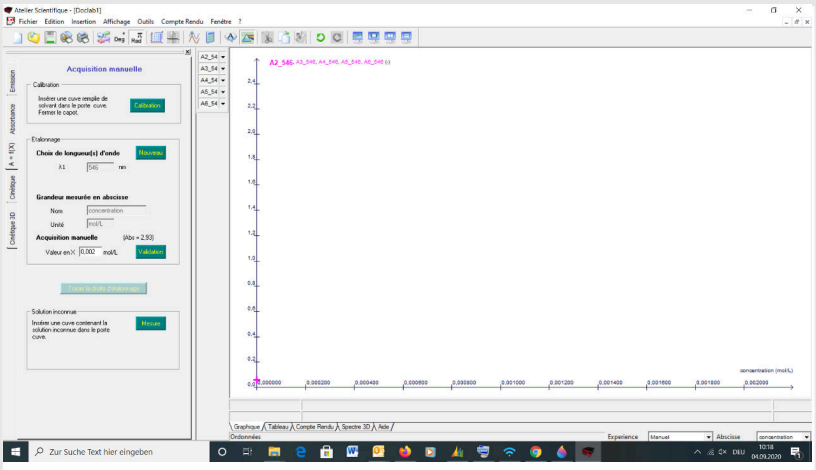
After performing the reference spectrum, you have to perfom the dark calibration.

The device is (by opening a window) telling you, when you have perfom this.

You take the black metal (included in the delivery) and put it in the light chamber in front of the cuvette.

Click than "ok" to beginn with the measurement of the dye

Procedure (4/4)



Measurement of a series of dyes

After the calibration is done, put the dark metal out of the light chamber.

Enter in the software the wavelength at which you want to work (Fuchsine, about 546 nm). Fill in the characteristic size and its unit, generally the concentration of the solutions analysed (e.g. "concentration" and "mol/L" or "g/mL")

Enter the value of the concentration of each sample and click on 'Validation'.

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Evaluation

Evaluation (1/3)

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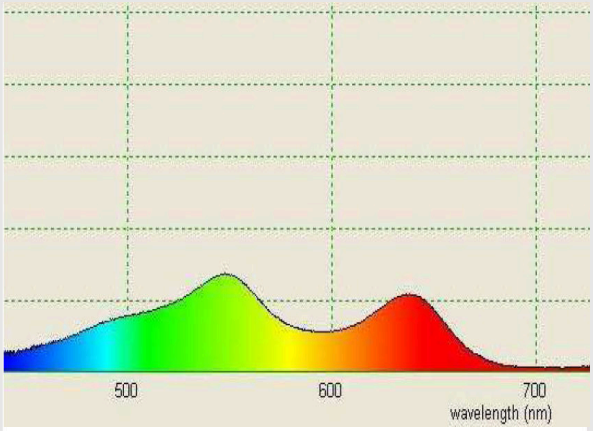

Wavelanght scan ot the mixture

The absorption maxima of two dye substances (Patent-blue and Fuchsine) differ substantially from each other and do not influence each other. So it is possible to carry out simultaneous photometric analysis of this solution without first separating each substance out of the mixture.

Fig. left shows the absorption spectra of Fuchsine acid and Patent blue V in solution. There is a difference between the absorption maxima of these two substances of approx. 100 nm.

The residual absorption of each dye at the absorption maximum of the other dye has no influcence. The law of Beer and Lambert can be used to examine the concentration.

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Evaluation (1/3)

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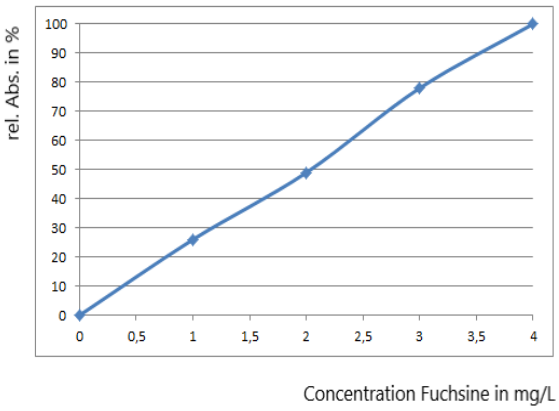

Diagramme of Fuchsine

When the red fuchsine acid solution is mixed with the blue patent blue V solution, it produces a purple coloration of the solution.It is possible to determine the proportion (concentration) of Fuchsine acid in this purple solution (at the absorption max. at 546 nm)

For this purpose, a solution series for fuchsine acid is analysed and a calibration curve drawn up be entering extinction values as a function of the proportion off Fuchsine acid stock solution.

The determination of the extinction is at 546 nm. The concentration of Fuchsine acid solution is direcly proportional to the extinction / absorbance. At any value of the extinction / absorbance the concentration of Fuchsine in the soltion can be read out of the diagramme.

Evaluation (3/3)

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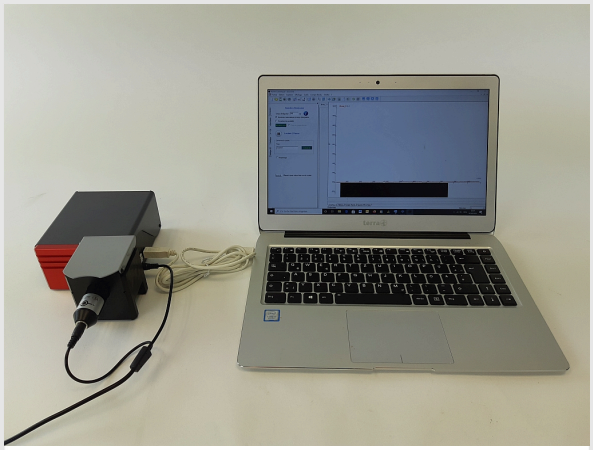
The formula

$$E = \lambda \cdot d \cdot c$$

belongs to?

- ○ The law of Lambert-Beer
- ○ The law of Heisenberg
- ○ The law of Wolfgang

Check



Experiment Setup

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Slide	Score/Total
Slide 22: The law of ...	0/1

Total Score 0/1

Show solutions Retry

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