Determination of the practical applicability range of Beer–Lambert Law

Literature: –
Type of the practice: Individual.
Aim of the practice: The experiment demonstrates the determination of the absorbance range, in which a given spectrophotometer can be applied for quantitative measurements. After appropriate modifications, this process can be also applied for the determination of the accuracy of other molecular spectroscopic instruments.

1 Introduction

In spectrophotometry, the relation between the measured signal (either light intensity, or absorbance) and the concentration of the colored compound is described by the Beer–Lambert law:

$$A^{\lambda} = \lg \frac{I_0^{\lambda}}{I^{\lambda}} = \varepsilon^{\lambda} \cdot c \cdot \ell, \tag{1}$$

where the λ superscript denotes the used wavelength,

A^{λ} is the absorbance,

I_0^{λ} is the intensity of the incident light,

I^{λ} is the intensity of the transmitted light,

ε^{λ} is the molar absorbance (i.e., the value of absorbance in a solution with unit concentration) of the colored compound, mostly given in $M^{-1}cm^{-1}$ units,

c is the concentration of the colored compound and

ℓ is the length of the light path in the cuvette.

If there are more colorful compounds in the measured sample, the measured absorbance is the sum of the individual absorbances, and therefore the general form of the Beer–Lambert law should be valid:

$$A^{\lambda} = \left(\sum_{i=1}^{n} \varepsilon_i^{\lambda} \cdot c_i \right) \cdot \ell, \tag{2}$$

where n is the number of the colored species.[1]

Equation (1) suggests that the measurement is performed according to Figure 1(a). This way, the measured signal is the sum of absorbance of the cuvette, the solvent and the dissolved colored compound(s). However, in the Beer–Lambert law, ε_i and c_i refer only to the colored compound. Therefore, the measurement is carried out in a different way. As seen in Figure 1(b), only the intensity of the passed light (and

[1] For the sake of simplicity, indication of λ is omitted (but implicitly implied) in the following equations.

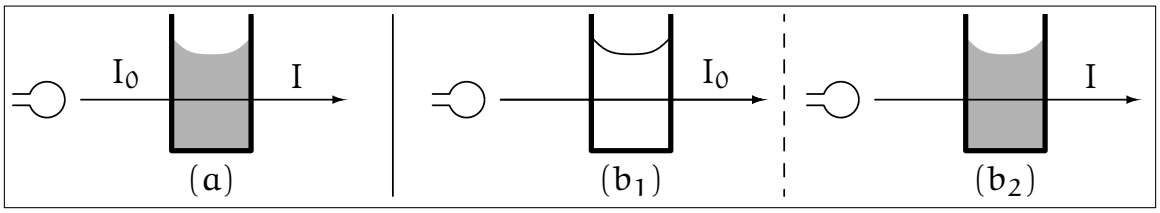

Figure 1: Possible arrangements of absorbance measurements; see the text for explanation.

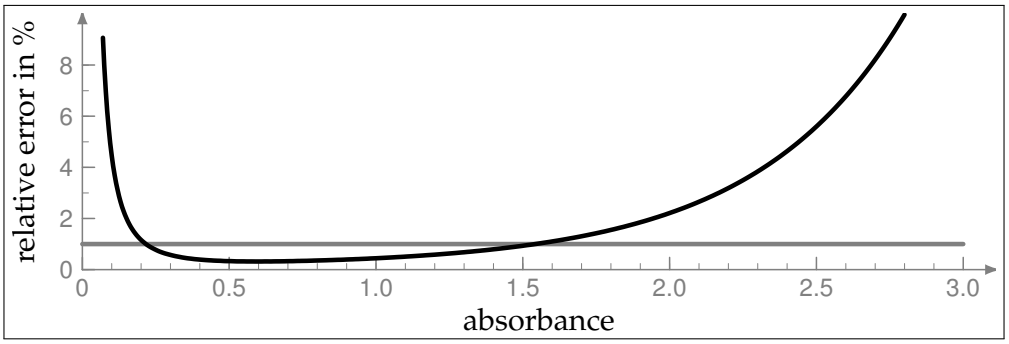

Figure 2: Relative error of the absorbance measurement as a function of the measured absorbance provided that the temporal uncertainty of the intensity is 0.1 %.

not the intensity of the light source) is detected in a spectrophotometric measurement. I_0 is determined by filling the cuvette with the solvent (Figure 1(b_1)) and measuring the intensity of the transmitted light. This way we can eliminate the "background" effect (absorbance of the cuvette and the solvent), and determine the initial light intensity. The cuvette is then filled with the solution, and the absorbance is measured (Figure 1(b_2)). Any decrease in the light intensity in this case is related only to the light absorbance of the dissolved colored compound, and therefore equation (1) or (2) can be applied. In spectrophotometers, having a single light path, measurement of I_0 and I happens after each other, while these parameters can be determined simultaneously by double beam spectrophotometers.

1.1 Technical aspects

In practice, measurement of the absorbance is limited by several technical parameters. Beyond the quality of the optical components, the most important accuracy determining part of an instrument is the light intensity measuring device, the detector. Just for example, when the absorbance of the sample is 3, $I_0/I = 1000$ can be calculated from (1), meaning a 1000 times lower transmitted light intensity (compared to the intensity of the incident light). To measure, for example, values $A = 3$ and $A = 0.5$ with the same accuracy, such detector would be needed, which can measure I_0 and a 1000 times lower intensity with the same relative error. However, use of such high-quality detectors is still not prevalent in basic laboratory instruments.

Accuracy of the measurement of the light intensity can be discussed similarly to the measurement of the mass. In the latter case, it is obvious, that 20.00 g and 0.02 g cannot be measured with the same accuracy by using a normal precision balance. Similarly, it can be proven (but not detailed here), that the relative error of the absorbance, calculated from the measured light intensity changes according to Figure 2. in function of the absorbance, assuming 0.1% precise measurement of the light intensity. In case of a more precise instrument, the curve shifts to lower values, while it shifts upwards in case of a less precise instrument.

In Figure 2 there is a straight line at 1% relative error, defining the limits of the quantitative analytical application. The measured absorbance can be used for quantitative analysis if it falls in the range, where the curve is below the straight line. Since technical details of the detectors are usually not provided by the manufacturers, this range must be determined experimentally.

Beyond the light intensity dependent relative error of the detector, two more technical aspects must be considered: *(1)* both the light intensity, *(2)* and the relative error of the detector depend on the wavelength of the light. Since these effects are not significant in a narrow wavelength range, and are automatically corrected in case of modern instruments, we disregard of these during the experiment.

The aim of the student's measurement is the determination of the upper limit of the absorbance range, in which the spectrophotometer can be applied for quantitative analysis, by measuring the concentration dependent absorbance of the solution of a given colored compound.

2 Steps of the measurement

To determine the absorbance range within which a spectrophotometer can be quantitatively used according to the Beer–Lambert law, a dilution series is prepared from a suitable colored compound, and the absorbance of these solutions is measured. By plotting the measured absorbance values in function of the concentration, a linear with 0 intercept can be fitted in the range, in which the Beer–Lambert law is valid. At higher concentrations, the measured data points deflect from this linear trend. In case of a general spectrophotometer, the validity range of the Beer–Lambert law falls mostly between the absorbance values of $0-2.5$.

Not only the too large absorbance can lead to deviation from the linear trend, it can be also caused by some chemical processes (e.g. (de)protonation, dissociation, decomposition, etc.), therefore the examined colored compound must be selected carefully. Moreover, appropriate circumstances must be provided, in which the Eq. (1) form of the Beer–Lambert law is valid (instead of the general form, given by Eq. (2) for mixtures containing more than one colored component). In case of spectrophotometers, operating in the visible range of light, basic potassium dichromate or neutral potassium ferricyanide aqueous solutions can be used. Both solutions are yellow colored, therefore wavelengths below 500 nm are used for the determination of the applicability (quantitative) range of the spectrophotometer. To measure at higher wavelengths, dilute aqueous solutions of different *p*H indicators are generally used. Note however, that both the color and the stability of these compounds are very sensitive to the *p*H value, therefore it must carefully be adjusted in case of such measurements. Moreover, the large adsorption tendency of indicator molecules to plastic surfaces, absorbance measurement of these solutions should be performed in glass cuvettes.

3 Student's measurement

The students' measurements consist of the preparation of a stock solution, a solution series by dilution of it, and the measurement of the absorbance of these solutions at two different wavelengths. By plotting the measured absorbance values as the function of concentrations, the absorbance range within which a spectrophotometer can be quantitatively used according to the Beer–Lambert law (in which the function is linear), and additionally the molar absorbances of the compound must be determined. The first wavelength (λ_1) is chosen to get absorbance values outside the validity range of the Beer–Lambert law in case of some concentrated solutions, while the second wavelength (λ_2) is chosen to get absorbance values for all solutions within this range (even for the stock solution).

At the beginning of the experiment, the instructor defines *(1)* the compound used (K_2CrO_4, $K_3[Fe(CN)_6]$, bromophenol blue, or methyl orange) and *(2)* the proposed absorbance ($5.5-6.5$) of the stock solution to be made later. If there are no specific instructions, $K_3[Fe(CN)_6]$ must be used and the proposed absorbance value is 6.0.

Prepare 250 cm^3 solution of the selected compound (this is not the later stock solution) whose concentration is 10^{-3} M for the salts and 5×10^{-5} M for the indicators. Solutions of the *p*H indicators must be prepared by dilution of the given stock solutions, while the others must be prepared by dissolving a precise amount of the solid chemicals. The K_2CrO_4, bromophenol blue, and methyl orange solutions must contain 0.50 g $Na_2CO_3\times10H_2O$ to adjust the *p*H, while the $K_3[Fe(CN)_6]$ solution can be prepared without any further modification. Relative molar mass values of K_2CrO_4 and $K_3[Fe(CN)_6]$ can be calculated from the relative atomic masses presented in the Appendix. In case of the indicators, the initial concentration of the solutions is indicated on the bottles (0.0015 M for bromophenol blue and 0.003 M for methyl orange).[2]

The next step is to record the spectrum of the prepared solution within the wavelength range of visible light ($350-700$ nm). Given that the absorbance stays below ca. 2 within the investigated range, search the wavelength corresponding to the absorbance maximum (λ_1). Save the spectrum from the spectrophotometer, or take a photo of it, and attach it to your report. If the absorbance overcomes 2, prepare a more diluted solution and repeat the process. Calculate the concentration of the stock solution, which must be prepared for

[2]Before measurements, please check these concentrations on the bottles carefully.

the following measurements, by knowing the concentration of the just measured solution, its absorbance at λ_1, and the proposed absorbance of the stock solution to be prepared. Then, prepare 250 cm^3 stock solution. When preparing the stock solution of the given compound, add $Na_2CO_3 \cdot 10H_2O$ as described above.

The second wavelength (λ_2) is to be chosen by the student according to recorded spectrum in order to get an expected absorbance of 2.5 for the stock solution prepared according to the above. For the above-described reasons (most importantly the wavelength dependent relative error of the detector) the second wavelength should be chosen close to the first one, preferably avoiding the regions where the spectrum changes rapidly.

The next step is to prepare the dilution series from the stock solution. The stock solution with the volume of 250 cm^3 is diluted. Care should be taken during dilutions to ensure that the solution released from the burette is sufficient to prepare all 20 samples! Do not waste it! Also wash the burette with stock solution with only a few cm^3 of solution and for the setting of the burette signal, do not place the residue in the waste, but collect it in a separate, clean beaker and we can later refill the burette with these solutions. If there are no further instructions, then one after the other 1, 2, ..., 20 cm^3 of the stock solution must be measured into a 25 cm^3 volumetric flask by using a burette, and then filled up with distilled water. Absorbance of the diluted solutions must be measured at the two chosen wavelengths. All the diluted solutions must be prepared by using the same volumetric flask[3], and the absorbance must be measured immediately after the preparation of samples. Finally, absorbance of the stock solution must be also recorded. *The experimental error can be minimized by starting with the most dilute solution and proceeding to continuously increasing concentrations.*

Steps of the absorbance measurement are the following. First, the cuvette is filled up to its 2/3 volume by distilled water, and then the absorbance is adjusted to 0.000 at the first wavelength.[4] The cuvette is then leached 3 times with 1.0 – 1.5 cm^3 large portions of the first solution (having the lowest concentration). Subsequently, the cuvette is filled up to its 2/3 with the solution, and its absorbance is measured. The spectrophotometer is then set to the second wavelength, and the absorbance is again adjusted to 0.000, by using properly cleaned cuvette, filled with distilled water. Accordingly, to the above-described method, absorbance of the solution is then measured at this wavelength. Absorbance measurement of the next solution can be started at the second wavelength (and therefore the adjustment of the absorbance of distilled water to 0.000 can be circumvented, making the measurement significantly faster). Then the wavelength is set to the first wavelength, and the absorbance is again adjusted to 0.000, by using properly cleaned cuvette, filled with distilled water. *When changing the wavelength, the absorbance should be always adjusted to 0.000 by using distilled water filled cuvette, since the absorbance of both the cuvette and the solvent is highly dependent on the wavelength!*

By following this method, the whole experiment can be performed by using only one cuvette. However, the cuvette must be carefully washed numerous times, which is both time consuming, and offers too many chances to make experimental mistakes. Usage of two cuvettes makes the measurement faster and more precise. In this case, one of the cuvettes is filled with distilled water, and just used to adjust the absorbance to 0.000 at every wavelength changing, while the other cuvette is filled with the solutions to be measured. In case of using this technique, the measured absorbance values must be corrected with the difference between the cuvettes. This is determined by filling both cuvettes with distilled water, adjusting the absorbance to 0.000 using the first cuvette, and measuring the absorbance of the other cuvette used for the measurement of the samples. This procedure has to be performed at both used wavelengths! These correction values ($A^{\lambda_1}_{\text{cuvette}}$, and $A^{\lambda_2}_{\text{cuvette}}$ must be then subtracted from the measured absorbance values. It is highly recommended to perform this measurement both before the measurement of the first, and after the last sample.

If there are no further instructions, and two cuvettes are supplied, the technique applying two cuvettes must be used. If both plastic and glass cuvettes are at service, glass cuvettes should be used for experiments with the indicators.

[3]The error caused by the slight changes in the volume of the different volumetric flasks can be avoided this way.

[4]The user manual of the spectrophotometer, containing the necessary steps of the absorbance measurement is supplied as part of the students' inventory.

Removal of the indicator traces from a laboratory equipment is often difficult. If the measurement is performed with indicators, at the end of the experiment the volumetric flask, the burette, the cuvettes and the used pipettes and beakers must be subsequently washed 5 – 6 times with tap water, distilled water and finally with the available ∼1 M base solution. The base solution used for cleaning is applicable for several times, so we can pour back into the storing bottle.

4 Evaluation of the measured data

1. All the experimental conditions, the measured absorbance values, and the concentrations of the solutions should be summarized according to Table 1.

Table 1: Summary of the measured and calculated data

Investigated compound=..., $\lambda_1 = \ldots$, $\lambda_2 = \ldots$, $A^{\lambda_1}_{\text{cuvette}} = \ldots$, $A^{\lambda_2}_{\text{cuvette}} = \ldots$

V_{diluted} (cm^3)	c (M)	$A^{\lambda_1}_{\text{measured}}$	$A^{\lambda_2}_{\text{measured}}$	$A^{\lambda_1}_{\text{corrigated}}$	$A^{\lambda_2}_{\text{corrigated}}$

The corrected absorbance values must be calculated only if the technique using two cuvettes was applied.

2. Plot the $A^{\lambda_1}_{\text{measured}}$ (or $A^{\lambda_1}_{\text{corrigated}}$ in case of the technique with two cuvettes) values as the function of c. Fit a line through the origin to the linear range of the curve and determine the molar absorbance and its standard deviation of the color material at λ_1 (ε^{λ_1}) wavelength according to the Eq. (1) (Excel LINEST function, Origin, QtiPlot, etc.). From the linear range the curve determined by the experimental points differs better at higher absorbances. Because of this, skip the data points with the largest absorbance values one after the other, and examine if the remaining points are all lying on the fitted straight line or not (aside from the experimental errors).
3. Perform the previously described procedure for the second wavelength also.
4. Based on the created figures, give the upper limit of the absorbance range, in which the Beer–Lambert law is valid on the used spectrophotometer.
5. Calculate the molar absorbance values for both wavelengths and compare them to the literature; give the source of the literature data. Discuss the possible reason of the differences (if there is any compared to, e.g., the determined standard deviations)!

Questions

1. Give the relationship between the absorbance and the light intensity!
2. Give the Beer–Lambert law for one, and for more than one light absorbing compound!
3. How do we eliminate the absorbances of the solvent and the cuvette?
4. What are the technical limitations, leading to an absorbance range within which a spectrophotometer can be quantitatively used according to the Beer–Lambert law?
5. Sketch the change of the relative error of the absorbance in function of the absorbance values!
6. How can be the practical applicability range of Beer–Lambert law experimentally determined?
7. What conditions must be fulfilled for a colored compound to be suitable for the experiment?
8. Upon what considerations should the used wavelengths be selected at the beginning of the experiment?
9. What are the advantages and disadvantages of applying the technique with one cuvette?
10. Why and how should be the measured absorbance values corrected in case of applying the technique with two cuvettes?
11. 250 cm^3 0.0025 M potassium ferricyanide ($K_3[Fe(CN)_6]$) solution is to be prepared. How many grams of the solid material must be measured to make the solution? $A_r(C) = 12.01$, $A_r(N) = 14.00$, $A_r(K) = 39.10$ és $A_r(Fe) = 55.85$.
12. In separate volumetric flasks, 1, 5, and 10 cm^3 of a 0.0025 M potassium ferricyanide solution is diluted to 25 cm^3. Calculate the concentration of the diluted solutions!
13. Molar absorbance of the potassium dichromate solution is 607.7 $M^{-1}cm^{-1}$ at 420 nm. Calculate the concentration of the solution, of which absorbance is 3.0 (measured in a cuvette with 1 cm light path length)!
14. Molar absorbance of the basic solution of methyl orange is 16100 $M^{-1}cm^{-1}$ at 500 nm. How large volume of the stock solution, having a concentration of 0.003M has to be diluted to 250 cm^3, to get a solution of which absorbance is 3.0 (measured in a cuvette with 0.5 cm light path length)?