LABORATORY SAFETY

WORKING IN THE LABORATORY REQUIRES STRICT ADHERENCE TO LABORATORY REGULATIONS AND OCCUPATIONAL HEALTH AND SAFETY RULES. THESE DOCUMENTS ARE FREELY AVAILABLE ON THE WEBSITE OF THE INSTITUTE OF MEDICAL BIOCHEMISTRY AND LABORATORY DIAGNOSTICS:

HTTPS://ULBLD.LF1.CUNI.CZ/FILE/3143/SAFETY2017.PDF

BEFORE ENTERING THE LABORATORIES, ALL STUDENTS ARE REQUIRED TO FAMILIARIZE THEMSELVES THOROUGHLY WITH THESE DOCUMENTS.

PHOTOMETRIC DETERMINATION OF SOLUTION CONCENTRATION

Theoretical introduction

Dispersion systems

A **dispersion system** is defined as a system composed of two or more chemically pure substances. One of the components (usually the one present in the greatest quantity) is called the **solvent**. Molecules or ions of the other substances, referred to as **solutes**, are dispersed within the solvent. The particles of these substances are thoroughly mixed and do not react with one another.

Based on the size of the dispersed particles, dispersion systems can be classified as analytical (referred to as **true solutions**), colloidally dispersed, and coarsely dispersed. True solutions constitute a homogeneous, single-phase system in which the size of the solute particles is less than 1 nm. Colloidally dispersed systems are systems in which small particles – either solid or liquid – with sizes ranging from 1 nm to 1 μm, are homogenously dispersed. Significant types of colloidally dispersed systems include micellar and colloidal systems.

Micellar solutions are dispersions of a solvent and tiny spherical particles known as micelles. Micelles are organized structures composed of aggregated substances that possess both a polar and a non-polar region; due to this dual nature, they are classified as amphiphilic substances. Micellar solutions play a role in lipid digestion, a process involving the targeted secretion of amphiphilic substances into the digestive tract to emulsify dietary lipids. They are also used in cosmetics in the form of "micellar water," where the micelles can incorporate substances with hydrating, moisturizing, anti-inflammatory, or astringent properties.

Colloidal systems contain other kinds of particles ranging in size from 1 nm to 1000 nm. Colloidal solutions exhibit a number of specific properties:

1. Since the particle size in a colloidal system is comparable to the wavelength of visible light (390-760 nm), radiation passing through the system is scattered, forming a coloured cone of light – a phenomenon known as the **Tyndall effect** (Fig. 1).
2. Particles of a colloidally dispersed system pass freely through filter paper and therefore cannot be separated from the solvent by conventional filtration. However, colloidal particles are unable to pass through specific membranes and thus **can be separated using dialysis**.
3. Particles of a colloidally dispersed system are in constant motion due to interactions with solvent molecules (Brownian motion) and consequently do not **undergo sedimentation**.

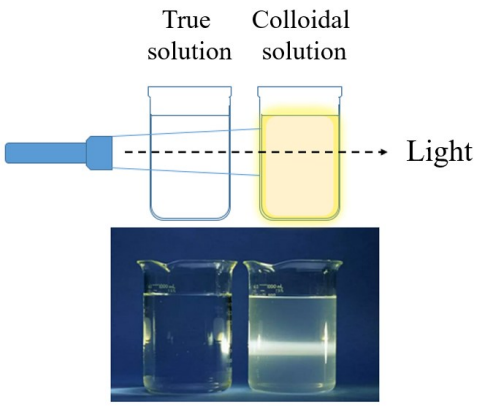


Figure 1: Tyndall effect

Due to the particle size in a colloidal dispersion system, radiation passing through the solution is scattered, creating a cone of light. True solutions do not scatter radiation.

Colloidal systems are of two types: either consist of metal micro-crystals finely dispersed in a liquid medium, causing a characteristic colour to the colloidal solution, or the "dissolved" particles are clusters of organic molecules (macromolecules, such as proteins). Colloidal solutions represent an intermediate state between true solutions and mixtures such as suspensions and emulsions.

In case a solid substance with particles larger than 1 μm (e.g., graphite dust) is dispersed in a solvent, the mixture is referred to as a **suspension**; in the case of a dispersed liquid (for instance tiny oil droplets in water) the mixture is referred to as an **emulsion**. The particles constituting a suspension or emulsion can usually be detected using an optical microscope. Over time, the components of both suspensions and emulsions tend to separate – a process known as sedimentation. This process can be accelerated by centrifugation.

Photometry and spectrophotometry

Photometry is a fundamental analytical method enabling both quantitative and qualitative sample analysis. The principle of photometry lies in the interaction of radiation with sample molecules, giving rise to various optical phenomena such as absorption, emission, and scattering. When radiation of a single specific wavelength is used for analysis, the technique is referred to as photometry; when radiation of varying wavelengths (a spectrum) is used, it is called spectrophotometry (Fig. 2).

a) **Photometry:** measurement of light absorption by a sample that takes place at **one or several specific wavelengths.**

b) **Spectrophotometry:** measurement of light absorption by a sample that takes place over a specific **continuous range of wavelengths.**

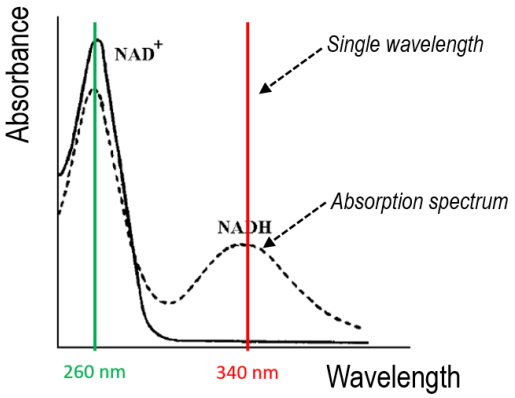


Figure 2: Photometric and spectrophotometric analysis:

*During **photometric** analysis, absorbance is determined at a **single specific wavelength** (260 nm for NAD^+ and at 340 nm for NADH). The result is therefore a **single absorbance** value. During **spectrophotometric** analysis, measurements are performed over a **continuous range of wavelengths** (in this case, from 240 to 450 nm). The result is therefore a curve – **absorption spectrum**.*

One of the most common applications of photometry is quantitative analysis – that is, the determination of the exact concentration of a substance in a solution. Quantitative analysis relies on the principle described by the **Lambert-Beer law** (Fig. 3):

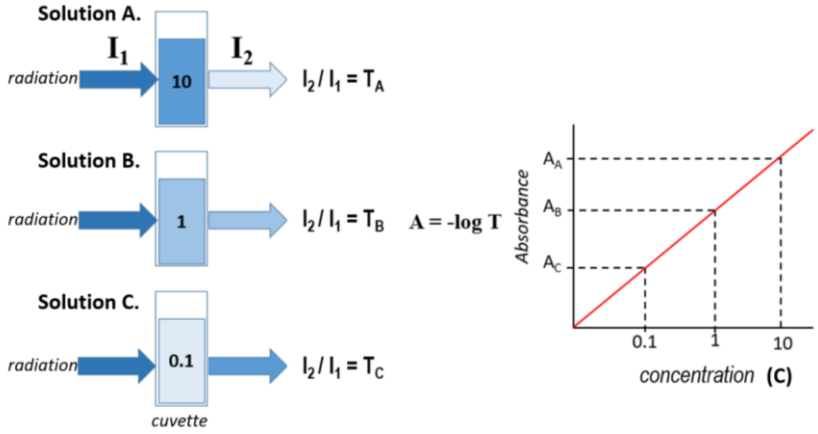


***Figure 3: Lambert-Beer law** is a mathematical expression of the dependence of the absorption of the radiation on the properties of the substance through which the radiation passes. The ratio of radiation intensities after (I2) and before (I1) passing through the solution is referred as **transmittance (T)**. For most quantitative analyses, the **negative logarithm of T** (referred to as **absorbance A**) is used as the evaluation parameter. **Absorbance is directly proportional to the sample concentration (c)** and the thickness of the layer through which the radiation passes (l, corresponding to the cuvette width). The specific absorption properties of each substance are characterized by the molar absorption coefficient (ε). A plot of absorbance (A) values for samples of known concentration (A, B, C) is called a **calibration curve**; for **true solutions**, this **curve is linear** within a certain range. The concentration ranges over which the linear relationship between absorbance and concentration holds is referred to as linearity.*

$$A = -\log T = \varepsilon \cdot c \cdot l$$

A *= absorbance at the given wavelength*
T *= transmittance at the given wavelength*
ε *= molar extinction coefficient of the particular compound at the given wavelength*
c *= molar concentration of the analysed solution in mol/L*
l *= thickness of the measured layer (cuvette) in cm*

In quantitative analysis, the concentration of an unknown sample can be determined in the following ways:

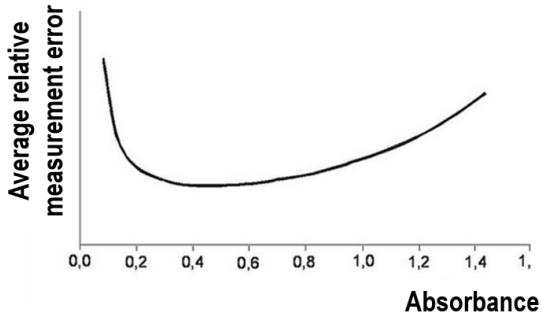
a) by calculation using the Lambert-Beer law, if the molar absorption coefficient is known
b) by reading from a calibration curve
c) by calculation using a calibration factor
d) by measuring the absorbance of a standard solution of known concentration and the absorbance of the sample solution, followed by calculation using the relationship: $\mathbf{A_{st}/A_{sample} = c_{st}/c_{sample}}$
where A_{st} is the absorbance of the standard solution
A_{sample} is the absorbance of the analysed solution
c_{st} is the molar concentration of the standard solution
c_{sample} is the molar concentration of the analysed solution

Regarding the fact that radiation absorption occurs at the level of molecular orbitals, the Lambert-Beer law cannot be applied to systems where other optical phenomena – such as scattering – occur alongside absorption. In such cases, the degree of transmittance (or absorbance) is influenced not only by the variables ε·c·l but also by other factors, such as the size and structure of dispersed particles, the rate of sedimentation, and so on. **Consequently, under certain conditions, colloidally dispersed systems do not conform to the Lambert-Beer law**.

Turbidimetry and nephelometry are analytical methods based on measuring the intensity of scattered radiation. These methods are primarily used to analyse colloidally dispersed systems. The only difference between them is the position of the detector: turbidimetry measures the attenuation of radiation intensity after it passes through the sample – the more turbid (concentrated) the sample, the less light it transmits; nephelometry measures the intensity of scattered radiation – the more turbid (concentrated) the sample, the more light it scatters.

Accuracy of Photometric Methods
Most photometers can measure absorbance in the range of 0 to 3 or 4. However, this does not mean that measurements across this entire range are advisable. The definition of absorbance implies that an absorbance value of 1 corresponds to 90% radiation absorption, a value of 2 to 99% absorption, and so on. Consequently, at absorbance levels above 1.5, measurements are highly sensitive to the quality of the procedure, and results may be subject to significant error, leading to inaccuracy. Optimal results are achieved when the sample absorbance falls within the 0.2 to 0.8 range; a range of 0.2 to 1.2 is also considered acceptable. If the absorbance exceeds the upper limit, it is advisable to dilute the sample. While specific values depend on the characteristics of the photometer used, the trend of the

average relative error as a function of absorbance remains consistent (varying only in the "spread" of the curve; Fig. 4).



T	Passed (%)	Absorbed (%)	A
1	100	0	0
0.99	99	1	0.004
0.90	90	10	0.05
0.50	50	50	0.3
0.10	10	90	1.0
0.01	1	99	2.0
0.001	0.1	99.9	3.0
0.0001	0.01	99.99	4.0

Figure 4: *The dependence of the average relative error of the photometric analysis on the absorbance. Precise numerical expression of the passed/absorbed light on the transmittance/absorbance.*

Blank – sometimes referred as a zero or blind sample – is a solution that has exactly the same composition like the analysed sample but does not contain the substance being determined. It is used to subtract the influence of the solvent and the cuvette from the measurement. At the start of each measurement, the photometer must first be set to a zero value; by measuring the absorbance of the blank, the resulting reading is established as zero. Subsequent measurements record the difference relative to this value – representing a form of relative quantification that displays the change from the set zero point.

Question*: Is it possible, under certain circumstances, to obtain a negative absorbance value during measurement?*

The **linearity range** in spectrophotometry is the range of sample concentrations within which the measured value is directly proportional to the concentration of the analyte. In practice, this means that the spectrophotometer yields accurate results only when the sample concentration falls within this linearity range. If this range is exceeded, absorbance ceases to follow the Beer-Lambert law, thereby affecting measurement accuracy.

The **absorption maximum** represents the peak in the absorption spectrum. The wavelength of the absorption maximum (in nm) is therefore the wavelength at which a given substance interacts most with (absorbs) radiation.

Measurement accuracy is also significantly influenced by sample and cuvette preparation. The most significant factors affecting the accuracy of spectrophotometric determinations are:

- **Certain measurements must be performed in a suitable cuvette**; the selected wavelength must fall within the range for which the cuvette is designed.

- **Cuvettes used for both samples and blanks must be clean**. This can be verified by ensuring by measuring the absorbance of the cuvette filled with distilled water – all the cuvettes have to perform equally.
- Handle cuvettes carefully to avoid contaminating the optical surfaces (e.g., by touching them with your fingers).
- The outer surface of the cuvette must be dry, there must be no air bubbles inside, and the solution being measured must be thoroughly mixed. Droplets running down the outside of the cuvette, air bubbles, or floating precipitates in the solution usually manifest as constantly fluctuating absorbance readings.
- **The cuvette must be sufficiently filled** with the sample (the light beam usually pass the cuvette in the middle of its height).
- When measuring multiple samples sequentially in the same cuvette, minimize errors caused by residues of the previous solution. Typically, the cuvette is rinsed with distilled water between samples and dried as thoroughly as possible. More accurate results are obtained by rinsing the cuvette with a small amount of the sample itself after washing (discarding this rinse) before filling it with the actual volume required for measurement. When working with several similar solutions, it may be more accurate not to rinse the cuvette with distilled water between them, but simply to empty and dry it as thoroughly as possible.
- Ideally, every measurement should be performed as a set of replicates to minimize error. In such a case, the result is reported as the arithmetic mean of the measured values ± the standard deviation.

Multiple Dilution

Solution concentration is very often expressed as a multiple of the target concentration. In practice, a "10×" designation means that the solution is ten times more concentrated than the target concentration. Thus, if the target concentration is 0.1 mol/L, a 10× concentrated solution has a concentration of 1 mol/L. This notation also implies that the solution at this concentration represents a specific fraction of the total volume of the solution at the target concentration. To prepare a solution at the target concentration from a 10× concentrated solution, one-tenth of the final volume of the 10× concentrate solution is to be mixed with nine-tenths of the final volume of solvent – representing a 1:9 dilution.

Example:

Calculate the volumes of water and 10× concentrated NaCl stock solution required to prepare 0.5 L of a 1× concentrated working solution.

Solution:

The stock solution is 10 times more concentrated than the working solution. Therefore, the stock solution constitutes one-tenth of the final volume of the working solution.

Thus, the working solution is prepared by mixing 50 mL (1/10 of the final volume 0.5 L) of the stock solution with 450 mL of water.