WTW
a xylem brand

Photometry Compendium

in cooperation with
G.I.T. LABORATORY JOURNAL

The Basics of Photometric Measurement

Part 1: Principles, Optics, AQA, Test Kits I

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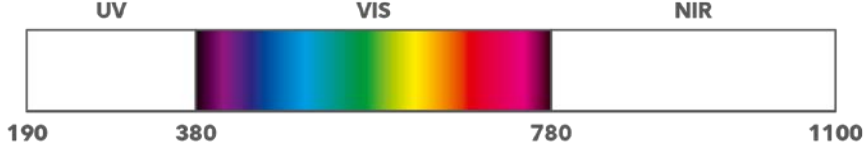
Introduction

Phos (Greek) for light => Photometry is a measurement method to analyse (aqueous) solutions by means of a light source.

Light (physical) is a spectra of electromagnetic waves, divided into different ranges: Visible light (white light) ranges from approx. 380 – 780 nm



WTW photometric range (190-1100 nm)

Photometric / Colorimetric Analysis:

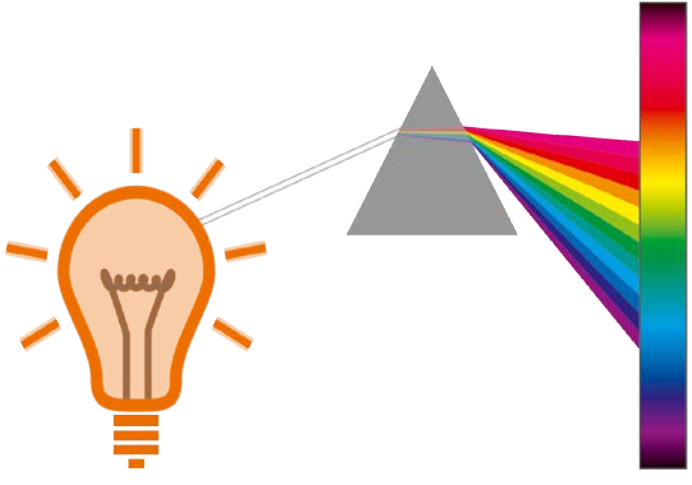
Determination of substances by their specific colour reaction and light absorbance in dependance of their chemical properties at a specific wavelength.

Introduction – Light Sources and Optics

Specific Wavelengths are obtained by

Different light sources

- LEDs (λ_x) = lowest power consumption, lower light intensity
- Tungsten (white light halogene lamp) for VIS range
- Xenon (UV-VIS) => Flash lamp with long life span
- Deuterium (UV) => special lamp, expensive



Different optical techniques, such as

- Monochromators
- Polychromators
- Filters
- LED

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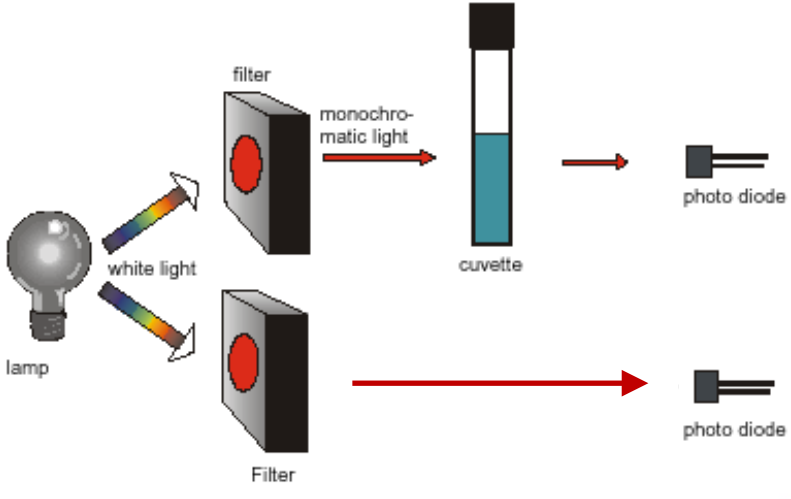
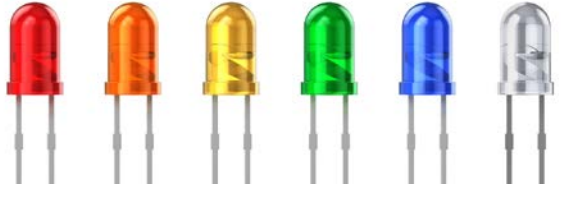
Optics: Filter and LED Photometer

Filter photometer with reference beam: photoLab® S6/S12



LED λ + optical Filter – single beam: photoFlex® Series



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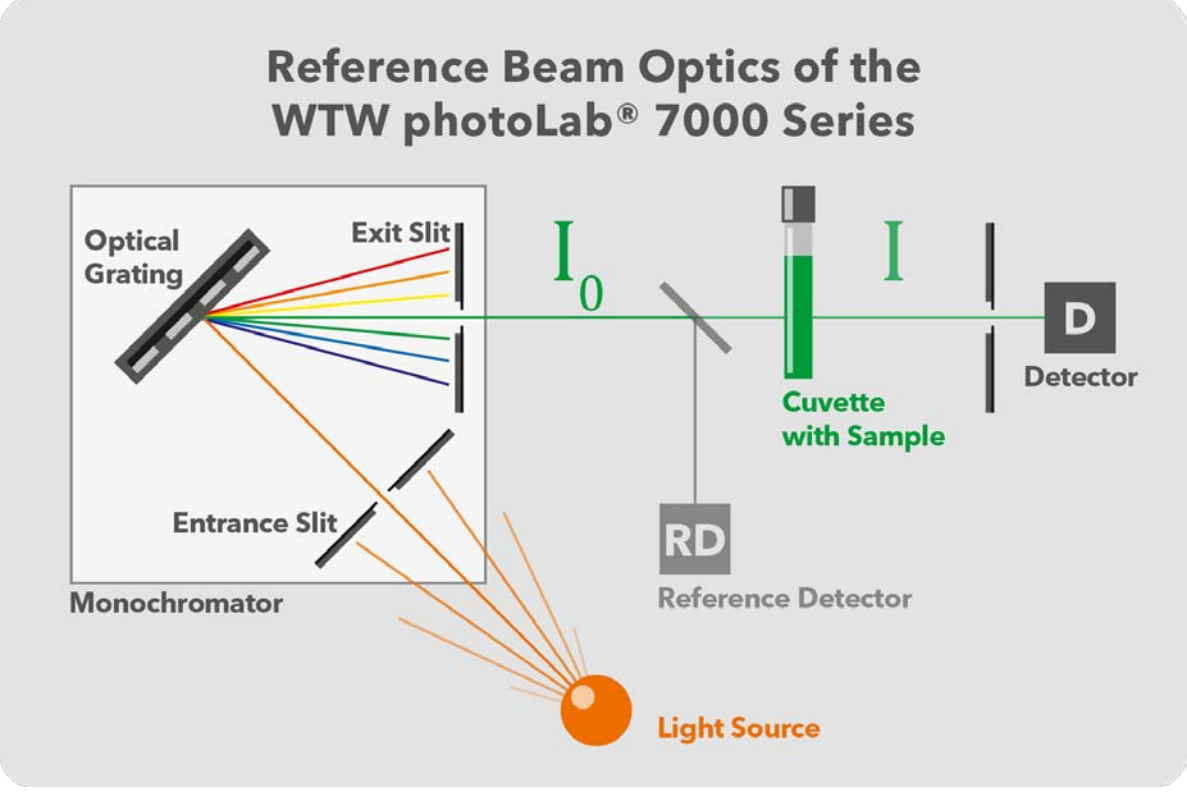
Monochromator of photoLab® 7000 Series



Measurement modes

What type of measurement is performed in with a photometer?

=> 3 Measurement modes of photometric analysis and their relation

1) **Transmission T(%)**: Ratio of light intensity after cuvette (I) and before (I_o)

2) **Absorbance:** $Abs_\lambda = - \log_{10} (T_\lambda)$
or „extinction of light“ passing the cuvette

3) **Concentration**: quantitative analysis of a substance (mg/l, ppm,…)
at a defined wavelength based on a calibration curve

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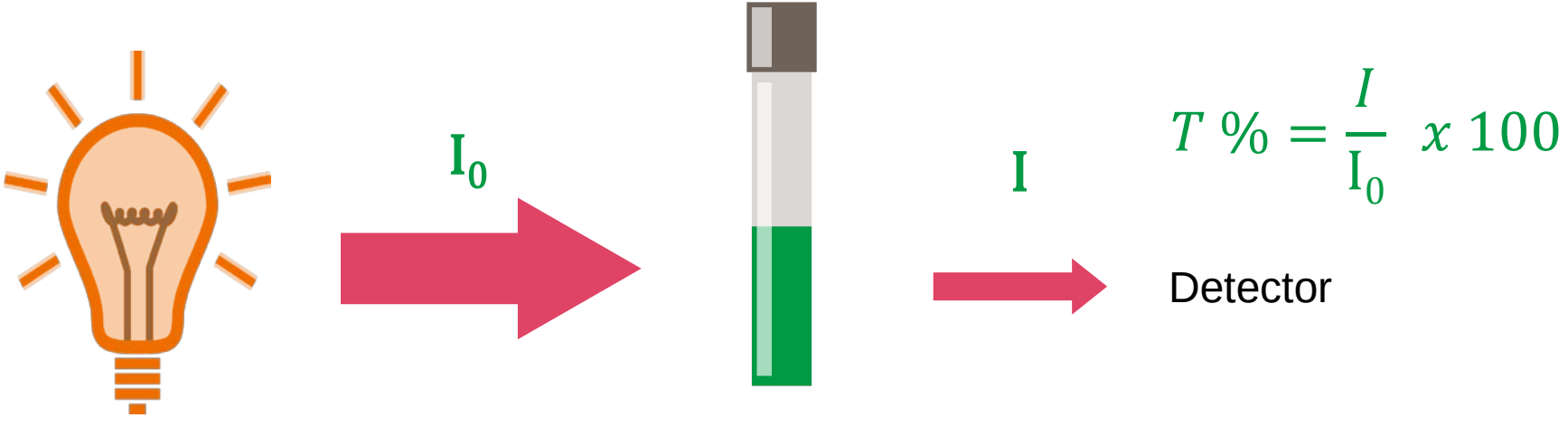
Transmission measurement



Transmission is the ratio of passed light **I** / initial light $\mathbf{I_0}$:

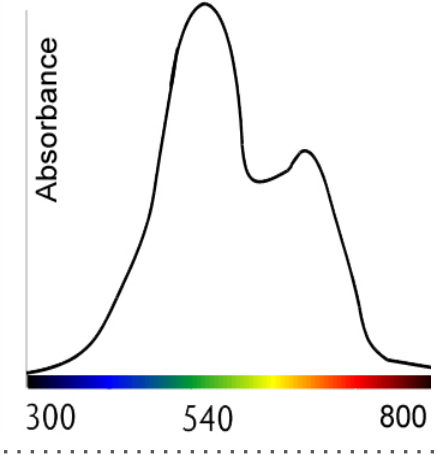
Transmission measurement is also being used to measure **turbidity at 180° angle** (unit FAU, e.g. for quality control) and for turbidity correction in concentration measurement.

Absorbance : Concentration Measurement

Absorbance = „Extinction of light“:

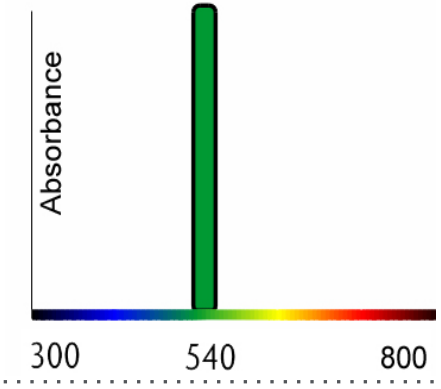
Each substance has a specific spectra with absorbance peak(s).

=> Run spectrum to define maximum or optimal peak = wavelength definition for concentration measurement



Concentration measurement:

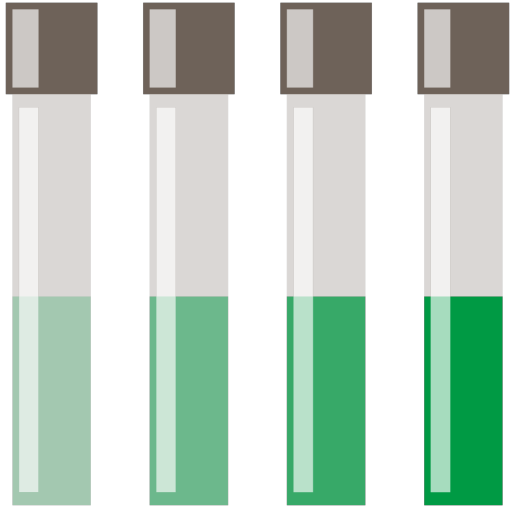
Measurement at specific wavelength, obtained by either matching LED, optical filters from white light or mono-chromator



Relation %T : Absorbance : Concentration

Transmission measurement:

The transmission of a sample varies **exponentially** with thickness and concentration



Absorbance measurement:

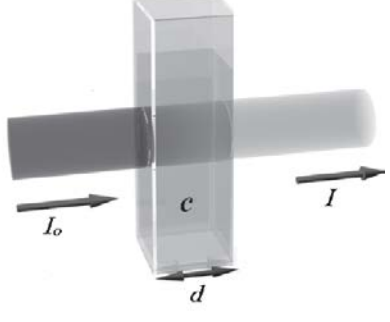
Absorbance of a sample is **proportional** to thickness of the sample and concentration

Transmission (T%)	100	10	1	0,1	=> Logarithmic correlation
Absorbance A = $-\log_{10}$ (T)	0	1	2	3	=> linear correlation
Concentration (mg/l)	0	4	8	12	

Relation %T : Absorbance : Concentration

Lambert-Beer's law

Experiments by BOUGUER (1698–1758) and LAMBERT (1728–1777) showed that the absorbance is dependent on the thickness of the absorbing layer of the cell used. The relationship between the absorbance and the concentration of the analyte in question was discovered by BEER (1825–1863). The combination of these two natural laws led to the derivation of *Lambert-Beer's law*, which can be described in the form of the following equation:



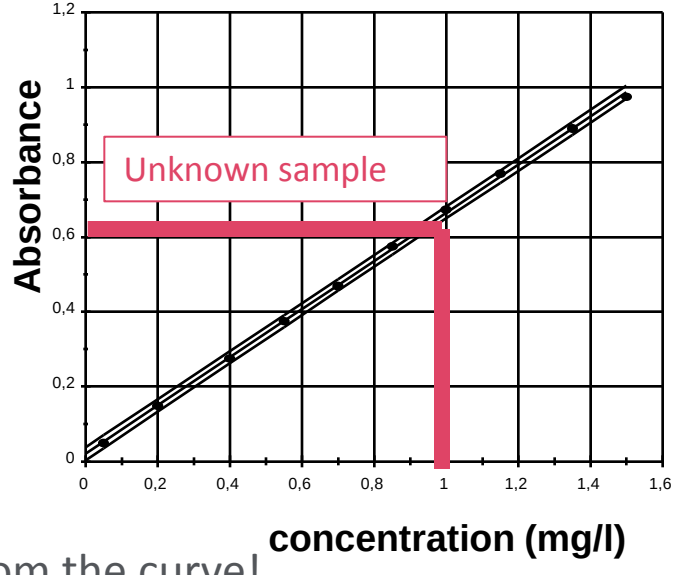
$$A = \varepsilon_\lambda \cdot c \cdot d$$

ε_λ = molar absorptivity, in l/mol x cm
d = Path length of the cell, in cm
c = Concentration of the analyte, in mol/l

Source: Operating Instructions of photoLab® S12, Part 1: General Information (www.wtw.com)

Concentration Measurement

The correlation of absorbance/concentration is determined by setting up a characteristic calibration curve for each substance (parameter). The chemical reaction must be known:



Dilution series with defined concentrations, measured at defined λ and cuvette size (pathlength)

⇒ Characteristic (calibration) curve

⇒ Unknown sample concentration can be „read“ from the curve!

Methods/ programs in photometers contain all data and compute result automatically, including various cuvette sizes. Barcoded test kits additionally call up the respective method=program.

Method data / Program for each parameter

Programmed data for comfortable concentration measurement are consisting in:

λ matching the absorbance for determination

Reagent blank E_0 = coloration of reagent

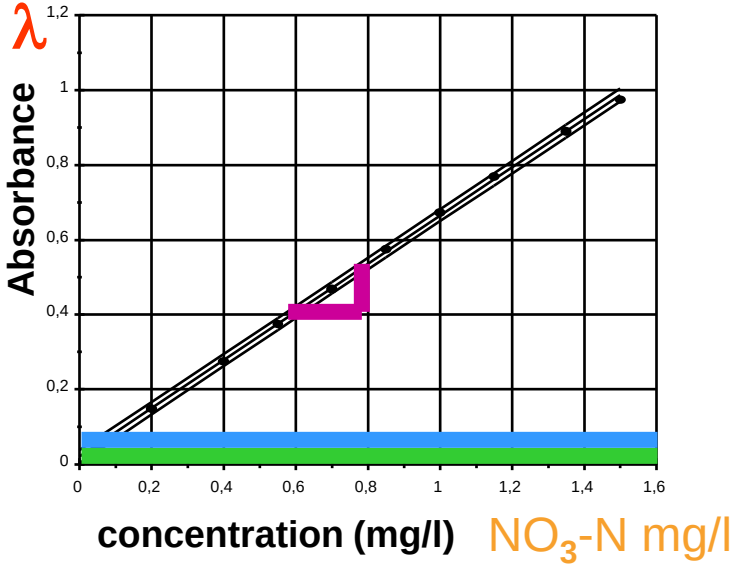
Slope of calibration curve for calculation

Citation & unit (e.g. NO_3-N mg/l)

Factors for citation & unit switch (e.g. NO_3; mmol/l)

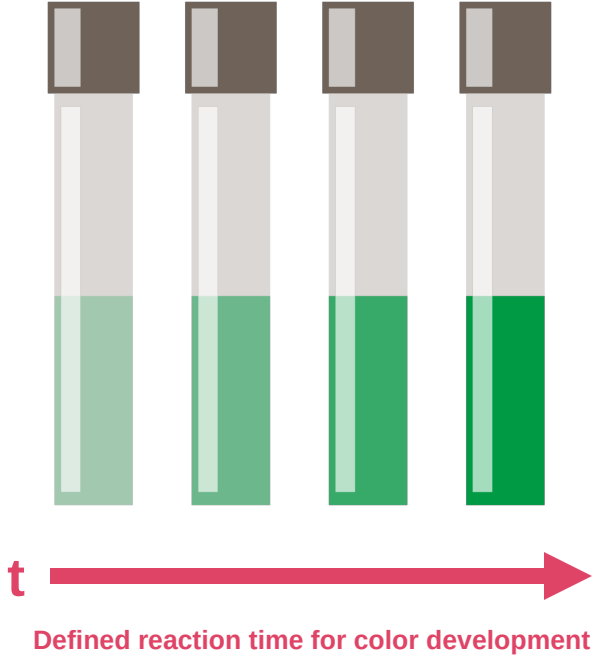
Sample blank (e.g. coloration of the sample) is **not** included!

„E_{sample}“ comes on top of E_0 : individually for sample, for small volumes mostly negligible



Prerequisites for concentration measurement

- Coloured solution contains dissolved dye
- Absorption of light leads to a coloration(complementary to λ)
- Color intensity is correlating with concentration
- The chemical reaction of analyte leads to building or disappearance (e.g. COD 4-40 mg/l) of dye in defined reaction time
- Reaction must be selective for the analyte – no cross reaction with other **disturbing** substances
- The developed dye must be stable for time of measurement => e.g. reading within 10 minutes after reaction time w/o color deterioration (see analytical instructions)



Instrument Check – AQA

- Self calibration/check & warm up time (especially for kinetics and spectra)
- AutoCheck: photoLab® levels meter vs air in the background
- Zeroing/Baseline: correcting the meter to „E_0“ => especially after transport or in changing conditions (temperature…) meters E_0 „drift“ some mAbs
  => readings become inaccurate (mostly too high)
- AQA Tools:
  - Optical or liquid filters
  - Color solutions, e.g. PhotoCheck®
  - Selected unscratched zero cuvettes
  - Control standards of the substance

FAQ Commercial test kits in brief

Measurement range: (MR)

The range is meter (optics) dependent, the reaction has detection limits. MR-values are reaching approx. ± 2 - 2,5 Abs (test dependent!)

At the lower end detection limits and tolerances of the procedure have the biggest influence on accuracy of readings: Limitation of chemical procedure, confidence interval and accuracy are often at the lower limit.

=> Scratches, pipette faults etc. additionally affect the accuracy of readings! Readings at the lower end become more inaccurate!

Measure in the middle of the MR, if possible!

Test: A6/25 Ammonium (WTW)

2. Measuring range and numb

Measuring range
0.20 - 8.00 mg/l NH_4-N
0.26 - 10.30 mg/l NH_4^+

a): Low end 0,20 mg/l

confidence interval: ± 0,20 mg/l

Characteristic data of the procedure:

Sensitivity: Absorbance 0.010 A corresp...	0.04
Accuracy of a measurement value (mg/l NH_4-N)	max. ± 0.20

b): Accuracy: ± 0,20 mg/l

FAQ – The importance of “photometric Zero”

Performing a zero (see manuals!)

LED meters, e.g. pHotoFlex® Series

Portable meters require a zero due to changing conditions, transportation and optics.

Filter photometers, e.g. photoLab® S12

In lab with stable conditions, slow drift and often stabilizing reference beam requires less zeroing.

Spectrophotometers, e.g. photoLab® 7000 Series

Zero/base line is required for many functions of spectral tasks, concentration mode is similar to filter photometers with reference beam

Influence of meter drift: => Zeroing

Package leaflet of many tests show sensitivity by correlating absorbance A (E=A) to mg/l.

Influence can be seen directly:

For COD test 14560, 4-40 mg/l COD, an absorbance of 10 mE means 0.4 mg/l COD. 10 mE drift without zeroing means 0.4 mg/l or 10% evitable miss-reading in the low end!

Characteristic data of the procedure:

	1.14560.	1.14540.
Number of lots	23	27
Sensitivity 0.010 E (absorbance) ≙ mg/l COD	0.4	2
Accuracy of a measurement value (mg/l COD)	max. ± 1.8	max. ± 6