Photometric Measurement

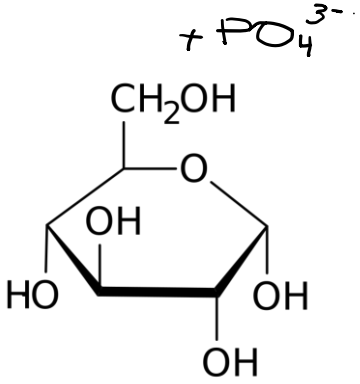
Presentation by Rebecca Butollo and Panna Polák

Oxidation, Reduction

- gain or loss of electrons in a chemical reaction
- Oxidation: loss of electrons, reduction: gain of electrons
- Processes are linked

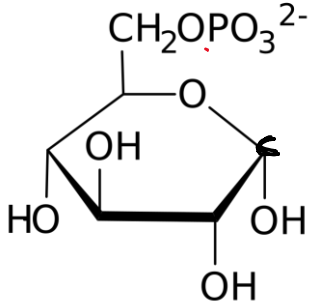
Photometric Measurement of Glucose

ATP → ADP



Glucose

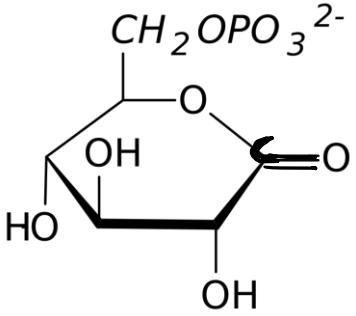
Hexokinase



Glucose-6-Phosphate

Oxidation
−2e⁻

Glucose-6-Phosphate Dehydrogenase



6-Phosphogluconate

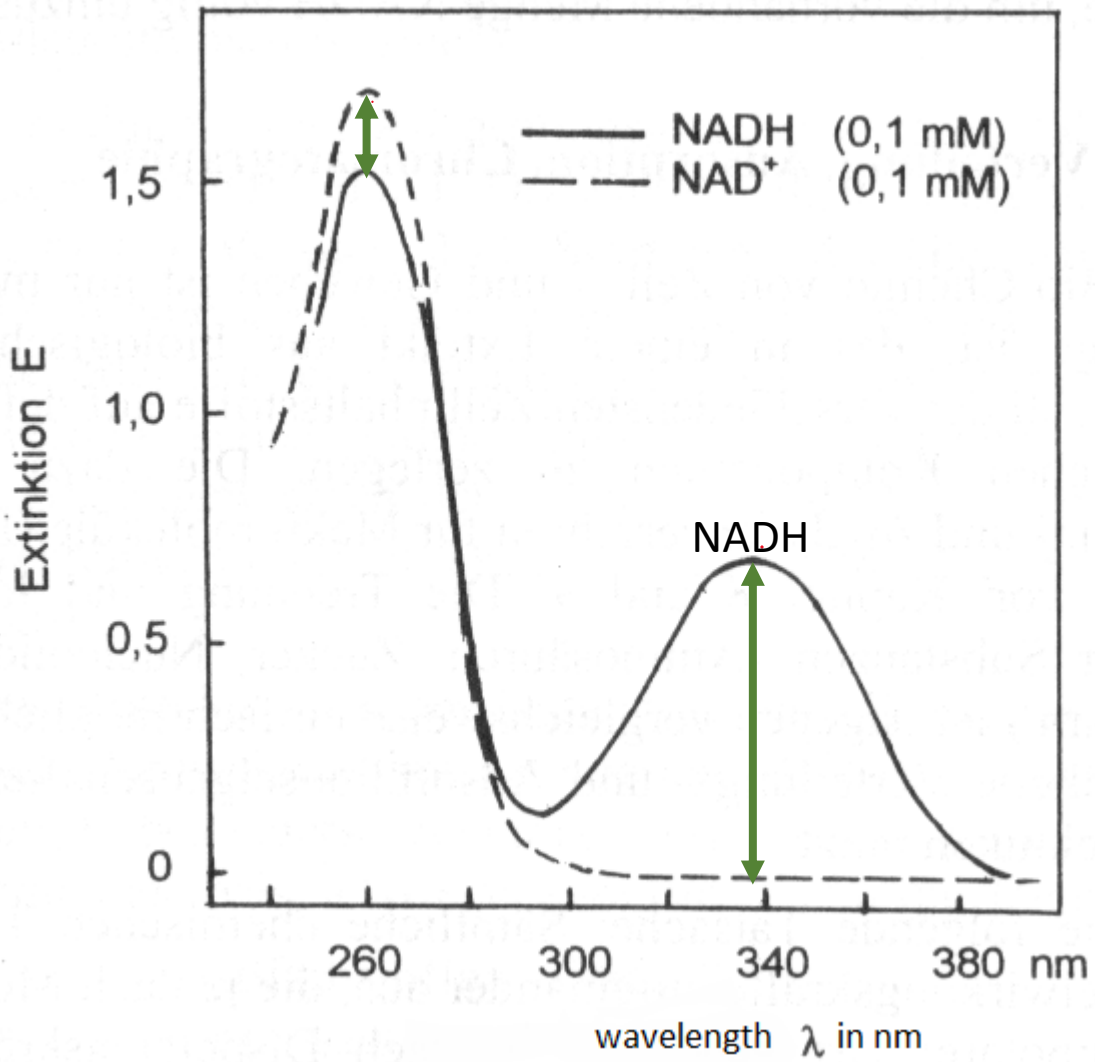
\+

NAD^+ → $NADH + H^+$

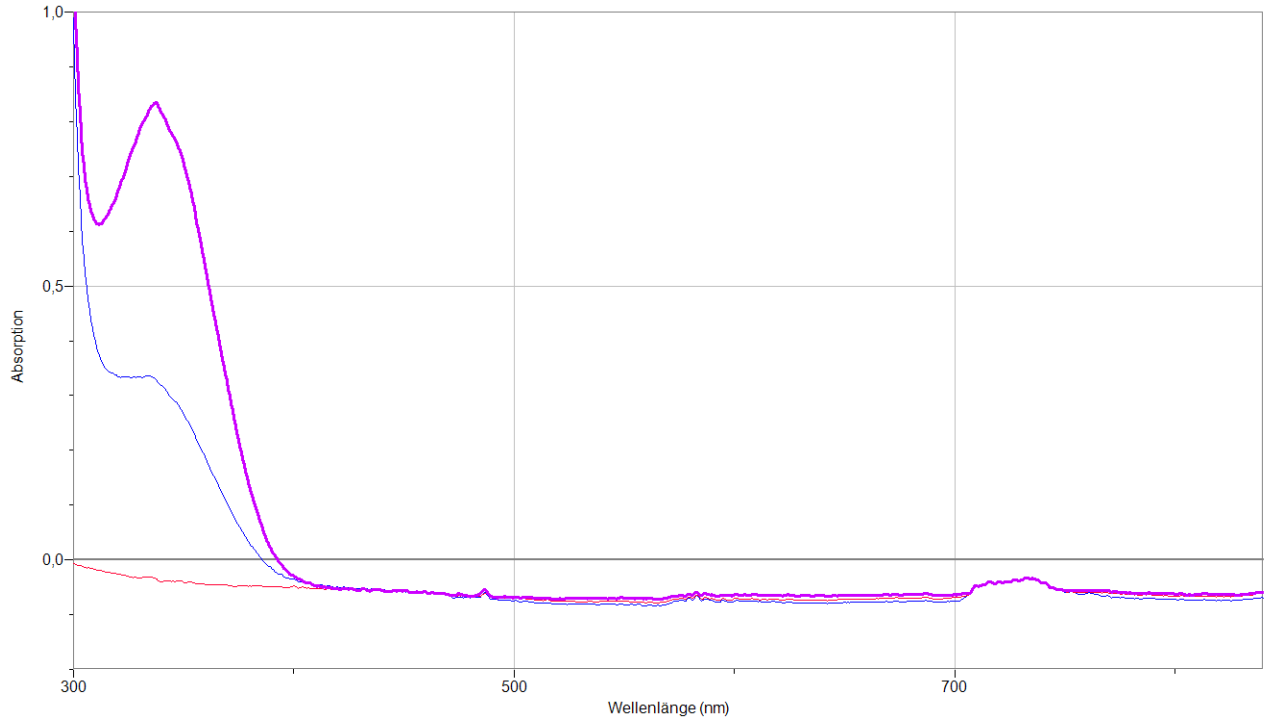
+ 2e⁻

Reduction

Determination by measuring the Cofactor in the Reaction



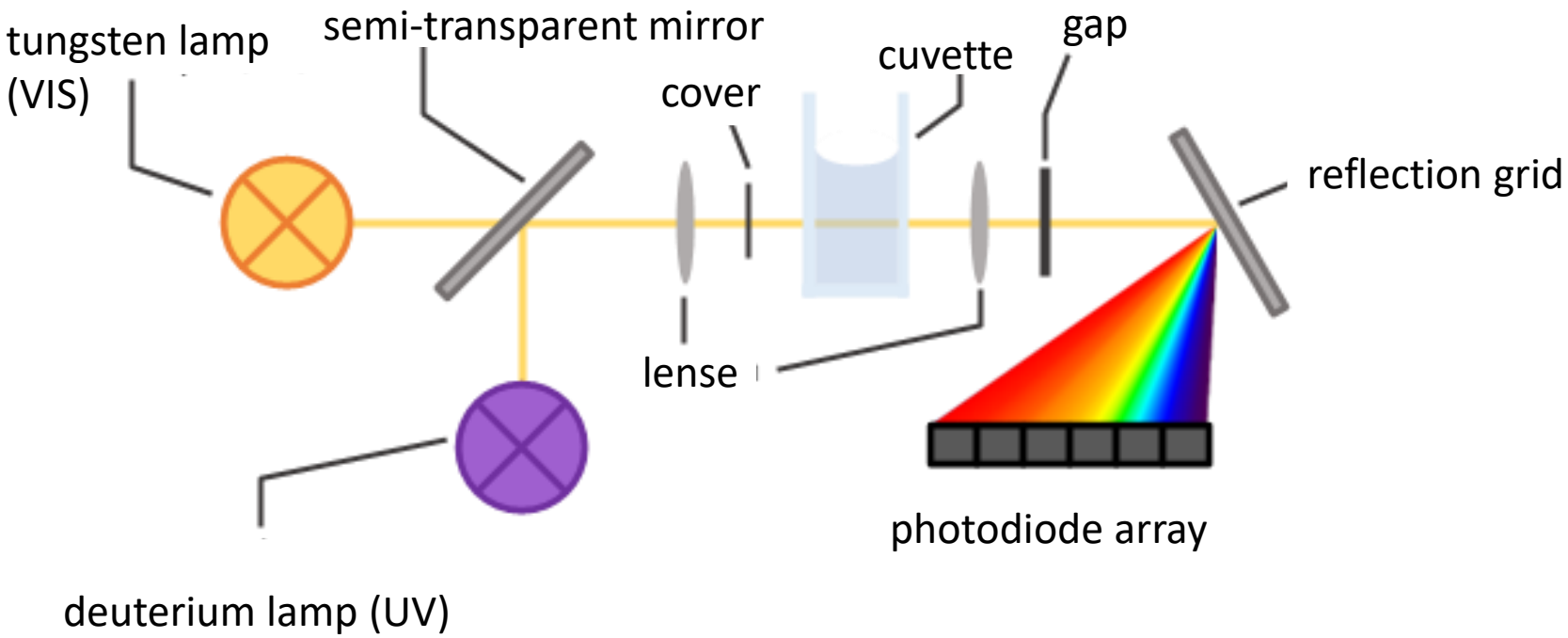
Photometric Measurement of Glucose



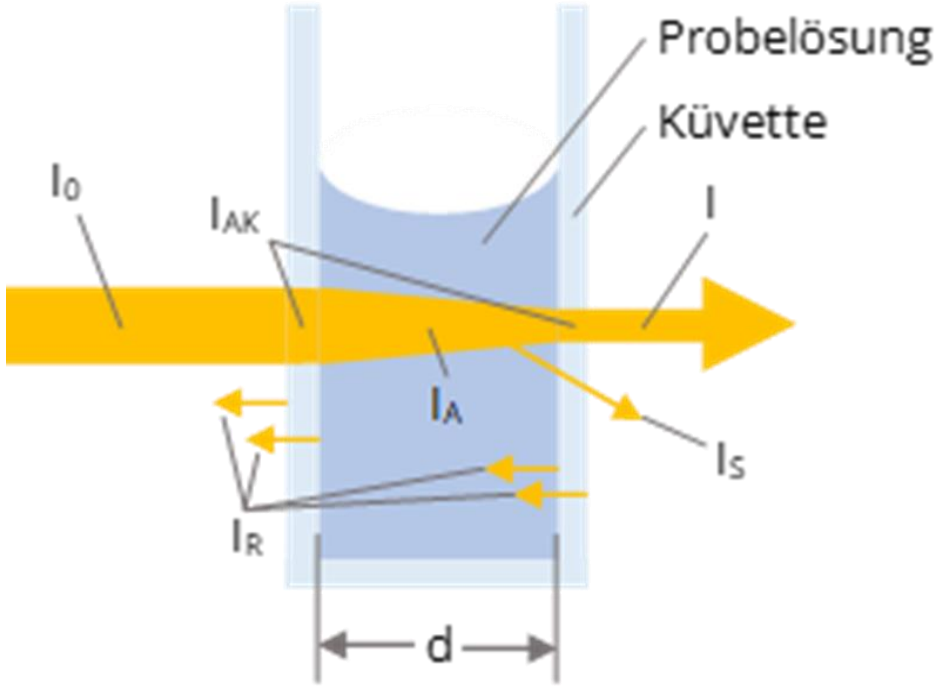
Change in absorbance with increasing NADH

Glucose Estimation with the Photometer

Construction of a Photometer



Calculations used in Connection with Photometric Measurement



$$T(\lambda) = \frac{I(\lambda)}{I_0(\lambda)}$$

transmission for wavelength λ

$$E(\lambda) = \log\left(\frac{1}{T(\lambda)}\right) = \log\left(\frac{I_0(\lambda)}{I(\lambda)}\right)$$

absorbance for wavelength λ

Calculations used in Connection with Photometric Measurement

Lambert-Beersche Law

$$E(\lambda) = \varepsilon(\lambda) \bullet d \bullet c$$

Concentration of the substance measured in mol/l

Molar decadic extinction coefficient in L/mol-cm; constant specific to wavelength and substance

Thickness of the cuvette / sample solution in cm

$$c = \frac{E(\lambda)}{\varepsilon(\lambda) \bullet d}$$

Concentration formula based on LBL

Calculations used in Connection with Photometric Measurement

calculation of glucose concentration

$$c = \frac{(E - E_{control}) * 11 * 180,2 * D}{\varepsilon * d * 1000}$$

$$c = 0,319 * (E - E_{control}) * D$$

- E: Extinktion of probe (Tube P)
- $E_{control}$: E of Tube 0 + $E_{chemicals}$. $E_{chemicals}$ 180,2: molecular mass of glucose;
- ε: extinction coeffizient for the reactant
- d: length of cuvette

→ summarised to 0,319.

- D: dilution factor if necessary
- c: concentration of glucose [mg/mL)

Sources

- VWA Johannes Berndorfer: Lactatherstellung aus Glucose mit Hilfe von Lactobacillus Sp. (Abbildung 10: Schematische Darstellung der Lichtverläufe und Lichtintensitäten einer Küvette)
- VWA Johannes Berndorfer: Lactatherstellung aus Glucose mit Hilfe von Lactobacillus Sp. (Abbildung 8: Schematischer Aufbau eines Spektralphotometers)
- VWA Johannes Berndorfer: Lactatherstellung aus Glucose mit Hilfe von Lactobacillus Sp. (S.22-24)
- Berndorfer Johannes: Lactatherstellung aus Glucose mit Hilfe von Lactobacillus Sp. VWA am Kollegium Kalksburg. 2020.
- Fischill Paul: Biochemische Erzeugung von elektrischer Energie aus Zellulose. VWA am Kollegium Kalksburg. 2019
- Hampel, Bruno: Absorptionsspektroskopie im Ultravioletten und sichtbaren Spektralbereich. 1. Aufl. Wiesbaden: Springer-Verlag, 1962, S. 37f.
- Otto, Matthias: Analytische Chemie. 2. Aufl. Weinheim: WILEY-VCH Verlag GmbH, 2000, S. 270.
- Fischill, Paul; Raschke, Felix; Robanser Regina: Biotechnologische Gewinnung von Ethanol aus Zellulose. Chemie&Schule 4/2019