Supelco®
Analytical Products

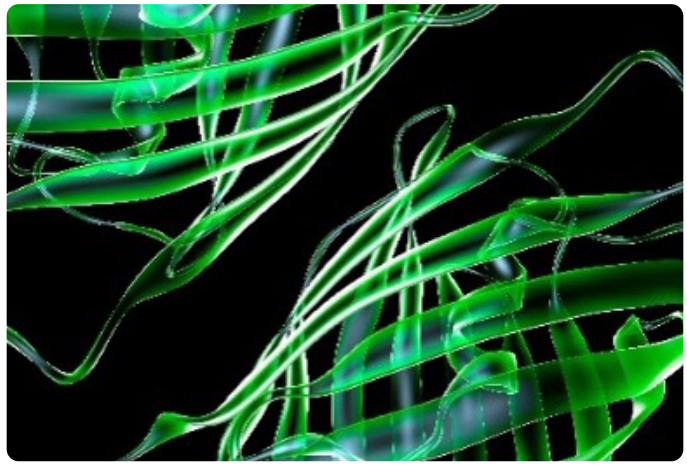
Application Note

Protein (OD280)

UV spectrophotometric measurement at 280 nm

Introduction

A simple method for estimating the protein content of a solution is the spectrophotometric measurement of the intrinsic absorption of a sample at 280 nm. Dissolved proteins absorb UV light at an absorption maximum of 280 nm and 200 nm. The ratio between the protein concen-tration and the absorption at 280 nm is linear and the measurement can be made without any additional reagents. In the technical sphere, this procedure is generally known as the "OD_{280}" method. The absorption at 280 nm is primarily caused by aromatic ring structures of the amino acids of the proteins. These amino acids include phenylalanine, tryptophan, histidine, and tyrosine. Secondary, tertiary, and quaternary structures of the molecule also have an impact on the absorption. It is important to consider that, due to variations in the proportions of these amino acids, each protein or protein mixture has its own characteristic absorption properties. Proteins with a low content of amino acids with aromatic ring structures have a lower absorption at 280 nm than do proteins with a high content of these amino acids [1, 2]. This method is hence primarily used in the analysis of protein solutions with a known composition, as is the case, for example, in the purification or isolation of enzymes or anti¬bodies. If the composition is known, the protein content can be calculated by factoring the absorption measured at 280 nm with the specific absorption coefficient of the protein of protein mixture in question. Specific absorption coefficients for proteins can be taken from the technical literature [3, 4] or else can be determined using reference proteins. One disadvantage of this method is that not only proteins, but also nucleic acids show a strong absorption at 280 nm. When a sample contains RNA and/or DNA, this results in strongly elevated absorption values at 280 nm and thus potentially to false-high results for the protein content. For this reason, the technical literature refers to the option of correcting the calculation by making an additional measurement at 260 nm [1, 2].



Experimental

This Application Note describes the determination of the OD_{280} value of protein-containing solutions. The analysis is quick and easy without any use of additional reagents. The method is preprogrammed on the corresponding Spectroquant® Prove photometers with firmware version 1.5 or above.

Method

- UV spectrophotometric measurement at 280 nm

Measuring range

- -0,020–2,000 OD_{280}

Sample material

- Protein-containing solutions

MERCK

Reagents, Instruments and Materials:

Instruments

For the dsDNA measurement one of the following Spectroquant® photometers is necessary:

Cat. No.	Description
1.73028	Spectroquant® UV/VIS Spectrophotometer Prove 600 plus
1.73027	Spectroquant® UV/VIS Spectrophotometer Prove 300 plus

Also first generation Prove instruments are compatible and preprogrammed with this method.

Software for data maintenance

The Spectroquant® Prove Connect to LIMS software package provides an easy way to transfer your data into a preexisting LIMS system. This software can be purchased under:

Cat. No.	Description
Y11086	Prove Connect to LIMS

Materials

Cat. No.	Description
100784	Rectangular quartz cell 10 mm
Z600288	Semi-micro rectangular quartz cell 10 mm* Pipettes

Pipettes

*Due to the optical characteristics of the Prove Spectrophotometers the use of 10 mm micro cells is not possible. Plus, due to the automatic cell detection of the Prove instruments it is important to use semi-micro cells with complete side walls.

Analytical approach

Sample preparation

- Homogenize samples by swirling carefully
- Avoid too vigorous shaking, vigorous shaking may cause denaturation and loss of protein
- Analyze clear solutions only
- Removing of turbidity by filtration or centrifugation may cause loss of protein.

Measurement

- Open the method list **(<Methods>)** and select Method No. 312 **"OD_{280}"**.
- For each measurement series, a zero adjustment is required. It is recommended to use the same cell for zero adjustment and for sample measurement.
- The zero-adjustment procedure must be selected by opening **<Settings>** and clicking on the selection button **"ZERO ADJUSTMENT"**.
- For zero adjustment fill the 10-mm rectangular quartz cell with the used solvent (distilled water or buffer solution). After prompting, place the filled rectangular quartz cell in the cell compartment, the zero adjustment is executed automatically. Confirm the implementation of zero adjustment with **"OK"**. The zero adjustment is valid for the entire measurement series.
- After zero adjustment fill the measurement sample into the same or a matched 10-mm rectangular quartz cell and insert the cell into the cell compartment. Please note that the **minimum sample volume is 0.6 mL for the 10 mm semi-micro cell or 1.5 mL for the 10 mm cell.** The measurement starts automatically.
- Read off the results from the display.

Data transfer Prove spectrophotometers

- After measurement transfer the values measured on the Prove spectrophotometer using Prove Connect to LIMS

Influence of foreign substances

The technical literature mentions several substances with a potential impact on the measurement of the absorption of proteins at 280 nm [1], primarily nucleic acids. Nucleic acids such as RNA or DNA show a strong absorption band at the 280 nm wavelength and can thus have a strong impact on the measurement. Hints on how to ascertain whether there are any interferences due to nucleic acids and how these can be compensated are described in the technical literature [1, 2].

In addition, the composition of the buffer solution used can also have an impact on the absorption measurement. Buffer salts, ionic strength, detergents, and the pH may have an impact on the tertiary structure of the proteins or also show an intrinsic absorption at 280 nm [1, 2]. An intrinsic absorption of the buffer solution is compensated by zeroing the measurement. Effects on the tertiary structure and any alterations of the absorption results that these may cause can be tested by making comparative measurements using reference proteins.

Calculating the protein concentration

The protein concentration can be calculated from the absorption result measured at 280 nm by using the specific absorption coefficient. If the specific absorption coefficient or the composition of the solution is not known, in practice an absorption coefficient of 1.0 mg-1*mL*cm-1 is frequently used to calculate the protein concentration.

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$$\text{Concentration} \left[\frac{\text{mg}}{\text{mL}}\right] = \frac{OD_{280}}{\text{"specific absorption coefficient} \cdot \text{optical path length"}}$$

Example (analysis of a solution of bovine serum albumin):

Specific absorption coefficient for bovine serum albumin (BSA), (1 mg/mL in 1 cm cell)

= 0.66 mg-1*mL*cm-1

Absorption measured at 280 nm (OD_{280}) = 1.050

$$\text{Concentration} = \frac{1.050}{\text{"0.66 mg}^{-1*\text{mL*cm-1*}}\text{1cm}} = 1.59 \text{ mg/mL}$$

Conclusion

The measurement of the optical density at 280 nm is a fast and easy way to quantify the protein content of your sample without any use of additional reagents.

The method is preprogrammed in the Spectroquant® Prove 300 and 600 with firmware version 1.5 or above.

For more information visit,
SigmaAldrich.com/photometry

References

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2. Layne, E. Spectrophotometric and Turbidimetric Methods for
3. Measuring Proteins. Methods in Enzymology 3: 447-455. 1957.
4. Practical Handbook of Biochemistry and Molecular Biology, Fasman, D.G., Ed. (1992). CRC Press, Boston.
5. Gill, S.C.; von Hippel, P.H. (1989). Calculation of protein extinction coefficients from amino acid sequence data. Anal. Biochem. 182:319-26.

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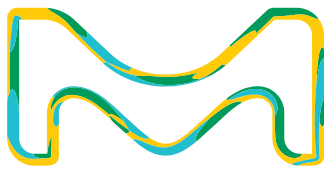

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