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Protein concentration, Refractometry and NanoCuvette™ One

Protein concentration determination is fast and easy with the novel NanoCuvette™ One.

1. Proteins

Proteins are macromolecules consisting of one or more strands of amino acids. The human genome specifies 20 different amino acids. The sequence of these is the main differentiating factor of proteins and affects their folding into specific three-dimensional structures. Proteins perform a vast variety of vital functions in organisms, including catalysis, transportation and signal transmittance. Additionally, many proteins are produced industrially for a variety of applications, including medication.

2. Concentration determination

A selection of bioassays is available for determining the concentration of purified protein. However, these bioassays require the experimenter to make a standard curve, which, if using the same protein, can be very expensive, and if using a different protein, may induce large systematic errors. Alternatively, some proteins can be quantified by absorption spectroscopy. A few amino acids (tryptophan, tyrosine and phenylalanine) are aromatic and absorb UV light at 280 nm. Depending on the amount of aromatic amino acids a protein is comprised of, it will absorb more or less light at 280 nm, i.e. have a higher or lower extinction coefficient. The extinction coefficient can be calculated theoretically, but this may give values up to 10 % off. Alternatively it can be determined experimentally. This requires making a standard curve for the specific protein on the specific spectrophotometer.

Whereas extinction coefficients of proteins vary greatly, it has been shown that proteins have very similar values of refractive index increment, i.e. the protein refractive index dependence on protein concentration[1]. Thus the measure of protein refractive index is a quick and easy manner of obtaining the protein concentration without the need of making a standard curve. As a further advantage, this method also allows for direct quantification of your protein without prior dilution.

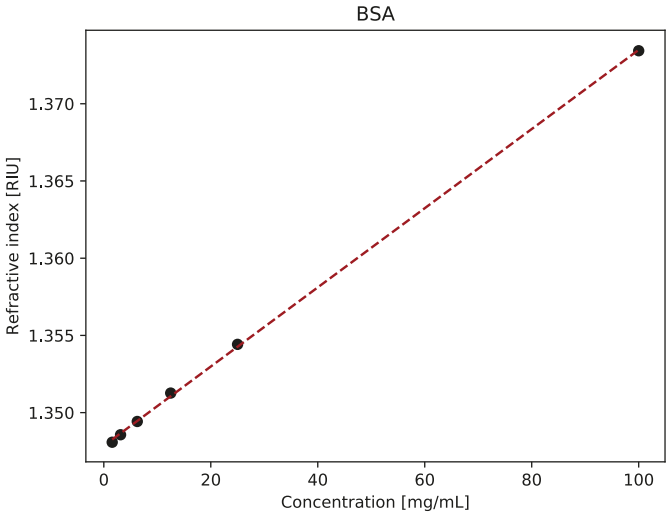

Figure 1 The sensor response, shown as refractive index, is linearly proportional to BSA concentration, which the free software will display.

3. Principle

Traditionally a specialized refractometer has been required for determination of the refractive index. However, with the innovative NanoCuvette™ One, a nanosensor is installed in a cuvette, allowing determination of the refractive index to be carried out in a standard spectrophotometer. This way even proteins with few or no aromatic residues can be measured with a conventional spectrophotometer.

4. Safety precautions

This method does not entail any safety precautions. Please refer to common laboratory practices.

5. Measurement

5A. Materials and apparatus

The only apparatus required is a standard spectrophotometer and a computer with internet access. For each measurement a NanoCuvette™ One is required.

5B. Sample preparation

Bovine serum albumin (BSA) is dissolved in phosphate buffered saline (PBS) at a concentration of 100 mg/mL. When fully dissolved, the stock is serial diluted in PBS from 100 to 0.2 mg/mL.

5C. Measurement procedure

The spectrophotometer and computer are switched on and the free NanoCuvette™ One software is opened. For each sample a new NanoCuvette™ One is used according to the software guidance. Following the last measurement the software will present the data as a variable of absorbance, refractive index or concentration, depending on the experimenter's preferred choice of representation (Figure 1).

6. References

[1] Zhao H, Brown P. H. and Schuck P. 2011, On the Distribution of Protein Refractive Index Increments. Biophysical Journal, 100, 2309-2317.

Figure 2 NanoCuvette™ One measures refractive index and absorbance on the same sample, using only a standard spectrophotometer and the free NanoCuvette™ One software.

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