

Protein Determination by UV Absorption

Alastair Aitken and Michèle P. Learmonth

1. Introduction

1.1. Near UV Absorbance (280 nm)

Quantitation of the amount of protein in a solution is possible in a simple spectrometer. Absorption of radiation in the near UV by proteins depends on the Tyr and Trp content (and to a very small extent on the amount of Phe and disulfide bonds). Therefore the A_{280} varies greatly between different proteins (for a 1 mg/mL solution, from 0 up to 4 [for some tyrosine-rich wool proteins], although most values are in the range 0.5–1.5 [1]). The advantages of this method are that it is simple, and the sample is recoverable. The method has some disadvantages, including interference from other chromophores, and the specific absorption value for a given protein must be determined. The extinction of nucleic acid in the 280-nm region may be as much as 10 times that of protein at their same wavelength, and hence, a few percent of nucleic acid can greatly influence the absorption.

1.2. Far UV Absorbance

The peptide bond absorbs strongly in the far UV with a maximum at about 190 nm. This very strong absorption of proteins at these wavelengths has been used in protein determination. Because of the difficulties caused by absorption by oxygen and the low output of conventional spectrophotometers at this wavelength, measurements are more conveniently made at 205 nm, where the absorbance is about half that at 190 nm. Most proteins have extinction coefficients at 205 nm for a 1 mg/mL solution of 30–35 and between 20 and 24 at 210 nm (2).

Various side chains, including those of Trp, Phe, Tyr, His, Cys, Met, and Arg (in that descending order), make contributions to the A_{205} (3).

The advantages of this method include simplicity and sensitivity. As in the method outlined in **Subheading 3.1.** the sample is recoverable and in addition there is little variation in response between different proteins, permitting near-absolute determination of protein. Disadvantages of this method include the necessity for accurate calibration of the spectrophotometer in the far UV. Many buffers and other components, such as heme or pyridoxal groups, absorb strongly in this region.

2. Materials

1. 0.1 M K₂SO₄ (pH 7.0).
2. 5 mM potassium phosphate buffer, pH 7.0.
3. Nonionic detergent (0.01% Brij 35)
4. Guanidinium-HCl.
5. 0.2-μm Millipore (Watford, UK) filter.
6. UV-visible spectrometer: The hydrogen lamp should be selected for maximum intensity at the particular wavelength.
7. Cuvets, quartz, for <215 nm.

3. Methods

3.1. Estimation of Protein by Near UV Absorbance (280 nm)

1. A reliable spectrophotometer is necessary. The protein solution must be diluted in the buffer to a concentration that is well within the accurate range of the instrument (*see Notes 1 and 2*).
2. The protein solution to be measured can be in a wide range of buffers, so it is usually no problem to find one that is appropriate for the protein which may already be in a particular buffer required for a purification step or assay for enzyme activity, for example (*see Notes 3 and 4*).
3. Measure the absorbance of the protein solution at 280 nm, using quartz cuvetts or cuvetts that are known to be transparent to this wavelength, filled with a volume of solution sufficient to cover the aperture through which the light beam passes.
4. The value obtained will depend on the path length of the cuvet. If not 1 cm, it must be adjusted by the appropriate factor. The Beer-Lambert law states that:

$$A \text{ (absorbance)} = \epsilon c l \quad (1)$$

where ϵ = extinction coefficient, c = concentration in mol/L and l = optical path length in cm. Therefore, if ϵ is known, measurement of A gives the concentration directly, ϵ is normally quoted for a 1-cm path length.

5. The actual value of UV absorbance for a given protein must be determined by some absolute method, e.g., calculated from the amino acid composition, which can be determined by amino acid analysis (4). The UV absorbance for a protein is then calculated according to the following formula:

$$A_{280} \text{ (1 mg/mL)} = (5690n_w + 1280n_y + 120n_c)/M \quad (2)$$

where n_w , n_y , and n_c are the numbers of Trp, Tyr, and Cys residues in the polypeptide of mass M and 5690, 1280 and 120 are the respective extinction coefficients for these residues (*see Note 5*).

3.2. Estimation of Protein by Far UV Absorbance

1. The protein solution is diluted with a sodium chloride solution (0.9% w/v) until the absorbance at 215 nm is <1.5 (*see Notes 1 and 6*).
2. Alternatively, dilute the sample in another non-UV-absorbing buffer such as 0.1 M K₂SO₄, containing 5 mM potassium phosphate buffer adjusted to pH 7.0 (*see Note 6*).
3. Measure the absorbances at the appropriate wavelengths (either A_{280} and A_{205} , or A_{225} and A_{215} , depending on the formula to be applied), using a spectrometer fitted with a hydrogen lamp that is accurate at these wavelengths, using quartz cuvetts filled with a volume of solution sufficient to cover the aperture through which the light beam passes (details in **Subheading 3.1.**).

4. The A_{205} for a 1 mg/mL solution of protein ($A_{205}^1 \text{ mg/mL}$) can be calculated within $\pm 2\%$, according to the empirical formula proposed by Scopes (2) (see **Notes 7–10**):

$$A_{205}^1 \text{ mg/mL} = 27 + 120 (A_{280}/A_{205}) \quad (3)$$

5. Alternatively, measurements may be made at longer wavelengths (5):

$$\text{Protein concentration } (\mu\text{g/mL}) = 144 (A_{215} - A_{225}) \quad (4)$$

The extinction at 225 nm is subtracted from that at 215 nm; the difference multiplied by 144 gives the protein concentration in the sample in $\mu\text{g/mL}$. With a particular protein under specific conditions accurate measurements of concentration to within 5 $\mu\text{g/L}$ are possible.

4. Notes

1. It is best to measure absorbances in the range 0.05–1.0 (between 10 and 90% of the incident radiation). At around 0.3 absorbance (50% absorption), the accuracy is greatest.
2. Bovine serum albumin is frequently used as a protein standard; 1 mg/mL has an A_{280} of 0.66.
3. If the solution is turbid, the apparent A_{280} will be increased by light scattering. Filtration (through a 0.2- μm Millipore filter) or clarification of the solution by centrifugation can be carried out. For turbid solutions, a convenient approximate correction can be applied by subtracting the A_{310} (proteins do not normally absorb at this wavelength unless they contain particular chromophores) from the A_{280} .
4. At low concentrations, protein can be lost from solution by adsorption on the cuvet; the high ionic strength helps to prevent this. Inclusion of a nonionic detergent (0.01% Brij 35) in the buffer may also help to prevent these losses.
5. The presence of nonprotein chromophores (e.g., heme, pyridoxal) can increase A_{280} . If nucleic acids are present (which absorb strongly at 260 nm), the following formula can be applied. This gives an accurate estimate of the protein content by removing the contribution to absorbance by nucleotides at 280 nm, by measuring the A_{260} which is largely owing to the latter (6).

$$\text{Protein (mg/mL)} = 1.55 A_{280} - 0.76 A_{260} \quad (5)$$

Other formulae (using similar principles of absorbance differences) employed to determine protein in the possible presence of nucleic acids are the following (7,8):

$$\text{Protein (mg/mL)} = (A_{235} - A_{280})/2.51 \quad (6)$$

$$\text{Protein (mg/mL)} = 0.183 A_{230} - 0.075.8 A_{260} \quad (7)$$

6. Protein solutions obey Beer-Lambert's Law at 215 nm provided the absorbance is <2.0 .
7. Strictly speaking, this value applies to the protein in 6 M guanidinium-HCl, but the value in buffer is generally within 10% of this value, and the relative absorbances in guanidinium-HCl and buffer can be easily determined by parallel dilutions from a stock solution.
8. Sodium chloride, ammonium sulfate, borate, phosphate, and Tris do not interfere, whereas 0.1 M acetate, succinate, citrate, phthalate, and barbiturate show high absorption at 215 nm.
9. The absorption of proteins in the range 215–225 nm is practically independent of pH between pH values 4–8.
10. The specific extinction coefficient of a number of proteins and peptides at 205 nm and 210 nm (3) has been determined. The average extinction coefficient for a 1 mg/mL solution of 40 serum proteins at 210 nm is 20.5 ± 0.14 . At this wavelength, a protein concentration of 2 $\mu\text{g/mL}$ gives $A = 0.04$ (5).

References

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