

Extinction Coefficients

A guide to understanding extinction coefficients, with emphasis on spectrophotometric determination of protein concentration

TR0006.4

Introduction

In many applications involving peptides or proteins it is important either to identify fractions containing protein or to estimate the concentration of a purified sample. Amino acids containing aromatic side chains (i.e., tyrosine, tryptophan and phenylalanine) exhibit strong UV-light absorption. Consequently, proteins and peptides absorb UV-light in proportion to their aromatic amino acid content and total concentration. Once an absorptivity coefficient has been established for a given protein (with its fixed amino acid composition), the protein's concentration in solution can be calculated from its absorbance.

For most proteins, UV-light absorption allows detection of concentration down to 100µg/mL. Nevertheless, estimation of protein concentration by UV-light absorption is not accurate for complex protein solutions (e.g., cell lysates) because the composition of proteins with different absorption coefficients is not known. In addition, proteins are not the only molecules that absorb UV-light, and complex solutions will usually contain compounds like nucleic acids that interfere with protein concentration determination by this method. However, for aqueous protein solutions commonly used in the research laboratory setting, interference from other compounds is minimized by measuring absorbances at 280nm.

Only the amino acids tryptophan (Trp, W) and tyrosine (Tyr, Y) and to a lesser extent cysteine (Cys, C) contribute significantly to peptide or protein absorbance at 280nm. Phenylalanine (Phe, F), which was mentioned above, absorbs only at lower wavelengths (240-265nm).

Absorbance and Extinction Coefficients

The ratio of radiant power transmitted (P) by a sample to the radiant power incident (P₀) on the sample is called the transmittance, T:

$$T = P/P_0$$

Absorbance (A), then, is defined as the logarithm (base 10) of the reciprocal of the transmittance:

$$A = -\log T = \log (1/T)$$

In a spectrophotometer, monochromatic plane-parallel light enters a sample at right angles to the plane-surface of the sample. In these conditions, the transmittance and absorbance of a sample depends on the molar concentration (c), light path length in centimeters (L), and molar absorptivity (ε) for the dissolved substance at the specified wavelength (λ).¹

$$T_\lambda = 10^{\epsilon c L} \quad \text{or} \quad A_\lambda = \epsilon c L$$

Beer's Law states that molar absorptivity is constant (and the absorbance is proportional to concentration) for a given substance dissolved in a given solvent and measured at a given wavelength.² For this reason, molar absorptivities are called *molar absorption coefficients* or *molar extinction coefficients*. Because transmittance and absorbance are unitless, the units for molar absorptivity must cancel with units of measure in concentration and light path. Therefore, molar absorptivities have units of M⁻¹ cm⁻¹. Standard laboratory spectrophotometers are fitted for use with 1cm-width sample cuvettes; hence, the path length is generally assumed to be equal to one and the term is dropped altogether in most calculations.

$$A_\lambda = \epsilon c L = \epsilon c \quad \text{when } L = 1\text{cm}$$

The molar absorption coefficient of a peptide or protein is related to its tryptophan (W), tyrosine (Y) and cysteine (C) amino acid composition. At 280nm, this value is *approximated* by the weighted sum of the 280nm molar absorption coefficients of these three constituent amino acids, as described in the following equation:^{3,4}

$$\epsilon = (nW \times 5500) + (nY \times 1490) + (nC \times 125)$$

where *n* is the number of each residue and the stated values are the amino acid molar absorptivities at 280nm.

Determining the Protein Concentration of a Solution from its Absorbance

Solving the expression of Beer's law for concentration, one can easily see what values are needed to determine the concentration of a peptide or protein solution:

$$c = A / \epsilon L \quad (= A / \epsilon \text{ when } L = 1 \text{ cm})$$

Dividing the measured absorbance of a peptide or protein solution by the calculated or known molar extinction coefficient yields the molar concentration of the peptide or protein solution. The peptide or protein amino acid composition must be known to calculate the molar extinction coefficient using the formula stated in the previous section.

A brief reading of the cited articles^{3,4} makes one appreciate that there is no single correct extinction coefficient value for a complex molecule like a peptide or protein. Even minor differences in buffer type, ionic strength and pH affects absorptivity values at least slightly. Most protein preparations, even those of equal purity, differ slightly in conformation and extent of modifications, such as oxidation, and these also affect absorptivity. Therefore, the best extinction coefficient value is one that is determined *empirically* using a solution of the study protein of known concentration dissolved in the same buffer as the sample (e.g., see discussion of Pierce Albumin Standards to follow).

Alternatively, absorption coefficients (i.e., extinction coefficients) for many proteins have been compiled from the literature and reported in the Practical Handbook of Biochemistry and Molecular Biology.⁵ These values provide sufficient accuracy for most routine laboratory applications that require an assessment of protein concentration. Most sources report extinction coefficients for proteins measured at or near a wavelength of 280nm in phosphate or other physiologic buffer.

Molar Extinction Coefficients vs. Absorbances for 1% Solutions

Application of a *molar* extinction coefficient in the calculation yields an expression of concentration in terms of *molarity*:

$$A / \epsilon_{\text{molar}} = \text{molar concentration}$$

However, many sources, including the reference cited above, do not provide molar extinction coefficients. Instead, they provide absorbance ($A_{280\text{nm}}$) values for 1% (= 1g/100mL) solutions measured in a 1 cm cuvette. These values can be understood as *percent solution* extinction coefficients ($\epsilon_{\text{percent}}$) having units of (g/100mL)⁻¹ cm⁻¹ instead of M⁻¹cm⁻¹. Consequently, when these values are applied as extinction coefficients in the general formula, the units for concentration, c, are percent solution (i.e., 1% = 1g/100mL = 10mg/mL).

$$A / \epsilon_{\text{percent}} = \text{percent concentration}$$

If one wishes to report concentration in terms of mg/ml, then an adjustment factor of 10 must be made when using these percent solution extinction coefficients (i.e., one must convert from 10 mg/ml units to 1 mg/ml concentration units).

$$(A / \epsilon_{\text{percent}}) 10 = \text{concentration in mg/ml}$$

The relationship between molar extinction coefficient (ϵ_{molar}) and percent extinction coefficient ($\epsilon_{\text{percent}}$) is as follows:

$$(\epsilon_{\text{molar}}) 10 = (\epsilon_{\text{percent}}) \times (\text{molecular weight of protein})$$

Still other sources provide protein absorbance values for 0.1% (= mg/mL) solutions, as this unit of measure is more convenient and common for protein work than percent solution. This variation in reporting style underscores the importance of carefully reading stated values to be sure that the unit of measure is understood and applied correctly.

Examples

A. Proteins and Protein Mixtures with Unknown Extinction Coefficients

If no extinction coefficient information exists for a protein or protein mixture of interest, and a rough estimate of protein concentration is required for a solution that has no other interfering substances, assume $\epsilon_{\text{percent}} = 10$. Most protein extinction coefficients ($\epsilon_{\text{percent}}$) range from 4.0 to 24.0.⁵ Therefore, although any given protein can vary significantly from $\epsilon_{\text{percent}} = 10$, the average for a mixture of many different proteins likely will be approximately 10.

B. Immunoglobulins

Most mammalian antibodies (i.e., immunoglobulins) have protein extinction coefficients ($\epsilon_{\text{percent}}$) in the range of 12 to 15.

Therefore, for typical antibody solutions, assume $A_{280\text{nm}}^{1\%} = 14$ or $A_{280\text{nm}}^{0.1\%} = A_{280\text{nm}}^{1\text{mg/ml}} = 1.4$.

For a typical IgG with MW = 150,000, this value corresponds to a molar extinction coefficient (ϵ) equal to 210,000M⁻¹ cm⁻¹.

C. Bovine Serum Albumin (BSA)

Thermo Scientific™ Pierce™ Albumin Standard Ampules (Product No. 23209) are provided as 2mg/mL solutions of purified bovine serum albumin (BSA) in 0.9% NaCl. The product is calibrated by direct comparison of the absorbance at 280nm to a known concentration of a BSA standard from the National Institute of Standards and Technology (NIST). Numerous values for the absorptivity of BSA have been reported in the literature but are generally ~6.6 for a 1% solution at 280nm.

Therefore, the predicted absorbance at 280nm for the Albumin Standard, assuming exactly 2mg/mL and $\epsilon_{\text{percent}} = 6.6$ is

$$\epsilon_{\text{percent}} c L / 10 = A$$

$$[(6.6)(2.000)(1)] / 10 = 1.320$$

Suppose that relative to a water reference a researcher obtains a 280nm absorbance reading of 1.346 for the Albumin Standard. The calculated concentration, assuming the stated percent absorptivity value, is as follows:

$$(A / \epsilon_{\text{percent}}) \times 10 = c_{\text{mg/ml}}$$

$$(1.346 / 6.6) \times 10 = 2.039\text{mg/mL}$$

Assuming a MW = 66,400, the molar extinction coefficient at 280nm for BSA is approximately $43,824\text{M}^{-1} \text{cm}^{-1}$.

Using Thermo Scientific Pierce Albumin Standards

If you plan to use a Pierce Albumin Solution (Product No. 23209 or 23210) as an absorbance standard, assume its concentration to be accurate (that's the whole point of a standard!) and use it to calculate your own "system-specific" extinction coefficient. Do this by measuring the absorbance of the provided solution (or several dilutions thereof, ideally prepared in duplicate or triplicate) and then applying the formula $A / c L = \epsilon$. As in the above examples, this ϵ that you calculate will be in terms of the units you used for c. The resulting "system-specific" extinction coefficient will be accurate for your particular buffer, spectrophotometer and cuvette, etc., allowing the albumin to function as an accurate reference standard for protein samples of unknown concentration. Without also knowing the extinction coefficient for the proteins in the sample, you will not know whether the same concentrations of BSA and sample protein will have the same absorbance. (For example, 1mg/mL IgG has nearly twice the absorbance of 1mg/mL BSA.) However, you will be able to use the BSA standard as a uniform reference to compare and normalize multiple samples to each other.

Related Thermo Scientific Pierce Protein Research Products

23209	Albumin Standard Ampules, 2mg/mL , 10 × 1mL ampules containing bovine serum albumin (BSA) at a concentration of 2.0mg/mL in 0.9% saline and 0.05% sodium azide
23210	Albumin Standard Ampules, 2mg/mL , 50mL, containing bovine serum albumin (BSA) at a concentration of 2.0mg/mL in 0.9% saline and 0.05% sodium azide
23212	Bovine Gamma Globulin Standard Ampules , 2mg/mL, 10 × 1mL
23225	BCA Protein Assay Kit , sufficient reagents for 500 test tube or 5000 microplate assays
23235	Micro BCA Protein Assay Kit , working range of 0.5-20µg/mL
23236	Coomassie Plus (Bradford) Assay Kit , working range of 1-1500µg/mL

References

1. Lange's Handbook of Chemistry, 14th Edition, Dean, J.A., Ed. (1992). McGraw-Hill, Inc., New York.
2. Handbook of Chemistry and Physics, 56th Edition, Weast, R.C., Ed. (1975). CRC Press, Cleveland.
3. Gill, S.C. and von Hippel, P.H. (1989). Calculation of protein extinction coefficients from amino acid sequence data. *Anal. Biochem.* **182**:319-26.
4. Pace, C.N., *et al.* (1995). How to measure and predict the molar absorption coefficient of a protein. *Protein Sci.* **4**:2411-23.
5. Practical Handbook of Biochemistry and Molecular Biology, Fasman, D.G., Ed. (1992). CRC Press, Boston.

Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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