

# Extinction Coefficients

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

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## Introduction

In many applications involving 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, phenylalanine and tryptophan) 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 100 µg/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 unknown absorption coefficients is not known. In addition, proteins are not the only molecules that absorb UV-light and complex solutions will usually contain compounds such as nucleic acid that will interfere with determination of protein concentration 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 280 nm.

## Absorbance

The ratio of radiant power transmitted (P) by a sample to the radiant power incident (P<sub>0</sub>) 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.<sup>1</sup>

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

Beer's Law states that molar absorptivity is constant and the absorbance is proportional to concentration for a given substance dissolved in a given solute and measured at a given wavelength.<sup>2</sup> Accordingly, molar absorptivities are commonly called molar extinction coefficients. Since transmittance and absorbance are unitless, the units for molar absorptivity must cancel with units of measure in concentration and light path. Accordingly, molar absorptivities have units of M<sup>-1</sup>cm<sup>-1</sup>. Standard laboratory spectrophotometers are fitted for use with 1 cm width sample cuvettes; hence, the path length is generally assumed to be equal to one and the term is dropped altogether in most calculations.

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

## Molar Extinction Coefficients

Molar absorptivities (= molar extinction coefficients) for many proteins are provided in the Practical Handbook of Biochemistry and Molecular Biology.<sup>3</sup> Expressed in this form, the extinction coefficient allows for estimation of the molar concentration of a solution from its measured absorbance.

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

Extinction coefficients for proteins are generally reported with respect to an absorbance measured at or near a wavelength of 280 nm. Although the absorption maxima for certain proteins may be at other wavelengths, 280 nm is favored because proteins absorb strongly there while other substances commonly in protein solutions do not.

## Absorbances for 1% Solutions

Many sources, including the reference cited above, provide absorbance ( $A_{280\text{nm}}$ ) values for 1% (= 1 g/100 ml) solutions measured in a 1 cm path length. These values can be understood as percent solution extinction coefficients ( $\epsilon_{\text{percent}}$ ) having units of  $(\text{g}/100 \text{ ml})^{-1} \text{ cm}^{-1}$  instead of  $\text{M}^{-1} \text{ cm}^{-1}$ . Consequently, when these values are applied as extinction coefficients in the general formula, the units for concentration,  $c$ , are percent solution (i.e., 1% = 1 g/100 ml = 10 mg/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 ( $\epsilon_{\text{molar}}$ ) and Percent Extinction Coefficient ( $\epsilon_{\text{percent}}$ ) is as follows

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

## 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 ( $\epsilon_{\text{percent}}$ ) fall in the range 4.0-24.0.<sup>3</sup> Therefore, although any given protein can vary significantly from  $\epsilon_{\text{percent}} = 10$ , the average for a mixture of many different proteins will likely be close to 10.

### B. Immunoglobulins

Most mammalian antibody types (i.e., immunoglobulins) have protein extinction coefficients ( $\epsilon_{\text{percent}}$ ) in the range 13-15. Therefore, rough estimation of antibody concentration should be made assuming  $\epsilon_{\text{percent}} = 14$ .

### C. Bovine Serum Albumin (BSA)

Pierce Albumin Standard Ampules (Bovine Albumin Fraction V, Product No. 23209) are provided as a 2 mg/ml solution in 0.9% NaCl. The product is calibrated by absorbance at 280 nm to a BSA Fraction V standard from the National Institute of Standards and Technology (NIST) having a reported percent solution absorbance (=  $\epsilon_{\text{percent}}$ ) equal to 6.67.

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

$$\begin{aligned} \epsilon_{\text{percent}} c L / 10 &= A \\ [(6.67)(2.000)(1)] / 10 &= 1.334 \end{aligned}$$

Suppose that relative to a water reference a researcher obtains a 280 nm absorbance reading of 1.346 for the Albumin Standard. The calculated concentration is

$$\begin{aligned} (A / \epsilon_{\text{percent}}) 10 &= c_{\text{mg/ml}} \\ (1.346 / 6.67) 10 &= 2.018 \text{ mg/ml} \end{aligned}$$

## Using Pierce Albumin Standards

If you plan to use the Pierce Albumin Standard (Product Nos. 23209 or 23210) as a 280 nm absorbance standard, it is suggested that you 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 absorption of the provided solution (or several known dilutions thereof, ideally prepared in duplicate or triplicate) and then applying the formula  $A / (c L) = \epsilon$ . As in the above examples, this  $\epsilon$  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 spectrophotometer, cuvette, etc., allowing the Albumin to function as an accurate reference standard for protein samples of unknown concentration.

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**Related Pierce Products**

<b>23209</b>	<b>Albumin Standard Ampules, 2 mg/ml</b> , 10 x 1 ml ampules containing bovine serum albumin (BSA) at a concentration of 2.0 mg/ml in 0.9% saline and 0.05% sodium azide
<b>23212</b>	<b>Bovine Gamma Globulin Standard Ampules</b> , 2 mg/ml, 10 x 1 ml
<b>23225</b>	<b>BCA Protein Assay Reagent Kit*</b> , sufficient reagents for 500 test tube or 5,000 microplate assays
<b>23227</b>	<b>BCA Protein Assay Reagent Kit*</b> , sufficient reagents for 250 test tube or 2,500 microplate assays
<b>23235</b>	<b>Micro BCA™ Protein Assay Reagent Kit*</b> , working range of 0.5-20 µg/ml
<b>23236</b>	<b>Coomassie® Plus Protein Assay Reagent Kit</b> , working range of 1-1,500 µg/ml
<b>23215</b>	<b>Compat-Able™ Protein Assay Preparation Reagent Set</b> , sufficient reagents to pre-treat 500 samples to remove interfering substances before total protein quantitation

**References**

1. Lange's Handbook of Chemistry, 14<sup>th</sup> Edition, Dean, J.A., Ed. (1992). McGraw-Hill, Inc., New York.
2. Handbook of Chemistry and Physics, 56<sup>th</sup> Edition, Weast, R.C., Ed. (1975). CRC Press, Cleveland.
3. Practical Handbook of Biochemistry and Molecular Biology, Fasman, D.G., Ed. (1992). CRC Press, Boston.

\*U.S. Patent # 4,839,295.

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