Biology and Biochemistry Department
BIOC311
Biochemistry Lab

Experiment #4

Title: Ultraviolet Absorption of Proteins and Amino Acids.

Student name: Melak Ottallah
Student number: 1190389

Partners: Ali Milhem, AbdelFattah Tanneneh, Donna Taye ,Abdalrahman Sroor.

Instructor: Dr. Amanie Abed
Teacher's assistant: Aseel Mhani

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Objective:

The objective of this experiment is to observe the UV absorption of different proteins and amino acids and detect which are the best UV absorbers.

Introduction:

Protein UV absorption is primarily attributed to the amino acids tyrosine and tryptophan. Both share a molar absorption coefficient at 280 nm, but the protein's peak absorbance is largely determined by the order and amount of these individual amino acids within the linear chain. As a result, protein UV absorption peaks and valleys vary depending on amino acid composition. This provides a fundamental approach for assessing the purity of known proteins and the number of amino acids present in unknown samples or protein mixtures.

The most direct approach to quantify the quantity of protein in solution is to use a spectrometer to measure UV-absorbance. While samples are exposed to UV light with wavelengths ranging from 260 to 280 nm, the degree of absorption is recorded. This technique is suitable for measuring pure proteins containing tyrosine and tryptophan aromatic chains in a simple and fast manner. It's useless for evaluating protein properties in samples that don't include tyrosine or tryptophan. UV-Vis spectroscopy is commonly used to spectroscopically investigate such refractory materials at peak wavelengths of 205 nm. (1)

Materials:

1. Proteins (5 g/L albumins and casein)
2. Amino acids (1 mM tyrosine, tryptophan, and phenylalanine in water adjusted to pH 7)
3. Ultraviolet spectrophotometer
4. Quartz cuvette
5. Distilled water

Methods:

1. A spectrophotometer was blanked on air.

2. a clean Quartz cuvette was filled with double distilled water.

3. The absorbance spectrum of this "blank" was taken every 5 nm from 200 - 450 nm.

4. The water was removed and the cuvette was dried of the excess liquid and filled with the above compounds, one at a time. The absorbance was measured every 5 nm from 200 - 450nm. Each time the compound is changed, the cuvette was washed with water and dried.

5. The blank `` spectra" will only be done once and was subtracted by the actual readings at each point from the blank measurements.

6. The absorption spectra were plotted of the above compounds over the range of 200 - 450 nm.

Data and results:

Chart 1 absorbance vs wavelength of albumin, tyrosine, tryptophan, phenylalanine, casein, and double-distilled water from 200-450nm.

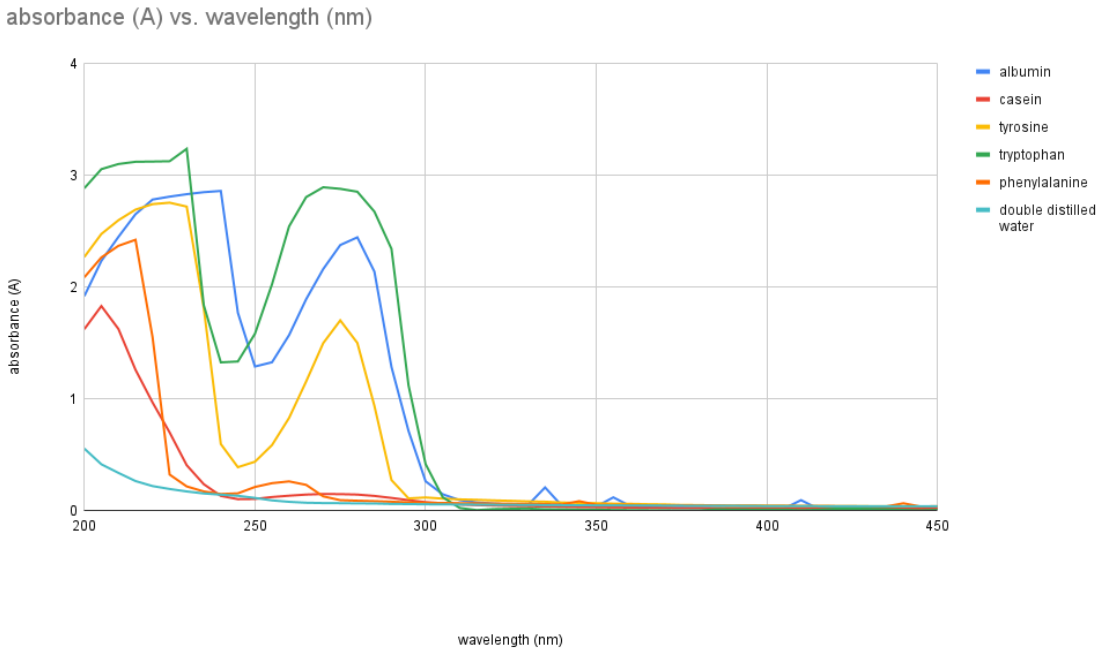


chart 2 absorbances vs wavelength double distilled water from 200-450nm

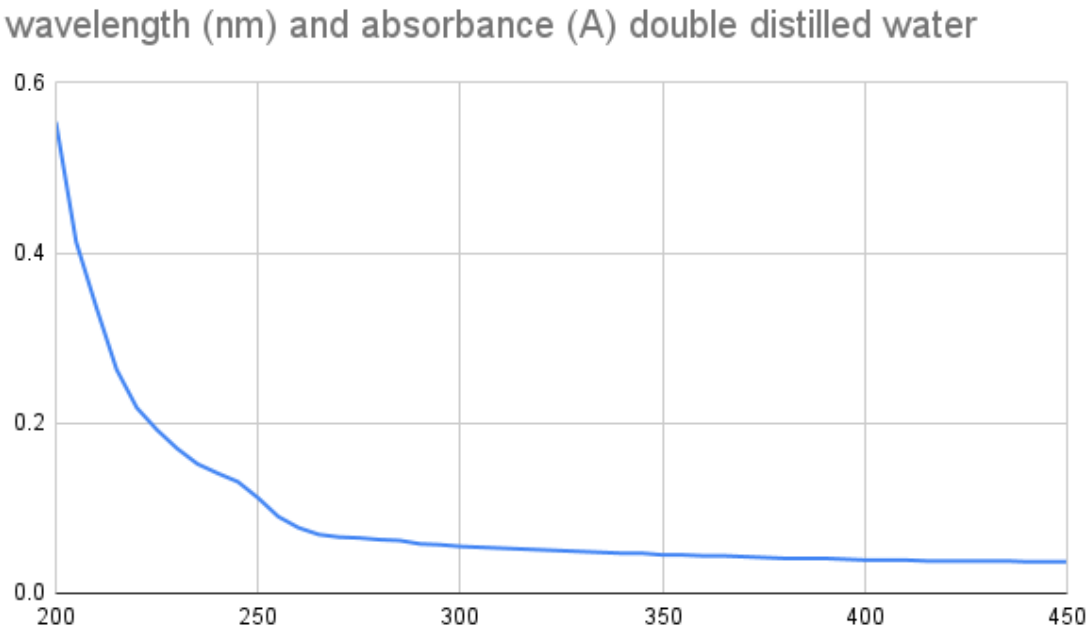


Chart 3 absorbance vs wavelength of phenylalanine from 200-450nm

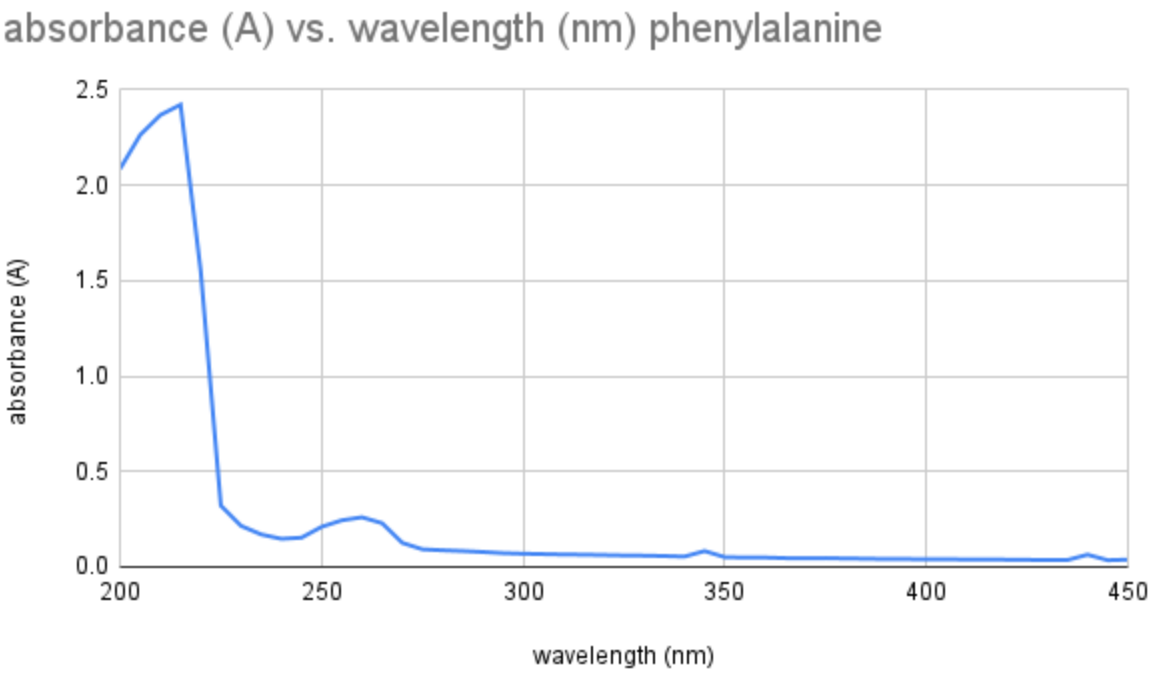


chart 4 absorbance vs wavelength of albumin from 200-450nm

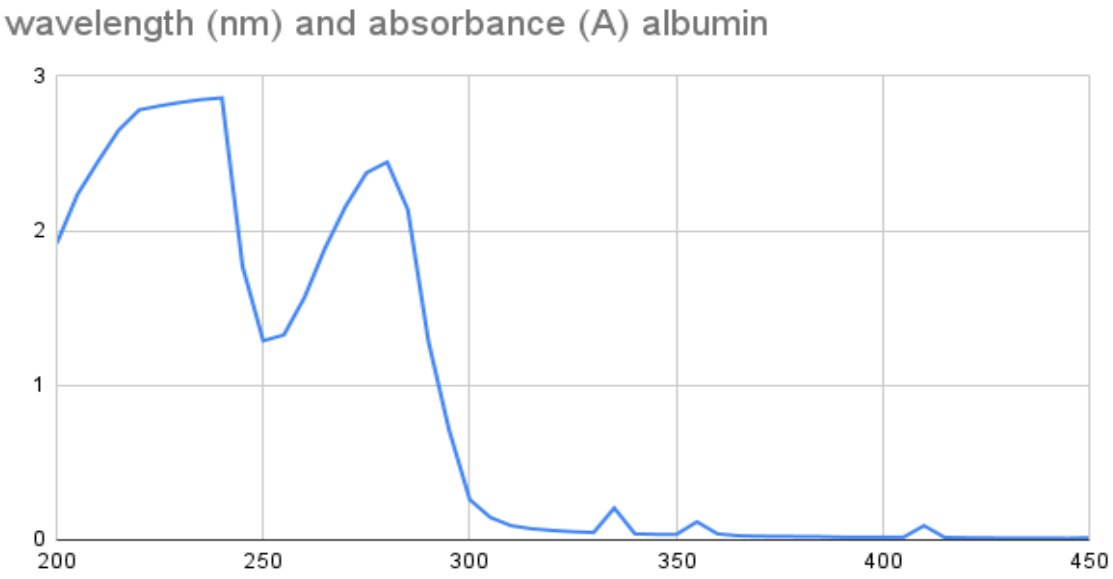


chart 5 absorbance vs wavelength casein from 200-450nm

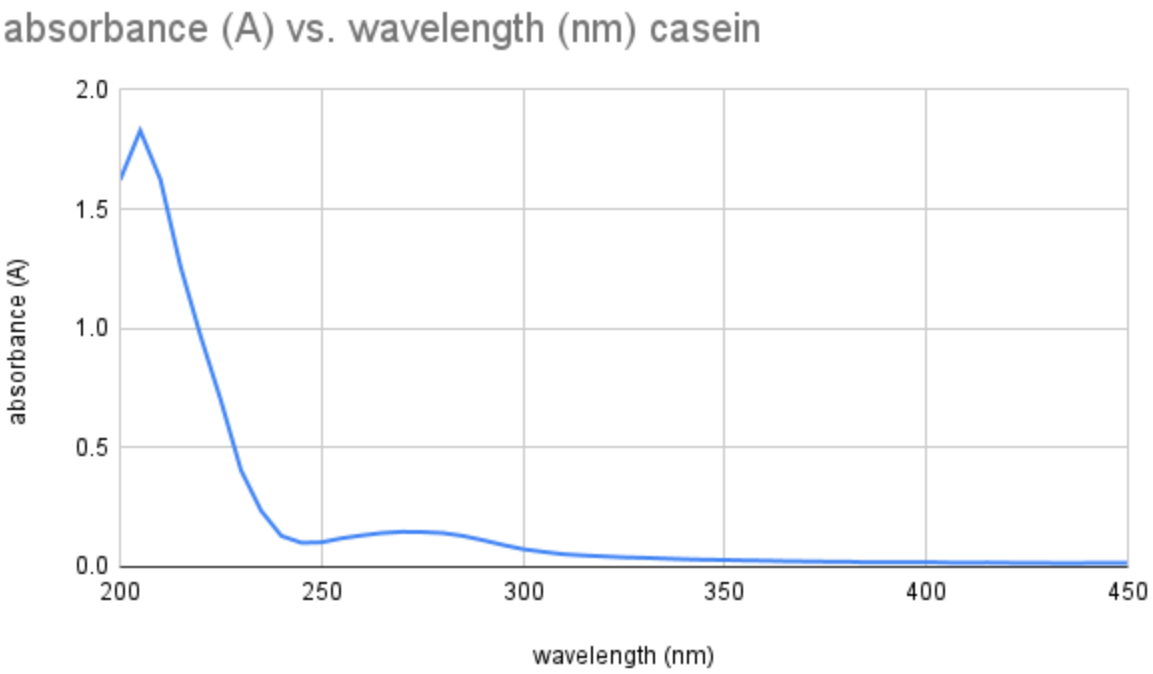


chart 6 absorbance vs wavelength of tryptophan from 200-450nm

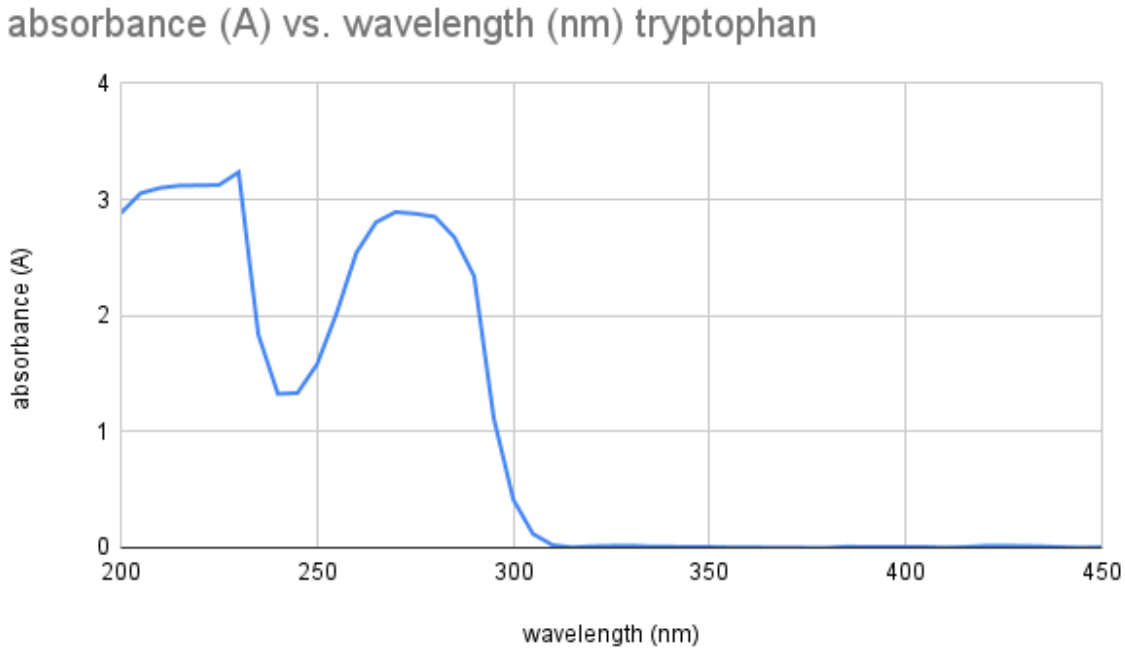
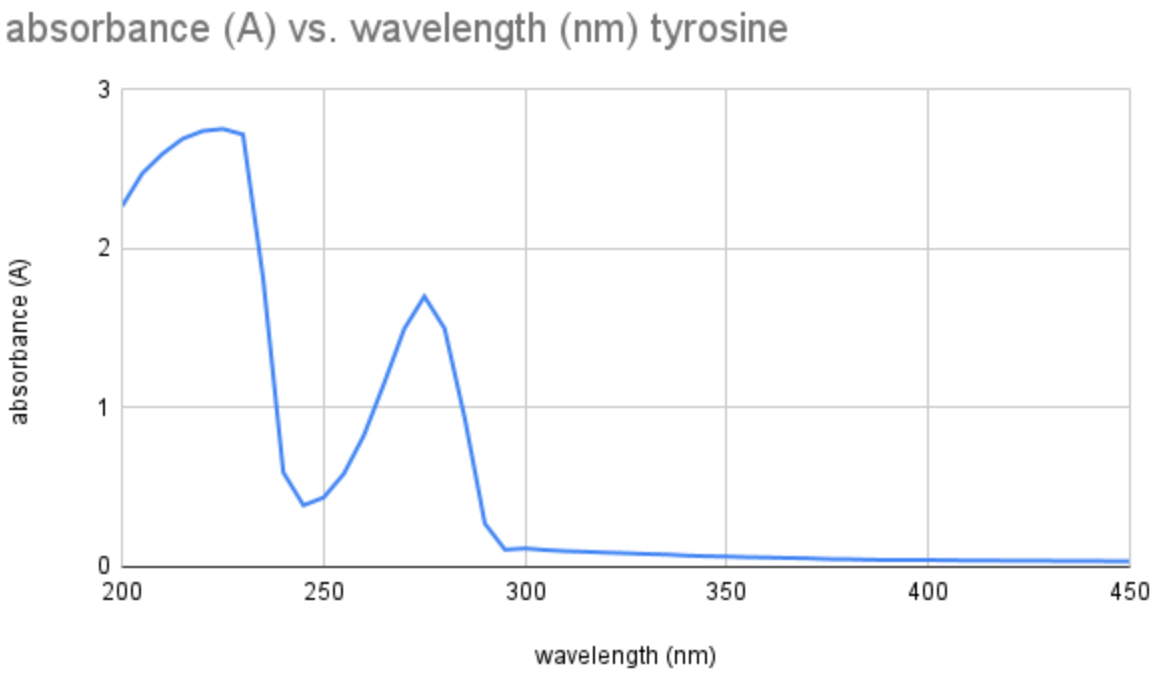


chart 7 absorbances vs wavelength tyrosine from 200-450nm.



Discussion:

In this experiment, we discuss the factors that increase absorbance within amino acids and proteins. It is proven that amino acids with aromatic rings and proteins with tyrosin are highly absorbent at 250nm - 280nm. But, if the protein contained neither, its peak absorbance would most likely be at 205nm.

Let's start with chart 2, water. Water is not a protein, so, its absorbance was logarithmic. The more the UV the less the absorbance. We used water in this experiment to subtract it from the proteins for a more accurate result.

Chart 3, phenylalanine, is an amino acid that has proven to not have tyrosine or tryptophan, hence, its peak is at around 205nm.

Chart4, albumin, is a protein that contains an abundance of the amino acid tyrosine. (2) this could be sought out through looking at the chart and recognizing albumins' second peak at around 280nm. Albumins purity was sought out to be 1.6.

Chart 5, casein is a protein with a low amount of tryptophan (about 1.25%). This is why the first peek at around 205nm is way higher than the peak around 280nm. The purity of casein is 1.1. (3)

Chart 6 and 7, tryptophan and tyrosine are the main amino acids responsible for the absorbance of UV light. Their peak absorptivity is at 280nm. Without these essential amino acids, proteins will not be able to absorb higher than 205nm.

Conclusion:

Tyrosine and tryptophan are responsible for the UV absorbance of proteins and amino acids at 280nm.

References:

1. *UV Vis Absorbance in Proteins* https://www.azom.com/article.aspx?ArticleID=19610
2. *Access NCBI through the World Wide Web (WWW). (1995). Molecular Biotechnology, 3(1), 75.* https://doi.org/10.1007/bf02821338
3. *UV/Vis+ Spectra Database. (2010). Chemistry International -- Newsmagazine for IUPAC, 32(6).* https://doi.org/10.1515/ci.2010.32.6.22

Appendix:

Purity of protein = A280/A260