Questions for Absorption Spectroscopy Laboratory:

1.

Beer Lamberts law:

$$OD \equiv \log\left(\frac{I_0}{I}\right) = A(\lambda) * c * l$$

Part of law	Meaning	unit
OD	Optical density is the amount of attenuation, or gradual intensity loss that occurs when light passes through on optical component	No unit
I_0	Intensity in on the solution	W/m^2
I	Intensity out from the solution	W/m^2
A(λ)	The molar extinction coefficient	$(cm * M)^{-1}$
c	Concentration of macromolecule in the solution	Mol/L = M
l	Light length through the solution	cm

2.

$A_{1\%}^{cm}$ is defined as OD at a wavelength λ for 1% (10 mg/mL) solution l = 1 cm light length.

Extinction coefficient is calculated from measured values of OD on a sample with concentration, c, like:

$$A_{1\%}^{1\,cm}(\lambda) = OD * (10\frac{mg}{ml})/(c(in\frac{mg}{ml})$$

The unit is, from the formula, given in mg/ml. The mg/ml replaces M in the extinction coefficient so we get, (10 mg/mL * cm)^-1.

3.

In this question we are supposed to explain how the molecules are absorbing the light.

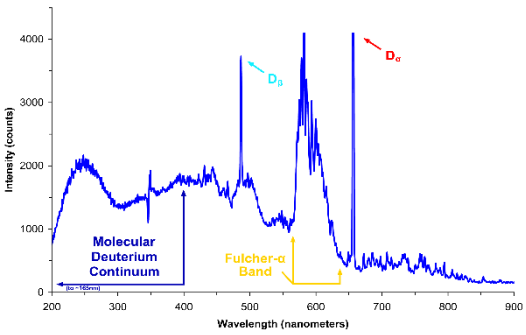
Absorption of ultraviolet or visible light by a molecule depends on electron transitions between molecular orbital energy levels. Energy is absorbed only when the amount of energy provided matches the difference in energy, ΔE, of 2 energy levels. Every molecule has a characteristic electronic spectrum depending on its characteristic $\Delta E's$. We can mix different compounds that will give a particular spectrum due to this.

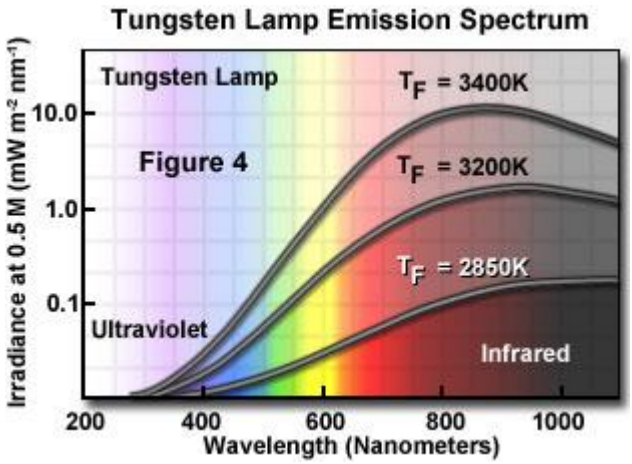
4.

Deuterium lamps gives intensity in area λ = 185-350nm and is called a UV-lamp

because it gives a colour of purple and below (violet).

The wavelength of an electromagnetic wave gives a certain colour. The Wolfram = tungsten gives intensity in the area 300-1000nm and is called a glow lamp. We use both of them because different molecules have different absorption energies.





Above is the spectrum from deuterium lamp Second figure is from a tungsten lamp

5.

Those have an absorption peak at 280nm because they are aromatic.

Aromatic amino acids are amino acids that include an aromatic ring and they absorbs at that wavelength because they have delocalized pi-electrons.

6.

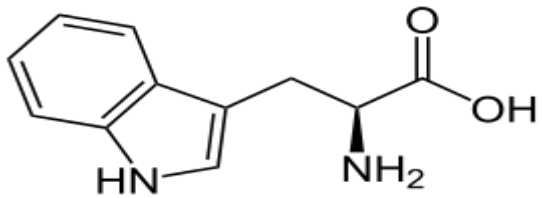
A modern spectrophotometer can measure the absorption in solutions in wavelength area from 200 nm and above.

Wavelengths below his rate are absorbed strongly in air. From values of 200 nm to 250 nm the ordinary spectrophotometer is unreliable. In our demonstration we measured from 260 nm and above.

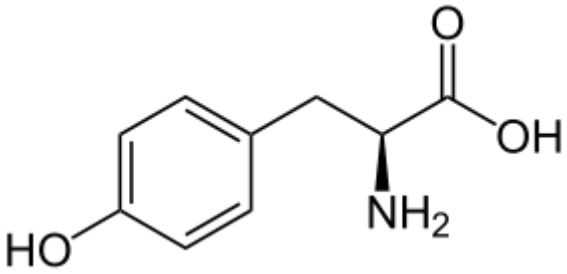
7.

We are supposed to find the chemical structure of 3 amino acids. These were Tryptophan, Tyrosine and glutamic acid.

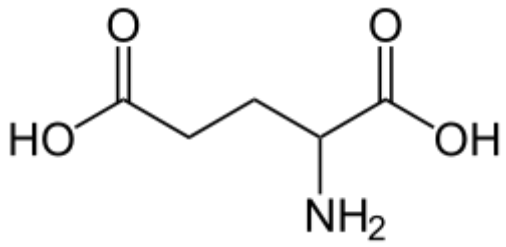
Tryptophan:



Tyrosine:



Glutamic acid:



Tryptophan is an aromatic amino acid, so I expect to see an absorbance peak at 280 nm. Tyrosine is also aromatic, so I sort of expect it to have an absorbance peak at 280 nm as well. Glutamic acid is not an aromatic acid. I expect its peptide-bonds to be responsible for its absorbance and that should give an absorbance peak at about 200 nm.

8.

A blank cuvette is a cuvette that only contains de-ionized water.

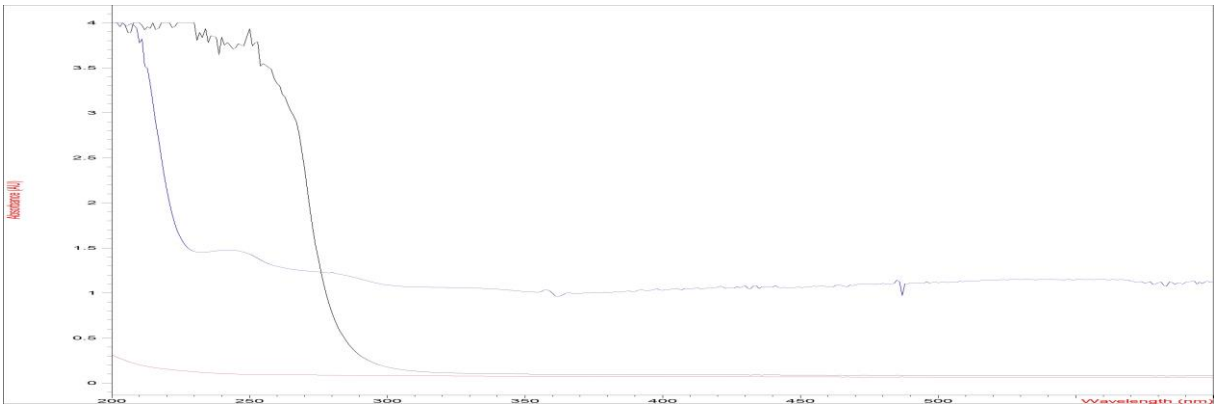
This is done to make it a sort of reference point, so that when you measure the sample, you subtract the blank. This gives you the net absorption for the sample.

9.

The flushing of cuvettes with the sample repeatedly was done to improve the concentration of the sample in the cuvette since it was first filled with water and other compounds (we only used two cuvettes for several samples).

10.

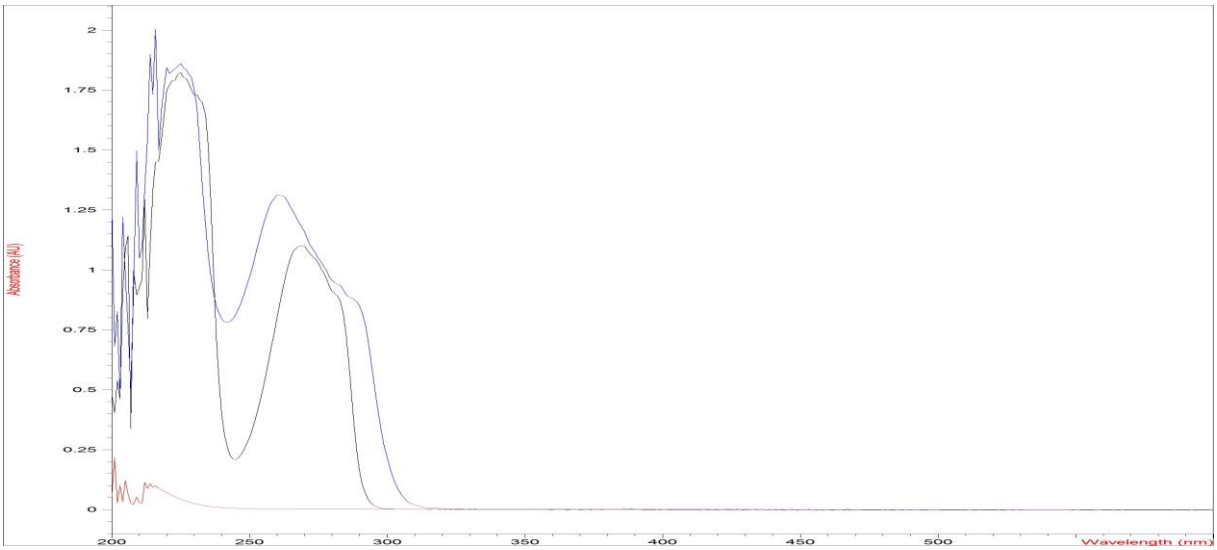
Following are the absorption spectra for the two cuvettes they had in the laboratory: glass and quartz. We had one glass cuvettes and two different type of quartz cuvettes (the expensive 1000 kroner one and the disposable UV quartz cuvette). This is why we have three graphs in the spectra.



Red is the expensive quartz, purple is the glass quartz and blue is the disposable quartz. We used the disposable quartz because it was much cheaper than the expensive one and our group were worried about the possible expenditure in case of unforeseen accident. The glass cuvette was never considered because it absorbed UV-radiation strongly.

11.

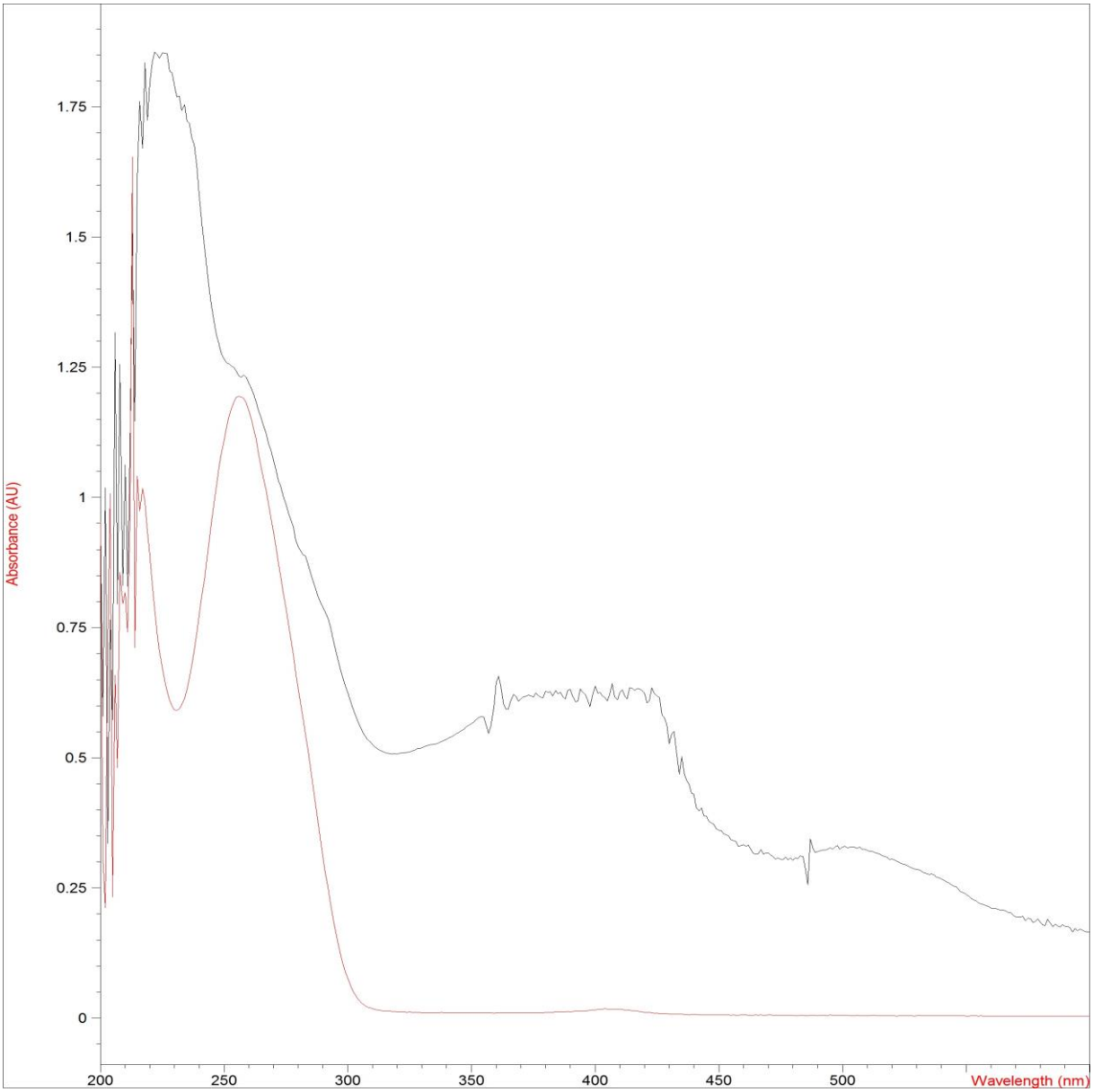
In this assignment we are supposed to include the image of the spectra obtained for amino acids and compare them to our prediction in assignment 7.



In the function Red = glutamic acid, Purple = Tyrosine and Blue = Tryptophan. With regards to the comparison to my earlier prediction, we can see that Tyrosine and Tryptophan both have "local" peaks in the 270 nm – 280 nm range. This means that they are slightly off the 280 nm prediction, this could be due to calibration, machine deviations and other factors. The glutamic acid does have peaks at about 200 nm, but since, as earlier mentioned, the machine is not too reliable at that wavelength it's difficult to say if its correct. Although it does look right. The main problem with this Absorption spectrum is that there are peaks before the 250 nm wavelength, which is not supposed to be the case. It should not be that the aromatic acids absorb more at 220 nm wavelength than 280 nm. According to Uttam Pal at researchgate.net, the high absorptionenergy at 230 nm or below is due to the n→pi^* or pi→pi^* transitions of the carboxylic group or amide in protein. The pi^* $is\ the$ antibonding molecular orbital and has as its main function that if its occupied by electrons it weakens the bonding between two atoms.

12.

We have the DNA and haemoglobin in the same function, so we have to do question 12 and 13 based on the same plot.



Red is the DNA and blue is the haemoglobin. An another important aspect to consider is the table values:

Sample/Result Table

#	Name	Abs<280nm>	Abs<260nm>
1	hemoglobin	0.90572	1.21940

2 DNA 0.63364 1.16650

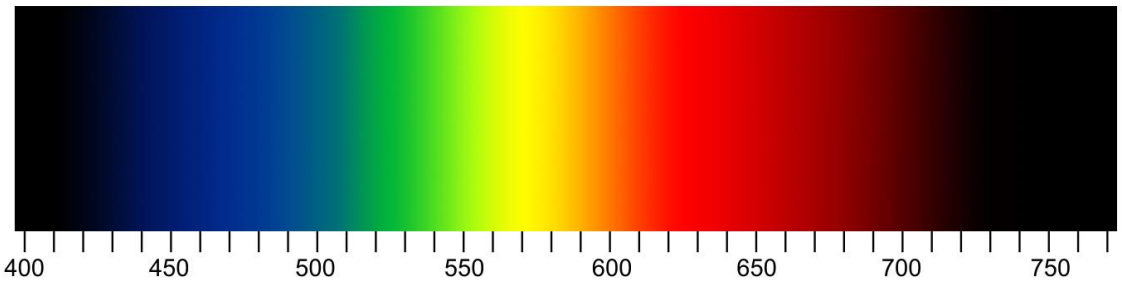
DNA is supposed to have a pronounced peak at about 265 nm. From the graph we can see that this seems to be true. With regards to the concentration we use something called the Chomczynski protocol. This protocol is based on the relationship between $\frac{Abs<260nm>}{Abs<280nm>}$. If the answer is between 1.6 and 1.8 it means that the DNA is purely concentrated and that there is no contamination from protein.

$$\frac{1.16650}{0.63364} = 1.84095$$

We see that the answer is slightly above what we were supposed to find. But it would have to, we can conclude that it is pure DNA. If the answer had been closer to 2.00 it would have been classified as RNA. If the ratios had been below 1.6, then we could have concluded that there had been protein contamination. Since its not, there is no contamination.

13.

We can see from the spectrum that violet, green and yellow colours are absorbed, while red from 600 to 700 nm has no absorption. The colour scheme is show below, remember that violet is up to 400 nm.



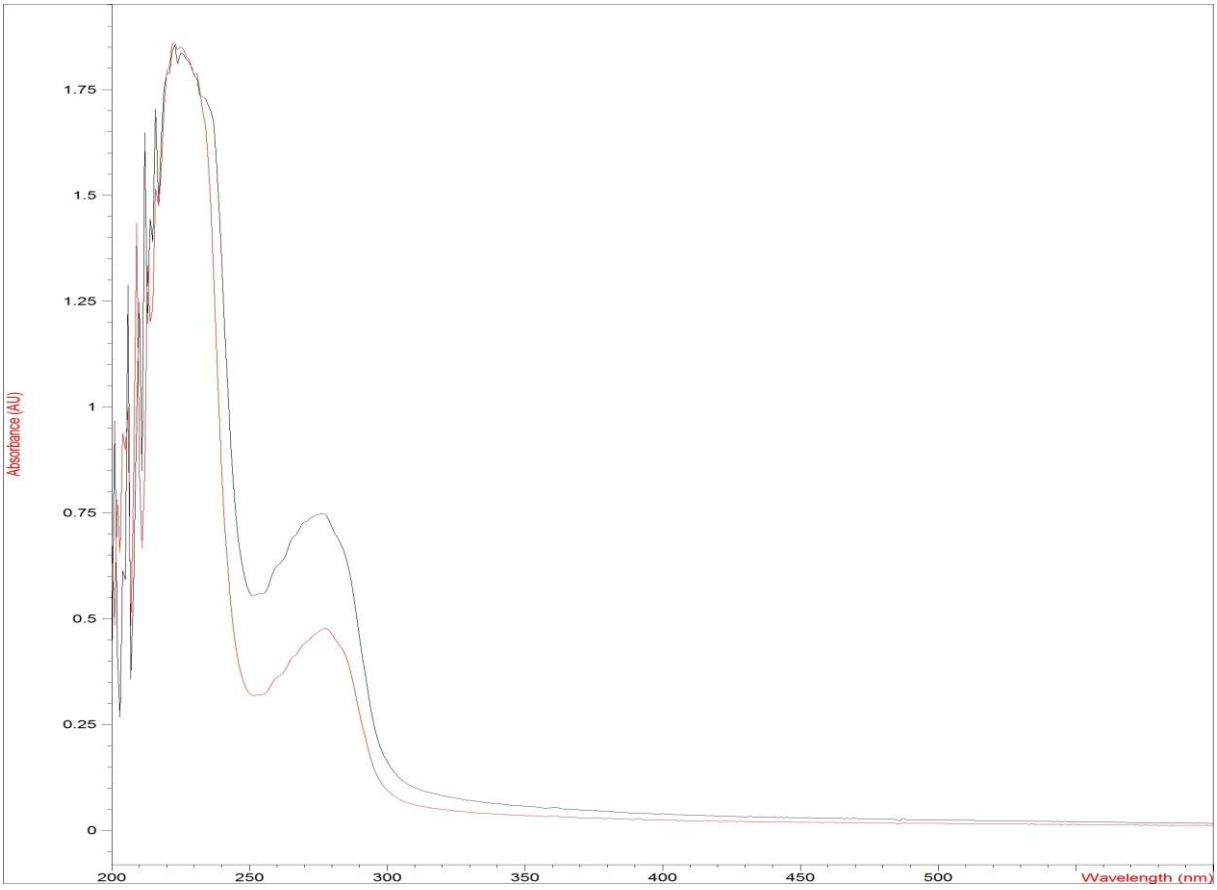
This gives that haemoglobin transmits a red colour. It is no surprise because blood has the colour red and haemoglobin is responsible for that.

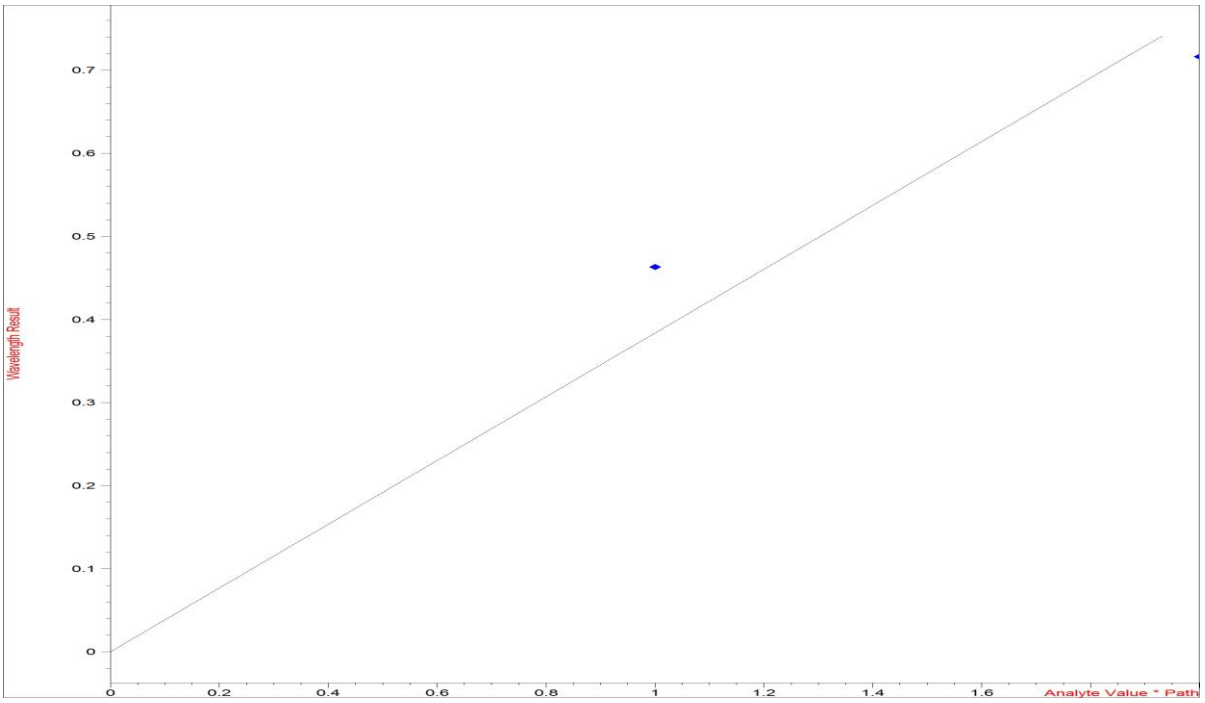
14.

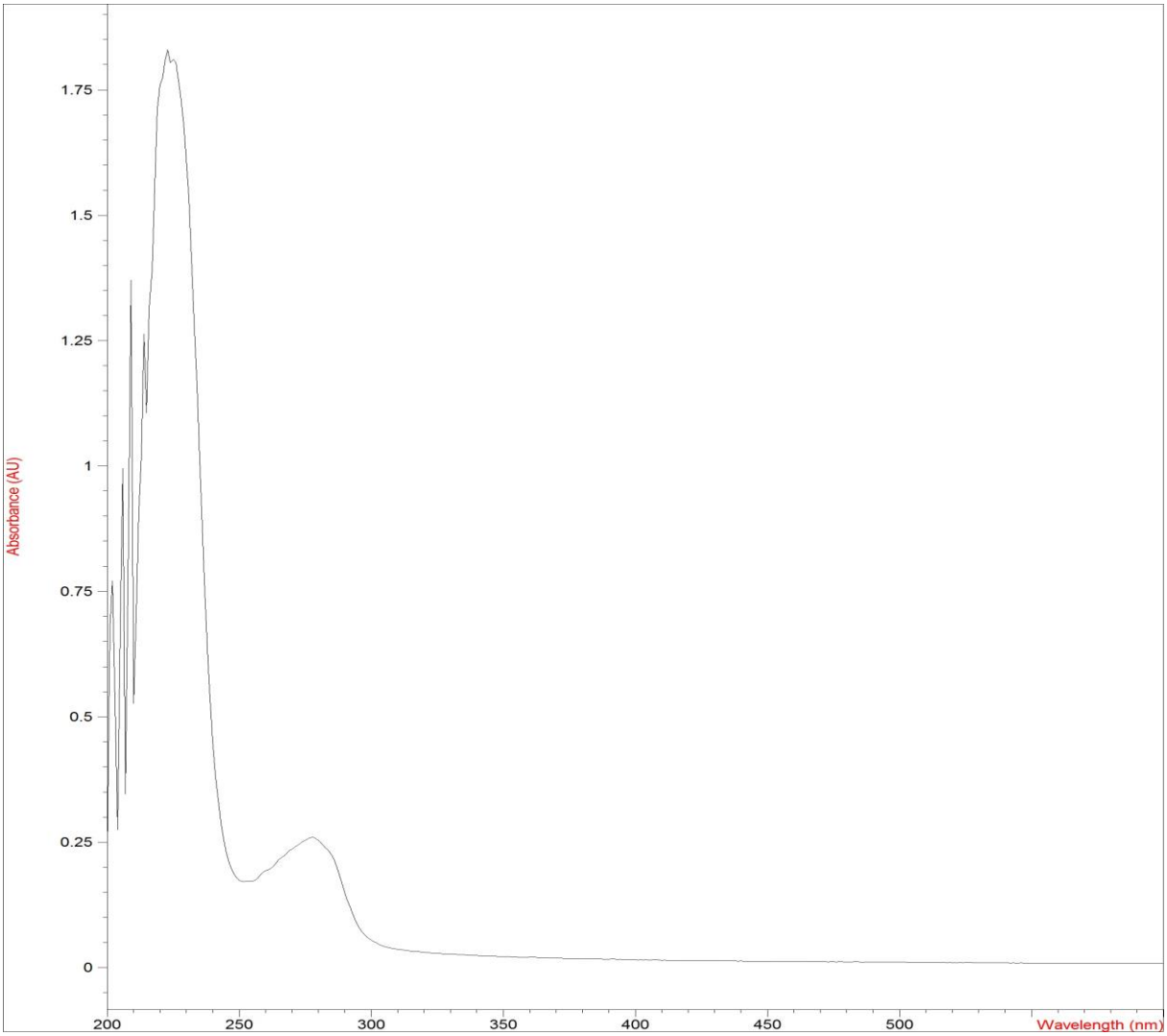
A standard solution is a solution in which the analyte concentration is accurately known. The absorbance of the standard solutions are measured and used to prepare a calibration curve. Then we analyse an unknown solution and compare it with the standard solution.

15.

We are to add the spectra of calibration standards, bovine serum albumin and the spectra of "unknown" samples. In addition to that, we will also add table of concentrations.







Calibration Table

#	Standard Name	Concentration(mg/ml)	Abs<280nm>	%Error
1	BSA	2.00000	0.71617	7.17
2	BSA	1.00000	0.46311	-17.14

Sample/Result Table

#	Name	Dilut. Factor	Concentration(mg/ml)	Abs<280nm>
1	BSA should be 0.	1.00000	0.66089	0.25361

The first graphs are of the calibration values and the last one is our unknown sample. Our intention was to have a concentration of 0.5 mg/ml. Obviously our answer is 0.66089 and not anything close to what we were supposed to have. Especially since the other groups had concentration values at about 0.54 mg/ml. The error could either be due to sample, the machinery or personal mistake in combining by the person who did it in the laboratory. Its hard to say where the mistake is. We would probably need an another sample done by the same person who made the first one. Regardless where the mistake were, the results are not good enough.