

Chapter 8: spectroscopic analysis

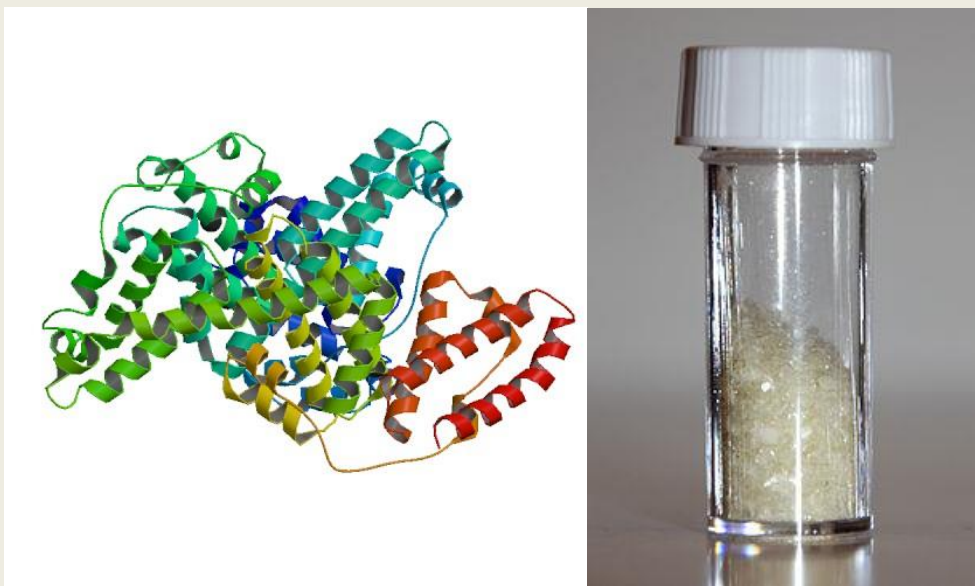
ACTIVITY 1 : Spectrophotometry: using a calibration curve

This activity was started in 1ere STL.

DOCUMENT 1: BSA protein

Source: [Wikipedia](#)

Bovine serum albumin (also known as **BSA** or "Fraction V") is a **serum albumin** protein derived from cows. It is often used as a protein concentration standard in lab experiments.



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DOCUMENT 2: Spectrophotometry measurements with proteins using Brilliant Blue dye (Bradford method)

Source: [Wikipedia](#)

The dye (Bradford reagent) associated with BSA protein has an absorption spectrum maximum at 595 nm. The increase of absorbance at 595 nm is proportional to the amount of bound dye, and thus to the concentration of protein present in the sample.

The procedure for Bradford protein assay is very easy and simple to follow. It is done in one step where the Bradford reagent is added to a test tube along with the sample. After mixing well, the mixture almost immediately changes to a blue color and the absorbance can be read at 595 nm using a spectrophotometer.



DOCUMENT 3: Procedure for standard assay

Source: [Wikipedia](#)

Procedure (Standard Assay, 20-150 μg protein; 200-1500 $\mu\text{g/mL}$)

- Prepare a series of protein standards diluted with 0.15 M NaCl to final concentrations of 0 (blank = NaCl only), 250, 500, 750 and 1500 $\mu\text{g/mL}$. Also prepare serial dilutions of the unknown sample to be measured.
- Add 100 μL of each of the above to a separate test tube
- Add 5.0 mL of dye to each tube and mix by vortex, or inversion.
- Adjust the spectrophotometer to a wavelength of 595 nm, and blank using the tube which contains no protein.
- Wait 5 minutes and read each of the standards at 595 nm wavelength.
- Plot the absorbance of the standards vs. concentration.

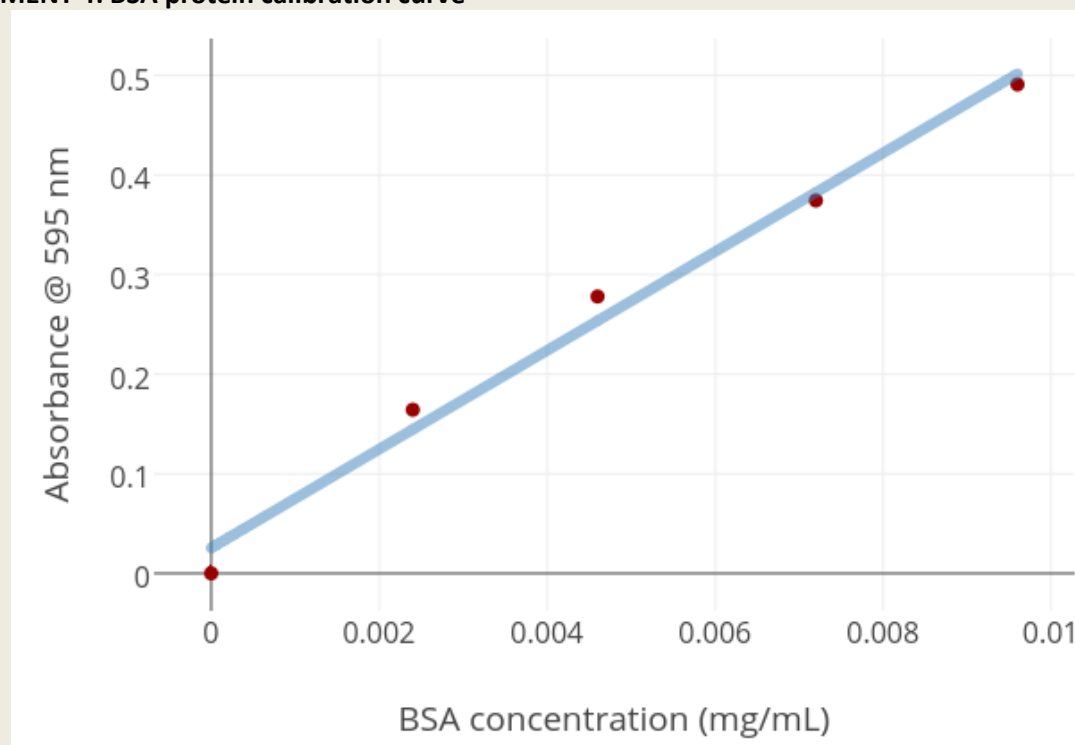
DOCUMENT 4: BSA protein calibration curve

Figure: Calibration curve - generated from BSA samples measured at 595nm

Source: [Wikipedia](#)

Presentation (5min)

Using the calibration curve, explain how you would determine the concentration of an unknown solution of BSA protein:

Activity summary

What you must remember:

- how to calculate a concentration using a calibration curves

Skills linked to the curriculum:

Compétences	Capacités à maîtriser
<ul style="list-style-type: none">– COM– APP	Concevoir et mettre en œuvre un protocole pour déterminer la concentration d'une espèce à l'aide d'une droite d'étalonnage établie par spectrophotométrie.