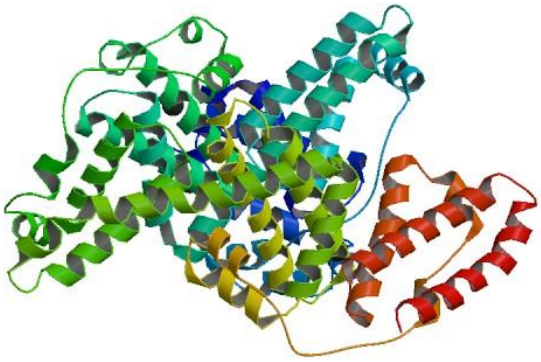
CH9: titrations using calibration

ACTIVITY 1 : Spectrophotometry : using a calibration curve

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 portein 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 µg/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 µg/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 and each of the samples at 595 nm wavelength.
- Plot the absorbance of the standards vs. concentration.

DOCUMENT 4: BSA protein calibration curve

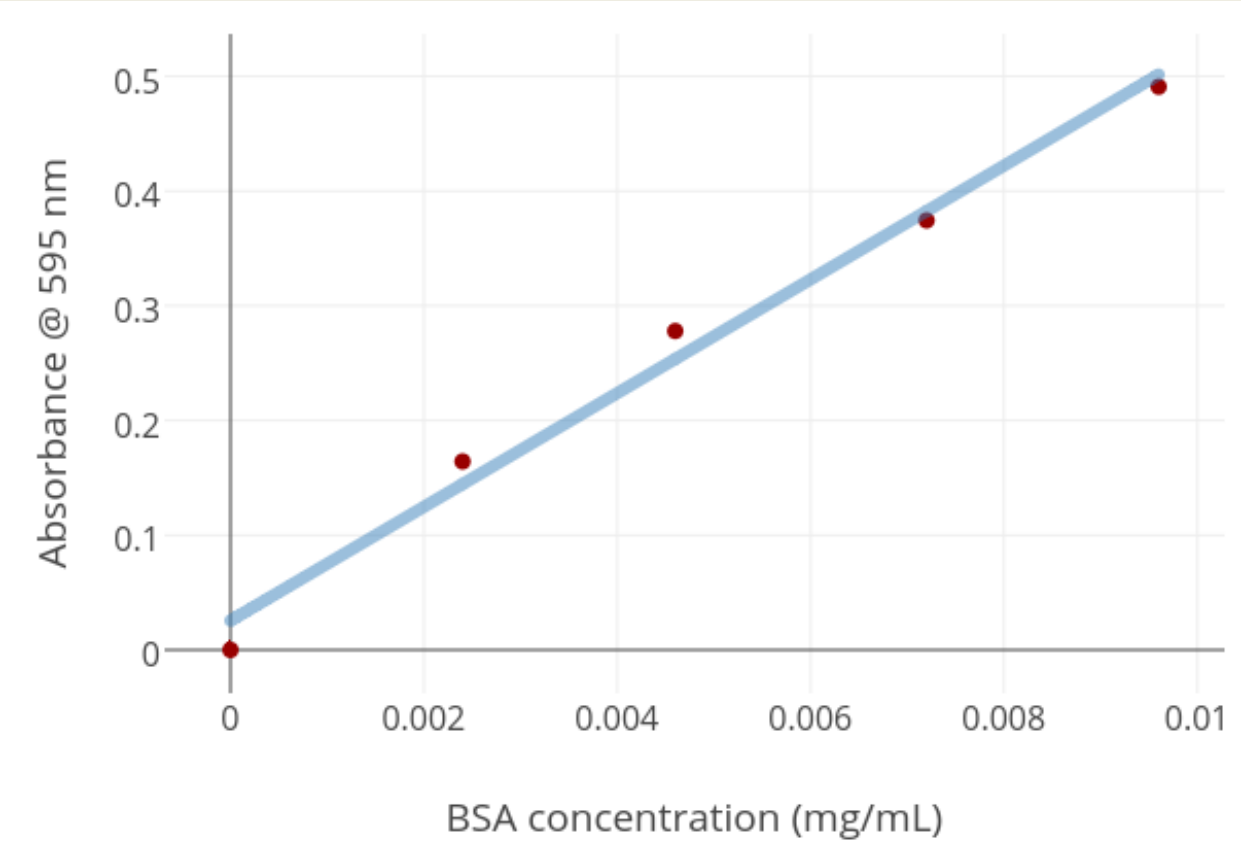

Figure: Calibration curve - generated from BSA samples measured at 595nm
Source: Wikipedia

Vocabulary

Using the previous documents, fill in the blanks:

French	English
	lab experiments
	wavelength
une mesure	
un essai	
un échantillon	
un colorant	
	(protein) bound dye
	the process
absorbance	
une courbe d'étalonnage	
	reagent

Presentation

Prepare a short 2min presentation :

Using the knowledge acquired in chemistry class and document 3, recap the procedure carried out during spectrophotometry measurements?

_____
_____
_____
_____

Questions

At which wavelength were measurements carried out on BVA protein? Explain.

_____
_____
_____

According to **document 2**, how was this value chosen?

_____
_____
_____
_____
_____

An unknown sample has an absorbance of A = 0,3, work out the BSA concentration in the sample

_____
_____

Activity summary

What you must remember:

- vocabulary associated with spectrophotometry mesasurements
- how to explain spectrophotometry measurements
- how to calculate a concentration using a calibration curves

Skills linked to the curriculum:

Compétences	Capacités à maitriser
– COM – APP	– Savoir expliquer un protocole
– APP – ANA	– Savoir exploiter une courbe d'étalonnage