

Experiment 3 on protein determination by the Bradford method.

①

• protein: BSA

#8 i) Calibration curves: 7 solutions of SPS (Standard protein solution) of increasing concentrations

e.g. soln no. 3

Bradford reagent: 2.00 mL

dH₂O: 0.80 mL

200 µg/mL SPS: 0.20 mL

~~init~~ $V_i = 0.20$ mL

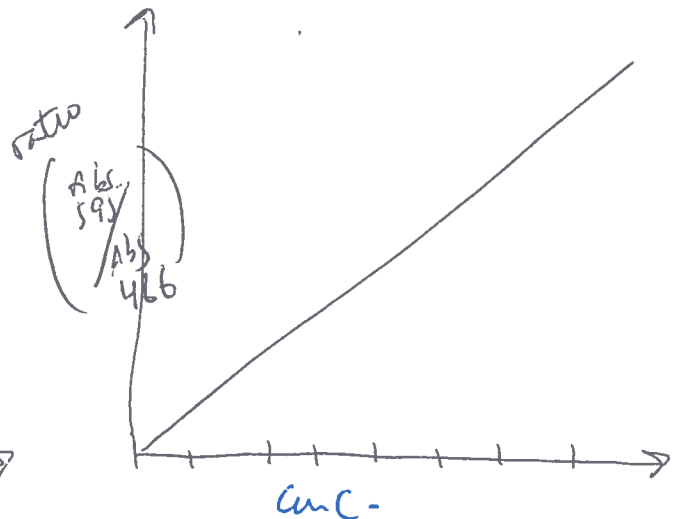
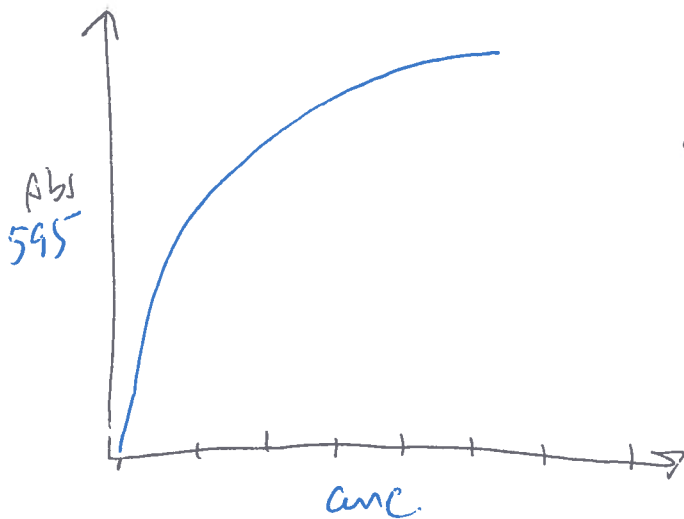
$V_f = 3.00$ mL

$$DF = \frac{3}{0.2} = 15$$

$$\text{new conc} = \frac{200 \mu\text{g/mL}}{15} = 13.33 \mu\text{g/mL}$$

#9 note: for each solution you make 2 measurements, at 466 and 595 nm

You get 2 cal. curves:



(L) BSA unknown (#10)

(2)

1 mL BSA stock solution

from this, 100 μ L (V_1)

are mixed with 4900 μ L of H_2O

$$D.f. = \frac{5000}{100} = 50$$

#11

solution to be measured for unknown:

1 mL of solution above

+ 2 mL of Bradford reagent

$$D.f. = \frac{3}{1} = 3.$$

measure abs. at 466 and 495 nm.

#12

re-measure all solutions (including blank and calibrator)
in single λ mode at 595 nm

