Bradford assay: standard quantitative method determination

The Bradford assay is a standard quantitative method for the determination of protein concentrations. Bradford reagent used in the assay contains Coomassie Blue which produces a characteristic blue colour upon binding to proteins in solution (Bradford, Anal. Biochem. 72: 248, 1976).. Using a spectrophotometer the absorbance of the coloured product can be determined. Analysis of multiple samples may involve the use of a microtitre plate so that samples can be replicated and more reliable results obtained. The use of microtitre plates also automates the process so results are produced very quickly. Samples are pipetted into multi-well plates and the absorbance values measured using a multi-well microtitre plate reader equipped with a 595 nm filter.

Part 1 – Calibration Data for the Bradford Assay

A calibration curve for the Bradford assay in the range 0. 2- 1. 0 mg/ml was obtained using three replicates for each concentration, using a microtitre plate. These data are shown in Figure 1 below. Linear Regression Analysis was performed and the equation of the line of best fit, y= mx +c, was produced (where y represents absorbance, x is the concentration of protein, m is the slope of the line of best fit and c is the intercept of the line on the y-axis).

Q1. Describe the relationship between protein concentration and absorbance for the Bradford assay calibration curve.

Q2. What is meant by a ‘ line of best fit’?

Q3. Comment on the value of R2

Using the values of the equation of the line of best fit, the absorbance value for each standard was used to calculate the actual concentration of protein in each replicate. The mean, standard deviation and coefficient of variation ((mean/SD)x100%) were calculated and are presented in Table 1 below:

Comment on the reproducibility of the assay in the range 0. 2 to 1. 0mg/ml of protein.

Part 2 – Investigation of the effects of detergents on the absorbance of a set concentration of protein, using the Bradford Assay.

Background

You are part of a research team that is looking at the different proteins involved in a cell signalling pathway which leads to the increased synthesis of a specific protein, P. One technique used in this work involves the use of cells kept under tissue culture conditions. These cells are treated with inhibitors, after which they are broken open by solubilisation of their membranes (cell lysis) and the soluble cell contents taken up into solution. Before analysis of the solution for levels of P, the total protein content of each lysate has to be determined. One of the reagents in the buffer solution that can be used to solubilise the cell membranes is a detergent. Detergents can cause interference in a protein assay. In an investigation using the Bradford assay, two detergents at different concentrations were exposed to a set concentration of protein (0. 5mg/ml). These are sodium dodecyl sulphate (SDS) and Triton X. The absorbance values were converted into protein concentrations using the equation of the line of best fit from the

calibration curve and the data subjected to statistical analysis (One Way ANOVA). Mean data are compared in Tables 2 and 3, and Figures 2 and 3.