Determining concentrations with spectrophotometer

To learn how to make a spectrophotometer accurately and interpret the data recorded to construct a graph and obtain a standard curve using excel.

To learn how mathematical calculations of the absorbance readings and of unknowns to get the standard curve of a concentration value from the curve.

Method:

Refer to Proc 2048 Biochemical Engineering Lab Manual

Exercise 1- The Spectrophotometer

Absorbance readings of Methyl Orange and Bormophenol Blue were recorded for a range of wavelengths from 400 to 700nm intervals, zeroing the apparatus with a distilled water blank after each change in wavelength.

Exercise 2: Determination of Glucose Concentration

Each of the standard glucose solutions and the unknown solutions were tested in spectrophotometer using a wavelength of 580nm and absorbance readings were taken for each.

Exercise 3- Determination of Yeast concentration

Similarly to the glucose experiment absorbance readings of different standard and unknown yeast concentrations were recorded at a wavelength of 600nm. Unknown solutions U and V were also diluted to a 1: 2 ratio with distilled water as the concentrations are too high and fall outside the acceptable absorbance range.

Calculations:

Unknown glucose calculations

y = 0. 1836x – 0. 008, Solve for x gives

Subbing in absorbance values for each unknown glucose solution gives:

Unknown yeast calculations

y = 0. 557x + 0. 003 Solve for x gives

Subbing in absorbance values for each unknown glucose solution gives:

U and V need to be multiplied by 2 after calculation as they were diluted in a 1: 2 dilution

Discussion:

In exercise 1 different wavelengths was used and the absorbance in the graph increasing then decreases and then slightly increasing. The colour contributes to where the max absorbance occurs and the concentration affects the intensity of the peaks. For instant the parts blue and the parts yellow light is a green colour. This is the light that we see, and therefore the wavelengths of light to pass through the absorbance with the minimum in terms of all other waves of light absorbance higher.

Spectrum analysis of pure sugar solution would be impossible for any absorbance that can happen is the solution to be transparent and any suspended particles. This means that solutions need glucose to the reaction with 1 ml of 3. 5 acid Dinitrosalicylic (Domain Name System) to form amino 3, 5 – Nitrosalicylic acid, a compound color absorbs light strongly in all parts

of 580nm. This enables us to use spectral analysis to determine the focus. This applies only if the intensity of the color of the product is directly proportional to the concentration of the reactants. In this case, glucose concentration is directly proportional to the amount of amino – 3, 5 – Nitrosalicylic acid producers such as the Stoichiometry of the reaction is 1: 01, and most of this focus is not to reach a balance It is important that the blank or zero concentration used for this experiment is not just distilled water but 1ml of DNS and distilled water made up to the same volume as the other samples, as the unreacted DNS in our glucose solutions is contributing to the colour of the solution as well as the 3-Amino, 5-Nitrosalicylic Acid.

In exercises 3 yeast is basic in terms of chemistry is based on the physics. By increasing the concentration, the absorbance will increases in yeasts solutions however they are not coloured but they are block and scatter, so some the light will not go through them. And this because we are dealing with suspended particulate matter, and not resolved ions. Blot out the light commensurate with the focus so that we can find the concentrations of unknown values of absorbance. It is important to shake well before taking the sample absorbance reading such as yeast, particles and settle to the bottom, that mean if we are not shaken them, so they will give us a lower absorbance reading.

Questions

Exercise 2

The cuvettes have different surfaces for two reasons. The frosted ridged sides are so no light escapes out the sides of the cuvette giving a false

reading. The other reason they have 2 different sides is so that you don’t handle the transparent sides directly as oils or dirt from your fingers could increase the absorbance and give inaccurate results

Particles in solution (just like in the yeast experiment) affect the absorption reading by blocking or deflecting light away from the detector therefore the particles in a coloured would increase the absorbance and give inaccurate results, unless the concentration and size of the particles is constant with all tests conducted then it would not affect the calculated results.

A standard curve in spectrophotometric analysis is a linear trendline that fits through your experimental data. It is calculated by measuring absorbance readings at a range of different concentrations and plotting them against each other. A linear regression done my excel or other means is calculated for the points and an equation in terms of absorbance (Y) and concentration (X) is formed and you can use this equation to calculate unknown concentrations from absorbance readings.

Exercise 3

The cuvettes have different surfaces for two reasons. The frosted ridged sides are so no light escapes out the sides of the cuvette giving a false reading. The other reason they have 2 different sides is so that you don’t handle the transparent sides directly as oils or dirt from your fingers could increase the absorbance and give inaccurate results

Firstly dilute the dye to an adequate concentration with distilled water . Find the maximum absorbance of the diluted dye by testing absorbance’s at a

range of different wavelengths ensuring you zero with distilled between each wavelength. Take some of the dye and dilute it with distilled water to about 6 – 8 different concentrations i. e. 1: 100 ïƒ 1: 10, depending on what absorbance readings you get adjust the dilutions to fit in a range of 0 ïƒ 0. 8 as that is where the Beer-Lambert Law applies. Using the max absorption wavelength you would then prepare a standard curve for the dye by measuring absorbance of each of the diluted concentrations. Plot the absorbance vs concentration and use a linear regression to form an equation. Take a sample of the waste water and filter off any suspended particles to increase the accuracy of the absorbance reading. Finally measure the absorbance of the sample diluting accordingly if absorbance is not in the required range. Using this absorbance value in your standard curve equation calculate the concentration and multiply by your dilution factor if the sample was diluted.

Assuming you already have a standard curve and equation for the nitrate complex for a range of 0 ïƒ 1. 5 mg/L. All you need to do is dilute your sample from your suspected 55 mg/L to fall in the concentration range of 0 ïƒ 1. 5 mg/L. So a 1: 50 or a 1: 100 dilution would be enough to give you 1. 1 mg/L or 0. 55 mg/L respectively. Then the sample is ready for spectrophotometer analysis.

Conclusion:

All of the yeast and sugar were obtaining accurate test results with the value of R2 (0. 99) for each of the trend lines and graphs. 99% accuracy meaning that lines fits for most the points. The process was a good knowledge to

learn and it is useful to understand the Beer-Lambert law and his applications.