Wavelength functions of a spectrophotometer

The graph of absorption spectrum which is the graph of absorbance against corresponding wavelength plotted is a bell shape. From the graph, it can be seen that the absorbance readings increases from 0. 091 to 0. 919 as the wavelength increases from 470nm to 590nm. However, the absorbance readings show a decrease as the wavelengths continue to increase to 680nm. The graph shows maximum absorbance reading at wavelength of 590nm as indicated by the peak of the curve. This implies that the maximum absorption of light of bromophenol blue occur in wavelength of 590nm.

Part 2

Amax of bromophenol blue, which is at 590 nm is set in the spectrophotometer to determined the absorbance readings of different concentration of bromophenol blue. A standard concentration curve of absorbance versus concentration of bromophenol blue is plotted. The graph obtained is a straight lined graph. This indicated that the absorbance of bromophenol blue is directly proportional to the concentration of bromophenol blue. Therefore, the graph is said to obey the Beer-Lambert Law where A= Î» bc.

However, the line of the standard concentration curve of absorbance versus concentration of bromophenol blue does not pass through origin. This shows that random error might occur during the experiment as the graph should pass through origin since the first reading of the absorbance was distilled water. Distilled water should shows zero absorbance reading. The random error that might occur was that traces of chemical were present in the cuvette thus contaminating the distilled water which in turn giving the

distilled water an absorbance reading. This random error can be reduced by rinsing the cuvette with distilled water before use.

Part 3

The absorbance of the two bromophenol blue solutions (Tube A & B) of unknown concentration at the Amax of bromophenol blue (590nm) were measured. The concentrations of the two unknown solutions were determined using two methods. The first method used was interpolating the graph 2 while the second method used was using the formula of Beer-Lambert Law to calculate the concentrations of the two unknowns.

From the first method, the concentration for the Tube A bromophenol blue is 3. 65 mg/L while the concentration of Tube B bromophenol blue is 1. 40 mg/L. While, from the second method using the Beer-Lambert Law, A = Î» bc, the concentration of Tube A bromophenol blue obtained is 3. 833 mg/L while the concentration of Tube B bromophenol blue is 1. 627 mg/L. Concentration result for each unknown obtained using two different methods mention above give different result. The difference in results might due to random errors that occur in the experiment. The random error that might occur was that the surface of the cuvette was not clear. Fingerprints might be left on the surface of the cuvette thus affecting the absorbance reading of bromophenol blue. This random error can be reduced by ensuring that the surface of the cuvette was wiped with paper towel before placing it into the spectrophotometer. Furthermore, the orientation of the cuvette may be inserted wrongly into the spectrophotometer when the absorbance reading is taken.

Part 4

The absorbance of bromophenol blue solutions in tube 1-6 from Part 2 is measured at Amax of methyl orange (460nm), while the absorbance of different concentration of methyl orange solutions were also measured at Amax of methyl orange (460nm) and Amax of bromophenol blue (590nm). According to the results, all the graph of standard concentration curve shows straight line. This indicates that the absorbance of bromophenol blue and methyl orange is directly proportional to the concentration of bromophenol blue and concentration of methyl orange respectively. Hence, the law of Beer-Lambert is obeyed.

However, the standard concentration curve of bromophenol blue in Amax of methyl orange (460nm) shows scattered points. These results might be due to random error such as poor pipetting technique that can cause inaccuracy in the absorbance of bromophenol blue. Besides that, the wavelength used to measure the absorbance of the bromophenol blue was 460nm which was not the Amax of bromophenol blue can also affect the absorbance reading of bromophenol blue. The choice of wavelength for quantitative analysis of a particular solution is very vital as the choice of wavelength will affect the sensitivity of analysis and whether or not Beer’s Law is obeyed.

The concentration of bromophenol blue and methyl orange in the two mixture solutions of tube C was determined using the formula, Atotal = K1C1+K2C2. By solving simultaneous equation, the concentration of bromophenol blue in the mixture C was 7. 158 mg/L and the concentration for methyl orange in the mixture was found to be 3. 706 mg/L.

Conclusion

Part 1, the Amax of bromophenol blue is at the wavelength of 590nm.

Part 2, the absorbance of bromophenol blue is directly proportional to the concentration of bromophenol blue as the standard concentration curve of absorbance versus concentration of bromophenol shows a straight line.

Part 3, two methods were used to determined the concentration of the two unknowns (tube A & B). By using interpolation of the graph, the concentration of bromophenol blue in Tube A is 3. 65 mg/L and the concentration of bromophenol blue in Tube B is 1. 40 mg/L. While by using the formula of Beer – Lambert Law, the concentration of bromophenol blue in Tube A is 3. 833 mg/L and the concentration of bromophenol blue in Tube B is 1. 627 mg/L.

Part 4, the molar absorbtivity coefficient of methyl orange in Amax of bromophenol blue is 0. 005L mg-1cm-1 and in Amax of methyl orange is 0. 0743 L mg-1cm-1. The concentration of bromophenol blue in Tube C is 7. 158 mg/L and the concentration of methyl orange in Tube C is 3. 706 mg/L.