Lab sheet #1

-Scanning spectrophotometry and spectrophotometric determination of concentration-

-Objectives:

- Drawing the absorption spectrum of bromophenol blue and determination of λ_{max}.
- Determination the concentration for unknown concentration of bromophenol blue using a standard curve and Beer-Lambert Law.

A. Absorption spectrum:

-Method:

1. Take a test tube and add the following reagents:

Reagent	Volume (ml)
0.1 M citrate buffer, pH 2.4	9.0
7.5 x 10^{-4} M bromophenol blue	0.2
95% ethanol	0.8

2. Mix and measure the absorbance of the solution from 320 to 620 nm at 20 nm intervals against a wat/er blank. Use quartz cuvettes. (Use a scanning spectrophotometer to measure the absorbance if available).

-Results:

Wavelength (nm)	Absorbance
320	
340	
360	
380	
400	
420	
440	
460	
480	
500	
520	
540	
560	
580	
600	
620	

1. Record the absorbance in the table.

2. Using Excel, plot a graph of absorbance against wavelength (absorption spectrum curve), then determine the λ_{max} for bromophenol blue at pH 2.4.

3. From the graph, λ_{max} for bromophenol blue at pH 2.4 = _____ nm.

Related questions:

1. What type of cuvettes you used? Why?

    _____

2. If you change the pH to 7, do you think you will get the same absorption spectra? Why?

    _____

3. How you will determine the λ_{max} ?

    _____

B. Determination of concentration of unknown:

1. Standard curve and determination of concentration of unknown:

-Method:

1. Set up 7 test tubes as following:

Tube No.	0.1 M Citrate buffer [pH 2.4] (ml)	7.5x10^{-4} M Bromophenol blue (ml)	95% Ethanol (ml)	Unknown sample (ml)
1	9	0.1	0.9	-
2	9	0.2	0.8	-
3	9	0.4	0.6	-
4	9	0.6	0.4	-
5	9	0.8	0.2	-
6	9	1.0	-	-
Unknown	9	-	-	1.0

2. Mix and measure the absorbance of all the tubes at 430 nm (why?) against a distal water blank.

-Results:

1. Record the absorbance in the table below:

Tube No.	Molar concentration of bromophenol blue x 10^{-5}	Absorbance at 430 nm
1		
2		
3		
4		
5		
6		
Unknown	-From the standard curve-	

2. Calculate the concentration of the standard solutions (1-6) using C1 x V1 = C2 x V2 formula.

3. Plot a standard curve of absorbance against molar concentration of bromophenol blue using Excel.

4. From the curve determine the molar concentration of the unknown solution of bromophenol blue (using TREND formula).

2. Determination of concentration of unknown by Beer-Lambert law:

1. Using any tube of the standard solutions (1-6), calculate the molar extinction coefficient (ε) of bromophenol blue with a pH 2.4:

_____

_____

2. Calculate the molar concentration (c) of unknown bromophenol blue sample using Beer-Lambert law:

_____

_____

Related questions:

1. What is the relationship between the absorbance at 430 nm and bromophenol blue concentration?

_____

2. Which method (2.1 or 2.2) is more accurate for determination the unknown concentration? Why?

_____