**Lab #1,[BCH 333]**

**Method:**

1. **Absorption Spectrum:**
2. 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 |

1. Mix and measure the absorbance of the solution from 320 to 620 nm at 20 nm intervals against a water blank. Remember to zero the instrument at each wavelength setting.
2. Use a scanning spectrophotometer to measure the absorbance. [And suitable cuvettes].

**Results:**

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

-Plot a graph of absorbance against wavelength (absorption spectrum) From the graph or spectrum from a scanning spectrophotometer determine λmax for bromophenol blue at pH 2.4

λmax for bromophenol blue at pH 2.4 =……………. nm.

**2. Standard curve**

a) Set up 8 test tubes as follows:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Tube No.** | **0.1 M citrate buffer [pH 2.4] (ml)** | **7.5x10 - 4M bromophenol blue (ml)** | **95% ethanol (ml)** | **Unknown**  **sample**  **(ml)** | **Molar concentration of**  **bromophenol blue x 10 - 4** |
| **1** | 9 . 0 | 0.05 | 0.95 | - | 0.38 |
| **2** | 9 . 0 | 0.1 | 0 . 9 | - | 0.75 |
| **3** | 9 . 0 | 0.2 | 0.8 | - | 1.5 |
| **4** | 9 . 0 | 0.4 | 0.6 | - | 3 |
| **5** | 9 . 0 | 0.6 | 0.4 | - | 4.5 |
| **6** | 9 . 0 | 0.8 | 0.2 | - | 6 |
| **7** | 9 . 0 | 1.0 | - | - | 7.5 |
| **8 [unknown]** | 9 . 0 | - | - | 1.0 | ? |

b) Mix and measure the absorbance of all the tubes at 430 nm against a water blank.

**Results:**

a-Record the absorbance in the table below:

|  |  |  |
| --- | --- | --- |
| **Tube No.** | **Molar concentration of**  **bromophenol blue x 10 - 4** | **Absorbance at 430 nm** |
| **1** | 0.38 |  |
| **2** | 0.75 |  |
| **3** | 1.5 |  |
| **4** | 3 |  |
| **5** | 4.5 |  |
| **6** | 6 |  |
| **7** | 7.5 |  |
| **8[unknown]** | ?............. |  |

b-Plot a standard curve of absorbance against Molar concentration of bromophenol blue x10 **– 4**

c-From the curve determine the molar concentration of the unknown (tube 8)

Molar concentration of bromophenol blue in unknown =………………… x 10 – 4

**Calculations:**

-Calculate the concentration of the unknown also from the absorbance of one standard, tube 3.

i) As = εlCs for tube 3

ii) Au = εlCu for tube 8 ( s = standard, u = unknown)

Cu = CsAu/ As

Calculated molar concentration of bromophenol blue in unknown=………….

- Calculation of molar extinction coefficient:

Calculate the molar extinction coefficient by using absorbance and molarity values from tubes 4 and 5,

* A4 = εlC4

Molar extinction coefficient for tube 4 = A4/lC4

= A4/ 1x 3x10-4

=………. M-1 cm-1

* A5 = εlC5

Molar extinction coefficient for tube 5 = A5/lC5

= A5/ 1x 4.5 x 10-4

=……….. M-1 cm-1

Average molar coefficient of bromophenol blue at 430 nm, pH 2.4

=……….. M-1 cm-1