**BCH 333**

**Lab 1**

**Absorption spectrum and spectrophotometric determination of concentration**

**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 - 5 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, using a scanning spectrophotometer, against a water blank. Remember to zero the instrument at each wavelength setting.

Note: Use suitable cuvettes at sets of wavelengths .

**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 curve), From the graph or spectrum 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.** | **7.5x10 - 5M bromophenol blue (ml) [std.]** | **95% ethanol (ml)** | **0.1 M citrate buffer [pH 2.4] (ml)** | **Unknown**  **sample**  **(ml)** |
| **1** | 0.1 | 0 . 9 | 9 . 0 | - |
| **2** | 0.2 | 0.8 | 9 . 0 | - |
| **3** | 0.4 | 0.6 | 9 . 0 | - |
| **4** | 0.6 | 0.4 | 9 . 0 | - |
| **5** | 0.8 | 0.2 | 9 . 0 | - |
| **6** | 1.0 | - | 9 . 0 | - |
| **7 [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 - 6** | **Absorbance at 430 nm** |
| **1** | 0.75 |  |
| **2** | 1.5 |  |
| **3** | 3 |  |
| **4** | 4.5 |  |
| **5** | 6 |  |
| **6** | 7.5 |  |
| **7[unknown]** | ................... |  |

b-Plot a standard curve of absorbance against Molar concentration of bromophenol blue x10 **– 6.**

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

- Molar concentration of bromophenol blue in unknown sample (tube 7).=………………… x 10 – 6

**Calculations:**

-Calculate the concentration of the unknown [tube 7]using the given information corresponding to tube4.

1st Calculation of molar extinction coefficient:

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2nd Calculated molar concentration of bromophenol blue in unknown "tube 7" using calculated extinction coefficient.

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