

# Uv of sodium aminosalicylate biology essay



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Specific absorbance of the sodium aminosalicylate can be determined by using the Beer-Lambert law equation with the presence of the value of concentration of the sodium aminosalicylate and the absorbance values which can be obtained through UV spectrophotometry. The concentration of 'Unknown' solutions can also be determined by getting the absorbance values through the calibration graph, absolute method and comparative method.

## **Introduction**

UV spectrophotometry is a technique which is based on measuring the absorption of near-UV or visible radiation by molecules. [1] It acts by where the electrons in the bonds within the molecule become excited to reach up to a higher quantum state and in the process to absorb some of the energy passing through the solution. The more loosely the electrons are held within the bonds of the molecules, the longer will the wavelength of the radiation (lower the energy) be absorbed. [2] Radiation in this wavelength region causes the characteristics of the molecular structure of the molecule to be determined. [1]

As there is a broad absorption bands due to different vibrational and rotational energy levels, UV spectrophotometry is used mainly for quantitative purposes rather than identification.[3] Spectrophotometer works by where the sample of the solution is inserted into the spectrometer for the UV/visible light to pass through the sample to get the value of absorbance/transmittance. The value of the absorbance indicates the amount of light being absorbed by the sample. The measurement of light absorption of molecules can be governed by Beer-Lambert law equation,  $A =$

e. c. l. With the presence of the absorbance value (A), concentration of the sample (c) and the pathlength of the cell (l), the molar absorptivity ( $\epsilon$ ) can therefore be determined.

Concentration of an unknown solution can also be determined by measuring its absorbance using the UV spectrophotometry at the selected wavelength. The determination of the concentration of the unknown can be determined by using three methods, which are by using the Beer-Lambert graph, comparative method and absolute method.

In this experiment, sodium aminosalicylate will be used as the sample as it is a good absorber of ultraviolet light which allows it to be conveniently to be analysed using spectrophotometry. The purpose of this experiment is to find the specific absorbance of the sodium aminosalicylate solution by using different concentrations of it and also to determine the concentration of the unknown solutions by using calibration graph of Beer-Lambert, comparative method and absolute method.

## **Experimental**

### (a) Absorption Spectrum of Sodium Aminosalicylate in 0. 1M NaOH

The spectrum of a 1-cm layer of 0. 001% solution of sodium aminosalicylate in 0. 1M NaOH over the wavelength range 235 to 325nm was been scanned and examined. The wavelengths ( $\lambda_{max}$ ) and the absorbance values at these wavelengths of the two maxima was then been measured from the spectrum. Approximate specific absorbance [i. e. the absorbance of a 1-cm layer of a 1% solution,  $A(1\%, 1cm)$ ] was then been calculated at each of these maxima using the Beer-Lambert equation ;

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$$A = A(1\%, 1\text{cm}) \cdot c \cdot l$$

Where A = Absorbance

$A(1\%, 1\text{cm})$  = Specific absorbance of a 1 cm layer of a 1% solution

c = Concentration (%)

l = path length (cm)

#### (b) Beer's Law, Specific Absorbance and Molar Absorptivity

50mL volumes of 0.0002, 0.0004, 0.0006 and 0.008 % solutions of sodium aminosalicylate in 0.1M NaOH from the 0.0010 % stock solution had been prepared. One of the  $\lambda_{\text{max}}$  from (a) had been selected and the wavelength was set to the selected value. The solvent (0.1M NaOH) was been placed in both cells and the absorbance reading of the single beam spectrophotometer was been set to zero. The absorbance was been checked a few nm each side of the selected wavelength using the 0.0010 % and been recorded. The new  $\lambda_{\text{max}}$  from the table of absorbance values that had been recorded was been selected and the spectrophotometer was been set to that wavelength. The absorbance of a 1-cm layer of each of the five solutions of sodium aminosalicylate that had been prepared earlier were been recorded and replicate readings were been obtained. A graph of absorbance against concentration had been plotted and the specific absorbance had been determined using the gradient of the graph. The molar absorptivity of the sodium aminosalicylate in 0.1M NaOH at the selected maximum was been calculated.

#### (c) Determination of Concentrations

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### (i) Calibration Graph

The absorbance of a 1-cm layer of ' Unknown 1' was been determined. ' Unknown 1' was then been diluted so that its absorbance is in the mid range of the graph of absorbance against concentration. The graph was then been used to determine the concentration of the diluted solution and hence the concentration of ' Unknown 1' was been calculated.

### (ii) Absolute Method

The absorbance of a 1-cm layer of ' Unknown 2' was been recorded. The concentration of ' Unknown 2' was been calculated using the A (1%. 1cm) value which had been determined previously.

### (iii) Comparative Method

The wavelength was set to the second  $\lambda_{max}$  which had been determined in (a). The absorbance of a 1-cm layer of the 0. 0010 % and a 1-cm layer of ' Unknown 2' was been recorded. The concentration of ' Unknown 2', C2, using the relationship below,

=

## Results

(a)

Wavelength / nm

Absorbance Reading

264

0. 630

300

0. 430

Wavelengths ( $\lambda_{\max}$ ) = 264nm and 300nm

Concentration) = 0. 001 %

**At 264nm,**

Specific Absorbance =

=

= **630**

**At 300nm,**

Specific Absorbance =

=

= **430**

(b)

$\lambda_{\max}$  which been selected from (a) = 264nm

**Wavelength / nm**

**Absorbance Reading**

259

0. 584

260

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0.588

261

0.588

262

0.582

263

0.573

264

0.559

265

0.546

### **Table of Absorbance Values**

New  $\lambda_{\text{max}}$  selected from table of absorbance = 261nm

### **Concentration of sodium aminosalicylate solution / %**

#### **First Absorbance Reading**

#### **Second Absorbance Reading**

#### **Average Absorbance Reading**

0.001

0.608

0.606

0.607

0.0008

0.477

0.482

0.480

0.0006

0.360

0.364

0.362

0.0004

0.243

0.243

0.243

0.0002

0.119

0.123



0. 121

**Specific Absorbance (Gradient) =**

**= 606. 06**

From the graph of absorbance reading of 0. 46,

Concentration of Sodium Aminosalicylate = 0. 00076 %

RMM of sodium aminosalicylate = 211. 15

**0. 00076 % = 0. 00076 g/100mL**

**= 0. 0076 g/1000mL**

**= 0. 000036 mol/1000mL**

**= 0. 000036M**

By using the Beer Lambert equation,  $A = e \cdot c \cdot l$ ,

**e =**

**=**

**= 12777. 78**

**Molar absorptivity (e) of sodium aminosalicylate in 0. 1M NaOH at the selected maximum = 12777. 78**

(c) (i)

## Type of Solution

### First Absorbance Reading

### Second Absorbance Reading

### Average Absorbance Reading

Unknown 1

1. 187

1. 188

1. 188

Diluted Unknown 1 (Unknown 1 +  $\frac{3}{4}$  0. 1M NaOH)

0. 286

0. 287

0. 287

**Concentration of Diluted ‘ Unknown 1’ Solution from the graph = 0. 00048 %**

Using the formula  $C_1V_1 = C_2V_2$  ,

$$(0. 00048) (100) = (C_2) (25)$$

**$C_2$  (Concentration of Unknown 1) = 0. 00192 %**

(ii)

**Type of Solution****First Absorbance Reading****Second Absorbance Reading****Average Absorbance Reading**

Unknown 2

0.525

0.525

0.525

Using Beer-Lambert equation,  $A = A(1\%, 1\text{cm}) \cdot c \cdot l$

$A(1\%, 1\text{cm}) = 606.06$

$l = 1.00$

**Concentration of ‘Unknown 2’ (c) =**

**= 0.000866 %**

(iii)

Wavelength ( $\lambda_{\text{max}}$ ) selected from (a) = 300nm

**Type of Solution****First Absorbance Reading****Second Absorbance Reading****Average Absorbance Reading**

0.0010 % sodium aminosalicylate

0.337

0.336

0.337

'Unknown 2'

0.300

0.300

0.300

=

**C2 (Concentration of 'Unknown 2') = 0.000890 %**

## **Discussion**

From the experiment (a), we can see that the absorbance reading decreases with the increase of the wavelength where 0.630 absorbance value had been recorded at 264nm wavelength and 0.430 absorbance at 300nm wavelength. When wavelength increases, the amount of energy of the molecules of sodium aminosalicylate will decrease as well. Therefore, the amount of light that will be absorbed by the sodium aminosalicylate will decrease which resulting in the decrease in the value of the absorbance. By using the Beer-Lambert law equation, we can know that the specific absorbance of the sample decreases with the decrease of the absorbance value with a fixed concentration of sodium aminosalicylate and pathlength of the cell.

In experiment (b), new  $\lambda_{\text{max}}$  had been selected from the table of absorbance which is 261nm because it had the highest absorbance reading among all values which had been obtained. Repeated readings of absorbance had been recorded when measuring the absorbance values of each of the different concentrations of sodium aminosalicylate. This is to prevent getting an error in the measurement of the absorbance reading in the experiment. When plotting the graph of average absorbance against concentration of sodium aminosalicylate, a linear line was obtained. This shows that the value of absorbance increases with the increase of the concentration of sodium aminosalicylate in the experiment. The value of specific absorbance that had been obtained from the gradient of the graph of average absorbance against concentration of sodium aminosalicylate is 606.06. The value is quite typical which is in the range of 20-1,000. However, the value of the molar absorptivity of the sodium aminosalicylate obtained from the graph is 12777.78 which is more than the typical values ranging from 1,000-100,000. This may be due to some errors produced during the experiment.

In experiment (c)(i), the concentration of 'Unknown 1' obtained from the spectroscopy and calibrating graph are almost the same. However, the concentration of 'Unknown 1' obtained through the calibrating graph is slightly higher than that of the one obtained from spectroscopy. This might be due to some errors in calibrating the graph. Error in the graph will result in getting an incorrect absorbance reading which will be used to calculate the concentration of the unknown solution. However, several measurements can be recorded to minimize the errors.

In experiment (c)(ii) and (iii), the results showed that the concentration of 'Unknown 2' obtained using absolute method is almost the same with the value of concentration obtained using comparative method. Absolute method can be carried if the value of specific absorbance is known with the presence of the value of absorbance. It works exactly based on the Beer-Lambert law equation and is a preferred method in BP. If the specific absorbance of the solution is not known, then comparative method is preferably to be used as it only needs the absorbance reading of the standard solution and the unknown solution. This is a preferred method in USP and works the best if the standard and sample concentrations are close.[3] As the specific absorbance of sodium aminosalicylate used in (c)(ii) is obtained from experiment (b), there might be some errors in the value of the concentration of 'Unknown 2' if errors had occurred when calibrating the graph in (b) which resulting in the value of specific absorbance to be not accurate. Compared to comparative method, only the absorbance reading of the standard and sample solutions obtained from the spectroscopy are required in order to calculate the concentration of 'Unknown 2'. Therefore, in this case the concentration of 'Unknown 2' using absolute method is not as accurate as the one obtained by using comparative method in this experiment.

## **Conclusion**

UV spectrophotometry is an universal technique where it can find the value of specific absorbance of a sample using the Beer-Lambert equation with the presence of the other 3 factors value and also to identify the concentration

of an unknown either through methods of calibration, absolute and comparative.