

Evaluation of three different types of spectroscopy - lab report example

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Evaluation of Three Different Types of Spectroscopy

Laboratory Report: Evaluation of Three Different Types of Spectroscopy

INTRODUCTION PRACTICAL Atomic Absorption Spectroscopy

Atomic Absorption Spectroscopy (AAS) is an analytical technique used to measure the concentration of atoms of elements. Ground state atoms absorb light energy of a specific wavelength hence causing these atoms to become excited. There is a direct proportion between the number of atoms and amount of light absorbed, therefore, we can determine the concentration of the sample by measuring the amount of light absorbed (Karabegov, 2011). Samples have to be heated to extremely high temperatures in order to atomise them. The sample goes through a nebulizer which removes solvents to produce aerosol. The sample is then heated via flame, plasma or electricity; this causes desolvation, liquefaction, vaporization and finally atomization (Sagi & Rathnam, 2013).

There are two basic types of atomic absorption instruments: single-beam and double-beam.

Figure 1: Single beam Atomic Absorption Spectrometer.

Components include; a) The light source which is usually a hollow cathode lamp. Atoms of different elements absorb specific wavelengths of light therefore in order to analyse a sample for a specific element, the light source used must correspond to the wavelength of that element. b) A compartment in which samples are atomised via (flame, graphite furnace, MHS cell, FIAS cell, FIMS cell). c) A monochromator that disperses the light. d) A detector, which measures the light intensity and amplifies the signal. e) A display that shows the reading.

Figure 2: Double-Beam Atomic Absorption Spectra.

It contains the same components as figure 1. The difference being that the light source is divided into two, one for the reference cell and the other for the sample cell.

AAS has various uses such as analysis of bodily fluids like blood and urine for heavy metal poisoning and monitoring of the environment by measuring levels of certain elements in lakes, food like fish and the air (Karabegov, 2011).

PRACTICAL 2

Fluorescence Spectroscopy

Fluorescence is a photon emission process. Absorption of light by some molecules causes movement of electrons from the ground state to an excited state. This excited molecule will return to a lower energy level by dispersing part of its energy. It will then return to the ground state by emission of a photon (fluorescence). As a result of this loss of energy, Stokes shift is observed. Stokes shift is when there is a difference in wavelength of the absorption and emission spectra (Ge, et al., 2010)

Only molecules containing a functional group which has the ability to absorb energy of a certain wavelength and re-emit it at a different but specific wavelength (fluorophore) will exhibit fluorescence (Ge, et al., 2010)

Figure 3: Shows the essential components of a fluorescence spectrometer

a) The light source, an example of the first type used is the tungsten-halogen lamp and later on, mercury lamps were used. However, now xenon lamps are mainly used. b) A Monochromator used to select both the excitation and emission wavelength. c) Sample holders, the cuvette is placed in the

direction of the incident beam. Resulting in fluorescence being given off equally in all directions. The fluorescence emission is collected at right angle to the incident beam or in-line with the incident beam. Some instruments allow the user to choose which collection method is used. d) Detectors which are usually photomultiplier tubes (Lakowicz, 2007).

UV-VIS Spectroscopy

When light is absorbed by substances, electrons are propelled from the ground state to the excited state. The transition of these electrons is associated with a specific amount of energy. The energy from the highest molecular orbital (excited state) to the lowest unoccupied molecular orbital (ground state) usually corresponds to the ultraviolet (UV) or visible (VIS) region of the electromagnetic spectrum with wavelengths between 100nm and 800nm. UV-VIS Spectroscopy is the measure of the amount of visible or ultraviolet radiation absorbed by a substance in solution (Amendola & Meneghetti, 2009).

Organic molecules that absorb UV-VIS radiation usually do so due to the presence of a functional group called chromophores.

Figure 4: Shows how the UV-VIS Spectrometer works.

A hydrogen or deuterium discharge lamp is used for ultraviolet spectra while a tungsten filament is used for the visible spectra. Radiation of the same frequency and intensity is passed through the reference and sample cells. A photomultiplier converts the photons into electrical currents and the spectrometer compares the differences between the current intensity for the reference and sample cells to produce a spectrum graph.

UV-VIS is usually used for analysing substances such as drug metabolite and

trace metal quantification (Forster, 2004).

AIMS

PRACTICAL 1

The aim of this practical was to determine the concentration of Lead (Pb) in blood plasma. This was done by plotting a calibration graph of absorbance at 283.3nm versus concentration of Lead standard (mg/L). An equation was obtained and used to calculate the unknown concentration of the blood plasma. (Note: Instrument used Perkin Elmer AAS 800).

PRACTICAL 2

Fluorescein and Eosin are chemicals used to detect latent blood stain (Goodard, 2014). The aim of this practical was to determine which analytical technique (Fluorescence or UV-VIS Spectroscopy) is more appropriate for detecting fluorescein and eosin. (Note: Instrument used Varian Cary Eclipse for fluorescence and Shimadzu -UV-1650pc for UV-VIS)

MATERIALS AND METHODS

The practical handbook contains the methods and materials used (Goodard, A. 2014).

Changes made:

For analysis of blood plasma using AAS, the total volume used was changed from 20ml to 50ml.

RESULTS

Practical 1: Data for AAS

Calibration standard Lead concentration (mg/L)

Absorbance at 283.3nm (arbitrary units)

Average Absorbance Value

1st Reading

2nd Reading

3rd Reading

0

0.006

0.004

0.006

0.005

1

0.015

0.015

0.015

0.015

10

0.203

0.208

0.205

0.205

20

0.382

0.373

0.378

0.378

40

0.577

0. 583

0. 580

0. 580

Table 1: Absorbance values of Lead calibration standard.

Unknown blood plasma

Absorbance at 283. 3nm (arbitrary units)

Average Absorbance Value

1st Reading

2nd Reading

3rd Reading

Sample1

0. 471

0. 474

0. 478

0. 474

Sample 2

0. 460

0. 473

0. 459

0. 464

Sample 3

0. 481

0. 479

0. 478

0. 479

Table 2: Absorbance values of the unknown blood plasma Lead concentration.

Reference standard Lead concentration (mg/L)

Absorbance at 283.3nm (arbitrary units)

Average Absorbance Value

1st Reading

2nd Reading

3rd Reading

10

0.2034

0.2021

0.2041

0.2032

Table 3: Absorbance of the reference standard concentration (10mg/L).

Figure 5: Calibration graph of absorbance of Lead against Lead standard concentration (mg/L).

The quadratic equation ($y = ax^2 + bx + c$) $y = -0.0002x^2 + 0.0231x - 0.0017$

was used to calculate the concentration of Lead in the blood plasma for each repeat as well as the lead concentration for the reference standard.

Where y = absorbance and x = concentration (mg/L).

Calculations

The quadratic equation obtained from the calibration graph (figure 1) is $y = -0.0002x^2 + 0.0231x - 0.0017$. To calculate the x values of the blood plasma and reference standard, the quadratic formula was used.

x = unknown Lead concentration (mg/L)

Y = absorbance value

$$a = -0.0002$$

$$b = +0.0231$$

$$c = -0.0017$$

Sample 1:

Two values for x will be obtained because the equation will be split into two and .

$$\text{For sample 1; } x_1 = 26.849 \text{ mg/L}$$

$$x_2 = 88.651 \text{ mg/L}$$

$$\text{Sample 2: } x_1 = 26.024 \text{ mg/L}$$

$$x_2 = 89.476 \text{ mg/L}$$

$$\text{Sample 3: } x_1 = 27.253 \text{ mg/L}$$

$$x_2 = 88.247 \text{ mg/L}$$

$$\text{Reference Standard: } x_1 = 9.682 \text{ mg/L}$$

$$x_2 = 105.818 \text{ mg/L}$$

For each of the calculated quadratic results, the x_1 values were accepted to be the correct concentration of Lead (mg/L) because they were within the calibration standard range. I. E the average absorbance values for the blood plasma samples were between the 20mg/L and 40mg/L average absorbance values for the Lead calibration standard concentration (mg/L). Hence we would expect the concentration of the blood plasma sample (repeats) to be between 20mg/L and 40mg/L.

Accuracy of the results

The calculated concentration of the reference standard was 9.682mg/L instead of its actual concentration of 10mg/L.

Therefore % Error = $|9.682 - 10| \div 10 \times 100 = 3.18\%$

Precision of the blood plasma =

= 1.655%

The average concentration of Lead in the blood plasma = 26.709 mg/L.

Therefore, the actual value of Lead in the blood plasma =

= 26.709

Practical 2

Data for Fluorescence Spectroscopy

Fluorescein (MW = 376.3 g/mol)

Calibration standard concentration (mg/L)

Molar concentration (mol/L)

Intensity (a. u.)

Average Intensity (a. u.)

1st Reading

2nd Reading

1×10^{-3}

2.657×10^{-9}

6.710

6.355

6.532

5×10^{-3}

1.329×10^{-8}

27.309

26.786

27.047

1×10⁻²

2. 657×10⁻⁸

72. 516

72. 748

72. 632

2. 5×10⁻²

6. 644×10⁻⁸

135. 543

135. 543

135. 543

5×10⁻²

1. 329×10⁻⁷

281. 420

280. 295

280. 857

Table 4: Shows the intensity values of the fluorescein calibration standard concentration (mg/L).

The table also shows the molar concentration (mol/L), this was calculated using .

Figure 6: Fluorescence Spectroscopy graph of intensity of fluorescence versus molar concentration (mol/L) of Fluorescein. Eosin (MW = 691. 9g/mol)

Calibration standard concentration

(mg/L)

Molar concentration (mol/L)

Intensity (a. u.)

Average Intensity (a. u.)

1st Reading

2nd Reading

1×10^{-3}

1. 445 $\times 10^{-9}$

6. 747

6. 969

6. 858

5×10^{-3}

7. 226 $\times 10^{-9}$

29. 244

29. 604

29. 424

1×10^{-2}

1. 445 $\times 10^{-8}$

60. 255

59. 682

59. 986

$2. 5 \times 10^{-2}$

3. 613 $\times 10^{-8}$

152. 261

151. 876

152. 068

5×10^{-2}

7. 2.26×10^{-8}

302. 480

300. 365

301. 422

Table 5: Shows the intensity values of the eosin calibration standard concentration (mg/L). The table also shows the molar concentration (mol/L), this was calculated using .

Figure 7: Fluorescence Spectroscopy graph of intensity of fluorescence versus molar concentration (mol/L) of Eosin.

Data for UV-VIS spectroscopy

Fluorescein (MW = 376. 3g/mol)

Table 6: Shows the absorbance values at 500nm for fluorescein calibration standard concentration (mg/L). The table also shows the molar concentration (mol/L), this was calculated using .

Figure 8: UV-VIS Spectroscopy graph of Absorbance at 500nm versus concentration (mol/L) of Fluorescein. The equation of the line when the intercept is set at zero is $y = 50431x$.

Eosin (MW = 691. 9g/mol)

Table 7: Shows the absorbance values at 525nm for eosin calibration standard concentration (mg/L). The table also shows the molar concentration (mol/L), this was calculated using .

Figure 9: UV-VIS Spectroscopy graph of Absorbance at 500nm versus concentration (mol/L) of Eosin. The equation of the line when the intercept is set at zero is $y = 89744 x$.

The aim of the practical was to determine which technique is more sensitive,

Fluorescence or UV-VIS Spectroscopy. Based on Beer-Lambert law $Abs = Ecl$.
 E = molar absorptivity, l = path way length which in this practical is 1cm
and c = concentration in mol/L.

If we set the intercept of the line of best fit we get $y = Mx$ where y =
absorbance and x = concentration in mol/L. This equation is similar to the
Beer-Lambert equation when l is excluded ($Abs = Ec$), hence we can infer
that the value of M (gradient) will be the same as that of E .

For fluorescein the equation of the line when the intercept is set at zero is y
= $50431x$ (figure 8), therefore $E = 50431$. For eosin the equation of the line
when the intercept is set at zero is $y = 89744 x$ (figure 9), therefore $E =$
 89744 . (Unit of E is $Lmol^{-1}cm^{-1}$)

For both fluorescein and eosin, the most sensitive method was Fluorescence.
The gradient of fluorescein when Fluorescence was used is 2×10^9 (figure 6)
while the gradient when UV -VIS Spectroscopy was used is 46544 (figure 8).
The ratio of sensitivity is = 42970 . Therefore Fluorescence is approximately
 43000 times more sensitive than UV -VIS Spectroscopy.

The gradient of eosin when Fluorescence was used is 4×10^9 (figure 7) while
the gradient when UV -VIS Spectroscopy was used is 80611 (figure 9). The
ratio of sensitivity is = 49621 . Therefore, for eosin Fluorescence is
approximately 50000 times more sensitive than UV -VIS Spectroscopy.

DISCUSSION

Practical 1

The aim of the practical was to calculate the concentration of Lead in the
blood plasma. The calculated Lead concentration is 26.709 with a
percentage error of 3.18% and a precision of 1.655% . The precision is less

than 5%, therefore, the results of the analysis can be trusted (Malainey, 2010).

2

In accordance with the aim of this experiment, blood cells had to be removed so that only blood plasma was left. As McRae et al., (2013) notes, blood cells can introduce an unusually high quantity of cellular lead into the blood plasma. Hence, false results are obtained. For this reason, blood plasma had to be extracted from the blood before it was analysed (McRae, et al., 2009).

3

The concentration of lead in blood plasma is determined by factors such as recurrent exposure and its absorptive capacity into the blood plasma (Okuonghae, et al., 2011). Hence, its concentration in blood plasma is a show of the exposure that occurred several months ago (Pizzorno & Murray, 2012). As per the results obtained, the concentration of the blood plasma in sample, 1, 2, & 3 was 26. 849mg/L, 26. 024mg/L, and 27. 253mg/L respectively.

Hence, getting the average of this gives:

$$(26. 849 + 26. 024 + 27. 253)/3 = 26. 209\text{mg/L}$$

Hence, any concentration of lead in blood plasma above this level is toxic.

Practical 2

1

Fluorescence is more sensitive compared to UV/VIS because it can detect minute levels of the compound. Cao et al., (2010), asserts this by noting that fluorescent is more sensitive than UV/VIS spectroscopy in the detection of Gabapentin (Cao, et al., 2010). It has a higher specificity than UV/VIS as it only detects fluorescent molecules. UV/VIS is not highly specific because of

the overlapping of the bands of absorption. The bands are also broad, hence, this explains the reason for some of the graphs lacking peaks or poorly formed peaks. As per the results, the approximate ratio of how fluorescence is more sensitive than UV/VIS is 1: 43000.

2

Fluorescence spectroscopy is the better method for eosin and fluorescence. As noted above, the sensitivity for fluorescence is 43000 times better than for UV/VIS while that of eosin is 50000 times more sensitive than UV/VIS. UV/VIS is plagued by various limitations. According to Pompidor et al., (2013), they include the low specificity of the spectra for certain compounds, the solution conditions can determine the absorption ability, and the mixing of molecules is an issue because of overlapping of the spectra (Pompidor, et al., 2013).

Conclusion

Fluorescence spectroscopy is more sensitive than UV/VIS.

Lead blood plasma levels depicts the exposure a person has had.

Fluorescence spectroscopy is better than UV/VIS for fluorescence and eosin.

The results of UV/VIS are highly impacted by the overlapping of the produced spectra.

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