Example of report on determination of concentration using spectrophotometry

Law

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Introduction

Light is a form of electromagnetic radiation that also includes microwaves and radio waves. Light energy is made up of photons which shows the properties of a wave in that they oscillate up and down as they move through space (Fritzsch, 2005). The main factor that separates the different types of electromagnetic radiations is that each of them has a different wavelength. For instance, the visible light is comprised of radiation with wavelengths ranging from 400 to 700 nm. The difference in these wavelengths is perceived by the human eye as different colors of light. Colors, such as purple green blue, yellow, orange and red all have different wavelength ranges. Purple has the shortest and red has the longest wavelength. When visible light falls on an object, some of the light colors may be absorbed while others are reflected (Rose, 2013).

Absorption of all the light colors means no color will be reflected, and the color will appear black. Conversely, if none of the wavelengths is absorbed the color will appear white. If a substance that is red in color, it means that it has absorbed all the other colors and reflected the red color. An object that absorbs some or all the wavelengths also absorbs the energy that is in the absorbed light waves. This is the reason behind black objects being warmer than the white ones; they absorb more wavelengths of light than the white

material (Meinhold, 2007).

The phenomenon of reflecting light occurs on opaque matter. However, in transparent materials, there is no reflected light and instead the light is transmitted. If a solution absorbs the red color, it will then transmit all the other colors. The content in the solution will, therefore, determine the amount of light that will be transmitted, and that will be absorbed. This property can be used to find the concentration of a solution. The solutions that have high levels of concentration will absorb more light and transmit fewer wavelengths.

Experiments to determine the absorbance of a solution are usually performed using a single wavelength rather than using the white light. The choice of the wavelength is dependent on the one that the sample being analyzed is able to absorb strongly. An instrument called spectrophotometer measures the amount of the light absorbed (NMENV, 2011).

The set-up of a spectrophotometer is composed of a light source, a monochromatic, a combination of mirrors and prisms that are used to give a specific wavelength, a detector and a meter. Light passes through the monochromatic before passing through the sample. The light amount that is transmitted or not absorbed is detected (NMENV, 2011). The light transmitted is expressed either as a percentage of the total light that was emitted or in the form of absorbance. The absorbance (A) and the percent transmittance are related by a relationship:

$A = \log_{10} \frac{100}{\%T}$

The relationship between the concentration of a compound and the light absorption is linked together using the equation $A = \varepsilon bc$. This equation is

derived to construct the Beer's Law plot, which indicates that the relationship between the concentration of a compound and the light absorption is linear (Sheffield Hallam University, 2013). In the equation, A represents the absorbance, b the path length, c concentration of the sample while ε is the absorptivity (Blauch, 2009). By measuring a series of solutions of various concentrations of a compound and plotting their absorbance data, obtained with a spectrophotometer, on a graph, the linear relationship between absorbance and concentration can be visualized. This relationship may then be used in determining the concentration or absorbance of any other solution of the same compound, as long as the other variable is known.

This experiment was thus aimed to determine the concentration of a solution using the spectrometry technique.

Method

The initial step was the preparation of standard solution where five large test tubes were washed and clearly labeled. Two burettes were rinsed, set-up and one filled with deionized water while the other one was filled with stock solution. The spectrophotometer was operated using the Logger Pro program. A cuvette was prepared with a blank solution of deionized water in order to remove any impunity. The colorimeter was set to 470 nm wavelength light and the colorimeter calibrated. The distilled water was used to calibrate the colorimeter setting the base for the analyte ensuring that any reading was due to the sample.

The Beer's Law plot was constructed using the absorbance of the standard solutions and their concentration. This was to get a linear relationship

between the absorbance and concentration to facilitate the calculation of concentration for the unknown samples.

The absorbance of the unknown samples was measures using the colorimeter and their absorbance used in the Beer's Law plot to calculate the concentration for the unknown samples as shown below.

Results and Calculations

Five standard solutions of various concentrations of water and the Congo Red that were prepared are shown in Table 1 below. The solution used as the standard solution was the Congo Red with a concentration of 2. 267E-2 g/L.

The solutions were mixed through vortexing and the concentration for each burette recorded in Table 2 below.

The absorbance, volume and the concentration of standard solution were recorded in Table 2. The stock solution has a concentration of 2. 27E-02 g/L from which the other samples were diluted. For sample number 3, the concentration of stock solution was 2. 27E-02 g/L and a volume of 5. 98 ml while the total volume was 9. 97ml of the diluted solution. Using the formula $C_1 \times V_1 = C_2 \times V_2$, the sample solution (C2) was calculated as follows:

0. 0227×5. 98 ml= C2×9. 97 ml= 0. 0227g/L×5. 98 ml9. 97 ml

= 1. 36e-2g/L

The concentration and the absorbance of the standard solutions were used to plot a graph of absorbance against concentration as in Figure 1 below.

Figure 1: A graph of absorbance against concentration of standard solution

The equation for the line of best fit was y = 43. 211x + 0. 0453 with a slope of 43. 22 and y intercept of 0. 045. This equation was used to calculate the

concentration of the unknown and the known concentrations. The concentration was calculated as illustrated below where y is the absorbance and x concentration.

$y= 43.211x+0.0453$

$x= y-0.045343.211$

For unknown sample 1 with absorbance of 0.311, Concentration= 0.311-0.045343.211

$= 6.149E-02$ g/L

For unknown sample 2 with absorbance of 0.716,

Concentration= 0.716-0.045343.211

$= 1.55E-02$ g/L

For known sample 3 with absorbance of 0.565, Concentration= 0.565-0.045343.211

$= 1.202E-02$ g/L

The actual concentration of the known sample number 3 was 1.23E-02 g/L while the concentration calculated was 1.202E-02 g/L. The percentage error in the experiment was calculated using the formula.

% Error= Actual concentration-calculated concentrationActual concentration ×100

$= 0.0123-0.0120.0123 ×100$

$= 2.22%$

Discussion

The purpose of this experiment was to determine the concentration of an unknown sample through spectrometry. The concentration of the unknown

solution was determined and compared the value of the concentration of a known sample with the calculated one. This was to determine the reliability of the experiment in the determination of concentration of a compound.

The Beer-Lambert law, which is also referred to as the Beer's law, is the linear relationship that exists between the concentration of a substance that absorbs an electromagnetic radiation and the absorbance and concentration of an absorber of electromagnetic radiation. A beer's plot that is of good quality is the one whose point all lie on the line of best fit. Points that are away from the line tend to pull the line towards them and hence resulting in false results. The plot from the experiment was of good quality since most of the points were lying on the line best-fit. However, the point 2. 27E-2, 0. 995 did not lie perfectly on the line of best-fit. This point may have pulled the line downwards resulting in the reduction of gradient of the line. This deviation may have also resulted in the low value of R2.

The value of R2 gives the proportion of variance in the dependent variable which can be predicted using the independent variable. A perfect dependence is given by a R2 value of 1. In the experiment, the value of R2 was 0. 993, which is a close to the perfect relationship between the dependent variable and the independent one. This shows that the Beer's Law plot was a reliable model for the determination of the concentration of the unknown sample.

Another way to determine the credibility of the Beer's Law in the determination of the concentration of an unknown sample is to test for the concentration of a known sample. This helps in the determination of the percentage error of the experiment. From the concentration of known

sample and that which was calculated, the experimental had an error of 2. 22 %. The concentration of the unknown sample may, therefore, be said to be 97. 78% correct. This shows that the technique had a very high percentage of accuracy which also indicates that the technique is reliable. The concentration of the unknown sample was calculated to be 1. 55E-02 g/L a value that was exactly the same as that of a colleague who used the same technique but in a different set up. The colleague reported that the concentration of the unknown sample 3 which was also used in this experiment to be 1. 55E-02 g/L (Sokol, 2013). This fact indicates the reproducibility of the technique, as well as, the accuracy when all procedures are followed correctly.

The experiment went on smoothly, and no major problems were encountered. Minor problem may have resulted from inaccurately measuring of samples which have led to the difference in the actual and the calculated concentration for the known sample. This may be reduced by measuring the volumes of the solution more accurately as well as avoiding contaminations by washing the equipments thoroughly. The spectrophotometer technique is used in the forensic laboratories, in detecting and characterization of poisons and other compounds (Grippo, 2001). Reference

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