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Experiment 18 – Spectrophotometric Analysis of Blood Glucose

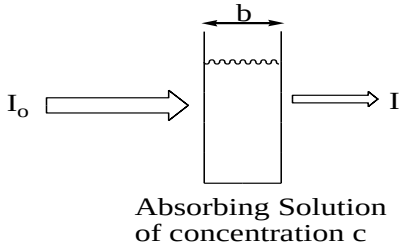
Discussion

The normal concentration of glucose in the blood should lie within the range 65 – 95 mg glucose per 100 mL blood. The units typically used in clinical analysis are mg/dL. The blood glucose level may decrease temporarily during strenuous exercise because the glucose may not be replenished rapidly enough from liver glycogen or by gluconeogenesis. A condition of low blood glucose is called hypoglycemia. It is characterized by a rapid heartbeat, general weakness, trembling, perspiration, whitening of the skin, and loss of consciousness. The loss of consciousness is due to the deprivation of brain cells of the necessary glucose.

A condition of high blood glucose is called hyperglycemia. The digestion of carbohydrates may result in absorption of glucose into the blood faster than glycogen can be formed by the process of glycogenesis. As the blood glucose level increases, the body also transforms glucose into fat and stores the fat as adipose tissue. When blood glucose levels reach 140 – 160 mg per 100 mL of blood, neither glycogen nor fat can be formed rapidly enough to decrease the glucose level. The condition in which glucose is then excreted by the kidneys and eliminated in the urine is called glucosuria.

Tests for the concentration of glucose in blood and urine are done in clinical laboratories. Modern methods use automated analytical procedures that rapidly produce colored products that can be analyzed by spectrophotometers. If a liquid is colored, it is because some component of the liquid absorbs visible light of a certain wavelength. In a solution, the greater the concentration of the light-absorbing substance, the more light absorbed and the greater the intensity of the solution. The quantity of light absorbed by a substance can be measured using a spectrophotometer. The instrument consists of: (1) a source that emits all wavelengths of light in the visible region (wavelengths 400 to 700 nm); (2) a monochromator which selects a given wavelength of light; (3) a sample holder for the solution being measured; and (4) a detector which compares the intensity of incident light I_o to the intensity of light after it has passed through the sample I. When a beam of light passes through a substance, some of the energy is often absorbed by the substance. This causes a decrease in the intensity of the transmitted beam. The ratio I / I_o is called the transmittance, T, a measure of the fraction of light that passes through the sample holder (or cuvette) which contains the absorbing solution. The amount of light absorbed by the solution is given by the absorbance, A, where:

$$A = -\log (I / I_o) = -\log T$$



$T = I/I_o$

$A = -\log (I/I_o) = -\log T$

$\%T = 100\% T$

The distance, b, the light travels through the solution (in cm) and the concentration, c, of the absorbing species are represented in the schematic above. A beam of parallel radiation with an intensity is shown before (I_o) and after (I) it has passed through a layer of solution with a measured thickness at a certain concentration. The Beer-Lambert law is the basis for using spectroscopy in quantitative analysis which relates absorbance (A) to the concentration of the absorbing solution (c) and the path length of the cuvette (b). That is:

$$A = \varepsilon b c$$

where ε is the molar absorptivity or the molar extinction coefficient. Each pure substance has its own unique extinction coefficient. Note that during the experiment, the same cuvette should be used for all measurements.

This experiment uses specifically prepared aqueous glucose solutions (rather than body fluids) and Benedict's solution. Glucose reacts with the Cu^{+2} complex ion of Benedict's solution to give solid Cu_2O. As a result of this reaction, the concentration of the Cu^{+2} ion decreases. The resulting decrease in the absorbance of the solution at the wavelength of maximum intensity for the Cu^{+2} is directly proportional to the glucose concentration. You will determine the linear relationship between absorbance (y-axis) and concentration (x-axis in units of mg glucose per 100 mL) using solutions generated by the reaction of various known concentrations of glucose with Benedict's solution. Using this calibration line (to be constructed using Microsoft Office Excel®), you can then determine the concentration of a solution of glucose of unknown concentration.

Procedure

Turn on the power switch by rotating it clockwise. The pilot light will glow red when the machine is on. Note that the power switch is also the zero control knob (left side knob). Set the wavelength control to 730 nm and allow the spectrophotometer to warm up for 15 minutes. Adjust the zero control knob so that it reads 0% T.

Prior to making all absorbance readings, a spectrophotometer must be calibrated using a blank solution, which is comprised of a cuvette filled with 2/3 full D.I. water for this experiment. Insert the cuvette into the sample holder, aligning the mark on the test tube with the line on the sample compartment and close the cover. Adjust the transmittance/absorbance control (right side knob) until the meter reads 100% T.

Obtain an unknown solution sample from your instructor. Mark the unknown code on your data sheet.

Prepare and number six test tubes, and carefully pipet 5.00 mL of the dilute Benedict's solution into each tube. Now obtain a different pipet for each subsequent addition: into tube 1, pipet 5.00 mL of D.I. water; into tubes 2 through 5, pipet 5.00 mL of the different standard glucose solutions found in the hood. Into tube 6, pipet 5.00 mL of your unknown glucose solution.

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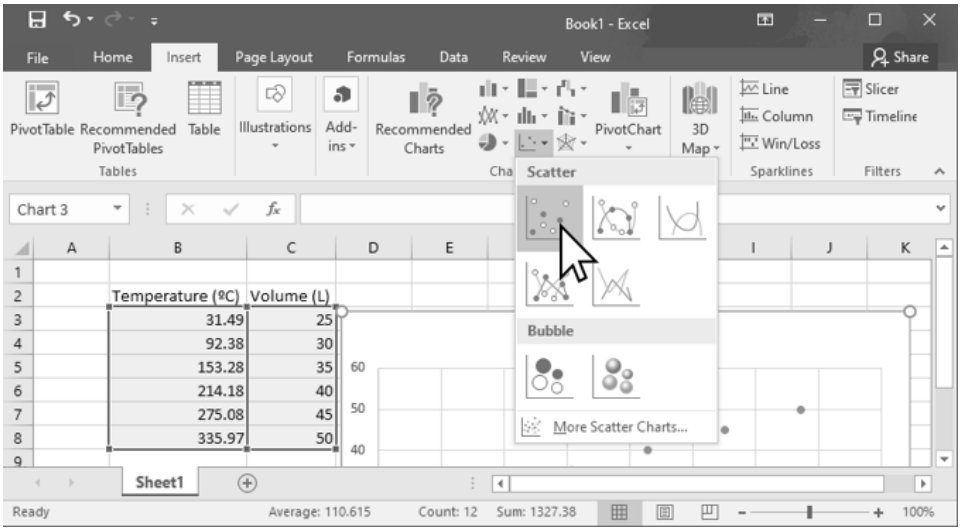
Gently agitate each test tube to ensure complete mixing. Place all tubes in a 400 mL beaker containing 200 mL of hot water maintained at a gentle boil via a Bunsen burner with boiling chips. After 30 minutes, remove the test tubes and place them in a test tube rack to cool. If the Cu_2O formed in the reaction settles to the bottom of the test tubes, it will be possible to decant the solution; otherwise, you will have to centrifuge the mixture to separate the solid from the solution before decanting.

Obtain two cuvettes from your instructor, calibrate the spectrophotometer with the blank solution, and record your absorbance measurements for the six solutions. Make certain to rinse the sample cuvette several times with small amounts of the new solution prior to recording its absorbance. Record your results in the data section of this report.

Excel® Guidelines

Note that various versions of Excel® may function a bit differently from the directions outlined below (which work on department-owned laptop computers):

Put the title for your x-axis (include units) in one Excel® cell (box). In the cell to the right, put the title for your y-axis. Using these boxes as headings, input the numeric data (like a table) in the cells under these titles (each box should contain one number; each row represents one data point in x,y format). Click and drag your mouse to highlight just the numeric boxes. From the "Insert" tab, choose a "Scatter" plot. (See example, below.)



Your graph must include a meaningful Chart Title and Axis Titles (with units). These Chart Elements can be added to your graph by clicking on the "+" icon in the upper right corner of your graph. Your instructor may request additional Chart Elements.

To add a Trendline, right click on any data point on your graph and choose "Display Trendline" from the menu that appears. The format trendline pane will appear on the right side of your screen. Linear should be selected by default. From this pane, you should check the box next to "Display Equation on chart." Your instructor may also ask you to check the box for "Display R-squared value on chart."

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Data and Calculations for Experiment 18

Test Tube	Initial glucose concentration (from bottle)	Absorbance at 730 nm
1		
2		
3		
4		
5		
Unknown Code _____	**To be determined in Question #1 below**	

Based on your Excel® graph, what is the equation of the line? _____

Questions

1. Using your unknown absorbance value and calibration line, what is the concentration of your unknown solution? Show your work below.

2. The following absorbance values for four solutions with known MnO_4^- concentrations were measured using a spectrophotometer:

Solution	$[MnO_4^-]$	Absorbance
1	7.00×10^{-5} M	0.175
2	1.00×10^{-4} M	0.250
3	2.00×10^{-4} M	0.500
4	3.50×10^{-4} M	0.875

Plot a graph of Absorbance vs. Concentration of MnO_4^- using Microsoft Excel® (be sure to include your graph with this report). Determine the concentration of an unknown MnO_4^- sample whose absorbance is found to be 0.780.

3. In your own words, write a logical, coherent conclusion which demonstrates a thorough working knowledge and understanding of important concepts and underlying chemical principles pertinent to this experiment, forms appropriate conclusions based on interpretations of results, includes applications of and improvements in the experiment, and demonstrates accountability by providing justification for any errors. If additional space is needed, please use the back of this page. (For additional guidelines on writing this conclusion, please refer to the **Moorpark College Chemistry Department Laboratory Report Rubric** found in the lab manual and department website.)