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Biuret colour change

Assignment 3: Biochemical Assays and Cells Due September 4 Part 1-Biochemical Assays Use of qualitative measurements As an example of a simple qualitative assay, you will compare the amount of protein in several solutions using the biuret assay. In this assay, a reagent is added that reacts with protein to give a violet color. The biuret reagent contains copper (II) sulfate which gives it a light blue color. When added to a solution containing protein, the copper ion of the reagent forms a complex with the peptide bonds of the protein. This changes the light blue color to violet or a blue-violet color; the more protein in the solution, the darker the color. Procedure (work in pairs) 1. Number test tubes 1 through 5 and place them in the test tube rack. 2. Using the appropriate dropper pipets, add 1 ml of each of the following solutions to the tubes: Tube 1 Tube 2 Tube 3 Tube 4 Tube 5 Solution water albumen 1 albumen 2 albumen 3 milk Protein (mg) 0 4 mg 200 mg ? ? 3. Add 2 drops of biuret reagent to each tube and mix by gentle shaking. 4. Wait 3-5 min for the violet color to fully develop, then estimate the amount of protein in tubes 4 and 5. Since you know how much protein is in tubes 2 and 3, you can estimate the amount of protein in tubes 4 and 5 (the unknowns) based on color intensity. Make your best estimates, then answer question 1. Use of quantitative measurements You will now use a quantitative assay to precisely measure the amount of glucose in an unknown sample. This method will utilize a spectrophotometer to quantify the color produced in a reaction of tris-glucose oxidase reagent (TGO) with glucose. The components of TGO react with glucose to form an orange color. The darker the color, the more glucose is present. Since this assay is quantitative, you will use an instrument to measure the exact degree of color in glucose solutions and calculate the amount of glucose in an unknown sample by using a standard curve. The spectrophotometer An instrument commonly used to measure the color of solutions is the spectrophotometer. A spectrophotometer works by shining light of a specific wave length through a glass tube (cuvette) that contains the solution of interest. A detector on the other side of the cuvette records the intensity of the light. The colored product within the solutions will absorb some of the light so that less light enters the detector. The instrumentation then converts light intensity to an absorption value. Thus the more intense the color, the higher the absorption. An orange solution absorbs light in the blue-green part of the spectrum. (If we can see orange, the red-yellow light has not been absorbed and thus enters our eyes.) For this assay, we will set the spectrophotometer to emit a wavelength of light in the blue-green region, 420 nm, to increase the sensitivity of the absorption measurement. Before use, a spectrophotometer must calibrated to display an absorption value of zero when the cuvette is empty and/or when a solution containing no color (such as water) is in the cuvette. A solution with water plus reagents is often used as an assay control (also called a blank) to set the absorption to zero. View this image of a typical spectrophotometer to learn its parts. Then find each part in the spectrophotometer at your table. The glucose assay The glucose assay is simple; the unknown sample is mixed with the TGO reagent. When color has developed, the absorption is measured in the spectrophotometer. In order to calculate the amount of glucose in the unknown, a standard curve is needed. This consists of several solutions of known glucose content as shown in the table below. Tube 0 serves as the assay blank; it contains water instead of a glucose solution. Note that 1 microgram (ug) = .001 milligram. Procedure (work in pairs) 1. Number test tubes 1 through 7 and place them in the test tube rack. 2. Using the micropipet, add glucose standard (200 ug/ml) and water to each of the following solutions to the tubes as indicated below. Add 200 ul of unknowns A and B to tubes 6 and 7. Use a separate pipet tip for water, glucose, and each unknown. Tube 1 Tube 2 Tube 3 Tube 4 Tube 5 Tube 6 Tube 7 Glucose standard 0 50 ul 100 ul 150 ul 200 ul Glucose unknowns 200 ul of A 200 ul of B Water 200 ul 150 ul 100 ul 50 ul 0 0 0 Glucose (ug) 0 10 ug 20 ug 30 ug 40 ug ? ? 3. Use the pump dispenser on the counter to add 2 ml of TGO solution to each tube. 4. Place you tubes in the water bath at 37°C and wait 20-30 min for the color to fully develop. While the color is developing, your TA will discuss the use of the spectrophotometer and how to construct a standard curve. 5. Go to a spectrophotometer and obtain 7 cuvettes. Note that one side of the cuvette has an arrowhead at the top. This side of the cuvette should be on the left when you place the cuvette into the holder. Carefully pour the contents of tube 1 (the blank) into a cuvette and place the cuvette in the first position of the cuvette holder. Pour the contents of tubes 2-7 into the other cuvettes and place them into consecutive spaces in the holder. Close the top of the chamber, then press "auto zero".This will set the absorption of the blank to 0. (Be sure that the blank, B, is in place when you auto zero.) Now press "measure". The holder will move the next cuvette into the light beam and give an absorption reading for solution 2. Press measure again to obtain absorption readings for the solution in each of the cuvettes. When you have obtained all values, press the "cell down" key until the B position is in place again. Remove the cuvettes, pour the solutions down the drain, and rinse the cuvettes several times with tap water. Leave them on the counter to dry. 6. Use graph paper to draw a standard curve and use it to determine the amount of glucose in tubes 6 and 7. In theory, a standard curve should be a straight line when amount of glucose (categories) is graphed against absorption (values). However, due to inevitable slight technical or mechanical errors, the points may not all fall on a straight line. Draw the best straight line through all of the points on your graph, including zero. HINT: To determine the amount of glucose in the "unknowns", you can mark where each unknown absorption falls on your standard curve, then "draw" a perpendicular line to the x axis. Submit the glucose values of tubes 6 and 7 to WebAssign for question 2. Write your names on your graph and turn it in to the TA (you will not receive credit for question 2 unless the paper graph is turned in). Now use the on-line plotter to graph your standard curve. Use a regression line to determine the slope of the standard curve. Capture an image of your graph and the table below and submit it to WebAssign for question 3. Then calculate the glucose concentration of tubes 6 and 7 mathematically: absorption of unknown/slope of standard curve = ug glucose . Submit the values for tubes 6 and 7 that you calculated mathematically to WebAssign for question 4. Copyright © 2007 - 2021 Revision World Networks Ltd. In order to continue enjoying our site, we ask that you confirm your identity as a human. Thank you very much for your cooperation. Definition noun A chemical test that detects any compound containing two or more peptide bonds Supplement Biuret test is used for detecting compounds with peptide bonds. A biuret reagent may be used to test the aqueous sample. This blue reagent is made by combining sodium hydroxide and copper sulfate solutions. A few drops of this reagent will turn the aqueous sample containing compounds with peptide bonds from pale to intense violet colour. The violet colour intensity depends on the number of peptide bonds in the sample. The biuret test is also used to detect proteins. That is because proteins are made up of polypeptides, which in turn, are made of amino acids joined by peptide bonds. The longer the polypeptide chain is, the more peptide bonds there are, and therefore, the more intense the violet colour will be when biuret test is applied. It also follows that a pale violet or pinkish colour indicates shorter polypeptide chains or fewer peptide bonds. A negative result (lack of violet colour formation) may mean lack of protein, or the presence of free amino acids (without peptide bonds). The test, however, gives positive result to any compound containing two carbonyl groups attached to a nitrogen or carbon atom. Thus, it may not be completely protein-specific. Performing other protein tests may be necessary. See also: If the balance of nature is left untouched, landscapes can change dramatically over time. A previous ecosystem is supers.. Regulation of Biological Systems tutorials are focused on the modulation of biological systems from cell to population l.. A still body of water may be disturbed by a variety of factors. One of them is wind. In fact, it is considered as the pr.. Bryophytes (nonvascular plants) are a plant group characterized by lacking vascular tissues. They include the mosses, th.. Hormones are chemical messengers produced by specialized glands and they were produced by switching on the genes designe.. The gastrointestinal system breaks down particles of ingested food into molecular forms by enzymes through digestion and.. Proteins Like the complex carbohydrates, proteins (or polypeptides) are large molecules made up of smaller subunits which have been bonded together. In this case, amino acids joined by peptide bonds. April 20, 2018 Gaurab Karki Protein and Amino acid tests, Tests 0 Biuret test is a general test for compounds having a peptide bond. Biuret is a compound formed by heating urea to 180° C. When biuret is treated with dilute copper sulfate in alkaline condition, a purple colored compound is formed. This is the basis of biuret test widely used for identification of proteins and amino acids. This test is given by compounds containing two or more peptide bond (CO-NH group). Since all proteins and peptides possessing at least two peptide linkage ie. tripeptide gives positive biuret test. The principle of biuret test is conveniently used to detect the presence of proteins in biological fluids. Alkaline CuSO4 reacts with compounds containing two or more peptide bonds to give a violet colored product which is due to formation of co-ordination complex of cupric ions with un-shared electron pairs of peptide nitrogen and O2 of water. Requirements for Biuret test: 1 % alanine, 5 % egg white (albumin) Biuret reagent Water bath Dry test tubes Pipettes Biuret reagents: Copper sulfate (CuSO40 Sodium hydroxide (NaOH) Sodium potassium tartarate (commonly known as Rochelle salt) Preparation Biuret reagent Biuret reagent is prepared by adding NaOH in CuSO4 solution, making it alkaline. To prepare 1000ml of Biuret reagent Take 1.5 gram of pentavalent copper sulphate (CuSO4) and 6 gram of Sodium potassium tartarate and dissolve them in 500 ml of distilled water **Sodium potassium tartarate is a chelating agent and it stabilize the copper ion Take 375 ml of 2 molar Sodium hydroxide Mix both the solution in volumetric flask and make it final volume to 1000 ml by adding distilled water. Procedure for Biuret test Take 1 ml of test solutions in dry test tubes and in another tube take 1 ml distilled water as control. Add 1 ml of biuret reagent to all test tubes, mix well. Look for the development of blue colors Result: Biuret test positive: color changes to purple all peptides and protein give the test positive Histidine is the only amino acid that give biuret test positive. Biuret test negative: Precautions: Presence of magnesium and ammonium ions interfere in biuret test. This can be overcome by using excess alkali. A. Bianchi-Bosisio, in Encyclopedia of Analytical Science (Second Edition), 2005The biuret method is a colorimetric technique specific for proteins and peptides. Copper salts in alkaline solution form a purple complex with substances containing two or more peptide bonds. The absorbance produced is proportional to the number of peptide bonds that are reacting and therefore to the number of protein molecules present in the reaction system. Thus, the biuret reaction with proteins is suitable for the determination of total protein by spectrophotometry (at 540–560 nm). The method is used extensively in clinical laboratories, particularly in automated analyzers in which protein concentration can be measured down to 0.1–0.15 g l−1. The use of bovine or human serum albumin to standardize the biuret method is well established. High-purity albumin contains only amino acids; its nitrogen content is a constant fraction of its molecular mass and the number of peptide bonds per molecule is known. Since the peptide bond is the biuret-reacting unit in all proteins and the number of peptide bonds determines the absorbance of the colored product, albumin is a reasonable peptide bond standard for all proteins in the mixture.F. Sánchez Rojas, ... J.M. Cano Pavón, in Encyclopedia of Analytical Science (Third Edition), 2019There are three prime methods for quantification of proteins based on derivatization reactions: the biuret, Lowry, and Bradford methods.The biuret method is based on the fact that proteins (and, as a rule, all substances containing two or more peptidic bonds) react with copper to form a colored complex whose absorption (λmax= 454 nm), in the presence of excess copper, is proportional to the amount of protein present. The reagent is obtained by dissolving 1–5 g of copper(II) sulfate and 6 g of sodium potassium tartrate tetrahydrate in 3% sodium hydroxide. Bovine serum albumin is used as standard. The most serious drawback of this method is its poor sensitivity.The Lowry method, more sensitive than the biuret method, affords the determination of protein at the microgram per milliliter level. The procedure involves two reactions: that of the protein with alkaline copper solution (the biuret reaction) and reduction of the Folin–Ciocalteau reagent by tyrosine and tryptophan residues of the protein. The Folin–Ciocalteau reagent is prepared by boiling a solution containing sodium tungstate, sodium molybdate, and phosphoric and hydrochloric acids, which produces molybdophosphate and tungstophosphate ions. The absorbance is measured at 750 nm. The Lowry method has also been applied to proteins in whole cells. Like the biuret method, it is subject to some limitations and interferences (particularly those from ammonium salts, glycine, and mercaptans). In addition, variations in the tyrosine and tryptophan contents from protein may introduce some uncertainty in the determinations, so the method is more practical for determining concentration changes than absolute protein concentrations. The Lowry method has also been applied by exploiting solid-phase spectrophotometric techniques.The Bradford method is based on the absorbance of Coomassie Brilliant Blue on the protein to be determined, which results in a spectrum shift of the dye from 465 to 595 nm. The absorbance at 595 nm is proportional to the protein concentration. Bovine serum albumin is used as standard. The reaction is susceptible to interferences from surfactants and alkalinity of the solution. The main advantage of the method is rapidity, which enables efficient application to many samples.A micromethod has been developed using the Bradford method for studying the proteins present in cell membranes.Proteins can also be determined by 1% bicinchoninic acid solution in alkaline medium, to produce a red compound. The absorbance is measured at 562 nm against a reagent blank. Proteins can be determined in the interval 0.5–10 μg ml− 1. The method offers a one-step alternative to the methods of Lowry and Bradford and is less subject to interferences.In recent years, diverse chelating agents have been introduced for proteins quantification: Arsenazo III, fuchsine acid, methyl blue, bromophenol blue, methylene blue, etc.Another common determination is that of haemoglobin, a major component of red blood cells. The method involves oxidation of haemoglobin with hexacyanoferrate(III) to form methaemoglobin, and further reaction with cyanide ion to form cyanmethaemoglobin that presents maximal absorptivity at 540 nm (ε= 4.4×104 l mol− 1 cm− 1). The procedure is carried out by using Drabkin's reagent, a solution containing potassium hexacyanoferrate(III), potassium cyanide, sodium phosphate, and a surfactant (e.g., Triton X-100). The method can also be applied to whole blood samples.Haemoglobin can also be determined after complex formation with inositol hexaphosphate that is quantified by differential spectrometry to avoid the strong absorbance of the protein.Alison Rodger, Karen Sanders, in Encyclopedia of Spectroscopy and Spectrometry (Third Edition), 2017While the biuret method is sensitive in the range 0.5 to 2.5 mg protein per assay, the Lowry method is 1 to 2 orders of magnitude more sensitive (5 to 150 μg). The main disadvantage of the Lowry method is the number of interfering substances; these include ammonium sulfate, thiol reagents, sucrose, EDTA, Tris, and Triton X-100.The final colour in the Lowry method is a result of two reactions. The first is a small contribution from the biuret reaction of protein with copper ions in alkali solution. The second results from peptide-bound copper ions facilitating the reduction of the phosphomolybdic-tungstic acid (the Folin reagent) which gives rise to a number of reduced species with a characteristic blue colour. The amino acid residues which are involved in the reaction are tryptophan and tyrosine as well as cysteine, cystine and histidine. The amount of colour produced varies slightly with different proteins. In this respect it is a less reliable assay than the biuret method, but it is more reliable than the absorbance method since A280 may include contribution from other species, and also the absorption of a given residue is dependent on its environment within the protein.Two solutions are required for the Lowry method. For the alkaline copper solution, mix 50 cm3 Na2CO3 (2% w/v) in NaOH (0.1 M) with 1 cm3 of CuSO4.5H2O (0.5% w/v) and 1 cm3 of sodium potassium tartrate (1% w/v). This solution must be discarded after 1 day. The Folin reagent (phosphomolybdic-tungstic acid) may be made by diluting the concentrated Folin reagent obtained from, for example Sigma with an equal volume of water so that it is 1 N (i.e. 1 M H+).To perform an assay add x cm3 of sample (where x