The lowry method essay

Design

* Highly susceptible to taint by buffers, biological stuffs and salts* Protein amino acid composing is highly of import, therefore the pick of a criterion is really hard, particularly for purified proteins* Absorbance is to a great extent influence by pH and ionic strength of the solution.

General Considerations* This is frequently used to gauge protein concentration prior to a more sensitive method so the protein can be diluted to the correct scopeQuantitative Procedure* Zero the spectrophotometer with a buffer space* Make a criterion curve utilizing your criterion of pick in the expected concentration scope, utilizing the same buffer that your unknown sample is in.* Take the optical density values at 280 nanometers in a vitreous silica cuvette* Place sample into quartz cuvette (do certain concentration is in the scope of 20 µg to 3 milligrams* Take optical density at 280 nanometersAppraisal Procedure* Zero spectrophotometer to H2O (or buffer)* Take the optical density at 280 nanometers in a vitreous silica cuvette* Change wavelength to 260 nanometers and nothing with H2O (or buffer)* Take soaking up at 260 nanometers in a vitreous silica cuvette* Use the undermentioned equation to gauge the protein concentration[Protein] (mg/mL) = 1. 55*A280 – 0.

76*A260DiscussionDetermination of protein concentration by ultraviolet soaking up (260 to 280 nanometers) depends on the presence of aromatic amino acids in proteins. Tyrosine and tryptophan absorb at about 280 nanometers. Higher orders of protein construction besides may absorb UV visible radiation or modify the molar absorption factors of tyrosine and tryptophan and therefore the UV sensing is extremely sensitive to pH and ionic strength at which measuring is taken. Many other cellular constituents,

and peculiarly nucleic acids, besides absorb UV visible radiation. The ratio of A280/A260 is frequently used as a standard of the pureness of protein or nucleic acid samples during their purification. The existent advantages of this method of finding protein concentration are that the sample is non destroyed and that it is really rapid. Although different proteins will hold different amino acid composings and therefore different molar absorption factors, this method can be really accurate when comparing different solutions of the same protein.

To do an accurate finding of protein concentration, you will hold to bring forth a standard curve (A280) with known sums of purified protein. You will besides hold to supply a space that is appropriate for the sample and contains the same concentrations of buffer and salts as the sample. It is frequently convenient to dialyse the sample and step the optical density of the retentate (still in the dialysis poke) utilizing the dialysate as the space. Care must be taken to utilize quartz cuvettes, since glass absorbs UV visible radiation. A ready to hand equation to gauge protein concentration that is frequently used is[Protein] (mg/mL) = 1. 55*A280 – 0. 76*A260However, it is besides a good thought to ever utilize a standard curve and suggested that you evaluate the understanding of the consequences utilizing the above equation with consequences utilizing a standard curve.

This method is the least sensitive of the methods discussed here. For increased sensitiveness, the wavelength can be lowered to the scope of 210 to 225 nanometer. This measures the amide bond in proteins. However it is much more capable to interference from many more biological constituents and compounds used to do buffer solutions.

If you do n’t cognize what the protein concentration of an unknown sample is likely to be, the UV method might be a good starting point. Fix a standard curve for the optical density at 280 and 260 nanometer. After you have the informations for the standard curve, rezero the spectrophotometer with H2O. Put your samples into a dry 1 milliliter vitreous silica cuvette and read the optical density. If the A280 of your unknown sample is less than 2, you should likely non thin your sample farther.

If the optical density is & A ; gt ; 2, dilution will be required. When you are finished with the first measuring, the unknown can be returned to its original tubing with minimum loss. The Lowry method relies on two different reactions. The first is the formation of a Cu ion composite with amide bonds, organizing reduced Cu in alkalic solutions. This is called a “ Biuret ” chromophore. The 2nd is the decrease of Folin-Ciocalteu reagent (phosphomolybdate and phosphotungstate) by tyrosine and tryptophan residues. The decreased Folin-Ciocalteu reagent is bluish and therefore noticeable with a spectrophotometer in the scope of 500-750 nanometer.

The Biuret reaction itself is non all that sensitive. Using the Folin-Ciocalteu reagent to observe reduced Cu makes the assay about 100 times more sensitive than the Biuret reaction entirely. The check is comparatively sensitive, but takes more clip than other checks and is susceptible to many interfering compounds. The undermentioned substances are known to interfere with the Lowry check: detergents, saccharides, glycerin, Tricine, EDTA, Tris, K compounds, sulfhydryl compounds, disulfide compounds, Mg and Ca.

Most of these meddlesome substances are normally used in buffers for fixing proteins. This is one of the major restrictions of the check. The Lowry check is sensitive to fluctuations in the content of tyrosine and tryptophan residues. If the protein you are assaying has an unusual content of these residues, an appropriate replacement criterion is required. The standard curve is additive in the 1 to 100 ug protein part. The optical density can be read in the part of 500 to 750 nanometers.

Most research workers use 660 nanometers, but other wavelengths besides work and may cut down the effects of taint (e. g. chlorophyll in works samples interferes at 660 nanometer, but non at 750 nanometer) .