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A New Colorimetric Method for the Determination of Proteins

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Abstract: A new colorimetric method for the determination of proteins was investigated using the local dye "*Uri isi*". Serial dilution of a solution of bovine serum albumin (BSA) was made. Protein assay was carried out photo-metrically with BSA using both the biuret reagent and the local dye. The results showed that the optical density decreased from 1.269 to 0.189 with an increase in the concentration of protein in the case of biuret reagent and also decreased from 0.276 to 0.174 with increase in the concentration of protein in the case of the local dye. The results suggest that the local dye was more sensitive than biuret reagent for assaying proteins since the bovine serum albumin was diluted 100 times before the local dye could be used for the assay.

Key words: Colorimetric method · Uri isi · Biuret reagent · BSA and Protein

INTRODUCTION

Proteins are highly diversified class of biomolecules. Differences in their chemical properties, such as charge, shape, size and solubility, enable them to perform many biological functions. These functions include – enzyme catalysts, metabolic regulation, binding and transport of small molecules, gene regulation, immunological defense and cell structure.

The cellular activities and functions involve one or more proteins. Their central place in the cell is reflected in the fact that genetic information is ultimately expressed as proteins, [1].

The basic building blocks of proteins are amino acids. There are about 20 amino acids found in proteins, all of which share certain structural features. These features are:

- Carboxyl (acid) (-COOH) group
- An amino (basic) ($-NH_2$) group

They differ from each other with respect to their side chains. Amino acids of proteins are linked together by peptide bonds between their carboxyl and α–amino group to form linear polymers [2].

Proteins have 3 or 4 levels of structural organization and complexity. The primary structure of a protein is the sequence of amino acids in its polypeptide chain or chains. Secondary structure is formed and stabilized by the interaction of amino acids that are fairly close to one another on the polypeptide chain. The polypeptide with its primary and secondary structure can be coiled or organized along three axes to form a more complex, three dimensional shape. Thus, level of organization is the tertiary structure [2].

A number of colorimetric and photometric methods are used for the determination of proteins. Photocolorimetric methods are based on the so called "colour" reactions for functional group of protein molecules. Among these are reactions for peptide groups and folin's test for amino acid aromatic radicals (tyrosin and trypthophan). The biuret test is more specific since peptide bond occurs only in proteins and peptides. It is widely used in clinico-biochemical examination. The Lowry's method, based on folin's reaction is highly sensitive but of low specificity, since free aromatic amino acids and numerous materials containing a phenolic group produce a similar colouration [3]. Photonephelometric methods for protein concentration determination are based on the estimation of the degree of turbidity (or clouding) of a protein suspension in solution. These methods have not gained wide acceptance in practice [4].

Spectrophotometric methods are sub-divided into direct and indirect methods. The latter method represents a sensitive and accurate variant of the photocolorimetrically techniques. After the induction of the colour reaction of a protein, the coloured solution is measured spectrophotometrically and the protein

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concentration is estimated by the percentage of monochromatic light energy absorbed by the colour solution, according to [2].

The direct method is based on the measure of light absorption by protein solution in the ultra violet spectra region at 200-220nm (characteristic absorption due to aromatic amino acid radicals, chiefly tryphotophan and tyrosine). These methods are easy to handle and require no preliminary colouration of the solution to be induced by a chromogenic agent [5]. The 200-220nm spectrophotomery is more specific than that at 230nm since in the later case, the additional absorption due to various low molecular aromatic compound, which are found in biological materials that interferes with the measurement accuracy.

The local dye "*Uri isi*" which was purchased at Nsukka market is used locally for dying grey hair. It is believed to undergo some reactions with certain chemical components of the hair in the presence of hydrogen peroxide. When applied on the hair in the presence of hydrogen peroxide, the grey colour of the hair is changed to dark colour.

Preliminary screening showed that the dye reacts with proteins to produce a change in colour.

The present study attempts to design a new colorimetric method for estimation of proteins based on the colour reaction between local dye "*uri isi*" and proteins.

MATERIALS AND METHODS

MATERIAL:

- Sp8.100 Ultraviolet spectrophotometer (By Pye Unican)
- Measuring scale
- Refrigerators
- Test tubes
- Pipette
- Conical flasks
- Measuring cylinders
- Hydrogen peroxide
- Bovine serum albumin
- Sodium potassium tartarate
- Sodium hydroxide (May and Baker Ltd, England)
- Copper sulphate
- Local dye (Nsukka market)
- Distilled water (Crop Science, UNN)
- Biuret reagent (BCH Lab, UNN)
- Protein albumin
- Carbohydrate i.e. lactose
- Starch
- Test tube racks
- Beakers

Method

Preparation of Reagents

Biuret Reagent: About 9g of sodium potassium tartarate was dissolved in 50ml of 0.2N NaOH. Also, 3g of copper sulphate was dissolved in water and it was mixed with the mixture of sodium potassium tartarate and sodium hydroxide. Then, it was preserved in a refrigerator [6].

Bovine Serum Albumin (BSA): About 10g of (BSA) Bovine Serum Albumin was mixed with 1m of water and was used for the experiments. Some quantity of prepared BSA was put into different test tubes and diluted accordingly as shown in the Table 2.

Local Dye Reagent: Some quantity of local dye about 5g was mixed with 1ml of hydrogen peroxide and this was used for the experiments as in the Table 3.

Preliminary Screening for Colour Reactions of Various Chemical Compounds with the Local Dye Reagent: Samples of carbohydrates and proteins were respectively mixed with some quantity of the local dye solution and observed for any colour change.

Determination of Proteins (BSA)

Use of Biuret Reagent: Using different concentration of BSA in distilled water, biuret reagent was used to determine the protein content according to the method of [6]. Both the BSA and the reagent were added to several test tubes (as in table 2) and then allowed to stand for 20 minutes.

The optical density was then determined for each mixture at 550nm using a spectrophotometer against distilled water blank.

Use of Local Dye: This was carried out with local dye solution in hydrogen peroxide. Different concentrations of the BSA (which was diluted a hundred fold) were used to determine the protein content.

Both the BSA and the local dye reagent were added to several test tubes as shown in Table 3.

These were allowed to stand for 20 minutes before reading the absorbance at 550nm with a spectrophotometer against distilled water blank.

RESULTS

Determinations of Bsa Using Local Dye Reagent: Table 2 shows the optical density values obtained after mixing the local dye reagent with different concentrations of BSA.

The result of the preliminary screening revealed that carbohydrates did not produce any change in colour with the local dye reagent while there was a change in colour to blue-black with proteins.

The results presented in Tables 2 and 3 show that both the biuret and the local dye reagent caused a decrease in optical density at 550nm as the concentration of the protein (BSA) decreased in each case.

DISCUSSION AND CONCLUSION

In the present study, serial dilution of a solution of bovine serum albumin (BSA) was made and protein assay was carried out photometrically with BSA using both the hiuret reagent and the local dye reagents.

The result presented in Table 1 shows that only proteins caused a change in colour when the local dye was added to samples of carbohydrates and proteins. This result suggests that the local dye reagent is a local chromogenic reagent which is capable of reacting with proteins to give a dark blue colour. This appears to the principle behind the use of this local dye as a hair dye.

The result in both Tables 2 and 3 showed a similar pattern of absorbance with increase in the concentration of protein. In the case of local dye reagent, dilution factor of hundred was introduced and this made the local dye reagent method a more sensitive method for the estimation of proteins.

Table 1:

Chemical Compound	Colour Change
Lactose	Colourless
Starch	Colourless
Glucose	Colourless
Protein	Blue-black

The reagents used in various standard methods for the estimation of protein are not easy to come by and are very expensive when they are available. This new method, (using the local reagents) for the estimation of proteins will go a long way towards solving the problem of unavailablity due to expensive nature of the reagents since the local dye could be sourced locally. The sensitivity of the local dye reagent was confirmed by the fact that the bovine serum albumin (BSA) was diluted hundred times before the local dye reagent could be used for the assay.

Table 3 shows clearly that the optical density decreased from 1.269 to 0.189 with an increase in the concentration of protein using biuret reagent for determine the proteins present in the solution bovine serum albumin. The biuret reagent is a standard reagent for the estimation of proteins and photocolorimetric estimation of protein using biuret is a standard method employed for both qualitative and quantitative determination of proteins. The result obtained here is in accordance with the results obtained in previous studies where BSA was used for standard calibration curve [7].

The results presented in Table 2 shows that the optical density decreased from 0.276 to 0.174 with the increase in concentration of protein using the local dye reagent.

Table 2: The optical density values for different concentrations of BSA using local dye reagent.

Test Tube	BSA (ml)	Distilled H_2O	Local Dye	Dilution Factor	Conc. Mg/ml	Optical Density 550nm
1	1	-	4	100	$10x10^2$	1.269
2	0.9	0.1	4	100	$9 x10^2$	0.900
3	0.8	0.2	4	100	$8 x10^2$	1.025
4	0.7	0.3	4	100	$7 x10^2$	1.352
5	0.6	0.4	4	100	$6 x10^2$	1.280
6	0.5	0.5	4	100	$5 x10^2$	1.025
7	0.4	0.6	4	100	$4 x10^2$	0.927
8	0.3	0.7	4	100	$3 x10^2$	0.617
9	0.2	0.8	4	100	$2 x10^2$	0.516
10	0.1	0.9	4	100	$1 x10^2$	0.144

Table 3: Optical density values for the different concentrations of BSA after using the biuret reagent

Test Tube	BSA (ml)	Distilled H_2O	Biuret Reagent	Conc. Mg/ml	Optical Density 550nm
1	1	-	4	10	0.267
2	0.9	0.1	4	9	0.261
3	0.8	0.2	4	8	0.244
4	0.7	0.3	4	7	0.237
5	0.6	0.4	4	6	0.236
6	0.5	0.5	4	5	0.233
7	0.4	0.6	4	4	0.205
8	0.3	0.7	4	3	0.198
9	0.2	0.8	4	2	0.919
10	0.1	0.9	4	1	0.176

The present study has demonstrated that the local dye "*Uri isi*" which is used locally for dying grey hair could be used as a cheap and a readily available reagent for the determination of proteins. The results further suggested that the local dye reagent method is a more sensitive method than the biuret method for estimation of protein.

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