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Spectrophotometric Measurement Of The Stoichiometry Of Formaldehyde And Plasma Albumin Reaction In Water Solution Using The Method Of Continous Variation (Job's Method) And The Mole Ratio Method

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Abstract: *The stoichiometry of the reaction of plasma albumin and formaldehyde was determined by the spectrophotometric method. Measurements were made at pseudo –first order condition of formaldehyde using the method of continuous variation (Job's method) and the mole ratio method at varying concentrations of formaldehyde and plasma albumin at their respective determined λ max and at constant conditions. The ionic strength of the reactants μ, was kept constant at 0.5moldm^3 (NaCl), and at constant temperature of 37^{0}C. The results showed a stoichiometric ratio of 1: 2 of plasma albumin to that of formaldehyde in aqueous medium*

Keywords: *Spectrophotometric, Stoichiometry, Formaldehyde, Plasma Albumin, Water solution, Jobs's method, mole ratio method*

I. Introduction

A human albumin molecule is reported to have a molecular weight of 66483 and consists of a single and quite long, compactly folded polypeptide chain of about 582 amino acid residues in length. It is also reported to be with an almost spherical configuration with seventeen disulphide bonds with one Aspartic amino acid terminal group and six amino acid residues comprising of Glutamic acid, Alanine, Histidine, Lysine, Serine and Glutamic acids as side chains [1,2]. Formaldehyde has a chemical formula of CH_2O and is the most simple known aldehyde. It is colourless gas with a boiling point of -21^{0}C and readily polymerized at room temperature and pressure and has a relative molecular mass of 30.03g/mol with a pungent odour. It is found to have a dipole moment of 2.33D which makes it highly soluble in water. Formaldehyde is also known as formalin, embalming fluid, or formol. The term formalin refers to the aqueous solutions. It can be obtained from its cyclic trimer trioxane and polymer paraformaldehyde and exist mostly in water as the hydrate, $H_2C(OH)_2$. Formalin is known to also occur in solid state as polymers (paraformaldehyde and trioxymethylene). The aqueous solution of formaldehyde usually referred to as formalin (100% w/v) consist of formaldehyde of about 40 v/v or 37% w/v in water with small amount of stabilizer usually methanol in addition to limit oxidation and polymerization. A typical commercial grade formalin may contain 10-12% percent methanol in addition to metallic impurities such as aluminium (3 mg/L), iron (1mg/L) and copper(1mg/L) [3]

Formaldehyde is found to cross-link, inactivate, stabilize, or immobilize proteins. It has been reported to react with the amino groups of the N-terminal amino acid residue and the side –chains of argine, cystein, histidine, and lysine residues when treated with proteins. Other series of investigations have found that under mild conditions formaldehyde irreversibly combine with the basic groups of proteins forming cross-links between the following pairs of groups: amine and amide; amine and phenol; amine and indole. Formaldehyde cannot, in their opinion, form a stable link between two amine or two amide group.[4] The formaldehyde treatment has been found to result in a large variety of chemical modifications in proteins, such as the formation of methylol groups, Schiff- bases, and methylene bridges. Depending on the peptide sequence, methylool groups, Schiff bases, and methylene bridges are reported to be formed and the most important modification of proteins induced by formaldehyde is reported to be the formation of stable methylene bridges [3].

Reactions of higher values of aqueous formaldehyde with proteins are reported to be based on classical carbonyl-amine reaction chemistry. Amines and related nucleophiles are found to react with aqueous formaldehyde to form various chemicals and intermediates with ultimately methylene bridging (-CH_2-) resulting in fixation or tanning type action. At any rate, primary amines are known to react to form intermediate hydroxymethyl groups that drives a basicity loss with pKa drops of about 4-5 units. Subsequently, but slowly, dehydration or condensation reaction occurs by loss of a molecule of water and a methylene bridge forms. Also possibly, after initial reactions are dimethylene ether linkages and the reduction of the hydroxymethyl groups by formaldehyde itself to methyl groups with production of formic acid as an endpoint product. These reducing

properties of formaldehyde are reported to be accelerated in alkaline conditions where formaldehyde is known to precipitate the metals of various salts, such as bismuth, copper and silver [3]

To date, there are a few scientific studies reporting the detailed mechanisms of the reaction of formaldehyde with bio-molecules, especially proteins. In fact [5] were the only ones to report the kinetics and mechanisms of the reaction of formaldehyde with a human protein – protein tau using a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) method. Their study found that protein tau was crosslinked and aggregated by formaldehyde via a second order mechanism thus indicating formaldehyde is neurotoxic. Formaldehyde in the human body is distributed by blood and its possible reaction with plasma albumin is worth exploring hence we devised this study to establish the mole ratio of low values of aqueous formaldehyde with plasma albumin in their reaction.

II. Materials and method

The Job's Method also called the method of continuous variation was employed at constant conditions, the ionic strength of the reactants μ, was kept constant at 0.5moldm^3 (NaCl), and at constant temperature of 37^{0}C. Then 0,1,2,3,4,5,6,7,8,9, and 10 mL of formaldehyde solutions were accurately transferred into a series of 50 mL measuring flasks. To each flask 5mL of sodium acetate buffer was added followed by 2.0 mL of hydroxylamine hydrochloride. Also 10,9,8,7,6,5,4,3,2,1, and 0 mL of the plasma albumin solution were added into the above flasks as in first step respectively, each flask now containing 17mL of the mixture. Each of the flasks was then diluted to mark with distilled water and the solution allowed to stand for 10 minutes and the absorbance of each of the solutions was recorded using water as reference at λ max 235nm.. The absorbance of the solutions was plotted against the mole fractions of formaldehyde to find the stoichiometric ratio the stoichiometry of the adduct from the resulting curve [6].

The mole ratio method

In the mole ratio method the concentration of plasma albumin was kept constant 5.1x 10^{-4} mol dm^{-3} while that of HCHO was varied between 2.7x 10^{-2} 2.7 x 10^{-3} , 2.7x 10^{-4} and 2.7 x 10^{-5} mol dm^{-3}, the ionic strength μ, was kept constant at 0.5 moldm^{-3} (NaCl), and at constant temperature of 37^{0}C. for each run 2 mL of the plasma albumin were taken in a quartz cuvette and kept in a spectrophotometer, while 2.0 mL each of the HCHO previously treated with 0.1 moldm^{-3} NaOH solution were taken in a 50 mL beaker and thoroughly mixed and was warmed in a in a water bath up to 37^{0}C then 2 mL of the formaldehyde mixture were taken and quickly mixed with the plasma albumin in the quartz cuvette using a syringe and the spectrophotometer light shield closed. The increase in absorbance was recorded at intervals of five seconds and the reaction was allowed to go to completion as indicated by little or no change in absorbance at λ max 235nm. The stoichiometry was then determined from a plot of mean absorbance against the mole ratio [HCHO]/[albumin] [7].

III. Results Discussion

Table 1.0 shows the absorbances with their corresponding mole fractions values obtained using the continuous variation method that was used in finding the stoichiometry of plasma albumin and formaldehyde reaction. Fig. 1.0 was obtained by plotting the absorbance versus mole fraction values shown in (Tables 1.0) based on the method of continuous variation [6]. From the plot obtained, tangents drawn on both sides of the maximum and perpendicular line from the middle point to the x- axes represented the mole fraction of the plot. From (Fig.1.0) a value of 0.26 was observed, this value is less than 0.5 which gives the mole ratio of the plasma albumin to formaldehyde as 1: 2 [6]. Using the similar procedure based on mole ratio method [7] the absorbance versus mole ratio values shown in (Table 1.1) were plotted and a value of 2 was observed in (Fig. 1.1) confirming that using both the methods, the mole ratio of the plasma albumin to formaldehyde was 1: 2.

IV. Conclusion

The stoichiometry of the reaction of plasma albumin and formaldehyde determined by the spectrophotometric measurement at pseudo –first order condition of formaldehyde using the methods of continuous variation (Job's method) and mole ratio method at λ max of 235nm at constant conditions, the ionic strength of the reactants was kept constant at 0.5moldm^3 (NaCl), and at constant temperature of 37^{0}C.The results showed a stoichiometric ratio of 1: 2 of plasma albumin to that of formaldehyde in aqueous medium. The result shows that for every mole of plasma albumin , two moles of formaldehyde are required to take up the free amino acids in this reaction. This information could go a long way in proposing the mechanism of this reaction.

Table 1.0 Stoichiometry of plasma albumin (0.03 moldm^{-3}) and (0.03 moldm^{3}) formaldehyde by continous variation method at λ max 235nm

Absorbance	Mole fraction
0.1	0.792
0.2	1.547
0.3	1.383
0.4	1.00

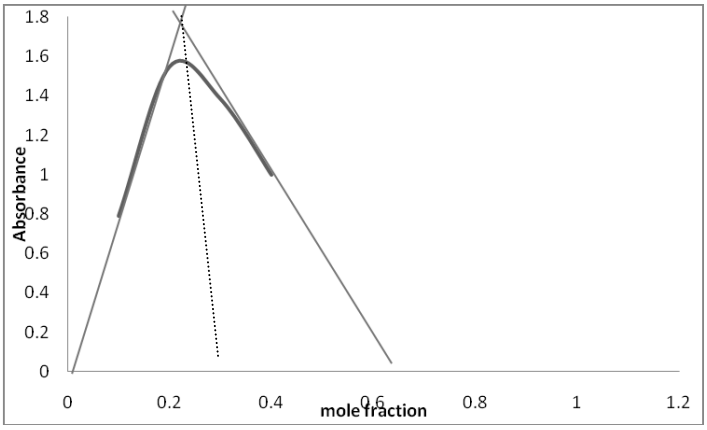

Fig. 1.0 Stoichiometry of plasma albumin (0.03 moldm^{-3}) and (0.03 moldm^{3}) formaldehyde by continous variation method

Table 1.1 Stoichiometry of formaldehyde and plasma albumin by mole ratio method at λ max ,235nm.

Mean absorbance	Mole ratio
0.0037	52.94
0.0029	5.29
0.0043	0.53
0.0028	0.05

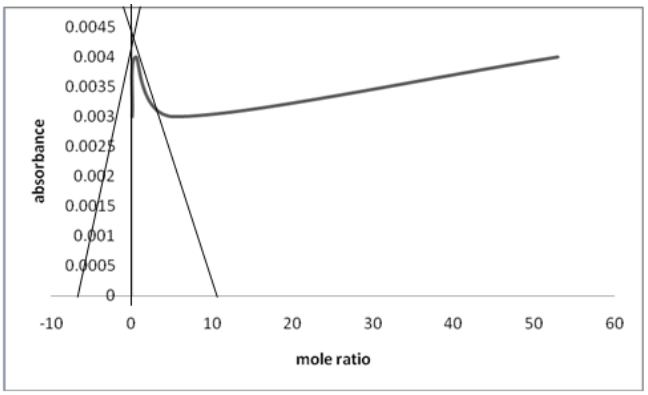

Fig. 1.1 Stoichiometry of plasma albumin by mole ratio method

Abbreviations:
HCHO : Formaldehyde
$PABNH_2$: Plasma albumin adduct
$H_2C(OH)_2$: Methylene glycol
$PABNHCH_2OH$] : Hydroxy methylol adduct
λ :Wavelenght of maximum absorption.

Competing interests:
The Authors wish to declare that there are no conflicts of interest associated with this paper.

Authors' contributions:
Professors Uzairu, A; Kwanashie, H.O and Idris,S.O conceived and designed the research as well as carefully proof read the manuscript. Ugye, TJ performed the analyses and prepared the draft manuscript.

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