Biuret Reaction of Transfusion Gelatin. I

Spectrophotometric Studies on the Interaction of Copper and Nickel with Transfusion Gelatin

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With 2 Figures

Summary

Absorption measurements of mixtures of copper-transfusion gelatin and nickel-transfusion gelatin were carried out with changing metal: protein ratio and at different pH-values (10—12.4). The addition of minute quantities of protein to cupric chloride gave a violet colour, and gradually increasing amounts of protein brought a change in colour from violet to red. The violet complex absorbed maximum light at 550 mμ whereas red complex absorbed at 525 mμ. The shift in λ max. with a change in metal: protein ratio has been ascribed to the formation of various types of chelate structure during the copper-transfusion gelatin biuret reaction. On the other hand nickel-transfusion gelatin biuret reaction has been characterised by the formation of only one type of complex corresponding to the violet complex of copper. λ max. for nickel complex was found to lie in the vicinity of 430 mμ.

Recent studies by MALIK and co-workers[3–11]) on metaltransfusion gelatin complexes have provided some valuable informations regarding the mode and extent of combination of the heavy metal ions to the different available sites of a fibrous protein molecule. The studies are generally made in the lower pH range and in some cases[3])[9]) up to pH 10, where the possibility of biuret reaction does not arise. It was, therefore, thought worthwhile to

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[3]) WAHID U. MALIK and SALAHUDDIN, J. Electroanal. Chem. **5**, 147 (1963).
[4]) WAHID U. MALIK and SALAHUDDIN, Nature **200**, 1204 (1963).
[5]) WAHID U. MALIK and SALAHUDDIN, Naturwiss. **50**, 401 (1963).
[6]) WAHID U. MALIK and M. MUZAFFARUDDIN, Naturwiss. **51**, 84 (1964).
[7]) WAHID U. MALIK and M. MUZAFFARUDDIN, J. Electroanal. Chem. **6**, 214 (1963).
[8]) WAHID U. MALIK and M. MUZAFFARUDDIN, J. prakt. Chem. **22**, 108 (1963).
[9]) WAHID U. MALIK and SALAHUDDIN, Indian. J. Chem. **1**, 203 (1963).
[10]) WAHID U. MALIK and SALAHUDDIN, Chemistry and Industry **17**, 706 (1964).
[11]) SALAHUDDIN and WAHID U. MALIK, J. Ind. chem. Soc. **40**, 703 (1963).

investigate the biuret reaction of this well characterised protein[12]). Copper and nickel were selected for the test metals, since transfusion gelatin readily combined with these metal ions in the vicinity of pH 12 and the resulting complexes gave a sharp absorption maxima in the visible region. The influence of the factors viz; concentration of the reactants, pH and ionic strength on the extent of the metal-protein combination are discussed in the present communication.

Experimental

Apparatus

Light absorption measurement were carried out by means of BAUSCH and LOMB Spectronic 20 and pH-values were measured on BECKMAN Model G-pH meter using glass electrode.

Reagents

Transfusion gelatin supplied by the Director, National Chemical Laboratory, Poona, India, was used throughout these investigations. Chemically pure (A.R.) samples of cupric chloride and nickel chloride were employed as source of metal ions. Metal content of the stock solution was estimated by complexometric titration[13]). Carbonate free potassium hydroxide and potassium chloride (A.R.) solutions were used to maintain the pH and ionic strength of the reaction mixtures respectively.

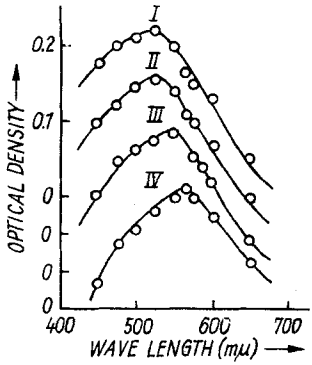

Fig. 1. Absorption spectra of copper-transfusion gelatin complex at pH 12.0. Curves I, II, III and IV for 1×10^{-3} M Cu + 2.1%, 1.8%, 0.9% and 0.3% protein respectively

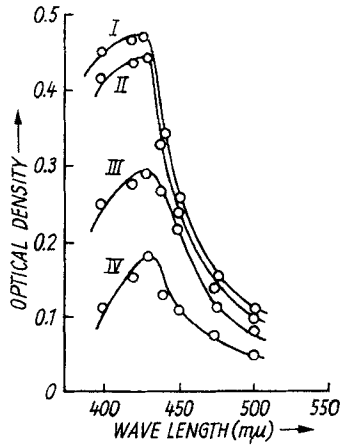

Fig. 2. Absorption spectra of nickel-transfusion gelatin complex at pH 12.0. Curves I, II, III and IV for 1.5×10^{-3} M Ni + 2.1%, 1.8%, 0.9% and 0.3% protein respectively

[12]) S. L. KALRA, G. SINGH and M. RAM, Ind. J. Med. Res. **46**, 171 (1958).

[13]) G. SCHWARZENBACH and H. IRVING, "Complexometric Titration". Interscience Publishers Inc. New York 1957.

Procedure

The procedure was essentially the same as outlined in the previous communications[8][14]. The molar extinction coefficient E of the metal ions was calculated by means of the expression $\log \frac{I_0}{I} = E\,Cd$ where "C" is the molar concentration of the metal ions, "d" is the depth of the cell (1/2") and $\log \frac{I_0}{I}$ is the observed optical density at λ max. The results are summarised in Tables 1 and 2. Figs 1 and 2 illustrate the effect of protein concentration on the λ max.

Discussion

The absorption studies carried out in a wide pH range (6.8 to 12.4 for copper-transfusion gelatin and 8.1 to 12.3 for nickel transfusion gelatin systems) at different wave lengths gave a maximum at 550 mμ for copper and at 430 mμ for nickel complex, showing thereby the binding of these metal ions to the peptide nitrogen atom. [The characteristic peak for copper-biuret complex[15]) is 565 mμ and for nickel-biuret complex[15]) is 433 mμ].

Table 1

Effect of pH, ionic strength metal and protein concentration on the extent of copper transfusion gelatin interaction

Concentration of cupric chloride: 1.0×10^{-3} M
Concentration of transfusion gelatin: 0.6%
Ionic strength: 0.15

A. Effect of pH

pH	6.8	8.9	10.5	11.4	11.8	12.0	12.2	12.4
λ max. (mμ)	625	565	550	550	550	550	550	550
E-values	76.0	100.0	132.0	136.0	140.0	142.0	145.0	147.2

B. Effect of ionic strength (T/2), λ max. 550, pH 12.0

T/2	0.075	0.150	0.225	0.300	0.450	0.60		
E-values	132.0	142.0	141.6	143.0	144.0	148.0		

C. Effect of metal concentration T/2: 0.15, pH: 12.0

Metal conc. $\times 10^{-3}$ M	0.2	0.5	1.0	1.5	2.0	2.5	3.0	4.0
λ max.	525	525	550	550	550	550	550	550
E-values	240.1	163.7	142.4	128.0	128.0	134.4	136.0	136.0

D. Effect of protein conc., T/2: 0.15, pH 12.0

Protein conc. %	0.3	0.6	0.9	1.2	1.5	1.8	2.1
λ max.	565	550	550	550	525	525	525
E-values	128.0	142.4	148.0	156.0	160.0	164.0	176.0

[14]) Wahid U. Malik and M. Muzaffaruddin, J. prakt. Chem. **28**, 129 (1965).

[15]) M. Kato, Y. Komuro and K. Sone, Nippon Kagaku Zasshi **77**, 308 (1956); M. Kato, Y. Komuro and K. Sone, J. chem. Soc. Japan, Pure Chem., Sect. **76**, 1034 (1955); ibid. **75**, 1134 (1954).

Table 2

Effect of pH, ionic strength metal and protein concentration on the extent of nickel transfusion gelatin interaction

Concentration of transfusion gelatin: 0.6%
Concentration of nickel chloride: 1.5×10^{-3} M
Ionic strength: 0.15

A. Effect of pH, λ max.: 430 mμ

pH	8.1	9.1	10.5	11.3	12.0	12.3		
E-values	54.4	96.0	138.7	141.4	144.0	144.0		

B. Effect of ionic strength, pH 12.0, λ max.: 430 mμ

Ionic strength	0.075	0.150	0.225	0.300	0.450	0.600		
E-values	142.9	144.0	144.0	145.1	146.7	148.3		

C. Effect of metal concentration, pH 12.0, ionic strength 0.15, λ max.: 430 mμ

Metal conc. $\times 10^{-3}$ M	0.30	0.65	1.50	2.25	3.0	3.75	4.50	6.00
E-values	266.0	184.6	144.0	128.0	122.7	128.0	121.8	114.7

D. Effect of protein concentration, pH 12.0, ionic strength: 0.15, λ max.: 430 mμ

Protein conc. %	0.3	0.6	0.9	1.2	1.5	1.8	2.1
E-values	96.0	144.0	154.6	165.4	191.5	234.7	256.4

Metal: protein ratio appears to exert a profound influence on the binding of copper and nickel to the protein. From the results (tables 1 and 2) it may be concluded that the presence of a large proportion of metal ions in the reaction mixture brings about a decrease in the binding capacity. On the other hand, the presence of larger amounts of protein invariably brings about an increase in the binding of metal ions (as evident from the increase in molar extinction coefficient values). An interesting feature of the copper-transfusion gelatin biuret reaction is that, the addition of small amounts of protein to a fixed amount of cupric chloride gave a violet coloured complex. This complex has an absorption maximum at 550 mμ. The absorption maximum gradually shifted towards shorter wave length with a change in colour from violet to red when an increasing amounts of protein has been added gradually to the cupric chloride solution (Fig. 1). It is, therefore, concluded that copper forms various types of chelate structure[16]) during the copper-transfusion gelatin biuret reaction. Among the several possible structures the two prominent types namely violet and red could be realised by spectrophotometric method. It appears plausible that copper combines primarily with two or more nitrogen atoms of the adjacent polypeptide chane at low protein concentration, and when the protein presents in larger quantities the participation of oxygen atoms (enolised peptid chane) in the binding process

[16]) A. C. JENNINGS, Aust. J. Chem. **16**, 989 (1963); ibid. **16**, 1006 (1963).

increases progressively, thus the colour of the comples changed violet to red and also λ max. shifted towards the shorter wave lengths.

Nickel transfusion gelatin biuret reaction exhibits an altogether different phenomenon. The increase in protein concentration although failed to bring about any significant change in λ max. (Fig. 2) the molar extinction coefficient has been greatly enhanced. This leads to the conclusion that nickel-transfusion gelatin biuret reaction is characterised by the formation of only one type of complex. This complex, of course, may have a structure similar to that of violet complex of copper. The increase in the molar extinction coefficient may easily be understood, since greater proportion of protein provides a large number of active sites through which metal ions can be fixed.

It is evident from tables 1B and 2B that, the ionic strength has got very insignificant effect on both the reaction, except that cupric ion binding is facilitated more in presence of larger amounts of potassium chloride than nickel binding. The molar extinction coefficient of copper transfusion gelatin complex exhibited a 12% change when ionic strength is changed from 0.075 to 0.600 where as nickel-transfusion gelatin reaction showed only 4% change.

Thanks are due to Prof. A. R. KIDWAI for providing facilities and to Prof. K. VENKATARAMAN, Director, National Chemical Laboratory, Poona, India, for the supply of transfusion gelatin.

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Bei der Redaktion eingegangen am 18. August 1964.