# Spectrophotometric Studies on the Binding of Cr(iii) to Bovine Serum Albumin

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With 2 Figures

## Summary

Spectrophotometric studies of mixtures containing Cr(iii) and bovine serum albumin were carried out to know the binding of chromic ion to the serum protein. Experiments performed at varying metal:protein ratio and at different pH values gave a maximum at 580 mμ. An inflexion was obtained in molar extinction coefficient-pH curves at a pH value corresponding to the pK values of carboxyl groups. All these go to prove that the carboxyl groups are likely to be the Prinzipal sites through which the fixation of chromic ions have been affected. Experiments carried out in the neutral pH region gave no evidence regarding the binding of metal ions to the imidazole groups of bovine serum albumin. The lesser reactivity of bovine serum albumin in comparison with that of transfusion gelatin (previously studied) has been attributed to the lack of effective multipoint sites, due to its folded structure, so as to fix several chromium atom intramicellarly.

Chromic ions are known to play an important role in chrome tanning. Although the chromium gelatin system was studied previously by GUSTAVSON[1][2] and KUNTZEL[3][4] their investigations were limited to the protein which were not well characterised in terms of hydrogen ion equilibria and molecular weight. Therefore, it was thought necessary to reconsider the problem critically employing a simpler variety of gelatin. In a recent communication[5] evidence has been presented for the irreversible fixation of chromic ions through carboxyl groups of transfusion gelatin. These studies however preceded by an extensive investigation on hydrogen ion binding of

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[1]) K. H. GUSTAVSON, "Advances in protein chemistry" **5**, 353 (1949).

[2]) K. H. GUSTAVSON, J. Amer. chem. Soc. **74**, 4608 (1955).

[3]) A. KUNTZEL and C. RIESS, Collegium 138 (1936).

[4]) A. KUNTZEL, Kolloid. Z. **19**, 152 (1940).

[5]) WAHID U. MALIK and M. MUZAFFARUDDIN, J. prakt. Chem. [4] **22**, 108 (1963).

transfusion gelatin[6]). During these investigations it occurred to us that the spectrophotometric method could be extended to still more simpler system like-chromium-bovine serum albumin-and valuable information could be made available regarding the mode of chromic ion binding to the proteins. Hence the detailed spectrophotometric studies on the chromium-bovine serum albumin interaction dealing with a number of factors viz., effect of pH, anions of the buffer solution, ionic strength and concentration of the reactants, on the extent of metal-protein interaction are incorporated in the present communication.

## Experimental

Apparatus: Light absorbtion measurements were carried out by means of Beckman DU spectrophotometer using tungsten lamp as the light source and corex cell (1 cm depth). Beckman Model G. pH meter was employed for measurement of pH.

Reagents: Crystalline bovine serum albumin (donated by Dr. R. C. KAPOOR, Allahabad, India) was used and its solution was prepared by direct weighing. Chemically pure sample of chromic chloride (Baker A. R.) was dissolved in triply distilled water (distilled in all glass apparatus). Metal content of the stock solution was determined by spectrophotometric method as recommended by GREEN and ANG[7]). A. R. potassium hydroxide and potassium chloride were used to prepare their solutions. These solutions were used to maintain pH and constant ionic strength respectively. Walpole acetate buffers were prepared from 0.2 M solutions of acetic acid and sodium acetate and their pH-values were checked.

Procedure: i-Bovine serum albumin (1.0%) and chromic chloride ($1 \cdot 10^{-2}$ M) were mixed in a number of Pyrex boiling tubes, their pH-values were adjusted to 3.7, 4.4, 4.8, 5.2, 5.5, 5.9 and 6.3 by the addition of buffers. Total volume was made upto 10 ml. ionic strength being kept at 0.15. Another set in which the pH-values were adjusted by the addition of dilute potassium hydroxide instead of buffers was also analysed spectrophotometrically.

(ii) At a fixed pH (5.5) and ionic strength (0.15) direct and reverse titrations were carried out. The mixture for direct titration contained fixed amount of chromic chloride and varying amounts of protein, whereas the mixtures for reverse titration contained fixed amount of protein and varying amounts of chromic chloride.

(iii) The effect of ionic strength was also investigated, taking mixtures of varying concentration of potassium chloride (0.15, 0.2, 0.4 and 0.5 M) at fixed metal and protein concentrations.

The molar extinction coefficient "E" of chromic ion was calculated by means of the expression

$$\log \frac{I_0}{I} = ECd$$

where "c" is the molar concentration of chromium, "d" is the depth of the cell (1 cm.) and $\log I_0/I$ is the observed optical density at $\lambda_{max} = 580$ mμ. The results are summarised in the following tables.

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[6]) WAHID U. MALIK and SALAHUDDIN, J. Electroanal. Chem. **5**, 68 (1963).

[7]) R. W. GREEN and K. P. ANG, J. Amer. chem. Soc. **77**, 5482 (1955).

Table 1
Effect of pH, metal and protein concentration on the chromium-bovine serum albumin interaction

A. Protein concentration = 1.0%, $[Cr^{+3}] = 1 \cdot 10^{-2}$ M, $\mu = 0.15$

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| pH-values (acetate buffers) | 3.7 | 4.4 | 4.8 | 5.2 | 5.5 | 5.9 | 6.3 |
| E-values (at 580 mμ) | 15.2 | 15.8 | 18.3 | 19.8 | 21.6 | 22.6 | 23.0 |
| pH-values (with KOH) | 3.6 | 4.1 | 4.4 | 4.65 | 4.8 | 5.15 | |
| E-values (at 580 mμ) | 22.0 | 22.5 | 23.5 | 24.8 | 25.9 | 27.6 | |

B. Protein concentration = 1.0%, pH = 5.5, $\mu = 0.15$

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $[Cr^{+3}] \cdot 10^{-2}$ M | 0.5 | 1.0 | 1.5 | 2.0 | 3.0 | | |
| E-values at 580 mμ | 29.0 | 21.6 | 18.1 | 16.4 | 15.8 | | |

C. $[Cr^{+3}] = 1 \cdot 10^{-2}$ M, pH = 5.5, $\mu = 0.15$

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Protein concentration % | 0 | 0.25 | 0.5 | 0.75 | 1.25 | 1.5 | |
| E-values (at 580 mμ) | 14.8 | 15.6 | 17.4 | 19.8 | 22.6 | 23.2 | |

Table 2
Effect of ionic strength on chromium-bovine serum albumin interaction
Protein concentration = 1.0%, $[Cr^{+3}] = 1.0 \cdot 10^{-2}$, pH = 5.5

| | | | | |
|---|---|---|---|---|
| Ionic strength (μ) | 0.15 | 0.2 | 0.4 | 0.5 |
| E-values (at 580 mμ) | 21.6 | 22.0 | 22.6 | 22.8 |

## Discussion

The absorption studies carried out in the pH range 3.7 to 6.3 at different wave lengths (Fig. 1) gave a maximum at 580 mμ [8]), indicating thereby that the metal ions get bound through the carboxyl groups of bovine serum albumin. The participation of imidazole groups in the interaction process

[8]) M. MUZAFFARUDDIN, SALAHUDDIN and WAHID U. MALIK, J. Ind. Chem. Soc. **40**, 467 (1963).

seems to be less probable, since even at pH 6.3 the metal-protein mixtures show no detectable shift in absorption maximum. This clearly shows that the nature of metal-protein linkage is not radically different in the entire experimental pH range. Furthermore, the molar extinction coefficient values when plotted against pH (Fig. 2 A and B), gave S-shaped curves with an inflexion near about pH 5.0. These results again lead to the conclusion that, the carboxyl groups offer themselves as the principal sites for chromic ion

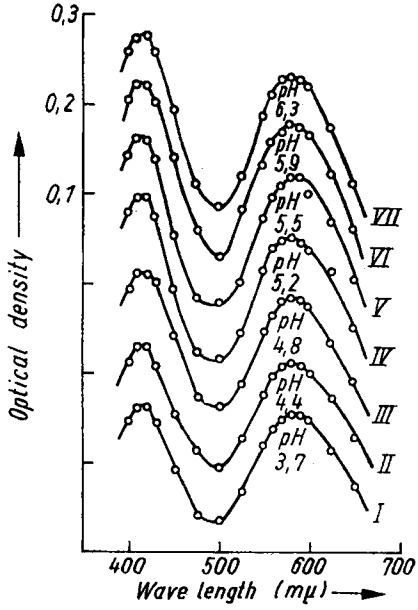


Fig. 1. Absorption spectra of chromium-bovine serum albumin complex. Curves I to VII for $1 \times 10^{-2}$ M $Cr^{+3} + 1.0\%$ protein at different pH-valves in accetate buffer

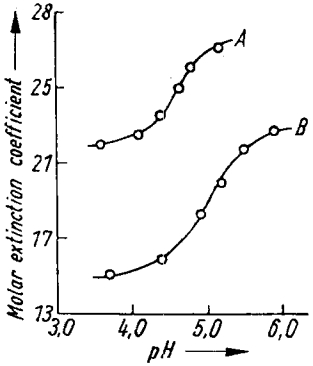


Fig. 2. Effect of pH on molar extinction coefficient. Curve A with KOH and curve B with acetate buffer; protein concentration 1.0% and chromium concentration, $1 \times 10^{-2}$ M

binding. The lesser reactivity of chromic ions in acetate medium (as indicated by the low extinction coefficient values Fig. 2, Curve B) in comparison with that of KOH medium may, however be attributed to the tendency of chromic ions to form a uncharged chloro species [9]) $(Cr_2(OH)_2Cl)^0$ in acetate medium. The bulky uncharged chloro complex seems to have got very little affinity for carboxyl groups.

Metal protein ratio also seems to be a predominant factor which controls the reaction. As reported earlier [5])[10])[11]) the variation in molar extinction

[9]) K. H. Gustavson, J. Colloid. Sci. **1**, 397 (1946).

[10]) Wahid U. Malik and Salahuddin, Naturwiss. **50**, 401 (1963).

[11]) Wahid U. Malik and Salahuddin, Ind. J. Chem. **1**, 203 (1963).

coefficient values of two sets of mixtures (Table 1 B and C) points towards the fact that the larger amount of protein in the reaction mixture greatly facilitates the reaction whereas the larger proportion of metal ions in the reaction mixture considerably decreased the extent of metal-protein combination. The effect of neutral salt concentration is evident from the increase in molar extinction coefficient values with increase in the concentration of potassium chloride (only a 5% increase in E-values is noted over 3 fold increase in neutral salt concentration Table 2).

One of the most interesting features of the chromic ion binding to the two different types of proteins (i. e. transfusion gelatin and bovine serum albumin) is that, bovine serum albumin with its greater number of carboxyl groups[12]) (100) has got very less binding capacity in comparison with that of transfusion gelatin[5]). The lesser reactivity of bovine serum albumin itself reveals the structural difference between the fibrous and globular protein. The fibrous protein with its peptide chain being more or less extended and oriented in parallel pattern may form a intra- or intermicellar cross linking. This type of linkage as demonstrated by GUSTAVSON[1]) may contain several chromium atoms by means of two or more carboxyl groups of adjacent protein chains. In fact KUNTZEL[13]) has shown that intramicellar combination occurred between collagen and vegetable tannins. On the other hand, globular proteins have a compact and folded structure and such a multipoint combination may not be possible in case of serum albumin. Hence low binding capacity of bovine serum albumin may be attributed to the lack of such effective multipoint sites, so as to fix several chromium atoms intramicellarly.

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[12]) C. TANFORD, S. A. SWANSON and W. S. SHORE, J. Amer. chem. Soc. **77**, 6414 (1955).
[13]) A. KUNTZEL, Collegium 207 (1929).

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