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# Studies on Interaction Between Molybdenum(VI) and Human Serum Albumin By pH Displacement Method

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The interaction of Mo(VI) has been studied with human serum albumin using pH displacement method at two different temperatures and pH values. The apparent association constants and binding sites have been calculated with the help of Scatchard method. The binding data are suggested to be non-linear behaviour at pH 5.38 and 7.50 while linear behaviour at pH 9.85 and 11.50. The order of binding parameter and thermodynamic constants suggested that the interaction is pH and temperature dependents. The results are concluded that the guanidinium sites are major molybdenum ion binding sites.

**Key Words: Human serum albumin, Molybdenum(VI), pH Displacement method.**

## INTRODUCTION

Owning to several biological implications of molybdenum[1-9], its interaction with proteins have been extensively investigated[10-20]. However, a literature survey indicates that its interaction with human serum albumin (HSA) has not been studied so far. The present paper describes the results on the binding of molybdenum(VI) with human serum albumin using pH displacement measurements. The effect of pH and temperature on the binding constants has been discussed.

## EXPERIMENTAL

A solution of human serum albumin (HSA) (Sigma Chemical, m.w. 69,000) was prepared in double distilled water. The strength of the protein solution was determined by drying its known volume in an air oven at 105-110 °C. Sodium molybdate (E. Merck) was dissolved in double distilled water and its molybdenum content was determined gravimetrically. Solutions of hydrochloric acid and potassium hydroxide were prepared from reagent grade chemicals. A potassium chloride solution was used for the maintenance of ionic strength of the reaction mixtures. The pH measurements were made on an Elico digital pH-meter using a wide range combined electrode. The instrument was standardized against 0.05 M potassium phthalate (pH 4.0) and 0.05 M borax (pH 9.2) for the acidic and basic ranges, respectively. 10 mL of 0.3 % HSA solution of different initial pH values were titrated against 0.1 M

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sodium molybdate solution of the same initial pH as that of protein. The pH values of the mixtures were recorded immediately after mixing. These measurements at pH 5.35, 7.50, 9.85 and 11.50 were carried out at 25 and 40 °C, respectively.

## RESULTS AND DISCUSSION

The pH values of different experiments are shown in Fig. 1. This shows a progressive fall in the initial pH of HSA with increasing molybdenum concentration between pH 5.35 to 11.50. The difference in pH of protein and protein-molybdenum mixtures is used to indicate ΔpH. The negative ΔpH values are in line with those earlier reported by Arora *et al.*[13]. The negative or positive sign of ΔpH indicates the interaction of molybdenum to HSA either as cation or anion. The difference in pH of the solution was taken as the index of molybdenum binding to the reactive groups of HSA. The number moles of molybdenum bound to per mole of HSA was calculated by the following relation of Scatchard *et al.*[21].

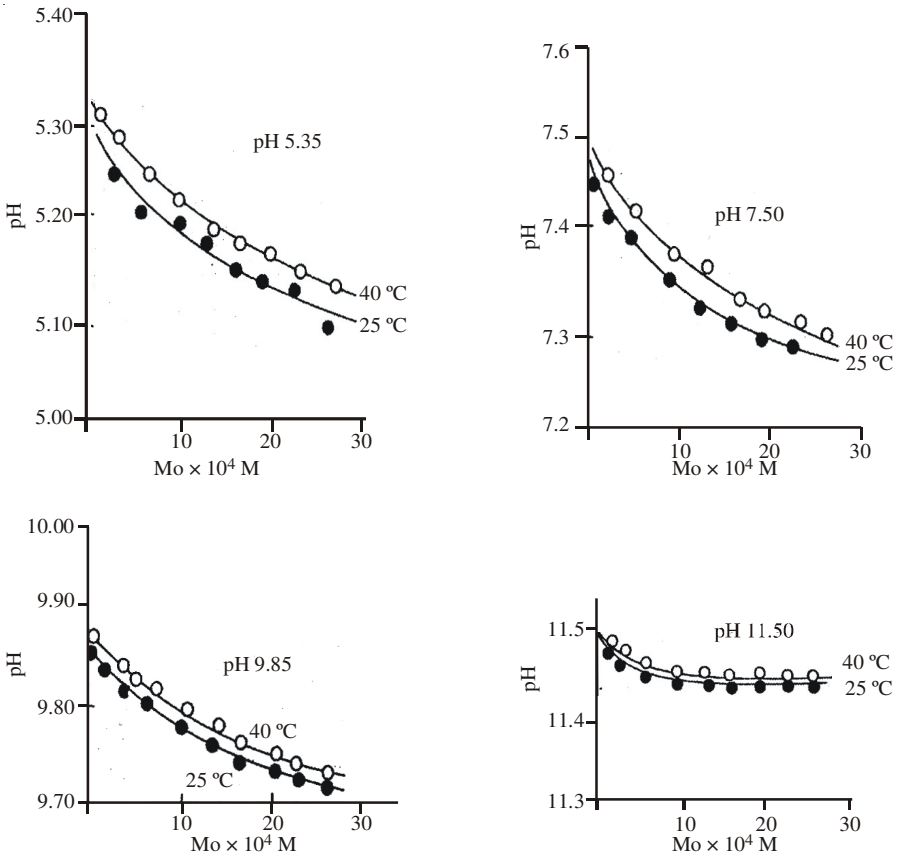


Fig. 1. pH plotted against Mo(VI) × $10^{-4}$ M concentration in HSA-Mo(VI) system at 25 ºC and 40 ºC

$$\Delta pH = \frac{2W}{2.3}\Delta V \qquad \text{and} \qquad \Delta V = \frac{2.3}{2W}\Delta pH$$

where $\Delta V$ is the moles of molybdenum bound per mole of HSA (*i.e.*, $V_M$) and W is the electrostatic interaction factor[22].

The binding data are shown in Figs. 2-5 in the form of logarithmic plots *i.e.*, $V_M$ is plotted against free log concentration of molybdenum. These binding isotherms at pH 5.35 and 7.50 are sigmoidal due to the attainment of saturation at higher log free concentrations of molybdenum. The $V_M$ values at the saturation limit correspond to the maximal number of binding sites (n) occupied by the molybdenum(VI) on HSA surface. The value of log free concentration at half saturation corresponds to the logarithm of association constant (log K) of interaction. The value of log K and n binding sites evaluated from sigmoidal isotherms compare favourably with those determined by other methods. However, the nature of isotherms at pH 9.85 and 11.50 were found to be different than at other pH values. The differences between the two pH levels indicated nonidentical set of sites beyond these pH values. Thus, the nature of HSA sites as well as that of molybdenum species appears to be different at varying conditions of pH values.

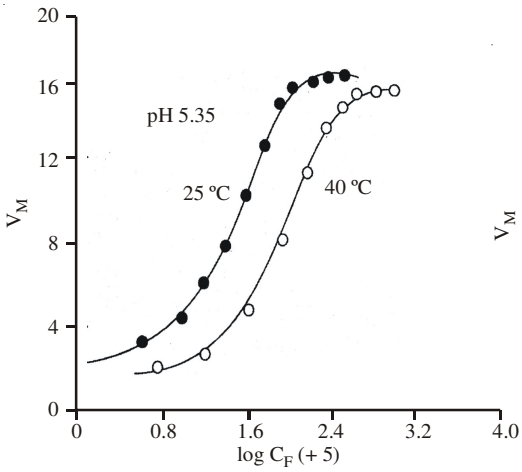


Fig. 2. Moles of Mo(VI) bound/mol of HSA ($V_M$) against log free concentration (log $C_F$ + 5) at pH 5.35 at 25 ºC and 40 ºC

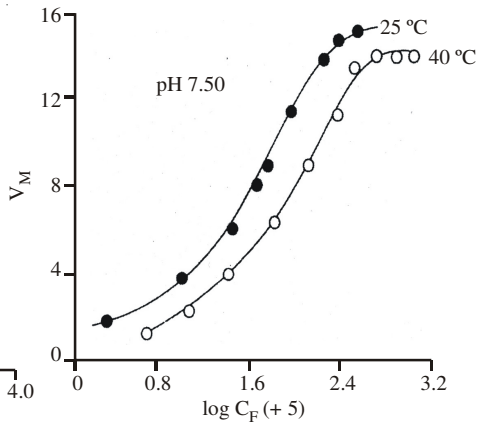


Fig. 3. Moles of Mo(VI) bound/mol of HSA ($V_M$) against log free concentration (log $C_F$ + 5) at pH 7.50 at 25 ºC and 40 ºC

The binding data, *viz.*, $V_M$ and $C_F$ were analyzed by means of Scatchard equation[23] in the form of $V_M/C_F = n \cdot K - K \cdot V_M$, where $V_M$ and $C_F$ represents to mole of ligand bound per mole of HSA and free molar concentration of molybdenum(VI) at equilibrium, K is the average apparent association constant for binding at each site and n is the average maximal number of binding sites on protein molecule with the same association constant K. If all the binding sites are equivalent and independent, a

plot of $V_M/C_F$ against a function of $V_M$ would give a straight line such that the intercept on the $V_M/C_F$ is nK as $V_M$ approaches to zero as a limit. The results in the form of $V_M/C_F$ *versus* $V_M$ plots are shown in Fig. 6. These plots at pH values 5.35 and 7.50 exhibited nonlinear nature. The anomalous nature of plots reflects the occurence of nonidentical sites[24]. Furthermore, the combination of the electrostatic factor to the interaction and the changes in the ionic species of the interacting ligand may also produce above type of anomalous plots[25]. The occurrence of heterogeneous, non-saturable sites of low binding affinity or of nonspecific. Yet saturable site will produce such deviation[13].

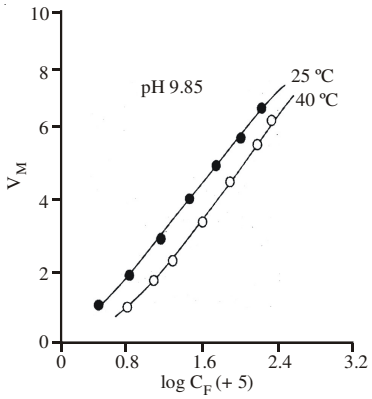


Fig. 4. Moles of Mo(VI) bound mol of HSA ($V_M$) against log free concentration (log $C_F$ + 5) at pH 9.85 at 25 ºC and 40 ºC

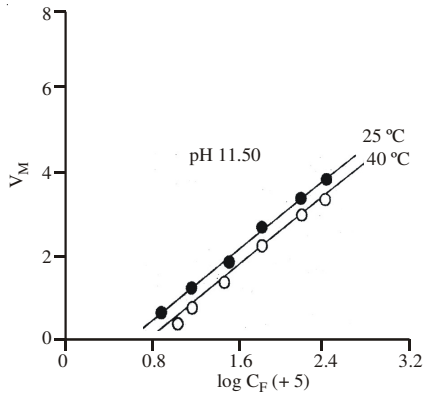


Fig. 5. Moles of Mo(VI) bound mol of HSA ($V_M$) against log free concentration (log $C_F$ + 5) at pH 11.50 at 25 ºC and 40 ºC

A linear relationship was found to exist in Scatchard plots at pH values 9.85 and 11.50, which revealed the involvement of single class of sites in the interaction process. If a second class of site exists, it should have a much smaller association constant than expected for the primary binding sites. The maximal number of binding sites (n) and their logarithm of average association constants (log K) are compiled in Table-1. In case of non-linear plots the values of $n_1$, $n_2$ *etc*. and their association constants could be determined from modified Scatchard method.

TABLE-1
BINDING CONSTANTS OF HSA-Mo(VI) INTERACTION
FROM pH DISPLACEMENT MEASUREMENTS

| pH | 25 ºC | | | | | 40 ºC | | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | log K | ΔGº (kcal/mol) | ΔHº (kcal/mol) | ΔSº (cal/deg/mol) | n | log K | ΔGº (kcal/mol) | ΔSº (cal/deg/mol) |
| 5.35 | 18 | 3.691 | -5.015 | -3.686 | 4.459 | 16 | 3.561 | -5.082 | 4.460 |
| 7.50 | 14 | 3.505 | -4.762 | -3.402 | 4.563 | 12 | 3.385 | -4.831 | 4.565 |
| 9.85 | 9 | 3.387 | -4.602 | -3.119 | 4.976 | 8 | 3.277 | -4.677 | 4.977 |
| 11.50 | 4 | 3.352 | -4.554 | -3.260 | 4.342 | 4 | 3.237 | -4.620 | 4.345 |

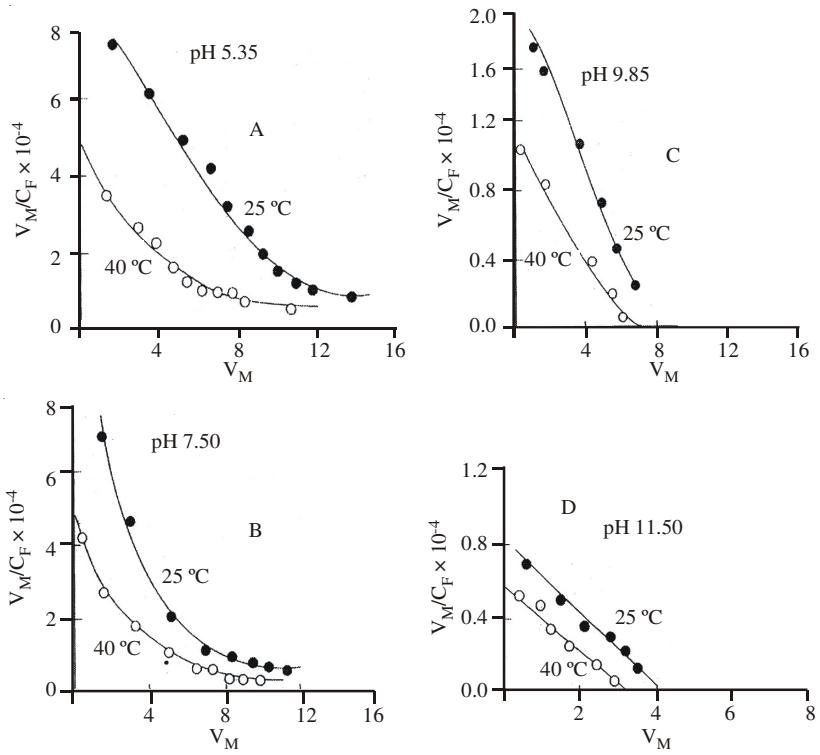


Fig. 6. Moles of Mo(VI) bound mol of HSA ($V_M$) against $V_M/C_F$ at pH 5.35 (A) 7.50 (B), 9.80 (C) and 11.50 (D) at 25 ºC and 40 ºC

It is evident from the data of Table-1 that the value of n decreases with increasing of pH, hence free cationic groups on HSA surface are the main binding sites. The availability of a fewer binding sites show that all the cationic groups are not exposed to interact with molybdate ions. Infact out of the total cationic sites only 18, 14, 9 and 4 are involved at pH 5.35, 7.50, 9.85 and 11.50, respectively at 25 ºC. The reason for the involvement of a smaller number of binding sites, may be a conformational one. This protein possesses a significant structure brought about by hydrophobic interactions hydrogen bonding and other intersegmental cohesive forces[26,27]. However, since the folded core of the globular protein is known to be inaccessible to water, a rough estimate of 40-60 % of the total sites may be available to interact with ligand molecules. Considering this constraint imposed by the chain folding of HSA, it appears that ion binding to this protein occurs in a restricted way[28]. The lesser value of binding sites (n) at pH 9.85 and 11.50 could also be explained on the basis of successive deprotonation of protein groups. The pK values for histidyl and lysyl amino acid residues are 6.90 and 9.85, while for the guanidinium of arginyl residue, it is greater than pH 12[29]. Thus, at pH 9.85 and 11.50 the sites involved in molybdenum(VI) interaction must be the guanidinium groups of HSA. However, the involvement of a smaller number of guanidinium groups could be due to a large

amount of electrostatic repulsion between anionic protein and the molybdate ions and also some of these groups may be irreversibly bonded to the carboxylate residues[13].

The molybdenum(VI) ion binding by HSA is observed to be greatly modified by pH and temperature. The number of binding sites progressively decreases with increasing pH and temperature but log K values remains nearly in the same vicinity. The significance of the uniformity of association constants is that only a single primary class of site is reacting and the occurrence of their different number is therefore not responsible for diminished interaction which may then be due to the decreased availability of the same class of sites owing to irreversible effects in the HSA structure. Klotz *et al.*[30] have suggested that protein becomes either swollen or unfolded when the negative charge increases. As the pH increases and the charge becomes more negative, electrostatic repulsion causes an even greater opening up of the protein until a maximum is reached, as suggested by the diminished molybdenum ion binding sites. The free energy (ΔG°), enthalpy (ΔH°) and entropy (ΔS°) changes of the above interaction were computed by the following standard expressions:

$$\Delta G^{o} = -2.303\ RT \log K$$

$$\Delta H^{o} = \frac{2.303RT_1T_2}{T_2 - T_1} \log \frac{K_2}{K_1}$$

$$\Delta S^{o} = \frac{\Delta H^{o} - \Delta G^{o}}{T}$$

where $K_1$ and $K_2$ are average association constants at $T_1$ and $T_2$ absolute temperatures, respectively. The values of thermodynamic parameters are given in Table-1. The negative values of enthalpy is attributed to covalent linking between nitrogen and molybdenum atoms[29,31]. The negative values of enthalpy (ΔHº) of the interaction of Mo(VI) and HSA indicated that the binding is mainly enthalpy driven[32]. The negative ΔHº value observed can not be mainly attributed to electrostatic interactions since for electrostatic interaction ΔHº is very small, almost zero[33]. The entropy (ΔSº) value was unfavourable for it. A positive ΔSº value is frequently taken as a typical evidence for hydrophobic interaction from the point of view of water structure. The extent of entropy values explained the changes in the structure of the molybdate species. The positive sign of entropy shows the release of water molecules from hydrated HSA and molybdenum species and that the configuration of the HSA is changing to a random coil as the complex formation occurs. The higher values of entropy and enthalpy changes at pH 9.85 possibly indicate that all the above mentioned factors may be involved at this pH.

The results of pH displacements revealed an interaction between molybdate ion and positive loci of HSA molecule. It is concluded that the guanidinium sites are major molybdenum ion binding sites. The order of affinity is found to be: guanidinium > imidazolium > ε-ammonium, which is inclose agreement with reported polarographic and equilibrium dialysis results[13,19,20]. However, owing to the complex structure of HSA molecule, it is difficult to ascertain which groups are really involved in the binding with molybdate species.

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