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Interaction Between Soyabean Protein and Molybdate Ions

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The interaction between soyabean protein and molybdate ions has been studied employing transmittance measurements. In the acidic range, the stoichiometry of the combination between molybdate ion and protonated nitrogen groups of soyabean was found to be one-to-one in the pH range 5.0 to 7.0 and higher ratio at pH-values. The pH-dependent binding result has been ascribed to the existance of anionic as well as cationic species of molybdenum(VI). The effect of pH on the formation constants and free energy changes of molybdenum(VI)-soyabean system has been discussed.

Key Words: Soyabean, Molybdenum, Transmittance.

INTRODUCTION

Many workers have studied polyanion-polycation combinations to throw some light on the formation of cell matrix in livings. A few workers[1,2] have made use of increased intensity and salt tolerance to prepare essentially insoluble polyanion-polycation complex from polyions of very high charge density. The complexes of polysaccharides with proteins[3] and proteins with DNA[4] also fall in the same category. Other similar reactions have been carried out because of the possible role of proteins as regulatory substances in inducible enzyme systems[5-7]. The precipitation of BSA by anionic polyelectrolytes like polymethyl acrylic acid has been used for its fractionation[8]. Polyacrylic acid has also been used to separate catalase from other proteins with which it is normally associated[9]. The precipitation of proteins by cationic surfactants is used to isolate proteins from human serum[10]. Malik and coworkers[11,12] and Arora *et al.*[13] made detailed physico-chemical studies of insoluble complex formation between proteins and molybdic acid. These workers also made systematic investigations on the interaction of silicic acid with proteins in the wide pH range[14].

This paper reports the study of the reactions of molybdic acid with soyabean protein (SBP), a vegetable protein, with the help of physico-chemical methods. Of the several methods tried, only turbidimetric (transmittance) method was found to be suitable for the quantitative determination of the binding of Mo(VI) with this

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protein. The significance of this technique is well established in may ways. It has been used for the estimation of microamounts of sulphur in proteins and related compounds[15] at one hand, and for the study of protein-polysaccharide complexes on the other[16]. The appearance of turbidity with time in many reactions has been used for measuring the kinetics of the reaction. The present study was under taken in view to determine whether the precipitation of protein as Mo(VI) salts could provide the basis for an analysis of the components of protein mixtures. In this paper we have calculated the quantity of SBP and Mo(VI), both in solution and in the precipitate over a wide range of initial reagent concentrations.

EXPERIMENTAL

Sodium molybdate (E. Merck) was dissolved in distilled water and its molybdenum content was determined gravimetrically. Buffers and other solutions were prepared from reagent grade chemicals. A potassium chloride (BDH) solution was used for maintenance of ionic strength of the reaction mixtures.

Protein solution: Soyabean protein (SBP) was extracted from soyabean powder (BDH) by alkali extraction followed by the gradual addition of HCl to lower the pH to the isoelectric point[17]. It was dissolved in dilute alkali solution and centrifused to obtain the clean solution. The concentration of protein solution was determined by colorimetric method. It was stored in a refrigerator and purified toluene was added to check its surface denaturation.

Transmittance measurements: The transmittance of SBP-Mo(VI) mixtures, which can be taken as an index of the turbidity developed, was measured by means of the Elico Spectrophotometer of 375 nm at 30 ºC. Three types of experiments were prepared.

(i) 0.178g of SBP was mixed with different amount of sodium molybdate in different boiling tubes. The pH was adjusted to 1.99, 3.09, 3.95 and 4.59 with the help of buffers keeping total volume 10 mL (ionic strength 0.15 M). (ii) Fixed amount of sodium molybdate and different concentrations of SBP (0.178 to 2.0 %) were taken as in (i). The pH was again adjusted as in (i) keeping the total volume 10 mL. (ionic strength 0.15M). (iii) A fixed amount of both SBP (1.78 g/L) and Mo(VI) (30×10^{-4} M) were mixed and the pH-values of the mixtures were adjusted with the help of desired buffers. (iv) In sets (i) and (iii) there was complete separation of the precipitate. The separated protein was first removed by centrifugation and soluble protein and Mo(VI) concentrations of the supernatent solutions were determined by the usual methods. The Mo(VI) bound to the soluble fraction was calculated in the usual way from the free Mo(VI) concentrations.

RESULTS AND DISCUSSION

Soyabean protein (SBP) gave insoluble complexes with Mo(VI) below its isoelectric point (IEP). The different aspects of the complex formation such as pH, protein and Mo(VI) concentrations are critically discussed in this paper.

Effect of pH on transmittance: The plots of SBP revealed a minimum at pH 4.50 (Fig. 1) which corresponds to the isoelectric point of this protein. On adding Mo(VI), the transmittance decreases sufficiently and the minimum changes from pH 4.50 to 4.20. This shift can be explained in terms of the reaction of Mo(VI) anions with the cationic groups of SBP. Due to the binding Mo(VI) ions to positively charged nitrogen groups, the total positive charge on SBP molecule decreases. This explains the displacement of its IEP towards more lower pH value.

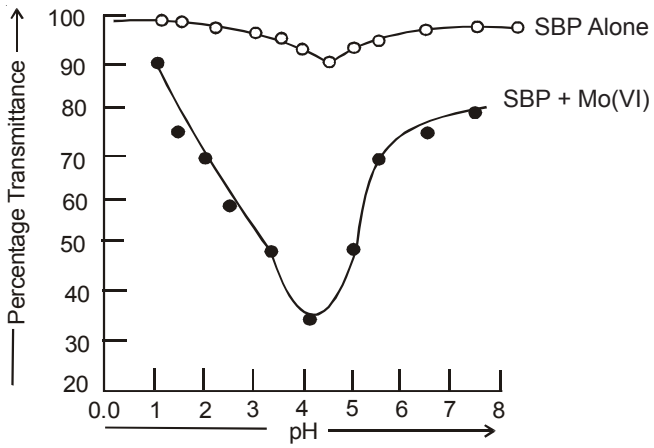

Fig. 1. Effect of pH on the transmittance of SBP and SBP + Mo(VI) systems

Stoichiometry from transmittance titrations: The binding of Mo(VI) to SBP could be measured by means of direct (Fig. 2) and reverse titrations (Fig. 3). The inflection in log T *vs.* concentration curves show the point where the formation of insoluble complex is complete like those in conductometric and amperometric titrations involving precipitation[18]. Further, if this does not indicates completion of reaction than with the addition of more Mo(VI) there would be an even larger decrease of transmittance (T). From the inflections in the curves, the number of moles of Mo(VI) bound per 10^5 g of SBP (V_M) were computed using the relationship, $V_M = C_B/[P]$, where C_B is the molar concentration of bound Mo(VI) and [P] the molarity of SBP (Table-1). In these calculations, it is assumed that the complete consumption of SBP or Mo(VI) depending upon the type of titration (direct or reverse).

Instability constant of precipitation reaction: For a reaction involving precipitation, the equilibriuim can be shown by the following reaction[19].

$$mP^{z+} + ZA^{m-} \longrightarrow P_mA_Z$$

where P is a positively charged species and A, the anion which reacts with it to form a precipitate. In acidic solutions, the SBP contains several positive charges whereas Mo(VI) shows various anionic species[20]. The equilibrium constant of such a reaction can be expressed as follows:

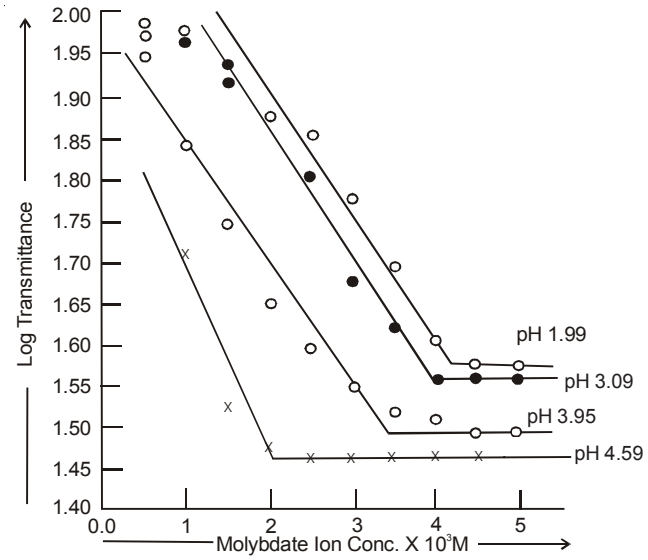

Fig. 2. Plots of log transmittance *vs.* conc. of molybdate ion for fixed conc. of SBP (0.178 %) and varying conc. of molybdate ion

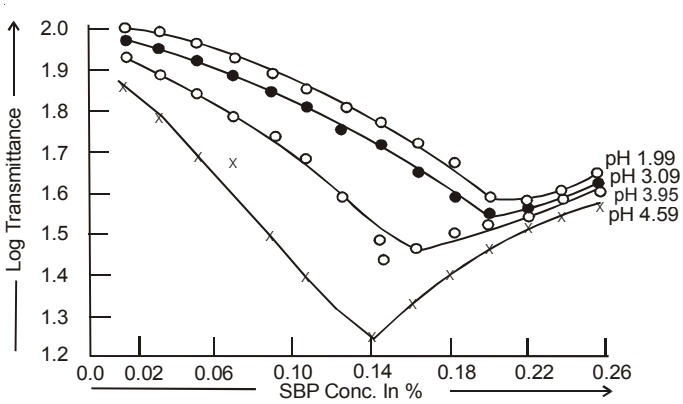

Fig. 3. Plots of log transmittance *vs.* conc. of SBP for fixed conc. of molybdate ion (5×10^{-3} M at pH 1.99, 4×10^{-3} M at pH 3.09, 3×10^{-3} M at pH 3.95 and 1.75×10^{-3} M at pH 4.59) and varying conc. of SBP

TABLE-1
MOLES OF Mo(VI) BOUND PER MOLE OF SBP

pH	V_M from		V_M in ppt	'n' from Scatchard plot
	Direct titration	Reverse titration		
1.99	107.6	107.2	25.5	72.5
3.09	99.7	90.9	25.0	71.5
3.95	86.0	84.5	24.0	70.0
4.59	53.1	54.3	23.8	61.0

$$Ks = \frac{1}{[P]^m[A]^z}$$

$$\text{or } - \log Ks = m \log [P] + z \log [A]$$

where Ks is the reciprocal of solubility product (S_o) at the given ionic strength

$$S_o = [P]^m [A]^z$$

where m and z represent the number of moles of each of the reacting species at the stoichiometric point. If the value of Ks or S_o is known and the precipitate formed is a strong electrolyte, the position of the equilibrium and the amount of the components in the precipitate and in solution under the conditions of the precipitation can be determined.

The Mo(VI)-SBP insoluble complex does not solubilized in excess of Mo(VI) solution but excess of SBP solublized the complex. This supported by an increase in transmittance at constant pH and Mo(VI) concentration (Fig. 2). This solubilization may be due to unequal amount of charge on SBP which shifted the solubility product of Mo(VI)-SBP complex. The formation constants calculated as reciprocal of solubility product along with free energy changes are compiled in Table-2.

TABLE-2
EFFECT OF pH ON THE FORMATION CONSTANTS AND FREE ENERGY CHANGES OF Mo(VI)-SBP SYSTEM

pH	Direct titration		Reverse titration	
	log Ks	ΔG^o Kcal/mol	log Ks	ΔG^o Kcal/mol
1.99	6.774	-9.150	6.634	-8.952
3.09	6.815	-9.194	6.753	-9.114
3.95	6.874	-9.275	6.975	-9.411
4.59	7.112	-9.565	7.325	-9.883

An observation of the above data show that formation constant and free energy changes values successively diminished with increasing acidity. This pattern of data shows that the extent of reaction rises as the positive charge on SBP molecule increase. It is interesting to note that addition of increasing amount of SBP results in the solubilization of insoluble complex. It appears that Mo(VI)-SBP complex exists as colloidal precipitate which undergo sedimentation on keeping. However, with excess of the protein, a protective layer is formed keeping this is a stable colloidal state. There are evidences[21,22] of the protection of many hydrophobic colloidal systems by the adsorption of gelatin on the surface of the sol particle through the undissociated carboxyl groups.

Determination of maximum number of binding groups from Mo(VI) and SBP precipitated: When equivalence point was attained, the reaction mixtures were centrifuged and the amount of Mo(VI) and SBP in the supernatant were measured. The data are plotted in form of ratio of SBP and Mo(VI) conc. in supernatant against V_M which indicated that after the equivalence point has attained, the limiting value

was obtained. A good relationship is also obtained by plotting SBP in precipitate against V_M. A few workers[23] reported similar linear relation between moles lysozyme precipitated and moles dye orange II precipitated in their study of the mechanism of dye uptake in protein dye salts. The present data deviated from linearity because of the complicated pH and concentration dependent polymerization-depolymerisatin reactions of Mo(VI). In this study, our assumption is that the simple ionic species of Mo(VI) are involved. The result of plots of ratio of SBP and Mo(VI) conc. is precipitate against V_M shows a linear relationship at all pH-values at higher SBP to Mo(VI) mixing ratios, thus exhibiting the constancy of composition of the precipitate throughout the process of mutual precipitation. The stoichiometry determined from these curves is nearly the same as derived from inflections in concentration *vs.* transmittance curves.

Mo(VI) uptake by SBP-Mo(VI) precipitate: From the free concentration of Mo(VI) and SBP in the supernatent, it can be assumed that the precipitation reaction is not simply a case of coagulation. It is proposed that the mechanism is an ion-exchange process between buffer anions and Mo(VI) anions at the cationic sites of the protein. A similar mechanism has been proposed in orange II-lysozyme reaction by some workers[23]. Assuming that all the Mo(VI) binding sites of protein have the same apparent dissociation constants and the electrostatic effects are negligible at the ionic strength used, we can describe the binding of Mo(VI) by the precipitate in simple mass action terms. The data of present study are plotted according to Scatchard[24] (Fig. 4) and the n value are given in Table-1. In lower pH the interaction between Mo(VI) ion with SBP seems to be of co-operative nature. Similar behaviour of this type was also reported by Karush[25] who found a sigmoid V_M/C_F *vs.* V_M plots for the binding of d-form of an optically anionic azo dye by serum albumin. Such a curve indicates that in a certain region of free dye concentration, the intrinsic constant increased with the equilibrium amount of the free dye. The effect was attributed by Karush[25] to 'opening up', of the protein molecule resulting from breakage of secondary intramolecular linkage. It is seen that the co-operative hydrogen bonding explains such 'opening up' of sites without the necessity of assuming any gross geometric changes in the molecule. The present curves also follow the same pattern hence a similar explanation could be offered from the abnormal binding of Mo(VI) to this protein.

The present transmittance data of Mo(VI)-SBP interaction agrees with the results of NMR and polarometric studies of Spence and Lee[26] who suggested 1:1 ratio between Mo(VI) and histidine amino acid in the pH range 5.0-7.0 and higher ratios at lower pH values. Similar results were reported with L-histidine methyl ester but no evidence of complex formation with N-acetyl histidine. This showed that the nitrogen atoms and not the carboxyl groups that are involved. The enhanced binding in the lower pH range than at higher pH can be explained in terms of electrostatic attraction between the cationic protein and the anions of the poly acid. This suggested an interaction between Mo(VI) anions and protonated nitrogens as proposed by

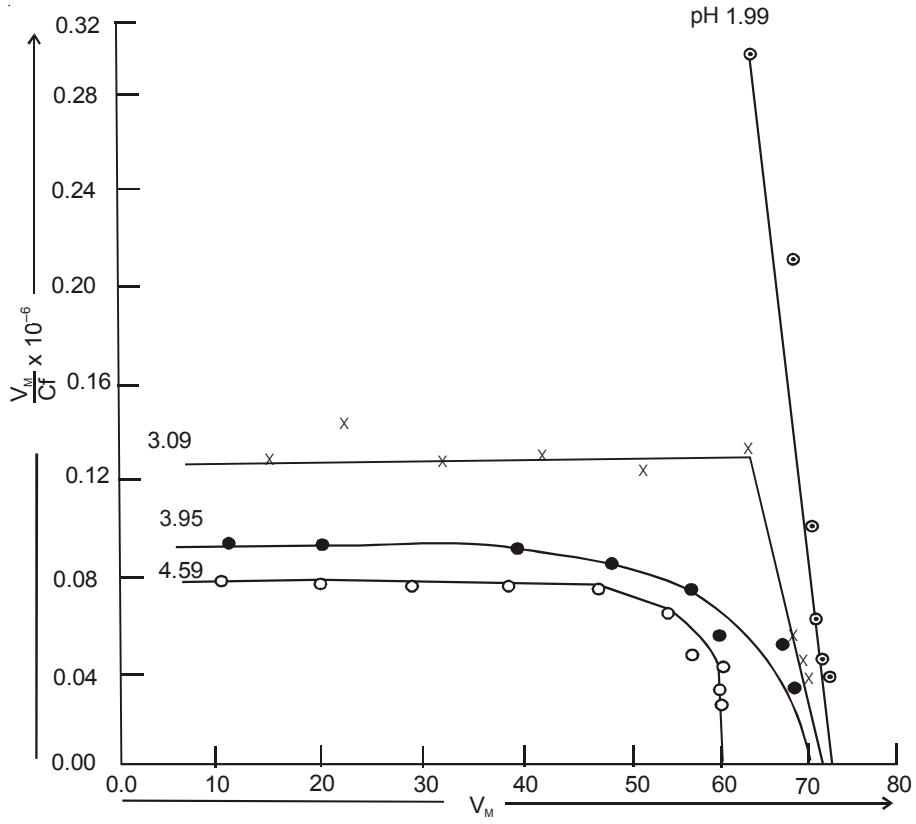

Fig. 4. Plots of V_M/C_F *vs.* V_M for SBP-Mo(VI) system at different pH values

Malik *et al.*[12] as well as by Sterinhardt *et al.*[27,28] and Smith *et al.*[29] in detergent-protein interactions and by other workers in precipitation reactions of proteins[30-31]. However, unlike other metals, the binding of molybdenum decreases with rising pH. It may be concluded that in the higher pH range due to progressive deprotonation of imidazole from histidine and ε-amino from lysine of SBP provided lesser cationic sites for combination. On the other hand, in the lower pH regions due to the existence of polymeric species of Mo(VI) and on account of the destruction of hydrogen bonding between cationic nitrogen groups and the carboxyl groups of SBP, the extent of Mo(VI) bound becomes equal to the total number of nitrogen groups present in the SBP molecule. It may be concluded that the intensity of the insoluble complex formation decreases with increase in the acidity of the system.

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