

pH-Metric Studies of the Binding of Hydrous Oxide Sols With Pepsin

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The binding of hydrous oxide sols of aluminium, iron and chromium with pepsin was studied by pH-metric method. The shifts in the titration curves was used as a means to explain the interaction of metals in the hydrous oxide sols with the various ionizable groups of pepsin. The interaction of chromium and aluminium is assumed to take place through the carboxyl groups of pepsin, since the flat segment of the pepsin sol curve lies between pH 3.0 to 6.0 where the carboxyl groups are deprotonated. The similar segment of iron oxide sol-pepsin curve lies in the acid range. Hence, the interaction could not be expected with carboxyl groups, but the phenolic groups of tyrosine may be involved in the binding interaction. The quantitative calculation revealed higher binding in low pH than in higher pH which is in accordance that pepsin contained larger anionic carboxylic groups than basic groups. The order of linking is acidic and basic ranges were identified.

Key Words: Pepsin, Aluminium, Chromium, Iron.

INTRODUCTION

The study of interaction of metallic cations with proteins has for years been one of the central problem of bio-chemistry and soil chemistry. Among these interaction the colloidal-colloidal types comprise a separate field of investigation which provide explanation for the activity of drugs, transportation of biological fluids and are also extremely useful from technological point of view. Chromium salts being polyvalent have been widely used for tanning process. Poly, isopoly and heteropoly furnishing an example of colloidal-colloidal combinations. Several references on this problem are available in the existing literature involving the use of animal proteins¹⁻⁴ and plant proteins⁵. Pauli⁵ reported that complex formation involved nitrogen atoms of the dyes and carboxylic groups of the proteins. Malik and coworkers^{6,7} have shown the availability of different ionizable groups of the proteins for interaction with the hydrous oxide sols. They have also reported the formation of adsorption complexes between gelatin and hydrous oxide sols viscometrically⁸. The binding of ovalbumin, globulin and lysozyme with chromium hydrous oxide sol in aqueous media as a function of the pH at constant ionic strength has been reported by Johnson

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and Matifev⁹. The interaction of α -amylase with hydrous titanium (IV) oxide¹⁰, enzyme lipase with hydrous niobium oxide¹¹, different proteins with copper hydrous oxide¹², hydrous ferric oxide¹³ and aluminium hydrous oxide¹⁴ has been given in literature.

In the present paper pH-metric method has been used to study the reaction of hydrous oxide sols with pepsin. The results are supported by flow measurement. The extent of binding as well as the mode of interaction has been critically discussed.

EXPERIMENTAL

Preparation of alumina sol: It was prepared by the method of Weiser¹⁵. Aluminium chloride (BDH) was dissolved in double distilled water and then heated to boiling. Aluminium hydroxide was precipitated with slight excess of ammonia and the mixture was boiled for 5 min. The repeatedly washed precipitate was suspended in double distilled water by boiling and peptized by adding gradually 0.2 N HCl. The pH of the sol was adjusted to 1.50. The concentration of the sol was determined gravimetrically as Al₂O₃.

Preparation of ferric oxide sol: Ferric oxide sol was obtained by the method of Krecke¹⁶. For its preparation a ferric chloride solution was gradually added to the boiling water with constant stirring. The colloidal solution was dialyzed while still hot. The pH of the sol was adjusted to 1.50. The iron content of the sol was determined volumetrically by KMnO₄ method.

Preparation of chromium oxide sol: Chromium oxide sol was obtained by Graham's method¹⁷. The precipitate of chromium hydroxide, obtained by addition of ammonia to chromic chloride solution, was repeatedly washed with double distilled water and finally suspended in water. The suspension was peptized by adding 0.1 M chromic chloride solution and the pH was brought to 1.50 by dialysis. Its chromium contents were determined colorimetrically by oxidising with sodium peroxide and comparing the optical density at 373 nm with that of standard chromate solutions¹⁸. The charge on the colloidal particles was determined by means of Burton type of electrophoretic tube. All the three hydrous oxide sols of aluminium, iron and chromium were found to be positively charged.

BDH samples of ferric chloride, aluminium chloride and chromium chloride were used as source of metal ions. Their solutions were prepared by dissolving required amount of each in double distilled water and their concentrations determined by usual methods. The pH values of all these solutions were adjusted at pH 1.50.

Protein solution: Pepsin was purchased from Sigma Chemicals and its solution of required concentration was prepared in double distilled water. The concentration of stock solution was determined by colorimetric biuret method. The pH of a 2.0 % pepsin solution was adjusted to 11.50 by means of an Elico pH-meter.

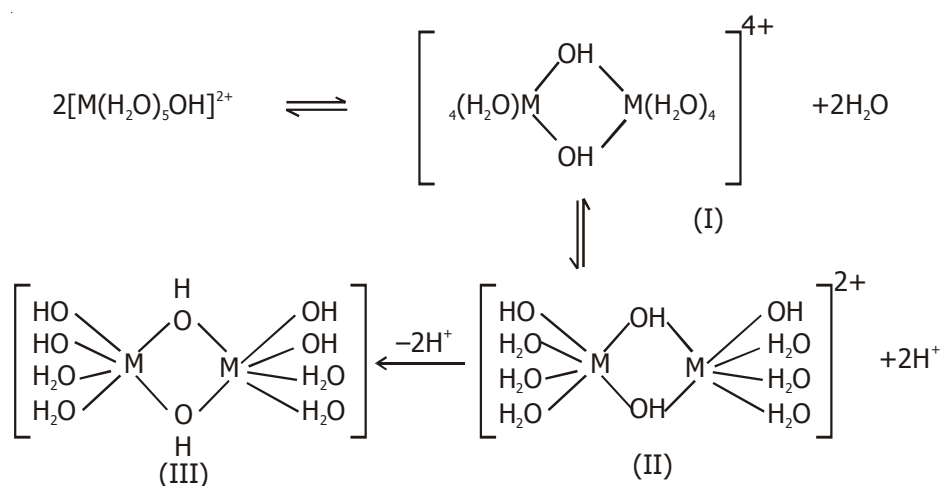
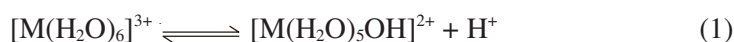
Other solutions: Dilute solution of KOH (pH = 11.5) was prepared by adding 0.10 M KOH in double distilled water. Hydrochloric acid solution was prepared from AR sample and its pH was adjusted at pH 1.50.

Procedure: Varying volumes as shown in curves of hydrous metal oxide sols or electrolytes were mixed with 4.0 mL of pepsin of pH 11.50 in different boiling tubes and the total volume was made 20 mL by adding the required amount of distilled water. Similar sets for all these hydrous oxide sols and electrolytes were also arranged replacing protein by KOH solution of the same pH. The pH of these sets were measured which are shown in the corresponding curves.

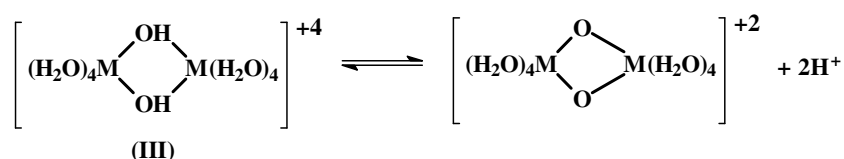
The pH-measurements were carried out using an Elico pH-meter in conjunction with a wide range glass electrode. The instrument was calibrated against 0.05 M potassium hydrogen phthalate (pH 4.0) and 0.05 M sodium borate (pH 9.20) for the acidic and basic ranges, respectively.

RESULTS AND DISCUSSION

The initial and final pH values for the different systems obtained by titrating anionic pepsin or KOH of the same initial pH are given in Figs. 1-3. The hydrous metal oxide sols under investigation are known to contain colloidal micelles responsible for the colloidal nature and are produced as a result of several complicated reactions¹⁹ in the following ways:



In the above equations equilibrium exists between (I) and (II) while (III) represents the coagulated state of the sol. The formation of an oxo compounds can be shown as follows:



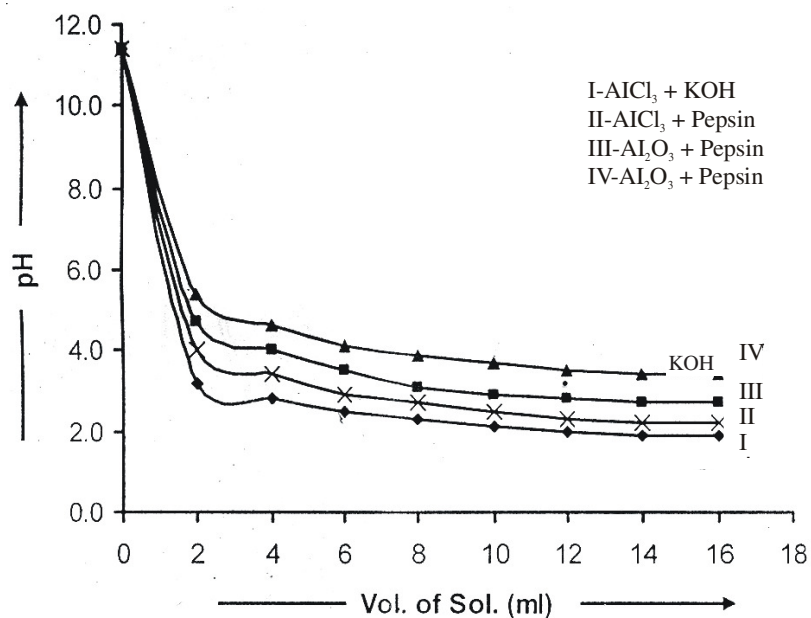


Fig. 1. pH-Titration curve of pepsin in presence of AlCl₃ and Al₂O₃ and similar curves of sols and KOH

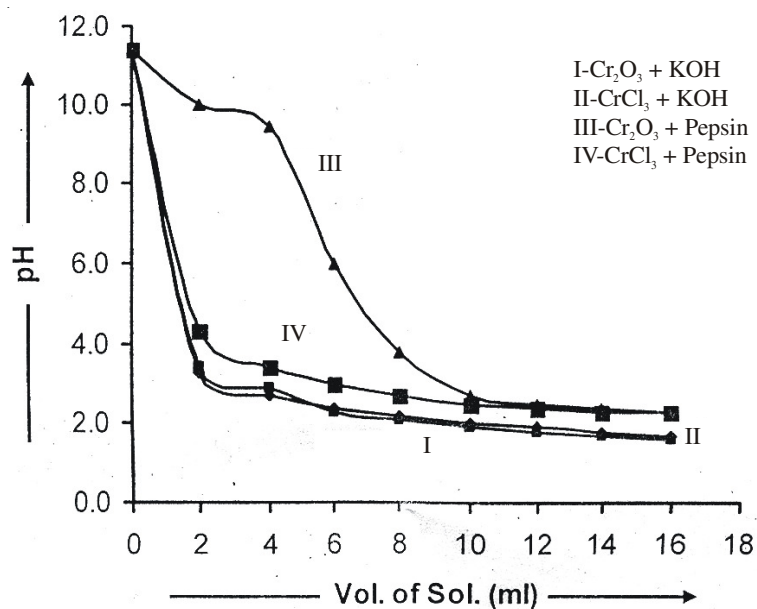


Fig. 2. pH-Titration curve of pepsin in presence of CrCl₃ and Cr₂O₃ and similar curves of sols and KOH

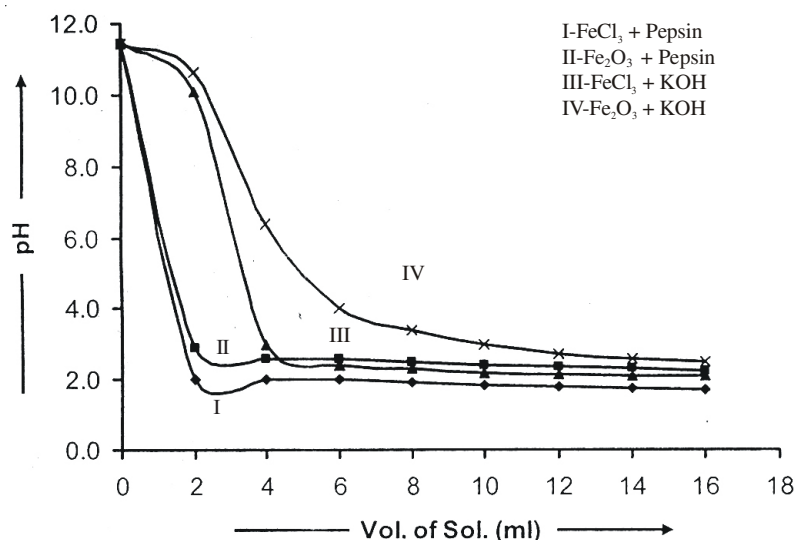


Fig. 3. pH-Titration curve of pepsin in presence of FeCl₃ and Fe₂O₃ and similar curves of sols and KOH

The formation of the oxo complex is favoured in the case of aluminium oxide and chromium oxide sols in comparison to ferric oxide sol. The formation of iron micelles are reported by Granick and Hahn²⁰ and Loewus and Fineberg²¹ in the case of apoferritin. They have also suggested the presence of ferric oxyhydroxide core in ferritin. These micelles may polymerize and consequently may be fixed by the reactive surfaces of the other polymeric compounds.

The above mentioned colloidal micelles are positively charged and therefore combination with anionic protein may be affected either by means of mutual adsorption or through purely chemical forces, where the metal ions from the inner part of the double layer make themselves available for reaction with the respective reactive sites of the pepsin molecules. In these experiments, the titration curves of pepsin in the presence of metal oxide sols lie above the corresponding curves of sols and KOH. In all the cases, binding takes place between pH 3.0 to 6.0 where ionized carboxyl groups from aspartate and glutamate residues are made available for the interaction. The extent with which sol-alkali or electrolyte-alkali curves are displaced by the addition of pepsin to the sol or electrolyte, may be assumed as a qualitative measure of the extent of metal protein binding. In general, the complexes are formed through chelation to carboxyl groups especially at lower pH-values. The pK of carboxyl group is well below pH 6.0 and the dominating species is pepsin anion. Chromium also showed combination with amino groups as became evident from the shifts of curves towards more basic side. Green and Ang¹⁹ have presented evidences about the interaction of chromium with alanine. Aluminium excludes the possibility of reaction with the basic groups from the nature of titration curves.

However, the titration curves of ferric oxide sol-pepsin are different from those of other two sols, though from the slight shift of curves in higher pH range binding was assumed to take place with the phenolic groups of the tyrosine residues as conalbumin²² and casein²³ combines with iron through their tyrosine residues. Although iron shows appreciable tendency to coordinate with amino groups, but in the present case an insignificant binding was observed between iron and the four basic residues of pepsin. From the position of titration curves, it seems possible that ferric ion is readily micellised and required more hydrogen ions for micelle formation. Brady *et al.*²⁴ from physical studies have shown that the structure of ferric hydroxy nitrate is closely similar to the core of ferritin. In the present case several iron atoms may join to form the above type of complicated micelle.

The combination between positively charged micelles of hydrous metal oxide sols and anionic pepsin can be greatly enhanced through electrostatic forces. The molecule of pepsin (m.w. 34,500) contains 37 carboxylic groups, 4 basic amino acid residues, therefore, at the starting of titration all the 37 carboxylic groups are in the ionized form and the molecule as a whole is a negative polymer. The higher positive charge on the colloidal micelles would exert a greater electrostatic attraction for pepsinate ion and hence favoured enhanced interaction. This mode of linking is however, be limited to a specific pH range. In highly acidic solutions, where pepsin is totally cationic due to protonation of basic groups, amide nitrogen and carboxylate residues, there can be no possibility of combining with positively double layer of the hydrous oxide sol micelles. In the same way, the possibility of combination in higher pH range will reduce due to sensitization of the sol micelles in the presence of excess of hydroxyl ions. Infact, in presence of excess of alkali, the colloidal micelles would be converted into chromate, aluminate and ferrate ions, which being negatively charged like pepsinate ion will be a subject of electrostatic repulsions. This is the reason that in the middle pH range most of the linking with the carboxylate groups of the pepsin would be taking place.

The amount of different hydrous oxide sols bound to per gram of pepsin was determined from the difference of sols taken up by alkali at any specified pH and that consumed by pepsin at the same pH. A comparative statement of the binding data are given in Table-1.

TABLE-1
AMOUNT OF DIFFERENT SOLS BOUND per g OF PEPSIN

pH	Fe ₂ O ₃ (g)	Al ₂ O ₃ (g)	Cr ₂ O ₃ (g)
2.5	0.525	0.355	0.322
3.5	0.350	0.248	0.292
5.5	0.252	0.122	0.265
7.5	0.165	0.062	0.215
9.5	0.100	0.028	0.135

It is evident from Table-1 that the pepsin binds larger amount of hydrous metal oxide sols in the low pH than in the higher pH. This is probably due to the formation

of polynuclear complexes²⁵, a point contradictory that binding should be lesser on account of like charges on protein and the colloidal micelles. This point also justifies that pepsin contains a large number of anionic carboxyl groups than the basic groups. The order of linking in acidic and basic ranges followed the following orders:

Fe > Al > Cr (acidic range)

Cr > Fe > Al (basic range)

The above order postulates higher affinity of iron for carboxyl group, while chromium least, similarly chromium binds more strongly with basic groups while aluminium least.

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