

TRANSACTIONS
OF THE
Nova Scotian Institute of Science.

SESSION OF 1911-1912

ON THE BEHAVIOUR OF IRON SALTS, IN THE PRESENCE OF ALBUMENS AND OTHER ORGANIC SUBSTANCES, TOWARDS CERTAIN REAGENTS.—BY HENRY JERMAIN MAUDE CREIGHTON, M. A., M. SC., DR. SC., *Lecturer on Physical Chemistry, Dalhousie University, Halifax, N. S.**

I. INTRODUCTION.

In the course of another investigation, it was observed by the writer that, under certain conditions, solutions of soluble Prussian blue were decolorized by white of egg. As the writer was unable to obtain any satisfactory information regarding this behaviour, the present investigation was carried out with a view of throwing new light on the associations of iron with native and derived proteins.

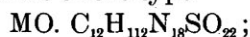
The prevention of many reactions by the presence of certain organic substances is well known. For example, the precipitation of ferric and aluminium hydroxides is prevented by the presence of small quantities of non-volatile organic acids, notably tartaric acid¹, of sugar, of glycerine, and of other organic substances. The cause of this is to be found in the formation

* Contributions from the Science Laboratories of Dalhousie University—[Chemistry].

1. Staedeler and Krause: *Jahresb.*, 746, (1854).

of a soluble complex ion brought about by the entrance of the metal into the hydroxyl group of these substances.

It has long been known that albumen manifests a marked tendency to hinder many chemical reactions. The cause of this may be either physical or chemical. *von Meyer* and *Lottermoser*¹ have shown, for instance, that small quantities of egg albumen prevent the precipitation of silver hydrosol by salts. The effect of albumen on the ionization of silver nitrate has been investigated by *Galeotti*² who found, that small quantities greatly diminish the concentration of the silver ions. It is possible that albumen may, in some cases, diminish the ionization of a substance to such an extent as to prevent its recognition by the usual tests. Albumen readily forms complexes with salts of both the alkali and the heavy metals, as well as with many bases and all the mineral acids except ortho- and pyro-phosphoric acid. These albumen compounds may be divided into two classes: those in the which the metal is present as a simple cation; and those in which it forms part of a complex anion, and in which, for this reason, its presence cannot be demonstrated by ordinary reagents. Complexes formed by albumen with HCl, HNO₃, H₂SO₄, NaOH, KOH, NaCl, KCl, MgCl₂, CaCl₂, (NH₄)₂SO₄, MgSO₄, Na₂SO₄, and K₂SO₄ have recently been studied by *Mayer*³. The existence of the complex can often be readily shown. For example, when hydrochloric acid is added to a solution of white of egg and the mixture filtered so as to remove the precipitate which forms, it is found that no silver chloride is thrown down on the addition of silver nitrate to the filtrate. With salts of most of the heavy metals albumen forms compounds of the type⁴



but with iron salts the compound is more complex⁵. These

-
1. *von Meyer, E., and A. Lottermoser* : J. pr. Chem., **56**, (2) 214, (1897).
 2. *Galeotti, G.* : Zeitschr. physiol. Chem. **42**, 330, (1904).
 3. *Mayer, A.* : Compt. rend., **143**, 515, (1906).
 4. *Piotrowski* : Jahrsber. über die Fortschritt der Chemie, **534**, (1857).
 5. *Brücker* : Zeitchr. für Chemie, **61** (1871).

compounds are unstable and the albumen may be recovered by treatment with acids, when it is precipitated and the metal goes into solution.

Physiologists have long divided iron compounds into two classes; those which contain "organic" iron, and those which contain "inorganic" iron. *Macallum*¹ has shown that haematoxylin may be used to distinguish between these two classes of compounds; and in the experiments which follow I have employed this reagent to demonstrate the condition of the iron.

The terms "bound" and "unbound", as used by Prof. Fraser Harris are, I think, to be preferred to the more ambiguous "organic" and "inorganic". As even better than "bound" and "unbound", however, I would suggest the use of the terms "*non-ionic*" and "*ionic*"; for inorganic or unbound denotes the simple ionic state, while organic or bound denotes a condition which is not elementary, but often quite complex. A compound containing "ionic" iron would be one in which the iron exists as a cation; and in a compound containing "non-ionic" iron the iron would not be present as a simple ion, but would exist as *part* of a complex ion, usually as part of the anion. This classification is justified by experiment, for compounds such as ferric chloride, ferrous sulphate, ferric acetate, and potassium ferri-ferrocyanide (soluble Prussian blue), all of which contain a simple iron cation, give a deep blue black or violet black colouration with haematoxylin; while, on the other hand, compounds such as potassium ferricyanide or potassium ferrocyanide, in which the iron is not present as a simple ion, but as part of a complex anion, give no reaction with haematoxylin.

II. EXPERIMENTAL.

Albumen: A 15% white of egg solution, a 10% solution of Merck's "Egg Albumen granular", and a 10% solution of Merck's "Serum Albumen" were used in the following experiments. The egg and serum albumen were dissolved in very

1. *Macallum, A. B. : J. Physiol., 22, 92, (1897-1898).*

dilute sodium chloride solution. These solutions were filtered before using.

Haematoxylin: A 0.5% solution of haematoxylin was used in the following experiments.

(a) *Experiments with ferric chloride.*

To 10 c. c. of 1.0% ferric chloride solution 10 c. c. of the white of egg solution were added and the mixture divided into two parts. One portion was allowed to stand at room temperature for a few minutes, and the other kept at 60° for the same length of time. The two portions were divided into several parts and treated with different reagents. The following results were obtained:

With NaOH no precipitate formed.

“ NH_4OH no precipitate formed.

“ $\text{K}_3\text{Fe}(\text{CN})_6$ no change.

“ $\text{K}_3\text{Fe}(\text{CN})_6$ slight blue precipitate formed.

“ KCNS deep red colouration.

“ $(\text{NH}_4)_2\text{S}$ black precipitate formed.

“ Haematoxylin violet black colouration appeared.

Similar results were obtained when 10 c. c. of either the serum albumen or the egg albumen solutions were employed instead of the white of egg solution. It was found with these substances that some of the reagents caused albumen to separate from the solution. Control experiments, in which the albumen solution was replaced by an equal volume of distilled water, were carried out. In these it was found that sodium hydroxide, ammonium hydroxide, potassium ferricyanide, and potassium ferrocyanide all threw down the usual precipitates.

On addition of the albumen solutions to the ferric chloride, it was observed that the brownish colour of the mixtures gradually deepened on standing, thus suggesting that albumen increased the degree of hydrolysis of the iron salt. This result was also observed when a mixture of white of egg and ferric

chloride was warmed. To 5 c. c. of 1.0% ferric chloride solution 5 c. c. of the white of egg were added in one case, and in another the chloride was diluted with 5 c. c. of distilled water. These solutions were kept at 65° for ten minutes. At the end of this time the solution containing the albumen was found to be of a deeper colour than the other. To determine whether the non-formation of a precipitate of ferric hydroxide or of Turnbull's blue, in the foregoing experiments, was due to the conversion of the iron into colloidal ferric hydroxide by hydrolysis, sodium acetate was added drop by drop to a solution of ferric chloride, until its colour was somewhat deeper than that of the mixture of white of egg and ferric chloride that had been warmed; on adding ammonia to the acetate solution a heavy precipitate of ferric hydroxide was thrown down, thus proving that only a small quantity of the iron in the egg solution was in the form of colloidal hydroxide.

Hydrochloric acid was found to produce a precipitate when added to a solution of ferric chloride and albumen. To a mixture of 5 c. c. of 1% ferric chloride and 10 c. c. of the white of egg solution a few drops of dilute hydrochloric acid were added. At the end of some minutes no precipitate was observed to have formed. With several c. c. of the acid, however, a precipitate was slowly thrown down. The precipitate was filtered off, and to different portions of the light yellow filtrate ammonia, haematoxylin, potassium ferrocyanide, and potassium sulphocyanide were added. In each case the usual reaction for ferric iron took place. Further addition of hydrochloric acid precipitated no more albumen from the filtrate. On boiling some of the filtrate, albumen coagulated and was thrown out of solution. This was filtered off and potassium ferrocyanide added to the almost colourless filtrate. The dark blue precipitate was removed by filtration. On boiling the colourless filtrate a light blue flocculent precipitate separated from the solution. The results obtained in this experiment point to the existence of a complex of the iron salt with the albumen. This complex is

stable towards heat. When potassium ferrocyanide is present, however, the complex is precipitated by boiling the solution.

A mixture consisting of equal parts of the white of egg and 1.0% ferric chloride solution, was found to be more stable towards heat than a pure albumen solution of the same concentration; whereas the mixture did not commence to coagulate until the temperature was raised to 65°, the pure albumen solution became cloudy at 61.5°

(b) *Experiments with potassium ferricyanide.*

White of egg solution, egg albumen, and serum albumen were all found to prevent the precipitation of potassium ferricyanide, in dilute solution, by ferrous salts. On the addition of a few drops of ammonium ferrous sulphate to a mixture consisting of 2 c. c. of 0.1% potassium ferricyanide and 5 c. c. of the white of egg solution only a very faint blue colouration was produced. When the concentration of the potassium ferricyanide was smaller than this, a blue colouration did not occur on the addition of the ammonium ferrous sulphate. 5.0 c. c. of 0.1% potassium ferricyanide were mixed with 25 c. c. of the white of egg solution. Ammonium ferrous sulphate solution was then added drop by drop to the pale yellow mixture until it became colourless. This disappearance of the yellow colour points to the occurrence of chemical change on the addition of the ammonium ferrous sulphate, and is probably due to the disappearance of the ion to which the colour of the solution is due. A blue black colouration appeared when a few drops of haematoxylin were added to part of the decolourised solution. The decolourised solution turned blue on the addition of a drop of dilute hydrochloric acid. Excess of hydrochloric acid caused a blue precipitate to separate slowly from the solution.

With solutions containing no albumen and the above concentrations of potassium ferricyanide, ammonium ferrous sulphate gave deep blue precipitates.

To a mixture of 5 c. c. of 0.1% potassium ferricyanide and 25 c. c. of the white of egg solution, sufficient dilute hydro-

chloric acid was added to throw down a white precipitate. The precipitate was filtered off and small portions of the filtrate were tested with ammonium ferrous sulphate, and haematoxylin. The former gave a deep blue precipitate, but no reaction occurred with the latter reagent. To the remainder of the filtrate more hydrochloric acid was added, but no further precipitation took place; on boiling, however, a white precipitate separated from the solution. It was found that the light yellow filtrate from this precipitate could be boiled without further precipitation taking place. To the cooled filtrate a few drops of ammonium ferrous sulphate were then added. The solution turned blue, and on standing a deep blue precipitate separated out; on boiling the solution the precipitate became flocculent, resembling a precipitate of aluminium hydroxide. The precipitate when heated on a platinum foil charred at a low temperature. This precipitate could not have consisted of simply Turnbull's blue; for when ammonium ferrous sulphate was added to a hydrochloric acid solution of potassium ferricyanide having the same degree of yellow colour as the above filtrate, and the mixture boiled, the precipitate which separated out was not flocculent, but finely divided. Moreover, on allowing it to settle and pouring off the supernatant liquid the blue precipitate was found to dissolve in water. The results of this experiment suggest the formation of a complex by the iron salt and albumen, which is stable towards heat, and which is precipitated by ammonium ferrous sulphate.

Like ferric chloride, potassium ferricyanide increases the coagulation temperature of albumen. A solution of potassium ferricyanide and white of egg, of one half the concentration previously employed, first became turbid at 64.5° ; while a pure white of egg solution, of the same concentration, became cloudy at 61.5° . When, besides the potassium ferricyanide and white of egg, a very small quantity of ammonium ferrous sulphate was present in the solution, coagulation did not take place below 75° .

(c) *Experiments with potassium ferric-ferrocyanide (soluble Prussian blue).*

It has already been mentioned in the introduction that, under certain conditions, white of egg solution is capable of decolourizing a solution of soluble Prussian blue. Neither the egg albumen nor the serum albumen were found to decolourize soluble Prussian blue as readily as the white of egg solution.

10 c. c. of 0.05% soluble Prussian blue were mixed with 10 c. c. of the white of egg solution and the mixture kept at 60° for an hour. At the end of this time the deep blue solution had become practically colourless. The fading of the blue colour took place gradually. With pure white of egg, or at a higher temperature, the decolourization of the soluble Prussian blue was found to proceed with greater rapidity. When a mixture of equal volumes of 0.05% soluble Prussian blue and the white of egg solution were kept at room temperature, no apparent change in the intensity of the blue colour of the solution was observed at the end of six hours. To some of the decolourized solution haematoxylin was added; *no precipitation occurred*, thus proving that the Fe^{III} ion of soluble Prussian blue was no longer present as such. The addition of dilute hydrochloric acid or concentrated salt solutions to some of the decolourized solution caused the precipitation of a white substance, which gradually turned to a deep green blue colour on standing or on treatment with hydrogen peroxide. No change was observed on the addition of hydrogen peroxide to some of the decolourized soluble Prussian blue mixture.

Like the foregoing mixtures of iron salts with albumen, a solution of 0.05% soluble Prussian blue and an equal volume of the white of egg, which had been decolourized by heating at 60°-70°, was stable to heat and could be boiled without the albumen coagulating. Indeed, it was found that the substances could be rapidly brought to boiling immediately after mixing without precipitation taking place. At this temperature the mixture became colourless in two or three minutes.

(d) *Experiments with gelatine.*

On account of the close relationship between the albumens and the albuminoids, the following experiments were carried out to determine the influence of the presence of the latter substances on reactions of certain iron salts. As a typical albuminoid gelatine was employed. A 10% solution was found to be quite fluid at 20°.

No precipitation of ferric hydroxide occurred on the addition of ammonia to a mixture consisting of equal volumes of 1.0% ferric chloride, 6% gelatine solution, and distilled water; haematoxylin, however, gave a violet black colouration. In a control experiment ferric hydroxide was precipitated by ammonia.

1 c. c. of 0.1% potassium ferricyanide and 5 c. c. of 6% gelatine solution were added to 5 c. c. of distilled water. Although the mixture turned blue on the addition of ammonium ferrous sulphate, no precipitate formed. The blue colour disappeared from this solution on boiling. With 8 c. c. of 10% gelatine solution no blue colouration appeared on the addition of ammonium ferrous sulphate to the mixture; if however, besides the ammonium ferrous sulphate, a few drops of hydrochloric acid were added, the solution turned blue and a precipitate of Turnbull's blue slowly formed. In the control experiments, in which the gelatine solution was replaced by an equal volume of distilled water, a deep blue precipitate was obtained on the addition of the ammonium ferrous sulphate.

It was found that a mixture containing 5 c. c. of 0.05% soluble Prussian blue and 5 c. c. 6% gelatine could be kept at 95°-100° for one and a half hours without the blue colour of the solution appreciably decreasing in intensity. With 7 c. c. of 10% gelatine, however, the colour of the Prussian blue faded completely under these conditions. The blue colour of the mixture returned on the addition of a few drops of either dilute hydrochloric acid or hydrogen peroxide. With hydrochloric

acid the blue colour was deeper than with hydrogen peroxide. When a decolourized solution was allowed to stand for twenty-four hours, the jelly, which formed on cooling, was found to be deep blue at the surface. The blue colour gradually decreased until, at a depth of three inches, the jelly was colourless.

(e) *Experiments with ferrous salts.*

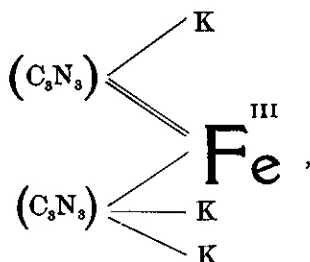
Dilute solutions of ammonium ferrous sulphate and potassium ferrocyanide were separately mixed with varying quantities of the white of egg solution, and allowed to stand some minutes. On adding ammonia or sodium hydroxide to the ferrous ammonium sulphate mixtures ferrous hydroxide was invariably precipitated. When the concentration of the white of egg was relatively large, the ferrous hydroxide precipitated somewhat slowly. The potassium ferrocyanide mixtures were tested with solutions of iron alum and copper sulphate. On the addition of a few drops of the alum solution to the mixture a deep blue precipitate was always produced, while with the copper sulphate solution a brick red precipitate of copper ferrocyanide was immediately formed. The same results were obtained when gelatine was used instead of white of egg.

(f) *Experiments with other organic substances.*

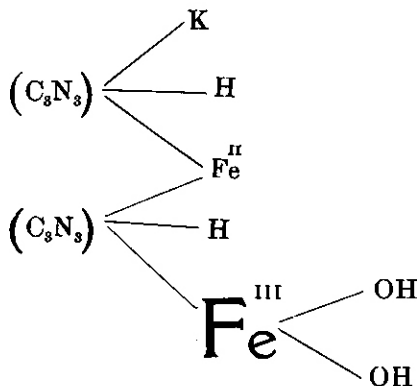
Dilute solutions of all the iron salt previously used were separately mixed with varying quantities of cane sugar, of tartaric acid, and of glycerine. The mixtures containing soluble Prussian blue were kept at 60° for an hour, while the others were allowed to stand at room temperature for some minutes. Apart from the prevention of the precipitation of ferric hydroxide on the addition of ammonia or sodium hydroxide to the mixtures containing ferric chloride, it was found that neither sugar, tartaric acid, nor glycerine apparently hindered the different iron salts from reacting with the various reagents previously used for their demonstration.

III. DISCUSSION OF RESULTS.

From the experiments described, it will be seen that albumens, as well as the closely related albuminoid gelatine, tend to prevent certain reactions of ferric chloride, potassium ferricyanide, and soluble Prussian blue; while on the other hand, the presence of albumen or gelatine does not appear to hinder reactions with ferrous ammonium sulphate or potassium ferrocyanide. The prevention of reactions of iron salts by albumen or derived proteins, such as gelatine, seems to be closely associated with the state of oxidation of the iron; for ferric chloride, potassium ferricyanide



and soluble Prussian blue



each contain at atom of trivalent iron. In potassium ferricyanide the trivalent iron forms part of the anion $\text{Fe}(\text{CN})_6^{\text{III}}$.

As to just how reactions of compounds containing trivalent iron are hindered or prevented by the presence of native or derived proteins, three possibilities present themselves: either the protein may decrease the dissociation of the iron compound to such an extent that ionic reactions are no longer possible; or it may exert a so called "protective action" on the iron salt, similar to that of gelatine on colloidal gold, which is due to the gelatine forming a very thin coating over each of the gold particles¹; or, lastly, the protein may be *intimately associated* with the iron salt. Intimate association of the protein and the iron salt may be brought about through the formation of a *chemical compound*, or by *adsorption*, giving rise to what may be looked upon as a *physical* compound.

If the phenomenon were to be ascribed to either decrease in dissociation or to protective action, we should expect proteins to hinder reactions of salts containing bivalent iron as well as those of salts containing trivalent iron. On the other hand the *specificity* of the proteins employed, points to their *intimate association* with trivalent iron.

It is well known that many colloids have a tendency to adsorb certain substances, which are in true solution, with the formation of so called adsorption compounds. Since such compounds do not possess a constant composition they cannot be looked upon as chemical. The formation of such compounds depends on several factors of which the following are the more important: the nature and structure of the colloid, the nature of the solvent, the nature of the dissolved substance, the condition of the molecule of the dissolved substance, and lastly, the temperature.

In the foregoing experiments the nature and structure of the colloidal albumen and gelatine are not very dissimilar, while those of the ferric and ferrous salts employed are greatly so. In view of what has been said we should expect to find

1. Mines, G. R. : Proc. Physiol. Soc., November 18th, 1911.

ferrous, as well as ferric salts, adsorbed by albumen and gelatine. That this is not the case at once suggests that the selective adsorption of trivalent iron may be due to some electrical effect. In support of this it may be mentioned it has recently¹ been pointed out, that while the adsorption of non-electrolytes is probably due to surface tension phenomena, that of electrolytes is probably of electrical origin.

Additional evidence of the adsorption of ferric compounds by native and derived proteins is afforded by the fact that the coagulation temperature of albumen is increased by these substances. This behaviour is in accordance with that observed by *Pauli* and *Handovsky*², who found that small concentrations of alkali salts retarded the coagulation of albumen, i. e. raised the coagulation temperature.

The supposition of the formation of a chemical compound is supported by the raising of the coagulation temperature of albumen when trivalent iron is present; by the disappearance of the colour of soluble Prussian blue on the addition of albumen or gelatine to its solution; and by the fact that the rate of fading of the blue colour increases as the temperature of the solution is raised. This last is a further argument against the hindering of the reactions being brought about by the protein lessening the degree of dissociation of the iron compounds. The fact that hydrochloric acid when added to mixtures of potassium ferricyanide and albumen or gelatine, such as used in the foregoing experiments, liberates the ferricyanide so that it is demonstrable by ammonium ferrous sulphate and, in the case of albumen mixtures, also precipitates the albumen; the return of the blue colour to solutions of soluble Prussian blue that have been decolorized with gelatine, on the addition of hydrochloric acid, hydrogen peroxide, or on exposure to

1. Lachs, H. and L. Michaelis: *Zeitschr. Elektrochem.*, **17**, 1, (1911); *ibid.* **17**, 917, (1911).

2. Pauli, W. and H. Handovsky: *Beitrage z. chem. Physiol. u. Pathol.*, **11**, 415, (1908).

the air for some time; the white or pale blue precipitates thrown down by hydrochloric acid from solutions of soluble Prussian blue decolorized by albumen; and the fact that this latter precipitate turns to a deeper blue on treatment with hydrogen peroxide or on exposure to the air, constitute additional evidence of the existence of a chemical complex. The return of the blue colour to colourless solutions of soluble Prussian blue and gelatine, and to the substance precipitated by hydrochloric acid from colourless solutions of soluble Prussian blue and albumen, indicates that in the soluble Prussian blue protein complex the trivalent iron of the soluble Prussian blue has undergone reduction and is present in the bivalent condition.

The results obtained in this investigation indicate that native and derived proteins prevent the ordinary reactions of substances containing trivalent iron, owing to the formation of associations between the protein and the iron salt. There is reason to believe that this phenomenon is partly physical and partly chemical: physical in that the colloid attracts the iron salts and forms adsorption compounds; and chemical in that the proteid actually combines with the iron salt. These physical and chemical complexes are readily broken down by hydrochloric acid. Complexes of soluble Prussian blue with gelatine are also decomposed in solution by hydrogen peroxide, but those with albumen are not. On the other hand, these complexes seem fairly stable towards heat, and in the case of those formed with soluble Prussian blue a temperature of 100° does not effect decomposition. Through the formation of complexes of proteins with soluble Prussian blue, the trivalent iron of the latter is probably reduced to the bivalent condition. No indication that complexes are formed by proteins and salts containing bivalent iron has been obtained. Neither cane sugar, glycerine, nor tartaric acid appear to form chemical or adsorption compounds with either ferro- or ferri-salts.

It is the intention of the writer to extend these experiments.

In conclusion it may be pointed out that the results of this paper are not without physiological significance, for the ready formation of chemical or physical complexes between native and derived proteins and compounds containing trivalent iron, either as cation or as part of the anion, may possibly throw new light on the metabolism of iron¹.

Department of Chemistry, Dalhousie University.
Halifax, Nova Scotia,
March 2nd, 1912.

1. See the following paper in this Journal, by Professor Fraser Harris.