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COLORIMETRIC DETERMINATION OF IRON WITH ORTHOPHENANTHROLINE AT HIGH ELECTROLYTE CONCENTRATIONS

by

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In order to investigate the corrosion rates of steels, *Johansson* (1941) developed a colorimetric method for measuring the concentration of dissolved iron in an aqueous phase. As reagent, he used ortho-phenanthroline which he added to the corrosive salt solution and which reacted instantaneously with the divalent Fe forming a coloured complex $(C_{12}H_8N_2)_3Fe^{++}$. *Saywell & Cunningham* (1937) conclude that ortho-phenanthroline possesses advantages over other reagents because it gives (a) a relatively more intense colour, (b) great working sensitivity, and (c) a much more stable colour. One of the great advantages of this reagent is that it can be used in acid solution, thus avoiding the precipitation of hydroxides and hydrated oxides in alkaline solution (*Pepi* 1946, *West* 1951).

Johansson's method may, however, disturb the corrosion process occurring on non-stainless steels since the ortho-phenanthroline prevents the precipitation of iron hydroxides and thus only the initial phase of the corrosion can be studied in this way. At high temperatures and pressures existing in cooking and autoclave experiments, in which the corrosive medium during the corrosion test moreover consists of steam and condensate, it is clear that the method devised by Johansson cannot be employed. It must be modified so that the determination of Fe in the corrosion

products is carried out after the corrosion experiment has been finished.

The modified method is characterized by the following features,

(1) The corrosion products, which were formed under the hot, moist conditions in the cooking apparatus and autoclave, were found after the corrosion test to be mostly situated on the surface of the sample in the form of hydro-oxides and hydroxides.

(2) The corrosion products were removed by ultrasonic treatment (*Holmlund* 1963).

(3) In order to convert them into an ionic form, the corrosion products were then dissolved in hydrochloric acid. The acidification was strong so as to make quite certain that all of the Fe was dissolved. The pH in the resulting solution was corrected to a suitable value by neutralization and buffering. The solutions obtained by this procedure thus contained a high concentration of electrolytes.

(4) Any possible trivalent Fe was converted to divalent Fe by adding a suitable reducing agent.

(5) Ortho-phenanthroline was employed as colour reagent.

(6) The colorimetric determination was performed in a Baeckman B spectrophotometer with an Agner micro-attachment. The same apparatus was used during the whole of this investigation.

REAGENTS

Conc. HCl 36.4 % pro analysi (E. Merck A.G., Darmstadt).

4N NaOH pro analysi (Elektrokem. AB, Bohus, Sweden).

1N HCl.

Citrate buffer pH = 6.0 (420.16 g citronic acid + 2N NaOH to 2000 ml). ($C_6H_8O_7 + H_2O$ pro analysi E. Merck A.G., Darmstadt).

Ascorbic acid pro analysi (E. Merck A.G., Darmstadt).

0.6 % o-Phenanthrolinium chloride (E. Merck A.G., Darmstadt).

(All solutions made in distilled water).

PROCEDURE

(1) (a) *Test:* 5 ml sample + 2 ml conc. HCl,

(b) *Blank:* 5 ml distilled water + 2 ml conc. HCl.

The acidified solutions were shaken for 14 h (see note 1).

(2) *Alternative A:* (a) 2 ml solution 1 a + 1.4 ml NaOH,
(b) 2 ml solution 1 b + 1.4 ml NaOH.

(2) *Alternative B:* In the 1:5 and 1:10 dilutions used here, no pH correction was necessary (see note 2). In order to obtain the same volume, the dilution was performed with 1.4 ml distilled water instead of 1.4 ml NaOH (alt. A).

(a) 2 ml of the 1:5 or 1:10 dilution of solution 1 a + 1.4 ml distilled water,

(b) 2 ml of the 1:5 or 1:10 dilution of solution 1 b + 1.4 ml distilled water.

(2) *Alternative C:* In the 1:20 and 1:30 dilutions used here, the pH in the solution was corrected using 1.4 ml 1N HCl (see note 2).

(a) 2 ml of the 1:20 or 1:30 dilution of solution 1 a + 1.4 ml 1N HCl,

(b) 2 ml of the 1:20 or 1:30 dilution of solution 1 b + 1.4 ml 1N HCl.

(3) To the solutions (2) alt. A—C, 40 mg ascorbic acid was then added (see note 3).

(4) The solutions prepared according to (3) were then treated with 1 ml ortho-phenanthroline (see note 4).

(5) To the solutions made according to (4), 1 ml citrate buffer was added (see note 5).

(6) The readings were performed in the Baeckman B spectrophotometer with an Agner micro-attachment at a wavelength of 515 mμ. The tests were performed with sample cells having a light path of 5 cm.

(7) The Fe content in the sample expressed in μg/ml was calculated using the formula $K \times (A_{test} - A_{blank})$ where K is a constant, and A_{test} and A_{blank} the absorption values read in the spectrophotometer for the sample solution and the blank solution respectively. The value of K has been determined for this particular spectrophotometer by using a standard solution of ferroammonium sulphate with a known iron content. The factor K is constant for Fe concentrations up to 8 μg/ml. The coloured complex thus follows Lambert-Beers' law.

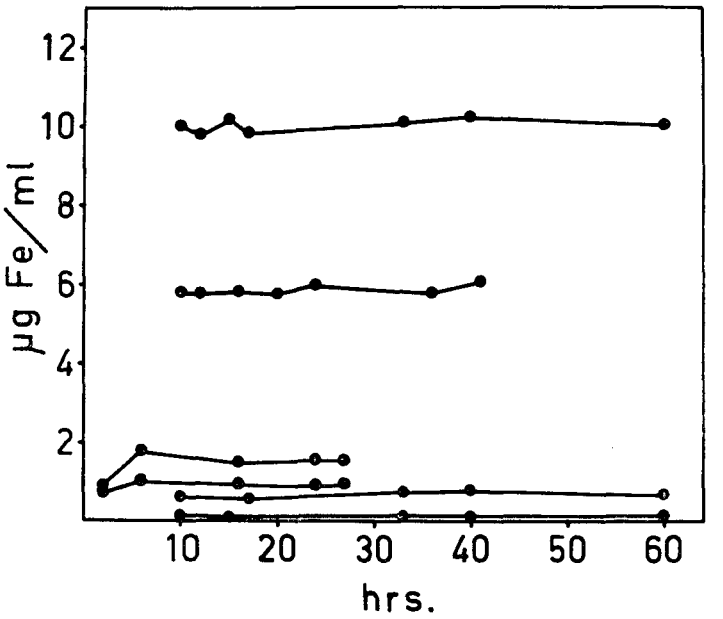

Fig. 1. Acidification time.

Note 1.

1. *Shaking:* The corrosion products are dissolved faster if the acidified solution is shaken. *Johansson* (1941) and *Mehlig & Hulett* (1942) also point out the importance of shaking for the dissolution in acid.

2. *Acidification:* In order to determine the Fe content in the corrosion products colorimetrically, it is necessary to convert the Fe into an ionic form. This is performed by acidifying with conc. HCl. Different amounts of Fe powder (pro analysi) were treated with HCl at the particular concentration used in the method. After shaking for 14 h, the Fe determination was carried out. All of the Fe corresponding to the maximum concentrations used in this investigation was dissolved.

3. *Acidification time:* The "solutions" obtained containing different amounts of corrosion products were acidified with 40 ml conc. HCl/100 ml. The solutions were shaken for 14 h and the Fe content determined after different times. Fig. 1 shows that the amount of dissolved iron becomes constant 10 h after the acidification.

Note 2.

Fortune & Mellon (1938) state that the intensity of the orthophenanthroline — Fe^{++} complex is unchanged between pH 2.0 and 9.0 provided that foreign ions do not interfere (see also Discussion). The strongly acidified solutions must therefore be cor-

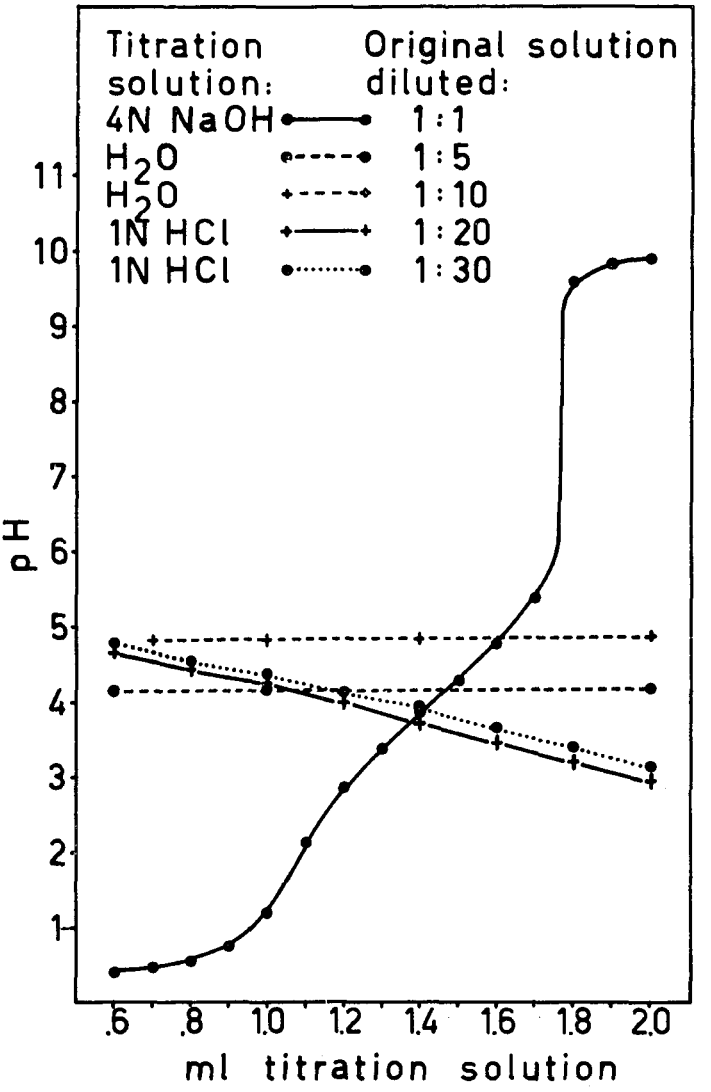

Fig. 2.

rected to a suitable pH. The risk for ion interference is also least at pH 2.5—6.0. The pH and volume of the solutions have been corrected by adding NaOH, HCl or distilled water. Of the solution prepared according to Procedure 1 (5 ml distilled water + 2 ml conc. HCl), 2 ml undiluted liquid and 2 ml of the dilutions 1: 5, 1: 10, 1: 20 and 1: 30 were taken for the experiments. To these were added 40 mg ascorbic acid, 1 ml ortho-phenanthroline and 1 ml citrate buffer. The undiluted solution was titrated with 4N NaOH, the dilutions 1: 5 and 1: 10 with distilled water, and the dilutions 1: 20 and 1: 30 with 1N HCl. Fig. 2 shows how the pH changed.

In this investigation, the pH of the solutions was corrected to the range 3.7—4.8 by adding either 1.4 ml NaOH, distilled water or HCl.

Note 3.

Any trivalent iron present must be reduced to the divalent state in order for it to react with the ortho-phenanthroline. As effective reducing agents, hydroxylamine hydrochloride and sodium hypophosphite (*Johansson* 1941) and ascorbic acid (*Moss & Mellon* 1942) have been suggested. To avoid an alteration in

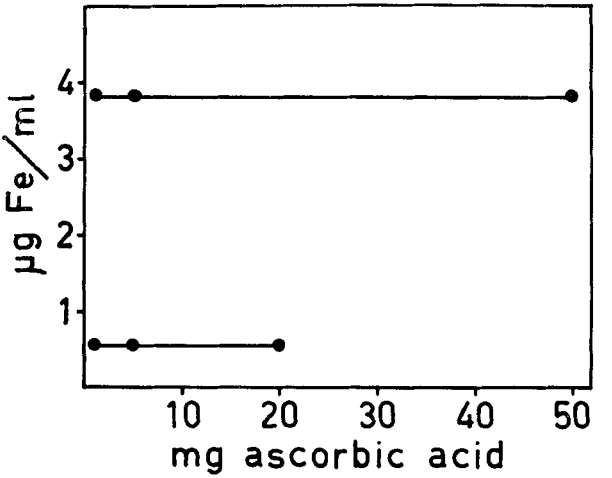

Fig. 3. Amount of ascorbic acid required for a complete reaction between Fe and ortho-phenanthroline.

the volume of the solution due to the reducing agent, the present author chose ascorbic acid pro analysi for this purpose. In order to determine the amount required, 1, 5, and 50 mg ascorbic acid were added to a solution containing a large amount of corrosion products while 1, 5, and 20 mg ascorbic acid were added to a

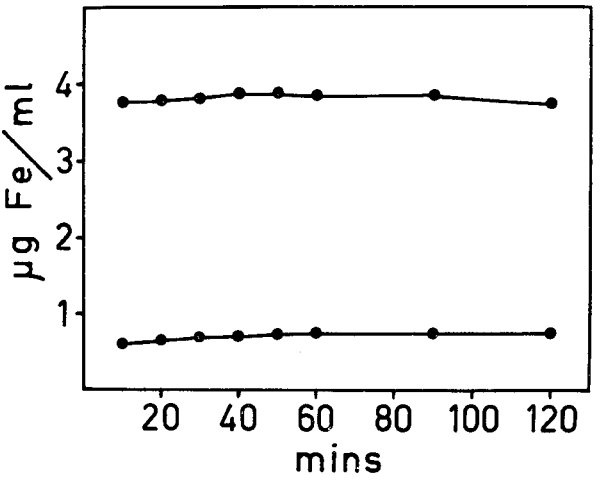

Fig. 4. Stability of the coloured complex.

solution containing only a small quantity of these products. Fig. 3 indicates that 40 mg ascorbic acid corresponds to a large excess. Fig. 4 shows that the coloured complex is stable during the actual time of measurement.

Note 4.

Ortho-phenanthroline reacts with divalent iron forming an orange-coloured complex $(C_{12}H_8N_2)_3Fe^{++}$. The colour intensity in a solution is dependent on the amount of complex-bound Fe ions if the ortho-phenanthroline is present in excess. If too small amounts of ortho-phenanthroline are used, the colour intensity will be weaker. In this investigation, the demand for a sufficient ortho-phenanthroline excess has been satisfied since 1.0 ml 0.6 % ortho-phenanthroline chloride was used (*Fortune & Mellon* 1938).

Note 5.

A final correction of the pH was made by adding sodium citrate. *Blandemer & Schaible* (1944) have studied the effect of sodium acetate, sodium citrate, and potassium citrate as buffers in the spectrophotometric determination of Fe using ortho-phenanthroline. Sodium acetate gave a cloudy solution which required centrifugation whereas those obtained with the citrates were clear. All three gave correct Fe values. The rate of colour development with sodium citrate was the same as that with sodium acetate but was somewhat slower than that with potassium citrate. In general, if the adjusting solution, whether acetate or citrate, was added before the reducing agent and ortho-phenanthroline, poor recoveries were obtained. When the acetate or citrate was added last, recoveries were complete. A possible explanation of this interfering effect is that complex formation occurs between Fe and the buffers and that this complex-bound Fe then reacts only slowly with the ortho-phenanthroline. The citrate buffer was therefore always added after the ascorbic acid and ortho-phenanthroline.

DISCUSSION

Fortune & Mellon (1938) performed an extensive investigation of the ortho-phenanthroline method. They examined various reducing agents and also list interference effects due to the presence of foreign ions. The following ions do not interfere: lithium, sodium, potassium, ammonium, arsenate, barium, calcium, lead, magnesium, manganese and strontium. Bismuth and silver must be completely absent since they form precipitates with the reagent. Cadmium, zinc, and mercury form sparsely soluble complexes. Copper is non-interfering between pH 2.5 and 4.0. Nickel causes a change in hue. Acetate, chloride, citrate, nitrate, sulphate and sulphite can be present up to 500 ppm without interfering. More than 20 ppm dichromate alters the shade of the solution.

In the present investigation, more than 500 ppm sodium, chloride and citrate have, however, been present in the reaction solution. Because of this, the interference of these ions at the particular concentrations used here was studied. The solutions used to investigate this point were prepared in the following manner:

Solution A: 2 ml Fe solution + 2 ml 25 % NaCl + 40 mg ascorbic acid + 1 ml ortho-phenanthroline + 1 ml citrate buffer.

Solution B: 2 ml Fe solution + 2 ml distilled water + 40 mg ascorbic acid + 1 ml ortho-phenanthroline + 1 ml citrate buffer diluted 1: 100.

In solution A, 2 ml 25 % NaCl corresponds to approximately a 10 % excess of electrolytes relative to the sample solutions. Solution A has the same citrate concentration as the solutions used in the Fe determination while, on the other hand, solution B has a citrate concentration less than 500 ppm. According to *Fortune & Mellon* (1938) the latter does not give any interference. The results in Table 1 show that no interference occurs at these high concentration of sodium, chloride and citrate.

In previously reported investigations on the spectrophotometric determination of iron in foods and biological materials (*Saywell*

Table 1

Interference at different electrolyte concentrations.

Amount of Fe = I; II; III; IV
M = mean value μg Fe/ml; n = no. of determinations;
SD = standard deviation.

A

	I	II	III	IV
M =	0.79 μg/ml	1.55 μg/ml	4.54 μg/ml	7.52 μg/ml
n =	10	10	10	10
SD =	0.01 μg/ml	0.02 μg/ml	0.03 μg/ml	0.05 μg/ml

B

	I	II	III	IV
M =	0.78 μg/ml	1.54 μg/ml	4.48 μg/ml	7.49 μg/ml
n =	10	10	10	10
SD =	0.01 μg/ml	0.02 μg/ml	0.02 μg/ml	0.11 μg/ml

& Cunningham 1937, *Hummel & Willard* 1938,*Blandemer & Schaible* 1944), only relatively small Fe contents have been under investigation. *Pepi* (1946) has used this method to determine Fe in aluminium alloys while *Mehlig & Hulett* (1942) have employed it to determine Fe in various ores. In addition, *Johansson* (1941) determined Fe contents in his corrosion experiments on steels. In all of these cases, however, the Fe contents were small.

In the present investigation, the spectrophotometric determination of iron using ortho-phenanthroline as reagent has been shown to be suitable for the high Fe contents existing in the corrosion products formed during the cooking and autoclave experiments. The method has been modified so that the iron in these products is converted into the ionic state after the corrosion test has been finished. This results in high electrolyte concentrations. No interference between ortho-phenanthroline and the electrolytes present has been detected.

SUMMARY

A colorimetric method using ortho-phenanthroline as reagent for the determination of iron in solutions containing high electrolyte concentrations has been described.

The iron in corrosion products formed during cooking and autoclave experiments was converted into the ionic state after the corrosion test had been finished. These experiments resulted in large amounts of iron and high electrolyte concentrations.

When ortho-phenanthroline was added as reagent in sufficient amounts, the spectrophotometric determination was shown to be satisfactory, and no interference between ortho-phenanthroline, foreign ions, and the electrolytes present was detected.

RÉSUMÉ

DÉTERMINATION COLORIMÉTRIQUE DE LA PRÉSENCE DU FER PAR L'ORTHO-PHÉNANTHROLINE, LA CONCENTRATION DE L'ÉLECTROLYTE ÉTANT ÉLEVÉE

L'auteur décrit une méthode colorimétrique utilisant l'ortho-phénanthroline comme réactif pour déceler le fer dans des solutions contenant des concentrations élevées l'électrolyte.

Le fer contenu dans les produits de corrosion formés pendant les expériences d'ébullition et de traitement à l'autoclave passe à l'état d'ions après la terminaison du test de corrosion. De cette expérience résultent de grandes quantités de fer et des concentrations élevées d'électrolyte.

Si on ajoute de l'ortho-phénanthroline comme réactif en quantités suffisantes, la détermination par spectrophotométrie s'est révélée appropriée, et il n'a pas été décelé d'interférence entre l'ortho-phénanthroline, les autres ions, et les électrolytes présents.

ZUSAMMENFASSUNG

KOLORIMETRISCHE BESTIMMUNG VON EISEN MIT ORTHOPHENANTHROLIN BEI HOHER ELEKTROLYTKONZENTRATION

Eine kolorimetrische Methode, bei welcher Ortho-phenanthrolin als Reagenz zur Bestimmung von Eisen in Lösungen mit hohen Elektrolytkonzentrationen angewandt wurde, wird beschrieben.

Das Eisen der Korrosionsprodukte, experimentell durch Kochen und Autoklavierung gebildet, wird, nachdem der Korrosionstest beendet ist, in den ionischen Zustand umgewandelt. Dieser Versuch resultiert in grossen Eisenmengen und hohen Elektrolytkonzentrationen.

Wenn Ortho-phenanthrolin in ausreichender Menge als Reagenz hinzugefügt wurde, erwies sich die spektrophotometrische Bestimmung als ausreichend, und es konnte keine Interferenz zwischen Ortho-phenanthrolin, fremden Ionen und gegenwärtigen Elektrolyten entdeckt werden.

REFERENCES

Agner, R.: Personal communication.

Blandemer, Selma L. & P. J. Schaible, 1944: Determination of iron. A study of the o-Phenanthroline method. Ind. Eng. Chem. Anal. Ed. *16:* 317.

Fortune, W. B. & M. G. Mellon, 1938: Determination of iron with o-Phenanthroline. A spectrophotometric study. Ind. Eng. Chem. Anal. Ed. *10:* 60.

Holmlund, L. G., 1963: Standardization, by means of ultrasonic treatment of test samples for laboratory steam-corrosion tests. Acta odont. scand. *21:* no. 4.

Hummel, F. C. & H. H. Willard, 1938: Determination of iron in biological materials. The use of o-Phenanthroline. Ind. Eng. Chem. Anal. Ed. *10:* 13.

Johansson, S., 1941: Metod för undersökning av korrosionshastigheten hos stål. Jernkontorets Annaler *125:* 599.

Mehlig, J. P. & H. R. Hulett, 1942: Spectrophotometric determination of iron with o-Phenanthroline and with Nitro-o-Phenanthroline. Ind. Eng. Chem. Anal. Ed. *14:* 869.

Moss, M. L. & M. G. Mellon, 1942: Color reactions of 1,10-Phenanthroline derivatives. Ind. Eng. Chem. Anal. Ed. *14:* 931.

Pepi, M. S., 1946: Rapid photometric determination of iron in aluminium alloys. Ind. Eng. Chem. Anal. Ed. *18:* 111.

Saywell, L. G. & B. B. Cunningham, 1937: Determination of iron. Colorimetric o-Phenanthroline method. Ind. Eng. Chem. Anal. Ed. *9:* 67.

West, T. S., 1951: Colorimetric determination of iron. A review of known methods — 1. Metallurgia, *43:* 204.