LXII. A MACRO-METHOD FOR THE DETERMINATION OF IRON IN BIOLOGICAL MATERIAL BY A MODIFIED PERMANGANATE TITRATION.

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In the course of investigations on the metabolism of iron, it became necessary to determine relatively large amounts of iron in the presence of such interfering agents as organic matter and phosphate. It was found that the errors introduced by colorimetric micro-methods became enormously exaggerated when amounts of iron between 1 and 10 mg., or more, were determined. A survey of the existing methods which could be used for such amounts of iron showed many disadvantages.

Destruction of organic matter by ignition was extremely time-consuming; incineration with sulphuric acid was considered to present the advantages both of quickness and of removal of halogens which would interfere in subsequent steps in the procedure.

The gravimetric estimation of iron required either the removal of phosphate by molybdate, or the use of the basic acetate separation. These modifications, together with the general undesirability of using ammonia in the laboratory, ruled out this method of investigation.

The use of a sulphuric acid digestion made possible the volumetric estimation of iron. The permanganate method of Margueritte [Treadwell and Hall, 1924] was chosen because of the ease of determining the end-point and the fact that permanganate offers a self-contained indicator. The iron for this method must be in the ferrous condition. This was assured by reduction by nascent hydrogen in the presence of an excess of zinc and sulphuric acid.

The method has proved to be very satisfactory and extremely accurate within the limits of its application. It has been used in some 400 estimations of iron during the past year with uniform success. The results obtained by the method will be published elsewhere, the present paper giving the details of the modifications employed, the precautions and a few examples of its accuracy and possible applications.

Experimental.

Reagents.

Sulphuric acid. "Baker's Analysed," "C.P." brand, low in heavy metals.

Perchloric acid. Merck's brand 60 % perchloric acid, sp. gr. 1·54, low in heavy metals.

Zinc. "Baker's Analysed" zinc, "C.P." brand, 30 mesh granular, low in lead and iron.

Potassium permanganate N/*10 and* N/*100.* Standardised according to Treadwell and Hall [1924] against pure sodium oxalate.

Glassware. All glassware was of pyrex, extracted with 2 % HCl, chromic acid and rinsed in many changes of distilled water. The distilled water should be checked for the presence of iron and if necessary redistilled from treated glass vessels.

Destruction of organic matter.

(*a*) *Solutions of soluble iron compounds.* An amount of the iron solution containing 5–10 mg. of iron was measured into an 800 ml. Kjeldahl flask. 10 ml. of concentrated sulphuric acid and two glass beads were added and digestion commenced in a Kjeldahl digestion apparatus over a low flame. After charring was complete and a blood-red, apparently homogeneous solution was obtained, 0·5 ml. of perchloric acid was added. Digestion was then continued for one hour. Oxidation was invariably complete at this time and the solution was free from chlorides.

(*b*) *Faeces.* 1 g. samples were used when patients were being treated by some form of iron therapy. When a low iron content of the faeces necessitated the use of much larger samples than this, it was deemed advisable to use one of the many micro-methods. The faeces were prepared for analysis by careful drying, first on the water-bath and later in a desiccator. The sample should be kept as finely divided as possible during the drying process. The sample for analysis was further finely ground and sampled by quartering. 1 g. samples of this finely ground material were transferred directly to an 800 ml. Kjeldahl flask and completely washed into the flask by distilled water. 10 ml. of sulphuric acid and two glass beads were added. The digestion was allowed to proceed very slowly, the flasks being rotated from time to time until homogeneous mixtures, free from foam, were obtained. At this point the speed of digestion was increased until golden-red, translucent solutions resulted. 0·5 ml. of perchloric acid was then added and the digestion continued for a further hour.

(*c*) *Whole blood.* 2–10 ml. of whole blood were used for the analysis, depending on the degree of anaemia. The digestion was carried out in the same way as for faeces. (To facilitate digestion the Kjeldahl flasks containing the blood and sulphuric acid may be allowed to stand overnight before the digestion commences.)

(*d*) *Insoluble iron compounds.* Insoluble iron compounds were analysed in the same way as for faeces. The amount taken for analysis however should contain at least 5 mg. of iron as the metal.

Reduction.

The contents of the Kjeldahl flasks were diluted with 15 ml. of water and raised to the boiling-point with constant rotation. The sulphuric acid solution was then transferred quantitatively to 125 ml. Erlenmeyer flasks. 10 ml. of distilled water were added to the Kjeldahl flasks, boiled and transferred quantitatively to the Erlenmeyer flasks. The volume of transfer fluid should be kept as low as possible. Boiling out the Kjeldahl flasks was found to be absolutely necessary since, even with the greatest care, some etching of the glass occurs.

The contents of the Erlenmeyer flasks were allowed to cool completely, and 5 g. of granulated zinc were added. The flasks were then fitted with Bunsen valves and allowed to stand overnight.

When the reaction between the zinc and sulphuric acid was complete, a small sample of the contents of the flask was removed by means of a capillary tube and the fraction of a drop so removed tested for ferric iron with ammonium thiocyanate, using a porcelain spot plate.

Titration.

When reduction was complete the contents of the Erlenmeyer flasks were filtered quantitatively through No. 42 Whatman filter-papers. The flasks were washed by decantation and the final residue transferred by a stream of water to the filter-paper. The filtrate, including the washing, was collected in 400 ml. pyrex beakers. Washing and transferring were done in the cold as quickly as possible to avoid re-oxidation of the iron.

2 ml. of concentrated sulphuric acid were added to the contents of the beakers and titration with 0·1 *N* potassium permanganate carried out. During the titration the beakers were surrounded by a white background and the end-point taken as the first recognisable pink flush persisting for 30 seconds.

When the amount of iron was less than 10 mg. it was found advisable to use 0·01 *N* potassium permanganate. In this case all transfers from the beginning of the procedure should be made with the minimum amount of wash fluid. To diminish further the dilution of the iron, the final transfers and washings may be made with cold 1 % sulphuric acid. The addition of further sulphuric acid to the solution before titration may then be omitted. The final titration was done in white porcelain casseroles which facilitated identification of the end-point with certainty. It was found advisable to use a standard end-point at first, but when the eye became accustomed to the recognition of the end-point, this precaution was omitted. All titrations were done with a Folin micro-sugar burette of 5 ml. capacity, graduated in 0·02 ml. to facilitate splitting of drops.

Blank.

A blank was run on all reagents by carrying through the exact procedure without the addition of the iron-containing material. This blank was subtracted from the total titration figure.

Precautions.

The oxidation of organic matter and the removal of interfering agents by sulphuric acid digestion was found to be straightforward if the directions were closely followed. Time was saved by digesting as slowly as possible at first and only increasing the temperature after all danger of foaming was over.

Much time was spent in determining a satisfactory form of zinc to employ in reduction. The 30-mesh zinc suggested was found to be the right size to prevent floating, if added after the solutions to be reduced were cool. 5 g. of zinc are more than adequate for the usual amounts of iron handled and this amount of zinc will keep the iron in a reduced condition for 12 hours, if the Bunsen valves are made air-tight.

From the time the reducing flasks are opened, no delay should be made. Re-oxidation of the iron was found to be quite rapid; however, if the titration follows the transfer within 15 minutes, no appreciable change in the titre can be found. In order to reduce the time still further, at this point, filtration may be made through platinum cones if such are available.

Experimental results.

The method has been used for the analysis of iron in such compounds as ferric ammonium citrate, in the presence of organic matter, phosphates and chlorides. It has also been used in the estimation of iron in such biological

material as faeces and blood. The data presented in Tables I and II indicate the degree of duplication and accuracy which has been in general obtained by the method.

Table I. *Recovery of added iron from dried faeces.*

Weight of faeces g.	Iron content of faeces mg.	Iron added mg.	Total iron found mg.	Iron recovered mg.	Error mg.
1·000	9·90	9·98	19·95	10·05	+0·07
1·000	9·90	9·98	19·85	9·95	−0·03
1·000	9·90	9·98	19·85	9·95	−0·03
1·000	9·90	5·00	14·80	4·90	−0·10
1·000	9·90	5·00	14·83	4·93	−0·07

Table II. *Analyses of 1 % and 0·1 % ferric ammonium citrate solution containing iron-free organic matter*.*

ml. of solution	%	Iron present mg.	Iron found A mg.	Iron found B mg.	Iron found Average mg.	Error mg.
50	1	88·95†	88·95	88·95	88·95	±0·00
25	1	44·47	44·35	44·25	44·30	−0·17
10	1	17·79	18·15	18·05	18·10	+0·31
5	1	8·89	9·05	9·15	9·10	+0·20
50	0·1	8·89	8·60	8·55	8·57	−0·32
25	0·1	4·45	4·21	4·27	4·24	−0·21
10	0·1	1·78	1·70	1·71	1·705	−0·07
5	0·1	0·89	0·80	0·62	0·71	−0·18

* Filter-paper. † Calculated from repeated estimations of ferric ammonium citrate.

In Table I recovery of added amounts of a standard iron solution (ferric chloride prepared from pure iron wire) from faeces is given. For amounts of iron above 5 mg., recoveries to within 0·1 mg. were obtained. The duplication of results was good; variations between duplicates of 0·03 mg. of iron were found. The iron values for the solutions given were obtained by the customary gravimetric method.

Table II presents data obtained from the analysis of standard solutions of ferric ammonium citrate. This compound was used in the investigation for which this method was devised and represents iron attached to an organic radical. Further organic matter was added in the form of filter-paper during the digestion. The error in mg. varied from 0·07 to 0·32 throughout the range of dilutions. The amount of iron present varied from 0·89 to 88 mg.

Table III is included as an example of the use of the method for general biological analyses. The patients had varying degrees of anaemia. The haemoglobin value was determined in two cases by direct estimation from the oxygen capacity and in the third, by use of the Sahli haemoglobinometer standardised against the oxygen capacity. The volumes of blood taken varied from 2 to 10 ml.

Table III. *Determination of the iron content of haemoglobin.*

Patient	ml. blood	Iron mg./100 ml. blood	O_2-capacity vol./100 ml. blood	Hb g./100 ml. blood	% iron in Hb
1	6	53·2	19·95	14·82	0·359
2	10	38·2	—	11·39*	0·345
3	2	45·8	17·91	13·30	0·342

* Sahli.

The determined percentage of iron in haemoglobin found in all cases checked to within 0·02 %. The values found for the percentage of iron in haemoglobin were slightly higher than those calculated from the figures of Murphy *et al.* [1931], who used a micro-method, but in close agreement with those of Hufner [1894]. From the duplication of results it appears that the method lends itself very well to whole blood determinations.

Reports by Brock and Taylor [1934] show that the method may be satisfactorily used in dialysis and metabolic studies of iron, when this metal is present in gross amounts.

SUMMARY.

1. A detailed description is given of a modification of the permanganate titration method for the determination of iron.
2. The method is applicable to the determination of iron in the presence of organic material and phosphate.
3. Data are presented showing the degree of accuracy and duplication of the results.
4. The satisfactory use of the method in the determination of the percentage of iron in haemoglobin is demonstrated.
5. The method is recommended for the determination of iron in all biological material in which samples can be obtained containing more than 2 mg. of iron per g.
6. Within the limits specified for its application, iron may be determined within $\pm 0{\cdot}3$ mg.

One of us (J. F. B.) was Leverhulme Research Scholar of the Royal College of Physicians of London at the time that the work was done, and expresses his appreciation to the Science Committee of the College for the privilege of spending the year in Boston.

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