

# The determination of micro quantities of manganese in plant material

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## Summary

After a liquid-liquid extraction with 2-thenoyltrifluoroacetone, micro quantities of manganese in plant material can be determined with formaldoxime without interference from other elements.

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## 1. Introduction

For research work on plant material it was necessary to develop a method for determining micro quantities of Mn in plant material with a very high Fe content. One of the most sensitive determinations of Mn is a method in which Mn catalyses the oxidation of tetramethyldiaminodiphenylmethane (SINGLE, 1957; CORNFIELD and POLLARD, 1950). As with all catalytic processes the method calls for a rigid temperature and time control. Nevertheless unaccountable variations associated with changes in the rate of colour development seem to occur (SINGLE, 1957). Fe interferes and must be extracted in advance (SNELL and SNELL, 1959).

Another reaction catalyzed by Mn is the oxidation of leucomalachitegreen (4,4'-tetramethyldiaminotriphenylmethane). However, this very sensitive reaction (KOCH and KOCH-SEDCI, 1964; FERNANDEZ *et al.*, 1963) is severely influenced by the presence of Fe (III). The procedure of FERNANDEZ *et al.* (1963) is furthermore very time consuming because the extinction of each sample has to be measured five times at intervals of one hour. The slope of the extinction-time curve is a linear function of the Mn concentration.

1-(2-pyridylazo)-naphthol (PAN) was first proposed by CHENG and BRAY (1955) as a very sensitive reagent for the determination of several metals. BERGER and ELVERS (1959/60) and especially SHIBATA (1960; 1961) have published on this subject.

It however proved not altogether possible to get reproducible results with the methods described. This can perhaps be attributed to acids contained in the chloroform which set free oxidation products that "bleach" the coloured Mn complex. This suggestion of BERGER and ELVERS (1964) was however, at that moment, not known to the authors. The determination of Mn with formaldoxime is less sensitive than the methods mentioned above but it is much more sensitive than the permanganate method (PINTA, 1962; SANDELL, 1959; SNELL and SNELL, 1959). According to MARCZENKO (1964) formaldoxime forms highly-coloured complexes with Mn, Fe, Ni, Ce, V, Cu and Co.

Received for publication 19th August, 1965.

To test the interference of some of these metals spectrograms were made of 50  $\gamma$  quantities of Mn, Fe, Ni, Co and Cu with formaldoxime in a  $\text{NH}_4\text{OH}$  medium at a final volume of 25 ml. These are presented in the FIGURE.

At 450 nm., interference can be expected from Fe and Ni. Contrary to the statement of MARCZENKO, the influence of Cu and Co proved to be negligible at these concentrations. The level of Co, Ce and V in plant material (PINTA, 1962) is so low that interferences by these elements are not to be expected.

The interference caused by Ni is readily masked by cyanide. The masking of high amounts of Fe however constitutes a problem because all the masking agents tested for this purpose resulted in the formation of a yellow-coloured complex which, in its turn, interferes. It was therefore necessary to separate the Fe from the Mn. If this procedure is worked out in such a way that Mn is concentrated at the same time, the formaldoxime method can be applied to very low Mn levels.

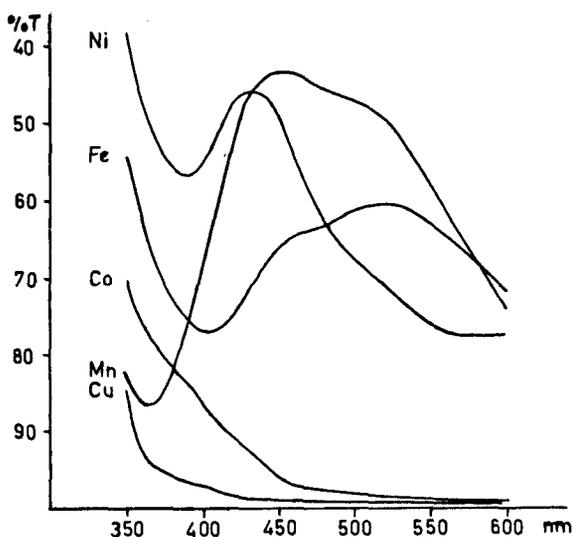


FIGURE  
Spectrograms of Mn-, Fe-, Ni-,  
Co- and Cu-formaldoxime  
complexes

ECKERT (1955/56, 1957) and BODE and TUSCHKE (1957) use a sodium-diethyldithiocarbamate extraction in chloroform or carbontetrachloride. A back-extraction of the organic phase with a zinc solution liberates all of the Mn and only a small part of the Fe (BODE and TUSCHKE, 1957). At a 1000 : 1 Fe/Mn ratio the separation did not prove to be sufficiently selective.

It was tried to prevent the extraction of Fe, under the circumstances mentioned above, by complexation with *o*-phenanthroline. At the high pH levels tested a part of the orange-coloured Fe complex always contaminated the back-extraction phase. Washings with the medium used for the extraction of the Mn eliminated this contamination, but resulted in the loss of some Mn.

CHENG (1961) mentions the use of 2-thenoyltrifluoroacetone (TTA) as a means of extracting Mn selectively at pH 6—10 in the presence of tartrate with 4-methyl-2-pentanone. According to this author only Co will accompany Mn and this can probably be prevented by triethylenetetramine. JOHNSON and LOTT (1963) extract Mn with

TTA in a mixture of 2-octanon and 4-methyl-2-pentanone at pH 8. The flamephotometric determination of Mn directly in the organic phase is however influenced by Ce, Co, Cr, Fe (II), (III), Ni and Th. DE and RAHAMAN (1963) extract the same complex in an acetone-benzene mixture at pH 6.7—8.0. Colour reactions are reported with Cu, Ni, Cr, U. Co interferes seriously. Fe is removed beforehand. The determination in the organic phase as proposed by these authors is not sensitive enough for our purpose.

It therefore seemed logical to back-extract the organic phase under slightly acidic circumstances. The iron will then stay in the organic phase (MORISON and FREISER, 1957) whereas the Mn will be transferred into the water phase where it now can be determined with formaldoxime.

## 2. Experiments

The experiments were started using 4-methyl-2-pentanone as the organic phase. The separation of the two phases was however time consuming. A 1:1 mixture of the 4-methyl-2-pentanone with n-dibutylether led to a fast and clear separation of the two phases. It furthermore proved profitable, for the same reason, to introduce  $(\text{NH}_4)_2\text{SO}_4$ .

Because of the rather high pH tartrate was added to prevent precipitations.

An extraction with an acetone-benzene mixture as proposed by DE (1963) led to a turbid water phase under our circumstances. The yield of the extraction was as high as with the 4-methyl-2-pentanone and n-dibutylether mixture.

In the presence of tartrate and  $(\text{NH}_4)_2\text{SO}_4$  the optimal pH for extraction was determined. The highest yield was obtained at pH 7.7—8.3 but errors caused by a higher pH up to 8.8 were negligible.

The extraction of 20  $\gamma$  of Mn was optimal after 2 minutes shaking using a shaking machine with a stroke of 9 cm and 150 oscillations/minute.

For the determination of Mn, VAN DEN HENDE and COTTENIE (1960) use a metol-sulphite-reducing agent. This mixture has a much longer shelf life as the more commonly used ascorbic acid. The back extraction of Mn into a water phase could readily be achieved with diluted  $\text{HNO}_3$  or  $\text{H}_2\text{SO}_4$ . To cut down on the number of additions this acid was mixed with the metol-sulphite-reducing agent. Lower blanks could, in this case, be obtained with  $\text{H}_2\text{SO}_4$  as compared with other acids tested ( $\text{HCl}$  and  $\text{HNO}_3$ ).

A shaking time of three minutes sufficed for a reproducible extraction.

The back-extracted Mn was determined with formaldoxime according to the method described by VAN DEN HENDE and COTTENIE (1960).

## 3. Reagents

Only analytical grade chemicals have been used.

a. metol-sulphite-sulphuric-acid solution: mix before use equal volumes of reagents a.1 and a.2.

a.1. dissolve 34 g of  $\text{Na}_2\text{S}_2\text{O}_5$ , 2.5 g of  $\text{Na}_2\text{SO}_3 \cdot 7 \text{ aq}$  and 0.25 g of metol (p-methylaminophenolsulphate) and make up to a volume of 500 ml.

a.2. diluted sulphuric acid: dilute 14 ml of  $\text{H}_2\text{SO}_4$  (s.w. 1.84) to a final volume of 1 litre.

- b. buffer solution: dissolve 50 g of KNa tartrate .4 aq, 125 g of  $(\text{NH}_4)_2\text{SO}_4$  and 10 ml of  $\text{NH}_4\text{OH}$  (s.w. 0.91) and make up to a final volume of 1 litre.
- c. TTA solution: dissolve 3.3 g of 2-thenoyltrifluoroacetone in 1 litre of a 1 + 1 mixture of *n*-dibutylether and 4-methyl-2-pentanone. This solution can be stored for at least one month.
- d. alkaline KCN solution: dissolve 1.7 g of KCN and 22.4 ml of  $\text{NH}_4\text{OH}$  (s.w. 0.91) and make up to a final volume of 100 ml.
- e. formaldoxime reagent: dissolve 5 g of hydroxylamine-HCl and 5 g of formaldehyde 40 % and make up to a final volume of 100 ml.
- f. ammonia 1.6 n: dilute 12 ml  $\text{NH}_4\text{OH}$  (s.w. 0.91) and make up to a final volume of 100 ml.
- g. standard solution of 10 ppm Mn: dissolve in a 600-ml beaker 0.288 g of  $\text{KMnO}_4$  in about 200 ml of distilled water to which has been added 45 ml of  $\text{H}_2\text{SO}_4$  s.w. 1.84). Reduce the  $\text{KMnO}_4$  with a few drops of  $\text{H}_2\text{O}_2$  30 %. Boil to expel the excess of  $\text{H}_2\text{O}_2$ . Rinse the cooled solution into a 1-litre volumetric flask and make up to volume. Pipette 100 ml of this solution into a 1-litre volumetric flask and make up to volume.

#### 4. Procedure

Digestion of the organic material (SCHAUMLÖFFEL, 1960): Weigh out 1 g of air-dry plant material in a 50-ml volumetric flask. Next add 10 ml of an acid mixture ( $\text{HNO}_3 : \text{HClO}_4 : \text{H}_2\text{SO}_4 = 10 : 1 : 0.25$  volume parts) and, if possible, keep overnight to prevent excessive foaming. Heat moderately on a hotplate. It should take 40 minutes or more for the  $\text{HNO}_3$  to evaporate. Afterwards the heat is raised to full. Destruction is complete when only the sulphuric acid is left. If a colour persists, the flask should be cooled and some drops of  $\text{HClO}_4$  60 % added. Reheat the flask until all the  $\text{HClO}_4$  is evaporated. Add 10—20 ml of distilled water and some drops of  $\text{H}_2\text{O}_2$  30 % to reduce Mn oxides which might have formed, and boil during 10 minutes. (BRADFIELD (1964) suggests the use of  $\text{NaNO}_2$  for this purpose which seems a better approach since traces of  $\text{H}_2\text{O}_2$  might interfere with other determinations in the same digest). Cool the flasks and fill up to the mark with distilled water.

Determination of the manganese:

Pipette 5 — 10 — 20 or 35 ml of the digest (containing less than 20  $\gamma$  of Mn) into a separating funnel. Then add 10 ml of buffer solution and bring to pH 7.8—8.8 with ammonia 1.6 n (1 ml of ammonia 1.6 n for each 10 ml of digested sample serves the purpose). Add distilled water to a total volume of 50 ml. Add 20 ml of TTA solution and shake mechanically during 2 minutes (stroke length 9 cm, 150 oscillations/minute). Wait until the phases have separated and discard the water phase. Next, add to the organic phase 4 ml of the metol-sulphite-sulphuric-acid mixture and shake on a mechanical shaker for 3 minutes. Wait until the phases have separated and leave off the water phase, quantitatively, into test tubes. Add to the contents in the test tubes 1 ml of the alkaline KCN solution and 3 drops of the formaldoxime reagent respectively. Wait 30 minutes and filter the solution before measurement in 1-cm cuvettes at 450 nm.

TABLE 1. Reproducibility of added manganese

$\gamma$ of Mn added	$\gamma$ of Mn recovered							
5	5.1	5.0	4.9	5.2	5.0	4.8		
10	9.7	9.9	9.9	9.7	9.3	10.0	10.1	10.2
	10.0	9.8	9.5	9.9				
20	19.8	20.6	21.0	20.8	20.0			

### 5. Interferences

No interferences within experimental error were found with 4000  $\gamma$  of Fe (II) or Fe (III), 20  $\gamma$  of Co, 500  $\gamma$  of Cu and 500  $\gamma$  of Ni with the determination of 10  $\gamma$  of Mn. Part of the Fe originally present accompanied the Mn in the organic phase but it was back extracted to a negligible fraction which could easily be masked by the KCN present.

Co interfered (about 14 %) at the 100  $\gamma$  level. Following CHENG's suggestion (1961), triethylenetetramine was tried as a masking agent for Co. 0.4 ml of a 1 + 9 diluted triethylenetetramine solution masked the Co influence at the 100  $\gamma$  level but 200  $\gamma$  of Co had a positive influence on the results (about 6 %). No further work was done on this point because higher quantities of Co are not expected in plant material.

The iron salt used for testing the interference of Fe ( $\text{FeCl}_3 \cdot 6 \text{ aq}$ ) appeared to be contaminated with some Mn. The iron was therefore separated by a liquid-liquid extraction with ether from a 6 n HCl medium and back extracted in 1.2 n HCl.

### 6. Reproducibility

The results follow BEER's law up to at least 20  $\gamma$  of Mn. The blank, under our circumstances, varied between 0.030—0.070 extinction units, depending on the shelf-life of the reagents.

Considering the minor quantities involved, the reproducibility of the method is satisfactory, as shown in TABLE 1. Applied to the determination of Mn in plant material the results proved to be satisfactory. Different quantities of plant material with and without an addition of Mn have been wet-ashed with 20 ml of a 10 : 1 : 0.25 v/v

TABLE 2. Mn content (ppm) of plant material after wet ashing of 0.5, 1.0 and 1.0 g of plant material with Mn added

	0.5 g	1.0 g	1.0 g + Mn
Potato tubers	8.1	8.4	9.0
Peas	15	15	15
Barley grain	21	21	22
Apple leaves	37	37	36
Cucumber leaves	41	41	40
Valeriana officinalis	74	72	72
Grass	88	89	86
Oats straw	146	148	—

mixture of  $\text{HNO}_3$ ,  $\text{HClO}_4$ ,  $\text{H}_2\text{SO}_4$  respectively. After subtraction of the Mn added, the results are as presented in TABLE 2.

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DETERMINATION OF MICRO QUANTITIES OF MANGANESE IN PLANT MATERIAL

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