making the total solution to 400 cc. The solution is then electrolyzed hot, using 1.5 amperes at 6 volts. A rotating cathode is used. The end of the revolving spindle carries a rubber stopper over which a clean weighed platinum crucible is slipped. The apparatus which has been found convenient for carrying out the electrolysis of a number of determinations at the same time is shown in front elevation in Fig. 1 and in side elevation in Fig. 2. From one to four hours is necessary to complete an electrolytic run, two hours being generally sufficient, except in cases in which the tin content is very high. At the end of a run the crucibles are cleaned by heating in a solution made by mixing 100 cc. of 10 per cent. oxalic acid with 100 cc. of concentrated nitric acid.

Numerous experiments were made with the electrolytic apparatus which showed that tin could be recovered with a fair degree of accuracy from an ammonium sulfide solution. Some of these results are given in Table I.

TABLE I

Sample No.	Tin added mg	Tin found mg	Time taken
1	1.0	1.1	1 hour
2	1.0	1.0	1 hour
3	10.0	9.8	2¼ hours
4	10.0	9.9	2¼ hours
5	10.0	10.0	2⅓ hours
6	10.0	10.0	2⅓ hours
7	25.0	24.6	2¾ hours
8	50.0	50.5	4 hours

The next step was to study the effect of organic food material on the method. Tomatoes were put into glass bottles with 50 cc. of a ten per cent. sodium chloride solution, and 10 mg. of tin in solution were then added to each bottle. The bottles were corked and autoclaved under pressure in a manner similar to the processing of canned foods. The results of analysis are given in Table II.

TABLE II

No.	Tin added mg.	Tin recovered mg.	Time of electrolysis
1	10	9.9	2 hours
2	10	9.2	2 hours
3	10	10.1	2 hours
4	10	9.9	2 hours

In run No. 2 the cork blew out of the bottle during processing and it was known that a slight amount of the contents had been lost. These results were very encouraging as the determination of small amounts of tin in the presence of an excess of organic food material must be but a fair approximation of the truth at the best. The next step was to try the method on a large variety of food products. The results of a number of duplicate runs are given in Table III.

TABLE III

Material	Tin found Mg. per kilo Run I	Run II	Time, hours
Apple butter A	178	180	3
Apple butter B	142	142	2.5
Apple butter A	168	168	2.5
Apple butter B	142	142	2.5
Blackberries	28	26	2.5
Rhubarb A	170	174	2.5
String beans	72	64	2.5
Beef soup(a) (solid)	18	22	2.5
Tomato soup	74	74	2.5
Bouillon soup	28	26	2.5
Oxtail soup (solid)	66	76	2.5
Vegetable soup (solid)	22	36	2.5
Mock turtle soup (solid)	20	20	2.5
Baked beans with tomato sauce	40	42	2.5
Baked beans without tomato sauce	8	10	2.5

(a) All the soups except the bouillon and tomato were solid and contained large amounts of meat and vegetables.

The results given in Table III show that it is possible to produce satisfactory check results when working in duplicate by the method, and, in fact, the checks are generally much closer than can be expected when working with the longer gravimetric method. In each analysis the insoluble residue, consisting of food pulp, was carefully examined for tin, but if any was present it was in such small amount that it could not be found by qualitative tests.

In Table IV the results are given of a series of analyses of various foods when the gravimetric and electrolytic methods were both used.

TABLE IV

Material	Tin found Mg. per kilo Electrolytic method	Gravimetric method
Tomato soup	145	130
Rhubarb	112	102
Squash	316	314
Spinach	35	22
String beans (liquid portion)	94	70
String beans (solid portion)	340	356
String beans (total)	229	226
Beets (solid portion)	70	27(a)
Cuthbert raspberries	248	222
Sweet potatoes	40	39
Pork and beans	64	73
Sauer-kraut	12	13
Apple-butter	142	112
Blackberries	27	17

(a) Result probably low.

An inspection of Table IV shows a generally satisfactory agreement between the results obtained by the two methods, and it should be stated that these are not selected results but are given directly in the order in which they were obtained by two different workmen. Only about three hours are required to get out a series of results by the electrolytic method, the number in the batch being limited only by the number of electrolytic cells available in the laboratory. With the gravimetric method usually the best part of three days is required to produce a batch of determinations, the number being in this case limited by the hood and flue space available. In the saving of time, acids and other reagents consumed and in destruction to flues and the laboratory generally, the advantage is all on the side of the electrolytic method. If considerable work of this kind has to be done, the first cost of the electrolytic apparatus is fully justified.

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POTASSIUM PERMANGANATE IN THE QUANTITATIVE ESTIMATION OF SOME ORGANIC COMPOUNDS[1]

By C. M. PENCE

Potassium permanganate has been most generally used in the volumetric estimation of iron. Some uncertainties formerly existed since it was impossible to obtain a chemically pure article and insufficient data were

[1] Read before the Indiana Section of the American Chemical Society, May 10, 1912.

at hand as to proper methods of preparation and standardization of its solutions.

At present, these objections have been largely overcome and almost all of our text-books on quantitative analysis contain an extended treatise on proper means of preparation and standardization of volumetric permanganate solutions.

One of the most commonly known organic compounds that is quantitatively determined by the use of volumetric permanganate is oxalic acid. Now oxalic acid and iron are determined in acid solution, but the procedure most applicable for the oxidation of all types of aromatic compounds as well as carbohydrates and hydrocarbons is with alkaline permanganate. Oxidations in acid solution are less energetic than those with alkaline $KMnO_4$ and in the latter case the final product of a completed decomposition of the organic compound is oxalic acid instead of CO_2 and H_2O.

Among the substances mentioned in the literature[1] as being oxidized to oxalic acid are propylene, isobutylene, amylene, acetone, fatty acids; butyric, lactic, succinic, and tartaric acids; dextrose, sucrose, glycerol and phenol. Now when an organic compound is oxidized to oxalic acid, a further oxidation to CO_2 and H_2O readily follows upon acidifying and warming the solution. Such a procedure forms the nucleus of a method for the determination of the compounds previously enumerated. Tocher made use of this method and found that phenol could be determined. His method was substantially as follows: Dissolve 1 gram phenol in 1000 cc. distilled water and take 10 cc. for titration. Add 3–4 grams $NaHCO_3$ together with a little distilled water. Then add 50 cc. $KMnO_4$ and boil for five minutes. Set aside to cool and gradually add dilute H_2SO_4 to decided excess; warm to 60° C. and titrate the excess of $N/10$ $KMnO_4$ with $N/10$ oxalic acid.

This method was found to be open to the following objection: that the manganese dioxide formed as a result of the action of $N/10$ $KMnO_4$ upon the phenol did not reduce readily enough with consequent solution upon direct titration with $N/10$ oxalic acid. Thus the solution was full of oxide which not only obscured but rendered the end point of little value, in that the oxide was not completely reduced before the permanganate end point was obtained.

The following modification of Tocher's method was found to give good results:

Dissolve 0.4 gram phenol in 1000 cc. distilled water. Place 50 cc. $N/10$ $KMnO_4$ and 3 to 4 grams $NaHCO_3$ in a 500 cc. glass-stoppered Erlenmeyer flask. Add 25 cc. of the phenol solution with gentle rotation. Boil 5–10 minutes (with stopper removed). Cool flask to about 60° C. Acidify with dilute H_2SO_4, let stand about 2 minutes; cool to room temperature. Dilute with distilled water, add 5 cc. 20 per cent. KI and titrate the liberated iodine with $N/10$ thiosulfate solution, using starch as indicator. The number of cc. of $N/10$ thiosulfate subtracted from the number of cc. $KMnO_4$ originally added = no. cc. of $KMnO_4$ consumed by the phenol.

1 cc. $N/10$ $KMnO_4$ = 0.000336 gram phenol.

If a glass-stoppered Erlenmeyer flask is not available an ordinary Erlenmeyer may be used and its contents transferred to a glass-stoppered bottle before acidifying. Any oxide adhering to the Erlenmeyer is easily removed by the addition of a little distilled water acidified with H_2SO_4 and containing a few drops of 20 per cent. KI.

In considering the nature of the oxidation with $KMnO_4$ in acid and alkaline solutions it is observed that each molecule of $KMnO_4$ in acid solution liberates 2.5 atoms of oxygen according to following equation:

$$2KMnO_4 = K_2O + 2MnO + 5O$$

or

$$2KMnO_4 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 3H_2O + 5O$$

Now in alkaline solutions the two molecules of MnO are immediately oxidized to 2 MnO_2 at the expense of 2 atoms of oxygen, so that we actually have

$$2KMnO_4 = K_2O + 2MnO_2 + 3O$$

Hence, for each molecule of $KMnO_4$ used, only $1^1/_2$ atoms of oxygen are available for our oxidation process. This fact must be recognized in providing sufficient $KMnO_4$ to readily complete the oxidation process, and it would necessarily enter into a calculation of the value of $N/10$ $KMnO_4$ in terms of phenol if the MnO_2, or rather its hydrated form, were filtered from the solution before acidifying and adding the KI. But since the procedure is not lengthened by a filtration the MnO_2 is reduced to its manganous form with the liberation of free iodine, and we must calculate our factor by considering that reaction proceeds as in acid solution with 2.5 atoms of oxygen available per molecule of $KMnO_4$ although such is not literally the truth.

To completely oxidize phenol, 14 atoms of oxygen are required, according to the following equation:

$$C_6H_5OH + 14O = 6CO_2 + 3H_2O$$

Since only 2.5 atoms of oxygen are available per molecule of $KMnO_4$ then 5.6 molecules of $KMnO_4$ would be required for every molecule of phenol and the factor for $N/10$ $KMnO_4$ in terms of phenol becomes:

$$\frac{\text{Mol. wt. phenol}}{2.5 \times 2 \times 10 \times 5.6 \times 1000}$$

Experiments with a phenol solution containing 0.0005 gram of phenol, per cc. as determined by the Koppeschaar bromine method, resulted as follows:

Exp. No.	Grams phenol taken	Per cent. phenol found
1.....	0.0050	99.65
2.....	0.0050	100.18
3.....	0.0075	99.65
4.....	0.0076	99.86
5.....	0.0100	99.86
6.....	0.0100	99.72

Now when the cresols were run in the same manner as phenol it was found that they were not completely

[1] "Oxidation of Organic Compounds with Alkaline Permanganate," Eduard Donath and Hugo Ditz, *J. prakt. Chem.*, [2] **60,** 566–576 (1899); through *J. Chem. Soc.*, [1] **78,** 197 (1900). "Contribution to the Knowledge and Determination of the Carbohydrates," J. König, W. Greifenhagen and A. Scholl, *Z. Nahr. Genussm.*, **22,** 705–723; through Abstr. *J. Am. Chem. Soc.*, **6,** 901 (1912). "Volumetric Determination of Phenol," Jas. F. Tocher, *Pharm. J.*, **66,** 360.

oxidized and that they varied slightly as to the rate with which oxidation proceeded; hence, any permanganate method for their accurate determination must depend upon definitely fixed conditions.

Likewise, it was obvious that commercial creosote and guaiacol could not be determined by this procedure, since they are mixtures of several more or less related phenols that are not present in like proportion in different specimens. However, with single solutions of several common phenols and closely related compounds, fairly gratifying results were obtained. Pyrogallol, pyrocatechin, resorcinol and hydroquinone, from all of which the CH_3 group is absent, were readily and completely oxidized.

Benzoic acid was very slightly attacked while under similar conditions salicylic acid and salol were completely oxidized. Thus it would seem that the phenolic OH group predisposes towards a complete oxidation and that many uninvestigated phenols and closely related compounds would give analogous reactions. In making up solutions of the several phenols, sufficient $N/2$ NaOH was added when necessary, to insure ready solution.

The following table is self-explanatory:

Substance	Percentage Found	Substance	Percentage Found
Pyrogallol	100.4 100.1	Hydroquinone	99.49 99.56
Pyrocatechin	100.2 99.9	Salicylic acid	99.79 100.2
Resorcinol	100.5 100.4	Salol	99.77 99.91

The alkaline permanganate method is especially applicable for the quantitative estimation of the above compounds when they occur individually in very small amounts in single solutions or in conjunction with substances not readily oxidized.

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SOME PROPERTIES OF KOJI-DIASTASE

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The properties of the saccharifying enzyms of koji (a culture of aspergillus oryzae on steamed rice) have been studied by many investigators and are supposed to be comparatively well understood. There are, however, certain obscurities which exist in regard to the identification and classification of these enzyms. They must be cleared up on account of their scientific and practical importance.

I have lately been occupied in a series of investigations relative to Soya or Shoyu (Japanese sauce fermented from beans, wheat and salt) making, and incidentally cleared up certain of the above points.

1. Is saccharification of starch by koji-diastase carried out by two different enzyms, diastase (amylase) and glucase, successively?

It has been generally thought that koji contains two kinds of saccharifying enzyms, *viz.*, diastase (amylase) and glucase, and it is supposed that the glucose present in a liquid saccharified by means of koji is produced by the two enzyms above mentioned. It has been affirmed by some investigators that while the glucase present in koji does not act rapidly, yet, the sugar finally produced in the saccharification of starchy matters with koji is invariably glucose, and that maltose is present only in a negligible quantity. Evidence in support of the above assertions is still meager and I was led to think that there may be present in koji another new enzym, which is altogether different from glucase, and which can convert starch directly to glucose without intermediate steps. The following experiments were made:

Experiment 1.—To each 200 cc. of a 2 per cent. solution of maltose (moisture = 4.25 per cent.) and also starch (moisture = 16.99 per cent.), 20 cc. of the solution of enzyms (corresponding to 2 grams of koji) were added. After allowing the enzyms to act for three hours at 50° C., the reducing action of the digested liquid on Fehling's solution was tested and a rough estimation of glucose was made by means of the glucosazone reaction.

In the determination of the osazone, 100 cc. of each liquid were boiled $1^1/_2$ hours with 4 grams of phenylhydrazine hydrochloride and 6 grams of sodium acetate, filtered and washed with 100 cc. boiling water. This, of course, cannot be said to be a perfect method either for the isolation or determination of glucosazone, but is sufficiently accurate for comparison of results.

Solution acted upon by enzyms	Starch	Maltose
Cc. saccharified solution required to reduce 50 cc. Fehling's solution	18.6	21.0
Grams of glucosazone obtained from 100 cc. of saccharified solution	0.2575	0.1164

Experiment 2.—A like experiment was performed with Taka-diastase. In this case each 100 cc. of a 2 per cent. solution of maltose and starch, respectively, was hydrolyzed with 20 cc. of a 1 per cent. solution of the enzym. For the determination of glucosazone 50 cc. of each solution were taken:

Kind of solution acted upon by enzyms	Starch	Maltose
Cc. saccharified solution required to reduce 50 cc. Fehling's solution	30.0	23.6
Grams of glucosazone obtained from 100 cc. of saccharified solution	0.2088	0.0772

As will be seen in the above experiment, the reducing power of the starch solution after digestion is so strong that we can hardly compare the rate of hydrolysis of maltose to that of starch. Moreover, the quantity of glucosazone from the starch solution is very much higher. The presence of dextrine in the production of glucosazone does not seem to possess remarkable influence, but the presence of maltose has a tendency to give an increased quantity of glucosazone when phenylhydrazine hydrochloride is used in excess. Therefore, in the hydrolysis of starch by means of koji-diastase, it is evident that glucose is not produced principally by the action of glucase, but directly without the aid of it. Dr. Nishisaki[1] asserts that in the case of saccharifying starch by means of koji, the sugar produced thereby is composed in its first stage, of maltose, on account of the weak activity

[1] *J. Chem. Soc., Tokyo*, **29**, 325.