MODIFICATION OF THE CHLORATE DIGESTION METHOD  
FOR MICRODETERMINATION OF IODINE IN  
BIOLOGICAL MATERIALS  

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In a previous paper\(^1\) a method for the microdetermination of iodine was described. This consisted of digesting the organic material in sulfuric acid and potassium chlorate. The method, although very rapid, had certain limitations. By modifying it practically all the disadvantages have been overcome. One of the limitations was that not more than 30 mg. of solid matter could be used in each sample. According to the new method as much as 80 mg. of solid matter can be used for analysis. The second disadvantage was the interference of iron and manganese, which limited the determination to products that did not contain these materials. The cause of this trouble has also been eliminated. The third limitation was the restricted period of titration which was caused by the liberation of iodine from potassium iodide owing to high acidity. This has been avoided by the addition of phosphate, which actually has a 2-fold purpose, as will be explained in the following paragraph.

In the modified method, the material is digested in a solution consisting of perchloric acid, sodium chlorate,\(^2\) and disodium hydrogen phosphate. During the digestion iodine is oxidized to iodic acid. When the digestion is complete, the liquid is made alkaline by the addition of sodium hydroxide to precipitate iron, calcium, and copper in the form of phosphate salts which are removed by centrifugation. The clear liquid is then acidified with hydrochloric acid to a pH of 1.2, with thymol blue as an indicator. At this pH in the presence of phosphate, ferric salts do not oxidize potassium iodide and, therefore, even if iron is not removed from the solution by centrifugation it will not interfere with the iodide-iodate reaction. The indicator is then decolorized with a solution of chlorine in carbon tetrachloride, which also oxidizes the reducing impurities that might have been present in the added reagents. After the chlorine is removed by boiling, the solution is cooled, a crystal of phenol is added, and the solution allowed to stand for 5 minutes; such concentrations of manganese salts as are found in tissues will then be reduced by the phenol after this time.  

\(^1\) Shahrokh, B. K., \textit{J. Biol. Chem.}, \textbf{147}, 109 (1943).  
\(^2\) Potassium salts cannot be used because on their addition to perchloric acid-potassium perchlorate precipitates, which interferes with the determination.
dine is then liberated by the addition of potassium iodide solution. Soon after, the pH of the solution is adjusted to about 6 by the addition of disodium hydrogen phosphate solution. This change in pH has two functions. First, at this pH no iodine will be liberated from the potassium iodide solution and the titration can be carried out as slowly as desired; secondly, an almost neutral solution will safeguard against the oxidation of potassium iodide by slow oxidizing agents that might be present in the solution as impurities.

This method takes longer than the original method. It will take about 3 hours to determine the iodine content of twelve samples, if they are digested together, which will give an average of about 15 minutes for each determination. If the centrifugation of the precipitate is not necessary, as is the case with most tissues, the time can be reduced to about 2½ hours for twelve samples. There is almost 100 per cent recovery of iodine by this method if the directions are thoroughly followed. The accuracy of the method, therefore, is limited by the accuracy of titration. 0.5 γ of iodine is the limit of accuracy for the writer.

Reagents——
1. Digestion mixture. Disodium hydrogen phosphate (anhydrous) 2.5 gm., sodium chlorate 30.0 gm., distilled water 135.0 ml., 60 per cent perchloric acid 90.0 ml. First sodium chlorate and disodium hydrogen phosphate are dissolved in distilled water. Then, while the mixture is shaken slowly, perchloric acid is added. If the liquid becomes warm, it is cooled under tap water. The mixture should be kept at room temperature. Higher temperatures cause speedy decomposition of the reagents.
2. 13 per cent sodium hydroxide solution.
3. Thymol blue indicator. 0.04 per cent thymol blue in 50 per cent alcohol.
4. Solution of chlorine in carbon tetrachloride. (a) If chlorine gas is available, chlorine solution can be obtained by slowly bubbling chlorine through carbon tetrachloride. (b) If chlorine gas is not available, chlorine can be obtained from sodium hypochlorite solution. 100 ml. of 5 per cent sodium hypochlorite (commercial products like Clorox are satisfactory) are placed in a 250 ml. separatory funnel, followed by 25 ml. of carbon tetrachloride. The solution is then slowly acidified with 6 N HCl. After each addition the separatory funnel is shaken. When the liquid becomes deep green, the solution is ready for extraction. There will be evolution of CO₂ gas during and after acidification. This gas should be allowed to escape. The mixture is shaken slowly to dissolve the chlorine in the carbon tetrachloride. The shaking is continued until the hypochlorite solution loses its green color. Carbon tetrachloride is then drained out. The green tetrachloride solution is washed only once with 25 ml. of distilled
water. If the solution remains turbid, it is centrifuged in a stoppered 50 ml. centrifuge tube. This solution is stored in a glass-stoppered bottle and must be prepared fresh every

5. 1 per cent potassium iodide solution.
6. 5 per cent disodium hydrogen phosphate solution.
7. 0.001 N sodium thiosulfate solution freshly prepared
8. 1 per cent starch indicator.

Method

1 ml. of sample is pipetted into micro-Kjeldahl digestion flasks. It is preferable to use the type of digestion flasks which can also be used for centrifuging. Then, 6 ml. of the digestion mixture are added to the sample, followed by a few small pieces of pumice stone. The addition of pumice stone is essential to prevent the vigorous bumping which may occur. The flasks are heated on a digestion rack until the liquids start to turn green, when the micro flames are lowered and the flasks are heated very gently. During this part of the digestion the liquids should simmer and not boil. Speedy heating may cause minor explosions. The heating is continued in this manner until no more chlorine gas is seen in the neck of the flasks. This may take from 30 minutes to 1 hour, depending on the quantity of organic material present. When chlorine gas disappears, the flames are raised slowly and the liquids are allowed to boil. Vigorous boiling should be avoided in all stages of digestion. When the contents of the flasks are reduced to about 1 ml. to 2 ml., the solutions become colorless and fumes start to evolve. Heating is continued for another 2 to 3 minutes, after which the flasks are allowed to cool. 4 ml. of 13 per cent NaOH are then added to each sample. The flasks are cooled to room temperature with tap water. 1 drop of thymol blue is then added and sufficient 13 per cent NaOH to turn the indicator blue. If at this stage a visible precipitate appears, the liquid should be centrifuged for 1 minute. The supernatant liquids are poured in 50 ml. Erlenmeyer flasks, the precipitates are washed twice, each time with 5 ml. of distilled water, and the washings added to the original liquid. Sufficient 1 N hydrochloric acid is added to each flask to give a definite pink color. A few drops of a solution of chlorine in carbon tetrachloride are added to each flask to decolorize the liquids. The samples are then boiled until about 15 ml. of liquid are left in each flask, after which they are cooled in a stream of running water. When the temperature reaches about 40° (at higher temperatures phenol reduces iodic

The author prefers to omit the centrifuging unless copper is present. If the solution is not centrifuged, small quantities of precipitate will remain in the liquid. However, in none of the biological materials encountered has the amount of precipitate been large enough to interfere with the accuracy of the titration.
acids), a crystal of phenol is added to each flask and, after cooling for at least 5 minutes, 1 ml. of 1 per cent potassium iodide solution is added to each flask. At a pH of approximately 1.2 the iodide-iodate reaction goes on at a comparatively slow rate and about 30 seconds should be allowed for complete liberation of iodine. When the reaction is complete, 1 ml. of a 5 per cent solution of disodium hydrogen phosphate is added, and the iodine is titrated with a freshly prepared 0.001 N solution of sodium thiosulfate, 1 drop of 1 per cent starch being used as an indicator.

When a large number of samples are being analyzed, the time for determination can be reduced if, while one series is boiling in Erlenmeyer flasks, another series is being prepared in digestion flasks and if while the titrations are being made for the first batch the second series can be digested.

**SUMMARY**

The chlorate digestion method for the microdetermination of iodine is modified so that this method can be used for all the biological materials containing more than 0.01 per cent of iodine.
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