3. G. Galeotti, "Über die Kondensierung der Aminosäuren vermittelst des Formaldehyds," *Biochem. Z.*, **53**, 474–492 (1913).

4. R. A. Gortner, "The Origin of the Humin Formed by the Acid Hydrolysis of Proteins. II. Hydrolysis in the Presence of Carbohydrates and of Aldehydes," *J. Biol. Chem.*, **26**, 177–204 (1916).

5. R. A. Gortner and M. J. Blish, "On the Origin of the Humin Formed by the Acid Hydrolysis of Proteins," THIS JOURNAL, **37**, 1630–36 (1915).

6. E. B. Hart and B. Sure, "The Influence of Carbohydrates on the Accuracy of the Van Slyke Method in the Hydrolysis of Casein," *J. Biol. Chem.*, **28**, 241–9 (1916).

7. P. A. Levene and W. A. Beatty, "Analyse der Spaltungsprodukte der Gelatine," *Z. physiol. Chem.*, **49**, 252–261 (1906).

8. W. Löb, "Die Methylierung des Glykokolls mittels Formaldehyd," *Biochem. Z.*, **51**, 116–127 (1913).

9. T. B. Osborne and D. B. Jones, "A Consideration of the Sources of Loss in Analyzing the Products of Protein Hydrolysis," *Am. J. Physiol.*, **26**, 305–28 (1910).

10. M. L. Roxas, "The Reaction between Amino Acids and Carbohydrates as a Probable Cause of Humin Formation," *J. Biol. Chem.*, **27**, 71–93 (1916).

11. Hugo Schiff, "Über Methyleneasparagine, *Ann.*, 310, 25–44 (1900).

12. Hugo Schiff, "Trennung von Amin-und Säurefunktion in Lösungen von Eiweisskörpern, *Ann.*, **319**, 287–303 (1901).

13. Hugo Schiff, "Trennung von Amin-und Säurefunktionen mittles Formaldehyde, III," *Ann.*, **325**, 348–354 (1902).

14. S. P. L. Sorensen, "Enzymstudien," *Biochem. Z.*, **7**, 45–101 (1908).

15. D. D. Van Slyke, "The Analysis of Proteins by Determination of the Chemical Groups Characteristic of the Different Amino Acids, "*J. Biol. Chem.*, 10, 15–55 (1911).

16. D. D. Van Slyke, "The Quantitative Determination of Aliphatic Amino Groups, II," *J. Biol. Chem.*, **12**, 275–284 (1912).

17. D. D. Van Slyke, "Improvements in the Method for Analysis of Proteins by Determination of the Chemical Groups Characteristic of the Different Amino Acids," *J. Biol. Chem.*, **22**, 281–285 (1915).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE MEDICAL COLLEGE OF VIRGINIA.]

OBSERVATIONS ON THE McLEAN-VAN SLYKE IODOMETRIC METHOD FOR THE TITRATION OF SMALL AMOUNTS OF HALIDES, IN ITS APPLICATION TO CHLORIDES.

BY ROBERT F. MCCRACKEN AND MARY D. WALSH.

Received June 11, 1917.

For the purpose of determining small amounts of chlorides more accurately than could be done by the Volhard method, McLean and Van Slyke devised an iodometric method.[1] To the chloride solution they added an excess of a standard solution of silver nitrate in nitric acid, and titrated the excess of silver nitrate with potassium iodide, in an aliquot part, using sodium nitrite and soluble starch as an indicator. To regulate the acidity they added trisodium citrate. They showed the method to be satisfactory for the determination of halides, and it was found

[1] THIS JOURNAL, **37**, 1128 (1915).

especially useful in analyzing small amounts of blood and urine for chlorides.[1] It was used by McLean in his investigation of the "Numerical Laws Governing the Rate of Excretions in Man,"[2] in normal cases, and later in his investigations concerning "The Influence of Pathological Conditions and of Drugs on Excretions."[3]

Efforts to apply the method in the Physiological Chemistry Laboratory of this institution showed that the end point was at times difficult to recognize, due to the development of a starch-iodide color before the titration was complete. We undertook to find a way to determine the end point with greater certainty, and to test the method in the analysis of pure sodium chloride solutions.

The standard silver nitrate and potassium iodide solutions used by McLean and Van Slyke were an acid solution of silver nitrate, 1 cc. of which was equivalent to 2 mg. of NaCl or 0.034 *N*, and a solution of potassium iodide, 1 cc. of which was equivalent to 1 mg. of NaCl or 0.017 *N*.[4]

After precipitating a halide by an excess of the silver nitrate solution they say:

> "In either the entire filtrate, washed through the filter, or in an aliquot part, passed through a dry filter, the excess of silver is titrated with 0.02 *N* or 0.01 *N* potassium iodide, which has been standardized against the silver solution. Just before titration one adds from a buret for each g. of free nitric acid present (1 g. approximately, or 16 mg. molecules, is contained in 1 cc. of nitric acid sp. gr. 1.42) 4 cc. of the following solution:

Trisodium citrate, $Na_3C_6H_5O_7 + 5^1/_2H_2O$..........	446.0 g.	$^5/_4$ gram-molecules or $^{15}/_4$ equivalents
Sodium nitrite................................	19.0	($^1/_4$ gram-molecules)
Soluble starch................................	2.5	
Water to....................................	1000.0 cc.	

> "One cc. of this solution contains four milligram-equivalents of sodium. The solution, because of its nitrite, serves to liberate iodine; the citrate regulates the acidity of the titrated solution; and the starch is present for the end reaction."[5]

Their directions for standardizing the potassium iodide solution against the silver nitrate solution are to add 5 cc. of the standard silver nitrate solution to 5 cc. of the citrate solution and to titrate with the potassium iodide solution to the blue end point. The iodide solution is then diluted to such a degree that 10 cc. are exactly equivalent to 5 cc. of the silver solution.[4]

We thought the water of crystallization in the sodium citrate a likely source of error and proceeded to investigate along that line. The eighth pharmacopoeia of the United States required that the salt have $5^1/_2$

---

[1] McLean and Van Slyke, *J. Biol. Chem.*, **21**, 361 (1915).

[2] *J. Exp. Med.*, **22**, 212 (1915).

[3] *Ibid.*, **22**, 366 (1915).

[4] McLean and Van Slyke, *J. Biol. Chem.*, **21**, 363 (1915).

[5] McLean and Van Slyke, THIS JOURNAL, **37**, 1131 (1915).

molecules of water while the ninth edition required 2 molecules. Analyses of three of the best grades of sodium citrate on the market, each supposed to contain $5^1/_2$ molecules of water of crystallization, showed them to contain 2.43, 5.56, and 5.66 molecules of water, respectively. The portions analyzed were ignited to the carbonate and titrated with standard sulfuric acid.

The authors stated that the amounts of sodium citrate used might vary 10% above or below the exact nitric acid equivalent without affecting results.[1] They used 446 g. per liter of the sodium citrate containing $5^1/_2$ molecules of water of crystallization. The equivalent weight of the salt with 2 molecules of water of crystallization is 367 g. The excess of 446 over 367 is about 21%, and 10% of 446 additional would make about 33% in excess. Our potassium iodide solution was standardized against the silver nitrate solution according to the directions of the authors, and the standard was then determined in the presence of amounts of sodium citrate that varied from 90% to 121% of the equivalent of the nitric acid present. The concentrations of the nitric acid and of the sodium citrate were known exactly, the stock solutions having been analyzed. These experiments led to the following conclusions:

1. Varying the amount of sodium citrate from 10% below to 21% above the equivalent of the nitric acid present has but slight effect on the end point when the titrations are completed without delay. As the acidity of citric acid is already very slight, the use of an excess of sodium citrate doubtless makes the acidity still less. In case time is allowed for the silver iodide to collect at the bottom and to occlude silver nitrate the effect of reducing the acidity may be noticeable. In that case, as the potassium iodide is added equilibrium seems to be established a little more slowly than when the total acidity of the citric acid or of a little of the nitric acid comes into play.

2. When the potassium iodide is added rapidly the end point can be determined with greater certainty than when it is added very slowly. When from 50% to 75% of the necessary amount of potassium iodide to titrate 5 cc. of the silver nitrate was added and the solution allowed to stand, a greenish blue color soon developed. In a few minutes it was bluer than the end point color, and in half an hour it was several times as blue. When 98% of the potassium iodide was added at once the depth of color that developed on standing was inappreciable. As titrations were continued with solutions that had been allowed to stand, the blue colors became very faint just before the end points were reached. When titrations were stopped exactly at the end points the colors neither disappeared nor deepened appreciably on standing half an hour.

3. The settling out of silver iodide may occlude silver nitrate and make the

---

[1] McLean and Van Slyke, This Journal, **37**, 1131 (1915).

end point uncertain. When titrations were completed without delay the silver iodide did not usually settle out but gave an opalescent solution. When they were carried out more slowly and with vigorous stirring the silver iodide did sometimes collect at the bottom in considerable quantity. In such cases what was thought to be the end-point color often disappeared on standing a few minutes. Due to this occlusion of silver nitrate the end point may be taken from 0.05 to 0.15 cc. too soon. Treadwell is authority for the statement that the precipitate occludes silver nitrate.[1]

We found that the addition of 10 cc. of 1% soluble starch solution just before beginning the titration held the silver iodide in solution so that the end point could be obtained in a clear solution. Van Slyke thinks this use of more starch a distinct addition to the method.[2]

Experimental.

The method, substantially as given by McLean and Van Slyke, was applied to the determination of widely differing amounts of sodium chloride, care being taken in each case to see that the silver nitrate to be titrated was not more than the amount in 5 cc. of the standard solution.

After standardizing our silver nitrate solution containing 250 cc. of concentrated nitric acid per liter, and adjusting it so that the silver nitrate was equivalent to 2 g. of sodium chloride per liter, we determined the concentration of the acid and used the exact equivalent of trisodium citrate in preparing our citrate solution, which was 380.2 g. per liter. The potassium iodide solution was adjusted so that 2 cc. were equivalent to 1 cc. of the silver nitrate solution. A solution containing 2 g. of freshly ignited sodium chloride per liter was prepared and portions used for analysis. Instead of mixing the trisodium citrate, soluble starch, and sodium nitrite in one solution as directed by McLean and Van Slyke, we prepared each solution separately.

In the method of procedure, definite weight of sodium chloride was placed in a small volumetric flask. An excess of silver nitrate was added and after diluting to the mark with distilled water and mixing thoroughly it was allowed to stand a few minutes. It was then filtered through a dry filter. To an aliquot part of the filtrate was added trisodium citrate equivalent to the acid in the aliquot part, as well as the amounts of sodium nitrite and soluble starch that would have been added with the trisodium citrate if the three had been prepared in one solution according to the directions of McLean and Van Slyke. Finally it was titrated with the standard solution of potassium iodide until the first definite blue color was reached. From the potassium iodide used the excess of silver nitrate

---

[1] Treadwell and Hall, "Analytical Chemistry," 2, 546 (1909).

[2] Private communication from Donald D. Van Slyke.

was found, and from the original amount of silver nitrate less the excess left in the total solution the sodium chloride was calculated.

ANALYSES.

NaCl. Mg.	$AgNO_3$. Cc.	Dilution. Cc.	Used. Cc.	KI. Cc.	Calc. NaCl. Mg.	Error. Mg.	Error, %.
6	5	20	10	1.98	6.04	0.04	0.67
6	5	20	10	2.00	6.00	0.00	0.00
18	10	50	25	1.00	18.00	0.00	0.00
18	10	50	25	0.98	18.04	0.04	0.22
30	20	50	25	5.10	29.80	0.20	0.66
30	20	50	25	5.00	30.00	0.00	0.00
46	25	100	75	3.00	46.00	0.00	0.00
46	25	100	75	2.96	46.05	0.05	0.12
66	35	100	50	1.88	66.24	0.24	0.36
78	45	100	50	6.10	77.80	0.20	0.26
90	50	100	50	5.00	90.00	0.00	0.00
100	55	100	50	5.12	99.76	0.24	0.24

The method was then applied with an increased amount of starch. The method of procedure in this series was to add an excess of the standard silver nitrate solution to a definite weight of sodium chloride in solution, to filter, wash the precipitate free of silver nitrate, and then to titrate the whole filtrate with potassium iodide. Just before titrating, the equivalent amount of sodium citrate solution, containing the amounts of sodium nitrite and soluble starch given by McLean and Van Slyke, was added, and then 10 cc. of a 1% soluble starch solution.

ANALYSES.

$AgNO_3$. Cc.	KI. Cc.	Mg. NaCl present.	Mg. NaCl found.	Error. Mg.	Error, %.
5	4.10	6	5.90	0.10	1.66
5	4.00	6	6.00	0.00	0.00
10	10.05	10	9.95	0.05	0.50
10	10.06	10	9.94	0.06	0.60
15	10.02	20	19.98	0.02	0.10
15	10.03	20	19.97	0.03	0.15
15	4.90	25	25.10	0.10	0.40
15	5.02	25	24.98	0.02	0.08
29	7.90	50	50.10	0.10	0.20
29	7.94	50	50.06	0.06	0.12
55	10.00	100	100.00	0.00	0.00
55	10.04	100	99.96	0.04	0.04

Summary.

1. When a titration is made very slowly in the McLean-Van Slyke Iodometric Method for the Determination of Chlorides, a starch-iodide color that might be mistaken for the end point, sometimes develops before the titration is complete.

2. This color, even when several times as deep as the end-point color, gradually disappears as the end point is approached.

3. The end point can be obtained in a clear solution instead of in an opalescent solution by supplementing the starch present with 10 cc. of a 1% soluble starch solution just before titration.

4. The original method and the method as applied with the use of additional starch both give satisfactory results.

RICHMOND, VA.

[CONTRIBUTION FROM THE ARKANSAS AGRICULTURAL EXPERIMENT STATION.]

THE DETERMINATION OF PHYTIN PHOSPHORUS IN PLANT PRODUCTS.[1]

BY J. B. RATHER.

Received August 13, 1917.

No satisfactory method for the direct determination of phytin phosphorus in plant products exists. The amount of phytin phosphorus in such materials has been estimated by Posternak[2] by separating the crude acids in a state more or less free from bases, by Suzuki and Yoshimura[3] by precipitating the acid extract of the plant with absolute alcohol and determining the phosphorus in this product, and by Funatsu[4] by determining the phosphorus in the acid extract of the plant. The exclusion of inorganic phosphorus, organic forms of phosphorus other than phytin, and enzymatic hydrolysis was, apparently, not considered by these workers.

Heubner and Stadler[5] determine phytin phosphorus in pharmaceutical products by titrating with ferric chloride under certain definite conditions. These workers show that inorganic phosphorus and glycerophosphates do not interfere with the titration. Briefly the method is as follows: The volume of the solution to be titrated is, at the beginning of the titration, 100 cc. and contains 0.6% hydrochloric acid and 0.03% ammonium sulfocyanate. It is titrated with standard ferric chloride solution in 0.6% hydrochloric acid, the solution containing from 0.05 to 0.2% Fe, until the supernatant liquor above the whitish iron precipitate develops a pale red color persisting for 5 minutes. One mg. Fe corresponds to 1.19 mg. phytin P. The factor was derived from experiments on a sample of commercial phytin.

On account of its simplicity the method of Heubner and Stadler was made the basis of work by the present writer.

Experimental.

In testing the applicability of the ferric chloride titration method for

---

[1] Full details of this work will be published as a Bulletin of the Arkansas Experiment Station.

[2] *Compt. rend.*, 137, 202 (1903).

[3] *Bull. Coll. Agr. Tokyo*, 7, 495–502 (1907).

[4] *Ibid.*, 7, 457.

[5] *Biochem. Z.*, 64, 422–437 (1914).