THE DETERMINATION OF CHLORIDE IN MILK

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The determination of chloride in milk is one of the standard tests used in the diagnosis of mastitis in dairy cows. The methods most commonly used in this country are modifications of Mohr's direct titration method, in which chloride is titrated with a standardized solution of silver nitrate in the presence of potassium chromate indicator. Direct titratable chloride values above 0.14 (20) or 0.16 (11) per cent have been cited as signifying abnormality in milk caused by a diseased condition of the udder. In an intensive study of the mastitis problem in these laboratories, covering a period of several years and involving tests on more than 3000 quarter-udder samples taken periodically throughout several lactations, our results have indicated that in the vast majority of samples, when a strictly quantitative method for chloride is used, 0.12 per cent chloride is a surer criterion of mastitis than the values quoted above. We shall publish later a paper containing evidence that the chloride test, when carried out quantitatively on quarter-udder samples, gives a very useful indication of mastitic conditions in the udder. In much of the diagnostic work done in the past, investigators apparently have been at a disadvantage because of their use of methods that are not sufficiently precise. Because of lack of precision apparent in the direct titration method, the writer found it desirable at an early stage in the mastitis work to survey the literature and conduct experiments in an attempt to find a method which would not be too laborious and which would be sufficiently precise to detect small changes in chloride content, as well as small differences in chloride values between different quarters.

REVIEW OF LITERATURE

The direct titration of chloride with silver nitrate and with potassium chromate indicator, in neutral chemical solutions in which interfering substances are absent, was proposed by Mohr (14) in 1861. His data indicate that a slight excess of silver nitrate is always required to produce a visible endpoint. His method has been applied to the chloride test in milk by Richmond (18), Hammer and Bailey (8), Rosell (20), Sharp and his co-workers (24), and others. In Hammer and Bailey’s method, 10 volumes of water are added to 1 volume of milk before the titration, and the titration is carried out in a white porcelain dish in order to make the endpoint more visible. They reported that, although the direct titration gave results which were always higher than those obtained by titrating chloride in ashed samples, it was “a satisfactory index of the chloride content.”

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Hammer and Bailey found that the presence of casein is an important cause of positive error in the direct titration, an error which Poetschke (16) attributed largely to the presence of phosphates and to the evident fact that the endpoint is very indefinite. Poetschke reported much higher results by direct titration than by the ashing method; while he found that the positive error was reduced considerably and the visibility of the endpoints increased by titrating undiluted rather than diluted samples, the endpoints were still so indistinct that it was impossible to secure satisfactory results.

Sharp and his co-workers (24) devised a direct titration method in which samples were titrated without first being diluted with water. They found that the positive error was relatively great when the amount of indicator used was small and when water was added before titration. They found that the endpoints were not satisfactory and they stated that, since the extent of the positive error was not known, values obtained by direct titration should be referred to as "direct titratable chloride values" rather than as percentages of chloride. Blood and Rowlands (2) stated that the percentage found by direct titration was always absurdly high; high results have been likewise reported by everyone who has made comparisons, including Weiss (33), Meyer (13), Drost (7), and others. W. L. Davies (6), who used and recommended a wet digestion and Volhard titration method, stated that the high results obtained by direct silver nitrate titration in milk are caused by chemical combination of silver ions with protein.

Recently the use of an adsorption indicator, dichlorofluorescein, has been proposed by Kolthoff et al. (12) for direct titrations with silver nitrate. The color of a clear solution changes from a fluorescent greenish hue to a bright pink when the endpoint is reached, and the result in a clear solution appears to be strictly quantitative when interfering substances are not present; however, Collier (4) has shown that, in biological materials, the pH of the sample has an influence on the endpoint, and should be 7.0 to 7.5. Rose (19) studied the use of this indicator for titrating chloride in milk, and reported that accuracy of results not only depends upon pH but is influenced by the presence of protein. If more than 3 per cent protein was present there was a proportional positive error and it was necessary to remove the protein before titrating. Hastings and Beach (10) state that the ashing method gives the lowest and most accurate values, the use of dichlorofluorescein gives somewhat higher results, and the use of chromate gives the highest values. They believed that a wet ashing method would be best for quantitative work.

Of the strictly quantitative methods, the Volhard (30) titration has been used generally in physiological studies and has been used in analyses of milk principally by foreign workers. It has been demonstrated that, since milk does not contain significant amounts of colored substances which might interfere, it is feasible to omit the wet digestion procedure and thus materially shorten the procedure for determining chloride accurately by the Volhard
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method. Moreover, it has been shown that the reagents added before titrating may be combined, thus further simplifying the method.

In such a procedure, as proposed for urine studies by Strauss (26), a standardized solution of silver nitrate containing nitric acid and ferric ammonium sulfate (ferrie alum) indicator, known as the "Martius-Lüttke solution," is added to the test material and the mixture is titrated with standardized potassium sulfocyanate. Drost (7) has shown that the presence of proteins and other constituents in milk does not affect the results. He, and also Munchberg (15), obtained the same results in undigested milk samples by using this method as by using other quantitative methods. Although the writer has found no reference in the literature of this country to the Volhard titration of undigested milk, it is described in foreign literature as the simplest and quickest of the accurate methods (22) (23) (13). A modification of such a method has been published recently, in abstract form, by the writer (21).

In a consideration of the chemical factors on which the usefulness of this method in milk is based, it is desirable to review work in which the open Carius (wet digestion) method, followed by the Volhard titration, has been used; such a procedure has been used successfully and is advocated principally by foreign workers (6) (3) (15). It was proposed by von Korányi (31) for use in physiological fluids, and has been shown to yield quantitative results when used in milk (36) (2) (32) and other dairy products (25) (13). In the wet digestion method, the sample is digested by boiling in nitric acid and potassium permanganate or persulfate (3), in the presence of a known amount of silver nitrate, more than sufficient to combine with all of the chloride. The chloride combines with silver and the amount of excess silver is determined by titration, in the presence of ferric alum indicator, with a standardized solution of potassium sulfocyanate. The endpoint color changes from pure white to blood red and is comparatively distinct and easily read.

The applicability of the Volhard method is based on the fact that sulfocyanate has a greater affinity for silver than it has for iron, and combines with all of the excess silver to form a white precipitate of silver sulfocyanate. When the silver is completely used, the sulfocyanate combines with ferric alum indicator to form ferric sulfocyanate, which is soluble and has a distinct blood red color. With nitric acid present, interference from the presence of phosphate or other salts which precipitate silver in neutral but not in acid solutions is eliminated; moreover, there is no interference caused by copper, iron, and other common metals, as there is with chromate in Mohr’s method.

Van Slyke and Donleavy (29) precipitated protein and chloride in plasma with silver nitrate, nitric acid, and picric acid. The material was then filtered and titration was carried out in an aliquot of the filtrate with
standardized potassium iodide in the presence of an indicator containing starch, nitrite, and citrate. This procedure, slightly modified, was used recently by Reder (17) for determining chloride in milk. However, it has been shown by Van Slyke (28) and his co-workers that the Van Slyke-Donleavy method when applied to whole blood gave data which were 30 to 40 per cent too high, and they indicated that some component of the mixture other than chloride has the property of binding silver. They showed that in the case of whole blood, as is described below by the writer in the case of milk, the silver nitrate must be added after, rather than before, filtration. Van Slyke discarded the protein precipitation and potassium iodide titration method in favor of the wet digestion and Volhard titration method, and confirmed the precision of the latter.

Sunderman and Williams (27) found that, in blood analyses, the wet digestion and Volhard titration method is quantitatively accurate in comparison with standard gravimetric procedures. In analyzing solid tissues, however, they obtained concordant values for chloride only when they subjected the material to a preliminary alkaline digestion to prevent volatilization of chloride.

Conditions necessary for an accurate Volhard titration have been prescribed by Harvey (9) and by Whitehorn (34); they showed that it is not necessary to filter off the silver chloride precipitate before titrating, and that an essential consideration is the use of the proper amount of nitric acid, which decolorizes the untitrated indicator and also causes a maximum coagulation of the silver chloride crystals, with a consequent reduction of the amount of surface exposed for reaction with sulfocyanate. In addition, they showed that the presence of an abundance of ferric alum indicator retards any possible reaction between the precipitated silver chloride and the sulfocyanate and results in a deepening of the endpoint color. Whitehorn found further that accuracy was not increased by dilution, but that the volume of liquid should be small in order to insure sharpness of endpoint.

Numerous workers have used protein precipitation and filtration methods for the preparation of milk sera in which to determine chloride values. Poetschke (16) precipitated the proteins by means of copper sulfate and sodium hydroxide, filtered, and determined chloride in an aliquot by the Volhard method. Weiss (33) used aluminum sulfate and sodium hydroxide as the precipitant and titrated chloride in the filtrate with chromate indicator; Blood and Rowlands (2) used picric acid, and Drost (7) used nitric acid as the precipitant. The writer has used the method of Weiss in comparison with other methods and found that it gives lower and more accurate values than are obtained by Mohr's direct titration method. However, there is some error in protein precipitation methods if correction is not made for volume of precipitate (16) (6), and the work of making the material up to volume and filtering makes these methods so laborious that it did not seem advisable to include them in this study.
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The dry ashing method is too laborious for routine or general laboratory work. Moreover, it has been indicated by numerous workers (3) (7) (5) that there is a considerable loss of chloride in the ashing method unless a base is added to prevent its volatilization (32) (36), and there is some evidence that a slight loss of chloride may occur even when compounds containing sodium are added (5) (3). According to H. E. Davies (5), the loss may occur mostly during the early stages of heating, and, in the absence of a sufficient amount of base, chloride passes off as hydrochloric acid.

In the official method of the Association of Official Agricultural Chemists (1) for determining ash in milk, nitric acid is added to aid in oxidizing organic material, and no mention is made of the addition of a base to bind the negative elements such as chlorine and phosphorus. It is evident, in view of the results of H. E. Davies mentioned above, and in view of the loss of chloride in ashing milk reported by Poetschke (16) and others, that the official method is deficient in these two respects—acid should not be added, and a basic material should be incorporated in the material to be ashed.

METHODS

1. Volhard titration without preliminary digestion: To 10 cc. of milk is added a measured quantity of the 0.0291 N special silver nitrate solution described below, more than sufficient to combine with all of the chloride; ordinarily 15 cc. is the amount used. The mixture is titrated with a 0.0291 N solution of potassium sulfoeyanate. The number of cc. of silver nitrate solution used minus the titration value, multiplied by 0.01, equals the percentage of chloride.

Special silver nitrate solution: Exactly 4.9438 grams of C. P. Silver nitrate is dissolved in water, about 200 cc. of concentrated nitric acid and about 300 cc. of a saturated solution of ferric ammonium sulfate indicator are added, and the mixture is cooled and made up to 1 liter. C. P. silver nitrate can be used with reasonable accuracy as a primary standard, and the sulfoeyanate should be standardized against it before use.

2. Open Carins (wet) digestion and Volhard titration: To 10 cc. of milk in a 300 or 500 cc. Erlenmeyer flask are added, first, 15 cc. of a 0.0291 N (4.9438 grams in 1 liter) solution of silver nitrate, or an amount more than sufficient to combine with all of the chloride, and then 15 to 20 cc. of concentrated nitric acid and about 50 cc. of water. The material is heated to boiling and 2 successive 5-cc. portions of a saturated solution of potassium permanganate are added slowly, drop by drop, to aid in digestion. The mixture is boiled (b. p. about 105–109° C.) slowly until it is clear yellow and contains no sediment other than coagulated silver chloride. It is then cooled, 5 cc. of a saturated solution of ferric ammonium sulfate indicator is added, and titration of excess silver is carried out with a 0.0291 N (2.8280 grams in 1 liter) solution of potassium sulfoeyanate, until a trace of blood red color remains for at least 1 minute. Chloride is calculated as in method No. 1.
The 0.0291 N solutions described are of such concentration that each cc. equals 0.01032 gram of chlorine; the percentage of chloride may be read directly, in analysis of 10 cc. of milk and with solutions of this normality, if one assumes that the specific gravity of the milk is 1.032. In the early part of the work described herein, 0.1711 N solutions were used, for the reason that, with solutions of this concentration, direct readings for sodium chloride were made in analyzing weighed samples of cheese in a method proposed by the writer and published previously (25) (35). With the higher concentration of reagents, 15-cc. samples of milk were used and the correction factor used was 0.0392. If 0.10 N solutions are used with 10-cc. samples, the factor is 0.03436; with 10-gram samples it is 0.03546.

3. Direct titration with silver nitrate: In titrating with potassium chromate indicator, 10 cc. of milk was measured accurately into a porcelain dish and 50 cc. of water and 1 cc. of a 10 per cent solution of potassium chromate were added. The titration was then carried out with a 0.0291 N solution of silver nitrate until a definitely visible brick-red color became evident.

In titrating with dichlorofluorescein indicator, the procedure outlined above was used, except that 5 drops of indicaor was added; the titration was carried out until a faint pink tint was evident, as described by Hastings and Beach. The indicator was made up by dissolving 0.1 gram of the reagent in 100 cc. of 70 per cent ethyl alcohol.

4. Dry ashing: Ten cc. of milk was measured accurately into a platinum crucible, dried over night under vacuum at 100°, and ashed in a muffle at a very low red heat, at not over 600°; in some cases the samples were ashed in porcelain dishes over a free flame, and in some cases it was necessary to remove the charred material by washing it with water onto a filter and complete the ashing of the filter paper and its contents. The method is essentially the same as that of the Association of Official Agricultural Chemists (1) except that it was found necessary to add a base to prevent loss of chloride, and the use of nitric acid as an oxidant was dispensed with; the base used was sodium hydroxide, 5 cc. of a normal solution being added to the sample before it was dried.

RESULTS

In the present work, the Volhard method has been taken as a standard for quantitative accuracy for the reason that, among all the references which the writer has found in which it has been used in comparison with other methods and in which the conditions prescribed by Volhard and by others mentioned herein have been adhered to, the accuracy of results obtained has not been questioned.

Data obtained in analyses of several milk samples by the methods outlined above, and representative of a large number of comparative tests, are shown in Table 1. The preliminary alkaline digestion method of Sunder man and Williams, referred to above, was used with the Volhard titration
TABLE 1

Percentage of chloride in milk samples as determined by different methods

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>Potassium chromate indicator</td>
</tr>
<tr>
<td>Right front (mastitis)...........</td>
<td>0.310</td>
<td>0.306</td>
<td>0.309</td>
<td>0.346</td>
</tr>
<tr>
<td>Right rear (normal)..............</td>
<td>0.092</td>
<td>0.090</td>
<td>0.092</td>
<td>0.130</td>
</tr>
<tr>
<td>Left front (normal)..............</td>
<td>0.110</td>
<td>0.110</td>
<td>0.110</td>
<td>0.141</td>
</tr>
<tr>
<td>Left rear (mastitis)...............</td>
<td>0.208</td>
<td>0.206</td>
<td>0.206</td>
<td>0.250</td>
</tr>
<tr>
<td>Mixed herd milk..................</td>
<td>0.079</td>
<td>0.078</td>
<td>0.078</td>
<td>0.111</td>
</tr>
<tr>
<td>Same plus 0.05% Cl...............</td>
<td>0.128</td>
<td>0.128</td>
<td>0.128</td>
<td>0.160</td>
</tr>
<tr>
<td>Milk cont’g. 2.1% casein.........</td>
<td>0.085</td>
<td>0.083</td>
<td>0.083</td>
<td>0.110</td>
</tr>
<tr>
<td>Milk cont’g. 2.7% casein.........</td>
<td>0.090</td>
<td>0.089</td>
<td>0.088</td>
<td>0.135</td>
</tr>
</tbody>
</table>

A Open Carius (wet) digestion with HNO₃, KMnO₄, and an excess of AgNO₃ followed by titration with KCNS, with ferric alum indicator.
B Preliminary alkaline digestion followed by open Carius method.
as an added precaution against the volatilization of chloride in digesting the samples for which data are shown under method 2B. The concordance of these results with results obtained by the ordinary open Carius (wet digestion) method followed by the Volhard titration, shown under method 2A, shows that there is no loss of chloride in the ordinary wet digestion method. It was found, however, that as Collier (4) and others (32) have pointed out, a considerable loss of chloride occurred when milk was boiled with nitric acid without the previous addition of an excess of silver nitrate. It was noted also that there was no difference in results whether or not the silver chloride precipitate was first removed by filtration before titration.

Results obtained by the Volhard titration in undigested samples by the use of the combined silver-acid-alum reagent (method No. 1), shown in Table 1, were consistently concordant with results obtained by the open Carius (wet digestion) method taken as a standard.

The Volhard titration, conducted with the combined reagent and in undigested milk, is characterized not only by accuracy but also by ease and speed of operation; once the two standardized solutions have been prepared, the test is carried out nearly as quickly as is the direct titration with chromate and silver nitrate. As a result of the accuracy with which the Volhard titration was conducted in undigested samples with the combined silver-acid-alum reagent, the writer recommends this procedure—method No. 1 described above—for determining chloride in milk, in cases in which accuracy is sufficiently important to justify the slight amount of extra time which may be required in comparison with the direct Mohr method.

In the titration, a definite change in endpoint color is apparent when 3 to 5 extra drops of titrating solution are added, equal to 0.15 to 0.25 cc., which amount is equivalent to 0.0015 to 0.0025 per cent chloride. The titration values were about 0.1 cc. higher when samples being titrated were diluted with 50 cc. of water in comparison with undiluted samples—an amount equivalent to 0.001 per cent chloride. Under the conditions specified, endpoint errors do not exceed 0.3 cc. in titration value, or 0.003 per cent chloride.

Results obtained with chromate indicator in a large number of tests were usually 0.02 to 0.045 per cent higher than those obtained by the Volhard titration; results with dichlorofluorescein were about midway between the normal values for the Volhard titration and the abnormally high values obtained with chromate. Similar results were reported by Hastings and Beach. It is shown in the lower two lines in Table 1 that the positive error for both chromate and dichlorofluorescein is increased when the percentage of casein in the sample is high. This fact indicates that the subtraction of a constant correction factor would lead to erroneously variable results.

Attempts were made to increase the accuracy of the direct titration by analyzing samples prepared as follows: (a) Proteins were precipitated by adding to the undiluted sample an equal volume of a saturated solution of
zinc sulfate in 70 per cent ethyl alcohol; (b) Casein was removed by adding rennet and filtering, and an aliquot was titrated; (c) Samples were titrated without being diluted with water. Data secured in these tests are shown in Table 2.

**TABLE 2**

*Effects of dilution of sample, precipitation of protein, and removal of casein, in the direct titration of chloride in milk*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volhard titration</th>
<th>Direct titration with silver nitrate</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Potatochromate indicator</td>
<td>Dichlorofluorescein indicator</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>undiluted with 50 cc. water</td>
<td>undiluted with 50 cc. water</td>
<td></td>
</tr>
<tr>
<td>Mixed herd milk</td>
<td>0.081</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Same, protein precipitated&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td>0.107</td>
<td>0.120</td>
<td></td>
</tr>
<tr>
<td>Whey from above milk (casein removed)</td>
<td>0.083</td>
<td>0.097</td>
<td>0.109</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Ten cc. of a saturated solution of zinc sulfate in 70 per cent ethyl alcohol was added to 10 cc. of milk, as a protein precipitant, before titration.

The results indicate that, in the case of either indicator, chloride values can be reduced slightly by titrating the samples undiluted rather than diluted with water, and by precipitating protein or removing casein. While these modifications add to the accuracy of the method, the values are still considered to be too high and the endpoints too indistinct in the case of both indicators.

The fact that chemical combination occurs between silver and milk protein, as claimed by W. L. Davies in work cited above, was demonstrated by analyzing samples of milk serum prepared as follows:

(A) To 20 cc. of milk in a 100 cc. volumetric flask there were added 50 cc. of water and 10 cc. of nitric acid, the flask was filled to the 100 cc. mark with water, the contents were mixed, and the material was filtered. To 50 cc. of the filtrate there was added 15 cc. of 0.0291 N silver nitrate solution and the Volhard titration was then carried out; (B) Thirty cc. of 0.0291 N silver nitrate solution and 10 cc. of ferrie alum indicator were added to 20 cc. of milk and after the sample was made up to volume and filtered as described above, 5 cc. of nitric acid was added to 50 cc. of the filtrate and the Volhard titration was carried out. The titration values in the filtrates were 7.00 cc. and 5.20 cc. of 0.0291 N sulfocyanate, respectively. It was apparent that a portion of the silver had combined with the protein precipitate in the second sample, resulting in a low value for silver in the filtrate and a positive error for chloride in the analysis. The error is similar to that found by Van Slyke in blood analyses. The combination of silver with protein indicates that, if a filtration method is used, the silver nitrate should be added to the serum.
after filtration rather than to the milk before filtration. It is likewise a verification of the belief, expressed by W. L. Davies, that the positive errors obtained in direct titrations with silver nitrate are caused partially by the tendency of silver ions to combine with protein. The validity of this conclusion is strengthened by the fact, shown in Table 1, that errors in direct titrations are greatest in those samples which contain the most protein. In the Volhard titration of undigested milk, the results indicate that the affinity of silver for sulfocyanate is sufficiently greater than that for milk protein to overcome this difficulty.

It was noted that when a pure solution of sodium chloride was titrated with a pure solution of silver nitrate of the same normality, with chromate indicator, a slight excess of silver nitrate solution was always required to produce a visible endpoint; the average of the excess titrations, with 0.0291 N solutions, was 0.15 cc.; similar results were reported by Mohr. This error, which did not occur when dichlorofluorescein was used, serves to point out the necessity of standardizing the silver nitrate solution against a pure solution of sodium chloride before use.

There was a considerable loss of chloride in the dry ashing method, as shown in Table 1,—a loss which varied markedly and which apparently was not materially greater in high-chloride than in low-chloride samples; there was some evidence of a slight loss even when a base was added to bind the chloride. Poetschke (16) recommends the use of sodium carbonate for this purpose, in an amount equal to 5 per cent of the organic matter present. In this work, however, it was found that less difficulty was encountered because of foaming, and less time was required to burn the charred material, if sodium hydroxide was substituted. Results of tests in several milks, in which varying amounts of sodium hydroxide were added to samples of each milk before ashing, showed that an addition of 5 cc. of normal, chloride-free solution to 10 cc. of milk was more than sufficient to produce a maximum retention of chloride.

**SUMMARY**

The direct titration of chloride in milk by modifications of Mohr's method, with silver nitrate and with potassium chromate indicator, yields results which are so erroneously high and variable that this method, even with a correction factor, is not considered suitable for the accurate determination of chloride in milk. The direct titration method with dichlorofluorescein indicator yields somewhat lower results, but results which are still considered too high and erratic to be relied upon. Indistinct endpoints with either indicator, and the adsorption of silver by protein, are pointed out as important sources of error.

An easy and quantitatively precise modification of the Volhard method is proposed for determining chloride in milk. One combined reagent, containing standardized silver nitrate, nitric acid, and ferric alum indicator, is
added to the sample and the percentage of chloride is determined by titrating with standardized potassium sulfocyanate.

Sources of error and methods of improvement in other methods, as well as conditions necessary for accurate Volhard titrations, are described.

REFERENCES


