A SIMPLE AND ACCURATE METHOD FOR THE DETERMINATION OF CHLORIDE IN BIOLOGICAL FLUIDS

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Several modifications of the classical Volhard method (1) for the determination of chloride have been proposed but none is entirely free from the drawbacks of the original procedure. One of the principal causes of error is that the end-point fades and is uncertain when the silver chloride precipitate is not removed before titration. This is due to the fact that AgCl is more soluble than AgSCN and therefore reacts with thiocyanate: AgCl + SCN' = AgSCN + Cl'.

In order to avoid this reaction and to make an accurate determination possible, AgCl has to be filtered off. This, however, introduces a new error, as, according to Kolthoff (2), a definite loss in silver ions occurs through adsorption on the precipitate. The possibility of retarding the reaction with the precipitate so that the end-point lasts long enough to afford an accurate result was studied by a number of authors, some of them being quoted by Peters and Van Slyke (3).

While a satisfactory modification of Volhard's method without removal of the silver chloride has not yet been published, efforts to replace his procedure by methods based on different principles have led to promising results.

A number of those newer methods have been investigated and, while many of them give reliable results, only a few are at the same time simple enough to compete with the traditional Volhard procedure and its modifications. Consequently it has been concluded that the mercurimetric determination of chloride offers an especially favorable method for a rapid, simple, and accurate determination.
As with silver ions, chloride combines also with mercuric ions according to the equation, \(2\text{Cl}^- + \text{Hg}^{2+} = \text{HgCl}_2\), without, however, forming a precipitate. Mercuric chloride is only very slightly dissociated and the end-point of the titration is therefore recognized by the appearance of mercuric ions in the solution. Liebig (4), who discussed the possibility of a mercurimetric determination of chloride, used urea as an indicator. As sodium nitroprusside gives a turbidity with mercuric ions, this was used subsequently by some investigators as an indicator (5-7), but it did not become very popular, as there seems to be a large subjective range in the observation of the appearance of "the first" turbidity.

The situation became more attractive with the introduction by Dubsky and Trtilek (8) of diphenylcarbazide and diphenylcarbazone as indicators, forming intensive violet-blue-colored complex salts with mercuric ions. Lang (9) recommended the use of their method for the determination of chloride in blood filtrates.

There are, however, several important factors upon which depends the success or failure of the mercurimetric method. Once they are known, they can be easily controlled and as they now have been worked out in detail, a method has been developed which is accurate and free from technical difficulties.

EXPERIMENTAL

Reagents---

Mercuric nitrate solution. 2.0 to 3.0 gm. of mercuric nitrate (c.p. Baker's Analyzed) are dissolved in a few hundred ml. of water with the addition of 20 ml. of 2 N HNO₃. The solution is made up with water to 1000 ml.

Indicator. 100 mg. of diphenylcarbazide (Eastman Kodak No. 4459) are dissolved in 100 ml. of 95 per cent alcohol and stored in the dark, preferably in a refrigerator.

Chloride standard. Sodium chloride c.p. is dried at 120° and 584.5 mg. are dissolved in water and made up to 1000 ml. The solution contains 10 milliequivalents of chloride per liter. It is used for the standardization of each new batch of mercuric nitrate solution.
Procedure

Protein-Free Solutions—The following procedure is used for the determination of chloride in Folin-Wu filtrates of serum or blood. This technique may be used on other fluids with low protein content. To 2 ml. of filtrate (= 0.2 ml. of serum) in a 25 ml.

<p>| TABLE I |</p>
<table>
<thead>
<tr>
<th>Standardization of Approximately 1/60 Mercuric-Nitrate Solution against 2 Ml. of 0.01 N Sodium Chloride Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Hg(NO}_3)_2$ used</td>
</tr>
<tr>
<td>ml.</td>
</tr>
<tr>
<td>1.13 (0)</td>
</tr>
<tr>
<td>1.14 (0)</td>
</tr>
<tr>
<td>1.13 (0)</td>
</tr>
<tr>
<td>1.13 (0)</td>
</tr>
<tr>
<td>1.14 (0)</td>
</tr>
<tr>
<td>Average</td>
</tr>
</tbody>
</table>

The figures in parentheses indicate estimation to the third decimal place.

<p>| TABLE II |</p>
<table>
<thead>
<tr>
<th>Determination of Chloride in Serum and Protein-Free Serum Filtrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum No.</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>ml.</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Erlenmeyer flask is added 0.06 ml. (= 4 drops) of indicator solution. Mercuric nitrate is added from a microburette calibrated in 0.01 ml. intervals. The size of the drops should be such that 1 ml. equals about 100 drops. The clear and colorless solution turns an intensive violet-blue on the addition of the 1st drop of mercuric nitrate solution in excess.
Determination of Chloride

Protein-Containing Solutions—The removal of the proteins intensifies the color change at the end-point, but deproteinization is not absolutely necessary. To 1.8 ml. of water in a 25 ml. Erlenmeyer flask are added 0.2 ml. of serum and 0.06 ml. of indicator. The color of the slightly turbid mixture is first salmon-red and changes, after titration has been started, to deep violet. As more mercuric nitrate is added, the solution becomes clear and pale yellow to colorless. At the end-point there is a sharp change to pale violet which can be seen without difficulty. The results are 1 to 2 milliequivalents per liter higher than those obtained with serum filtrates. A probable explanation is that a small amount of chloride is lost by adsorption on the protein precipitate in the preparation of a Folin-Wu filtrate.

Standardization of Mercuric Nitrate—2 ml. of NaCl standard solution are titrated as described for protein-free solutions. For routine work a factor \( F \) is calculated from the result of this titration, by which the amount of mercuric nitrate solution (expressed in ml.) used for the titration of 2 ml. of Folin-Wu filtrate has to be multiplied to give directly the result in milliequivalents of chloride per liter of serum or blood. \( F = \frac{100}{z} \), where \( z \) is the amount of mercuric nitrate solution used for the titration of 2 ml. of NaCl standard.

Tables I and II give examples of the standardization of the mercuric nitrate solution and of some determinations on serum and protein-free serum filtrates.

Discussion

The method described has been used during the past 2 years for over 2000 chloride determinations and has proved satisfactory for serum filtrates, blood filtrates, cerebrospinal fluids, and urine. The recovery of sodium chloride added to blood or serum is quantitative. All results were within 1 per cent of the calculated amounts. The simplicity of the procedure, the sharp and permanent end-point, and the fact that only one standardized solution is required make this method superior to Volhard's technique.

The following precautions must be observed in order to get satisfactory results. In preparing the mercuric nitrate solution the specified amount of nitric acid should be added. If more or less acid is used, the end-point will not be sharp. It is also of
importance to use the right amount of indicator and to keep the
diphenylcarbazone solution cool and away from light. In day-
light the orange-red solution turns yellow in a few days and cannot
be used. Even in the dark its color changes slowly and after about
2 months becomes cherry-red and no longer gives a sharp end-point.
Consequently a fresh solution is prepared each month. The use
of diphenylcarbazide as an indicator instead of diphenylcarbazone
is not recommended, as the end-point is not as sharp. Hypo-
dermic needles on the tips of the microburettes are unsatisfactory,
as the metal reacts with mercuric nitrate to cause errors up to 25
per cent, depending on the type of metal and on the time the solu-
tion remains in contact with it. If single drops of an available
microburette should amount to more than 0.01 ml., such a burette
can be made suitable for the titration by sliding a short glass
tube, the end of which is drawn to a capillary, over its tip and
attaching it with a piece of rubber tubing.

By this method the chloride concentration of normal human
serum has been found to be 100 to 110 milliequivalents per liter,
which is the same range given by Myers and Muntwyler (10).
The majority of normal sera contains 103 to 107 milliequivalents.
For whole blood the normal values are 77 to 88 milliequivalents
per liter.

SUMMARY

A method for the mercurimetric determination of chloride in
biological fluids is described. The procedure is simple, fast, and
accurate and requires only one standardized solution. Depro-
teinization of the fluids to be titrated is not necessary but increases
the intensity of the color change at the end-point and therefore
facilitates the titration.

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