DETERMINATION OF THE SALT CONTENT OF FISH

A SIMPLE APPARATUS AND PROCEDURE

BY

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The procedure generally used for the determination of the salt content of salt fish is the following: The salt is extracted from the sample by blending with water, or a buffer solution, followed by heating and filtration; the salt content of the extract is then determined by titrating with a standardized solution of silver nitrate and the amount of salt present is calculated from the amount of silver nitrate used. The end-point of the titration is detected with dichlorofluorescein as the indicator.

In the hands of an experienced operator, this method produces very good results, but for those who only occasionally carry out this analysis, the detection of the end-point is very difficult. The indicator, which is first yellow, and in solution, at the end-point is absorbed to the bluish-white precipitate of silver chloride and gives that a pink colour. The analyst must decide whether or not the precipitate in the yellow, turbid solution has become faintly pink. Obviously this decision is subject to wide variations and hence, the precision with which the salt content of the sample is determined is low.

To avoid these difficulties, a procedure was developed in which the use of a coloured indicator is avoided: the end-point of the titration is detected electrometrically. A suitable electrode-system was selected and a simple, inexpensive potentiometric apparatus was constructed for this purpose. Then a routine procedure was worked out and a number of analyses were done to check the reliability of the new method. Very accurate results were obtained, even by inexperienced technicians, and the new method and apparatus, which will be described below, are recommended for routine use.

EQUIPMENT AND APPARATUS

A. Potentiometric Apparatus

The circuit used in the potentiometric apparatus is a conventional compensation circuit with a microammeter as the null-detector. A diagram and a list of the parts necessary for its construction are given in Figure 1.

The instrument knob, given in the parts list, was made into a pointer by gluing a 2-inch plastic pointer to its base (this may also be done by fitting a 2-inch pin in a small hole drilled into the collar of the knob). A special scale was made for the 500-Ohm wirewound potentiometer as follows: a circle with 4-inch diameter was drawn on a small plate of 1/16-inch aluminum; the potentiometer was mounted in its center and the pointer knob attached to its shaft. The
FIG. 1: CIRCUIT AND PARTS OF POTENTIOMETRIC APPARATUS

1. MICRO AMMETER 25-0-25 μA, D.C., 3".
2. POTENTIOMETER, WIRE WOUND, 500 OHMS, 2 WATTS.
3. BAKELITE INSTRUMENT KNOB, 1½".
4. RESISTOR 1500 OHMS, 2 WATTS, 10%.
5. BAKELITE BINDING POSTS (3).
6. DRY-CELL BATTERY, 1.5 VOLTS, SCREW TERMINALS.
7. TOGGLE SWITCH, S.P.S.T.
8. BANANA JACKS, INSULATED (2).
9. BANANA PLUGS, INSULATED (2).
10. PRACTICE KEY FOR MORSE CODE.

FIG. 2: POTENTIOMETRIC APPARATUS FOR SALT DETERMINATIONS
FIG. 3: ELECTRODES USED IN SALT TITRATIONS
FIG. 4: TITRATION ASSEMBLY
positions of the pointer with the knob turned fully anti-clockwise and fully clockwise were then marked on the circle and the larger segment between these two points was divided into 100 equal parts. The divisions were marked permanently on the aluminium plate with a small centre punch.

It was thought advisable, though not absolutely necessary to mount the components in a small cabinet. A photograph of the completed arrangement is given in Figure 2. The instrument can be made in any small workshop; the total cost should not be over forty dollars.

B. Electrodes

Many different electrode systems have been tested; the best results were obtained with platinum-copper. The electrodes were of a very simple construction, as shown in Figure 3.

C. Titration Assembly

Details of the arrangement used for routine titrations, are given in Figure 4. The set-up proved very easy to operate routinely.

REAGENTS AND CHEMICALS

(all chemicals used are reagent grade, unless stated otherwise)

1. Sodium chloride solution 0.1000 N: Dissolve 2.923 grams of NaCl in water; make up to 500.0 ml.
2. Silver nitrate standard solution: Dissolve 7.26 grams of AgNO₃ in water; make up to 500 ml. Standardize this solution against the sodium chloride solution: 1.00 ml. is equivalent with approximately 5 mg NaCl. Store the solution in a cool dark place.
3. Acetate buffer pH 4.5: Dissolve 8.2 grams sodium acetate, cryst. and 5.7 ml acetic acid, glacial, in water; make up to 500 ml.
4. N-Sulphuric acid: Pipette 13.5 ml concentrated H₂SO₄ into about 250 ml water; mix well and then make up to 500 ml.
5. Nitric acid, 6N: Dilute 190 ml HNO₃ 70% with water to 500 ml.
6. Gelatin solution, 0.2%: Dissolve 280 mg gelatin, USP., in 100 ml water.
7. Methanol 99.5% reagent.
8. Distilled water: chloride-free (all reagents are made up with distilled water).
PROCEDURE

Two different titration procedures may be followed: the first is a complete potentiometric titration, which will supply results of the highest precision; the second is a rapid procedure which uses the potentiometric apparatus as the end-point indicator and allows rapid titrations with only a slight loss of precision. A step-by-step description of both methods follows:

A. Precision method

   a) weigh 10 grams of fish into a 150 ml beaker,
   b) add 25 ml buffer pH 4.5,
   c) add distilled water to make about 75 ml,
   d) cover the beaker with a watch glass,
   e) boil the mixture gently for about 5 minutes,
   f) cool and dilute with distilled water to 500 ml.

2. Set up the titration apparatus as shown in Figures 1, 2, 3, and 4, but do not attach the copper electrode.

3. Prepare the copper electrode as follows (to be repeated before each determination; it was found very convenient to have two or three copper electrodes, one of which is being used while the others are being prepared for use):
   a) immerse the lower 4 inches of the copper wire in 6N-nitric acid for about 5 seconds,
   b) rinse with distilled water,
   c) heat the electrode in a flame to dull-red,
   d) immerse the whole electrode immediately in cool methanol and stopper the container (test tube, 25 x 200 mm, with cork).

4. Immediately before use, remove the prepared copper electrode from the methanol, place it in its position in the titration assembly and connect it to the potentiometric apparatus.

5. Pipette an aliquot of the sample solution (obtained under 1f) containing 2.5 to 15 mg salt (usually 2.00 to 5.00 ml) into a titration vessel.

6. Add 4 ml N-sulphuric acid.

7. Add distilled water to make to total volume about 10 ml.

8. Attach the vessel to the titration assembly.

9. Allow a slow stream of air to enter through the air inlet-tube (adjust the flow rate until the stirring is thorough but not vigorous).

10. Balance the bridge circuit of the potentiometric apparatus as follows: depress the key very briefly and turn the potentiometer knob until a short depression of the key does not cause a deflection of the microammeter pointer. Record the reading of the potentiometer dial at this point.

11. Alternately add a small amount of standard silver nitrate solution from the burette and balance the bridge circuit, as under 10. Each time, record the amount of titrant added
FIG. 5: POTENTIOMETRIC TITRATION CURVE OF SALT-FISH EXTRACT
and the reading of the potentiometer dial. Continue this operation until the dial reading, which was originally low, has gone through an increase and then become practically constant at a higher level.

12. Plot a titration curve of the dial-reading as a function of the amount of silver nitrate solution added (see Figure 5).

13. Find the end-point of the titration at the point of greatest inflection of the curve.

14. Calculate the salt content of the fish sample as follows:

\[
\% \text{ salt} = 5.000 \times \text{Std} \times \frac{\text{ml AgNO}_3}{\text{ml sample}} \quad \cdots \cdots (1)
\]

where Std. is the standard value found for the silver nitrate solution when titrated against the standard sodium chloride solution (see standardization procedure); ml AgNO\textsubscript{3} is the volume of silver nitrate solution added to reach the end-point (found in step 13) and ml sample is the volume of the sample extract added to the titration vessel (step 5).

B. Rapid method

1. Carry out steps 1 to 9 inclusive of the precision method.

2. Set the potentiometer knob to the reading for the end-point. (read from a titration curve as in Figure 5, obtained in earlier titrations; this value is practically constant).

3. Alternately add silver nitrate solution from the burette and briefly depress the key of the potentiometric apparatus until the pointer of the microammeter does not show a deflection.

4. Record the amount of silver nitrate solution added to reach the end point.

5. Calculate the salt content of the sample with equation (1) (see step 14 of the precision method), where now ml AgNO\textsubscript{3} is the volume of silver nitrate solution found under 4.

STANDARDIZATION OF SILVER NITRATE SOLUTION
(to be carried out weekly)

1. Carry out steps 2, 3 and 4 of the precision method.

2. Pipette 2.00 ml. 0.1N-sodium chloride solution into a titration vessel.

3. Add 1 ml 0.2% gelatin solution.

4. Carry out steps 6 to 13 inclusive of the precision method.

5. Calculate the standard value for the silver nitrate solution from

\[
\text{Std.} = \frac{11.70}{\text{ml AgNO}_3}
\]

6. Repeat this sequence at least once and use the mean of two or more determinations as the value for Std. in equation (1)
RESULTS OF SALT DETERMINATIONS

To demonstrate the reliability of the apparatus and methods, series of determinations were carried out on salt fish extracts. The analyses were done in four ways: 1) using the precision method, 2) using the rapid method, 3) using a conventional titration with dichlorofluorescein as the indicator; carried out by an experienced chemist, and 4) the conventional titration; carried out by an inexperienced technician.

Table I
Results of determinations of the salt content of fish extract and statistical information regarding these results, obtained by four titration methods

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Potentiometric method</th>
<th>Conventional method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precision</td>
<td>Rapid</td>
</tr>
<tr>
<td>1.</td>
<td>24.93%</td>
<td>25.09%</td>
</tr>
<tr>
<td>2.</td>
<td>25.01%</td>
<td>25.03%</td>
</tr>
<tr>
<td>3.</td>
<td>25.05%</td>
<td>25.01%</td>
</tr>
<tr>
<td>4.</td>
<td>25.11%</td>
<td>25.13%</td>
</tr>
<tr>
<td>5.</td>
<td>25.09%</td>
<td>25.13%</td>
</tr>
<tr>
<td>6.</td>
<td>25.03%</td>
<td>25.09%</td>
</tr>
<tr>
<td>7.</td>
<td>25.07%</td>
<td>24.93%</td>
</tr>
<tr>
<td>8.</td>
<td>25.11%</td>
<td>24.97%</td>
</tr>
<tr>
<td>9.</td>
<td>25.01%</td>
<td>25.15%</td>
</tr>
<tr>
<td>10.</td>
<td>25.07%</td>
<td>25.05%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>% salt</td>
<td>25.05</td>
<td>25.06</td>
<td>25.09</td>
<td>25.06</td>
</tr>
<tr>
<td>S.E. of mean</td>
<td>.018</td>
<td>.023</td>
<td>.031</td>
<td>.810</td>
</tr>
<tr>
<td>S.E. of mean %</td>
<td>.070</td>
<td>.093</td>
<td>.124</td>
<td>3.23</td>
</tr>
</tbody>
</table>

From the results given in Table I, it is quite clear that the potentiometric titrations (both the rapid and the precision method), even though they may be carried out by inexperienced operators, produced very accurate results. The values found by the experienced analyst using the conventional titration, were certainly acceptable, although not quite as accurate as those obtained by potentiometric titration, but the results of the work of the inexperienced technician showed such wide variation as to make them unacceptable; even the mean value of the 10 analyses was subject to gross error.

Undoubtedly, on the basis of these results, the use of a potentiometric method and apparatus may be strongly recommended.