STANDARD METHODS
FOR THE
EXAMINATION
OF
WATER AND SEWAGE

THIRD EDITION
Revised by committees of the American Public Health Association,
American Chemical Society, and referees of the Association of Official Agricultural Chemists

AMERICAN PUBLIC HEALTH ASSOCIATION
120 MASSACHUSETTS AVENUE
BOSTON
1917
Copyright, 1917

By the American Public Health Association
## CONTENTS

<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preface to Third Edition</td>
<td>vii</td>
</tr>
<tr>
<td>Preface to Second Edition</td>
<td>viii</td>
</tr>
<tr>
<td>Preface to First Edition</td>
<td>x</td>
</tr>
<tr>
<td>Introduction to 1897 Bacteriologic Report</td>
<td>xiii</td>
</tr>
<tr>
<td>Preface to 1897 Bacteriologic Report</td>
<td>xv</td>
</tr>
<tr>
<td>Collection of Samples</td>
<td>1</td>
</tr>
<tr>
<td>Quantity of Water Required for Analysis</td>
<td>1</td>
</tr>
<tr>
<td>Bottles</td>
<td>2</td>
</tr>
<tr>
<td>Time Interval between Collection and Analysis</td>
<td>2</td>
</tr>
<tr>
<td>Representative Samples</td>
<td>3</td>
</tr>
<tr>
<td>Physical Examination</td>
<td>4</td>
</tr>
<tr>
<td>Temperature</td>
<td>4</td>
</tr>
<tr>
<td>Turbidity</td>
<td>4</td>
</tr>
<tr>
<td>Turbidity Standard</td>
<td>4</td>
</tr>
<tr>
<td>Platinum Wire Method</td>
<td>5</td>
</tr>
<tr>
<td>Turbidimetric Method</td>
<td>7</td>
</tr>
<tr>
<td>Coefficient of Fineness</td>
<td>8</td>
</tr>
<tr>
<td>Color</td>
<td>9</td>
</tr>
<tr>
<td>Comparison with Platinum-Cobalt Standards</td>
<td>9</td>
</tr>
<tr>
<td>Comparison with Glass Disks</td>
<td>10</td>
</tr>
<tr>
<td>Comparison with Nessler Standards</td>
<td>10</td>
</tr>
<tr>
<td>Lovibond Tintometer</td>
<td>11</td>
</tr>
<tr>
<td>Odor</td>
<td>12</td>
</tr>
<tr>
<td>Cold Odor</td>
<td>12</td>
</tr>
<tr>
<td>Hot Odor</td>
<td>12</td>
</tr>
<tr>
<td>Expression of Results</td>
<td>12</td>
</tr>
<tr>
<td>Chemical Examination</td>
<td>14</td>
</tr>
<tr>
<td>Expression of Results</td>
<td>14</td>
</tr>
<tr>
<td>Forms of Nitrogen</td>
<td>15</td>
</tr>
<tr>
<td>Ammonia Nitrogen</td>
<td>15</td>
</tr>
<tr>
<td>Determination by Distillation</td>
<td>15</td>
</tr>
<tr>
<td>Measurement of Ammonia Nitrogen</td>
<td>16</td>
</tr>
<tr>
<td>Comparison with Ammonia Standards</td>
<td>16</td>
</tr>
<tr>
<td>Comparison with Permanent Standards</td>
<td>17</td>
</tr>
<tr>
<td>Modification for Sewage</td>
<td>18</td>
</tr>
<tr>
<td>Determination by Direct Nessleration</td>
<td>19</td>
</tr>
<tr>
<td>Albuminoid Nitrogen</td>
<td>20</td>
</tr>
<tr>
<td>Organic Nitrogen</td>
<td>21</td>
</tr>
<tr>
<td>Nitrite Nitrogen</td>
<td>22</td>
</tr>
<tr>
<td>Nitrate Nitrogen</td>
<td>23</td>
</tr>
<tr>
<td>Phenolsulfonic Acid Method</td>
<td>23</td>
</tr>
<tr>
<td>Reduction Method</td>
<td>24</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>25</td>
</tr>
<tr>
<td>Oxygen Consumed</td>
<td>25</td>
</tr>
<tr>
<td>Recommended Method</td>
<td>26</td>
</tr>
<tr>
<td>Other Methods</td>
<td>27</td>
</tr>
<tr>
<td>Residue on Evaporation</td>
<td>29</td>
</tr>
<tr>
<td>Total Residue</td>
<td>29</td>
</tr>
<tr>
<td>Fixed Residue and Loss on Ignition</td>
<td>29</td>
</tr>
</tbody>
</table>
# Table of Contents

- **Suspended Matter** ............................................... 30
  - Determination with Gooch Crucible .......................... 30
  - Determination by Filtration .................................. 30
  - Determination of Volume ...................................... 30
  - Fixed Residue and Loss on Ignition ......................... 30
- **Hardness** ......................................................... 30
  - Total Hardness by Calculation ............................... 31
  - Total Hardness by Soap Method .............................. 31
  - Total Hardness by Soda Reagent Method .................... 34
  - Temporary Hardness by Titration with Acid ................. 34
  - Non-Carbonate Hardness by Soda Reagent Method .......... 34
  - Non-Carbonate Hardness by Soap Method ................... 35
- **Alkalinity** ....................................................... 35
  - Procedure with Phenolphthalein ............................ 36
  - Procedure with Methyl Orange .............................. 37
  - Procedure with Lactmoid .................................... 37
  - Procedure with Erythrosine ................................ 37
  - Bicarbonate .................................................... 37
  - Normal Carbonate ............................................. 38
  - Hydroxide ....................................................... 38
  - Alkali Carbonates ............................................. 39
- **Acidity** .......................................................... 39
  - Total Acidity ................................................ 40
  - Free Carbon Dioxide ......................................... 40
  - Free Mineral Acids .......................................... 41
  - Mineral Acids and Sulfates of Iron and Aluminium ...... 41
- **Chloride** ......................................................... 41
- **Iron** .............................................................. 43
  - Total Iron ..................................................... 44
    - Colorimetric Method ....................................... 44
    - Comparison with Iron Standards ......................... 45
    - Comparison with Permanent Standards ................... 46
    - Volumetric Method ......................................... 46
  - Dissolved Iron ................................................. 47
  - Suspended Iron ................................................ 47
  - Ferrous Iron .................................................. 47
  - Ferric Iron .................................................... 48
- **Manganese** ....................................................... 48
  - Persulfate Method ............................................ 48
  - Bismuthate Method ............................................ 49
- **Lead, Zinc, Copper, and Tin** ................................ 50
  - Lead ............................................................ 51
  - Zinc ............................................................. 52
  - Copper .......................................................... 53
  - Tin ............................................................... 54
- **Mineral Analysis** ................................................ 56
  - Residue on Evaporation ....................................... 56
  - Alkalinity and Acidity ........................................ 56
  - Chloride .......................................................... 56
  - Nitrate Nitrogen .............................................. 56
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separation of Silica, Iron, Aluminium, Calcium, and Magnesium</td>
<td>56</td>
</tr>
<tr>
<td>Silica</td>
<td>56</td>
</tr>
<tr>
<td>Iron and Aluminium</td>
<td>57</td>
</tr>
<tr>
<td>Calcium</td>
<td>57</td>
</tr>
<tr>
<td>Magnesium</td>
<td>57</td>
</tr>
<tr>
<td>Separation of Sulfate, Sodium, and Potassium</td>
<td>58</td>
</tr>
<tr>
<td>Sulfate</td>
<td>58</td>
</tr>
<tr>
<td>Sodium, Potassium and Lithium</td>
<td>59</td>
</tr>
<tr>
<td>Potassium</td>
<td>59</td>
</tr>
<tr>
<td>Lithium</td>
<td>60</td>
</tr>
<tr>
<td>Bromine, Iodine, Arsenic, and Boric Acid</td>
<td>61</td>
</tr>
<tr>
<td>Bromine and Iodine</td>
<td>61</td>
</tr>
<tr>
<td>Arsenic</td>
<td>63</td>
</tr>
<tr>
<td>Boric Acid</td>
<td>63</td>
</tr>
<tr>
<td>Hydrogen Sulfide</td>
<td>63</td>
</tr>
<tr>
<td>Chlorine</td>
<td>64</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>64</td>
</tr>
<tr>
<td>Ether—Soluble Matter</td>
<td>69</td>
</tr>
<tr>
<td>Relative Stability of Effluents</td>
<td>69</td>
</tr>
<tr>
<td>Biochemical Oxygen Demand of Sewages and Effluents</td>
<td>71</td>
</tr>
<tr>
<td>Relative Stability Method</td>
<td>71</td>
</tr>
<tr>
<td>Sodium Nitrate Method</td>
<td>72</td>
</tr>
<tr>
<td>Analysis of Sewage Sludge and Mud Deposits</td>
<td>73</td>
</tr>
<tr>
<td>Collection of Sample</td>
<td>73</td>
</tr>
<tr>
<td>Reaction</td>
<td>73</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>74</td>
</tr>
<tr>
<td>Moisture</td>
<td>74</td>
</tr>
<tr>
<td>Volatile and Fixed Matter</td>
<td>74</td>
</tr>
<tr>
<td>Total Organic Nitrogen</td>
<td>74</td>
</tr>
<tr>
<td>Ether—Soluble Matter</td>
<td>75</td>
</tr>
<tr>
<td>Ferrous Sulfide</td>
<td>76</td>
</tr>
<tr>
<td>Biochemical Oxygen Demand</td>
<td>76</td>
</tr>
<tr>
<td>Analysis of Chemicals</td>
<td>77</td>
</tr>
<tr>
<td>Reagents</td>
<td>77</td>
</tr>
<tr>
<td>Sulfate of Aluminium</td>
<td>78</td>
</tr>
<tr>
<td>Insoluble Matter</td>
<td>78</td>
</tr>
<tr>
<td>Oxides of Aluminium and Iron</td>
<td>87</td>
</tr>
<tr>
<td>Total Iron</td>
<td>79</td>
</tr>
<tr>
<td>Ferric Iron</td>
<td>79</td>
</tr>
<tr>
<td>Ferrous Iron</td>
<td>80</td>
</tr>
<tr>
<td>Basicity Ratio</td>
<td>80</td>
</tr>
<tr>
<td>Lime</td>
<td>80</td>
</tr>
<tr>
<td>Sulfate of Iron</td>
<td>81</td>
</tr>
<tr>
<td>Insoluble Matter</td>
<td>81</td>
</tr>
<tr>
<td>Iron as Ferrous Sulfate</td>
<td>81</td>
</tr>
<tr>
<td>Acidity</td>
<td>81</td>
</tr>
<tr>
<td>Soda Ash</td>
<td>82</td>
</tr>
<tr>
<td>Insoluble Matter</td>
<td>82</td>
</tr>
<tr>
<td>Available Alkali</td>
<td>82</td>
</tr>
<tr>
<td>Chemical Bibliography</td>
<td>82</td>
</tr>
</tbody>
</table>
# Table of Contents

**Microscopical Examination** ............................................. 89  
**Microscopical Bibliography** ........................................... 91  
**Bacteriological Examination** .......................................... 93  

## Apparatus
- Sample Bottles ..................................................... 93  
- Pipettes ............................................................. 93  
- Dilution Bottles .................................................... 93  
- Petri Dishes ........................................................ 93  
- Fermentation Tubes ................................................. 93  

## Materials
- Water ................................................................. 93  
- Meat Extract ....................................................... 93  
- Peptone .............................................................. 94  
- Sugars ............................................................... 94  
- Agar ................................................................. 94  
- Gelatin ............................................................... 94  
- Litmus ............................................................... 94  

## General Chemicals ...................................................... 94  

## Methods ....................................................................... 94  

## Preparation of Culture Media ......................................... 94  
  - Titration ............................................................ 94  
  - Sterilization ....................................................... 95  
  - Nutrient Broth ..................................................... 95  
  - Sugar Broths ....................................................... 95  
  - Nutrient Gelatin .................................................. 96  
  - Nutrient Agar ...................................................... 96  
  - Litmus or Azolitmin Solution ..................................... 96  
  - Litmus-Lactose-Agar ............................................. 96  
  - Endo's Medium ..................................................... 97  

## Collection of Sample .................................................. 98  

## Storage and Transportation of Sample ............................. 98  

## Dilutions .................................................................... 98  

## Plating ...................................................................... 99  

## Incubation .................................................................. 99  

## Counting .................................................................... 99  

## The Test for the Presence of Members of the B. Coli Group 100  
  - Presumptive Test .................................................... 100  
  - Partially Confirmed Test .......................................... 101  
  - Completed Test ..................................................... 102  
  - Application of these Tests ....................................... 102  
  - Expression of Results ............................................. 103  
  - Summary of these Tests ........................................... 104  
  - Interpretation of Results .......................................... 106  

## Differentiation of Fecal from Non-fecal Members of the B. Coli Group ......................................................... 106  
  - Methyl Red Test ..................................................... 107  
  - Voges Proskauer Test .............................................. 107  

## Routine Procedure for Bacteriological Examination .......... 108  

## Bacteriological Bibliography .......................................... 110  

## Index ......................................................................... 112
PREFACE TO THIRD EDITION.

When the second edition of Standard Methods for the Examination of Water and Sewage was issued in 1912, the committees were continued and it was understood that a revised report should be prepared for publication at the end of about five years.

The Committee on Chemical Methods was modified immediately after the final presentation of the report by the appointment of Robert Spurr Weston as chairman; the reappointment of Earle B. Phelps and Edward Bartow and the appointment of R. B. Dole and W. F. Monfort as members of the committee. In 1913 Robert Spurr Weston resigned, Edward Bartow was appointed chairman and Arthur Lederer was made a member of the committee.

The Committee on Bacteriological Methods was continued until 1914 when F. P. Gorham was made chairman, G. W. Fuller, Roger Perkins, S. C. Prescott, and L. A. Rogers were made members. In 1915 S. C. Prescott resigned and W. H. Frost was made a member of the committee.

The two committees have worked independently in the preparation of the two sections of the present volume. The Chemical Committee, of the American Chemical Society, by action of the section on water, sewage and sanitation was authorized to co-operate with that society through a similar committee, which originally consisted of Profs. L. P. Kinnicutt, chairman, and W. P. Mason, H. E. Jordan, E. H. S. Bailey, and Edward Bartow, members. In 1911* Dr. J. H. Long was made chairman. In December, 1911, Dr. J. H. Long resigned, Edward Bartow was made chairman of the committee and W. W. Skinner made member of the committee. Progress reports have been made to the Council of the American Chemical Society.† The American Chemical Society authorized its committee to co-operate with the committee of the American Public Health Association. Both committees were authorized to co-operate with the referees of the Association of Official Agricultural Chemists, W. W. Skinner and H. P. Corson. This volume is, therefore, the result of the combined labors of the three committees. The various methods were divided for study among the ten members of the associated committees. Members of the Laboratory Section of the American Public Health Association and the Division of Water, Sewage and Sanitation of the American Chemical Society were asked to assist. Many responded and were assigned to sub-committees under the direction of the members of the main committees. It is impossible to mention all who have given assistance, but the committee desires to express its appreciation of the valuable assistance rendered. The referees of the Association of Official Agricultural Chemists have prepared a report which has been published in the Journal of Official Agricultural Chemists.‡

At the Rochester meeting of the American Public Health Association, the committee was authorized to publish the additions and modifications and after

*Proc. Am. Chem. Soc. for the year 1911, 33, p. 102, 1911; for the year 1912, 34, p. 40, 1912.
time for criticism, to publish the complete report. The proposed alterations were published in the American Journal of Public Health.*

The committee considered all criticisms and took final action at the meeting of the American Chemical Society, April 17–21, 1916, where the report was received and adopted. The 1912 edition has been modified by the addition of methods for the examination of sewage sludge and muds, the analysis of chemicals used in the treatment of water, and the determination of chlorine. Some changes have been made in the technique of existing methods. There have been omitted from the report the silver nitrate method for caustic alkalinity, the determination of total acidity by titration with sodium carbonate in the presence of phenolphthalein, and Knorre's volumetric persulphate method for the determination of manganese. The diction has been carefully revised and methods have been rearranged in groups in order to make the arrangements more logical.

The Committee on Bacteriological Methods has worked in co-operation with a committee appointed by the Society of American Bacteriologists in 1915. The bacteriological portion of the 1912 report has been entirely revised and much of the material included in that report, which had only a limited bearing upon water analysis, has been eliminated. The committee felt that such subjects as the isolation and identification of specific organisms other than members of the B. coli group, the determination of species of bacteria in general, and methods of general bacteriological technique, did not come within the province of a report upon standard methods of water analysis. The present report, therefore, concerns itself only with the standardization of apparatus, materials, and methods which are at present used in bacteriological water analysis.

PREFACE TO SECOND EDITION.

The report issued in 1912 is a revised edition of the volume issued in 1905 under the title "Report of the Committee on Standard Methods of Water Analysis to the Laboratory Section of the American Public Health Association." A full history of the origin of the movement toward standard methods of water analysis and of the work of the various committees which have had that matter in hand since 1895 is given in the letter of transmittal of the original report. The committee having in charge the preparation of that report was continued in office after its final presentation at the Boston meeting in 1904, and it was the expressed opinion of those present at that meeting that progress reports should be made from time to time looking toward a revision of the standard text when such revision seemed desirable. The question of a second edition of the report was definitely settled at the Atlantic City meeting in 1908. This task was placed in the hands of two new committees to whom were referred, respectively, methods for the chemical analysis of water and sewage, and methods for the bacteriological tests of water and sewage. The former committee was made up of Messrs. Earle B. Phelps, Chairman; Robert Spurr Weston, Leonard P. Kinnicut, Edward Bartow, and A. E. Kimberly; and the latter of D. D. Jackson, Chairman; Wm.

Royal Stokes, J. A. Amyot, Fred P. Gorham and Stephen DeM. Gage. These two committees have worked quite independently in the preparation of the two sections of the present volume.

The work of the committees during the three years that have elapsed since their appointment has been contained in the progress reports which have been submitted at the annual meetings. These reports have been in the nature of proposed changes in or additions to the existing text. It has been thought best to consider these annual reports to be the actual working reports of the committees, and in the final incorporation of the committees' recommendations in the text of this volume to change the title of the latter so that it no longer appears as a committee report.

The changes which have been incorporated in the chemical section of the report have been minor changes in technique in certain of the determinations; a considerable rearrangement of the material into what is considered a somewhat more convenient form for use; the substitution of entirely new matter in the case of a few of the determinations, notably that for nitrates in sewage, copper, and some minor determinations; a substitution of the term "non-carbonate hardness" for the rather troublesome term "incrustants" heretofore used; a method of statement for carbon dioxide heretofore reported as "free," "half-bound," and "bound" carbon dioxide, which statement it is believed brings the results of this determination more nearly into line with those of other mineral constituents now commonly expressed in the ionic form; and in the addition of certain new material, notably methods for manganese, hydrogen sulfide, bromine, and iodine. The principal addition to the text, however, consists in a complete method for the so-called "mineral analysis of water," the analysis for industrial purposes and for use in boilers. Methods for the determination of putrecibility or relative stability in sewage and rapid approximate methods known as "lime value," and "soda value," intended for use in connection with water softening plants to determine the relative amounts of these two reagents necessary for softening, have also been added; and a new table of oxygen saturation values, including fresh and saline water and with a correction formula for altitude, is incorporated.

The changes proposed by the Committee on Bacteriological Methods have consisted in a few minor changes in the text, together with the addition of much new material. In order to study the various bacteria of fecal origin a study has been made of the B. coli group, and this group has been divided into four subdivisions, as the text of the report will show. The quantitative tests for the B. coli group have been considered, and the dilution method in fermentation tubes has been recommended. In quantitative tests for general gas-producing bacteria in water or sewage the use of liver broth has been suggested, and a number of comparative studies are set forth in the report concerning the rejuvenation of attenuated forms by this method. Tests for B. sporogenes and B. typhi in drinking water are also considered, and the report ends with the consideration of the isolation of specific disease germs from water supplies and a careful set of directions concerning the technique of sterilization and the manufacture of various culture media. A list of references is appended.

This volume on Standard Methods is presented in the belief that it represents the best methods for the various determinations that are available. It is in no sense the work of the committee members alone, but throughout the work of
revision every effort has been made to obtain a consensus of opinion upon all matters. It is believed that the procedures described represent such a consensus of opinion and fairly represent the best practice in American laboratories. These, however, are not in any sense regarded as final. It must be obvious that standard methods of this kind in order to be useful must, while maintaining sufficient rigidity to serve as a satisfactory guide to laboratory procedure, be at the same time sufficiently elastic to be readily if somewhat slowly moulded by new developments in the best practice, to the end that the Standard Methods themselves shall always reflect what is best in laboratory procedure rather than arbitrarily fix such procedure.

The members of the two committees wish to express their thanks to those who have rendered assistance in the preparation of this report, and to beg for their continued interest, support, and co-operation in the future work of the laboratory section along these lines.

PREFACE TO THE FIRST EDITION.

New York, December 19, 1904.

To the Chairman and Members of the Laboratory Section of the American Public Health Association.

Gentlemen: The final report of your Committee on Standard Methods of Water Analysis is submitted herewith.

It is now more than ten years since there arose in North America a movement for securing the adoption of more uniform and efficient methods for water analysis, particularly of bacteriological methods. Earlier accomplishments related largely to uniform methods of recording chemical results. The late Dr. Wyatt Johnston of Montreal was the first one to call the attention of the Association to this line of work, and at the Montreal meeting in 1894 the first step in this direction was taken. By invitation of a sub-committee of the Committee on Pollution of Water Supplies, a convention of American bacteriologists assembled in New York in June, 1895, and appointed a committee to draw up procedures for the study of bacteria in a uniform manner and with special reference to the differentiation of species. This committee submitted a report at the Philadelphia meeting of the Association in 1897. It was published early in 1898 and has been widely used in various laboratories in this country. The demand for copies of it still continues, although for some time it has been out of print.

At the Minneapolis meeting in 1899 the present committee was appointed with a view to extending the standard procedures to include not only the determinations of species of bacteria, but all the other lines of investigation involved in the analysis of water.

The committee undertook first to ascertain the views of the analysts of America regarding not only the bacteriological, but also the chemical, physical, and microscopic examinations of water. Circular letters were sent to all the principal laboratories, and much co-operative work was done in connection with the differentiation of species of bacteria.

Progress reports were made at meetings held at Indianapolis, Buffalo, and New
Orleans in 1900, 1901, and 1902, the two latter being published in the *Proceedings*
of the Association for those years, and the former in *Science*. In 1901 this committee was instructed to revise the 1897 report of the Bacteriological Committee in order to exclude from it those features not found to have been of general service and to include such new matter as later developments had justified.

In 1902 the committee suffered a severe loss through the death of one of its most valued members, the late Dr. Wyatt Johnston of Montreal, who is credited by all with the initiation of this movement. Dr. Adolph Gehrmann of Chicago resigned from the committee in that year. At the New Orleans meeting the full membership of the committee was restored by the appointment of Mr. R. S. Weston of Boston and Mr. J. W. Ellms of Cincinnati.

The past decade has been a transitional period for water analysis. As this field of sanitary investigation has been extended to cover in a more thorough manner the conditions existing throughout this country, it has been found that the methods applicable to the fairly clear waters of the Atlantic seaboard and to many European waters have not been adequate for the analysis of the muddy waters of the South and West. Conversely, new methods used in certain sections of the country are for similar reasons not always applicable to the conditions existing where the earlier studies were made.

The purification of water supplies has received a great impetus during the past decade, and the water analyst is more and more being placed in charge of the operation of large public filter plants. In work of this character, especially when muddy waters are being treated, he thus uses some methods of analysis which are not generally required for sanitary work; while, on the other hand, he omits with propriety various tests which form an essential part of the customary sanitary analysis.

The presence of objectionable amounts of iron in various ground water supplies and the introduction of special processes for its removal have in some instances severely taxed the resources of the water analyst, and necessitated modifications of old methods. Methods for determining lead and copper are being used with more frequency than was the case in earlier years, when less attention was given to all the aspects of public health.

Treatment of sewage, to prevent gross nuisances and to prevent the pollution and infection of streams, has recently received a marked stimulus through the development of the so-called rapid biological filters, the septic tank treatment, etc. Special tests and analyses have been required in connection with these lines of work—notably those for the determination of the putrescibility of the effluents of sewage works. The study of the longevity of disease germs in connection with the question of infection of water supplies drawn from streams at various distances below the discharge of outfall sewers has brought into prominence new lines of bacteriological investigation.

Enough has been said in outline of the new and varied requirements made of the water analyst to show the need of a broad and substantial basis for his methods and for his work under present conditions. Some of the older methods, used for the study of the general sanitary quality of unfiltered water supplies, are becoming less and less important, while the newer ones, used in the operation of purification plants, are becoming of more value. This is in keeping with the modern tendency
of the analyst to become more and more an important factor in connection with the operation of plants for the purification of water and sewage, indeed, unpurified sources of water supply are becoming fewer and fewer, as hygienic demands are being met by the rapid introduction of purification works, as evidenced by the best practice both in Europe and in America.

The methods of analysis presented in this report as "standard methods" are believed to represent the best current practice of American water analysts, and to be generally applicable in connection with the ordinary problems of water purification, sewage disposal and sanitary investigations. Analysts working on widely different problems manifestly cannot use methods which are identical, and special problems obviously require the methods best adapted to them; but, while recognizing these facts, it yet remains true that sound progress in analytical work will advance in proportion to the general adoption of methods which are reliable, uniform, and adequate.

It is said by some that standard methods within the field of applied science tend to stifle investigation, and that they retard true progress. If such standards are used in the proper spirit this ought not to be so. The committee strongly desires that every effort shall be continued to improve the technique of water analysis, and especially to compare current methods with those herein recommended, where different, so that the results obtained may become still more accurate and reliable than they are at present.

In Table No. 1 are given the more essential determinations which, in the opinion of the committee, should be applied to each of the principal lines of analytical work in connection with the ordinary problems of water supply and sewage disposal. It is realized that some of the older laboratories are hardly in a position at the present time to follow out these suggestions in a literal manner, although on new work it is believed that they could follow them to advantage.

Some of these suggestions may seem radical, and in special instances they may be indeed inexpedient, as already mentioned, but on the whole it is believed that they indicate the lines along which the water analyst may direct his efforts to best advantage and with the feeling that he is obtaining all the data necessary, while doing little or nothing that is needless.

It will be noted that the bacteriological determinations, including the tests for B. coli, are given much more prominence than was formerly the case, and that less attention is given to the organic matter as determined by chemical analysis. This is because of the inability of the chemical methods to separate that portion of the organic matter which is of no sanitary significance from that which is associated with pollution or infection.

The most substantial steps in advance relate to improvements in the physical and chemical methods required in connection with the operation of plants for the improvement and purification of water supplies.

Detailed descriptions of the various methods recommended are given in concise form, covering the essential features of each determination. It is assumed that those using these directions are thoroughly grounded in the fundamental principles of chemistry and biology, and that they are also familiar with the leading literature upon the subject. So many satisfactory textbooks upon chemical analysis in general and on water analysis in particular are in existence that it is unnecessary to give a complete detailed description of all procedures; but it is
fully recognized that in many cases the adherence to certain details is an essential matter, and hence for the newer methods they are incorporated in this report.

Some of the methods described are known in different parts of America by different proper names, hence it has been the endeavor of the committee to describe them with sufficient clearness to make plain what procedures are meant without reference to the name of the author, but to give due credit for the method by referring to his published work in the bibliography at the end of the report.

The bibliography is by no means a full list of important works on water analysis. It is simply a list of references to the works most consulted in America, arranged for the purpose of assisting the reader in getting in touch with the general aspects of a method, including its history and application, together with full technical details of the procedure as now practiced.

No attempt is made to report upon the interpretation of the results of water analyses, or upon the classification of bacteria, as these subjects are receiving the attention of other committees of the Association.

This report does not deal with any of the numerous phases of applied bacteriology in the domains of medicine or industrial science. It is hoped, however, that workers in these fields may find useful portions dealing with the preparation of media, and that published descriptions of bacteria associated with disease or with various industrial processes will be made to conform with the procedures herein recommended.

Very respectfully,
(Signed) George W. Fuller, Chairman,
George C. Whipple, Secretary,
H. W. Clark,
Edwin C. Jordan,
H. L. Russell,
J. W. Ellms,
Robert Spurr Weston.

INTRODUCTION TO THE 1897 REPORT OF THE BACTERIOLOGICAL COMMITTEE.

As explained by Dr. Smart in the preface to this report, a convention of bacteriologists from the United States and Canada assembled in the city of New York, on June 21 and 22, 1895, in response to the invitation of a sub-committee of the Committee on the Pollution of Water Supplies of the American Public Health Association. The proceedings of this convention, including the papers read and their discussion, were published in the Journal of the American Public Health Association, October, 1895. These papers and discussions related mainly to technical procedures to be followed in the systematic study of bacteria, with especial reference to their description and identification. There was general agreement of opinion as to the importance of securing greater precision and uniformity in the methods of studying and describing bacterial species. A committee of members of the convention was therefore appointed to prepare a report, to be presented to the Water Committee of the American Public Health Association, this report to
contain recommendations concerning bacteriological methods based partly upon the deliberations of the convention and partly upon a wider study of the subject. The members selected for this committee were Drs. J. George Adami, William T. Sedgwick, George W. Fuller, Charles Smart, Alexander C. Abbott, T. M. Cheesman, Theobald Smith, and William N. Welch.

A first draft of a report was drawn up by Dr. Adami and submitted to the members of the committee, who made various suggestions. The final preparation of the report was undertaken by Dr. T. H. Cheesman, Instructor in Bacteriology in the College of Physicians and Surgeons, Columbia University, New York. The following statement by Dr. Adami well expresses the aims and manner of preparation of the report:

Naturally, with a committee, the members of which are so widely scattered, it has been found impossible to hold frequent meetings, but at these meetings the members have found themselves singularly in accord upon everything relating to the main points at issue. Naturally, also, correspondence and the circulation of the report in its various stages have not been found entirely satisfactory in eliciting the opinions of every member upon matters of detail. But all these means accomplished much, and it was eventually found possible to place the final drafting of the recommendations in the hands of one member. We cannot sufficiently express our indebtedness to Dr. Cheesman for the amount of time, and indeed of independent work which he has devoted to this task.

The recommendations thus do not indicate the previous procedure in all details of any single member of the committee, but are a concord of what has appeared to be the best in the methods and technique of all the members of bacteriologists generally. To have indicated in the following pages wherein any single member found himself unable to accept in its entirety any one of the many recommendations would have counteracted our main object, that, mainly of inducing uniformity and precision in procedure in the study and descriptions of species. Each member, therefore, to attain this object, has voluntarily refrained from demanding that one or other method, to which from long employment he has become firmly attached, should be inserted in these pages. The committee freely admits that there may be other and better methods than those here detailed. It has, on the other hand, striven to recommend what in the present state of our knowledge would seem to be the best and most likely to gain acceptance. It does not demand of bacteriologists in general—it does not promise for its own members in particular—that these and only these methods shall be employed. It does but ask that where new species are being studied for publication the procedure here recommended be given a trial, and that, for the direction of other workers, where it has been employed a note be given to that effect, e.g., “cultures in broth (Method B.C.) presented the following characters” or, “save where otherwise indicated, the B.C. Methods have been used.”

In short, the committee recognizes fully that these recommendations must of necessity be provisional. It publishes them in the hope that by this act it will direct attention to the urgent need now existing for full and accurate descriptions of species of bacteria in which the items have been determined by methods common to the main body of workers, and as a consequence are capable of verification and control.

The report is not intended to be a complete treatise upon bacteriological technique. Its purpose is to make certain recommendations concerning methods to be pursued in the study of bacteria, with the view of securing greater uniformity and exactness in the determination and description of the characters of bacterial species. When one considers the difficulty, often the impossibility, of the identification of many bacterial species or varieties described in literature, in consequence of imperfections and carelessness in the determination and description
of their characters, it is evident that the attainment of the purpose aimed at in this report is greatly to be desired.

The report deals especially with certain ordinary and fundamental procedures in bacteriological technique, and it does not attempt to cover fully the entire field. In a science so rapidly developing as bacteriology, it need scarcely be said that any attempt to present the best technical procedures can apply only to the existing state of the science, and that much will be added and much corrected in the near future. It is hoped that the recommendations in this report may prove useful to workers in bacteriology, and especially may lead to greater accuracy and fullness and uniformity in the determination and description of the characters of bacteria.

(Signed) WILLIAM H. WELCH.

PREFACE TO THE 1897 REPORT OF THE BACTERIOLOGICAL COMMITTEE.

At the meeting of the American Public Health Association in Montreal, Canada, in 1894, the Committee on the Pollution of Water Supplies closed its report with the suggestion of a co-operative investigation into the bacteriology of water supplies as a means of bringing order out of the chaotic state of the literature of water bacteria and of throwing light from the bacteriology side on questions of practical sanitation. This suggestion was approved by the Association and the Chairman of the Committee was authorized to build up a committee for collective bacteriological investigation. The bacteriologists promptly acceded to the proposition. They recognized that such an investigation would give an immense impetus to bacteriological work; that it would do much to clear away the confusion surrounding species, and to increase and systematize our knowledge; and that practical results might also be expected, particularly as regards the typhoid and colon bacilli, the unwholesomeness of water supplies and the means of lessening the prevalence of typhoid fever and diarrheal diseases. A sub-committee consisting of Professor J. George Adam, Dr. Wyatt Johnston, Mr. George W. Fuller and myself, appointed to determine methods of laboratory procedure to be adopted by the committee in the practical work of the investigation, found it impossible to formulate a satisfactory scheme of work until certain questions, mostly relating to technique, had been discussed fully and settled in accordance with the most advanced knowledge of the subjects concerned. An effort to effect this by correspondence developed so much variation in the practice of the different laboratories that it became needful to call a convention for a thorough discussion of the points at issue. The convention was held in the Academy of Medicine, New York City, June 21 and 22, 1895. Most of the prominent bacteriologists of the United States and Canada were present, but although the members were informed beforehand of the subjects that were to be brought up for settlement, and although full discussion was given to each under the chairmanship of Professor Welch of Johns Hopkins University, many of the points presented so much difficulty that the whole series was referred to a committee, with the understanding that the convention would accept its decision.
This committee consisted of J. George Adami, McGill University, Chairman; A. C. Abbott, University of Pennsylvania; T. M. Cheesman, College Physicians and Surgeons, New York; George W. Fuller, Louisville Water Company; W. T. Sedgwick, State Board of Health, Massachusetts; Charles Smart, U. S. Army; Theobald Smith, Harvard University; W. H. Welch, Johns Hopkins University.

The committee met in New York City in February, 1896, to digest its material and outline its report which was presented to the American Public Health Association at its meeting in Buffalo, New York, in September of that year. The report was subsequently withdrawn for further criticism and amendment, and was finally submitted for publication at the meeting of the Association in Philadelphia, Pennsylvania, September, 1897.

(Signed) CHARLES SMART.
AMERICAN PUBLIC HEALTH ASSOCIATION.

LABORATORY SECTION.

STANDARD METHODS FOR THE EXAMINATION OF WATER AND SEWAGE.

Compiled and revised by committees of the American Public Health Association and the American Chemical Society and referees of the Association of Official Agricultural Chemists.

COLLECTION OF SAMPLES.

QUANTITY REQUIRED FOR ANALYSIS.

The minimum quantity necessary for making the ordinary physical, chemical, and microscopical analyses of water or sewage is 2 liters; for the bacteriological examination, 100 cc. In special analyses larger quantities may be required.

BOTTLES.

The bottles for the collection of samples shall have glass stoppers, except when physical, mineral, or microscopical examinations only are to be made. Jugs or metal containers shall not be used.

Sample bottles shall be carefully cleansed each time before using. This may be done by treating with sulfuric acid and potassium bichromate, or with alkaline permanganate, followed by a mixture of oxalic and sulfuric acids, and by thoroughly rinsing with water and draining. The stoppers and necks of the bottles shall be protected from dirt by tying cloth, thick paper or tin foil over them.

For shipment bottles shall be packed in cases with a separate compartment for each bottle. Wooden boxes may be lined with corrugated fibre paper, felt, or similar substance, or provided with spring corner strips, to prevent breakage. Lined wicker baskets also may be used.
Collection of Samples

Bottles for bacteriological samples shall be sterilized as directed on page 98.

Interval Before Analysis.

In general, the shorter the time elapsing between the collection and the analysis of a sample the more reliable will be the analytical results. Under many conditions analyses made in the field are to be commended, as data so obtained are frequently preferable to data obtained in a distant laboratory after the composition of the water has changed.

The time that may be allowed to elapse between the collection of a sample and the beginning of its analysis cannot be stated definitely. It depends on the character of the sample, the examinations to be made, and other conditions. The following are suggested as fairly reasonable maximum limits.

Physical and chemical analysis.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Maximum Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground waters</td>
<td>72 hours</td>
</tr>
<tr>
<td>Fairly pure surface waters</td>
<td>48 hours</td>
</tr>
<tr>
<td>Polluted surface waters</td>
<td>12 hours</td>
</tr>
<tr>
<td>Sewage effluents</td>
<td>6 hours</td>
</tr>
<tr>
<td>Raw sewages</td>
<td>6 hours</td>
</tr>
</tbody>
</table>

Microscopical examination.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Maximum Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground waters</td>
<td>72 hours</td>
</tr>
<tr>
<td>Fairly pure surface waters</td>
<td>24 hours</td>
</tr>
<tr>
<td>Waters containing fragile organisms</td>
<td>Immediate examination</td>
</tr>
</tbody>
</table>

Bacteriological examination.

Samples kept at less than 10°C. 24 hours

If a longer period elapses between collection and examination the time should be noted. If sterilized by the addition of chloroform, formaldehyde, mercuric chloride, or some other germicide samples for sanitary chemical examination may be allowed to stand for longer periods than those indicated, but as this is a matter which will vary according to circumstances, no definite procedure is recommended. If unsterilized samples of sewage, sewage effluents, and highly polluted surface waters are analyzed after greater intervals than those suggested caution must be used
in interpreting analyses of the organic content, which frequently changes materially upon standing.

Determinations of dissolved gases, especially oxygen, hydrogen sulfide, and carbon dioxide, should be made at the time of collection in order to be reasonably accurate, in accordance with the directions given hereafter in connection with each determination.

REPRESENTATIVE SAMPLES.

Care should be taken to obtain a sample that is truly representative of the liquid to be analyzed. With sewages this is especially important because marked variations in composition occur from hour to hour. Satisfactory samples of some liquids can be obtained only by mixing together several portions collected at different times or at different places—the details as to collection and mixing depending upon local conditions.
PHYSICAL EXAMINATION.

TEMPERATURE.

The temperature of the sample, if taken, shall be taken at the time of collection, and shall be expressed preferably in degrees Centigrade, to the nearest degree, or closer if more precise data are required. The thermophone is recommended for obtaining the temperature of water at various depths below the surface.

TURBIDITY.

The turbidity of water is due to suspended matter, such as clay, silt, finely divided organic matter, microscopic organisms, and similar material.

TURBIDITY STANDARD.

The standard of turbidity shall be that adopted by the United States Geological Survey, namely, a water which contains 100 parts per million of silica in such a state of fineness that a bright platinum wire 1 millimeter in diameter can just be seen when the center of the wire is 100 millimeters below the surface of the water and the eye of the observer is 1.2 meters above the wire, the observation being made in the middle of the day, in the open air, but not in sunlight, and in a vessel so large that the sides do not shut out the light so as to influence the results. The turbidity of such water is arbitrarily fixed at 100 parts per million.

For preparation of the silica standard dry Pear's "precipitated fuller's earth" and sift it through a 200-mesh sieve. One gram of this preparation in 1 liter of distilled water makes a stock suspension which contains 1,000 parts per million of silica and which should have a turbidity of 1,000. Test this suspension, after diluting a portion of it with nine times its volume of distilled water, by the platinum-wire method to ascertain if the silica has the necessary degree of fineness and if the suspension has the necessary degree of turbidity. If not, correct by adding more silica or more water as the case demands.*

Standards for comparison shall be prepared from this stock suspension by dilution with distilled water. For turbidity readings

*This method of correction very slightly alters the coefficient of fineness of the standard, but does not noticeably affect its use.
below 20, standards of 0, 5, 10, 15, and 20 shall be kept in clear glass bottles of the same size as that containing the sample; for readings above 20, standards of 20, 30, 40, 50, 60, 70, 80, 90, and 100 shall be kept in 100 cc. Nessler tubes approximately 20 millimeters in diameter.

Comparison with the standards shall be made by viewing both standard and sample sidewise toward the light by looking at some object and noting the distinctness with which the margins of the object can be seen.

The standards shall be kept stoppered, and both sample and standards shall be thoroughly shaken before making the comparison.

In order to prevent any bacterial or algal growths from developing in the standards a small amount of mercury bichloride may be added to them.

**PLATINUM WIRE METHOD.**

This method requires a rod with a platinum wire 1 mm. in diameter inserted in it about 1 inch from one end of the rod and projecting from it at a right angle at least 25 mm. Near the other end of the rod, at a distance of 1.2 meters from the platinum wire, a small ring shall be placed directly above the wire through which, with his eye directly above the ring, the observer shall look when making the examination.

The rod shall be graduated as follows: The graduation mark of 100 shall be placed on the rod at a distance of 100 mm. from the center of the wire. Other graduations shall be made according to Table 1, which is based on the best obtainable data. The distances recorded in Table 1 are intended to be such that when the water is diluted the turbidity readings will decrease in the same proportion as the percentage of the original water in the mixture. These graduations are those on what is known as the U. S. Geological Survey Turbidity Rod of 1902.
Table 1.—Graduation of turbidity rod.

<table>
<thead>
<tr>
<th>Turbidity (parts per million)</th>
<th>Vanishing depth of wire (mm.)</th>
<th>Turbidity (parts per million)</th>
<th>Vanishing depth of wire (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1095</td>
<td>70</td>
<td>138</td>
</tr>
<tr>
<td>8</td>
<td>971</td>
<td>75</td>
<td>130</td>
</tr>
<tr>
<td>9</td>
<td>873</td>
<td>80</td>
<td>122</td>
</tr>
<tr>
<td>10</td>
<td>794</td>
<td>85</td>
<td>116</td>
</tr>
<tr>
<td>11</td>
<td>729</td>
<td>90</td>
<td>110</td>
</tr>
<tr>
<td>12</td>
<td>674</td>
<td>95</td>
<td>105</td>
</tr>
<tr>
<td>13</td>
<td>627</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>587</td>
<td>110</td>
<td>93</td>
</tr>
<tr>
<td>15</td>
<td>551</td>
<td>120</td>
<td>86</td>
</tr>
<tr>
<td>16</td>
<td>520</td>
<td>130</td>
<td>81</td>
</tr>
<tr>
<td>17</td>
<td>493</td>
<td>140</td>
<td>76</td>
</tr>
<tr>
<td>18</td>
<td>468</td>
<td>150</td>
<td>72</td>
</tr>
<tr>
<td>19</td>
<td>446</td>
<td>160</td>
<td>68.7</td>
</tr>
<tr>
<td>20</td>
<td>426</td>
<td>180</td>
<td>62.4</td>
</tr>
<tr>
<td>22</td>
<td>391</td>
<td>200</td>
<td>57.4</td>
</tr>
<tr>
<td>24</td>
<td>361</td>
<td>250</td>
<td>49.1</td>
</tr>
<tr>
<td>26</td>
<td>336</td>
<td>300</td>
<td>43.2</td>
</tr>
<tr>
<td>28</td>
<td>314</td>
<td>350</td>
<td>38.8</td>
</tr>
<tr>
<td>30</td>
<td>296</td>
<td>400</td>
<td>35.4</td>
</tr>
<tr>
<td>35</td>
<td>257</td>
<td>500</td>
<td>30.9</td>
</tr>
<tr>
<td>40</td>
<td>228</td>
<td>600</td>
<td>27.7</td>
</tr>
<tr>
<td>45</td>
<td>205</td>
<td>800</td>
<td>23.4</td>
</tr>
<tr>
<td>50</td>
<td>187</td>
<td>1000</td>
<td>20.9</td>
</tr>
<tr>
<td>55</td>
<td>171</td>
<td>1500</td>
<td>17.1</td>
</tr>
<tr>
<td>60</td>
<td>158</td>
<td>2000</td>
<td>14.8</td>
</tr>
<tr>
<td>65</td>
<td>147</td>
<td>3000</td>
<td>12.1</td>
</tr>
</tbody>
</table>

Procedure.—Lower the rod vertically into the water as far as the wire can be seen and read the level of the surface of the water on the graduated scale. This will indicate the turbidity.

The following precautions shall be taken to insure correct results:

Observations shall be made in the open air, preferably in the middle of the day and not in direct sunlight. The wire shall be kept bright and clean. If for any reason observations cannot be made directly under natural conditions a pail or tank may be filled with water and the observation taken in that, but if this is done care shall be taken that the water is thoroughly stirred before the observation is made, and no vessel shall be used for this purpose unless its diameter is at least twice as great as the depth to which the wire is immersed. Waters which have a turbidity greater than 500
shall be diluted with clear water before the observations are made, but if this is done the degree of dilution shall be reported.

TURBIDIMETRIC METHOD.

Several forms of turbidimeter or diaphanometer have been suggested for use. The simplest and most satisfactory form is the candle turbidimeter. This consists of a graduated glass tube with a flat polished bottom, enclosed in a metal case. This is supported over an English standard candle and so arranged that one may look vertically down through the tube at the flame of the candle. The observation is made by pouring the sample of water into the tube until the image of the flame of the candle just disappears from view. Care shall be taken not to allow soot or moisture to accumulate on the lower side of the glass bottom of the tube so as to interfere with the accuracy of the observations. The graduations on the tube correspond to turbidities produced in distilled water by certain numbers of parts per million of silica standard. In order to insure uniform results it is necessary to have the distance between the top rim of the candle and the bottom of the tube constant, and this distance shall be 7.6 cm. or 3 inches. The observations shall be made in a darkened room or with a black cloth over the head.

It is allowable to substitute for the candle an electric light. Calibrate the apparatus to correspond with the United States Geological Survey scale. The figures in Table 2 on page 8 are believed to be approximately correct for the candle turbidimeter but should be checked by the experimenter. It is allowable to calibrate the tube of the instrument with waters of known turbidity prepared by making a series of dilutions of the silica standard with distilled water. From the figures obtained in calibrating plot a curve from which the turbidity of a sample may be read when the depth of water in the tube has been obtained.
The results of turbidity observations shall be expressed in whole numbers which correspond to parts per million of silica and recorded as follows:

<table>
<thead>
<tr>
<th>Turbidity between 1 and 50 recorded to nearest unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot; &quot; 1 and 50 &quot; &quot; 100 &quot; &quot; 5</td>
</tr>
<tr>
<td>&quot; &quot; 101 &quot; &quot; 500 &quot; &quot; 10</td>
</tr>
<tr>
<td>&quot; &quot; 501 &quot; &quot; 1000 &quot; &quot; 50</td>
</tr>
<tr>
<td>&quot; &quot; 1001 &quot; greater &quot; &quot; 100</td>
</tr>
</tbody>
</table>

COEFFICIENT OF FINENESS.40

The quotient obtained by dividing the weight of suspended matter in the sample by the turbidity, both expressed in the same unit, shall be called the coefficient of fineness. If the quotient is greater than unity the matter in suspension is coarser and if it is less than unity it is finer than the standard.
COLOR

COLOR.

The "color," or the "true color," of water shall be considered the color that is due only to substances in solution; that is, it is the color of the water after the suspended matter has been removed. In stating results the word "color" shall mean the "true color" unless otherwise designated.

The "apparent color" shall be considered as including not only the true color but also any color produced by substances in suspension. It is the color of the original unfiltered sample.

The platinum-cobalt method of measuring color shall be considered as the standard, and the unit of color shall be that produced by 1 part per million of platinum.

COMPARISON WITH PLATINUM-COBALT STANDARDS.

Reagents.—Dissolve 1.246 grams of potassium platinic chloride (PtCl₄·2KCl), containing 0.5 gram platinum, and 1.00 gram crystallized cobalt chloride (CoCl₂·6H₂O), containing 0.25 gram of cobalt, in water with 100 cc. concentrated hydrochloric acid, and dilute to 1 liter with distilled water. This solution has a color of 500. Dilute this solution with distilled water in 50 cc. Nessler tubes to prepare standards having colors of 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, and 70. Keep these standards in Nessler tubes of such diameter that the graduation mark is between 20 and 25 cm. above the bottom and of such uniformity that they match within such limit that the distance from the bottom to the graduation mark of the longest tube shall not exceed that of the shortest tube by more than 6 mm. Protect the tubes from dust and light when not in use.

Procedure.—The color of a sample shall be observed by filling a standard Nessler tube to the height equal to that in the standard tubes with the sample and by comparing it with the standards. The observation shall be made by looking vertically downward through the tubes upon a white or mirrored surface placed at such angle that light is reflected upward through the column of liquid.

Water that has a color greater than 70 shall be diluted before making the comparison, in order that no difficulties may be encountered in matching the hues.

Water containing matter in suspension shall be filtered, before the color observation is made, until no visible turbidity remains.
COLOR

If the suspended matter is coarse, filter paper may be used for this purpose; if the suspended matter is fine, the use of a Berkefeld filter is recommended. The Pasteur filter shall not be used as it exerts a marked decolorizing action.

The apparent color, if determined, shall be determined on the original sample without filtration. The true and the apparent color of clear waters or waters with low turbidities are substantially the same.

The results of color determinations shall be expressed in whole numbers and recorded as follows:

<table>
<thead>
<tr>
<th>Color between</th>
<th>1 and 50</th>
<th>recorded to nearest unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;</td>
<td>51 &quot;</td>
<td>100 &quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>101 &quot;</td>
<td>250 &quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>251 &quot;</td>
<td>500 &quot;</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

COMPARISON WITH GLASS DISKS.  106

As the platinum-cobalt standard method is not well adapted for field work, the color of the water to be tested may be compared with that of glass disks held at the end of metallic tubes through which they are viewed by looking toward a white surface. The glass disks are individually calibrated to correspond with colors on the platinum scale. Experience has shown that the glass disks used by the U. S. Geological Survey give results in substantial agreement with those obtained by the platinum determinations, and their use is recognized as a standard procedure.

COMPARISON WITH NESSLER STANDARDS.

Inasmuch as the Nessler scale 62 and the natural water scale 27 49 which agrees with it except for colors less than 20, have been largely used in the past, the old results may be converted 117 into terms of the platinum standard by means of the ratios in Table 3, but they must not be considered as universally applicable as the variable sensitiveness of the Nessler solution introduces an uncertain factor.
Table 3.—VALUES FOR CONVERTING COLORS BY THE NATURAL WATER SCALE INTO COLORS BY THE PLATINUM STANDARD IN PARTS PER MILLION.*

<table>
<thead>
<tr>
<th>Modified Nessler or natural-water standard</th>
<th>0.00</th>
<th>0.01</th>
<th>0.02</th>
<th>0.03</th>
<th>0.04</th>
<th>0.05</th>
<th>0.06</th>
<th>0.07</th>
<th>0.08</th>
<th>0.09</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platinum-robalt standard color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.00</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>.10</td>
<td>18</td>
<td>19</td>
<td>20</td>
<td>21</td>
<td>23</td>
<td>24</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.20</td>
<td>26</td>
<td>27</td>
<td>28</td>
<td>30</td>
<td>30</td>
<td>31</td>
<td>32</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.30</td>
<td>33</td>
<td>34</td>
<td>35</td>
<td>35</td>
<td>36</td>
<td>37</td>
<td>37</td>
<td>38</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>.40</td>
<td>39</td>
<td>40</td>
<td>41</td>
<td>42</td>
<td>42</td>
<td>43</td>
<td>44</td>
<td>45</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>.50</td>
<td>46</td>
<td>47</td>
<td>48</td>
<td>49</td>
<td>50</td>
<td>50</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.60</td>
<td>52</td>
<td>53</td>
<td>54</td>
<td>55</td>
<td>55</td>
<td>56</td>
<td>57</td>
<td>57</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>.70</td>
<td>58</td>
<td>59</td>
<td>59</td>
<td>60</td>
<td>60</td>
<td>61</td>
<td>62</td>
<td>62</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>.80</td>
<td>63</td>
<td>64</td>
<td>65</td>
<td>66</td>
<td>67</td>
<td>68</td>
<td>69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.90</td>
<td>70</td>
<td>71</td>
<td>72</td>
<td>73</td>
<td>74</td>
<td>75</td>
<td>77</td>
<td>78</td>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td>1.00</td>
<td>81</td>
<td>82</td>
<td>83</td>
<td>84</td>
<td>85</td>
<td>86</td>
<td>87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.10</td>
<td>88</td>
<td>89</td>
<td>90</td>
<td>91</td>
<td>92</td>
<td>93</td>
<td>94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.20</td>
<td>95</td>
<td>96</td>
<td>96</td>
<td>97</td>
<td>98</td>
<td>98</td>
<td>99</td>
<td>100</td>
<td>101</td>
<td>101</td>
</tr>
<tr>
<td>1.30</td>
<td>102</td>
<td>103</td>
<td>103</td>
<td>104</td>
<td>105</td>
<td>105</td>
<td>106</td>
<td>107</td>
<td>108</td>
<td>108</td>
</tr>
<tr>
<td>1.40</td>
<td>109</td>
<td>110</td>
<td>110</td>
<td>111</td>
<td>112</td>
<td>112</td>
<td>113</td>
<td>114</td>
<td>115</td>
<td>115</td>
</tr>
<tr>
<td>1.50</td>
<td>116</td>
<td>117</td>
<td>117</td>
<td>118</td>
<td>119</td>
<td>120</td>
<td>120</td>
<td>121</td>
<td>121</td>
<td>121</td>
</tr>
<tr>
<td>1.60</td>
<td>122</td>
<td>123</td>
<td>123</td>
<td>124</td>
<td>125</td>
<td>125</td>
<td>126</td>
<td>127</td>
<td>128</td>
<td>128</td>
</tr>
<tr>
<td>1.70</td>
<td>129</td>
<td>130</td>
<td>130</td>
<td>131</td>
<td>132</td>
<td>132</td>
<td>133</td>
<td>134</td>
<td>135</td>
<td>136</td>
</tr>
<tr>
<td>1.80</td>
<td>136</td>
<td>137</td>
<td>137</td>
<td>138</td>
<td>139</td>
<td>139</td>
<td>140</td>
<td>141</td>
<td>142</td>
<td>142</td>
</tr>
<tr>
<td>1.90</td>
<td>143</td>
<td>144</td>
<td>144</td>
<td>145</td>
<td>146</td>
<td>146</td>
<td>147</td>
<td>148</td>
<td>149</td>
<td>149</td>
</tr>
<tr>
<td>2.00</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Zero on the true Nessler scale is about 15 on the platinum scale.

LOVIBOND TINTOMETER.

The value of the readings of tint and shade by the Lovibond tintometer has not been commensurate with the labor involved, but it is necessary to make a record of the reflected tint and shade of some waters. The standard color disks used in teaching optics may be used for the purpose.

Procedure.—The white disk supports three movable standard color sectors, red, yellow, and blue, and one movable black sector. All are mounted on a device which can be revolved rapidly, blending the colors into a uniform tint or shade. A scale around the circumference of the disk is used to indicate the percentage of each color or white or black in the blend.

Place the sample in a battery jar on a white ground; adjust the sectors so that when blended the tint or shade will match the reflected tint or shade of the sample. Report the percentages of red, yellow, blue, white, and black in the blended tint or shade.
The observation of the odor, cold and hot, of samples of surface water is important as the odors are usually indicative of organic growths or sewage contamination or both. The odor of some ground waters is caused by the earthy constituents of the water-bearing strata. The odor of a contaminated well water is often contributory evidence of its pollution. A study of the organisms as directed under Microscopical Examination (p. 90) is a valuable adjunct to physical and chemical examination of water. Certain odors distinguish or identify certain organisms, as, for example, the "fishy" odor of *Uroglena*, the "aromatic" or "rose geranium" odor of *Asterionella* and the "pig pen" odor of *Anabaena*. Observe and record the odor, both at room temperature and at just below the boiling-point, as follows:

**COLD ODOR.**

Shake the sample violently in one of the collecting bottles, when it is half to two-thirds full and when the sample is at room temperature (about 20° C.). Remove the stopper and smell the odor at the mouth of the bottle.

**HOT ODOR.**

Pour about 150 cc. of the sample into a 500 cc. Erlenmeyer flask. Cover the flask with a well-fitting watch glass. Heat the water almost to boiling on a hot plate. Remove the flask from the plate and allow it to cool not more than five minutes. Then agitate it with a rotary movement, slip the watch glass to one side, and smell the odor.

**EXPRESSION OF RESULTS.**

Express the quality of the odor by a descriptive epithet like the following, which may be abbreviated in the record:

- a—aromatic
- C—free chlorine
- d—disagreeable
- e—earthy
- f—fishy
- g—grassy
- m—moldy
- M—musty
- p—peaty
- s—sweetish
- S—hydrogen sulfide
- v—vegetable.

Express the intensity of the odor by a numeral prefixed to the term expressing quality, which may be defined as follows:
<table>
<thead>
<tr>
<th>Numerical value</th>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>No odor perceptible.</td>
</tr>
<tr>
<td>1</td>
<td>Very faint</td>
<td>An odor that would not be detected ordinarily by the average consumer, but that could be detected in the laboratory by an experienced observer.</td>
</tr>
<tr>
<td>2</td>
<td>Faint</td>
<td>An odor that the consumer might detect if his attention were called to it, but that would not attract attention otherwise.</td>
</tr>
<tr>
<td>3</td>
<td>Distinct</td>
<td>An odor that would be detected readily and that might cause the water to be regarded with disfavor.</td>
</tr>
<tr>
<td>4</td>
<td>Decided</td>
<td>An odor that would force itself upon the attention and that might make the water unpalatable.</td>
</tr>
<tr>
<td>5</td>
<td>Very strong</td>
<td>An odor of such intensity that the water would be absolutely unfit to drink. (A term to be used only in extreme cases.)</td>
</tr>
</tbody>
</table>
CHEMICAL EXAMINATION.

EXPRESSION OF RESULTS.

The results of chemical analyses shall be expressed in parts per million, which in most analyses is practically equivalent to milligrams per liter. In some laboratories other forms of expression have been used. Results expressed in parts per 100,000 or in grains per gallon may be transformed to parts per million, or conversely, by the use of the following table:

Table 4.—FACTORS FOR TRANSFORMING RESULTS OF ANALYSES.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Equivalent.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grains per U. S. gallon.</td>
</tr>
<tr>
<td>1 grain per U. S. gallon.</td>
<td>1.000</td>
</tr>
<tr>
<td>1 grain per Imperial gallon.</td>
<td>.835</td>
</tr>
<tr>
<td>1 part per 100,000</td>
<td>.585</td>
</tr>
<tr>
<td>1 part per million</td>
<td>.058</td>
</tr>
</tbody>
</table>

The following general rules shall govern the use of significant figures in the expression of results:

1. If the results show quantities greater than 10 parts per million use no decimals; record only whole numbers. If the quantities reach hundreds and thousands of parts record only two significant figures.

2. If the results are between 1 and 10 parts do not retain more than one decimal place.

3. If the results are between 0.1 and 1 part do not retain more than two decimal places.

4. Estimates of ammonia, albuminoid, and nitrite nitrogen alone justify the use of three decimals.

5. If the results of analyses are tabulated ciphers should not be added at the right of the decimal point to make the column uniform.
AMMONIA NITROGEN

FORMS OF NITROGEN.

Nitrogenous organic matter passes through several intermediate compounds during its natural decomposition, and that which does not gasify ultimately forms nitrate. Nitrogen in organic matter is determined by the Kjeldahl process. An indication of the amount present is obtained by the albuminoid nitrogen determination. It has not been found possible to differentiate the nitrogen in the organic matter that readily decomposes from that in stable or non-putrescible compounds. Decomposition of organic matter produces nitrogen combined in ammonia, which is the first step between nitrogenous organic matter and the completely mineralized nitrate. Ammonia nitrogen may be determined by distillation and Nesslerization or by direct Nesslerization of the clarified sample. The next step is oxidation to nitrite, and the final step, oxidation to nitrate. It is recommended that all forms of nitrogen be reported as the element nitrogen (N).

AMMONIA NITROGEN.

There are two methods for estimating ammonia nitrogen—distillation and direct Nesslerization. Distillation is recommended for most waters and direct Nesslerization is recommended for sewages, sewage effluents, and highly polluted surface waters.

DETERMINATION BY Distillation.

Procedure.—Use a metal or a glass flask connected with a condenser so that the distillate may drop from the condenser tube directly into a Nessler tube or a flask. Free the apparatus from ammonia by boiling distilled water in it until the distillate shows no trace of ammonia. After this has been done empty the distilling flask and measure into it 500 cc. of the sample, or a smaller portion diluted to 500 cc. with ammonia-free water. If the sample is acid or if the presence of urea is suspected add about 0.5 gram of sodium carbonate before distillation. Omit this if possible as it tends to increase "bumping." Apply heat so that the distillation may proceed at the rate of not more than 10 cc. nor less than 6 cc. per minute. Collect the distillate in four Nessler tubes, 50 cc. to each tube, or if the nitrogen is high in a 200 cc. graduated flask. These receptacles contain the ammonia nitrogen to be measured as hereafter described.
AMMONIA NITROGEN

Use Nessler tubes of such diameter that the graduation mark is between 20 and 25 cm. above the bottom and of such uniformity of diameter that the distance from the bottom to the graduation mark of the longest tube shall not exceed that of the shortest tube by more than 6 mm. The tubes must be of clear white glass with polished bottoms.

MEASUREMENT OF AMMONIA NITROGEN.

The amount of ammonia in the distillates may be measured either by (1) comparison of the Nesslerized distillates with Nesslerized solutions containing known quantities of nitrogen as ammonium chloride, or by (2) comparison of the Nesslerized distillates with permanent standard solutions in which the colors of Nesslerized standard ammonia solutions are duplicated by solutions of platinum and cobalt chlorides.

COMPARISON WITH AMMONIA STANDARDS.

Reagents.—1. Ammonia-free water.
2. Standard ammonium chloride solution. Dissolve 3.82 grams of ammonium chloride in ammonia-free water and dilute to 1 liter; dilute 10 cc. of this to 1 liter with ammonia-free water. One cc. equals 0.00001 gram of nitrogen.
3. Nessler reagent. Dissolve 50 grams of potassium iodide in a minimum quantity of cold water. Add a saturated solution of mercuric chloride until a slight precipitate persists permanently. Add 400 cc. of 50 per cent solution of potassium hydroxide, made by dissolving the potassium hydroxide and allowing it to clarify by sedimentation before using. Dilute to 1 liter, allow to settle, and decant. This solution should give the required color with ammonia within five minutes after addition and should not produce a precipitate with small amounts of ammonia within two hours.

Procedure.—Prepare a series of 16 Nessler tubes containing the following amounts of the standard ammonium chloride solution, diluted to 50 cc. with ammonia-free water, namely: 0.0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.4, 1.7, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 6.0 cc. These solutions will contain 0.00001 gram of nitrogen for each cubic centimeter of the standard solution.

Nesslerize the standards and the distillates by adding approximately 1 cc. of Nessler reagent to each tube. Do not stir the contents of the tubes. The temperature of the tubes should be
practically the same as that of the standards; otherwise the colors
will not be directly comparable. Allow the tubes to stand at least
10 minutes after Nesslerizing. Compare the color produced in the
tubes with that in the standards by looking vertically downward
through them at a white or mirrored surface placed at an angle in
front of a window so as to reflect the light upward.

If the color obtained by Nesslerizing the distillates is greater than
that of the darkest tube of the standards, mix the contents of the
tube thoroughly, pour out half of the liquid, and dilute the remainder
to the original volume with ammonia-free water; then make the
color comparison and multiply the result by two. If the color is
still too dark after pouring out half the liquid, repeat this process
of division until a reading can be made. The process of dilution
may be shortened by mixing together the distillates from one sample
before making the comparison and comparing an aliquot portion
with the standards.

After the readings have been recorded add the results obtained
by Nesslerizing each portion of the entire distillate. If 500 cc.
of the sample is distilled this sum, expressed in cubic centimeters
and multiplied by 0.02, will give the number of parts per million
of ammonia nitrogen in the sample. If x cc. of sample is used
multiply the sum of the readings by $\frac{10}{x}$.

If the ammonia is known to be high the distillate may be collected
in 200 cc. flasks and an aliquot part Nesslerized.

Comparison with permanent standards.

Reagents.—Platinum solution. Dissolve 2.00 grams of potassium
platinic chloride (PtCl₄.2KCl) in a small amount of distilled water,
add 100 cc. of strong hydrochloric acid, and dilute to 1 liter.

Cobalt solution. Dissolve 12 grams of cobaltous chloride
(CoCl₂.6H₂O) in distilled water, add 100 cc. of strong hydrochloric
acid, and dilute to 1 liter.

Prepare standards by putting various amounts of these two solu-
tions into Nessler tubes and diluting to the 50 cc. mark with distilled
water as indicated in Table 5. These standards may be kept for
several months if protected from dust.
### Table 5.—Preparation of permanent standards for the determination of ammonia.

<table>
<thead>
<tr>
<th>Value in standard ammonium chloride.</th>
<th>Solution of platinum.</th>
<th>Solution of cobalt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>0.0</td>
<td>1.2</td>
<td>0.0</td>
</tr>
<tr>
<td>.1</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>.2</td>
<td>2.8</td>
<td>0.0</td>
</tr>
<tr>
<td>.4</td>
<td>4.7</td>
<td>1.0</td>
</tr>
<tr>
<td>.7</td>
<td>5.9</td>
<td>2.0</td>
</tr>
<tr>
<td>1.0</td>
<td>7.7</td>
<td>5.0</td>
</tr>
<tr>
<td>1.4</td>
<td>9.9</td>
<td>1.1</td>
</tr>
<tr>
<td>1.7</td>
<td>11.4</td>
<td>1.7</td>
</tr>
<tr>
<td>2.0</td>
<td>12.7</td>
<td>2.2</td>
</tr>
<tr>
<td>2.5</td>
<td>15.0</td>
<td>3.3</td>
</tr>
<tr>
<td>3.0</td>
<td>17.3</td>
<td>4.5</td>
</tr>
<tr>
<td>3.5</td>
<td>19.0</td>
<td>5.7</td>
</tr>
<tr>
<td>4.0</td>
<td>19.7</td>
<td>7.1</td>
</tr>
<tr>
<td>4.5</td>
<td>19.9</td>
<td>8.7</td>
</tr>
<tr>
<td>5.0</td>
<td>20.0</td>
<td>10.4</td>
</tr>
<tr>
<td>6.0</td>
<td>20.0</td>
<td>15.0</td>
</tr>
<tr>
<td>7.0</td>
<td>20.0</td>
<td>22.0</td>
</tr>
</tbody>
</table>

The amounts in Table 5 are approximate, and the actual amount necessary will differ with the character of the Nessler solution, the color sensitiveness of the analyst’s eye, and other conditions. The final test of the standard is best obtained by comparing it with Nesslerized standards and modifying the tint accordingly. Such comparison should be made for each new batch of Nessler solution and should be checked by each analyst.

**Procedure.**—In comparison with permanent standards, Nesslerize the distillates in the manner above described and compare the resulting colors at the end of about 10 minutes with the permanent standards. The method of calculating results is precisely the same as with the ammonia standards.

**Modification for sewage.**

Ammonia nitrogen and albuminoid nitrogen in sewages, soils, and other materials of high nitrogen content may be satisfactorily determined by diluting the sample with ammonia-free distilled water and proceeding as described in the preceding sections, but it is permissible to distil with steam.\(^4\)}
Ammonia Nitrogen

Procedure.—Use a 200 cc. long-necked Kjeldahl flask connected with a condenser so that the distillate may drop from the condenser tube directly into a Nessler tube or a flask. Connect the Kjeldahl flask with a steam generator by a tube reaching almost to the bottom of the flask.

After the apparatus is freed from ammonia put the sample to be tested into the flask. Use 10 to 100 cc. of the sample according to its ammonia content. Pass ammonia-free steam through the liquid in the Kjeldahl flask and collect the distillate in the usual way. It is usually convenient to collect the distillate in a 200 cc. flask and to take an aliquot part of it for Nesslerization. Compare with standards and calculate the nitrogen content in the usual manner.

This method has the advantage, when the sample is treated with an alkaline solution of potassium permanganate, of avoiding bumping, permitting the assay of solid matter, and yielding the ammonia more rapidly than by the ordinary process of distillation.

Determination by Direct Nesslerization.

Reagents.—1. Ten per cent solution of copper sulfate (CuSO₄. 5H₂O).

2. Ten per cent solution of lead acetate (Pb(C₂H₃O₂)₂.

3. Fifty per cent solution of sodium hydroxide (NaOH) or potassium hydroxide (KOH).

Procedure.—To 50 cc. of the sample to be tested, diluted if necessary with an equal volume of ammonia-free water, in a short tube, add a few drops of the copper sulfate solution. After thoroughly mixing, add 1 cc. of the alkali hydroxide solution and again thoroughly mix. Allow the tube to stand for a few minutes, when a heavy precipitate should fall to the bottom, leaving a colorless supernatant liquid. Nesslerize an aliquot part. Compare with standards and compute the ammonia nitrogen in the same manner as in the distillation procedure.

Samples containing hydrogen sulfide may require the use of lead acetate in addition to the copper sulfate. Some samples may require a few trials before the right combination of the three solutions to bring about the best results can be found.

Instead of adding copper sulfate to sewages of high magnesium content satisfactory clarification of the sample can be obtained by mixing it with the alkali hydroxide alone.
ALBUMINOID NITROGEN.

The addition of an alkaline permanganate solution to liquids containing nitrogenous organic matter causes the formation of ammonia, which can be distilled and determined by Nesslerization of the distillate. The nitrogen of the ammonia, thus obtained, is called albuminoid nitrogen. As the ratio of nitrogenous organic matter to the ammonia obtained by distillation is decidedly variable in sewages and other substances containing much nitrogenous organic matter albuminoid nitrogen results on such substances are less accurate than organic (Kjeldahl) nitrogen. Therefore in sewage work, including analysis of influents and effluents of purification plants and the water of highly polluted streams, it is recommended that determinations of organic nitrogen be substituted for determinations of albuminoid nitrogen. For ground waters and surface waters containing but little pollution, the albuminoid nitrogen is approximately one-half the organic nitrogen; accordingly the continuance of albuminoid nitrogen determinations for this class of work is approved.

Reagents.—Alkaline potassium permanganate. Pour 1,200 cc. of distilled water into a porcelain dish holding 2,500 cc., boil 10 minutes, and turn off the gas. Add 16 grams of C. P. potassium permanganate and stir until solution is complete. Then add 800 cc. of 50 per cent clarified solution of potassium hydroxide or an equivalent amount of sodium hydroxide and enough distilled water to fill the dish. Boil down to 2,000 cc. Test this solution for ammonia by making a blank determination. Correct determinations by the amount of this blank.

Procedure.—After the collection of the distillate for ammonia nitrogen described on page 15 add 50 cc. (or more if necessary to insure the complete oxidation of the organic matter) of alkaline potassium permanganate and continue the distillation until at least four portions, and preferably five portions, of 50 cc. each, of distillate have been collected in separate tubes. Determine the albuminoid nitrogen in the distillate by Nesslerization. If the albuminoid nitrogen is known to be high it is convenient to collect the distillate in a 200 cc. flask and to Nesslerize an aliquot part of it.

Dissolved albuminoid nitrogen may be determined in a sample from which suspended matter has been removed by filtration either through filter paper or through a Berkefeld filter. Suspended
albuminoid nitrogen is the difference between the total and the dissolved albuminoid nitrogen.

**ORGANIC NITROGEN.**

*Procedure for water.*—Boil 500 cc. of the sample in a round-bottomed flask to remove ammonia nitrogen. This usually causes the loss of 200 cc. of the sample, which may be collected for the determination of ammonia nitrogen. Add 5 cc. of nitrogen-free concentrated sulfuric acid and a small piece of ignited pumice. Mix by shaking and place over a flame under a hood. Digest until copious fumes of sulfuric acid are given off and the liquid finally becomes colorless or pale straw color. Remove from the flame, and add potassium permanganate crystals in small portions until a heavy green precipitate persists in the liquid. Cool. Dilute to about 300 cc. with ammonia-free water. Make alkaline with 10 per cent ammonia-free sodium hydroxide. Distill the ammonia, collect the distillate in Nessler tubes, Nesslerize, and compare with standards as described (pp. 16-18).

*First procedure for sewage.*—Distill the ammonia nitrogen directly from 100 cc. or less of the sample, diluted to 500 cc. with nitrogen-free water. Collect the distillate and determine the ammonia nitrogen in it. Add 5 cc. of nitrogen-free sulfuric acid and 1 cc. of 10 per cent nitrogen-free copper sulfate, and digest the liquid for half an hour after it has become colorless or pale straw color. Add 0.5 gram of potassium permanganate crystals to the hot acid solution, and dilute to 500 cc. with ammonia-free water. Dilute 10 cc. or more of this liquid, in a Kjeldahl distilling flask, to about 300 cc. with ammonia-free water. Make alkaline with 10 per cent sodium hydroxide, distill, and Nesslerize. With some samples direct Nesslerization may be used. (See p. 19.)

In this determination care must be taken to digest thoroughly, to add potassium permanganate to the point of precipitation, to sample carefully after dilution, and to add enough sodium hydroxide to insure the separation of the ammonia from the precipitated manganese hydroxide. Potassium permanganate should not be added during digestion because it causes loss of nitrogen.

*Second procedure for sewage.*—Omit the separation of ammonia nitrogen and determine the ammonia nitrogen and organic nitrogen together. Determine the ammonia nitrogen in a separate sample
by direct Nesslerization as described on page 19. The organic nitrogen is equal to the difference.

**Reagents.**—1. Sulfanilic acid solution. Dissolve 8.00 grams of the purest sulfanilic acid in 1,000 cc. of 5 N acetic acid (sp. gr. 1.041) or in 1,000 cc. of water containing 50 cc. of concentrated hydrochloric acid. This is practically a saturated solution.

2. \(\alpha\)-naphthylamine acetate or chloride solution. Dissolve 5.00 grams solid \(\alpha\)-naphthylamine in 1,000 cc. of 5 N acetic acid or in 1,000 cc. of water containing 8 cc. of concentrated hydrochloric acid. Filter the solution through washed absorbent cotton or an aluminium filter.

3. Sodium nitrite stock solution. Dissolve 1.1 gram silver nitrite in nitrite-free water; precipitate the silver with sodium chloride solution and dilute the whole to 1 liter.

4. Standard sodium nitrite solution. Dilute 100 cc. of solution 3 to 1 liter, then dilute 50 cc. of this solution to 1 liter with sterilized nitrite-free water, add 1 cc. of chloroform, and preserve in a sterilized bottle. One cc. = 0.0005 mg. nitrogen.

5. Fuchsin solution. 0.1 gram per liter.

**Procedure.**—Place in a standard Nessler tube 50 cc. of the sample, decolorized if necessary with nitrite-free aluminium hydroxide (see p. 42) or a smaller amount diluted to 50 cc. At the same time prepare in Nessler tubes a set of standards, by diluting to 50 cc. with nitrite-free water, various amounts of the standard nitrite solution. The following amounts of standard solution are suggested: 0.0, 0.1, 0.2, 0.4, 0.7, 1.0, 1.4, 1.7, 2.0, and 2.5 cc. Add 1 cc. of the sulfanilic acid solution and 1 cc. of the \(\alpha\)-naphthylamine acetate or hydrochloride solution to the sample and to each standard. Mix thoroughly and allow to stand 10 minutes; then compare the sample with the standards. Do not allow the sample to stand more than one-half hour before making the comparison. If the color of the sample is deeper than that of the highest standard repeat the test on a diluted sample. If 50 cc. of the sample is used 0.01 times the number of cc. of the standard matched equals parts per million of nitrite nitrogen. Satisfactory results can be obtained by using either hydrochloric or acetic acid in preparing the test solutions, but the speed of the reaction is more rapid if acetic acid is used.\(^{12}\)
NITRATE NITROGEN

Permanent standards may be prepared by matching the nitrite standards with dilutions of the fuchsiné solution. Fuchsiné standards have been found to be sufficiently accurate for waters high in nitrite and for sewage. The standards should be checked once a month and kept out of bright sunlight.

Two methods are recommended for the determination of nitrate nitrogen in water, sewage, and sewage effluents.

PHENOLDISULFONIC ACID METHOD.

Reagents.—1. Phenoldisulfonic acid. Dissolve 25 grams of pure white phenol in 150 cc. of pure concentrated sulfuric acid. Add 75 cc. of fuming sulfuric acid (15 per cent SO₃), stir well, and heat for 2 hours at about 100°C.

2. Potassium hydroxide solution. Prepare an approximately 12 N solution, 10 cc. of which will neutralize about 4 cc. of the phenoldisulfonic acid.

3. Standard nitrate solution. Dissolve 0.72 gram of pure recrystallized potassium nitrate in 1 liter of distilled water. Evaporate cautiously to dryness 10 cc. of the solution on the water bath. Moisten residue quickly and thoroughly with 2 cc. of phenoldisulfonic acid and dilute to 1 liter. This is the standard solution, 1 cc. of which equals 0.001 mg. of nitrate nitrogen.

4. Standard silver sulfate solution. Dissolve 4.4 grams of silver sulfate free from nitrate in 1 liter of water. One cc. of this solution is equal to 1 mg. of chloride.

Procedure.—The alkalinity, chloride, and nitrite content, and color of the sample must first be determined. If the sample is highly colored decolorize it with freshly precipitated aluminium hydroxide. Measure into an evaporating dish 100 cc. of the sample, or if nitrate is very high such volume as will contain about 0.01 mg. of nitrate nitrogen. Add sufficient N/50 sulfuric acid nearly to neutralize the alkalinity. Then add sufficient standard silver sulfate to precipitate all but about 0.1 mg. of chloride. The removal of chloride may be omitted if the sample contains less than 30 parts per million of chloride. Heat the mixture to boiling, add a little aluminium hydroxide, stir, filter, and wash with small amounts of hot water. Evaporate the filtrate to dryness, and add 2 cc. of the phenoldisulfonic acid, rubbing with a glass rod to insure intimate
NITRATE NITROGEN

contact. If the residue becomes packed or appears vitreous because of the presence of much iron, heat the dish on the water bath for a few minutes. Dilute the mixture with distilled water, and add slowly a strong solution of potassium hydroxide or ammonium hydroxide until the maximum color is developed. Transfer the solution to a Nessler tube, filtering if necessary. If nitrate is present a yellow color will be formed. Compare the color with that of standards made by adding 2 cc. of strong potassium hydroxide or ammonium hydroxide to various amounts of standard nitrate solution and diluting them to 50 cc. in Nessler tubes. The following amounts of standard nitrate solution are suggested: 0, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0, and 40.0 cc. These standards may be kept several weeks without deterioration. If 100 cc. of water is used the number of cubic centimeters of the standard multiplied by 0.01 is equal to parts per million of nitrate nitrogen.

Standards that will remain permanent for several years if stored in the dark may be prepared from tripotassium nitrophenoldisulfonate.

If nitrite nitrogen is present in excess of 1 part per million it should be oxidized by heating the samples a few minutes with a few drops of hydrogen peroxide free from nitrate repeatedly added or by adding dilute potassium permanganate in the cold until a faint pink coloration appears; the nitrogen equivalent of the nitrite thus oxidized to nitrate is then subtracted from the final nitrate nitrogen reading.

REDUCTION METHOD

Reagents.—1. Sodium or potassium hydroxide solution. Dissolve 250 grams of the hydroxide in 1.25 liters of distilled water. Add several strips of aluminium foil and allow the evolution of hydrogen to continue over night. Concentrate the solution to 1 liter by boiling.

2. Aluminium foil. Use strips of pure aluminium about 10 cm. long, 6 mm. wide, and 0.33 mm. thick and weighing about 0.5 gram.

Procedure.—To 100 cc. of the sample in a 300 cc. casserole add 2 cc. of the hydroxide solution and concentrate by boiling to about 20 cc. Pour the contents of the casserole into a test tube about 16 cm. long and 3 cm. in diameter, or of approximately 100 cc. capacity. Rinse the casserole several times with nitrogen-free water and add the rinse water to the liquid already in the tube,
thus making the contents of the tube approximately 75 cc. Add a strip of aluminium foil. Close the tube by means of a rubber stopper through which passes a bent glass tube about 5 mm. in diameter. Put the shorter arm of the tube flush with the lower side of the rubber stopper and let the longer arm extend below the surface of distilled water in another test tube. This apparatus serves as a trap through which the evolved hydrogen escapes freely. The small amount of ammonia escaping into the trap may be neglected. Allow the action to proceed for a minimum period of four hours or over night. Pour the contents of the tube into a distilling flask, dilute with 250 cc. of ammonia-free water, distil, collect the distillate in Nessler tubes, and Nesslerize. If the nitrate content is high collect the distillate in a 200 cc. flask and Nesslerize an aliquot part. If the supernatant liquid in the reduction tube is clear and colorless the solution may be diluted to a definite volume and an aliquot part Nesslerized without distillation.

TOTAL NITROGEN. 10

In sewage work it is frequently of assistance to know the total nitrogen content. This is ordinarily computed by adding together the organic, ammonia, nitrite, and nitrate nitrogen, each of which is determined as already described.

OXYGEN CONSUMED. 24 07 04 85 06 101 102

Oxygen consumed means the oxygen that the oxidizable compounds of sewage and water consume when treated in an acid solution with potassium permanganate. The expression is synonymous with oxygen required, oxygen absorbed, and oxygen-consuming capacity. It should not be confused with biochemical oxygen demand.

As the carbon, not the nitrogen, in organic matter is oxidized by potassium permanganate, oxygen consumed is considered by some an indication of the amount of carbonaceous organic matter present. The determination indicates, however, only part of the carbon, the proportion varying in different samples because the carbon in nitrogenous matter is not so readily oxidized as that in carbonaceous organic matter. Furthermore, it does not directly differentiate the carbon present in unstable organic matter from that in fairly stable organic matter, such as is sometimes referred
to as residual humus matter. As nitrite nitrogen, ferrous iron, sulfide, and other oxidizable mineral substances reduce potassium permanganate, corrections for them should be made in the determination.

RECOMMENDED METHOD.

Reagents.—1. Dilute sulfuric acid. Dilute 1 part of concentrated sulfuric acid with 3 parts of distilled water and free the solution from oxidizable matter by adding potassium permanganate until a faint pink color persists after the solution has stood several hours.

2. Standard ammonium oxalate. Dissolve 0.888 gram of the pure salt in 1 liter of distilled water. One cc. is equivalent to 0.1 mg. of oxygen. An equivalent quantity of oxalic acid or sodium oxalate may be used.

3. Standard potassium permanganate. Dissolve 0.4 gram of the crystallized salt in 1 liter of distilled water. Add 10 cc. of the dilute sulfuric acid and 10 cc. of this solution of potassium permanganate to 100 cc. of distilled water, and digest 30 minutes. Add 10 cc. of the ammonium oxalate solution, and then add potassium permanganate till a pink coloration appears. This destroys the oxygen-consuming capacity of the water used. Now add another 10 cc. of ammonium oxalate solution and titrate with potassium permanganate. Adjust the potassium permanganate solution so that 1 cc. is equivalent to 1 cc. of ammonium oxalate solution or 0.1 mg. of available oxygen.

Acid digestion.—Place in a flask 100 cc. of the water, or, if the water is of high organic content, a smaller portion diluted to 100 cc. Add 10 cc. of sulfuric acid solution and 10 cc. of standard potassium permanganate and digest the liquid exactly 30 minutes in a bath of boiling water the level of which is kept above the level of the contents of the flask. If the quantity of permanganate is insufficient for complete oxidation repeat the digestion with a larger quantity; at least 5 cc. excess of the standard permanganate should be present when the ammonium oxalate solution is added. Remove the flask, add 10 cc. of the ammonium oxalate solution, and titrate with the standard permanganate until a faint but distinct color is obtained. If 100 cc. of water is used the number of cubic centimeters of potassium permanganate solution in excess of the
number of cubic centimeters of ammonium oxalate solution is equal to parts per million of oxygen consumed.

If oxidizable mineral substances, such as ferrous iron, sulfide, or nitrite, are present in the sample corrections should be applied as accurately as possible by suitable procedures. Direct titration of the acidified sample in the cold, using a three-minute period of digestion, serves this purpose quite well for polluted surface waters and fairly well for purified sewage effluents. Few raw sewages containing no trade wastes need such a correction, but raw sewages containing "pickling" liquors do need it. If the sample contains both oxidizable mineral compounds and gaseous organic substances the latter should be driven off by heat and the sample allowed to cool before applying this test for the correction factor. If such corrections are made the fact should be stated with the amount of correction.

Period and temperature of digestion.—As the practice in regard to the period and temperature of digestion has varied widely it is difficult to compare the results obtained at one laboratory with those obtained at another. None of the methods gives absolute results. They are all relative at best. Digesting 30 minutes at the boiling temperature is herein designated the recommended method. If samples are analyzed by any other method the method should be noted, and, representative results by the standard method should be placed on record for purposes of comparison.

OTHER METHODS.

Additional reagents.—1. Potassium iodide solution. Ten per cent solution, free from iodate.

2. Standard sodium thiosulfate. Dissolve 1.0 gram of the pure crystallized salt in 1 liter of distilled water. Standardize this solution against the standard potassium permanganate. As the thiosulfate solution does not keep well determine its actual strength at frequent intervals.

3. Starch indicator. Prepare as directed in the section on dissolved oxygen (pp. 65–66).

4. Sodium hydroxide solution. Dissolve 1 part of pure sodium hydroxide in 2 parts of distilled water.

Certain widely practiced deviations from the standard procedure just described are noted in the following paragraphs.

1. Heat the acidified sample to boiling, add the permanganate
solution, and digest for two minutes at boiling temperature. This procedure is facilitated by agitating the liquid constantly with a small current of air to guard against bumping.

2. Same method as No. 1 except that the period of digestion is five minutes.\textsuperscript{121a}

3. Same method as No. 2 except that the permanganate solution is added to the acidified sample when cold, and digestion is continued five minutes after the sample reaches the boiling point. The advantage of this method is that there is included the oxygen-consuming power of the volatile matter present in some sewages and sewage effluents, which is driven off by heat and thus escapes when the test is made in accordance with procedures 1 and 2.

4. Same method as No. 3 except that the period of digestion is 10 minutes.\textsuperscript{63 59a}

5. Digestion of the sample after the acid and permanganate solutions are added is carried out abroad, especially in England, at approximately the room temperature,\textsuperscript{34a 59a 94f 100a} apparently to guard against decomposition\textsuperscript{17} of permanganate in the presence of high chloride, for periods of three minutes, fifteen minutes, and four hours; many observers record the oxygen consumed after all three periods, while some record the result only for the four-hour period. At the end of the period of digestion, add 0.5 cc. of potassium iodide solution to discharge the pink color; mix; titrate the liberated iodine with thiosulfate until the yellow color is nearly destroyed, then add a few drops of starch solution and continue titration until the blue color is just discharged. The number of cubic centimeters of potassium permanganate solution in excess of the number of cubic centimeters of sodium thiosulfate solution is equal to parts per million of oxygen consumed.

6. Digestion in alkaline solution\textsuperscript{104} is preferable to digestion in acid solution for brines or waters high in chlorine. Place in a flask 100 cc. of the sample, or if it is of high organic content a smaller portion diluted to 100 cc. Add 0.5 cc. of sodium hydroxide solution and 10 cc. of standard potassium permanganate and digest exactly 30 minutes. Remove the flask, add 5 cc. of sulfuric acid and 10 cc. of the standard ammonium oxalate, and titrate with the standard potassium permanganate as in the acid digestion.
RESIDUE ON EVAPORATION.

TOTAL RESIDUE.\textsuperscript{18}

Ignite and weigh a clean platinum dish, and measure into it 100 cc. of the thoroughly shaken sample. Evaporate to dryness on a water bath. Then heat the dish in an oven at 103° C. or 180° C. for one hour. Cool in a desiccator and weigh. The temperature of drying should be mentioned in the report. The increase in weight gives the total solids or residue on evaporation. If 100 cc. of the sample was taken this weight expressed in milligrams and multiplied by 10 is equal to parts per million of residue on evaporation. The residue from waters low in organic matter but relatively high in iron may be used, as a matter of convenience, for the determination of iron.

FIXED RESIDUE AND LOSS ON IGNITION.\textsuperscript{19}

The residue from sewages and waters high in organic matter may be ignited to burn off the organic matter, which, with some volatile inorganic matter, constitutes the loss on ignition.

Procedure.—Ignite the residue in the platinum dish at a low red heat. If great accuracy is desired this should be done in an electric muffle furnace or in a radiator, which consists of a platinum or a nickel dish large enough to allow an air space of about half an inch between it and the dish within it, the inner dish being supported by a triangle of platinum wire laid on the bottom of the outer dish. A disc of platinum or nickel foil large enough to cover the outer dish is suspended over the inner dish to radiate the heat into it. The larger dish is heated to bright redness until the residue is white or nearly so. Allow the dish to cool, and moisten the residue with a few drops of distilled water. Dry the residue in the oven, cool in a desiccator, and weigh. The fixed residue on evaporation is the difference between this weight and the weight of the dish.

The loss on ignition is the difference between the total residue on evaporation and the fixed residue on evaporation.

If the odor and color on ignition of some residues give helpful clues to the character of the organic matter record them.
SUSPENDED MATTER—HARDNESS

SUSPENDED MATTER. 10

DETERMINATION WITH GOOCH CRUCIBLE.

Reagent.—Prepare a dilute cream of asbestos fibre which has been finely shredded, thoroughly ignited, treated with strong hydrochloric acid for at least 12 hours, and washed with distilled water till free from acid.

Procedure.—1. Prepare a mat of the asbestos fibre 1/16 inch thick in a Gooch crucible. Dry it in an oven at 103 or 180° C., cool and weigh. Filter 1,000 cc. of samples having a turbidity of 50 parts per million or less. If the turbidity is higher use sufficient water to obtain 50 to 100 mg. of suspended matter. Dry for one hour at 103 or 180° C., cool and weigh. Report the temperature at which the residue was dried. If 1,000 cc. is filtered the increase in weight expressed in milligrams is equal to parts per million of suspended matter.

DETERMINATION BY FILTRATION.

The difference between the total solids in filtered and unfiltered portions of a sample may be used as a basis for calculating suspended matter.

DETERMINATION OF VOLUME.

The determination of the volume 9 of suspended matter in sewages has received considerable attention abroad. Imhoff recommends the use of conical glass vessels holding 1 liter with the lower portions graduated in cubic centimeters. Others recommend centrifuges with sediment tubes.

FIXED RESIDUE AND LOSS ON IGNITION.

Treat the total residue from a filtered sample in the same manner as described for the total residue, and obtain the loss on ignition due to dissolved matter, and by difference the loss on ignition due to suspended matter.

HARDNESS. 10

A water containing certain mineral constituents in solution, chiefly calcium and magnesium, which form insoluble compounds with soap, is said to be hard. Carbon dioxide in water increases the solubility of calcium and magnesium carbonates, forming
bicarbonate. If carbon dioxide is removed from the water by boiling the bicarbonate is decomposed and calcium and magnesium are partly precipitated. The proportion of calcium or magnesium carbonate that a water can hold in solution depends on the concentration of carbon dioxide, which in turn depends on the temperature of the water and the proportion of carbon dioxide in the atmosphere with which the water has been in contact. Consequently, when the carbon dioxide is removed from the water by boiling or otherwise the carbonates of calcium and magnesium are partly, but not completely, precipitated, and the hardness of the water is thus diminished and the water is softened to the extent to which these substances are precipitated. The hardness thus removed is called temporary hardness. The hardness which still remains after boiling is due mainly to calcium and magnesium in equilibrium with sulfate, chloride, and nitrate, and residual carbonate, and it is called permanent hardness. Non-carbonate hardness is the hardness caused by sulfates, chlorides, and nitrates of calcium, magnesium, iron, and other metals that form insoluble soaps.

**TOTAL HARDNESS BY CALCULATION.**

The most accurate method of ascertaining total hardness is to compute it from the results of determinations of calcium and magnesium in the sample. (See methods, pp. 57–58.) Iron and other metals must be included in the calculation if they are present in significant amounts. Total hardness as CaCO₃ equals 2.5 Ca plus 4.1 Mg.

**TOTAL HARDNESS BY SOAP METHOD.**

The determination of hardness by the soap method roughly approximates the amount of calcium and magnesium in a water, though it actually measures the soap-consuming power of the water. *Reagents.*—1. Standard calcium chloride solution. Dissolve 0.2 gram of pure calcite (calcium carbonate) in a little dilute hydrochloric acid, being careful to avoid loss of solution by spattering. Evaporate the solution to dryness several times with distilled water to expel excess of acid. Dissolve the residue in distilled water and dilute the solution to 1 liter. One cc. of this dilution is equivalent to 0.2 mg. of calcium carbonate.

2. Standard soap solution. Dissolve 100 grams of dry white Castile soap in 1 liter of 80 per cent alcohol, and allow this
solution to stand several days before standardizing. Pure potassium oleate made from lead plaster and potassium carbonate may be used in place of Castile soap.

*First method of standardization.*—Dilute 20 cc. of the calcium chloride solution in a 250 cc. glass-stoppered bottle to 50 cc. with distilled water which has been recently boiled and cooled. Add soap solution from a burette, 0.2 or 0.3 cc. at a time, shaking the bottle vigorously after each addition until a lather remains unbroken for five minutes over the entire surface of the water while the bottle lies on its side. Then adjust the strength of the stock solution with 70 per cent alcohol so that the resulting diluted soap solution will give a permanent lather when 6.40 cc. of it is properly added to 20 cc. of standard calcium chloride solution diluted to 50 cc. Usually 75 to 100 cc. of the stock soap solution is required to make 1 liter of the standard soap solution. The quantity of calcium carbonate equivalent to each cubic centimeter of the standard soap solution consumed in the titration is indicated in Table 6.

**Table 6.—Total hardness in parts per million of CaCO₃ for each tenth of a cubic centimeter of soap solution when 50 cc. of the sample is titrated.**

<table>
<thead>
<tr>
<th>Cubic centimeters of soap solution</th>
<th>0.0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0</td>
<td>1.6</td>
</tr>
<tr>
<td>1.0</td>
<td>4.8</td>
<td>6.3</td>
<td>7.9</td>
<td>9.5</td>
<td>11.1</td>
<td>12.7</td>
<td>14.3</td>
<td>15.6</td>
<td>16.9</td>
<td>18.2</td>
</tr>
<tr>
<td>2.0</td>
<td>19.5</td>
<td>20.8</td>
<td>22.1</td>
<td>23.4</td>
<td>24.7</td>
<td>26.0</td>
<td>27.3</td>
<td>28.6</td>
<td>29.9</td>
<td>31.2</td>
</tr>
<tr>
<td>3.0</td>
<td>32.5</td>
<td>33.8</td>
<td>35.1</td>
<td>36.4</td>
<td>37.7</td>
<td>38.0</td>
<td>40.3</td>
<td>41.6</td>
<td>42.9</td>
<td>44.3</td>
</tr>
<tr>
<td>4.0</td>
<td>45.7</td>
<td>47.1</td>
<td>48.6</td>
<td>50.0</td>
<td>51.4</td>
<td>52.9</td>
<td>54.3</td>
<td>55.7</td>
<td>57.1</td>
<td>58.6</td>
</tr>
<tr>
<td>5.0</td>
<td>60.0</td>
<td>61.4</td>
<td>62.9</td>
<td>64.3</td>
<td>65.7</td>
<td>67.1</td>
<td>68.6</td>
<td>70.0</td>
<td>71.4</td>
<td>72.9</td>
</tr>
<tr>
<td>6.0</td>
<td>74.3</td>
<td>75.7</td>
<td>77.1</td>
<td>78.6</td>
<td>80.0</td>
<td>81.4</td>
<td>82.9</td>
<td>84.3</td>
<td>85.7</td>
<td>87.1</td>
</tr>
<tr>
<td>7.0</td>
<td>88.6</td>
<td>90.0</td>
<td>91.4</td>
<td>92.9</td>
<td>94.3</td>
<td>95.7</td>
<td>97.1</td>
<td>98.6</td>
<td>100.0</td>
<td>101.5</td>
</tr>
</tbody>
</table>

This table does not provide for the use of so large volume of soap solution for a single determination as former ones because the end-point becomes somewhat obscured in the presence of magnesium if more than 7 cc. is used.

*Second method of standardization.*—Dilute 100 cc. of the stock soap solution to 1 liter with 70 per cent alcohol. This dilute solution should be of such strength that approximately 6.4 cc. of it will give a permanent lather when 20 cc. of standard calcium chloride
solution diluted to 50 cc. with distilled water is titrated with it. Determine the amount of soap solution required to give a permanent lather with 50 cc. of distilled water and with 5, 10, 15, and 20 cc. of standard calcium chloride solution diluted to 50 cc. with distilled water. Finally plot on cross-section paper a curve showing the relation of various quantities of soap solution to corresponding quantities of standard calcium carbonate solution and therefore to parts per million of hardness.

Procedure.—Measure 50 cc. of the water into a 250 cc. bottle and add to it soap solution in small quantities in precisely the same manner as described under the standardization of the soap solution. From the number of cubic centimeters of soap solution used obtain from Table 6 or from the plotted curve the total hardness of the water in parts per million of calcium carbonate.

To avoid mistaking the false or magnesium end-point for the true one when adding the soap solution to waters containing magnesium salts, read the burette after the titration is apparently finished, and add about 0.5 cc. more of soap solution. If the end-point was due to magnesium the lather will disappear. Soap solution must then be added until the true end-point is reached. Usually the false lather persists for less than five minutes.

If more than 7 cc. of soap solution is required for 50 cc. of the water take less of the sample and dilute it to 50 cc. with distilled water which has been recently boiled and cooled. This step reduces somewhat the disturbing influence of magnesium, which consumes more soap than an equivalent weight of calcium.

At best the soap method is not a precise test on account of the different relative amounts of calcium and magnesium in different waters. For hard waters, especially in connection with processes for purification and softening, it is advised that this method be not exclusively used. If the same water is frequently analyzed it may be of assistance to standardize the soap solution against a mixture of calcium and magnesium salts, the relative proportions of which approximate those found in the water.

The strength of the soap solution should be determined from time to time, to make sure that it has not materially changed. Record all results in parts per million of calcium carbonate.

One English degree of hardness, Clark’s scale, is equivalent to 1 grain per Imperial gallon of calcium carbonate. One French degree of hardness is equivalent to 1 part per 100,000 of calcium carbonate.
TEMPORARY HARDNESS

One German degree of hardness is equivalent to 1 part per 100,000 of calcium oxide, and multiplied by 17.9 gives parts per million of calcium carbonate. The relations of these various scales are indicated in Table 7.

Table 7.—CONVERSION TABLE FOR HARDNESS.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Equivalent.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parts per million.</td>
</tr>
<tr>
<td>One part per million</td>
<td>1.00</td>
</tr>
<tr>
<td>One Clark degree</td>
<td>14.3</td>
</tr>
<tr>
<td>One French degree</td>
<td>10.0</td>
</tr>
<tr>
<td>One German degree</td>
<td>17.9</td>
</tr>
</tbody>
</table>

TOTAL HARDNESS BY SODA REAGENT METHOD.47 74 94d

Add standard sulfuric acid to 200 cc. of the sample until the alkalinity is neutralized. (See Procedure with methyl orange, p. 37.) Then apply the non-carbonate hardness method (pp. 34–35). This method gives fairly satisfactory estimates of total hardness of hard waters.

TEMPORARY HARDNESS BY TITRATION WITH ACID.

Determine the alkalinity in presence of methyl orange (see p. 37) in the original sample and also in the sample after boiling, cooling, restoring to the original volume with boiled distilled water, and filtering. The difference between the two, if any, is the temporary hardness. This is the most accurate method of determining the temporary hardness of ordinary waters. Iron bicarbonate is included as a part of the temporary hardness.

NON-CARBONATE HARDNESS BY SODA REAGENT METHOD.47 74 94d

The use of soda reagent does not avoid entirely the error due to solubility of the salts of calcium and magnesium; consequently, if much depends on the results, as in water softening, gravimetric determinations of the calcium and magnesium that remain in solution should be made and a correction should be applied for those amounts.

Reagent.—Prepare soda reagent from equal parts of sodium
hydroxide and sodium carbonate. It should be approximately tenth normal.

Procedure.—Measure 200 cc. of the sample and 200 cc. of distilled water into 500 cc. Jena or similar glass Erlenmeyer flasks. Treat the contents of each flask in the following manner. Boil 15 minutes to expel free carbon dioxide. Add 25 cc. of soda reagent. Boil 10 minutes, cool, rinse into 200 cc. graduated flasks, and dilute to 200 cc. with boiled distilled water. Filter, rejecting the first 50 cc., and titrate 50 cc. of each filtrate with N/50 sulfuric acid in the presence of methyl orange or erythrosine indicator. The non-carbonate hardness in parts per million of calcium carbonate is equal to 20 times the difference between the number of cubic centimeters of sulfuric acid required for the soda reagent in distilled water and the number of cubic centimeters of N/50 sulfuric acid required for the soda reagent in the sample.

Water naturally containing bicarbonate and carbonate in excess of calcium and magnesium requires a larger amount of acid to neutralize the sample after it has been treated than is required to neutralize the volume of soda reagent originally added. (See p. 39.)

NON-CARBONATE HARDNESS BY SOAP METHOD.

Non-carbonate hardness may be calculated for waters which are soft or moderately hard in a fairly satisfactory manner by deducting the total alkalinity from the total hardness by the soap method (pp. 31–34). For waters that are very hard, and particularly those that contain much magnesium, this method is not advised.

ALKALINITY.\textsuperscript{11 13 67 97}

The alkalinity of a natural water represents its content of carbonate, bicarbonate, borate, silicate, phosphate, and hydroxide. Alkalinity is determined by neutralization with standard sulfuric acid or potassium bisulfate in the presence of phenolphthalein and either methyl orange, erythrosine, or lacmoid as indicators. Methyl orange may be used except in waters containing aluminium sulfate or iron sulfate. The relations between estimates in presence of these indicators and the carbonate, bicarbonate, and hydroxide radicles are indicated in Table 8. The alkalinity of carbonates in the presence of phenolphthalein is different from that in the presence of methyl orange, partly because of loss of carbon dioxide.
and partly because of defects in phenolphthalein as an indicator in such conditions.

Table 8.—RELATIONS BETWEEN ALKALINITY TO PHENOLPHTHALEIN AND THAT TO METHYL ORANGE, ERYTHROSIINE, OR LACMOID, IN PRESENCE OF BICARBONATE, CARBONATE, AND HYDROXIDE.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>P = 0</td>
<td>T</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P &lt; 1/4 T</td>
<td>T - 2 P</td>
<td>2 P</td>
<td>0</td>
</tr>
<tr>
<td>P = 1/4 T</td>
<td>0</td>
<td>2 P</td>
<td>0</td>
</tr>
<tr>
<td>P &gt; 1/4 T</td>
<td>0</td>
<td>2(T - P)</td>
<td>2 P - T</td>
</tr>
<tr>
<td>P = T</td>
<td>0</td>
<td>0</td>
<td>T</td>
</tr>
</tbody>
</table>

* T = Total alkalinity in presence of methyl orange, erythrosine, or lacmoid.
* P = Alkalinity in presence of phenolphthalein.

Reagents.—1. Sulfuric acid or potassium bisulfate. A N/50 solution.

2. Phenolphthalein indicator. Dissolve 5 grams of a good quality of phenolphthalein in 1 liter of 50 per cent alcohol. Neutralize with N/10 potassium hydroxide. The alcohol should be diluted with boiled distilled water.

3. Methyl orange indicator. Dissolve 0.5 gram of a good grade of methyl orange in 1 liter of distilled water. Keep the solution in the dark.

4. Lacmoid indicator. Dissolve 2.0 grams of lacmoid in 1 liter of 50 per cent alcohol. Dilute the alcohol with freshly boiled distilled water.

5. Erythrosine indicator. Dissolve 0.5 gram of erythrosine (the sodium salt) in 1 liter of freshly boiled distilled water.

PROCEDURE WITH PHENOLPHTHALEIN.

Add 4 drops of phenolphthalein indicator to 50 or 100 cc. of the sample in a white porcelain casserole or an Erlenmeyer flask over a white surface. If the solution becomes colored, hydroxide or normal carbonate is present. Add N/50 sulfuric acid from a burette until the coloration disappears.

The phenolphthalein alkalinity in parts per million of calcium carbonate is equal to the number of cubic centimeters of N/50
sulfuric acid used multiplied by 20 if 50 cc. of the sample was used, or by 10 if 100 cc. was used.

PROCEDURE WITH METHYL ORANGE.

Add 2 drops of methyl orange indicator to 50 or 100 cc. of the sample, or to the solution to which phenolphthalein has been added. In a white porcelain casserole or an Erlenmeyer flask over a white surface. If the solution becomes yellow, hydroxide, normal carbonate, or bicarbonate is present. Add N/50 sulfuric acid from a burette until the faintest pink coloration appears. The methyl orange alkalinity in parts per million of calcium carbonate is equal to the total number of cubic centimeters of N/50 sulfuric acid used multiplied by 20 if 50 cc. of the sample was used, or by 10 if 100 cc. was used.

PROCEDURE WITH LACMOID.

Add 4 drops of lacmoid indicator to 50 or 100 cc. of the sample in a porcelain casserole or an Erlenmeyer flask. Add N/50 sulfuric acid from a burette until within 1 or 2 cc. of the amount necessary for neutralization has been added. Heat the solution until bubbles of steam begin to break at the surface. Remove the dish from the source of heat and continue the titration until a drop of the acid striking the surface of the liquid and sinking to the bottom of the vessel produces no change in the uniform reddish or purple color of the solution. The calculation is the same as for phenolphthalein alkalinity.

PROCEDURE WITH ERYTHROSINE.

Add 5 cc. of neutral chloroform and 1 cc. of erythrosine indicator to 50 or 100 cc. of the sample in a 250 cc. clear glass-stoppered bottle. If the chloroform becomes rose colored on shaking, hydroxide, bicarbonate, or normal carbonate is present. Add N/50 sulfuric acid from a burette until the chloroform becomes colorless. A white surface behind the bottle facilitates detection of a trace of color as the end-point is approached. The calculation is the same as with phenolphthalein alkalinity.

BICARBONATE.

Bicarbonate is present if the alkalinity to phenolphthalein is less than one-half the alkalinity to methyl orange, erythrosine, or lac-
mold. The alkalinity to methyl orange, erythrosine, or lacmoid is
due entirely to bicarbonate if there is no phenolphthalein alkalinity.
If there is phenolphthalein alkalinity the bicarbonate, in terms of
calcium carbonate, is equal to the methyl orange, erythrosine, or
lacmoid alkalinity minus twice the phenolphthalein alkalinity.
Bicarbonate, carbon dioxide as bicarbonate, and half-bound carbon
dioxide can be calculated as follows:

Bicarbonate \((\text{HCO}_3^-) = 1.22\) times the bicarbonate expressed in
terms of calcium carbonate.

Carbon dioxide \((\text{CO}_2)\) as bicarbonate \(= 0.88\) times the bicarbonate
expressed in terms of calcium carbonate.

Half-bound carbon dioxide \((\text{CO}_2) = 0.44\) times the bicarbonate
expressed in terms of calcium carbonate.

NORMAL CARBONATE.\(^{30 \, 94}\)

Normal carbonate is present if the alkalinity to phenolphthalein
is greater than zero but less than the alkalinity to methyl orange,
erythrosine, or lacmoid. If the phenolphthalein alkalinity is exactly
equal to one-half the methyl orange, erythrosine, or lacmoid alka-
linity the alkalinity is due entirely to normal carbonate. If the
phenolphthalein alkalinity is less than one-half the methyl orange,
erythrosine, or lacmoid alkalinity normal carbonate expressed in
terms of calcium carbonate is equal to twice the phenolphthalein
alkalinity. If the phenolphthalein alkalinity is greater than one-
half the methyl orange, erythrosine, or lacmoid alkalinity the
normal carbonate is equal to twice the difference between the
methyl orange, erythrosine, or lacmoid alkalinity and the phenolph-
thalein alkalinity. The carbonate, carbon dioxide as carbonate,
and bound carbon dioxide can be calculated as follows:

Carbonate \((\text{CO}_3^-) = 0.6\) times the normal carbonate expressed in
terms of calcium carbonate.

Carbon dioxide as carbonate \((\text{CO}_2) = 0.44\) times the normal
carbonate expressed in terms of calcium carbonate.

Bound carbon dioxide \((\text{CO}_2)\) is the sum of the carbon dioxide as
carbonate and one-half that as bicarbonate.

HYDROXIDE.\(^{39 \, 94}\)

If hydroxide, or caustic alkalinity, is present the alkalinity to
phenolphthalein is greater than one-half the alkalinity to methyl
ALKALINITY—ACIDITY

orange, erythrosine, or lacmoid; the alkalinity is due entirely to hydroxide if the phenolphthalein alkalinity is equal to the methyl orange, erythrosine, or lacmoid alkalinity. If the phenolphthalein alkalinity is more than half and less than all the methyl orange, erythrosine, or lacmoid alkalinity, hydroxide, expressed in terms of calcium carbonate, is equal to twice the phenolphthalein alkalinity minus the methyl orange, erythrosine, or lacmoid alkalinity.

ALKALI CARBONATES.

Waters which contain sodium or potassium carbonates or bicarbonates contain all of their calcium and magnesium as carbonates or bicarbonates. That is, they possess no non-carbonate hardness (sulfates, nitrates or chlorides of calcium and magnesium).

The most accurate method is to determine the total alkalinity by titration with N/50 sulfuric acid, using methyl orange, erythrosine, or lacmoid as an indicator; then determine the calcium and magnesium content; and subtract from the total alkalinity the computed alkalinity due to the calcium and magnesium expressed in terms of calcium carbonate. The remainder is the alkalinity due to carbonates and bicarbonates of sodium and potassium.

This determination may also be made by applying the method, for non-carbonate hardness with soda reagent (see p. 35), and by noting the excess of acid required to neutralize the alkaline carbonates originally present.

With present information as to solubilities of the normal carbonates of calcium and magnesium, it is difficult in their presence to measure slight quantities of carbonates of sodium or potassium.

ACIDITY. 144 17

Waters may have an acid reaction because of the presence of free carbon dioxide, mineral acids, or some of their salts, especially those of iron and aluminium.

Reagents.—1. N/50 sodium carbonate. Dissolve 1.06 grams of anhydrous sodium carbonate in 1 liter of boiled distilled water that has been cooled in an atmosphere free from carbon dioxide. Preserve this solution in bottles of resistant glass protected from the air by tubes filled with soda-lime. One cc. is equivalent to 1 mg. of CaCO₃.
2. N/22 sodium carbonate. Dissolve 2.41 grams of anhydrous sodium carbonate in 1 liter of boiled distilled water that has been cooled in an atmosphere free from carbon dioxide. Preserve this solution in bottles of resistant glass protected from the air by tubes filled with soda-lime. One cc. is equivalent to 1 mg. of $\text{CO}_2$.

3. Phenolphthalein indicator (see p. 36).

4. Methyl orange indicator (see p. 36).

**TOTAL ACIDITY.**

*Procedure.*—Add 4 drops of phenolphthalein indicator to 50 or 100 cc. of the sample in a white porcelain casserole or an Erlenmeyer flask over a white surface. Add N/50 sodium carbonate until the solution turns pink. The total acidity in parts per million of calcium carbonate is equal to the number of cubic centimeters of N/50 sodium carbonate used multiplied by 20 if 50 cc. of the sample was used, or by 10 if 100 cc. was used.

**FREE CARBON DIOXIDE.**

Carbon dioxide may exist in water in three forms—free carbon dioxide, bicarbonate (pp. 37–38), and carbonate (p. 38). One-half the carbon dioxide as bicarbonate is known as the half-bound carbon dioxide. The carbon dioxide as carbonate plus one-half that as bicarbonate is known as the bound carbon dioxide.

*Procedure.*—Pour 100 cc. of the sample into a tall narrow vessel, preferably a 100 cc. Nessler tube. Add 10 drops of phenolphthalein indicator, and titrate rapidly with N/22 sodium carbonate, stirring gently, until a faint but permanent pink color is produced. The free carbon dioxide ($\text{CO}_2$) in parts per million is equal to 10 times the number of cubic centimeters of N/22 sodium carbonate used.

Because of the ease with which free carbon dioxide escapes from water, particularly when the gas is present in large amount, a special sample should be collected for this determination, which should preferably be made at the time of collection. If the analysis cannot be made at the time of collection approximate results with water not too high in free carbon dioxide may be obtained on samples collected in bottles completely filled so as to leave no air space under the stopper. Bottled samples should be kept, until tested, at a temperature lower than that of the water when collected. If mineral acids or certain salts are present correction must be made.
At best, the results of the titration are uncertain because the proper end-point for correct results differs in color with different types of water.

FREE MINERAL ACIDS.

Procedure.—Add 2 drops of methyl orange indicator to 50 or 100 cc. of the sample in a white porcelain casserole or an Erlenmeyer flask over a white surface. Add N/50 sodium carbonate from a burette until the pink coloration of the solution disappears. The acidity due to free mineral acids, expressed in terms of calcium carbonate, is equal to the number of cubic centimeters of N/50 sodium carbonate used multiplied by 20 if 50 cc. of the sample was used, or by 10 if 100 cc. was used.

MINERAL ACIDS AND SULFATES OF IRON AND ALUMINIUM.

Procedure.—Modify the method for free mineral acids by titrating the water at boiling temperature in the presence of phenolphthalein indicator. The acidity due to free mineral acids and sulfates of iron and aluminium, expressed in terms of calcium carbonate, is equal to the number of cubic centimeters of N/50 sodium carbonate used multiplied by 20 if 50 cc. of the sample was used, or by 10 if 100 cc. was used.

The acidity due to sulfates of iron and aluminium is equal to the acidity due to mineral acids and sulfates minus the acidity due to mineral acids. The acidity due to ferrous and ferric sulfate can be calculated from the determined amount of these salts (pp. 43–48). The acidity due to aluminium sulfate is equal to the acidity due to total acid sulfates minus that due to iron sulfates.

Acidity shall be reported in parts per million of calcium carbonate (CaCO₃). Sulfate (SO₄) equals parts per million of calcium carbonate multiplied by 0.96.

Carbon dioxide (CO₂) equals parts per million of calcium carbonate multiplied by 0.44.

CHLORIDE.

Chloride in water and sewage has its origin in common salt, from mineral deposits in the earth, from ocean vapors carried inland by the wind, or from polluting materials like sewage and trade wastes, which contain the salt used in the household and in manufacturing.
Comparison of the chloride content of a water with that of other waters in the vicinity known to be unpolluted frequently affords useful information as to its sanitary quality. If, however, the chloride normally exceeds 20 parts per million because of chloride-bearing mineral deposits the chloride content of a water has little sanitary significance.

Reagents.—1. Standard sodium chloride solution. Dissolve 16.48 grams of pure fused sodium chloride in 1 liter of distilled water. Dilute 100 cc. of this stock solution to 1 liter in order to obtain a standard solution each cubic centimeter of which contains 0.001 gram of chloride.

2. Standard silver nitrate solution. Dissolve about 2.40 grams of silver nitrate crystals in 1 liter of distilled water. Standardize this with the standard salt solution, and adjust, correcting for volume (see p. 43), so that 1 cc. will be exactly equivalent to 0.0005 gram of chloride.

3. Potassium chromate indicator. Dissolve 50 grams of neutral potassium chromate in a little distilled water. Add enough silver nitrate to produce a slight red precipitate. Filter and dilute the filtrate to 1 liter with distilled water.

4. Aluminium hydroxide. Electrolyze ammonia-free water, using aluminium electrodes. Wash the precipitate until it is free from chloride, ammonia, and nitrite. Or dissolve 125 grams of potassium or ammonium alum in 1 liter of distilled water. Precipitate the aluminium by adding cautiously ammonium hydroxide. Wash the precipitate in a large jar by successive additions and decantations of distilled water until free from chloride, nitrite, and ammonia.

Procedure.—Add 1 cc. of potassium chromate indicator to 50 cc. of the sample in a 6-inch white porcelain evaporating dish or a 150 cc. Erlenmeyer flask over a white surface. Titrate with the silver nitrate solution under similar conditions of volume, light, and temperature as were used in standardizing the silver nitrate until a faint reddish coloration is perceptible. The detection of the end-point is facilitated by comparison of the contents of the porcelain dish with those of another dish containing the same quantity of potassium chromate indicator in 50 cc. of distilled water. Some analysts prefer to make the titration in a dark-room provided with a yellow light. The end-point is very sharp by electric light and also by daylight with photographic yellow glass. The titration may
be made in Nessler tubes if the solutions are standardized under similar conditions.

If the amount of chloride is very high use 25 cc., or even a smaller quantity, diluting the volume taken to 50 cc. with distilled water. If the amount of chloride is very low concentrate 250 cc. of the sample to 50 cc. by evaporation. Rotate the liquid to make sure that no residue remains undissolved on the walls of the dish, and, if necessary, use a rubber-tipped glass rod to assist in this operation.

Chloride is determined by some observers by extracting with hot distilled water the residue in the platinum dish in the determination of the residue on evaporation and proceeding as just described. This is permissible if a little sodium carbonate is added before evaporation to prevent loss of chloride through decomposition of magnesium chloride in the residue.

If the sample has a color greater than 30 it should be decolorized by shaking it thoroughly with washed aluminium hydroxide (3 cc. to 500 cc. of the sample) and allowing the precipitate to settle. Make the determination on a portion of the clarified sample, filtered if necessary. If the sample is acid neutralize it with sodium carbonate; if hydroxide is present add dilute sulfuric acid until the cold liquid will just discharge the color of phenolphthalein. If the presence of sulfide and sulfocyanate renders it necessary make proper corrections or modifications in treatment.

Make correction for the error due to variations in the volume of the liquid and precipitate by means of the formula, in which is the correction in cubic centimeters of silver nitrate solution and is cubic centimeters of liquid at the end of the titration. If 50 cc. of the sample is titrated chloride (Cl) in parts per million is equal to the number of cubic centimeters of silver nitrate solution multiplied by 10. The correction to be applied is 0.2 cc. unless unusual accuracy is required.

Iron occurs in natural waters in both ferrous and ferric condition, depending on the source of the sample. In ground waters the iron is usually in an unoxidized and soluble condition, sometimes combined with carbonic or sulfuric acid, and also in combination with organic matter. Many waters, especially those that have been
exposed to the air, contain the iron in the form of a colloidal hydroxide. Silt-bearing waters often contain much iron in suspension, usually in an oxidized form. Sewages and sewage effluents, particularly those receiving manufacturing wastes, contain various forms of iron of different degrees of solubility, oxidation, and coagulation.

TOTAL IRON

COLORIMETRIC METHOD.

Reagents.—1. Standard iron solution. Dissolve 0.7 gram of crystallized ferrous ammonium sulfate in 50 cc. of distilled water to which 20 cc. of dilute sulfuric acid has been added. Warm the solution slightly and add potassium permanganate until the iron is completely oxidized. Dilute the solution to 1 liter. One cc. of the standard solution equals 0.1 mg. Fe.

2. Potassium sulfocyanide solution. Dissolve 20 grams of the salt in 1 liter of distilled water.

3. Dilute hydrochloric acid. One volume of acid (Sp. gr. 1.2) and one volume of distilled water. This shall be free from nitric acid.

4. N/5 potassium permanganate. Dissolve 6.30 grams of the salt in distilled water and dilute to 1 liter.

5. Hydrochloric acid. Concentrated, free from iron.


7. Nitric acid. 5N, free from iron.

First procedure.—Evaporate 100 cc. of the water to dryness, or use the residue left after the determination of residue on evaporation (p. 29). Ignite the residue at a low red heat taking care not to heat it hot enough to make the iron difficultly soluble. Cool the dish and add 5 cc. of concentrated hydrochloric acid. Moisten the inner surface of the dish. Warm the solution for two or three minutes, and again moisten the inner surface of the dish by permitting the hot acid to flow over it. Wash the hot solution from the dish into a 50 cc. Nessler tube, filtering if necessary through paper that has been washed with hot water. Dilute to 50 cc., and add 3 drops of potassium permanganate solution. Add 5 cc. of potassium sulfocyanide solution, mix, and compare with standards.

If it is not convenient to use the residue on evaporation and if the sample is relatively free from organic matter boil 50 cc. of the sample with 5 cc. of 5N nitric acid for five minutes. Add a few drops of permanganate and 5 cc. of potassium sulfocyanide and
compare with standards, using nitric acid in place of hydrochloric acid in the standards. This method is excellent for ground waters. The permanganate and acid liberate chlorine in water high in chloride, and produce a permanent yellow color which interferes with the determination, unless the sample is first diluted to 50 cc. An excess of permanganate, reacting with hydrochloric acid, causes similar trouble. The amounts of hydrochloric acid, 5 cc., and of sulfocyanide, 5 cc., should be approximately measured because more acid lightens the color whereas more sulfocyanide deepens it. This is especially important if permanent standards are used.

Second procedure.—For surface waters containing small amounts of organic matter, the method of Klut is recommended. Samples containing small amounts of iron should be concentrated, if possible, until at least 0.5 mg. of iron is present in the volume tested. Boil the sample in a beaker with 2 to 3 cc. of concentrated nitric acid free from iron, adding permanganate if necessary to destroy the organic matter. To the hot liquid add ammonia in slight excess and warm until the smell of ammonia is hardly discernible. Filter and wash with water at 70° to 80° C. containing a little ammonia. Dissolve the iron in the beaker and on the filter paper in 5 cc. of concentrated hydrochloric acid, and wash with hot water until the iron is all dissolved, collecting the filtrate in a 50 cc. Nessler tube. Dilute to 50 cc. Add potassium sulfocyanide and determine the iron by comparison with standards.

Comparison with iron standards.

First procedure.—Prepare standards containing amounts of standard iron solution ranging from 0.05 to 4 cc. according to the quantity of iron in the sample. Dilute these amounts with water to about 40 cc. Add 5 cc. of dilute hydrochloric acid and 3 drops of potassium permanganate to each tube and dilute to 50 cc. Add 5 cc. of the potassium sulfocyanide to each of the standard solutions at the same time that it is added to the samples of water under examination, and compare immediately after mixing. If 100 cc. of the sample is used the iron in parts per million is equal to the number of cubic centimeters of the standard iron solution in the standard that the sample matches.

Second procedure.—For a small number of determinations it is more convenient to run the standard iron solution into a Nessler
tube containing the acid, distilled water, and potassium sulfocyanide until the color matches that of the sample tested. When determining iron in three or four samples the colors may be matched in the order of their intensity and the volumes of standard iron solution required for each tube may be read from the burette.

**Comparison with Permanent Standards.**

**Reagents.**—1. Platinum solution. Dissolve 2 grams of potassium platinic chloride (PtCl₄ 2KCl) in distilled water, add 100 cc. of concentrated hydrochloric acid, and dilute to 1 liter with distilled water.

2. Cobalt solution. Dissolve 24 grams of dry cobaltous chloride crystals (CoCl₂ 6H₂O) in a small amount of distilled water, add 100 cc. of strong hydrochloric acid, and dilute to 1 liter with distilled water.

**Procedure.**—Prepare a series of permanent standards by diluting to 50 cc. with distilled water the amounts of platinum and cobalt solutions, in 50 cc. Nessler tubes, indicated in Table 9. Compare the sample with these standards, and calculate the parts per million of iron.

**Table 9.—Preparation of Permanent Standards for the Determination of Iron.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>cc.</td>
<td>cc.</td>
</tr>
<tr>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>.1</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>.3</td>
<td>6</td>
<td>3.0</td>
</tr>
<tr>
<td>.5</td>
<td>10</td>
<td>5.0</td>
</tr>
<tr>
<td>.7</td>
<td>14</td>
<td>7.5</td>
</tr>
<tr>
<td>1.0</td>
<td>20</td>
<td>11.0</td>
</tr>
<tr>
<td>1.5</td>
<td>28</td>
<td>17.0</td>
</tr>
<tr>
<td>2.0</td>
<td>35</td>
<td>24.0</td>
</tr>
<tr>
<td>2.5</td>
<td>39</td>
<td>32.0</td>
</tr>
<tr>
<td>3.0</td>
<td>39</td>
<td>43.0</td>
</tr>
<tr>
<td>3.5</td>
<td>40</td>
<td>55.0</td>
</tr>
</tbody>
</table>

**Volumetric Method.**

Some samples of sewage and water mixed with trade wastes and mine drainage contain so much iron that it is preferable to use the volumetric method described on page 57 for the determination of
both total and dissolved iron, rather than to work with quantities small enough to permit application of the colorimetric methods just described. If iron is present in large quantities in suspension, as in some sewages and septic tank effluents, it may be filtered off and the residue washed, ignited, and fused with potassium and sodium carbonate. The fusion is then extracted with hydrochloric acid and the iron determined as on page 57.

Samples containing much organic matter should be evaporated to dryness with 0.5 cc. of concentrated sulfuric acid and the residue then ignited before estimation of iron.

**DISSOLVED IRON.**

Determine, by the method described for total iron, the iron in the sample after filtration. Iron may precipitate from some samples during filtration.

**SUSPENDED IRON.**

The suspended iron is the difference between total iron in the unfiltered sample and dissolved iron in the filtered sample.

**FERROUS IRON.**

Determine the total ferrous iron in an unfiltered sample and the dissolved ferrous iron in a filtered sample.

*Reagents.*—1. Standard iron solution. Dissolve 0.7 gram of crystallized ferrous ammonium sulfate in a large volume of freshly boiled distilled water to which 10 cc. of dilute sulfuric acid has been added and dilute to 1 liter. This solution should be freshly prepared when needed. One cc. of this standard solution contains 0.1 mg. of Fe.

2. Potassium ferricyanide solution. Dissolve 5 grams of the salt in 1 liter of distilled water. Use a freshly prepared solution.

3. Dilute sulfuric acid. Dilute 1 part of sulfuric acid, specific gravity 1.84, with 5 parts of distilled water.

*Procedure.*—Add 10 cc. of dilute sulfuric acid to 50 cc. of the sample, remove the suspended matter by filtration if necessary, and add 15 cc. of potassium ferricyanide solution. Dilute the solution to 100 cc. with distilled water. Compare the color developed in the sample with that in standards made at the same time from the ferrous iron solution. Place in 100 cc. Nessler tubes, in the following order, 75 cc. of distilled water, 10 cc. of dilute sulfuric
acid, and 15 cc. of potassium ferricyanide solution, and mix well
the contents of each tube. Prepare as many tubes in this way as
are needed. Add various quantities of standard ferrous iron solu-
tion to several tubes, mix well, and compare the resulting colors
with the samples immediately.

FERRIC IRON.

The amount of ferric iron in solution and suspension is equal to
the difference between the total iron and the ferrous iron obtained
by the methods described.

MANGANESE.

If the sample contains less than 10 parts per million of manganese,
use a colorimetric method in which the manganous salt is oxidized
to permanganate and the color produced thereby is compared with
that of a standard solution similarly treated. The persulfate
method and the bismuthate method are suitable. If the sample
contains more than 10 parts per million of manganese it is some-
times preferable to use a volumetric or gravimetric method.

PERSULFATE METHOD.

Reagents.—1. Nitric acid. Dilute concentrated nitric acid with
an equal volume of distilled water. Free the diluted acid from
brown oxides of nitrogen by aeration.

2. Silver nitrate. Dissolve 20 grams of silver nitrate in 1 liter
of distilled water.

3. Standard manganous sulfate. Dissolve 0.288 gram of purest
potassium permanganate in about 100 cc. of distilled water. Acidify
the solution with sulfuric acid and heat to boiling. Add slowly a
sufficient quantity of dilute solution of oxalic acid to discharge the
color. Cool and dilute to 1 liter. One cc. of this solution contains
0.1 mg. of manganese.


Procedure.—Use an amount of the sample that contains not
more than 0.2 mg. of manganese. Add 2 cc. of nitric acid and
boil down to about 50 cc. Precipitate the chloride with silver
nitrate solution, adding at least 1 cc. in excess. Shake and heat to
coagulate the precipitate, and filter. A sample that contains much
chloride should be evaporated with a few drops of sulfuric acid until
MANGANESE

white fumes appear and then diluted before the nitric acid and silver nitrate are added as directed above. If the sample is highly colored by organic matter it should be evaporated with sulfuric acid, and the residue ignited and dissolved in dilute nitric acid. Add about 0.5 gram of ammonium persulfate crystals and warm the solution until the maximum permanganate color is developed. This usually takes about ten minutes. At the same time prepare standards by diluting portions of 0.2, 0.4, 0.6 cc., etc. of the standard manganous sulfate solution to about 50 cc. and treating them exactly as the sample was treated. Transfer the sample and the standards to 50 cc. Nessler tubes, and compare the colors immediately. Manganese in parts per million is equal to the number of cubic centimeters of standard manganous sulfate solution in the tube that the sample matches multiplied by 100, divided by the number of cubic centimeters of the sample used.

BISMUTHATE METHOD. * 112

Reagents.—1. Nitric acid. Dilute 1 part of concentrated nitric acid with 4 parts of distilled water. Free the dilute acid from brown oxides of nitrogen by aeration.

2. Sulfuric acid. Dilute 1 part of concentrated sulfuric acid with 3 parts of distilled water.

3. Dilute sulfuric acid. Dilute 25 cc. of concentrated acid to 1 liter with distilled water. Add enough permanganate solution to color faintly the dilute acid.

4. Standard manganous sulfate. The standard solution of manganous sulfate prepared as described under persulfate method (p. 48) should be used and the standards should be prepared by following the same procedure as is used for the sample. This solution is more permanent than a solution of potassium permanganate, which may, however, be used. To prepare it dissolve 0.288 gram of potassium permanganate in distilled water and dilute the solution to 1 liter.

5. Sodium bismuthate. Purest dry salt.

Procedure.—Use an amount of the sample that contains not more than 0.2 mg. of manganese. Add 0.5 cc. of sulfuric acid and evaporate to dryness. Heat until the sulfuric acid is volatilized and ignite the residue. Dissolve in 40 cc. of nitric acid, add about 0.5 gram of sodium bismuthate, and heat until the permanganate color disappears. Add a few drops of a solution of ammonium or
sodium bisulfate to clear the solution and again boil to expel oxides of nitrogen. Remove from the source of heat, cool to 20° C., again add 0.5 gram of sodium bismuthate, and stir. When the maximum permanganate color has developed, filter through an alundum or Gooch crucible containing an asbestos mat ignited and washed with potassium permanganate. Wash the precipitate with dilute sulfuric acid until the washings are colorless. Transfer the filtrate to a 50 cc. Nessler tube and compare the color of it with that of standards prepared from the potassium permanganate solution. To prepare the standards, dilute portions of 0.2, 0.4, 0.6 cc., etc. of the permanganate solution to 50 cc. with dilute sulfuric acid. The content of manganese is calculated as described under persulfate method (p. 49).

**LEAD, ZINC, COPPER, AND TIN.**

Determinations of lead, zinc, copper, and tin are important in certain mining regions and in places where the water has a solvent action on pipes and other containers. The use of certain "germicides" also makes it necessary to test for some of these metals.

Lead, zinc, and copper may be determined colorimetrically or electrolytically. The colorimetric methods are not so accurate as a combination of both, and are chiefly of value as qualitative tests.

It is possible to make a rough estimation of the amount of lead in clear waters by acidifying with acetic acid, saturating with hydrogen sulfide, and comparing the color produced with that produced by standard lead solutions in Nessler tubes, treated in similar manner. This method, however, is not applicable if the water is colored or contains iron.

**Reagents.**—1. Standard lead solution. Dissolve 1.60 grams of lead nitrate (Pb(NO₃)₂) in 1 liter of distilled water. One cc. of this solution contains 1 mg. of lead (Pb). As a check it is desirable to determine lead as sulfate in a measured portion of this solution.

2. Standard copper solution. Dissolve about 0.8 gram of copper sulfate crystals (CuSO₄·5H₂O) in water and, after the addition of 1 cc. of concentrated sulfuric acid, dilute the solution to 1 liter. Determine the copper in 100 cc. of this solution in the usual way by electrolytic deposition. Dilute the solution so that 1 cc. contains 0.2 milligram copper (Cu). This solution is permanent.

3. Ammonium chloride. Twenty-five per cent solution.

4. Ammonium acetate. Fifty per cent solution.
5. Ammonium hydroxide. (Sp. gr. 0.96.)
7. Potassium sulfide. An alkaline solution of potassium sulfide made by mixing equal volumes of 10 per cent potassium hydroxide and a saturated aqueous solution of hydrogen sulfide.
10. Alcohol. Ninety-five per cent.
11. Alcohol. Fifty per cent.
14. Nitric acid. Dilute 1 part of the concentrated acid to 10 parts with distilled water.
15. Hydrochloric acid. (Sp. gr. 1.20.)
16. Sulfuric acid. Concentrated acid (Sp. gr. 1.84).
17. Sulfuric acid. Dilute the concentrated acid with an equal volume of distilled water.

LEAD.

Concentrate (1)* rapidly by boiling in a 7-inch porcelain dish over a free flame 3 or 4 liters of the sample to be tested, or more if very small amounts of the metals are present, to a volume of about 30 cc. Add 10 or 15 cc. of ammonium chloride solution to assist in the separation of the sulfides, then add a few drops of concentrated ammonium hydroxide, and saturate with hydrogen sulfide. Allow to stand some time, preferably over night, add a little more ammonium hydroxide and hydrogen sulfide, boil the contents of the dish a few minutes, and filter. The precipitate (2) may consist of lead, zinc, copper, and iron sulfides and the suspended organic matter. The soluble coloring matter is in the filtrate (3). Wash the precipitate a few times with hot water, place the precipitate and the filter paper in the original dish and boil with dilute nitric acid, rubbing down the sides of the dish, if necessary, to detach any adhering sulfide precipitate. After again filtering and washing several times with hot water, evaporate the filtrate and washings in the original dish to a bulk of 10 to 15 cc., cool, add 5 cc. of concentrated sulfuric acid, and heat until copious fumes of sulfuric acid are evolved.

*The numbers in parentheses refer to tables 10–12, pages 55–56.
If lead is present dilute the contents of the dish slightly with water, and treat them with 150 cc. of 50 per cent alcohol, in which the lead sulfate is insoluble. Allow to stand some time, preferably over night, filter off the lead sulfate, and wash it with 50 per cent alcohol. Save the filtrate for the determination of zinc.

Dissolve the precipitate of lead sulfate by boiling the filter containing it in ammonium acetate solution in a porcelain dish. (4). Filter into a 50 cc. Nessler tube and wash the filter with boiling water containing a little ammonium acetate. Divide this filtrate in halves and treat one-half with saturated hydrogen sulfide water in order to get an approximation of the amount of lead present. To the other half, or an aliquot portion, if a large amount of lead is present, add a few drops of acetic acid, then an excess of saturated hydrogen sulfide solution, and compare the color with that of standards made by treating known amounts of the standard lead solution with a little acetic acid, ammonium acetate, and hydrogen sulfide.

**ZINC.**

If zinc is present and copper is absent concentrate the filtrate from the lead sulfate to expel the alcohol, and remove the iron by adding an excess of ammonium hydroxide. Filter, wash, and acidify the filtrate with sulfuric acid. Concentrate the filtrate to about 150 cc. and transfer to a weighed platinum dish. Add 2 grams of potassium oxalate and 1.5 grams of potassium sulfate. Deposit the zinc electrolytically by means of a current of about 0.3 ampere for three hours. After deposition is complete and while the current is on, siphon off the solution and at the same time run into the dish a stream of distilled water in order to expel the free sulfuric acid, which might dissolve some of the zinc if the circuit were broken. After the acid has been removed break the circuit, wash the dish with water, then with 95 per cent alcohol, dry at 70° C., cool, and weigh it. The difference between this weight (10) and the weight of the platinum dish equals the amount of metallic zinc. Some difficulty has been experienced in this determination in obtaining pure reagents. It is therefore advisable to make blank determinations with each new lot of reagents and to correct the results if necessary.

If copper also is present (5) concentrate the filtrate from the lead sulfate until the alcohol is expelled, and add an excess of
ammonium hydroxide. (6) Remove any iron precipitate by filtration. Neutralize the filtrate (7) with sulfuric acid, and add 2 cc. of concentrated sulfuric acid and 1 gram of urea. Electrolyze the solution and determine copper colorimetrically as described in the procedure for copper (p. 54). After the copper has been deposited add ammonium hydroxide to the solution containing the zinc until nearly all the sulfuric acid has been neutralized, concentrate to slightly less than the capacity of the platinum dish, add 1.5 grams of potassium sulfate and 2 grams of potassium oxalate, and electrolyze for zinc. As this solution is usually saturated with ammonium salts due to neutralizing the large quantity of sulfuric acid, it is frequently impossible to get the zinc deposited firmly on the dish before the salts interfere by crystallization. To avoid this difficulty, dilute half the solution and electrolyze it for zinc; or, if the amount of zinc is very small, precipitate the zinc as sulfide in acetic acid solution, wash, ignite to oxide, and weigh the precipitate. This difficulty will not be encountered if copper is absent as there will then be no excess of ammonium salts.

If lead and copper are known to be absent and zinc alone is to be determined (13), after treating with sulfuric acid for separation of lead, slightly dilute the contents of the dish. Add an excess of ammonium hydroxide to precipitate iron and filter. Make the filtrate slightly acid with sulfuric acid, concentrate to about 150 cc., transfer to a weighed platinum dish, add potassium oxalate and sulfate, and electrolyze the solution as described for deposition of zinc.

COPPER."

Use 1 liter of a sample containing 0.1 to 1.0 part per million of copper, and proportionate amounts for other concentrations. Evaporate to about 75 cc., and wash into a 100 cc. platinum dish. Add 2 cc. of dilute sulfuric acid for clear and soft waters; add more acid to very alkaline waters to offset the alkalinity; add 5 cc. of acid to waters carrying much organic matter or clay to insure the formation of a soluble copper salt. Then place the dish as the anode in a direct current circuit, suspend a spiral wire cathode in the solution so that it is parallel to and about half an inch from the bottom of the dish, and close the circuit.

Electrolyze for about four hours with occasional stirring, or over night, if convenient. The current may be supplied by two gravity
cells in series, yielding a current through the solution of about 0.02 ampere. Lift out the cathode without previously opening the circuit, and immerse the spiral in a small amount of dilute nitric acid previously heated to boiling. Wash off the wire and evaporate the nitric acid solution to dryness on the water-bath. If the presence of silver is suspected add a few drops of hydrochloric acid before evaporation. Dissolve the residue in water and wash it into a 50 cc. Nessler tube. Dilute to 50 cc. and add 10 cc. of the potassium sulfide solution. The color of the copper sulfide develops at once and is fairly permanent, lasting at least several hours. Add 10 cc. of the potassium sulfide solution to a similar tube containing 50 cc. of distilled water, and then add to it standard copper solution in 0.2 cc. portions until the colors of the two tubes match. If 1 liter of the sample is used copper in parts per million is equal to the number of cubic centimeters of standard copper solution required to match the color of the sample multiplied by 0.2.

TIN.

Small quantities of tin are occasionally found in waters that have passed through tin or tin-lined pipes. This metal, if present, is precipitated with the iron by ammonia in the lead, zinc, and copper separations. In the method for copper alone, it is removed in the same way and may be further avoided by dissolving the sulfides in concentrated nitric acid. Any tin present will then separate as an insoluble compound, which may be ignited and weighed as the oxide (SnO₂).

The following schematic tables illustrate the procedures given.
Table 10.—Scheme for the Separation of Lead, Zinc, and Copper.

1. Concentrate sample. Add 10 cc. NH₄Cl, a few drops NH₄OH and saturate with H₂S. Allow to stand, add more NH₄OH and H₂S. Boil, filter, and wash.

2. Dissolve the precipitate in dilute HNO₃. Filter and wash. Evaporate to 10 or 15 cc. Cool. Add 5 cc. concentrated H₂SO₄ and heat until white fumes are given off. Dilute slightly and treat with 150 cc. of 50 per cent alcohol. Allow to stand; filter, and wash with 50 per cent alcohol.

3. Reject the filtrate which contains the coloring matter.

4. The precipitate contains the Pb. Dissolve in NH₄C₂H₃O₂ solution. Filter into a 50 cc. Nessler tube and wash with water containing NH₄C₂H₃O₂. Divide filtrate in halves. Saturate one half with H₂S. Determine the Pb in the other half by adding HCl,H₂O and H₂S and comparing with standards containing known amounts of Pb.

5. The filtrate contains the Zn and Cu. Concentrate to expel alcohol. Add excess of NH₄OH, filter and wash precipitate.

6. Reject the precipitate which contains the Fe.

7. The filtrate contains the Zn and Cu. Neutralise with H₂SO₄. Add 10 cc. concentrated H₂SO₄ and 1 g. urea. Electrolyze for two hours with a current of 0.5 ampere. Break circuit, empty dish and wash.

8. The deposit is Cu. Immerse the cathode in a small amount of hot, dilute HNO₃; wash off and evaporate to dryness. Take up in water and wash into a Nessler tube. Make up to mark, and add 10 cc. of potassium sulfoend solution. Compare with standard. If large amount is present, dry and weigh as Cu.

9. The solution contains the Zn. Nearly neutralize with NH₄OH. Concentrate to less than the capacity of the dish. Add 2 g. K₂C₂O₄ and 1.5 g. K₂SO₄. Electrolyze for 3 hours with a current of 0.3 ampere. Siphon off solution, break circuit, wash with water, then alcohol, dry at 70° C., cool and weigh.

10. The weighed residue is metallic Zn.

Table 11.—Scheme for Determination of Copper Only.

11. Concentrate sample to 75 cc. Add 2 cc. conc. H₂SO₄ for clear, soft waters and 5 cc. for alkaline or turbid waters. Electrolyze following procedure in 7 and 8.
Table 12.—Scheme for determination of zinc only.

13. Follow scheme for all three metals as given in Table 10 through section 5. Nearly neutralize the filtrate with H₂SO₄, concentrate to less than the capacity of the dish and electrolyze as directed in section 9.

MINERAL ANALYSIS.

RESIDUE ON EVAPORATION.

See description of method (p. 29). The residue should be dried one hour at 180° C. Turbid waters should be filtered, and the composition of the suspended matter should be determined separately or the amount of it reported as suspended matter.

ALKALINITY AND ACIDITY.

See description of method (pp. 35–41).

CHLORIDE.

See description of method (pp. 41–43).

NITRATE! NITROGEN.

See description of method (pp. 23–25).

SEPARATION OF SILICA, IRON, ALUMINIUM, CALCIUM, AND MAGNESIUM.¹⁰ ⁴⁸

SILICA.

Evaporate in platinum 100 to 1,000 cc. of the sample or sufficient if possible to form a residue weighing 0.4 to 0.6 gram, and preferably containing 0.1 to 0.2 gram of calcium. When the residue is nearly dry add 1 cc. of hydrochloric acid (1 to 1) and, after moistening the sides of the dish, evaporate to dryness. Dry at 180° C. and if much organic matter is present char it in a radiator. Moisten the residue with dilute hydrochloric acid and expel the excess of acid by heating on the water bath. Add a few drops of hydrochloric acid, dissolve in hot water, and filter. Wash the residue with hot water. Evaporate the filtrate to dryness, repeat the filtration, and combine the two residues. If great accuracy is not required the second evaporation with hydrochloric acid may be omitted. Ignite and weigh the insoluble residue. Add 2 drops of concentrated sulfuric acid and a little hydrofluoric acid, volatilize the acids, ignite, and weigh again. Report the loss in weight.
as silica (SiO₂). A weight of non-volatile matter exceeding 0.5 mg. should be analyzed.

IRON AND ALUMINIUM.

Heat to boiling the filtrate from the insoluble residue, oxidize with concentrated nitric acid or bromine, and concentrate to about 25 cc. Add ammonium hydroxide in slight excess, boil one minute, and filter. Dissolve the precipitate on the filter in a small amount of hot dilute hydrochloric acid. Reprecipitate with ammonium hydroxide, filter, and wash. Unless very accurate results are necessary this solution and reprecipitation may be omitted. Unite the two filtrates for determination of calcium. Ignite and weigh the precipitate. It will comprise oxides of iron and aluminium and phosphate. If much phosphate is present it should be determined in a separate sample and a correction for the amount applied; otherwise it may be neglected. Determine the iron in the ignited precipitate by fusion with sodium or potassium pyrosulfate, reduction with zinc, and titration with potassium permanganate. Aluminium (Al) is calculated as follows:

\[ Al = 0.53[(Fe₂O₃ + Al₂O₃) - 1.43 \text{ Fe}] \]

CALCIUM.

Concentrate the filtrate from the separation of iron and aluminium to about 100 cc., and add an excess of concentrated solution of ammonium oxalate, little by little, to the hot ammoniacal solution. Keep the solution warm and stir at intervals till the precipitate settles readily and leaves a clear supernatant liquid. Filter, dissolve the precipitate in a little hot dilute hydrochloric acid, and reprecipitate with ammonium hydroxide and ammonium oxalate. If great accuracy is not required this solution and reprecipitation may be omitted, and the first precipitate may be washed clean with hot water. Save the filtrate for determination of magnesium. Ignite the precipitate and weigh it as calcium oxide, 71.5 per cent of which is the equivalent of calcium (Ca); or dissolve the precipitate in hot 2 per cent sulfuric acid and titrate with a standard solution of potassium permanganate.

MAGNESIUM.

Acidify with hydrochloric acid the filtrate from the separation of calcium and concentrate it to about 100 cc. Add 20 cc. of a saturated solution of microcosmic salt, cool, and make slightly but
distinctly alkaline by adding ammonium hydroxide, drop by drop. Allow the solution to stand four hours, then filter and wash with 3 per cent ammonium hydroxide. Dissolve the precipitate, especially in the presence of large amounts of sodium or potassium, in a slight excess of dilute hydrochloric acid and reprecipitate the magnesium with ammonium hydroxide and a few drops of microcosmic salt solution. If great accuracy is not required this solution and reprecipitation may be omitted. Ignite the precipitate and weigh it as magnesium pyrophosphate \((\text{Mg}_2\text{P}_2\text{O}_7)\), 21.9 per cent of which is the equivalent of magnesium \((\text{Mg})\). If manganese is present it is precipitated with the magnesium and a correction for it should be applied after having determined manganese in a separate sample. The weight of manganese pyrophosphate \((\text{Mn}_2\text{P}_2\text{O}_7)\) is 2.58 times the weight of manganese.

**SEPARATION OF SULFATE, SODIUM, AND POTASSIUM.**

**SULFATE.**

Evaporate to dryness 100 to 1,000 cc. of the sample, or sufficient to obtain a residue weighing 0.4 to 0.6 gram and containing preferably 0.05 to 0.2 gram of sodium. Acidify the residue with hydrochloric acid and remove the silica, iron, and aluminium (pp. 56–57). Make acid and add a hot solution of barium chloride in slight excess to the hot filtrate, and warm it, stirring at intervals for one-half hour, until the precipitate settles readily and leaves a clear supernatant liquid. Dry, ignite, and weigh the precipitate of barium sulfate, 41.1 per cent of which is equal to the content of sulfate \((\text{SO}_4)\).

**SODIUM, POTASSIUM, AND LITHIUM.**

Evaporate to dryness the filtrate from the precipitation of barium sulfate. Add a few cubic centimeters of hot water and then a saturated solution of barium hydroxide until a slight film collects on the top of the solution. Filter and wash the precipitate with hot water. Add to the filtrate an excess of ammonium hydroxide and ammonium carbonate solution. Filter, evaporate the filtrate to dryness, dry, and ignite at low red heat to expel ammonium salts. Repeat the operations including the addition of barium hydroxide until no precipitate is obtained by barium hydroxide or by ammonium hydroxide and ammonium carbonate. Evaporate the final filtrate to dryness in a weighed platinum dish, dry, cool,
and weigh the residue. Dissolve the residue in a few cubic centimeters of water, filter, wash the filter paper twice with hot water, then ignite the filter paper in the platinum dish. Cool and weigh the residue. Subtract this weight from the first weight including the residue. The difference is the weight of the chlorides of sodium and potassium and lithium. If it is not desired to separate sodium and potassium the weight of sodium and potassium as sodium may be calculated from this difference by multiplying it by 0.394.

POTASSIUM.

First procedure.—Add to the solution of the chlorides of sodium and potassium a few drops of dilute hydrochloric acid (1 to 3) and 1 cc. of 10 per cent platinic chloride (PtCl₄) for each 30 mg. of the combined chlorides. Evaporate to a thick syrup on the water bath, then remove dish and allow it to come to dryness at laboratory temperature. Treat the residue cold with 80 per cent alcohol and filter. Wash the precipitate with 80 per cent alcohol until the filtrate is no longer colored. Dry the precipitate and dissolve it in hot water. Evaporate the solution to dryness in a platinum dish and weigh it as potassium platinic chloride (K₂PtCl₆). The weight of potassium (K) is 16.1 per cent of this weight and the equivalent of potassium chloride (KCl) is 30.7 per cent of this weight. Subtract the equivalent weight of potassium chloride from the weight of the combined chlorides. The weight of the sodium is 39.4 per cent of the difference.

Second procedure.—Add to the hot solution of the combined chlorides 20 per cent perchloric acid (HClO₄) slightly in excess of the amount required to combine with the bases. One cubic centimeter of 20 per cent perchloric acid is equivalent to 90 mg. of potassium. Evaporate the solution to dryness, dissolve the residue in 10 cc. of hot water and a small amount of perchloric acid, and again evaporate to dryness. Repeat the addition of water, perchloric acid, and evaporation until white fumes appear on evaporating to dryness. Add to the residue 25 cc. of 96 per cent alcohol containing 0.2 per cent of perchloric acid (1 cc. of 20 per cent perchloric acid in 100 cc. of 98 per cent alcohol). Break up the residue with a stirring rod. Decant the supernatant liquid through a weighed Gooch crucible that has been washed with 0.2 per cent perchloric acid in alcohol. If the precipitate is unusually large dissolve it in hot water and repeat the evaporation with perchloric acid. Wash
the precipitate once by decantation with the 0.2 per cent perchloric acid in alcohol, transfer the precipitate to the crucible, and wash it several times with a 0.2 per cent perchloric acid in alcohol. Dry the crucible at 120-130° C. for one hour, cool, and weigh it. The increase in weight is potassium perchlorate (KClO₄). The equivalent weight of potassium is 28.2 per cent and the equivalent weight of potassium chloride is 53.8 per cent of the potassium perchlorate. Calculate the content of sodium by difference.

**LITHIUM.**

Use a large quantity of the sample. Obtain the combined chlorides of sodium, potassium, and lithium (see pp. 58-59). Transfer the combined chlorides to a small Erlenmeyer flask (50 or 100 cc. capacity) and evaporate the solution nearly, but not quite, to dryness. Add about 30 cc. of redistilled amyl alcohol. Connect the flask, the stopper of which carries a thermometer, with a condenser* and boil until the temperature rises approximately to the boiling point of amyl alcohol (130° C.), showing that all the water has been driven off. Cool slightly and add a drop of hydrochloric acid to convert small amounts of lithium hydroxide to lithium chloride. Connect with the condenser and continue the boiling to drive off again all water and until the temperature reaches the boiling point of amyl alcohol. The content of the flask at this time is usually 15 to 20 cc. Filter through a small paper or a Gooch crucible into a graduated cylinder and note exact quantity of filtrate, which determines the subsequent correction. Wash the precipitate with small quantities of dehydrated amyl alcohol. Evaporate the filtrate and washings in a platinum dish to dryness on the steam bath, dissolve the residue in water, and add a few drops of sulfuric acid. Evaporate on a steam bath and expel the excess of sulfuric acid by gentle heat over a flame. Repeat until carbonaceous matter is completely burned off. Cool and weigh the dish and contents. Dissolve in a small quantity of hot water, filter through a small filter, wash, and return filter to dish, ignite, and weigh. The difference between the original weight of dish and contents and the weight of the dish and small amount of residue equals the weight of impure lithium sulfate. The purity of the lithium sulfate should be tested by adding small amounts of

---

*The amyl alcohol may be boiled off without the use of a condenser, but the vapors are very disagreeable.
ammonium phosphate and ammonium hydroxide, which will precipitate any magnesium present with the lithium sulfate. Any precipitate appearing after standing over night should be collected on a small filter and weighed as magnesium pyrophosphate, calculated to sulfate, and subtracted from the weight of impure lithium sulfate. From this weight subtract 0.00113 gram for every 10 cc. of amyl alcohol filtrate exclusive of the amyl alcohol used in washing residue because of the slight solubility of solid mixed chlorides in amyl alcohol. Calculate lithium from the corrected weight of lithium sulfate. Dissolve the mixed chlorides from flask and filter with hot water, evaporate to dryness, ignite gently to remove amyl alcohol, filter and thoroughly wash; concentrate the filtrates and washings to 25 to 50 cc.

To the weight of potassium chloride add 0.00051 gram for every 10 cc. of amyl alcohol used in the extraction of the lithium chloride, which corrects for the solubility of the potassium chloride in amyl alcohol. Calculate to potassium.

The weight of sodium chloride is found by subtracting the combined weights of lithium chloride and potassium chloride (corrected) from the total weight of the three chlorides. Calculate sodium chloride to sodium.

**BROMINE, IODINE, ARSENIC, AND BORIC ACID.**

Evaporate to dryness a large quantity of the sample to which a small amount of sodium carbonate has been added. Boil the residue with distilled water, transfer it to a filter, and thoroughly wash it with hot water. Dilute the alkaline filtrate to a definite volume, and determine bromine and iodine, arsenic, and boric acid in aliquot portions of it.

**BROMINE AND IODINE.**

*Reagents.*—1. Sulfuric acid. 1 to 5.
2. Potassium nitrite or sodium nitrite. Two per cent solution.
4. Iodine standards. Acidify with dilute sulfuric acid measured quantities of a standard solution of potassium iodide in small tubes. Add 3 or 4 drops of the potassium nitrite solution and extract with carbon bisulfide as in the actual determination. Transfer to small flasks the standards from which the iodine has been removed.
6. Bromine standards. Add measured quantities of a standard solution of a bromide to the liquid in each of the small flasks from which the iodine has been removed. Add to each 5 cc. of purified carbon bisulfide, and proceed exactly as with the sample.

Procedure.—Evaporate to dryness an aliquot portion of the alkaline filtrate. Dissolve the residue in 2 or 3 cc. of water, and add enough absolute alcohol to make the percentage of alcohol about 90. Boil and filter and repeat the extraction of the residue with alcohol once or twice. Add 2 or 3 drops of sodium hydroxide to the combined alcoholic filtrates and evaporate to dryness. Dissolve the residue in 2 or 3 cc. of water and repeat the extraction with alcohol and the filtration. Add a drop of sodium hydroxide to the filtrate and evaporate it to dryness. Dissolve the residue in a little water. Acidify this solution with dilute sulfuric acid, adding 3 or 4 drops excess, and transfer it to a small flask. Add 4 drops of the solution of potassium nitrite or sodium nitrite and about 5 cc. of carbon bisulfide. Shake the mixture until all the iodine is extracted. Separate the acid solution from the carbon bisulfide by filtration. Wash the flask, filter, and contents with cold distilled water, and transfer the carbon bisulfide containing the iodine in solution to Nessler tubes by means of about 5 cc. of pure carbon bisulfide. In washing the filter, dilute the contents of the tube to a definite volume, usually 12 or 15 cc., and compare the color with that of known amounts of iodine dissolved in carbon bisulfide in other tubes.

Transfer to a small flask the sample from which the iodine has been removed. Add saturated chlorine water, 1 cc. at a time, shaking after each addition until all the bromine is freed. Care must be taken not to add much more chlorine than that necessary to free the bromine, since an excess of reagent may form a bromochloride that spoils the color reaction. Separate the water solution from the carbon bisulfide by filtration through a moistened filter, wash the contents of the filter two or three times with water, and then transfer them to a Nessler tube by means of about 1 cc. of carbon bisulfide. Repeat this extraction of the filtrate twice, using 3 cc. of carbon bisulfide each time. The combined carbon bisulfide extracts usually amount to 11.5 to 12 cc. Add enough carbon bisulfide to the tubes to bring them to a definite volume, usually 12 to 15 cc., and compare the sample with the standards. If much bromine is present it is not always completely extracted.
by the amounts of carbon bisulfide recommended. If the extraction is incomplete, therefore, make one or two extra extractions with carbon bisulfide, transfer the extracts to another tube, and compare the color with that of the standards.

**ARSENIC.**

Evaporate to dryness an aliquot portion of the alkaline filtrate (p. 61). Acidify the residue with arsenic-free sulfuric acid, and subject it to the action of arsenic-free zinc and sulfuric acid in a Marsh-Berzelius apparatus. Compare the mirror obtained with a mirror obtained from an arsenious oxide solution of known strength.

**BORIC ACID.**

Evaporate to dryness an aliquot portion of the alkaline filtrate (p. 61), treat the residue with 1 or 2 cc. of water, and slightly acidify the solution with hydrochloric acid. Add about 25 cc. of absolute alcohol, boil, filter, and repeat the extraction of the residue. Make the filtrate slightly alkaline with sodium hydroxide, and evaporate it to dryness. Add a little water, slightly acidify with hydrochloric acid, and place a strip of turmeric paper in the liquid. Evaporate to dryness on the steam bath, and continue the heating until the turmeric paper is dry. If boric acid is present the turmeric paper becomes cherry red. It is not usually necessary to determine quantitatively boric acid; the quantitative method devised by Gooch is recommended.

**HYDROGEN SULFIDE.**

Hydrogen sulfide should be determined preferably in the field; the procedure as far as the final titration with sodium thiosulfate must be carried out in the field.

*Reagents.*—1. N/100 sodium thiosulfate.

2. Standard iodine. A N/100 solution containing potassium iodide standardized against the N/100 sodium thiosulfate. To standardize, add 10 cc. of the iodine solution to 500 cc. of boiled distilled water. Add about 1 gram of potassium iodide, and titrate with N/100 sodium thiosulfate in the presence of starch indicator. One cc. of N/100 iodine is equivalent to 0.17 mg. H₂S.


4. Starch. A freshly prepared solution for use as indicator.
Procedure.—Add 500 cc. of the sample to 10 cc. of the standard iodine solution and 1 gram of potassium iodide in a large glass-stoppered bottle or flask. If the sample is to be collected from a tap lead the water into the bottle through a rubber tube extending to the bottom of the bottle so as to eliminate errors due to aeration. Shake the bottle, allow it to stand for a few minutes, and then titrate the excess of iodine with sodium thiosulfate in the presence of starch indicator. Hydrogen sulfide (H₂S) in parts per million is equal to 0.34 times the difference in cubic centimeters between the amount of iodine solution added and the amount of N/100 thiosulfate used in the titration.

CHLORINE.

In waters that have been treated with calcium hypochlorite or liquid chlorine it is frequently advisable to ascertain the presence or absence of chlorine. As the reagents which have been proposed for its detection are not specific for chlorine but give similar or identical reactions with oxidizing agents or reducible substances care must be exercised in interpreting the results of such tests; nitrites and ferric salts are of common occurrence, and chlorates also may lead to misinterpretation in waters treated with calcium hypochlorite.

Reagents.—1. Tolidin solution. One gram of o-tolidin, purified by being recrystallized from alcohol, is dissolved in 1 liter of 10 per cent hydrochloric acid.

2. Copper sulfate solution. Dissolve 1.5 grams of copper sulfate and 1 cc. of concentrated sulfuric acid in distilled water and dilute the solution to 100 cc.

3. Potassium bichromate solution. Dissolve 0.025 gram of potassium bichromate and 0.1 cc. of concentrated sulfuric acid in distilled water and dilute the solution to 100 cc.

Procedure.—Mix 1 cc. of the toolidin reagent with 100 cc. of the sample in a Nessler tube and allow the solution to stand at least 5 minutes. Small amounts of free chlorine give a yellow and larger amounts an orange color.

For quantitative determination compare the color with that of standards in similar tubes prepared from the solutions of copper sulfate and potassium bichromate. The amounts of solution for various standards are indicated in Table 13.
Table 13.—Preparation of Permanent Standards for Content of Chlorine.

<table>
<thead>
<tr>
<th>Chlorine.</th>
<th>Solution of copper sulfate.</th>
<th>Solution of potassium bichromate.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parts per million.</strong></td>
<td><strong>cc.</strong></td>
<td><strong>cc.</strong></td>
</tr>
<tr>
<td>0.01</td>
<td>0.0</td>
<td>0.8</td>
</tr>
<tr>
<td>0.02</td>
<td>0.0</td>
<td>2.1</td>
</tr>
<tr>
<td>0.03</td>
<td>0.0</td>
<td>3.2</td>
</tr>
<tr>
<td>0.04</td>
<td>0.0</td>
<td>4.3</td>
</tr>
<tr>
<td>0.05</td>
<td>0.4</td>
<td>5.5</td>
</tr>
<tr>
<td>0.06</td>
<td>0.8</td>
<td>6.6</td>
</tr>
<tr>
<td>0.07</td>
<td>1.2</td>
<td>7.5</td>
</tr>
<tr>
<td>0.08</td>
<td>1.5</td>
<td>8.7</td>
</tr>
<tr>
<td>0.09</td>
<td>1.7</td>
<td>9.0</td>
</tr>
<tr>
<td>0.10</td>
<td>1.8</td>
<td>10.0</td>
</tr>
<tr>
<td>0.20</td>
<td>1.9</td>
<td>20.0</td>
</tr>
<tr>
<td>0.30</td>
<td>1.9</td>
<td>30.0</td>
</tr>
<tr>
<td>0.40</td>
<td>2.0</td>
<td>38.0</td>
</tr>
<tr>
<td>0.50</td>
<td>2.0</td>
<td>45.0</td>
</tr>
</tbody>
</table>

**Reagents.**—1. Sulfuric acid, concentrated. (Sp. gr. 1.83–1.84.)

2. Potassium permanganate. Dissolve 6.32 grams of the salt in water and dilute the solution to 1 liter.

3. Potassium oxalate. A 2 per cent solution.

4. Manganous sulfate. Dissolve 480 grams of the salt in water and dilute the solution to 1 liter.

5. Alkaline potassium iodide. Dissolve 700 grams of potassium hydroxide and 150 grams of potassium iodide in water and dilute the solution to 1 liter.

6. Hydrochloric acid. Concentrated (Sp. gr. 1.18–1.19).

7. Sodium thiosulfate. A N/40 solution. Dissolve 6.2 grams of chemically pure recrystallized sodium thiosulfate in water and dilute the solution to 1 liter with freshly boiled distilled water. Each cc. is equivalent to 0.2 mg. of oxygen or to 0.1395 cc. of oxygen at 0° C. and 760 mm. pressure. Inasmuch as this solution is not permanent it should be standardized occasionally against a N/40 solution of potassium bichromate. The keeping qualities of the thiosulfate solution are improved by adding to each liter 5 cc. of chloroform and 1.5 grams of ammonium carbonate before diluting to the prescribed volume.

8. Starch solution. Mix a small amount of clean starch with cold water until it becomes a thin paste and stir this mass into 150 to 200 times its weight of boiling water. Boil for a few minutes,
then sterilize. It may be preserved by adding a few drops of chloroform.

*Collection of sample.*—Collect the sample in a narrow-necked glass-stoppered bottle of 250 to 270 cc. capacity. The following procedure should be followed in order to avoid entrainment or absorption of atmospheric oxygen. In collecting from a tap fill the bottle through a glass or rubber tube extending well into the tap and to the bottom of the bottle. To avoid air bubbles allow the bottle to overflow for several minutes, and then carefully replace the glass stopper so that no air bubble is entrained. In collecting from the surface of a pond or tank connect the sample bottle to a bottle of 1 liter capacity. Provide each bottle with a two-hole rubber stopper having one glass tube extending to the bottom and another glass tube entering but not projecting into the bottle. Connect the short tube of the sample bottle with the long tube of the liter bottle. Immerse the sample bottle in the water and apply suction to the outlet of the liter bottle. To collect a sample at any depth arrange the two bottles so that the outlet tube of the liter bottle is at a higher elevation then the inlet tube of the sample bottle. Lower the two bottles, in any convenient form of cage properly weighted, to the desired depth. Water entering during the descent will be flushed through into the liter bottle. When air bubbles cease rising to the surface raise the bottles. Finally replace the perforated stopper of the sample bottle with a glass stopper in such manner as to avoid entraining bubbles of air.

*Procedure.*—Remove the stopper from the bottle and add, first, 0.7 cc. of the concentrated sulfuric acid, and then 1 cc. of the potassium permanganate solution. These and all other reagents should be introduced by pipette under the surface of the liquid. Insert the stopper and mix by inverting the bottle several times. After 20 minutes have elapsed destroy the excess of permanganate by adding 1 cc. of the potassium oxalate solution, the bottle being at once restoppered and its contents mixed. If a noticeable excess of potassium permanganate is not present at the end of 20 minutes, again add 1 cc. of the potassium permanganate solution. If this is still insufficient use a stronger potassium permanganate solution. After the liquid has been decolorized by the addition of potassium oxalate add 1 cc. of the manganous sulfate solution and 3 cc. of the alkaline potassium iodide solution. Allow the precipitate to settle. Add 2 cc. of the hydrochloric acid and mix by shaking.

The procedure to this point must be carried out in the field, but
after the acid has been added and the stopper replaced there is no further change, and the rest of the test may be performed within a few hours, as convenient. Transfer 200 cc. of the contents of the bottle to a flask and titrate with N/40 sodium thiosulfate, using a few cubic centimeters of the starch solution as indicator toward the end of the titration. Do not add the starch solution until the color has become faint yellow, and titrate until the blue color disappears.

The use of potassium permanganate is made necessary by high nitrite or organic matter. The procedure outlined must be followed in all work on sewage and partly purified effluents or seriously polluted streams or samples whose nitrite nitrogen exceeds 0.1 part per million. In testing other samples the procedure may be shortened by beginning with the addition of the manganous sulfate solution and proceeding from that point as outlined, except that only 1 cc. of alkaline potassium iodide need be added.

Calculation of Results.—Oxygen shall be reported in parts per million by weight. It is sometimes convenient to know the number of cubic centimeters per liter of the gas at 0° C. temperature and 760 mm. pressure and also to know the percentage which the amount of gas present is of the maximum amount capable of being dissolved by distilled water at the same temperature and pressure. If 200 cc. of the sample is taken the number of cubic centimeters of N/40 thiosulfate used is equal to parts per million of oxygen. Corrections for volume of reagents added amount to less than 3 per cent and are not justified except in work of unusual precision. To obtain the result in cubic centimeters per liter multiply the number of cubic centimeters of thiosulfate used by 0.698. To obtain the result in percentage of saturation divide the number of cubic centimeters of thiosulfate by the figure in Table 14 opposite the temperature of the water and under the proper chlorine figure. The last column of Table 14 permits interpolation for intermediate chlorine values. At elevations differing considerably from mean sea level and for accurate work attention must be given to barometric pressure, the normal pressure in the region being preferable to the specific pressure at the time of sampling. The term "saturation" refers to a condition of equilibrium between the solution and an oxygen pressure in the atmosphere corresponding to 158.8 millimeters, or approximately one-fifth atmosphere. The true saturation or equilibrium between the solution and pure oxygen is nearly five times this value, and
consequently values in excess of 100 per cent saturation frequently occur in the presence of oxygen-forming plants.

Table 14.—Solubility of Oxygen in Fresh Water and in Sea Water of Stated Degrees of Salinity at Various Temperatures When Exposed to an Atmosphere Containing 20.9 Per Cent of Oxygen Under a Pressure of 760 MM. \(^*\)

(Calculated by G. C. Whipple and M. C. Whipple from measurements of C. J. Fox.) \(^ {119} \)

<table>
<thead>
<tr>
<th>Temperature ( ^\circ \text{C.} )</th>
<th>Chloride in sea water (milligrams per liter)</th>
<th>Difference per 100 parts of chloride</th>
<th>Parts per million.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.</td>
<td>5000.</td>
<td>10000.</td>
</tr>
<tr>
<td>0.</td>
<td>14.62</td>
<td>13.79</td>
<td>12.97</td>
</tr>
<tr>
<td>1.</td>
<td>14.23</td>
<td>13.41</td>
<td>12.61</td>
</tr>
<tr>
<td>2.</td>
<td>13.84</td>
<td>13.05</td>
<td>12.28</td>
</tr>
<tr>
<td>3.</td>
<td>13.48</td>
<td>12.72</td>
<td>11.98</td>
</tr>
<tr>
<td>4.</td>
<td>13.13</td>
<td>12.41</td>
<td>11.69</td>
</tr>
<tr>
<td>5.</td>
<td>12.86</td>
<td>12.09</td>
<td>11.39</td>
</tr>
<tr>
<td>6.</td>
<td>12.48</td>
<td>11.79</td>
<td>11.12</td>
</tr>
<tr>
<td>7.</td>
<td>12.17</td>
<td>11.51</td>
<td>10.85</td>
</tr>
<tr>
<td>8.</td>
<td>11.87</td>
<td>11.24</td>
<td>10.61</td>
</tr>
<tr>
<td>9.</td>
<td>11.59</td>
<td>10.97</td>
<td>10.36</td>
</tr>
<tr>
<td>10.</td>
<td>11.33</td>
<td>10.73</td>
<td>10.13</td>
</tr>
<tr>
<td>11.</td>
<td>11.06</td>
<td>10.49</td>
<td>9.92</td>
</tr>
<tr>
<td>12.</td>
<td>10.83</td>
<td>10.28</td>
<td>9.72</td>
</tr>
<tr>
<td>13.</td>
<td>10.60</td>
<td>10.05</td>
<td>9.52</td>
</tr>
<tr>
<td>14.</td>
<td>10.37</td>
<td>9.85</td>
<td>9.32</td>
</tr>
<tr>
<td>15.</td>
<td>10.15</td>
<td>9.65</td>
<td>9.14</td>
</tr>
<tr>
<td>16.</td>
<td>9.95</td>
<td>9.46</td>
<td>8.96</td>
</tr>
<tr>
<td>17.</td>
<td>9.74</td>
<td>9.26</td>
<td>8.78</td>
</tr>
<tr>
<td>18.</td>
<td>9.54</td>
<td>9.07</td>
<td>8.62</td>
</tr>
<tr>
<td>19.</td>
<td>9.35</td>
<td>8.89</td>
<td>8.45</td>
</tr>
<tr>
<td>20.</td>
<td>9.17</td>
<td>8.73</td>
<td>8.30</td>
</tr>
<tr>
<td>21.</td>
<td>8.99</td>
<td>8.57</td>
<td>8.14</td>
</tr>
<tr>
<td>22.</td>
<td>8.83</td>
<td>8.42</td>
<td>7.99</td>
</tr>
<tr>
<td>23.</td>
<td>8.68</td>
<td>8.27</td>
<td>7.85</td>
</tr>
<tr>
<td>24.</td>
<td>8.53</td>
<td>8.12</td>
<td>7.71</td>
</tr>
<tr>
<td>25.</td>
<td>8.38</td>
<td>7.96</td>
<td>7.56</td>
</tr>
<tr>
<td>26.</td>
<td>8.22</td>
<td>7.81</td>
<td>7.42</td>
</tr>
<tr>
<td>27.</td>
<td>8.07</td>
<td>7.67</td>
<td>7.28</td>
</tr>
<tr>
<td>28.</td>
<td>7.92</td>
<td>7.53</td>
<td>7.14</td>
</tr>
<tr>
<td>29.</td>
<td>7.77</td>
<td>7.39</td>
<td>7.00</td>
</tr>
<tr>
<td>30.</td>
<td>7.63</td>
<td>7.25</td>
<td>6.86</td>
</tr>
</tbody>
</table>

\(^*\) Under any other barometric pressure, \( B \), the solubility can be obtained from the corresponding value in the table by the formula:

\[
\frac{B}{760} \times 29.92 = B' 
\]

where:

- \( B' \) = Solubility at \( B \) or \( B' \)
- \( S' \) = Solubility at 760 mm. or 29.92 inches
- \( B \) = Barometric pressure in mm.
- \( B' \) = Barometric pressure in inches.
ETHER-SOLUBLE MATTER.

Evaporate 500 cc. of the sample in a porcelain evaporating dish to a volume of about 50 cc. By means of a rubber-tipped glass rod remove to the bottom of the dish the solid matter attached to the sides, and add normal sulfuric acid to neutralize the alkalinity. Do not use an excess of acid. Then evaporate the contents of the dish to dryness. Treat the dry residue with boiling ether, rubbing the bottom and sides of the dish to insure complete solution of fat. Three extractions with ether are required. Filter the ether solution through a 5 cm. filter into a weighed flask having a wide mouth. Evaporate the ether slowly, and dry the flask at 100° C. for 30 minutes. The increase in weight of the flask gives the amount of fats, or, in more precise language, the ether-soluble matter.

An excess of acid gives too high results because of the formation of fatty-acid residues.

RELATIVE STABILITY OF EFFLUENTS.

Reagent.—Methylene blue solution. A 0.05 per cent aqueous solution of methylene blue, preferably the double zinc salt or commercial variety.

Collection of sample.—Collect the sample in a glass-stoppered bottle holding approximately 150 cc. If the dissolved oxygen is low observe precautions similar to those used in collecting samples for dissolved oxygen (p. 66).

Procedure.—Add 0.4 cc. of the methylene blue solution to the sample in the 150 cc. bottle. As methylene blue has a slightly antiseptic property be careful to add exactly 0.4 cc. Add the methylene blue solution preferably below the surface of the liquid after filling the bottle with the sample. If the methylene blue is added first do not allow the liquid to overflow as coloring matter will thus be lost. Incubate the sample at 20° C. for ten days. Four days' incubation may be considered sufficient for all practical purposes in routine plant-control work. If quick results are desired incubate the sample at 37° C. for five days using suitable stoppers to prevent the loss and reabsorption of dissolved oxygen. The bacterial flora at 37° C. is different from that at 20° C. The lower temperature is more nearly the average temperature of surface waters and therefore the higher temperature should be used only when quick approximate results are essential.
Observe the sample at least twice a day during incubation. Give a sample in which the methylene blue becomes decolorized a relative stability corresponding to the time required for reduction (see Table 15). For routine filter control ordinary room or cellar temperature gives fairly satisfactory results. For accurate studies, room temperature incubation is very undesirable, as the fluctuations in temperature which are ordinarily not noticed are responsible for appreciable deviations from the true values of relative stability. If the samples are incubated less than 10 days at 20° C. and are not decolorized place a plus sign after the stability value in order to indicate that the stability might have been higher if more time had been allowed. In applying this test to river waters it often happens that the blue coloring matter is removed either partly or completely through absorption by the clay which many rivers carry in suspension. True relative stabilities cannot be obtained for such waters except by determining the initial available oxygen at the start and the biochemical oxygen demand on incubation at 20° C. for 10 days (pp. 71–73). Germicides, such as hypochlorite of lime, if present in sufficient quantity, vitiate the results. If a sample contains free chlorine, therefore, store it about 2 hours, or until the chlorine is gone, and then add methylene blue.

Table 15 gives the relation between the time in days to decolorize methylene blue at 20° C. ($t_{20}$) and the relative stability number or ratio of available oxygen to oxygen required for equilibrium, expressed in percentage (S).

Table 15.—Relative Stability Numbers.

<table>
<thead>
<tr>
<th>Time required for decolorization at 20° C.</th>
<th>Relative stability.</th>
<th>Time required for decolorization at 20° C.</th>
<th>Relative stability.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>11</td>
<td>8.0</td>
<td>84</td>
</tr>
<tr>
<td>1.0</td>
<td>21</td>
<td>9.0</td>
<td>87</td>
</tr>
<tr>
<td>1.5</td>
<td>30</td>
<td>10.0</td>
<td>90</td>
</tr>
<tr>
<td>2.0</td>
<td>37</td>
<td>11.0</td>
<td>92</td>
</tr>
<tr>
<td>2.5</td>
<td>44</td>
<td>12.0</td>
<td>94</td>
</tr>
<tr>
<td>3.0</td>
<td>50</td>
<td>13.0</td>
<td>95</td>
</tr>
<tr>
<td>4.0</td>
<td>60</td>
<td>14.0</td>
<td>96</td>
</tr>
<tr>
<td>5.0</td>
<td>68</td>
<td>16.0</td>
<td>97</td>
</tr>
<tr>
<td>6.0</td>
<td>75</td>
<td>18.0</td>
<td>98</td>
</tr>
<tr>
<td>7.0</td>
<td>80</td>
<td>20.0</td>
<td>99</td>
</tr>
</tbody>
</table>
The theoretical relation is, \( S = 100 (1 - 0.794t_{80}) \)

The relation between the time of reduction at 20° C. and that at 37° C. is approximately two to one, but if an observer incubates at 37° C. he should work out his own comparative 37° C. table or factor.

A relative stability of 75 signifies that the sample examined contains a supply of available oxygen equal to 75 per cent of the amount of oxygen which it requires in order to become perfectly stable. The available oxygen is approximately equivalent to the dissolved oxygen plus the available oxygen of nitrate and nitrite. Nitrite in sewage is usually so low as to be negligible.

**BIOCHEMICAL OXYGEN DEMAND OF SEWAGE AND EFFLUENTS.**

**RELATIVE STABILITY METHOD.**

The relative stability method may be employed to obtain a measure of the putrescible material in sewages and effluents in terms of oxygen demand.

*Procedure for effluents.*—Divide the total available oxygen, including the oxygen of nitrite and nitrate, by the relative stability expressed as a decimal.

*Procedure for sewages.*—Make one or two dilutions with fully aerated distilled water of known dissolved oxygen content. Tap water may be employed if it is free from nitrates. Vary the relative proportions of sewage and water to be employed to give a relative stability of 50 to 75. Unless seals are used bring the water as well as the sewage to the temperature at which the mixtures are to be incubated before preparing the dilutions. During the manipulation avoid aeration. Having made the proper dilutions, determine the relative stability of each.

Calculate the oxygen demand in parts per million by the formula:

\[
\text{Oxygen demand} = \frac{O (1 - p)}{R_p}
\]

In this formula, \( O \) is the initial dissolved oxygen of the diluting water, \( p \) is the proportion of sewage, and \( R \) is the relative stability of the mixture. Ordinarily the available oxygen in crude sewages, septic tank effluents, settling tank effluents, and trade wastes can be neglected.
SODIUM NITRATE METHOD.

For the determination of the biochemical oxygen demand the sodium nitrate method may be used. The method is based on the biochemical consumption of oxygen from sodium nitrate by a sewage or polluted water during an incubation period of ten days at 20° C. A reasonable excess of sodium nitrate does not give a higher oxygen demand, as do higher dilutions with aerated water. The oxygen absorbed from the air in applying the method to sewages is negligible.

Reagent.—Sodium nitrate solution. Dissolve 26.56 grams of pure sodium nitrate in 1 liter of distilled water. One cc. of this solution in 250 cc. of sewage represents 50 parts per million of available oxygen. The strength of the sodium nitrate solution may be varied to suit conditions.

Procedure for sewages.—Ordinarily disregard the initial available oxygen as it is very small compared with the total biochemical oxygen demand. Add measured amounts of the sodium nitrate solution to the sewage in bottles holding approximately 250 cc. which have been completely filled and stoppered. Incubate for 10 days at 20° C. A seal is not required during incubation. The appearance of a black sediment and the development of a putrid odor during incubation indicates that too little sodium nitrate has been added. Methylene blue solution in proper proportion may be added at the start to serve as an indicator during the incubation. Domestic sewage usually varies in its oxygen demand from 100 to 300 parts per million, approximately 30 per cent of which is used up at 20° C. in the first 24 hours. At the end of the incubation period determine the residual nitrite and nitrate. Determine the nitrate by the aluminium reduction method and direct Nesslerization. To convert the nitrogen into oxygen equivalents, multiply the nitrite nitrogen by 1.7 and the nitrate nitrogen by 2.9. The difference between the available oxygen added as sodium nitrate and that found as nitrite and nitrate at the end of the incubation period is the biochemical oxygen demand.

Procedure for industrial wastes.—Employ the same procedure using larger quantities of the sodium nitrate solution. Make the reaction alkaline to methyl orange and acid to phenolphthalein. Adjust an acid reaction with sodium bicarbonate and a caustic alkaline reaction with weak hydrochloric acid. If the liquid is
devoid of sewage bacteria seed it with sewage after adjusting the reaction.

Procedure for polluted river waters.—Determine the initial available oxygen. Unless the river water is badly polluted add 10 parts per million of sodium nitrate oxygen. Collect carefully, avoiding aeration, three samples in 250 cc. bottles. To one sample add a definite quantity of sodium nitrate solution and incubate. Incubate the other two samples for the determination of the residual free oxygen, nitrite, and nitrate. If there is free oxygen left, the bottle containing the sodium nitrate solution may be discarded. If there is no free oxygen determine residual nitrite and nitrate as directed under the procedure for sewage (p. 72) and calculate the oxygen demand.

ANALYSIS OF SEWAGE SLUDGE AND MUD DEPOSITS.

COLLECTION OF SAMPLE.

Collect a representative sample of the material. In general more than one sample should be taken from a spot and a large number of samples should be collected rather than a few large samples. If the surface layer is darker and a lower layer consists of pure clay sample only the surface layer. Samples may be analyzed either separately or as composites of careful mixtures. After the sample has settled a few minutes roughly drain or siphon the excess water. Allow sewage sludge to stand for one hour before draining it free from excess water unless it is essential to determine the moisture content of the sample originally collected. If sludge cannot be analyzed within twenty-four hours it is better not to use air-tight bottles and to add small quantities of chloroform and keep in the ice box to retard decomposition. At the time of collection carefully examine mud from the bottom of surface water for evidence of sewage pollution and macroscopic and microscopic animal and plant organisms. Record the predominant species. Note the physical appearance of the material, particularly its color, odor, and consistency. Express all analytical results in percentage on a dry basis.

REACTION.

Determine the reaction by diluting a definite quantity of the wet sludge and titrating by the methods given under alkalinity and acidity (pp. 35–39 and 39–41).
SPECIFIC GRAVITY.

Weigh to the nearest tenth of a gram a wide-mouthed flask of 100 to 300 cc. capacity, according to the quantity of material available. Then completely fill the flask with distilled water to the brim and weigh it again. Empty the flask and fill it completely with fresh sewage sludge or mud. If the material is of such consistency that it flows readily fill the flask to the brim and weigh. The specific gravity is equal to the weight of the sludge or mud divided by the weight of an equal volume of distilled water.

If the material does not flow readily fill the weighed flask as completely as possible without exerting pressure during the procedure. Weigh and then fill the flask to the brim with distilled water. Let it stand for a few minutes, until trapped air has escaped, then add more water if necessary and weigh. Subtract the weight of the added water from the weight of the water that completely fills the flask; the specific gravity is equal to the weight of the material divided by this difference. Record the specific gravity only to the second decimal place.

MOISTURE.

Heat approximately 25 grams of sludge or mud in a weighed nickel dish on the water bath until it is fairly dry. Dry the residue in an oven at 100° C., cool, and weigh. Repeat to approximate constant weight. The loss in weight is moisture.

VOLATILE AND FIXED MATTER.

Ignite, at dull red heat in a hood, the residue from the determination of moisture until all the carbon has disappeared. Cool the residue in a desiccator and weigh it. The residue is the fixed matter. The volatile matter is the difference in weight between the original dried sludge and the ignited sludge.

TOTAL ORGANIC NITROGEN.

Preparation of sample.—For the determination of organic nitrogen and fats dry approximately 50 to 75 grams of the sludge or mud in a porcelain dish first on the water bath and finally in the hot-water oven until all the moisture has disappeared. Grind the dry material to a fine powder and keep it in a glass-stoppered bottle.
Reagents.—1. Sulfuric acid. Concentrated, nitrogen free.
2. Copper sulfate solution. Ten per cent.

First procedure.—Weigh accurately 0.5 gram of dried sludge or 5.0 grams of dried mud and put it into a 500 cc. Kjeldahl flask. Digest it with 20 cc. of sulfuric acid, or more if necessary, and 1 cc. of copper sulfate solution to assist the oxidation. Boil for several hours until the liquid becomes colorless or slightly yellow. Oxidize the residue with 0.5 gram of potassium permanganate, and follow the “Procedure for Sewage” (pp. 21–22).

Second procedure.—The following method is convenient for routine work at sewage disposal plants. After digestion as described in the first procedure, cool, transfer to a glass-stoppered 100 cc. flask, dilute with distilled water to 100 cc., and mix well. Transfer 50 cc. with a pipette into another 100 cc. volumetric flask, and make this portion alkaline with 50 per cent sodium hydroxide, testing a drop of the liquid on a porcelain plate with phenolphthalein to insure neutralization. The formation of a floc usually indicates that neutralization is complete. Dilute the solution to 100 cc., pour it into a small glass-stoppered bottle and permit it to stand until the next day. Nesslerize an aliquot portion of the clear supernatant liquid, and calculate the percentage of nitrogen in the material.

ETHER-SOLUBLE MATTER.

Fats are usually determined only on sewage sludge, but some mud deposits contain small quantities due to the presence of trade wastes.

Procedure.—Weigh 0.5 to 25 grams of dry material according to the quality of the sludge or mud. Add water to the weighed portion in a porcelain dish and acidify the mixture with N/50 sulfuric acid in the presence of litmus tincture or azolitmin solution as indicator. Avoid adding too much acid as an excess gives too high results on account of fatty acid residues. Evaporate the acidified mixture to dryness on the water bath, and heat it in the hot air oven at 100° C. two to three hours. Extract the dry residue with boiling ether, rubbing the sides and bottom of the dish to insure complete solution of the fat. Three extractions with ether are usually sufficient. Filter the ether solution through a 5 cm. filter paper into a small flask. Evaporate the ether slowly, dry the fatty
extract for half an hour at 100° C., cool in a desiccator, and weigh. If it is desirable, particularly with certain industrial wastes, to determine the quantity of saponified fat determine the fats with and without the addition of acid. The difference between the quantities found by the two determinations is the content of saponified fat.

FERROUS SULFIDE.

The liberation of hydrogen sulfide on adding dilute hydrochloric acid to a sludge indicates the presence of ferrous sulfide. As ferrous sulfide quickly oxidizes on exposure to air a quantitative determination of this constituent must be made immediately after collection of the sample.

Procedure.—Heat a definite portion of the sludge with hydrochloric acid in a flask. Pass the liberated gas through bromine water or hydrogen peroxide. Determine gravimetrically the sulfate in the oxidizing solution, and calculate the equivalent of ferrous sulfide by multiplying the weight of barium sulfate by 0.376.

BIOCHEMICAL OXYGEN DEMAND.

The quantity of river mud most suitable for the determination of the biochemical oxygen demand ranges within certain limits, largely according to the amount of oxidizable matter present. For examinations of river mud prepare a 1 per cent stock suspension in distilled water or tap water saturated with oxygen and free from nitrate; use in the test a dilution of this stock suspension equivalent to a concentration of 1 to 10 grams per liter of mud. For examinations of fresh sewage sludge prepare a 1 per cent stock suspension in a similar manner, but use in the test a dilution equivalent to only 0.1 to 1.0 gram per liter of wet material. For examinations of dried sludges, which have undergone more or less oxidation higher concentrations may be required.

Procedure.—Place a measured portion of the sample, or the proper amount of the 1 per cent stock suspension of the sample, in a 300 cc. narrow-mouth glass-stoppered bottle, and dilute it to the desired concentration with water saturated with oxygen. Determine the oxygen content at 20° C. of the waters that are used for dilution. This determination must be made before the mud or sludge is added because iron sulfide in the mud or sludge rapidly consumes part of the dissolved oxygen. Incubate at 20° C. for five days.
ANALYSIS OF CHEMICALS

Shortly before the determination of the oxygen remaining in solution at the end of five days rotate the bottle once or twice to mix its contents and allow sedimentation for about 30 minutes. Siphon the greater part of the liquid through a narrow-bore siphon into a 150 cc. bottle, which has been filled with carbon dioxide. Reject the first 25 cc. of the siphoned liquid and allow a little to overflow at the end of siphoning. Determine the oxygen content of the solution in the bottle in the usual way (pp. 65–68). Report the oxygen demand in percentage of the dried mud or sludge.

ANALYSIS OF CHEMICALS.

The following sections describe the accepted methods for the analysis of the chemicals commonly used in the treatment of water.

REAGENTS.

1. Distilled water. In practically all the tests of chemicals it is necessary to use exclusively distilled water that has been freshly boiled to free it from carbon dioxide and oxygen.
3. Hydrochloric acid, N/2.
4. Hydrochloric acid, N/10.
5. Ammonium hydroxide. Redistilled; sp. gr. 0.90.
6. Dilute sulfuric acid. Dilute 1 part of concentrated sulfuric acid with 3 parts of freshly boiled distilled water.
7. Methyl orange indicator. See page 36.
10. Stannous chloride, N/20. This should be frequently standardized by titration against a standard iron solution. One cc. of N/20 stannous chloride is equal to 0.0028 gram of iron (Fe) estimated in the ferrous state.
11. Sodium hydroxide, N/1. Free from carbonate. This should be frequently standardized by titration against a standard acid solution in presence of phenolphthalein indicator. One cc. of N/1 sodium hydroxide is equal to 0.049 gram of sulfuric acid (H₂SO₄), or to 0.03645 gram of hydrochloric acid (HCl).
13. Standard potassium permanganate. A N/10 solution. One
SULFATE OF ALUMINIUM.

Determine and report insoluble matter, aluminium oxide (Al₂O₃), ferric oxide (Fe₂O₃), ferrous oxide (FeO), basicity ratio, and, if present, free acid as H₂SO₄. If the material is what is known as "granular" sulfate mix it well before sampling. If it is in lump form crush it to 1/8 to 1/4 inch size, mix, and sample it. It is unnecessary to grind the sample to a fine powder, but it is preferable to have the particles fairly uniform in size.

INSOLUBLE MATTER.

Treat 10 grams of the sample with 100 cc. of distilled water and digest one hour at boiling temperature. Filter through a weighed Gooch crucible and wash the insoluble matter with hot water freshly boiled to free it from carbon dioxide. Dry the crucible to constant weight at 100° C., cool, and weigh. Report the percentage of insoluble matter.

OXIDES OF IRON AND ALUMINIUM.

Dilute the filtrate from the determination of insoluble matter to 500 cc. with water free from carbon dioxide and thoroughly mix the solution. Transfer 50 cc. of the solution to a 250 cc. beaker, add about 150 cc. of water and 5 cc. of concentrated hydrochloric acid, and heat to boiling. Add ammonium hydroxide in slight excess; when the solution has been almost neutralized it is convenient to add a drop of methyl orange indicator and then to add about 0.5 cc. of ammonium hydroxide after the solution is neutral to the indicator. Digest at about 100° C. for a few minutes and filter. Some analysts prefer to wash this gelatinous precipitate with hot water by decantation, and some to wash it evenly distributed over the surface of a filter paper; either method may be used. It is difficult to free it completely from impurities and it is not necessary to do so unless unusual quantities of calcium, magnesium, sodium, or potassium are present. While the precipitate is being washed do not allow it to become dry, as it then packs and can not
be washed clean. After most of the water has drained drying the
filter may be hastened by placing it on a sheet of blotting paper.
If much iron is present completely dry the precipitate, remove it
from the paper, and ignite the paper separately. Finally, blast
the precipitate, with free access of air to the crucible, for five or
ten minutes, cool, and weigh as oxides of iron and aluminium
\((\text{Fe}_2\text{O}_3 + \text{Al}_2\text{O}_3)\).

Subtract the content of total iron, expressed as ferric oxide
\((\text{Fe}_3\text{O}_4)\), from the weight of the combined oxides and report the
difference as aluminium oxide \((\text{Al}_2\text{O}_3)\), in percentage.

TOTAL IRON.

As filter alum usually contains 0.2 to 0.3 per cent of iron use a
10 gram sample for the determination of total iron. Treat the
sample with 50 cc. of freshly boiled distilled water and add 5 cc. of
concentrated hydrochloric acid and 1 cc. of bromine water. Evapo-
rate the solution to dryness, dissolve the residue in water, and wash it
into a flask with sufficient water to make the volume about 50 cc.
Add 50 cc. of concentrated hydrochloric acid, boil to expel oxygen,
and titrate, as hot as possible, with N/20 stannous chloride.

If a 10 gram sample is used the percentage of iron \((\text{Fe})\) is equal
to the number of cubic centimeters of stannous chloride used
multiplied by 0.028, and the percentage of iron expressed as ferric
oxide is equal to the number of cubic centimeters of stannous
chloride used multiplied by 0.040.

FERRIC IRON.

As filter alum usually contains 0.02 to 0.04 per cent of ferric iron
use a 20 gram sample. Boil 50 cc. of distilled water to expel
oxygen, add 50 cc. of concentrated hydrochloric acid, and add the
sample while the solution is boiling. Keep it boiling till the sample
is dissolved. The flask should be kept filled with carbon dioxide
during this process by dropping in occasionally small amounts of
sodium carbonate. When solution of the sample is complete
titrated it hot immediately with N/20 stannous chloride.

If a 20 gram sample is used the percentage of ferric oxide \((\text{Fe}_3\text{O}_4)\)
is equal to the number of cubic centimeters of stannous chloride
used multiplied by 0.020.
FERROUS IRON.

The content of ferrous iron is the difference between total and ferric iron. The percentage of ferrous oxide (FeO) is, therefore, equal to 0.90 times the difference between the percentage of total iron expressed as ferric oxide and the percentage of ferric iron expressed as ferric oxide. Report the percentage of ferrous oxide (FeO).

BASICITY RATIO.

Transfer 50 cc. of the filtrate from the determination of insoluble matter to a 200 cc. casserole and dilute it to 100 cc. Boil the solution and titrate it at boiling temperature with N/1 sodium hydroxide in presence of phenolphthalein indicator. The percentage of acidity in equivalent of sulfuric acid (H₂SO₄) is equal to the number of cubic centimeters of sodium hydroxide used multiplied by 4.9. In this titration iron and aluminium are precipitated as hydroxides and any free acid is neutralized.

Calculate the percentage of sulfuric acid equivalent to the determined percentages of aluminium oxide, ferric oxide, and ferrous oxide by the following formula:

$$2.88 \text{ Al}_2\text{O}_3 + 1.83 \text{ Fe}_2\text{O}_3 + 1.36 \text{ FeO}.$$  

If this percentage of acid equivalent is less than that found by titration report the difference as percentage of free acid. If the percentage of acid equivalent is greater than that found by titration the difference divided by 2.88 is the percentage equivalent to the excess of aluminium oxide present. Divide this excess by the percentage of total aluminium oxide and report the quotient as the basicity ratio.

LIME.

Mix well the sample, which should contain no lumps. If foreign matter is present grind the sample to pass a 100-mesh sieve.

Place 20 grams of granulated cane sugar and 1 gram of the sample in a 250 cc. glass-stoppered bottle, tightly stopped, and mix the mass by rolling. Do not shake hard as much of the lime could thus be lost as dust. Then add 187.4 cc. of distilled water freshly boiled to expel carbon dioxide. This makes 200 cc. of sugar solution. The lime is mixed dry with the sugar and the water added later to keep the lime from lumping. After shaking the sugar solution one hour titrate 50 cc. of it with N/2 hydrochloric
SULFATE OF IRON

acid in presence of methyl orange indicator. The acid used is equivalent to the carbonate and hydroxide in 0.25 gram of the sample.

Filter the remainder of the sugar solution, discarding the first 25 cc. of filtrate. Titrate 50 cc. of the filtrate with N/2 hydrochloric acid in presence of methyl orange indicator. The acid used is equivalent to the hydroxide in 0.25 gram of the sample.

If a 1 gram sample is used the percentage of calcium oxide (CaO) is equal to 5.6 times the number of cubic centimeters of hydrochloric acid used in the second titration; and the percentage of calcium carbonate (CaCO₃) equivalent to the carbonate present is equal to 10 times the difference in cubic centimeters between the results of the two titrations.

SULFATE OF IRON.

INSOLUBLE MATTER.

Treat 10 grams of the sample with 100 cc. of freshly boiled distilled water cooled to 30° C. or less. When solution is complete filter through a weighed Gooch crucible, wash, dry, cool, and weigh. Report the weight of the residue, in percentage, as insoluble matter.

IRON AS FERROUS SULFATE.

Dissolve 1 gram of the sample and dilute to 200 cc. with freshly boiled distilled water cooled to 30° C. or less. Add 5 cc. of dilute sulfuric acid (1 to 3) to a 50 cc. portion of the solution and titrate with N/10 potassium permanganate. The percentage of ferrous sulfate (FeSO₄·7H₂O) is equal to 11.12 times the number of cubic centimeters of potassium permanganate used.

ACIDITY.

Shake 12.25 grams of the sample in a 150 cc. bottle with 75 cc. of 95 per cent alcohol for ten minutes. Run a blank. Filter rapidly both sample and blank and wash rapidly with alcohol sufficient to make 100 cc. of filtrate. Titrate with N/20 sodium hydroxide in presence of phenolphthalein and subtract the result of titrating the blank from that of titrating the solution of the sample. The percentage of acidity, expressed as sulfuric acid (H₂SO₄), is equal to 0.02 times the number of cubic centimeters of sodium hydroxide used.
SODA ASH, Chemical Bibliography

SODA ASH.

INSOLUBLE MATTER.

Treat 5.305 grams of the sample with 200 cc. of freshly boiled and cooled distilled water. When solution is complete filter through an asbestos mat in a weighed Gooch crucible, dry, cool, and weigh. Report the weight of the residue, in percentage, as insoluble matter.

AVAILABLE ALKALI.

Dilute the filtrate from the determination of insoluble matter to 1,000 cc. and thoroughly mix. Titrate 25 cc. of this dilution with N/10 hydrochloric acid in presence of methyl orange indicator. The percentage of available alkali, expressed as sodium carbonate (Na₂CO₃), is equal to 4 times the number of cubic centimeters of hydrochloric acid used.

CHEMICAL BIBLIOGRAPHY.

The subjoined bibliography comprises the publications cited in the text of this report. The references are arranged alphabetically by authors' names and under each author in order of dates of publication. When different pages of a single work are cited letters are used in connection with the number that refers to the work.


15. ——— and Hazen, Allen. A report of the chemical work done at the Lawrence Experiment Station: *Examinations by the State Board of Health of water supplies of Mass. 1887-90*, pt. 2, Purification of sewage and water, pp. 707-34, 1890.


22. Fowler, G. J. Sewage works analyses, pp. 21-37, John Wiley & Sons, New York, 1902; (a) pp. 31-4; (b) pp. 58-60; (c) pp. 86-9; (d) pp. 89-95; (e) pp. 96-7; (f) pp. 98-100.


30. ————- Experiments upon the purification of sewage and water at the Lawrence Experiment Station: Report Mass. State Board of Health, pp. 447–700, 1894.


44. ————- Experiments on the purification of sewage at the Lawrence Experiment Station: Report Mass. State Board of Health, pp. 393–448, 1892.


60. Leach, A. E. Food inspection and analysis, pp. 493, 495, and 497, John Wiley & Sons, New York, 1904.


65. LEVY, D. D. *Ann. de l’Observatoire de Mont-Souris*, 1883 et seq.


70. PALMER, A. W. Chemical survey of the waters of Illinois, Report for years 1897–1902, pp. 27–8, Univ. Ill., 1903.

71. ————. Report of the University of Illinois [in Report of streams examination, Sanitary Dist. Chicago], p. 60, Chicago, 1903; (a) p. 58; (b) pp. 61–4.


92. Stearns, F. P., and Drown, T. M. Discussion of special topics relating to the quality of public water supplies: Examinations by the State Board of Health of water supplies of Mass., 1887–90, pt. 1, Examination of water supplies, pp. 740–9, 1890.
100. ————. The examination of water and water supplies, p. 200, Philadelphia, 1904; (a) p. 219; (b) p. 195; (c) p. 282.


MICROSCOPICAL EXAMINATION.

The microscopical examination of water consists of the enumeration of the kinds of microscopic organisms (Plankton), and an estimation of their quantity.

It may serve any one or more of the following purposes:

1. To explain the presence of objectionable odors and tastes.
2. To indicate the progress of the self purification of streams.
3. To indicate the presence of sewage contamination.
4. To explain the chemical analysis.
5. To identify the source of a water.
6. To aid in the study of the food of fish, shellfish, and other aquatic organisms.

The term "Microscopic Organisms" shall include all organisms microscopic or barely visible to the naked eye, with the exception of the bacteria. It includes the diatomaceae, chlorophyceae, cyanophyceae, fungi, protozoa, rotifera, crustacea, bryophyta, and spongidae found in water.

Fragments of organic matter, silt, mineral matter, zoöglea, etc., shall be considered as amorphous matter. The recording of amorphous matter usually serves no useful purpose and shall not be considered a part of the standard method.

Apparatus.—1. A cylindrical funnel about two inches in diameter at the top, with a straight side for nine inches, narrowed over a distance of three inches to a bore of one-half inch in diameter, and terminating in a straight portion of this diameter two and one-half inches in length. The capacity of this funnel is 500 cc. It shall be provided at the bottom with a tightly fitting rubber stopper with a single perforation and a disk of silk bolting cloth over the hole about three eighths of an inch in diameter.

2. A counting cell consisting of a brass rim closely cemented to a plate of optical glass. The shape and size of this cell are not essential but its depth shall be one millimeter. A convenient capacity is about one cubic centimeter.

3. An ocular micrometer ruled as follows: The ocular micrometer is commonly of such a size that with a 16 mm. objective and a suit-
able tube length, the largest square cuts off one square millimeter on the stage.

Procedure.—Filter 250 cc. of the water (more or less according to the clearness of the sample) through a one-half inch layer of quartz sand (washed and screened between 60 and 120 mesh sieves) supported by the disk of bolting cloth and rubber stopper at the bottom of the funnel. Suction may be applied to hasten the filtration.

Remove the stopper and catch the plug of sand and its entrained organisms in a small beaker or test tube, washing down the inside of the funnel into the beaker with 5 cc. of clean (preferably distilled) water. Agitate the mixture of sand, water, and organisms to detach the latter from the sand grains, and quickly decant the water and the organisms in suspension to a test tube. If desired the sand may then be again washed with 5 cc. water and the wash water added to the first portion.

Cover the cell partially with a cover glass, and by means of a pipette run the concentrate under the cover glass until the cell is completely filled.

Cover and place on the microscope stage in a horizontal position for examination.

Count the organisms in twenty fields, i.e., twenty cubic millimeters, estimating their areas in terms of Standard Units.

The Standard Unit is the smallest square in the ocular micrometer, and represents an area $20\mu \times 20\mu$, or 400 square microns on the stage.

Results shall be expressed in the number of Standard Units of each kind of micro-organism per cc. and also the total number of standard units of all kinds per cc. The general directions as to significant figures given under Turbidity shall apply also to the microscopical examination.

Caution.—Many micro-organisms, especially some of those causing odors, are so fragile that they are broken up in filtration, especially if the agitation of the filtrate is too vigorous. A direct examination of a fresh sample is therefore a useful supplementary procedure. For the same reason the concentrate should not stand long before examination. Also some organisms are carried by specific gravity to the top of the cell which should be scrutinized as well as the bottom layer each time.

It is always better to examine the micro-organisms in the field when possible, and for this purpose the sling filter has been devised
consisting of a metal funnel slung to a pivoted handle, with a disk of wire gauze in the detachable lower end to support the sand. Filtration is hastened by imparting a whirling motion to the whole and utilizing the centrifugal force thus generated.

THE OCULAR MICROMETER.

MICROSCOPICAL BIBLIOGRAPHY.

c. ————. A report of the biological work of the Lawrence Experiment Station: Examinations by the State Board of Health of water supplies of Mass., 1887–90, pt. 2, Purification of sewage and water, pp. 793–862, 1890.
d. Parker, G. H. Report upon the organisms, excepting the bacteria found in the waters of the State: Examinations by the State Board of Health of water supplies of Mass., 1887–90, pt. 1, Examination of water supplies, pp. 579–620, 1890.


i. ________. Microscopy of drinking water, 3d ed., John Wiley & Sons, New York, 1914. (Contains bibliography.)
BACTERIOLOGICAL EXAMINATION.

I. APPARATUS.

1. Sample Bottles.—Any size, shape or quality of bottle may be used for a bacterial sample, provided it holds a sufficient amount to carry out all the tests required and is such that it may be properly washed and sterilized and will keep the sample uncontaminated until the analysis is made. Four- or eight-ounce, ground-glass-stoppered bottles are recommended. These should be protected by being wrapped in paper, or their necks covered with tin-foil, and should be placed in proper boxes for transportation.

2. Pipettes.—Pipettes may be of any convenient size or shape provided it is found by actual test that they deliver accurately the required amount in the manner in which they are used. The error of calibration shall in no case exceed 2 per cent. Protecting the pipettes with a cotton stopper is recommended.

3. Dilution Bottles.—Bottles for use in making dilutions should preferably be of tall form, of such capacity as to hold at least twice the volume of water actually used. Close-fitting ground-glass stoppers are preferable, but tight fitting cotton stoppers may be used, provided due care is taken to prevent contamination and to avoid loss of volume through wetting of the stopper before mixing has been accomplished.

4. Petri Dishes.—Petri dishes ten centimeters in diameter shall be used with glass or porous tops as preferred. The bottoms of the dishes shall be as flat as possible so that the medium shall be of uniform thickness throughout the plate.

5. Fermentation Tubes.—Any type of fermentation tube may be used provided it holds at least four times as much medium as the amount of water to be tested.

II. MATERIALS.

1. Water.—Distilled water shall be used in the preparation of all culture media and reagents.

2. Meat Extract.—Liebig’s meat extract shall be used in place of meat infusion. Other brands may be substituted for Liebig’s when comparative tests have shown that they give equivalent results.

3. Peptone.—Witte’s peptone shall be the standard. Other brands may be substituted for Witte’s when comparative tests have shown that they give equivalent results. At the present time
Armour's, Digestive Ferments Co's, and Fairchild's Peptones have been found to give equivalent results.

4. **Sugars.**—All sugars used shall be of the highest purity obtainable.

5. **Agar.**—The agar used shall be of the best quality and shall be dried for one-half hour at 105° C. before weighing. Much of the agar on the market contains considerable amounts of sea salts. These may be removed by soaking in water and draining before use.

6. **Gelatin.**—The gelatin used shall be of light color, shall contain not more than a trace of arsenic, copper, sulfids, and shall be free from preservatives, and of such a melting point that a 10 per cent. standard nutrient gelatin shall have a melting point of 25° C. or over. Gelatin shall be dried for one-half hour at 105° C. before weighing.

7. **Liquor.**—Reagent liquor of highest purity (not liquor cubes) or azolitmin (Kahlbaum) shall be used for all media requiring a liquor indicator.

8. **General Chemicals.**—Special effort shall be made to have all the other ingredients used for culture media chemically pure.

### III. METHODS.

#### 1. PREPARATION OF CULTURE MEDIA.

##### a. Titration.

Phenolphthalein shall be the indicator used in obtaining the reaction of all culture media.*

Titration shall be made as follows:

In a white porcelain dish put 5 cc. of the medium to be tested, add 45 cc. of distilled water. Boil briskly for one minute. Add 1 cc. of phenolphthalein solution (5 grams of commercial salt to one liter of 50 per cent. alcohol). Titrate immediately with a n/20 solution of sodium hydrate. A faint but distinct pink color marks the true end point. This color may be precisely described as a combination of 25 per cent. of red (wave length approximately 658) with 75 per cent. of white as shown by the disks of the standard color top made by the Milton Bradley Educational Co., Springfield, Mass.

All reactions shall be expressed with reference to the phenolphthalein neutral point and shall be stated in percentages of normal acid or alkali solutions required to neutralize them. Alkaline media

---

* The Committee is of the opinion that a new method of titrating and adjusting the reaction of culture media which will give a more accurate indication of the hydrogen ion concentration is desirable. Until further studies are made and simpler methods available, it is thought unwise to change the present standard methods. It is recommended, however, that as far as possible the true reaction or hydrogen ion concentration be recorded. See Bibliography Nos. 1, 4, 5, 6, 7, 8, 10, 13, 17, 18, 19, 22, 23, 24, 26.
shall be recorded with a minus (−) sign before the percentage of normal acid needed for their neutralization and acid media with a plus (+) sign before the percentage of normal alkali solution needed for their neutralization.

The standard reaction for culture media for water analysis shall be +1.0 per cent., as determined by tests of the sterilized medium. As ordinarily prepared, broth and agar will be found to have a reaction between +0.5 and +1.0. For such media no adjustment shall be made. The reaction of media containing sugar shall be neutral to phenolphthalein. Whenever reactions other than the standard are used, it shall be so stated.

b. Sterilization.

All media and dilution water shall be sterilized in the autoclav at 15 lbs. (120°C.) for 15 minutes after the pressure reaches 15 lbs. All air must be forced out of the autoclav before the pressure is allowed to rise. As soon as possible after sterilization the media shall be removed from the autoclav and cooled rapidly. Rapid and immediate cooling of gelatin is imperative.

Media shall be sterilized in small containers, and these must not be closely packed together. No part of the medium shall be more than 2.5 cm. from the outside surface of the glass. All glassware shall be sterilized in the dry oven at 170°C. for at least 1 ½ hours.

c. Nutrient Broth. To Make One Liter.

1. Add 3 grams of beef extract and 5 grams of peptone to 1,000 cc. of distilled water.
2. Heat slowly on a steam bath to at least 65°C.
3. Make up lost weight, titrate, and if the reaction is not already between +0.5 and +1 adjust to +1.
5. Distribute in test-tubes, 10 cc. to each tube.
6. Sterilize in the autoclav at 15 lbs. (120°C.) for 15 minutes after the pressure reaches 15 lbs.

d. Sugar Broths.

Sugar broths shall be prepared in the same general manner as nutrient broth with the addition of 1 per cent. of the required carbohydrate just before sterilization. The removal of muscle sugar is unnecessary as the beef extract and peptone are free from any fermentable carbohydrates. The reaction of sugar broths shall be neutral to phenolphthalein. Sterilization shall be in the autoclav at 15 lbs. (120°C.) for 15 minutes after the pressure reaches 15 lbs.
e. *Nutrient Gelatin. To Make One Liter.*

1. Add 3 grams of beef extract and 5 grams of peptone to 1,000 cc. of distilled water and add 100 grams of gelatin dried for one-half hour at 105° C. before weighing.

2. Heat slowly on a steam bath to 65° C. until all gelatin is dissolved.*

3. Make up lost weight, titrate, and if the reaction is not already between +0.5 and +1, adjust to +1.

4. Filter through cloth and cotton until clear.

5. Distribute in test-tubes, 10 cc. to each tube, or in larger containers as desired.

6. Sterilize in the autoclave at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs.

f. *Nutrient Agar. To Make One Liter.*

1. Add 3 grams of beef extract, 5 grams of peptone and 12 grams of agar, dried for one-half hour at 105° C. before weighing, to 1,000 cc. of distilled water. Boil over a water bath until all agar is dissolved, and then make up the loss by evaporation.

2. Cool to 45° C. in a cold water bath, then warm to 65° C. in the same bath, without stirring.

3. Make up lost weight, titrate, and if the reaction is not already between +0.5 and +1, adjust to +1.

4. Filter through cloth and cotton until clear.

5. Distribute in test-tubes, 10 cc. to each tube, or in larger containers, as desired.

6. Sterilize in the autoclave at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs.

g. *Litmus or Azolitmin Solution.*

The standard litmus solution shall be a 2 per cent. aqueous solution of reagent litmus. Powder the litmus, add to the water and boil for five minutes. The solution usually needs no correction in reaction and may be at once distributed in flasks or test-tubes and sterilized as is culture media. It should give a distinctly blue plate when 1 cc. is added to 10 cc. of neutral culture medium in a Petri dish.

The standard azolitmin solution shall be a 1 per cent. solution of Kahlbaum's azolitmin. Add the azolitmin powder to the water and boil for five minutes. The solution may need to be corrected

---

*The solution of the gelatin will be facilitated by allowing it to soak in the cold one-half hour before heating.*
in reaction by the addition of sodium hydride solution so that it will be approximately neutral and will give a distinctly blue plate when 1 cc. is added to 10 cc. of neutral culture medium in a Petri dish. It may be distributed in flasks or test-tubes and sterilized as is culture media.

h. Litmus-lactose-agar.

Litmus-lactose-agar shall be prepared in the same manner as nutrient agar with the addition of 1 per cent. of lactose just before sterilization. Its reaction shall be neutral. One cc. of sterilized litmus or azolitmin solution shall be added to each 10 cc. of the medium just before it is poured into the Petri dish, or the mixture may be made in the dish itself.

i. Endo’s Medium. To make One Liter.

1. Add 5 grams of beef extract, 10 grams of peptone and 30 grams of agar dried for one-half hour at 105° C. before weighing, to 1,000 cc. of distilled water. Boil on a water bath until all the agar is dissolved and then make up the loss by evaporation.

2. Cool the mixture to 45° C. in a cold water bath, then warm to 65° C. in the same bath without stirring.

3. Make up lost weight, titrate, and if the reaction is not already between neutral and +1 adjust to neutral.

4. Filter through cloth and cotton until clear.

5. Distribute 100 cc. or larger known quantities in flasks large enough to hold the other ingredients which are to be added later.

6. Sterilize in the autoclave at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs.

7. Prepare a 10 per cent. solution of basic fuchsin in 95 per cent. alcohol, allow to stand 20 hours, decant and filter the supernatant fluid. This is a stock solution.

8. When ready to make plates, prepare a 10 per cent. solution of anhydrous sodium sulphite. To 10 cc. of the sodium sulphite solution add 2 cc. of the fuchsin stock solution and steam five minutes in the Arnold or on a water bath. To each 100 cc. of the agar mixture, add 1 gram of lactose, dissolve in streaming steam or on a water bath and add $\frac{1}{2}$ cc. of the fuchsin sulphite solution. The lactose used must be chemically pure and the sulphite solution must be made up fresh every day.

9. Pour plates and allow to harden thoroughly in the incubator before use.
2. COLLECTION OF SAMPLE.

Samples for bacterial analysis shall be collected in bottles which have been cleansed with great care, rinsed in clean water, and sterilized with dry heat for at least one hour and a half at 170° C., or in the autoclav at 15 lbs. (120° C.) for 15 minutes or longer after the pressure reaches 15 lbs.

Great care must be exercised to have the samples representative of the water to be tested and to see that no contamination occurs at the time of filling the sample bottles.

3. STORAGE AND TRANSPORTATION OF SAMPLES.

Because of the rapid and often extensive changes which may take place in the bacterial flora of bottled samples when stored even at temperatures as low as 10° C., it is urged, as of importance, that all samples be examined as promptly as possible after collection.

The time allowed for storage or transportation of a bacterial sample between the filling of the sample bottle and the beginning of the analysis should be not more than six hours for impure waters and not more than twelve hours for relatively pure waters. During the period of storage, the temperature shall be kept as near 10° C. as possible. Any deviation from the above limits shall be so stated in making reports.

4. DILUTIONS.

Dilution bottles shall be filled with the proper amount of tap water so that after sterilization they shall contain exactly 9 cc. or 99 cc. as desired. The exact amount of water can only be determined by experiment with the particular autoclav in use. If desired, the 9 cc. dilution may be measured out from a flask of sterile water with a sterile pipette.

Dilution bottles shall be sterilized in the autoclav at 15 lbs. (120° C.) for 15 minutes after the pressure reaches 15 lbs.

The sample bottle shall be shaken vigorously 25 times and 1 cc. withdrawn and added to the proper dilution bottles as required. Each dilution bottle after the addition of the 1 cc. of the sample, shall be shaken vigorously 25 times before a second dilution is made from it or before a sample is removed for plating.
5. PLATING.

All sample and dilution bottles shall be shaken vigorously 25 times before samples are removed for plating. Plating shall be done immediately after the dilutions are made. One cc. of the sample or dilution shall be used for plating and shall be placed in the Petri dish, first. Ten cc. of liquefied medium at a temperature of 40° C. shall be added to the 1 cc. of water in the Petri dish. The cover of the Petri dish shall be lifted just enough for the introduction of the pipette or culture medium, and the lips of all test-tubes or flasks used for pouring the medium shall be flamed. In making litmus-lactose-agar plates, 1 cc. of sterile litmus or azolitmin solution shall be added to each 10 cc. of culture medium either in the Petri dish or before pouring into the Petri dish. The medium and sample in the Petri dish shall be thoroughly mixed and uniformly spread over the bottom of the Petri dish by tilting or rotating the dish. All plates shall be solidified as rapidly as possible after pouring and gelatin plates shall be placed immediately in the 20° C. incubator and the agar plates in the 37° C. incubator. Endo plates shall be made by placing one loopful of the material to be tested on the surface of the plate and distributing the material with a sterile loop or glass rod.

6. INCUBATION.

All gelatin plates shall be incubated for 48 hours at 20 C. in a dark, well-ventilated incubator in an atmosphere practically saturated with moisture. All agar plates shall be incubated for 24 hours at 37° C. in a dark, well-ventilated incubator in an atmosphere practically saturated with moisture. Glass covered plates shall be inverted in the incubator. Any deviation from the above described method shall be stated in making reports.

7. COUNTING.

In preparing plates, such amounts of the water under examination shall be planted as will give from 25 to 250 colonies on a plate, and the aim should be always to have at least two plates giving colonies between these limits. Where it is possible to obtain plates showing colonies within these limits, only such plates should be considered in recording results, except where the same amount of water has been planted in two or more plates, of which one gives colonies
within these limits, while the others give less than 25 or more than 250. In such case, the result recorded should be the average of all the plates planted with this amount of water. Ordinarily it is not desirable to plant more than 1 cc. of water in a plate; therefore, when the total number of colonies developing from 1 cc. is less than 25, it is obviously necessary to record the results as observed, disregarding the general rule given above.

Counting shall in all cases be done with a lens of 2½ diameter's magnification, 3½X. The Engraver's Lens No. 146 made by the Bausch & Lomb Optical Company fills the requirements, and is a convenient lens for the purpose.

8. THE TEST FOR THE PRESENCE OF MEMBERS OF THE B. COLI GROUP.

It is recommended that the B. coli group be considered as including all non-spore-forming bacilli which ferment lactose with gas formation and grow aerobically on standard solid media.

The formation of 10 per cent. or more of gas in a standard lactose broth fermentation tube within 24 hours at 37° C. is presumptive evidence of the presence of members of the B. coli group, since the majority of the bacteria which give such a reaction belong to this group.

The appearance of aerobic lactose-splitting colonies on lactose-litmus-agar or Endo’s medium plates made from a lactose-broth fermentation tube in which gas has formed confirms to a considerable extent the presumption that gas-formation in the fermentation tube was due to the presence of members of the B. coli group.

To complete the demonstration of the presence of B. coli as above defined, it is necessary to show that one or more of these aerobic plate colonies consists of non-spore-forming bacilli which, when inoculated into a lactose-broth fermentation tube, form gas.

It is recommended that the standard tests for the B. coli group be either (A) the Presumptive, (B) the Partially Confirmed, or (C) the Completed test as hereafter defined, each test being applicable under the circumstances specified.

A. PRESUMPTIVE TEST.

1. Inoculate a series of fermentation tubes with appropriate graduated quantities of the water to be tested.
2. Incubate these tubes at 37° C. for 48 hours. Examine each
tuberculosis at 24 and 48 hours, and record gas-formation. The records should be such as to distinguish between:

(a) Absence of gas-formation.
(b) Formation of gas occupying less than ten per cent. (10%) of the closed arm.
(c) Formation of gas occupying more than ten per cent. (10%) of the closed arm.

More detailed records of the amount of gas formed, though desirable for purposes of study, are not necessary for carrying out the standard tests prescribed.

3. The formation within 24 hours of gas occupying more than ten per cent. (10%) of the closed arm of fermentation tube constitutes a positive presumptive test.

4. If no gas is formed in 24 hours, or if the gas formed is less than ten per cent. (10%), the incubation shall be continued to 48 hours. The presence of gas in any amount in such a tube at 48 hours constitutes a doubtful test, which in all cases requires confirmation.

5. The absence of gas formation after 48 hours’ incubation constitutes a negative test. (An arbitrary limit of 48 hours’ observation doubtless excludes from consideration occasional members of the B. coli group which form gas very slowly, but for the purposes of a standard test the exclusion of these occasional slow gas forming organisms is considered immaterial.)

B. PARTIALLY CONFIRMED TEST.

1. Make one or more Endo’s medium or lactose-litmus-agar plates from the tube which, after 48 hours’ incubation, shows gas formation from the smallest amount of water tested. (For example, if the water has been tested in amounts of 10 cc., 1 cc., and 0.1 cc., and gas is formed in 10 cc., and 1 cc., not in 0.1 cc., the test need be confirmed only in the 1 cc. amount.)

2. Incubate the plates at 37° C., 18 to 24 hours.

3. If typical colon-like red colonies have developed upon the plate within this period, the confirmed test may be considered positive.

4. If, however, no typical colonies have developed within 24 hours, the test cannot yet be considered definitely negative, since it not infrequently happens that members of the B. coli group fail to form typical colonies on Endo’s medium or lactose-litmus-agar plates, or that the colonies develop slowly. In such case, it is always necessary to complete the test as directed under “C” 2 and 3.


C. COMPLETED TEST.

1. From the Endo's medium or lactose-litmus-agar plate made as prescribed under "B," fish at least two typical colonies, transferring each to an agar slant and a lactose broth fermentation tube.

2. If no typical colonies appear upon the plate within 24 hours, the plate should be reincubated another 24 hours, after which at least two of the colonies considered to be most likely B. coli, whether typical or not, shall be transferred to agar slants and lactose broth fermentation tubes.

3. The lactose broth fermentation tubes thus inoculated shall be incubated until gas formation is noted; the incubation not to exceed 48 hours. The agar slants shall be incubated at 37° C. for 48 hours, when a microscopic examination shall be made of at least one culture, selecting one which corresponds to one of the lactose broth fermentation tubes which has shown gas-formation.

The formation of gas in lactose broth and the demonstration of non-spore-forming bacilli in the agar culture shall be considered a satisfactory completed test, demonstrating the presence of a member of the B. coli group.

The absence of gas-formation in lactose broth or failure to demonstrate non-spore-forming bacilli in a gas-forming culture constitutes a negative test.

APPLICATION OF PRESUMPTIVE, PARTIALLY CONFIRMED, AND COMPLETED TESTS.

A. The Presumptive Test.

1. When definitely positive, that is showing more than 10 per cent. (10%) of gas in 24 hours, is sufficient:
   (a) As applied to all except the smallest gas-forming portion of each sample in all examinations.
   (b) As applied to the smallest gas-forming portion in the examination of sewage or of water showing relatively high pollution, such that its fitness for use as drinking water does not come into consideration. This applies to the routine examinations of raw water in connection with control of the operation of purification plants.

2. When definitely negative, that is showing no gas in 48 hours, is final and therefore sufficient in all cases.
3. When doubtful, that is showing gas less than 10 per cent. (10%) (or none) in 24 hours, with gas either more or less than 10 per cent. in 48 hours, must always be confirmed.

B. The Partially Confirmed Test.
1. When definitely positive, that is, showing typical plate colonies within 24 hours, is sufficient:
   (a) When applied to confirm a doubtful presumptive test in cases where the latter, if definitely positive, would have been sufficient.
   (b) In the routine examination of water-supplies where a sufficient number of prior examinations have established a satisfactory index of the accuracy and significance of this test in terms of the completed test.

2. When doubtful, that is, showing colonies of doubtful or negative appearance in 24 hours, must always be completed.

C. The Completed Test.
The completed test is required as applied to the smallest gas-forming portion of each sample in all cases other than those noted as exceptions under the "presumptive" and the "partially confirmed" tests.
The completed test is required in all cases where the result of the confirmed test has been doubtful.

9. EXPRESSION OF RESULTS.

In order to avoid fictitious accuracy and yet to express the numerical results by a method consistent with the precision of the work, the numbers of colonies of bacteria per cubic centimeter shall be recorded as follows:

<table>
<thead>
<tr>
<th>Number of bacteria per cc.</th>
<th>From 1 to</th>
<th>50</th>
<th>shall be recorded as found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>51</td>
<td>100</td>
<td>&quot; &quot; to the nearest 5</td>
</tr>
<tr>
<td></td>
<td>101</td>
<td>250</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>251</td>
<td>500</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>501</td>
<td>1,000</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>1,001</td>
<td>10,000</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>10,001</td>
<td>50,000</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>50,001</td>
<td>100,000</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>100,001</td>
<td>500,000</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>500,001</td>
<td>1,000,000</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>1,000,001</td>
<td>10,000,000</td>
<td>&quot; &quot; &quot; &quot; &quot; &quot; &quot; &quot;</td>
</tr>
</tbody>
</table>

This applies to the gelatin count at 20° C. and to the agar count at 37° C.
## B. Coli

### Summary of steps involved in making presumptive, partially confirmed and completed tests for B. coli.

<table>
<thead>
<tr>
<th>Steps in procedure.</th>
<th>Further procedure required.</th>
</tr>
</thead>
</table>
| I. Inoculate lactose broth fermentation tubes; incubate 24 hours at 37° C.; observe gas-formation in each tube.  
   1. Gas-formation, 10 per cent. or more; constitutes positive presumptive test.  
      (a) For other than smallest portion of any sample showing gas at this time, and for all portions, including smallest, of sewage and raw water this test is sufficient.  
      (b) For smallest gas-forming portion, except in examinations of sewage and raw water.  | None |
| 2. Gas-formation less than 10 per cent. in 24 hours; inconclusive.  | III |
| II. Incubate an additional 24 hours, making a total of 48 hours' incubation; observe gas-formation.  
   1. Gas-formation, any amount; constitutes doubtful test, which must always be carried further.  | III |
| 2. No gas-formation in 48 hours; constitutes final negative test.  | None |
| III. Make plate from smallest gas-forming portion of sample under examination; incubate 18 to 24 hours; observe colonies.  
   1. One or more colonies typical in appearance.  
      (a) If only "partially confirmed" test is required.  
      (b) If completed test is required, select two typical colonies for identification.  | None |
| 2. No typical colonies.  | V |
| IV. Replace plate in incubator for an additional 18 to 24 hours; then, whether colonies appear typical or not, select at least two of those which most nearly resemble B. coli.  | IV |
| V. Transfer each colony fished to:  
   1. Lactose broth fermentation tube; incubate not more than 48 hours at 37° C. Observe gas-formation.  | None |
| 2. Agar slant; incubate 48 hours at 37° C.  
      (a) If gas formed in lactose broth tube inoculated with corresponding culture.  
      (b) If no gas formed in corresponding lactose broth tube, test is completed and negative.  | None |
| VI. Make stained cover-slip or slide preparation, and examine microscopically.  
   1. If preparation shows non-spore-forming bacilli in apparently pure culture, demonstration of B. coli is completed.  | None |
| 2. If preparation fails to show non-spore-forming bacilli or shows them mixed with spore-bearing forms or bacteria of other morphology.  | VII |
| VII. Replate, to obtain assuredly pure culture, select several colonies of bacilli and repeat steps V and VI.  | |
In order that tests for B. coli may have quantitative significance, the following general principles and rules should be observed:

Ordinarily not less than three portions of each sample should be tested, the portions being even decimal multiples or fractions of a cubic centimeter; for example, 10 cc., 1 cc., .1 cc., .01 cc., etc. It is essential that the dilutions should be such that the largest amount gives a positive test (unless the water is such as to give negative tests in 10 cc.), and the smallest dilution, a negative result. To insure this result, it is often necessary to plant four or five dilutions, especially in the examination of a sample of entirely unknown quality. The quantitative value of a series of tests is lost, unless all or at least a large proportion of the smallest dilutions tested have given negative results.

In reporting a single test, it is preferable merely to record results as observed, indicating the amounts tested and the result in each, rather than to attempt expression of the result in numbers of B. coli per cc. In summarizing the results of a series of tests, however, it is desirable, for the sake of simplicity, to express the results in terms of the numbers of B. coli per cc., or per 100 cc. To convert results of fermentation tests to this form, the result of each test is recorded as indicating a number of B. coli per cc. equal to the reciprocal of the smallest decimal or multiple fraction of a cubic centimeter giving a positive result. For example, the result: 10 cc. +; 1 cc. +; 0.1 cc. −; would be recorded as indicating one B. coli per cc. An exception should be made in the case where a negative result is obtained in an amount larger than the smallest portion giving a positive result; for example, in a result such as: 10 cc. +; 1 cc. −; 0.1 cc. +. In such case, the result should be recorded as indicating a number of B. coli per cc. equal to the reciprocal of the dilution next larger than the smallest one giving a positive test, this being a more probable result.

Where tests are made in amounts larger than 1 cc., giving average results less than one B. coli per cc., it is more convenient to express results in terms of the numbers of B. coli per 100 cc.

The following table illustrates the method of recording and averaging results of B. coli tests:
Interpretation—B. Coli Group

<table>
<thead>
<tr>
<th>Result of Tests in Amounts Designated</th>
<th>Indicated Number of B. coli.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cc.</td>
</tr>
<tr>
<td>10 cc. 1 cc. 0.1 cc. .1 cc.</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>0.1</td>
</tr>
<tr>
<td>+ +</td>
<td>1.0</td>
</tr>
<tr>
<td>+ + +</td>
<td>10.0</td>
</tr>
<tr>
<td>+ +</td>
<td>100.0</td>
</tr>
<tr>
<td>+</td>
<td>10.0</td>
</tr>
<tr>
<td>Totals (for estimating averages)</td>
<td>121.1</td>
</tr>
<tr>
<td>Average of 5 tests</td>
<td>24.0</td>
</tr>
</tbody>
</table>

The above method of expressing results is not mathematically altogether correct. The average number of B. coli per cc., as thus estimated, is not precisely the most probable number calculated by application of the theory of probability. To apply this theory to a correct mathematical solution of any considerable series of results involves, however, mathematical calculations so complex as to be impracticable of application in general practice. The simpler method given is therefore considered preferable, since it is easily applied and the results so expressed are readily comprehensible.

In order that results as reported may be checked and carefully valuated, it is necessary that the report should show not only the average number of B. coli per cc., but also the number of samples examined; and, for each dilution, the total number of tests made, and the number (or per cent.) positive.

10. Interpretation of Results.

While it is not within the province of this report to suggest the proper interpretation of results obtained by the use of the methods herein specified as standard, the committee feels that a word of caution should be given regarding the significance of the presence in a water of members of the B. coli group as defined in this report. Recent work seems to indicate that the B. coli group as herein defined consists of organisms of both fecal and non-fecal origin. Therefore care must be exercised in judging the sanitary quality of a water solely from the determination of the presence of members of the group.

11. Differentiation of Fecal from Non-Fecal Members of the B. Coli Group.

(1) At least 10 cultures should be used. If possible these should be subcultured from plates made direct from the water since all of the cultures obtained by plating from fermentation tubes may be
descendants of a single cell in the water. If cultures from water plates are not available those obtained from plates made as prescribed under B (p. 101) may be used.

(2) Inoculate each culture into dextrose potassium phosphate broth,* adonite broth, and gelatin. For additional confirmatory evidence inoculation may be made into tryptophane broth,† and saccharose broth. The dextrose broth must be incubated at 30°. Other sugar broths may be incubated at 30° or 37° as convenient. Gelatin should be incubated at 20°.

(3) After 48 hours record gas formation in adonite and saccharose broths. Determine indol formation in tryptophane broth by adding drop by drop, to avoid mixing with the medium, about 1 cc. of a 2 per cent. alcoholic solution of p-dimethyl amido-benzaldehyde, then a few drops of concentrated hydrochloric acid. The presence of indol is indicated by a violet color.

(4) After 5 days apply methyl red test and Voges-Proskauer test to dextrose broth.

_Methyl Red Test._

Indicator solution.—Dissolve 0.1 gram methyl red in 300 cc. alcohol and dilute to 500 cc. with distilled water.

Procedure in test.—1. To 5 cc. of each culture add 5 drops of methyl red solution.

2. Record distinct red color as methyl red +, distinct yellow color as methyl red −, and intermediate colors as ?.

_Voges-Proskauer Test._

To the remaining 5 cc. of medium add 5 cc. of a 10 per cent. solution of potassium hydroxide. Allow to stand over night. A positive test is indicated by an eosin pink color.

(5) Gelatin tubes should not be pronounced negative until they have been incubated at least 15 days.

---

*Medium for Methyl Red Test. To Make One Liter.*
1. To 800 cc. distilled water add 5 grams Witte's peptone (other peptones should not be substituted), 5 grams c. p. dextrose, and 5 grams dipotassium hydrogen phosphate (K₂HPO₄). A dilute solution of the K₂HPO₄ should give a distinct pink with phenolphthalein.
2. Heat with occasional stirring over steam for twenty minutes.
3. Filter through folded filter paper, cool to 20° C. and dilute to 1,000 cc. with distilled water.
4. Distribute 10 cc. portions in sterilized test tubes.
5. Sterilize by the intermittent method for 20 minutes on three successive days.

†Tryptophane Broth for Indol Test.
To 1,000 cc. of distilled water add 0.3 gram tryptophane, 5 grams dipotassium hydrogen phosphate (K₂HPO₄), and 1 gram peptone. Heat until ingredients are thoroughly dissolved, tube (6 to 8 cc.), and sterilize in autoclave for 15 minutes after the pressure reaches 15 pounds. Some American peptones are standardized to contain a uniform amount of tryptophane. If such peptone is used the tryptophane in the above formula may be omitted and the peptone increased to 5 grams.
The following group reactions indicate the source of the culture with a high degree of probability:

\[
\begin{align*}
\text{Methyl red +} & \quad \text{Voges-Proskauer -} \\
\text{Gelatin -} & \quad \text{Adonite -} \\
\text{Indol, usually +} & \quad \text{Saccharose, usually -} \\
\end{align*}
\]

\[
\begin{align*}
\text{B. coli of fecal origin.} \\
\end{align*}
\]

\[
\begin{align*}
\text{Methyl red -} & \quad \text{Voges-Proskauer +} \\
\text{Gelatin -} & \quad \text{Adonite +} \\
\text{Indol, usually -} & \quad \text{Saccharose +} \\
\end{align*}
\]

\[
\begin{align*}
\text{B. aërogenes of fecal origin.} \\
\end{align*}
\]

\[
\begin{align*}
\text{Methyl red -} & \quad \text{Voges-Proskauer +} \\
\text{Gelatin -} & \quad \text{Adonite -} \\
\text{Indol, usually -} & \quad \text{Saccharose +} \\
\end{align*}
\]

\[
\begin{align*}
\text{B. aërogenes, probably not of fecal origin.} \\
\end{align*}
\]

\[
\begin{align*}
\text{Methyl red -} & \quad \text{Voges-Proskauer +} \\
\text{Gelatin +} & \quad \text{Adonite +} \\
\text{Indol, usually -} & \quad \text{Saccharose +} \\
\end{align*}
\]

\[
\begin{align*}
\text{B. cloacae, may or may not be of fecal origin.} \\
\end{align*}
\]

12. ROUTINE PROCEDURE FOR EXAMINATION OF SAMPLES OF WATER.

**First Day:**

1. Prepare dilutions as required.
2. Make two (2) gelatin plates from each dilution, and incubate at 20° C.
3. Make two (2) agar plates from each dilution, and incubate at 37° C.
4. Inoculate lactose broth fermentation tubes with appropriate amounts for B. coli tests, inoculating two (2) tubes with each amount.
Note:—Where repeated tests are made of water from the same source, as is customary in the control of public supplies, it is not necessary to make duplicate plates or fermentation tubes in each dilution. It is sufficient, in such circumstances, to make duplicate plates only from the dilution which will most probably give from 25 to 250 colonies per plate.

Second Day:
1. Count the agar plates made on the first day.
2. Record the number of lactose broth fermentation tubes which show 10 per cent. (10%) or more of gas.

Note:—In case only the presumptive test for B. coli is required, fermentation tubes showing more than 10 per cent. (10%) of gas at this time may be discarded.

Third Day:
1. Count gelatin plates made on first day.
2. Record the number of additional fermentation tubes which show 10 per cent. (10%) or more of gas.
3. Make a lactose-litmus-agar or Endo’s medium plate from the smallest portion of each sample showing gas. Incubate plate at 37° C.

Note:—In case the smallest portion in which gas has been formed shows less than 10 per cent. (10%) of gas, it is well to make a plate also from the next larger portion, so that, in case the smallest portion gives a negative end result it may still be possible to demonstrate B. coli in the next larger dilution.

Fourth Day:
1. Examine Endo’s medium or lactose-litmus-agar plates. If typical colonies have developed, select two and transfer each to a lactose broth fermentation tube and an agar slant, both of which are to be incubated at 37° C.
2. If no typical B. coli colonies are found, incubate the plates another 24 hours.

Fifth Day:
1. Select at least two colonies, whether typical or not, from the Endo’s medium or lactose-litmus-agar plates which have been incubated an additional 24 hours; transfer each to a lactose
broth fermentation tube and an agar slant, and complete the test as for typical colonies.

2. Examine lactose broth fermentation tubes inoculated from plates on the previous day. Tubes in which gas has been formed may be discarded after the result has been recorded. Those in which no gas has formed should be incubated an additional 24 hours.

Sixth Day:

1. Examine lactose broth fermentation tubes reincubated the previous day.

2. Examine microscopically agar slants corresponding to lactose fermentation tubes inoculated from plate colonies and showing gas-formation.

Respectfully submitted,

F. P. Gorham,
W. H. Frost,
R. G. Perkins,
G. W. Fuller,
L. A. Rogers.

BACTERIOLOGICAL BIBLIOGRAPHY.


INDEX.

A.
Acidity, determination of, 39.
Acids, mineral, 41.
Agar, nutrient, 94, 96.
lacto-litmus, 97.
Alkali carbonates, 39.
Alkalinity, determination of, 35.
Albuminoid nitrogen, 20.
Aluminium sulfate, determination of, 41.
analysis of, 78.
Aluminium and iron, determination of, 57.
Ammonia nitrogen, determination of, 15.
Apparatus, bacteriological, 93.
Application of colon group tests, 102.
Arsenic, determination of, 63.
Azolitmin solution, 96.

B.
Bacillus aërogenes, reactions, 108.
cloacae, reactions, 108.
coli, reactions, 108.
B. coli group, tests, 100.
application of, 102.
fecal and non-fecal, 106.
summary of tests, 104.
Bacteriological examination, 93.
bibliography, 110.
Bacitracin, determined, 90.
Bibliography,
bacteriological, 110.
chemical, 82.
microscopical, 91.
Biochemical oxygen demand, 71.
in sludge and mud, 76.
Bismuthate method (Mn), 49.
Boric acid, 63.
Bottles, sample, 1, 93.
di ution, 93.
Bromine and iodine, determination of, 61.
Broth, nutrient, 95.
sugar, 95.

C.
Calcium, determination of, 57.
Carbon dioxide, determination of, 40.
Chemical analysis, water and sewage, 1.
bibliography, 82.
Chemicals, analysis of, 77.
Chloride, determination of, 41.
Chlorine, determination of, 64.
Coefficient of fineness, 8.
Collection of samples, bacteriological, chemical, 1.
Colon group, tests (see "B. coli"), 100.
Color, determination of, 9.
Copper, determination of, 53, 55.
Counting (bacterial), 99.
Cultural characters of colon-group, 108.
Culture media, 94.
azolitmin solution, 96.
Endo's medium, 97.
litmus-lactose agar, 97.
litmus solution, 96.
methyl red test, 107.
nutrient agar, 96.
nutrient broth, 95.
nutrient gelatin, 96.
sterilization, 95.
sugar broth, 95.
titration, 94.
tryptophane broth, 107.

D.
Dilution (bacteriological), 98.
Dissolved oxygen, 65.

E.
Effluents, relative stability of, 69.
biochemical, oxygen, demand of, 71.
Endo's medium, 97.
Erythrosine indicator, 36.
Ether—Soluble matter, 69.
in sludge and mud, 75.
Evaporation, 29.
Expression of results (see under "Re-
sults").

F.
Fat, determination of, 69, 75.
Fecal and non-fecal members, colon
group, 106.
Fermentation tubes, 93.
Ferrous sulfide in sludge and mud, 76.
Ferric iron, determination of, 47.
Fineness, coefficient of, 8.
INDEX

G.
Gelatin media, 94, 96.

H.
Hardness, determination of, 30.
bicarbonate, 37.
carbonate, 38.
hydroxide, 38.
non-carbonate, 34.
temporary, 34.
Hydrogen sulfide, determination of, 63.

I.
Ignition, loss on, 30.
Incubation, 99.
Indol test, broth for, 107.
Indicators, 36, 94, 107.
Iodine and bromine, determination of, 61.
Iron and Aluminium, separation, 57.
analysis of, 79.
Iron, determination of, 43.
standards, 45.
sulfate, determination of, 41.
analysis of, 81.

L.
Lacmoid indicator, 36.
Lead, determination of, 51, 55.
Lime, analysis of, 80.
Lithium, determination of, 60.
Litmus reagent, 94.
lactose-agar, 97.
solution, 96.

M.
Manganese, determination of, 48.
Materials, bacteriological, 93.
Meat extract, 93.
Media, culture (see "Culture media"), 94–7, 107.
Methyl orange indicator, 37.
Methyl red media, 107.
test, 107.
Microscopical bibliography, 91.
examination, 89.
Mineral analysis, 56.
Moisture in sludge and mud, 74.
Mud deposits, analysis, 73.

N.
Nessler's reagent,
color standards, 10.
ammonia determination, 19.
Nitrogen, 15.
ammonia, 15.
albuminoid, 20.

Nitrogen, in sludge and mud, 74.
nitrate, 23.
nitrite, 22.
organic, 21.
total, 25.
Nutrient media (see "Culture media"), 94, 107.

O.
Odor, 12.
Organic nitrogen, 21.
Oxygen consumed, 25.
demand, biochemical, 71.
dissolved, 65.
in fresh and
sea water (table) 68.

P.
Peptone, authorized brands, 93.
Per sulfate method (Mn), 48.
Petri dishes, 93.
Phenolsulfonic acid method (nitrate), 23.
Phenolphthalein indicator, 36, 94.
Physical examination, 4.
Pipe, bacteriological, 93.
Plating, bacteriological, 99.
Platinum—cobalt color standard, 9.
wire turbidity, 5.
Potassium, determination of, 59.
Presumptive tests, colon group, 102.

R.
Reactions of colon group, 108.
Reaction of culture media, 94.
of sludge and mud, 73.
Reduction method (nitrate), 24.
Relative stability method, 71.
Residue on evaporation, 29.
Results, expression of,
bacteriological, 103.
chemical examination, 14.
color, 8.
odor, 12.
Results, interpretation of (bacteriological), 106.
Routine procedure (bacteriological), 108.

S.
Samples,
bacterial, 93.
bottles, 1.
chemical, 1.
time before analysis of, 2.
quantity required, 1.
representative, 3.
sludge and mud, 73.

Sewage sludge, analysis, 73.
Silica, determination of, 76.
<table>
<thead>
<tr>
<th>Index</th>
<th>115</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodas ash, analysis of, 82.</td>
<td></td>
</tr>
<tr>
<td>Soap method (hardness), 31.</td>
<td></td>
</tr>
<tr>
<td>Sodium and potassium, 58.</td>
<td></td>
</tr>
<tr>
<td>Solids, total, fixed, volatile, 29.</td>
<td></td>
</tr>
<tr>
<td>Specific gravity of sludge and mud, 74.</td>
<td></td>
</tr>
<tr>
<td>Stability, relative, of effluents, 69.</td>
<td></td>
</tr>
<tr>
<td>method, relative, 71.</td>
<td></td>
</tr>
<tr>
<td>Standards,</td>
<td></td>
</tr>
<tr>
<td>ammonia, 17.</td>
<td></td>
</tr>
<tr>
<td>chlorine, 65.</td>
<td></td>
</tr>
<tr>
<td>color, 9.</td>
<td></td>
</tr>
<tr>
<td>hardness, 32.</td>
<td></td>
</tr>
<tr>
<td>iron, 45.</td>
<td></td>
</tr>
<tr>
<td>Nessler, color, 10.</td>
<td></td>
</tr>
<tr>
<td>platinum cobalt, 10.</td>
<td></td>
</tr>
<tr>
<td>turbidity, 4.</td>
<td></td>
</tr>
<tr>
<td>Sterilization of media, 95.</td>
<td></td>
</tr>
<tr>
<td>Storage of samples, 2, 98.</td>
<td></td>
</tr>
<tr>
<td>Sugars for media, 94.</td>
<td></td>
</tr>
<tr>
<td>Sugar broths, 95.</td>
<td></td>
</tr>
<tr>
<td>Sulfate, K and Na, 58.</td>
<td></td>
</tr>
<tr>
<td>Suspended matter, 30.</td>
<td></td>
</tr>
<tr>
<td>T.</td>
<td></td>
</tr>
<tr>
<td>Tin determination of, 54, 55.</td>
<td></td>
</tr>
<tr>
<td>Tintometer, Lovibond, 11.</td>
<td></td>
</tr>
<tr>
<td>Titration of media, 94.</td>
<td></td>
</tr>
<tr>
<td>Total nitrogen, 25.</td>
<td></td>
</tr>
<tr>
<td>residue on evaporation, 29.</td>
<td></td>
</tr>
<tr>
<td>Tryptophane broth, 107.</td>
<td></td>
</tr>
<tr>
<td>Turbidity, 4.</td>
<td></td>
</tr>
<tr>
<td>coefficient of fineness, 8.</td>
<td></td>
</tr>
<tr>
<td>platinum wire method, 5.</td>
<td></td>
</tr>
<tr>
<td>rod, graduation, 6.</td>
<td></td>
</tr>
<tr>
<td>standard, 4.</td>
<td></td>
</tr>
<tr>
<td>turbidimetric method, 7.</td>
<td></td>
</tr>
<tr>
<td>turbidimeter, graduation, 8.</td>
<td></td>
</tr>
<tr>
<td>V.</td>
<td></td>
</tr>
<tr>
<td>Voges-Prokauer test, 107.</td>
<td></td>
</tr>
<tr>
<td>Volatile matter, 29.</td>
<td></td>
</tr>
<tr>
<td>in sludge [74.</td>
<td></td>
</tr>
<tr>
<td>and mud [74.</td>
<td></td>
</tr>
<tr>
<td>Z.</td>
<td></td>
</tr>
<tr>
<td>Zinc, 52.</td>
<td></td>
</tr>
</tbody>
</table>