Standard Solutions
(Microtitration Techniques)

In general, standard solutions for microchemical work are prepared and standardized in the same manner as those for macroanalytical work. Methods for the latter are found in many works on analytical chemistry. Where macromethods use normal and tenth normal solutions, microanalysis employs one or two hundredth normal solutions. The reader is referred to the above-mentioned works if there exist any doubts regarding the preparation of standard solutions required, other than those discussed below.

Fortunately, practically all of the standard solutions are commercially available and have proved satisfactory in the author's laboratory. Where the standards are prepared in the laboratory, freshly boiled, distilled water is generally used.

Standard solutions of hydrochloric acid, sodium hydroxide, potassium biiodate, iodine, sodium thiosulfate, potassium sulfate and barium chloride are the most frequently used. Potassium biiodate (also called biniodate) may be used either as an acid in alkalimetric and acidimetric or in iodometric titrations as shown by the following equations:

(1) Alkalimetric—Acidimetric
\[
\text{KH} (\text{IO}_3)_2 + \text{NaOH} \rightarrow \text{NaIO}_3 + \text{KIO}_3 + \text{H}_2\text{O}
\]

(2) Iodometric
\[
\text{KH} (\text{IO}_3)_2 + 10\text{KI} + 11\text{HCl} = 11\text{KCl} + 6\text{H}_2\text{O} + 6\text{I}_2
\]
\[
6\text{I}_2 + 12\text{Na}_2\text{S}_2\text{O}_3 = 12\text{NaI} + 6\text{Na}_2\text{S}_4\text{O}_6
\]

The normality of a solution of potassium biiodate differs depending on whether it is to be used as an acid or in iodometric titrations, the same solution being twelve times as strong for the latter as for the former, as seen from the equations.

Dissolved carbon dioxide must be removed before proper end points can be obtained in acid-base titrations. Consequently, the solutions should be boiled for about 30 seconds before beginning the titration and then again for a few seconds toward the end. (The one exception to this is the titration of the
dissolved ammonia in boric acid, obtained in the Kjeldahl determination of nitrogen—see Chapter 8.)

**Reagents**

**STARCH SOLUTION**

Starch solution may be prepared by any of the methods generally used in macroanalysis (see above-noted references), or by that suggested by Elek, which is as follows:

An approximate 1% solution is made by triturating one gram of water-soluble starch with a few milliliters of cold distilled water and adding this mixture to about 95 ml. of boiling 20% aqueous solution of sodium chloride. The resulting mixture is boiled for 5 minutes, cooled and filtered. The solution should be kept in a refrigerator.

An alternate reagent is prepared according to the following which is recommended by the Association of Official Agricultural Chemists:

Two grams of finely powdered potato starch is mixed with a little cold distilled water to form a thin paste. About 200 ml. of boiling distilled water is added with constant stirring and the resulting mixture is immediately allowed to cool. One milliliter of mercury is added as a preservative.

**THYMOLPHTHALEIN**

A 0.1% solution in 95% ethanol is prepared.

**SODIUM ALIZARIN SULFONATE**

A 0.035% solution in water is prepared and stored in a glass-stoppered flask or bottle. The material is also known as Alizarin Red S.

**BROMOCRESOL GREEN—METHYL RED**

Five parts of 0.2% bromocresol green and 1 part of 0.2% methyl red, both in 95% ethanol, are mixed and stored in a dropping bottle.

**METHYL RED—METHYLENE BLUE**

Two parts of 0.2% methyl red and 1 part of 0.2% methylene blue, both in 95% ethanol are mixed and stored in a dropping bottle.

**METHYL RED**

In a flask are placed 0.15 gram of methyl red powder and 40 ml. of 0.01N sodium hydroxide solution. The mixture is shaken and then filtered and the filtrate stored in a glass-stoppered dropping bottle.

* A 0.2% aqueous solution of amylose, was found to be an excellent indicator by Aluise, Hall, Staats and Becker.
PHENOLPHTHALEIN\textsuperscript{144,145,194}

A 1\% solution of phenolphthalein in 95\% ethanol is prepared.

TETRAHYDROXYQUINONE (THQ)\textsuperscript{4,25,87,150,194,198,210,212}

This is kept in the solid state and used as such. It is used together with the glass filter listed below.

**Apparatus**

**Burettes**

Automatic filling microburettes of the type shown in Figs. 69 and 70, are preferred for storing and titrating standard solutions, although storage in ordinary bottles or volumetric flasks and titrating from the hand-filled type of burette is permissible. The automatic burette\textsuperscript{88,84,144,145,168-171,194} shown in Fig. 69 has a storage reservoir of 0.5–1.0 liter. The solution is forced up into the top of the burette when the pressure in the reservoir is increased by pumping the hand-operated bulb. The level of the solution is automatically adjusted to coincide with the 0.00-ml. mark since the delivery tube is at this mark and the tube is so constructed that it acts as a siphon to remove the excess. The flow of solution from the burette is controlled by means of a glass bead inside the rubber tube, a pinch clamp, or a ground glass stopcock. The burettes are usually 10-ml. capacity, graduated to 0.05-ml. The solution in the burette and in the reservoir is protected from carbon dioxide of the air by the Ascarite tubes at the top and between the reservoir and bulb, respectively. The stopcock between the reservoir and the bulb should be closed at all times except when the solution is being pumped up into the burette. Solution which has remained in the burette for more than an hour or two should be discarded. Obviously, the reservoir should be shaken each day to mix any condensate that occurs on standing and which alters the normality of the solution, unless mixed in.

The type\textsuperscript{196,194} of burette shown in Fig. 70 is available in sizes of 1, 2, 5, and 10 ml. This type is graduated in 0.01 or 0.05 ml. and has its reservoir at the top. Manipulation of the three-way stopcock allows the solution to flow from the reservoir into the burette. The capacities of the reservoirs are usually approximately 100 ml.

**Illuminated Titration Stand Assembly\textsuperscript{78,150,194,198}**

The titration stand shown in Fig. 71 is used for standardizing BaCl\textsubscript{2} solution using tetrahydroxyquinone (THQ) indicator together with the orange-brown glass filter plate described below. The stand is illuminated from below by means of fluorescent lighting. The base is a frosted glass plate. This should be
covered with the masking plate or with black paper which has an oblong section cut from it, just large enough for the filter plate (see below) and cuvette (Fig. 72) to set in, side by side. The cuvette which is prepared from glass has the dimensions, $20 \times 40 \times 60$ mm. high, and has graduation marks indicating the 15- and 30-ml. capacities.

**ORANGE-BROWN GLASS FILTER PLATE**

Glass color filter, approximately $2.5 \times 26 \times 45$ mm. having a spectral transmission, uncorrected for reflectance, of 37% at 550 mm$\mu$ ± 2 mm$\mu$ is required.
pH METER

Zeromatic pH meter, No. 9600\textsuperscript{16} or comparable instrument is used in the adjustment of the pH of solutions, such as in the standardization of thorium nitrate.

PHOTOELECTRIC FILTER PHOTOMETER\textsuperscript{127,132,137,189}

This instrument is of particular value in the titration of fluoride with thorium nitrate using sodium alizarin sulfonate as the indicator, although it undoubtedly
can be found to be useful in other titrations in which the color change at the end point is gradual. It also makes possible the handling of larger volumes of solutions of low concentrations. The instrument has a small lamp in the center with a Meyer Trioplan lens (3 inches, f 2.8, provided with adjustable iris diaphragm) and green filter (maximum transmittance, 520 μm) on each side. The light from the lamp, on each side, passes through the lens and filter, then through a titrating cell containing solution, and finally impinges onto a photocell (Weston photronic photocell, model 594, Weston Instruments Division of Daystrom Inc., Newark, New Jersey). The two photocells are connected in opposition, a mirror galvanometer being mounted in parallel. The latter is shunted (by Ayrton shunt, Fisher Scientific Company, New York, and Pittsburgh) which permits stepwise variation of the sensitivity over a range. Provision is made for magnetic stirring and titrating. For direct titration, a blank solution and the indicator are placed in the left side titrating cell while the solution to be titrated and the indicator are placed in the right side titrating cell or vice versa. The various stages of operation of the instrument are described below in the standardization of thorium nitrate. Figure 73 shows
a commercially available instrument, the type used in the author’s laboratories (photoelectric filter photometer, model PFP-1, Marley Products Co., Hyde Park, New York).182 It is completely encased. The switch on the extreme left controls the galvanometer light, light source and magnetic stirrers. The next control, the second from the left, controls the speed of the magnetic stirrers. The third switch from the left shorts out the galvanometer and photocells when not in use. The switch on the extreme right is a three-stage shunt for varying the sensitivity. At the top are the galvanometer knob for zero adjusting and two diaphragm adjusting wheels which are used to balance the light source to the photocells. Two clear plastic dishes (ordinary refrigerator dishes) are used as titrating cells.

**Procedures**

It should be remembered that the detecting of an end point is a personal matter, which is more exaggerated when working with one hundredth normal solutions. Therefore, the analyst who is going to perform the particular volumetric procedure should also standardize the solutions, or at least make certain that he sees the same end point as the one who does the standardization.

**SODIUM THIOSULFATE, 0.01N**

A liter of distilled water is brought to a boil to remove carbon dioxide and cooled while loosely covered. Sodium thiosulfate, \( \text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O} \) (2.48 grams) is dissolved in the water and the solution made up to 1000 ml. It should be transferred to a brown bottle equipped with a rubber stopper. One ml. of chloroform is added and the contents of the bottle shaken for a few minutes. The chloroform acts as a preservative.08,184 The use of a rubber stopper over a ground glass one is preferred as the former absorbs chloroform, increasing the efficiency of the preservative. This solution is quite stable as long as a pool of chloroform is present, but should be standardized at intervals of about one month. In the absence of chloroform, standardization should be done every few days.74 Instead of the rubber-stoppered brown bottle, an automatic burette of the type shown in Fig. 69 may be used. If so, it is preferable to use a brown bottle, but if clear glass is used, the solution should be standardized more frequently. Obviously, chloroform must be present at all times, regardless of the setup, or standardization must be done daily.

**Standardization:**

About 2–3 mg. of solid potassium biiodate is accurately weighed (using a porcelain boat or charging tube—see Chapter 3), transferred to a 125-ml. glass-stoppered Erlenmeyer flask, and dissolved in 5 ml. distilled water. To this is
added 1.5 ml. concentrated hydrochloric acid, followed by 1 ml. of freshly prepared 4% solution of potassium iodide, and the flask stoppered. After 2 minutes, the solution is diluted with water to 20 ml. and the liberated iodine is titrated with the sodium thiosulfate solution. When the iodine color has almost disappeared (yellow) several drops of the starch solution is added. Thiosulfate is added until the blue color has been converted to a faint pink at most (end point). If it is easier for the analyst to detect the end point by titrating to a colorless solution, such action is permissible, but being consistent is an absolute necessity.

Calculation:

From the equations shown at the beginning of the chapter,

\[
\frac{\text{mg. } \text{KH}(\text{IO}_3)_2}{\text{mg. Na}_2\text{S}_2\text{O}_3} = \frac{1522.92}{1897.368}
\]

\[
\begin{align*}
\text{mg. Na}_2\text{S}_2\text{O}_3 \times 158.114 & = N_{\text{Na}_2\text{S}_2\text{O}_3} \\
389.928 \times \text{ml. Na}_2\text{S}_2\text{O}_3 \times 158.114 & = N_{\text{Na}_2\text{S}_2\text{O}_3} \\
\text{ml. Na}_2\text{S}_2\text{O}_3 \times 0.03077 & = N_{\text{Na}_2\text{S}_2\text{O}_3}
\end{align*}
\]

**IODINE, 0.01N**

Approximately 1.27 grams of resublimed iodine crystals are dissolved in a solution of 4 grams of potassium iodide in 4 ml. of water. (Note: Iodine dissolves rather rapidly in a concentrated solution of potassium iodide, but very slowly in a dilute solution.) After about 20–30 minutes (first making certain that solution is complete) the concentrated solution is transferred to a 1-liter volumetric flask and diluted to the mark, with freshly boiled distilled water.

**Standardization:**

The resulting solution is standardized by titration against the 0.01N thiosulfate (prepared above) using starch indicator.

This solution is not stable and should be standardized daily.
POTASSIUM SULFATE, 0.01N

Exactly 0.8713 gram of reagent grade potassium sulfate is placed in a 1-liter volumetric flask, dissolved in a little distilled water and the resulting solution diluted to the mark, with distilled water.

BARIUM CHLORIDE, 0.01N

Approximately 1.04 grams of barium chloride is dissolved in water and made up to 1 liter. The solution is standardized against the potassium sulfate, prepared above, using the illuminated titration stand, orange-brown filter plate, cuvette (see above), and tetrahydroxyquinone indicator as follows: Three to 5 ml of standard potassium sulfate, 0.01N, is placed in the cuvette, one or two drops of phenolphthalein indicator solution added, and enough 0.1N sodium hydroxide to render the solution alkaline after which it is back-titrated with 0.01N hydrochloric acid just to expel the color. (The titration using THQ indicator must be done in a fairly neutral solution. Although the potassium sulfate solution is already neutral, the above procedure of making alkaline and back-titrating is recommended since this technique is used in the actual titration of sulfate in the determination of sulfur—see Chapter 10—and therefore the BaCl₂ solution is standardized under the identical conditions as when used.) Enough water is added to bring the volume to 15 ml and this is followed by adding 15 ml of 95% ethanol. One-half scoop (provided with the indicator) of dry THQ indicator is added and dissolved by stirring with a glass rod protected on the end by a rubber sleeve (or policeman) to prevent scratching of the cuvette. The container is then placed on the illuminated titration stand alongside the orange-brown filter plate so that these are illuminated from below and the rest of the stand is blacked out to avoid interference with the visual comparison of the solution with the filter plate. The barium chloride solution is added to the yellow contents of the cuvette, with stirring, until the color gradually shifts towards the red and matches that of the filter plate when viewed from the top, looking through the entire depth of solution.† This is taken as the end point and is very sharp, an additional drop changing the color markedly more toward the red. If the end point has been overstepped the solution cannot be back-titrated. The same solution will have a different normality factor for each filter plate used since the titration would be to a slightly different end point for each. However, this is of no consequence as long as the factor for the particular filter is determined.

* The amount is regulated so that the appearance of solution plus BaSO₄ at the end point is about the same as the filter glass. Considerably more or less BaSO₄ present does not give a mixture comparable in appearance to the filter.

† Note: Barium salt of indicator is purple-red.
SODIUM HYDROXIDE, 0.01N

This solution may be obtained, commercially, and as such is quite satisfactory. Otherwise, it is prepared from carbonate-free sodium hydroxide as follows: A 50% solution (about 20N) of reagent grade of sodium hydroxide is prepared and stored in a stoppered paraffin-lined bottle until the carbonate has settled out, which usually takes some weeks. This is then decanted and diluted with freshly boiled distilled water to about 0.01N and standardized as follows. About 12 to 14 mg. of reference-standard potassium acid phthalate, KHC₈H₄O₄, is weighed into a 125-ml. Pyrex Erlenmeyer flask. This is dissolved in about 5 ml. of distilled water, a few drops of phenolphthalein indicator added, and the mixture boiled for about 30 seconds to drive out dissolved carbon dioxide. The hot solution is titrated with the above-prepared sodium hydroxide to a faint pink end point.

\[
\text{KHC}_8\text{H}_4\text{O}_4 + \text{NaOH} \rightarrow \text{KNaC}_8\text{H}_4\text{O}_4 + \text{H}_2\text{O}
\]

Calculation:

\[
\frac{1\text{NaOH}}{39.999} = \frac{1\text{KHC}_8\text{H}_4\text{O}_4}{204.228}
\]

\[
\frac{\text{mg. NaOH}}{39.999} = \frac{\text{mg. KHC}_8\text{H}_4\text{O}_4}{204.228}
\]

or

\[
\text{mg. NaOH} = \frac{\text{mg. KHC}_8\text{H}_4\text{O}_4 \times 39.999}{204.228}
\]

Now 1 ml. of N NaOH contains 39.999 mg. of NaOH

\[
\frac{\text{mg. NaOH}}{\text{ml. NaOH} \times 39.999} = \text{Normality of NaOH}
\]

\[
\frac{\text{mg. KHC}_8\text{H}_4\text{O}_4 \times 39.999}{204.228 \times \text{ml. of NaOH} \times 39.999} = N_{\text{NaOH}}
\]

\[
\frac{\text{mg. KHC}_8\text{H}_4\text{O}_4}{204.228 \times \text{ml. of NaOH}} = N_{\text{NaOH}}
\]

Similarly, potassium biiodate or benzoic acid may be used. For the latter, neutral 95% ethanol (see Chapter 15) is employed as the solvent. For both of these acids, one molecule of acid is equivalent to one molecule of sodium hydroxide:
HYDROCHLORIC ACID, 0.01N

This solution may be obtained commercially\(^1\) or it may be prepared from more concentrated solution, diluting with freshly boiled distilled water and standardized against standard, 0.01N sodium hydroxide solution, using phenolphthalein as the indicator. Just before the end point is reached, the mixture is boiled for at least 30 seconds to expel carbon dioxide. (Note: If the acid is titrated with the alkali, at the end point, boiling increases the color, so that the end point should be approached only with a hot, well-boiled mixture.)

SODIUM FLUORIDE, 0.01N\(^{127,187,190}\)

Exactly 0.4200 gram of reagent grade sodium fluoride is placed in a one-liter volumetric flask, dissolved in a small amount of distilled water and the resulting solution diluted to the mark, with distilled water.

THORIUM NITRATE, 0.01N\(^{127,187,190}\)

Approximately 1.38 grams of thorium nitrate tetrahydrate, Th(NO\(_3\))\(_4\)·4 H\(_2\)O, are dissolved in water and the resulting solution diluted to one liter. This is standardized against the sodium fluoride, prepared above, using sodium alizarin sulfonate as the indicator with the aid of the photoelectric filter photometer described above. (Before performing actual titrations, the proper sensitivity for the instrument must be selected. This is accomplished by doing a blank determination and determining which sensitivity setting gives a deflection of 25 units upon the addition of 0.05 ml. of the thorium nitrate when the iris openings are the same as during the actual determination, preferably as large as possible.\(^{132}\)

One-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, and 10-ml. portions of 0.01N sodium fluoride are titrated with the thorium nitrate so that a plot may be made of ml. of thorium nitrate vs. mg. of fluorine. Each of the above-mentioned portions of sodium fluoride are treated in the following manner: The fluoride is transferred to the clear plastic titrating cell and diluted with distilled water to a volume

\[
\begin{align*}
1\text{NaOH} & \quad \Leftrightarrow \quad 1\text{KH(IO}_3\text{)}_2 \\
\text{M.W. 39.999} & \quad \Leftrightarrow \quad \text{M.W. 389.928} \\
1\text{NaOH} & \quad \Leftrightarrow \quad 1\text{C}_6\text{H}_5\text{COOH} \\
\text{M.W. 39.999} & \quad \Leftrightarrow \quad \text{M.W. 122.125} \\
\therefore \quad \frac{\text{mg. KH(IO}_3\text{)}_2}{389.928 \times \text{ml. of NaOH}} & = N_{\text{NaOH}} \\
\text{and} \\
\frac{\text{mg. C}_6\text{H}_5\text{COOH}}{122.125 \times \text{ml. of NaOH}} & = N_{\text{NaOH}}
\end{align*}
\]
of 450 ml. This solution is adjusted to pH 3.0±0.05 (using 0.1N hydrochloric acid), and 2 ml. of 0.035% sodium alizarin sulfonate indicator is added. A duplicate clear plastic titrating cell containing 450 ml. of distilled water adjusted to pH 3.0±0.05 and 2 ml. of 0.035% sodium alizarin sulfonate indicator is placed in one compartment of the photoelectric filter photometer and the cell containing the fluoride solution in the other. (Note: Since sodium alizarin sulfonate is also an acid-base indicator, all pH adjustments are most critical.) With the third switch from the left in the position that both the galvanometer and photocells are shorted out (short) and the lights and stirrers on, the galvanometer is adjusted to the zero mark by rotating the galvanometer adjusting knob at the top of the instrument. (Note: The lights should be turned on in advance of the time that the instrument is to be used.) The third switch from the left is then turned to the “on” position to connect the galvanometer and photocells. The switch on the extreme right should be turned to the position of predetermined sensitivity (maximum preferred). The galvanometer is again adjusted to the zero mark this time by means of the diaphragm adjusting wheels (usually only one—that on the side of the fluoride solution). The fluoride is then titrated with the thorium nitrate until a deflection of 25 units on the galvanometer is obtained. (Note: The color change is from a yellow to a pink.) After a number of different size portions of sodium fluoride have been titrated, a curve is plotted showing the relationship between milliliters of thorium nitrate and milligrams of fluorine titrated. This is preferable to labeling the fluoride as “so-much” normal, since a straight-line function does not exist except possibly over a small range of amount of fluorine and then only under certain limited conditions.

**ADDITIONAL INFORMATION FOR CHAPTER 5**

Although the volumetric procedures described in this chapter as well as throughout this entire book call for the use of burettes of the types shown in Figs. 69, and 70, the analyst encounters problems and procedures elsewhere calling for the use of other volumetric glassware. For the sake of completeness, pieces are shown here for which recommended specifications have been published by the Committee on Microchemical Apparatus of the Division of Analytical Chemistry of the American Chemical Society. These include microvolumetric flasks (Fig. 74), pipettes (Fig. 75) to be used with the flasks, and a centrifuge tube (Fig. 61, Chapter 4).
## Fig. 74. Microvolumetric flask—details of construction.

<table>
<thead>
<tr>
<th>Capacity (ml.)</th>
<th>Inside diameter (mm.)</th>
<th>Inside diameter (mm.)</th>
<th>(Approx.) (mm.)</th>
<th>(Maximum) (mm.)</th>
<th>(Maximum) (mm.)</th>
<th>Tolerance (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.2–4.6</td>
<td>8.0–8.5</td>
<td>10</td>
<td>70</td>
<td>37</td>
<td>±0.010</td>
</tr>
<tr>
<td>2</td>
<td>5.0–5.4</td>
<td>10.5–11.0</td>
<td>13</td>
<td>70</td>
<td>39</td>
<td>±0.015</td>
</tr>
<tr>
<td>3</td>
<td>5.0–5.4</td>
<td>13.25–13.75</td>
<td>14</td>
<td>72</td>
<td>39</td>
<td>±0.015</td>
</tr>
<tr>
<td>4</td>
<td>6.2–6.6</td>
<td>13.75–14.25</td>
<td>18</td>
<td>75</td>
<td>39</td>
<td>±0.020</td>
</tr>
<tr>
<td>5</td>
<td>6.2–6.6</td>
<td>15.5–16.0</td>
<td>18</td>
<td>75</td>
<td>39</td>
<td>±0.020</td>
</tr>
</tbody>
</table>

To be marked "T.C. (capacity) 20° C."

1-ml. size to weigh less than 19 grams empty (stopper included).

Shape of bases may be either round or hexagonal. Dimensions given in column E are maximum permitted for distance between parallel sides of hexagonal bases and are maximum diameters of round bases.
### Fig. 75. Micropipette with cylindrical tip—details of construction.

<table>
<thead>
<tr>
<th>Capacity (ml.)</th>
<th>Subdivision (ml.)</th>
<th>Interval graduated (ml.)</th>
<th>Lining</th>
<th>Number at 0 and each ml.</th>
<th>Tolerance (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.01</td>
<td>0 to 0.18</td>
<td>0.02</td>
<td>0.02</td>
<td>±0.005</td>
</tr>
<tr>
<td>0.5</td>
<td>0.01</td>
<td>0 to 0.45</td>
<td>0.05</td>
<td>0.01</td>
<td>±0.01</td>
</tr>
<tr>
<td>1</td>
<td>0.02</td>
<td>0 to 0.90</td>
<td>0.1</td>
<td>0.1</td>
<td>±0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>0 to 1.75</td>
<td>0.25</td>
<td>0.05</td>
<td>±0.04</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>0 to 2.70</td>
<td>0.25</td>
<td>0.05</td>
<td>±0.06</td>
</tr>
</tbody>
</table>

No graduations to appear in tapered portion.
Tip may be tapered at junction with body, but outside diameter at this point may not exceed 4.5 mm.
Tip outlet to be glazed, with least possible constriction.
Calibrated to deliver at 20° C. touching off last drop.
The volumetric flasks\textsuperscript{105} are of a new design which combines convenience in use with accuracy. The wide base affords greater stability against upset (Fig. 74). In the case of the one-ml. size, the diameter of the base is small enough to permit placement on the microchemical balance pan and the weight is restricted to a maximum of 19 grams when empty so that when full of a solution of specific gravity of one or less, the capacity of the balance is not exceeded.

In order to increase the usefulness of the above flasks, special measuring pipettes (Fig. 75) were designed.\textsuperscript{106} The long narrow delivery stems of the pipettes with cylindrical tip (0.2-, 0.5-, 1-, 2-, and 3-ml. sizes, Fig. 75) reach to the bottom of the flasks, permitting almost complete withdrawal of the contents. Actually, all but a few hundredths of a milliliter can be withdrawn.

The microliter pipettes\textsuperscript{106} shown in Figs. 76, and 77 also have dimensions such that they too may be used with the microvolumetric flasks. These types, sizes, and dimensions were recommended after a study was made of the returns from a questionnaire sent out to interested parties. Returns were received from 56 individuals, 48 of whom were users of this type of pipette, and a number of whom have since tested the finished items and found them to be quite satisfactory. The following procedure is recommended for calibration of these microliter pipettes:\textsuperscript{106}

"Standard procedure for micropipette calibration consists of filling the pipette with mercury, discharging the mercury into a porcelain dish, weighing the mercury, and making the appropriate weight-temperature-volume calculation. This method has been compared with that of weighing the pipette both empty and mercury filled, and has been found less difficult and equally precise."

(Note: For calibration purposes where mercury is used, the edge of the mercury meniscus should coincide with the top of the line.)

Micro washout pipettes\textsuperscript{107} of the types shown in Figs. 78 and 79, respectively, are used extensively. They are designated as micropipettes instead of as microliter pipettes because of their particular applications and not because of their range. With both, adhering material is transferred by means of wash liquid, being sucked up through the tip in the case of the Folin-type\textsuperscript{65} and added to the cuplike top of the other.\textsuperscript{84}

The density-type pipettes\textsuperscript{2,197} described in Chapter 22, Fig. 217, will also prove useful, particularly because they have ground tips and caps.

Self-filling, self-adjusting polyethylene type of measuring units developed by Sanz are commercially available.\textsuperscript{17}

As an aid to pipetting, hypodermic syringes are attached to microliter pipettes by means of plastic tubing. This affords a means of better control.

The burette\textsuperscript{9} shown in Fig. 80 may be used as either a micro- or ultramicroburette. It has a 3.5-ml. reservoir through which passes a power driven
Fig. 76. Microliter pipette for 1- to 4-μl sizes—details of construction.

<table>
<thead>
<tr>
<th>Size (Calibd. to contain) (μl.)</th>
<th>I.D. tubing at end (mm.)</th>
<th>I.D. at end (mm.)</th>
<th>Min. cap. safety bulb (μl.)</th>
<th>Vol. tol.* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 140 ± 5</td>
<td>0.12–0.16</td>
<td>0.10–0.20</td>
<td>50</td>
<td>±1</td>
</tr>
<tr>
<td>2 140 ± 5</td>
<td>0.16–0.25</td>
<td>0.15–0.25</td>
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* Closer volumetric standardization must be carried out by user with substances under actual conditions of use.
FIG. 77. Microliter pipette for 5- to 500-μl sizes—details of construction. For dimensions see next page.
### Fig. 77. (cont.)  
Microliter pipette for 5- to 500-μl. sizes—dimensions.

<table>
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<tr>
<th>Size (Calibd. to contain) (μl.)</th>
<th>Over-all length (mm.)</th>
<th>O.D. tubing (mm.)</th>
<th>I.D. tubing (mm.)</th>
<th>Approx. length (mm.)</th>
<th>Max. O.D. tubing length (mm.)</th>
<th>Min. length at end (mm.)</th>
<th>Wall at end (mm.)</th>
<th>I.D. at end (mm.)</th>
<th>Min. cap. safety bulb (μl.)</th>
<th>Vol. tol. (%)</th>
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<td>0.18-0.25</td>
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<td>4</td>
<td>55</td>
<td>0.5-0.7</td>
<td>0.15-0.25</td>
<td>50</td>
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<td>50</td>
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<td>0.50-0.75</td>
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<td>4</td>
<td>55</td>
<td>0.5-0.7</td>
<td>0.30-0.50</td>
<td>75</td>
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<tr>
<td>19</td>
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<td>5-6</td>
<td>0.75-1.00</td>
<td>65</td>
<td>4</td>
<td>55</td>
<td>0.5-0.7</td>
<td>0.40-0.60</td>
<td>100</td>
<td>±0.3</td>
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<tr>
<td>20</td>
<td>145 ± 10</td>
<td>5-6</td>
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<td>65</td>
<td>4</td>
<td>55</td>
<td>0.6-0.8</td>
<td>0.40-0.60</td>
<td>100</td>
<td>±0.2</td>
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<td>5-6</td>
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<td>65</td>
<td>4</td>
<td>55</td>
<td>0.6-0.8</td>
<td>0.40-0.60</td>
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</tr>
<tr>
<td>22</td>
<td>145 ± 10</td>
<td>5-6</td>
<td>0.75-1.00</td>
<td>65</td>
<td>4</td>
<td>55</td>
<td>0.6-0.8</td>
<td>0.40-0.70</td>
<td>200</td>
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<td>23</td>
<td>150 ± 10</td>
<td>6-7</td>
<td>1.00-1.25</td>
<td>70</td>
<td>6</td>
<td>60</td>
<td>0.6-0.8</td>
<td>0.40-0.70</td>
<td>200</td>
<td>±0.2</td>
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<tr>
<td>24</td>
<td>160 ± 10</td>
<td>6-7</td>
<td>1.25-1.50</td>
<td>70</td>
<td>6</td>
<td>60</td>
<td>0.6-0.8</td>
<td>0.40-0.70</td>
<td>200</td>
<td>±0.2</td>
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* Closer volumetric standardization must be carried out by user with substances under actual conditions of use.
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Fig. 78. Micro washout pipettes—details of construction.

Fig. 79. Micropipettes, Folin-type—details of construction.
Fig. 80. Aminco automatic power-driven burette.
Vycor\textsuperscript{*} plunger. A revolution counter is so calibrated that it registers to the nearest 0.001 ml. and a graduated drum mounted on the input shaft to the counter registers 0.0001 ml. per division. Meniscus reading has been eliminated. The delivery type is immersed in the solution to be titrated.

Fig. 81. Rehberg burette. (A) Separate delivery tube with ground joint to facilitate cleaning. (B) Removable glass tip. (C) Gas bubbling tube for insertion in solution being titrated to provide stirring action.

Besides the types of burettes described and shown in the preceding pages, many others have been described, particularly for measuring smaller quantities of solutions. Figures 81 and 82 show two of these capillary burettes\textsuperscript{120,163}

\textsuperscript{*} Silica glass (96\%) No. 790.\textsuperscript{46}
which require micrometer screw type of manipulation. For descriptions of these, the reader is referred to the book by Kirk.98

A steaming apparatus of the type shown in Fig. 83 is useful for cleaning flasks in connection with volumetric procedures.145-147

For complete information regarding titration curves, equilibrium for acid and for base dissociation, pH, buffer action, the theory and choice of indicators, electrometric titrations, etc., the reader is referred to the treatise by Clark.44

Fig. 82. (Left) Linderstrøm-Lang and Holter burette.

Fig. 83. (Right) Steaming apparatus, showing method of cleaning flask by flushing with steam.
TABLE 15
ADDITIONAL INFORMATION ON REFERENCES RELATED TO CHAPTER 5

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