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Text Book
for
Basic Operations in Chemical Analysis
- Analysis Video Series for Environmental Technology Transfer -

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Overseas Environmental Cooperation Center, Japan

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INTRODUCTION

This video series was produced by Overseas Environmental Cooperation Center, Japan sponsored by the Environmental Agency of Japan in 1998 to provide audiovisual assistance for experts who are teaching basic laboratory techniques in developing countries. This video, entitled “Basic Operations in Chemical Analysis”, aims to teach the basic techniques for environmental analysis. It covers four primary themes: washing and storing glassware, making pure water and checking the water purity, diluting standard solutions, and some basic techniques for titration.

In the production of this video, a committee was established to examine and discuss the contents of the video. The members of the committee are as follows:

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1. Contents of the Video:

1) Prologue:

- Discusses the need for objective monitoring and environmental analysis in the context of environmental policy.
- Explains the necessity of proper laboratory techniques in obtaining accurate environmental data.
- Gives an overview of the contents of the video.

2) Washing and storing glassware:

- Refers to the importance of washing glassware.
- Explains that environmental analysts are responsible for properly instructing personnel on washing techniques and the necessity of washing glassware.
- Describes the basic techniques for washing, rinsing glassware.

- Describes the basic techniques to wash, rinse, dry, and store pipettes.
 - Explains the manner to wash using ultrasonic cleaner and the manner to soak dirty items over night in a rinsing bath.
- 3) Making pure water and checking the water purity:
 - Describes the construction and use of a water distilling device made of glassware, a lower-cost technique that can be used for water purification in developing countries.
 - Describes commercially available water purification systems (image only).
- 4) Diluting standard solutions
 - Explains the basic characteristics of volumetric glassware and basic techniques of handling pipettes and flasks in diluting the standard solution.
 - Presents an example of spectrophotometric analysis of nitrite, to demonstrate a typical application of diluted standard solutions.
- 5) Basic techniques for titration
 - Explains techniques for using burettes in volumetric analysis.
 - Presents an example of iodometry to assess dissolved oxygen in water.

2. Contents of Attached Text (Appendix to Video):

Part ENGLISH NARRATION

- Prologue
- Washing and keeping of glassware
- Pure water making and purity check
- Dilution of standard solutions
- Absorptiometric analysis of nitrite ion
- Titration
- Epilogue

Part DETAILED EXPLANATION NOT GIVEN IN THE VIDEO

Chapter 1. : Prologue: Necessity and importance of environmental analysis

Chapter 2. : Washing and storing glassware

Chapter 3. : Making pure water and checking the water purity:

Chapter 4. : Diluting standard solutions

Chapter 5. : Basic techniques for titration

PART

ENGLISH NARRATION

- **Prologue**
- **Washing and keeping of glassware**
- **Pure water making and purity check**
- **Dilution of standard solutions**
- **Absorptiometric analysis of nitrite ion**
- **Titration**
- **Epilogue**

BASIC OPERATIONS IN CHEMICAL ANALYSIS

ANALYSIS VIDEO SERIES FOR ENVIRONMENTAL TECHNOLOGY TRANSFER

PROLOGUE

Explanation 1

A beautiful and abundant natural environment is essential for a healthy life.

To be able to protect and hand down the natural environment of today, we must push forward research and carry out various environmental protection policies.

Explanation 2

For that, it is necessary to understand objectively the present condition of the environment and get accurate scientific data.

One of the most effective ways to know how much pollution is in the environment is environmental analysis.

Explanation 3

In order to understand the data and interpret the environmental analysis correctly, it is important to choose appropriate methods for all environmental study processes, such as planning, sampling, pretreatment of the sample, chemical analysis, and data processing.

Explanation 4

We collect the sample in the field, bring it into the laboratory, do the pretreatment necessary for the testing method for the target components, and then analyze them.

Explanation 5

In this video, we will discuss the four basic but very important areas that we must understand to do analysis in the laboratory.

WASHING AND KEEPING OF GLASSWARE

Explanation 6

Glassware must be clean at all times, and contamination reduced as much as possible for correct and precise chemical analysis.

Analysts are responsible for washing experimental apparatuses.

Explanation 7

The analysts, in theory, wash and manage glass apparatuses by themselves in analytical chemistry.

However, in reality the washing is often done by other personnel. In this case, the analysts are still responsible for proper instruction and supervision to ensure that it is done correctly.

If good data is not obtained because the washing was inadequate, the analysts are responsible.

Explanation 8

Used glassware apparatuses need to be sorted and the proper washing method selected for intended use.

Explanation 9

If used glassware is left dirty the glass surface adsorbs material and is hard to clean.

At a minimum, throw away the contents in the correct place and rinse with water immediately after every use.

First, let's look at basic glassware washing methods.

Explanation 10

Glass apparatuses for environmental analysis should be washed with a sponge or a soft brush in diluted laboratory detergent.

Explanation 13

Rinse glassware that was washed with cleanser with tap or warm water to remove all detergent and make sure that there are no bubbles left.

Also, not only the inside but also the outside of apparatuses must be kept clean.

Explanation 14

To make sure that oil or stains have been removed from the surface hold up the apparatus and make sure that the surface is uniformly covered by a thin water film.

If water drops bead on the surface, the washing is inadequate.

Stains come out much more easily with warm water.

Explanation 15

Using a washing bottle, rinse the inside and rim of washed glassware with pure water three times.

Carefully using small amounts of pure water several times, cleans better than using a large amount of water once and it doesn't waste pure water.

Explanation 11

When you clean places like the inside of a flask with a brush, that brush should fit the apparatus's shape and size.

You should not use worn-out brushes or powdered cleansers because they will scratch the glass surface.

Explanation 12

Soaking very dirty items over night in a rinsing bath with laboratory detergent is effective.

In heavy metal analysis, apparatuses are sometimes soaked in dilute nitrate or hydrochloric acid to remove metallic elements on the glass surface.

Explanation 16

Put rinsed glassware in a dish drainer, and then store it on a drying shelf or dry it in a clean and relatively dust-free area.

Explanation 17

Dirty samples should never be left in a drying oven in case it needs to be used.

Explanation 18

Use an ultrasonic cleaner to wash apparatuses too small to clean by brush.

They should be washed in a rinsing bath with a small amount of diluted cleanser and water.

Explanation 20

Used pipettes should always be quickly rinsed with tap water while they are wet.

Explanation 21

If you use a pipette cleaner, the pipettes should be placed pointing upward in a basket to prevent the tip from breaking.

Explanation 22

Rinse pipettes with tap water, and don't leave them soaking in the rinsing bath too long.

Rinse the washed pipettes with pure water.

Explanation 23

Volumetric glassware such as pipettes and volumetric flasks expand if heated and shrink if cooled, resulting in a change in size.

Thus always dry them at room temperature and never dry them in an oven.

Explanation 24

Care must be taken to protect carefully cleaned apparatus from room dust.

Keep glassware in a cabinet with a door.

Explanation 25

To protect the inside of glassware from dust, they should be placed upside down on paper towels

on a shelf.

Protect small apparatuses by keeping them in a small box.

They can be kept in air-tight containers depending upon the how they are to be used.

PURE WATER MAKING AND PURITY CHECK

Explanation 26

You cannot get correct results if the water used for chemical analysis contains impurities.

Various water purification production systems are available.

But the most important thing to remember is that it is expensive to maintain pure water quality.

Explanation 27

This is a still which is comparatively easy to assemble.

Distilled water can be made by putting a large flask with a condenser on a gas range.

Fill it with water to 80% of flask capacity, and add several boiling stones to prevent sudden boiling.

Explanation 28

Add a little potassium permanganate to decompose and remove organic materials in tap water.

By adding tap water at the rate of production water can be distilled for an extended period of time.

But, if there are scales in a distillation flask, the efficiency of distillation drops.

In that case, wash the inside of the apparatus with acid.

Explanation 29

There are many other types of commercially available water purification systems.

Purified water can be produced using the best system for the intended use, combining processes such as activated carbon adsorption, ion exchange, reverse osmosis, and filtration.

Explanation 30

The cartridges, used in activated carbon or filtration systems, have to be replaced on a regular basis.

Explanation 31

The quality of purified water produced can be checked with an electric conductivity meter.

Commercial systems have indicators that show when conductivity ratio exceeds a set value and the cartridge must be changed.

DILUTION OF STANDARD SOLUTIONS

Explanation 32

In general stock solutions are highly concentrated.
They need to be diluted to the proper concentration for each specific use.

First, let's look at the basic operation for diluting a stock solution to the proper concentration for absorption spectrophotometric analysis.

Explanation 33

Volumetric glassware such as graduated cylinders, pipettes, volumetric flasks, and burettes are available to measure a set amount of a solution.

Explanation 34

Use a volumetric pipette or measuring pipette to measure solution.
There are various sizes available to fit a variety of needs.

Explanation 35

When you need to measure a specific amount of solution for operations like making a standard solution, use a volumetric pipette.
Exactly 10 ml of solution can be obtained by drawing in fluid up to the line in this volumetric pipette.

Explanation 36

This measuring pipette has a scale on it.
It is useful for adding an approximate amount of solution.

Explanation 37

Some pipettes are accurate down to the tip, while others have been graduated for partial delivery.
So, check the scale of a pipette before using it.

Explanation 38

Use a pipetter when you draw a solution into a pipette.
When you use a rubber ball, squeeze it to push out the air, and then put it on the end of the pipette.

Explanation 39

Be careful with the tip of the pipette when drawing in a solution.
It should be inserted into the solution.
You must be very careful, because the tip comes out of the liquid while drawing in solution, some of it will get into the rubber ball.

Explanation 40

Draw in the solution up to a little more than the line, tilt the pipette, quickly take off the rubber ball, and then cover the end of the pipette with your index finger.

Explanation 41

Normally, a pipette must be dry.

If it's wet, drain it as much as possible and then rinse it by drawing in a small amount of the solution to be measured.

This is called washing with the solution to be measured and is repeated three times.

Explanation 42

To bring the drawn solution precisely to the line, hold the pipette vertically while looking at the solution level slowly release your index finger and allow the solution to fall to the target line.

Explanation 43

Next, put the tip of the pipette on the inside surface of the volumetric flask, and completely release your index finger.

The solution will drain out naturally.

Explanation 44

To get the last drop from the pipette, once again cover the edge of the pipette with your index finger, and hold the bulge with your other hand.

Your body heat will raise the air pressure and force out all of the solution out.

Explanation 45

We will look at volumetric flasks.

Volumetric flasks are generally used to make a solution with a given concentration and come in various sizes.

Explanation 47

First, add 80% of the purified water for the dilution.

Then, draw up 5 ml of solution with a pipette, add it to the volumetric flask and mix gently.

Explanation 48

Then, put a stopper on the volumetric flask, hold the stopper with your finger repeatedly, turn the flask upside-down and mix the solution thoroughly.

Explanation 49

Add purified water up to the line, and mix it again the same way as before.

This is the diluted solution.

The stock solution is diluted 20 fold by this process.

The concentration of the standard solution can be further changed by repeating this dilution process.

ABSORPTIOMETRIC ANALYSIS OF NO₂ ION

Explanation 50

Next, let take a look at a spectrophotometric analysis of nitrate as an example.

Explanation 51

Three concentrations of nitrate, 0.6, 0.2, and 0.06 milligram per liter were made in stages from a stock nitrate solution.

Explanation 52

These are two water samples which have unknown concentrations.

Purified water has also been prepared as a blank.

Explanation 53

Then, 1 ml of surfanil amide solution is added to each sample, under acidic conditions, mixed and allow to stand for five minutes.

Explanation 54

Then, 1 ml of naftylethylenediamine solution is added and mixed.

Experimental conditions have to be the same for all samples, standards, and blank.

After allowing the solutions to stand for twenty minutes, we see the color change toward a reddish purple, proportional to the concentration of nitrate.

Explanation 55

A spectrophotometric check of the concentration of the color changed solution is made by measuring the amount of light absorption at the wavelength of 540 nanometers.

TITRATION

Explanation 56

Next, let's look at how to do a titration analysis as an example of measuring solution concentration.

Titration is widely used in various fields because it requires only basic apparatus and gives precise results.

Explanation 57

The burette for titration is one important type of glass volumeter.

Choose the size of the burette, to fit the amount of reagent used for the particular titration.

Use an amber glass burette when using light-sensitive reagents such as potassium permanganate and silver nitrate.

Explanation 58

Place the burette in a stand, and slowly pour the standard solution from the upper part of the funnel to a little above the line.

Explanation 59

Both the inside of the burette and funnel must be well rinsed with standard solution.

Discard the standard solution used in rinsing.

Repeat this process three times.

Explanation 60

Allow the rinsed burette to stand, and then reduce the solution to the line on the burette.

Explanation 61

For a 25 ml burette, eyeball the position of level of the solution up to 0.01 milliliter and record the results in your field notes.

Explanation 62

Let's look at an example of iodometry for dissolved oxygen in water.

Assume a sample has been collected and pretreated for example by fixing dissolved oxygen in water.

Here 25 millimoles per liter sodium thiosulfate solution is used as a standard.

Explanation 63

Put the sample in an Erlenmeyer flask and hold it with your right hand, grasp the cock of the burette with your left hand and open it slightly.

While the standard solution is dripping in, shake the flask with your right hand.

Explanation 64

A magnetic stirrer is generally used for uniform mixing.

Choose the appropriate stirring rod, rinse with purified water, put it in a conical beaker or an Erlenmeyer flask and adjust the speed for proper stirring.

Explanation 65

Slowly add the standard solution from the burette, and stop adding at the moment when the yellow color fades.

It will turn blue when you add 1 ml of starch solution, as an indicator, because of the starch-iodine reaction.

Explanation 66

Add the standard, two to three drops at a time.

The volume of one drop is about 0.03 ml.

Practice in advance to achieve the reproducibility within a range of 0.05 ml when you repeat the same titration.

When the color grows faint, add the standard solution a drop at a time.

When the blue color disappears the end point of the reaction has been reached.

Explanation 67

Read and record the burette's scale at the end point to 0.01 ml in your field notes.

EPILOGUE

Explanation 68

Even though you use highly advanced analytical apparatus, you will never get correct analytical data for environmental analysis without correctly doing the basic operations introduced here.

To increase the reliability of research and investigation, never skip simple but essential work such as washing glassware.

It is important to train and guide people who do analysis so they can do it correctly.

To obtain analytical data close to true values, you have to do analysis with as much precision as possible.

For environmental protection, it is important that these results are reflected in environmental policy.

Doing the important work of environmental analysis correctly helps protect the natural environment of a nation and the health of its citizens.

PART

DETAILED EXPLANATION NOT GIVEN IN THE VIDEO

Chapter 1: Prologue: Necessity and Importance of Environmental Analysis

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Chapter 5 : Basic Techniques for Titration

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Reference

JIS K0050 "General rules for chemical analysis"

JIS K0557 "Water used for industrial water and wastewater analysis"

JIS K0102 "Testing method for industrial wastewater"

JIS R3505 "Volumetric glassware"

1. Prologue: Necessity and Importance of Environmental Analysis

1.1 Introduction

Japan has successfully undergone extensive economic development in recent decades. However, this development can have adverse impacts on the environment, with increases in water and air pollution that can be detrimental to both human health and natural environment. The nation, local governments, and corporations have jointly addressed the pollution issue, and have been able to achieve moderate improvements.

However, developing countries are currently faced with the difficult task of enhancing economic strength and making infrastructure improvements in cities, while attempting to curb pollution and preserve the local and global environment. Measures are needed to achieve environmental improvements in every economic sector, including residences and the transportation sector particularly in urban settings. Experts who are sent to these countries can be quite useful, as they can use their experiences in addressing environmental issues to determine policies and techniques that are feasible and suitable for these countries.

This text provides some basic information that can be presented to people primarily engaged in environmental analysis, to help understand environmental issues and techniques. The primary focus of this text is on techniques for laboratory analysis and water purification, necessary steps for comprehensive environmental monitoring. We expect that this text can be used to help train environmental counterparts in developing countries, and can also be used to help familiarize experts with the analytical techniques (in the training before they are dispatched overseas.).

1.2 Recognizing environmental changes and indices of change

We are surrounded by a range of environmental settings in different media, including air, water, and soil. Environmental analysis is needed to identify changes in the composition of these media, both qualitatively and quantitatively. As an example of the types of analysis that can be conducted, we consider changes to a body of water (the aquatic environment).

There are a number of characteristics of the aquatic environment that can influence the health of fish and plants, which we can measure with relatively simple indices. Understanding these indices and their evolution over the short run and the long run can help understand the health of the body of water. Some important characteristics include the color of the water, its temperature and turbidity, and the

prevalence of plants and aquatic life.

To help understand how a body of water can change and how this can affect aquatic health, let us consider a scenario in which fish are found floating on the surface of the water. Clearly, this scenario is a consequence of drastic changes in the habitat of the fish.

Let us suppose that the following changes have been documented for this body of water:

- The water volume has reduced
- The depth of the water has decreased
- Wind is minimal
- The water color has not changed
- The sun is shining directly on the water
- Fish are floating on the surface of the water with their mouths open

These characteristics of the water point toward a low level of oxygen in the water. This status can be confirmed by measuring the level of dissolved oxygen in the water.

However, there could be additional causes of fish kills, and a thorough investigation is needed. An environmental analyst should also measure the water temperature and pH, and catch some of the fish to examine physiological responses. If any abnormalities are documented in the gills of the fish, toxic substances could potentially be adversely affecting the health of the fish. Especially in circumstances when the body of water is used for drinking water, measurements should be taken immediately to determine the condition of the water. Following are some typical measurements that can be made in water or to fish to quantitatively determine the water condition:

1) Dissolved oxygen (DO):

- DO is defined as the amount of oxygen that is dissolved in water. Oxygen is typically dissolved in water at a concentration of 9 mg/L at standard air pressure and at 20 degrees Celsius. The higher the air pressure and the lower the temperature, the greater the level of DO. Dissolved oxygen in water is essential for any creatures or plant life living in the water; decreases in DO lead to corresponding decreases in the quantity and diversity of species found.

2) Hydrogen ion concentration:

- Hydrogen ion concentration (also called hydrogen ion concentration index or hydrogen ion index) is best known by its abbreviation: pH. The pH of a substance is defined as $\log_{10}(1/[H^+])$. (*)

* $[H^+]$ means molar concentration of hydrogen ion.

Water is considered neutral at a pH of 7; lower pH corresponds to higher concentrations of hydrogen ion (more acidic water). Fish are typically able to survive in water with pH ranging between 4 and 10, provided that other stressors are not present. When pH rises to around 10, fish are unable to swim smoothly, and viscous liquid seeps from their skin, gills or mouth, turning the water turbid.

3) Changes to fish gills:

- When fish are killed by toxic substances, the gills tend to blacken. Depending on the substance, the color of the fish might change as well. Some typical pollutants and their corresponding colors include:

Cyanogen	vivid red at high concentrations(0.25 mg/L)
Lead	pale
Hydrogen sulfide	dark red
Shortage of oxygen	brown or white
Acid	brown
Sulfate plating solution	blue and white

These colors change as time passes since the death of the fish.

4) Organic contamination:

- The increase in water hyacinth populations and changes in fish habitats over the course of a few years show organic contamination is caused. Such contamination is typically caused by factors such as population increase near the body of the water, operation of factories emitting organic effluent, and organic fertilizer for cultivation of farmland. Organic contamination can affect the capacity of the water to self-purify, leading to changes in plant and fish populations. Organic contamination can be quantitatively determined by measuring oxidation-reduction potential(*), chemical oxygen demand (COD), or biochemical oxygen demand (BOD).

*Oxidation-reduction potential

Oxidation-reduction potential is measured by placing a non-reactive metal electrode and a standard electrode into a solution containing an oxidant and reductant of some substance. The result is the generation of a potential difference, with larger potential differences corresponding to a high concentration of organics.

5) COD (Chemical oxygen demand)

- COD is defined as the volume of oxygen that is equivalent to the volume of a given oxidizer that is used to oxidize organic substances in the water. High COD corresponds to elevated organic concentrations.
- It should be noted that in Japan, potassium permanganate (KMnO_4) is used as the oxidizer, while potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) is used in most other countries. The COD value obtained by using the latter is higher than that using the former. It must therefore be noted which oxidizer is used.

6) BOD (Biochemical oxygen demand)

- BOD is defined as the volume of dissolved oxygen in water that is consumed by aerobic microorganisms as they decompose organic material. This occurs through the oxidation of organics, and the level of BOD depends on the temperature and time of consumption. As above, higher levels of BOD correspond to greater organic contamination.

Along with these environmental measurements, detailed and regular observation of creatures in the river can be used to construct another index of the degree of contamination in the water. Algae and benthos are useful indices. Clearly, the plant and animal species prevalent in each body of water will vary, depending on the ability of species to thrive in the local environment. This demonstrates the need to track conditions across time, so that changes from a baseline can be determined.

In summary, to understand environmental changes, monitoring of environmental conditions and indices is required on a regular basis. Changes in the environment can only be determined by paying due attention to the environment and being sensitive to environmental changes.

1.3 The role of analysis in environmental monitoring

As described above, changes in the environment are measured quantitatively in environmental analysis. To help preserve and improve the environment, it is

necessary to understand current conditions and trends. Quantitative data can be compared over time, as well as across regions and countries, to help in the development of regulations and policies. Clearly, the data collected from the field must be credible. An environmental analyst must be able to decide on the methodology and parameter to evaluate environmental change, and must balance the tradeoffs among different methods. While evaluation by determining multiple parameters may be theoretically desirable, costs and reliability concerns necessitate environmental evaluation using minimum necessary parameters.

In many situations, the concentrations of targeted substances are quite small, and cannot be detected unless the basic fieldwork and labwork are done to perfection. This involves the use of proper devices to measure the specified substance, but also involves a number of other stages. Containers holding the samples and glassware used to extract the specified substances must be clean and stable, and purified water must be used to avoid sample contamination. Despite the importance of this step, little attention is typically devoted to the cleaning of glassware ñ many people wash sample containers like they are washing glasses at home! It is also important to address the purity of the water, both in terms of the quality of the water supplied to the water purification system and the resulting purified water.

2. Washing and Storing Glassware

As described above, it is crucial to use clean glassware for chemical analysis. If a dirty apparatus were used, the results of the analysis would be incorrect. Even if the preparation, prior treatment and measurement of the reagents are properly implemented, the results of the analysis would be incorrect if dirty apparatus were used. In addition, even if the apparatus were carefully cleaned, it could still be contaminated if stored inappropriately.

In many countries, analysts often ask other personnel to wash glassware. If good data are not obtained due to inadequate or improper washing, the analyst must take responsibility. It is always important to remember that the first step in chemical analysis is washing glassware.

2.1 Methods to wash glassware

Some glassware can be sufficiently cleaned using only water, while other pieces might require more comprehensive methods. The first point to remember is to wash the glassware immediately after use, since the glass surface can adsorb the dirty material if left unwashed for long periods of time, making it quite hard to clean. The actual methods for washing the glassware vary by the chemicals that have been used:

- 1) Glassware used for water-soluble agents can be cleaned by washing with water 4-5 times, using either a brush or a sponge. If the surface is stained by oily material, detergent must be used to clean it.
- 2) Glassware used for non-polar reagents, samples, or viscid materials must be washed using detergent ¹⁾ and a sponge or brush which fits the shape and size of the apparatus. Once the detergent has been used, the glassware must be rinsed with water. Warm water should be used to remove the detergent more quickly. If the glassware is quite dirty, you should soak it in detergent overnight ²⁾, and then wash with a brush and rinse with water. Vigorous scrubbing of the glassware can harm the glass surface. After soaking the glassware, an ultrasonic cleaner can be used if desired.
- 3) It is important to rinse the inside surface of the apparatus with some purified water after rinsing, in order to remove any salt contained in the water, especially high concentration in hard water.

¹⁾ Laboratory detergent must be used and handled with care. Neutral household detergent can be used, but detergent should be selected that does not contain phosphorus, since this

can cause eutrophication of rivers, lakes, and the ocean. Household detergent should also be rinsed completely, since it can contain additives such as perfume.

- 2) To remove metallic material, the apparatus should be soaked in dilute nitric acid or hydrochloric acid. In the past, chromic acid mixture in which potassium dichromate was diluted into concentrated sulfuric acid was used due to its cleansing ability, but this mixture pollutes the environment and should not be used.

2.2 Drying glassware

Glassware can be dried either at room temperature or an oven. In either case, the apparatus must be carefully protected from room dust (i.e., by using a dry shelter). If an oven is used, a different oven should be chosen for drying clean glassware than for drying samples. An oven should not be used for any volumetric glassware such as pipettes and volumetric flasks, because they expand and will not measure properly if heated.

2.3 Storing glassware

It is most desirable to store washed glassware in an enclosed cabinet. Otherwise, glassware should at least be placed upside-down in a shelf, either with a door or some other cover (Aluminum foil should be used, if it is placed in an open space.). Regardless of the storage, glassware should be washed prior to use, since it can become contaminated over time.

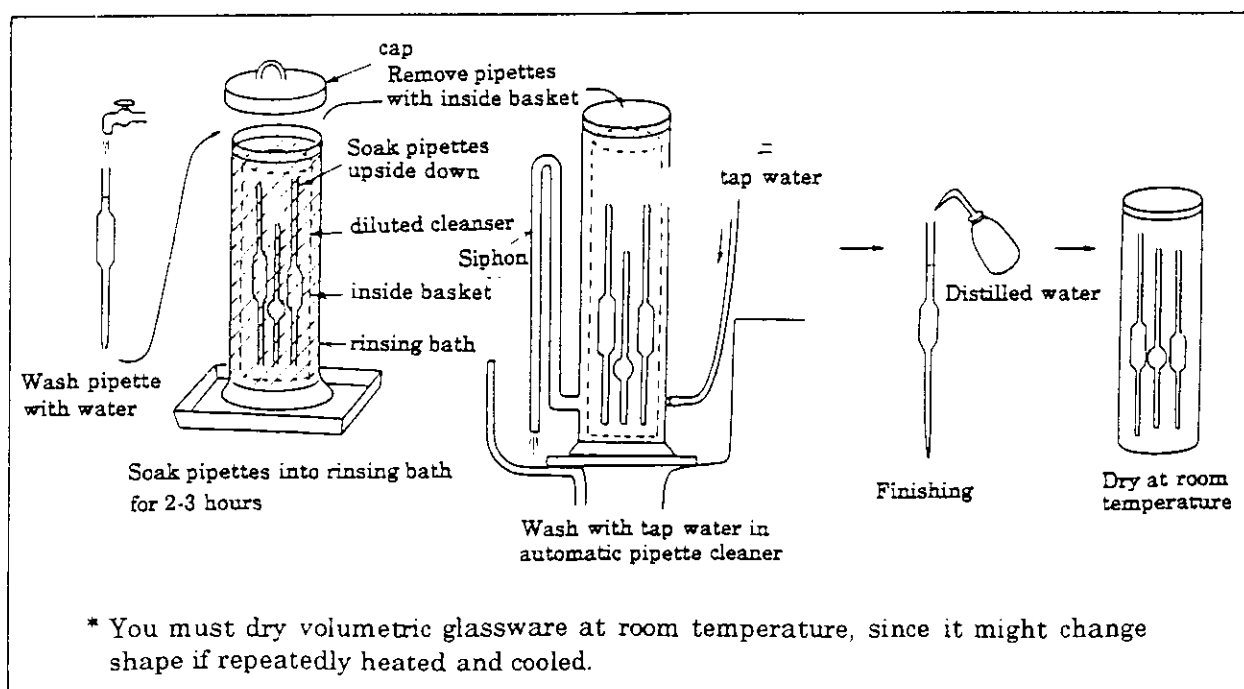


Figure2-1 Cleaning pipette

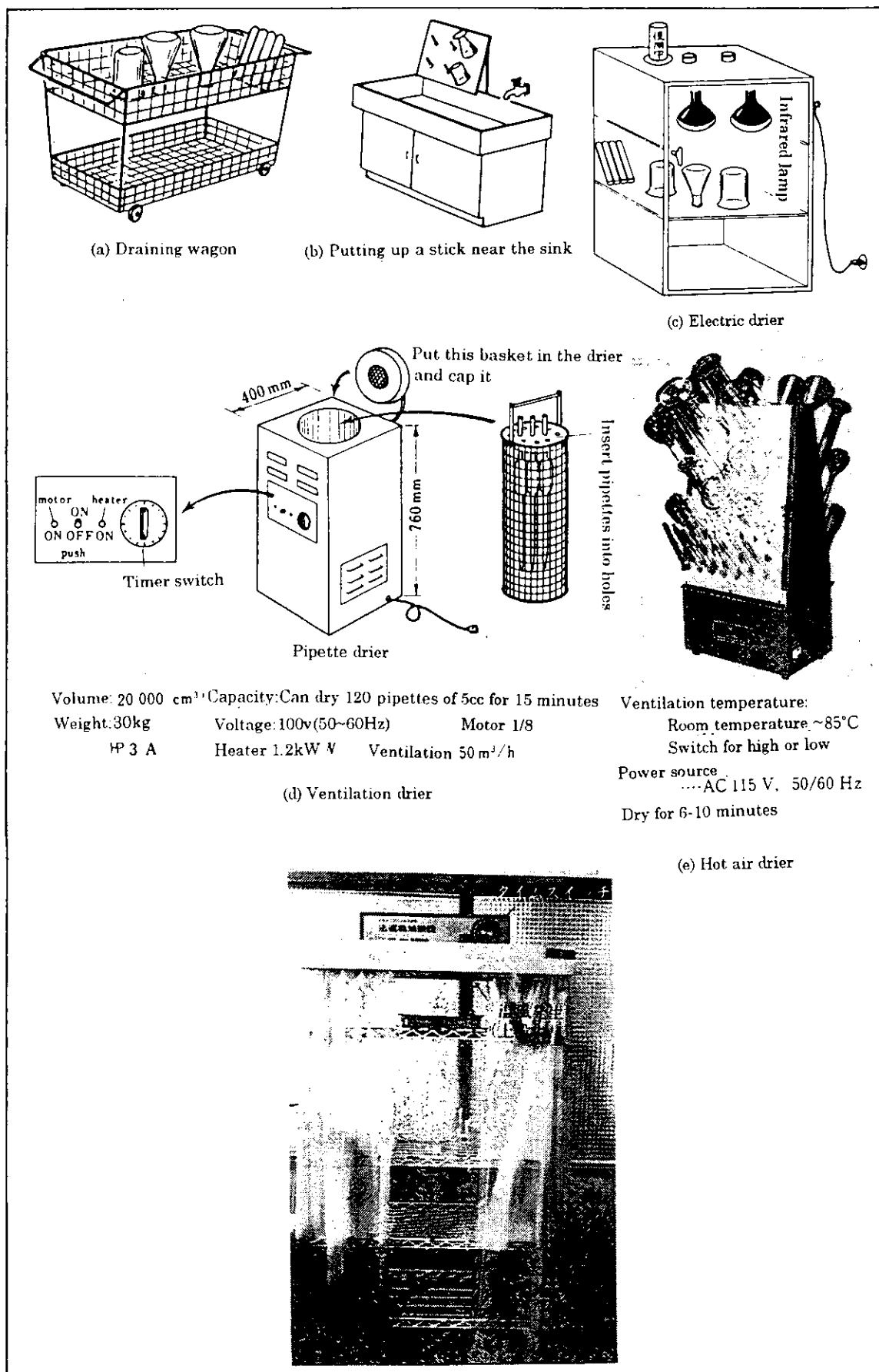


Figure 2-2 Instruments for drying glassware

Reference: Ryuzo Nishiyama, "Illustration. Basic chemical experiments", 1983, Ohm-sha

3. Making Pure Water and Checking the Water Purity

3.1 Distilling water using glassware

Purified water for chemical analysis is usually made using various water purification systems, which need to be maintained and managed to work correctly. These systems are expensive and maintenance costs are high, which may inflict heavy burdens on local analyzing laboratories in developing countries. Therefore, we describe the construction and use of a handmade water distiller, which is cheap and easy to maintain and manage. The necessary glassware for the apparatus can be procured at a lower price in developing countries.

(1) Materials

- 1) Power source to heat water: Gas cylinder containing LPG (commercially available)
- 2) Gas range
- 3) Water supply: Tap water (to be distilled and used as cooling water)
- 4) Glassware (Ground glass for connecting portion)
 - A. Large flask with flat bottom (3-5 L flask, shaped like a turnip, \$45/50)
 - B. Spherical condenser (\$45/50)
 - C. Adapter (\$19/38)
 - D. Bottle to gather distilled water
- 5) Glass bottle to store distilled water(20L)
- 6) Rubber hose to connect
- 7) Potassium permanganate, boiling stones, etc.

(2) Assembling the device

Connect propane gas cylinder to gas range with rubber hose. Put a large flat-bottom flask on gas ring. Connect a spherical condenser on the flask and an adapter to the condenser. Put a triangle flask under the adapter. Insert rubber hose into the tap and connect the other side of the hose to the condenser.

(3) Making the distilled water

- 1) Place a small amount of potassium permanganate in the large flask to remove organic substances contained in the water as impurities. When the bright violet color of potassium permanganate has faded, add more of the chemical.
- 2) Place boiling stones in the large flask to prevent abrupt boiling.

- 3) Pour water to 80% of flask capacity. The heat level should be adjusted to prevent abrupt boiling.
- 4) Adjust the bypass cock connecting the condenser to the flask, so that the same amount of tap water as distilled water can be supplied.
- 5) Dispose of the first 100 mL of water that has been distilled. After that point, keep the distilled water in the triangle flask, and then move the distilled water to the storing bottle.
- 6) When the device is not in use, cap the gathering flask with a beaker to avoid contamination.

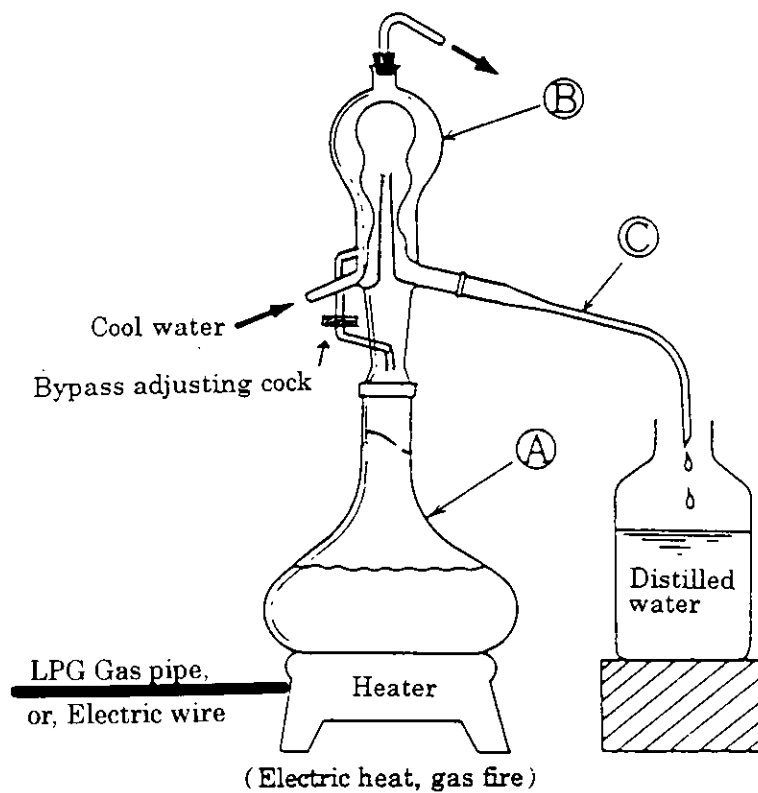


Figure 3 Glass water distiller

A device of this size can distill about 1.5 liters of water per hour.

3.2 Commercially available water purification systems

Commercially available purification systems use a number of different purification methods, including distillation, adsorption by activated carbon, ion exchange, reverse osmosis, and filtration. These techniques can provide a range of water classes, suitable for different analytical purposes.

(1) Classification of purified water

A classification scheme for purified water is provided in JIS K 0557 (1998). Water is classified into four categories, ranging from A1 to A4. The potential applications of different categories of water and the methods used to purify the water are as follows:

A1: Used for washing and as material for higher degree water (A2-A4). Made by ion exchange.

A2: Used for general chemical analysis. Made by refining A1 using a combination of ion exchange and precision filtration.

A3: Used for preparing reagents and microanalysis. Made by refining A1 or A2 using distillation.

A4: Used for testing organic substances (TOC, COD etc.) and microanalysis of organic substances. Made by distilling A1 or A2 and adding potassium permanganate.

Characteristics of each category of purified water are provided in Table 3-1.

Table 3-1 Quality of water used for chemical analysis ¹⁾

Item	Rank			
	A1	A2	A3	A4
Electric conductivity mS/m(25 °C)	under 0.5	under 0.1	under 0.1	under 0.1
Total Organic Carbon (TOC)mgC/L	under 1.0	under 0.5	under 0.2	under 0.05
Zinc μgZn/L	under 0.5	under 0.5	under 0.1	under 0.1
Silica μgSiO ₂ /L	-	under 50	under 5.0	under 2.5
Chloride ion μgCl ⁻ /L	under 10	under 2	under 1	under 1
Sulfate ion μgSO ₄ ²⁻ /L	under 10	under 2	under 1	under 1

1) JIS K 0557 (1998) Water used for industrial water and wastewater analysis

(2) Techniques for purifying water (Reference 2)

1) Reverse osmosis

- Can remove anion, cation and organic substances. Using an improved film module, over 99% of NaCl and almost 100 % of organic substances with molecular weights over 300 can be removed. Reverse osmosis cannot remove substances with low molecule weights, suspensoids, and dissolved gases such as CO₂.

2) Ultrafiltration

- Involves passing water through a filter with a hole diameter of 1-10 nm. Ultrafiltration is typically used to remove colloidal substances, and at least 90-95% of impurities can be removed. Polysulfone, polyacrylonitrile, and polyethersulfone are used as the film modules.

3) Adsorption

- Activated carbon, activated alumina, or a molecular sieve is used to adsorb dissolved macromolecular substances and some dissolved inorganic substances. The absorption process lasts only for a short time.

4) Ion exchange

- Involves the use of a strong acidic cation exchange resin and a strong basic anion exchange resin (the ion exchangers). Usually used in a mixed bed system, in which the cation and anion resins are mixed at the rate of 1 to 2.

5) Distillation

- Involves the use of a non-boiling distilling device.

6) Precision filtration

- Porous high molecule such as acetate cellulose, polysulfone/polypropylene, and nylon are used as the filtration film. When placed on the back end of an ion exchanger, a precision filtration element with a hole diameter of 0.2-0.45 μ m is used.

7) Heating

- Used to remove bacteria and endotoxin with high efficiency. Ultraviolet rays are used to remove bacteria such as *Legionella*, which are typically found in pipes.

Having highly purified water is important when eluting solutions in high performance liquid chromatography (HPLC). Generally, tap water is filtered through a pre-cartridge filter, and purified water is obtained by a combination of reverse

osmosis and deionization. To make the purified water output alkaline, add KMnO_4 and distill. Then, to make ultra-pure water, distill again using a device made of quartz glass. Ultra-pure water should not be stored, since it can easily be contaminated by the container and the surrounding environment.

In general, maintaining the quality of purified water requires appropriate maintenance and management of the systems regardless of the purification method.

(3) Examples of commercially available water purification systems

Some commercially available systems filter water through an activated carbon filter, undergo ion exchange, and then distill to produce the purified water. Other devices first distill the water and then use ion exchange. The water purification system should be chosen to yield the desired purity for the given usage. Some examples of commercially available systems and the quality of the purified water they yield include:

1) ADVANTEC, Aquarius GS-200

Raw water	Activated carbon filter	Ion exchange resin	Distilling	Storage tank
		Ion-exchanged water(A2)		Distilled water(A3)

*Distilling device (boiler, condenser) is made of super hard glass.

2) ADVANTEC, Aquarius GSH-500

Raw water	Activated carbon filter	Ion exchange resin	Distilling	High purity ion exchange resin	Membrane filter
			Distilled water (A3)	Ion-exchanged water(A3)	

3) ADVANTEC, Aquarius GS-990

Raw water	Filtration	Distilling	Storage	High purity ion exchange resin	Membrane filter
		Distilled water (A3)		Ion-exchanged water (A3)	

4) ADVANTEC, Super water purification production systems CPW-200

Treated water	Activated carbon filter	Highly purity Ion exchange resin	High purity ion exchange resin	Resin to remove little impurity	Membrane filter
					Purified water (A4)

5) ORGANO, Cartridge water deionizer

Raw water Ion exchange resin Purified water

6) MILLIPORE , Water purification production systems , Elix series Ex-3

Raw water Proguard Reverse Osmosis Continual ion exchange Purified water (A3)
 preparation (RO) EDI module

*Put ion exchange film over ion exchange resin to electrically remove ion. Maintenance and running cost is reduced because regeneration process of ion exchange resin is not necessary.

4. Diluting Standard Solutions

4.1 Handling pipettes

(1) Types of pipettes

There are two types of pipettes: volumetric pipettes and measuring pipettes. The choice of pipette depends on the desired volume of the solution.

Measuring pipettes have a capacity between 1 and 50 mL. 1 mL pipettes have scales that indicate every 0.01 mL, while 10-25 mL pipettes are scaled on 0.1 mL increments. These pipettes can therefore be used to measure a small amount of liquid or a precise quantity for a solution.

These scales represent the volume of water at 20 °Celsius; different ambient conditions would affect the precision of the measurements. Two grades of allowance for the scale, class A and class B, are provided by Japan Industrial Standards (JIS R3505-1993). Recently, a color code has been provided to indicate the volume.

Generally, a pipette has a mark of “TD (to deliver)” or “Ex”. This does not mean that the quantity of liquid poured into the pipette will be precisely 10 mL, even when liquid is filled to the 10 mL line. Instead, when the liquid is transferred from the pipette, 10 mL will be dispensed. This scale is marked on the pipette and takes into account the fact that some liquid will be left on the surface of the pipette. So, the liquid remaining on the inside surface does not need to be completely removed.

(2) Drawing up liquid with a pipette

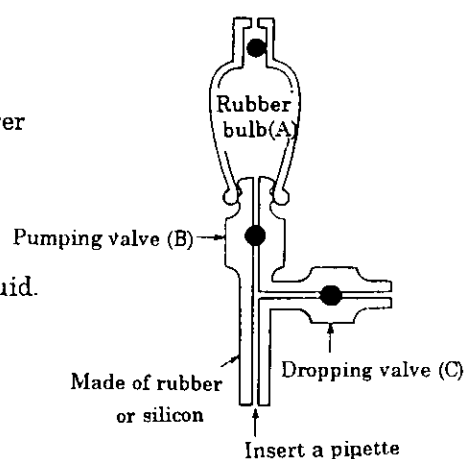
- 1) Insert the tip of a pipette deeply into the solution. If the tip is not completely in the solution, some liquid might enter the pipette.
- 2) When the liquid has reached the point slightly over the marked line, cover the end of the pipette with your index finger.
- 3) Loosen your index finger to slowly reduce the level of the liquid. When the liquid is lowered to the target line, cover tightly again with your index finger. At that time, you should look at the height of the liquid to confirm that it is at the target level. The bottom of the curved surface of the liquid (the meniscus) should be at the target line.
- 4) Place the tip of the pipette on the inner wall of the volumetric flask. Then, release your finger to empty the liquid into the flask.
- 5) Keep the pipette in position for about 10 seconds after all of the solution has been emptied. You should not blow to make the liquid drop more quickly.

finger and grasp the bulge of the pipette with your other hand. Your body heat will expand the air in the pipette, and the liquid will completely drain.

(3) Pipetter

For some chemicals, you can draw up the solution using your mouth. However, you must use a pipetter to draw any solution which vaporizes easily, is acidic or alkaline, or contains a hazardous chemicals. This video explains how this can be done using a rubber bulb, which would be readily available in developing countries. Alternatively, a safety pipetter with a valve is also convenient. The following instructions detail the handling of a safety pipetter:

- 1) Insert a pipette into the safety pipetter.
- 2) Press and dent the rubber bulb (A).
- 3) Press the pumping valve (B) with your finger to pump the liquid. Release your finger to stop the liquid.
- 4) Press dropping valve (C) to drain out the liquid.



4.2 Handling volumetric flasks

Volumetric flasks are used to prepare solutions with given concentrations at room temperature. This involves first pouring water or other solvents slightly below the target line, and then adding the remaining amount of solution using a pipette or washing bottle to reach the target line. This process should be done at a fixed temperature, to avoid volume fluctuations.

Flasks are typically made of either glass or polyethylene, with capacities including: 5, 10, 20, 25, 50, 100, 200, 250 and 500 mL, and 1 and 2 liters. Of these capacities, sizes between 50 and 500 mL are most often used.

Unlike pipettes, volumetric flasks have marks indicating "TC (To contain)" or "In". This means that when the liquid is poured to the line of "250 mL", the volume of the liquid is exactly 250 mL. The JIS standard for glass volume measurements provides the accuracy of the scale.

provides the accuracy of the scale.

4.3 Making standard solutions for environmental analysis

This subject is documented in “Pollution analysis series I, Standard substances and pollution measurement”, written by Yoshikuma Nagasawa et al., Japan Standard Association, 1980.

5. Basic Techniques for Titration

Titration is a key quantitative analytical method for environmental analysis. Titration is also known as volumetric analysis, in contrast with gravimetric analysis.

5.1 Summary of titration techniques

In titration, a given concentration of a standard solution is mixed into a given volume of a testing solution to cause a reaction. The amount of the constituent contained in the testing solution is determined (based on the chemical equivalent) by the concentration and volume of the standard solution needed for the reaction to occur.

(1) Conditions

The following conditions must be satisfied for titration to occur:

- 1) The reactions should be chemically quantitative.
- 2) The reaction should be relatively fast.
- 3) No reactive constituent other than the target one must coexist in the solution.
- 4) The end point of the reaction should be able to be clearly and easily identified.

(2) Equivalent point and end point

The equivalent point is a point when the equivalent volume of titration reagent (standard solution) is added to the target constituent in the testing solution. When the equivalent point is obtained experimentally, it is called the end point. The experimental end point may not necessarily agree with the theoretical equivalent point. The difference between these values (the experimental end point minus the theoretical equivalent point) is called titration error.

(3) Error in volumetric analysis

If all procedures are followed carefully in the titration process, errors in the volumetric analysis can be on the order of 0.1%. Typical errors occur due to the following reasons:

- 1) Accuracy of volumeter
- 2) Impact of temperature
- 3) Error in reading
- 4) Error in confirming the end point

(4) Types of titration methods

Titration methods can be classified by the reactions they utilize as follows:

1) Neutralization titration

This method utilizes a neutralization reaction between an acid and a base (alkali). Acidimetry or acidimetric titration measures an alkali with an acidic standard solution, while alkalimetry or alkalimetric titration measures an acid with an alkaline standard solution.

2) Oxidation-reduction titration

In this reaction, oxidation and reduction occur simultaneously. Oxidation titration utilizes a reaction that oxidizes the target constituent. In this type of titration, potassium permanganate solution, potassium dichromate solution, or iodine solution (starch used as an indicator) is used. Reduction titration measures by reducing the target constituent, using arsenious acid, stannous chloride, and ferrous chloride.

3) Precipitation titration

This method utilizes reaction of formation and disappearance of a deposit. One common method involves measuring chloride ion using silver nitrate (called the Mohr method, using potassium dichromate solution as an indicator).

4) Compleximetry or Complexometric titration

Compleximetry involves the creation of complexes in the solution, and is used mainly for measurement of metals. For example, chelatometric titration using ethylenediaminetetraacetic acid (EDTA) is often used for measuring potassium or magnesium in the solution or measuring concentrations of heavy metal solutions.

(5) Methods for confirmation of the end point

To confirm the end point of titration, you can either use visual confirmation using an indicator or by assessing the color of the reacted solution without using indicators. For example, color change can be measured optically (photometric titration). In potentiometric titration, coulometric titration and conductometric titration changes in the electrochemical characteristics of the solution at the equivalent point can also be measured.

5.2 Handling volumeters

Volumetric flasks, pipettes, and burettes are used as volumeters in volumetric analysis. Although these glasswares are designed to provide accurate

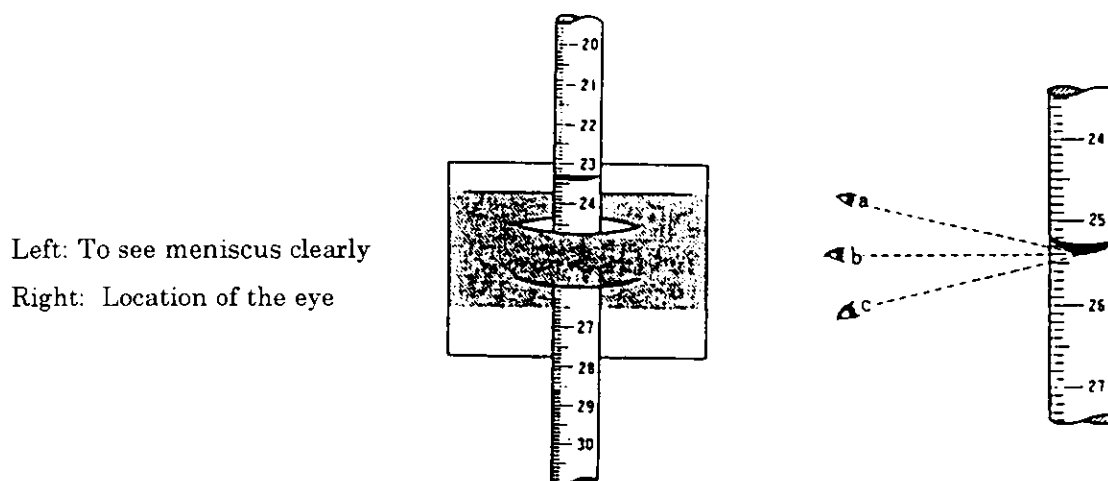
This is an important step in any volumetric analysis, since accurate findings are dependent on the precise volumes of the solutions. The following factors will help maintain the accuracy of these glasswares:

- 1) Volumeters must not be heated to over 50° Celsius. Glassware never returns to its original volume once it has been heated.
- 2) Any substance that reacts to glass should not be put in volumeters. For example, hydrofluoric acid reacts quickly to glass, and alkali solutions and EDTA solutions react gradually to glass. These substances should be stored in polyethylene containers.
- 3) Volumeters should not be scrubbed with a hard brush, since this can damage the glass.

5.3 Reading the scale

Because of surface tension, the surface of a liquid in a thin tube (such as a burette) is in the form of a curved surface (meniscus). Thus, depending on where the eye of the analyst is located, the scale will be read differently, which can cause measurement errors (parallax). The scale should be read at the bottom of the meniscus, with the eye placed at the same level as the meniscus (Point b on Figure 5-1). When the eye is located at Point a, the upper scale should be read, and at Point c, the lower scale should be read.

A burette should be held vertically when reading the scale. If the meniscus is not seen clearly, you can surround the burette by a sheet of white paper, the middle of which is colored black (Figure 5-1).



Left: To see meniscus clearly

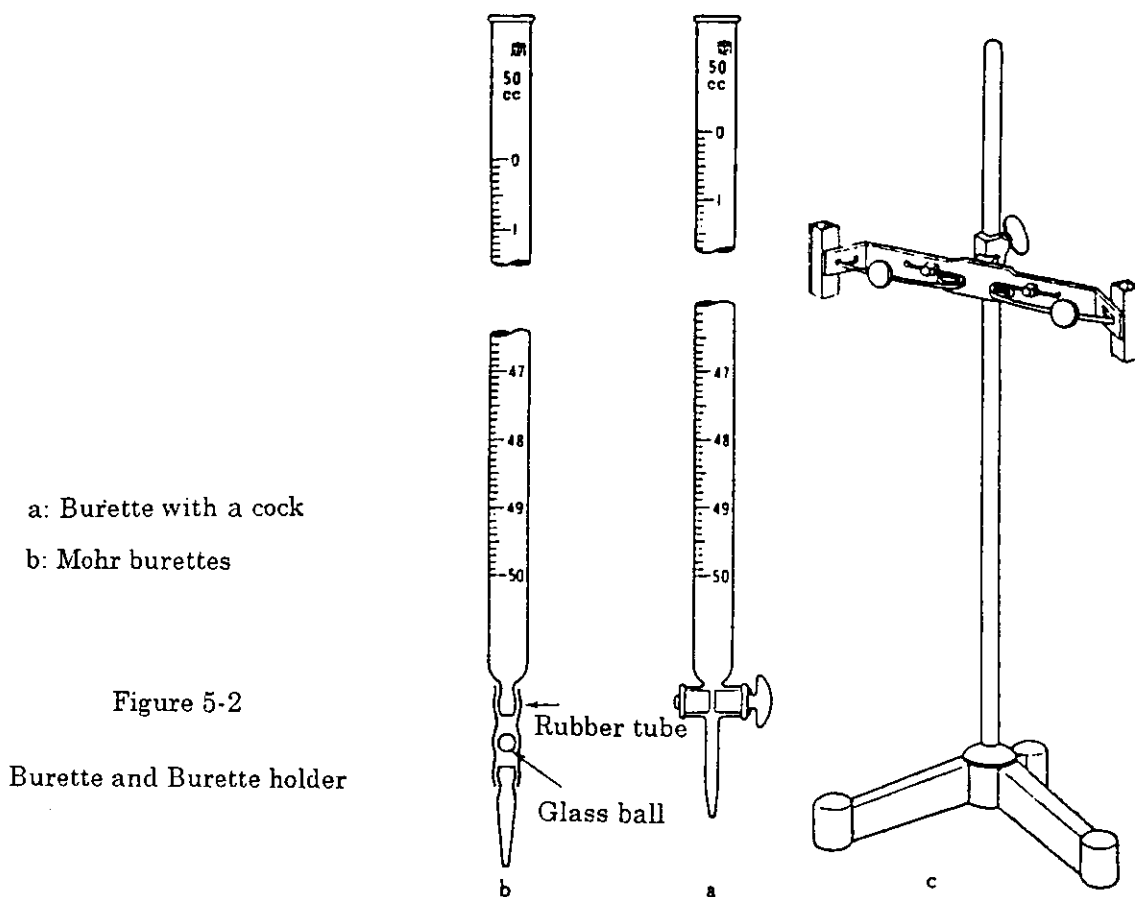
Right: Location of the eye

Figure 5-1 Reading a burette

5.4 Types of burettes

The scale of the burette refers the volume of the liquid that is moved from the burette to another container. There are many different available scales of burettes.

Figure 5-2 illustrates a popular type of burette. Burette (a) in Figure 5-2 can contain 25 or 50 mL of liquid, and is scaled by 0.1 mL increments. A cock is attached to the bottom of the burette. In some burettes, the cock is connected to a rubber tube and closed by a pinch cock. Some burettes (Mohr burettes) have cocks with a glass ball.



A burette holder is used to keep a burette vertical. The burette should be held tightly, to avoid having the burette fall and collapse.

Another type of burette can contain 10 mL of liquid and is scaled by 0.02 mL increments. These burettes have very thin tubes, and are suitable for precise titration at low titration volumes. However, care is needed not to drop too quickly since the thin tube can lead to large errors due to "wake" (liquid remaining on the surface of upper part of the burette).

A burette for small volumes of liquid is shown in Figure 5-3a. This burette can

part of the burette).

A burette for small volumes of liquid is shown in Figure 5-3a. This burette can contain 2 or 5 mL, and is scaled by 0.01 or 0.02 mL increments. Standard solutions for titration are put into the spherical part.

The burette shown in Figure 5-3b is connected to a container bottle. Air is introduced with double bulb controller to send titration solution from a bottle to the burette. This system works more easily than an ordinary burette. It is used for titration of alkali solutions like sodium hydroxide, to prevent dissolution of carbon dioxide in the air.

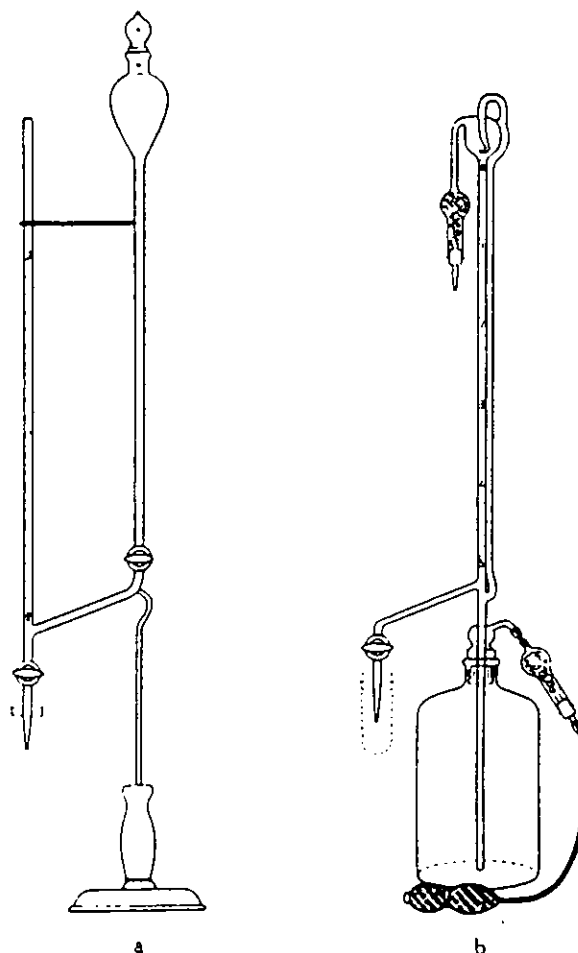
In a burette with a cock, the parts where cock rotates is carefully made so that the cock and the inside surface of the part is rubbed when the cock is rotated in order to help cock to rotate smoothly and to prevent leakage of the solution. Rotating the cock when dry can potentially harm the sliding surface of the cock. To avoid this, grease or vaseline should be applied to the cock. To do so, remove the cock from the burette, apply a small amount of grease to the sliding surface, wipe with paper, return to the burette, adjusting holes of inner and outer parts of the cock and then rotate the cock to thinly and uniformly spread the grease to the entire area. Take care not to apply too much grease, since this will plug the hole and become difficult to remove. When a suitable amount of grease is applied, sliding surface turns transparent. If it is not transparent, remove the cock, add small amount of grease, return the cock and confirm the transparency of the sliding surface.

Grease and vaseline are organic substances, so they should not be used when solutions that react to them (e.g., potassium permanganate, potassium dichromate, silver nitrate) are put in the burette. If chromic acid mixture is applied to the burette, which has been used before, grease and vaseline should be carefully wiped out. Otherwise, the tip of the burette can be clogged with the decomposed substance of grease and vaseline.

The burette need not be dried before use. After rinsing wash it three times with the solution, filling one-fourth of the burette.

When solution is put in a burette, air sometimes remains in the bottom of the cock as a bubble. The bubble should be removed by opening the cock fully. In addition, the scale should be read one minute after putting the solution in, since any remaining solution at the upper part of the burette may be added gradually after putting the solution in. This type of error can also occur when the solution is drained out, and can be avoided by not draining the solution quickly. For a 50 mL burette, you should drain the solution at no more than 0.5 mL per second. For smaller diameter burettes, you

Figure 5-3 Special burette



5.5 General techniques for titration

- 1) Draw up the required volume of the sample solution with a pipette and pour in a conical beaker. Add the designated reagent and indicator. Place a white sheet of paper beneath the beaker so that you can see changes in the color clearly.
- 2) Fill the burette with the standard solution. Usually, titration starts from a scale of zero. If the titration begins from a different scale, read up to the one-tenth digit of the minimum scale and record it.
- 3) Open the cock as illustrated in Figure 5-4 to drip the solution to the beaker. Inexperienced analysts should hold the outside of the cock with their left hand and open the cock with the right hand to prevent failure. Experienced personnel might be comfortable opening and closing the cock both with their left hands, shaking the beaker with their right hands.
- 4) As you drip the solution into the beaker, watch carefully to detect changes in the color of the solution. The color of the solution changes locally where the solution is added, but shaking the beaker smoothly and drawing a circle horizontally to mix the solution will remove these changes. As the end point approaches, continue to drip slowly and mix well drop by drop. It often takes experience with titration to determine precisely when the color changes.
- 5) Just before reaching the end point, stop titration for ten seconds and mix the

solution well. You should wait until the chemical reaction ends and any remaining liquid passes through the inside wall of the burette.

- 6) Then open the cock slightly so that a small drop hangs from the tip of the burette. As shown in Figure 5-5, tilt the beaker so that this drop falls to the inner wall slightly over the liquid surface, and tilt the beaker to mildly mix. Repeat this operation and stop titration when the color changes to that indicated in the analysis manual. The volume of one drop is usually 0.03 mL. Practice in advance to achieve reproducibility within 0.05 mL when you repeat the same titration.
- 7) Place your eye at the same level as the liquid surface (meniscus) and read the scale by reading to the one-tenth digit of the minimum scale. To more easily see the meniscus, place a white sheet of paper behind the burette. For a Schellbach burette, the thinnest part of the blue line is considered as the meniscus.
- 8) Based on the concentration of the standard solution and the volume required to reach the end point, calculate the volume of the target constituent in the testing solution.
- 9) Clean the devices. The remaining solution in the burette should be disposed and should not be returned to the bottle. Wash and dry used devices with tap water, cleanser, acid and distilled water.

In Japan, 13.4.2 of JIS K 0050 provides the standard, which should be referred to in any titration exercise.

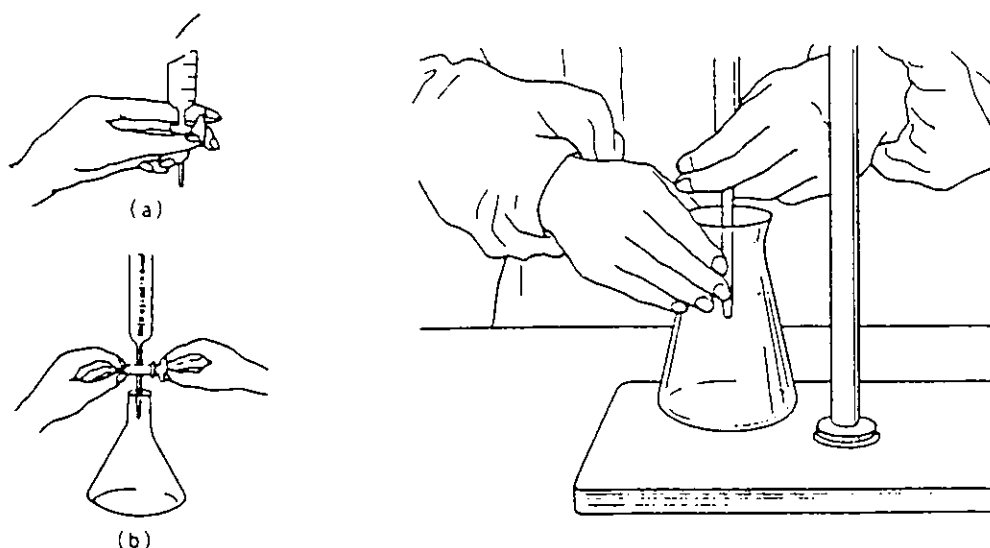
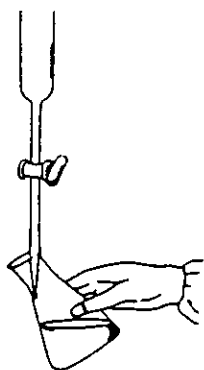


Figure 5-4 Operation of cock



When approaching the end point, hang very small drop from the tip of the burette, put it on the inner wall of the burette and mix.

Figure 5-5 Dropping very small drop

5.6 Calibrating the scale of the burette

The capacity of all measuring devices will be identical to the values marked on the device at the standard temperature only. In Japan, 20° Celsius is adopted as the standard temperature. Strictly speaking, this implies that the scale of a volumeter made in Japan is only correct at 20°C. Therefore, at other ambient temperatures, adjustment is required. Since volumetric analysis requires extremely accurate measurements, knowing the correct volume is critical.

Some volumetric devices may have relatively large errors even if they satisfy standards. Furthermore, glassware often changes when it grows old. It is important to calibrate the volumetric devices, to understand the differences between the scale and the actual volume. Calibration is especially necessary for burettes.

To calibrate, you should measure the volume of the liquid (typically water) that is equivalent to each scale, and adjust for the temperature and pressure to convert to volume at standard temperature and pressure. This will allow you to obtain the difference between the true value and the value on the scale. This calculation requires a number of coefficients, which can be obtained from 9.3.3 of JIS K 0050.¹⁾

1) JIS K 0050(1991) "General rules for chemical analysis"

Text Book for Basic Operations in Chemical Analysis
- Analysis Video Series for Environmental Technology Transfer -

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