(3) Observation of components in the Description
In each monograph, description is usually given of the outer portion and the inner portion of a section in this order, followed by a specification of cell contents. Observation should be made in the same order. In the case of a powdered sample, description is given of a characteristic component or a matter present in large amount, rarely existing matter, and cell contents in this order. Observation should be made in the same order.

12. Determination of Specific Gravity and Density

The density ρ (g/mL or g/cm^3) means the mass per unit volume, and the relative density means the ratio of the mass of a sample specimen to that of an equal volume of a standard substance. The relative density is also called the specific gravity.

The specific gravity, $d_t^{t'}$, means the ratio of the mass of the sample specimen at t'°C to that of an equal volume of water (H_2O) at t°C . Unless otherwise specified, the measurement is to be performed by Method 1, Method 2 or Method 4. When the specified value is accompanied with the term "about" in the monograph, Method 3 is also available.

Method 1. Measurement using a pycnometer

A pycnometer is a glass vessel with a capacity of usually 10 mL to 100 mL, having a ground-glass stopper fitted with a thermometer, and a side inlet-tube with a marked line and a ground-glass cap.

Weigh a pycnometer, previously cleaned and dried, to determine its mass W. Remove the stopper and the cap. Fill the pycnometer with the sample solution, keeping them at a slightly lower temperature by 1°C to 3°C than the specified temperature t'°C, and stopper them, taking care not to leave bubbles. Raise the temperature gradually, and when the thermometer shows the specified temperature, remove the portion of the sample solution above the marked line through the side tube, cap the side tube, and wipe the outside surface thoroughly. Measure the mass W_1 of the pycnometer filled with the sample solution. Perform the same procedure, using the same pycnometer containing water, and note the mass W_2 at the specified temperature t°C. The specific gravity $d_t^{t'}$ can be calculated by use of the following equation.

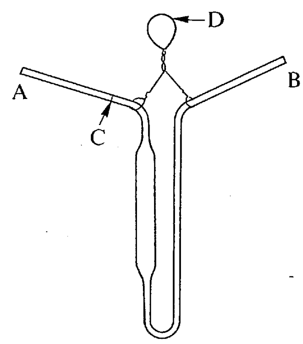
$$d_t^{t'} = \frac{W_1 - W}{W_2 - W}$$

Further, when measurements for a sample solution and water are performed at the same temperature (t°C = t'°C), the density of the sample solution at the temperature t'°C ($\rho_T^{t'}$) can be calculated from the measured specific gravity $d_{t'}^{t'}$ and the density of water at the temperature t'°C ($\rho_{S1}^{t'}$) indicated in the attached Table by using the following equation.

$$\rho_T^{t'} = \rho_{S1}^{t'}\, d_{t'}^{t'}$$

Method 2. Measurement using a Sprengel-Ostwald pycnometer

A Sprengel-Ostwald pycnometer is a glass vessel with a capacity of usually 1 mL to 10 mL. As shown in the figure, both ends are thick-walled fine tubes (inside diameter: 1 – 1.5 mm, outside diameter: 3 – 4 mm), one of which, tube A,



has a line C marked on it. Determine the mass of a pycnometer, W, previously cleaned and dried, by hanging it on the arm of a chemical balance with a platinum or aluminum wire D. Immerse the fine tube B in the sample solution, which is at a lower temperature by 3°C to 5°C than the specified temperature t'°C. Attach rubber tubing or a ground-glass tube to the end of A, and suck up the sample solution until the meniscus is above the marked line C, taking care to prevent bubble formation. Immerse the pycnometer in a water bath kept at the specified temperature t'°C for about 15 minutes, and then, by attaching a piece of filter paper to the end of B, adjust the level of the sample solution to the marked line C. Take the pycnometer out of the water bath, wipe thoroughly the outside surface and determine the mass W_1. By use of the same pycnometer, perform the same procedure for the standard solution of water. Weigh the pycnometer containing water at the specified temperature t°C, and note the mass W_2. Calculate the specific gravity $d_t^{t'}$ according to the equation described in Method 1.

Further, when measurements of specific gravity for a sample solution and water are performed at the same temperature (t'°C = t°C), the density of sample solution at temperature t'°C can be calculated by using the equation described in Method 1.

Method 3. Masurement using a hydrometer

Clean a hydrometer with ethanol (95) or diethyl ether. Stir the sample well with a glass rod, and float the hydrometer in the well. When the temperature is adjusted to the specified temperature t'°C and the hydrometer comes to a standstill, read the specific gravity $d_t^{t'}$ or the density $\rho_T^{t'}$ at the upper brim of the meniscus. Here the temperature t°C indicates the temperature at which the hydrometer is calibrated. If specific instructions for reading the meniscus are supplied with the hydrometer, the reading must be in accordance with the instructions.

Further, when measurement of the specific gravity for a sample solution is performed at the same temperature (t'°C = t°C), at which the hydrometer is calibrated, the density of a sample solution at t'°C, $\rho_T^{t'}$, can be calculated by using the specific gravity $d_t^{t'}$ and the equation shown in Method 1.

Method 4. Measurement using an oscillator-type density meter

Density measurement with an oscillator-type density meter is a method for obtaining the density of liquid or gas by measuring the intrinsic vibration period T (s) of a glass tube cell filled with sample specimen. When a glass tube containing a sample is vibrated, it undergoes a vibration with an intrinsic vibration period T in proportion to the mass of the sample specimen. If the volume of the vibrating part of the sample cell is fixed, the relation of the square of intrinsic oscillation period and density of the sample specimen shall be linear.

Before measuring a sample density, the respective intrinsic oscillation periods T_{S1} and T_{S2} for two reference substances (density: ρ_{S1}, ρ_{S2}) must be measured at a specified temperature t'°C, and the cell constant $K_{t'}$(g·cm^{-3} s^{-2}) must be determined by using the following equation.

$$K_{t'} = \frac{\rho^{t'}_{S1} - \rho^{t'}_{S2}}{T_{S1}{}^2 - T_{S2}{}^2}$$

Usually, water and dried air are chosen as reference substances. Here the density of water at t'°C, $\rho^{t'}_{S1}$, is taken from the attached Table, and that of dried air $\rho^{t'}_{S2}$ is calculated by using the following equation, where the pressure of dried air is at p kPa.

$$\rho^{t'}_{S2} = 0.0012932 \times \{273.15/(273.15 + t')\} \times (p/101.325)$$

Next, introduce a sample specimen into a sample cell having a cell constant $K_{t'}$, the intrinsic vibration period, T_T, for the sample under the same operation conditions as employed for the reference substances. The density of a sample specimen at t'°C, $\rho^{t'}_T$, is calculated by use of the following equation, by introducing the intrinsic oscillation period T_{S1} and the density of water at a specified temperature t'°C, $\rho^{t'}_{S1}$, into the equation.

$$\rho^{t'}_T = \rho^{t'}_{S1} + K_{t'}\,(T_T{}^2 - T_{S1}{}^2)$$

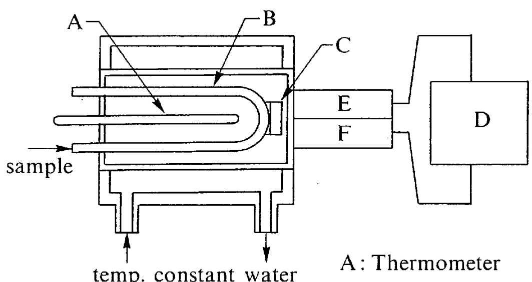
Further, the specific gravity of a sample specimen $d^{t'}_t$ against water at a temperature t°C can be obtained by using the equation below, by introducing the density of water at a temperature t°C, ρ^t_{S1}, indicated in the Table.

$$d^{t'}_t = \frac{\rho^{t'}_T}{\rho^t_{S1}}$$

Apparatus

An oscillator-type density meter is usually composed of a glass tube cell of about 1 mL capacity, the curved end of which is fixed to the vibration plate, an oscillator which applies an initial vibration to the cell, a detector for measuring the intrinsic vibration period, and a temperature controlling system.

A schematic illustration of the apparatus is depicted in the Figure.



A: Thermometer
B: Sample cell
C: Vibration plate
D: Amplifier
E: Detector
F: Vibrator

Procedure

A sample cell, water, and a sample specimen are previously adjusted to a specified temperature t'°C. Wash the sample cell with water or an appropriate solvent, and dry it thoroughly with a flow of dried air. Stop the flow of dried air, confirm that the temperature is at the specified value, and then measure the intrinsic oscillation period T_{S2} given by the dried air. Separately, the atmospheric pressure p (kPa) must be measured at the time and place of the examination. Next, introduce water into the sample cell and measure the intrinsic oscillation period T_{S1} given by water. Using these values of the intrinsic oscillation period and the atmospheric pressure, the sample cell constant $K_{t'}$ can be determined by use of the above-mentioned equation.

Next, introduce a sample specimen into the glass cell, confirm the specified temperature, and measure the intrinsic oscillation period T_T given by the sample specimen. Using the intrinsic oscillation periods for water and the sample specimen, the density of water $\rho^{t'}_{S1}$, and the cell constant $K_{t'}$, the density of the sample specimen $\rho^{t'}_T$ can be obtained by use of the above equation. If necessary, the specific gravity of the sample specimen $d^{t'}_t$ against water at a temperature t°C, can be calculated by using the density of water ρ^t_{S1} shown in the attached Table.

In this measurement, avoid the occurrence of bubble formation in the sample cell, when a sample specimen or water is introduced into the cell.

Density of water

Temp. °C	Density g/mL	Temp. °C	Density g/mL	Temp. °C	Density g/mL	Temp. °C	Density g/mL
0	0.999 84	10	0.999 70	20	0.998 20	30	0.995 65
1	0.999 90	11	0.999 61	21	0.997 99	31	0.995 34
2	0.999 94	12	0.999 50	22	0.997 77	32	0.995 03
3	0.999 96	13	0.999 38	23	0.997 54	33	0.994 70
4	0.999 97	14	0.999 24	24	0.997 30	34	0.994 37
5	0.999 96	15	0.999 10	25	0.997 04	35	0.994 03
6	0.999 94	16	0.998 94	26	0.996 78	36	0.993 68
7	0.999 90	17	0.998 77	27	0.996 51	37	0.993 33
8	0.999 85	18	0.998 60	28	0.996 23	38	0.992 97
9	0.999 78	19	0.998 41	29	0.995 94	39	0.992 59
10	0.999 70	20	0.998 20	30	0.995 65	40	0.992 22

* In this Table, although the unit of density is represented by g/mL in order to harmonize with the unit expression in the text, it should be expressed in g/cm^3 seriously.

13. Digestion Test

The Digestion Test is a test to measure the activity of digestive enzymes, as crude materials or preparations, on starch, protein and fat.

(1) Assay for Starch Digestive Activity

The assay for starch digestive activity is performed through the measurement of starch saccharifying activity, dextrinizing activity, and liquefying activity.

(i) Measurement of starch saccharifying activity

The starch saccharifying activity can be obtained by measuring an increase of reducing activity owing to the hydrolysis of the glucoside linkages when amylase acts on the starch. Under the conditions described in Procedure, one starch sac-