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.

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

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

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

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, $\rho_{S1}^{t'}$, into the equation.

$$\rho_T^{t'} = \rho_{S1}^{t'} + K_{t'}\,(T_T^2 - T_{S1}^2)$$

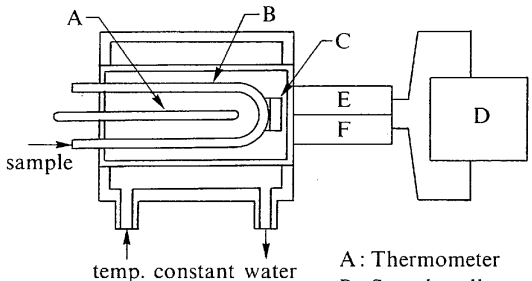
Further, the specific gravity of a sample specimen $d_t^{t'}$ against water at a temperature t°C can be obtained by using the equation below, by introducing the density of water at a temperature t°C, $\rho_{S1}^{t'}$, indicated in the Table.

$$d_t^{t'} = \frac{\rho_T^{t'}}{\rho_{S1}^{t}}$$

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 $\rho_{S1}^{t'}$, 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_{S1}^{t} 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-

charifying activity unit is the amount of enzyme that catalyzes the increase of reducing activity equivalent to 1 mg of glucose per minute.

Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the reducing activity increases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.4 to 0.8 starch saccharifying activity unit/mL. Filter if necessary.

Preparation of Substrate Solution

Use potato starch TS for measuring the starch digestive activity. If necessary, add 10 mL of buffer or salts solution specified in the monograph, instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0.

Procedure

Pipet 10 mL of the substrate solution, stand at 37 ± 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Allow this solution to stand at 37 ± 0.5°C for exactly 10 minutes, add exactly 2 mL of alkaline tartrate solution of the Fehling's TS for amylolytic activity test, and shake immediately. Then, add exactly 2 mL of copper solution of the Fehling's TS for amylolytic activity test, shake gently, heat the solution in a water bath for exactly 15 minutes, and then immediately cool to below 25°C. Then, add exactly 2 mL of concentrated potassium iodide TS and 2 mL of diluted sulfuric acid (1 in 6), and titrate the released iodine with 0.05 mol/L sodium thiosulfate VS to the disappearance of the blue color produced by addition of 1 to 2 drops of soluble starch TS (a mL). Separately, pipet 10 mL of water instead of the substrate solution and titrate in the same manner (b mL).

$$\text{Starch saccharifying activity (unit/g)} = \text{amount (mg) of glucose} \times \frac{1}{10} \times \frac{1}{W}$$

$$\text{Amount (mg) of glucose} = (b - a) \times 1.6$$

W: Amount (g) of the sample in 1 mL of the sample solution

(ii) Measurement of starch dextrinizing activity

The starch dextrinizing activity can be obtained by measuring a decrease in starch coloration by iodine resulting from hydrolysis of the straight chain component (amylose) in starch when amylase acts on the starch. Under the conditions described in Procedure, one starch dextrinizing activity unit is the amount of enzyme required to reduce the coloration of potato starch by iodine by 10% per minute.

Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water or a buffer or salts solution specified in the monograph so that the coloration of starch by iodine decreases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.2 to 0.5 starch dextrinizing activity unit/mL. Filter if necessary.

Preparation of Substrate Solution

Prepare the substrate solution in the same manner as the substrate solution in the measurement of starch saccharifying activity.

Procedure

Pipet 10 mL of the substrate solution, stand at 37 ± 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Allow this solution to stand at 37 ± 0.5°C for exactly 10 minutes. Pipet 1 mL of this solution, add it to 10 mL of 0.1 mol/L hydrochloric acid TS, and shake immediately. Pipet 0.5 mL of this solution, add exactly 10 mL of 0.0002 mol/L iodine TS, and shake. Determine the absorbance A_T of this solution at the wavelength of 660 nm as directed under the Ultraviolet-visible Spectrophotometry. Separately, using 1 mL of water instead of the sample solution, determine the absorbance A_B in the same manner.

$$\text{Starch dextrinizing activity (unit/g)} = \frac{(A_B - A_T)}{A_B} \times \frac{1}{W}$$

W: Amount (g) of the sample in 1 mL of the sample solution

(iii) Measurement of starch liquefying activity

The starch liquefying activity can be obtained by measuring a decrease in the viscosity of starch solution resulting from the hydrolysis of molecules when amylase acts on the starch. Under the conditions described in Procedure, one starch liquefying activity unit is the amount of enzyme required to reduce the viscosity of the substrate solution equivalent to 1 g of potato starch from 200% to 100% of that of the 50% sucrose standard solution.

Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the viscosity decreases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.15 to 0.25 starch liquefying activity unit/mL. Filter if necessary.

Preparation of Substrate Solution

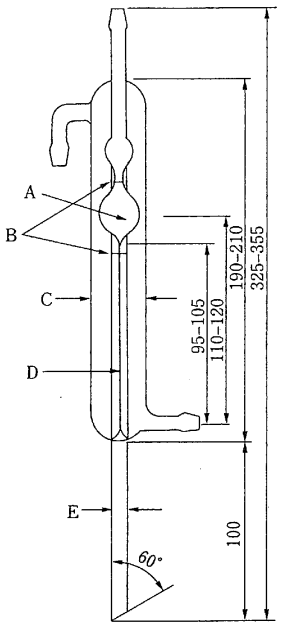
Weigh accurately about 1 g of potato starch, and measure the loss of drying at 105°C for 2 hours. Weigh exactly potato starch equivalent to 15.00 g calculated on the dried basis, add 300 mL of water, then add gradually 25 mL of 2 mol/L sodium hydroxide TS under thorough shaking, until the mixture forms a paste. Heat the mixture in a water bath for 10 minutes, shaking it occasionally. After cooling, neutralize the mixture with 2 mol/L hydrochloric acid TS, and add 50 mL of the buffer solution specified in the monograph and water to make exactly 500 g. Prepare before use.

Preparation of 50% Standard Sucrose Solution

Dissolve 50.0 g of sucrose in 50.0 mL of water.

Procedure

Put 50 mL of the 50% standard sucrose solution in a 100-mL conical flask, and allow it to stand in a thermostat at 37 ± 0.5°C for 15 minutes. Fix a viscometer shown in the figure so that its lower end almost touches the bottom of the flask and that the water in the thermostat circulates around the outer cylinder of the viscometer. After slowly drawing off the 50% standard sucrose solution by suction to the middle of the upper bulb of the viscometer, let it flow down by gravity, measuring the time taken for the solution to fall from the upper to the lower indicators (t_1 seconds). Take exactly 50 g of the substrate solution in another 100-mL conical flask, and



A: Bulb volume: 5 mL
B: Indicators
C: Outside diameter: 30 mm
D: Capillary inside diameter: 1.25 – 1.30 mm
E: Outside diameter: 8 mm

The figures are in mm.

stand it in another thermostat at 37 ± 0.5°C for 20 minutes. Add exactly 1 mL of the sample solution to it, and shake the flask immediately. Fix a viscometer vertically so that its lower end almost touches the bottom of the flask and that the water in the thermostat circulates around the outer cylinder of the viscometer. Occasionally draw the reaction solution off by suction to the middle of the upper bulb slowly, then let it flow down by gravity, measuring the time taken for the solution to fall from the upper to the lower indicators (t seconds).

Repeat this operation until t becomes shorter than t_1. At each measurement, record the time (T' seconds) from the moment that the sample solution is added to the moment that the solution surface in the flask passes the upper indicator. $(T' + t/2)$ is the reaction time (T) corresponding to t. Draw a curve for both t and T. Obtain T_1 and T_2that correspond to t_1 and $(2 \times t_1)$ by interpolation.

$$\text{Starch liquefying activity (unit/g)} = \frac{60}{(T_1 - T_2)} \times \frac{1.5}{W}$$

W: Amount (g) of the sample in 1 mL of the sample solution

(2) Assay for Protein Digestive Activity

The protein digestive activity can be obtained by the colorimetric measurement, making use of Folin's reaction, of the amount of acid-soluble low-molecular products, which is increased owing to the hydrolysis of the peptide linkages when protease acts on casein. One protein digestive activity unit is the amount of enzymes that produces Folin's TS-colorable substance equivalent to 1 μg of tyrosine per minute under the conditions described in Procedure.

Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the amount of non-protein, Folin's TS-colorable substances increase in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 15 to 30 protein digestive activity unit/mL.

Tyrosine Calibration Curve

Weigh exactly 0.050 g of Tyrosine Reference Standard, previously dried at 105°C for 3 hours, and dissolve in 0.2 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 1 mL, 2 mL, 3 mL and 4 mL of this solution separately, and add 0.2 mol/L hydrochloric acid TS to each solution to make them exactly 100 mL. Pipet 2 mL of each solution, and add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to each solution, shake immediately, then stand them at 37 ± 0.5°C for 30 minutes. Determine the absorbances, A_1, A_2, A_3 and A_4, of these solutions at 660 nm as directed under the Ultraviolet-visible Spectrophotometry, using a solution prepared with exactly 2 mL of 0.2 mol/L hydrochloric acid TS in the same manner as the blank. Then, draw a calibration curve with the absorbances, A_1, A_2, A_3 and A_4 as the ordinate, and with the amount (μg) of tyrosine in 2 mL of each solution as the abscissa. Obtain the amount (μg) of tyrosine for the absorbance 1.

Preparation of Substrate Solution

Substrate solution 1: Weigh accurately about 1 g of milk casein, and measure the loss on drying at 105°C for 2 hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 12 mL of lactic acid TS and 150 mL of water, and warm to dissolve in a water bath. After cooling in running water, adjust to the pH specified in the monograph with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200 mL. Prepare before use.

Substrate solution 2: Weigh accurately about 1 g of milk casein, and measure the loss on drying at 105°C for 2 hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 160 mL of 0.05 mol/L disodium hydrogenphosphate TS, and warm to dissolve in a water bath. After cooling in running water, adjust to the pH specified in the monograph with the 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200 mL. Prepare before use.

Preparation of Precipitation Reagent

Trichloroacetic acid TS A: Dissolve 7.20 g of trichloroacetic acid in water to make 100 mL.

Trichloroacetic acid TS B: Dissolve 1.80 g of trichloroacetic acid, 1.80 g of anhydrous sodium acetate and 5.5 mL of 6 mol/L acetic acid TS in water to make 100 mL.

Procedure

Pipet 5 mL of the substrate solution specified in the monograph, stand at 37 ± 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. After standing this solution at 37 ± 0.5°C for exactly 10 minutes, add exactly 5 mL of trichloroacetic acid TS A or B as specified in the monograph, shake, stand it at 37 ± 0.5°C for 30 minutes, and then filter. Discard the first 3 mL of the filtrate, exactly measure the subsequent 2 mL of the filtrate, add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to the solution, shake well, and stand it at 37 ± 0.5°C for 30 minutes. Determine the absorbance A_T of this solution at 660 nm as directed under the

Ultraviolet-visible Spectrophotometry, using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS A or B to the solution as specified in the monograph, and shake. To this solution add exactly 5 mL of the substrate solution specified in the monograph, shake immediately, and stand it at 37 ± 0.5°C for 30 minutes. Follow the same procedure for the sample solution, and determine the absorbance A_B at 660 nm.

Protein digestive activity (unit/g)

$$= (A_T - A_B) \times F \times \frac{11}{2} \times \frac{1}{10} \times \frac{1}{W}$$

W: Amount (g) of the sample in 1 mL of the sample solution

F: Amount (μg) of tyrosine for absorbance 1 determined from Tyrosine Calibration Curve

(3) Assay for Fat Digestive Activity

The fat digestive activity can be obtained by back titration of the amount of fatty acid produced from the hydrolysis of the ester linkage, when lipase acts on olive oil. One fat digestive activity unit is the amount of enzymes that produces 1 μmole of fatty acid per minute under the conditions described in Procedure.

Preparation of Sample Solution

Dissolve or suspend the sample in an appropriate amount of cold water, or a buffer or salts solution specified in the monograph so that the amount of fatty acid increases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 1 to 5 fat digestive activity unit/mL.

Preparation of Substrate Solution

Take 200 to 300 mL of a mixture of emulsifier and olive oil (3:1) in a blender, and emulsify it at 12,000 to 16,000 revolutions per minute for 10 minutes, while cooling the solution to a temperature below 10°C. Stand this solution in a cool place for 1 hour, and make sure before use that the oil does not separate.

Preparation of Emulsifier

Dissolve 20 g of polyvinyl alcohol specified in the monograph in 800 mL of water by heating between 75°C and 80°C for 1 hour while stirring. After cooling, filter the solution if necessary, and add water to make exactly 1000 mL.

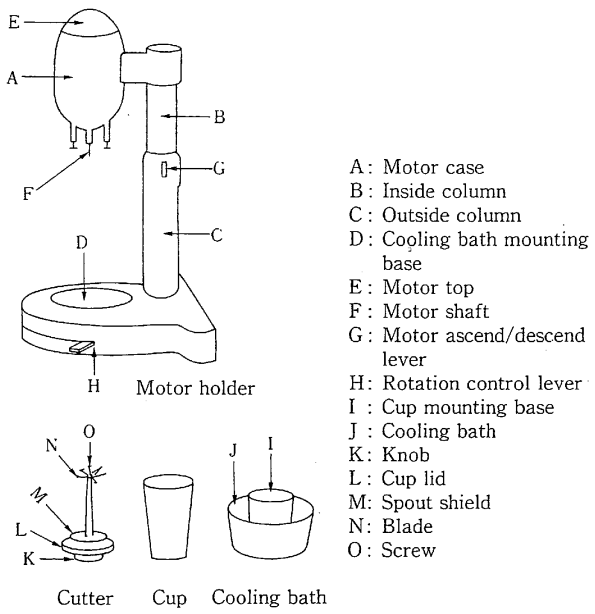
Procedure

Pipet 5 mL of the substrate solution and 4 mL of the buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing the mixture at 37 ± 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Stand this solution at 37 ± 0.5°C for exactly 20 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Then add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Titrate the excess sodium hydroxide with 0.05 mol/L hydrochloric acid VS (b mL) (indicator: 2 to 3 drops of phenolphthalein TS). Separately, pipet 5 mL of the substrate solution and 4 mL of buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing it at 37 ± 0.5°C for 10 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), then add exactly 1 mL of the sample solution, and shake. Add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, and titrate in the same manner (a mL).

Fat digestive activity (unit/g)

$$= 50 \times (a - b) \times \frac{1}{20} \times \frac{1}{W}$$

W: Amount (g) of the sample in 1 mL of the sample solution



14. Disintegration Test

The Disintegration Test is a method to determine the resistance or disintegration of solid preparations for internal use in the test fluids. Unless otherwise specified, tablets, tablets coated with suitable coating agents, pills, capsules, granules or enteric coated preparations comply with the test described below. This test method, however, is not applied for preparations exceeding 20.0 mm in diameter, for sustained release preparations, or for preparations which are subject to the Dissolution Test.

Apparatus

The apparatus consists of a basket-rack assembly, a beaker about 110 mm in inside diameter and about 155 mm in height, a suitable thermostatic arrangement for heating, and a motor. Auxiliary disks or auxiliary tubes are used as directed in the Procedure.

Basket-rack assembly: The basket-rack assembly, as illustrated in Fig. 1, consists of 6 open-ended glass tubes D, each 77.5 ± 2.5 mm long, 21.5 ± 0.5 mm in inside diameter and 23.5 mm in outside diameter; the tubes are held in a vertical position by two plastic plates A, each about 90 mm in diameter and 6 mm in thickness, with 6 holes, each 24 mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached by screws to the under surface of the lower plate is an acid-resistant wire gauze B, having openings of 2.0 mm and wire diameter of 0.6 mm.