metals or organic substances, or phase transition point of crystalline inorganic salts or oxides. Melting points of Indium for thermal analysis and/or Tin for thermal analysis are usually employed for calibration.

2. Heat-quantity calibration for DSC

For accurate estimation of a quantity of heat change (enthalpic change) of a sample specimen, caused by a certain physical change accompanying a temperature change, it is necessary to calibrate the apparatus by using appropriate reference substances. As indicated in the section of Temperature calibration, heat-quantity calibration for DSC apparatus can be performed by using appropriate reference substances having a known definite enthalpic change caused by such physical changes as melting of pure metals and/or organic substances, or phase transition of crystalline inorganic salts. Melting points of Indium for thermal analysis and/or Tin for thermal analysis are usually employed for calibration.

Notes on operating conditions

When DTA or DSC measurements are made, the following items must be recorded: sample size, discrimination of open- or closed-type sample container, heating or cooling rate, measuring temperature range, and kind and flow rate of atmospheric gas.

Method 2 Thermogravimetry (TG)

Apparatus The construction of a TG apparatus is fundamentally similar to that of DTA or DSC apparatus. However, the detector for TG is a balance, called a thermobalance, which can be classified to hanging-, Roberval's-, and horizontal-type balances. The TG apparatus is designed to detect small mass changes of a specimen, placed at a fixed position on a thermobalance, caused by temperature change of the furnace under a controlled temperature program. Mass change with time and/or temperature is recorded continuously.

Procedure

A specimen is put in a sample container, which is placed at a fixed position of the thermobalance, then the heating furnace is run under a controlled temperature program. During this temperature change of the furnace, the mass change of a specimen with time and/or temperature is recorded continuously. Apparatus equipped with a data-processor is operated according to the instruction manual provided with the instrument.

When TG is used as an alternative method for "Loss on Drying" or "Water Determination", the measurement starts at room temperature and ends at a temperature above which no further mass change due to drying and/or vaporization of water can be observed. The standard heating rate is usually 5°C/min, and a linear heating program is recommended. However heating conditions (rate and time span) can be changed as necessary, depending on the kind of specimen and the extent of the measuring temperature range. Further, in TG measurement, dry air or dry nitrogen is usually passed through the heating furnace to ensure rapid elimination of evolved water or other volatile components and to avoid the occurrence of any chemical reaction, such as oxidation. By analyzing the TG curve plotted against time and/or temperature, absolute mass change and/or relative mass change with respect to the initial quantity(%) is obtained.

When the mass change caused by oxidation or degradation of a specimen is measured, a specific temperature range has to be determined beforehand so that stable baselines can be obtained before and after a targetted chemical reaction. Subsequent operating procedures are the same as described above.

Calibration of the apparatus

1. Temperature calibration

The Curie temperature of a ferromagnetic substance such as pure Nickel can be used for temperature calibration for TG, based on the occurrence of an apparent mass change at the Curie point. In the case of a TG apparatus capable of simultaneously conducting DSC and DTA, the same reference substances as those for DTA and DSC can be adopted.

2. Scale calibration and confirmation

The scale calibration for TG must be done by using reference masses for chemical balances and/or semi-microbalances in the appropriate range. This is called a primary scale calibration, and is performed under ordinary temperature and pressure when the apparatus is set up initially and periodically, thereafter.

In usual measurement by TG, scale calibration or confirmation is done by using Calcium Oxalate Monohydrate Reference Standard to take account of such effects as buoyancy and convection due to atmospheric gas flow in the real measurement state. This is called secondary scale calibration, and is performed under the standard operation conditions stated below by using the above-mentioned Reference Standard, with a certified water content. When the difference of water content between the measured value and the certified one for the Reference Standard is less than 0.3%, normal operation of the apparatus is confirmed. However, when the difference is more than 0.3%, scale calibration for TG must be done, based on the certified water content of the Reference Standard.

The standard operation conditions are as follows,

Amount of Calcium Oxalate Monohydrate Reference Standard: 0.01 g

Heating rate: 5°C/min

Temperature range: room temperature—250°C

Atmospheric gas: dried Nitrogen or dried Air

Flow rate of atmospheric gas,

hanging- or Roberval's-type balance: 40 mL/min

horizontal-type balance: 100 mL/min

Notes on operating conditions

In TG measurement, the following operation conditions must be recorded: sample size, heating rate, temperature range, kind and flow rate of atmospheric gas, etc.

63. Thin-layer Chromatography

Thin-layer Chromatography is a method to separate each ingredient by developing a mixture in a mobile phase, using a thin-layer made of a suitable stationary phase, and is applied for identification, purity test, etc. of substances.

Preparation of thin-layer plate

Generally, proceed by the following method.

A smooth and uniformly thick glass plate having a size of 50 mm × 200 mm or 200 mm × 200 mm is used for preparing a thin-layer plate. Using a suitable apparatus, apply a

water suspension of powdered solid substance for the stationary phase, directed in the monograph, on one side of the glass plate to make a uniform layer of 0.2 to 0.3 mm in thickness. After air-drying, dry further by heating at a fixed temperature between 105°C and 120°C for 30 to 60 minutes. A suitable plastic plate may be used instead of the glass plate. Preserve the dried plate with protection from moisture.

Procedure

Unless otherwise specified, proceed by the following method.

Designate a line about 20 mm distant from the bottom of the thin-layer plate as the starting line, spot 2 to 6 mm in diameter the directed volumes of the sample solution or the standard solution in the monograph using micropipets at points on this line, separated by more than 10 mm, and air-dry. Unless otherwise specified, attach the filter paper along with the inside wall of the container, and wet the filter paper with the developingt solvent. In the container, the developing solvent is placed up to about 10 mm in height from the bottom beforehand, seal the container closely, and allow it to stand for 1 hour at ordinary temperature. Place the plate in the container, avoiding contact with the inside wall, and seal the container. Develop it at ordinary temperature.

When the solvent front has ascended from the starting line to the distance directed in the monograph, remove the plate from the container. Immediately put a mark at the solvent front. After air-drying, observe the location, color, etc., of each spot by the method specified in the monograph. Calculate the Rf value by using the following equation:

$$Rf = \frac{\text{distance from the starting line to the center of the spot}}{\text{distance from the starting line to the solvent front}}$$

64. Ultraviolet-visible Spectrophotometry

The Ultraviolet-visible Spectrophotometry is a method to measure the degree of absorption of light between the wavelengths of 200 nm and 800 nm by substances for the tests of their identity and purity, and for assay. When an atomic absorption spectrophotometer is used for these purposes, proceed as directed under the Atomic Absorption Spectrophotometry. When monochromatic light passes through a substance in the solution, the ratio of transmitted light intensity I to incident light intensity I_0 is called transmittance t; transmittance expressed in the percentage is called percent transmission T, and common logarithm of the reciprocal of transmittance is called absorbance A.

$$t = \frac{I}{I_0} \quad T = \frac{I}{I_0} \times 100 = 100t \quad A = \log\frac{I_0}{I}$$

The absorbance A is proportional to the concentration c of a substance in the solution and the length l of the layer of the solution through which light passes.

$$A = kcl \quad (k\text{: constant})$$

The absorbance, calculated on the basis that l is 1 cm and c is 1 mol/L, is called molar absorption coefficient ε. The molar absorption coefficient at the wavelength of maximum absorption is expressed as ε_{max}.

When a light beam passes through a substance in the solution, the absorbance by the sample differs depending on the wavelengh of the light. So, an absorption spectrum is obtained by determining the absorbances of a light beam at various wavelengths and by graphically plotting the relation between absorbance and wavelength. From the absorption spectrum, it is possible to determine the wavelength of maximum absorption λ_{max} and that of minimum absorption λ_{min}.

The absorption spectrum of a substance in the solution is characteristic, depending on its chemical structure. Therefore, it is possible to identify a substance by comparing the spectrum of a sample within the specified wavelength range with the Reference Spectrum or the spectrum of Reference Standard, by determing the wavelengths of maximum absorption, or by measuring the ratio of absorbances at two specified wavelengths. For the purpose of assay, the absorbance by a sample solution with a certain concentration is measured at the wavelength of the maximum absorption λ_{max} and compared it with the absorbance of a standard solution with a certain concentration.

Apparatus and adjustment

A spectrophotometer or a photoelectric photometer is used for the measurement of absorbance.

After adjusting the spectrophotometer or photoelectric photometer based on the operation manual of the apparatus, it should be confirmed that the wavelength and the transmission rate meet the specifications of the tests described below.

The calibration of wavelength should be carried out as follows. Using an optical filter for wavelength calibration, measure the transmission rate in the vicinity of the standard wavelength value shown in the test results form, under the test conditions given in the test results form attached to each of the filters. When performing a test to determine the wavelength which shows minimal transmission rate, the difference between the measured wavelength and the standard wavelength value should be within ± 0.5 nm. When the measurement is repeated three times, each value obtained should be within the mean ± 0.2 nm. It is also possible to carry out the test using a low-pressure mercury lamp at bright line wavelengths of 253.65 nm, 365.02 nm, 435.84 nm and 546.07 nm, or a deuterium discharge lamp at bright line wavelengths of 486.00 nm and 656.10 nm. In the case of these tests, the difference between the measured wavelength and the wavelength of the bright line should be within ± 0.3 nm. When the measurement is repeated three times, each value obtained should be within the mean ± 0.2 nm.

The calibration of transmission rate or absorbance should be carried out as follows. Using an optical filter for transmission rate calibration, determine the transmission rate at the standard wavelength value under the test conditions given in the test results form attached to each of the filters. The difference between the measured transmission rate and the standard transmission rate value should be within the range of from 1% larger of the upper limit to 1% smaller of the lower limit for the relative accuracy shown in the test results form. When the measurement is repeated three times, each absorbance obtained (or calculated from the transmission rate) should be within the mean ± 0.002 when the absorbance is not more than 0.500, and within the mean ± 0.004 when the absorbance is more than 0.500. In addition, it will be desirable to confirm the linearity of transmission rate at