PULSED SPECTROMETRY

Set the spectrometer controls, e.g. pulse flip angle, pulse amplitude, pulse interval, spectral width, number of data points (resolution) and data acquisition rate, as indicated in the manufacturer's instructions and collect the necessary number of free induction decays. After mathematical transformation of the data by the computer, adjust the phase control in order to obtain as far as possible a pure absorption spectrum and calibrate the spectrum relative to the resonance frequency of the chemical shift internal reference compound. Display the spectrum stored in the computer on a suitable output device and, for quantitative measurements, process the integral according to the facility of the instrument.

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2.2.34. THERMAL ANALYSIS

Thermal analysis is a group of techniques in which the variation of a physical property of a substance is measured as a function of temperature. The most commonly used techniques are those which measure changes of mass or changes in energy of a sample of a substance.

THERMOGRAVIMETRY

Thermogravimetry is a technique in which the mass of a sample of a substance is recorded as a function of temperature according to a controlled temperature programme.

Apparatus. The essential components of a thermobalance are a device for heating or cooling the substance according to a given temperature program, a sample holder in a controlled atmosphere, an electrobalance and a recorder. In some cases the instrument may be coupled to a device permitting the analysis of volatile products.

Temperature verification. Check the temperature scale using a suitable material according to the manufacturer's instructions.

Calibration of the electrobalance. Place a suitable quantity of a suitable certified reference material in the sample holder and record the mass. Set the heating rate according to the manufacturer's instructions and start the temperature increase. Record the thermogravimetric curve as a graph with temperature, or time, on the abscissa, increasing from left to right, and mass on the ordinate, increasing upwards. Stop the temperature increase at about 230 °C. Measure the difference on the graph between the initial and final mass-temperature plateaux, or mass-time plateaux, which corresponds to the loss of mass. The declared loss of mass for the certified reference material is stated on the label.

Method. Apply the same procedure to the substance to be examined, using the conditions prescribed in the monograph. Calculate the loss of mass of the substance to be examined from the difference measured in the graph obtained. Express the loss of mass as per cent $\Delta m/m$.

If the apparatus is in frequent use, carry out temperature verification and calibration regularly. Otherwise, carry out such checks before each measurement.

Since the test atmosphere is critical, the following parameters are noted for each measurement: pressure or flow rate, composition of the gas.

DIFFERENTIAL SCANNING CALORIMETRY

Differential Scanning Calorimetry (DSC) is a technique that can be used to demonstrate the energy phenomena produced during heating (or cooling) of a substance (or a mixture of substances) and to determine the changes in enthalpy and specific heat and the temperatures at which these occur.

The technique is used to determine the difference in the flow of heat (with reference to the temperature) evolved or absorbed by the test sample compared with the reference cell, as a function of the temperature. Two types of DSC apparatuses are available, those using power compensation to maintain a null temperature difference between sample and reference and those that apply a constant rate of heating and detect temperature differential as a difference in heat flow between sample and reference.

Apparatus. The apparatus for the power compensation DSC consists of a furnace containing a sample holder with a reference cell and a test cell. The apparatus for the heat flow DSC consists of a furnace containing a single cell with a sample holder for the reference crucible and the test crucible.

A temperature-programming device, thermal detector(s) and a recording system which can be connected to a computer are attached. The measurements are carried out under a controlled atmosphere.

Calibration of the apparatus. Calibrate the apparatus for temperature and enthalpy change, using indium of high purity or any other suitable certified material, according to the manufacturer's instructions. A combination of 2 metals, e.g. indium and zinc may be used to control linearity.

Operating procedure. Weigh in a suitable crucible an appropriate quantity of the substance to be examined; place it in the sample holder. Set the initial and final temperatures, and the heating rate according to the operating conditions prescribed in the monograph.

Begin the analysis and record the differential thermal analysis curve, with the temperature or time on the abscissa (values increasing from left to right) and the energy change on the ordinate (specify whether the change is endothermic or exothermic).

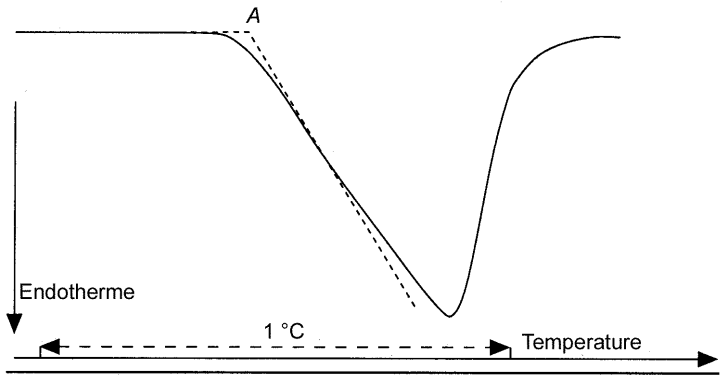

Figure 2.2.34.-1. – *Thermogram*

The temperature at which the phenomenon occurs (the onset temperature) corresponds to the intersection (A) of the extension of the baseline with the tangent at the point of greatest slope (inflexion point) of the curve (see Figure 2.2.34.-1). The end of the thermal phenomenon is indicated by the peak of the curve.

The enthalpy of the phenomenon is proportional to the area under the curve limited by the baseline; the proportionality factor is determined from the measurement of the heat of fusion of a known substance (e.g., indium) under the same operating conditions.

Each thermogram may be accompanied by the following data: conditions employed, record of last calibration, sample size and identification (including thermal history), container, atmosphere (identity, flow rate, pressure), direction and rate of temperature change, instrument and recorder sensitivity.

Applications

Phase changes. Determination of the temperature, heat capacity change and enthalpy of phase changes undergone by a substance as a function of temperature.

solid - solid transition:	allotropy - polymorphism glass transition desolvation amorphous-crystalline
solid - liquid transition:	melting
solid - gas transition:	sublimation
liquid - solid transition:	freezing recrystallisation
liquid - gas transition:	evaporation

Changes in chemical composition. Measurement of heat and temperatures of reaction under given experimental conditions, so that, for example, the kinetics of decomposition or of desolvation can be determined.

Application to phase diagrams. Establishment of phase diagrams for solid mixtures. The establishment of a phase diagram may be an important step in the preformulation and optimisation of the freeze-drying process.

Determination of purity. The measurement of the heat of fusion and the melting point by DSC enables the impurity content of a substance to be determined from a single thermal diagram, requiring the use of only a few milligrams of sample with no need for repeated accurate measurements of the true temperature.

In theory, the melting of an entirely crystalline, pure substance at constant pressure is characterised by a heat of fusion ΔH_f in an infinitely narrow range, corresponding to the melting point T_0. A broadening of this range is a sensitive indicator of impurities. Hence, samples of the same substance, whose impurity contents vary by a few tenths of a per cent, give thermal diagrams that are visually distinct (see Figure 2.2.34.-2).

The determination of the molar purity by DSC is based on the use of a mathematical approximation of the integrated form of the Van't Hoff equation applied to the concentrations (not the activities) in a binary system [$\ln(1 - x_2) = -x_2$ and $T \times T_0 = T_0^2$]:

$$T = T_0 - \frac{RT_0^2}{\Delta H_f} \times x_2 \qquad (1)$$

x_2 = mole fraction of the impurity i.e. the number of molecules of the impurity divided by the total number of molecules in the liquid phase (or molten phase) at temperature T (expressed in kelvins),

T_0 = melting point of the chemically pure substance, in kelvins,

ΔH_f = molar heat of fusion of the substance, in joules,

R = gas constant for ideal gases, in joules·kelvin^{-1}·mole^{-1}.

Hence, the determination of purity by DSC is limited to the detection of impurities forming a eutectic mixture with the principal compound and present at a mole fraction of less than 2 per cent in the substance to be examined.

This method cannot be applied to:

- amorphous substances,
- solvates or polymorphic compounds that are unstable within the experimental temperature range,
- impurities forming solid solutions with the principal substance,
- impurities that are insoluble in the liquid phase or in the melt of the principal substance.

During the heating of the substance to be examined, the impurity melts completely at the temperature of the eutectic mixture. Above this temperature, the solid phase contains

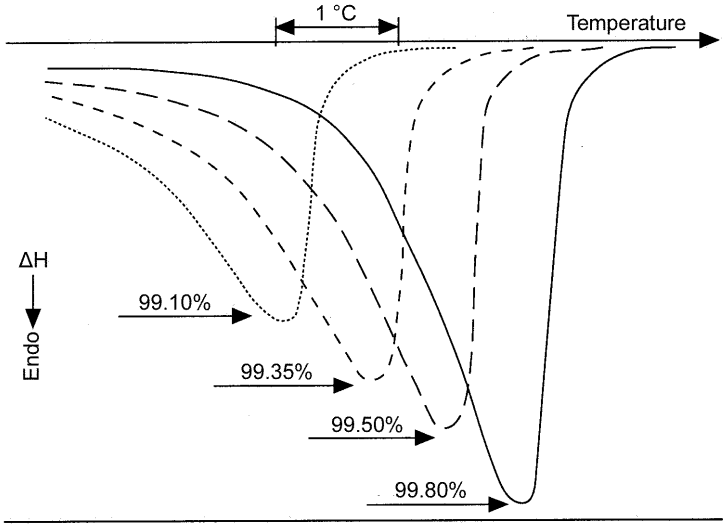

Figure 2.2.34.-2. – *Thermal diagrams according to purity*

only the pure substance. As the temperature increases progressively from the temperature of the eutectic mixture to the melting point of the pure substance, the mole fraction of impurity in the liquid decreases constantly, since the quantity of liquified pure substance increases constantly. For all temperatures above the eutectic point:

$$x_2 = \frac{1}{F} \times x_2^* \qquad (2)$$

F = molten fraction of the analysed sample,

x_2^* = mole fraction of the impurity in the analysed sample.

When the entire sample has melted, $F = 1$ and $x_2 = x_2^*$.

If equation (2) is combined with equation (1), the following equation is obtained:

$$T = T_0 - \frac{x_2^* R T_0^2}{\Delta H_f} \times \frac{1}{F}$$

The value of the heat of fusion is obtained by integrating the melting peak.

The melting point T_0 of the pure substance is extrapolated from the plot of $1/F$ versus the temperature expressed in kelvins. The slope α of the curve, obtained after linearisation, if necessary, corresponding to $RT_0^2 \frac{x_2^*}{\Delta H_f}$ allows x_2^* to be evaluated.

The fraction x_2^*, multiplied by 100 gives the mole fraction in per cent for the total eutectic impurities.

THERMOMICROSCOPY

Phase changes may be visualised by thermomicroscopy, a method which enables a sample subjected to a programmed temperature change to be examined, in polarised light, under a microscope.

The observations made in thermomicroscopy allow the nature of the phenomena detected using thermogravimetry and differential thermal analysis to be clearly identified.

Apparatus. The apparatus consists of a microscope fitted with a light polariser, a hot plate, a temperature and heating rate and/or cooling rate programmer and a recording system for the transition temperatures. A video camera and video recorder may be added.

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2.2.35. OSMOLALITY

Osmolality is a practical means of giving an overall measure of the contribution of the various solutes present in a solution to the osmotic pressure of the solution.

An acceptable approximation for the osmolality ξ_m of a given aqueous solution is given by:

$$\xi_m = \upsilon m \Phi$$

If the solute is not ionised, $\upsilon = 1$; otherwise υ is the total number of ions already present or formed by solvolysis from one molecule of solute.

m = molality of the solution, that is the number of moles of solute per kilogram of solvent,

Φ = molal osmotic coefficient which takes account of the interactions between ions of opposite charge in the solution. It is dependent on the value of m. As the complexity of solutions increases, Φ becomes difficult to measure.

The unit of osmolality is osmole per kilogram (osmol/kg), but the submultiple milliosmole per kilogram (mosmol/kg) is usually used.

Unless otherwise prescribed, osmolality is determined by measurement of the depression of freezing point. The following relationship exists between the osmolality and the depression of freezing point ΔT:

$$\xi_m = \frac{\Delta T}{1.86} \times 1000 \text{ mosmol/kg}$$

Apparatus. The apparatus (osmometer) consists of:

– a means of cooling the container used for the measurement,

– a system for measuring temperature consisting of a resistor sensitive to temperature (thermistor), with an appropriate current or potential-difference measurement device that may be graduated in temperature depression or directly in osmolality,

– a means of mixing the sample is usually included.

Method. Prepare reference solutions as described in Table 2.2.35.-1, as required. Determine the zero of the apparatus using *water R*. Calibrate the apparatus using the reference solutions: introduce 50 µl to 250 µl of sample into the measurement cell and start the cooling system. Usually, the mixing device is programmed to operate at a temperature below that expected through cryoscopic depression to prevent supercooling. A suitable device indicates attainment of equilibrium. Before each measurement, rinse the measurement cell with the solution to be examined.

Table 2.2.35.-1. – *Reference solutions for osmometer calibration*

Mass in grams of *sodium chloride R* per kilogram of *water R*	Real osmolality (mosmol/kg)	Ideal osmolality (mosmol/kg)	Molal osmotic coefficient	Cryoscopic depression (°C)
3.087	100	105.67	0.9463	0.186
6.260	200	214.20	0.9337	0.372
9.463	300	323.83	0.9264	0.558
12.684	400	434.07	0.9215	0.744
15.916	500	544.66	0.9180	0.930
19.147	600	655.24	0.9157	1.116
22.380	700	765.86	0.9140	1.302

Carry out the same operations with the test sample. Read directly the osmolality or calculate it from the measured depression of freezing point. The test is not valid unless the value found is within two values of the calibration scale.