

spectrometers, this value is between 100 and 2000, 2000 and 10,000, and greater than 10,000, respectively.

If one electron is removed or added to a neutral molecule, a molecular ion of essentially the same molecular weight as the parent molecule results. It is often possible to determine the mass of this ion with sufficient precision to enable the empirical formula of the compound to be calculated. Molecular masses may be determined accurately by using high resolution instruments or by peak-matching measurements using reference compounds.

Fragment ions are those produced from the molecular ion by various bond cleavage processes. Numerous papers in the literature relate bond cleavage patterns (fragmentation patterns) to molecular structure.

In addition to measurement of the mass of a molecular ion and its associated fragment ions, mass spectrometers are also used to quantitate compounds with a high degree of selectivity, precision, and accuracy. Compounds are introduced into the mass spectrometer either via direct insertion probe, gas inlet, or, as is more common, via gas or liquid chromatographic interfaces, which provide sample purification. Ionization may be by EI, CI, FAB, thermospray, or electrospray and mass separation by magnetic sector, quadrupole, or quadrupole ion-trap mass spectrometers. Quantitative mass spectrometry involves measuring the abundance of a specific ion, or set of ions, and relating the response to a known standard. External or internal standards may be used, but the latter are preferred for greater accuracy.

For mass spectrometry, internal standards may be either structural or stable isotope analogs. The former have the advantage of lower cost and availability while precision and accuracy are typically achieved by use of a stable isotope (^2H , ^{13}C , ^{15}N) labeled analog of the analyte. The only requirements for labeling the analyte are that the ion monitored for the internal standard must retain an isotopic label after ionization and the label must not be exchangeable under the sampling, separation, or ionization conditions. Stable isotope internal standards are often required for acceptable quantitation, particularly with FAB and LC/MS techniques such as thermospray and electrospray.

Relative abundances of the analyte and internal standard ions are typically determined by selected ion monitoring, by which only specific ions due to the analyte and the internal standard are monitored. This technique has the advantage over scanning the full mass range in that more time is spent integrating the ion current at a selected mass-to-charge ratio, thereby increasing sensitivity. Chromatographic peak area or amount of analyte in a sample is calculated from the ratio of analyte to internal standard peak area (or height) and the regression parameters as determined by a calibration curve, using standard techniques.

(741) MELTING RANGE OR TEMPERATURE

For Pharmacopeial purposes, the melting range, melting temperature, or melting point is defined as those points of temperature within which, or the point at which, the first detectable liquid phase is detected to the temperature at which no solid phase is apparent, except as defined otherwise for *Classes II* and *III* below. A melting transition may be instantaneous for a highly pure material, but usually a range is observed from the beginning to the end of the process. Factors influencing this transition include the sample size, the particle size, the efficiency of heat diffusion, and the heating rate, among other variables, that are controlled by

procedure instructions. In some articles, the melting process is accompanied by simultaneous decomposition, which is visually evidenced as a side event like darkening of the material, charring, bubbling, or other incident. The visual impact of this side reaction frequently obscures the end of the melting process, which it may be impossible to accurately determine. In those circumstances, only the beginning of the melting can be accurately established; and it is to be reported as the melting temperature. The accuracy of the apparatus to be used as described below should be checked at suitable intervals by the use of one or more of the available USP Melting Point Reference Standards, preferably those that melt nearest the melting temperatures of the compounds being tested (see *USP Reference Standards* (11)).

Eight procedures for the determination of melting range or temperature are given herein, varying in accordance with the nature of the substance. When no class is designated in the monograph, use the procedure for *Class Ia* for crystalline or amorphous substances and the procedure for *Class II* for waxy substances.

The procedure known as the mixed-melting point determination, whereby the melting range or temperature of a solid under test is compared with that of an intimate mixture of equal parts of the solid and an authentic specimen of it, e.g., the corresponding USP Reference Standard, if available, may be used as a confirmatory identification test. Agreement of the observations on the original and the mixture constitutes reliable evidence of chemical identity.

Apparatus I—An example of a suitable melting range *Apparatus I* consists of a glass container for a bath of transparent fluid, a suitable stirring device, an accurate thermometer (see *Thermometers* (21)),* and a controlled source of heat. The bath fluid is selected with a view to the temperature required, but light paraffin is used generally and certain liquid silicones are well adapted to the higher temperature ranges. The fluid is deep enough to permit immersion of the thermometer to its specified immersion depth so that the bulb is still about 2 cm above the bottom of the bath. The heat may be supplied by an open flame or electrically. The capillary tube is about 10 cm long and 0.8 to 1.2 mm in internal diameter with walls 0.2 to 0.3 mm in thickness.

Apparatus II—An instrument may be used in the procedures for *Classes I, Ia, and Ib*. An example of a suitable melting range *Apparatus II* consists of a block of metal that may be heated at a controlled rate, its temperature being monitored by a sensor. The block accommodates the capillary tube containing the test substance and permits monitoring of the melting process, typically by means of a beam of light and a detector. The detector signal may be processed by a microcomputer to determine and display the melting point or range, or the detector signal may be plotted to allow visual estimation of the melting point or range.

Procedure for Class I, Apparatus I—Reduce the substance under test to a very fine powder, and, unless otherwise directed, render it anhydrous when it contains water of hydration by drying it at the temperature specified in the monograph, or, when the substance contains no water of hydration, dry it over a suitable desiccant for not less than 16 hours.

Charge a capillary glass tube, one end of which is sealed, with a sufficient amount of the dry powder to form a column in the bottom of the tube 2.5 to 3.5 mm high when packed down as closely as possible by moderate tapping on a solid surface.

Heat the bath until the temperature is about 30° below the expected melting point. Remove the thermometer, and quickly attach the capillary tube to the thermometer by wetting both with a drop of the liquid of the bath or otherwise, and adjust its height so that the material in the capillary is level with the thermometer bulb. Replace the thermometer, and continue the heating, with constant stirring, sufficiently to cause the temperature to rise at a rate of

* ASTM Method E77 deals with "Verification and Calibration of Liquid-in-glass Thermometers."

about 3° per minute. When the temperature is about 3° below the lower limit of the expected melting range, reduce the heating so that the temperature rises at a rate of about 1° to 2° per minute. Continue heating until melting is complete.

The temperature at which the column of the substance under test is observed to collapse definitely against the side of the tube at any point indicates the beginning of melting, and the temperature at which the test substance becomes liquid throughout corresponds to the end of melting or the melting point. The two temperatures fall within the limits of the melting range. If melting occurs with decomposition, the melting temperature corresponding to the beginning of the melting is within the range specified.

Procedure for Class Ia, Apparatus I—Prepare the test substance and charge the capillary as directed for *Class I, Apparatus I*. Heat the bath until the temperature is about 10° below the expected melting point and is rising at a rate of $1 \pm 0.5^\circ$ per minute. Insert the capillary as directed under *Class I, Apparatus I* when the temperature is about 5° below the lower limit of the expected melting range, and continue heating until melting is complete. Record the melting range as directed for *Class I, Apparatus I*.

Procedure for Class Ib, Apparatus I—Place the test substance in a closed container and cool to 10°, or lower, for at least 2 hours. Without previous powdering, charge the cooled material into the capillary tube as directed for *Class I, Apparatus I*, then immediately place the charged tube in a vacuum desiccator and dry at a pressure not exceeding 20 mm of mercury for 3 hours. Immediately upon removal from the desiccator, fire-seal the open end of the tube, and as soon as practicable proceed with the determination of the melting range as follows: Heat the bath until a temperature $10 \pm 1^\circ$ below the expected melting range is reached, then introduce the charged tube, and heat at a rate of rise of $3 \pm 0.5^\circ$ per minute until melting is complete. Record the melting range as directed for *Class I, Apparatus I*.

If the particle size of the material is too large for the capillary, precool the test substance as directed above, then with as little pressure as possible gently crush the particles to fit the capillary, and immediately charge the tube.

Procedure for Class I, Apparatus II—Prepare the substance under test and charge the capillary tube as directed for *Class I, Apparatus I*. Operate the apparatus according to the manufacturer's instructions. Heat the block until the temperature is about 30° below the expected melting point. Insert the capillary tube into the heating block, and continue heating at a rate of temperature increase of about 1° to 2° per minute until melting is complete.

The temperature at which the detector signal first leaves its initial value indicates the beginning of melting, and the temperature at which the detector signal reaches its final value corresponds to the end of melting, or the melting point. The two temperatures fall within the limits of the melting range. If melting occurs with decomposition, the melting temperature corresponding to the beginning of the melting is within the range specified. In the event of dispute, only the melting range or temperature obtained as directed for *Class I, Apparatus I*, is definitive.

Procedure for Class Ia, Apparatus II—Prepare the test substance and charge the capillary as directed for *Class I, Apparatus I*. Operate the apparatus according to the manufacturer's instructions. Heat the block until the temperature is about 10° below the expected melting point and is rising at a rate of $1 \pm 0.5^\circ$ per minute. Insert the capillary as directed under *Class I, Apparatus I* when the temperature is about 5° below the lower limit of the expected melting

range, and continue heating until melting is complete. Record the melting range as directed for *Class I, Apparatus I*. If melting occurs with decomposition, the melting temperature corresponding to the beginning of the melting is within the range specified. In the event of dispute, only the melting range or temperature obtained as directed for *Class Ia, Apparatus I*, is definitive.

Procedure for Class Ib, Apparatus II—Place the test substance in a closed container and cool to 10°, or lower, for at least 2 hours. Without previous powdering, charge the cooled material into the capillary tube as directed for *Class I, Apparatus I*, then immediately place the charged tube in a vacuum desiccator, and dry at a pressure not exceeding 20 mm of mercury for 3 hours. Immediately upon removal from the desiccator, fire-seal the open end of the tube, and as soon as practicable proceed with the determination of the melting range as follows: operate the apparatus according to the manufacturer's instructions. Heat the block until the temperature is about $10 \pm 1^\circ$ below the expected melting range, then introduce the charged tube, and heat at a rate of rise of $3 \pm 0.5^\circ$ per minute until melting is complete. Record the melting range as directed for *Class I, Apparatus I*.

If the particle size of the material is too large for the capillary, precool the test substance as directed above, then with as little pressure as possible gently crush the particles to fit the capillary, and immediately charge the tube. In the event of dispute, only the melting range or temperature obtained as directed for *Class Ib, Apparatus I*, is definitive.

Procedure for Class II—Carefully melt the material to be tested at as low a temperature as possible, and draw it into a capillary tube, which is left open at both ends, to a depth of about 10 mm. Cool the charged tube at 10°, or lower, for 24 hours, or in contact with ice for at least 2 hours. Then attach the tube to the thermometer by suitable means, adjust it in a water bath so that the upper edge of the material is 10 mm below the water level, and heat as directed for *Class I, Apparatus I* except, within 5° of the expected melting temperature, to regulate the rate of rise of temperature to 0.5° to 1.0° per minute. The temperature at which the material is observed to rise in the capillary tube is the melting temperature.

Procedure for Class III—Melt a quantity of the test substance slowly, while stirring, until it reaches a temperature of 90° to 92°. Remove the source of the heat, and allow the molten substance to cool to a temperature of 8° to 10° above the expected melting point. Chill the bulb of a suitable thermometer (see *Thermometers* (21)) to 5°, wipe it dry, and while it is still cold dip it into the molten substance so that approximately the lower half of the bulb is submerged. Withdraw it immediately, and hold it vertically away from the heat until the wax surface dulls, then dip it for 5 minutes into a water bath having a temperature not higher than 16°.

Fix the thermometer securely in a test tube so that the lower point is 15 mm above the bottom of the test tube. Suspend the test tube in a water bath adjusted to about 16°, and raise the temperature of the bath at the rate of 2° per minute to 30°, then change to a rate of 1° per minute, and note the temperature at which the first drop of melted substance leaves the thermometer. Repeat the determination twice on a freshly melted portion of the test substance. If the variation of three determinations is less than 1°, take the average of the three as the melting point. If the variation of three determinations is 1° or greater than 1°, make two additional determinations and take the average of the five.

(751) METAL PARTICLES IN OPHTHALMIC OINTMENTS

The following test is designed to limit to a level considered to be unobjectionable the number and size of discrete metal particles that may occur in ophthalmic ointments.

Procedure—Extrude, as completely as practicable, the contents of 10 tubes individually into separate, clear, flat-bottom, 60-mm Petri dishes that are free from scratches. Cover the dishes, and heat at 85° for 2 hours, increasing the temperature slightly if necessary to ensure that a fully fluid state is obtained. Taking precautions against disturbing the melted sample, allow each to cool to room temperature and to solidify.

Remove the covers, and invert each Petri dish on the stage of a suitable microscope adjusted to furnish 30 times magnification and equipped with an eye-piece micrometer disk that has been calibrated at the magnification being used. In addition to the usual source of light, direct an illuminator from above the ointment at a 45° angle. Examine the entire bottom of the Petri dish for metal particles. Varying the intensity of the illuminator from above allows such metal particles to be recognized by their characteristic reflection of light.

Count the number of metal particles that are 50 µm or larger in any dimension: the requirements are met if the total number of such particles in all 10 tubes does not exceed 50, and if not more than 1 tube is found to contain more than 8 such particles. If these results are not obtained, repeat the test on 20 additional tubes: the requirements are met if the total number of metal particles that are 50 µm or larger in any dimension does not exceed 150 in all 30 tubes tested, and if not more than 3 of the tubes are found to contain more than 8 such particles each.

(755) MINIMUM FILL

The following tests and specifications apply to articles such as creams, gels, jellies, lotions, ointments, pastes, powders, and aerosols, including pressurized and nonpressurized topical sprays that are packaged in containers in which the labeled content is not more than 150 g or 150 mL.

PROCEDURE FOR DOSAGE FORMS OTHER THAN AEROSOLS—For containers labeled by weight, select a sample of 10 filled containers, and remove any labeling that might be altered in weight during the removal of the container contents. Thoroughly cleanse and dry the outside of the containers by a suitable means, and weigh individually. Quantitatively remove the contents from each container, cutting the latter open and washing with a suitable solvent, if necessary, taking care to retain the closure and other parts of each container. Dry, and again weigh each empty container together with its corresponding parts. The difference between the two weights is the net weight of the contents of the container. For containers labeled by volume, pour the contents of 10 containers into 10 suitable graduated cylinders, and allow to drain completely. Record the volume of the contents of each of the 10 containers. The average net content of the 10 containers is not less than the labeled amount, and the net content of any single container is not less than 90% of the labeled amount where the labeled amount is 60 g or 60 mL or less, or not less than 95% of the labeled

amount where the labeled amount is more than 60 g or 60 mL but not more than 150 g or 150 mL. If this requirement is not met, determine the content of 20 additional containers. The average content of the 30 containers is not less than the labeled amount, and the net content of not more than 1 of the 30 containers is less than 90% of the labeled amount where the labeled amount is 60 g or 60 mL or less, or less than 95% of the labeled amount where the labeled amount is more than 60 g or 60 mL but not more than 150 g or 150 mL.

PROCEDURE FOR AEROSOLS—Select a sample of 10 filled containers, and remove any labeling that might be altered in weight during the removal of the container contents. Thoroughly cleanse and dry the outsides of the containers by suitable means, and weigh individually. Remove the contents from each container by employing any safe technique (e.g., chill to reduce the internal pressure, remove the valve, and pour). Remove any residual contents with suitable solvents, then rinse with a few portions of methanol. Retain as a unit the container, the valve, and all associated parts, and heat them at 100° for 5 minutes. Cool, and again weigh each of the containers together with its corresponding parts. The difference between the original weight and the weight of the empty aerosol container is the net fill weight. Determine the net fill weight for each container tested. The requirements are met if the net weight of the contents of each of the 10 containers is not less than the labeled amount.

(761) NUCLEAR MAGNETIC RESONANCE

Nuclear magnetic resonance (NMR) spectroscopy is an analytical procedure based on the magnetic properties of certain atomic nuclei. It is similar to other types of spectroscopy in that absorption or emission of electromagnetic energy at characteristic frequencies provides analytical information. NMR differs in that the discrete energy levels between which the transitions take place are created artificially by placing the nuclei in a magnetic field.

Atomic nuclei are charged and behave as if they were spinning on the nuclear axis, thus creating a magnetic dipole of moment μ along this axis. The angular momentum of the spinning nucleus is characterized by a spin quantum number (I). If the mass number is odd, I is $1/2$ or an integer plus $1/2$; otherwise, it has a value of 0 or a whole number.

Nuclei having a spin quantum number, $I \neq 0$, when placed in an external uniform static magnetic field of strength, H_0 , align with respect to the field in $(2I + 1)$ possible orientations. Thus, for nuclei with $I = 1/2$, which include most isotopes of analytical significance (Table 1), there are two possible orientations, corresponding to two different energy states. A nuclear resonance is the transition between these states, by absorption or emission of the corresponding amount of energy. In a static magnetic field the nuclear magnetic axis precesses (Larmor precession) about the external field axis. The precessional angular velocity, ω_0 , is related to the external magnetic field strength through the equation:

$$\omega_0 = \gamma H_0$$

in which γ is the magnetogyric ratio and is a constant for all nuclei of a given isotope. If energy from an oscillating radio-