Designation: E2603 – 15 (Reapproved 2023)

Standard Practice for Calibration of Fixed-Cell Differential Scanning Calorimeters[1]

This standard is issued under the fixed designation E2603; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers the calibration of fixed-cell differential scanning calorimeters over the temperature range from –10 °C to +120 °C.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in Section 7.

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E473 Terminology Relating to Thermal Analysis and Rheology

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E967 Test Method for Temperature Calibration of Differential Scanning Calorimeters and Differential Thermal Analyzers

E968 Practice for Heat Flow Calibration of Differential Scanning Calorimeters (Withdrawn 2023)[3]

E1142 Terminology Relating to Thermophysical Properties

3. Terminology

3.1 Specific technical terms used in this practice are defined in Terminologies E473 and E1142, including *differential scanning calorimeter, enthalpy, Kelvin,* and *transformation temperature*.

4. Summary of Practice

4.1 This practice covers calibration of fixed-cell differential scanning calorimeters. These calorimeters differ from another category of differential scanning calorimeter in that the former have generally larger sample volumes, slower maximum temperature scan rate capabilities, provision for electrical calibration of heat flow, and a smaller range of temperature over which they operate. The larger sample cells, and their lack of disposability, make inapplicable the calibration methods of Practices E967 and E968.

4.2 This practice consists of heating the calibration materials in aqueous solution at a controlled rate through a region of known thermal transition. The difference in heat flow between the calibration material and a reference material, both relative to a heat reservoir, is monitored and continuously recorded. A transition is marked by the absorption or release of energy by the specimen resulting in a corresponding peak in the resulting curve.

4.3 The fixed-cell calorimeters typically, if not always, have electrical heating facilities for calibration of the heat-flow axis. Despite the use of resistance heating for calibration, a chemical calibration serves to verify the correct operation of the calibration mechanism and the calorimeter. The thermal denaturation of chicken egg white lysozyme is used in this practice for verification of the proper functioning of the instrument's systems. The accuracy with which the denaturation enthalpy of chicken egg white lysozyme is currently known, ±5 %, is such that it should be rare that a calorimeter provides a value outside that established in the literature for this reference material.

5. Significance and Use

5.1 Fixed-cell differential scanning calorimeters are used to determine the transition temperatures and energetics of materials in solution. For this information to be accepted with confidence in an absolute sense, temperature and heat calibration of the apparatus or comparison of the resulting data to that of known standard materials is required.

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[1] This practice is under the jurisdiction of ASTM Committee E37 on Thermal Measurements and is the direct responsibility of Subcommittee E37.09 on Microcalorimetry.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] The last approved version of this historical standard is referenced on www.astm.org.

5.2 This practice is useful in calibrating the temperature and heat flow axes of fixed-cell differential scanning calorimeters.

6. Apparatus

6.1 Apparatus shall be:

6.1.1 *Differential Scanning Calorimeter (DSC),* capable of heating a test specimen and a reference material at a controlled rate and of automatically recording the differential heat flow between the sample and the reference material to the required sensitivity and precision.

6.1.2 *DSC Test Chamber,* composed of:

6.1.2.1 A device(s) to provide uniform controlled heating or cooling of a specimen and reference to a constant temperature or at a constant rate within the applicable temperature range of this method.

6.1.2.2 A temperature sensor to provide an indication of the specimen temperature to ±0.01 K.

6.1.2.3 Differential sensors to detect a heat flow (power) difference between the specimen and reference with a sensitivity of ±0.1 µW.

6.1.3 A *Temperature Controller*, capable of executing a specific temperature program by operating the furnace(s) between selected temperature limits at a rate of temperature change of 0.01 K/min to 1 K/min constant to ±0.001 K/min or at an isothermal temperature constant to ±0.001 K.

6.1.4 A *Data Collection Device*, to provide a means of acquiring, storing, and displaying measured or calculated signals, or both. The minimum output signals required for DSC are heat flow, temperature, and time.

6.1.5 *Containers,* that are inert to the specimen and reference materials and that are of suitable structural shape and integrity to contain the specimen and reference in accordance with the specific requirements of this test method. These containers are not designed as consumables. They are either an integral part of the instrument, whether or not user-removable for replacement or, in some implementations, are removable and reusable. Container volumes generally range from 0.1 ml to 1 ml, depending on the instrument's manufacture.

6.2 *Analytical Balance,* capable of weighing to the nearest 0.1 mg, for preparation of solutions.

6.3 *UV spectrophotometer or UV/Vis spectrophotometer,* capable of scanning the UV spectrum in a region about 280 nm.

6.4 *Reagents:*

6.4.1 *Phosphatidylcholines,* 1,2-ditridecanoyl-sn-glycero-3-phosphocholine (DTPC) CAS Number 71242-28-9 and 1,2-ditetracosanoyl-sn-glycero-3-phosphocholine (DLPC) CAS Number 91742-11-9 are the minimum required.

6.4.2 *Aqueous buffer solutions,* 0.01 Molar, pH 7 aqueous solution of $Na_2HPO_4 - NaH_2PO_4$ and 0.1 Molar, pH (2.4 ± 0.1) aqueous solution of HCl + glycine.

6.4.3 *Chicken egg white lysozyme.*

7. Precautions

7.1 This practice assumes linear temperature indication. Care must be taken in the application of this practice to ensure that calibration points are taken sufficiently close together so that linear temperature indication may be approximated.

8. Calibration Materials

8.1 Phosphatidylcholines: 1,2-ditridecanoyl-sn-glycero-3-phosphocholine (DTPC) CAS Number 71242-28-9; and 1,2-ditetracosanoyl-sn-glycero-3-phosphocholine (DLPC) CAS Number 91742-11-9. Purities are to be 0.99 or better. Additional calibration materials are listed in Table 1.

8.1.1 Aqueous suspensions of the phosphatidylcholines are prepared as follows. Weighed amounts of a 0.01 Molar, pH 7 solution of the buffer $Na_2HPO_4 - NaH_2PO_4$ and DTPC are combined so to give a solution of 1 mass percent of the phosphatidylcholine. This procedure is repeated for DLPC. The solutions are heated in a hot water bath to 5 K above the transition temperatures. A vortex mixer is used to shake the solutions at their respective temperatures until the lipid appears to have been completely suspended. The solutions may be stored in a refrigerator until use for up to a week.

8.2 Chicken egg white lysozyme with purity of at least 95 % mass percent.

8.2.1 Weighed amounts of the lysozyme and of a 0.1 M HCl – glycine buffer at pH = (2.4 ± 0.1) are combined to obtain a solution of approximately 3 mass percent.

8.2.2 The concentration of lysozyme in this solution is calculated from UV absorbance at a wavelength of 280 nm, using a 1 cm cell and the optical density of 2.65 for a 1 mg mL^{-1} solution.

8.2.2.1 Fill a 1 cm optical cell with buffer solution and another 1 cm cell with the lysozyme solution. Follow the instrument's directions for establishing baseline, and if needed, calibration of the absorbance scale. Insert both of the filled cells in the UV spectrometer if the spectrometer is a dual beam instrument. Scan through the 280 nm region and note the absorbance at 280 nm. If the spectrometer is a single beam instrument, the buffer is measured first, then the lysozyme solution is measured and the difference in the recorded absorbances is used to calculate the concentration. Concentration is calculated as:

$$c = A/(2.65\ \mathrm{mL\ mg^{-1}})$$

where:

A = absorbance, and
c = concentration in mg mL^{-1}.

NOTE 1—Different concentrations may be used between 1 and 10 mass percent, the concentration used shall be included in the report.

TABLE 1 Melting Temperature of Calibration Material

NOTE 1—The uncertainties for the temperatures are ±0.1 K.

Calibration Material	Melting Temperature	
	°C	K
1,2-ditridecanoyl-*sn*-glycero-3-phosphocholine (DTPC)	13.25	286.4
1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine (DMPC)	23.75	296.9
1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine (DPPC)	41.45	314.6
1,2-dioctadecanoyl-*sn*-glycero-3-phosphocholine (DSPC)	54.85	328.0
1,2-dieicosanoyl-*sn*-glycero-3-phosphocholine (DAPC)	65.05	338.2
1,2-didocosanoyl-*sn*-glycero-3-phosphocholine (DBPC)	73.35	346.5
1,2-ditetracosanoyl-*sn*-glycero-3-phosphocholine (DLPC)	80.55	353.7