

Melting Point Determination

Application Note #1

Introduction

A few basic guidelines must be carefully followed to avoid errors during melting point determinations with OptiMelt. The way in which the sample is prepared and the instrument is programmed have the greatest influence on the accuracy and reproducibility of a melting point measurement. Subjective interpretation of the changes observed in the sample (visually and/or automatically) during the analysis can also lead to unreliable results.

Background

The melting point of a substance is the temperature at which the material changes from a solid to a liquid state. Pure crystalline substances have a clear, sharply defined melting point. During the melting process, all of the energy added to a substance is consumed as heat of fusion, and the temperature remains constant.

A pure substance melts at a precisely defined temperature, characteristic of every crystalline substance and dependent only on pressure (though the pressure dependency is generally considered insignificant).

Determining the MP is a simple and fast method used in many diverse areas of chemistry to obtain a first impression of the purity of a substance. This is because even small quantities of impurities change the melting point, or at least clearly enlarge its melting range. Melting point determinations are more than just a classroom exercise in the organic chemistry lab. The test is still an important technique for gauging purity of organic and pharmaceutical compounds.

The determination of melting points is one of the oldest identification and test methods for organic substances. The melting point is easy to measure, tabulate and classify. Extensive collections of tables give the exact values of many pure, inorganic and organic compounds. The MP determination is a fast and cost-effective technique which remains a strong link to the vast pre-instrumental chemistry literature.

Capillary Method

The procedural rules for melting point determinations are defined in the pharmacopeias. The medical handbooks include minimum requirements for the

design of the melting point apparatus and for performing the measurements. Automated melting point determination procedures are generally included. Very often, the pharmacopeias also list special methods for difficult or unusual cases of melting point determination.

The pharmacopeias regard the capillary method as the standard technique for melting point determination. In this methodology, a thin glass capillary tube containing a compact column of the substance to be determined is introduced into a heated stand (liquid bath or metal block) in close proximity to a high accuracy thermometer. The temperature in the heating stand is ramped at a user-programmable fixed rate until the sample in the tube transitions into the liquid state. While determining a melting point, several observations and the temperatures are recorded.

Tips

- The metal heating stand of OptiMelt can accommodate three capillary tubes so that up to three independent samples can be analyzed at the same time. A platinum resistance thermometer, in close proximity to the sample slots, is used to read the temperatures during the melt.
- Your OptiMelt includes a vial of precision melting point capillaries specifically designed to fit the sample slots and provide the most uniform and repeatable results.

The capillary method described by most pharmacopeias relies on a visual detection of the melt. However, some modern instruments, such as OptiMelt, enable automated detection of the melting point and melting range, while at the same time providing a view of the sample during the process.

The accuracy of a melting point record is assured by: (a) careful sample preparation, (b) proper instrument setup, and (c) routine calibration of the instrument's temperature scale against certified melting point standards.

Sample Preparation

Careless preparation of a sample is the leading cause of inaccurate and irreproducible results in melting point determinations.

Any substance being loaded into a melting point capillary must be:

1. Fully dry
2. Homogeneous
3. In powdered form



Moist samples must be dried first—48 hours over P_2O_5 , in a dessicator, usually gets the job done.

The primary requirement for good melting point determination is that the sample be in a fine powder form. This makes the heat transfer into the sample more efficient and reproducible, and also enhances the overall reflectivity of the sample for easier automated detection of the melt. Coarse crystalline and non-homogeneous samples must be crushed into a fine powder in a mortar. An agate, glass or alumina mortar and pestle are recommended.



Solid samples, mortar and pestle, and capillary tubes—the basic ingredients for sample preparation.

To fill a capillary tube with a sample, the open end of the capillary is pressed gently into the substance several times. The powder is then pushed to the bottom of the tube by repeatedly pounding the bottom of the capillary against a hard surface (preferred method). Alternatively, the capillary tube can be dropped onto a table through a glass tube of ≈ 1 m in length. A sample packing wire can be used at the end to further compact the sample and improve the reproducibility of the measurements.

In addition to tight packing, maintaining a fixed level in the fill is also a very important requirement. Taller samples take extra heat to completely melt and usually display larger melting ranges than their shorter counterparts.

A sample height between 2.0 mm and 3.0 mm is recommended for optimum results and reproducibility.

If your sample is hygroscopic, or sublimates at high temperatures, the open end of the capillary tube must be sealed by heating. Hygroscopic samples must be stored in a desiccator between tests. This is particularly critical in humid environments or even rainy days.

The sample tubes are loaded into OptiMelt by inserting them into one of the sample position slots located on top of the instrument. Up to three samples can be accommodated by the heating block simultaneously. For improved reproducibility of results, it is recommended that three tubes be inserted even if each capillary does not contain a sample. Since up to three different samples can be determined at the same time, loading three capillaries with the same substance and averaging their MP temperatures provides the fastest and simplest way to improve the repeatability and accuracy of all MP determinations.

Tips

- It is generally considered good practice to wipe the outside surface of capillary tubes with a clean cloth before inserting them into the heating stand. Over time, dust can accumulate on the glass window of the heating block reducing overall visibility of the melt.
- Tight compaction of capillary samples with packing wires can result in excessive bubble formation during the melt that can compromise proper detection of meniscus and clear points.
- Most pharmacopeias list recommended drying procedures for melting point samples and certified reference standards.
- Make sure OptiMelt is plugged in and set to a start temperature below the expected MP of the sample(s) before placing any capillaries into the sample slots.
- Use the same batch of capillaries for calibration and for routine measurements to assure the repeatability of results. **NOT ALL CAPILLARIES ARE MADE EQUAL!**
- The standard OptiMelt package includes a vial of precision melting point capillaries specifically designed to (1) fit the OptiMelt heating stand and (2) provide the most uniform and repeatable results. Replacement capillaries can be purchased directly from SRS.
- Never force a capillary into the heating block! Once the capillary is inserted into a sample hole, it should literally drop down to the bottom of the stand.
- Some chemists prefer to make their own capillary tubes. This is not recommended for accurate and reproducible results. The use of commercial capillaries, with tight manufacturing tolerances, is strongly recommended instead.
- For precision measurements, the optimum filling height of 2 mm to 3 mm must be strictly observed.

- Whenever possible, use a packing wire to compact the sample plug at the bottom of the capillary tube. A packing wire is included with each package of capillaries purchased from SRS.

Tube Cleaning

Failure to clean the tubing before making capillary tubes is one of the chief causes of low melting points and wide melting ranges. The presence of alkali on the surface of the sample tubes is one of the main problems. This is not generally an issue with pre-made, commercially available melting point capillaries.

Important! If you must make your own tubes, make sure the stock glass is cleaned by rubbing the inside with dilute solution of a neutral detergent, rinsing with dilute (10%) HCl, and finally rinsing thoroughly with distilled water.

Instrument Setup

Along with proper sample preparation, careful selection of the instrument settings is also essential for accurate and reproducible melting point determinations.

The modern trend in melting point instrumentation is towards small aluminum ovens. Small ovens minimize overshooting, which allows researchers to set the initial temperature closer to the melting temperature and thus reduce analysis time. A typical oven can hold three capillaries and the thermal mass around the three tubes is very close. Deviations as small as 0.02 °C to 0.1 °C (temperature dependent) are typical between the three tubes during a melt.

The main advantage of a small metal oven is the lack of an overshoot. This lets you park the unit at a start temperature <5 °C below the expected melting point of the compound, makes heating and cooling the unit fast and allows determinations that only last 2 to 3 minutes practical.

The prototypical pharmacopeia melting point determination procedure, followed by virtually every modern instrument, involves four basic steps:

- Step 1.** The heating stand is rapidly preheated to a user-specified start temperature, selected just a few degrees below the expected melting point of the samples.
- Step 2.** Once the temperature is stable, up to three sample capillaries are inserted into the oven and after the temperature stabilizes (i.e. thermal soak) a heating ramp is immediately launched.
- Step 3.** The temperature of the samples continues to rise at the user-specified ramping rate until a user-specified stop temperature is reached. Automated and/or visual observations of the melting point, melting

range, and other thermal related processes are tagged during this time.

- Step 4.** At the end of the heating ramp, the capillaries are discarded and the heater stand is rapidly cooled down back to the start temperature in preparation for a new determination.

Correct selection of the start temperature, ramp rate and stop temperature is absolutely essential to prevent inaccuracies due to a heat increase in the sample that is incorrect or too fast.

Start Temperature (OptiMelt range: 30 °C to 400 °C)

This is the temperature at which the sample capillaries are introduced into the heating stand, and serves as the starting temperature for the heating ramp. The start temperature is usually programmed 5 °C to 10 °C below the expected melting point of the substance.

Tip

- The start temperature must be at least 10 °C above ambient temperature to assure proper stabilization.

Ramp Rate (OptiMelt range: 0.1 to 20 °C/min)

This is the fixed rate of temperature rise between the start and final temperatures for the heating ramp. User-adjustable ramp rates are standard in modern automated melting point instrumentation.

The ramp rate is the most important instrumental parameter affecting the accuracy of melting point determinations.

Since the melting point temperature is not measured directly within the substance, but rather outside the capillary tube (i.e. inside the heating stand) the results are dependent on the heating rate. The temperature in a pure melting substance remains constant until the sample has completely melted. However, this takes a finite amount of time, and the oven temperature continues to increase depending on the heating rate chosen (i.e. thermal lag). The temperature displayed does not correspond to the exact temperature in the melting substance but to that of the oven. Consequently, higher values are obtained whereby the difference between the melting point measured and the true melting temperature is greater the more rapid the rise in oven temperature. These heating rate dependent temperatures are referred to as “according to pharmacopeia”. Some melting point instruments (such as the SRS OptiMelt) can

compensate for the oven ramp rate and provide corrections for the temperature readings obtained according to pharmacopeia so that the “true thermodynamic” melting point of a pure substance can be reported.

Misuse of fast ramp rates is the main cause of inaccuracies in MP measurements.

Rates of temperature increase up to 2 °C/min are reasonable for routine determinations. Higher rates are only recommended for quick determinations on substances with unknown melting points. Purity determination and precision measurements are performed at a maximum heating rate of ≈ 0.5 °C/min, though the recommendation is to stay at 0.1 to 0.2 °C/min., whenever feasible. Samples that start to decompose at temperatures below their melting point are usually measured at ramp rates above 5°C/min to avoid contamination from byproducts. Mixed melting point determinations (described below) can be performed with ramp rates as large as 10°C/min.

Tips

- Following most pharmacopeia recommendations, the heating rate should always be included in a melting point record, along with the melting range, to enable proper reproduction of the results.
- It is often time-saving to run a preliminary (i.e. fast) melting point determination, ramping the temperature rapidly (10 to 20 °C/min). After the approximate melting point is known, a second determination is performed at a much smaller ramp rate and with a start temperature 5 °C below the expected melting point. A fresh sample must be used for the second determination.

Stop Temperature

(OptiMelt range: [Start Temp +5°C] to 400°C)

This is the temperature at which the heating ramp is terminated. At the end of the ramp, the capillaries are discarded. The results are saved and displayed in a summary test report and the heater stand is automatically cooled down back to the start temperature in preparation for a new determination.

Samples cannot be re-melted!! Always start a new determination with fresh capillaries.

Calibration

Before any melting point measurement system is put into operation its temperature scale must be calibrated using appropriate reference substances with melting points that are known exactly.

Analytical quality control (QC) laboratories must also test their melting point instrumentation on a regular basis against certified reference standards (CRSs), to determine the acceptability of their instruments. These tests must be done according to specific QC requirements set forth by local, national and international standards and pharmacopeia laboratories.

In order to comply with QC certification requirements and Good Laboratory Practices (GLP), the OptiMelt system includes a menu-driven calibration procedure. The temperature calibration of the OptiMelt system should be checked, and readjusted if necessary, every six months (minimum) to maintain the instrument within its factory-certified accuracy specifications. (The aging of Pt/RTD resistor thermometers has been well documented in the engineering and scientific literature and is the leading cause for loss of accuracy in most modern melting point instrumentation.)

Calibration of OptiMelt is recommended every 6 months.

The calibration procedure is very straightforward. The melting points of three CRSs, with melting points around 75, 125 and 225 °C, are measured and then compared with their certified values. If the two sets of numbers (measured vs. nominal) deviate from one another beyond the accuracy of the instrument, the temperature measurement in the heating block must be recalibrated. Step-by-step calibration instructions and an option to correct the instrument's temperature scale (if necessary) at the end of the procedure are available through the front panel interface.

Attempts to cover the entire range of RT to 400 °C with any automated melting point apparatus combined with calibration curves has failed and, as a result, most automated systems function with temperatures under 300 °C (or quote larger errors at >300 °C). A visual inspection must be combined with the automated determination for substances that sublime at >300 °C.

A CRS kit (SRS Part# O100MPS), traceable to WHO International pharmacopeia standards, is available directly from Stanford Research Systems and is recommended for recalibration and for determination of acceptability of your OptiMelt system.

Visual Observations

Several noticeable changes take place in the capillaries during a melting point determination. Subjectivity in the interpretation of the physical and chemical changes observed during the heating ramp can be an important factor affecting the reproducibility of melting point results.

The following events should be noted, and their temperatures recorded to provide a complete record of the changes observed in the samples during the melt.

First signs of change

Record the first signs of change in the samples. Early changes may be due to:

- (1) Loss of solvent (dehydration)
- (2) Change in crystallization state (shriveling)
- (3) Slow onset of decomposition (darkening or change of color)
- (4) Condensation of solvent in the coolest points of the tube
- (5) Individual isolated crystals starting to melt without the liquid showing up as a cohesive phase, i.e. *Sintering Point*.

Onset Point

The onset point is generally considered the “official” start of the melt; liquid clearly appears for the first time as a separate phase in coexistence with the crystals. It must not be confused with the “sintering point” which corresponds to just isolated drops due to a few surface crystals melted.

Tips

- The onset point corresponds to the low temperature record in the MP Range of a substance.
- The US Pharmacopeia describes the Onset Point as “the temperature at which the column of the substance under test is observed to collapse definitely against the side of the tube”. This is known as the collapse point of the sample.
- For an automated instrument relying on bulk absorption or reflection, the US Pharmacopeia describes the Onset Point as “the temperature at which the first change in detected signal is observed during the melt”.
- Simple automated systems relying on optical absorption and bulk reflection cannot readily detect the onset of a melt. They usually report temperatures for the start of the melt that are large compared to what is detected visually. This is because it takes a significant change in sample appearance for the measurement to detect a change in bulk absorption or reflection. The error in the determination of the onset point leads to a

reduced melting range report which is a cause of concern in some analytical and QC applications.

- OptiMelt can automatically detect and record the onset point of a sample. The built-in camera is sensitive to even the slightest changes in the physical appearance of the samples, closely matching the sensitivity of your own eyes. A user-adjustable threshold (Onset %) is available to carefully match the visual and automatic values of the onset point for each substance.

Meniscus Point

The meniscus point corresponds to the stage along the melt when the meniscus for the liquid becomes visible; there is a solid phase at the bottom and a clear liquid phase on top with a well-defined and visible meniscus. This point is readily detectable except occasionally when air bubble(s) from below push unmelted solid to the surface.

Since the meniscus point represents the time during which liquid and solid coexist, it is often considered a good approximation to the “thermodynamic” MP of a substance (especially at very low ramping rates).

Tips

- The meniscus point is often the temperature listed in European MP tables and the preferred value of the British pharmacopeia methodology.
- The meniscus point is one of the three values (onset, meniscus and clear point) recorded for each of its melting point standards by the Laboratory of the Government Chemist (LGC). In an attempt to remove subjectivity from its detection, the LGC defines the meniscus point as “the point where a definite meniscus is visible, and there is equal volumes of solid and liquid in the capillary”.
- The meniscus point is not specifically mentioned by the US Pharmacopeia Melting Point methods (Method <741> of USP25-NF20). The clear point (described below) is identified as the “melting point” of a substance instead. Notice that this is a big difference in interpretation between the British and US Pharmacopeias.
- The SRS OptiMelt can automatically detect and record the meniscus point of a sample. A user-adjustable threshold (Single %) is available to match the visual and automatic records of the meniscus point.

Clear (or Liquefaction) Point

The clear point corresponds to the stage along the melt at which the substance becomes completely liquid — no more solid is left (i.e. the last crystals are melted).

The clear point is more dependent on the ramping rate than the onset point. In general, the clear point increases with increasing ramping rates (see Table 1).

Table 1. Clear point of phenacetin at various ramp rates (OptiMelt system).

| Ramp Rate, r [°C/min] | Clear Point [°C] |
|--------------------------|---------------------|
| 0.1 | 134.2 |
| 0.2 | 134.4 |
| 0.5 | 134.9 |
| 1 | 135.4 |
| 2 | 136.2 |
| 5 | 137.9 |

Tips

- The clear point record corresponds to the high temperature record in the MP range of a substance.
- The clear point is most often the single temperature melting point listed in melting point tables.
- The clear point is the temperature most often listed in US-based MP tables and the only one accepted by the US Pharmacopeia as the “single” melting point of a substance.
- In an automated system, the clear point is usually identified as the temperature at which the last change in detected signal is observed during the melt.
- Simple automated systems relying on optical absorption and bulk reflection give a number that is best correlated to the clear point.
- The SRS OptiMelt can automatically detect and record the clear point of a sample. A user-adjustable threshold (Clear %) is available to match the visual and automatic records for the clear point.

Last Signs of Change

Any changes in the sample composition before, during and after the clear point should also be recorded. Common events include:

Sublimation: Crystals appear in the protruding part of the capillary tube.

Decomposition: Sample bubbles or changes in color during and after the melt.

Melting Point Range

In a dynamic melting point determination, where true equilibrium between solid and liquid phase is never achieved, the Melting Point Range—defined as the interval between the onset and clear points- is a valuable indicator of purity of a solid compound.

The Melting Point Range is the most popular melting point record listed in scientific papers, standard procedures, reference tables and MP standards. It is always advantageous to record the entire melting range of a substance, especially with (1) unknown or new compounds, (2) impure samples, (3) mixtures with large melting intervals and (4) polymorphous compounds. The observed range is an aid in identifying the substance and drawing conclusions about its purity and heat stability.

Reporting the melting range [onset point, clear point] of a solid sample, along with the ramping rate, is the preferred way to report the results of a melt, and it is much more reliable than a single number report.

If a single number must be used, please specify whether clear or meniscus point was used.

The ramping rate affects the melting point range record and must always be specified for full compliance with GLP specifications.

Table 1. Melt point range of phenacetin at various ramp rates (OptiMelt system).

| Ramp Rate, r [°C/min] | Onset Point- Clear Point [°C] | Temp. Range [°C] |
|--------------------------|----------------------------------|---------------------|
| 0.1 | 133.7-134.2 | 0.5 |
| 0.2 | 133.8-134.4 | 0.6 |
| 0.5 | 134.0-134.9 | 0.9 |
| 1 | 134.1-135.4 | 1.3 |
| 2 | 134.3-136.2 | 1.9 |
| 5 | 134.9-137.9 | 3.0 |

Notice the larger effect of ramping rate on the clear point than on the onset point.

Tips

- The SRS OptiMelt can automatically detect and record the onset and clear points of a sample. User-adjustable thresholds (Onset %, Clear %) are available to match the visual and automatic values for both points.
- A large majority of pure organic compounds melt neatly within a range of 1.5 °C or melt with decomposition over a narrow range of temperature

(≈ 2 °C) at heating rates below 0.5 °C/min. Many organic compounds (aminoacids, salts of acids, salts of amines, carbohydrates, etc.) melt with decomposition over a considerable range of temperature.

- Impure substances (i.e. mixtures) melt over a more or less large temperature range.

Test Report

A complete melt-point report should include enough information to make it possible for somebody else to reproduce the determination and compare results. Very useful reporting guidelines, compatible with modern GLP requirements, were set forth by Carter and Carter (J. Chem. Ed., 72 (1995) 647) and are reproduced here:

- ◆ Report all instrument settings, specifically heating rate, so they can be duplicated or reasonable adjustment made.
- ◆ Report onset and clear point temperatures to the nearest 0.1 °C (or at least 0.5 °C) for routine melting point ranges.
- ◆ Report onset, meniscus and clear point to nearest 0.1°C for important melting point ranges, such as those of new compounds.
- ◆ If a single temperature is to be reported as the melting point (not recommended), specify whether meniscus or clear point was used.
- ◆ Use well-known melting point standards (i.e. certified reference standards) for calibration.

Reference Tables

There is often some uncertainty as to what exactly is tabulated in MP tables, especially when a single-temperature is listed for a substance.

This confusion is based on the fact that while most chemists use the clear point to report the MP temperature of their samples, others prefer the meniscus point. The meniscus point is often regarded as closer to the true thermodynamic value, since it corresponds to a coexistence of liquid and solid in the capillary. However, there is no real thermodynamic justification for that assumption. Luckily, the difference between the two numbers (i.e. clear vs. meniscus point) is, in most cases, very small and within the accuracy of most determinations.

Melting Point Depression / Melting Range

Mixtures of substances whose components are insoluble in each other in the liquid phase, display a melting point depression, and instead of a sharp melting point, a melting range (interval).

The size of the melting point depression depends on the composition of the mixture. The depression in melting point is used for determining the purity and identity of compounds.

Rule-of-thumb

1 % of foreign substance will result in a 0.5 °C depression.

This is the main reason why recording the MP range is the preferred result of a melting point determination, and more useful than a single temperature melting point report.

A wide melting range usually indicates that a substance is impure, but it may also result from the fact that the pure substance undergoes some decomposition prior to reaching its phase transition. Pure substances that decompose during heating, form a mixture of the parent substance and the byproducts, and will also show a melting range. In some cases, the material undergoes a slight liquefaction and contraction at a temperature below the true melting point. In others, the material may decompose and discolor so badly that a definite melting point cannot be observed.

Purity Tracking

The phenomenon of melting point depression can be applied to the evaluation of purity of synthetic compounds.

In preparative organic chemistry, the purity of a substance often has to be evaluated without a pure reference sample being available. This is the case for example when a new chemical compound is manufactured. The raw product is generally subjected to a few re-crystallization steps, and the melting point is determined at each stage. The onset point continues to increase, and the melting range continues to decrease, until the substance is either pure or until it is as pure as it is going to get through the purification method being applied.

Tip

- It is common practice to re-crystallize synthetic products of reactions until no more changes are detected in their melting point range.

Mixed Melting Point

If two substances melt at the same temperature, a Mixed Melting Point determination can reveal if they are one and the same substance.



The phenomenon of melting point depression can be applied to the identification of unknown pure substances. For example, if you measure the melting point of a sample at 160 °C, you will find from the MP tables that this is the same melting point for several different reference compounds. The substance can be identified by determining its Mixed Melting Point—the sample is mixed one-by-one with small amounts of the references and the mixed melting point is determined in each case. Whenever the melting point of the sample is depressed by mixing a small amount of a reference with it, the two substances cannot be identical. If, however, the melting point of the mixture does not drop, the reference substance that was added was identical to the sample (i.e. the sample has been identified).

The mixed melting point technique is an important reason why all high-quality melting point instruments accommodate at least three capillaries in their heating blocks.

In its most common implementation, three melting points are determined: (1) sample, (2) reference and (3) reference and sample in a 1:1 mixing ratio. If the melting point of the mixture remains the same, the two substances are identical. If the melting point is depressed, they are two different substances.

Tips

- The requirements for precision and reproducibility are not as high here as when doing a high-precision, single MP determination. Heating rates as large as 10 °C/min are acceptable for mixed MP determinations.
- A few pairs of substances show no melting point depression when mixed, but more frequently the failure to depress may be observed only at certain compositions. It requires little additional effort to measure the melting point of several compositions. Typically a 20/80, 50/50 and 80/20 % mixture of sample and reference is prepared, and the three tubes are run in the MP apparatus. If the three melt at the same temperature, it is very likely the two compounds are one and the same!