Operation and Service Manual

MPA120

EZ-Melt Automated Melting Point Apparatus

Stanford Research Systems

Revision 1.4 (11/05)

Certification

Stanford Research Systems certifies that this product met its published specifications at the time of shipment.

Warranty

This Stanford Research Systems product is warranted against defects in materials and workmanship for a period of one (1) year from the date of shipment.

Service

For warranty service or repair, this product must be returned to a Stanford Research Systems authorized service facility. Contact Stanford Research Systems or an authorized representative before returning this product for repair.

Stanford Research Systems, Inc.
1290-C Reamwood Avenue
Sunnyvale, California 94089
www.thinksrs.com

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Safety and Preparation for Use

CAREFULLY READ THE IMPORTANT SAFETY INSTRUCTIONS AND NOTES INCLUDED IN THIS SECTION BEFORE USING THE EZ-MELT AUTOMATED MELTING POINT APPARATUS AND ANY OF ITS ACCESSORIES.

Within this section, the word **'product'** specifically refers to the **EZ-Melt Automated Melting Point Apparatus and any of its accessories**.

This product is designed and built for use in synthetic and analytical chemistry laboratories. It is intended to be used to determine melting temperatures and melting ranges between ambient and 400°C temperatures.

Safety risks are associated with all research and production activities. Though long experience has proven melting point instrumentation to be remarkably safe, hazards are always associated with analytical equipment. The most effective way to minimize risk to yourself and others is to read, and strictly follow, all safety instructions and warnings during the installation, operation and maintenance of all equipment used in your laboratory.

The intent of this section is to collect, in a single place, the most common risks associated to the installation, operation and maintenance of this product. The instructions are also repeated, with additional information, at the appropriate points throughout this manual.

This product has been designed with user safety as a priority and has been proven to show reasonably safe operation provided it is installed, operated and serviced in strict accordance with all the safety instructions included in its manual

Safety Instructions and Warnings

- **SAFETY PAYS!** Safety instructions must be strictly followed during all stages of installation, operation and service of this product. Failure to comply with these precautions and warnings violates the safety standards expected of users of this product.
- This Operations Manual is a component of the product and must remain readily available to all laboratory personnel with access to the EZ-Melt Melting Point Apparatus.
- If you have any doubts about how to use this product safely, contact Stanford Research Systems using the contact information provided in this manual.
- Do not use this product for any purpose other than its intended usage.
- Retain these safety and operating instructions for future reference.
- Identify and adhere to all warnings posted on the product and throughout this manual.
- Failure to comply with these instructions may result in serious personal injury, including death, as well as significant property damage.

- This instruction manual should form the basis of any training requiring the use of this product.
- Wear protective garments such as lab coat and goggles at all times.
- Refer servicing to qualified personnel only.

Product Placement Requirements

- Place this product on a stable, clean, level and even surface.
- Place the product away from water sources - faucets, safety showers, eyewashes, rain, etc. Do not allow the product to become wet.
- No containers, chemicals or other appliances should be placed behind the product.
- **Always operate the unit in its proper upright orientation**. Do not operate the unit on its side.
- To prevent damage to the product and ensure sufficient cooling in the electronic compartment, place the sidewalls of the unit at least 10 cm away from walls or other objects.
- Your EZ-Melt may produce some smoke the first time it is heated. The smoke is caused by residual oils coating the metal surfaces of the heater and surrounding areas. Once the oils burn away, the smoke will permanently cease.

Electrical Shock Risks

The most common risk associated with the operation of chemical instrumentation equipment is electrical shock.

- **It is your responsibility to install and operate this product in full conformance with local electrical codes.** Consult an experienced electrician if necessary.
- **If the power cord becomes damaged, replace it immediately.**
- Dangerous voltages capable of causing injury are present during the operation of this product. **Do not remove the covers while the unit is plugged into a live outlet.**
- Do not use this product if it has unauthorized modifications. Unauthorized modifications may result in fire, electric shock and other hazards.
- Do not install substitute parts or perform any unauthorized modifications to this instrument.
- **The line fuse is internal to the instrument and may not be serviced by the user.**
- **Always use an outlet which has a properly connected protective ground.** Consult with an experienced electrician if necessary.
- GFCI (Ground Fault Circuit Interrupter) protected outlets are often available in laboratory environments, particularly in proximity to water sources. GFCI's are

generally regarded as an important defense against electrocution. However, **the use of a GFCI in conjunction with the EZ-Melt must NOT be regarded as a substitute for proper grounding and careful connections.** GFCI's must also be tested regularly to verify their functionality. Always consult an electrician when in doubt.

- Do not use accessories not recommended in this manual as they may be hazardous.
- Keep all electrical wiring on your laboratory benches neatly organized and in good working conditions. Label and color-code all high voltage cables. Inspect all HV wires periodically for problems as part of your safety checkups.
- Use tie downs and cable channels to hold all electrical wiring in place - no dangling cables.
- Keep all electronic instrumentation neatly organized, and remove unconnected cables, power supplies and connectors from your laboratory benches and shelves.
- Do not push objects of any kind into this product through openings as they may come in contact with dangerous voltage points or short out parts that could result in a fire or electric shock.
- Operation of this product with line voltages other than those accepted by the power supply can cause damage to the instrument and injury to personnel.

Burn Risks

A common risk associated specifically with the operation of thermal analysis instrumentation equipment is burns.

- Observe, and respect, the **"HOT LID"** warning on the top surface of the product.
- Do not touch the heater block while hot.
- Check the temperature of the block before opening its compartment. **The block may still be hot from previous use even when the unit is off**. Turn the unit on to check the block temperature.

Explosion Risks

Injury due to explosion is another important safety concern during the operation of thermal analysis instrumentation.

- This product is not compatible with application environments requiring explosion-proof equipment nor with samples which may explode or ignite by heat, friction or spark.
- Do not use this product to analyze samples of unknown composition or contamination.

Contents

Front Panel Overview

Figure 1. Front Panel of EZ-Melt.

LED Display and Keypad

A simple front panel, including a 4 digit LED display with 6 button interface, displays the oven temperature, setup parameters and melting point results, as well as step-by-step status indications throughout the determination process.

Observation Window

A wide observation window, with a large magnification lens and internal illumination, allows users to observe the samples and determine their melting points visually.

Power Indicator

The 4 digit LED display and oven illumination is on whenever the unit is turned on.

Back Panel Overview

Figure 2. Back Panel of EZ-Melt.

Ceramic Sample Holder

Three sample capillaries may be inserted into the oven via the ceramic insulator visible at the top of the unit. The small metal oven is heated under tight microprocessor temperature control and in strict compliance with modern pharmacopeia methodologies. A precision platinum resistance thermometer provides accurate and reproducible temperature readings with 0.1°C resolution.

Glass Capillary Receptacles

Two separate glass capillary holders, located on top of the instrument, are available to store empty and discarded capillary tubes.

Power Entry Module

Connect the EZ-Melt to a properly grounded outlet using the power cord provided with the instrument. Consult an electrician if necessary.

EZ-Melt is turned on by flipping the power switch on the Power Entry Module.

Specifications

Operation

Start Temperature range	(Ambient + 10°C) to 396°C
Stop Temperature range	(Start temperature + 4°C) to 400°C
Temperature resolution	0.1°C
Temperature sensor	Platinum RTD
Temperature accuracy	±0.3°C (up to 100°C) ±0.5°C (up to 250°C) ±0.8°C (up to 400°C)
Reproducibility	0.2°C
Ramp rates	0.1, 0.2, 0.5, 1, 2, 5, 10 or 20°C per minute
Heat-up time	<10 minutes (50°C to 350°C)
Cool-down time	<10 minutes (350°C to 50°C)
Oven control	Microprocessor controlled closed loop PID
Automated determinations	Onset point and Clear point using Digital Image Processing
Manual determinations	Store up to 4 manual temperature recordings per capillary
User calibration	Single temperature offset, 0.1°C resolution

General

Temperature display	4 digit LED display
Capillaries	
Dimensions	1.4 mm to 2.0 mm outside dia., 100 mm length
Capacity	Up to 3 tubes simultaneously
Fill height	2 mm to 3 mm
Power	90 to 264 VAC, 47 to 63 Hz, 125 W
Operating temperature	0°C to 40°C, non-condensing
Weight	9 lbs.
Dimensions	7.5" x 10" x 8.5" (WxHxL)
Warranty	One year parts and labor on defects in materials and workmanship

Chapter 1

Getting Started

Unpacking

Before You Open the Box

Read the entire Safety and Preparation for Use section of this manual before starting any installation procedure.

Read and follow all installation and operation instructions in this manual to ensure that the performance of this instrument and the accuracy of your melting point determinations is not compromised.

Checklist

- Open the box(es) and inspect all components of your EZ-Melt Apparatus.
- Report any damage to Stanford Research Systems immediately.
- Compare the contents of the shipping boxes against your original order and the checklist below. Report any discrepancies to Stanford Research Systems immediately.

Standard Equipment Supplies

- EZ-Melt Melting Point Apparatus (SRS Part# MPA120)
- Operation and Service Manual
- One (1) Power Cord
- One pack of Capillary Tubes (100 count)

Optional Equipment

- Sample Capillaries (300 count) (SRS Part# O100MPC)
- Melting Point Standards Kit (O100MPS)
- Replacement Glass Window (SRS Part# O100GW)
- Replacement Capillary Holder (SRS Part# O100CH)

Before Using EZ-Melt

Remove any packing material and tape from the EZ-Melt unit before use.

Instrument Placement

- Place your EZ-Melt on a stable, clean, level and even surface.
- Place your EZ-Melt away from water sources - faucets, safety showers, eyewashes, rain, etc. Do not allow the unit to become wet.
- No containers, chemicals or other appliances should be placed behind the product.
- Always operate the unit in its proper upright orientation. Do not operate the unit on its side.
- To prevent damage to your EZ-Melt and ensure sufficient cooling in the electronic compartment, place the sidewalls of the unit at least 10 cm away from walls or other objects.
- Your EZ-Melt may produce some smoke the first time it is heated. The smoke is caused by residual oils coating the metal surfaces of the heater and surrounding areas. Once the oils burn away, the smoke will permanently cease.

Removable Parts

Capillary Holders

The glass Capillary Holders are inserted into the two openings located on the top.

Figure 3. Mounting the glass Capillary Holders.

The Capillary Holders are used to store unused and discarded capillaries. Once in place, they guard the unit against dust and small particles and protect the user from touching live electrical parts.

Warning!

Do not put your unit into operation without the Capillary Holders in place.

Ceramic Insulator

Remove the gray metal lid from the top of the unit to expose the oven. The Ceramic Insulator is located on top of the heating block as depicted in Figure 4.

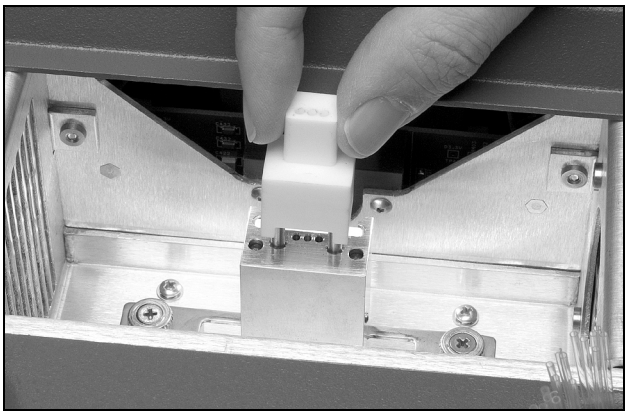


Figure 4. Placing the Ceramic Insulator on top of the metal heating block. Two metal pins, located on top of the oven, secure self-alignment of the assembly.

The Ceramic Insulator is designed to (1) reduce heat losses from the metal oven into ambient and (2) guide the capillary tubes into the metal heating block reducing the chances of capillary breakage inside the oven.

Glass Window

Remove the Ceramic Insulator to expose the glass window located in the vertical slot on the front face of the heating block as depicted in Figure 5.

The Glass Window is designed to (1) thermally insulate the sample capillaries from ambient, (2) allow uniform heating of the three capillary slots and (3) provide a clear view of the samples. The sliding assembly facilitates cleanup of the window and the block in the case of capillary breakage inside the oven.

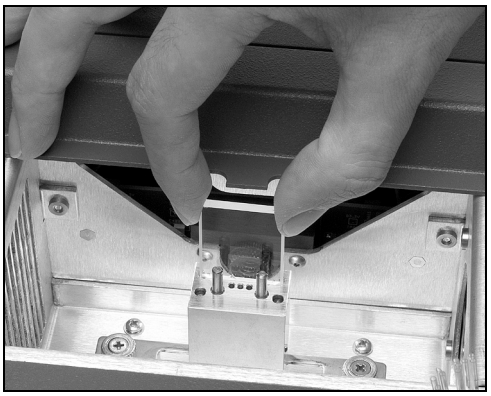


Figure 5. Inserting the Glass Window into the heating block.

Warning!
Do not operate your EZ-Melt without the Glass Window, Ceramic Insulator and metal lid in place.

Quick Start Instructions

This section includes Quick Start instructions for a melting point determination with sample(s) of known melting point.

1. Connect the power

With the rear panel power switch in the Off position, connect your EZ-Melt to a grounded outlet using the power cord provided. Turn the power switch on. The display shows the unit's firmware version briefly before displaying the oven temperature. The oven is off at this time.

2. Prepare the sample

EZ-Melt relies on the capillary method for melting point determinations. Capillary sample tubes are included with your EZ-Melt.

Any substance being loaded into a melting point capillary must be fully dry, homogeneous and in powdered form. Granular crystalline and non-homogeneous samples must first be crushed in a mortar. Load each capillary to to a height of 2–3 mm. All capillaries must be filled to the same height, and compacted in a similar way, to ensure comparable results. **Clean the outside of the capillaries to keep the oven and window clean.**

Do not insert sample capillaries into the EZ-Melt oven at this time.

3. Adjust the Start Temperature (°C)

This is the temperature at which the sample capillaries are introduced into the oven and is the starting point for the heating ramp. **The Start temperature should be at least 5°C below the known melting point of the sample.**

Press the yellow SET button once to display the Start temperature. The yellow SETUP LED turns on indicating that the display is NOT the current oven temperature. The START TEMP LED indicates that the Start temperature is displayed. Adjust the Start temperature with the RAISE↑ (START) and LOWER↓ (STOP) buttons. Hold RAISE↑ or LOWER↓ to modify by greater and greater amounts.

4. Choose a Ramp Rate (°C/min)

The Ramp Rate is the fixed rate of temperature rise between the Start and Stop temperatures for the heating ramp. **The Ramp Rate is the most important instrument parameter affecting the accuracy and reproducibility of measurement of melting points. A Ramp Rate of 1°C/min is appropriate for most routine determinations.**

Press the yellow SET button again to display the Ramp Rate. The RAMP RATE LED will be on. The yellow SETUP LED remains on indicating that the display is NOT the current oven temperature. Choose the Ramp Rate with RAISE↑ or LOWER↓.

5. Adjust the Stop Temperature (°C)

The Stop temperature is the temperature at which the heating ramp is terminated. **A Stop temperature at least 10°C higher than the Start temperature is recommended.**

Press the yellow SET button again to display the Stop temperature. The STOP TEMP LED will be on. The yellow SETUP LED remains on indicating that the display is NOT the current oven temperature. Adjust the Stop temperature with RAISE↑ or LOWER↓.

6. Finish setup

Press the yellow SET button again to finish setup. The yellow SETUP LED turns off and the display returns to the current oven temperature.

7. Preheat the oven to the Start Temperature

Press the green Start button to preheat the oven to the Start Temperature. The OPERATE LED turns on indicating that the oven is under microprocessor temperature control. The HEATING LED turns on indicating that the oven is heating up.

An audible tone is heard and the INSERT SAMPLES LED is turned on when the oven stabilizes at the Start temperature. EZ-Melt is now ready for a melting point determination.

8. Insert the sample capillaries

Insert the capillary sample tube(s) into the oven via the ceramic insulator on top of the unit and wait a few seconds for the temperature of the oven to stabilize. 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 heating stand.

9. Initiate the heating ramp

Press the green Start Button to initiate the temperature ramp and the automated melt determination process. The MELTING LED is on indicating that the oven is ramping from the Start to the Stop temperature. After a brief delay the oven temperature will start to rise at the specified Ramp Rate.

The capillary tubes must not be disturbed while this ramping takes place!

10. View the melt

During the heating ramp, EZ-Melt will make an automatic determination of the onset point and clear point for each capillary present. In addition, manual determinations may be recorded during the melt as well.

Important changes that take place in the capillary tubes can be manually flagged by pressing the blue SAMPLE buttons. Press only one SAMPLE button at a time. The three samples are identified as Left, Center and Right according to their physical location in the heating block. Store up to 4 temperature points for each sample.

11. Finish the ramp

The heating ramp is terminated when the Stop temperature is reached, or press the red STOP button to terminate the ramp early. The oven will cool back down to the Start temperature (COOLING LED on) in preparation for another experiment.

If temperatures were manually stored (using the blue SAMPLE buttons) or if EZ-Melt was able to make an automatic melting point determination, the DATA LED will flash indicating that melting temperature data is available and needs to be read.

12. Recall the information

When the DATA LED is flashing, there is new melting point data which needs to be read *before* starting the next measurement. Once the next measurement is started, the stored data is erased.

Press one of the SAMPLE buttons to see the results for that sample. The LED in the SAMPLE button lights indicating which sample's data is being displayed. Each press of the button shows the next recorded temperature in the following sequence - Auto1 (onset), Auto2 (clear), Manual1, Manual2, Manual3, Manual4. The AUTO LED is on when one of the automatic determinations is displayed. A '—' in the display indicates that there is no data for that entry in the sequence. No automated results are displayed for sample slots that do not contain capillaries or for samples that did not melt during the analysis.

Repeat this procedure for all of the samples analyzed in this experiment. Once any of the results are read, the DATA LED will turn off. The results remain in memory until another measurement is started.

Press SET, START or STOP to return to normal operation displaying the current oven temperature.

13. Prepare for the next melt

EZ-Melt automatically cools down to the Start temperature at the end of a melt. When the oven stabilizes at the Start temperature again, the audible tone is sounded and the INSERT SAMPLES LED turns on. Return to Step 8 to perform another melt (at the same temperature) or return to Step 3 to program a new set of temperatures.

14. Turn the oven off

Press the red STOP button to turn off the oven. The OPERATE LED will turn off indicating that the oven is cooling off to ambient.

If the unit is in the middle of a ramp then pressing STOP terminates the ramp and the oven will return to the Start temperature. In this case, press STOP again until the OPERATE LED turns off.

The EZ-Melt Apparatus was designed with safety as a top priority. EZ-Melt will hold at the Start temperature for at most 30 minutes without further user input. The oven will turn off after 30 minutes of holding with no user input.

Chapter 2

Using EZ-Melt

Principle of Operation

The EZ-Melt apparatus sets a new standard in ease-of-use. The easy to read LED display and the simple keypad interface are almost self explanatory. Distinct beeps and bright front panel indicators announce important events such as oven stabilization or end of melt.

Microprocessor control of both heating and cooling cycles and a small metal oven design provide fast and repeatable warmup and cool down cycles and tight temperature regulation (0.1°C resolution) during analysis. Microprocessor controlled temperature ramping eliminates the inconsistencies and overshoots typically associated with manually adjusted models.

EZ-Melt relies on the capillary method supported by virtually all pharmacopeia procedures for melting point determinations. The inclusion of three independent sample slots in the heating block allows simultaneous analysis of up to three samples, thus ensuring high sample throughput.

A typical analysis only takes a minute to set-up:

- Set a Start temperature a few degrees below the expected onset of melting
- Set a Ramping Rate from 0.1°C/min to 20°C/min
- Set a Stop temperature that exceeds the expected melting point range
- Start the measurement

The unit first heats up rapidly to the Start temperature and then holds at that temperature until the user is ready to proceed with the test. This reduces the analysis time considerably, provides more accurate results and minimizes the time delicate samples are exposed to damaging high temperatures.

After inserting the capillaries, the temperature is ramped, at the specified Ramping Rate, from the Start to the Stop temperature. Automated and/or visual analysis takes place during this time.

Once the ramping cycle is completed and the melts are detected (automatically and/or visually) the oven is cooled back down to the Start temperature in preparation for the next test.

When all tests have been completed, the oven is turned off.

Automated Analysis

The EZ-Melt unit is unique among low cost melting point analyzers in being the first to have a built-in digital camera to capture real-time images of the samples and using Digital Image Processing (DIP) technology to determine phase transitions from the analysis of those images.

The high-resolution camera can easily detect and interpret minute changes in the appearance of the capillary samples in a manner similar to your own eyes. This effectively eliminates the need for the user to be present during the analysis and avoids the subjectivity of visual melting point determinations.

The unattended melting points and melting point ranges determined by the EZ-Melt unit closely match visual results and provide a dramatic improvement over the measurements delivered by analyzers relying on more primitive and limited bulk optical absorption and reflection techniques.

The Digital Image Processing will determine 2 temperatures for each sample. These correspond to the Onset point and the Clear point. These temperature points are discussed in greater detail in Chapter 3.

Setup

Press the SET button to cycle the display through the Start temperature, Ramp Rate and Stop temperature and back to the current oven temperature.

Adjust the Start Temperature (°C)

Range: (Ambient + 10°C) to 396°C

Press the yellow SET button once to display the Start temperature. The yellow SETUP LED turns on indicating that the display is NOT displaying the oven temperature. The START TEMP LED is on indicating that the Start temperature is displayed. Adjust the Start temperature with the RAISE↑ (START) and LOWER↓ (STOP) buttons. Hold RAISE↑ or LOWER↓ to modify by greater and greater amounts.

Note that the Stop temperature must always exceed the Start temperature by 4°C. Increasing the Start temperature may cause the Stop temperature to be increased automatically to maintain this minimum gap.

The Start temperature is the temperature at which the sample capillaries are introduced into the oven, and serves as the starting point for the heating ramp. As a general rule, sample capillaries should be introduced into the heating block *after* the temperature is stabilized at the Start temperature. This is particularly critical for samples that melt with decomposition.

The Start temperature is usually programmed 5-10°C below the expected melting point of the sample substance. *Beware that some pharmacopeia guidelines have very specific requirements for Start temperature settings.*

EZ-Melt automatically returns to the Start temperature at the end of a melt in preparation for the next melting point determination.

Note
Substances with melting points between 20 and 40°C can be analyzed while operating EZ-Melt inside a refrigerator with a temperature above 0°C.

Choose the Ramp Rate (°C/min)

Range: 0.1 – 20°C/min

Press the yellow SET button again to display the Ramp Rate. The RAMP RATE LED will be on. The yellow SETUP LED remains on indicating that the display is NOT displaying the oven temperature. Choose the Ramp Rate with RAISE↑ and LOWER↓.

The Ramp Rate is the fixed rate of temperature rise between the Start and Stop temperatures for the heating ramp.

The Ramp Rate is the most important instrumental parameter affecting the accuracy and reproducibility of melting point measurements.

Ramp rates around 1°C/min are adequate for routine determinations and recommended by most pharmacopeias. Purity determination and precision measurements are performed at a maximum heating rate of ~0.5°C/min, though the recommendation is to use 0.1 or 0.2°C/min whenever feasible. Higher rates are only recommended for quick determinations on substances with unknown melting points.

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 can be performed with ramp rates as large as 10°C/min.

Note
The EZ-Melt is calibrated at the factory using a 1°C/min ramping rate for routine determinations. See Chapter 4 to change the calibration for routine determinations at a different ramping rate.

Adjust the Stop Temperature (°C)

Range: (Start Temperature + 4°C) to 400°C

Press the yellow SET button again to display the Stop temperature. The STOP TEMP LED will be on. The yellow SETUP LED remains on indicating that the display is NOT displaying the oven temperature. Adjust the Stop temperature with RAISE↑ and LOWER↓. Hold RAISE↑ or LOWER↓ to modify by greater and greater amounts.

Note that the Stop temperature must always exceed the Start temperature by 4°C. Decreasing the Stop temperature may cause the Start temperature to be decreased automatically to maintain this minimum gap.

The Stop temperature is the temperature at which the heating ramp is terminated. At the end of the ramp, the automatic melting point determinations are calculated and saved and the oven is automatically cooled back to the Start temperature. Any used capillaries must be discarded.

Press STOP to interrupt the melt before the programmed Stop temperature is reached.

Note
A Stop temperature at least 10°C higher than the Start temperature is recommended for this procedure.

Finish Setup

Press the yellow SET button again to finish setup. The yellow SETUP LED turns off and the display returns to the current oven temperature.

Oven States

The operating status of the EZ-Melt can be described in terms of Oven States. These states are Off, Preheat, Hold, Ramp and Cool. The typical progression of oven states is shown in Figure 6 following this section. During a normal measurement, the oven progresses from one state to another, either automatically or as a result of a button press. The state can be determined from the status LEDs. The current oven temperature is displayed.

In general, pressing the START button moves the oven to a state where the temperature will move to either the Start or Stop temperature. Pressing the STOP button will halt any oven heating. This either halts the temperature ramp and returns the oven to the Start temperature, or turns the oven off.

Off

The oven is off and will cool down to the ambient temperature. The OPERATE LED is OFF indicating that the oven temperature is not under microprocessor temperature control. If the oven is warm, the fan will turn on to assist the cooling. The fan will shut down below 40°C.

Preheat

While the oven is Off, press START to enter the Preheat state. The OPERATE LED is ON indicating that the oven temperature is being controlled by the microprocessor. If the Start temperature is above the current oven temperature, the oven will heat up to the Start temperature and the HEATING LED is ON. If the Start temperature is below the current oven temperature, the oven will cool down to the Start temperature and the COOLING LED will be ON.

Pressing STOP will take the oven to the Off state.

Hold

Once the oven has reached the Start temperature the oven will enter the Hold state where the oven is held at the Start temperature. The unit will beep to alert the user that the oven is ready to perform a melting point determination. The OPERATE LED is ON and the INSERT SAMPLES LED indicates that the user should now insert new sample capillaries.

If there is no user input for 30 minutes, the unit will enter the Off state. Pressing STOP will also take the oven to the Off state.

Ramp

While in the Hold state, press Start to enter the Ramp state where the oven temperature will ramp from the Start to the Stop temperature at the programmed Ramp Rate. The OPERATE and MELTING LEDs are ON.

During this time, the unit will make an automated melting point determination on the samples in the oven. Manual observations can also be recorded using the blue SAMPLE buttons.

No changes to the setup can be made in this state. The ramp progresses to the Stop temperature unless terminated using the STOP button. In either case, the oven will enter the Cool state.

Cool

In the Cool state, the oven is cooled back to the Start temperature. This may or may not require the fan. The OPERATE and COOLING LEDs are ON.

Once the oven temperature reaches the Start temperature, the oven enters the Hold state once again and is ready to make another melting point measurement.

Pressing STOP will take the oven to the Off state.

Recording Visual Observations

Sample Buttons (LEFT, CENTER and RIGHT)

During the temperature ramp, the user can watch the samples through the magnifying glass, detect any physical changes and flag relevant temperature values by pressing the blue SAMPLE buttons.

There is a dedicated sample button for each capillary, identified as LEFT, CENTER and RIGHT. Up to four temperatures can be recorded for each sample, usually associated with critical points such as onset of the melt, meniscus point, clear point, change in coloration, decomposition or sublimation. Each press of a sample button is acknowledged by a distinctive beep and confirmed by displaying a label ('l', 'c' or 'r' followed by '1', '2', '3' or '4' for left, center, right points 1, 2, 3 and 4). Press only one SAMPLE button at a time.

Reading the Results

Sample Buttons (LEFT, CENTER and RIGHT)

Upon completion of the temperature ramp, the DATA LED will flash if there is data available to be read, either automatic determinations or visually recorded data. This data needs to be read *before* starting the next measurement. Once the next measurement ramp is started, the stored data is erased.

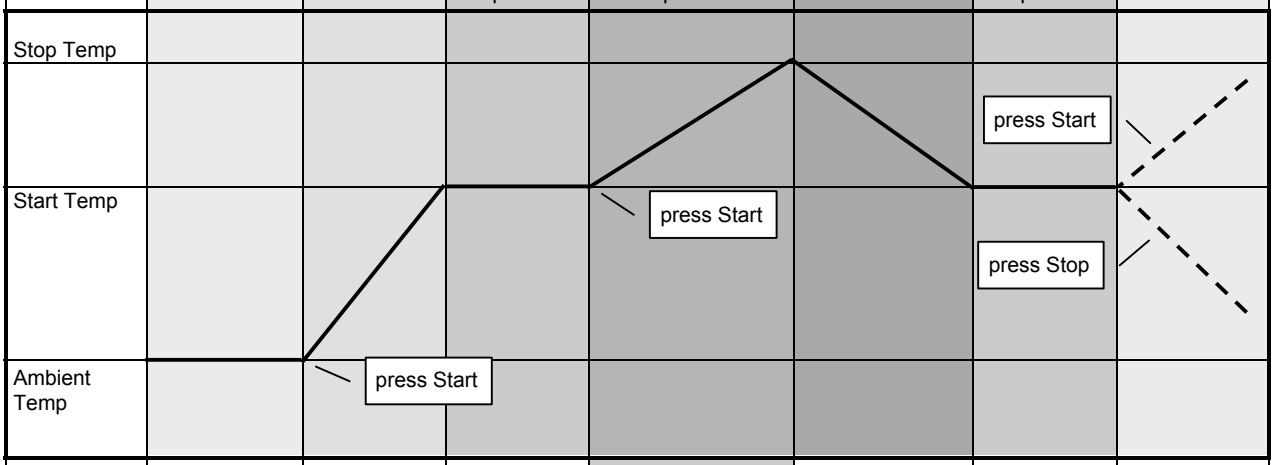
Press one of the blue SAMPLE buttons to see the results for that sample. The LED in the SAMPLE button lights indicating that this sample's data is being displayed. Each press of the button shows the next recorded temperature in the following sequence - Auto1 (onset point), Auto2 (clear point), Manual1, Manual2, Manual3, Manual4. The AUTO LED is on when one of the automatic determinations is displayed. A '—' in the display indicates that there is no data for that entry in the sequence. No automated results are displayed for sample slots that do not contain capillaries or for samples that do not melt during the analysis. Repeat this procedure for each sample. Press SET, START or STOP to return to normal operation displaying the current oven temperature.

Once any of the results are read, the DATA LED will turn off. The results remain in memory until another measurement is started.

Note

The melting point range of the samples is defined as the temperature interval bracketed by the Onset and Clear points listed in the automated results.

Typical Operation of EZ-Melt



MELTING LED	off	off	off	on	off	off	
HEATING LED	off	on	off	off	off	off	
COOLING LED	on/off with fan	off	off	off	on	off	
OPERATE LED	off	on	on	on	on	on	
INSERT SAMPLES LED	off	off	on	off	off	on	
DATA LED					flashes if new melting point data is available [3]		
Parameter change allowed?	yes	yes	yes [2]	no	yes	yes	
Press Start to...	preheat oven to start temp [1]		initiate ramp to stop temp			initiate ramp to stop temp	
Press Stop to...		turn oven off	turn oven off	stop ramp and cool oven to start temp again	turn oven off	turn oven off	
Press Sample keys to...				store temperatures based on visual observations	read melting point data if DATA LED is flashing [3]		
Fan	off below 40C				on if significant cooling needed		

[1] If start temp is below the current temp, start key will enter a cooling phase followed by a hold state at the start temp.

[2] Will enter preheat state if start temp is changed while in hold state.

[3] Read data (auto and manual) by pressing Left, Center and Right Sample keys repeatedly. Start key will erase data.

Oven turns off after 30 minutes of holding if Start key is not pressed.

Figure 6. Typical Operation of EZ-Melt.

Chapter 3

Melting Point Determination

This chapter includes basic guidelines and recommendations designed to maximize the accuracy of melting point determinations with EZ-Melt.

Introduction

A few basic guidelines must be carefully followed to avoid errors during melting point determinations with EZ-Melt. The way in which the sample is prepared and the instrument is configured both have a great influence on the accuracy and reproducibility of a melting point measurement. Subjective interpretation of the changes observed in the sample 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 throughout the phase transition.

A pure crystalline substance melts at a precise characteristic temperature dependent only on pressure (though the pressure dependency is generally considered insignificant).

Determining the melting point 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 clearly enlarge its melting range. Melting point determinations are more than just a classroom exercise in the organic chemistry laboratory, 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 melting point determination is a fast and cost-effective technique and 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-programmed fixed rate, until the sample in the tube transitions into the liquid state. While determining a melting point, several observations and the temperatures in each case, are recorded.

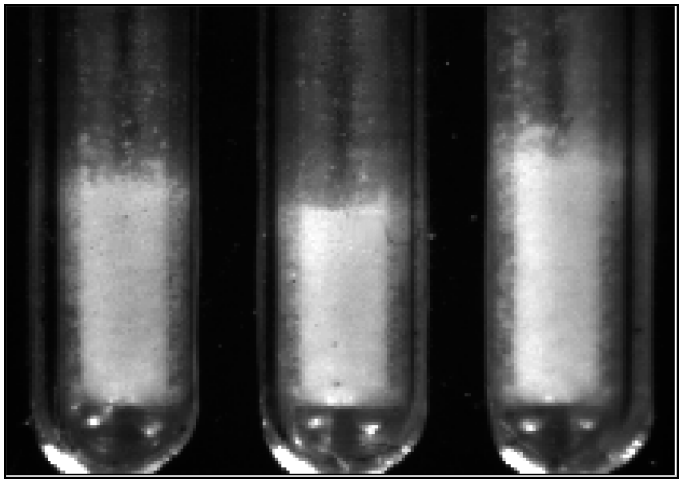


Figure 7. Capillary tubes with solid sample.

Tips

- The metal heating stand of the EZ-Melt can accommodate three capillary tubes and 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 EZ-Melt 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, EZ-Melt allows 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 temperature scale against certified reference standards.

Sample Preparation

Careless preparation of the sample is a 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 and (3) in powdered form.

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

The primary requirement for a good melting point determination is that the sample be in a fine powder form. This assures efficient and reproducible heat transfer into the sample and enhances the overall appearance of the sample for easier 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.

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 tapping 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 ~1m in length. A sample packing wire can be used at the end to further compact the sample.

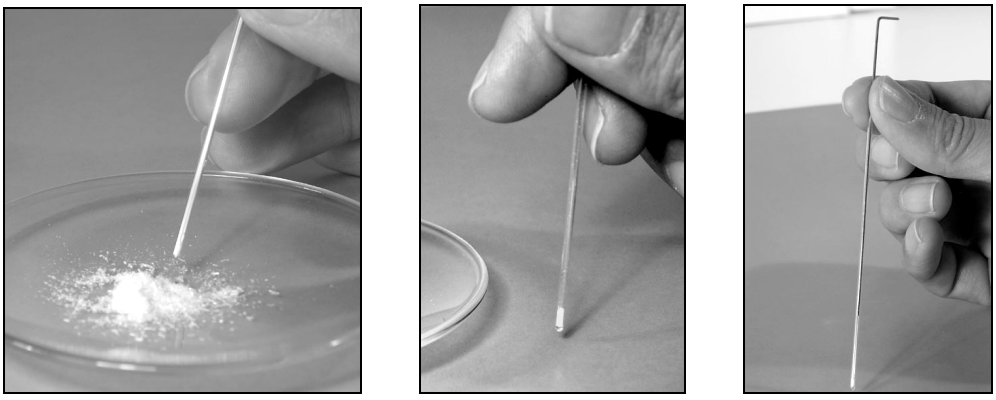


Figure 8. Loading a solid sample into a capillary tube, packing the tube by tapping, and using a packing wire.

In addition to tight packing, maintaining a fixed level in the fill is also a very important requirement. Taller samples require 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 in the EZ-Melt for optimum results and reproducibility.

Tip

It is considered good practice to **wipe the outside surface of the capillary tubes** with a clean cloth before inserting them into the heating stand. Dust from dirty tubes can slowly accumulate on the glass window of the heating block reducing overall visibility of the melt.

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 dessicator between tests, this is particularly critical in humid environments or during rainy days.

The sample tubes are loaded into the EZ-Melt 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. Loading three capillaries with the same substance and melting them at the same time and averaging their melting points provides the fastest and simplest way to improve the repeatability and accuracy of melting point determinations.

Figure 9. Loading capillaries into EZ-Melt.

Tips

- Most pharmacopeias list recommended drying procedures for melting point samples and certified reference standards.
- Make sure the EZ-Melt is holding at a Start temperature below the expected melting point of the sample(s) before placing any capillaries into the sample slots.
- Use the same batch of capillaries for calibration and for high accuracy measurements to assure the repeatability of results. NOT ALL CAPILLARIES ARE MADE EQUAL!
- The standard EZ-Melt package includes a vial of precision melting point capillaries specifically designed to (1) fit the EZ-Melt heating stand and (2) provide the most uniform and repeatable results. Replacement capillaries can be purchased directly from SRS (SRS Part# O100MPC).
- 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 choose 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 2-3 mm must be strictly observed.

- The use of a packing wire to compact a sample can lead to excessive bubble formation and trapping during the phase transition. Do not use a packing wire if excessive bubble formation interferes with the detection of the meniscus and clear points of your sample.

Tube Cleaning

Failure to clean the glass 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 generally not an issue with pre-made, commercially available melting point capillaries.

Important

If you must make your own tubes, make sure the glass stock 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. A typical oven can hold three capillaries and the thermal mass around the three tubes is very close so that deviations as small as 0.02 to 0.1ºC (temperature dependent) are kept between the three tubes during a melt.

The main advantage of a small metal oven is the lack of an overshoot that lets you preheat the unit to a start temperature <5°C below the expected melting point of the compound. This makes the heating and cooling of the unit a lot faster and determinations that only last a few 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, just a few degrees below the expected melting point of the samples.

Step 2

Up to three sample capillaries are inserted into the oven and once the temperature is stable (thermal soak), a heating ramp is 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 back down 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

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

Note

The Start Temperature must be at least 10°C above ambient temperature to assure proper stabilization of the oven.

Ramp Rate

This is the fixed rate of temperature rise between the Start and Stop 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 points.

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 according to 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, the difference between the measured melting point 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”. See Appendix A for information about compensating for the ramp rate and providing 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 melting point measurements.

The factory default setting is 1°C/min. Ramp Rates 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 0.1-0.2°C/min whenever feasible. Samples that start to decompose at temperatures below their melting point, are typically analyzed at ramp rates

above 5°C/min to avoid contamination from byproducts. Mixed melting point determinations (described later) 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 fast melting point determination, ramping the temperature rapidly (10-20°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. Samples cannot be remelted, a fresh capillary must be used for the second determination.

Stop Temperature

This is the temperature at which the heating ramp is terminated. At the end of the ramp, the capillaries are discarded, the results are read and the heater stand is cooled back to the start temperature in preparation for a new determination.

Samples cannot be remelted!! Always start a new determination with fresh capillaries.

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 and
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.

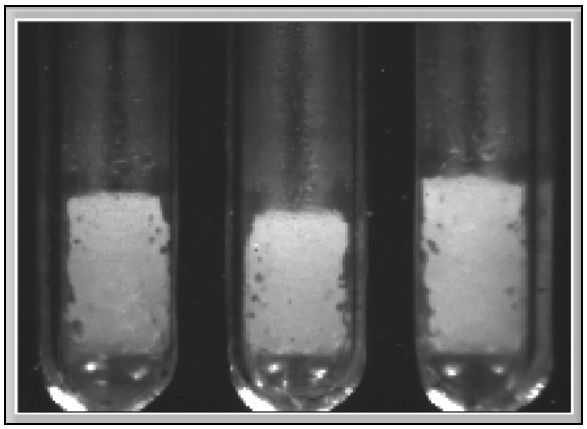


Figure 10. Onset point. The onset point is also often called collapse point of the sample.

Tips

- The onset point corresponds to the lower temperature recorded in the Melting Point Range of a substance.
- The US and International Pharmacopeias describe 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 also defined as the “collapse point”.
- Simple automated systems relying on optical absorption and bulk reflection cannot accurately detect the onset of a melt. They usually report temperatures for the start of the melt that are high compared to what is detected visually. This systematic error is because a significant change in sample appearance is required before the system detects 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.
- EZ-Melt can automatically detect and record accurate onset points. 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. This is the Onset Point and is the first or lowest automatic temperature value reported by EZ-Melt.

Meniscus Point

The meniscus point corresponds to the stage of the melt when the meniscus of the liquid becomes visible: there is a solid phase at the bottom and a clear liquid phase on top with a well defined visible meniscus. This point is readily detectable except occasionally when air bubbles from the bottom push unmelted solid to the surface.

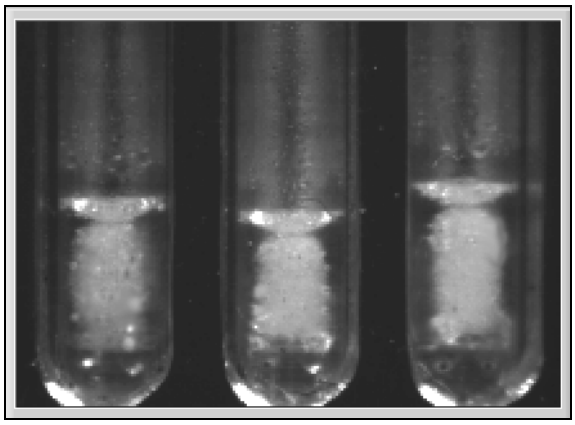


Figure 11. Meniscus Point.

Since the meniscus point represents the time during which liquid and solid coexist it is often considered a good representation of the "thermodynamic" melting point of a substance. However, this correlation is only accurate at very low ramping rates.

Tips

- The meniscus point is often the temperature listed in European Melting Point tables and the preferred value of the British Pharmacopeia methodology.
- In an attempt to remove subjectivity from its detection, the Laboratory of the Government Chemist (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 significant difference in interpretation between the British and US Pharmacopeias.
- The use of a packing wire during sample preparation can lead to excessive bubble formation and trapping for some samples. Bubbles can interfere with the automated determination. Do *not* use a packing wire if bubbles interfere with the detection of the meniscus point.

Clear or Liquefaction Point

The clear point corresponds to the stage of the melt at which the substance becomes completely liquid – the last crystals are melted and no more solid is left.

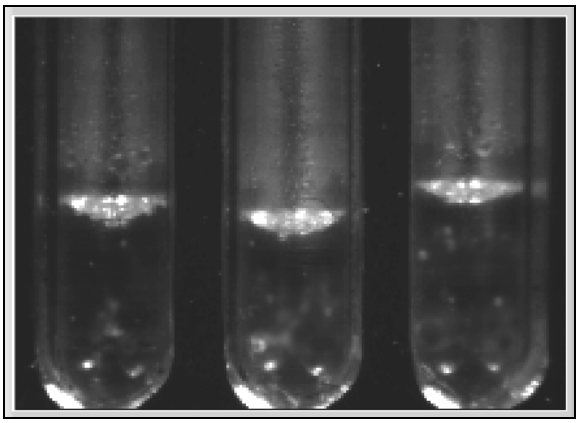


Figure 12. Clear Point.

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).

Ramp Rate (°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

Table 1. Clear Point of Phenacetin at various ramp rates (EZ-Melt Apparatus).

Tips

- The clear point corresponds to the high temperature record in the Melting Point 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 Melting Point tables and the only one accepted by the US and International Pharmacopeias 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 EZ-Melt can automatically detect and record the clear point of a sample. This is the second or higher automatic temperature value reported.

Last Signs of Change

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

Sublimation

Crystals appear in the protruding part of the glass tube.

Decomposition

Sample bubbles or changes in color or appearance 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 melting point 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 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 is much more reliable than a single temperature report.

If a single temperature must be used, specify whether it represents the clear or meniscus point.

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

Ramp Rate [°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

Table 2. Melt Point Range of Phenacetin at various ramp rates (EZ-Melt Apparatus).

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

The sample height inside the capillary also affects the melting point range. Since the temperature displayed by the melting point apparatus does not correspond to

the exact temperature in the melting substance but to that of the oven, higher clear point values are obtained for taller samples. The sample height recommended for the EZ-Melt units is 2-3 mm. Deviations from the recommended value can lead to errors in the clear point determination beyond the accuracy specifications of the instrument.

The capillary geometry (diameter and wall thickness) affects the melting point range. Thinner capillaries load smaller amounts of sample, but also provide decreased thermal coupling with the block.

Tips

- The dependence of the clear point on the sample height and capillary geometry is why it is so important to calibrate your EZ-Melt using capillaries and loading techniques identical to those applied during routine determinations.
- The SRS EZ-Melt can automatically detect and record the onset and clear points of a sample.
- A large majority of pure organic compounds melt neatly within a range of 1.25±5°C or melt with decomposition over a narrow range of temperature (~2°C) at heating rates about 1°C/min. Many organic compounds, melt with decomposition over a considerable range of temp: amino acids, salts of acids, salts of amines, carbohydrates, etc.
- Impure substances (i.e. mixtures) melt over a larger temperature range.

Melting Point Report

A complete melting 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 listed here:

- Report all instrument settings, especially heating rate, so they can be duplicated or reasonable adjustments 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 it represents the meniscus or clear point.
- Use well known melting point standards (i.e. certified reference standards) for calibration. The quality of your measurements are only as good as the quality of the standards used for calibration.

Reference Tables

There is often some uncertainty as to what really is tabulated in melting point 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 melting point 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, and is favored by some scientists. However, there is no real thermodynamic justification for that assumption. Luckily, the difference between the two temperatures (clear vs. meniscus point) is, in most cases, very small and within the accuracy requirements of most determinations.

Melting Point Depression

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 a foreign substance will result in a 0.5°C depression.

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

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 synthesized. The raw product is generally subjected to a few purification steps (i.e. recrystallization or resublimation) 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 as pure as it is going to get through the purification method being applied.

Tips

- It is common practice to recrystallize synthetic products of reactions until no more changes are detected in their melting point range.
- Careful reproduction of the sample preparation procedure is essential during Purity tracking determinations. Particular attention must be dedicated to grinding and drying all samples reproducibly.

Mixed Melting Point

If two compounds 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 melting point tables that this is the 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 different 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 the main reason why most high quality melting point measurement systems can accommodate a minimum of three capillaries in their heating blocks.

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

Tips

- The requirements for precision and reproducibility are not as high here as when doing a high precision single melting point determination. Heating rates as large as 10°C/min are acceptable.
- 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 melting point apparatus. If the three melt at the same temp it is very likely the two compounds are one and the same.

Chapter 4

Maintenance and Calibration

This chapter includes basic guidelines and recommendations for keeping your EZ-Melt calibrated and in excellent working condition. Detailed instructions are listed for:

(1) temperature offset calibration

(2) camera alignment

(3) replacing the illumination LEDs

(4) removal of broken capillaries

(5) cleaning the unit's exterior

(6) interpreting error codes

Temperature Offset Calibration

Chemical quality control (QC) laboratories must test their analytical instrumentation on a regular basis against Certified Reference Standards (CRSs) to determine the "acceptability" of their equipment according to specific QC requirements set forth by local, national and international standards and pharmacopeia laboratories.

EZ-Melt uses a *single* temperature offset to adjust its thermometer readings over its entire operating range. This offset should be determined at a temperature fairly close to the desired operating temperature. It may be necessary to use a different offset for measurements at widely different temperatures. An Acceptability Test is provided for the user to check the temperature calibration and to apply a correction if necessary.

To determine if your EZ-Melt is "Acceptable for Melting Point Determinations", follow the guidelines below and the flow chart in Figure 13.

- EZ-Melt units are calibrated at the factory prior to shipment against O100MPS CRS Kit standards (Appendix B) using 1°C/min ramping. There is usually no need to test the calibration of a brand new instrument unless: (1) specifically required by your incoming inspection protocols or (2) ramping rates much different than 1°C/min are required for your routine MP determinations.

- If the calibration of the unit is in doubt, or if no proper GLP documentation is available for the calibration data, perform an Acceptability Test on the instrument following the instructions in the next section.

- If the expiration period for the temperature calibration has expired, perform an Acceptability Test on the instrument following the instructions in the next section. A typical calibration expiration is 6 months. It is important to record when and how a calibration offset was computed to determine its applicability at the desired temperature and at the current time.

- If the instrument's heating block was modified in any way since the last calibration date, perform an Acceptability Test on the instrument following the instructions in the next section.

- If a new ramping rate, different from the one used for the last calibration, needs to be applied to upcoming routine MP determinations, perform an Acceptability Test on the instrument following the instructions in the next section and using the new ramping rate throughout the test.

Acceptability Test

The Acceptability Test will

(1) check the accuracy of your EZ-Melt's temperature readings

(2) determine if correction of the temperature offset is required

(3) adjust the temperature offset if necessary

During the Acceptability Test, the melting point of a Certified Reference Standard (CRS) is measured and a temperature offset correction is calculated. If the magnitude of the calculated temperature offset correction exceeds the published accuracy specifications of the instrument, the temperature offset must be updated. The temperature offset is adjusted from the front panel.

The step-by-step instructions listed in this section rely on the use of the SRS O100MPS CRS Kit (Appendix B) as the source of melting point standards. However, this procedure is also compatible with other Standards Kits obtained directly from local, national or international certification centers. The only compatibility requirement for a CRS is that its melting point temperature be fairly close to the desired operating temperature.

SRS O100MPS CRS Kit		
CRS	**Rated or Nominal MP (°C)**	**Operating Range (°C)**
Vanillin	83.0	40 – 110
Phenacetin	135.9	100 – 200
Caffeine	237.0	190 – 300

Table 3. SRS CRS Kit melting points and operating ranges.

In general, using one of the SRS CRSs within each operating range is suitable for a calibration. Note that no good standards exist above 300°C.

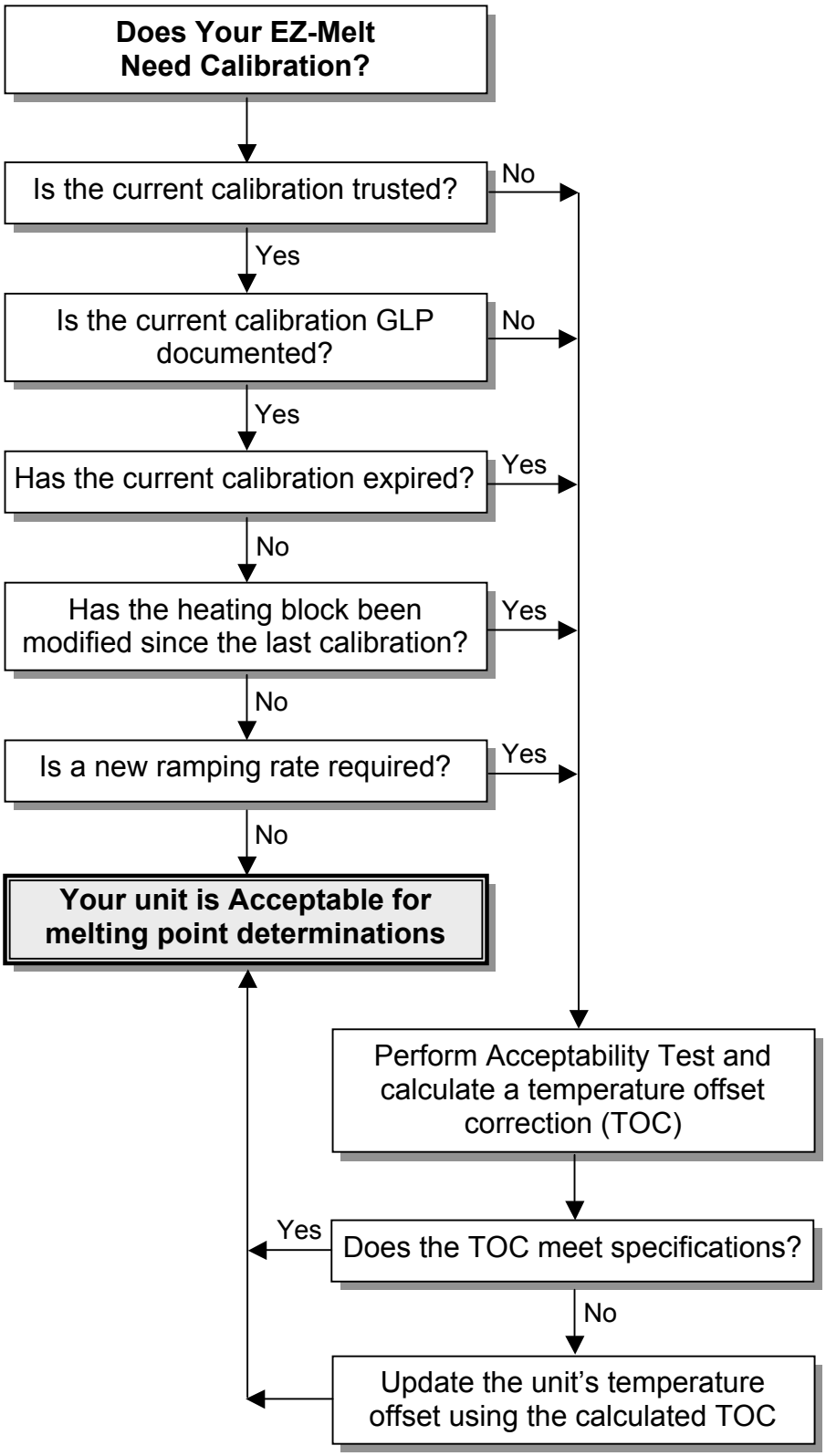


Figure 13. Temperature Offset Calibration Protocol.

Acceptability Test Procedure

Step 1 – prepare the certified reference sample

Collect the sample preparation information for the CRS which will be used for the calibration test. Look specifically for information on sample pretreatment, drying procedures, grinding, capillary tube dimensions, sample loading and rated melting points (Rated MPs).

The Rated MP of a CRS is usually the *clear point* of the substance determined by the Certifications Lab. If a temperature interval (MP Range) is indicated instead of a clear point, use its *mean* as the Rated MP.

Step 2 – setup the EZ-Melt

Set the Start temperature 5°C below the certified clear point of the CRS. Set the ramping rate to 1°C/min (or your preferred ramp rate) and the Stop temperature 5°C above the certified clear point of the CRS.

Preheat the oven to the selected temperature.

Step 3 – load the capillaries

Load *three* capillaries with a 2–3 mm column of the CRS and insert the tubes into the EZ-Melt's sample slots. Measurements for all three capillaries are carried out simultaneously.

To increase the reproducibility of results, load the capillaries using the same grinding, loading and compacting techniques used for your routine melting point determinations. Use the same column height as used for routine determinations.

Step 4 – start the melt

Start a melting point determination on the three identical samples.

Step 5 – observe and record the melt

All determinations in this procedure are made visually. Using the blue sample buttons record manual entries for the temperatures corresponding to the *onset* and *clear* points during the melt.

Step 6 – record the results

Once the melt is completed read the results from EZ-Melt and record them on paper. Compare the manual entries for clear point for the three capillaries against each other. The clear points for the three capillaries must fall within ±0.3°C of each other to be considered acceptable.

A spread in melting point values among the three samples larger than ±0.3°C could be symptomatic of a heating asymmetry in the oven block caused by unbalanced heaters. However, it is most often caused by subtle differences in sample packing, sample heights and sample placement in the slots. Repeat the measurement if a large spread is observed,

taking special care to prepare all three capillaries in the *exact same way*. Consult the factory for additional recommendations if no improvement is detected.

If this CRS has a single Rated MP, then calculate the *average of the three clear points* for this CRS. This is the Measured MP ($MP_{Measured}$) for this CRS.

If this CRS has a specified *melting point range*, instead of a single Rated MP, then calculate the mean melting point (average of onset and clear points) for each capillary. Use the *average of the three mean melting points* for this CRS as the Measured MP ($MP_{Measured}$).

Step 7 – check the melting range

Calculate the melting point ranges (MP Range = clear point - onset point) for the three capillaries. The MP Range must be less than 2°C (at 1°C/min ramping rate) to be acceptable.

If the measured MP Range exceeds 2°C (at 1°C/min ramping rate), check the ramping rate and the sample preparation procedure to eliminate any potential experimental errors and repeat the determination if necessary. If no improvement is observed consult the factory for additional information.

The melting point range for a pure substance melted at 1°C/min ramping rate is typically 1.25 ± 0.25°C. Larger ranges are observed at higher ramping rates (As a general rule, the MP Range scales with the square root of the ramping rate).

If a melting point range larger than 2°C (at 1°C/min ramping rate) is observed, and no sample contamination is suspected, a malfunction of the instrument should be suspected and the instrument should be serviced. Contact the factory or your local representative for additional information.

Step 8 – calculate the Temperature Offset Correction

Calculate the temperature offset correction (TOC) according to

$$TOC = MP_{Rated} - MP_{Measured}$$

where,

TOC is the temperature offset correction,

MP_{Rated} is the melting point temperature assigned to the CRS sample by the certification laboratory (if a melting point range is specified, use the mean of the range),

$MP_{Measured}$ is the melting point temperature from Step 6 as actually measured by the EZ-Melt unit during this Acceptability Test.

Rated MPs are listed in the Certificates of Measurement included in all CRS kits. Assigned MPs (clear points) are listed on the labels of the CRSs packaged in the O100MPS CRS Kit.

The Rated MP of a CRS is usually the clear point of the substance rated by the Certifications Lab. If a temperature interval (MP Range) is indicated instead of a clear

point, use its mean as the Rated MP and also use means to calculate the Measured MP to be consistent.

If the magnitude of the calculated TOC *exceeds* the published accuracy specifications of your EZ-Melt (below), the temperature calibration must be updated as described in Step 9. Otherwise, the unit is within specifications and should continue to be used without further adjustment.

Melting Point (°C)	Accuracy (°C)
<100	± 0.3
100 –250	± 0.5
>250	± 0.8

Table 4. EZ-Melt Temperature Accuracy.

Step 9 – adjust the Temperature Offset

Press the CENTER and RIGHT SAMPLE buttons simultaneously to display the current Temperature Offset. A small 'c' in the display indicates that the Offset Calibration is being displayed.

The Temperature Offset needs to be adjusted to a value equal to the displayed Offset *plus* the TOC (calculated above). If the measured melting point was too low, then the TOC is positive and the Offset needs to increase. If the measured melting point was too high, then the TOC is negative and the Offset needs to decrease. The Offset may be negative.

Use the RAISE↑ (START)and LOWER↓ (STOP) buttons to adjust the Temperature Offset to the new value. Press SET when finished to return to normal operation.

Record the new Offset along with the CRS used to determine it and the calibration date. Note that this Offset should only be relied on for melting point determinations at temperatures fairly close to the CRS melting point.

To return to the factory calibration, set the Offset to 0.0 or power the unit on while holding down CENTER and RIGHT, then press SET for normal operation.

The Temperature Offset can not be displayed during a melt.

Recommendations and Tips

- The accuracy of the melting point determinations performed with your EZ-Melt is only as good as the last temperature calibration and ultimately only as good as the accuracy of the standards used.
- Calibrating the temperature readings against a CRS accounts not only for inaccuracies in the thermometer but also for the unique heat conducting properties of each metal oven/capillary assembly. Alternative calibration methods relying on the use of reference thermometers or calibration baths, though adequate for checking the temperature accuracy of the thermometer by itself, are static measurements that do

not take into consideration the thermal lag between the thermometer and the capillary samples during the heating process.

- If in doubt about the EZ-Melt Temperature Offset, display the Offset on the front panel and compare it with the last recorded calibration. Adjust it if necessary.
- Remember that EZ-Melt uses a single Temperature Offset. A different Offset may be required for measurements at widely different temperatures.
- No reliable CRSs with melting points above 300°C are available.
- An important advantage of WHO traceable standards (such as the O100MPS CRS Kit) is that their assigned melting point temperatures are clear points with accuracies that closely match the accuracy specifications of EZ-Melt.
- It is not unusual to see differences between the results obtained for the same compound from liquid bath and metal ovens. This discrepancy is generally understood and accepted, and rarely exceeds the uncertainty of the measurement. A slight difference is to be expected, and is rarely beyond the accuracy of the standards.
- A list of compounds commonly used as Melting Point Standards is included in Appendix B of this manual.

Camera Alignment

The digital camera built into EZ-Melt faces the capillaries and relies on a simple optical system to image the samples. The Digital Image Processor (DIP) relies on camera alignment information, stored in the EZ-Melt's memory, to zoom in on the area of the images where the samples are located. Stable optical alignment between the heating block and the camera is essential for accurate and reproducible automated results. If this alignment changes for any reason, the position of the samples within the digital images will shift and the effectiveness of the DIP algorithms can be compromised.

Do not perform this alignment unless the automated determinations have become unreliable or the heating block is moved, fastened, cleaned or repositioned.

To update the camera alignment information, the following steps must be followed:

1. **Insert three sample tubes, with at least three millimeters of white powder into the sample slots.**
2. Press the LEFT and CENTER SAMPLE buttons simultaneously to start the Camera Optical Alignment. This can not be done while the unit is ramping.
3. If no error message is displayed, then the camera alignment was successful. Test the new alignment by performing a melting point determination and comparing the automated results with previously recorded acceptable automated results.
4. If an error message (Err1 or Err2) is displayed, try to clean or repack the sample tubes – or try 3 new tubes, and repeat the alignment.
5. If the error message Err3 is displayed, contact the factory for instructions.

To return the unit to the factory camera alignment, power the unit on while holding down LEFT and CENTER, then press SET for normal operation.

Replacement of Faulty LEDs

Internal lighting of the heating block is provided by high brightness white LEDs with expected lifetimes in excess of 50,000 hours. In the case of a fault, the LEDs must be replaced at the factory. Contact your local representative for service information.

Broken Capillaries

All broken capillaries and capillary pieces need to be removed from the heating block.

Wait for the heating block to cool down before touching it! The oven may still be hot even if the unit is turned off.

Remove the top ceramic insulator carefully (shown below) and carefully extract any capillary pieces left behind.

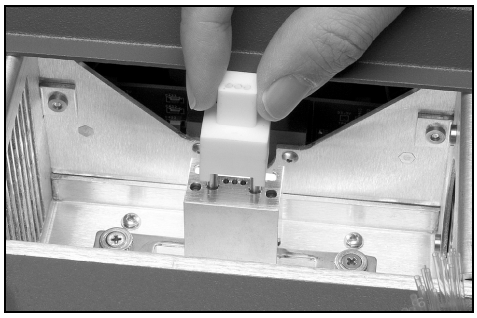


Figure 14. Removing the ceramic spacer.

Slide the front window out to expose the sample slots (shown below). Remove any broken glass pieces and spilled chemicals. Use a thin cotton swab or pipe cleaner, dipped in alcohol or similar solvent, to clean the capillary slots if necessary (careful to not drip solvent on the exterior paint). Wipe the window clean with alcohol or acetone.

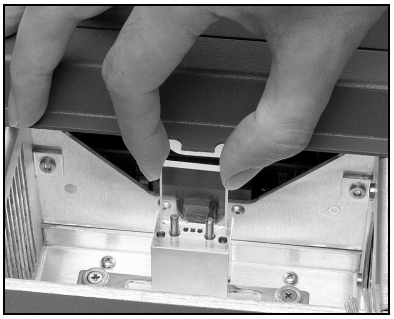


Figure 15. Removing the glass window.

If more debris remains, remove the block from its compartment to clean the internal slots. Loosen the two thumbscrews securing its base to the bottom of the box and pull on the block assembly to bring it out of the box. Air dry the block before inserting it back into the box. Tighten the thumbscrews to assure proper optical alignment of the samples

relative to the camera. Perform a camera alignment procedure (see earlier in this chapter) after each block cleaning procedure. Unless the block is completely replaced, there is no need for an Acceptability Test at this time.

Dirty Window

A dirty glass window can interfere with both automated and manual melting point determinations. The most common cause of a dirty window is failure to wipe the outside of the capillaries before inserting them.

Wait for the heating block to cool down before touching it! The oven may still be hot even if the unit is turned off.

To clean the window, remove the glass window from the heating block (shown above) and wipe it clean with alcohol or acetone. Replace the window with a new one if it will not come completely clean.

Exterior Cleaning

The housing of your EZ-Melt unit is coated with paint and should only be cleaned with a rag moistened in soapy solution. **Do not use halogenated solvents, acetone or any similar solvents to clean the magnifying lens!**

Error Codes

When EZ-Melt is turned on the firmware revision code is displayed briefly before the current oven temperature. If an error is detected at start up, an error code will be displayed instead of the firmware revision. The error codes are explained below.

Code	Meaning	User needs to...
Err6	User Camera Alignment lost, reverting to factory alignment	Perform Camera Alignment procedure
Err7	User Temperature Offset lost, reverting to factory calibration	Re-enter Temperature Offset or perform Acceptability Test
Err9	Factory calibrations lost	Contact factory for instructions

Table 5. EZ-Melt error codes.

Appendix A

Pharmacopeia vs. Thermodynamic Melting Point Determinations

Pharmacopeia Melting Point

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 technique** as the standard method for melting point determination. In this method, thin glass capillary tubes containing packed samples of the substance are introduced into a heating stand that is continuously being heated up.

The capillary technique is the standard method used for melting point determinations in most organic and pharmaceutical chemistry laboratories.

The procedures for melting point determinations in the pharmacopeias call for a fixed rate of temperature rise in the "heating stand" (liquid bath and/or metal block), typically between 0.2 and 2°C/min, within a temperature range that brackets the expected melting temperature of the compound. The oven continues to heat at a constant rate until the sample is completely melted – i.e. clear point. In determining the melting point – the temperature on the thermometer at the clear point (and/or sometimes at the meniscus point) is recorded. *This is not the temperature of the sample itself, but rather that of the heating stand where the thermometer is located – i.e. the temperature is determined by the heating medium.*

When different heating rates are used for determining the melting point of a sample, the values obtained for the clear point are dependent on the temperature ramping rate. Because the heat transfer from the heating stand to the sample cannot be increased proportionally to the temperature ramping rate, the temperature in the heating stand rises to a higher level with faster rates of heat increase than it does with lower rates. As a result, the faster the temperature is ramped, the higher the clear points are found to be. Due to this dependence on heating rate, *measurements taken for melting points are comparable with one another only if they were taken using the same ramping rates*. Any variance from the temperature ramping rates specified by pharmacopeia procedures must be properly recorded for Good Laboratory Practice documentation.

Table 6 is an example of the dependence of the clear point determination on ramping rate for phenacetin samples (MP: 135°C) analyzed with an EZ-Melt unit. As expected, the clear point increases with increasing ramping rates. The need to specify ramping rates along with the results of the melt is demonstrated by this data.

Ramp Rate (°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

Table 6. Clear Point of Phenacetin at various ramp rates.

The heating stands used by commercial melting point apparatuses are divided into two categories:

Liquid Bath
The capillaries are immersed in a liquid bath (typically silicone oil) that is continuously being heated up.

Metal Block
A small, dry thermal block (metal oven) has proven to be a good alternative to a liquid bath.

The melting point procedures described in early pharmacopeias were designed for “liquid bath” heating stands; however, in recent years most monographs have been updated and supplemented to include the more prevalent metal block setups.

Tip
A slight drift in melting point results is expected when the temperatures obtained with a liquid bath oven are compared to those obtained with a metal block stand. This difference is to be expected and generally ignored since it most generally falls within the intrinsic uncertainty of the measurement.

Thermodynamic Melting Point

The transition from solid to liquid does not take place instantaneously – it requires a finite amount of time. The melting process begins at the point where the first particles of the bulk substance turn into the liquid state – the **onset point**. The end of the melt is reached when the last solid particles have gone over into the liquid phase – the **clear point**. During the entire melting process of a pure compound: (1) the temperature of the pure substance remains constant (thermodynamic melting point) while (2) heat is constantly transferred from the heating stand to the sample and (3) the heating stand itself experiences a range of temperatures that depends on the selected heating rate.

When determining the melting point according to the US or International Pharmacopeias, the temperature of the heating stand at the end of the melt (clear point) is read. That single temperature record depends on the temperature ramping rate, it ignores the range between the start and the end of the melt and it is not the "true" thermodynamic melting point of the pure compound.

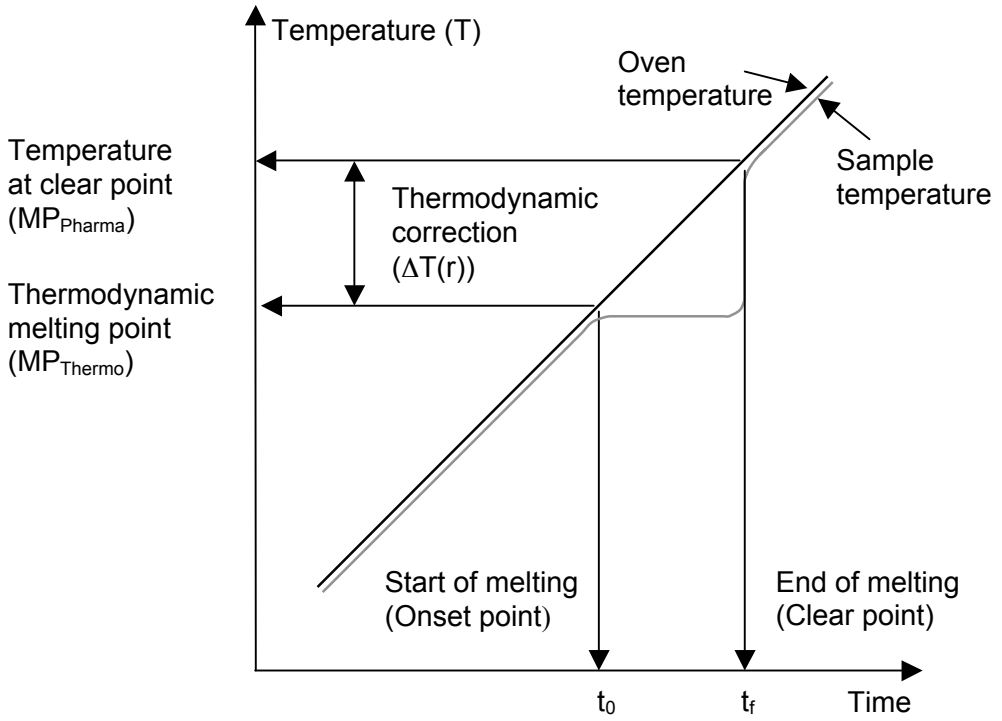


Figure 16. Graphical representation of the thermodynamic correction

Figure 16 is a simple representation of the sequence of events that take place during the melting of a pure substance. At the start of the melting (time = t_0), the block and the sample are at approximately the same temperature. As soon as the melt starts the sample temperature stabilizes while the block continues heating up. As the melt progresses, the sample remains at constant temperature (thermodynamic melting point, MP_{thermo}) while the block continues to heat up. Heat is constantly transferred from the block to the sample at a rate that is proportional to the temperature difference between the sample and the block. The temperature of the block at the end of the melt (time= t_f) is recorded as the

clear point (pharmacopeia melting point, MP_{pharma}). The thermodynamic correction is defined as:

$$\Delta T(r) = MP_{pharma} - MP_{thermo} \qquad \text{(eqn. 1)}$$

and must be expresed as a function of the ramping rate, r.

In order to obtain the thermodynamic melting temperature of a pure substance, it is necessary to calculate and subtract a thermodynamic correction from the detected clear point. This calculates back to the temperature at the beginning of the melt, so that the value obtained has virtually no dependence on the temperature ramping rate. A parametric derivation of that functional dependence is presented in the following section.

Thermodynamic Correction

At any given time, t, during a melt, the amount of heat, dQ(t), transferred from the heating stand to the sample during a time dt is:

$$dQ(t) = \alpha \cdot (T - MP_{thermo}) \cdot dt \qquad \text{(eqn. 2)}$$

where,

T is the temperature of the heating stand, [°C]

t is the time variable, [min]

α is the heat transfer constant for the melting point apparatus, [calories/(°C · min)]

r = dT/dt is the temperature ramping rate of the instrument, [°C/min].

Substituting dt with dT/r in eqn. 2, leads to:

$$dQ(t) = \frac{\alpha \cdot (T - MP_{thermo})}{r} \cdot dT \qquad \text{(eqn. 3)}$$

Integration of the heat transferred from the block to the sample, over the entire melting process, provides the "heat of fusion" of the sample, ΔH_f [Calories], which is dependent on its mass but independent of the ramping rate, r:

$$\Delta H_f = \int_{MP_{thermo}}^{MP_{pharma}} \left[\frac{\alpha \cdot (T - MP_{thermo})}{r} \right] \cdot dT \qquad \text{(eqn. 4)}$$

Calculation of the integral term leads to the analytical expression:

$$\Delta H_f = \left[\frac{\alpha}{2r} \right] \cdot (MP_{pharma} - MP_{thermo})^2 \qquad \text{(eqn. 5)}$$

which can be rearranged to provide an equation for the thermodynamic correction:

$$\Delta T(r) = MP_{pharma} - MP_{thermo} = \left[\frac{2\Delta H_f}{\alpha} \right]^{1/2} r^{1/2} = (ThermoCF) \cdot \sqrt{r} \qquad \text{(eqn. 6)}$$

According to eqn. 6 *the thermodynamic correction is directly proportional to the square root of the ramping rate*, and in order to calculate the thermodynamic melting temperature, it is necessary to know the value of the Thermodynamic Correction Factor, ThermoCF, for the melting point apparatus:

$$MP_{thermo} = MP_{pharma} - (ThermoCF)\sqrt{r} = MP_{clearpoint} - (ThermoCF)\sqrt{r} \quad \text{(eqn. 7)}$$

The above theory indicates that the value of the thermodynamic correction factor, ThermoCF, depends, among other things, on the (1) heat of fusion of the sample, (2) the thermal conductivity of the sample, (3) the thermal conductivity of the glass capillary, (4) the sample preparation/packing method, (5) sample size and (6) the geometry and construction of the oven. In practice, the value of ThermoCF is both compound and instrument dependent and must be calculated through empirical determination.

Since the same factors that affect the ThermoCF also affect the clear point, this derivation also confirms that calibration of the temperature offsets requires carefully reproducing the sample preparation procedures used during routine analysis.

Thermodynamic Correction with EZ-Melt

Experimental measurements have shown that in most cases a good approximation to the Thermodynamic Correction Factor, ThermoCF, for the SRS EZ-Melt is a value of ~1.0, when r is expressed in standard units of [°C/min]. However, empirical calculation of the Thermodynamic Correction Factor is recommended when more accurate results are required for a specific compound.

Calculation of Thermodynamic Correction Factor

The Thermodynamic Correction Factor, ThermoCF, is compound specific and depends on several factors:

- Specific heat of fusion of the sample
- Amount of sample
- thermal conductivity of the sample
- thermal conductivity of the glass capillary
- sample preparation method
- geometry of the oven

A step-by-step calculation procedure is described below:

Step 1

Perform complete pharmacopeia melting point determinations on your sample at six different temperature ramping rates: 0.1, 0.2, 0.5, 1, 2 and 5°C/min.

- Three samples are analyzed simultaneously for each ramping rate: Load three capillaries with a 2–3 mm column of sample, and insert the tubes into the three adjacent sample slots of the EZ-Melt oven.

- To increase the accuracy of results, load the capillaries using the **exact** same grinding, loading and packing techniques used for routine melting point determinations. Match all sample heights very carefully.
- For each ramping rate, average the detected "clear points" (according to pharmacopeia) for the three samples to obtain the recorded value.
- To increase the accuracy of results, use manual (automatic) clear point readings if manual (automatic) determinations are routinely performed in the lab.

Step 2

Plot the resulting clear points versus the square root of their corresponding temperature ramping rate (MP_{pharma} vs $r^{1/2}$) – a linear dependence should be observed. The slope of the straight line is the Thermodynamic Correction Factor, ThermoCF, of your EZ-Melt.

Step 3

Use the Thermodynamic Correction Factor to calculate the thermodynamic melting point of the sample from the measured clear points (eqn. 7). An agreement within the accuracy of measurement should be observed for all calculated thermodynamic melting points.

An example of this calculation procedure is included below.

ThermoCF Calculation Example

In order to demonstrate the simplicity of the calculation procedure described in the previous section, a series of melts were performed on a phenacetin sample with ramping rates 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0°C/min.

Figure 17 summarizes the results and shows the expected linear relationship between the clear point determinations and the square root of the ramping rate. The slope of the straight line corresponds to a thermodynamic correction factor, ThermoCF= 1.9 for phenacetin samples.

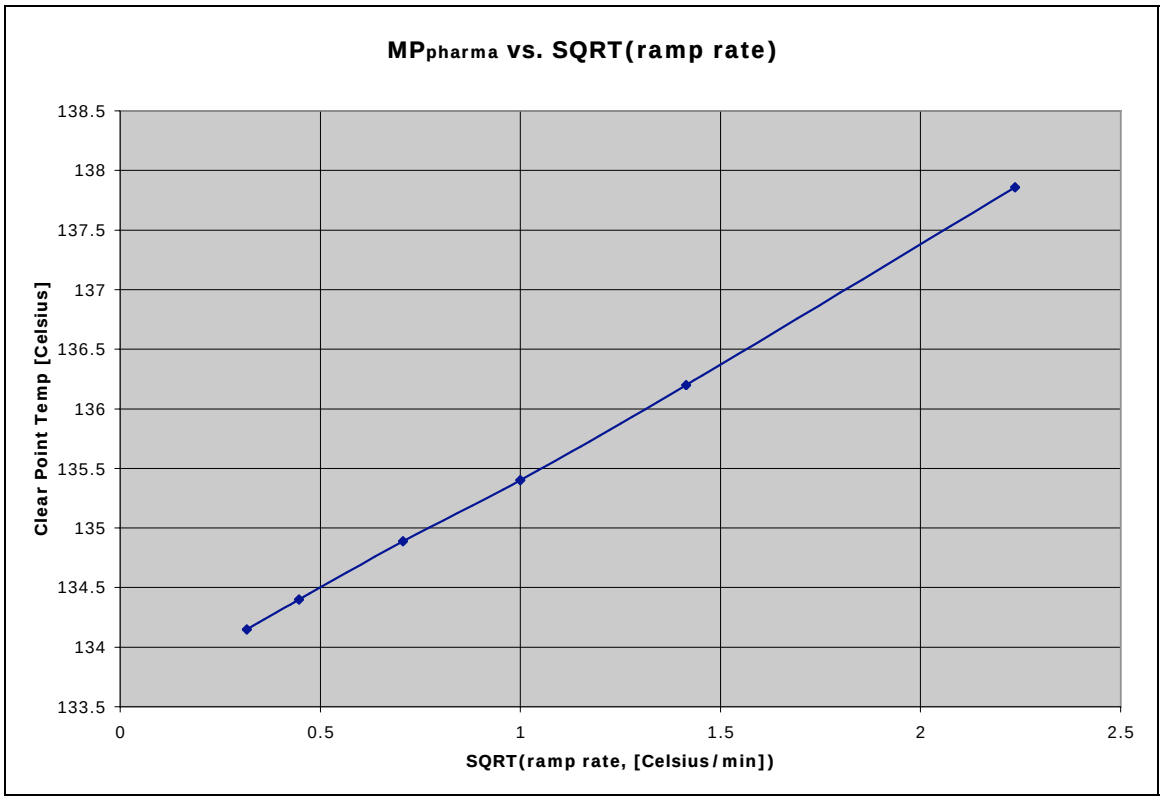


Figure 17. "Clear point temperature vs. square root of ramping rate" for a phenacetin sample melted at 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 ˚C/min. The slope of the straight line, ThermoCF=1.9, is programmed into the EZ-Melt as the thermodynamic correction factor, ThermoCF, for this compound.

Table 6 demonstrates the use of the thermodynamic correction factor to calculate the thermodynamic melting point of a phenacetin sample. In contrast to the clear point results, and within the accuracy of the measurement (±0.3°C), the thermodynamic melting point results are independent of the ramp rate as expected.

Ramp Rate, r (°C/min)	Clear Point (°C) (MP_{pharma})	$MP_{therm} = MP_{pharma} - ThermoCF \cdot r^{1/2}$ (°C)
0.1	134.2	133.6
0.2	134.4	133.4
0.5	134.9	133.6
1	135.4	133.5
2	136.2	133.5
5	137.9	133.6

Table 7. Clear and Thermodynamic Melting Point of Phenacetin at various ramp rates (ThermoCF = 1.9).

Appendix B

Melting Point Certified Reference Standards

What is a Certified Reference Standard?

Certified Reference Standards (CRSs) are high-purity chemicals certified by National, Local or International Standards Laboratories and Pharmacopeia Conventions, produced and tested according to well established and easy-to-reproduce procedures.

Use CRSs for calibration and determination of acceptability of melting point instruments including EZ-Melt.

General Guidelines and Recommendations

- Use CRSs obtained from reputable Standards and Pharmacopeia Laboratories.
- Use CRSs with Certificates of Measurement including records of:
  (1) Lot/Batch Identification number (for traceability)
  (2) Purity specification (analysis method, purity levels, etc.)
  (3) Detailed description of the instrumental setup used for the melting point determination: type of instrument, manual or automatic detection, hot bath or metal block oven, capillary tube dimensions, amount/height of sample packed, compaction method, etc.
  (4) Preconditioning of the sample (i.e. drying, grinding, etc).
  (5) Detailed heating conditions, including initial temperature, ramping rate and any special comments required to properly reproduce the melting conditions used by the standards laboratory to certify the compound. Alternatively, a reference to a standardized melting point determination method must be included.
  (6) Melting points and melting point ranges with well characterized uncertainty errors.
- Whenever possible, choose the CRSs with the smallest uncertainty errors. Remember that the accuracy of your EZ-Melt is only as good as the accuracy of the standards used for its temperature scale calibration. Note: As a general rule, avoid CRSs leading to uncertainty errors larger than 0.5°C.
- Do not assume that a CRS compatible with one specific pharmacopeia procedure is compatible with the requirements of a different pharmacopeia protocol. For example,

it is best to obtain CRSs directly from the US Pharmacopeia Convention in order to determine the usability of the EZ-Melt according to US Pharmacopeia protocols.

- Beware of Standards Laboratories offering CRSs compatible with multiple pharmacopeias. Ask for samples of Certificates of Measurement before ordering standards to assure the compatibility of their records with the pharmacopeia protocol you plan to follow. Contact the Laboratory director whenever in doubt. A reputable accreditation center will always be very helpful and forthcoming.
- Occasionally, accreditation centers will run out of stock of some CRSs. In that rare event, the standards lab should be able to recommend alternative sources for CRSs while they restock their supplies. It is always a good idea to have an emergency second source of standards identified in case a backup supplier is required.
- Some CRSs have expiration dates. Following strict GLP and GMP guidelines, QA labs must keep track of those dates, and never use expired CRSs for calibration or determination of acceptability of their melting point equipment.
- A slight drift in melting point results is expected when the melting point temperatures obtained with a liquid bath oven are compared to those obtained with a metal block oven under the same ramping conditions. This difference is to be expected and generally ignored since it generally falls within the intrinsic uncertainty of the measurement.
- Use capillary tubes of the same kind, and from the same supplier, to perform calibrations and to carry out all subsequent measurements. Beware that some pharmacopeia protocols include specific capillary requirements.
- Failure to clean the tubing before making capillary melting tubes is one of the most common causes of low melting points and broad melting point ranges. Whenever possible buy pre-made tubes from reputable sources, to assure the best reproducibility of results.
- Dry CRS samples carefully and store them in a vacuum dessicator between melting point determinations. Forty eight hours over P_2O_5 is a very common recommendation for drying standards. Follow all standard-specific recommendations.

Accreditation Centers

The following table is a list of accreditation centers which stock melting point CRSs. Notice that the scope of the centers ranges from local to worldwide.

Keep in mind that certification protocols are constantly being revised and modified. Use this table as reference only and access the Internet to update the records on this table if necessary.

Name	Address/Phone	E-Mail / Web
WHO Collaborating Center for Chemical Reference Substances (International)	Apoteket AB Produktion &Laboratorier Centrallaboratoriet, ACL Prismavägen 2, S-141 75 Kungens Kurva Sweden Telephone:(+ 46-8)466-1000 Fax: (+46-8) 740-6040	E-mail: who.apl@apoteket.se
U.S. Pharmacopeial Convention, Inc. (United States)	Reference Standards Order Department. 12601 Twinbrook Parkway Rockville, MD 20852 USA Telephone: US and Canada: (800) 227-8772 International : (+1-301) 881-0666 Fax: (+1-301) 816-8148	Web: www.usp.org
LGC PromoChem (US, UK, Europe and WHO standards)	LGC Promochem Queens Rd TEDDINGTON Middlesex TW11 0LY United Kingdom Telephone: +44 (0)20 8943 7000 Fax : +44 (0)20 8943 2767	Web: www.lgcpromochem.com E-mail: uk@lgcpromochem.com
National Physical Laboratory (United Kingdom)	Teddington TW11 0LW UK	Web: www.npl.co.uk.
European Directorate for the Quality of Medicines (Europe)	European Pharmacopeia Council of Europe B.P. 907, F-67029 Strasbourg Cedex 1 France. Telephone: (+33-(0)3) 88 41 20 35 Fax: (+33-(0)3) 88 41 27 71	E-mail: CRS@pheur.org Web: www.pheur.org
French Pharmacopeia Reference Substances (France)	Agence Française de Sécurité Sanitaire des Produits de Santé Direction des Laboratoires et des Contrôles Site de Montpellier-Vendargues 635 rue de la Garenne F-34740 Vendargues France Telephone: (+33-(0)4) 67 91 39 00 Fax: (+33-(0)4) 57 87 39 83	

WHO Melting Point Reference Substances

The World Health Organization (WHO) set of Melting Point Reference Substances contains thirteen compounds.

The WHO Melting Point Reference Substances are supplied primarily for calibration of different instruments and methods for determination of melting point temperatures against the method of the International Pharmacopeia (Int. Pharm.), 3rd Ed., Volume 1.

The nominal melting temperatures for the WHO Melting Point Reference Substances have been laid down on the basis of the results obtained in a collaborative study according to the capillary method of the Int. Pharm. 2nd Ed (H. Bervenmark, et. al., "WHO Melting Point Reference Substances", Bull. Wld Hlth Org. 28 (1963) 175-188).

The WHO set of Melting-Point Reference Substances contains the following 13 compounds:

WHO Melting-Point Reference Substances		
Substance	**Nominal Melting Range (°C)**	
Azobenzene	67.8	68.8
Vanillin (*)	81.7	83.0
Benzil	94.8	96.0
Acetanilide	114.4	115.7
Phenacetin (*)	134.7	135.9
Benzanilide	163.5	164.7
Sulfanilamide	164.7	165.9
Sulfapyridine	191.7	192.7
Dicyandiamide	209	210.2
Saccharin	227.2	229.3
Caffeine (*)	235.8	237.0
Phenolphthalein	261.5	263.0
(*) Compounds included in the O100MPS CRS Kit.		

Table 8. WHO reference substances.

The melting range is defined as the temperature range between the onset point (collapse point) and the clear point (liquefaction point). Heating Rate is 1°C/minute.

WHO Melting Point Reference Substances are packaged with detailed certificates including Control Number, Intended Use, Analytical Data, Storage, Directions For Use and Assigned Melting Point.

O100MPS CRS Kit

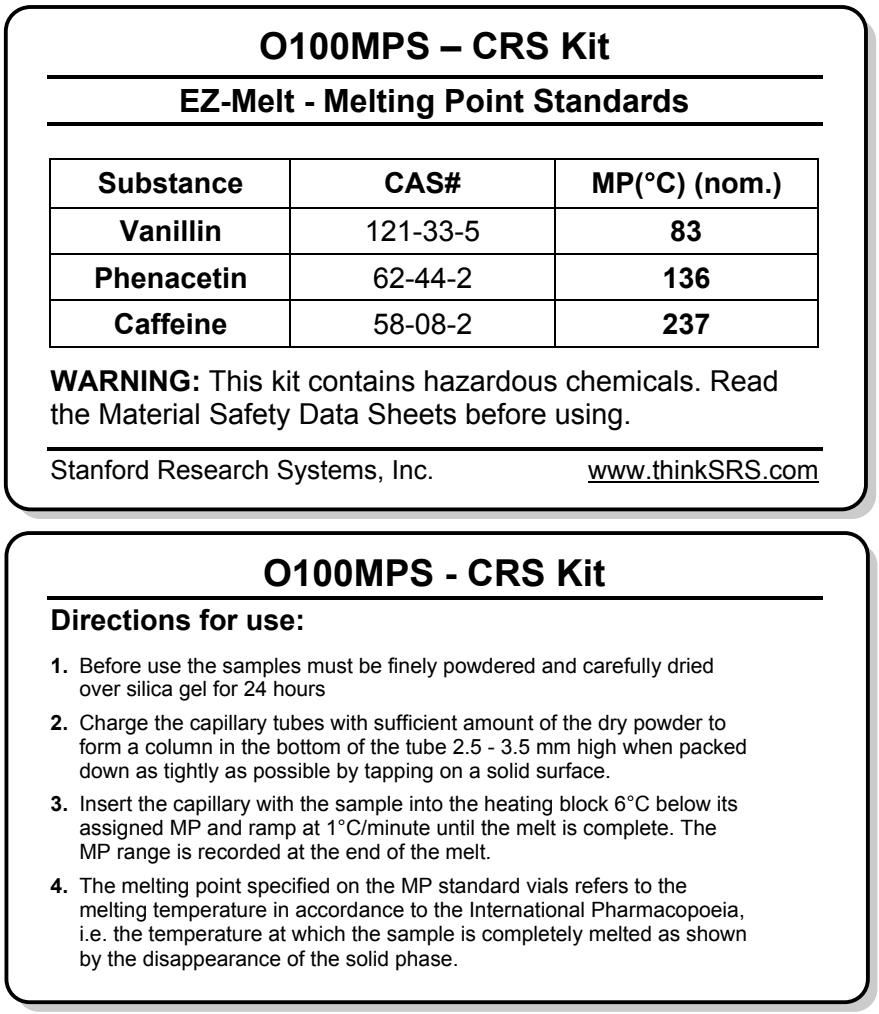
Stanford Research Systems supplies a calibration kit, SRS Part#O100MPS, consisting of three standard compounds with rated melting points traceable to WHO CRSs. The kit is specifically designed to check the temperature offset calibration of your EZ-Melt unit according to WHO certification requirements and to correct the temperature offset if necessary. For detailed calibration instructions see Chapter 4.

The three reference substances included in the O100MPS kit were carefully selected to match the calibration requirements of EZ-Melt.

SRS O100MPS-CRS Kit			
Substance	**Start / Stop (°C)**	**Clear Point (°C)**	**Operating Range (°C)**
Vanillin	78 / 88	83.0	40 - 110
Phenacetin	131 / 141	135.9	100 - 200
Caffeine	232 / 242	237.0	190 - 300
Ramping rate is 1°C/min for all compounds.			

Table 9. SRS O100MPS reference substances.

Each CRS Kit is packaged in a plastic box with two separate labels that (1) identify the chemicals and (2) list the recommended sample preparation procedure (See Figure 18). The rated melting points for the standards are listed on the labels attached to the bottles.



O100MPS – CRS Kit

EZ-Melt - Melting Point Standards

Substance	CAS#	MP(°C) (nom.)
Vanillin	121-33-5	**83**
Phenacetin	62-44-2	**136**
Caffeine	58-08-2	**237**

WARNING: This kit contains hazardous chemicals. Read the Material Safety Data Sheets before using.

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O100MPS - CRS Kit

Directions for use:

1. Before use the samples must be finely powdered and carefully dried over silica gel for 24 hours
2. Charge the capillary tubes with sufficient amount of the dry powder to form a column in the bottom of the tube 2.5 - 3.5 mm high when packed down as tightly as possible by tapping on a solid surface.
3. Insert the capillary with the sample into the heating block 6°C below its assigned MP and ramp at 1°C/minute until the melt is complete. The MP range is recorded at the end of the melt.
4. The melting point specified on the MP standard vials refers to the melting temperature in accordance to the International Pharmacopoeia, i.e. the temperature at which the sample is completely melted as shown by the disappearance of the solid phase.

Figure 18. Labels attached to the box of the O100MPS CRS Kit.

The rated melting points for the standard substances included in the kit are traceable to WHO International Pharmacopoeia CRSs and are determined at the factory based on the procedure described in the collaborative study according to the capillary method of the Int. Pharm. 2nd Ed. (H. Bervenmark, et. al., "WHO Melting Point Reference Substances", Bull. Wld Hlth Org. 28(1963)175-188.)