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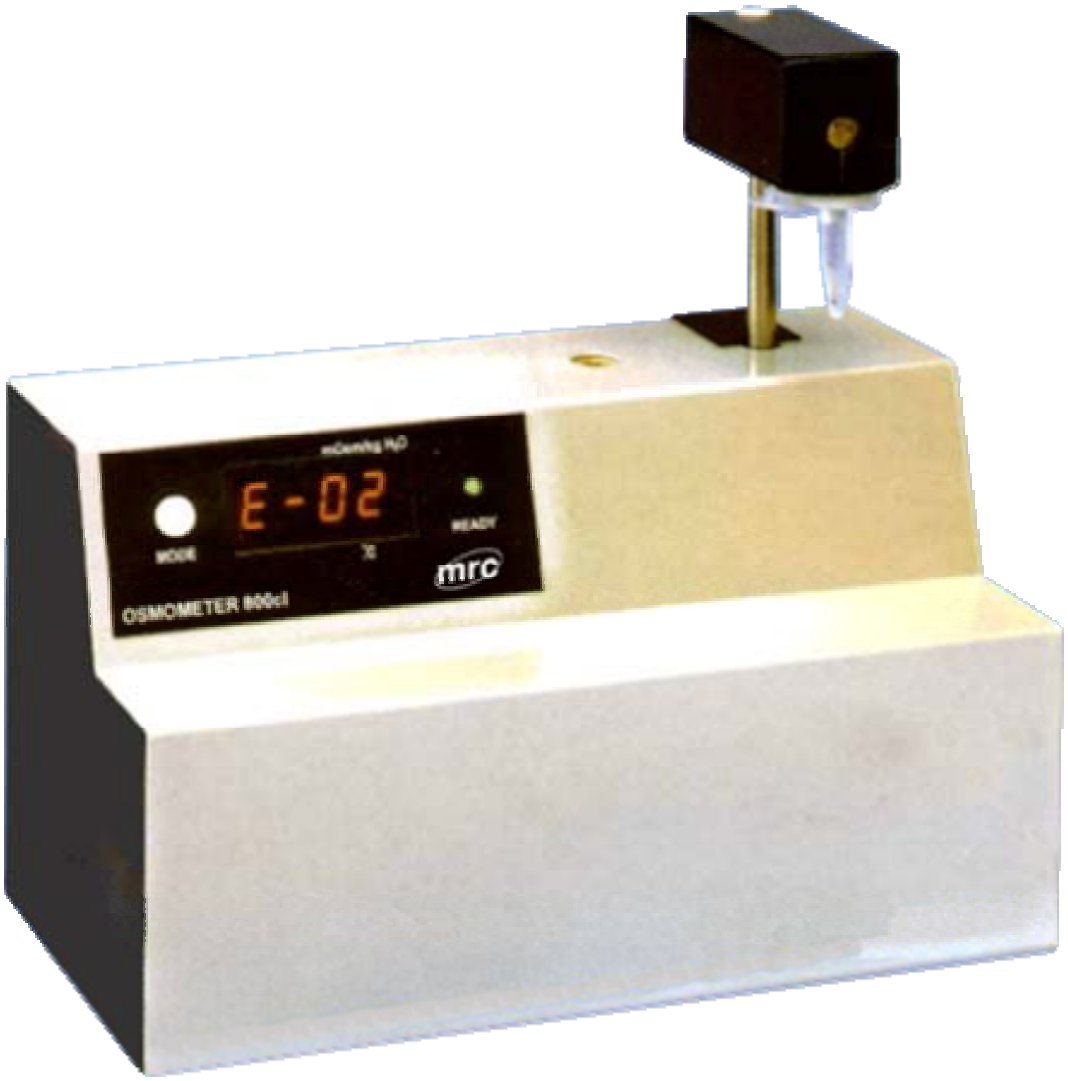
Freezing Point Analyser for Milk

Operation Manual

CRY-8



PLEASE READ THIS MANUAL CAREFULLY BEFORE OPERATION

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OPERATING INSTRUCTIONS

1. Introduction

The CRY 8 is an automatic freezing point analyzer that is used to identify the presence of extraneous water in milk and milk products. For each sample, a measurement result is produced consisting of the freezing point temperature and the corresponding percentage of extraneous water.

The device is very simple to operate, as it only requires a single-point calibration with distilled water. The sample is placed on the measuring head in sample tube which is **specially prepared** for the crystallization. The measuring head is then used to press it down into the cooling chamber. From this point the analysis runs automatically. Supercooling of the sample, initiation of the crystallization, measurement of the freezing temperature, and the conversion to the % extraneous water, with a display of results.

With its electronic components and microprocessor-based control for long-term stability, the CRY 8 offers exceptional reliability.

2. Method of measurement

Cryometry is based on a physical law, according to which the reduction of the freezing temperature of a solution relative to the freezing temperature of the pure solvent is proportional to the osmolality of the solution. The following figure shows the typical progression of the temperature of the sample during a measurement:

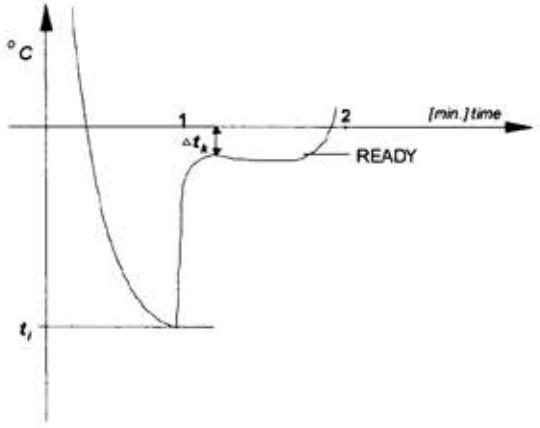

Figure 1 :
t_i - Temperature at which the crystallization is initiated in the supercooled solution,
t_k – Difference between the freezing temperature of the sample and the pure solution (distilled water).

The conversion formula for the non-linear relationship between t_k and the osmolality is saved in the microprocessor memory when the calibration is performed at the factory.

3. Scope and specifications

Scope	
Cry 8	1 unit
Sample tubes, specially prepared	250 units
Calibration solution 0°C (0 mOsm/kgH_2O)	50 ml
Spare stirring wire	1 unit

Power cable, power switch, and fuse are located on the rear

Specifications	
Calibration	Single-point calibration
Cooling	thermoelectric (Peltier effect)
Sample volume	100 µl
Measurement range	0÷ -3,600 °C
Resolution	+/- 0.001 °C
Precision	+/- 0.002 °C
Reproducibility	+/- 0.002 °C
Temperature of the cooling chamber	-12.00 °C
Temperature at initiation of crystallization	-7.00 °C
Measuring time	approx.1.5 min.
Time of operating readiness	5 min.
Power connection	230V/50-60 Hz, 50 VA
Dimensions	30 x 20 x 17 (29) cm
Weight	6 kg
Operating temperature	15 °C ÷ 35 °C
Relative humidity	max. 85% / 35 °C

4. Description of device

Face plate

Fig. 2: CRY 8 - face plate

MODE: Multi-function key

READY indicator light: A blinking light signals that the measurement is finished and the sample tube can NOT be removed from the holder of the measuring head because the sample is frozen.
A continuous light signals that the sample has melted, thus allowing the sample tube to be removed

Display: Four-character, 7-segment display on which are displayed the symbol of the device's current operating status, the working status, and the measurement result.

Measuring head

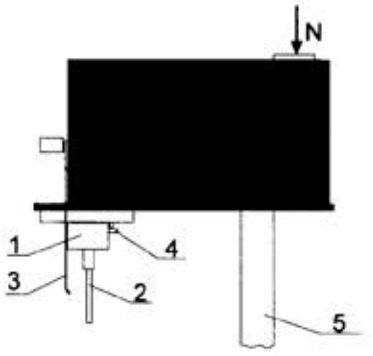

Fig. 3: CRY 8 – measuring head
1= mandrel, 2= thermistor probe, 3= stirring wire, 4= retaining spring, 5= slide bar, N= pressure point

5. Handling the measuring head

The sample tube is carefully moved from below onto the mandrel (1) until the retaining spring (4) engages. The thermistor probe (2) entering the sample tube and the stirring wire (3) must not get damaged while positioning the sample tube. The measuring head is lowered into the cooling chamber by applying pressure to the pressure point (N). The measuring head is mounted on the slide bar as it is being lowered. The measuring head is then held in bottom position electromagnetically.

6. Preparation for measurements

Before the CRY 8 is switched on, the cooling chamber must be wiped dry using a lintless tissue so that no condensation water freezes in it and no fibers are left behind. The device can then be switched on at the rear.

After around 7 seconds, the threshold values for the undiluted milk will appear in the display, e.g. -0.520 °C (comes preconfigured). When this value disappears, the display will indicate the amount of time remaining until the operating temperature of -12°C is reached in the cooling chamber; this takes approx. 5 minutes.

6.1. Calibration

When the CRY 8 is switched on, any previous calibration values will be deleted.
Thus the calibration must be repeated before each series of measurements. 100µl of distilled water is pipetted bubble-free and drip-free into a sample tube as a calibration solution. This sample tube is then attached to the measuring head as described in section 5. After the measuring head is pressed down, the calibration procedure will run automatically. The value -.000 appears in the display. The measuring head will move automatically in top position. The sample will now melt again. Once the green **READY** indicator light is illuminated continuously the sample tube can be carefully removed from the measuring head and lowered. Thermistor probe must now be carefully wiped dry using a lintless tissue. The milk sample can then be analyzed.

6.2. Measuring the milk sample

100µl of the milk sample is pipetted into the cleaned and dried sample tube. Here it is important to ensure that no bubbles are included, no drops remain on the wall of the sample tube wall, and primarily, that the gauge marks on the interior wall of the sample tube do not get wet. (The special gauge marking is used as crystallization seeds!).

The filled sample tube is attached to the measuring head as described in section 5. The measuring cycle starts as soon as the measuring head is pressed down and it ends when the results are displayed and the continuous green **READY** indicator light is illuminated. The measurement value is saved until the next measurement.

To prevent carry-over errors, the stirring wire and the thermistor probe must be carefully rinsed with water and immediately dried after each measurement procedure and before another sample tube is attached to the measuring head. The sample can be measured one after another in rapid succession. A longer pause should be allowed before performing another calibration, as described in section 6.1.

6.3 Completing the measurement

The device is switched off on the rear side. The thermistor probe and stirring wire are carefully rinsed with distilled water and dried. An empty sample tube must be moved onto the mandrel with the thermistor probe and stirring wire. The cooling chamber is dried and protected from contamination with an empty sample tube.

7. Important instructions

- The cooling chamber must always be dried off before the device is switched on as residual water will freeze in the cooling chamber, thus making it impossible to perform the measurements.

- Once the measurements are finished and the cooling chamber is melted, the chamber must always be dried using a lintless tissue.

- The only maintenance procedure consists of washing off the mandrel with the thermistor probe, and the stirring wire each time after completing the measurements.

- Between work procedures and after completion of measurement procedures, the empty sample tube is placed on the measuring head to protect the thermistor probe and stirring wire and to minimize the risk of damage.

- After completion of the task, the empty sample tube is inserted in the cooling chamber - this prevents any impurities from entering the cooling chamber that would make it impossible to complete the measurements.

- The cooling chamber will gradually be covered with frost during the measurements. If the frost layer becomes too thick, this can cause the sample tube to jam. If this occurs, the device is switched off and is dried with a lintless tissue when the cooling chamber has melted. The second preparation of the cryoscope for use follows the descriptions in the section **Preparation for measurements**.

- If the empty sample tube remains in the cooling chamber between measurements, this will significantly slow down the frost formation process on the cooling chamber.

- **Cleaning the slide bar:** Contamination will cause delays in the upward and downward movement of the measuring head, which can cause it not to reach its terminal point at the top. When the device is switched off, the slide bar and the slide box must be wiped several times with a cloth moistened with alcohol, which is done by manually moving the measuring head up and down.

- **Changing the stirring wire:** A deformed stirring wire can cause difficulties in getting the sample to freeze. To remove the defective stirring wire, the knurled screw must first be loosened and removed. The new stirring wire is inserted so that the wire end is placed with the thermistor probe (see Fig. 3)

- **Adjusting the threshold value:** A threshold value of -0.512 to -0.527 °C is assumed as a freezing temperature for the undiluted milk. The measured freezing temperatures are typically compared to the average of -0.520°C during conversion to the analysis results. However, to help the user in solving special problems, the CRY 8 offers the option to change the threshold value within the specified range in increments of 0.001 °C. The switch can be adjusted in the +/- direction on the left side of the device for this purpose. Once the device is switched on again, the new threshold value will appear in the display and will be stored in memory. The device must be recalibrated as described in section 6.1. Caution: The threshold value cannot be changed while a measurement or calibration is in progress! When a **printer** is connected, the configured threshold values are also recorded, along with the measurement results. After a change in the threshold value, the sample numbering is reset to 001.

8. Use with a printer

The CRY 8 has a RS 232 data output for connection of a thermal printer. When the cable connection is established between the two devices, the Cryoscope will switch on first, followed by the printer.

By pressing the MODE key on the cryoscope before beginning a series of measurements, a header line is generated. It includes the device description, date, and time, and the sample numbering is set to 001. The measurement results of samples are recorded with the sample number, result in °C, extraneous water in %H_2O, and the configured threshold value for 0% extraneous H_2O. These printouts are made automatically after each measurement. If no printouts are needed, the printer must be disconnected from the cryoscope.

The display and the printout for different temperature ranges of the freezing point are shown in the following table.

Freezing point temperature	Display	Printout (examples)
T>= 0°C	display without result conversion possibility	no printout
0,000°C >T> -0,256°C	-.- %	measurement 001: -0,255 °C (---%)
-0,255°C >T> -0,457°C	% extraneous water, precision 1%	measurement 001: -0,457 °C (10%) from 10% to 46 %
-0,458°C >T> -0,512°C	% extraneous water, precision 0,1%	measurement 001: -0,511 °C (0,0%) from 0,1% to 9,9%
-0,512°C >T> -0,699°C	0,0%	measurement 001: -0,699 °C (0,0%)
-0,700 °C >T	-.- %	measurement 001: -0,750 °C (---%)

9. Fault message

The microprocessor of the CRY 8 controls the sequence of the measurement program as well as the precision of the individual parameters. In case of deviations from the set point values, fault messages **E-...** will appear in the display. The meaning of the messages is provided in the following table:

Fault no.	Meaning	Cause of fault	Correction of error
E-91	damaged thermistor probe	broken, burst thermistor probe	service repair
E-01	incorrect temperature in the cooling chamber	heating of the chamber	remove heat source, press MODE after 30 sec.
		fault in the cooling system	service repair
E-02	faults in the crystallization of the sample	sample too highly concentrated Freezing temperature below -3.600 °C	outside the device range
		deformation of the stirring wire	replacement of the stirring wire
		damage to the stirring wire	service repair
		damage to the thermistor probe of the measuring head	service repair
		no crystallization seeds	repeat measurement in a new sample tube
E-03	measuring head cannot be inserted in the cooling chamber	sample tube jammed in the chamber	thawing and drying of cooling chamber
		damage to the lifting mechanism	service repair
		contamination of the measuring head guide	clean guide with alcohol, wipe off impurities, repeat cleaning procedure three times
E-04	unsatisfactory result of calibration measurement	incorrect calibration sample	use distilled water
E-94	the measuring head does not return to the top and remains below the cooling chamber	contamination of the measuring head guide	clean the guide
E-93	too much time required to melt the sample after the measurement	overcooling of the sample after a fault E-02	repeat measurement
E-92	too much time to cool the sample	wrong sample tube used damaged cooling system	use correct sample tube Inspect cooling system

10. Problematic samples

10.1 Unsatisfactory reproducibility

According to international standard IDF 108 B: 1991 (EN ISO 5764), reproducibility errors of +/- 0.004°C can be tolerated in the cryoscopic measurement, which means a conversion to a extraneous water of +/- 0.8% added to the milk. In case of discrepancies above the threshold of 0.004°C, a reason must be identified. In this case it is wise to begin by examining the sample material and the external measurement conditions. Experience has shown that this is the most common cause of the errors.

In the below description of functional procedure for the CRY 8, the assumption is made that the user has chosen the following configuration of the threshold temperature of the freezing point assumed for the undiluted milk (without extraneous water): - 0.512°C.

- moistening of gauge mark in the sample tube while dosing the sample
- incorrectly attached or bent stirring wire
- insufficient drying of the thermistor probe or the pipette after rinsing
- contamination of the thermistor probe by milk residues
- bacterial contamination of the milk
- milk acidification
- no mixing of the sample of the milk being examined before removal in the pipette, especially when the milk is diluted or the sample was stored in the refrigerator
- irregularity in the measurements, long delays between measurements
- in case of a sample that has been stored in the refrigerator over a longer period of time, the sample must be warmed in a water bath for at least 10 minutes at a temperature above 45 °C and then cooled to the ambient temperature
- damage to the thermistor probe - in this case a service repair is required.

The reproducibility of the CRY 8 should only be checked if the cause of the error is not found after going through the points listed. For this purpose, a 0.9% NaCl solution is used (available in any pharmacy as a physiological salt solution).

1. Switch on the CRY 8 and allow at least 10 minutes for it to reach operating temperature.
2. Dry the cooling chamber well, rinse and dry the thermistor probe and stirring wire well
3. Perform 4 measurements with distilled water, as described in 6.1. The last value is used for the calibration.
4. Perform 10 measurements with 0.9% NaCl solution as described in 6.2. The freezing temperature should be -0.512 °C.

If the difference between the measurement results does not exceed +/-0.004 C°, then the device is in proper working condition.

10.2 Prematurely freezing milk samples

If the milk sample being examined freezes automatically without initiation of crystallization by the stirring wire, then this means the following:

- the milk being examined is bacterially contaminated
- there is a high level of somatic cells
- the thermistor probe is contaminated by residual milk
- the acidification process has begun
- the gauge mark in the sample tube has got wet from the crystallization seeds while pipetting the sample into the measuring sample tube.

Assuming a threshold value of the freezing temperature of -0.512 C° was set on the CRY 8, then observed freezing points in a range between -4.0 and 6.9°C are not problematic. The device automatically corrects for the effects of premature freezing on the measurement results.

If less overcooling results in earlier crystallization within a temperature range of -1.2°C and -3.9°C, then the measurement result is not reliable and the measurement must be repeated. If the premature crystallization inside this temperature range is repeated, the sample can be "salvaged" by heating it in a water bath at a temperature of 45°C for at least 10 minutes and then cooling it to a temperature of approx. 20°C, then repeating the measurement.

If the crystallization occurs even earlier inside a temperature range of 0.0°C and -1.0°C, then the message E-02 will appear on the display of the cryoscope, which indicates an error in the crystallization.