OSMOMAT 010

FREEZING POINT OSMOMETER

INSTRUCTION MANUAL

CONTENTS

1 Freezing point Osmometer OSMOMAT 010

1.1 Measurement principle

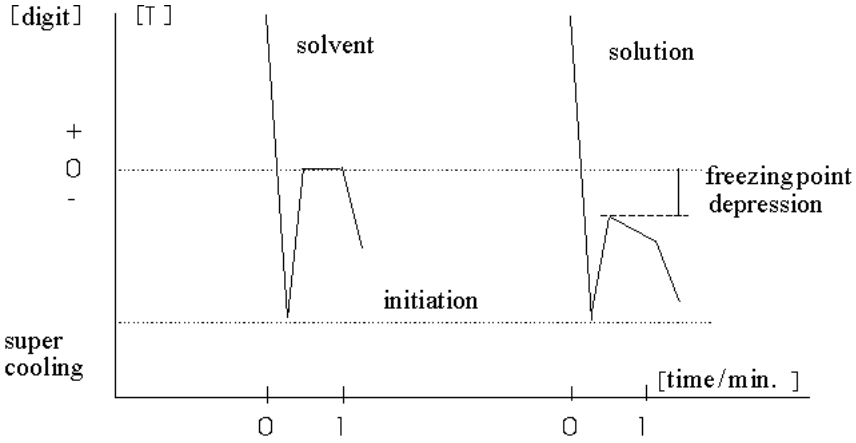
The determination of total osmolality in aqueous solution is performed by comparative measurement of the freezing point of pure water and of a solution.

Water has a freezing point of 0°C, a solution with a salt concentration of 1 Osmol/kg has a freezing point of -1.858°C.

1.2 Instrument measurement principle

The sample solution is cooled using a peltier element cooling system. At the same time the temperature of the sample is monitored electronically. When the sample reaches a defined temperature below the freezing point of pure water the crystallisation of the solution is automatically initiated.

A stainless steel needle is held well below 0°C such that water vapour in the air condensing on its tip freezes as tiny ice crystals; the needle tip, covered in tiny ice crystals, is stabbed into the super-cooled solution. Thus, initiation of crystallisation occurs by inoculation of the solution with ice crystals. Immediately after this the temperature of the solution begins to rise spontaneously as heat of crystallisation is released during the freezing process. The rise in temperature is measured with an accuracy of 1.858 x 10E-3°C (see picture 1).



picture 1

1.3 Applications

1.3.1 Measurement of the whole osmolality

The freezing point osmometer is particularly suitable for routine measurements in the medical field as well as for applications in industry and research. The OSMOMAT 010 determines the total osmolality in aqueous solutions with highest possible accuracy. The instrument requires an extremely small sample volume and is therefore suitable for measurements where sample volume is of critical importance. One measurement cycle is so fast that even a series of samples can be run in the shortest time possible. Some examples of user applications for which the OSMOMAT 010 provides years of problem-free analyses are:

General Medicine	Urology
RoutineMeasurements in Research	Nephrology
Legal Medicine	Haemodialysis
Electron Microscopy	Haemofiltration
Physiology	Botany
Clinical Laboratories	Veterinary Medicine
Intensive Care Stations	Pharmaceuticals
Pediatrics	Pharmacists
Chemists	Gynacology
In vitro Fertilisation	Culture Medim Product Control
Food/Drink Product Control	and many other areas.

1.3.2 Determination of the molar mass of substances soluble in organic solvents and purity determinations of this solvents

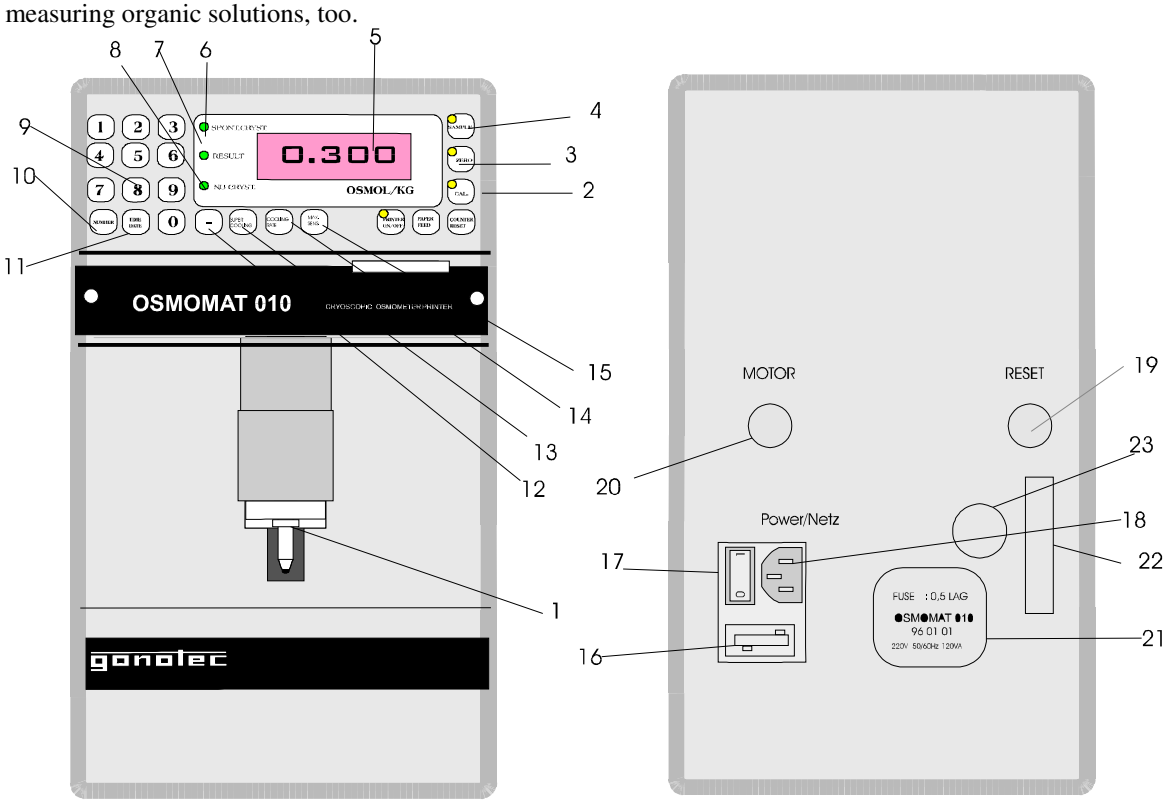
As solvent chiefly benzene is used, however p-xylol may be imployed, too. Fields of application:

molar mass determination	industry
	universities
	science / routine

2 Construction of the OSMOMAT 010

The OSMOMAT 010 is an universal freezing point osmometer which is suitable for aqueous and also certain organic solutions. Besides the normal operation keys for setting sample numbers, date, clock and calibration /sample measurement, the device has got additional keys to set the trigger temperature of the crystalisation, and the cooling power of the cooling system in a wide range. By this means it is possible to measure freezing points, which lie between +15°C and -5°C.

The automatical triggering of the crystalisation by inoculating water ice crystals proves to be very effectively in measuring organic solutions, too.



picture 2

1 measuring vessel holder
2 calibration key for calibration with calibration solution
3 calibration key for calibration with water
4 key for measurement of sample solutions
5 digital display
6 lamp indicating "spontaneous crystallisation"
7 lamp indicating "valid result"
8 lamp indicating "no crystallisation"
9 numeric keyboard for input of data
10 key for input of a 4-digit sample number
11 key for input of time, day/month and year
12 key for negative indication
13 key for trigger temperature
14 key for cooling power
15 key for maximum sensitivity
16 fuse box for 2-phase safeguard of the mains power supply, also for conversion to appropriate mains voltage supply
17 instrument power switch, on/off
18 power socket for connection to mains supply

19 "reset" key for resetting the microprocessor
20 "motor" key for activating the initiation needle
21 identification label indicating serial number, mains power supply, frequency and power consumption
22 RS232-output
23 analog output

3 Setting-up and operation

Place the instrument away from sources of vibration and protect it from direct sources of heat, like sunshine, radiators, laboratory ovens, etc. Make sure that the incoming air slot in the base of the instrument is not obstructed. The mains socket on the reverse side of the OSMOMAT 010 should be connected to the mains via the mains cable delivered with the instrument. Be careful to ensure an effective electrical earth contact with the grounding. If the mains cable is not compatible with the usual mains supply socket so that it must be changed ensure that the green/yellow cable is connected to the corresponding mains supply earth.

Check that the voltage of the mains power supply is in agreement with that indicated by the identification label on the back of the instrument.

The OSMOMAT 010 can then be connected to the mains supply by switching the mains switch (next to the mains supply socket) to the "On" position. Five dashes appear on the display. At the same time the control indicator lights 6,7 and 8 flash for 2 minutes. The OSMOMAT 010 is ready for measurement when these lights stop flashing. This waiting period is necessary for the formation of ice crystals on the upper cooling system.The waiting period can be cancelled by depressing any key. A measuring vessel has to be filled with 50 µl of sample solution and has to be fixed onto the measuring vessel holder. The thermistor (temperature sensor) is well within and is surrounded by the sample solution. The measuring vessel holder is pushed slowly but firmly down into the lower cooling system. The actual sample temperature is displayed in degrees Centigrade on the display. The cooling process can thus be directly monitored. As soon as the temperature reaches the super cooling temperature, the crystallisation is initiated. This is achieved by innoculation of the sample with ice crystals from the stainless steel needle held directly above the sample vessel. An acoustic signal tone can be heard. The process of ice formation in the sample results in release of heat which warms up the sample. The temperature rises to the freezing point of the solution where a temperature plateau is reached which is monitored via the thermistor probe and interpreted by the measurement electronics and electronically saved. The result is displayed in Osmol/kg or digits. This condition is indicated by the photodiode "RESULT". The measurement value in the memory remains on the display until the next measurement cycle has been started. The OSMOMAT 010 version with built-in printer automatically prints the result with appropriate time, date and a 4-digit sample number. After a measurement has been completed the measuring vessel holder is moved manually to the upper position and the measuring vessel with sample can be removed. The OSMOMAT 010 is then ready for the next measurement.

4 Measuring the total osmolality in aqueous solutions

4.1 Basic settings of the OSMOMAT 010

Prior to the calibration and measurement of the total osmolality of aqueous sample solutions, the basic settings of the OSMOMAT 010 relative to the trigger barrier and the cooling power have to be done. These settings are necessary only, if the device was programmed previously for the measurement of organic solutions. The corresponding program keeps stored until an alteration is made.

4.1.1 Setting the super cooling temperature

The supercooling temperature is the temperature at which the crystallisation is triggered automatically by inoculating ice crystals. This temperature may be set from 9.9 to -9.9°C. For aqueous solutions a temperature of -7°C is recommended and set in the following way: After pressing the key "SUPER COOLING" the digital display is flashing showing the hitherto set value. If needed, the display has to be corrected by means of the numeric keys as follows: For -7°C the keys "7", "0" and "-" have to be pressed. Afterwards the input will be entered by pressing "SUPER COOLING" again; the display will show the set supercooling temperature.

remark: Will more than two digits be dialed in the setting, the display will extinguish and the previously set value remains unchanged. The process has be possibly repeated then.

4.1.2 Setting the cooling power

The lower cooling system has got an electronic temperature regulator, which is controlled by a microprocessor. The cooling power and thus the cooling rate of the sample to be measured may be set between 5..and 100%. For measuring aqueous sample solutions, the setting 100% is recommended and done the following way: After pressing "COOLING RATE" the digital display will be flashing and showing the previously set value. The display now may be corrected by means of the numeric keys. For 100 % press "1", "0"and "0". Pressing "COOLING RATE" once more will enter the set cooling power; the display will show 100 %.

remark: Will more than two digits be dialed in, the display will extinguish and the previously set value will be kept unaltered.

Will digits larger than 100 up to 999 be dialed in, the value 100 will automatically be recognised. The process has to be repeated in this case.

4.2 Calibration of the OSMOMAT 010 for measuring the osmolality

Before measuring the total osmolality of sample solutions, the OSMOMAT has to be calibrated with water and a standard solution. First the OSMOMAT 010 must be adjusted to zero using water and then it will be calibrated to the value corresponding to the standard solution.

4.3 Calibration of zero with water

Pipette a 50µl volume of water into a clean, dry measuring vessel. Check that there are no visible air bubbles!
The measuring vessel is then pushed carefully onto the holder until the stop is reached. The thermistor probe is completely enveloped in water. Press the "ZERO" button before the measuring vessel holder is pushed down.
After the supercooling of the liquid is completed, crystallisation of the water is initiated and the freezing point of water is determined by the instrument. The measured value is automatically taken to be the "zero value" by the instrument and the display reads "0".
The switched on printer prints the message "ZERO: 0.000".
When calibrating the instrument it should be noted that the thermistor probe may be contaminated by the previously measured solution. A control measurement with water should be made to confirm the zero calibration. A new measuring vessel should be used for every measurement.

4.4 Measuring procedure at maximum sensitivity of the OSMOMAT 010

(not recommended for measuring the osmolality of aqueous solutions)
The OSMOMAT 010 permits measuring the freezing point at the highest possible sensitivity of the device. This state will be set by pressing the key "MAX SENS.". After pressing this key the display will show a flashing "9999". By pressing "MAX SENS." a second time, this state is entered. This means, that a previously stored calibration of the OSMOMAT 010 for the direct display of the osmolality is deleted! The OSMOMAT 010 then is adjusted to the maximum sensitivity, which is limited by electronicand physical conditions. The sensitivity now is about 10% higher than in the calibrated state with aqueous osmolal solutions. This mode is recommended exclusively for the determination of molal masses.

4.5 Direct calibration with a standard solution.

First a clean and dry measuring vessel will be filled by means of a pipette with 50µl of a standard solution. No air bubbles must be observable! The measuring vessel then will be inserted to the stop into the vessel holder. The temperature sensor is surrounded by the liquid. Before lowering the holder with the measuring vessel, the key "CAL" will be pressed whereupon the flashing display will show either "9999" or the value of the previously used standard solution. In this state the osmolality value of the new standard solution may be typed in using the numeric keys. Pressing "CAL" a second time will confirm the entered value. This way it is possible to preset any osmolality of a standard solution in the range of 0 to 2 500 mosmol/kg. Whereas normally a standard solution of 300mosmol/kg, bottled in vials, is available, the user may prepare further standard solutions himself. Corresponding concentrations for sodium chloride are listed at the end of this manual. The value of the solution to be used then will be taken for the calibration. After lowering the sensor the solution will be supercooled and the crystalisation will be triggered. The resulting measuring value automatically will be recognised as calibration value corresponding to the previous setting, and displayed digitally. At the integrated printer e.g. "CAL: 300" will be printed for a standard solution. When calibrating it must be observed, that the temperature sensor possibly is contaminated with sample residue of previous measurements. A control measurement with a new standard solution should confirm the correct setting after each calibration. If necessary, a new calibration has to be done. For each new measurement a new measuring vessel with fresh standard solution is required.
remark: The calibration has been executed only, if after choosing the key "CAL", a corresponding calibration measurement was carried out. Should the key "MAX SENS." has been pressed unintentionally, and the display shows flashing "9999", the calibration may be saved by pressing "RESET" at the back panel of the device. The "RESET" function sets the OSMOMAT 010 to the state just after switching on without deleting a measuring parameter. Thereby the flashing of the pilot lights 6,7 and 8 will be activated. By pressing any key e.g. "PRINTER ON/OFF" or "SAMPLE" the waiting cycle of 2 minutes may be broken off because the cooling systems have not been switched off. Pressing "CAL" a second time allowes to check, if the calibration is still kept. Should however the display show "9999", the calibration has to be repeated.

5 Measuring of the osmollity of aqueos sample solutions

Sample measurements can be made after calibration and confirming the calibration results.As measurements of samples take place under the same conditions as for the calibration solution 50µl of the corresponding sample has to be filled into a dry and clean measuring vessel. Check that there are no visible air bubbles! The measuring vessel is pushed carefully onto the holder until the stop is reached. The thermistor probe is completely enveloped in the calibration solution.

Press the "SAMPLE" button before the measuring vessel holder is pushed down. After the supercooling of the liquid is completed, crystallisation of the solution is initiated automatically. A freezing-point value is determined by the instrument. The measured value is converted to osmolality units, the instrument automatically takes into account the calibration value employed. The switched on printer prints the result together with a 4-digit sample number. After each sample measurement the thermistor probe should be cleaned thoroughly with a soft paper tissue in order to avoid contamination by the previously measured solution. If necessary, a soft paper tissue wetted with water can be used for cleaning.

5.1 Measurement series

The sample solutions to be measured can be pipetted into measuring vessels and the lids of the measuring vessels should be closed, being reopened just prior to measurement. The samples should be measured in a series making use of the full measurement speed of the OSMOMAT 030.

5.2 Repeating a sample measurement

In order to check measurement reproducibility, two measuring vessels (clean and dry) should be filled with the same sample solution and measured sequentially. The determination of two measurements with only one measuring vessel and the same sample solution is not possible as the solution freezes during the measurement and there can be no guarantee that the melted solution will have the same osmotic properties (due to protein precipitation etc.) as the original.

6 The determination of molar masses in selected organic solutions.

6.1 Introduction

The OSMOMAT 010 permits the freezing point measurement not only of aqueous solutions but also of selected organic solvents and their solutions. Precondition is, that the corresponding freezing points lie between 15°C and -5°C, the liquids may be supercooled to temperatures below their freezing points and crystalisation may be triggered by inoculating water ice crystals. For the determination of molar masses in the range of upto about 3 000g/mol, benzene with a freezing point of 5.51°C has proved very well. Further freezing point measurements of p-xylol with a freezing point of 13.2 C may be carried out. Both mentioned solvents show a measuring effect for the freezing point depression of approx. 3 digits per 1 mMol/kg (at the highest sensitivity). For a quick process monitoring, the determination of the purity of the both mentioned solvents is possible via the freezing point measurement too. The freezing point temperatures, measured by the OSMOMAT 010, will be determined at a defined supercooling. For this reason a calibration of the device with solutions of known molal concentration is required. For solutions with "ideal“ behaviour and a constant quotient measuring effect devided by the concentration, the measurement of just one sample solution is sufficient. This is valid for both the standard solutions and the sample solutions as well. In the case of solutions with nonlinear behaviour, at least 3 solutions with stepped concentrations of the same substance have to be measured whereby afterwards the influences of the concentration have to be eliminated by means of a statistical calculation.Considering these aspects, three possible ways of calibration are recommended:

A) single point calibration at maximum sensitivity

This calibration is to be used if the solutions behave "ideal" and the maximum sensitivity should be utilised.

B) calibration with "non ideal" solutions

This calibration mode is to be used, if the standard solutions behaves nonlinear in respect to the concentration.
In this case at least three solution with different concentrations of the same substance are measured. This measurements have to be carried out exclusively at the maximum sensitivity.

C) direct calibration of the OSMOMAT 010

This calibration mode is to be used, if the standard solutions behave "ideal" and the molality of the unknown solutions is to be displayed directly in mMol/kg at the digital display. This results however in a decrease of the sensitivity by a factor 3.

6.2 Basic settings of the OSMOMAT 010 for measuring of benzenic solutions

First, the basic setting of the OSMOMAT 010 in respect to the trigger barrier and cooling power has to be carried out.This settings are only necessary, if the device was programmed previously for the measurement of solutions of other solvents like water or p-xylol.

6.2.1 Setting the supercooling temperature

The supercooling temperature is the temperature at which the crystalisation is triggered automatically by inoculating ice crystals. This temperature may be set in the range of 9.9..to -9.9°C.For benzenic solutions a temperature of 0°C is recommended and may be set as follows: After pressing the key "SUPER COOLING" the digital display is flashing and shows the so far set value.

If necessary the display has to be corrected with the numeric keys as follows:
For 0°C the key "0" will be pressed once or twice. The leading "0" in front of the point is not displayed at values below "1.0". Afterwards the setting is confirmed by pressing "SUPER COOLING" again; the display will show the set supercooling temperature.
remark:
Are more than two numbers set when setting the supercooling temperature, the digital display will extinguish and the previously set value remains unchanged. The procedure has to be repeated eventually.

6.2.2 Setting the cooling power

The lower cooling system has got a microprocessor controlled temperature regulation. The cooling power, i.e. the cooling rate of the sample may be set between 5..and 100 %. For measuring benzenic sample solutions, a setting of 30-50 % is recommended and set as follows:
After pressing "COOLING RATE" the digital display is flashing and showing the hitherto set value. If needed, the display has to be corrected by means of the numeric keys as follows: For 50% press "5" and "0", confirm the setting by pressing "COOLING RATE" again. Afterwards the display will show the set cooling power of "50%".
remark: Are more than three digits entered when setting the cooling power, the display will extinguish and the previously set value remains unaltered. If numbers larger than 100 up to 999 will be entered, the value 100 will be recognised automatically.The setting possibly has to be repeated in this case.

6.3 Calibrating the OSMOMAT 010 for the determination of molar masses

Prior to the measurement of molar masses of substances solved in benzene, the OSMOMAT 010 has to be calibrated with pure benzene and standard solutions of known molal concentration. First the OSMOMAT 010 has to be calibrated to "Zero" with benzene and after that with a molal standard solution. To confirm the measuring results, the calibration should be checked prior to a new measuring series resp. after switching on the device the first time a day.

6.3.1 Zero calibration with benzene

First a clean and dry measuring vessel will be filled with 50 µl benzene by means of a pipette. No air bubbles have to be observeable! The vessel will be inserted into the holder to the stop. The sensor is surrounded by benzene. Before lowering the holder with the measuring vessel the "ZERO"- key will be pressed. After supercooling and triggering the crystalisation, the freezing point of benzene will be reached and recorded by the device. The egistered value will be automatically defined as "0" which is displayed. The integrated and started printer will print:
"NULL: 0,000" when calibrating the instrument it should be noted that the thermistore probe may be contaminated by sample residue of previous measurements. A control measurement with benzene should confirm the calibration, eventually a new measurement has to be carried out. For each measurement a new measuring vessel with fresh benzene is required.

6.3.2 Measuring under maximum sensitivity

The OSMOMAT 010 permits the measurement of the freezing temperature at the highest possible measuring sensitivity the device posesses. Although a direct calibration to (mosmol/kg) resp. (mmol/kg) is possible, a measurement under these conditions would mean a loss in senitivity and therefore a loss in precision by factor 3.

6.3.3 Setting the maximum measuring sensitivity

The maximum measuring sensitivity is adjusted by pressing the "MAX SENS" key. After pressing this key a flashing display "9999" arises. By pressing "MAX SENS" a second time, this mode is confirmed. Simultaneously a previously stored calibration of the OSMOMAT 010 for a direct display of the molal concentration is deleted!
The OSMOMAT 010 now is adjusted to the maximum sensitivity the device posesses limited by physical and electronical conditions. This calibration is recommended exclusively for the determination of molar masses.

6.4 Calibrating the OSMOMAT 010

Prior to the determination of molar masses the device has to be calibrated with solutions of known molality resp. osmolality.

6.4.1 Single point calibration under maximum sensitivity

The most simple way of calibration is a single point measurement of a standard solution at maximum sensitivity. A freezing point measurement of a previously prepared solution of a standard substance in benzene is carried out. The measuring result (digit) is devided by the molality resp. osmolality to calculate the device constant. The standard substance "benzil", which we would recommend, has got a molecular weight of 210,23 mol/kg, shows no concentration effects in the range up to 100*10E-3 mol/kg and is very well suitable for the single point calibration. The concentration

should be in the range of 10..to 100*10E-3 mol/kg, whereby a measuring result of 30 to 300 digit is achieved. The result of the calibration measurement (the device constant) will be calculated follows:

measuring effect/concentration = (digit * 10E3 /mol)

First a clean and dry measuring vessel is filled with 50µl of a standard solution by means of a pipette.
No air bubbles have to be observable!
The measuring vessel will be inserted into the measuring vessel holder to the stop. The temperature sensor is surrounded by the liquid. Before lowering the measuring vessel holder the key "MAX SENS" will be pressed, whereupon the flashing display shows "9999". By pressing "MAX SENS" again the input will be confirmed. After lowering the measuring vessel holder, the liquid will be supercooled and the crystalisation triggered. A measuring value (digit) is setting up which is shown on the display and printed at the printer as measuring value.This measuring value is the highest possible value the device can show under the present conditions. Although the determined measuring value is not stored directly digitally, the reproducibility and repeatability of this measurement in the sense of calibration is guaranteed because of the excellent long term stability of the electronics and especially of the amplifier. After pressing "CAL" twice, "9999" will be displayed, confirming, the device is adjusted to the maximum sensitivity. Second measurement of the same solution in a new vessel should confirm the first measurement.

6.5 Calibration of solutions with non linear behaviour at maximum sensitivity

A single point calibration is recommended only if the results do not depend on the concentration. If necessary this must be checked by measuring a concentration series of at least three concentrations of the same substance.
Concentration effects occur with rising molecular weight and limited solubility of the substances, chemical interactions substance/solvent and association effects of the substance. Does nonlinear behaviour of the measuring results in relation to the concentration appear, at least three solutions of the same substance in a stepped series must be measured. The quotients of the three measuring results (digit/concentration) will be extrapolated to "0" by means of a linear regression analysis. The constant gained this way is the corrected device constant, in which the nonlinear behaviour was eliminated. The measurement will be carried out the way discribed in the last chapter.

6.5.1 Direct calibration of the OSMOMAT 010

The device may also be calibrated with a standard solution of known molality. The advantage of this method is that later the measuring result of a sample solution direcly may be read from the display without any calculation. The disadvantage is the decrease of the measuring sensitivity by the factor 3. Further the direct calibration is usable only for solutions which behave ideal, i.e. they have no concentration dependence. First a clean and dry measuring vessel will be filled with 50µl of a standard solution by means of a pipette. No air bubbles have to be observable! The measuring vessel then will be inserted into the measuring vessel holder to the stop. The temperature sensor is surrounded by the liquid. Before lowering the measuring vessel holder, the key "CAL" will be pressed, so that the flashing display shows "9999" or the value of the previously used standard solution. In this state the molality of the standard solution that will be used, may be typed in by the numeric keys in (mMol/kg). The input will be confirmed by pressing "CAL" once more. It is possible to preset any molality values of corresponding standard solutions in the
range of 0..to appr. 100*10E-3 mmol/kg. After lowering the measuring vessel holder, the liquid will be supercooled and the crystalisation triggered. A measuring value is setting up which will automatically be accepted as calibration value and will be displayed in the digital display. The result will be printed as "CAL- Wert".
When calibrating it must be noted, that the sensor possibly is contaminated with sample residue from previous measurements. A control measurement with a new standard solution should be carried out after the calibration to confirm the exact setting. If needed, a second measurement has to be done. For each measurement a new measuring vessel with fresh standard solution is required.
remark: The calibration of the device has been executed only, if a calibration measurement was carried out after selecting "CAL"
In case, "MAX SENS" had been pressed unintentionally and the flashing display shows "9999", a calibration may be saved by pressing the "RESET" key at the back panel of the device. The "RESET" function sets the OSMOMAT 010 to the state just after switching on the device without deleting a measuring parameter. Simultanously the flashing of the three pilot lights 6.7 and 8will be activated. By pressing any key e.g. "PRINTER ON/OFF" or "SAMPLE" the waiting cycle of 2 minutes may be broken off because the cooling systems are still running in this case.By pressing "CAL" twice it may be checked, if the calibration has been kept. Should the display however show "9999", the calibration must be repeated.

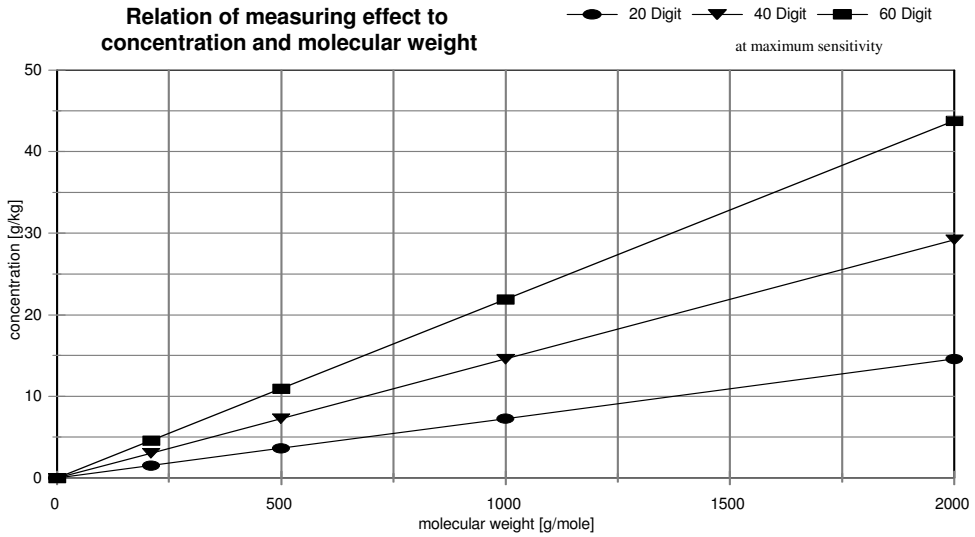
7 Sample measurement of substances solved in benzene

Precondition to the determination of molar masses is the previously carried out calibration of the OSMOMAT 010. The sample solutions will be measured under the same conditions like the previous calibration. Relative to the "ideal" or "non ideal" behaviour of the sample solutions, the same conditions are valid as discribed in the chapter "calibration". It

may be, however, that the OSMOMAT 010 has been calibrated by a single point calibration but, due to the "non ideal" behaviour of the sample solution, a concentration series of the same sample has to be measured and treated with a statistical calculation.

7.1 Preparing the sample solutions

Corresponding to the expectable molecular weight, solutions with concentrations up to 100g/kg benzene will be prepared (see diagramm "relation of measuring effect to concentration and molecular weight).



picture 3

Although the measurement requires only a few hundred µl of sample solution, at least 25 ml should be prepared to guarantee an acceptable weighing precision.

7.2 Carrying out the measurement

First a clean and dry measuring vessel will be filled with 50 µl of a sample solution. No air bubbles must be observable! Afterwards the measuring vessel will be inserted into the measuring vessel holder to the stop. The temperature sensor is surrounded by the liquid. After lowering the measuring vessel holder, the liquid will be supercooled and the crystalisation triggered. A measuring value is building up, which will be displayed as digital value. At the started printer the result will be printed together with time. When measuring it must be noted, that the temperature sensor may still be contaminated with residue of samples of a previous measurement. A control measurement with a second solution should confirm the measuring result in the sense of a double determination. For each measurement a new measuring vessel is required.

7.3 Evaluation

The result of a sample measurement will be read from the digital display in (digit).

7.3.1 Measuring under maximum sensitivity of the OSMOMAT 010

The calculation is done the same way as in single point measurements with "ideal" or "non ideal" solutions. The device constant, gained from the corresponding calibration measurement, will be devided by the quotient of the sample measurement (measuring effect/(g/kg)).

([digit]/[mol/kg*10E-3])/([digit]/[g/kg]) = g/mol*10E3

example:

device constant:	2.92*10E3 (digit/mol)
measuring result	60 digit for a concentration of 24 g/kg of the sample corresponding to 2.5 digit/(g/kg)
the molecular weight is therefore:	2.92*10E3 divided by 2.5 digit/(g/mol) = 1 186 g/mol

7.4 Measuring with direct calibrated OSMOMAT 010

The device has been calibrated with a molal standard solution in a way that the digital display shows the molality direcly in mMol/kg. The measuring result corresponds to the molality of the sample solution.
example:

measuring result 200 digit = 200 mmol at a concentration of 50g/kg

calculation:50/200*1 000 = 250 g/mol

8 Measuring the freezing point of p-xylol for the purity determination

The OSMOMAT 010 is suitable also for the determination of the freezing point of p-xylol. Thereby contaminants in the range of up to 1 weight percent are detectable, which are chemically similar to this solvent and have a molecular weight of approx. 100g/mol.(o- and m- xylol). Due to the high measuring sensitivity of the OSMOMAT 010 it is possible to detect this pollutions down to a concentration of 0.01 %. For the determination of molar masses p-xylol is not recommended because the solubility properties of this solvent are closely limited.

8.1 Basic settings of the OSMOMAT 010 for measuring p-xylol

Prior to calibrating and measuring p-xylol, in some cases the basic setting of theOSMOMAT 010 in respect to the trigger barrier and the cooling power has to be done first. This settings are necessary only, if the device was programmed before to the measurement of solutions of other solvents like water or benzene. The corresponding program is stored until a alteration will be made.

8.1.1 Setting the supercooling temperature

The supercooling temperature is the temperature, at which the crystalisation is triggered automatically by inoculating ice crystals. This temperature may be set in the range of 9.9 to -9.9°C. For p-xylol a temperature of 4°C is recommended and set following:
After pressing the key "SUPER COOLING" the digital display is flashing and showing the hitherto set value. If needed, the display may be corrected by means of the numeric keys: For 4°C the keys "4" and "0" have to be pressed. The input has to be entered by pressing "SUPER COOLING" once more; afterwards the display will show the entered supercooling temperature.
remark: Are more than two numbers set for the supercooling temperature, the digital display will extinguish and the previously set value is kept unchanged. In this case the setting has to be repeated.

8.1.2 Setting the cooling power

The lower cooling system has got an electronic temperature regulation, which is controlled by a microprocessor. The cooling power and thus the cooling rate of the sample may be set between 5.. and 100 %. To measure p-xylol and its sample solutions, a setting of 25 % is recommended and set as follows:
After pressing "COOLING RATE" the digital display will be flashing and showing the hitherto set value. If necessary, the display now may be corrected by means of the numeric keys: For 25 % the keys "2" and "5" have to be pressed. Afterwards the input will be confirmed by pressing "COOLING RATE" a second time. The display now will show the set cooling power of 25 %.
remark: Are more than 3 numbers set for the cooling power, the digital display will extinguish and the previously set value is kept unchanged. If numbers larger than 100 to 999 will be dialed in, the value 100 will be recognised automatically. If needed, the setting has to be repeated.

8.2 Calibrating the OSMOMAT 010 for purity determination

Before determining the purity of p-xylol, the OSMOMAT 010 must be calibrated with pure p-xylol and a solution with known concentration of contamination. First the OSMOMAT 010 will be calibrated with pure p-xylol to "zero" and following with an adjusted standard solution To ensure the measuring results, the calibration should be checked prior to a new measuring series and after switching on the device the first time a day.

8.3 Calibrating zero with p-xylol

Before measuring sample solutions, the OSMOMAT 010 must be calibrated with p-xylol and a standard solution. For this purpose the OSMOMAT 010 first must be calibrated to zero with p-xylol and then with a standard solution to the corresponding value. To confirm the measuring results, the calibration should be repeated prior to a new measuring series resp. after switching on the OSMOMAT 010 the first time a day. Prior to the measurement of the total osmolality of sample solutions, the OSMOMAT 010 has to be calibrated with p-xylol and a standard solution.

8.3.1 Measuring at maximum measuring sensitivity

The OSMOMAT 010 permits the measurement of the freezing point temperature at the highest possible measuring sensitivity of the device. This mode is recommended for the purity determination.

8.3.2 Setting the maximum measuring sensitivity

The maximum sensitivity is selected by pressing "MAX SENS". After pressing this key the digital display will be flashing and shows "9999". By pressing "MAX SENS" a second time, this mode is entered. By doing this, an eventually previously stored calibration of the OSMOMAT 010 for a direct display of the molal concentration is deleted!
The OSMOMAT 010 now is set to the maximum sensitivity the device permits and which is limited by electronic and physical conditions.

8.4 Calibration of the OSMOMAT 010

Before determining the purity of p-xylol, the device must be calibrated with solutions of known concentration.

8.4.1 Single point calibration at maximum sensitivity

The most simple way of calibration is the single point measurement of a standard solution at maximum sensitivity. This method is recommended especially for the purity determination of p-xylol. By this means a freezing point measurement of a previously prepared solution of a standard substance in p-xylol with a concentration of a certain percentage is carried out. The result (digit) represents in relation to the set concentration the degree of pollution ,i.e. the measuring result is a degree for the pollution.

$$\frac{\%\ \text{pollution}}{\text{digit}} = \left[\frac{\%}{\text{digit}}\right]$$

First a clean and dry measuring vessel is filled with 50 µl of a standard solution by means of a pipette. No air bubbles must be observable! The measuring vessel is then inserted into the measuring vessel holder to the stop. The temperature sensor is surrounded by the liquid. Before lowering the measuring vessel holder, the key "MAX SENS" is to be pressed if not yet done whereupon the flashing display will show "9999". The input now has to be entered by pressing "MAX SENS" once more. After lowering the measuring vessel holder, the liquid will be supercooled and the crystalisation triggered. A measuring value (digit) is setting up, which is diplayed and printed. This measuring value is the highest value the device can display for the measured solution under the preset conditions. Although the determined value is not stored directly digitally, the reproducibility and the repeatability of this calibration is granted due to the high long term stability of the electronics, especially the amplifier. After pressing "CAL" twice, the digital display will show "9999" confirming the device is set to the highest possible sensitivity. A second calibration measurement with the same solution should confirm the first one in the sense of a double determination.

8.5 Purity determination of p-xylol

Precondition to this measurement is the previously done calibration of the OSMOMAT 010. The sample solutions are measured under the same conditions like in the calibration.

8.5.1 The measuring procedure

First a clean and dry measuring vessel will be filled with 50 µl of a sample solution. No air bubbles must be observable! The measuring vessel will then be inserted into the measuring vessel holder to the stop. The temperature sensor is surrounded by the liquid. After lowering the measuring vessel holder, the liquid will be supercooled and the crystalisation triggered. A measuring value is building up, which will be displayed digitally. The printer will print this value together with the time and a 4-digit number. When measuring it has to be noted, that the temperature sensor may be contaminated with sample residue from previously made measurements. A control measurement with a further solution should be made for a double determination. For each measurement a new measuring vessel is required.

8.5.2 Evaluation

The result of a sample measurement may be read from the digital display in (digit). The device constant, gained from the calibration, (% contamination/digit) will be multiplied by the result of the solution to be checked.

result of the calibration measurement: $[\%] / [\text{digit}] = (\text{cell constant})$

result of the sample measurement: ([%] / [digit])*digit = [%]

example:
cell constant: 0.23 % / 68 digit = 0.0034 [% / digit]
measuring result: 60 digit for an unknown sample
calculation of the result: 0.0034 * 60 = 0.2 %

9 Error indications, reason and possible prevention

The examples listed here refer to the osmolality measurement in aqueous solutions. In the case of benzene and p-xylol these instructions are valid accordingly.
Errors are often caused by specific properties of some solutions. The instrument itself, through malfunction, can however also cause errors. Another reason for errors can also be the wrong calibration (e.g. calibration of zero with calibration solution). In this case the instrument does not show plausible measuring results anymore, the instrument has to be initiated newly. This can be done by the user himself. The OSMOMAT 010 has to be switched off. When switched on again the key "ZERO" has to be pressed for abt. 2 seconds. Afterwords the LEDs of the keys "ZERO" and "SAMPLE" are on. Then the key "ZERO" has to be pressed again and a calibration with water has to be effected. Afterwards the instrument can be calibrated as described in point 4.2.

9.1 Spontaneous crystallisation

In order to measure the freezing point of a sample it is first cooled, without the formation of ice-crystals, to a pre-defined temperature of -7°C. Crystallisation is initiated automatically by the addition of ice crystals to the water and a part of the water in the sample crystallises out of solution.
The large enthalpy of crystallisation associated with the formation of ice results in an immediate rise in the sample temperature up to the freezing point of the remaining solution, albeit slightly more concentrated (due to the loss of same of the water in the newly formed ice phase) than the starting sample solution. A characteristic freezing plateau temperature is then reached when ice formation continues in the presence of the remaining solution. Water continually lost from the sample to the newly forming ice phase, results in increasing sample concentration and hence increasing osmolality. This is reflected in an inflection point and an increasing slope away from the freezing plateau.
The determination of the total osmolality by the OSMOMAT 010 is performed using the temperature difference between the freezing point of water and that of the resulting inflection point in the freezing plateau of the sample solution. The quality of the total measurement depends on the maintenance of the conditions for supercooling the sample and the temperature of crystallisation initiation. The OSMOMAT 010 continually monitors the temperature during the supercooling phase and displays an error if crystallisation takes place before -7.0°C is reached, thus avoiding a false measurement result.

9.2 Methods to avoid errors caused by spontaneous crystallisation

The main cause of spontaneous crystallisation is the presence of crystallisation centres which do not allow a cooling down to the desired -7°C.
Crystallisation centres can be salt crystals, solutions super-saturated with gases, and the presence of ice crystals. Such crystallisation centres can be simply eliminated by warming the sample near to its boiling point followed by cooling again to room temperature.
Crystallisation centres which cannot be removed in this manner can often be removed by filtration.
Some samples may contain salts that are present close to their saturation limits and which crystallise out at lower temperatures resulting in the formation of crystallisation centres. In such case it is possible to dilute the sample, however extrapolation back to the original sample osmolality is not necessarily be expected to lead to an accurate result. (See Chapter "The osmolality is too high"). In addition the thermistor probe itself may have crystallisation centres sticking to its surface. These may be removed by careful washing with water and drying with a soft paper towel. If the crystallisation centre is a fine scratch on the surface of the thermistor probe which cannot be removed then the thermistor probe should be exchanged for a new one.Measuring vessels which are not clean (e.g. dust on the inner vessel wall) may also cause spontaneous crystallisation. Measuring vessels which are repeatedly used can cause this problem because fine cracks containing a thin film of solution may also act as crystallisation centres. For this reason it is not recommended to use the vessels more than once.

9.3 Crystallisation occurs too late or not at all

Crystallistion is automatically initiated by inoculation with ice crystals when the sample temperature has reached -7°C. In case of benzolic solution, the cooling power can be set to a lower rate in a range between 30 to 50%. There may be two causes should inoculation not lead to ice crystal formation in the sample.

9.3.1 Osmolality is too high

The crystallisation is, as described, initiated at -7°C. The OSMOMAT 010 is designed to measure osmolality up to about 3 Osmol/kg water. This osmolal concentration corresponds to a temperature of Osmol/kg water x molal freezing point depression)

3 x 1.858 = 5.574°C below the freezing point of water, i.e. -5.6°C

If the freezing point of a sample solution is close to the temperature at inoculation then the chance of a successful inoculation is not very high. This is particularly the case for viscous solutions. In this case the only help is to dilute the sample. However, the result after dilution must be carefully interpreted since the osmolality is not necessarily proportional to the dilution. For example, dissociation constants of electrolytes change depending on the electrolyte and their concentrations.
Nevertheless, for routine measurements, for example in quality control assurance, relative measurements made on diluted samples can be used for evaluation purposes.

9.3.2 Inoculation with ice crystalls is unsuccessful

There are three possible reasons why no crystallisation occurs in the ice crystal initiation phase even though the acoustic signal has indicated that ice crystal inoculation has taken place and although the sample osmolality is expected to be in the normal range. The transport of the needle covered in ice crystals may have been prevented from being lowered into the sample from the upper position.
A mechanical fault is preventing the movement of the needle.
Tip: The thermistor probe must be unscrewed. The needle can then be put back into its proper place and, after renewed construction, then tested to confirm normal mechanical function. Pressing the button "Motor" on the back plate of the instrument allows the testing of the proper needle movement. Despite normal mechanical functioning of the needle transport mechanism, the ice crystals on the stainless steel needle do not reach the super-cooled sample solution during initiation. The needle, covered with ice crystals, must pass through a small hole in order to reach the sample. This hole may contain a drop of water or may be very dirty (the ice melts/is removed before it reaches the sample solution).
The water in the hole may be derived from ice crystals on the needle which have stucked to the inner wall of the hole and melted.
Tip: A pasteur pipette with a long thin tube is a part of the accessories provided with the instrument. This can be used to blow out the water drop. It is recommended to remove the sample and to hold a paper tissue under the thermistor probe to catch the water drop.
With time the hole may be partially plugged with small amounts of sample material left behind by the needle. A wash bottle can be used to squirt water into the hole removing the debris present. The instrument does not have to be switched off for this procedure.
There is no ice on the needle due to low environmental humidity or the instrument is used too soon after being switched on.
Tip: The first measurement should not take place less than 2 min after switching the instrument on. When the humidity is very low (e.g. sometimes in heated rooms in winter) the cover in front of the needle cooling system should be opened.
Several important conditions must be respected to ensure good measurement reproducibility.

10 Reproducibility

10.1 Centering the thermistor Probe in a measuring vessel

Optimal reproducibility is achieved only when the thermistor probe is situated exactly in the middle of the measuring vessel. Attention to this must be particularly paid when replacing the thermistor probe with a new one.

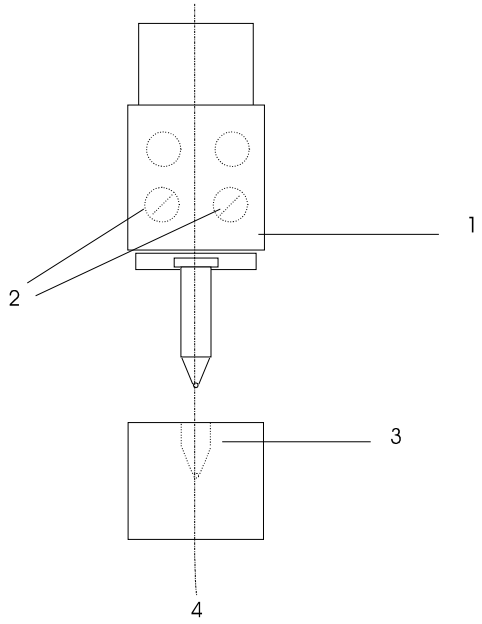
10.2 Changing a defective thermistor probe

Before starting remove the mains plug from the electricity mains supply.
Carefully slide the cover of the upper cooling system upwards (the cover on the measuring vessel holder support system). Two screws are now visible and must be unscrewed so that the complete cover can then be removed. Two more screws (2) can then be seen and also have to be unscrewed and removed. Disconnect the plug (black) from the connecting cable (green). The entire measurement vessel-thermistor-support with the holding plate (1) can now be removed from the initiation needle; the initiation needle slides out of its guide and the tube protecting the needle is also removed. Following this, the entire measurement vessel-thermistor support can be unscrewed from the holding plate and replaced by a new one. Reconstruction of the thermistor probe is basically a repeat of the above in reverse. The

initiation needle is placed into its guide tube and fixed onto the holding plate with the two screws. The connecting cable is plugged into the socket and the cable itself is placed in the grove on the side. The thermistor must now be fixed into the correct position. The precise position of the thermistor probe and the lower cooling system is very important for optimal reproducibility of the OSMOMAT 010 measurement results. A special tool is used to make the adjustment, it can be found in the accessories. The purpose of the adjustment is to bring into alignment the middle axis of the cooling shell (3) with the thermistor axis (4) (picture 3). The adjustment has to be made as follows:

- Place the adjustment tool in the lower cooling system taking care to ensure that it is securely in position.
- Carefully lower the thermistor probe. The thermistor probe must fit exactly into the hole in the adjustment tool. If this is not the case then the thermistor probe or the lower cooling system must be adjusted again. If the thermistor probe must be moved in the x-axis "left-right", then the screws on the thermistor probe should be loosened and an adjustment made. The procedure should be repeated until the glass pearl tip of the thermistor probe is precisely in the middle of the adjustment tool. The screws can now be firmly fixed in position.
- Remove the adjustment tool.

Fix the cover of the upper cooling system into position with the appropriate screws.



picture 4

If the thermistor probe must be adjusted in the direction of the y-axis then the lower cooling system will have to be adjusted. For this purpose remove the lower front plate by unscrewing the appropriate screws. The lower cooling system can then be adjusted to the middle position after loosening the screws in the bottom plate.

10.3 Use of the correct measuring vessels

The vessels used for measuring with the OSMOMAT 010 have been taken over from centrifuge technology. The measuring vessels employed for the OSMOMAT 010 possess particular qualities which enable very high reproducibility.For this reason we can only recommend measuring vessels supplied by GONOTEC because they comply precisely with our quality requirements.

11 Changing the main fuse

First remove the mains plug from the mains electricity supply. A small screwdriver can be used to remove the fuse-holder (16 - see picture 5) on the reverse of the instrument. Both fuses can then be changed. The instrument is 2-phase fused.The following fuses should be used:

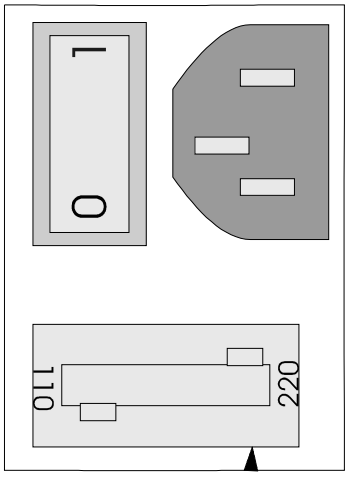
Mains supply 230 V: 0.5 A delay action/LAG

Mains supply 110 V: 1 A delay action/LAG

One set of fuses is to be found in the standard accessories.

ATTENTION!
Care should be taken to ensure that the fuse holder is put back into the same position as originally found.

The fuse holder is labelled with "110" and "220" opposite one another. The fuse holder must be positioned so that the appropriate mains supply voltage label (see instrument identity label) is directly under the instrument mains socket A small arrow indicates the appropriate position of the voltage (see diagram below).

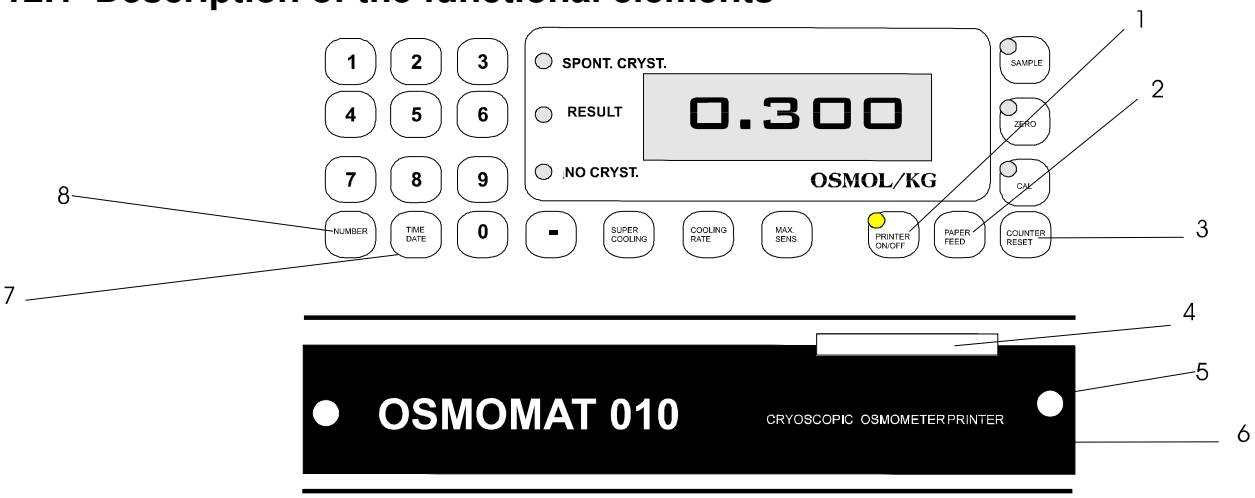


picture 5

12 Built-in printer

The OSMOMAT 010 is equipped with a matrix-printer. The printer works with standard paper and ink ribbon cassette. The printer paper as well as the endless ink ribbon cassette have to be exchanged after a certain period of time. The OSMOMAT 010 is also fully operable without the printer being switched on. After each measurement the result can be read on the digital display.

12.1 Description of the functional elements

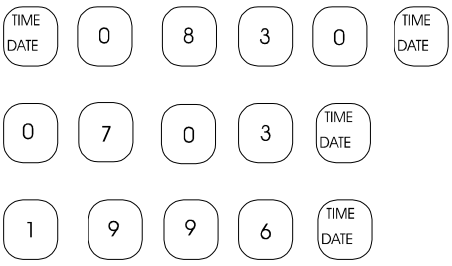


picture 6

1. Switch on/off for printer
2. Paper Feed
3. Counter Reset
4. Paper slot with sharp edge
5. Knurled-head screw
6. Plexiglass shield
7. Key for input of time, day/month and year
8. Key for input of 4-digit sample number

12.2 Input of time and date

The input of the time an the day is effected by pressing the key "TIME/DATE" (7) (picture 5) and the numeric keyboard. For the example 8:30 h 7th March 1996 the following pressing of keys is necessary :



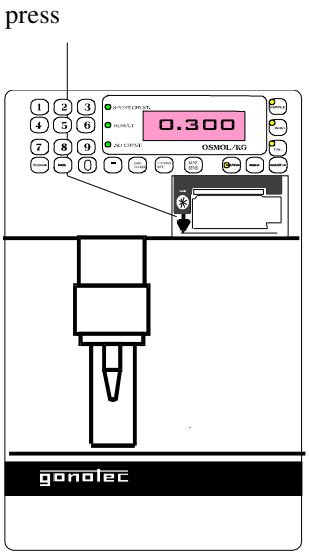
When pressing the last key date and time are stored.

The input of the sample number is effected by pressing the key "NUMBER" (8) and the numeric keyboard. For the example sample number "67" the following pessing of keys is necessary :

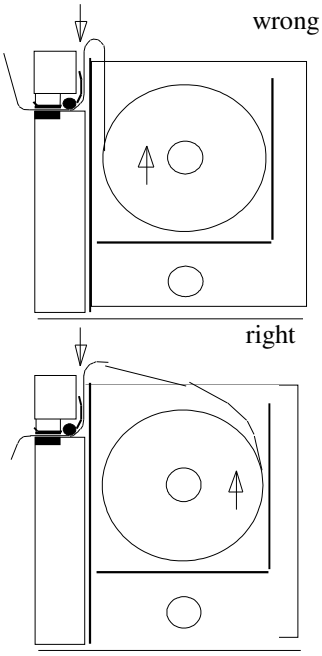
12.3 Starting the printer

The printer is switched on by single pressing on the key "PRINTER ON/OFF". The red LED is on when the OSMOMAT 010 is switched on. After the first switching on the head of the report is printed automatically.
The measuring results are automatically printed when the LED "RESULT" is on. The result is stored.
With the result a consecutive number is printed. By pressing the key "COUNTER RESET" a new head of report is printed and the numeration starts again from 1. Incorrect measurements which show no result on the digital display are not counted. The lamp indicating "spontaneous crystallisation" or "no crystallisation" lights up. The cause of the incorrect measurement is printed in clear. The paper tape can be pulled of neatly over the sharp edge of the paper slot.

12.4 Restoring of the printer paper



picture 7



picture 8

A) Removing of plexiglass shield (6) pict. 6 by screwing off the two knurled-screws (5) pict. 6.
B) Collapsing of the printer by light pressing of the finger on the lower, left part of the printer (picture 7).
C) Removing of the used up roll out of the paper box (picture 7). Doing this pull off the remaining paper tape. -Do not pull out paper tape backwards- Move the remaining paper tape out of the paper slot by pressing the key "PAPER FEED" (2) pict.6.
D) Insert new roll of paper Paper format broad : 43 mm, roll diameter : 34 mm Gonotec-article-no. 30.9.1010 (8 rolls/packing unit) Switch on OSMOMAT 010 and printer. Cut paper neatly and insert its end in paper slot from above. By pressing the push-button "PAPER FEED" (2) pict. 5 move the paper tape through the slot so that ca. 2

cms are outside the slot. Make sure that the paper roll has the right direction of unrolling (picture 8). Put the new paper roll in paper box and fold back printer.

E) Mount paper tape through slot of plexiglass shield (6) pict. 6 and rescrew shield. Before starting next measurements print head by pressing the key "COUNTER RESET" (3) pict.6.

12.5 Exchange of the ink ribbon cassette

The ink ribbon cassette has to be exchanged when the printing does not show enough contrast. The OSMOMAT 010 can be switched off. The OSMOMAT 010 can be switched off.

A) Before exchange of the ink ribbon cassette a paper tape which looks out of the paper slot has to be pulled off.
B) Removing of the plexiglass shield (6) pict. 6 by screwing off the two knurled-head screws (5) pict. 6.
C) Removing of the used up ink ribbon cassette by light pressing of the finger on the right side of the cassette.
D) Unpack new ink ribbon cassette Gonotec-article no. 30.9.1020
E) Tighten ink ribbon by twisting rotary button clockwise with finger.
F) Put new cassette in printer. The end of paper tape has to lie between ink ribbon and cassette.
G) Tighten ink ribbon again by twisting rotary button.
H) Rescrew plexiglass shield.

13 Serial digital data output RS 232 C

13.1 In general

The option RS allows a connection of OSMOMAT 010 to any personal or other computer with digital serial data output. A special part of the RS 232 C norm-data output was selected.

13.2 Technical specifications

Baud rate: The transmission speed is 1200 bits/sec.
Data form: 1 start bit, 8 data bits and 2 stop bits are sent.

Signals: TXD- Transmit Data (output)
Stand still level -3V >> U >> -7,5V
Active level +3V<< U << +7,5V
DTR - Data Terminal Ready (output)
This signal indicates the readiness of OSMOMAT 010 for transmission of data set.
Stand still level -3V >> U >> -7,5V
Active level +3V << U << +7,5V
DSR - Dataset Ready (input)
With this signal, the transmission of a data set can be prevented. The OSMOMAT 010 will start a transmission only when DSR is activated. The user does not have to connect this signal, as it is equipped internally with a 15 kOhm pull up resistor.
Stand still level -3V >> U >> -15V
Active level +3V << U << +15V

13.3 Format of dataset

After each measuring process a dataset is transmitted. The transmission of data is carried out in ASCII format. Depending on wether option D (matrix printer) is switched on, two different formats of dataset are transmitted.

13.3.1 The printer is switched on

If the printer is not turned on there is no possibilty to set sample number, date and time.only the masuring values is then transmitted.

13.3.2 Output format in case of incorrect measurements

In case of incorrect measurements, two different datasets can be transmitted:
SPONTANEOUS CRYSTALLISATION
NO CRYSTALLISATION

13.3.3 Pin connection on connection plug

On the attaching plug the following pins are used:
Pin 2 - TXD
Pin 6 - DSR
Pin 7 - GND

Pin 20 - DTR

14 OSMOMAT 010 analog output

Output voltage: 1 mV = 1 mOsmol
Max. output voltage: +/- 14 V
Min. ballast resistance: R= 4,7 kOhm

The output voltage is independant of the storage function of the "RESULT" display. The recorder cable supplied with the instrument is only needed for the analog output (output pocket pin 2 and pin 4).

15 NaCl-Calibration Solutions for Osmometry

Real Osmolality Milliosmol/kg H2O	Sodium Chloride Concentration g NaCl/kg H2O	Freezing Point Depression Δt in°C
100	3,0896	0,18580
200	6,2448	0,37160
300	**9,463**	**0,55740**
400	12,6872	0,74320
500	15,9350	0,92900
600	19,1860	1,11480
700	22,4456	1,30060
750	24,10	1,395
1200	38,76	2,23
1800	58,01	3,35
2500	79,97	4,65