

μ O S M E T T E™

Model 5004 Automatic Osmometer

O P E R A T I N G M A N U A L

PRECISION SYSTEMS INC.

16 Tech Circle
Natick, MA 01760-1029

Phone: 508-655-7010
Fax: 508-653-6999

TABLE OF CONTENTS

SECTION 1	INTRODUCTION	PAGE
1-1	GENERAL	1 - 1
1-2	INSTALLATION	1 - 2
1-3	PRINCIPLES OF OPERATION	1 - 2
1-4	CHEMICAL PRINCIPLES OF THE PROCEDURE	1 - 5
1-5	WARNING	1 - 6
1-6	SPECIFICATIONS	1 - 8
SECTION 2	INSTALLATION	
2-1	PACKING LIST	2 - 1
2-2	WARRANTY INFORMATION	2 - 1
2-3	SET-UP PROCEDURE	2 - 2
2-4	SHIPPING INSTRUCTIONS	2 - 3
SECTION 3	DESCRIPTION	
3-1	PURPOSE	3 - 1
3-2	PARTS	3 - 1
3-3	OPERATING HEAD	3 - 2
3-4	REFRIGERATOR	3 - 2
3-5	THERMOMETER	3 - 2
3-6	CONTROLS	3 - 3
SECTION 4	REAGENTS & SAMPLE HANDLING	
4-1	REAGENTS	4 - 1
4-2	WARNINGS AND PRECAUTIONS	4 - 1
4-3	REAGENT PREPARATION	4 - 1
4-4	REAGENT STORAGE AND STABILITY	4 - 2
4-5	INDICATIONS OF INSTABILITY OR DETERIORATION	4 - 2
4-6	PREPARATION FOR ANALYSIS	4 - 3
4-7	KNOWN INTERFERING SUBSTANCES	4 - 3
4-8	STORAGE AND HANDLING OF SAMPLES	4 - 3
SECTION 5	PRINCIPLES OF OPERATION	
5-1	GENERAL	5 - 1
5-2	THE OSMETTE CONTROLS THE THERMODYNAMICS	5 - 1
5-3	PERFORMANCE CHARACTERISTICS	5 - 2

SECTION 6 OPERATION

6-1	GENERAL	6 - 1
6-2	OPERATION	6 - 1
6-3	FACTORS AFFECTING REPRODUCIBILITY	6 - 3
6-4	PRE-SEEDING	6 - 3
6-5	RANGE EXTENDER	6 - 4
6-6	STANDARD SOLUTIONS	6 - 4
6-7	CALIBRATION	6 - 4
6-8	OPERATING PRECAUTIONS	6 - 6
6-9	LIMITATIONS	6 - 7
6-10	HAZARDS	6 - 7
6-11	STAND-BY POSITION	6 - 7

SECTION 7 PERFORMANCE DATA

7-1	PRECISION AND ACCURACY	7 - 1
7-2	REPRESENTATIVE VALUES	7 - 1
7-3	PRECISION EVALUATION ON AQUEOUS STANDARDS	7 - 1

SECTION 8 HAZARDS & CAUTIONS

8-1	GENERAL	8 - 1
8-2	ELECTRICAL	8 - 1
8-3	MECHANICAL	8 - 2
8-4	STANDARDS	8 - 2
8-5	SAMPLES	8 - 2

SECTION 9 MAINTENANCE

9-1	GENERAL	9 - 1
9-2	PERIODIC MAINTENANCE	9 - 1
9-3	SERVICE ADJUSTMENTS	9 - 1
9-4	PARTS REPLACEMENT	9 - 3
9-5	FUNCTIONAL CHECK	9 - 5
9-6	FUNCTIONAL CHECK PROCEDURE	9 - 5
9-7	TROUBLESHOOTING GUIDE	9 - 6

GENERAL CHEMISTRY REVIEW **i**

WARRANTY **ii**

CLAIM FOR SHIPPING DAMAGE **iii**

SECTION 1

INTRODUCTION

1-1 GENERAL

Thank you for purchasing an OSMETTE.

The μ OSMETTE is a precise instrument for measuring freezing point depression. Since the freezing point of a solution is a measure of the solution's concentration, the OSMETTE provides a simple, but very accurate method for concentration or osmotic pressure measurements. The OSMETTE is, therefore, a freezing point osmometer.

The uses of the OSMETTE include quality control of aqueous solutions during their manufacture, determination of the solute concentration of various biological solutions, and determination of average molecular weight of a solute weighed into a known weight of solvent. Since these determinations can be made by measuring the freezing point of a solution (a colligative property), the OSMETTE's function is to make a freezing point measurement in a very simple, precise and quick manner.

To obtain the best results, it is recommended that the user read this Operator Manual completely before attempting to operate the instrument. For those who have operated a freezing point osmometer before, a number of refinements which simplify and improve the operation of the instrument will be noted.

As in any precise measurement, the conditions under which the measurement is made should be reproduced as closely as possible. The careful duplication of the operating steps to achieve a high degree of precision may be called "technique". By unique design, the OSMETTE simplifies the technique required by reducing the number of variables which the operator must control. Disposable sample tubes provide additional user convenience.

The OSMETTE is designed with five modularized sections to simplify service through interchangeability of modules. The modules are: the Power Supply, the Front Panel, the Digital Control Board, the Operating Head, and the Refrigerator.

Many special applications of the instrument will be of interest to others or may require information which is not included in the Operator Manual. Precision Systems is interested in assisting you wherever possible, and in learning of new applications for the OSMETTE.

In the event of technical problems, refer first to **Section 9: Troubleshooting and Maintenance** of this manual. Then should you have problems which are not resolved, please call the Service Department at Precision Systems. **Please have ready the Instrument Model Number (5004) and the Serial Number.**

1-2 INSTALLATION

The OSMETTE is designed to be operated on a solid, flat laboratory bench approximately 2 feet (.6 m) in depth and at least 2 feet wide, located within 4 feet (1.2m) of a well-grounded electrical power outlet, with the ground connection of the receptacle and power which matches the OSMETTE's power specification and plug. A cool, dry, draft-free location should be selected, away from fans, heaters, radiators, sunlight, and equipment which generate electrical noise or vibration. **Do not use extension cords to which other equipment is connected.**

Further installation information is provided in **Section 2.**

1-3 PRINCIPLES OF OPERATION

The theory of concentration determination by freezing point measurement depends upon three phenomena. The first is that, in general, the more concentrated a solution is, the lower will be its freezing point. Common illustrations of this are the salting of roads in winter to melt ice, and the addition of alcohol or ethylene glycol to an automobile radiator to lower the freezing point of its coolant water so that it will not freeze up and damage the engine in winter.

The second phenomenon is that of supercooling. It has been observed that one may cool water to as low as -40° Celsius, and still have liquid water, provided no ice crystals, dust or other contamination is present, and the water is not agitated. The addition of salt, dust, or any other foreign particle, or violent agitation of supercooled water results in ice crystal growth on the nuclei introduced. Under certain conditions, this crystallization can be very rapid.

The third phenomenon is that when water freezes, heat is released at the rate of 80 calories per gram of water. This heat is called the Heat of Fusion, and will warm the sample or environment whenever freezing occurs.

Osmotic pressure, a reflection of the difference in solute concentration between two solutions separated by a semipermeable membrane, controls the flow of water across biological membranes; the greater the difference in solute concentration between the two solutions, the greater the tendency for water to flow across the membrane to equalize the total concentration on both sides of the membrane. In a closed system (i.e. no outlet to the atmosphere), this results in an increase in the pressure on the high solute side of the membrane. This increase in osmotic pressure due to dissolved substances is, for practical purposes, dependent only on the total number of dissolved solute molecules and is independent of the identity of the solute species. From this it can be seen that osmotic concentration (i.e. osmolality, or concentration of solute molecules contributing to osmotic pressure) of body fluids plays a significant role in the regulation of the hydrodynamic balance of the various body fluid compartments. In fact, measurement of osmotic concentration has been utilized extensively in the evaluation of fluid balance in humans, animals, and plants.

The dissolution of solutes in water also causes a change in three other physical properties of solutions which are dependent only on the total number of dissolved particles: 1) The freezing point, or the point at which pure ice and liquid water or solution exist in equilibrium, is lowered, 2) the vapor pressure, or tendency of water molecules to escape into the gas phase, is lowered, and 3) the boiling point is elevated.

All four of these properties, referred to as the colligative properties of solutions, are interrelated and are mathematically convertible. Therefore, the accurate determination of any one of these properties allows estimation of the other three, and is a measure of the osmotic concentration of the solution.

Because of practical considerations, direct measurement of osmotic pressure of ionic and biological solutions has not achieved widespread use. Instead, the measurement of freezing point depression, one of the oldest and easiest methods, has been used extensively for determination of the osmotic concentration of biological fluids, and is referred to as freezing point depression osmometry. By this method, the specimen to be analyzed is pipetted into a sample tube, which is then placed in the cooling chamber of the osmometer. The chamber is maintained at a temperature well below the range of freezing points to be encountered (for biological fluids, the bath temperature is usually -7° to -8°C).

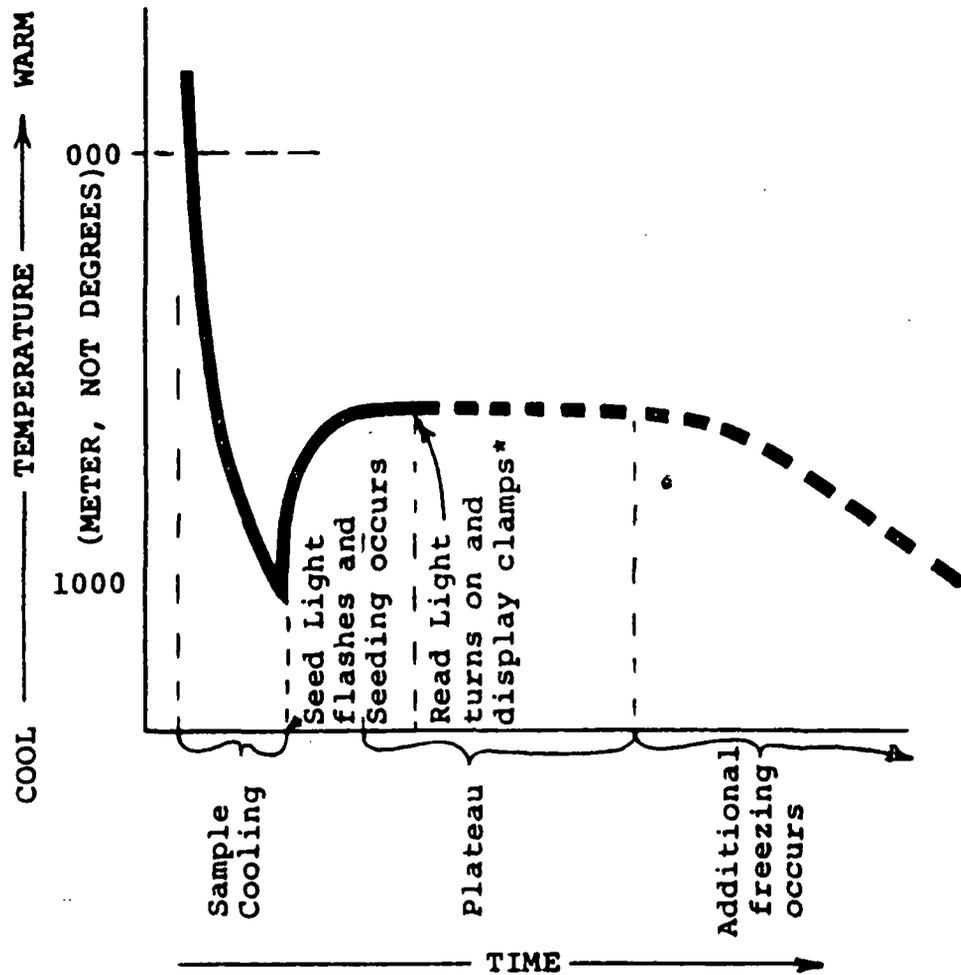
During the analysis cycle, the samples are evenly cooled to the same temperature, 1° to 2°C below the sample freezing point (i.e. supercooled), at which time crystallization is initiated, or the solution is "seeded". Seeding can be achieved by a variety of means including mechanical vibration, ultrasonic vibration, thermal shock, or addition of solid seed particles. The crystal formation results in release of the heat of fusion of water, causing the sample to warm to a point at which ice and solution exist in equilibrium, and the temperature remains constant (or reaches a plateau) for a period of time. This temperature plateau is the freezing point of the sample and is measured by use of thermistors (temperature-sensitive resistors). See **FIGURE 1-1**.

Osmotic concentration, or osmolality, is expressed in units of milliOsmoles (mOsm) per kg of water, where one mOsm is equivalent to one millimole of dissolved solute particles. A solution containing 1 Osmole (1000 mOsm) of dissolved solute per kg of water lowers the freezing point of water by 1.858°C . Therefore, the freezing point depression of the sample can be converted to units of osmolality, or osmotic concentration, by dividing by 1.858.

In practice, however, osmometers read directly in mOsm/kg H_2O by converting the thermistor readings by direct comparison with readings obtained using standard aqueous salt solutions of known osmolality.

Figure 1-1 shows an example of a typical cooling curve (variation of temperature with time) obtained with freezing point depression osmometry.

TYPICAL COOLING CURVE



*Display clamps only when **MODE** Switch is in **RUN**.

FIGURE 1

In terms of human physiology, osmolality has been of interest in the evaluation of fluid balance and the concentrating ability of the kidney, since the osmolality of the various body fluid compartments contributes significantly to the regulation of water excretion. Although urine osmolality varies with fluid intake, in general, a low urine osmolality can be indicative of impaired renal concentrating ability, as in chronic progressive renal disease or acute tubular necrosis. On the other hand, it can also be caused by congestive heart failure, diabetes insipidus, and severe burns.

Serum (or plasma) hyperosmolality generally results from decreased water intake, excessive water loss, or by accumulation of osmotically active substances such as glucose (in diabetes), sodium, alcohol, or drugs. However, because of the wide

variations found in urine osmolality under normal circumstances, it is usually more informative to examine the ratio of urine to serum osmolality, particularly after fluid restriction. With normal individuals this ratio may increase to as much as 4.7. In patients with renal tubular deficiency, the ratio drops below 1.0, and in cases of diabetes insipidus may fall to between 0.2 and 0.7 even after fluid restriction. In cases of neurogenic polyuria, the ratio is usually normal without fluid restriction and increases after fluid restriction.

1-4 CHEMICAL PRINCIPLES OF THE PROCEDURE

With freezing point depression osmometry, a sample of the specimen to be analyzed is pipetted into the sample tube and placed in the cooling chamber of the instrument. The sample is supercooled (cooled below the freezing point) and then seeded (crystallization initiated) by one of a number of methods (see **Paragraph 1-3 Principles of Operation**). The sample temperature rises due to the heat of fusion released during the freezing process until equilibrium; at this point, only a small fraction of the water is frozen, after which more ice freezes and the temperature begins to decrease again, resulting in a flat region, or plateau, in the cooling curve.

The temperature at the plateau is the freezing point of the sample and can be converted to units of osmolality (osmotic concentration) by observing that 1.0 Osmole depresses the freezing point of water by 1.858°C, where 1.0 Osmole = 1.0 mole of osmotically active particles = $\phi (n)(C)$,

where ϕ = osmotic coefficient

n = number of particles resulting from dissociation of each molecule in solution,

and C = concentration of each molecule in mol/kg water.

Using sodium chloride as an example, assume 29.0 g NaCl dissolved in 1.0 kg water, with

$$\phi = 0.93,$$

$n = 2$ dissolved particles (Na & Cl) per molecule

and $C = 0.5$ mol/kg H₂O;

$$\begin{aligned} \text{therefore, osmolality} &= \phi (n)(C) = 0.93(2)(0.5) = 0.93 \text{ Osmoles/kg H}_2\text{O} \\ &= 930 \text{ mOsm/kg H}_2\text{O}. \end{aligned}$$

Thus, freezing point depression = $0.93(-1.858^\circ\text{C/Osmole}) = -1.728^\circ\text{C}$

Therefore, the freezing point is = -1.728°C

Conversely, given an unknown sample with

freezing point = -0.278°C ,

freezing point depression = -0.278°C

and osmolality = $-0.278 \div -1.858$

= 0.150 Osmoles/kg H_2O

= 150 mOsm/kg H_2O .

However, the most commonly used technique for converting the voltage reading from the thermistor to units of mOsm/kg H_2O is direct mathematical comparison with standards of known osmolality by calibration of the instrument. (See **Paragraph 1-3 Principles of Operation**).

1-5 WARNING:

The OSMETTE is to be calibrated by using carefully prepared salt standards. As with any Standards used during calibration, the accuracy of subsequent assays depends upon the accuracy of the Standards used at the time of calibration and assay. Many factors can contribute errors in these materials. Some factors take place within the lab, and include such inappropriate acts as leaving tops off bottles, pipetting from bottles, using wet test tubes, using calibration material beyond its expiration date, using wrong materials, tampering, improper storage, freezing during storage, etc.

Other errors can occur prior to receipt by the lab, such as errors in manufacture, freezing of aqueous (and other) solutions during transportation or storage, overheating, tampering, etc.

Similarly, manufactured products for analytical work or biological applications are dependent upon avoidance of all these same problems and, of course, some additional ones, such as volume and/or weight errors. It is therefore necessary that all manufactured products and, where possible, their constituents, be validated prior to use, and that the final products also are validated.

An easy way to check many aqueous products is to use the OSMETTE as a Quality Control device. For example, where solutions are to be manufactured, one may choose to establish a proper osmolality value and range for the end product or intermediary product based on satisfactory pilot runs, and thereafter compare actual data with this previously established range. While such practice will not solve all possible errors, it does provide a quick one- to two-minute check, which will pick up many errors.

It is obvious, however, that the same concerns that exist in other standards and reagents equally apply to osmometry standards. To assure that the errors that can occur during the manufacturing process are eliminated at the source of errors, Precision Systems for more than 20 years has manufactured all the standards which it uses, thus

being able to quality control its standards by batch, rather than only by sampling some bottles from a shipment, as is the problem for other osmometer manufacturers.

To reduce the other errors prior to the standards' arriving in the laboratory, Precision Systems provides various values of CON-TROL™, which are standards in sealed glass ampules. These are recommended to all users needing accurate results. These ampules should be used either as calibrators or to verify, at least weekly, the standard solutions which Precision Systems provides in bottles. This recommendation is not because the standards prepared for the OSMETTE drift with time, but solely because of possible misuse within the lab or prior to reaching the lab.

One of the potential sources of errors in standard solutions during transportation relates to the fact that when an aqueous solution freezes, generally it is pure water which turns to ice, putting additional pressure, as the frozen water expands, on the remaining contents of the bottle. These liquid contents have increased concentration, since water has been pulled from the solution as ice. Thus, a more concentrated brine is under pressure to escape the bottle, and in fact will force its way out of even a tightly-capped container. The result is that when the solution subsequently thaws, it is more dilute. Thus, one should take particular notice of any salt accumulation inside the cap, and particular care to validate such solutions. Glass ampules, in contrast, will generally break upon freezing. Occasionally, a seal can be opened by increased pressure, resulting, again, in some change of concentration and salt on the outside of the ampule.

Care should be taken to use only Precision Systems standards and ampules, and they are recommended for all makes of true osmometers. There is a product, however, which calls itself an osmometer, and a vapor pressure osmometer, while it is, in fact, neither. This product measures dew point by a method which volatilizes, condenses, and re-volatilizes a portion of the solvent, and has been demonstrated to provide seriously erroneous data, particularly in the presence of volatiles, according to lab reports¹ and a number of papers.^{2 3 4 5}

It may not occur to the reader that biological solutions may contain volatiles. However, volatile solutes in blood samples, for example, can be of substantial importance. Two frequently encountered volatile components are carbon dioxide and alcohol. In fact, alcohol may be observed in fetal and newborn blood as the result of the fetal alcohol syndrome, which has been assessed as one of the largest assignable causes of mental retardation.⁶ As indicated by Rocco⁴, the dew point method results in errors of as much as 80 mOsm, while the freezing point method provides accurate results in the presence of alcohol, etc.

Similarly, not all osmometry standards are the same, as indicated by a letter to the editors of Clinical Chemistry, which indicates that the standards used on the dew point instrument are not the correct labelled values, which are listed in Standard Methods of CLINICAL CHEMISTRY.⁷

When introducing a new bottle of standard to the lab, it is recommended that it be compared with previous calibration standards believed to be accurate, or better, with the contents of sealed ampules.

Note that an ampule which has been opened will quickly change its value.

Be suspicious of any bottle of standard which has only a quarter to a third of its material remaining, or which, on receipt, has apparent loss of contents or salt in the cap.

To validate a newly-received instrument with its Standards, compare with salt standards prepared in accordance with specifications,⁷ and/or ampules. The inherent precision and calibratable accuracy of the OSMETTE are better than almost any instrument found in the lab.

Thus, the properly used OSMETTE can be a powerful, useful tool to assist in providing accurate analytical determinations for the laboratory and the manufacturer.

1-6 SPECIFICATIONS

Sample Size	50 μ l
Precision (1 SD)(mOsm/kg) <500	2
>500	.5%
Range (mOsm/kg)	0 - 3000
Calibration Standards	2
Calibration	User Acceptance of Calibration
Cooling Agent	Thermoelectric
Time/measurement	50 - 70 seconds
Time/initial cool-down	Approximately 5 minutes
Sample Tubes	Disposable Plastic
Display	Four-Digit Numeric
Power Requirements	115 v AC (\pm 10%), 50/60Hz
Dimensions	5"W x 15"D x 10"H (13 x 38 x 24 cm)
Weight	16.5 lbs. (7.5 kg)

REFERENCES

1. Weisberg, H.F., Data from ASCP Workshop on Osmolality, January 24, 1974.
2. Weisberg, H.F., Letter, **Clin Chem** 21: No. 8, 1975, p 1182.
3. Dorwart, W.V. et al, **Clin Chem** 21: No. 8, 1975, p 1185.
4. Rocco, R.M., Letter, **Clin Chem** 22: No. 3, 1976, p 399.
5. Champion, H.R. et al, Alcohol Intoxication and Serum Osmolality, **The Lancet**, June 28, 1975, 1402-1404.
6. Freier, E.F., et al, **Textbook of Clinical Chemistry**, ed. N.W. Tietz, W.B. Saunders Co., Philadelphia, PA (1986), pp 129-135, 40, 1251-1253, 1296-1297, 1300-1302, and 1838-1839.
7. Hanson, J.W. et al, Fetal Alcohol Syndrome, **JAMA** 235, 1976, p 1458-1460.
8. Johnson, R. B. et al, **Standard Methods of CLINICAL CHEMISTRY** 5, 1965, p 159

SECTION 2

INSTALLATION

2-1 PACKING LIST

Your μ OSMETTE was carefully packaged prior to shipment, and each accessory checked to assure that you would receive all parts necessary for proper operation. To avoid mislaying or throwing away these accessories, please check (\checkmark) that you have the following:

Cat. No.	MODEL 5004
() 5004	1 μ OSMETTE
() 2094	1 Instruction Manual
	Calibration Standards:
() 2101	1 100 mOsm/kg H ₂ O
() 2105	1 500 mOsm/kg H ₂ O
() 2202	1 CON-TROL™ 290
() 2014	1 Spare Temperature Probe
() 2033	1 pk Spare Seed Wires
() 2023	1 pk 50 μ l Disposable Test Tubes
() 2024	1 pk 200 Disposable Pipette Tips
() 2026	1 50 μ l Pipette
() 2044	1 Test Tube Rack
() 2073	1 Calibration Screwdriver

Check off the items on the packing list, and report any omissions to Precision Systems Inc. immediately. Call 508-655-7010 and ask for Customer Service.

2-2 WARRANTY INFORMATION

PLEASE RETURN YOUR WARRANTY CARD NOW. Its return will greatly assist in providing information and assistance to you, and in repairing and returning your instrument as quickly as possible, should a repair become necessary.

Your μ OSMETTE and its accessories are covered by a LIMITED WARRANTY, shown on page - i - at the back of this Manual.

If any problem develops, please take the following steps:

1. Check **SECTION 9: MAINTENANCE** of this Manual to isolate or solve the problem.

2. Notify us or the dealer from whom the instrument was purchased, giving details of the difficulty; include instrument name or model number, serial number, and date the instrument was received. You will be given service information or shipping instructions, or local service will be arranged.

ALL CLAIMS FOR SHORTAGE OR DEFECTIVE ITEMS MUST BE MADE TO PRECISION SYSTEMS WITHIN TEN (10) DAYS OF PURCHASER'S RECEIPT OF SHIPMENT. A RETURNED GOODS AUTHORIZATION NUMBER MUST BE OBTAINED BEFORE RETURNING ITEM.

2-3 SET-UP PROCEDURE

Installation can be performed by following the instructions below.

2-3-1 UNPACKING

Inspect the shipping container for visible damage. Shipping damage should be reported to the carrier immediately. Do not discard any damaged pieces of the container or packing material.

2-3-2 IMPORTANT ENVIRONMENTAL CONDITIONS

Remove instrument and accessory kit from box and place instrument on work surface.

NOTE: Since the principle of the OSMETTE is dependent on efficient cooling, allow at least 3 inches on both sides and back of the instrument.

DO NOT place near heat-generating devices or air conditioning outlets.

Work area should be free of vibration (avoid benches with centrifuges).

2-3-3 POWER SWITCH AND CORD

Check rear of instrument and place ON/OFF switch in the OFF position. Connect power cord to the proper power outlet after checking the requirements for voltage and frequency. This information can be found on the serial tag located at the rear of the instrument.
(See **SECTION 8: HAZARDS & WARNINGS**).

2-3-4 SET-UP

Because of its unique integrated design, your OSMETTE comes to you completely set up, ready for you to plug it in and run standardizing samples.

The Operating Head is raised by pressing the Head Release Button just to the right of the Head. (See **Figure 3-1.**) With the Operating Head raised, place a clean, dry test tube into the Refrigerator Well and turn on the OSMETTE with the Power Switch on the rear of the instrument (up is on, down is off). Note that the Digital Display and the red Cool Light turn on.

In a few minutes, the Cool Light will cycle on and off, indicating that the refrigeration system has reached operation temperature and the instrument is ready to use.

Do not attempt to use the OSMETTE until the light begins to cycle, as the refrigeration system will be too warm to give satisfactory results. Improved precision will be obtained if the OSMETTE is allowed about 15 minutes' warm-up to equilibrate all the circuitry.

****IMPORTANT** Always place a clean, dry test tube in the refrigerator well when not in use to prevent frost build-up.
(See SECTION 6-10.)**

Your instrument is now ready for operation.

Test tubes should be kept stored in such a way as to protect their cleanliness.

The instrument can be turned off when not in use, as there is no stand-by position required, and the life of the instrument will be extended in this manner. The Probe can be protected when the instrument is not in use by pressing down on the Operating Head to lower the Probe and Seed Wire into a clean, dry test tube.

**** IMPORTANT ** The μ OSMETTE was manufactured and intended for use with the Precision Systems disposable Test Tubes (#2023) only. Use of other tubes will result in poor performance and reliability.**

2-4 SHIPPING INSTRUCTIONS

Should it be necessary to ship your OSMETTE, pack the instrument carefully so that the packing material will not scratch the finish, and securely pack it in a sturdy carton. It is strongly recommended that this carton be packed in an outer carton with soft sponge or similar cushioning material between cartons and between the instrument and the inner carton.

Enclose Return Goods Authorization Number, together with reason for return and name, address and telephone number of the person returning the instrument.

For returns for service, please include a Purchase Order number for the repair authorization, details of the problems encountered, and date the instrument was originally received, together with name, address and telephone number of the person returning the instrument.

Mark the carton WELL with "FRAGILE" labels.

SECTION 3

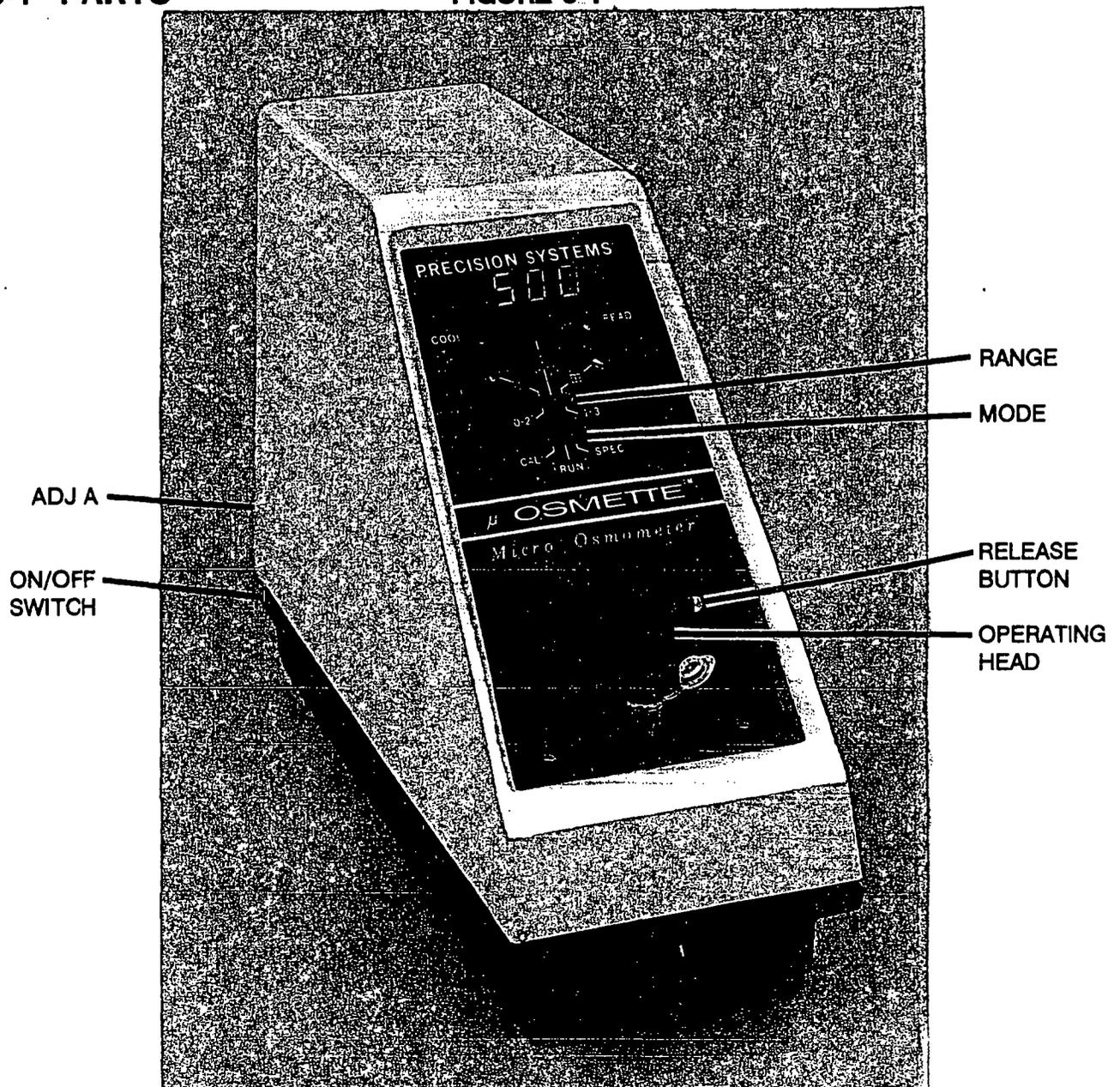
DESCRIPTION

3-1 PURPOSE

The μ OSMETTE is a complete system for holding a sample, cooling the sample to a definite temperature at a controlled rate, freezing the sample through vibration, isolating the sample in a cold environment, measuring the temperature of the sample during the entire process, and finally indicating the total concentration of the sample -- all automatically. Unique design of the μ OSMETTE makes each of these steps very reproducible through automation, and thereby eliminates operator technique, fatigue, and many sources of error.

3-1 PARTS

FIGURE 3-1



3-3 OPERATING HEAD

The Operating Head (**Figure 3-1**) is spring-loaded so that it will pop up automatically when a measurement cycle is completed, or when the Head Release Button is pressed.

Mounted under the Operating Head are the Seed Wire and the Temperature Probe. The black nosepiece centers the sample tube in the Refrigerator Well. The Seed Wire will vibrate to agitate the sample and cause it to freeze, if properly supercooled.

The tip of the Temperature Probe is its sensitive area, and should be in the center of a 50 μ l sample placed in one of the test tubes. Care should be taken to avoid bumping the Temperature Probe.

Lowering the Operating Head activates a micro switch which starts the measurement cycle. Raising the Head stops these functions and resets the measuring and control circuits for the next sample.

3-4 REFRIGERATOR

The Refrigerator Well is thermoelectrically cooled. This makes possible an entirely solid state Refrigerator, with no moving parts other than a small fan. The fan is located in the rear of the instrument. Be careful to avoid placing the instrument against a radiator or other heat source, and to avoid blocking the air outlet. Any such adverse positioning of the instrument will slow the cooling of the Refrigerator so that it will take longer to reach operating temperature. It is suggested that the OSMETTTE be placed in the coolest area of the lab. The air intake is under the front lip of the OSMETTTE.

The temperature of the Refrigerator is controlled through a proportional controller to maintain that temperature very precisely. The COOL Light is on during the cooling portion of the refrigerator cycle, and turns off when the Refrigerator Well is allowed to warm slightly. When working with samples of high concentration, a lower refrigeration temperature is desirable. Switching range from 0-2 to 1-3 automatically lowers the refrigeration temperature to a precisely controlled new temperature, and enables the operator to work with a highly reproducible and correct environment for each sample without having to fuss with thermometers and controls, as required by earlier instruments.

3-5 THERMOMETER

The temperature of the sample is read by a precision resistance temperature sensor, called a "thermistor", used in a Wheatstone Bridge circuit. The output of the Wheatstone Bridge operates the Digital Display and other circuitry. Thus, one may read the temperature of the sample continuously during the entire cooling, supercool, freezing and measuring operations.

3-6 CONTROLS

3-6-1 ON/OFF Switch The ON/OFF Switch is located on the left rear of the instrument. The Off position turns off all internal circuits, as no stand-by position is required. Up is ON, down is OFF.

3-6-2 ADJ A This control is also located on the rear of the μ OSMETTE, and controls the amplitude of seeding of the sample. Correct adjustment should be a minimum setting (counterclockwise direction when facing the rear of the instrument), with consistent freezing and reproducible osmolality. This minimum setting will increase seed wire life. (See **SECTION 7-3-3.**)

3-6-3 RANGE The Range Switch is the uppermost of the two 3-position slide switches located on the front panel. The OSMETTE incorporates two temperature ranges for its two overlapping concentration ranges: 0 to 2000 mOsm/kg H₂O and 1000 to 3000 mOsm/kg H₂O. For running samples, the Range Switch should be at either extreme left (**0-2, CAL I**), or extreme right (**1-3, CAL III**). The center position, **CAL II**, is used for evaluating stability of the instrument. (See **SECTION 3-6-5.**)

****IMPORTANT**** When taking osmolality reading with the Range Switch in the 1-3 position, add 1000 to the reading for correct value in mOsm/kg H₂O.

3-6-4 CALIBRATORS The calibrators are screwdriver adjustments located on the front panel above Roman numerals I, II, and III.

****IMPORTANT**** Complete step-by-step calibration procedures are explained in **SECTION 6-7** of this Manual.

1. First Range Calibration (0-2000 mOsm/kg H₂O)

The **CAL I** control is used to calibrate the OSMETTE for direct readout in milliosmoles with the 500 Standard. After **CAL I** has been adjusted to make the Display read 500, a second Standard – generally 100 mOsm -- may be frozen, and **CAL II** adjusted to make the Display read correctly for this Standard, also.

2. Second Range Calibration (1000-3000 mOsm/kg H₂O)

This control permits calibration of the **CAL III** range after initial calibration with **CAL I** and **CAL II** is complete. **Note that all readings on the second range must have 1000 added to the meter reading.** Generally, a 1500 mOsm/kg standard is used for calibrating with **CAL III**.

3-6-5 MODE The Mode Switch is the lower of the two front panel slide switches. Samples are normally measured in the **RUN** (center) position

Occasionally, one may wish to follow the entire cooling curve by disabling the normal locking of the Digital Display at the moment of reading. The **SPEC** (Special) position is provided for this purpose. (See **FIGURE 1-1.**) Secondly, the **SPEC** position is used in conjunction with checking the stability of the instrument, or when recalibrating.

The **CAL** position is used to assist in evaluating stability of the instrument by substituting three different resistors for the Temperature Probe by switching range to I, II, or III. The readings are arbitrary, but should be stable \pm one digit after instrument warm-up.

3-6-6 COOL Light The **COOL** Light is located on the front panel, and indicates the status of the Refrigerator Well. When the **COOL** Light is on, the Refrigerator is cooling; when off, the Refrigerator is slightly warming. *On/off/on/off* cycling means the unit is at the correct temperature for running samples.

3-6-7 READ Light Also located on the front panel is the **Read** Light, which turns on to indicate completion of the measurement and clamping of the Digital Display (in the **RUN** position of the Mode Switch), or the point at which clamping would normally have taken place in the **SPEC** Mode.

SECTION 4

REAGENTS & SAMPLE HANDLING

4-1 REAGENTS

Osmometry standards are aqueous solutions of known osmolality containing sodium chloride with a trace preservative. Standard osmolalities are indicated on the bottle label. Catalog numbers for available Standards are as follows:

100 mOsm/kg H ₂ O	#2101	125 ml
300 mOsm/kg H ₂ O	#2103	125 ml
500 mOsm/kg H ₂ O	#2105	125 ml
900 mOsm/kg H ₂ O	#2109	125 ml
1000 mOsm/kg H ₂ O	#2110	125 ml
1100 mOsm/kg H ₂ O	#2111	125 ml
1500 mOsm/kg H ₂ O	#2115	125 ml
2000 mOsm/kg H ₂ O	#2120	125 ml
2500 mOsm/kg H ₂ O	#2125	125 ml
3000 mOsm/kg H ₂ O	#2130	125 ml
100 mOsm/kg H ₂ O	#2201	5 ml Ampules (12/pk)
290 mOsm/kg H ₂ O	#2202	5 ml " (10+1 ea.100 & 500/pk)
500 mOsm/kg H ₂ O	#2205	5 ml " (12/pk)

4-2 WARNINGS AND PRECAUTIONS

These are high purity salt standards for use in calibrating freezing point Osmometers. To maintain the accuracy of the standards:

1. Pour, do **NOT** pipet, from the bottle into a **clean, dry** sample tube. Pipet from this tube.
2. Replace cap immediately. Standards and samples will change their values fairly quickly if left uncovered.
3. Do **NOT** dilute contents.
4. Discard solution after use; **NEVER** return it to the bottle.
5. Discard the bottle when only 20% of the contents remain.
6. **CAUTION: DO NOT ALLOW THE BOTTLE OF STANDARD TO FREEZE.**
Freezing will cause pure ice to form, squeezing more concentrated

solution from the bottle, leaving a more dilute solution. If the bottle may have been frozen or if salt is observed inside cap, check the standard for accuracy. Validate with CON-TROL™ ampules often.

7. Standards contain a preservative. Harmful if swallowed. Avoid contact with eyes, skin and clothing.
8. Refer to **Warnings in Paragraph 1-5.**
9. Use care in opening ampules to avoid sharp edges.
10. The samples and standards must be pipetted into clean, dry sample tubes, taking care that no bubble of air is left in the sample. Careless pipetting will result in erroneous readings or the necessity of re-running the samples.

4-3 REAGENT PREPARATION

Standards should be used as packaged without further preparation.

4-4 REAGENT STORAGE AND STABILITY

Store at room temperature. **Do not allow to freeze.** See product label for expiration date.

4-5 INDICATIONS OF INSTABILITY OR DETERIORATION

1. Inability to recover control values.

If a standard (or control) is left open the osmotic value will increase due to evaporation. Therefore, if the user runs the standard and cannot get the value that the standard is supposed to be, it may be because of evaporation of the standard or freezing of the bottle.

2. Turbidity or other indication of microbiological contamination.
3. Each standard is marked with an expiration date. Do not use after this date.
4. Use CON-TROL™ ampules.
5. See also **Paragraph 1-5.**

4-6 SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Serum or heparinized plasma is the blood specimen of choice. Oxalated plasma should not be used. Blood should be collected following the usual precautions observed in venipuncture for clinical assays. If serum is to be used, allow blood to clot and then centrifuge for 15 minutes at approximately 900 rcf.

Urine should be collected in clean, dry containers without preservatives. Centrifuge the specimen to remove particulate matter.

A specimen of 50 μ l is required for the μ OSMETTE.

4-7 KNOWN INTERFERING SUBSTANCES

1. Anticoagulants other than heparin.
2. Particulate matter will tend to cause sample to pre-seed when supercooled, interrupting the measurement cycle.
3. Water or solids in the sample tubes.

4-8 RECOMMENDED STORAGE AND HANDLING OF SAMPLES

Blood and urine specimens should be analyzed within two (2) hours of collection and separation. If longer delays are necessary, specimens should be refrigerated from 0° degrees to 4°C until the analysis is to be performed. Prior to analysis, refrigerated specimens should be warmed and gently mixed to allow dissolution of precipitated solutes. Any undissolved particulate matter should be removed by centrifugation.

Refrigerated samples must be tightly capped to avoid the drying effect of the refrigerator environment.

SECTION 5

PRINCIPLES OF OPERATION

5-1 GENERAL

It should be noted that the OSMETTE is capable of reading to almost $\pm .001^{\circ}\text{C}$. Because of this readability, care should be taken to follow the same procedure each time a measurement is made on either standards or unknowns, to assure precision. To assist the user in understanding the steps involved, the following paragraphs should be read with particular care.

Only Precision Systems sample tubes, #2023, should be used with the μ OSMETTE. Other brands of tubes have demonstrated poor precision, as well as other problems.

5-2 THE OSMETTE CONTROLS THE THERMODYNAMICS

A 50 μl sample in a Disposable Test Tube is placed in the Refrigerator Well under the Operating Head. The Operating Head is lowered until it latches down, pushing the Temperature Probe and Seed Wire into the sample.

When the sample cools, the Digital Display changes from a negative number toward 1000. When the Display reaches a value near 1000 (an arbitrary but reproducible point equivalent to several degrees of supercooling), the sample is then seeded by vibration. Very slow transfer of heat from the sample to its environment takes place, enabling the instrument to follow precisely the temperature changes of the sample, and at the appropriate moment, to measure the temperature.

Seeding the sample by a one-second, high-amplitude vibration of the Seed Wire initiates crystallization without contamination. Suddenly, the entire sample freezes. This freezing is not solid, but is, rather, a snowing or slush formation, in which approximately 2.5% of the solvent turns to ice. This slush blanket forms an environment around the temperature-sensing Probe, which is measuring the temperature of the ice/solution equilibrium very precisely.

After crystallization (freezing) of the sample takes place, there is liquid and ice in temperature equilibrium. For every milligram of ice that forms, 0.080 calories of heat are released. If the entire 50 μl sample froze, approximately $50 \times 0.080 = 4.0$ calories would be released. Because the sample tube is isolated from any good heat transfer medium after seeding, no appreciable amount of heat can leave the sample, and it warms up. If all the 4 calories were available to warm up the sample, it would get very warm indeed: about $+77^{\circ}\text{C}$. Obviously, no ice forms at such a high temperature. Thus, an equilibrium of just enough ice forms to warm the supercooled sample to the freezing temperature of the sample, and this temperature is then measured and displayed by the OSMETTE. Only about 2.5% of the solvent turns to ice.

Since the OSMETTE automatically senses the correct and reproducible amount of

supercooling, and since it also indicates the optimum moment to read the warmed ice/sample equilibrium (by the READ Light's turning on and the Display's clamping, if the MODE Switch is in the RUN position), the technique required in earlier instruments is minimized.

- CAUTION:**
- 1. ALWAYS TAKE THE READINGS ON THE SAME RANGE ON WHICH THE SAMPLE IS SEEDED.**
 - 2. Because of temperature conditioning of the Probe, best precision and accuracy are achieved by discarding the first samples and then running subsequent samples without interruption.**

After the measurement is completed, the Operating Head automatically pops up, if the MODE Switch is on RUN. The concentration of the sample remains locked on the Display until the Head is lowered for the next measurement.

To observe the part of the cycle shown by the dashed curve in **FIGURE 1-1**, place the MODE Switch in SPEC at the start of the sample run. The Head will remain in the down position and the meter will continue to reflect variations of the Probe temperature. The Head Release Button can be pressed when the next sample is to be introduced.

Should a sample read off-scale, thaw and re-freeze the sample with the Range Switch set on the correct range for the sample's concentration.

All serum and urine samples should be measured with the OSMETTE in RANGE 0-2 (Osmols, which equals 0 to 2000 mOsm/kg H₂O). **When operated in the higher range, 1-3 (1000 to 3000 mOsm/kg), addition of 1000 to the Display reading is necessary for calibrating and running samples.**

5-3 PERFORMANCE CHARACTERISTICS

The μ OSMETTE Refrigerator should cool to its cycle temperature in less than five minutes from the last turn-on in a 72°F ambient. Samples of 50 μ l typically require less than 90 seconds, when working on the first range. Some samples, such as milk, which do not form cool/freezing curves as sharply as salt stands, may take somewhat longer, and may best be run on SPEC.

The typical precision of the μ OSMETTE may be expected to be <2 mOsm/kg H₂O for 1 SD for serum, urine and salt standards for concentration below 500 mOsm, and <0.5% for readings above 500.

The range of concentrations which may be measured are from 0 to 3000 mOsm/kg in aqueous solutions. Modified OSMETTES are available for a wide variety of purposes.

Forty serum and urine samples from a hospital population were run on both a μ OSMETTE and an OSMETTE A. The data showed a Correlation Coefficient of .999893, a slope of .998331, and a Y-intercept of -3.42821.

SECTION 6

OPERATION

6-1 GENERAL

As discussed in the previous section, the use of a uniform technique to obtain a reproducible amount of supercooling is necessary for precision, requiring that measurements and calibration be made on the same range as that used for seeding the sample. In addition, seed amplitude should be adequate for reliable seeding every time. No attempt should be made to run samples until the COOL Light has cycled the first time, or the Refrigerator temperature will be too warm. This light, signifying whether the Refrigerator is being cooled or slightly warmed, corresponds to the cycling light on an oven.

For those samples where maximum accuracy is desired, duplicate readings to obtain mean values may be made. Temperature conditioning of the Probe by running one "throwaway" sample before quickly running a group of samples will provide improved precision. The use of **only #2023** Sample Tubes is required.

Daily check of readings on calibration standards should be made, even though daily recalibration may not be necessary. Periodic verification of calibration accuracy, using ampules of Precision Systems CON-TROL™ reference standards, is suggested at least as often as the lab receives survey samples, and preferably daily.

To increase the rate at which measurements can be made, some users like to place the rack with sample tubes in a tray of ice water for a few minutes before testing. This practice can contribute to sizable errors at times, however. For example, a solution that contains a component which tends to precipitate when the solution is kept at a reduced temperature will change its reading with time. A similar problem may be encountered with uncovered samples, as the solvent and any volatile substance will evaporate. The use of the caps on the disposable sample tubes will assist in preventing errors from this source. However, the tubes must be opened prior to lowering the Operating Head, or damage to the Probe and Seed Wire will result.

6-2 OPERATION

1. Plug the OSMETTE into a grounded A.C. outlet of the proper voltage. (See voltage label.)
2. Raise the Operating Head and place a clean, dry sample tube into the Refrigerator Well. This will eliminate frosting of the Refrigerator Well.
3. Switch **ON/OFF Switch** to **ON**, and allow the Refrigerator to cool down while samples are being pipetted into the sample tubes.

4. Place **Range Switch** in **0 - 2** position (the left position) to run samples from 0 to 2000 mOsm/kg for serum, urine, and samples and Standards of similar concentration. Place **MODE Switch** in **RUN** position. For samples from 1000 to 3000 mOsm/kg, switch to **RANGE 1 - 3**, and **add 1000 to the reading**.
5. Check that the **COOL Light** has cycled. Remove the clean, dry sample tube and place a sample tube with 50 μ l of sample into the Refrigerator Well.
6. Lower the Operating Head so that the Seed Wire and Probe enter the tube, and the Head latches in the down position without forcing it. (NOTE: Occasionally, a bit of excess plastic on the edge of the tube will prevent latching. Remove the excess plastic. Excess frost buildup in the well can also prevent latching. The frost can be melted by turning the **OSMETTE** off for several minutes, or with several drops of alcohol, and removed with a cotton swab.)

****IMPORTANT** ALWAYS PLACE A CLEAN, DRY SAMPLE TUBE IN THE REFRIGERATOR WELL WHEN NOT IN USE.**

7. Observe the Digital Display: The reading is a negative number when the Probe is at ambient temperature, and indicates cooling of the sample by becoming more positive -- to 000 -- and running to approximately 1000. (Time: Approximately 70 seconds.)
8. When the Digital Display reaches approximately 1000, the sample is automatically seeded by a one-second, high-amplitude vibration of the Seed Wire.
 - a) If the Digital Display does not approach 1000, and a one-second vibration does not occur, the **READ Light** will not turn on, and no reading can be made. Check the sample for ice or other particles. (For pre-seeding, see **Paragraph 6-3**.)
 - b) If, after seeding, the **READ Light** turns on and the digits read a negative number, 1, or overrange, the sample should be re-run with the Range Switch on the alternate range, using a fresh sample.
 - c) If, after seeding, the Display blanks out, see **Paragraph 9-3**.
9. The **READ Light** will turn on after the sample is seeded (vibrated), and the Head will pop up for removing the sample tube and introducing the next sample.

****IMPORTANT** When on the second range (1000-3000 mOsm), add 1000 to a positive reading. If a negative number is observed, switch to the first range (0-2000 mOsm) and re-run with a fresh aliquot of sample (or thaw and re-use the frozen sample, if a shortage of sample exists).**

The measurement is complete. Before placing a new sample in the Well, wipe off the Probe and Seed Wire with a clean, disposable wipe, such as Kimwipes. **NOTE: DO NOT USE GAUZE PADS!!**

6-3 FACTORS AFFECTING REPRODUCIBILITY

1. Always use **#2023 Sample Tubes** from Precision Systems.
2. The O-ring on the nosepiece must be in place and gently press on the top of the sample tube when the Operating Head is lowered, so that the sample tube does not change position during seeding.
3. Carefully pipet samples to avoid capturing air in the bottom of the sample tube.
4. Probe height and position are important. (See **Paragraph 9-4-2.**)
5. Minimum seed amplitude necessary to seed reliably is important.
6. Run a series of samples or Standards in quick succession, discarding the first reading, as it thermally conditions the Probe.
7. Use reduced seeing amplitude if samples tend to foam, or use a very small amount of Dow Antifoam B.
8. Avoid re-running the same sample in the same sample tube.
9. Replace the damaged Probe if its calibration drifts.
10. An electric power outlet that is not properly grounded or has electrical noise on the ground or power lines can cause poor precision.

6-4 PRE-SEEDING

Pre-seeding means that a supercooled sample freezes without the instrument's having seeded the sample by vibration.

To obtain reproducible readings, it is necessary for the instrument to seed each sample or Standard. If pre-seeding occurs, check:

1. Probe position: It must not touch walls or bottom of the sample tube, and must be in the center of the sample.
2. Seed Wire position and length: Bend the wire close to the Probe and position it to be of about the same length as the Probe, cutting it off, if necessary.
3. Sample and tube cleanliness and freedom from crystals or precipitates.

6-5 RANGE EXTENDER

The Range Switch is used to extend the range above 2000 mOsm/kg. **CAUTION: When running samples on the second range, add 1000 to the observed reading.** If many samples between 1000 and 2000 are to be run, the workload will be completed more quickly if done on the second range. When shifting from one range to another, as the result of more concentrated or dilute solutions, always thaw and re-run the sample so it is seeded on the same range as it is read.

6-6 STANDARD SOLUTIONS

The calibration of a freezing point Osmometer is based on precise salt standards. Care in handling these solutions so as to avoid contamination is therefore important.

1. **NEVER** pipet from the bottle of Standard.
2. Keep the bottle tightly capped when not in use.
3. Replace the Standards periodically, and whenever their validity is in doubt. Standard Solutions are inexpensive compare with the time and samples wasted with an inaccurately calibrated instrument.
4. Do not dilute Standards. Since the activity coefficient varies with concentration, the freezing point is not linearly related to the amount of dilution. It is linearly related to the total effective concentration of ions and molecules, as is the Osmotic Pressure and its effect on fluid transport across membranes.
5. Refer to **SECTION 4**.

In addition to the 125 ml bottles of Standards, CON-TROL™ Reference Standards are available in 5 ml glass ampules for 100, 290 and 500 mOsm/kg H₂O.

The Standards are made under sterile conditions with very precise gravimetric methods to assure stability and accuracy. Refrigeration is not required. It is recommended that Standards be replaced at least every six months, and whenever two-thirds of the bottle has been used.

Refer to the Price List at the back of this Manual for available Standards. For values not listed, write to Precision Systems Inc. for directions for making special values, or for prices on prepared Standards.

6-7 CALIBRATION

Your Precision Osmometer was checked and calibrated on both temperature ranges

before leaving the factory. To assure maximum accuracy, Calibration Standards should be checked daily, or whenever a series of unknowns is to be run.

Be certain that you have factory-fresh, uncontaminated Standard solutions, or that any standard which you make up has been checked against the factory-made Standard.

Allow 10 to 15 minutes' warm-up after initial turn-on to completely equilibrate the temperature of the OSMETTE. Follow the operation steps in **SECTION 6-2**.

When calibrating the OSMETTE, **always use the 500 mOsm Standard with CAL I first, and the 100 mOsm Standard with CAL II thereafter.**

6-7-1 FIRST RANGE CALIBRATION (0-2000 mOsm/kg H₂O)

1. Pipet several 500 mOsm/kg Standards into clean, dry sample tubes (**#2023 only**), and run as described in **SECTION 6-2**. If the values are not within specification (500 ± 2 mOsm/kg), follow the calibration steps below.
 - a) Set **RANGE Switch** in **CAL I** position.
 - b) Set **MODE Switch** in **SPEC** position.
 - c) Place the calibration screwdriver in CAL I adjustment port.
 - d) Place a fresh sample tube containing 500 mOsm/kg Standard into Refrigerator Well and lower the Head.
 - e) Observe the Digital Display. Seeding (vibration) will occur when meter reads approximately 1000. Just prior to the Read Light's coming on, begin adjusting CAL I so that the Display reads 500 just as the Read Light comes on.
 - f) Return **MODE Switch** to **RUN** and verify correctness of calibration by freezing a fresh 500 mOsm/kg Standard. Repeat 500 calibration, if necessary.
2. Pipet several 100 mOsm/kg Standards into clean, dry sample tubes, and run as described in **SECTION 6-2**. If the values are not within specification (100 ± 2 mOsm/kg), follow the calibration steps below.
 - a) Set **RANGE Switch** in **0 - 2** position.
 - b) Set **MODE Switch** in **SPEC** position.
 - c) **IMPORTANT:** Place calibration screwdriver in position **CAL II** adjustment port.

- d) Run several 100 mOsm/kg Standards as in **Paragraph 6-7-1, b-e**, remembering to adjust the Digital Display to read 100.
- e) Return the **MODE Switch** to **RUN**, and verify correctness of calibration by freezing a fresh 100 mOsm/kg Standard. Repeat 100 calibration, if necessary.

****IMPORTANT**** Before placing a new sample in the Well, wipe off the Probe and Seed Wire with a clean, disposable wiper. **DO NOT USE GAUZE PADS!!**

The OSMETTE is now calibrated on the First Range (0-2000 mOsm/kg H₂O).

6-7-2 SECOND RANGE CALIBRATION (1000 - 3000 mOsm/kg H₂O)

The Second Range Calibration control is used to calibrate the 1000-3000 range without interacting with or changing the First Range calibration.

1. Set **RANGE Switch** in **CAL III** position.
2. Set **MODE Switch** in **SPEC** position.
3. **IMPORTANT:** Place Calibration Screwdriver in position **CAL III** adjustment port.
4. Run several 1500 mOsm/kg Standards as described in **SECTION 6-6-1, b-e**, remembering to add 1000 to the digital reading, and therefore adjusting the Digital Display to read 500.
5. Return **MODE Switch** to **RUN** and verify correctness of calibration by freezing a fresh 1500 mOsm/kg Standard. Repeat calibration, if necessary.

****IMPORTANT**** The Second Range (1000-3000 mOsm/kg H₂O) has been checked and adjusted by the manufacturer. **Further calibration of the Second Range is not necessary if the First Range is to be used exclusively.** Accuracy on the ends of the Second Range can be improved by adjusting CAL I for an end-of-Range 1 - 3 Standard (e.g., 1100 or 2500 mOsm). When returning to Range 0 - 2, recalibration of CAL I will be required.

6-8 OPERATING PRECAUTIONS

The μ OSMETTE must be properly grounded through its line cord to a low-impedance-to-ground electrical connection at the wall outlet. Avoid setting containers of fluid on the instrument, and immediately clean up any fluid spilled on it or in it before turning on the μ OSMETTE. **DO NOT OPERATE THE INSTRUMENT WITHOUT ITS COVER ON SECURELY.**

6-9 LIMITATIONS

The μ OSMETTE is intended to measure aqueous solution concentrations from 0-3000 mOsm/kg. Other ranges are available on special order.

Viscous solutions may require dilution, although osmolality is not generally linear with dilution. Samples which tend to foam will give incorrect and non-reproducible answers. This problem can generally be eliminated by the addition of a very small amount of Dow Antifoam B to the sample, or by decreasing the seed amplitude.

Samples containing undissolved particles in suspension or precipitate may cause pre-seeding, which may be eliminated by heating, filtering or centrifuging the sample.

NO READING SHOULD BE TAKEN EXCEPT WHILE THE OSMETTE HAS THE SAMPLE ON THE FREEZING PLATEAU (AFTER THE READ LIGHT TURNS ON).

6-10 HAZARDS

Should fluid be spilled into the OSMETTE, **UNPLUG THE INSTRUMENT** and thoroughly clean up, rinsing with distilled water and thoroughly drying before turning on the power.

Since there is **HIGH VOLTAGE** in various circuits within the OSMETTE, **do not leave the cover off or loose. Unplug the line cord when removing the cover.**

Review **SECTION 8: HAZARDS & WARNINGS.**

6-11 STAND-BY POSITION

Since the Refrigerator Well will frost up eventually if exposed to room air, it is suggested that an empty sample tube be left in the Well whenever the instrument is not in use.

To protect the Temperature Probe from accidental breakage, the Operating Head may be lowered into the tube. If the OSMETTE is to be left turned off, the Head will remain down until shortly after it is turned on again or the Head Release Button is pressed.

If it is desired that the Operating Head remain down when the OSMETTE is on, switch **MODE Switch** to **SPEC**.

****IMPORTANT** DO NOT FORCE A SAMPLE TUBE INTO A FROSTED REFRIGERATOR WELL.** If the Refrigerator Well has accumulated frost, turn off the OSMETTE for several minutes to defrost, and remove accumulated moisture from the Well with a "Q-Tip". **DO NOT LOWER THE OPERATING HEAD ONTO A CAPPED SAMPLE TUBE OR A TUBE WITH ICE IN IT, as this will damage the Probe.**

SECTION 7

PERFORMANCE DATA

7-1 PRECISION AND ACCURACY

Precision and accuracy are measures of any analytical system. Obviously, without satisfactory precision, one cannot effectively obtain accuracy.

In one hospital lab, a series of twenty serum and twenty urine samples were run six times each on the μ OSMETTE. The sample standard deviation: s on all the serum samples was under 2.0, with a mean s of 1.09. All but two of the urine samples also had standard deviations of less than 2.0, with the worst case of 4.37 (sample Coefficient of Variation: $CV = .69\%$), and a mean s for urines of 1.68.

In a second hospital lab, ten serums and seven urines were run on a μ OSMETTE with similar results: average s for serum was 1.45, and for urine 1.63. CV 's were less than 1.0% for serum and all but the lowest urines (150 mOsm $CV = 1.6\%$).

In both labs, the same samples were run in duplicate on the older OSMETTE A's, which had been in use in those labs for some time.

In the first lab, the correlation coefficient was 0.99989, while the second reported a correlation coefficient of 0.994 on serum and 0.999 on urine.

7-2 REPRESENTATIVE VALUES

Controls were run in the three labs as follows:

SAMPLE	OSMETTE A	μ OSMETTE
CAPHyland Control #1	258	257
CAPHyland Control #2	324	325
Clinitrol™ 290	290	290
Gibco Frozen Human Serum	298	299

The literature on osmolality (now extending to thousands of papers) suggests a normal range for serum of 280 to 300 mOsm/kg H₂O. Some authors suggest 270 as the lower limit of the normal range. The human range appears to be approximately 220 to 480 for serum, and 50 to 1500 for urine.

7-3 PRECISION EVALUATION ON AQUEOUS STANDARDS

Whenever maintenance is performed on the μ OSMETTE, the precision of the instrument should be checked and verified to be within specification.

To evaluate the μ OSMETTE for reproducibility, the following will provide a good estimation of the μ OSMETTE CV , at 500 mOsm/kg H₂O only.

Using a freshly-opened bottle of 500 mOsm/kg H₂O aqueous standard, run a total of 11 determinations. Disregard the first value of the series. Calculate the **CV** according to the following method, and check against stated specifications. A hand calculator will greatly simplify the arithmetic.

$$s = \sqrt{\frac{\sum(X-\bar{X})^2}{n-1}}$$

s = Standard Deviation

X = an Individual Value

\bar{X} = Mean Value of Series

$\sum(X-\bar{X})^2$ = Sum of each difference from the mean, squared

n = the Number of Values

EXAMPLE:

Sample Number	Value (X)	(X- \bar{X})	(X- \bar{X}) ²
1 (omit 1st reading)	497	---	---
2	502	0.5	0.25
3	501	0.5	0.25
4	498	3.5	12.25
5	504	2.5	6.25
6	502	0.5	0.25
7	503	1.5	2.25
8	501	0.5	0.25
9	502	0.5	0.25
10	500	1.5	2.25
11	<u>502</u>	0.5	<u>0.25</u>

$$\sum X = \text{sum of } X = 5015$$

$$\sum(X-\bar{X})^2 = 24.50$$

$$\bar{X} = \text{Mean} = 501.5$$

$$n = 10$$

$$s = \sqrt{\frac{24.5}{9}} = 1.65$$

$$CV = \frac{100 \times s}{\bar{X}} = \frac{100 \times 1.65}{501.5} = 0.33\%$$

BIBLIOGRAPHY

- 1) Abele, J.E., **Am. J. of Med. Elec.**, Jan-Mar (1963), pp.32-41
- 2) Freier, E.F., et al, **Textbook of Clinical Chemistry**, ed. N.W. Tietz, W.B. Saunders Co., Philadelphia, PA (1986), pp. 129-135, 450, 1251-1253, 1296-1297, 1300-1302, and pp. 1838-1839
- 3) Wolf, A.V., **Aqueous Solutions and Body Fluids**, New York, Hoeber Medical Division, Harper and Row (1966)
- 4) **Clinical Diagnosis and Management by Laboratory Methods**, 16th ed., ed. J.B. Henry, M.D., W.B. Saunders Co., Philadelphia, Pa (1979), pp. 98-99
- 5) Haraway, A.W., Jr., et al, **J. Am. Med. Assoc.**, v. 205 (1968), pp. 506-512
- 6) Loeb, J.N., **N. Eng. J. Med.**, v. 290 (1974), p. 1184
- 7) Harrington, J.T., et al, **N. Eng. J. Med.**, v. 292 (1975) pp. 89-91
- 8) Lubowitz, H., et al, **Arch. Intern. Med.**, v. 134 (1974), pp. 1120-1124
- 9) McCluskey, R.T., et al, **N. Eng. J. Med.**, v. 282 (1973), pp. 564-570
- 10) Galambos, J.T., et al, Specific Gravity Determination: Fact or Fancy?, **N. Eng. J. Med.**, v. 270 (1964), pp. 506-508
- 11) Boyd, D.R., et al, Utilization of Osmometry in Critically Ill Surgical Patient, **Arch. Surg.**, v. 102 (1971), pp. 363-372
- 12) Glasser, L.G., et al, Serum Osmolality and Its Applicability to Drug Overdose, **A.J.C.P.**, v. 60 (1973), pp. 695-699

SECTION 8

HAZARDS & CAUTIONS

8-1 GENERAL

The OSMETTE incorporates safety features to protect the operator from injury, the instrument from damage, and the test results from inaccuracies.

Review Paragraph 1-5 for **Warnings**, and note the following:

8-2 ELECTRICAL

NOTE: THERE ARE HAZARDOUS VOLTAGES INSIDE THE OSMETTE. Therefore:

1. Connect the OSMETTE only to an electrical outlet with voltage shown on the OSMETTE's label.
2. The three-pronged line cord must be connected only to a matching three-wire grounded outlet. **Do not use an adaptor to connect the power plug to a two-pronged outlet.** If the electrical outlet will not accept the three-pronged line cord plug, notify qualified electrical maintenance personnel. They will supply the required electrical three-wire grounded outlet.

DO NOT try to force the line cord's plug into any receptacle.

DO NOT – UNDER ANY CIRCUMSTANCES – OPERATE THE INSTRUMENT UNTIL A PROPERLY GROUNDED ELECTRICAL RECEPTACLE IS PROVIDED *with* THE PROPER VOLTAGE FOR THE OSMETTE.

3. **DO NOT** connect the OSMETTE to an electrical outlet through an extension cord.
4. **DO NOT** use an outlet or electrical line which is used for centrifuges, ovens, refrigerators, or other equipment containing electrical noise-generating components.
5. **DO NOT** remove the cover of the OSMETTE without first disconnecting the line cord from the electrical outlet.
6. **DO NOT** operate the instrument without its cover.
7. Disconnect the power plug when performing any maintenance inside the OSMETTE case.

8-3 MECHANICAL

1. Place the instrument in a cool, dry, dust-free and draft-free environment, on a solid, level working surface. Leave space on each side and front and rear of the OSMETTE for air circulation.
2. Carefully wipe the Probe between samples.
3. If water or solutions are spilled onto or into the OSMETTE, **turn the instrument off immediately and immediately remove the electrical plug from the power receptacle** until clean-up is completed, to both the interior and the exterior of the instrument.
4. The Refrigerator Well must be clean, dry, and frost-free for proper results.
5. Always remove all pieces of broken Wire before installing new Wire.

8-4 STANDARDS

1. Standards can have the wrong value (See **Paragraph 1-5.**) Always check the accuracy of new Standards before accepting and using them.
2. Periodically -- daily or weekly -- revalidate the calibration of the instrument and the values of your Standards using CON-TROL™ ampules.
3. **DO NOT** use Standards beyond their expiration date.
4. No air bubbles should be in the bottom of the sample tubes.
5. When making an osmolality reading on Range 1 - 3 (1000 to 3000 mOsm), add 1000 to the number on the Display.
6. See **Paragraph 4-2** for additional cautions on handling Standards.

8-5 SAMPLES

1. Samples will evaporate and change value upon sitting or refrigeration, particularly when not tightly capped.
2. Observe all laboratory procedures and policies relevant to handling biological samples that may contain pathogens.
3. Dispose of all sample tubes in a manner that protects the user and all others from possible contact with pathogens.
4. Carefully pipet samples so that no bubble is included in the sample.
5. When making an osmolality reading on Range 1-3 (1000 to 3000 mOsm), add 1000 to the number on the Display.

SECTION 9

MAINTENANCE

9-1 GENERAL

The μ OSMETTE incorporates a number of unique features intended to simplify its operation, increase the average operator's precision, and reduce service problems. There is a minimum of mechanical parts, and no lamps to wear or burn out. However, proper care should be taken, as with any precision device, to assure long, useful life. Periodic maintenance, taking but a few minutes yearly, will help.

As previously discussed, the μ OSMETTE consists of five modular sections, any one of which can be replaced quickly to minimize "down time".

The fastest and most satisfactory maintenance is that provided by the user or the in-house service personnel, guided by the maintenance check list on the following pages. Thus, faster than you could receive instruction by phone or a visit from your local service person, you can probably pinpoint and correct the difficulty. Please, therefore, follow the points below before calling your Dealer service department or writing or calling the Service Department at Precision Systems.

9-2 PERIODIC MAINTENANCE

The μ OSMETTE should be checked periodically for dust accumulation on the rear grill, the front printed circuit board, the cooling fan, under the front edge of the instrument, and around the Refrigeration module. Should dust accumulate within the instrument, **unplug the power cord**, remove the cover, and clean completely, carefully using compressed air only.

Proper positioning of the Probe and Nosepiece should be checked.

DO NOT use bottles of Standards for more than six months after opening, or when less than one-quarter of the contents remain, or when accuracy is in doubt.

DO NOT save open ampules.

9-3 SERVICE ADJUSTMENTS

The μ OSMETTE has only three electronic controls which may require field adjustment. The first sets the temperature of the cooling module. The second provides the coarse adjustment of CAL I. The third is the adjustment of the seed amplitude (see **9-3-1** through **9-3-3** on the following pages).

9-3-1 Refrigerator Module Temperature Adjustment

****IMPORTANT**** Adjustment is needed only if the Refrigeration Module or the Logic Board (the vertical printed circuit board on the left of the instrument) is replaced.

1. Unplug the power cord and remove the cover.
2. Turn the module temperature control (trimpot) completely clockwise, and then turn it counterclockwise eight turns. (The temperature control trimpot is the one nearest the left rear corner of the instrument, on the vertical printed circuit board.)
3. Calibrate the instrument with 500 and 100 mOsm/kg H₂O Standards.
4. Fill the Well half full with alcohol.
5. Set **MODE** Switch to **SPEC**, and **RANGE** Switch to **0-2** position (extreme left).
6. Lower the Operating Head without a sample tube in the Well.
7. After seeding has occurred and the **READ** Light comes on, momentarily raise and lower the Operating Head to turn the **READ** Light off.
8. With the μ OSMETTE calibrated, and after a couple of minutes, the Digital Display should settle down to approximately 1400 as it indicates the refrigeration module temperature. (More positive numbers indicate colder temperatures.)
9. Adjust the temperature to 1600: turn the trimpot on the top rear edge of the logic board clockwise to raise the temperature, and counterclockwise to lower the temperature.
10. Repeat steps 1-8, readjusting **CAL** and trimpot, if necessary.

9-3-2 CAL I

CAL I is a 20-turn, fine adjustment control, and is used in conjunction with the coarse calibrator, a trimpot located on the top left corner of the front panel board. When a Probe cannot be calibrated on a 500 mOsm/kg H₂O Standard with **CAL I** alone, set **CAL I** 10 turns from either end (which puts the setting about in the middle of the trimpot), and calibrate roughly on a 500 mOsm/kg H₂O Standard with the coarse calibrator. Final calibrate with **CAL I**.

9-3-3 ADJ A (Seed Amplitude)

The correct setting of ADJ A will provide adequate seeding (freezing) and reduce Seed Wire breakage. The ADJ A potentiometer is a 3/4-turn control located outside, on the rear panel of the instrument, below the fan. Clockwise rotation (facing the rear of the instrument) decreases the vibration.

1. Set **ADJ A** at midpoint.
2. Run a 500 mOsm/kg H₂O Standard as described in **Paragraph 6-7-1**.
3. Observe the Display at seed point (meter reading +1000). The meter should immediately count down toward 500.
4. If the meter blanks, even momentarily, slightly increase ADJ A and run a fresh 500 Standard.
5. Repeat steps 2 through 4 until the sample seeds and freezes without the Display's blanking.

9-4 PARTS REPLACEMENT

9-4-1 Seed Wire replacement

****IMPORTANT**** Always remove all pieces of broken Wire before installing new Wire.

1. Remove the Operating Head Cover by removing the two screws.
2. Remove the block holding the Seed Wire in position.
3. Remove both parts of the broken Seed Wire.
4. **Unplug the instrument** and remove the instrument cover. Remove any broken pieces of Seed Wire inside the instrument.
5. Reverse the above procedure, installing a new Seed Wire. Be sure the block is square and its screw is tight, so that the Seed Wire does not bind during the seed pulse.
6. The Seed Wire should be bent slightly to be close to the Probe.
7. With the Operating Head down, the Wire should be completely free to move about in a loose position.

8. The length of the Seed Wire should be slightly shorter than the Probe. Cut the Seed Wire as necessary.

9-4-2 Probe Replacement

1. Remove the Operating Head Cover.
2. Observe the relative position of the Probe to that of the Seed Wire.
3. Loosen the Allen set screw on the front left side of the Operating Head.
4. Slide the Nosepiece up to expose the gold pins.
5. Unsolder the two leads from the Probe.

****IMPORTANT** Care should be taken not to overheat the gold pins and melt the Nosepiece.**

6. Loosen the front Allen set screw and remove the old Probe.
7. Insert the new Probe.
8. Adjust the Probe position in the sample as follows:
 - a) Place 50 μ l of sample in a test tube.
 - b) Holding the test tube up against the O-ring of the Nosepiece, compressing the O-ring slightly, adjust the position of the Probe until the tip is in the center of the sample vertically and horizontally.
9. Tighten the front set screw.
10. Solder the new Probe wires to the gold pins.
11. Adjust the position of the Nosepiece in relation to the test tube as follows:
 - a) Place an empty test tube in the Well.
 - b) Make sure the O-ring is still on the Nosepiece, and the left side Allen set screw for holding the Nosepiece is loosened.
 - c) Lower the Operating Head.
 - d) Press the Nosepiece down until it is slightly compressed against the top of the sample tube. (Seeding of samples should not cause the sample tube to bob about, or reproducibility will suffer.)
 - e) Tighten the allen screw firmly against the Nosepiece.
 - f) Make sure the wires from the Probe are not shorted to the inside of the Operating Head.
 - g) Replace the Operating Head Cover.

- h) Run a series of samples. Observe the reproducibility: Slight readjustment (raising or lowering) of the Probe may improve performance.
- i) See also **Paragraph 9-5**.

9-4-3 O-ring Replacement

Should the O-ring deteriorate or become overly compressed over time, replacement with a new O-ring (Precision Systems #2071) is recommended.

1. Remove the old O-ring from the instrument Nosepiece, using a screwdriver or similar tool. Be sure to remove the thin plastic ring attached to the foam rubber.
2. Place a new O-ring, **plastic ring up**, around the Seed Wire and Probe.
3. Raise and press into place.

9-5 FUNCTIONAL CHECK

The purpose of the functional check is to confirm the proper functioning of the electronic circuits and the proper temperature of the Refrigeration Module. This test should be made after a fifteen (15) minute warm-up period.

9-6 FUNCTIONAL CHECK PROCEDURE

If the proper indication is not obtained in any of the following steps, note the number of the step and the fault symptom, if service is required.

1. Set up the μ OSMETTE as follows:
 - a) Place the **ON/OFF Switch** in the **OFF** position (down).
 - b) Plug the instrument into a wall socket.
2. With the Operating Head raised, turn the **ON/OFF Switch** to **ON**.
 - a) **READ** Light may be on or off, and **COOL** Light will be on.
 - b) After about 20 seconds, the **READ** Light will go off (if it is on), and there will be a number on the Display.
3. Set **MODE** Switch to **CAL** position, and **RANGE** Switch to **0 - 2** position.
 - a) A number between 000 and 1000 should be on the Display.
 - b) The number should remain constant within ± 1 .

4. Set **RANGE** Switch to **II** position.
 - a) The display should read 400, ± 150 , more negative than position **I** number.
 - b) The number should remain constant within ± 1 .
5. Set **RANGE** Switch in **III** position.
 - a) The Display should read the same as position **I**, ± 100 .
 - b) This number should remain constant within ± 1 .
6. Set **MODE** Switch to **SPEC**, and **RANGE** Switch to **0 - 2**.
 - a) Do a Refrigerator Module temperature check as described in **Paragraph 9-3-1**.
 - b) The resulting number on the Display should be within ± 25 digits of the previously recorded reading, and approximately 1600 ± 150 .

9-7 TROUBLESHOOTING GUIDE

To assist the user, a number of possible malfunctions or problems are listed below, together with appropriate functions or operations to be checked.

9-7-1 There is nothing on the Display, and the COOL Light is off.

1. Is the instrument plugged in properly and the ON/OFF Switch ON?
2. Is there power at the wall outlet?
3. Is the fuse at the right rear corner of the instrument open?

9-7-2 The Refrigeration Module does not cool the sample. (The numbers on the Display do not increase when the Operating Head is lowered into the sample.)

1. Is the COOL Light cycling?
2. Check the Well temperature (see **Paragraph 9-3-3**).
3. Is the 6½-amp fuse mounted internally on the power transformer open?

****CAUTION**** Be sure the power is off and the plug is removed from the wall outlet before removing the instrument cover.

9-7-3 The Display goes blank after the seed pulse. (Sample did not freeze.)

1. Is the Seed amplitude control adjusted correctly? (See **Paragraph 9-3-3**.)

2. Is the Seed Wire broken? Remove the Operating Head Cover to check. (See Paragraph 9-7-3.)
3. Is the Seed Wire properly adjusted? (See Paragraph 9-4-1.)
4. Are the correct sample tubes (Precision Systems #2023 only) being used?

9-7-4 The Operating Head does not latch down when lowered.

1. Is there any foreign material in the Well?
2. Is there frost or ice in the Well? If so,
 - a) Turn off the μ OSMETTE for a few minutes.
 - b) Remove moisture with a "Q-Tip".
 - c) Turn on the instrument.
3. Has the Nosepiece been adjusted as in Paragraph 9-4-2, 11?

9-7-5 Poor reproducibility is observed.

1. You must use Precision Systems #2023 Disposable Sample Tubes, 50 μ l size. This tube is designed to conform to the μ OSMETTE's refrigerator size and thermodynamic specifications.
2. Does the O-ring on the Nosepiece gently press on the top of the sample tube when the Operating Head is lowered, so that the tube does not change position during seeding?
3. Has 50 μ l of sample been reproducibly pipetted into clean, dry sample tubes without entrapment of air bubbles?
4. Are the Probe and Seed Wire properly positioned so that the Probe is in the center of the sample, per Paragraph 9-4-1 & 9-4-2? To verify:

Vertical position -- Hold a sample tube containing 50 μ l of Standard around the Probe and the Nosepiece, and note that the black dot at the end of the Probe is centered in the sample vertically.

Horizontal position -- Seed a 100 mOsm/kg sample in the normal way. Immediately lift the Operating Head by pressing the Head Release Button. Quickly remove the sample tube with the sample still frozen, and note in the frozen sample the position of the indentation caused by the Probe and Seed Wire.

5. Has Paragraph 6-3 been reviewed?
6. Has the Probe been replaced? Does it need to be? (See Paragraph 9-4-2.)

9-7-6 The Operating Head does not pop up after the READ Light turns on.

1. Is there frost or ice in the Well? (See Paragraph 9-7-4, 2.)
2. Is the Nosepiece positioned correctly? (See Paragraph 9-4-2, 11.)
3. Is the MODE Switch in the SPEC position? It should be in the RUN position for the Head to pop up.

9-7-7 The Seed Wire Is broken.

This is indicated when, during a normal run, the Display goes blank after the seed pulse occurs.

1. **Shut off the instrument immediately.**
2. With the Head in the up position, look at the surface of the step casting between the posts which support the Head. If the Seed Wire is not visible between the step and the bottom of the Head, a piece has fallen into the instrument. If the Wire is visible, test it: It should not move more the 1/8" (3 mm) in any direction.
3. Remove the Operating Head Cover and examine the Wire for breaks.
4. If any of these checks indicate a broken Wire, remove the clamp (inside the Head) and withdraw the Wire. Compare the Wire to a new Wire. If it appears that any pieces are missing, **unplug the instrument**, remove the instrument cover, and remove the missing piece. Once assured that all pieces are accounted for, replace the Wire as detailed in Paragraph 9-4-1.
5. Adjust the Seed amplitude as in Paragraph 9-3-3.

NOTE: Currently, replacement Wires and those in later instruments have longer rear legs. With these new Wires, the broken piece may be seen sticking slightly above the step casting (Step 2, above). Try to grasp this piece with tweezers and remove it. This will save having to remove the instrument cover.

The **OSMETTE**, **OSMETTE S**, **OSMETTE A**, and **μOSMETTE** are manufactured by Precision Systems Inc., 16 Tech Circle, Natick, MA 01760, USA. Phone: 508-655-7010. Fax: 508-653-6999.

Last revision of this Manual: 1/92.

GENERAL CHEMISTRY REVIEW

When one substance is dissolved in another, the result is a solution.

A solution is made up of the **solvent** (the larger part, and generally a liquid) and the **solute** (the smaller part, and frequently a salt). When the amount of the solute dissolved varies, four colligative (related) properties will vary, generally with a linear relationship to concentration for a dilute solution. Thus, when the concentration of the solution increases, the osmotic pressure is increased, the freezing point is lowered, the vapor pressure is lowered, and the boiling point is raised. Therefore, a simple, precise measurement of any one of these properties is a measurement of the concentration, and also of the other three properties.

A convenient unit of measure in chemistry is the **mole**. A mole is the weighed amount of a substance expressed in grams, which is equal to the molecular (formula) weight. Thus, a mole of sodium would be 23 grams, and a mole of sodium chloride would be 23 + 35.5, or 58.5 grams.

When making up solutions, two common methods are used to express concentration:

A **molar** solution consists of one mole of the solute dissolved in enough solvent to make a total of one liter of solution.

A **molal** solution consists of one mole of solute dissolved in a kilogram of solvent, resulting in more than a liter of solution.

Thus, the molal solution is slightly more dilute than the molar solution.

The freezing point of a solution is the temperature at which ice just begins to form. When dilute solutions freeze, the ice which is formed is pure, or very nearly pure, frozen water. The more ice which freezes, the more concentrated will be the remaining solution, since part of the solvent is being frozen out of the solution. It is therefore very important that precise freezing point measurements always freeze the same fraction of the total solution. This is accomplished by carefully supercooling samples and standards to the same temperature.

For every additional particle dissolved in a solvent, the freezing point will be lowered by a uniform amount. It has been found that a mole of particles dissolved in a kilogram of water will lower the freezing point by 1.858°C. If a mole of a strong electrolyte (a substance which ionizes into two or more particles when dissolved) is dissolved, two or more moles of particles per kilogram of water will result, with a proportional lowering of the freezing point.

For example, in dilute solutions, a mole of NaCl will form almost two moles of particles: a mole of Na⁺ and a mole of Cl⁻. Thus, the freezing point is lowered almost 2 x 1.858°C, while a mole of CaCl₂ will form almost three moles of particles, lowering the freezing point almost 3 x 1.858°C.

WARRANTY

THIS WARRANTY IS EXPRESSLY IN LIEU OF ALL OTHER WARRANTIES EXPRESSED OR IMPLIED, AND ALL OTHER WARRANTIES, INCLUDING WARRANTIES AS TO MERCHANTABILITY OR FITNESS, ARE EXPRESSLY EXCLUDED.

Precision Systems Inc. carefully tests each instrument prior to shipment, and checks the packaging for all accessories, to assure that the instrument will be complete and in good condition when you receive it. Please check packing material thoroughly to be certain that no instrument parts or accessories are discarded accidentally.

Precision Systems Inc. warrants each purchased instrument and accessory manufactured by them to be free from defects in workmanship and material. Liability under this Warranty is limited to servicing or adjusting the instrument and replacing any defective parts or accessories thereof. In no event: 1) shall the cost of the remedy exceed the purchase price; 2) shall Precision Systems Inc. or its Dealer be liable for any special, indirect, incidental, consequential, or exemplary damages, irrespective of whether attributable to contract, warranty, negligence, strict liability, or otherwise.

This Warranty is effective for one year after delivery to the original purchaser, and is not transferable. (Light Bulbs, Tubing, Sample Tubes, Probes and Stirring Wires are not included under this Warranty, as they are considered consumables.) If the fault has been caused by misuse or abnormal conditions of operation, repairs will be billed at cost. In this case, an estimate, if requested, will be submitted before work is started.

Precision Systems Inc. neither assumes nor authorizes any other person to assume for it any other liability in connection with the sales of Precision Systems Inc. instruments, accessories, reagents, standards or replacement parts or other consumables.

Without limiting the generality of the foregoing, any inconsistent language contained in requests for quotation, buyers' purchase orders, shipping instructions, or like documents is specifically rejected by Precision Systems Inc.

Since the instrument may possibly be usable for many applications, it is required of the user to determine its suitability and fitness for each application prior to placing the instrument into service for that application.

Chemicals, reagents, and standards can change their values and effectiveness because of improper storage or shipment conditions. Therefore, verification of the materials must be made by the user before placing them in use.

To obtain no-charge replacement of missing or defective parts or accessories, notification of the Seller must be made in writing within ten (10) days of receipt of shipment.

If any fault develops, please take the following steps:

1. Check the Maintenance Section to isolate or solve the problem.
2. Notify the Dealer from whom the instrument was purchased, giving full details of the difficulty. Include instrument name or model number, serial number, and date instrument was received.

CLAIM FOR DAMAGE IN SHIPMENT

Your instrument should be unpacked and tested as soon as it is received. If it fails to operate properly, or is damaged in any way, the packing material should be saved, and a claim should be filed with the carrier immediately.

If the damage is evident in the exterior packaging, this should be noted on the shipping documents provided to the Carrier.

If the damage is concealed, a written claim report should be filed with the carrier immediately. (A delay can invalidate the claim against the carrier, making the purchaser responsible for repair costs. Since concealed damage reports need to be filed within hours of receipt of the package, the product should be unpacked and tested as soon as it is received.)

The completeness of your shipment must be validated by the receiver immediately upon receipt, reviewing both the packing slip and the packing list (if an instrument) contained in the Manual showing the accessories which are included, and any claim for shortages must be made to the Seller within ten (10) days of receipt.

A full report of the damage should be provided to the Carrier's Claim Agent, with a copy forwarded to the Dealer from whom the instrument was purchased. You will then be advised as to the disposition to be made of the equipment and arrangements for repair or replacement.

Include serial number when referring to this instrument for any reason.

PRECISION SYSTEMS INC.

e-mail precisionsystems@msn.com

16 Tech Circle Natick, MA 01760 USA

Phone 1-508-655-7010

Fax 1-508-653-6999

OSMOMETER PRICE LIST

Cat.No.	Ordering Specifications	List Price
5004	<p>AUTOMATIC HIGH SENSITIVITY MICRO-OSMETTE™, Laboratory Model Osmometer for 50 µl samples, featuring: fully automatic operation with high reliability, 0.5" LED readout which retains the sample answer until the next sample is introduced; operating head which automatically rises at the end of determination; non-interacting calibration controls; unique, triple-point calibration verification; latest integrated circuit techniques for low power consumption and reliability; 60 samples/hour capability; fast-acting, solid-state refrigerator, eliminating pumps and bath liquid for STAT 3-minute cooldown; bench-saver design: only 5"W x 10"H x 15"D. Power 115v,* 50/60Hz. Complete with instruction manual, disposable sample tubes, 100 & 500 mOsm/kg calibration standards, test tube rack, spare temperature probe, spare stir wires, pipette and tips. Shipped assembled and ready to use.....</p>	\$6,189.75
5002	<p>AUTOMATIC HIGH SENSITIVITY OSMETTE A™, Laboratory Model Osmometer for both 0.2 & 2.0 ml samples, featuring: fully automatic operation; all solid state circuitry; large seven-segment readout; 30-60 sample/hour capability; a thermoelectric refrigerator which reaches operating temperature in less than 15 minutes; special, fast-equilibrating 55ml bath ensuring exact temperature for each sample, controlled to ±0.1°C; automatic bath temperature lowering for high concentrations; precise air temperature environment during sample isolation; 2-step calibration; non-interacting calibration controls; third-point calibration; range switching without recalibration. Power: same as above. Complete with instruction manual, 24 2.0 ml sample tubes, 12 0.2 ml sample tubes, spare stirring wires, 1 bottle each of 100 & 500 standards and bath liquid, test tube rack, spare temperature probe. Shipped assembled and ready to use.....</p>	\$5,953.50
4002	<p>SEMI-AUTOMATIC HIGH SENSITIVITY OSMETTE S™, Laboratory Model Osmometer for both 0.2 & 2.0 ml samples, similar to Model 5002, featuring: semi-automatic operation requiring only one pushbutton step throughout the otherwise fully automatic cycle; automatic plateau indication; automatic digital readout displayed directly in concentration, with no manual adjusting or nulling required; unlike Models 5004 & 5002 above, operator must be attentive at the instrument while each sample is run. Other features, power, size and accessories same as Model 5002. Shipped assembled and ready to use.....</p>	\$5,607.00

*Add \$100 for voltage other than 110/115v.

Prices in U.S. currency, effective 01/01/01

Subject to change without notice

FOB Natick MA USA

Minimum Billing: \$50.00

PRECISION SYSTEMS INC.

e-mail precisionsystems@msn.co

16 Tech Circle Natick, MA 01760 USA

Phone 1-508-655-7010 Fax 1-508-653-6991

Cat. No.	ACCESSORIES	List Price
2012	High Isolation Probe for 0.2 & 2.0 ml samples.....	\$286.23
2014	High Isolation Probe for Model 5004.....	286.23
2021	Sample Tubes, 2.0 ml, 12/pk.....	31.06
2022	Sample Tubes, 0.2 ml, 12/pk.....	103.37
2023	Disposable Sample Tubes, 50 µl for Model 5004, 500/pk.....	65.60
2024	Disposable Pipette Tips, 50 µl for Model 5004, 1000/pk.....	41.77
2026	Pipettor, 50 µl.....	21.65
2027	Pipetting Station w/pipettor and 200 tips for Model 5004.....	73.90
2028	<i>CLEARVIEW</i> Disposable Sample Tubes, 0.2ml, 500/pk.....	112.19
2031	Stirring Wires for 2.0 ml tubes, 4/pk.....	31.06
2032	Stirring Wires for 2.0 & 0.2 ml tubes, 4/pk.....	34.81
2033	Stirring Wires for Model 5004, 4/pk.....	34.81
2041	Rack for 0.2 & 2.0 ml sample tubes.....	35.34
2044	Rack for 50 µl sample tubes.....	34.80
2047	<i>CLEANETTE</i> Wipe Sticks, 1000/pk.....	113.53
2052	Plastic Dust Cover for Models 4002 & 5002.....	31.06
2071	O-Rings for Model 5004, 4/pk.....	26.25
2092	Instruction Manual for OSMETTE Model 4002.....	53.81
2093	Instruction Manual for OSMETTE Model 5002.....	53.81
2094	Instruction Manual for OSMETTE Model 5004.....	53.81
2100	Bath Liquid, ready to use, 250 ml.....	14.99

CALIBRATION STANDARDS

2101	Standard Solution, 100 milliosmoles, 125 ml bottle.....	10.71
2103	Standard Solution, 300 milliosmoles, 125 ml bottle.....	10.71
2105	Standard Solution, 500 milliosmoles, 125 ml bottle.....	10.71
2109	Standard Solution, 900 milliosmoles, 125 ml bottle.....	10.71
2110	Standard Solution, 1000 milliosmoles, 125 ml bottle.....	10.71
2111	Standard Solution, 1100 milliosmoles, 125 ml bottle.....	10.71
2115	Standard Solution, 1500 milliosmoles, 125 ml bottle.....	10.71
2120	Standard Solution, 2000 milliosmoles, 125 ml bottle.....	10.71
2125	Standard Solution, 2500 milliosmoles, 125 ml bottle.....	10.71
2130	Standard Solution, 3000 milliosmoles, 125 ml bottle.....	10.71
2201	CON-TROL 100, 100 mOsm Reference Solution, 5 ampules, 12/pk.....	28.65
2202	CON-TROL 290, 100 mOsm Reference Solution, 5 ampules.....	
	including 10 of 290 mOsm plus 1 each of 100 & 500 mOsm.....	28.65
2205	CON-TROL 500, 100 mOsm Reference Solution, 5 ampules, 12/pk.....	28.65

Prices in U.S. currency, effective 01/01/01

Subject to change without notice

FOB Natick MA USA

Minimum Billing: \$50.00



PRECISION SYSTEMS, INC.

16 TECH CIRCLE • NATICK, MA 01760 • 508-655-7010 • FAX 508-653-6999

AE00

Page

1

DATE	NUMBER
6/25/01	S19403

THIS NUMBER MUST APPEAR ON ALL CORRESPONDENCE

BILLING ADDRESS:

Fisher Scientific Company
Accounts Payable
PO Box 535700
Pittsburgh, PA 15253-0001

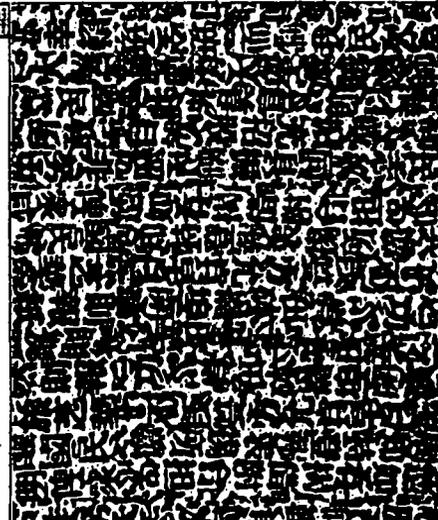
SHIPPING ADDRESS:

University of California
7835 Trade St., Suite 100
Attn: Shannon Hefler/UREY/7108
San Diego, CA 92121

PO# DR5948587

YOUR ORDER NO. DR5948587	SHIPPED VIA UPS 3rd Party	F.O.B. Factory	TERMS 1 & 10, Net 30 Days	PREPAID OR COLLECT
-----------------------------	------------------------------	-------------------	------------------------------	--------------------

QUANTITY	DESCRIPTION
1	'5004 Micro-OSMETTE, S/N EE06100 - Customer PO# 10200264



CLAIM FOR DAMAGE IN SHIPMENT

Your Instrument should be unpacked and tested as soon as it is received. If it fails to operate properly, or is damaged in any way, the packing material should be saved, and a claim should be filed with the carrier immediately.

If the damage is evident in the exterior packaging, this should be noted on the shipping documents provided to the Carrier.

If the damage is concealed, a written claim report should be filed with the carrier immediately. (A delay can invalidate the claim against the carrier, making the purchaser responsible for repair costs. Since concealed damage reports need to be filed within hours of receipt of the package, the product should be unpacked and tested as soon as it is received.)

The completeness of your shipment must be validated by the receiver immediately upon receipt, reviewing both the packing slip and the packing list (if an instrument) contained in the Manual showing the accessories which are included, and any claim for shortages must be made to the Seller within ten (10) days of receipt.

A full report of the damage should be provided to the Carrier's Claim Agent, with a copy forwarded to the Dealer from whom the instrument was purchased. You will then be advised as to the disposition to be made of the equipment and arrangements for repair or replacement.

Include serial number when referring to this instrument for any reason.