WESCOR®

Colloid Osmometer
Model 4420

Operator & Service Manual

M2054-3A

WARNINGS

Power source voltage for this instrument is indicated on the rear panel. Be sure to connect to the correct voltage source.

To prevent fire or shock hazard, do not expose this instrument to rain or any type of moisture.

IMPORTANT CAUTIONS

The pressure transducer used in this instrument is sensitive and delicate. Carefully read Section 2.2 for important information about the pressure transducer *before* attempting to operate the osmometer.

Never subject the instrument to freezing temperatures while liquid remains in the osmometer reference chamber cell. Serious damage could result.

Never leave colloid solution in the sample chamber after testing. Flush the sample chamber with saline after testing to promote membrane longevity.

U.S. Patent Number 4,150,564. U.K. Patent Number 2 018 430. Canadian Patent Number 1,122,033.

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SECTION 1 – Introduction and Specifications

Thank you for purchasing the Wescor Model 4420 Colloid Osmometer. We believe it is the finest instrument of its type available anywhere. Before you begin using the osmometer, please study the first three sections of this manual, which contain important information you must have to operate the instrument. The remaining sections offer more detailed information and procedures that you will need to refer to later.

We have attempted to make this operator's manual easy to read and convenient to use, so that you will want to refer to it often. The following is a brief description of the information in this section. Each section has a similar outline, to help you quickly locate the information you need.

SECTION 1 contains an overview of this operator's manual (1.1), a description of the Colloid Osmometer (1.2), a list of the instrument's features (1.3), a description of the osmometer's operating controls and connections (1.4), and instrument specifications (1.5).

1.1 – Operator's Manual Overview

This manual provides the information and procedures you need to set up and operate your Colloid Osmometer.

Section 2 contains important information about the pressure transducer and the installation and care of the semipermeable membrane.

Section 3 contains proper calibration procedures.

Section 4 contains proper operation procedures.

Section 5 contains information about the theory of operation.

Section 6 contains preventive maintenance information, as well as solutions for routine problems you may encounter.

Appendix A lists available supplies, accessories, and replacement parts.

Appendix B contains detailed technical information about the instrument electronics.

The Bibliography lists authors cited in this manual and sources for literature about colloid osmotic pressure measurement and applications.

The Index helps you quickly find the information you need.

1.2 – Instrument Description

The Model 4420 Colloid Osmometer is a compact, user-friendly instrument. It measures the colloid osmotic pressure (COP), or oncotic pressure, of high molecular weight blood solutes that are non-diffusible through the vascular membrane. The operating principle is based upon the movement of water molecules and diffusible solute particles through a synthetic semi-permeable membrane, a phenomenon known as transudation. The membrane separates the specimen solution (in the sample chamber) from a reference solution (in the reference chamber).

After a sample is injected, fluid from the reference chamber moves through the membrane and into the sample chamber until an equilibrium hydrostatic pressure is reached. This pressure is measured by a precise pressure transducer and associated electronic circuitry. Results are digitally displayed on the instrument front panel.

If you would like to learn more about how the Colloid Osmometer operates, refer to Section 5 for the theory of operation and additional descriptive detail.

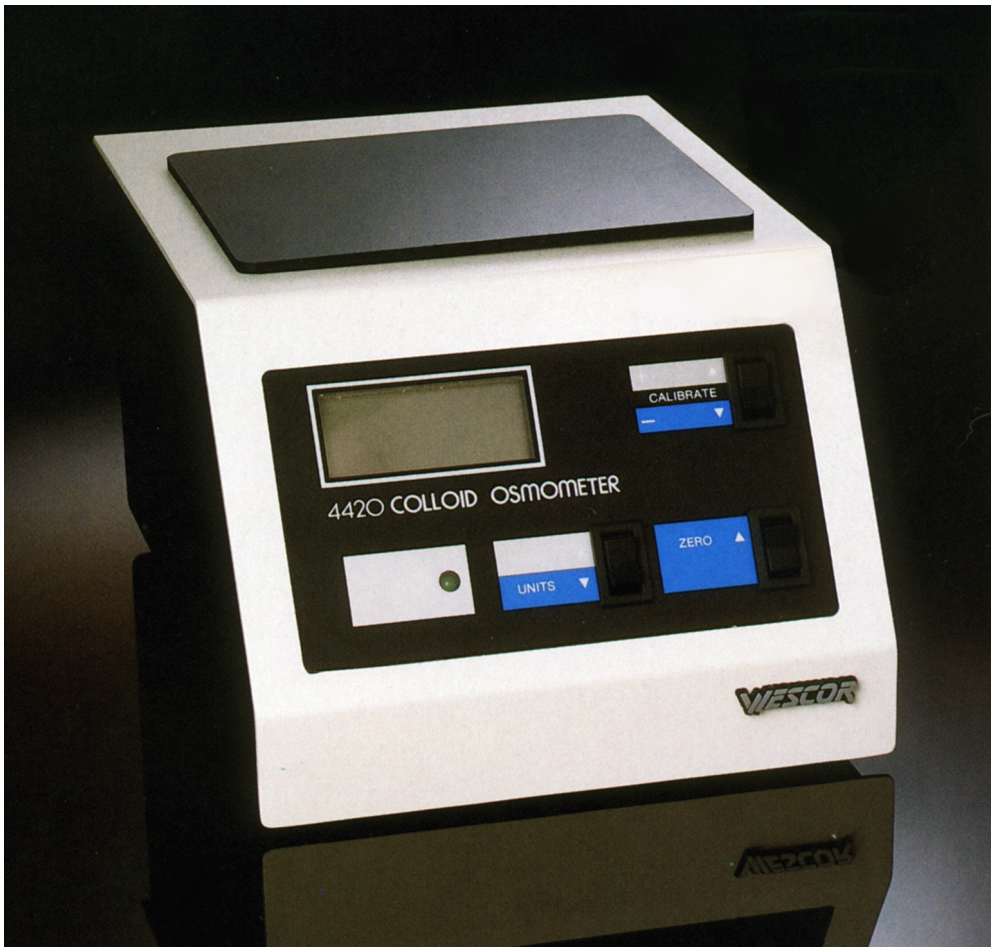


Figure 1-1 *The Wescor Model 4420 Colloid Osmometer*

1.3 – Features

The Colloid Osmometer offers many useful features, making it well suited for both routine clinical and research applications. Some of the features are listed below.

- ☞ Minimal sample volume.
- ☞ Simple operation and maintenance.
- ☞ Choice of manual or prompted operation.
- ☞ Choice of English, French, or German language displays.
- ☞ Colloid osmotic pressure values can be displayed in mmHg, cmH2O, or kPa.
- ☞ Built-in alarm helps prevent damage from excessive injection pressure.
- ☞ Reliability.
- ☞ Long membrane life.
- ☞ Built-in battery backup maintains calibration data if instrument power is interrupted.

Manual or prompted mode are selected by pressing **PROMPT** on the front panel (see Section 1.4, Controls and Connections). In the prompted mode, the instrument guides you through each sample injection with instructions on the alpha-numeric display. When the instrument detects a plateau condition, it displays the final result. The prompted mode works well for specimens such as heparinized whole blood, heparinized plasma, or serum.

In the manual mode, you determine the timing and volume of sample injections. You will make decisions based on the available sample size and by monitoring the display for plateau conditions. The manual mode works well for specimens such as hetastarch or synthetic serums.

When available sample size is very small, i.e. between 125 microliters and 350 microliters, you can use a special manual procedure which is described in Section 4.

The instrument can display the measured colloid osmotic pressure in millimeters of mercury (mmHg), kilopascals (kPa), or centimeters of water (cmH2O). Select **UNITS** on the front panel, you can set the current COP reading to be displayed in whichever unit of measurement you prefer.

1.4 – Controls and Connections

FRONT PANEL CONTROLS

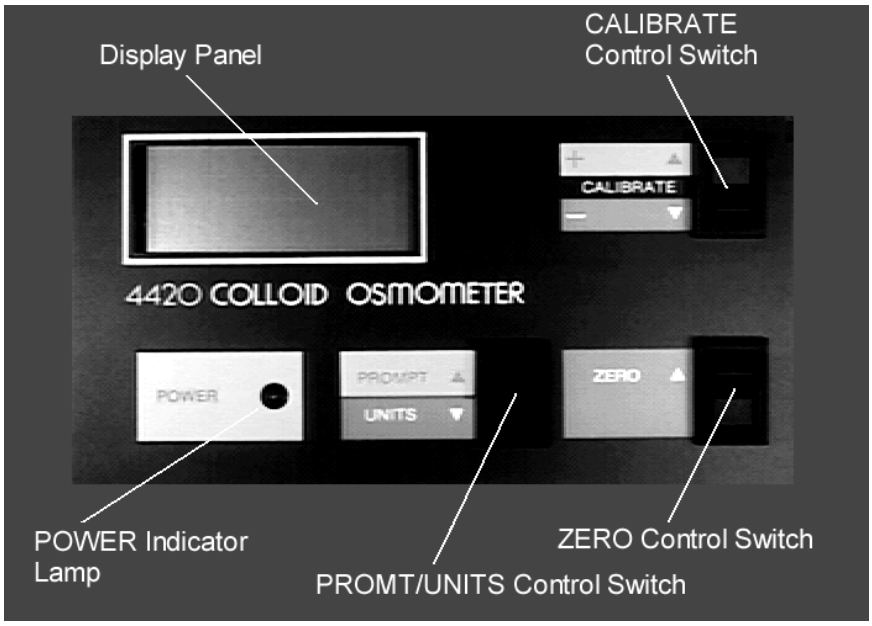


Figure 1-2 *Front Panel Controls*

POWER Indicator Lamp
Lights when the power is on.

Display Panel
4 line X 16 character alpha-numeric display provides specimen results, prompts, and other useful information. Display messages are described in Sections 2, 3, and 4.

PROMPT/UNITS Control Switch
A two-way rocker switch lets you select the prompted or manual mode of operation (**PROMPT**), or set the displayed units of COP to mmHg, kPa, or cmH2O (**UNITS**). See Section 3 for more information.

ZERO Control Switch
Resets the display to 0, clearing any offset. Used when the sample chamber contains saline solution. Refer to Section 3.2 for instructions.

CALIBRATE Control Switch
A two-way rocker switch to increase or decrease the instrument's reading, allowing you to calibrate the osmometer to the COP standard. See Section 3 for correct use of the **CALIBRATE** control.

INTERIOR CONTROLS and CONNECTIONS

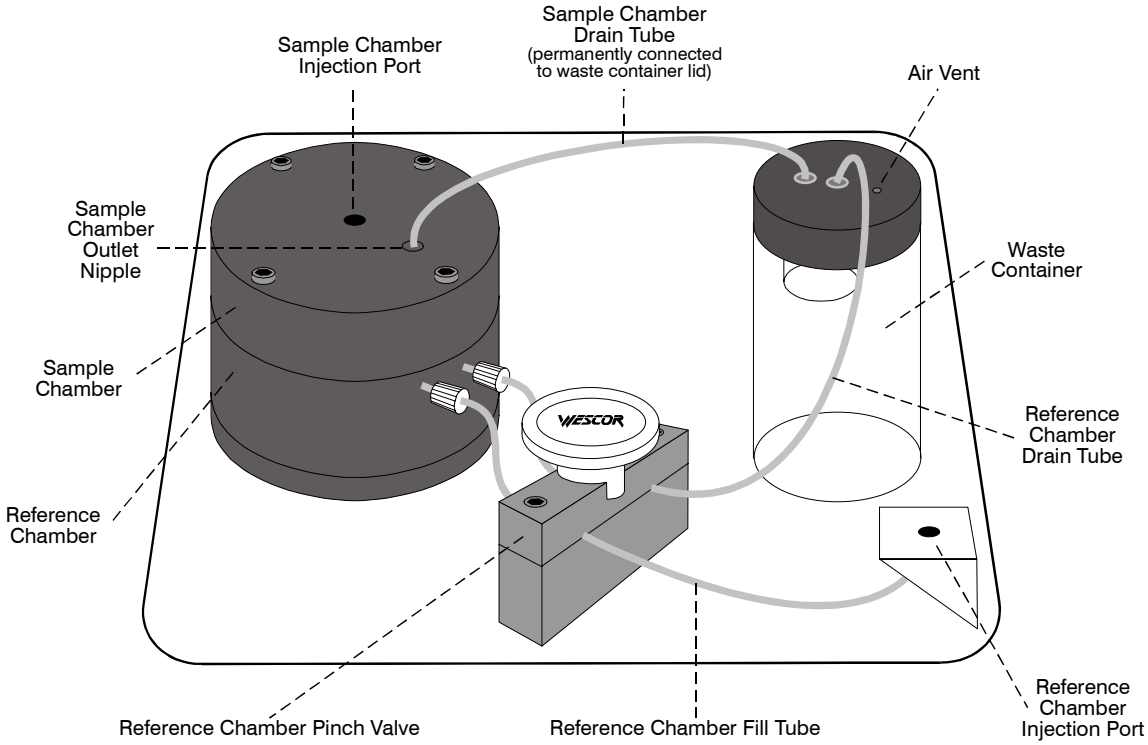


Figure 1-3 *Controls and Connections in the 4420's interior bowl*

Sample Chamber

The chamber that holds sample fluid after injection or normal saline between samples. Its inlet is the sample chamber injection port. As you inject additional sample fluid, the existing fluid flows through the sample chamber drain tube and into the waste container. The sample chamber must be filled with saline solution in order to set the **ZERO** offset (see Section 3). It should be flushed with saline whenever a membrane is in the instrument and you are not actually testing a sample.

Reference Chamber

The chamber that holds the reference fluid. This chamber must be filled with normal saline solution (0.9% NaCl) in order to test a sample, and whenever a membrane is in the instrument (see Section 2.3). The reference chamber is filled through the reference chamber fill tube. Both the reference chamber fill tube and drain are controlled by the reference chamber pinch-valve, so that the reference chamber can be sealed during measurement and to prevent evaporation of the saline solution.

Reference Chamber Fill Tube

Connects the reference chamber injection port to the reference chamber, passing through the reference chamber pinch-valve.

Port Plug
A pair of polyethylene plugs which seal the sample chamber injection port and reference chamber injection port.

Reference Chamber Injection Port
Facilitates injection of saline solution into the reference chamber. Accepts a standard non-locking syringe. (A plastic adapter must be used when injecting with locking syringes or metal tips, see Section 4.1).

Reference Chamber Drain Tube
Allows drainage of used saline solution from the reference chamber to the waste bottle.

Waste Container
A container for temporary storage of used sample fluids and saline solution from the sample chamber and reference chamber. Empty the waste container regularly to avoid the chance of a spill or overflow.

Air Vent
A small air vent in the waste container lid allows the air volume in the waste container to be displaced by fluid volume from the sample and reference chambers.

Sample Chamber Drain Tube
Drains used sample fluid from the sample chamber outlet nipple to the waste container.

Sample Chamber Injection Port
Accepts a standard non-locking tuberculin plastic syringe containing the sample fluid. (A plastic adapter must be used when injecting with locking syringes or metal tips, see Section 4.1). Inject the sample according to displayed prompts (in prompted mode) or user discretion (manual mode).

Sample Chamber Outlet Nipple
Facilitates connection of the sample chamber drain tube, to drain used sample fluid to the waste container when fresh sample or saline rinse is injected.

REAR PANEL CONTROLS and CONNECTIONS

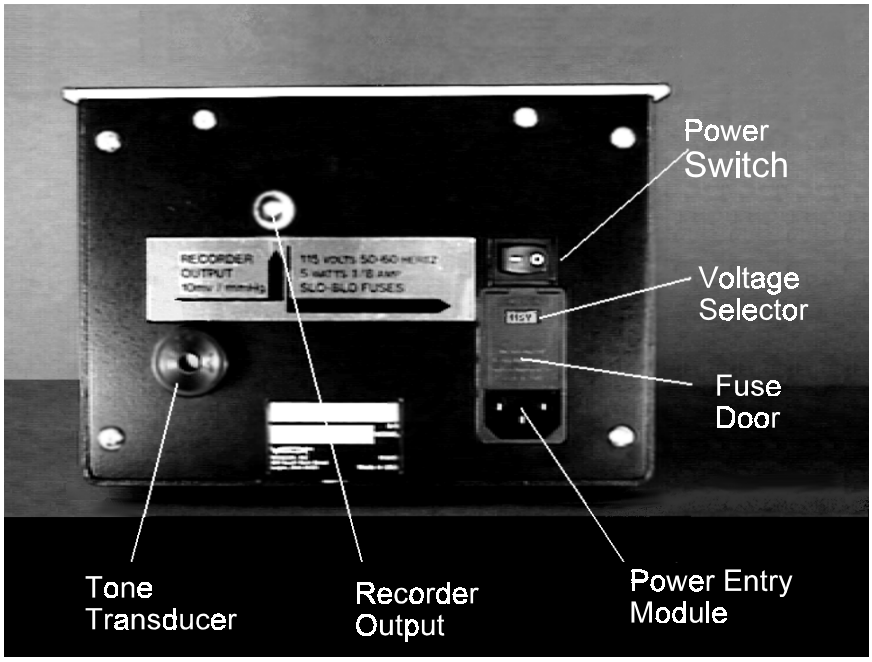


Figure 1-4 *Controls and connections on the rear panel*

Power Switch

Switches power mains for Colloid Osmometer on **(I)** or off **(0)**. When the instrument is connected to proper voltage source and the switch is on **(I)**, the front panel **POWER** indicator will be lit. It is normal to leave instrument power on for extended periods.

Fuse Door

Access the osmometer's main fuses by disconnecting the power cord and using a small screwdriver to open the fuse door (Figure 1-5). Before replacing the fuses for any reason, please refer to Section 6.2 for important safety precautions. For continued protection against fire hazard, replace fuses only with the correct type and rating.100 & 115 units: 1/8 Amp 'Type T," time-delay fuses (two required).
230 V units: 1/16 Amp 'Type T,' time-delay fuses (two required).

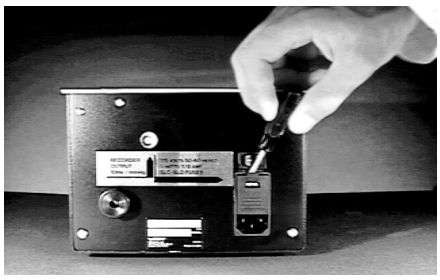


Figure 1-5 *Opening the fuse door*

Voltage Selector

The selector is set at the factory to 100V, 115V, or 230V. The voltage indicated may not agree *exactly* with your local source, but it should be within a range. For example, the 115V selector is safe for sources between 110 and 120 volts. The 230V selector is suitable for sources from 220 to 240 volts. Do not connect the unit to a voltage source outside the indicated range—such as a 115V unit to a 230V source. Serious damage could result.

Power Entry Module
Provides connection for the standard IEC 320 type power cord included with the instrument.

Recorder Output
Provides standard BNC connection for an external chart recorder. One mmHg on the display is equal to 10 mV at the recorder output.

Tone Transducer
Provides audible feedback for control operation and alarm for excessive injection pressure (see Section 2).

1.5 – Specifications

COLLOID OSMOMETER, Model 4420

Sample Volume	350 microliters nominal for routine clinical measurements in prompted mode; as little as 125 microliters using special manual technique.
Sample Loading	Direct syringe injection, flow-through system.
Reference Chamber	Direct syringe injection, flow-through system.
Standard Membrane	Selectively-impermeable to proteins exceeding 30,000 molecular weight. Wet-packed and pre-mounted for easy replacement (Wescor catalog # SS-030 (30,000 MW).
Membrane Life	Greater than 1,000 samples in routine applications, provided the membrane is properly maintained.
Response Time	3 to 7 minutes, depending upon membrane condition.
Clinical Range	0 to 35 mmHg.
Resolution	±0.1 mmHg.
Precision	±0.3 mmHg (Assuming proper membrane function).
Calibration	Osmocoll® N (Normal Level Colloid Osmotic Pressure Calibrator).
Readout	4 line by 16 character alphanumeric liquid crystal display (LCD).
Electronics	Solid state, microprocessor controlled.
Zero Offset Range	±30 mmHg.
Calibration Range	±2 mmHg.
Recorder Output	Standard BNC connector, 10 mv/mmHg, output impedance 500 ohms.
Electrical Requirements	115 to 120 Volt 50/60 Hz. 220 to 240 Volt 50/60 Hz. Factory Option:100 Volt 50/60 Hz.
Power Consumption	5 watts.
Internal Batteries	3 V Lithium Cell (Two Required) Eveready CR2025 or equivalent.

Fuses (2 Required)	1/8 Amp 'Type T', time-delay for 100V or 115V (P/N 39-0136) 1/16 Amp 'Type T', time-delay for 220-240V (P/N 39-0185)
Size and Weight	19 cm (7.5" wide x 14 cm (5.5") high x 28 cm (11") deep 3.2 kg (7 lb)
Waste Container Volume	120 mL (4 oz.)
Standard Accessories	AC-007 Membrane Scraper AC-012 Torque Indicating Screwdriver SS-025 Osmocoll N (Normal Level Colloid Osmotic Pressure Calibrator) SS-030 Wet-Packed, Pre-mounted Membranes Operator's Manual Tuberculin Syringes*: 2 each, 10 mL 25 each, 1 mL Cotton Swabs*, 2 packs Saline Solution, 500 mL (0.9% NaCl Irrigation USP)* Material Safety Data Sheet Return Forms Operator Warning Product Bulletin Power Cord

*Consumable not available for reorder from Wescor, Inc.
Specifications are subject to change without prior notice.

SPECIFICATION OF SAFE USE:

Using this instrument in a manner not specified by Wescor Inc may impair the safety protection designed into the equipment and may lead to injury.

SAFE USE ENVIRONMENT:

This equipment is designed to be safely operated at 5 to 35°C, maximum relative humidity 80%.

FUSES:
All fuses in this equipment are type T (SLO-BLO time-delay).

EXPLANATION OF SYMBOLS FOUND ON EQUIPMENT:

~	Alternating Current (AC)
I	Power On
O	Power Off

SECTION 2 – Getting Started

SECTION 2 familiarizes you with the Colloid Osmometer so that you can begin testing samples. Section 2.1 contains directions for installing the instrument. Sections 2.2 through 2.5 discuss the pressure transducer, membranes, and reference chamber. Preventive maintenance is covered in Section 2.6. After completing Section 2, you will be ready to begin testing samples with your Colloid Osmometer (Section 4).

2.1 – Installing the 4420

This section explains how to install the Colloid Osmometer in your lab and connect it to the correct power source. It describes the messages you will see on the display when you switch the osmometer on.

1. Locate the osmometer on a suitable laboratory bench or table with convenient access to the correct power source.
2. Attach the included power cord to the power connector on the rear panel (Figure 2-1).
3. Connect the power cord to the correct power source as indicated on the rear panel.

Figure 2-1 *Connecting Power*

4. Turn **ON (I)** the power switch located on the rear panel.

The **POWER** indicator should now be lit, followed by a short "beep" tone. The display will show a message similar to that in Figure 2-2. The default language for the display is English. Refer to Appendix B to change the language

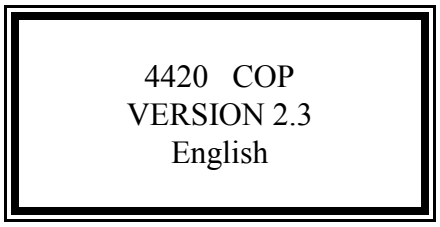


Figure 2-2 *The 4420 display as power is switched on*

If the **POWER** indicator does not light or you do not see this message, recheck your power connections and the rear panel power switch.

The display should remain for approximately two seconds, and then you will see one of the following displays:

Figure 2-3 represents the normal display indicating the 4420 is now ready for preparation, and that you can skip ahead to Section 2.2.

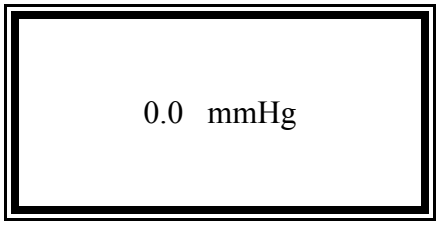


Figure 2-3 *The normal display when the 4420 is in manual mode (Units in mmHg, kPa, or cmH_20 according to your last selection.)*

The message in Figure 2.4 appears when the batteries fail, or when the osmometer has not been initialized after replacing batteries. The batteries power the memory settings for units, zero level and calibration data during power failure or disconnection.

Figure 2-4 *Message that appears when batteries must be replaced, or when the instrument is reset after new batteries are installed*

To clear the message and initialize the instrument, press **ZERO** as instructed. The osmometer will then initialize itself and return to the normal display as shown in Figure 2-3. After initializing, recalibrate before testing samples. Fresh batteries are installed at Wescor before shipment, and should provide approximately 5 years of service. See Appendix B for more information about replacing the internal batteries.

If the instrument will not power up or if you do not see either of the displays shown above, refer to Section 6, Solving Problems.

2.2 – The Pressure Transducer

The pressure transducer is a vital component of the osmometer system. It is integral with the reference chamber of the cell assembly. As a sensitive measuring instrument, the pressure transducer responds to minute pressure changes occurring within the reference chamber, producing a corresponding electrical signal.

Reference solution in the chamber is contiguous with the transducer diaphragm that is coupled to a precision semiconductor strain gauge. Pressure changes within the reference chamber deflect the diaphragm which changes the electrical signal from the strain gauge to the amplifier.

The cell assembly is designed to protect the pressure transducer, which is completely enclosed within the instrument to minimize the risk of shock damage through handling. The membrane can be installed and the reference chamber flushed without disassembling the transducer from the reference chamber. In normal operation and maintenance, the cell is always vented to the atmosphere to reduce the risk of inadvertent over-pressure resulting from injecting fluids into a “closed” system.

However, since the pressure transducer is highly sensitive and delicate, it is susceptible to damage if you do not observe the following cautions:

CAUTION

1. **Do Not** install membrane, inject any sample, standard or control solution into the instrument or attempt any operator functions unless instrument power is **ON**.

2. **Do Not Apply Excessive Injection Pressure**. The most common cause of transducer damage is excessive injection pressure. To help prevent this, the instrument has an automatic alarm which sounds when the instrument senses excessive injection pressure.

Always use a 10 or 12 mL syringe for injecting saline, and a 1 mL syringe for samples.

When injecting sample fluid into the sample chamber with a small (< 10 mL) syringe, you can generate sufficient fluid pressure to damage or destroy the pressure transducer. This can occur even if you feel no resistance.

Under certain conditions, such as injecting a sample very rapidly with a 1 mL syringe, you can damage the transducer before the built-in alarm can respond and sound the over-pressure warning tone.

If the drain tube from either the reference chamber or the sample chamber becomes obstructed*, the osmometer cell will become a closed system. Under such circumstances, damage to the pressure transducer can occur if you attempt to clear the blockage in the drain tube by applying pressure with a syringe at the sample injection port or at the reference chamber injection port.

You should be particularly cautious when using a syringe to apply pressure to a closed system*.

**This can happen if any outlet is left open to the atmosphere with saline solution in the cell. Evaporation of water will leave salt deposits in the bore. This can also happen if the pinch valve tubing remains pinched together after the pinch valve is open.*

NOTE: The negative reading on the instrument display when solutions are injected is an indicator of positive pressure generated in the cell. Hence, you can easily avoid pressures higher than 200 mmHg simply by injecting slowly and not driving the reading beyond -150 mmHg.

3. **Do Not Freeze.** If the osmometer must be stored or shipped in freezing temperatures, remove the membrane (see Section 2.3) and all liquid from the cell assembly to prevent damage to the pressure transducer.

4. **Do Not Ship** the Colloid Osmometer to Wescor unless it has been drained, cleaned, and decontaminated (see Appendix C, Customer Service, and Section 4.5, Disinfection Procedures).

2.3 – Membranes

The membrane is the heart of the colloid osmometer measurement system. This section explains procedures for installing a new membrane (and removing a used membrane, if in place). This step must be completed before the colloid osmometer can be used.

INSTALLING A MEMBRANE

The Colloid Osmometer is shipped from the factory without a membrane installed in the cell assembly. Before first-time use, you must install one of the membranes which are included with each new instrument.

Membranes are supplied in a disposable, preassembled plastic frame and packed in a saline solution. This method of packaging prolongs membrane shelf life.

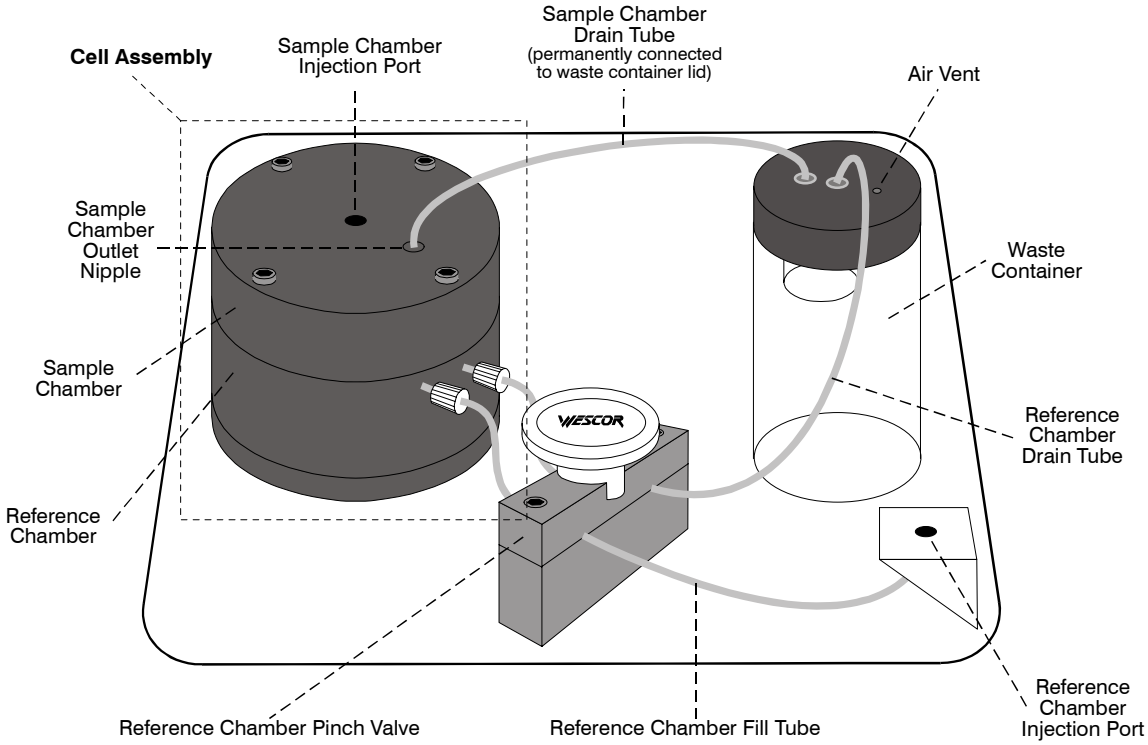


Figure 2-5 *The cell assembly in the interior bowl*

NOTE: Do not open the membrane package until preparing for installation. Do not allow the membrane to dry out.

1. Open the membrane wet-pack by cutting one end with scissors. Soak the membrane in a deionized water bath for at least 15 minutes. You may continue with the following steps during this soaking period.

2. Plug in the osmometer and turn the power on.

3. Remove the sample chamber drain tube from the sample chamber outlet nipple, shown in Figure 2-5.

NOTE: The sample chamber drain tube is permanently attached to the waste container lid.

4. Open the reference chamber pinch-valve (lift and turn so the knob stays in the upper position) to prevent any pressure buildup during membrane installation.

5. Remove the four socket-head cap screws from the osmometer cell assembly using a standard 9/64" hex driver. Do not use the AC-012 torque driver. The shock of screws breaking free can alter the zero adjustment of the torque driver.

6. Carefully lift the sample chamber straight up and away from the reference chamber. The used membrane usually adheres to the sample chamber.

7. Remove the old membrane frame from the sample chamber by separating it from the membrane as shown in Figure 2-6.

HINT: Use saline from a 10 mL syringe of saline solution to saturate the old membrane. Let the membrane soak in the saline for about two minutes. The membrane should lift off easily, leaving very little residue.

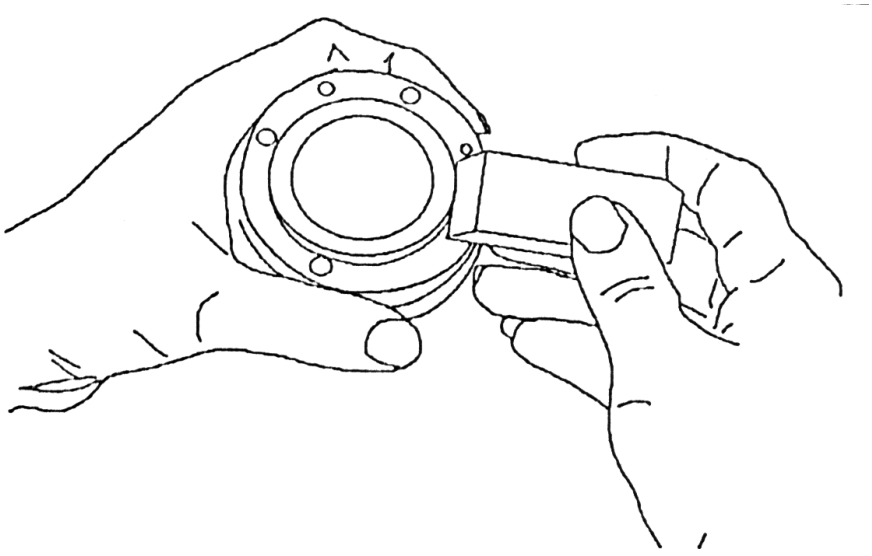


Figure 2-6 *Removing old membranes with the scraping tool*

8. Remove all residual membrane material from the sample chamber using the furnished plastic scraping tool. For convenience, you can do this while holding the sample chamber under running water.

CAUTION

USE ONLY A SOFT, NON-ABRASIVE IMPLEMENT TO AVOID DAMAGING THE PRECISELY MACHINED SURFACE.

9. Remove stubborn membrane particles from the sample and reference chambers using a cotton swab or lint-free tissue moistened with isopropyl alcohol followed by a pure water rinse. Make certain that no membrane particles clog the inlet or outlet ducts.

CAUTION

THE DELICATE MEMBRANE SURFACE CAN BE DAMAGED BY CONTACT. TOUCH ONLY THE PLASTIC FRAME WHILE HANDLING.

THE MEMBRANE CAN NOT BE REUSED IF DRIED OUT, REMOVED, OR REORIENTED.

10. Remove the membrane from the deionized water bath and then place the membrane in position on the reference chamber with the red ring (shiny side of membrane) up. Press down the membrane ring evenly into the chamber.

11. Install the sample chamber on the reference chamber. Be sure to align the offset index pins to assure correct alignment of the parts.

12. Holding the sample chamber down flat, replace the four socket-head cap screws and tighten them until the screw heads just touch the cell top. Back them off 1/8 turn. Using the torque driver (Figure 2-8), tighten each screw 1/8 turn following the pattern shown in Figure 2-7. Tighten each screw 1/8 turn following the same pattern (you should feel some resistance at this point). Make sure the driver is completely seated in the socket head screw before tightening.

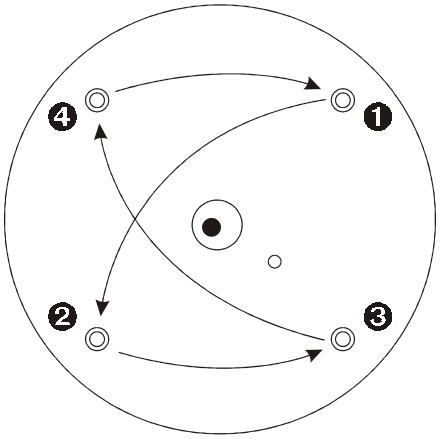


Figure 2-7 *Pattern for tightening the socket-head cap screws on the sample chamber*

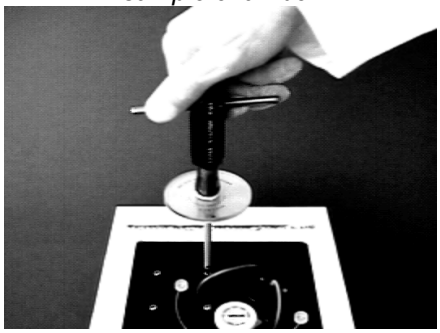


Figure 2-8 *Tightening the sample chamber screws with the AC-012 Torque Driver*

NOTE: A sudden slip while applying torque, may affect the driver zero torque setting and require readjustment. To readjust the zero: Make sure the setscrew near the driver tip is tight, then loosen the setscrew near the handle, turn the plastic dial to the zero position, and retighten the setscrew.

Tighten the screws following the pattern shown in Figure 2-7 in stages; first tighten all the screws to level A, then B, then C, and

finally to level D on the scale. After all screws have been tightened to level D, recheck each screw to make sure it does not advance further at torque C1/2 (halfway between C and D). This corresponds to 32 to 35 inch lbs.
NOTE: Poor plateau-holding performance or a low reading after injecting serum or colloid osmotic pressure standard into the sample chamber can indicate insufficient or uneven membrane clamping pressure due to improper screw tightening.

13. Close the reference chamber pinch-valve by turning the knob and lowering it into the closed position.

14. Replace the sample chamber drain tube removed in Step 1. The osmometer is now ready for chamber filling as instructed in the following sections. This should follow immediately to protect the newly installed membrane from drying out.

After installation, sample components build up on the sample side of the membrane forming a stable layer that remains after flushing with saline. This layer stabilizes in 24 to72 hours depending on how often samples are run. During this time you will notice the zero and calibration slowly change. Patient samples can be tested during this stabilizing period if you calibrate with Osmocoll® N (Section 3.4) just prior to testing. This assumes that you are using an SS-030 membrane and are only running human blood, Osmocoll® N, and 5% or 3% human albumin for samples. Other types of samples may adversely affect typical membrane performance.

2.4 – Filling the Reference Chamber

Fill the reference chamber with saline solution (0.9% NaCl irrigation) immediately after installing a new membrane in order to protect the membrane from drying out.

1. Make certain that all drain and fill tubes are connected as shown in Fig. 2-5. Instrument power should be on (**POWER** indicator will be lit).

2. Insert the tip of a 10 mL plastic syringe filled with normal saline solution into the reference chamber injection port. Use a slight rotation to ensure a tight connection (Figure 2-9).

3. Open the reference chamber pinch valve by lifting and turning the pinch-valve knob.

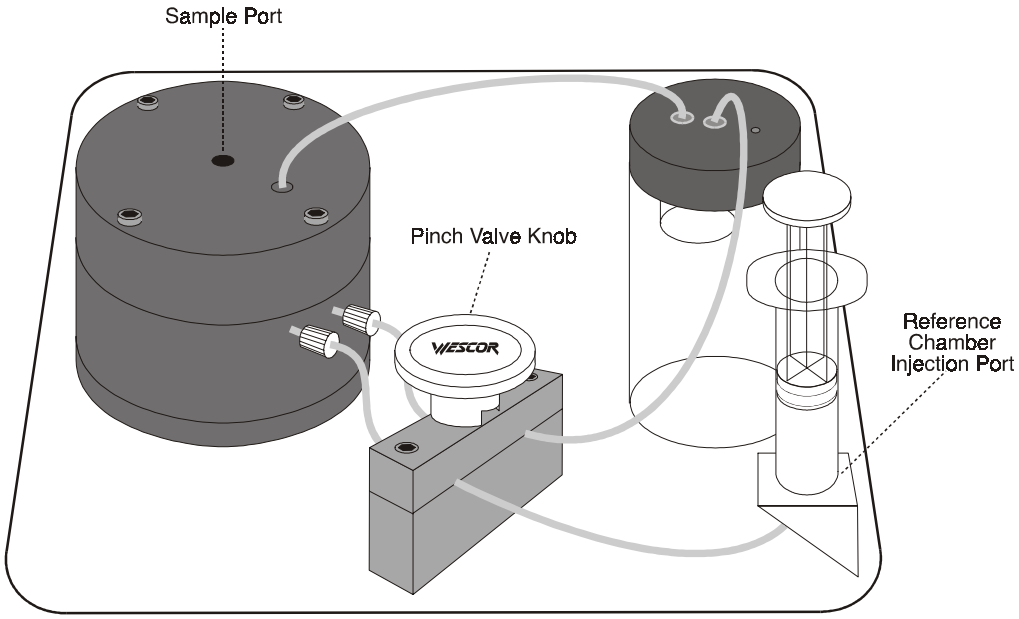


Figure 2-9 *Using a 10 mL syringe to inject saline solution into the reference chamber*

4. Using smooth, gentle pressure on the syringe, inject 5 to10 mL of saline solution into the reference chamber.

CAUTION! *Watch the display and do not exceed 150 mmHg or equivalent. If you observe bubbles in the lines, continue to inject solution until no bubbles are visible.*

Inject enough saline to fill the small inner cup in the waste collection bottle to prevent saline in the tubes from evaporating and leaving salt deposits in the lines. The reference chamber drain tube should be inserted approximately 1 inch into the center hole of the waste container lid.

NOTE: Solution should flow easily with gentle syringe pressure. If obstructed, do not attempt to clear the line by applying excessive pressure with the syringe. This can damage the pressure transducer (see Section 2.2).

5. Rotate and lower the pinch valve knob to seal the reference chamber.

6. Remove the syringe from the reference chamber injection port. Use a tissue or cotton swab to absorb any residual solution and prevent salt deposits around the plug. Insert the saline syringe into the sample port and inject 1 mL of saline solution.

NOTE: As a minimum, we recommend that you flush the reference chamber with fresh saline before and after each day's use of the colloid osmometer.

2.5 – Membrane Performance

Because the membrane is the heart of the osmometer's measurement system, it is important that you can clearly recognize whether a membrane is performing its role in the determination of colloid osmotic pressure. After injecting serum, plasma, or whole blood, regardless of the actual COP of the sample, the instrument response profile provides important diagnostic information. This information can help you determine the condition of the membrane and its suitability for measurement. Verifying appropriate membrane function is a prerequisite to calibration of the osmometer.

The two main factors of interest in assessing membrane performance are **speed of response** and **plateau-holding ability**. Of the two, plateau-holding ability is more important to accuracy. While rapid measurement is generally desirable, the speed of response is influenced by many variables, including both colloid and crystalloid solute makeup of the sample solution in addition to age and individual characteristics of the membrane itself. Membranes that can't hold equilibrium pressure plateau may appear to give rapid response times, but will show measurement inaccuracies.

When installed correctly, wet-packed membranes supplied by Wescor (Cat. No. SS-030), should produce the following characteristic instrument response after injecting the COP calibration solution, or human blood, serum, or plasma:

1. As the sample is injected into the cell, the readout will deflect sharply in the negative direction in response to the injection pressure (refer to CAUTIONS, Section 2.2).

2. After releasing sample injection pressure, the reading will immediately reverse direction, rising past zero to some positive value. The plateau will be reached typically between 30 and 120 seconds (insignificant increases may occur with some membranes even beyond 120 seconds).

3. The membrane should produce a rising display indication and stabilize at a plateau level for at least thirty seconds after sample injection. Any fall-off from plateau level within thirty seconds should be interpreted as marginal or submarginal performance. A good membrane can show a plateau decay at approximately 45 seconds. Non-parallel clamping of a good membrane can cause plateau decay in less than 5 seconds. Occasionally, a newly-installed membrane exhibits a tendency to fall from plateau. This will often stop when the membrane has been in the instrument for a few hours, if at least three samples have been tested during this period.

If the osmometer fails to perform as described, do not assume that the membrane is defective without first considering and eliminating all other possibilities. As stated in Section 2.3, the most common cause of poor performance in a newly-installed membrane is failure to properly tighten the osmometer cell screws. Use the provided torque-indicating driver to make certain that the screws are as evenly tightened as possible.

An air bubble in the reference chamber can produce erratic or unstable results. To reduce this possibility, use a 10 mL syringe to gently flush 3 or 4 mL of saline solution through the reference chamber. Then repeat the test.

Air leaks in the reference chamber will also cause failure to hold plateau reading.

If all of the above possibilities have been eliminated and the problem remains, check for a defective or damaged membrane.

A properly installed membrane should work well for several hundred samples. Resist changing zero or calibrate controls once the membrane has stabilized (see Section 2.3). Small positive shifts in zero usually indicate air in the reference chamber, which can be cured by flushing with saline (Section 2.4). When the membrane begins to deteriorate, its response time will tend to increase and the plateau level could decrease slightly, requiring you to increase the **CALIBRATE** setting to maintain calibration. These effects probably indicate a decrease in the number of pores available for transudation of fluid and an increase in the membrane cut-off (see Section 5). When you observe these changes, you should replace the membrane.

Section 2.6 (Preventive Maintenance) offers some important information to help keep the instrument operating properly. You can find instructions for calibrating the instrument in Section 3 and procedures for testing samples in Section 4.

2.6 – Preventive Maintenance

This section offers important preventive maintenance procedures to help you keep the Colloid Osmometer working well. These procedures are part of the routine operation of the instrument, and are not optional.

STORAGE BETWEEN USES

When the osmometer is idle, saline solution should fill both sample and reference chambers and the inner waste collection cup. The sample chamber injection port should be closed with a saline syringe and the pinch valve must be in the closed position.

EMPTYING THE WASTE CONTAINER

Empty the waste collection container whenever the liquid rises to a visible level. Add a small amount of sodium hypochlorite to the container before reinstallation into the osmometer to help control bacterial growth. After reinstallation, be sure to inject sufficient saline solution through the sample chamber to fill the inner cup of the waste collection system. This will prevent evaporation of water from within the osmometer cell that would otherwise allow the membrane to dry out and/or result in salt blockage of the chamber ducts. Make sure that the reference chamber drain tube is inserted approximately 1 inch into the center hole of the waste container lid.

MEMBRANE

The membrane must be kept wet during its life. Between uses, keep the sample chamber filled with saline solution and closed with a saline syringe. The reference chamber must likewise contain saline solution at all times. The pinch valve must be closed. These simple rules will promote membrane longevity.

> **NOTE: Never leave a colloid solution in the sample chamber when you are not actually testing a sample.** Use standard biohazard safety precautions when operating, maintaining, or decontaminating the instrument.

SECTION 3 – Setup & Calibration

This section offers procedures to prepare the osmometer for use and for calibrating it with the COP standard. The osmometer must be properly calibrated to have reliable COP readings. The system is very stable. After proper calibration and membrane stabilization, frequent recalibration is not required. Before attempting the procedures in this section, you should have read and followed the instructions in Section 2, Getting Started.

3.1 – Filling the Sample Chamber

CAUTION

Be sure instrument power is on before injecting solution or installing a membrane.

Before use, you must install a membrane and fill the reference chamber with saline as described in Sections 2.3 and 2.4. Once you have completed both those steps, you should fill the sample chamber with saline.

1. Insert the tip of a 10 mL plastic syringe filled with normal saline solution into the sample port. Use a slight rotation to ensure a tight connection.

2. Using smooth, gentle pressure, inject saline solution to clear all bubbles from the sample chamber and drain tube (typically about 2 mL). Leave the saline syringe in place if you are going to ZERO the 4420.

NOTE: Solution should flow easily with gentle syringe pressure. Do not attempt to clear an obstruction in the line by applying excessive pressure with the syringe. This can damage the pressure transducer (see Section 2.2). Use standard biohazard safety precautions when operating, maintaining, or decontaminating the instrument.

3.2 – Setting the Display ZERO

Before calibration, you must set the instrument to **ZERO**. Do this before entering the prompted mode, since the **ZERO** switch will not function in the prompted mode. The procedure is to flush the reference chamber, if needed, and then the sample chamber with normal saline solution (0.9% NaCl Irrigation, USP).

After the digital readout stabilizes (typically within 60 seconds), press **ZERO**. The display will now read zero.

> **NOTE:** Small positive shifts in zero usually indicates air in the reference chamber. If this is a problem, try flushing with saline (Section 2.4). A large negative offset (-30 mmHg or more) could indicate a problem with the transducer.

3.3 – Changing Displayed Units

You can set the display to colloid osmotic pressure readings in Millimeters of Mercury (mmHg), Centimeters of Water (cm H2O), or Kilopascals (kPa). To change displayed units, press **UNITS**. The display will update each time you press **UNITS**.

Changing units does not affect the zero or calibration of the instrument. Once you enter the prompted mode, the **UNITS** switch is disabled until the instrument reports a final result or until you abort the test by pressing **PROMPT**. At that time, the instrument will ask you to flush the sample chamber with saline. Once that is done, the instrument returns to manual mode and you can again change the displayed units.

Ready for Calibration

Once you have set the instrument zero and selected the desired units, the instrument is ready for calibration. Membranes may differ in response times; Calibration is always required after installing a new membrane.

3.4 – Colloid Osmotic Pressure Calibration/Control Solutions

The objective of colloid osmometer calibration is to set the amplifier gain (**CALIBRATE** control) so the instrument will accurately measure the true colloid osmotic pressure of the solution injected into the sample chamber. Since the measurement depends upon membrane function, calibration must involve an appropriate colloid solution whose colloid osmotic pressure has been accurately assayed.

Traditionally, COP calibration solutions for clinical work have been based upon assayed solutions of human albumin, a colloid material that is acceptable as a control.

A 5% solution of human albumin in saline prepared for IV infusion will typically have a COP of 19.3 ±1.4 mmHg.

Osmocoll® N (Normal Level Colloid Osmotic Pressure Calibrator) is a calibration solution for all colloid osmometers. Osmocoll is available from Wescor (Cat. No. SS-025) and comes packaged in six, 1 mL vials. The colloid osmotic pressure, osmolality range, lot number, and expiration date accompany each package. The package also includes instructions for use and storage, as well as product warranty information. Osmocoll is also useful as an osmolality control reference. Osmocoll HL (high and low level, Cat. No. SS-038) and Osmocoll HNL (high, normal, and low level, Cat. No. SS-039) are also available from Wescor. Each lot has a specific assayed control value and range, but will measure approximately 25, 20, and 15 mmHg for high, normal, and low levels.

CAUTION

Using standards or controls other than Osmocoll or human albumin can degrade membrane performance.

3.5 – Calibration Procedure Using the COP Calibration Solution

A membrane should have previously been properly installed and the cells recently flushed with saline. Use the information in Section 4 to inject the following sample using the prompted mode.

1. Press **PROMPT** on the front panel to set the osmometer to prompted mode. The display should appear as shown in Figure 3.1.

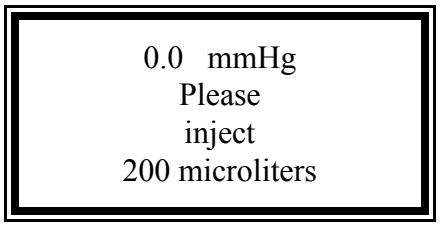


Figure 3-1 *The prompt to inject solution*

2. Follow the prompts to inject COP standard until the display indicates **FINAL RESULT**.

3. Press **CALIBRATE +** or **-** until the display indicates the assayed value of the COP calibration solution. (Repeat the calibration procedure two or three times to ascertain repeatability, if desired).

NOTE: Calibration by this procedure is a simple, one-step operation. However, the operator must ascertain proper membrane function (see Section 2.5).

SECTION 4 – Operating the Osmometer

SECTION 4 offers instructions for operating the Colloid Osmometer. It describes procedures for using either manual (Section 4.2) or prompted (Section 4.3) mode.

4.1 – Injecting Samples

The sample injection procedures in this section apply to both manual and prompted modes. Sections 4.2 and 4.3 cover manual and prompted modes in detail.

When ready to test a sample:

Figure 4-1 *Cleaning the sample injection port before injecting a sample*

1. Remove the saline syringe (do not be concerned if the zero reading drops slightly) and clean the injection port using a cotton swab as shown in Figure 4-1.

2. Remove any excess liquid from the port area with a swab or a tissue.

3. Use a fresh, clean plastic syringe to inject samples. The 1 mL size with a volume graduated scale will generally be most convenient. Avoid excessive injection pressure (Section 2.2) when using this type of syringe. Locking type syringes require a plastic adapter*. Make certain the sample is free of bubbles and insert the tip of the syringe firmly with a slight rotation.

*Plastic syringe adapters are available from: Industrial Specialties MFG., Inc. 2741 W. Oxford, Unit #6 Englewood, Colo. 80110 303-781-8486 (Part # IFML)

CAUTION

Never insert metal fittings into the sample port. A plastic adapter* can be used as an interface between the sample port and any metal fitting that must be used.

4. Use smooth, gentle pressure when injecting sample solution into the cell.

Note: Use standard biohazard safety precautions when operating, maintaining, or decontaminating the instrument.

Sample Volume and Injection Procedure.

With sufficient specimen volume (at least 350 µL), we recommend using the prompted mode, described below and in Section 4.3. With minimal specimen volume (but at least 125 µL), use the special procedure below and in Section 4.2 to test the specimen's COP. Also, if you prefer to operate in the manual mode for any reason, Section 4.2 lists the routine injection procedure as well.

Accurate measurements require complete displacement of any saline solution in the sample chamber by the injected sample. It is essential to avoid dilution of the incoming specimen, which would cause erroneously low COP indications. In theory, the advancing specimen fluid will displace saline from the sample chamber into the waste container, but because of turbulence, surface roughness and boundary layer effects, the process is not 100 percent efficient. To reduce dilution error, you must inject specimen in discontinuous steps, with pauses between steps to allow boundary-layer saline to diffuse into the specimen.

In prompted mode, the display instructs you to inject the specimen in a specific sequence. This reduces dilution error, and usually provides the quickest possible result with a nominal sample size of 350 µL. We recommend that you routinely use the prompted mode, sample size permitting, since it reduces operator error and ensures a consistent sampling technique. The prompted mode nominally requires 350 µL of specimen, but may require one or more additional specimen injections of 50 µL before reaching a final result. The time taken to actually inject the 50 µL should be approximately ½ second. 50 µL is a minimum injection. The injection could be as much as 100 µL or more if you have plenty of sample.

NOTE:
Run samples in the same mode that the instrument was calibrated in.

Selecting Manual or Prompted Mode

The default mode at power up is manual. The instrument stays in the manual mode until you press **PROMPT**. You must press **PROMPT** each time to begin a prompted test. After completing or aborting a test, the instrument returns to the manual mode. In the manual mode, the display shows only the colloid osmotic pressure in the selected units, as shown in Figure 4-2.

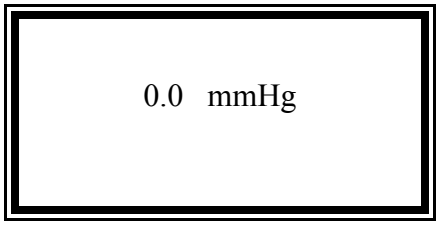


Figure 4-2 *A normal display in manual mode*

When entering the prompted mode, the display instructs you to inject 200 microliters of sample, as shown in Figure 4-3. If you press **PROMPT** again before injecting a sample, the instrument checks to see that the reading is at or near zero. If not, the display will prompt you to rinse the sample chamber with saline (Figure 4-4). If the reading is at or near zero, or after you inject sufficient saline, the display will revert to the manual mode display of Figure 4-2.

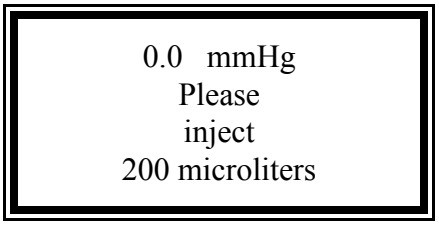


Figure 4-3 *The initial display in prompted mode*

Once you begin a prompted test by injecting specimen, the instrument remains in prompted mode until it reports a **FINAL RESULT**. To abort the prompted test, press **PROMPT**. The display will then direct you to flush the sample chamber with saline solution, as in Figure 4-4. When complete, the instrument reverts to manual mode. While in the prompted mode, the **UNITS** switch, **ZERO** switch, and **CALIBRATE** switch are not active.

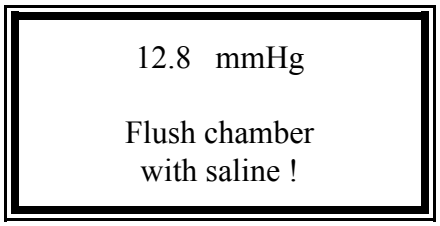


Figure 4-4 *A prompt to rinse the sample chamber when needed*

4.2 – The Manual Mode

This section describes recommended injection procedures for normal samples when using manual mode. The **UNITS**, **ZERO**, and **CALIBRATE** controls are all active during manual mode operation.

NORMAL SAMPLE VOLUME (at least 350 microliters)

Use a sample volume of 350 µL, injected as follows:

1. Inject 200 µL, then pause for 90 seconds. Immediately record reading and do Step 2.

2. Inject 50 µL, then pause for 45 seconds. Immediately record reading and do Step 3.

3. Inject 50 µL, then pause for 30 seconds . Record reading and repeat Step 3. When the previous reading is within 0.2 mmHg (or equivalent) of your current reading, you have reached equilibrium plateau. A typical sample requires 3 to 5 injections.

Inject Saline Solution

1. Promptly after the sample has been measured, remove the sample syringe from the injection port.

2. Insert a 10 mL syringe filled with saline into the sample injection port.

3. Inject 3 mL of saline through the sample chamber. Wait for a stable reading. Inject 1 mL of saline and wait for stable reading. You should now read virtually "Zero". If you still have a small positive offset after certain samples, flush another 1 mL of saline and wait for a stable reading.

The instrument should return to the within 20 to 90 seconds after you inject the saline. If the reading climbs after a few seconds, inject more saline through the sample chamber. Do not leave sample in the instrument.

SPECIAL PROCEDURE FOR MINIMAL SAMPLE VOLUME

If your specimen volume is limited, reasonably accurate measurements can still be made on as little as 125 microliters of sample solution if you employ the following procedure:

1. Before injecting the sample to be tested, inject 300 µL of a colloid solution having a colloid osmotic pressure similar to the anticipated value of the solution to be tested (a solution can be made up from pooled serum from the laboratory). After injecting this solution, pause until the displayed reading shows no change during a 10 second period.

2. Use a fresh, clean syringe to inject 125 µL of the test solution. To prevent an air bubble between the initial colloid solution and the specimen, use the syringe to fill the injection port level with the top of the sample chamber before inserting the specimen syringe. Since the difference between the colloid osmotic pressure of the solution previously in the chamber and that of the solution being tested will be small, dilution error that results from mixing of the two solutions will be correspondingly diminished.

With small volume samples, it is particularly important to use a uniform volume for each test, and to inject the sample at a uniform rate. Also, any air bubble in the system will tend to reduce the COP reading. For a valid reading, either inject the sample without introducing an air bubble (see below), or make sure you observe the air bubble (see below), or make sure you observe the air bubble leaving the sample chamber and moving toward the waste bottle.

Inject Saline Solution

1. Promptly after the sample has been measured, remove the sample syringe from the injection port.

2. Insert a 10 mL syringe filled with saline into the sample injection port.

3. Inject 3 mL of saline through the sample chamber. Wait for a stable reading. Inject 1 mL of saline and wait for stable reading. You should now read virtually "Zero". If you still have a small positive offset after certain samples, flush another 1 mL of saline and wait for a stable reading.

The instrument should return to the zero reference level within 20 to 90 seconds after you inject the saline. If the reading climbs after a few seconds, inject more saline through the sample chamber. **Do not leave sample in the instrument**.

Note: Use standard biohazard safety precautions when operating, maintaining, or decontaminating the instrument.

4.3 – The Prompted Mode

The prompted mode offers convenient, simple, and consistent sample testing in routine situations, such as in the clinical laboratory. COP results are determined, usually with no more than 350 microliters of specimen required. Once you begin a test in prompted mode, the **UNITS**, **ZERO**, and **CALIBRATE** switches will not function until the osmometer reports its final result, or until you abort the prompted test by pressing **PROMPT** and flushing the sample chamber as prompted.

The procedure below describes a typical specimen test in prompted mode. You may want to review Section 4.1, Injecting Samples, before beginning your test.

Insert the sample syringe into the sample injection port before selecting prompted mode. If the 4420 is in manual mode (only pressure shown on display), press **PROMPT** to enter the prompted mode. You will see a display like the one in Figure 4-5.

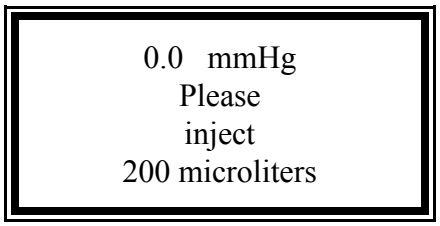


Figure 4-5 *The initial display in prompted mode*

1. Gently inject 200 µL of sample. The display will change to "Please wait" as the COP reading rises toward the plateau (see Figure 4-6).

2. After a delay, the osmometer chimes and the display will prompt you for a 50 µL injection.

3. Immediately inject 50 µL of sample. The display will change to "Please wait" as the reading rises.

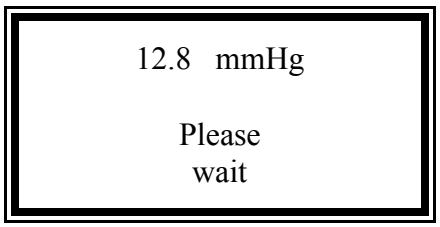


Figure 4-6 *Waiting for a plateau condition after the first sample injection*

The instrument continues to prompt you for 50 µL injections until it detects and displays the peak reading and **FINAL RESULT** (Figure 4-7). The microprocessor determines the final result when it detects a plateau condition on two consecutive 50 µL injections (within approximately 0.2 mmHg). A typical sample will require approximately three to five minutes to reach a final result in the prompted mode. In some cases it may take as long as seven minutes depending on the characteristics of the membrane.

Once **FINAL RESULT** appears on the display, you can change the units of measurement by pressing **UNITS**. You can also change the instrument calibration by pressing **CALIBRATE** (refer to Section 3 for proper calibration procedure). Both **UNITS** and **CALIBRATE** are inactive in prompted mode until the final result is obtained. The **ZERO** switch remains inactive until you return to manual mode by rinsing the sample chamber with saline solution.

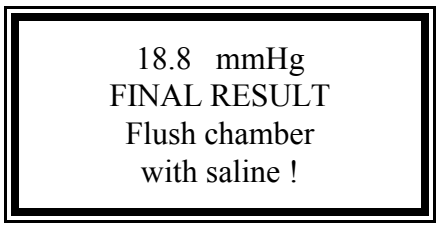


Figure 4-7 *Final result displayed with a reminder to rinse the sample chamber*

The display will now prompt you to flush the sample chamber

with saline solution, as in Figure 4-7. If you do not flush the sample chamber with sufficient saline within four minutes, the osmometer will sound an alarm to remind you to flush the chamber. As you flush the chamber with saline, the COP reading gradually falls below zero, the alarm will stop, and the “Flush chamber with saline!” prompt will be cleared from the display.

Inject Saline Solution

1. Promptly after the sample has been measured, remove the sample syringe from the injection port.

2. Insert a 10 mL syringe filled with saline into the sample injection port.

3. Inject 3 mL of saline through the sample chamber. Wait for a stable reading. Inject 1 mL of saline and wait for stable reading. You should now read virtually "Zero". If you still have a small positive offset after flushing out certain samples, flush another 1 mL of saline and wait for a stable reading.

The instrument should return to the zero reference level within 20 to 90 seconds after you inject the saline. Do not leave sample in the instrument.

Note: Use standard biohazard safety precautions when operating, maintaining, or decontaminating the instrument.

4.4 – Storage Procedures

STORAGE BETWEEN USES

When the osmometer is idle, saline solution should fill both sample and reference chambers and the inner cup of the waste collection system. The sample chamber injection port should be closed with a saline syringe and the pinch valve must be closed.

Empty the waste collection container after each use or whenever the liquid rises to a visible level. We recommend that you add a small amount of sodium hypochlorite to the container before installation in the osmometer to help control bacterial growth. After reinstallation, be sure to inject sufficient saline solution to fill the inner cup of the waste collection system. This will prevent evaporation of water from within the osmometer cell that would otherwise allow the membrane to dry out and/or result in salt blockage of the chamber ducts. Make sure that the reference chamber drain tube is inserted approximately 1 inch into the center hole of the waste container lid.

LONG-TERM STORAGE

If the osmometer will be idle for several weeks and you want to preserve the membrane: Flush both the reference chamber and the sample chamber with copious amounts of saline solution to remove any organic residues from the osmometer cell. Empty and dry the waste collection bottle. Remove the sample chamber drain tube from the sample chamber outlet nipple and connect the reference chamber drain tube in its place, as illustrated in Figure 4-8. Leave the pinch valve in the open position. Install plugs securely in the sample chamber injection port and the reference chamber injection port. With the osmometer cell thus sealed, the integrity of the membrane can be maintained over several weeks of storage.

CAUTION

DO NOT EXPOSE THE INSTRUMENT TO FREEZING TEMPERATURES WHILE LIQUID REMAINS IN THE OSMOMETER CELL

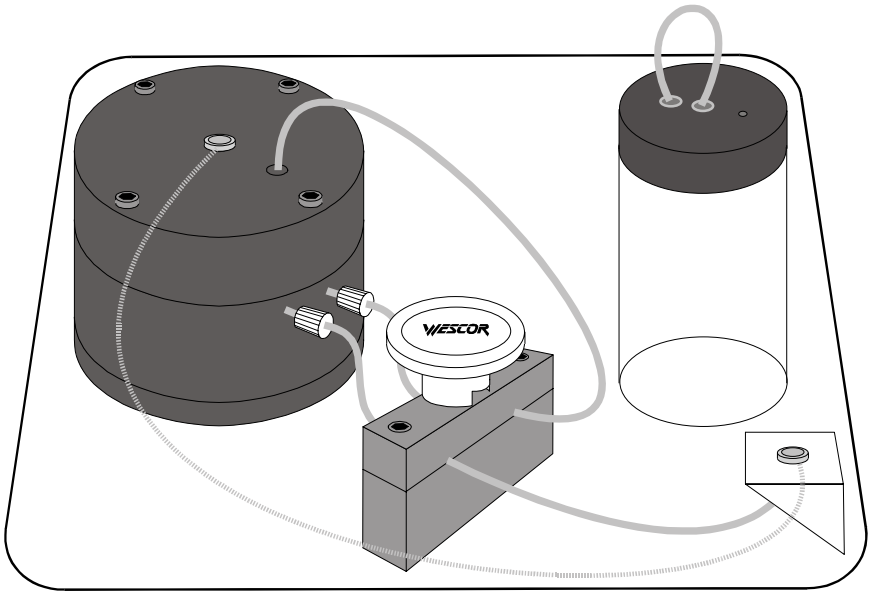


Figure 4-8 *Long-term storage with a membrane installed*

4.5 – Disinfection Procedures

Using the osmometer to measure an infectious sample contaminates the sample injection port, the internal tubing, the membrane, and the inside of the waste container. The saline syringe used for flushing the chambers also becomes contaminated when placed into the sample port and will in turn contaminate the saline when refilled from the saline container. Treat the saline flushing solution and the saline syringe as biohazards and dispose of them properly. We recommend the following procedures to disinfect the system:

1. Whenever you empty the waste container, you may add 5 mL of undiluted bleach to the empty waste container to help inactivate contaminated fluids and prevent microbial growth.

2. A solution with a 50/50 mix of isopropyl alcohol/distilled water and a contact time of 20 minutes will reduce contamination without causing significant damage to the materials used to manufacture the 4420. The solution can be wiped or lightly sprayed onto the affected surfaces for cleaning purposes. Avoid getting liquid into the electronics of the instrument.

CAUTION

Alcohol poses some fire hazard. Use appropriate biohazard, general health, and fire hazard precautions.

3. Other disinfectants may be used if you are *certain* that they will not damage materials used in the manufacture of the 4420.

CAUTION

Hydrogen peroxide can destroy aluminum. Bleach can destroy acrylic plastic. High concentrations of alcohol can destroy acrylic plastic.

4. When higher level disinfection or preparation for shipment is required, contact Wescor for current decontamination instructions and forms. (See Appendix C, Customer Service for contact information).

SECTION 5 – Theory of Operation

This section describes the theory of measuring colloid osmotic pressure, as applied in the Model 4420 Colloid Osmometer.

5.1 – Definition of Terms

The science of osmometry, as applied in clinical and research laboratories, includes the measurement of osmolality and of colloid osmotic pressure (COP), or oncotic pressure. Since there is often a degree of confusion regarding these and associated terms used in osmometry, it is appropriate to begin a theoretical discussion by reviewing the fundamental definitions and concepts.

Osmosis is the diffusion, or more specifically, the transudation of fluid through a semipermeable membrane that separates solutions of differing concentrations of solutes. Fluid transudes from the region of lower concentration to the region of higher concentration.

Osmolality is an expression of the total concentration (in mmol/kg of solvent) of dissolved particles in a solution without regard for the particle size, density, configuration, or electrical charge. Osmolality may be measured indirectly using laboratory instruments that determine either the vapor pressure depression of the solution or the freezing point depression of the solution. By definition, such measurements include both the so-called “colloid” particles and “crystalloid” particles.

“Crystalloid” and **“Colloid” Particles** are terms coined by Thomas Graham in 1861 and refer respectively to solute particles that are smaller or larger than an arbitrarily-decided particle weight which, in the specific case of body fluid components, is usually taken as 30,000 molecular weight (MW). Colloid particles (e.g. plasma protein molecules) are those that generally do not permeate the vascular membrane, while crystalloid particles (electrolytes and other small metabolites) freely permeate the vascular membrane.

Semipermeable Membranes used in the Wescor Colloid Osmometer have relatively uniform pore size so as to reject any solute particles having molecular weights above a certain limit. In physiological systems, this limit has been taken to be 30,000 MW, as noted above.

Osmotic Pressure can be a confusing term if used without qualification since it is identified as one of the colligative properties of a solution and is often used carelessly or by the uninformed as if synonymous with osmolality. Unlike the other colligative properties that are all intrinsic characteristics of the solvent, osmotic pressure is a relative characteristic of a solution with respect either to pure solvent or to another solution. While it can be calculated from mathematical considerations, it will arise as an actual pressure only when colloid particles are in differing concentrations in solutions separated by a semipermeable membrane.

The theoretical osmotic pressure of a solution with respect to its pure solvent can be calculated from the van't Hoff equation:

$$\pi = cRT$$

where π is the osmotic pressure,
c is the osmolality,
R is the universal gas constant and
T is the absolute temperature

This calculation assumes a hypothetical membrane having the ability to reject all solute particles while being freely permeable to solvent molecules. Obviously, such a membrane is an impossibility since the crystalloid particles are comparable in size to water molecules and hence will pass through any membrane that is permeable to water.

The van't Hoff relationship can be used to calculate the osmotic pressure that will exist across a semipermeable membrane if the term "c" is modified so as to represent the differential osmolality of colloid constituents on opposite sides of the membrane.

Colloid Osmotic Pressure is a physicochemical phenomenon that occurs whenever two solutions having different concentrations of colloid particles are separated by a semipermeable membrane. In general, colloid osmotic pressure measurements are not made relative to pure water, but rather with reference to normal saline solutions that more closely approximate the fluids present in the interstitial spaces of the body.

Colligative Properties are defined as those properties of a solution that bear a mathematically linear relationship to solution concentration, or osmolality. The four properties most frequently mentioned in this context are: vapor pressure, freezing point, boiling point, and osmotic pressure. The first three are cardinal properties of the solvent that are modified in direct proportion to the number of solute molecules added per unit mass of solvent. In general, the colligative relationships apply only to non-volatile solutes.

Solvent free-energy is the fundamental basis of the measurement of colloid osmotic pressure and the measurement of osmolality. Solvent free-energy is reduced whenever solute is added to the solvent. This in turn gives rise to corresponding changes in the colligative properties of the solution that afford a means for the determination of osmolality, i.e. vapor pressure depression or freezing point depression. The measurement accounts for all solute particles without discrimination as it is referenced to free-energy of the pure solvent.

On the other hand, the measurement of colloid osmotic pressure is discriminatory with respect to solute particle size because of the semipermeable characteristic of the membrane. Pressure results from the differential osmolality (differential solvent free-energy) that exists, at equilibrium, between the solutions on opposite sides of the membrane.

Because of electrical charge on some colloid molecules, diffusible charged particles present in solution will become involved in the development of colloid osmotic pressure, even though they can permeate the membrane. Known as the **Gibbs-Donnan Effect**, this phenomenon is discussed in Section 5.4.

5.2 – Fundamental Osmotic Pressure

Osmotic pressure can be demonstrated quite simply in the laboratory. Consider the classical experiment illustrated in Figure 5-1. The arms of a U-tube are separated by a semipermeable membrane. One arm is initially filled with pure solvent, while the other arm is filled to the same level with a solution made up of solvent and non diffusible (colloid) solute molecules.

Both solvent and solute molecules are in a state of constant random motion, due to their thermokinetic energy. Given time, a number of the solvent molecules will traverse the pores of the membrane in both directions, but there will be an initial net flow from the solvent side to the solution side of the membrane. This causes a rise in the liquid level in the solution arm as the level in the solvent arm falls.

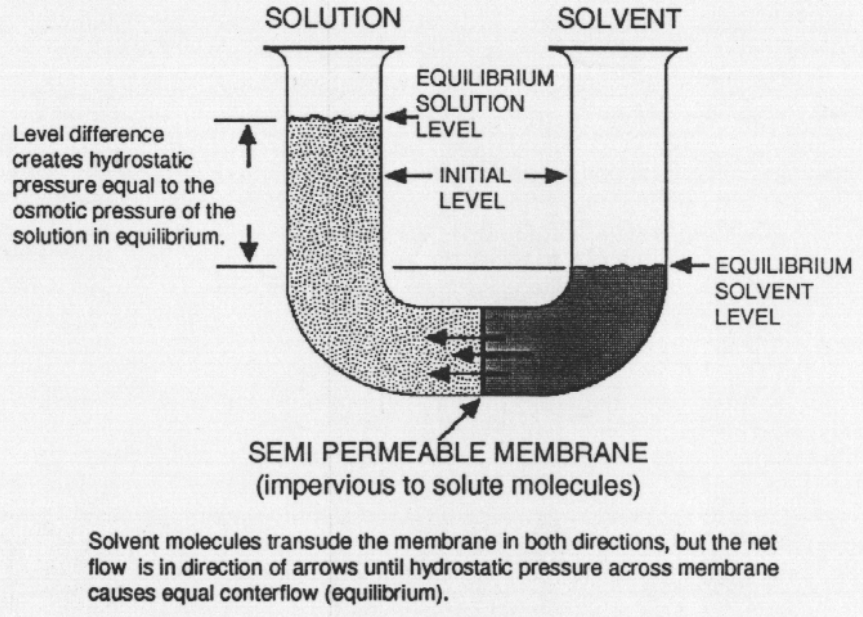


Figure 5-1 *An Open U-Tube Osmometer*

As the difference in level between the two arms increases, hydrostatic pressure builds across the membrane. The pressure acts to increase the flow of solvent molecules from the solution arm to the solvent arm, thus counteracting the osmotically-induced flow. The new flow reaches zero when solvent molecules transude the membrane equally in both directions. In this equilibrium, the solvent free-energy difference, or osmotic pressure, can be determined simply by measuring the difference in liquid levels in the arms of the tube. The osmotic pressure is equal to the level difference multiplied by the specific weight of the solution, and will agree with the value calculated using the van't Hoff relationship. Various theories have been postulated to explain the physicochemical mechanisms that come into play in the transport of water and diffusible solute molecules through membranes. A number of these have been reviewed by Kul (see bibliography).

While the open U-tube apparatus allows a simple demonstration of osmosis, it suffers from a number of practical shortcomings that prevent it from being a convenient laboratory instrument for routine testing applications. Some of these shortcomings are apparent on inspection:

1. Because the apparatus relies upon gravity to create hydrostatic pressure, a relatively large volume of solvent must transude the membrane into the solution side of the tube before equilibrium is attained. This takes considerable time and has the undesirable effect of significantly altering the concentration of the solution during the process.

2. Because of this dilution, a calculation must be performed to find the osmotic pressure of the original solution.

3. Since the membrane itself is structurally thin, the hydrostatic pressure tends to "balloon" the membrane away from the high-pressure side. This increases the time necessary to reach equilibrium and may also cause spurious permeability changes or even physical damage to the membrane because of the induced stress.

The apparatus illustrated in Figure 5-2 is improved in practical ways to eliminate the major shortcomings of the open U-tube. It provides a direct, rapid, and accurate readout of the osmotic pressure of the solution. In this configuration, a permeable support structure reinforces the membrane to reduce ballooning to a negligible level. The solvent arm of the U-tube is closed with a sensitive pressure-measuring device that is hydraulically coupled to the solvent.

The open arm of the U-tube is filled with solution, as before, so that the initial pressure in the solvent chamber is zero. (A zero pressure condition in the solvent chamber is unnecessary if correction can be made for quiescent initial pressure.) Solvent molecules transude the membrane, as in the previous example, but in this case, negative pressure develops rapidly in the solvent chamber.

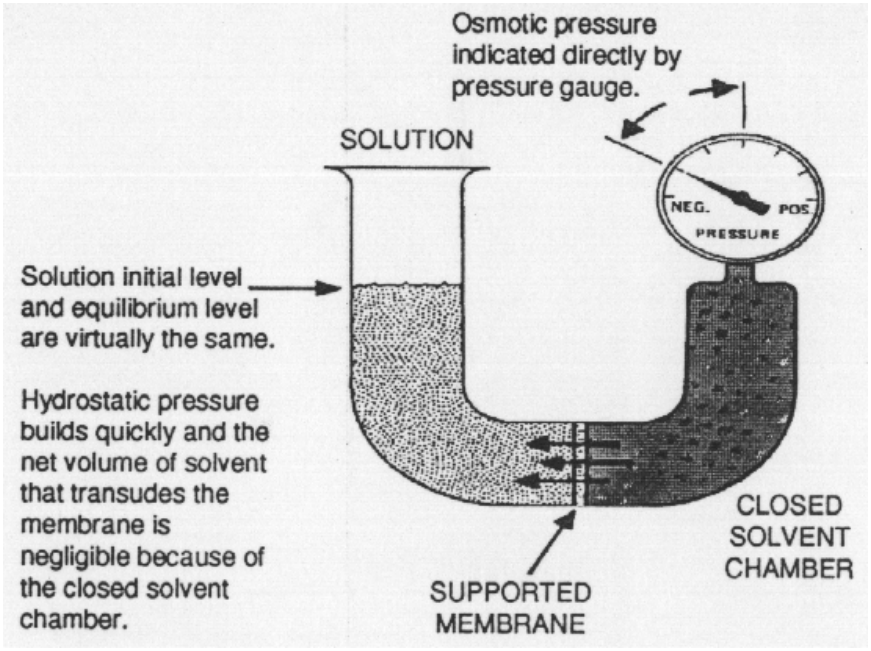


Figure 5-2 *A U-Tube Osmometer with Closed Chamber and Direct-Reading Pressure Transducer*

Theoretically, if there is negligible ballooning of the membrane and if the walls of the solvent chamber are perfectly rigid, then the volume of solvent that transudes the membrane to the solution side will be only that necessary to actuate the mechanism of the pressure indicator. Assuming this is small, so that the gravimetric effect of the increased liquid level in the solution arm is negligible, then the equilibrium hydrostatic pressure, as indicated by the negative reading on the pressure indicator, is the true osmotic pressure of the solution.

5.3 – Physiological Membrane Systems

For the sake of simplicity in the previous discussion, a single, uncharged, non diffusible (colloid) solute was considered. Thus, in this example the measured osmotic pressure is the colloid osmotic pressure of the solution with respect to pure solvent and is equal to the value given by the van't Hoff relationship where "c" is the osmolality of the solution. In applying this measurement concept to real physiological systems, we must take into account additional factors that influence the resultant measurement.

To begin, the osmotic pressure of interest will generally be the differential colloid osmotic pressure between two solutions rather than the absolute colloid osmotic pressure of a solution referred to pure solvent (water). Both solutions will contain mostly diffusible ionic and non-ionic solutes and a substantial number of colloid particles, mainly protein molecules. Furthermore, electrical charge on the colloidal protein will augment colloid osmotic pressure as a result of the Gibbs-Donnan Effect, detailed in the next section.

We must also recognize the practical limitations of membranes. Real membranes, whether natural or synthetic, will reject only solute molecules that are larger than the pore size of the membrane. Solute molecules and ions that are smaller than the pores will pass freely through the membrane along with solvent molecules. Furthermore, real membranes do not have perfectly uniform pores, but rather a distribution of pore diameters about a mean value. Therefore, even assuming globular solute particles, there will not be a precise point (in terms of molecular weight) above which all particles are rejected by the membrane, and below which all particles pass through the membrane. Instead, the membrane will exhibit a "rejection characteristic" that rises from 0 to 100 percent within a zone of increasing solute particle size.

By definition, membrane "cut-off" is the molecular weight at which the membrane will reject 90 percent of particles, as depicted in Figure 5-3. With synthetic membranes used for osmometry, suitability for a particular application requires that the cut-off be well below the lowest molecular weight of colloid particles of interest and that the pore size distribution be as narrow as possible. Membranes that meet these requirements will develop hydrostatic pressure and exhibit pressure holding ability approaching that of an "ideal" membrane.

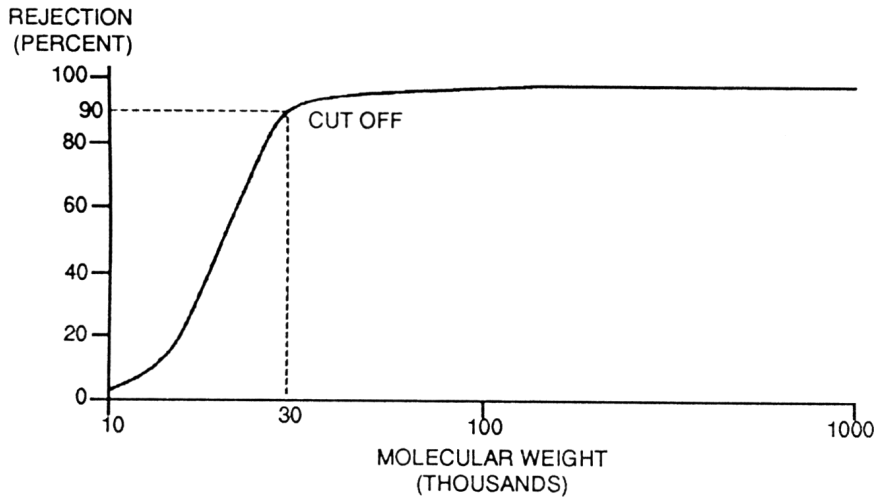


Figure 5-3 *Membrane Rejection Characteristic*

5.4 – The Gibbs-Donnan Effect

An important factor in biological systems is the contribution to colloid osmotic pressure from the net electrical charge of the protein molecules in the presence of charged membrane-diffusible ions. At normal blood pH levels, the net protein charge will be negative. Electroneutrality must be reached on either side of the membrane. The presence of non-diffusible negatively charged colloid particles requires that the concentration of positively charged diffusible ions must exceed that of the negatively charged diffusible ions on the colloid side of the membrane. The diffusible ions redistribute across the membrane so that, at equilibrium, the product of the concentrations of **diffusible** ions on each side of the membrane is equal.

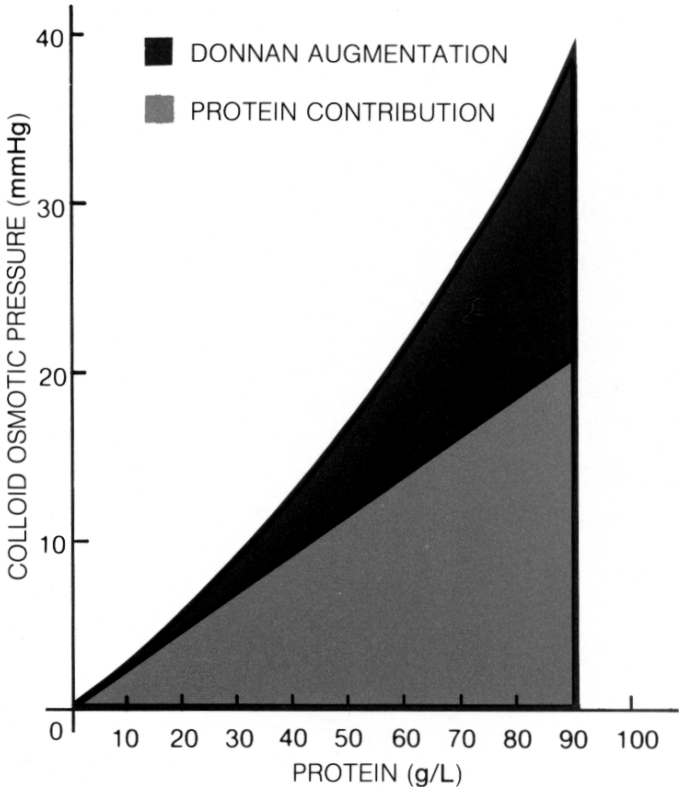


Figure 5-4 *COP vs. Protein Concentration*

Considering only the diffusible ions at equilibrium, the concentration on the colloid side of the membrane will be slightly greater than that on the non colloid side, producing an osmotic pressure difference that augments the pressure due to the concentration of the colloid particles per se, which is a linear function of the colloid concentration. The pressure component of the Gibbs-Donnan Effect is a function of the square of the electrical charge carried by the colloid component. It follows that since the total measured COP is made up of both contributions, it is non linearly related to colloid concentration. This relationship is illustrated in Figure 5-4.

5.5 – Calculated versus Measured COP

In recent years there has been controversy among medical professionals as to the need for laboratory measurement of COP. It has been demonstrated that a correlation exists between total measured protein and colloid osmotic pressure in normal blood samples. This fact is often put forth as an argument against the need to measure COP.

The actual value of COP in a given blood sample will be influenced by the blood pH as well as variation in the relative transportation of plasma proteins, as shown by the electrophoretic protein pattern of the sample. The formulas used to calculate COP from total protein measurements have been derived empirically and are based on blood samples where pH and electrophoretic patterns are normal. In the case of critically ill patients, these conditions may not apply. COP is becoming more widely recognized as a valuable laboratory test. A bibliography of relevant technical publications can be found at the end of this manual.

5.6 – The 4420 Colloid Osmometer

In the Wescor Colloid Osmometer, the vascular and interstitial compartments of the body are represented by the sample and reference chambers, respectively, of the osmometer test cell assembly. The synthetic membrane separating the two chambers simulates the vascular membrane.

The design of the instrument is based upon the concept illustrated in Figure 5-2, but with considerable refinement to satisfy the needs imposed by routine clinical testing, where minimal sample volume, ease of operation, and simple maintenance are mandatory.

The osmometer has five major parts or assemblies that function together as an integrated system, illustrated diagrammatically in Figure 5-5. The parts are separately detailed in the following.

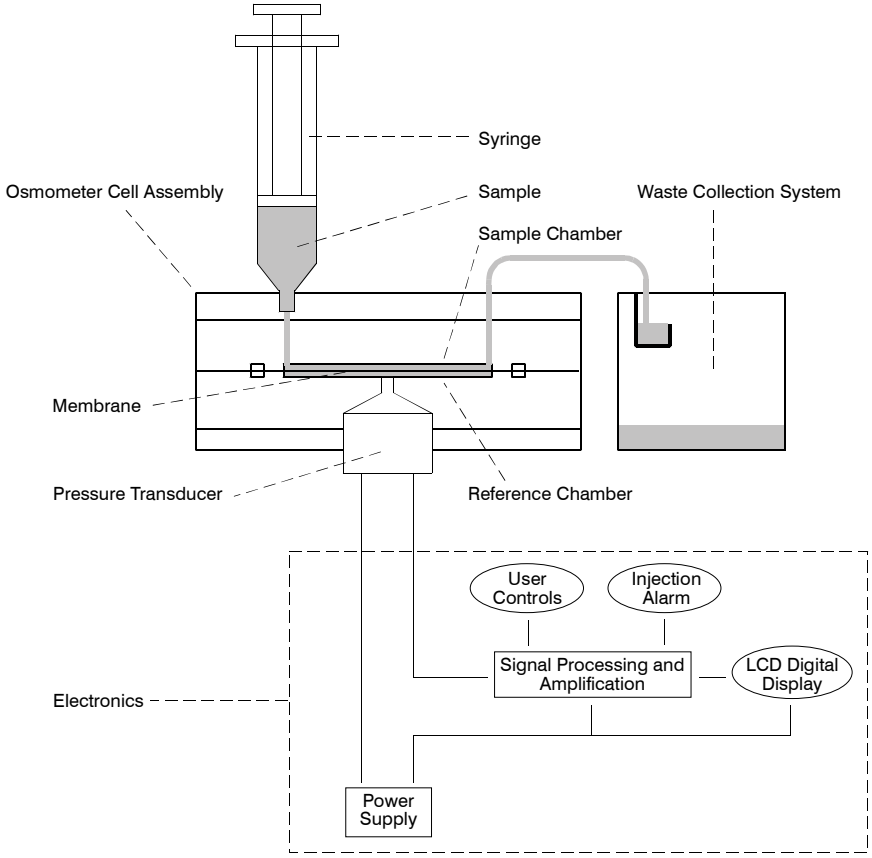


Figure 5-5 *The 4420 System*

1. Osmometer Cell Assembly

The osmometer cell assembly consists of two precisely machined cylinders having corrosion-resistant surfaces.

The reference chamber is machined in the face of the lower cylinder. It consists of a shallow spiral groove that communicates through ducts to the pressure transducer mounting port and the reference chamber inlet and outlet ports. An annular channel which receives the mounting frame of the membrane is also machined in the face of the lower cylinder.

The sample chamber has a spiral groove precisely matching that of the reference chamber. It is machined in the face of the upper cylinder of the osmometer cell assembly. The upper cylinder likewise has an annular channel to receive the membrane mounting frame. Opposite ends of the sample chamber groove are connected by ducts to the sample chamber luer inlet port and to the sample chamber outlet nipple on the top surface of the cylinder.

The reference and sample chamber cylinders are assembled with the membrane between them. Because the width of the grooves is small and the membrane is firmly held by the clamping action of the cylinders, membrane displacement (ballooning) is negligible.

2. Pressure Transducer

The pressure transducer is interfaced to the reference chamber cylinder of the osmometer cell assembly. Its sensing diaphragm is contiguous with the reference solution. The pressure transducer converts minute pressure changes in the reference chamber into electrical changes that are measured and displayed.

3. Membrane

The standard membrane has a cut-off of 30,000 MW and is only a few microns in thickness. It is formed on a polymeric substrate that is approximately 0.2 millimeters thick. A unique plastic mounting frame maintains flatness, provides a safe and convenient means of handling, and positions the membrane upon installation onto the reference chamber of the osmometer cell. Packed in saline solution to provide maximum shelf life, the membrane requires no preliminary preparation or conditioning prior to installation into the osmometer cell assembly.

4. Waste Collection System

The waste collection system is connected to the osmometer cell assembly and is an integral part of the sampling system of the osmometer. It helps assure accuracy of measurements by (a) providing a constant gravimetric pressure in the sample, and (b) eliminating capillary end-tension effects that would otherwise induce variable back pressure with concomitant measurement error.

5. Electronics

Most of the instrument electronics are mounted on a single, microprocessor controlled, printed circuit module that contains the signal conditioning and amplification circuits, zero, calibrate, and prompt/unit selection controls. The power supply, the over-pressure alarm transducer, and the digital display are mounted on separate printed circuit modules. In the unlikely event of a circuit component failure, the normal field servicing procedure is simply to replace the entire module. This can be accomplished in a matter of minutes. Refer to Section 6, Solving Problems for more information on servicing the instrument. Appendix B offers additional technical information about the instrument electronics.

5.7 – Operating Sequence

This section describes how the components of the instrument respond to your actions and work together to test a typical sample. This information is intended only as an overview of how the osmometer operates. For complete operating instructions, refer to Section 4, Operating the Osmometer.

You must initially install a membrane and fill both the reference chamber and sample chamber with normal saline solution. Normal saline is used in the reference chamber to produce approximately the same Gibbs-Donnan Effect as would normal interstitial fluid. Initial filling of the chambers calls for 10 cc of saline solution to fill the inner cup of the waste collection system as well, thereby bringing that system into operational readiness.

After filling both the reference and sample chambers with saline, press the **ZERO** switch on the front panel to clear any offset and adjust the display reading to zero.

Use a plastic syringe to inject sample solutions into the sample chamber. Leave the syringe in place during measurement to close the input end of the system. The sample chamber is vented to atmosphere at the waste collection end.

When a solution containing protein molecules is introduced into the sample chamber there is an immediate net migration of water molecules and diffusible solute ions from the reference chamber, through the membrane, and into the sample chamber. This is caused by the osmotic effect of the non-diffusible protein molecules and the Gibbs-Donnan Effect resulting from their electrical charge.

The resultant negative pressure in the reference chamber developed across the membrane is sensed by the pressure transducer. The inverted signal from the pressure transducer is converted directly into either millimeters of mercury (mmHg), centimeters of water (cmH2O) or kilopascals (kPa) depending upon the **UNITS** selection you have made. The pressure reading is displayed on the alpha-numeric display. The pressure difference will normally reach equilibrium within 30 to 120 seconds after the colloid solution is injected into the sample chamber. After the necessary number of injections, a final result (or plateau) is reached. Higher values typically require longer times and more injections to reach a final result.

Flush the colloid solution from the sample chamber with fresh saline solution promptly after making a colloid osmotic pressure measurement. When necessary, the reference chamber can be easily flushed with fresh saline solution following the procedure outlined in Section 2.4.

SECTION 6 – Solving Problems

SECTION 6 will help solve routine problems encountered in operating the osmometer. It includes troubleshooting procedures and additional information about the instrument electronics, transducer, membrane, and tubing. If these procedures fail to resolve a problem, contact Wescor's service department for help or to determine if your osmometer needs factory service. Refer to Appendix C, Customer Service.

> **Note: Do Not Ship** the Colloid Osmometer unless it has been drained, cleaned, and decontaminated (see Appendix C, Customer Service).

6.1 – Troubleshooting

This section will help you solve problems that you may encounter with the 4420. Try to locate a description of your problem in the left column, then use the possible solution listed in the right hand column.

PROBLEM	POSSIBLE SOLUTION
POWER lamp is not lit, display is blank.	Check the rear panel power switch, power connector, fuses, line power outlet, and source. Power cord must be completely inserted into power module. See Section 1.4, Figures 1-4 and 1-5. Make sure the source voltage matches the voltage indicated on the rear panel Voltage Selector; Section 1.4, Figure 1-4.
Scrambled language or messages appear on the display.	Switch the power off, wait 10 seconds, switch power on. If the problem remains, reset the language according to the instructions in Appendix B under heading **Software Language**.
Display indicates **BATTERY FAILURE**.	See **Batteries** in Section 6.2.
Cannot inject saline into the reference chamber injection port.	Make sure the reference chamber pinch-valve (Figure 1-3) is open. Check the tubing for salt deposits. Squeeze the tubing gently along the area normally inside the pinch valve. See Section 6.5.
Cannot inject fluid into the sample chamber.	Check the sample chamber drain system for salt deposits or blockage. Check the connections to the waste container, Figure 1-3. See Section 6.5

Osmometer repeats a loud tone.	Check the display–if it indicates "Flush chamber with saline!", immediately flush the sample chamber, Section 3.1.
Osmometer sounds a loud tone when you inject sample or saline, even though injection pressure is slight.	Check all tubing and waste container inner cup for salt deposits or blockage.
Instrument will not hold a plateau reading.	Check the tightness of the sample chamber screws–see Section 2.3, Steps 11,12, and 13, and Figures 2-7 and 2-8. Check for air bubbles in the sample or reference chambers. See the information in Sections 2.5 and 6.4.
No pressure displayed or pressure does not change with sample injection.	Be sure the pinch valve is closed. Transducer may be damaged- check the information in Sections 6.3 and 2.2. Contact Wescor for further assistance.
Readings increase.	Empty waste container. Membrane may be stabilizing.
Pressing "Zero" does not zero display when in manual mode.	Transducer may be damaged from excessive pressure. Contact Wescor.

6.2 – Electronics

Power Mains

Electronic malfunctions or failures are unlikely with the 4420. Two 'Type T', time-delay fuses protect the power mains to prevent the risk of catastrophic current surge and associated damage in the event of an internal short-circuit or malfunction. The fuses are in the power entry module on the rear panel. If a fuse fails, it may indicate a serious internal problem. You should always determine the cause of a fuse failure and correct the problem before replacing the fuse(s) and reconnecting the instrument to power.

The correct power source for your 4420 is indicated on a label on the rear panel. The selected power source is shown in the power entry module window. Be sure to connect the instrument to the correct power source to avoid serious damage or injury.

Internal Batteries

The 4420 uses two 3-volt lithium batteries to maintain power to the microprocessor's memory in case the external power source is interrupted. This eliminates the need to recalibrate the instrument or set the zero reference if power is interrupted. The batteries are mounted inside the instrument case, in a special module on the main circuit board. You will know the batteries have failed when the 4420 displays the message shown in Figure 6.1 at power up.

Figure 6-1 *Time to replace the 4420's batteries*

Normal battery life is about five years. Replacement batteries are available from Wescor under catalog number 30-0125 (two required, Eveready CR2025 or equivalent). See Appendix B for instructions to replace the batteries. If the batteries do fail, you may see scrambled information or a foreign language on the display. Refer to the troubleshooting chart in Section 6.1 for assistance.

6.3 – Transducer

As discussed in Section 2, the pressure transducer in the 4420 is an expensive, sensitive, and delicate component. The pressure transducer can be damaged by excessive injection pressure or improper cleaning procedures. Please read Section 2 carefully for correct operating procedures that will minimize the risk of damage to the transducer. Beyond the concerns listed above, the transducer is well protected. The cell assembly is designed to help protect the transducer from general shock or carelessness.

6.4 – Membrane

Most problems with the colloid osmometer arise from improper membrane installation and/or improper preventive maintenance procedures. The most common problem appears as a low response to a colloid osmotic pressure standard. This can arise from (a) insufficient clamping pressure on the membrane (refer to Section 2.3), (b) air leaks in the osmometer test cell assembly, (c) air bubbles in the sample or reference chambers or (d) cell top not parallel with the cell bottom when tightened (the most common cause of low response). There is also a tendency for certain membranes, when new, to give unsatisfactory plateau holding performance. In many cases the same membrane will perform normally after being left in the instrument overnight. Please refer to Sections 2.4, 2.5, and 2.6 for more information.

If, after following the procedures in this manual, you cannot resolve a problem, please contact Wescor (see Appendix C) to obtain prompt assistance.

CAUTION

Do Not Ship the Colloid Osmometer unless it has been drained, cleaned, and decontaminated. Contact Wescor for return authorization. (see Appendix C, Section 4.5, and Section 2.2).

6.5 – Tubing

If saline solution evaporates from the sample chamber or the drain lines, the resulting salt deposits can block the fluid ducts, making it impossible to inject solutions into the chamber. Proper preventive maintenance will preclude this, but if it does occur, remove the sample cell and membrane to allow safe removal of the obstructions.

If the instrument is idle for a long period with the pinch-valve closed, the tubing inside the valve may deform slightly. It then can remain "mashed" when the valve is opened, preventing fluid flow. In this happens reshape the tubing by squeezing it gently from the sides, along the length that lies inside the valve.

Salt deposits in the drain lines or in the spiral reference and sample chambers can be removed simply by rinsing with water. Clearing the smaller ducts may be more difficult, particularly in the reference chamber, which ordinarily remains attached to the interior of the instrument. These ducts can be cleared using a 0.038 inch diameter or smaller wire, such as a straight section of paper clip wire. It would be best to use soft copper wire such as a 1/4 watt resistor lead.

CAUTION

USE GREAT CARE WHEN USING THIS TECHNIQUE TO CLEAR THE VERTICAL BORE HOLE AT THE CENTER OF THE REFERENCE CHAMBER SPIRAL. THE PRESSURE TRANSDUCER DIAPHRAGM IS LOCATED DIRECTLY BELOW THE DUCT. MAKE A "SAFE" CLEARING TOOL FROM A LENGTH OF WIRE BY BENDING IT TO FORM A SHOULDER AT A DISTANCE OF 3 MILLIMETERS (1/8 INCH) FROM THE END (See Figure 6-2). THE BEND WILL PREVENT DAMAGE TO THE SENSITIVE DIAPHRAGM OF THE PRESSURE TRANSDUCER.

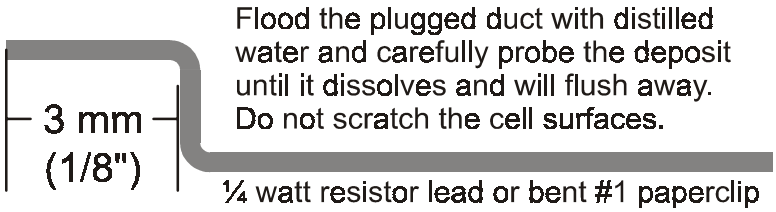


Figure 6-2 *A safe tool for clearing salt deposits from the reference chamber bore*

APPENDIX A – Accessories, Supplies, & Replacement Parts

Cat No.	Accessories
AC-007	Membrane Scraper
AC-012	Torque-Indicating Driver
	Supplies
SS-025	Osmocoll® N (Normal Level) COP Calibrator/Osmolality Control (package of 6, 1 mL vials) Approximately 20 mmHg COP*
SS-038	Osmocoll® HL (High and Low Level) COP Control References (package of 6, 1 mL vials, 3 High, 3 Low) High: Approximately 25 mmHg COP* Low: Approximately 15 mmHg COP*
SS-030	Membranes, Wet-packed, Premounted, 30,000 Molecular Weight Cutoff, PM Series (package of 3)
SS-050	Membranes, Wet-packed, Premounted, 10,000 Molecular Weight Cutoff, YM Series (package of 3)
SS-057	Membranes, Wet-packed, Premounted, 10,000 Molecular Weight Cutoff, PM Series (package of 3)
SS-123	Membranes, Wet-packed, Premounted, 3,000 Molecular Weight Cutoff, YM Series (package of 3)
	*Each Osmocoll lot has a specific control value and range.
	Replacement Parts
30-0125	3 Volt Lithium Battery, CR2025 (2 required)
RP-028	Port Plugs
RP-033	Reference Chamber Drain/Fill Tube Assembly (2 pieces, 1 white/1 red)
RP-034	Waste Container
RP-072	Pressure Transducer
RP-073	Cell Assembly
RP-128	Electronics Module Exchange
RP-129	Display Module
RP-130	Power Supply Module

APPENDIX B – Electronics

INSTRUMENT LAYOUT

The electronic components of the model 4420 Colloid Osmometer have been designed and assembled in a modular fashion. The main electronics module is mounted behind the instrument front panel. This module contains the control switches, power indicator, microprocessor, backup batteries, memory, signal processing, and associated circuitry. The power entry module is mounted on the instrument's inside rear panel, along with the injection alarm tone transducer and chart recorder output connector. The display module is mounted to the front panel between the case and the main electronics module. The power supply is located on the case bottom, directly beneath the bowl and cell area. Schematic diagrams for the 4420 electronics are included at the end of this appendix.

CIRCUIT DESCRIPTION

Power Supply and Regulation

The power supply board converts the line voltage to a dual unregulated DC voltage, typically this voltage would range from ±12 to 21 volts depending on the actual line voltage. Voltage regulation is performed by a total of four regulators located on the main board. Most of the circuitry receives power from the main ±5 volt regulators, two separate regulators are used for the pressure transducer and microcontroller memory.

Pressure Transducer

The pressure transducer is of the strain gauge type with the output signal magnitude proportional to the deflection of the diaphragm within the transducer. The phase of the output signal is indicative of either a positive or negative pressure.

Signal Amplification and Conversion

The output signal from the transducer is amplified by the main board to increase signal strength and reduce noise. This signal is then split and sent to a second amplifier for the chart recorder output and conversion to a digital signal for use by the microcontroller. A trim pot located on the main board provides correction of any (zero) offset of the transducer for the chart output, but no gain adjustment is provided by this circuitry.

Digital User Interface

The interface between the user and pressure transducer is provided by the microcontroller (68HC11), which is located on the main board. The microcontroller accepts the inputs from the front panel control switches, converts the transducer signal into the proper units, and displays the value. It uses stored offset and gain values to compensate for errors within the signal amplification and transducer itself. The microcontroller also detects injections and looks for the signal plateau by sensing the changing signal at the transducer output.

Internal Batteries

Two 3-volt lithium batteries are mounted on the main circuit board, on the left side of the instrument (behind the display). They provide back-up power to the microprocessor's RAM to maintain the stored calibration data, language selection, units, and zero offset in the event the external AC power is interrupted. Thus, recalibration is not required whenever the instrument is disconnected from line power. The batteries will normally last about five years. When the batteries fail, the osmometer's display will indicate **BATTERY FAILURE**. Replacement batteries are available from Wescor under catalog number RP-131 (two required). You may also use any convenient source for Eveready CR-2025 or direct replacement. To replace the batteries, use the following instructions, referring to Figures B-1, B-2, B-3, and B-4.

Remove the saline syringe, the sample syringe, and the waste container before proceeding.

1. Switch the rear-panel power switch off (O), then disconnect line power by removing the power plug from the rear panel.

2. Wait at least 30 seconds to allow the power supply's filter capacitors to discharge, then remove the two screws from the bottom-front and the two screws from the rear-top of the instrument (see Figure B-1).

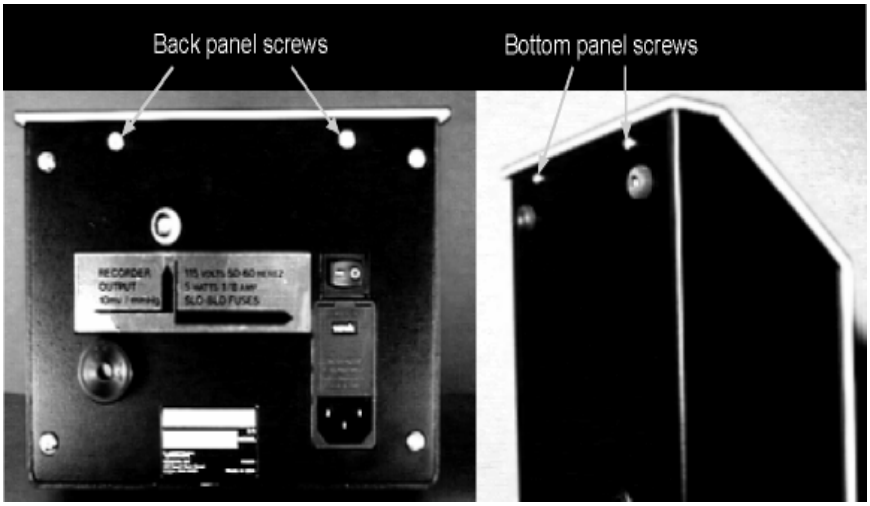


Figure B-1 *The four screws to be removed after disconnecting the 4420 from line power*

3. Gently lift the instrument's case top upward approximately one inch. The bowl and internal components will remain attached to the case top.

4. Now slide the case top assembly forward (away from the case back) about three inches.

5. You should now be able to see the batteries in their holder on the left side of the main circuit board (behind the display area).

6. Remove the small plastic connector from the pins just below the battery holder (labeled **Battery Disconnect**).

7. Gently lift the small spring away from the batteries and slide both batteries out the side of the holder.

8. Install two fresh batteries, being sure to match the polarity. The negative (-) surface of both batteries must face in, toward the circuit board. The positive (+) face of both batteries must face out, toward the battery holder clip (see Figure B-2). The battery holder clip must press against the top battery.

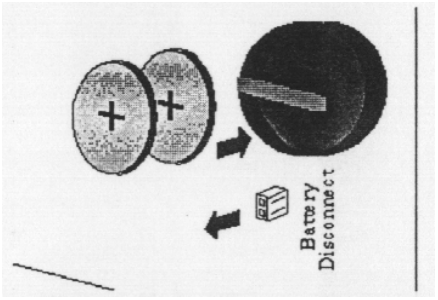


Figure B-2 *Correct polarity of batteries*

9. Replace the battery disconnect jumper that you removed in Step 6.

10. Lift the case top back into position and lower it carefully onto the case bottom. The plastic nipple from the waste container cup must fit into the rubber grommet on the case bottom. Do not try to force the case into position, since you could damage the waste cup. Also, be sure that none of the internal wires will be pinched by any case parts and that the wire connectors are still properly seated on the main board connectors.

11. Replace the four screws removed in Step 2 and reconnect line power.

You can now switch the instrument's power on. After indicating the software version and language, the instrument will display the message shown in Figure B-3.

BATTERY FAILURE
Re-calibration
required !!
Press 'ZERO'

Figure B-3 *Display after new batteries are installed (You must initialize and recalibrate the* osmometer.)

Press **ZERO** as prompted. The instrument performs internal initialization. You will briefly see the message shown in Figure B-4. Then the display shows the current COP reading. You must calibrate the instrument as described in Section3 and in Section 4 before attempting to test samples.

Software Language

Initializing

Figure B-4 *Message that appears for two seconds while the 4420 initializes*

The instrument is programmed to display prompts in your choice of English, French, or German. To change the current language, follow these instructions:

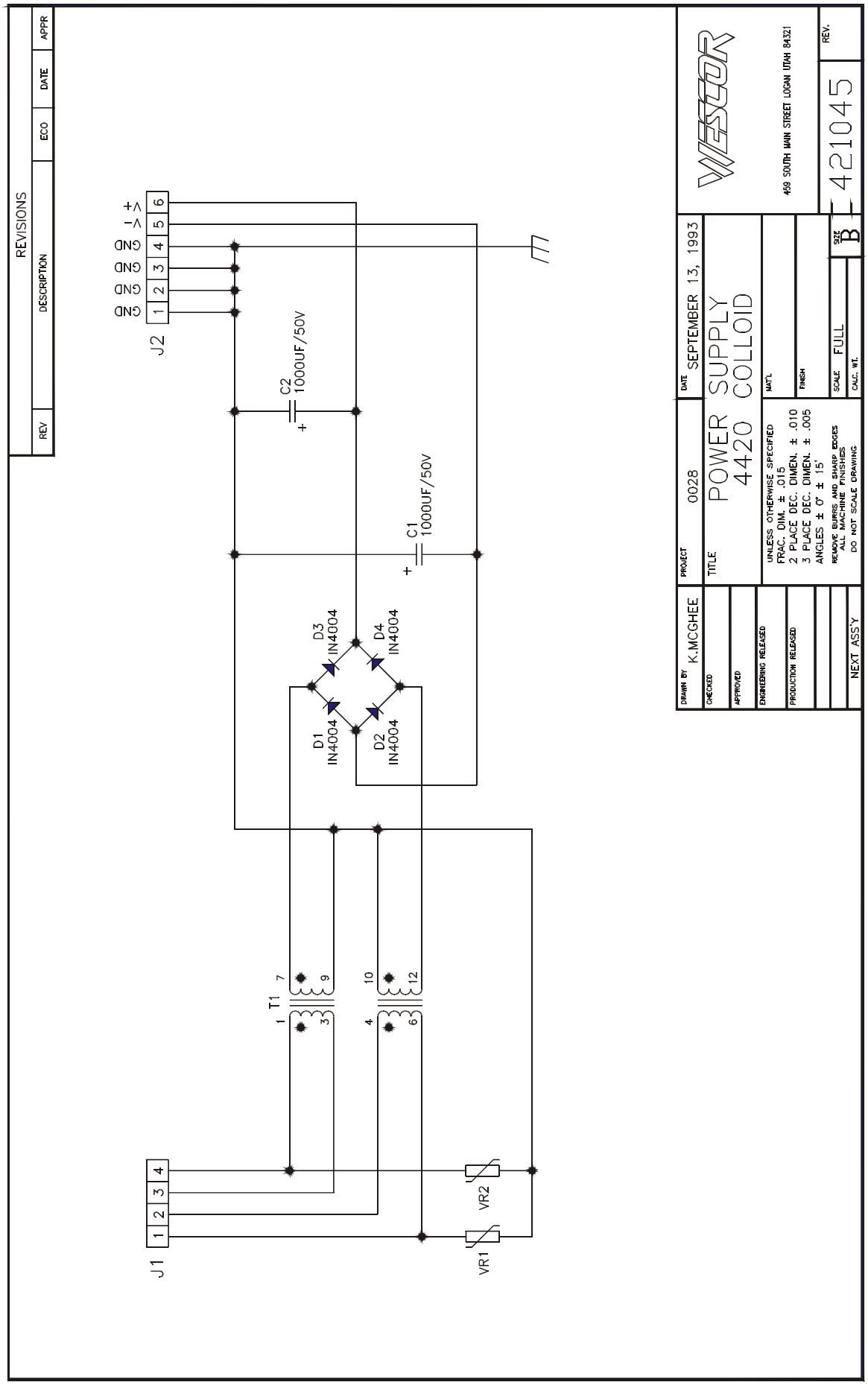
1. Switch the power off (O) at the rear panel switch. Wait at least five seconds, so that the internal voltage level can drop sufficiently for the microprocessor to reset.

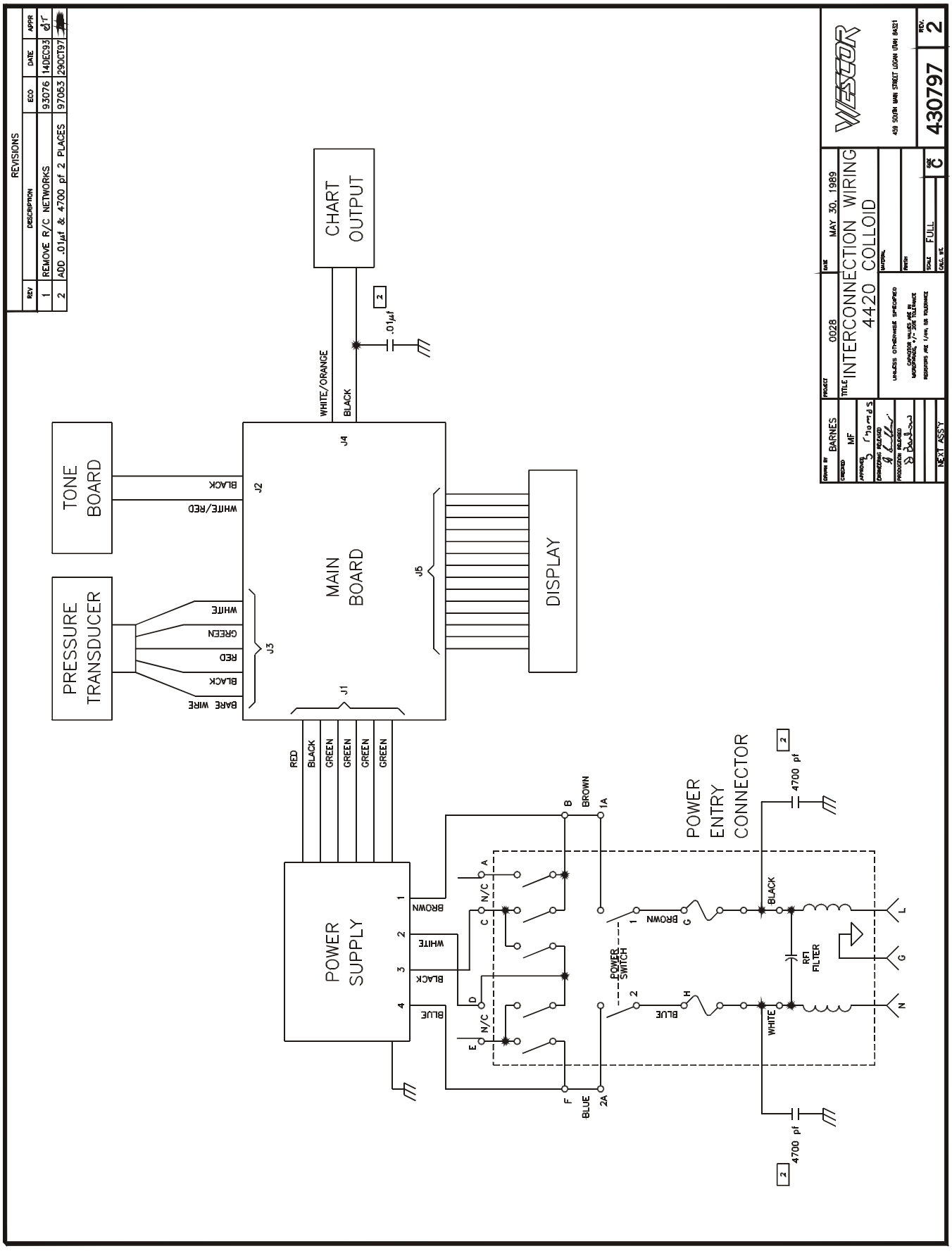
2. Switch the power back on, and **within one second** press and hold the **UNITS** switch down (while the software version and language are displayed).

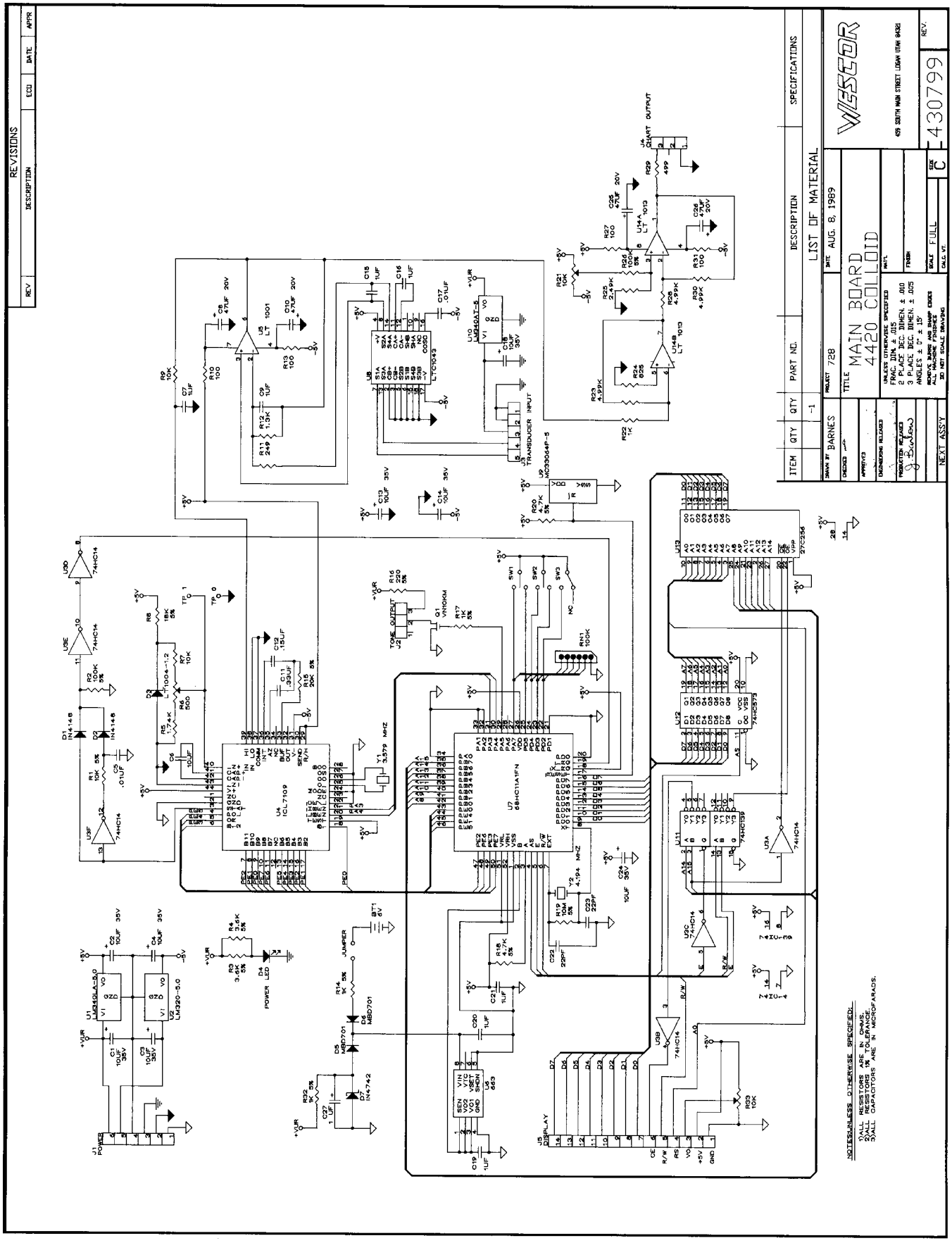
3. The display will change from the current language, cycling through **English, Francais,** and **Deutsch** as you press and continue to hold the **UNITS** switch down.

4. When the display indicates the desired language, release the **UNITS** switch. After two seconds, the display will change to the current COP reading. After this, you cannot change the language again unless you start at step 1.

If the line power is interrupted or is subject to heavy line noise, the display may show scrambled characters or a foreign language. If so, reset the language to your preference by repeating steps 1 through 4.


REVISIONS
REV
DESCRIPTION
ECO
DATE
APPR
J2
GND
GND
GND
GND
+V
+V
C2
1000UF/50V
C1
1000UF/50V
D1
IN4004
D2
IN4004
D3
IN4004
D4
IN4004
T1
J1
VR1
VR2
VESTOR
459 SOUTH MAIN STREET LOGAN UTAH 84321
DRAWN BY K.MCGHEE
CHECKED
APPROVED
ENGINEERING RELEASED
PRODUCTION RELEASED
NEXT ASS'Y
PROJECT 0028
TITLE POWER SUPPLY
4420 COLLOID
DATE SEPTEMBER 13, 1993
MATL
FINISH
SCALE FULL
CALC. WT.
SIZE B
REV.
421045
UNLESS OTHERWISE SPECIFIED
FRAC. DIM. ± .015
2 PLACE DEC. DIMEN. ± .010
3 PLACE DEC. DIMEN. ± .005
ANGLES ± 0° ± 15'
REMOVE BURRS AND SHARP EDGES
ALL MACHINE FINISHES
DO NOT SCALE DRAWING


REVISIONS
REV
DESCRIPTION
ECO
DATE
APPR
1
REMOVE R/C NETWORKS
93076
14DEC93
2
ADD .01µf & 4700 pf 2 PLACES
97053
29OCT97
CHART OUTPUT
.01µf
WHITE/ORANGE
BLACK
TONE BOARD
PRESSURE TRANSDUCER
MAIN BOARD
DISPLAY
J1
J2
J3
J4
J5
WHITE/RED
BLACK
BARE WIRE
WHITE
GREEN
RED
BLACK
RED
BLACK
GREEN
GREEN
GREEN
GREEN
POWER SUPPLY
BROWN
WHITE
BLACK
BLUE
POWER ENTRY CONNECTOR
4700 pf
4700 pf
POWER SWITCH
RFI FILTER
BROWN
BLUE
BLACK
WHITE
BROWN
BLUE
N/C
N/C
A
B
C
D
E
F
1A
2A
G
H
L
G
N
DRAWN BY BARNES
CHECKED MF
APPROVED
DIMENSIONS RELEASED
PRODUCTION RELEASED
PROJECT 0028
DATE MAY 30, 1989
TITLE INTERCONNECTION WIRING 4420 COLLOID
UNLESS OTHERWISE SPECIFIED
MATERIAL
FINISH
SCALE FULL
CAGE NO.
SIZE C
430797
REV. 2
459 SOUTH MAIN STREET LOGAN UTAH 84321
NEXT ASSY


REVISIONS
REV
DESCRIPTION
ECO
DATE
APPR
LIST OF MATERIAL
ITEM
QTY
PART NO.
DESCRIPTION
SPECIFICATIONS
DRAWN BY BARNES
PROJECT 728
DATE AUG. 8, 1989
CHECKED
APPROVED
TITLE MAIN BOARD 4420 COLLOID
WESTOR
459 SOUTH MAIN STREET LOGAN UTAH 84321
SIZE C
430799
REV.
SCALE FULL
NEXT ASSY
NOTES:UNLESS OTHERWISE SPECIFIED:
1)ALL RESISTORS ARE IN OHMS.
2)ALL RESISTORS ARE 1/4 WATT.
3)ALL CAPACITORS ARE IN MICROFARADS.

APPENDIX C – Customer Service

Wescor is ready to help you resolve any difficulty with the operation or performance of your Colloid Osmometer. If you cannot resolve a problem using the procedures in this manual, please contact us.

Customers within the United States are encouraged to contact us by telephone. Outside the U.S., many of our authorized dealers offer complete customer service and support. If factory service is required, instruments *must* be drained, cleaned, and decontaminated before being returned to Wescor. You will receive complete instructions and forms for decontaminating and shipping your osmometer once return is authorized.

Phone: (435) 752-6011
Toll Free: (800) 453-2725
Fax: (435) 752-4127
E-mail (Wescor): wescor@wescor.com
E-mail (Wescor Service and Repair): service@wescor.com
Web: www.wescor.com

Mailing Address:
Wescor, Inc
459 South Main Street
Logan, UT 84321 USA.

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