



# **SPI-DRY™ Critical Point Dryer Operation Manual**



SPI Supplies Part #13200JE-AB



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For further information regarding any of the other products designed and manufactured by SPI Supplies, contact your local representative or directly to SPI Supplies at the address above.

- Carbon and Sputter Coaters
- Plasma Reactor for ashing and etching
- High Vacuum Bench Top Evaporators
- Critical Point Dryers
- Electron Microscopy Consumables



## **Warranty**

The SPI Supplies unit you have purchased is guaranteed to be free of defects in workmanship on the day of shipment. This warranty covers parts and labor for a period of one year, excluding shipping charges or consumables. Breakage of glassware is specifically excluded from this warranty.

Proper use of your unit, according to the operation manual, should result in trouble-free operation. Any improper use of the SPI Supplies unit through modifications or unreasonable operating procedures will void this warranty.

## **Disclaimer**

SPI Supplies instruments are designed for simplicity of installation and operation. This manual provides full and complete information in both these areas. SPI Supplies therefore assumes no liability or responsibility of any kind for damage or injury resulting from incorrect installation or operation of the machine.

# 1 Contents

## 1.1 Manual Layout

This Operation Manual is divided up into the following major section, each section dealing with specific topics, as follows:

### **Section 1 - Contents**

### **Section 2 - Health and Safety**

General section which applies to all SPI Supplies products detailing the very important issues of Health and Safety applicable when using sample preparation equipment.

### **Section 3 - Introduction**

Introduces this manual.

### **Section 4 - General Description**

Identifies each of the equipment items and provides an overview of their functions and how they work.

### **Section 5 - Installation**

Instructions on how this instrument should be installed and the connections which should be made between the equipment items.

### **Section 6 - Operation**

Instructions on how to start-up and run the instrument.

### **Section 7 - Maintenance**

Instructions on routine maintenance checks ensure that the system is functioning correctly. Information on how to identify faults in the system, and how to rectify these faults.

### **Section 8 – Troubleshooting**

Spare parts and consumables

## 1.2 Section Contents

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## 2 HEALTH AND SAFETY

Safety is very important when using any instrumentation and all users of our equipment should read this section.

This section of the Manual applies to all specimen preparation equipment supplied by SPI Supplies, not just the particular instrument for which the manual refers.

Included in this section are details on warning notations and good working practices.

### 2.1 Safety Policy

This section contains important information relating to all health and safety aspects of the equipment. As such it should be read, and understood, by all personnel using the instrument whether as an operator or in a service capacity.

SPI Supplies is committed to providing a safe working environment for its employees and those that use its equipment.

SPI Supplies regularly reviews its operations to make environmental, health and safety improvements in line with applicable legislation.

The equipment has been designed as a free-standing bench mounted instrument. SPI Supplies cannot be held responsible for any damage, injury or consequential loss arising from the use of its equipment for any other purposes, or any unauthorized modifications made to the equipment.

All service work carried out on the equipment should only be undertaken by suitably qualified personnel. SPI Supplies is not liable for any damage, injury or consequential loss resulting from servicing by unqualified personnel. SPI Supplies will also not be liable for damage, injury or consequential loss resulting from incorrect operation of the instrument or modification of the instrument.

### 2.2 Conformity

This instrument is supplied in a form that complies with the protection requirements of the EC Electromagnetic Compatibility Directive **89/336/EEC** and the essential health and safety requirements of the low voltage directive **72/23/EEC** both as amended by **92/31/EEC**. Any modifications to the equipment, including electronics or cable layout may affect the compliance with these directives.

### 2.3 Servicing

#### 2.3.1 Disclaimer

All service work on the equipment should be carried out by qualified personnel. SPI Supplies cannot be liable for damage, injury or consequential loss resulting from servicing from unqualified personnel. SPI Supplies will also not be liable for damage, injury or consequential loss resulting from incorrect operation of the instrument or modification of the instrument.

#### 2.3.2 Operators and Service Engineers

A normal operator of the equipment will not be trained in or qualified for service work on the equipment and may cause a hazard to himself/herself or others if such work is attempted. Operators should therefore restrict themselves to the normal operation of the equipment and not remove covers from the electronic equipment or dismantling of the instruments.

Service Engineers who are suitably trained to assess and isolate electrical, mechanical and vacuum hazards should be the only personnel who access the equipment.

### 2.4 Hazard Signals and Signs

#### 2.4.1 Hazard Signal Words

The standard three hazard signal words are defined as follows:

- DANGER** - *imminently* hazardous situation or unsafe practice that, if not avoided, *will* result in death or severe injury.
- WARNING** - *potentially* hazardous situation or unsafe practice that, if not avoided, *could* result in death or severe injury.

**CAUTION** - *potentially* hazardous situation or unsafe practice that, if not avoided, *may* result in minor or moderate injury or damage to equipment.

## 2.4.2 Hazard Labels used on Equipment

Several hazard symbols may be found on the equipment, they are shown below with their meaning:

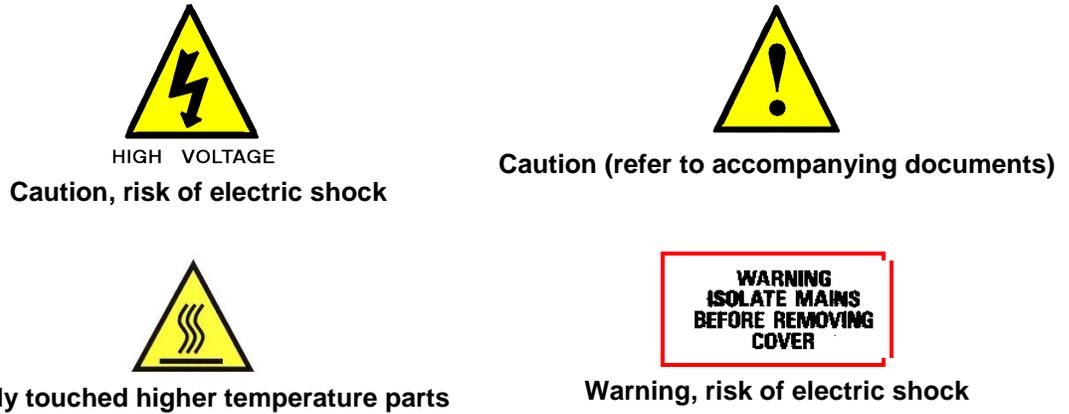


Figure 2.1 - Hazard Warning Symbols

## 2.4.3 Hazard Warning Labels used in Equipment Manuals

The international warning signs used in equipment manuals as shown in Figure 2.2.



Figure 2.2 - International Warning Symbols

Where appropriate these are used when a specific identifiable risk is involved in either using or maintaining the instrument. These take the form of warning triangles or signs with a graphical description of the hazard.

## 2.4.4 Instrument Functionality Signs

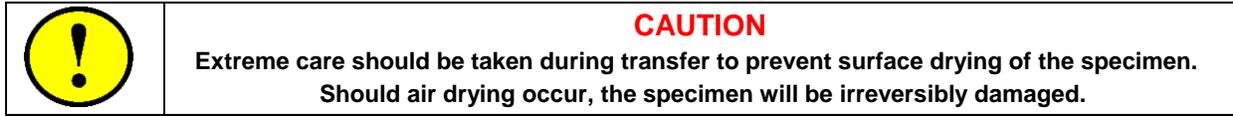


Figure 2.3 - Typical Warning sign as shown in this Manual

This typical sign applies to cautions where there is a risk to the functionality of equipment due to incorrect operation. These cautions or warnings will be contained in a box and be accompanied by a circular warning symbol as shown in Figure 2.3.

## 2.4.5 Serious Damage to Instruments



Figure 2.4 - Typical Warning sign as shown in this Manual

This typical caution sign is used where serious damage will be caused by incorrect operation of instrumentation. They will follow the same form as functionality warnings but with a triangular warning symbol as shown in Figure 2.4.

## 2.4.6 Hazard to Operator

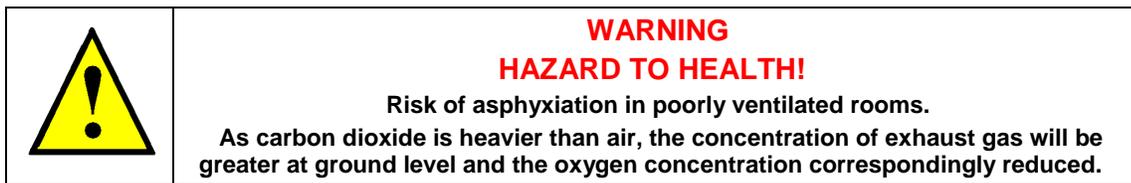


Figure 2.5 - Typical Warning as shown in this Manual

These warnings will generally occur in relevant installation and maintaining sections where there exists a potential hazard to the engineer working on the instrument. They will take the form of the triangular warning symbol accompanied by an international warning sign and bold type lettering beginning with **"WARNING-HAZARD TO HEALTH!"** as shown in Figure 2.5.

## 2.5 Risk Analysis

### 2.5.1 Personal Operational Risks

The following is a list of tasks carried out by both the operator and service engineer where recognized risks have been observed. Listed is the personnel protection equipment (PPE) which is suggested for use for various tasks on any surface analysis equipment and systems:

Task	Carried out by	Nature of Hazard	Recommended PPE
Cleaning of parts / samples with isopropanol (IPA)	Operator / Service engineer	Splash hazard to eyes, drying of skin	Protective goggles, protective gloves.
Use of Carbon Dioxide	Operator / Service engineer	Asphyxiation Frost Burns	Use in large room with good ventilation. Avoid exposure to CO2 being vented from the system.
Lifting of Heavy Items	Service engineer	Dropping on foot.	Protective footwear.

Table 2.1 - Personal Operational Risks

### 2.5.2 Hazardous Materials

#### Isopropanol (IPA)

For certain service tasks isopropanol is suggested for cleaning components before use in the vacuum system. It should be noted that isopropanol is a flammable liquid and as such should not be used on hot surfaces. In addition it is recommended that protective gloves be worn when using isopropanol.

#### Compressed Air

Compressed air can be a potential hazard if handled inappropriately. A compressed air line may be fed from some instruments to the customers supply, and the customer should ensure that this and any other service pipes and cables are maintained in good condition.

#### Nitrogen, Argon and Helium Gas Supplies

For instruments that use nitrogen, argon or helium gas supplies for their operation, the customer is responsible for maintaining the supply to the instrument. This supply should be regulated and kept to the lowest pressure and flow rate that is practical to minimise the effects of any leaks.

#### Hazardous Gases

SPI Supplies has no control over the gases used within the system. It is therefore viewed as the customers responsibility to assess the hazards involved and take appropriate precautions when using explosive, toxic or corrosive gases or gases which may result in hazardous products as a result of a chemical reaction.

## 2.6 Good Working Practices

It is essential that good hygienic working practices are adopted at all times especially in an ultra high vacuum or cleanroom environment and are generally of the “Common sense” type. Some simple good practice rules are:

If in doubt don't.

If in doubt ask.

When handling solvents wear face mask, gloves, apron and work only in a well ventilated area.

Mop up any spillages immediately.

When handling or decanting mineral oils wear protective clothing.

Aerosols of mineral oils, such as that produced by gas ballasting, can prove to be hazardous and an exhaust is recommended.

Before attempting to service electrical equipment, isolate from the mains.

Treat all unknown substances as hazardous.

Dispose of substances in an appropriate manner.

Use the correct tool for the job.

Keep a straight back and bend from the knees when lifting heavy objects.

Wear protective clothing when using liquid nitrogen.

Affix pressurised gas cylinders firmly to walls or racks. Use the correct regulating valves on gas cylinders and always transport cylinders using the appropriate specialist trolley.

Obey safety regulations regarding lifts, hoists and machine tools.

Always make sure you understand a procedure well before attempting it for the first time.

## 2.7 Critical Point Dryer Specific Safety Hazards

The following Safety Hazards are specific to the SPI Supplies 13200JE-AB Critical Point Dryers.

- (a) The equipment uses Liquid Carbon Dioxide (CO<sub>2</sub>) or alternatively Freon (CClF<sub>3</sub>). Ensure the area is adequately ventilated.
- (b) High pressures inside the chamber are generated during the drying process; the instrument is protected by a safety valve containing a thin diaphragm guaranteed to rupture at 1850 psi at 20°C.
- (c) All chambers and seals are pressure tested to 2500 psi by an independent authority before shipment to the customer. Each chamber bears the serial number of that test.

## 3 Introduction

This manual is intended for all users of the SPI Supplies **SPI-DRY™ Critical Point Dryer** (SPI Part #13200JE-AB) and provides information on the installation, operation and maintenance of the instrument.

Please note that the servicing and maintenance procedures should only be carried out by qualified service personnel, and it is essential that all users should read the **Health and Safety** section of this manual.

### 3.1 Return of Goods

If goods are to be returned to SPI Supplies for repair or servicing the customer should contact SPI Supplies or their local distributor before shipment. A "Return Authorisation Number" should be obtained in advance of any shipment. This number is to be clearly marked on the outside of the shipment. To obtain an RA#, contact our Customer Service Department and be sure to provide us with the following details:

- \* SPI Invoice Number and Invoice Date (if applicable)
- \* Method of shipment if applicable (post office, UPS, FedEx, Air Freight, etc.)
- \* Product(s) in question
- \* What is wrong with the product, or why do you want to make this return?

### 3.2 Returns Procedure

#### Warranty Claim

All components are sold with a **return to factory warranty** (unless otherwise stated) which covers failure during the first 12 months after delivery.

Returns must be sent courier paid, SPI Supplies will cover the return courier costs. This covers defects, which arise as a result of a failure in design or manufacturing. It is a condition of warranty that equipment must be used in accordance with the manufacturers instructions and not have been subjected to misuse. This warranty does not cover consumable items such as sputter coating targets and carbon evaporation material. To make a claim under the terms of this warranty provision contact the Customer Service Department at SPI Supplies.

#### Chargeable Repairs

Contact the Customer Service Department at SPI Supplies who will be able to provide an estimate of repair costs.

Service of equipment is generally completed within twenty working days after receipt of the equipment. A minimum evaluation fee is normally applied. Additional fees are charged as a per hour repair rate in addition to parts.

#### Returns

All returns to SPI Supplies are required to follow the procedure described above in Section 3.2.

All returned items are required to have a Return Authorization Number

#### Packaging and Shipping

All goods shipped to the factory must be sealed inside a clean plastic bag and packed in a suitable carton. If the original packaging is not available SPI Supplies should be contacted for advice. SPI Supplies will not be responsible for damage resulting from inadequate returns packaging or contamination of delicate structures by stray particles under any circumstances. All non-warranty goods returned to the factory must be sent courier pre-paid. They will be returned courier, pre-paid and added to the final invoice unless otherwise arranged.

## 4 Description

### 4.1 Equipment

Each SPI-DRY Critical Point Dryer when supplied as a complete package includes the basic instrument, specimen holder, high pressure CO<sub>2</sub> transfer hose, accessory kit, and an operation manual. Items can be ordered as a full package or separately against the following numbers:

**13200JE-AB** Jumbo Critical Point Dryer, consisting of the following:

Jumbo Critical Point Dryer.

Temperature and Pressure gauges

High pressure CO<sub>2</sub> transfer hose

Tubing for water connections

Tissue Baskets and Holder

Tool Kit

Spare Parts Kit

Operation Manual

#### 4.1.1 Accessories

The following are accessories available from SPI Supplies, which are used with our Critical Point Dryers.

**13201-AB** Grid Holder (3mm grids) for Regular Critical Point Dryer

**13203-AB** Grid Holder (2.3mm grids) holder for Regular Critical Point Dryer

**13202J-AB** Grid Holder (3mm grids) for Jumbo Critical Point Dryer

**13203J-AB** Grid Holder (2.3mm grids) holder for Jumbo Critical Point Dryer

Full list of consumables and spare parts is listed in Section 8.

## 4.2 Overview

The SPI Supplies SPI-DRY Critical Point Dryer (CPD) is designed to handle the dehydration of biological (as well as other materials) samples prior to examination the high vacuum environment of the scanning electron microscope (SEM).

Although a variety of liquid gases may be used for the critical point drying process, the procedures described in this document assume the use of liquid carbon dioxide (CO<sub>2</sub>), as it is the most commonly used and least expensive option. The SPI Supplies CPDs are designed to work at the critical temperature and pressure of CO<sub>2</sub>. The high pressure coupling hose supplied with the CPD has been provided with the appropriate connection to a carbon dioxide siphon cylinder.

Cooling water is applied to the water jacket to lower the temperature of the chamber to below 20C. Samples that have previously been chemically dehydrated are loaded into the chamber, which is then filled with liquid CO<sub>2</sub>. A series of flush cycles remove the dehydration liquid and replace it with the liquid CO<sub>2</sub>. Once the substitution is complete, the chamber is heated by flowing hot water through the jacket. This will raise the internal pressure, taking the CO<sub>2</sub> through its critical point. Once the critical point has been passed, the pressure is slowly released through the vent valve, and the samples removed.

## 4.3 Technical Specification

### 4.3.1 SPI-DRY Critical Point Dryer Specifications

Specimen Chamber:	13200JE-AB: 2.50" (60mm) ID x 3.12" (78mm) deep
Front Viewing Window:	1.00" (25mm) thick toughened glass with 0.50" (12.7mm) plastic shield
Pressure Chamber:	Water heated / cooled
Drying medium:	Carbon Dioxide (CO <sub>2</sub> ) or Freon 13 (CCIF <sub>3</sub> ).
Working Pressure:	1200 psi with a critical point of approximately 35°C using CO <sub>2</sub> .

## 4.4 Physical Description

The SPI Supplies SPI-DRY Critical Point Dryers are comprised of cylindrical, water heated/cooled pressure chambers, horizontally mounted, with attached gauges and valves.

Three manually operated valves, positioned around the chamber, are provided to control the flow of inlet and exhaust gases, as well as the venting of the system. The pressure system incorporates a bursting disc, rated at 1850 psi, located in the gas line between the chamber and the pressure gauge, see figure 7.1. The disc is designed to rupture in the unlikely event of an excessive build up of pressure in the chamber. Should this happen, all gas in the chamber is safely vented away.

**All chambers and seals are pressure tested by an independent authority before shipment to the customer. Each chamber bears the serial number of that test.**

Temperature and pressure gauges allow for constant monitoring of chamber conditions.

The complete assemble consists of the following:

- Pressure vessel assembly with integral water jacket, endplates (2), window, specimen loading door, and water connectors (2)
- Control valves (3)
- Thermometer
- Pressure Gauge
- Safety Valve
- Baseplate
- Sample holder assembly
- High pressure transfer hose
- Spare parts kit

### 4.4.1 Pressure Vessel

The pressure vessel is machined from a solid brass bar to form a cylindrical chamber with a wall thickness of approximately 1" (25mm). The vessel wall acts as a water jacket, with a series of narrow bores drilled lengthwise that allow water passage for heating and cooling. The ends of the water jacket are sealed with two annular end plates and gaskets. Two water connectors are screwed into the vessel wall (one at each end) to allow for connection of the water supply.

Both ends of the vessel are internally threaded. The specimen loading door screws into one end, with the viewing window located at the other end, held in place with a retaining ring. The window consists of a 1" (25mm) piece of toughened glass, covered by a ½" (12.7mm) plastic shield, which acts as a safety guard in the unlikely event of the glass cracking.

### 4.4.2 Control Valves

There are three high pressure valves (right-angle type) fitted to the pressure vessel using ¼" BSP threaded unions. The valves seal by contact between a ground steel cone and a brass knife edge.

***NOTE: Do not overtighten these valves, as doing so will impair the efficiency of the metal to metal contact.***

The **INLET** and **VENT** valves are screwed directly into the top of the pressure vessel, and seal against O-rings. The O-rings are located on their seats by small stainless steel inserts. When the valves are initially installed, they are adjusted so that they face the desired direction once they have been screwed into the vessel. Locking nuts ensure they do not rotate further.

The **DRAIN** valve located at the bottom of the vessel is installed in a slightly different manner. A ¼" BSP plug is sealed into the female part of the valve body with an adhesive, and this plug butts against an O-ring seal. This method allows the valve to be conveniently mounted in the horizontal position.

### 4.4.3 System Gauges

The thermometer is the bi-metallic type, and measures the temperature of the pressure vessel brass wall. It is attached to the vessel by means of a push-fit plug inserted in the vessel wall. The gauge fits

in such a way that the sensing head does not penetrate either the water jacket or the high pressure chamber.

The pressure gauge is a 0-2000 psi bronze Bourdon gauge, which screws into a ¼" BSP threaded port on the upper side of the vessel, and seals against a fiber washer.

***NOTE: By special arrangement with the manufacturers, this gauge was calibrated using methyl alcohol, rather than the usual mineral oil.***

#### **4.4.4 Safety Valve**

The stand pillar, which supports the vessel, also incorporates the safety valve. The top of the valve screws into the vessel and is sealed with a bonded seal. The valve exit is a small hole in the side of the pillar.

The valve uses a nickel fuse (bursting disc), which is a thin diaphragm guaranteed to rupture at 1850 psi (+/- 5%) at 20C. If the disc ruptures as a result of excess pressure, it must be replaced. A spare disc is included with the spare parts kit.

#### **4.4.5 Specimen Holder**

The standard specimen holder assembly consists of an aluminium alloy liquid transfer boat (with integral drain), mesh specimen baskets, and a gauze cover. The baskets slide into the channels on the gauze cover, ensuring they remain immersed when placed into the liquid transfer boat.

Sample preparation is carried out with the samples in the baskets to the point where final substitution is made (pure ethanol, acetone, amyl acetate, etc.). At this point, the boat is filled with the substitution liquid, and the basket assembly transferred to the boat.

When the liquid transfer boat is loaded into the pressure vessel, the post on the end of it fits into a hole drilled on the chamber door. This aligns the liquid transfer boat so that when the door is sealed, the drain valve is activated by a pin in the chamber floor. The valve aperture is small enough (.75mm) to ensure that the samples remain covered until the chamber is filled with liquid CO<sub>2</sub>.

#### **4.4.6 Microporous Specimen Capsules**

Microporous Specimen Capsules may be used as an alternative to the mesh baskets included with the CPD. These small, cylindrical containers protect fragile specimens during CPD, preserving three-dimensional form for later SEM observations. The sheltered environment also protects the fragile samples against the undesirable effects of turbulence. Both the capsule and its snug-fitting cap are solvent resistant (not harmed by alcohol, acetone, or chloroform). Capsules can also be used as convenient storage vessels for specimens under desiccation. The capsules measure 12mm in diameter by 11mm high and are available in three different pore sizes, the smallest at 30 µm. The inside diameter is 8mm, because of the roughly 2mm thickness of the wall.

## **5 Installation**

SPI Supplies carefully packs all instruments so that they will reach their destination in perfect working order. Do NOT discard any packing materials until the unit has been inspected for any transit damage and the instrument has been used to the customers satisfaction.

If any damage is found, notify the carrier and SPI Supplies (or local agent) immediately. If it is necessary to return the shipment, use the packaging as supplied and follow the instructions in this manual for return of goods paragraph 3.1.

### **5.1 Unpacking Checklist**

The Equipment package will normally be despatched from the factory in one box. Inside the box the following will be found, refer and check each item off against the supplied packing list.

- 13200JE-AB Unit, comprised of pressure vessel, pressure gauge, temperature gauge, three control valves, base/safety valve assembly
- High pressure transfer hose
- Tubing for water connections
- Liquid transfer boat with basket assembly
- Tool kit (window retaining ring spanner & tommy-bar for specimen door removal/sealing)
- Spare parts kit
- Operating Manual

#### **5.1.1 Site Selection**

The critical point dryer should be positioned in the laboratory convenient for:

- (a) Hot and cold water supply
- (b) Good ventilation
- (c) Space for carbon dioxide siphon cylinder

## 5.2 Connection of Services

Once unpacked and inspected, the critical point dryer should be secured to the surface where it will be used. There are two ¼" BSF tapped holes in the bottom of the base on a 5½" (127mm) PCD. These holes are to allow the CPD to be mounted to the bench top.

	<p style="text-align: center;"><b>WARNING</b></p> <p>If the apparatus is not secured, there is a risk that it could slide off the bench top in the event of the safety valve rupturing.</p>
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### 5.2.2 Water

Cut a suitable length of the included tubing to connect the water inlet port on the pressure vessel to the hot/cold water supply.

If connecting to individual hot and cold water taps, it may be more convenient to connect both taps to a "Y" adapter, and connect the adapter to the pressure vessel. This will allow for convenient changing from cold water to hot water, and also allow for fine control over the water temperature, which will give better control over the heating rate. Another possibility, if available, is the use of a water re-circulator that allows for precise control of the temperature.

Connect a second piece of tubing from the water outlet on the pressure vessel and run it to the drain (or to the water re-circulator, if using one).

**NOTE: Running mains water to waste may contravene local regulations. If in doubt, the user is advised to seek advice from the local water authority.**

### 5.2.3 Carbon Dioxide (CO<sub>2</sub>)

The critical point dryer requires a cylinder of liquid CO<sub>2</sub> fitted with a siphon tube. If there is any doubt regarding the presence of a siphon tube, advice should be sought from the gas supplier.

Using the included fiber washer, connect the high pressure transfer hose to the **INLET** valve of the pressure vessel. The fiber washer is necessary to ensure a good seal. Tighten with a ¾" wrench.

Connect the other end of the high pressure transfer hose to the CO<sub>2</sub> cylinder. Tighten with a 1 1/8" wrench.

Please note that cylinder connection threads may vary from country to country, and manufacturer to manufacturer. There is an included adapter that may or may not be necessary, depending on the threads on the CO<sub>2</sub> tank. If it is found to be necessary to fabricate an adapter, advice should be sought from a local supplier of high pressure gas fittings.

	<p style="text-align: center;"><b>CAUTION</b></p> <p>Before attempting to remove the high pressure hose, close the cylinder control valve and release any gas pressure in the pipe by opening the CPDA inlet and drain valves.</p>
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### 5.2.4 Carbon Dioxide (CO<sub>2</sub>) Exhaust

During operation of the Critical Point Dryer, it is usual to monitor the presence of intermediate fluid (acetone/ethanol) in the exhausting gas. For this reason it is unwise to make a permanent connection to the exhaust port. An arrangement incorporating an in-line type breakable connection would be more practical. With this method, except during gas monitoring, the exhausting CO<sub>2</sub> can be piped into a fume hood or out through an open window, if desired.

	<p style="text-align: center;"><b>WARNING</b> <b>HAZARD TO HEALTH!</b></p> <p style="text-align: center;">Risk of asphyxiation in poorly ventilated rooms.</p> <p>As carbon dioxide is heavier than air, the concentration of exhaust gas will be greater at ground level and the oxygen concentration correspondingly reduced.</p> <p>During the flushing, the CO<sub>2</sub> exhaust is very cold, and can cause frost burns.</p>
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## 6 Operation

### 6.1 Critical Point Drying Method

The phase diagram (Figure 6.1) shows the pressure to temperature ranges where solid, liquid and vapour exist. The boundaries between the phases meet at a point on the phase diagram called the triple point. Along the boundary between the liquid and vapor phases it is possible to choose a particular temperature, and corresponding pressure, where liquid and vapor can co-exist and hence have the same density. This is the critical temperature and pressure.

Critical Point Drying relies on this physical principle. The water in biological tissue is replaced with a suitable inert fluid whose critical temperature for a realizable pressure is just above ambient. The choice of fluids is severely limited and Carbon Dioxide (CO<sub>2</sub>) is universally used today despite early work with Freon 13 and Nitrous Oxide. With CO<sub>2</sub>, a critical point of approximately 35°C can be achieved at a pressure of around 1200 psi. Therefore, if the water is replaced with liquid CO<sub>2</sub> and the temperature then raised to above the critical temperature, the liquid CO<sub>2</sub> changes to vapour without change of density and therefore without surface tension effects which distort morphology and ultra-structure. Since liquid CO<sub>2</sub> is not sufficiently miscible with water, it is necessary to use an intermediate fluid, which is miscible with both water and liquid CO<sub>2</sub>. In practice, intermediate fluids commonly used are methanol, ethanol, amyl acetate and acetone.

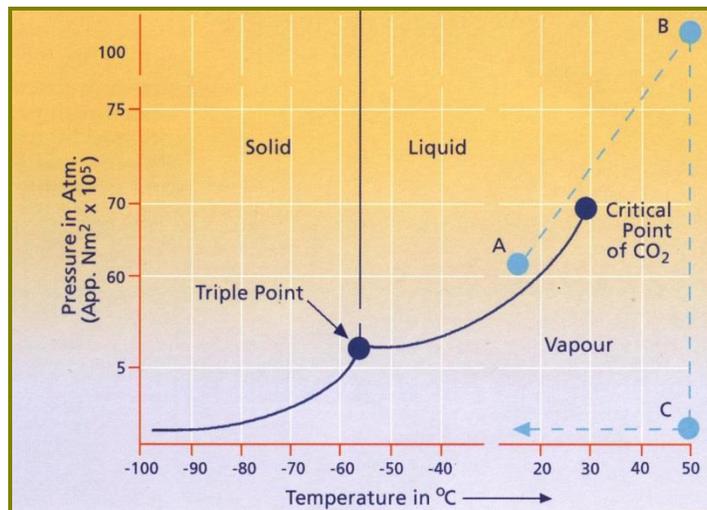


Figure 6.1 - Phase Diagram

#### 6.1.1 Drying Fluids

The two drying fluids in general use are liquid carbon dioxide and Freon 13. Their critical points are as follows:

Carbon Dioxide (CO <sub>2</sub> )	31.5° C, 1100 psi (75bar)
Freon 13 (CClF <sub>3</sub> )	28.8° C, 560 psi (38bar)

The critical point of Freon 13 is considerably lower than that of carbon dioxide but this gives no particular advantage. The usual deciding factor is the price. Freon 13, besides the environmental effects, is about twenty times more expensive than carbon dioxide and is not so readily available. These considerations have made carbon dioxide the most widely used drying fluid.

The transfer pipe provided with the critical point dryer is suitable for use with a 'siphon' type cylinder. Also, best results are obtained when using CO<sub>2</sub> with as little moisture content as possible. When ordering CO<sub>2</sub> tanks, make sure to specify "dry liquid CO<sub>2</sub> in a siphon tank".

**NOTE: It will be assumed in the following text that CO<sub>2</sub> is being used as the drying fluid.**

### 6.2 Trial Run

Before processing samples for the first time, it is suggested to run the CPD without any samples in order to gain familiarity with the process and the controls.



### WARNING

The CPDA is a pressure vessel and operates at pressures in the range of 800 - 2000 psi (54 - 136 bar) which is potentially dangerous if handled carelessly.



### WARNING

#### HAZARD TO HEALTH!

Risk of asphyxiation in poorly ventilated rooms.

As carbon dioxide is heavier than air, the concentration of exhaust gas will be greater at ground level and the oxygen concentration correspondingly reduced.

Ensure that all connections are properly made.

Close all valves on the pressure vessel (do not over-tighten, as this will damage the seals).

Turn on the cold water supply to cool the pressure vessel.

Open the CO<sub>2</sub> cylinder control valve.

Open the **INLET** valve on the pressure vessel. Listen for any sign of leaks. If a leak is detected, the associated connection should be re-tightened.



### CAUTION

Before attempting to re-tighten a transfer pipe union, close the cylinder control valve and release any gas pressure in the pipe by opening the CPDA inlet and drain valves.

With the **INLET** valve open, and the **VENT** and **DRAIN** valves closed, the pressure vessel will begin to fill with liquid CO<sub>2</sub>. The rate and extent of the filling can be increased by slightly opening the **VENT** valve on top of the unit. This allows any air trapped in the vessel to be flushed out.

At this point it is helpful to experiment with the manipulation of the various control valves. It should be noted that if the **INLET** and **DRAIN** valves are both opened, a constant level of liquid CO<sub>2</sub> can be maintained, as seen through the viewing window. This process is referred to as **FLUSHING**. Sometimes, it may be necessary to slightly open the **VENT** valve as well, if the liquid level begins falling.

Once comfortable with flushing and filling the chamber, adjust the liquid level so the chamber is half full, and then close all of the valves.

Turn off the cold water, and turn on the hot water (35C-40C) to slowly raise the temperature of the chamber. There may be some minor turbulence in the chamber. If there is excessive turbulence, this indicates that the chamber is heating too rapidly, and the water temperature should be lowered.

While the chamber is warming, observe the liquid level through the window, while also monitoring the **TEMPERATURE** and **PRESSURE** gauges. As the temperature of the chamber nears 30C, the pressure should rise to approximately 1100 psi. Once at this point, the surface of the liquid will start to dissolve. Once the pressure passes 1200 psi, the meniscus of the liquid will have disappeared completely, indicating that the CO<sub>2</sub> has passed through its critical point. There are actually three indications that critical point has been passed:

- The visible effect of the meniscus disappearing
- The **PRESSURE** gauge reading above 1200 psi
- The **TEMPERATURE** gauge reading above 32C (although this is the least reliable sign, due to the possibility of thermal lag)

**NOTE: The pressure reading will be unreliable if the liquid level is above the inlet to the PRESSURE gauge during the heating process.**

With the chamber above the critical point temperature (above 32 C), experiment with releasing the pressure by use of the **VENT** valve. If the pressure is released too quickly, re-condensation of the gas will occur by adiabatic cooling. The **VENT** valve should be adjusted to give a vent time of 4-5 minutes.

After a critical point run, cool the chamber to 20 C before attempting another run.

Repeat the above procedure several times, until comfortable with the workings of the critical point dryer.

## 6.3 Tissue Preparation

It is suggested that the user consults the appropriate literature before deciding on any particular techniques of sample preparation. A number of different methods are in use and the decision must be made based on which technique will give the best results for a given material. It must be stressed that the final result will depend almost entirely on the preparative technique carried out **BEFORE THE SPECIMEN GOES INTO THE PRESSURE CHAMBER.**

The following technique suggested by Dr. A. Boyd of University College, London. The sequence of operations outlined is very comprehensive. In many cases it may be possible to omit some of the stages. With difficult specimens however, care taken in fixation and dehydration produces much improved results.

The whole objective of the method is to obtain a specimen, which has been dried by the critical point method. During the preparatory stages, **THE SPECIMEN MUST BE GIVEN NO OPPORTUNITY TO DRY PREMATURELY. IT MUST BE KEPT WET AT ALL TIMES.**

The complete sequence involves the following steps:

- (a) Washing
- (b) Fixation
- (c) Dehydration
- (d) Substitution with CO<sub>2</sub> miscible liquid
- (e) Substitution with CO<sub>2</sub>
- (f) Heating to super-critical temperature
- (h) Pressure release

**NOTE:** *If dehydration is done with acetone, step (d) is omitted because acetone is miscible with CO<sub>2</sub>.*

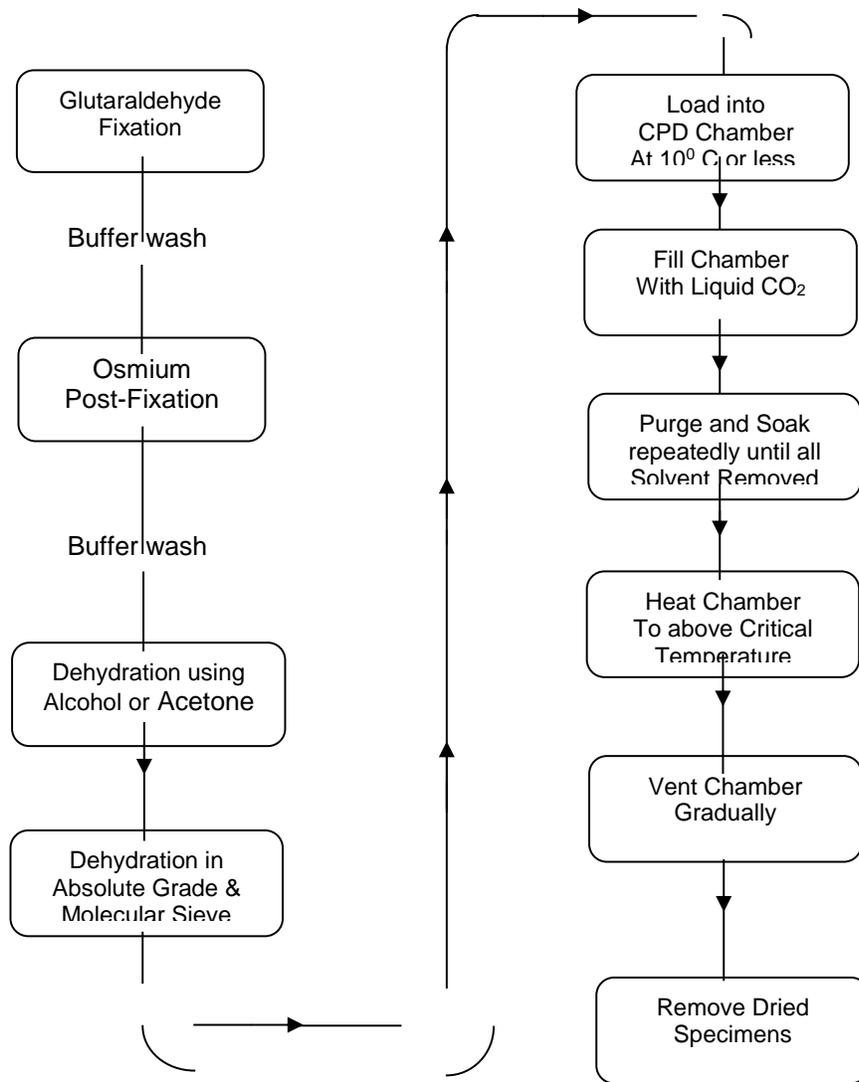


Figure 6.2 - Process Flow Chart

### 6.3.1 Washing

Specimens must be washed free of mucus, blood, serum or any other contaminant likely to be fixed on the surface. The washing medium must be physiological, i.e. must not cause any changes in shape or form of the tissue.

### 6.3.2 Fixation

If the specimen is pre-fixed in osmium tetroxide, it must be isotonic. This may then be followed by glutaraldehyde as the main fixative. If glutaraldehyde is used as the only fixative, it must be left for days rather than hours. Short periods of aldehyde fixation are not suitable, as they do not render tissues resistant to osmotic changes. Starting with 1% glutaraldehyde for 1 hour and following with 3% gives improved results.

### 6.3.3 Dehydration

It is recommended that, whenever possible, specimens are kept in the holders provided, or in the optional porous baskets, to reduce the risk of air drying when specimens are transferred to the Critical Point Dryer chamber.

Having fixed the tissue and washed it with distilled water to remove buffer salts and other electrolytes, it must have its water content replaced by a liquid miscible with CO<sub>2</sub> or Freon 13.

The routes in common use are:

- (a) Water - Acetone - CO<sub>2</sub>
- (b) Water - Acetone - Freon 13
- (c) Water - Ethanol - Amyl Acetate - CO<sub>2</sub>
- (d) Water - Ethanol - Freon 113 - Freon 13

It is not yet clear from the literature which routes, if any, give the best results. It may well be that they all give similar quality. The most convenient method is route (a) as it takes the shortest time and uses the cheapest transition fluid. Route (c) is also favoured as the strong smell of amyl acetate gives a good indication of whether or not all the intermediate fluid has been removed before the drying cycle is started.

If dehydration is to acetone, the following "Diffusion Dehydration" method can be used:

- (a) Put tissue in 20% acetone. (5ml)
- (b) Place on shelf of desiccator.
- (c) Fill desiccator with 100% acetone containing some anhydrous calcium sulfate.
- (d) Place watch glass of anhydrous CaCl<sub>2</sub> on shelf also.
- (e) Pump desiccator with water pump until acetone boils.
- (f) Seal under vacuum and leave over night. When the desiccator is opened, it will be found that the tissue is in 100% distilled acetone, having been fully and gently dehydrated. If dehydration is to ethanol, the following method is recommended for pieces of tissue up to 1mm in thickness:
  - a. Place in 30% ethanol for 15 minutes.
  - b. Place in 50% ethanol for 15 minutes.
  - c. Repeat with 70%, 80%, 90%, 95% and 100% each for 15 minutes.

***NOTE: The more care taken with this, the better the result. Rapid dehydration causes shrinkage of tissues. Larger pieces of tissue require proportionately longer periods of time.***

### 6.3.4 Substitution

After dehydration with ethanol, further substitution to amyl acetate or Freon 113 is necessary before putting tissue in the pressure chamber. We suggest that the substitution should not be carried out directly but through graded baths (25:75, 50:50 etc.) of the 2 liquids. 15 minutes per step should suffice.

### 6.4 Complete Operational Run

- (a) Prepare the samples using either the method outlined in section 6.
- (b) Place the samples into either the mesh baskets or the microporous specimen capsules, making sure not to allow them to air dry.
- (c) Place the mesh baskets or microporous specimen capsules into the liquid transfer boat, and fill it with the substitution liquid.
- (d) Ensure that the CPD is properly installed, and that all connections, including water lines the CO<sub>2</sub> high pressure transfer hose, are tight.
- (e) Start the flow of cold water to cool the chamber to 20 C or below.
- (f) Load the liquid transfer boat into the chamber, and secure the rear door.
- (g) After ensuring that all of the control valves are closed, open the CO<sub>2</sub> tank valve.

	<b>WARNING</b> The CPDA is a pressure vessel and operates at pressures in the range of 800 - 2000 psi (54 - 136 bar) which is potentially dangerous if handled carelessly.
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	<b>WARNING</b> <b>HAZARD TO HEALTH!</b> Risk of asphyxiation in poorly ventilated rooms. As carbon dioxide is heavier than air, the concentration of exhaust gas will be greater at ground level and the oxygen concentration correspondingly reduced.
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- (h) Open the **VENT** valve slightly, to release trapped air in the chamber, and then fully open the **INLET** valve to rapidly fill the chamber with liquid CO<sub>2</sub>.
- (i) Leave the **INLET** valve fully open, and the **VENT** valve slightly open to maintain the liquid level. Open the **DRAIN** valve to remove the substitution liquid. This flushing action should be continued for 4-5 minutes.
- (j) Once all of the substitution liquid has been flushed from the chamber, fill the chamber with liquid and close all of the gas control valves. Allow 1 hour for the liquid glass to impregnate the samples. Please note, this time is only a guide for tissue samples less than 1mm thick. For larger or denser samples, more time will need to be allotted for impregnation.
- (k) Repeat step (i)
- (l) Close the **INLET** valve and allow the liquid to fall to the level of the top of the liquid transfer boat.
- (m) Close all control valves, including the valve on the CO<sub>2</sub> cylinder.
- (n) Turn the cold water off, and turn the hot water (35 C – 40 C) on. Slowly raise the temperature of the chamber, and adjust the water temperature as needed.
- (o) While observing the CO<sub>2</sub> lever through the window, also monitor the **PRESSURE** and **TEMPERATURE** gauges.
- (p) Once the CO<sub>2</sub> has passed through the critical point (32 C, 1100 psi), turn off the hot water. **NOTE: To avoid any uncertainty in reaching the critical point caused by thermal lag, it is suggested to allow a suitable safety margin - ~36 C and 1200 psi.**
- (q) Gradually open the **VENT** valve to release the chamber pressure. To avoid re-condensation of the CO<sub>2</sub>, make sure to vent the chamber slowly (over a period of 4-5 minutes).
- (r) Once the chamber has de-pressurized, slowly open the **VENT** valve fully, to ensure the chamber reaches atmospheric pressure before attempting to remove the rear door.

- (s) Once there is no pressure in the chamber, remove the rear door and remove the liquid transfer boat with the now dried samples.
- (t) Mount the samples on the desired mount, and prepare them for examination in the SEM (or other instrument).

***NOTE: If the system is to be used again right away, the chamber must first be cooled to 20 C or below before another run is started.***

## 7

## Maintenance

These critical point dryers require very little in the way of routine maintenance, however, for optimum performance it is recommended that the following procedures be carried out on a regular basis.

- (a) Regularly clean the inside and outside surfaces of the pressure vessel including the O-ring and associated seating surfaces with isopropyl alcohol.
- (b) Regularly inspect all connection points for wear and/or leaks.

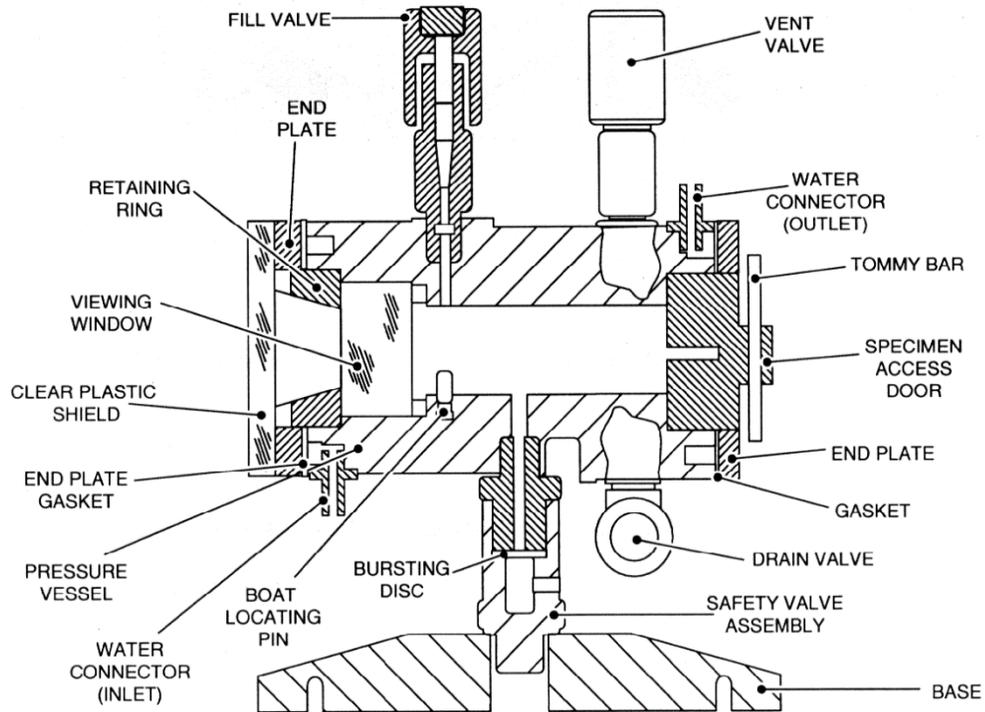


Figure 7.1: Cross-section view of the SPI-DRY Critical Point Dryer

## 7.1 Chamber Components

### 7.1.1 Viewing Window Maintenance

- (a) To remove the viewing window, remove the four hexagon socket screws, and remove the clear plastic safety shield.
- (b) Unscrew and remove the viewing window slotted retaining ring using the included spanner. Use care not to disturb the end plate covering the water jacket.
- (c) Clean and inspect the O-ring seal. Replace if the O-ring is hardened or damaged.
- (d) Remove the viewing window and bonded seal.
- (e) Clean and inspect the window and seal. Replace if damaged.
- (f)** Re-assemble in reverse order, taking care not to damage the window edges.

### 7.1.2 End Plate Maintenance

- (a) To remove the water jacket end plate, undo the four hexagon socket screws and pry the end plate away from the pressure vessel.
- (b) Thoroughly clean the end plate the mating surface on the pressure vessel. Discard the old cork gasket and nylon screw seal.
- (c)** Re-fit the end plate, using a new cork gasket and nylon screw seal.

### 7.1.3 Drain Valve Maintenance

- (a) To remove the **DRAIN** valve for cleaning, unscrew and separate the pressure vessel from the stand pillar by rotating the vessel counter-clockwise relative to the base.
- (b) Unscrew and remove the **DRAIN** valve and adapter plug.
- (c) Remove the insert and O-ring for inspection and cleaning.
- (d)** Re-assemble in reverse order, using a new O-Ring and bonded seal.

### 7.1.4 Gas Control valve Maintenance

- (a) **Gas Control Valve Adjustment.** If the action of one of the gas control valves is too tight or too loose, adjust it as follows:
  - a. Unscrew and remove the control valve knob retaining unit.
  - b. Lift the knob from the square keyed valve shaft.
  - c. Slightly loosed or tighten the shaft sleeve nut as required.
  - d. Re-assemble the valve and test the action.
  - e. If necessary, repeat the above steps until the valve operates in a satisfactory manner.
- (b) **Gas Control Valve Strip Down.**
  - a. With the valve removed from the vessel body, unscrew the knob retaining nut.
  - b. Lift the knob from the square keyed valve shaft.
  - c. Unscrew and remove the shaft sleeve nut.
  - d. Unscrew and extract the valve shaft, with O-ring seals, from the valve body.
  - e. Inspect and clean the seals, replacing as needed.
  - f.** Re-assemble in reverse order.

***NOTE: When refitting valves to the pressure vessel body, only tighten them by hand. Over tightening will damage the O-ring seals.***

### 7.1.5 Inlet / Vent Valve Maintenance

- (a) To remove the **INLET** or **VENT** valve, loosen the valve locking nut by one turn.
- (b) Remove the valve by rotating in a counter clockwise direction.
- (c) Remove the insert and O-ring for inspection and cleaning.

(d) Re-assemble in reverse order, using a new O-ring.

7.1.6

## Gauge Maintenance

There are two gauges on the CPD: A temperature gauge that reads from 0 – 100 C, and a pressure gauge that reads from 0 – 2300 psi (or 0 – 160 bar).

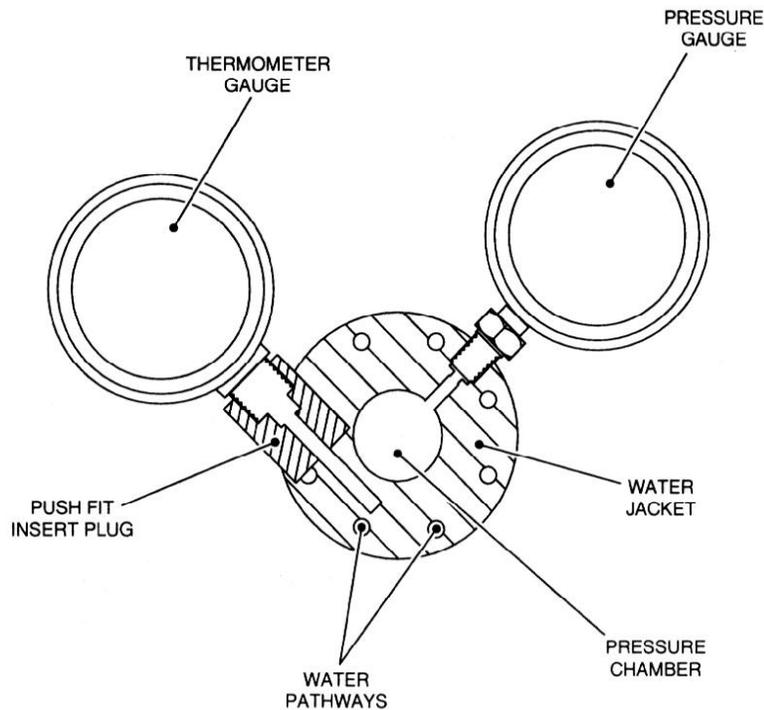


Figure 7.2: Gauge Mountings

### (a) Thermometer Gauge

- Remove the thermometer gauge and push-fit plug assembly, with O-ring seal, by rotating and pulling.
- Inspect and clean the O-ring, and replace if needed.
- Grease the O-ring lightly before re-fitting. If the thermometer is suspect, it can be checked in water against a laboratory thermometer.

**NOTE: In operation, there is a slight thermal lag between the true temperature inside the pressure vessel and the reading on the thermometer if heating or cooling is carried out rapidly. The use of a thermal transfer grease between the thermometer stem and the pressure vessel will reduce this lag.**

### (b) Pressure Gauge

- Using a well fitting, open-end wrench on the hexagonal sectioned gauge stem, unscrew and remove the pressure gauge. Do not use an over-sized wrench, or attempt to remove the pressure gauge by rotating the gauge head.
- Replace the fibre washer before refitting the pressure gauge. After replacing the pressure gauge, tighten the gauge stem to a torque of 20 ft. lb. It may be necessary to use a different thickness of fibre washer, or several washers, to achieve the correct tightness while maintaining the correct gauge orientation.

7.1.7

## Liquid Transfer Boat Maintenance

To prevent blockages, regularly strip and clean the transfer boat drain valve as follow:

- Remove the screw in the end of the transfer boat.
- Extract the valve slider and spring.
- Clean out the drain hole using a 1/32" diameter drill.

(d) Re-assemble in reverse order.

7.1.8

## Safety Valve Assembly

- To replace the Bursting Disc in the Safety Valve, unscrew and separate the pressure vessel from the stand pillar by rotating the pressure vessel counter clockwise relative to the base.
- Unscrew and remove the top of the Safety Valve using an appropriate size wrench.
- Remove and discard the Copper Ring and ruptured Bursting Disc from the Safety Valve body.
- Fit a replacement Bursting Disc and Copper Ring.
- Re-assemble in reverse order.

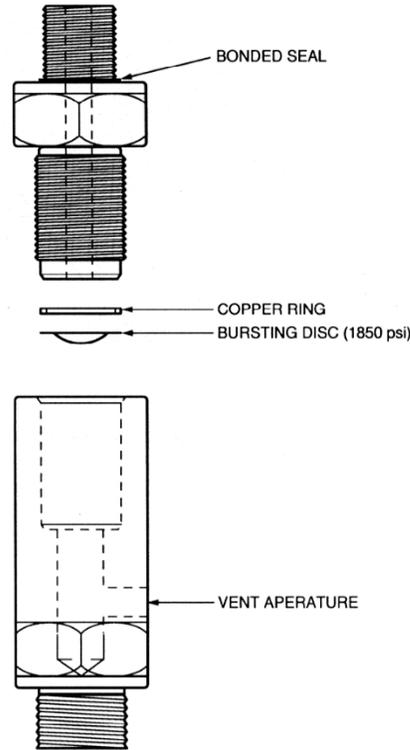


Figure 7.3: Safety Valve Assembly

## 7.2 Troubleshooting

We hope that you experience the minimum of problems throughout the lifespan of the instrument but inevitably problems may occur. Any known problems associated with this type of instrument have been listed below with the possible cause and suggestions to what to do. If problems continue to occur, contact the Customer Service Department at SPI Supplies or your local agent.

OBSERVATION	POSSIBLE CAUSE	REMEDY
Chamber will not fill completely	Insufficient cylinder gas	Replace gas cylinder
Chamber will not fill completely	Chamber temperature too high or gas cylinder temperature too low.	Allow longer pre-cooling time
Chamber will not reach critical pressure	Leaking gas connections, damaged door "O" ring gasket	Check and retighten connections as required, Replace damaged seals or gaskets as required

Chamber temperature will not rise or fall	Insufficient water temperature	Adjust water temperature as needed.
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**Table 7.1 - Trouble Shooting**

### 7.3 Preventive Maintenance

It is assumed that with a system in regular use and that the system was installed in a suitable environment and in regular use, faults will be repaired as they occur.

To maintain the equipment to the best operating conditions a maintenance schedule is suggested as part of a fault prevention programme, the following items are suggested to be included in such a programme. The frequency of checking will depend on the usage of the equipment.

ITEM	REGULARLY	OCCASIONALLY
Inspect all connections (gas and water) for signs of wear, any leakage from pipes and that they are securely retained in position.	X	
Inspect door sealing surface for any damage and the "O" ring is clean	X	
Cleaning	X	

**Table 7.2 – Preventive Maintenance**

## 8 Spare Parts & Accessories

Those parts, which due to wear and tear are more commonly required, are listed in the table as follows.

PART NUMBER	DESCRIPTION	QTY
13212-AB	Chamber Door Gasket Set – Regular – Nitrile	1
13213-AB	Rupture Disc & Copper Ring (pk/2)	2
13214-AB	Chamber Bonded Seal – 32mm – Nitrile (fits window of Regular and Jumbo, and rear door of Regular)	1
13214E-AB	Chamber Bonded Seal – 32mm – EPDM (fits window of Regular and Jumbo, and rear door of Regular)	1
13214J-AB	Chamber Bonded Seal – 67mm – Nitrile (fits rear door of Jumbo)	1
13214JE-AB	Chamber Bonded Seal – 67mm – EPDM (fits rear door of Jumbo)	1
13217E-AB	Safety Valve Bonded Seal – EPDM (fits both Regular and Jumbo)	1
13216-AB	Window O-Ring – Nitrile (fits both Regular and Jumbo)	1
13216E-AB	Window O-Ring – EPDM (fits both Regular and Jumbo)	1
13215-AB	Control Valve Seating O-Ring – Nitrile	1
13215E-AB	Control Valve Seating O-Ring – EPDM	1
13204-AB	High Pressure Transfer Hose	1
13207-AF	Gasket for High Pressure Transfer Hose	5

**Table 8.1 - Spare Parts for the SPI-DRY Critical Point Dryer**

PART NUMBER	DESCRIPTION	QTY
13201-AB	Holder for 3mm grids – Regular	1
13203-AB	Holder for 2.3mm grids – Regular	1
13202J-AB	Holder for 3mm grids – Jumbo	1
13203J-AB	Holder for 2.3mm grids – Jumbo	1
13208-AB	Liquid Transfer Boat w/ mesh baskets and screen (Regular)	1
13208A-AB	Replacement set of 3 mesh baskets, w/ screen (Regular)	1
13208B-AB	Replacement set of 3 mesh baskets (Regular)	1
13208J-AB	Liquid Transfer Boat w/ mesh baskets and screen (Jumbo)	1

**Table 8.2 - Accessories for the SPI-DRY Critical Point Dryer**