SUPPLIES

SPI SUPPLIES CRITICAL POINT DRYER MODELS 13200-AB and 13200J-AB

OPERATION AND MAINTENANCE MANUAL

SPI Supplies Division of **STRUCTURE PROBE, Inc.**

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WARRANTY

- ☐ SPI-Module Components™
- ☐ SPI SteadiCool Jr™
- ☐ SPI SteadiCool™
- ☐ SPI Plasma Prep II Plus™
- ☐ SPI Plasma Prep X™
- ☐ SPI Critical Point Dryer™
- ☐ SPI Jumbo Critical Point Dryer™
- ☐ SPI Vacu-Prep™

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

Proper use of your unit, according to this instruction 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. If questions arise, call SPI Supplies TOLL-FREE at 800-2424-SPI (in PA, 215-436-5400) for assistance. Also communicate via FAX (215-436-5755) or telex (835367).

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It has been found that when the Safety Valve blows, the instrument will slide off the table. To prevent this we have fitted rubber feet or, if for permanent fixture, there are two 1/4" holes drilled into the board for bolting to the table or bench.

1. DESCRIPTION OF APPARATUS

Introduction

The SPI Critical Point Drying Apparatus is a purely mechanical assembly. With the exception of the glass viewing window, pressure seals and gauges, it is constructed entirely from metal parts.

The construction of the apparatus is not complex and if it is found necessary to completely strip the unit for cleaning purposes, this operation could be carried out by any competent mechanical technician. It must be pointed out, however, that if any pressure seal is disturbed, **CARE MUST BE EXERCISED** in the reassembly and testing of that seal. One is dealing with **PRESSURES IN THE RANGE OF 800 to 2000 psi WHICH ARE POTENTIALLY DANGEROUS IF HANDLED CARELESSLY.**

Before delivery to the customer, the equipment undergoes the following tests:

1) On completion the unit is tested by an independent laboratory using a hydraulic pressure of 2500 psi.

2) The unit is then stripped and cleaned to remove traces of water.

3) It is then tested to 2000 psi using carbon dioxide. The safety valve is set to 1800 psi.

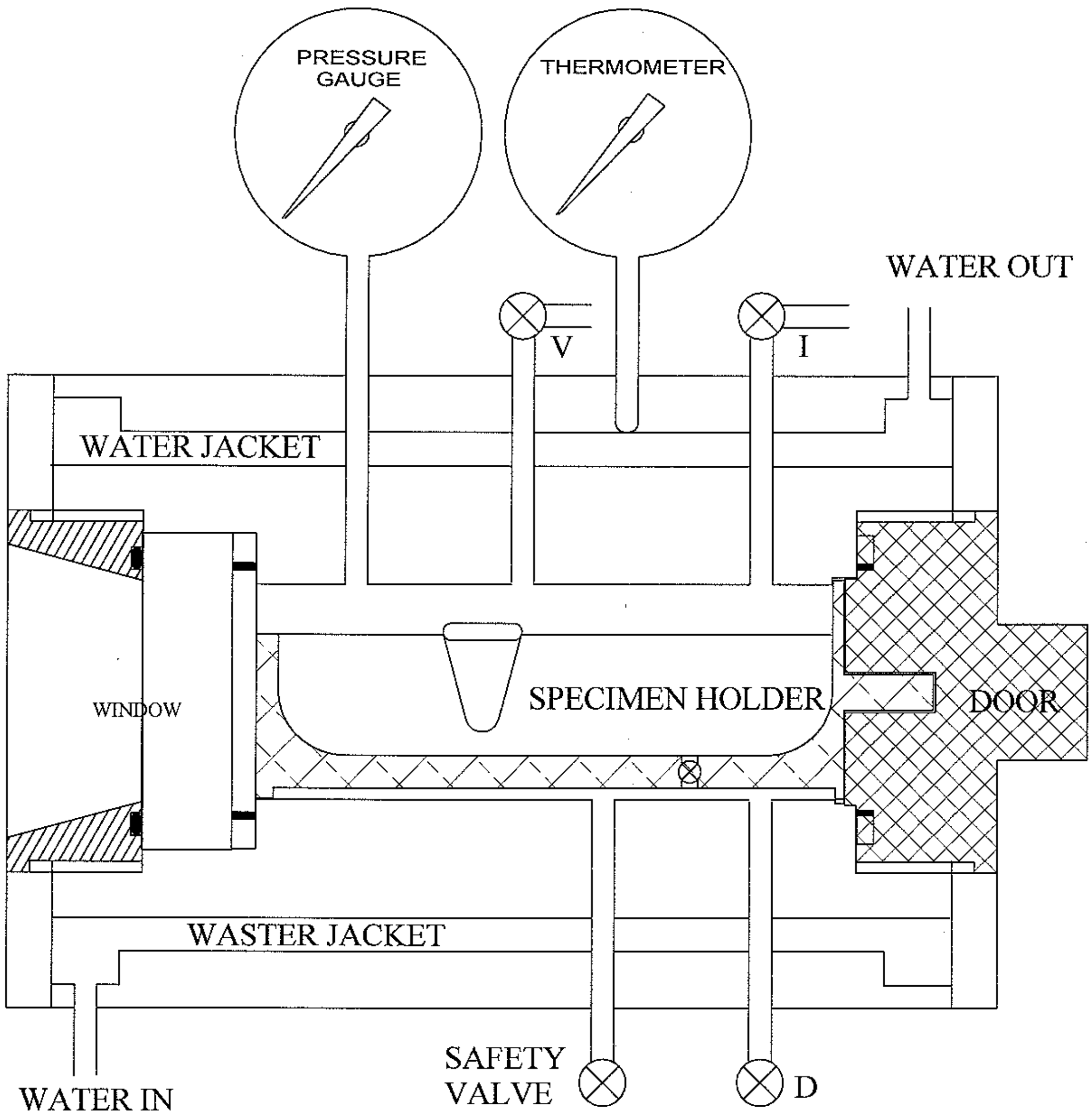
Following this procedure ensures that there are no defects or weaknesses in the mechanical parts.

The Complete Assembly

The apparatus comprises the following parts:

a) Pressure vessel assembly with integral water jacket
   endplates (2 off)
   window assembly
   specimen door
   water connectors (2 off)
b) Control Valves (3 off)
c) Thermometer
d) Pressure Gauge
e) Safety Valve
f) Baseplate
g) Specimen Holder Assembly
h) Transfer Pipe

These parts are identified in Figure 1 and Figure 2 overleaf.



Gas Handling Valves: V - Vent Valve
I - Inlet Valve
D - Drain Valve

Figure 1 - Semi-Scale schematic diagram of the SPI #13200 and 13200J Critical Point Drying Aparatus showing relative posistions of parts. The pressure vessel is to scale , valves and gauges are shown schematically.

The Pressure Vessel Assembly

Construction

The pressure vessel and water jacket are made from a solid brass cylinder of diameter 3-1/4" and length 5". Machining from solid gives reliable mechanical properties under high pressure conditions and the use of brass ensures good thermal transfer properties.

The internal diameter of the pressure vessel (i.e. the diameter of the pressure chamber) is slightly under 1-1/4" giving a wall thickness of over 1". The wall contains narrow-bore water passageways which act as an integral water jacket for heating and cooling. The ends of the water jacket are sealed by two annular end plates which do not cover the ends of the pressure chamber. Two connectors are screwed into the outside wall of the vessel and connect with the water channels.

Both ends of the pressure chamber are internally screw threaded. The specimen loading door screws into one end; the viewing window is held in the other end by a retaining ring. A 1/2" thick perspex shield acts as an explosion guard over the toughened glass (1" thick) viewing window.

Servicing

To replace glass viewing window:

a) undo hexagon socket screws (4) and remove perspex guard.

b) unscrew slotted retaining ring (DO NOT DISTURB THE CHROMIUM PLATED ANNULUS WHICH COVERS THE WATER JACKET).

c) check the 'O' ring in retaining ring.

d) remove viewing window and Dowty seal. If hardened or damaged they should be replaced.

e) reassemble in reverse order taking care not to damage edge of glass window.

To remove and replace water jacket end plate:

a) remove four hexagon socket screws.
b) prize end plate off body.
c) thoroughly clean all surfaces.
d) replace new cork gasket and nylon screw seal.
e) screw end plate firmly to body.

The Control Valves

Construction

The pressure vessel is drilled (1/4" BSP thread) to take three high pressure handling valves of the "right-angle" type. The valves seal by contact between a ground steel cone and a brass knife edge; this metal to metal contact should not be overtightened as this will impair the efficiency of the valve.

The INLET and VENT valves are screwed directly into the top of the pressure vessel and seal against an O-ring. The O-rings are located on their seats by small stainless steel inserts. When the valves are initially fitted, their lengths are adjusted so that they face the desired direction when they have been screwed onto the seal. A locking nut ensures that the valve does not rotate.

The DRAIN valve in the bottom of the vessel is fitted in a slightly different way. A 1/4" BSP plug is sealed into the female part of the valve body with "Locktite" and this plug butts against the O-ring seal. This method allows the valve to be mounted horizontally (for convenience) rather than vertically like the INLET and VENT valves.

Servicing

To loosen the action of a valve:

a) Unscrew nut in top of valve knob
b) remove knob from square keyed shaft
c) unscrew hexagonal sleeve nut slightly and retest the tightness of the action

To remove INLET or VENT valve:

a) unscrew locking nut (one turn)
b) remove valve by rotating in counterclockwise direction
c) remove insert and O-ring for checking and cleaning

To remove DRAIN valve:

a) remove pressure vessel from stand pillar by rotating pressure vessel counterclockwise relative to stand base
b) unscrew locking nut (one turn)
c) remove valve and plug by unscrewing
d) remove insert and O-ring for checking and cleaning

To completely strip valve:

a) unscrew nut and remove knob
b) unscrew sleeve nut and remove
c) unscrew keyed shaft and remove together with O-rings

NOTE: When refitting valves onto the vessel, it is only necessary to tighten the valve against the O-ring by hand. Overtightening can damage the O-ring.

Thermometer and Pressure Gauge

Construction

The thermometer is of the bimetallic type and measures the temperature of the brass wall of the pressure vessel. The sensing head of the thermometer does not break through into the water jacket or the pressure vessel.

The pressure gauge is a 0-2000 psi bronze tube Bourdon gauge. By special arrangement with the manufacturers, the gauges are calibrated using methyl alcohol rather than the usual mineral oil.

The pressure gauge is fitted into a 1/4" BSP port in the pressure vessel; the pressure seal is a fiber washer.

Servicing

To remove and refit thermometer:

a) The thermometer is screwed into a plug which is a push fit in the wall of the pressure vessel. The thermometer and plug (with compression O-ring) are removed as an assembly by rotating and pulling.

b) Before refitting, grease the O-ring slightly.

c) Should the thermometer fail to operate, first check it in water against a mercury thermometer. If malfunctioning, return to factory for replacement.

NOTE: There is a slight thermal lag between the true temperature inside the vessel and the reading on the thermometer if the heating or cooling is carried out rapidly. Using a thermal transfer grease between the thermometer stem and the pressure vessel will decrease this lag.

To remove and refit pressure gauge:

a) Find a well-fitting spanner for the nut on the stem of the gauge. We have found this size to be somewhat variable between 15mm and 5/8 AF. **DO NOT USE LOOSE SPANNER ON THIS NUT OR ATTEMPT TO ROTATE THE HEAD OF THE GAUGE.**

b) Unscrew the nut and remove pressure gauge.

c) Replace fiber washer before replacing gauge.

d) When tightening seal after refitting, use a torque of at least 20ft.lb. It may be necessary to use different thicknesses of washer (or a number of washers) before getting the correct pressure on the seal <u>and</u> have the gauge facing the correct direction.

e) In case of gauge failure, return to factory for replacement.

The Safety Blow-Off Valve

Construction

The stand pillar which supports the pressure vessel is also the safety blow-off valve. The top of the valve screws into the pressure vessel and is sealed with a nitrile/metal gasket. The exit of the valve is a small hole in the side of the pillar.

The valve is preset to operate if the pressure in the chamber should exceed 1850 psi. Once the valve has blown, the 'pressure fuse' must be replaced. A spare fuse is included with the apparatus as shipped.

<u>Replacing the Pressure Fuse</u>

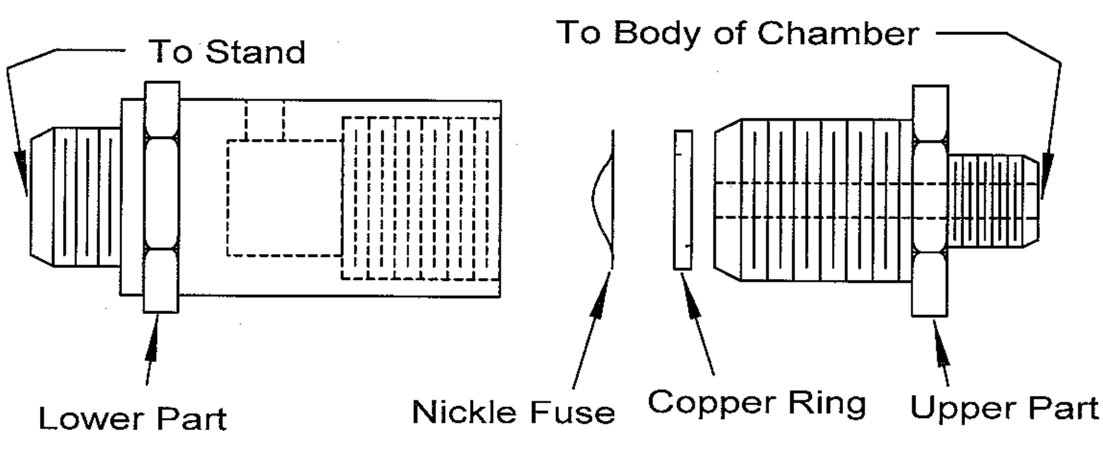
The pressure fuse is small nickel domed disc. To replace it proceed as follows:

a) Remove the body of the apparatus from the top of the Blow-off valve stem.

b) The upper part of the blow-off valve can be unscrewed using two spanners (turn counterclockwise).

c) Remove the copper annulus in the lower part of the valve and take out the damaged nickel fuse.

d) Replace fuse, copper ring and screw on the upper part of the valve tightly.



MK II Blow-off Valve Assembley

Specimen Holder

Construction

The specimen holder assembly consists of the following parts:

a) liquid transfer boat (aluminum alloy) with integral drain valve.

b) specimen holder baskets which slide into a gauze carrier. The carrier fits in the top of the liquid transfer boat.

Tissue preparation is carried out with the tissue in the baskets to the stage where the tissue is impregnated with the final substitution liquid (i.e. acetone, amyl acetate, Freon 113). The boat is then filled with the substitution liquid and the basket assembly is transferred rapidly to the boat. The boat can then be loaded into the pressure chamber of the apparatus.

When the specimen access door on the pressure chamber is closed, this actuates the drain valve in the liquid transfer boat. The drain valve aperture is only 0.75mm in diameter and this results in a sufficiently slow draining action for liquid carbon dioxide to enter and cover the specimen before the substitution liquid level has fallen.

Servicing

To strip and clean drain valve in transfer boat:

a) Undo screw in end of transfer boat
b) Remove valve slider and spring
c) Clean out drain hole using 1/32" diameter drill
d) Refit slider and spring, and retighten screw

2. OPERATION OF APPARATUS

Installation and Trial Run/Choice of Drying Liquid

The two drying liquids in general use are liquified carbon dioxide and liquified Freon 13. Their characteristics are:

Carbon dioxide critical point	31.5°C	1100 psi
Freon 13 ($CClF_3$) -"-	28.8°C	560 psi

The critical pressure 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 is about 20 times more expensive than carbon dioxide and is also not as readily obtainable. These considerations have made carbon dioxide the most widely used drying liquid.

The transfer pipe provided with the apparatus is suitable for use with a carbon dioxide cylinder of the siphon type. If a standard cylinder is used, it should be inverted to obtain liquid.

The liquid carbon dioxide should be essentially water-free. It is important to specify DRY carbon dioxide.

We will assume in the following notes that carbon dioxide is being used as the drying liquid.

IMPORTANT NOTE: Flexible high pressure coupling:

Cylinder connection threads tend to vary from country to country. If the customer finds it necessary to fabricate his own transfer pipe, he should take advice from a local supplier of high pressure fittings. The thread on the apparatus is 1/4" standard thread in the U.S.A. Copper piping with a bore of 1/8" is suitable for a transfer pipe.

The Water Supply

It is convenient to attach a water mixer at the hot and cold water supplies in the laboratory. The apparatus requires both heating and cooling water during the cycle: cooling to allow easy filling of the liquid carbon dioxide; heating to take the carbon dioxide above its critical point. Good control of the water temperature is of great assistance to the operator.

Installation

1) Choose a site for the apparatus which is conveniently near the hot and cold water supplies and the vertical standing carbon dioxide cylinder. The cylinder (5 feet high) will need changing regularly (every 20-30 runs), so leave some access to the cylinder. If mixer taps are not already fitted to the hot/cold water supply, it is useful to fit a 'Y piece.

2) Connect the flexible transfer pipe to the inlet valve on the apparatus. A fiber washer is provided to ensure a good seal. The spanner here is 7/16" BSF. Tighten to about 20ft.lb.

3) Connect the other end of the flexible transfer pipe to the cylinder. No gasket is required. Do not rotate the pipe while tightening the connection: A 1/4" BSF spanner is used to grip the pipe end while tightening the large nut (11/16" BSF).

4) These two unions can be tested by closing the inlet valve on the apparatus and then opening the tap on the cylinder. Any leaks will be audible (or visible if severe). Leaks in this area are cured by further tightening of the joints.

5) Connect the mixer tap or 'Y piece to the water inlet with 1/4" bore neoprene or polythene tubing. Connect the water outlet to a convenient drain.

Trial Run

1) Close all three valves on pressure vessel. Do not overtighten as this will damage the seats.

2) Open the tap on the liquid carbon dioxide cylinder.

3) Open the inlet valve on the pressure vessel. Listen for slight leaks. If any seals have loosened in transit, they should be tightened. (**NOTE:** Before disturbing seals first close the cylinder and vent all carbon dioxide from transfer pipe and vessel through drain valve with inlet valve open).

4) With the Inlet open and the other two valves closed the pressure vessel will fill slowly. The rate of filling can be increased by opening the Vent valve in the top of the apparatus. This removes the trapped volume of air.

5) It is useful at this stage to experiment with the manipulation of the control valves. It will be seen that if the inlet and drain valves are both opened, a constant level of liquid can be maintained. This is the flushing action. (It is sometimes necessary to slightly open the vent valve as well to prevent the liquid level from falling). It will also be noted that the pressure vessel is difficult to fill if the vessel is warmer than the cylinder; a constant throughput of liquid gas will, however, cool the vessel by adiabatic expansion at the drain valve.

6) Having experimented with filling and flushing the chamber, fill the chamber half full (the liquid level is visible at the window) and close all the valves. Slowly heat the body of the apparatus using a flow of water at about 35-40°C. At first, turbulence will be visible; if the turbulence is violent, the heating rate is excessive. When the temperature of the vessel is about 30°C the pressure will be about 1100 psi and, at this point, the surface of the liquid will start to "dissolve". When the pressure is greater than 1200 psi the liquid surface will have disappeared; the CO_2 is above its critical point:

NOTE: There are three indications that the CO_2 is above its critical point:

a) the visible effect
b) the pressure reading of above 1200 psi
c) the thermometer reading of above 32°C

The thermometer reading is the least reliable of the indications because of the possibility of thermal lag. It is useful to repeat the above experiment a number of times to become conversant with the critical point effect. **DO NOT HEAT WITH THE LIQUID LEVEL ABOVE THE INLET TO THE PRESSURE GAUGE AS THIS GIVES UNRELIABLE PRESSURE READINGS.**

7) When the chamber contains gas at a temperature above its critical point (36°C gives a suitable margin) experiment with the release of pressure through the vent valve. Note that an over rapid release of pressure causes recondensation of the gas by adiabatic cooling. This condensation will spoil a specimen if it occurs during a drying run. The vent valve should only be opened slightly to give a venting time of about 3-4 minutes.

8) After a drying run the chamber should be recooled to 20°C before attempting to reuse.

Tissue Preparation

General Comments

It is suggested that the user consults the literature before deciding on any particular technique of sample preparation. A number of different methods are in use and the customer must decide which technique will give the best results for his material. It must be stressed that the final result will depend almost entirely on the preparative technique carried out **BEFORE THE TISSUE GOES INTO THE PRESSURE VESSEL.**

The following technique is that recommended by Dr. A. Boyd of University College, London. The sequence of operations outlined is very comprehensive; in many cases it may be found possible to omit some of the stages; e.g., with botanical specimens it has been found that if the dehydration is carried out very carefully, it is not necessary to pre-fix the tissue. With difficult specimens, however, care taken in fixing and dehydration results in a much improved result.

NOTE: THE WHOLE OBJECT OF THE TECHNIQUE IS TO OBTAIN A SPECIMEN WHICH HAS BEEN DRIED BY THE CRITICAL POINT TECHNIQUE. 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_2 miscible liquid
e) substitution with CO_2
f) heating to super-critical temperature
g) pressure release

The method involved in f) and g) has been described in the previous section. If dehydration is done with acetone, step d) is omitted because acetone is miscible with CO_2.

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. not cause any changes in shape or form of the tissue.

Fixation

If the specimen is pre-fixed in osmium tetroxide this must be isotonic. This may then be followed by glutaraldehyde as the main fixative.

If glutaraldehyde is used as the first and 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.

Dehydration

Having fixed the tissue and washed it with distilled water to remove buffer salts and other electrolytes, it must then have its water content replaced by a liquid with a convenient critical point. This is not done directly as water is not miscible with carbon dioxide or Freon 13. The routes in common use are:

1) water - acetone - carbon dioxide
2) water - acetone - Freon 13
3) water - ethanol - amyl acetate - carbon dioxide
4) water - ethanol - Freon 113 - carbon dioxide
5) water - ethanol - amyl acetate - Freon 13
6) water - ethanol - Freon 113 - Freon 13

It is not yet clear from the literature which route, if any, gives the best results. It may well be that they all give similar quality.

The most convenient method is the first one listed as it takes the shortest time and uses the cheapest transition fluid. Route number 3 is also favored 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 dehydrating to acetone, the 'Diffusion Dehydration' method can be used:

a) put tissue in 20% acetone (5ml)
b) place on shelf of desiccator with 100% acetone containing some anhydrous calcium sulphate
c) place watch glass of anhyd. $CaCl_2$ on shelf also
d) pump desiccator with water pump until acetone boils
f) seal under vacuum and leave overnight

When the desiccator is opened it will be found that the tissue is in 100% distilled acetone and has been fully and gently dehydrated.

When dehydrating to ethanol, the following method is recommended for pieces of tissue up to 1mm in thickness:

Place in 30% ethanol for 15 minutes
Place in 50% ethanol for 15 minutes

Repeat with 70%, 80%, 90%, 95%, 100%, 100%, each for 15 minutes. The more care taken with this the better the result. Rapid dehydration causes shrinkage of tissues. Larger pieces need longer times.

Substitution

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

A Complete Run

1) Wash tissue (see previous section for details).

2) Fix tissue.

3) Dehydrate with acetone or ethanol.

4) If using ethanol, substitute with amyl acetate or Freon 113.

5) Place tissue in baskets taking care not to allow drying; this operation should be carried out under the solvent.

6) Fill transfer boat with transfer liquid (acetone, amyl acetate, Freon 113) and put baskets in boat.

7) Ensure apparatus is correctly installed (Section 2.1.3) and run cold water to cool chamber to 20°C.

8) Load specimen boat, close door and open inlet valve. Filling with liquid gas should be rapid; open vent valve to avoid back-pressure.

9) Leave inlet valve fully open (with vent valve slightly open to maintain liquid level) and open drain valve to remove substitution liquid. This flushing action should be kept up for 3-5 minutes. If the noise is found to be objectionable, a 'silencer' in the form of a length of plastic pipe may be fitted to the drain valve; this also serves to direct the flow of exhausting solvent.