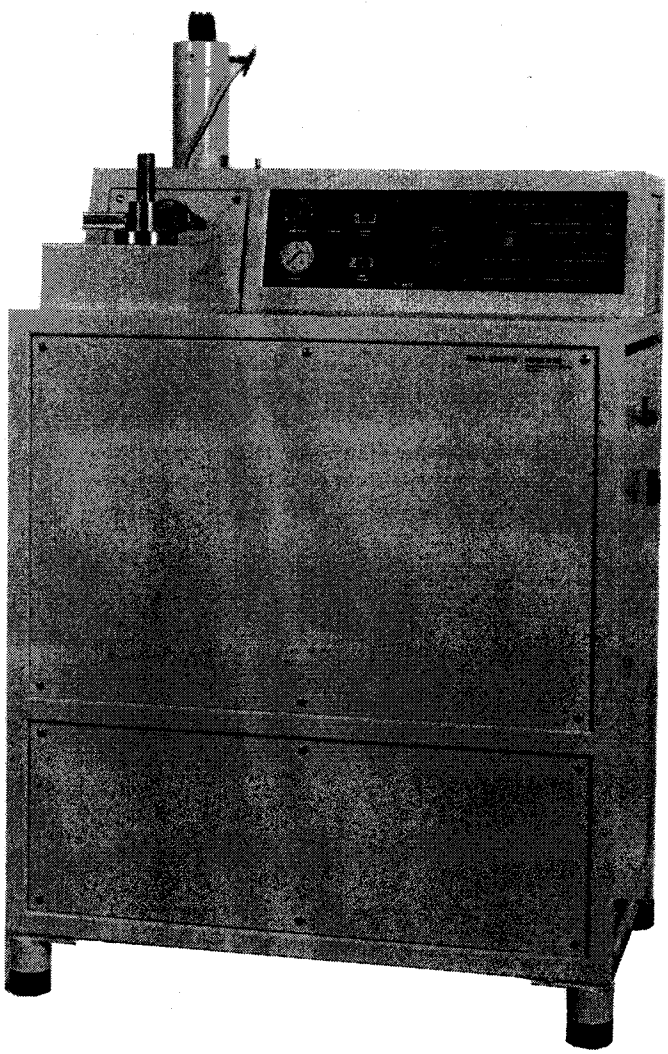
BAL-TEC

Operating Instructions

High Pressure Freezing Machine HPM 010



BU 800 127 BE

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Inspect shipment for possible damage and notify shipper if necessary.

Compare shipment with shipping papers and notify supplier of possibly missing pieces.

Save packing material. If possible, device should be shipped in the original packing for inspection or repair.

This manual is valid from series '96, serial number 128 on

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1. HIGH PRESSURE FREEZING

For preparing water containing samples with the aid of the electron microscopic cryo-preparation method, the flawless, if possible amorphous freezing of the cell fluid is one of the main prerequisites to maintain the cellular ultra-structure, and thus to obtain good and reproducible results.

Theoretical calculations have shown that there are physical limitations to the freeze fixation at **normal pressure** due to the poor thermal conductivity of the already frozen water in the sample.

Freezing under **high pressure** is a method which allows to freeze also thicker specimens without the formation of larger ice crystals which would destroy the fine structure of the cells. Due to the high hydrostatic pressure of 2100 bar, the physical features of the water are changed. Thus, the freezing point, for example, is lowered considerably and by increasing the water viscosity, the rate of crystal growth is drastically reduced. Thus, the formation of ice crystals inside the specimen to be frozen is largely prevented.

2. SAFETY INSTRUCTIONS

2.1. General Safety Instructions

The installation/setting up of the system must be reported to the authorities.
The system is constructed according to SVTI-regulations.
The system may only be operated with installed shrouding, installed protective cover, and installed sound absorbers.
Operation only in ventilated rooms.
The exhaust openings and the vents of the connecting pipes may neither be reduced (in diameter) nor completely closed.
Alcohol vapours must be drawn off.
Before and during service work, the mains switch at the side of the unit must always be set to "0", and the hydraulic accumulator must be relieved via the manual valve.
Protective eye-wear and ear protection must always be worn when working with the system.
High pressure fittings must never be re-tightened when under pressure.
There must be no welding, shaping or deforming of parts that are exposed to pressure.
During thermostatization, the parts must never be heated to more than 150° C.
Parts must only be replaced with original parts.

2.2. Special Safety Instructions

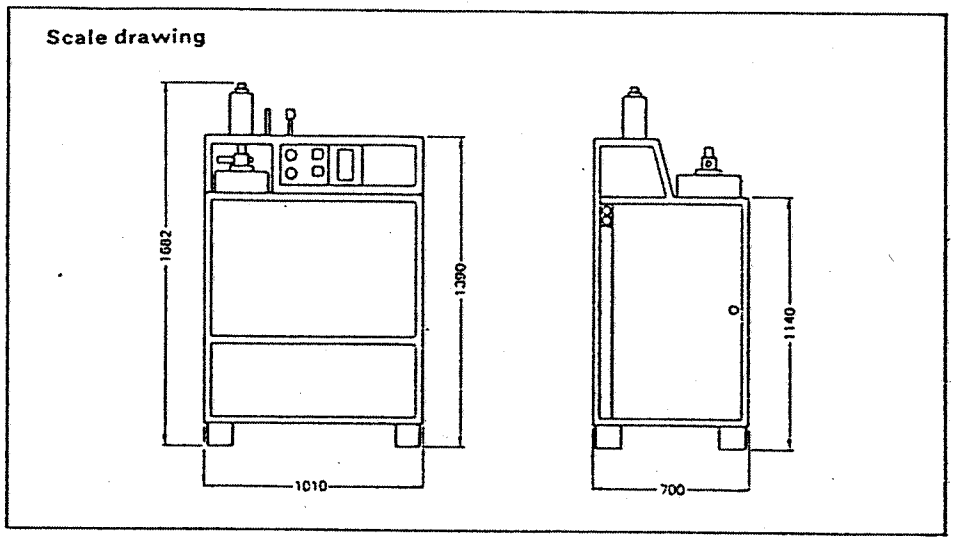
- Accumulator and accumulator safety valve with TUEV-approval,
- hand wheel on the accumulator safety valve,
- safety valve in the hydraulic control section (screw-in cartridge),
- material certificate according to EN 10204/ DIN 55049 31 B for high pressure cylinder,
- locking bolts and monitoring via initiator,
- thick-walled protective cover and shrouding sheets.

Furthermore, only materials are used which are especially suitable for low temperatures and high pressures.

3. DESCRIPTION OF THE SYSTEM

3.1. Machine and Connection Data

Dimensions	width	1010 mm
	depth	700 mm
	height	1682 mm
	transport tray	1200 x 950 mm



Weight of the unit	approx. 450 kg

Recommended surface for setting up the unit	width	2500 mm
	depth	1700 mm

- Power consumption
  Hydraulic motor 1,5 kW
  Thermostat 1 kW
  Control 0,2 kW
- Hydraulic system max. 315 bar
- Accumulator prestressing pressure 180 – 250 bar
- Hydraulic oil 40 l (Shell Tellus Oil T68/Pourpoint –39° C)
- Conversion 1 : 10
- High pressure system 3150 bar
- Piston displacement 200 mm
- Piston diameter 25.6 mm
- Nitrogen filling, hydraulic cylinder 100 cm
- Dewar content 7 l
- Liquid nitrogen consumption 10 – 20 l/hour
- Cooling of the system 15 min.
- Max. shooting cadence 1 shot/90 sec.

Electrical connection

Connection voltage	3 x 380/230 V or 3 x 208 V
Frequency	50 - 60 Hz
Power consumption	3000 VA
Mains cable	5-core, with free ends, length 5 m (**without** mains plug!)

Liquid nitrogen connection

Hose connection	G 1/4" outer thread (on medium supply)
Connection hose	copper hose, diam. 8x1 mm, length 1m, with armaflex hose insulation (contained in the accessory set)
Liquid nitrogen dewar	e.g. 60 l LN2-dewar (**not contained** in the scope of delivery) article no. B 8010 120 01
Inlet pressure	0.6 bar above atmospheric pressure

Compressed air connection

Hose connection	G 1/4" outer thread (on medium supply)
Connection hose	polyamide hose, blue, diam. 4/6 mm, length 3 m (contained in the accessory set)
Compressed air supply	compressed air cylinder with gas reducing valve or domestic compressed air supply
Inlet pressure	5 bar above atmospheric pressure

Warm water connection

The hose connections which are required for connecting the thermostatic bath are already connected to the specimen pressure chamber in the unit. They merely have to be connected to the suitable hose nipples of the thermostatic bath.

Operational start-up

If the unit is to be set up by a service engineer of the supplies firm or its representation, the customer has to ensure that the installations for the media which are required for operating the unit, such as current, liquid nitrogen and compressed air, are available as close as possible to the space where the unit is to be set up. Suitable connection nipples, G 1/4" outer thread (G 1/4" inner thread on the side of the hose), for the connection hoses have to be prepared on the domestic side.
To complete the mains connection cable (5 x 4 mm^2), it has to be fitted with a suitable mains plug 3P+N+E, which fits the domestic socket.

3.2. Converting the System to 3 x 208V, 60 Hz

Any system can be converted to 3 x 208 V, 60 Hz by a change of the wiring. See diagram.

The motor control from star to delta circuit - and the wiring of the transformer, as well as the name plate of the hydraulic motor have to be changed.

3.3. Operating Controls

Mains switch (at side of unit):
In position "0", the entire system is without power. If the mains switch is in position "1", the system can be activated with the key switch ON. The high-current part of the control unit and the two temperature displays as well as the alcohol display are in operation.

Key switch (at side of unit):
If the key switch is in position OFF and the mains switch in position "1", the system is without power, with the exception of the thermostat and the dewar lid heating. The delay for the complete shut-down can be set to max. 10 hours. After the set delay has elapsed, the entire system is without power.

START (main switch at front of unit):
The microprocessor control (MP) is activated. If the piston is not in starting position, the hydraulic system starts up, moves the piston automatically upwards and then switches off again. Keys MANUAL, AUTO, NITROGEN, PISTON UP, PISTON DOWN and ALCOHOL are activated.

STOP key, EMERGENCY-OFF:
The control can be switched off at any time. A "jet" will be interrupted immediately.
Thus, this key also covers the EMERGENCY-OFF function.

NITROGEN key:
The nitrogen supply to the dewar is released or interrupted, respectively.
In MANUAL operation, the NITROGEN function automatically becomes inactive. The key NITROGEN is also used to render the automatic nitrogen control operational.

DRIVE IN key:
As soon as the dewar is filled with nitrogen and the red control lamp NITROGEN LEVEL goes out, the DRIVE IN function can be started. The piston automatically moves back and forth three times.

AUTO key:
When this function is in operation, the system automatically moves into ready (stand-by) position and the accumulator pressure is maintained in the fixed range of 300 to 315 bar.
The system automatically returns to stand-by position after each "jet".

JET key:
This key is used to release the automatic freezing process.
The JET-key is only active if READY lights up.

MANUAL key:
This function is only important for service work.
The keys ALCOHOL, PISTON UP, PISTON DOWN are activated. The keys JET and DRIVE IN are blocked.
If the keys AUTO or NITROGEN have been selected, the function MANUAL is eliminated.

ALCOHOL key:
While this key remains pressed, the alcohol valve remains switched on, i.e. alcohol is blown into the object head.

PISTON UP key:
While this key remains pressed, the piston continues to move upwards (accumulator pressure must be < 250 bar).

PISTON DOWN key:
While this key remains pressed, the piston continues to move downwards (accumulator pressure must be < 250 bar).

3.4. Monitoring and Control Functions

READY control lamp:
If READY lights up, a "jet" can be released. READY lights up if the following conditions are met:

- pressure higher than 300 bar (lower pressure switch);
- initiator specimen head is active, i.e. specimen holder and safety bolt are inserted;
- NITROGEN LEVEL does not light up, the dewar is filled with liquid nitrogen;
- PISTON IN POSITION, hydraulic cylinder is in top position;
- NITROGEN must be pressed.

PRESSURE OVER control lamp:
PRESSURE OVER lights up two seconds after the start of the pressure converter, irrespective of the "jet"-duration.

TIME OVER control lamp:
The control lamp TIME OVER indicates the period of time within which the frozen sample must be removed from the object head and placed in liquid nitrogen, so that the sample is not warmed up above -100° C.

TIME OVER lights up for 7 seconds, irrespective of the "jet"-duration or the actual temperature of the sample.

PISTON IN POSITION control lamp:
PISTON IN POSITION indicates that the piston is withdrawn and in starting position (top).

HEATING control lamp:
As soon as the start key has been pressed, HEATING flashes for 30 seconds. After 30 seconds HEATING lights up permanently.
The flashing is a reminder to check whether the thermostat is filled with a sufficient quantity of water and whether it is switched on at all.

NITROGEN LEVEL control lamp:
NITROGEN LEVEL lights up as soon as the NITROGEN key has been pressed and goes out as soon as the dewar has been filled with liquid nitrogen. A thermosensor on the exhaust of the dewar monitors the nitrogen level.

ALCOHOL PRESSURE (manometer):
ALCOHOL PRESSURE indicates the air pressure in the alcohol system (4 to 5 bar).

OIL PRESSURE:
OIL PRESSURE indicates the oil pressure in the hydraulic system.

ALCOHOL MIN (sight glass):
If the ALCOHOL MIN-level is reached at the sight glass, alcohol must be refilled. Prior to doing so, it is essential that the pressure in the alcohol system is reduced to atmospheric pressure.

OBJECT HEAD TEMPERATURE:
This monitoring indicator (controller) shows the object head temperature, without having any influence upon it. A limit value of 000 should be set, so that the relay function of the "controller" is not activated in vain. Temperature is preset on warm water thermostat bath.

EXHAUST TEMPERATURE:
This monitoring indicator shows the exhaust temperature. The liquid nitrogen level in the dewar is monitored directly via the exhaust temperature (alarm value: -185° C, depending on the used system). Setting procedure: see Operational Start-up, section 4.1.

DEWAR TEMPERATURE:
This monitoring indicator shows the temperature of the electric dewar lid heating. It is set at 70° C. If the electric heating fails, a pulsating alarm signal starts up: Check fuse F 31 and fuse switch F 16 (alarm value 15° C).

AIR HEATER key:
If this switch is pressed, compressed air is blown through the air heater into the dewar. Consecutively the pneumatic valve and the air heater are activated. The air temperature is set at 80° C.

AIR HEATER PRESSURE (manometer):
The indication AIR HEATER PRESSURE shows the air pressure in the air heater (The setting at delivery of 0.5 bar must not be surpassed!).
The indication only shows up if the air heater is switched on.

4. INITIAL OPERATIONAL START-UP

Shots without nitrogen in the high pressure cylinder, i.e. without resistance, should be avoided. Such very fast movements may damage the hydraulic system and the hydraulic cylinder in particular.

4.1. Preparing the System

- Fill thermostat bath with water and set thermostat temperature to 45° C (See Operating Instructions for thermostat).

- Check oil level in the hydraulic system. If oil has been drained off for the transport of the system, fill in approx. 40 l of oil. Recommended oil: Shell Tellus Oil T 68 (Pourpoint -39° C).

- Prestress hydraulic accumulator with nitrogen gas: Remove protective caps of accumulator. Mount filling device between accumulator and nitrogen bottle (see Operating instructions for bladder-type accumulator charging and testing device). The prestress pressure of the accumulator should be 140 to 250 bar.

  From a minimal prestress pressure of 140 bar, the functioning of the system is guaranteed.

  If the accumulator prestress pressure reaches 250 bar - as compared to 180 bar - the high pressure side of the system will achieve a pressure pattern which is approx. 100 bar higher.

- Fill 0.5 l of isopropanate into the alcohol reservoir. Compressed air must be disconnected.

- Connect compressed air, max. 6 bar.

- Connect liquid nitrogen and set pressure to max. 0,6 bar. For the supply line a copper tube of 8 x 1 mm in diameter, with insulation, is included in the scope of delivery.

- Set mains switch at side of unit to "1".

- Set key switch to ON: thermostat is running, dewar lid heating is switched on.

- Set DEWAR HEATING to a maximum of 70° C.

- Check rotating direction of hydraulic aggregate motor: press "START"-key.

  The hydraulic system starts to run; check rotating direction of motor. If the rotation direction is not correct, change two phases of the mains connection line.

- Press STOP key.

  It is recommended to leave the system in this position for at least 4 hours. In this time the system is heated and the remaining condensation water in the cooling system evaporates.

- Press START key:

  The hydraulic system starts and moves the pressure converter to starting position. Indication PISTON IN POSITION lights up. The hydraulic system switches off again.

- Vent hydraulic cylinder with vent screws (Setting at delivery).

- Open safety valve on hydraulic aggregate until pressure indication OIL PRESSURE comes down to < 20 bar.

- Press MANUAL key.

- Vent cylinder at side of piston:

  Press PISTON DOWN until the manometer shows a rise in pressure. Slightly loosen upper vent screw on cylinder.

  Press PISTON UP until all air has left the cylinder and oil flows out. Repeat this procedure if necessary.

- Vent cylinder at side of piston rod:

  Press PISTON UP until the manometer shows a rise in pressure. Loosen lower vent screw on cylinder.

  Press PISTON DOWN until all air has left the cylinder and oil flows out. Repeat this procedure if necessary.

- Bake out system:

  Press key AIR HEATER; key lights up. Compressed air is blown through the air heater. Set temperature to a maximum of 80° C.

  Set manometer AIR HEATR PRESSURE to a maximum of 1 bar.

  Warning! Check that air moves through the exhaust pipe at the back.
  The air heater must not be operated without air moving through.

  Bake out for at least one hour.

- Press key AIR HEATER; air heater is switched off.

- Fill in liquid nitrogen:

  Press key NITROGEN. NITROGEN MIN lights up. MANUAL key becomes inactive. Hydraulic system moves piston into starting position.

- Set EXHAUST TEMPERATURE controller.

  Set limit value (Setting at delivery: 185° C):
  If liquid nitrogen is blown through the exhaust, the controller shows the actual nitrogen temperature. If the limit value of 185° C (-185° C) is reached, the nitrogen supply is interrupted. In principle, the limit value should be set so that the nitrogen supply is interrupted immediately if liquid nitrogen is blown through the exhaust.

- NITROGEN MIN goes out.

  The dewar is filled with liquid nitrogen. If formation of ice can be observed on the vent of the connecting pipe, the high pressure cylinder is also filled.

- Press DRIVE IN key:

  The hydraulic system automatically moves the piston back and forth 3 times in order to vent the volume of the high pressure cylinder.

- Press AUTO key, NITROGEN must also be pressed:

  The system moves into "ready position" and loads the hydraulic accumulator.

  As soon as READY lights up, the system is ready for high pressure freezing.

Prior to starting with the actual high pressure freezing, 2 to 3 test "jets" should be carried out. When doing so, care must be taken that specimen carriers have been mounted on the specimen holder

4.2. Handling during the Freezing Process

The safety instructions under section 2.1. must be observed.

It is advisable to lightly grease the O-ring, pos. 29, of the insert, pos. 5 (object head, complete, 0.50018.0), with vacuum grease prior to each operational start-up, so that the insert can be easily guided into and removed from the object head.

- Insert prepared sample holder into object head;
- insert locking bolt;
- press JET key, freezing process is started;
- pull out locking bolt as soon as PRESSURE OVER lights up;
- pull out specimen holder from object head and quickly plunge its tip with the frozen specimen into liquid nitrogen;
- remove specimen sandwich from specimen holder in liquid nitrogen.

4.3. Continuous Operation

It is no problem to use the system in continuous operation. When doing so, it is important that the thermostat bath is always filled with a sufficient amount of water. The water level may be max. 2 cm below the lid.

4.4. Switching off the System

The system should only be switched off if the pressure converter is in starting position and if PISTON IN POSITION lights up. It is of particular importance to comply with this "rule" after a "jet".

The nitrogen supply and the hydraulic system are rendered inoperative with the STOP-key. If the key switch is set to OFF, the system, with the exception of the thermostat and dewar lid heater, is without power.

Warning! If the mains switch is set to "0", the entire system is without power. It may only be set to "0" after the delay for the complete shut-down for thermostat and dewar lid heater has elapsed (at least 4 hours).

The delay for the shut-down must be set so that the entire liquid nitrogen can evaporate from the dewar, thus preventing the formation of water condensation.

4.5. Emergency-OFF

With the emergency-off key the pressure converter can be stopped at any time.

5. TYPICAL FREEZING DATA

When using nozzles of 0.4 mm in diameter, a prestressing pressure of 180 bar and a hydraulic pressure of 315 bar prior to the jet, the following system values are typical:

PRESSURE MAINTENANCE	pressure duration P > 2100 bar	550 ms +/-20 ms
pressure level		> 2600 bar maximum >2100 bar end of plateau
COOLING TIME	cooling speed 0 to –50° C	10 ms +/-2 ms
TEMPERATURE MAINTENANCE	duration t < -100° C	7 sec. +/-2 sec.
cooling	0° C transition	p = 2500 +/-100 bar

The thermo element is subject to great stress. The wear resulting thereof causes increasingly shorter cooling speeds to be measured until the thermo element finally breaks. The above indicated temperature values have been measured with a new thermo element.

6. MAINTENANCE

6.1. Maintenance Intervals

During the first week of operation, the high pressure fittings of the nitrogen system have to be retightened every day before operational start-up.

After this period, these fittings have to be checked weekly and retightened if necessary.

> High pressure fittings must never
> be retightened while under pressure.

The oil level of the hydraulic system has to be checked weekly. With relieved accumulator, the oil level must be visible through the upper sight glass.

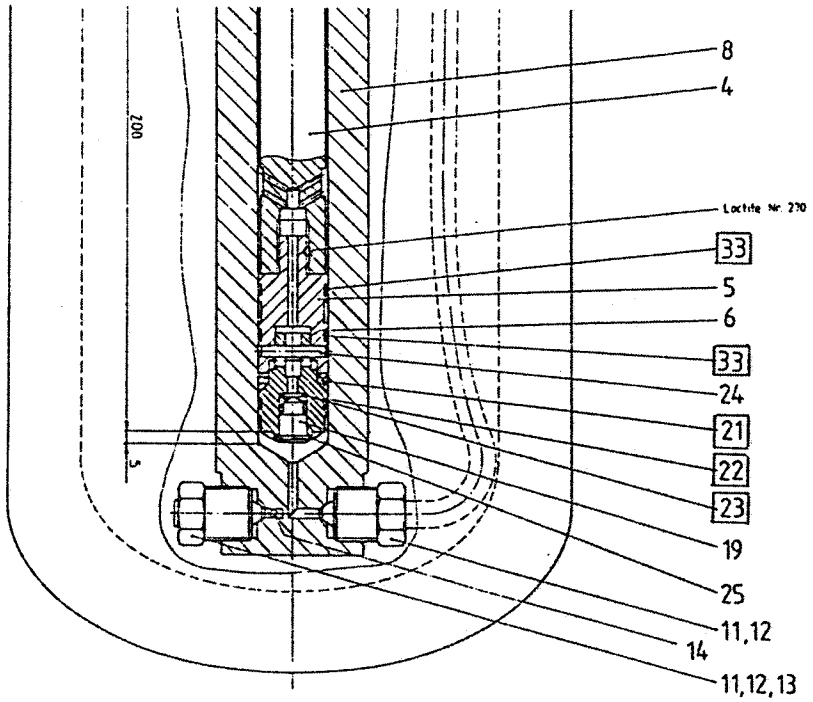
The hydraulic oil must be changed every two years or after 10,000 shots.

The electrical system requires no maintenance.

6.2. Wear Parts

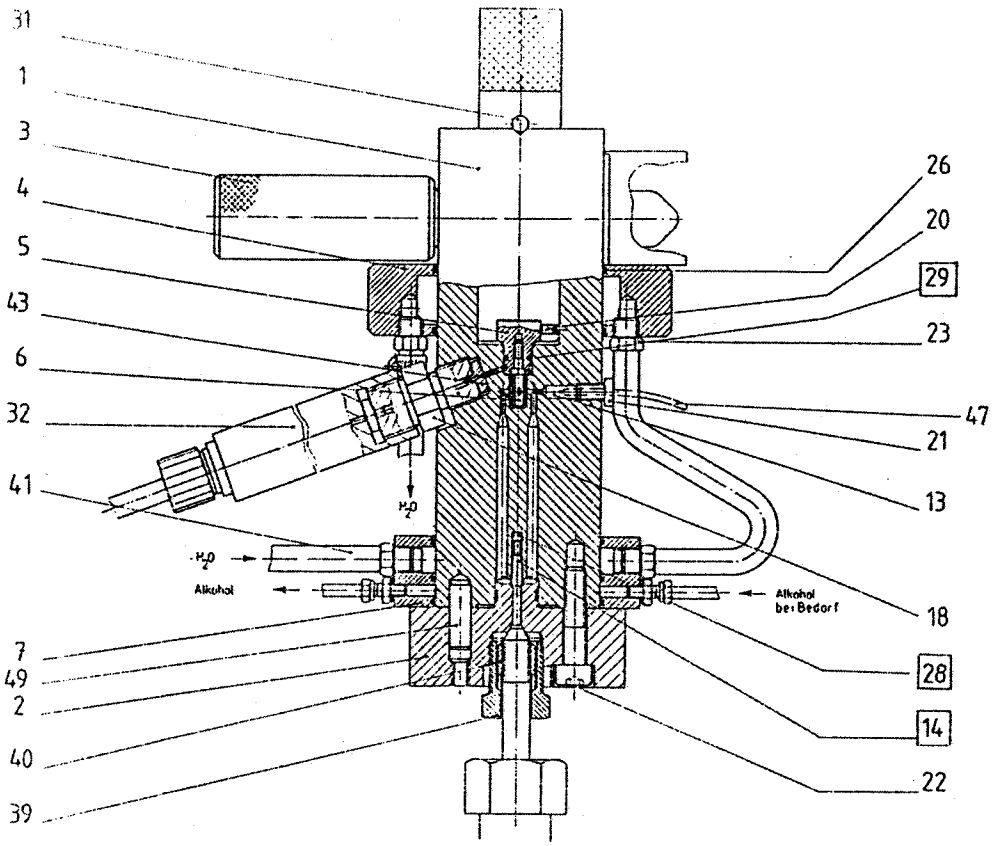
The wear of the wear-parts depends directly on the number of shots.

The following parts of the pressure converter are wear-parts:



Pos. 21	Piston seal	B 8010 124 56
Pos. 22	Slide ring	B 8010 124 57
Pos. 23	Pressure spring	B 8010 125 32
Pos. 33	Slide ring	B 8010 123 79

The following parts of the object head are wear-parts:



Pos. 14	Injector slide	BU 8010 125 30
Pos. 28	Injector spring	BU 8010 125 34
Pos. 29	O-ring, diam. 6.75 x 1.78	BU 8010 077 71

The O-ring, B 8010 077 71, at the sample holder must be replaced after every 50 to 100 shots or if it shows visible damage.

If the duration of pressure and/or the pressure level fall below the minimum requirements, piston seal, B 8010 124 56, and slide rings, B 8010 123 79 and B 8010 124 57, have to be replaced.

If the injector freezes or if the sample heats up too fast, the injector slide, B 8010 125 30, as well as the injector spring, B 8010 125 34, may have to be replaced. These parts normally last for 2000 to 3000 shots.

The highest wear in the hydraulic system – in particular in the hydraulic cylinder – is caused by so-called "free shots". Shots without nitrogen in the high pressure cylinder, i.e. without resistance, must therefore be avoided if possible.

7. TROUBLE SHOOTING

> It is essential that the general safety instructions mentioned under section 2.1. are observed during trouble shooting and repair.

7.1. Pressure Pattern

If the deviations of the pressure pattern are consistently 10 percent outside the limit values given in the enclosed test protocol, the problem must be located according to the following checklist. It is a prerequisite, however, that the system is equipped and operated according to section 4.

Pressure duration too long, pressure level slightly increased

Drawing: Object head, complete 0.50018.0

Nozzle is blocked: remove and clean nozzle.
Nozzle bore has become smaller: replace nozzle.

Pressure duration too long, pressure level slightly lower

Drawing: Object head, complete 0.50018.0

The high pressure system is restricted at some point. A particularly vulnerable spot is the change of cross-section of the line from diameter 3.2 to 1.6 mm in the object head (section A-A).
It is possible that the ball, pos. 37, has come loose and is caught in the bore. If the ball is jammed there, it must be removed by eroding.

Pressure duration too short, pressure level normal or slightly lower

Drawing: Object head, complete 0.50018.0
Drawing: Pressure converter, complete 1.50017.0

1. Replace O-ring, Pos. 29 (B 8010 077 71), in drawing 0.50018.0.

2. Retighten all high pressure fittings.

at object head:	nozzle holder	pos. 11
	pressure sensor	pos. 32
	blind plug	pos. 13
	1 high pressure screw joint	pos. 39
at pressure converter:	2 high pressure screw joint	pos. 11, 12

3. Check O-ring, Pos. 27 (B 4070 201 PV) and back-up ring, pos. 17 (B 8010 125 35) at the object head flange, pos. 2 (drawing 0.50018.0), and replace these parts if necessary.

4. The check valve in the high pressure cylinder or in the piston is stuck, i.e. the high pressure cylinder is no longer completely filled with liquid nitrogen. There is no or only a very weak emission of gas from the vent of the connecting pipe.

   As a first attempt, the exhaust of the LN2 dewar at the rear of the system can be blocked with a rubber plug during a filling cycle in order to loosen the jammed check valves (Check: gas emanating from ventilation of connection pipe).

   If the problem cannot be amended in this way, the system must be defrosted, the check valves must be removed and cleaned or possibly replaced.

   This trouble can be caused by humidity, oil or solid particles in the nitrogen.

5. If approx. 2000 shots have already been executed with the system, it is likely that the piston seal, pos. 21 of the drawing 1.50017.0 (B 8010 124 56) has to be replaced.

   If ingress of leaked oil into the connection pipe keeps causing operating problems, the hydraulic cylinder must be replaced.

7.2. Heating of Sample Takes Place too Fast

Drawing: Object head, complete 0.50018.0

The injector valve, consisting of pos. 14 and 28, leaks.

Provided that the system has cooled down, the tightness of this valve can be checked as follows:

While in MANUAL operation, inject alcohol into the object head by pressing the ALCOHOL key, until the sample chamber is filled. Change system again to AUTOMATIC operation. Look into the object head from above and check whether air bubbles rise through the alcohol. Only approx. 1 bubble should rise per 1 to 2 seconds. The leak can be observed best at the moment when liquid nitrogen flows into the dewar.

If the valve leaks, replace injector slide, pos. 14 (B 8010 125 33), and pressure spring, pos. 28 (B 8010 125 34). Please be aware that the new injector slide will leak initially, as it has to "work itself into position" first.

7.3. Freezing of the System

Drawing: Object head, complete 0.50018.0

Object head

If the injector valve leaks, alcohol can get into the cooled high pressure pipe, where it will freeze immediately, completely blocking the line. No nitrogen can be ejected. During a "jet", the pressure in the hydraulic system only drops by half the normal rate.

Press STOP key and defrost system for at least 4 hours. Press AIR HEATER key and bake out cooling system for approx. 1 hour. Carry out revision of injector valve at object head.

Dewar lid

If the dewar lid heating is defective, the system must be switched off and the nitrogen supply as well as the connection pipe have to be thermostatized, using a hot-air blower. The blower must not be set to above 150° C.

Warning: It is important to de-ice the connection pipe immediately, so that the seals in the hydraulic cylinder are not damaged.

7.4. Trouble in General

Special attention must be given to the screwed fittings. The strong vibrations may loosen the screws, although each one is secured with Loctite or with locking washers.

The hose between the alcohol valve and the object head flange may burst, if the ball valve – due to contamination or wear – does not close immediately.

It is important to ensure that no water enters the nitrogen system, since water in the system creates the danger that the valves will freeze stuck during a subsequent operational start-up.

It is absolutely necessary to remove any water prior to the following cooling of the system (hot-air blower, max. 150° C).

Electrical trouble has to be traced with the aid of the electrical diagram. The control programme is fixed and cannot be changed. A programme change requires a new EPROM.

The operating instructions enclosed to the units must be observed.

7.5. Assembly/Installation Instructions

7.5.1. Removing Protective Hood

- Remove initiators with initiator holders.
- Disconnect one water hose from the thermostat bath and blow through with compressed air until the entire water has been removed from the circulation system.
- Disconnect water hose from upper heating ring.
- Pull upper heating ring upwards over the object head.
- Loosen and remove protective hood.

7.5.2. Replacing the Nozzels

- Remove protective cover (see 7.5.1.).
- Remove nozzle holder.
- The nozzle has a M4 internal thread at the exit side. The nozzle can be picked up and pulled out with the help of a long M4 screw.

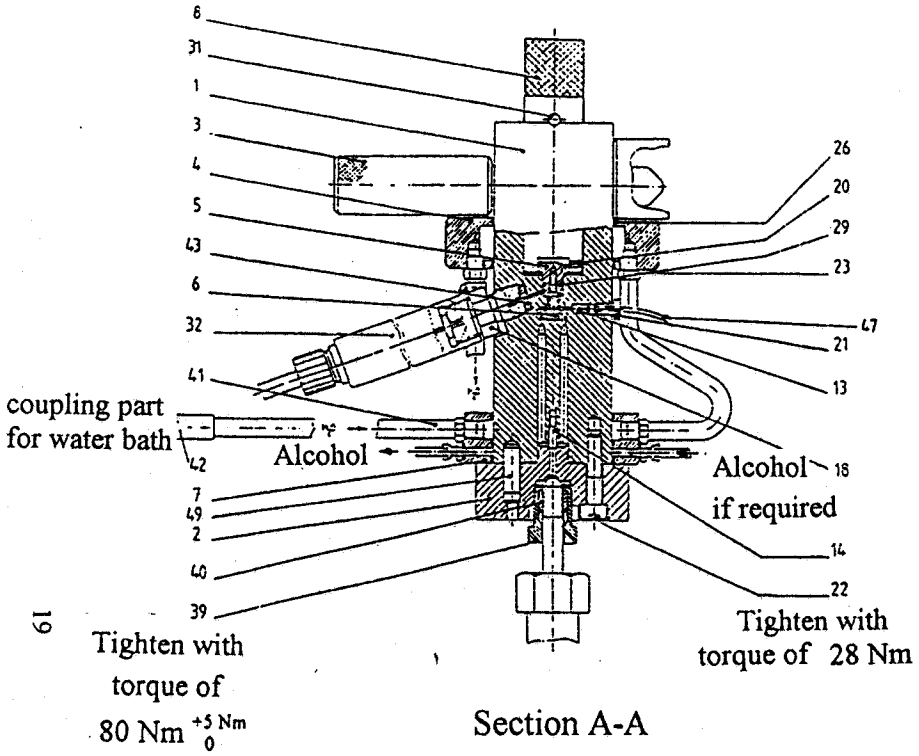
7.5.3. Maintenance of Object Head:

This job may only be performed by specially trained persons.

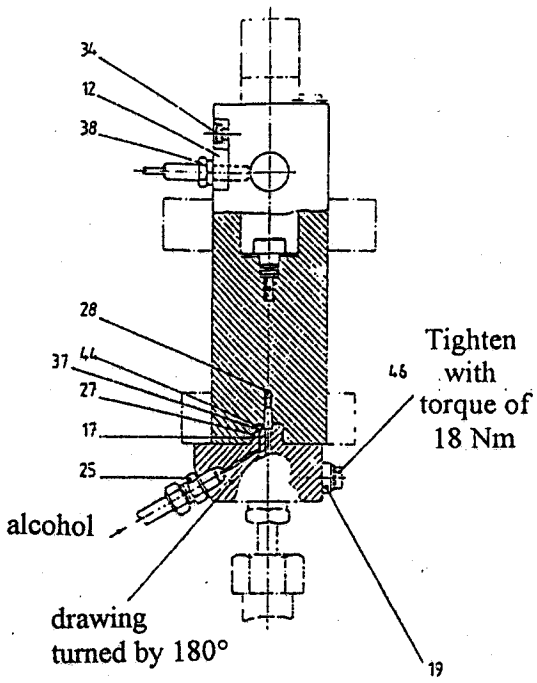
Drawing: Object head, complete 0.50018.0

- **Disassembly/removal of object head**

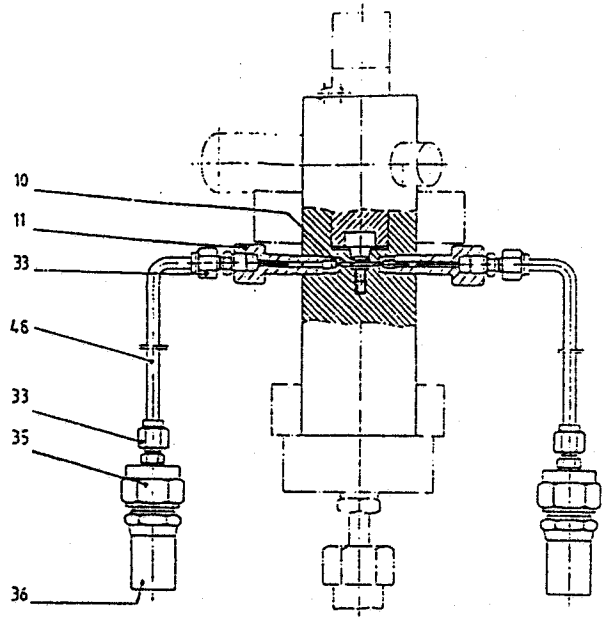
- Set mains switch (at side of unit) to position "0":
- Relieve hydraulic accumulator using the hand wheel of the accumulator safety valve. Check pressure on OIL PRESSURE manometer.
- Loosen screws, pos. 33, of both exhaust pipes and remove entire exhaust.
- Loosen screws, pos. 39, of the high pressure line.
- Disconnect supply lines for water and alcohol.
- Loosen thermo element, Pos. 47, from object head.
- Disconnect cable from pressure receiver, pos. 32.
- Loosen object head from holder, pos. 9.
- Remove four screws, pos. 22, from object flange, pos. 2.
- Pull object flange from object head.
- Replace injector slide, pos. 14, and injector spring, pos. 28. Check that injector slide moves easily.
- Replace support ring, pos. 17, and O-ring, pos. 27.
  Warning: Don't change positions. These parts are preferably removed using a wooden tool (e.g. tooth pick), so that the sealing surface isn't scratched.
- Check alcohol check valve (pos. 37/B 8010 125 36).



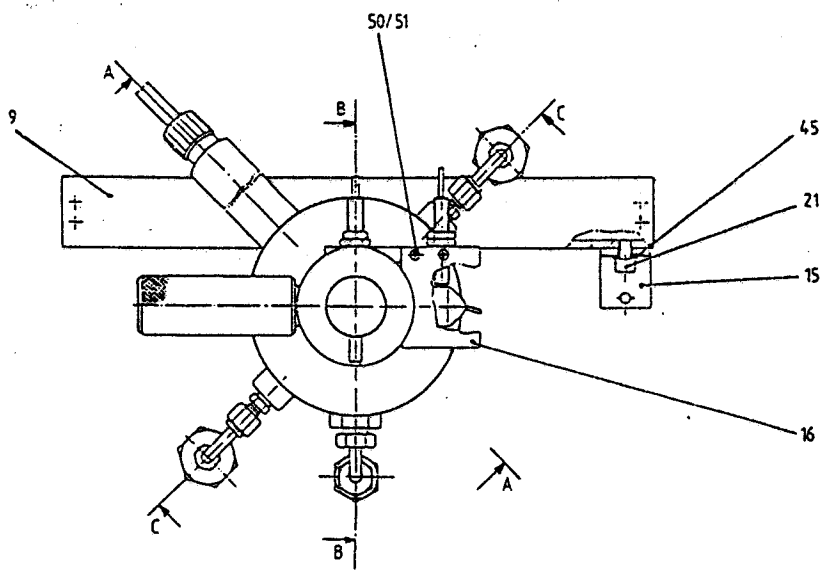
Section A-A



Section B-B



Section C-C



Object head 0.50018

Assembly of object head

Only greaseless and cleaned parts may be assembled.

The assembly is carried out in the reversed sequence of the above described disassembly.

7.5.4. Maintenance of Pressure Converter

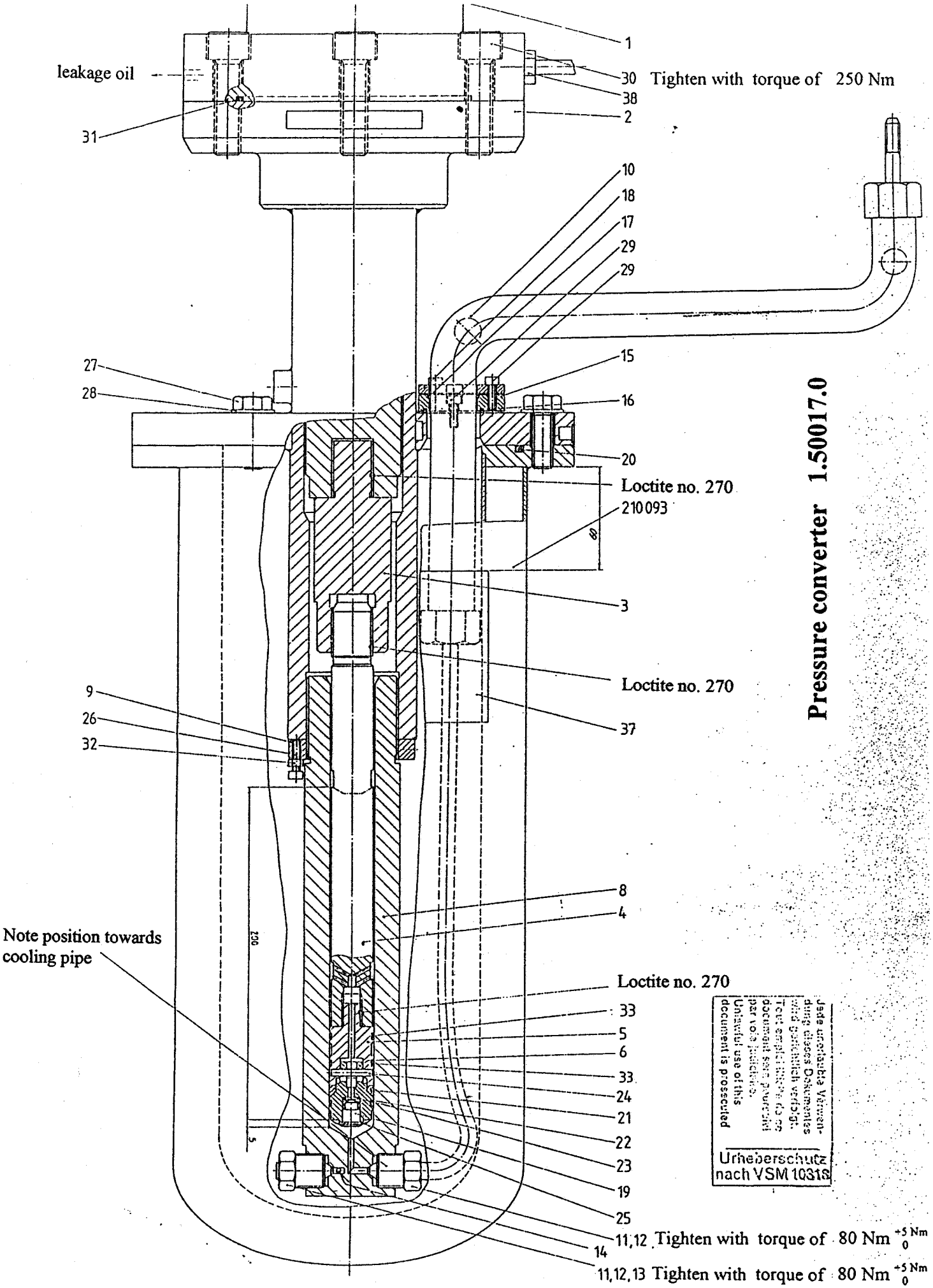
> This job may only be performed
> by specially trained persons.

Drawing: pressure converter, complete 1.50017.0

Disassembly of pressure converter

Move pressure converter back to the stop (PISTON IN POSITION)

- Set mains switch (at side of unit) to position "0":
- Relieve hydraulic accumulator using the hand wheel of the accumulator safety valve. Check pressure on OIL PRESSURE manometer.
- Disassemble dewar.
- Loosen both screwed connections, pos. 11.
  Warning: ball, pos. 14 (diam. 2.5 mm) lies loosely in the bore!
- Loosen threaded pin, pos. 26.
- Prior to disassembling the high pressure cylinder, pos. 8, the distance between the lower edge of the high pressure cylinder and the threaded ring, pos. 9, must be measured. This measure must be checked and exactly kept the same during the subsequent assembly.
- Loosen threaded ring, pos. 9, with hook wrench (contained in the tool set) and open slightly.
- Using a rope, pull high pressure pipe towards the front side of the system and tie it down so that there is enough room to take out the high pressure cylinder. Do not bend the pipe of the high pressure system.
- Remove high pressure cylinder, turning it by hand or with fork wrench SW 52.
- Weigh down piston rod, pos. 4, with 5 kg weight (wood).
- Knock out spring cotter, pos. 24, with punch of 3.8 mm in diameter. Take care that piston rod, pos. 4, and piston guide, pos. 5, as well as piston, pos. 6, are not damaged.
- Pull bronze insert downwards from the piston rod.
- Replace seal, pos. 21 (B 8010 124 56).
  The length of worn seals is reduced to 4 mm. The seal must be replaced if it is torn and/or if the seal length is shorter than 5.5 mm.
- Check if piston check valve functions correctly. Should this not be the case, remove, clean and, if necessary, replace valve.
- Check attachment of piston rod:



Pressure converter 1.50017.0

- Check visually, whether hydraulic oil from the hydraulic cylinder has flown into the connection pipe. If so, all traces of oil must be removed. If leaked oil impedes the operation of the system, the hydraulic cylinder must be repaired or replaced.

Assembly of pressure converter

Only greaseless and cleaned parts may be assembled.

- Install seal and piston.
- Move pressure converter back until the uppermost slide ring, pos. 33, is just barely visible.
- Switch off system with mains switch. Relieve accumulator.
- Spray Molykote onto the threads of the high pressure cylinder, prior to its installation (do not use any grease).
- Re-install slide rings, pos. 22 and pos. 23. If 5000 shots have already been made, these should be replaced.
- Manually hold slide ring in position, while slowly moving high pressure cylinder over piston, seal and slide rings.
- Screw in high pressure cylinder until the distance between the lower edge of the high pressure cylinder and the threaded ring, pos. 9, which has been noted down during disassembly, has again been reached. Care must also be taken that the high pressure connection (without any restriction) exactly points in the direction of the high pressure pipe.
- Tighten screwed fitting, pos. 11, of high pressure pipe.
- Tighten threaded ring, pos. 9, and threaded stud, pos. 26.
- The subsequent assembly is carried out in the reversed sequence of the earlier disassembly.

8. TRANSPORTATION OF THE SYSTEM

The transportation of the system requires special caution.

The centre of gravity of the system is slightly off centre. In addition, the system is relatively high (1390 mm) in comparison to its small floor surface of 1010 x 700 mm.

If the system is transported by air freight, the pre-stressing pressure of the accumulator must be reduced to < 10 bar, using the accumulator charging and testing device (B 8010 125 31).

9. CERTIFICATES AND TEST REPORTS

- TUEV Acceptance Certificate for the accumulator,
- calibration report for the pressure measuring sensor,
- test protocol for the complete system.

10. SPARE PARTS

10.1. Object head, complete, drawing 0.50018.0

Pos. 10	exhaust nozzle, diam. 0.4 mm	B 8010 120 72
Pos. 14	injector slide	B 8010 125 33
Pos. 17	support ring	B 8010 125 35
Pos. 27	O-ring, diam. 12.42 x 1.78	B 4070 201 PV
Pos. 28	injector spring	B 8010 125 34
Pos. 29	O-ring, diam. 6.75 x 1.78	B 8010 077 71
Pos. 37	ball, diam. 2 mm	B 8010 125 36

10.2. Pressure converter, complete, drawing 1.50017.0

Pos. 14	ball, diam. 2.5 mm	B 8010 124 54
Pos. 21	piston seal	B 8010 124 56
Pos. 22	guide sling	B 8010 124 57
Pos. 23	piston spring	B 8010 125 32
Pos. 33	guide sling	B 8010 123 79

11. TOOLS

Hook wrench	B 8010 124 80
Straddling drift	B 8010 125 30
Accumulator filling device	B 8010 125 31

12. MEASURING UNIT HPC 010 FOR PRESSURE- AND TEMPERATURE PATTERN

12.1. Documentation of the System

12.1.1. Description of the System

The measuring unit for the high pressure freezing system monitors specific time patterns of pressure and temperature in the specimen chamber (see fig. 1.1-1).

Pressure and temperature must reach specific discrete values within a measuring cycle. The time periods between these values are measured and indicated. They are:

- **Pressure Maintenance:**
  time between the moment when the pressure exceeds 2100 bar and the moment when it falls below this value again. The range lies between 100 ms and 9.99 seconds.

- **Cooling Time:**
  time between the moment when the temperature is below 0° C and -50° C. The range is between 0.1 ms and 99.9 ms.

- **Temperature Maintenance:**
  time between the moment when the temperature falls below -100° C and the moment when it returns above this value. The range is between 1 second and 99 seconds.

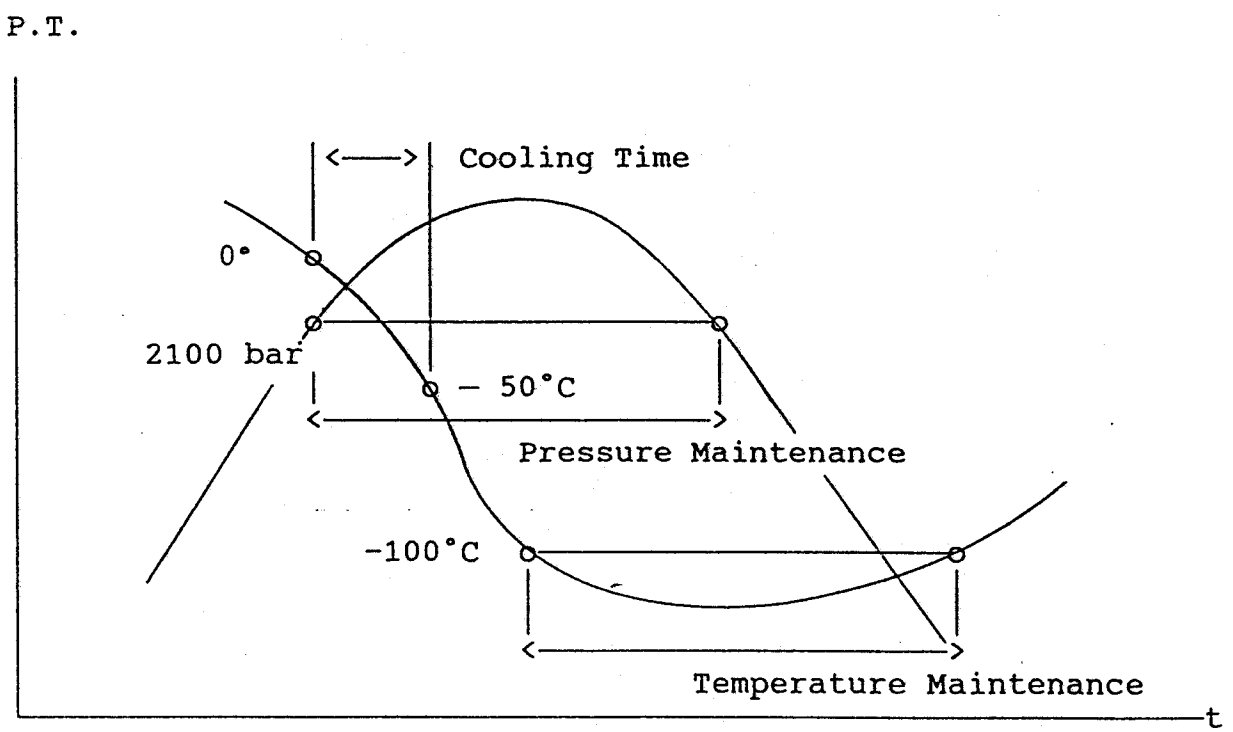


Fig. 1.1-1 Pressure and temperature pattern

The block diagram, fig. 1.1-2, shows the design of the measuring unit. It shows the measuring circuits for pressure and temperature, the comparator, the microprocessor and the LED display.

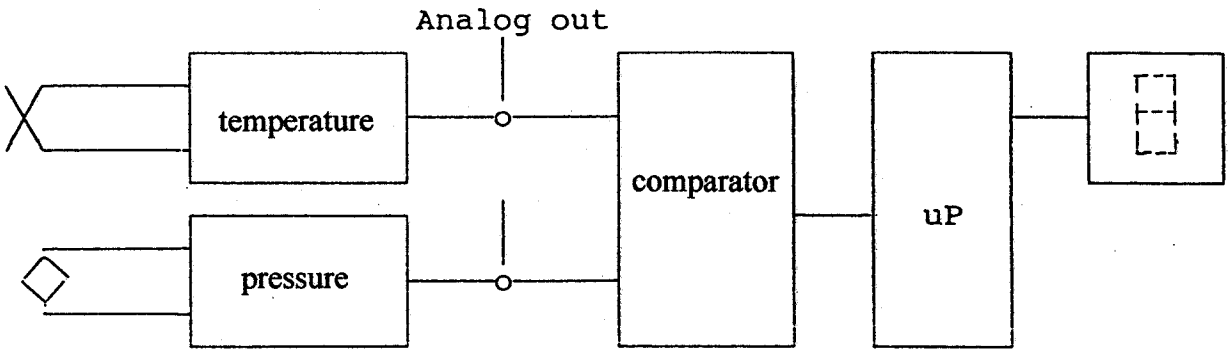


Fig. 1.1-2 Block diagram of the measuring unit

The pressure and temperature measuring circuits adapt the sensor signals to the input of the comparator. The comparator is programmable. This makes it possible to monitor the discrete pressure and temperature values, and their reference to time can be registered by the microprocessor. The microprocessor monitors the process and displays the respective times.

The circuitry is located on a printed circuit board, which is contained in a plastic housing. The LED display is behind the front plate. The sensors are connected with one-meter-long connector cables with sockets.

Pressure and temperature patterns must meet specific requirements in order to complete a valid measuring cycle. Such a cycle is started when the pressure exceeds 2100 bar. The temperature must be above 0° C and must drop blow 0° C within 100 ms. In a next step the temperatures of -50° C and -100° C must be reached. When -100° C have been reached, the pressure may again drop below the 2100 bar mark. Only after this has been accomplished, may the temperature again exceed -100° C. Deviations from this pattern, caused by a mix-up of measuring points or due to the fact that the maximum permissible time periods are exceeded, result in error messages.

Technical data:

connection voltage		24 V=
power consumption during lamp test		250 mA
accuracy of time basis		quartz-stable
sampling rate		34 µs
measuring accuracy:	pressure	1 %
	temperature	+/- 1° C
operating temperature range		0 – 70° C

Three processes are implemented in the software, namely:

- **SCI Clock**
  The comparator requires a clock signal. This signal is generated with the SCI/Timer 2 of the microprocessor. Whenever eight clock cycles have elapsed, a new cycle is started in the SCI interrupt service routine.

- **System Time and Display Driver**
  This interrupt service routine is started cyclically with the Timer Interrupt. In this routine, the system time is recorded and the display is set to the current value.

- **Main**
  The main programme is started after a system reset. It initialises the system and then waits for the start of a measuring cycle. The comparator is programmed during a measuring cycle, and the time periods between the respective pressure and temperature values are measured. Then these values are displayed and the programme waits for a new cycle.

12.1.2. Description of the Interfaces

- **Pressure sensor**
  The type 8219-R by Burster, Gernsbach, is used as pressure sensor in the system. The electrical circuit consists of a balanced half-bridge and must be fed from a constant voltage source.

- **Temperature sensor**
  A type K (Chromel-Alumel) thermo element is used to measure the temperature.

12.1.3. Handling of Errors

Errors may occur during the measuring cycles, if the pressure or temperature pattern does not follow the nominal procedure.

If an error occurs, the pressure time will be displayed and, instead of the cooling time, the error code will appear. The error code is described in detail in chapter 12.3. "Operation and maintenance".

12.2. Operational Start-up

12.2.1. Connecting the System

The unit is delivered with the connection cables for the sensors. They merely have to be connected, if the device hasn't already been built into the system. A mix-up is not possible because of the different plugs. Please note that the shield of the connection cable for the thermo element is grounded at the cold end (see connection plan 85 206.9 in the detailed description).

The power supply is connected to the terminals 11 and 12. The cable which is required for this purpose, is lead through the same strain relief as the measuring cable.

The measuring unit is now ready for operation. After it has been switched on, an automatic lamp test is carried out. All segments of the display light up for 5 seconds. The test is repeated every time the unit is switched on.

Possible errors during operational start-up:

- The lamp test is not carried out immediately after the unit has been switched on. Trouble:

  power supply defective.

- The measuring cycles are not started or end with error messages. Trouble:

  Connection of the pressure and/or temperature sensor is incorrect. It is recommended to check the pressure and temperature pattern with the help of the analog outputs.

12.2.2. Calibrating the System

The measuring unit is pre-set at the factory, but a calibration with connected sensors is necessary during the operational start-up.

The rear cover of the housing has to be removed so that the potentiometers for the calibration of the pressure and temperature measuring circuits are accessible.

When calibrating the pressure measuring circuit or the temperature circuit both the thermo element (test head) and the pressure measuring sensor must be connected to the measuring unit HPC 010, because both measuring circuits mutually influence each other.

- Calibrating the pressure measuring circuit

  Entirely relieve pressure sensor and connect voltammeter to analog output pressure. Alter potentiometer R32 until the voltage lies within +/- 26 mV. The pressure measuring circuit is now calibrated.

- Calibrating the temperature measuring circuit

  In order to compensate for tolerances and deviations of the grounded thermo element from the data in the charts, the actual pattern is adapted to the nominal pattern in two plotted points. These two points are at 0° C and at –195,7° C (boiling point of nitrogen).

  As far as the circuitry is concerned, these two points have a mutual dependence, so that the calibration has to be performed in an iterative process.

  Procedure during calibration:

  1. Hold the thermo element into liquid nitrogen and, using the potentiometer R57, set the voltage to 0.044 V (17 mV – 70 mV).

  2. Cool thermo element down to 0° C (in water-ice mixture) and set the voltage with the potentiometer R52 to 3.927 V (3.90 V – 3.95 V).

  3. Repeat steps 1 and 2 until both levels correct. The temperature measuring circuit is calibrated to an accuracy of +/- 1° C, if the voltage is within the following ranges:

     0° C: 3.90 V – 3.95 V
     -195.7° C: 17 mV – 70 mV

  Care must be taken that there is a stable temperature at the respective measuring points. It is especially important to de-ice the thermo element when changing from liquid nitrogen to the ice-water bath.

12.3. Operation and Maintenance

12.3.1. Summary

During normal operation, the measuring unit HPC 010 for the high pressure freezing machine operates completely automatic as display unit.

A self-check occurs after the unit has been switched on. Immediately afterwards, the unit is ready for operation and waits for the start of a measuring cycle, which is marked by the display of an "A". When the cycle has been completed, the measured times, or possibly any errors which might have occurred, are displayed until a new cycle starts.

12.3.2. Operation

Switching the unit on and off, interrupting the voltage

A lamp test occurs immediately after the unit has been switched on. All segments of the display light up for 5 seconds. As soon as they go out, the unit is ready for operation and waits for the start of a measuring cycle.

The displayed values disappear when the unit is switched off. Any interruption of the voltage has the same effect.

Measuring cycle

The measuring cycle can be explained with fig. 3.2-1.

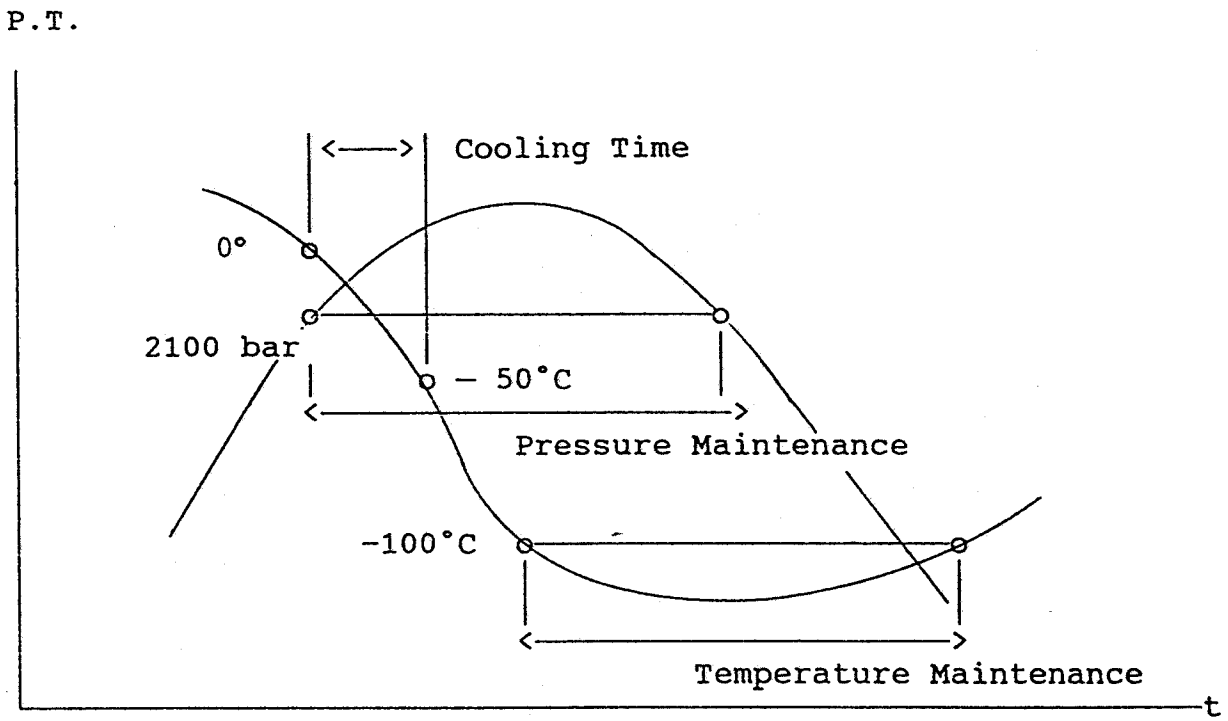


Fig. 3.2-1 Pressure- and temperature pattern of a measuring cycle

A measuring cycle is started when the pressure exceeds 2,100 bar. The displayed values are cancelled and an "A" appears. The measurement of the pressure time is started.

Immediately afterwards, the unit checks whether the temperature is above 0° C. If this is the case, it waits until 0° C has been reached and then the measurement of the cooling time starts. This continues until the temperature is below -50° C.

When the temperature reaches the -100° C mark the measurement of the temperature time is started. If the pressure now falls below the 2,100 bar mark, the measurement of the pressure time is stopped. Finally, when the temperature climbs above the -100° C mark, the temperature time measurement is stopped.

The measuring cycle has now been completed, and the recorded values of pressure maintenance, cooling time and temperature maintenance are displayed. These values are rounded. The displayed values remain visible until a new cycle is started.

The following errors may occur during a measuring cycle:

- The error indication **E0** appears if the temperature has already reached 0° C or less at the start of a cycle.
- The error indication **E4** appears if the temperature climbs above -100° C before the pressure falls below 2,100 bar.
- The error indication **E2** appears if the temperature of -100° C is not reached within the maximum displayable pressure time.
- The error indication **E3** appears if the pressure time exceeds the maximum displayable value.
- The error indication **E5** appears if the temperature time exceeds the maximum displayable value.

If an error occurs during a measuring cycle, only the pressure time and the respective error indication are displayed. If the pressure time exceeds the range that can be represented, the highest possible value is displayed.

A cycle can only be re-started when the pressure falls below 2,100 bar.

- Error indications:
  EO: At the start of a measuring cycle the temperature is at 0° C or below, or the thermo element may be defective.
  E1: The time between the moment when the pressure point of 2,100 bar is exceeded and the temperature of 0° C is reached is > 9.99 seconds.
  E2: The temperature of -100° C has not been reached within the maximum displayable pressure time.
  E3: The pressure has not dropped below the value of 2,100 bar again within the maximum displayable pressure time.
  E4: The temperature has reached or exceeded the value of -100° C, before the pressure has exceeded 2,100 bar.
  E5: The temperature time has exceeded the maximum displayable value.
  E6: The temperature of -100° C has not been reached.

- Analog output pressure

  The analog output of the pressure has the following scale:

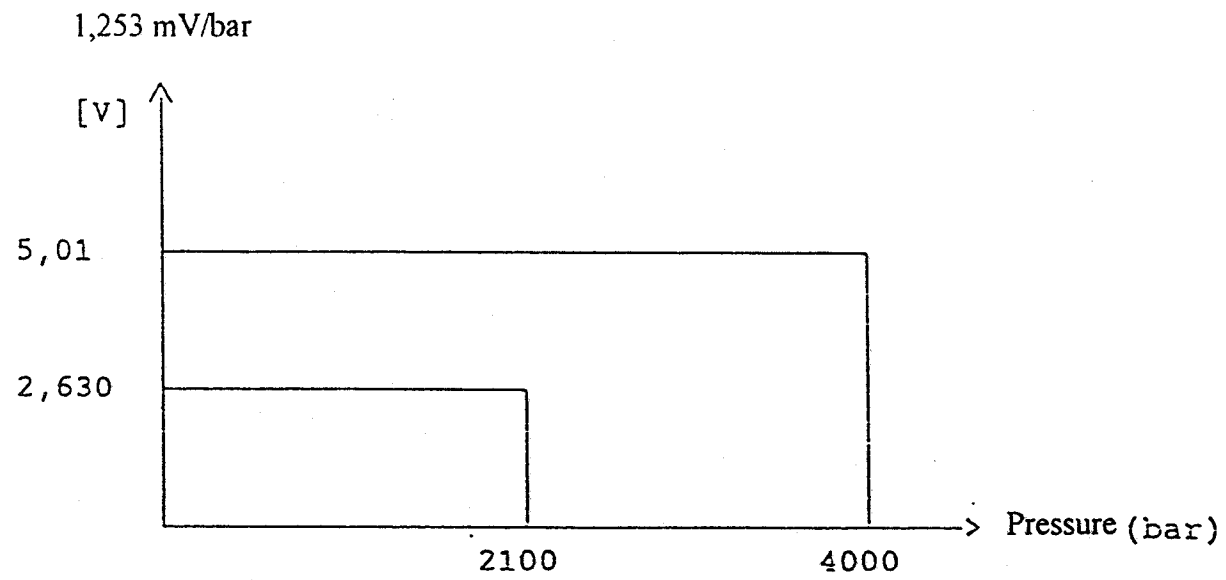


Fig. 3.2-2 Analog output pressure maintenance.

- Analog output temperature

  The analog temperature signal is not linear, except within the range of 0 – 40° C.

  Analog out = 669.936 * e +3.927 V, where e = thermo voltage in Volts.

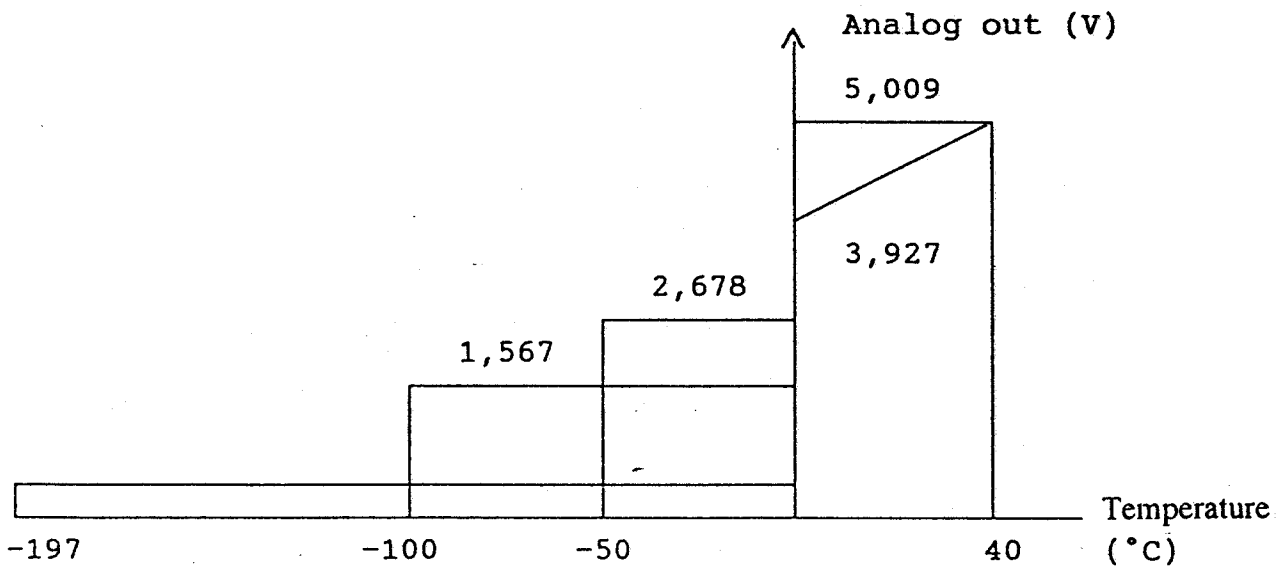


Fig. 3.2-3 Analog output temperature maintenance.

Analog Out Temperature (V)

Temperature (°C)	0	1	2	3	4	5	6	7	8	9
-200	-0.020									
-190	0.088	0.077	0.067	0.056	0.045	0.034	0.024	0.013	0.002	-0.009
-180	0.209	0.197	0.185	0.173	0.161	0.149	0.137	0.124	0.112	0.100
-170	0.340	0.327	0.314	0.301	0.288	0.275	0.261	0.248	0.235	0.222
-160	0.483	0.469	0.454	0.440	0.426	0.412	0.397	0.383	0.369	0.354
-150	0.363	0.621	0.606	0.509	0.575	0.560	0.544	0.529	0.514	0.498
-140	0.799	0.783	0.767	0.750	0.734	0.718	0.701	0.685	0.669	0.653
-130	0.973	0.955	0.938	0.921	0.903	0.886	0.868	0.851	0.834	0.816
-120	1.155	1.137	1.118	1.100	1.082	1.064	1.045	1.027	1.009	0.991
-110	1.346	1.327	1.308	1.289	1.270	1.251	1.231	1.212	1.193	1.174
-100	1.547	1.527	1.507	1.487	1.467	1.447	1.427	1.407	1.386	1.366
-90	1.755	1.734	1.713	1.693	1.672	1.651	1.630	1.609	1.588	1.568
-80	1.971	1.949	1.928	1.906	1.885	1.863	1.841	1.820	1.798	1.777
-70	2.195	2.172	2.150	2.127	2.105	2.083	2.060	2.038	2.016	1.993
-60	2.424	2.401	2.378	2.355	2.332	2.309	2.286	2.263	2.241	2.218
-50	2.661	2.638	2.614	2.590	2.567	2.543	2.519	2.495	2.472	2.448
-40	2.904	2.880	2.856	2.831	2.807	2.783	2.758	2.734	2.710	2.686
-30	3.153	3.128	3.103	3.078	3.053	3.028	3.003	2.979	2.954	2.929
-20	3.406	3.381	3.356	3.330	3.305	3.280	3.254	3.229	3.203	3.178
-10	3.664	3.639	3.613	3.587	3.561	3.535	3.510	3.484	3.458	3.432
-0	3.927	3.901	3.874	3.848	3.882	3.796	3.769	3.743	3.717	3.691
+0	3.927	3.954	3.980	4.007	4.033	4.060	4.087	4.113	4.140	4.166
+10	4.193	4.220	4.247	4.274	4.300	4.327	4.354	4.381	4.408	4.435
+20	4.462	4.489	4.516	4.543	4.570	4.597	4.624	4.652	4.679	4.706
+30	4.733	4.760	4.788	4.815	4.842	4.870	4.897	4.924	4.952	4.979
+40	5.006									

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