AUTOMATIC AMINO ACID ANALYSER

AAA 400

User manual

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1. TECHNICAL CHARACTERISTICS

Amino acid analyser is a special compact liquid chromatograph designed for the analysis of amino acids on a ion exchanger column with a post-column derivatisation by means of ninhydrin and for the determination of biogenic amines.

1.1 Parameters

Sensitivity	50 pmol
Reproducibility at 10 nmol	better than 1.5 %
Glass column, dimension	0.37 x 45 cm
Heating within the range	35 to 95 ^{0}C
Filling for the id. of hydrolysates	Ostion Lg ANB
Filling for the id. of free AA	Ostion Lg FA
Pumping system	Derived from LCP5020
Flow rate	0.01 to 10 ml/min
Pressure	0 to 20 MPa
Automatic dispenser with the cooling of samples	
No. of samples with the cassette A	25 x 1.5 ml
No. of samples with the cassette B	40 x 0.5 ml
Two-channel detector	440 and 570 nm
Cell volume	5 μl
Reactor	50 to 150 ^{0}C
Processing and print of results	PC with program `Chromulan`
Communication with PC	RS485
Power supply	230 V, $\pm$10%, 50 Hz
Power input	230 V x 1.6 A
Category of over-voltage within installations	II.
Dimensions w x h x d	700 x 600 x 550
Weight without bottles	55 kg

1.2 Environment

The apparatus is designed for the environment with a temperature of 15 to 30 ^{0}C; humidity up to 80 % without any acid and caustic vapours. For the reasons of ventilation a free area of 0.15 must be available behind the apparatus.

1.3 Operation

During operation it is necessary that side covers be fitted, column door be closed and waste systems be correctly connected (2.3.1).

1.4 Handling

The apparatus can be carried manually by two persons who grasp it with two hands at the bottom part of side frames.

1.5 Analytic cycle control

Analytic cycle control and processing of results require the connection of a PC equipped with the program `Chromulan`.

The program `Chromulan` contains a help facility designed for the selection and preparation of all operational solutions. Programs for the basic types of analyses, including all settings are delivered together with the program.

1.6 Chemicals

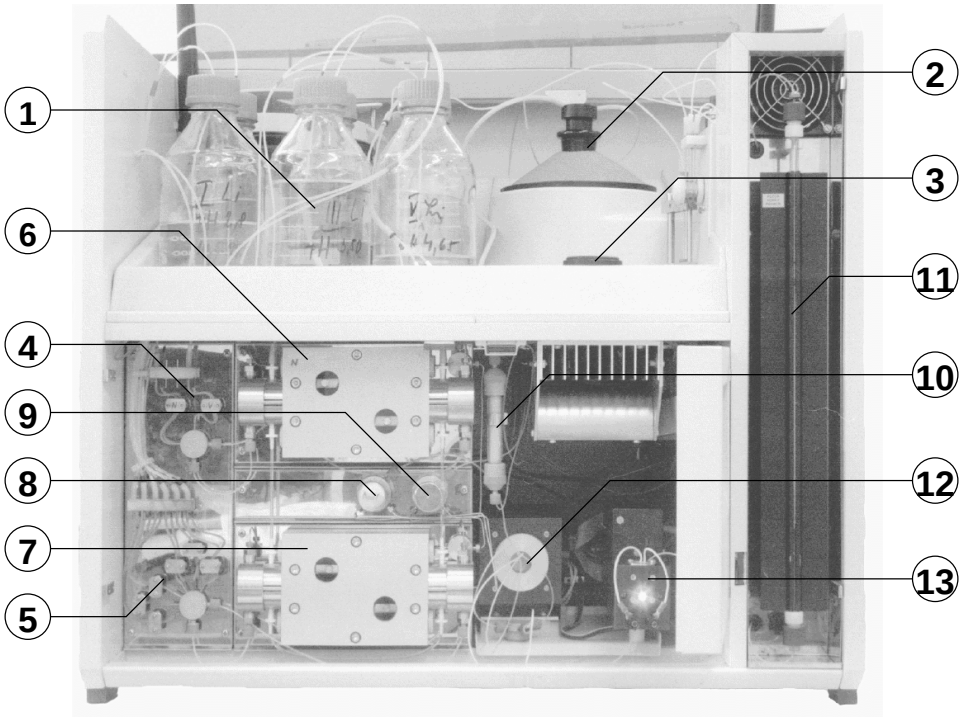
On the basis of an order it is possible to deliver chemicals for the production of operational solutions, but it is not a precondition for the operating of AAA400.

The apparatus is equipped with a chemical part of the operating instructions which is designed for the work with chemicals and compilation of analytical programs and contains the description of all issues relating to the chemical properties, sample preparation and tuning of the analytical cycle.

1.7 Safety

The amino acid and biogenic amines analyser cannot be used in any way other than those provided for in the operating instructions in order not to violate safety regulations.

2. GETTING STARTED WITH THE ANALYSER



1. *Buffers*
2. *Ninhydrin*
3. *Dispenser disk*
4. *Valves for the switching of NHD/ H_2O*
5. *Valves for the switching of the buffers*
6. *Ninhydrin pump 2*
7. *Buffer pump 1*
8. *Air release valve for ninhydrin*
9. *Air release valve for buffers*
10. *Pre-column*
11. *Column*
12. *Reactor*
13. *Detector*

Fig. 1. Layout of individual parts of AAA 400

The area under the upper cover serves for the placement of bottles with buffers, distilled water (H_2O) and cooled bottles with the reagent (ninhydrin, hereinafter referred to as NHD). This area also contains a dispensing system with a stock of cooled samples. The front part of the apparatus contains in its lower left part a panel fitted with electromagnetic valves which choose one of six buffers for Pump 1 (buffer type) situated in the lower part and also switch H_2O and NHD for Pump 2 situated in the upper part.

The air release valves are situated on the panel between the pumps, Pump 1 valve being on the right and Pump 2 valve being on the left.

The air release valves situated behind the panel contain tensometric pressure sensors.

The middle area of the lower part of the apparatus contains a door with a keyboard and a display. The area behind the door contains a reactor with a replaceable cartridge and a detector with a through-flow cell.

The right front part of the apparatus contains, behind the transparent door, a column designed for the separation of amino acids. The column is gripped by a metal bed which heats it. The cooling of the bed with the column is carried out by a fan located in the upper part of the area with the column.

Side panels of the apparatus are easily removable and thereby you can get access to most mechanisms and electronic equipment.

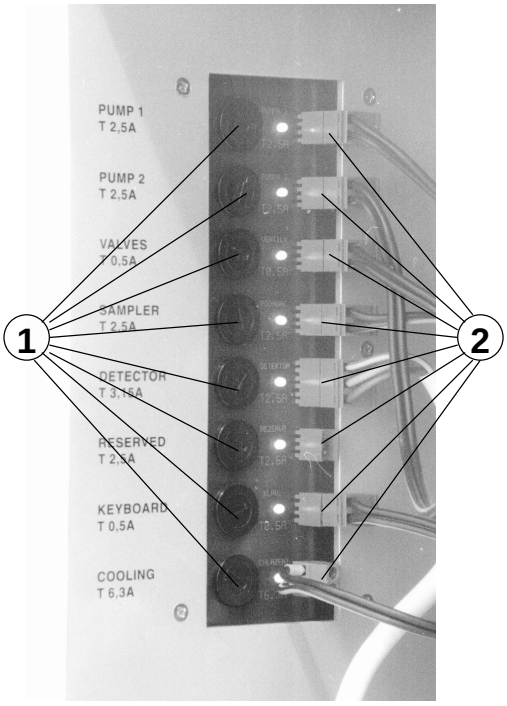
The left side of the apparatus is fitted with the main switch. After removal of the left side panel you will get access to the fuses of the power supply of both the network inlet and for individual modules of the apparatus.

In order to clarify the function, design and layout of the individual units of the apparatus, the entire apparatus is, for the purpose of further description, divided into the following parts:

- Power supply and fuses
- Pumps
- Hydraulic system
- Dispensing system
- Columns
- Detector with reactor.
- Key board

2.1 Power supply and fuses

Power supply is situated in a closed box. All outlet connectors and terminals transfer only safe voltages. The source contains a special fuse for each unit, see fig. 2.



1. Fuses of individual units. The value of the fuse is provided for on the description card next to the fuse.
2. Connectors for the power supply of individual units. All connectors feature a voltage of 24V.

Fig. 2. Power supply - Fuses and outlet connectors

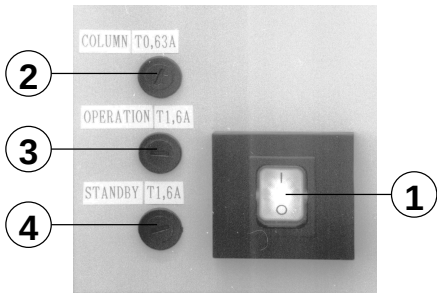
The source contains a transformer for the heating of the column 7 V AC and two switched sources which separate the mains voltage of 230 V AC from the voltage of individual modules of the apparatus through a safety voltage of 24 V DC. In the STANDBY mode only one source designed for the power supply of the cooling system and keyboard is operating.

2.1.1 Mains connection

The mains connection is situated on the rear panel of the apparatus. Also the main fuse (value T2.5A) is situated on the same place.

2.1.2 Secondary mains fuses

After removal of the left panel you will see secondary mains fuses, see fig. 3. T0.63A with the description COLUMN. T1.6A for the mode STANDBY and T1.6A OPERATION for the operation of other units of the analyser.



1. *Power switch*
2. *Fuse of the column heating system*
3. *Fuse of the operation mode*
4. *Fuse of the Standby mode*

Fig. 3. Power supply - Switch and fuses

2.1.3 Front panel of the source

The front panel of the source contains connectors designed for the power supply of the modules of the apparatus, its signalling lamps and fuses. The assignment and values of the fuses are given by the description. PUMP 1 T2.5A, PUMP 2 T2.5A, VALVES T0.5A, SAMPLER T2.5A, DETECTOR T3.15A, RESERVED T2.5A, KEYBOARD T0.5A, COOLING T6.3A.

2.1.4 Fuses in the switched sources

Besides the above specified fuses the apparatus contains inaccessible fuses situated in the switched sources. Each source has one fuse F4A.

2.1.5 Power supply control

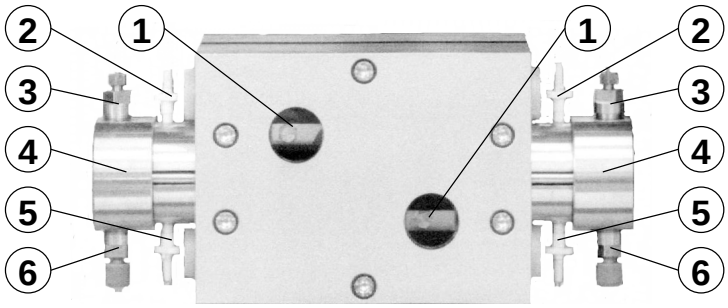
Through the DIN7 connector the apparatus receives a repeated command for operation (OPERATION) and a regulation command of heating of the column for the switching of the 230 V AC transformer whose outlet terminals (7 V, 11 A) are also situated on the front panel of the source.

2.2 Pumps

The pumps are pulse-free and feature an even power output and their use guarantees conformity of the retention times of individual peaks. The software equipment controlling

the units of the pumps during the switching ON of the analyser will set the pressure limit of pump 1 to 8 MPa and pump 2 to 3 MPa. The maximum pressure permitted for the analyser is 20 MPa. The upper pressure limit will be set after the activation of the program `Chromulan` 2 MPa above the level specified in the program.

The pumps feature the flush of the piston behind a high-pressure gland. This makes it possible to pump crystallising substances (buffers) without the glands and pistons being damaged. The flush is ensured by the second section of the peristaltic pump (2.4). PUMP 1 permits the pumping at a maximum flow rate of 10 ml/min. PUMP 2 features a half stroke of the pistons in order to ensure a perfect function during the pumping of NHD, and therefore the real maximum flow rate is 5 ml/min.



1. *Inspection windows where it is possible to see movement of the pistons*
2. *Piston flush inlets*
3. *Output valves*
4. *Heads*
5. *Piston flush outlets*
6. *Input valves*

Fig. 4. Pumping block

2.3 Hydraulic system

Hydraulic system is formed by bottles for 6 buffers, a bottle for H_2O and a cooled bottle with NHD. All liquids are conducted from the bottles to electromagnetic valves situated in the lower left part of the analyser. Hoses leading from individual buffers are conducted to them. These hoses bear colour marking at the outlet of the hoses to the panel above the valves. They are factory-set in an order from the left to the right. The buffer no. 1 hose is situated on the left, the last hose belongs to the buffer no. 6. Then the system continues with NHD and H_2O. H_2O is used also for the flushing of the dispensing valve and dispenser needle by the first section of the peristaltic pump and for the flushing of pump pistons by means of circulation through the second section of the peristaltic pump.

2.3.1 Waste

Waste originating from the rinsing of the dispensing system, condensed water from cooling samples and NHD are conducted to a single outlet situated on the rear part of the apparatus, which is connected to the waste canister.

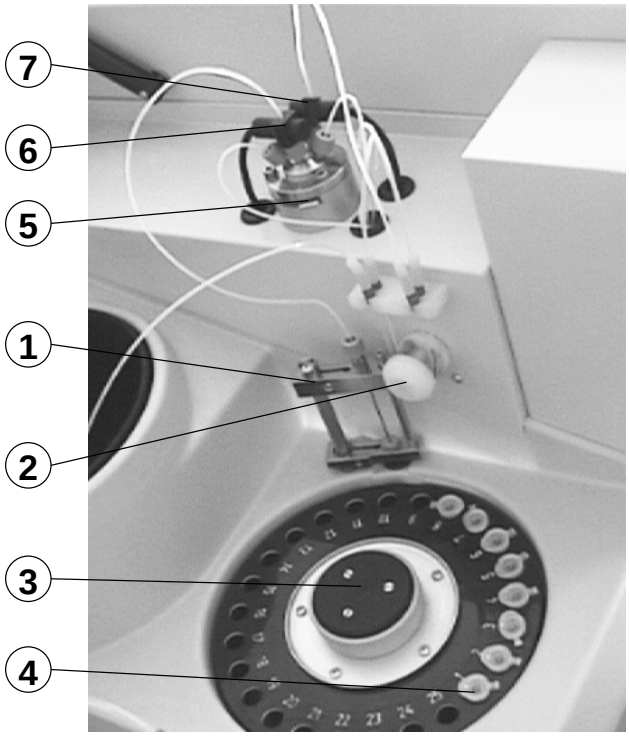
The outlet of the buffers consumed and NHD is conducted out from the cell by means of a capillary tube featuring a clearance of 0.3 mm up to the waste canister.

2.3.2 NHD

The bottle with NHD is situated in the metal pit which is cooled by means of thermoelectric cooling modules.

2.4 Dispensing system

For the dispensing system see fig. 5. The system consists of a removable disk designed for 25 or 40 micro test tubes closed with plugs, an arm with a needle, loop dispensing valve, optical sensors of the through-flow of the liquid through a hose (sensor 1 and sensor 2) and peristaltic pump. The disk fitted with the micro test tubes is stored in a cooled metal ring and thus the stability of samples is ensured for a long time.



1. *Arm with needle*
2. *Peristaltic pump*
3. *Dispensing disk*
4. *Micro test tube with a sample*
5. *Loop dispensing valve*
6. *The first sensor of the liquid*
7. *The second sensor of the liquid*

Fig. 5. Dispenser

The system is controlled by its own microprocessor, which performs the commands of the control computer. The computer issues commands for the preparation of a nominal sample and a command for the start of the analysis.

During preparation the peristaltic pump flushes the dispensing valve, hose and needle with distilled water. By applying the reverse procedure it dries all with air.

The disk brings the micro test tube selected to the arm. The arm rotates and pricks the micro test tube plug through with a needle.

The filling of the dispensing loop is controlled by optical sensors which recognise whether there is a liquid or air in the hose passing through the sensor.

The peristaltic pump draws a sample behind Sensor 1, the needle leaves the micro test tube and by means of the rotating of the arm it returns to its position above the waste.

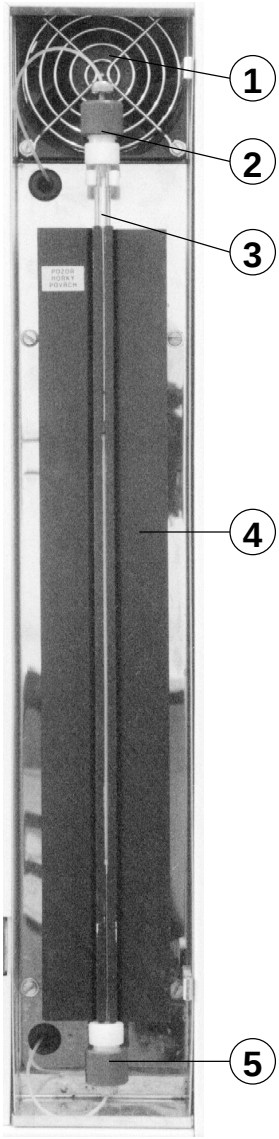
The peristaltic pump finishes pumping the sample through the loop of the dispensing valve to Sensor 2.

At the moment of the start of the analysis the dispensing valve turns from the LOAD position to the INJECT position. After a certain time the valve returns back.

The peristaltic pump will flush the loop of the dispensing valve and the H_2O needle.

2.5 Columns

There are two columns at this type of the apparatus. Pre-column and analytic column. The pre-column is made of Plexiglas and its lower and upper closures are the same.



1. *Cooling fan of the column*
2. *Upper closure of the column*
3. *Column*
4. *Heating of the column*
5. *Lower closure of the column*

Fig. 6. Column

The pre-column can be operated from both directions. The pre-column serves for the absorption of ammonia which gets to the column from acid buffers. This ammonia is washed out by means of alkaline buffers featuring a higher ionic strength and cancels the identification of arginine. For the purpose of accelerated identifications where no arginine

assessment is carried out this pre-column is not necessary. The analytical column is of a glass type with plastic stands serving for the attachment of closures. The upper closure makes it possible to shorten the active length of the column. The pre-column is situated in the holder behind the keyboard. The analytical column is situated in the heating metal bed which ensures a controlled temperature mode for the column.

CAUTION: The door of the column must be closed during the operation.

2.6 Detector with reactor

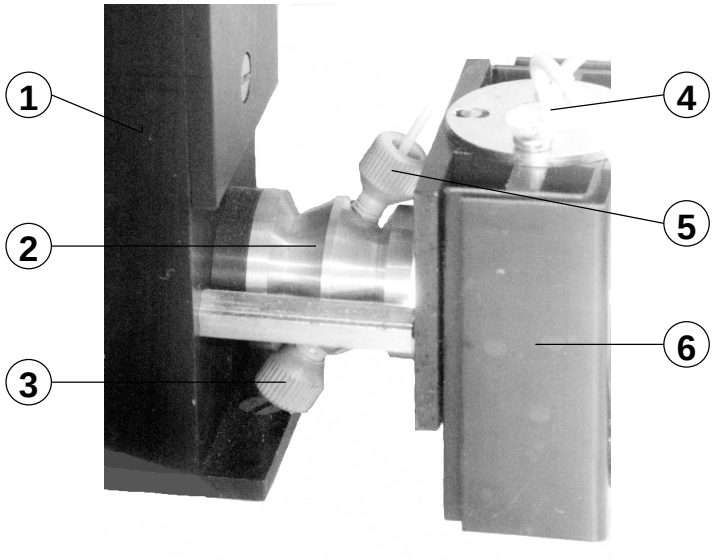
Detector with reactor are, together with the control unit, situated on the removable plate behind the keyboard.

2.6.1 Reactor

The reactor consists of a box fitted with a heating element and removable cartridge with an embedded Teflon pipe. The cartridge is held in the heating body by means of a bayonet closure.

2.6.2 cell

The detector cell is attached between the detector body and halogen lamp cooler, see fig. 7. The light which passed through the cell is, after a reflection from the concave holographic grillage, read by the photocells placed on the positions of 440 nm and 570 nm.



1. *Detector*
2. *Cell*
3. *Liquid line from the reactor to the cell*
4. *Halogen lamp*
5. *Drainage of liquid from the cell to the waste system*
6. *Lamp cooler*

Fig. 7. Cell attachment

The lamp is calibrated in the mop board by the manufacturer in factory and in the case of replacement it does not require, apart from the inserting and screwing into the cooler, any adjustment. Be careful to handle with the pin.

The capillary tube from the column is connected to a T-union attached in a removable way in the plastic bed attached in front of the reactor. Also the capillary tube leading from the pump 2 (NHD, H_2O) is connected to this union.

One end of the capillary tube from the rector cartridge is connected at the outlet of the T-union. The other end of this capillary tube is connected to the detector cell.

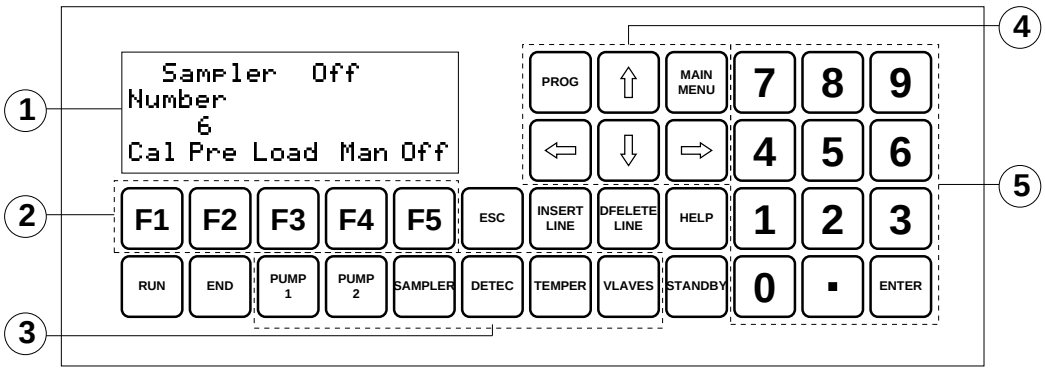
CAUTION: The cell must be attached in such a way that the inlet from the reactor heads obliquely downwards, to the detector body, see fig. 7

2.6.3 Outlet from the cell

The outlet from the cell is realised through a capillary tube, whose length is at least 3 m, of a clearance 0.3 mm, and it is recommended to conduct this capillary tube up to the waste canister.

2.7 Keyboard

The apparatus is fitted with a keyboard and a display. The keyboard serves for the control of all units during debugging, servicing and controlling AAA400. It is also convenient during the flooding of the hydraulic system. From the keyboard it is possible to receive all current information about the modules of the apparatus.



1. *Display*
2. *Function keys*
3. *Keys for switching between displays*
4. *Cursor keys*
5. *Numeric keys for the entering of numeric data*

Fig. 8. Keyboard

Individual modules of the apparatus can be inspected and possibly also controlled from this keyboard. On the activation of the program `Chromulan` all the default values pre-set by the manufacturer or by the operators from the keyboard will be overwritten by the values entered into a particular program by the author of the analytical program.

2.7.1 Switching on of the apparatus

The keyboard provides information concerning the switching ON of the apparatus and the putting aside to a STANDBY mode, which is indicated by the yellow signalling lamp of the key.

If the apparatus is activated from the STANDBY mode by means of pressing this key or through a computer program `Chromulan`, it is possible to get access to individual modules by pressing the keys | PUMP 1 | PUMP 2 | SAMPLER | DETEC | TEMPER | VALVES |

By pressing each of the above mentioned keys the display will provide the information concerning the module selected by the key in question. If the module is active, the corresponding LED on the keyboard is lit. The emergency condition is indicated by the flashing of this LED. A failure in the module can generally be reset by using the command Off | F5 |. It is necessary to remove the cause and to activate the unit in question again.

If you want to select another value, it is necessary to move cursor to the position of the number to be changed (it is done by horizontal arrows of the keyboard). After entering the number you must confirm it by pressing ENTER, should you make a mistake, you can delete the number edited by pressing the key ESC.

The area below the bottom line of the display contains the keys | F1 | F2 | F3 | F4 | F5 |. The meaning of these keys is given by the bottom line of the display activated for the module selected by the key.

2.7.2 Pumps PUMP 1, PUMP 2

With the key | PUMP 1 | (PUMP 2) it is possible to open the display of the pump 1 (2).

```
Pump 1 On
FLOW P-H P-L P MPa
0.30  12 0.0   4.5
=== === Purg On  Off
```

From the display you can see:

On (Purge, Error, Off) - immediate state of the pump.

Flow pre-set flow rate (usually 0.30 and 0.20 ml/min).

P-H emergency pressure limit (factory set as 8 MPa for PUMP 1 and 3 MPa for PUMP 2).

P-L lower pressure limit (usually 0 MPa).

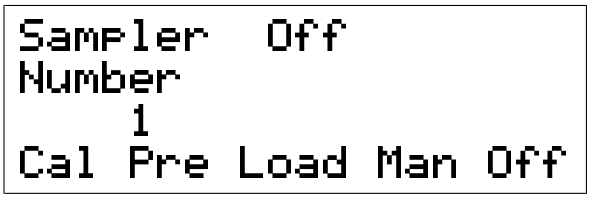
P MPa current pressure.

The meaning of the function keys is as follows::

F3	**Purge**	The pump is pumping at a rate of 8 ml/min and possible exceeding of the pressure limit 1 MPa is checked.
F4	**On**	Starts pumping at a flow rate given under Flow.
F5	**Off**	Stops pumping or possibly resets after an error indicated by the flashing of the corresponding LED.

2.7.3 Dispensing system SAMPLER

Through the key SAMPLER it is possible to open the display of the dispensing system.



From the display it is possible to see:

bf Off (On, Error, Prepare) - immediate state of the dispenser.

Number The number of the sample which has been dispensed most recently.

The meaning of the function keys is as follows:

F1 CAL Performs the flooding of the dispensing valve, needle and calibration of sensors of the liquid in the hoses of the dispensing system. (This function is performed automatically at a transfer from the STANDBY mode and during the activation by the program `Chromulan`.

F2 PRE Takes a sample from the test tube selected, (XX + ENTER). Preparation for dispensing.

F3 LOAD Dispenses a sample into the system, the sample must be prepared by using the key PRE (F2), or, if the function PRE has not been used in advance, it will perform both preparation and dispensing.

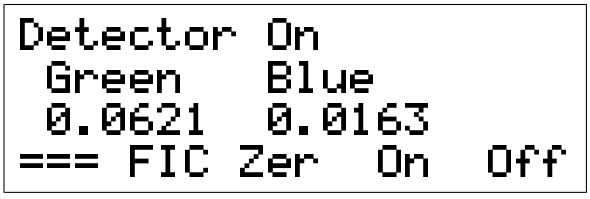
F4 MAN Turns the dispensing valve. The valve must be returned into its position by repeating MAN (F4).

F5 OFF Mainly for resetting after an error. (The LED is flashing).

Note: The LED SAMPLER is lit during the time of preparation of the dispensing and flushing up to the complete end of the dispensing sequence.

2.7.4 Detector DETEC

The display is listing current absorbance values for the green channel (Green 570 nm) and blue (Blue 440 nm).



From the display you can see:

On (Off, Error) - immediate state of the detector.

Green Absorbance for the green channel.

Blue Absorbance for the blue channel.

The meaning of the function keys is as follows:

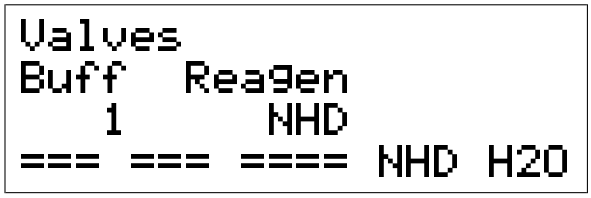
F4 On Turns the lamp ON.

F5 Off Turns the lamp OFF, resets an error.

F2 FIC Performs the basic calibration of the electronic installations of the detector. It is carried out automatically every time when turned on.

F3 Zer Resets both channels.

2.7.5 Electromagnetic valves VALVES



From the display it is possible to see:

Buff Number of the buffer valve which is open. Zero means that all valves are closed.

Reagen NHD/H2O identification of the valve which is open.

The meaning of the function keys is as follows:

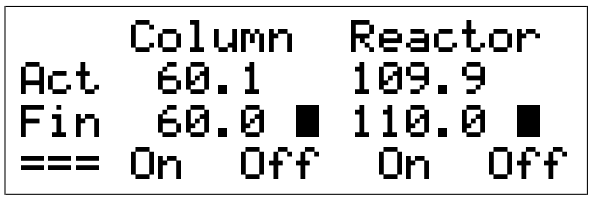
F4 NHD Opens the valve for ninhydrin.

F5 H2O Opens the valve for water.

The number of the buffer can be selected, the selection shall be confirmed by the key ENTER. By selecting the non-connected valve 7 or 8 the display shows 0 and all buffer valves are closed.

2.7.6 Thermostat of the column and reactor TEMPER

Through the key TEMPER you will open the display with information on the temperatures of the column and reactor.



From the display you can see:

Act Current temperatures of the column and reactor.

Fin Required temperatures of the column and reactor.

■ This sign behind the temperature required means that the heating is switched on.

The meaning of the function keys is as follows:

F2 On Switches the heating of the column ON.

F3 Off Switches the heating of the column OFF.

F4 On Switches the heating of the reactor ON.

F5 Off Switches the heating of the reactor OFF.

In the case that at least one regulation is ON, the LED of the key TEMPER is lit.

3. PUTTING INTO OPERATION

It is presupposed that the operators have been made familiar with analytical issues and with the use of the program `Chromulan`.

The apparatus can only work without any surveillance if it is controlled by a computer equipped with the program `Chromulan`. This program perfectly protects the column from pressure overloads, it is able to detect any leakage of liquid on the basis of pressure loss. Thus it ensures safety of operation of the electric equipment containing thermostats, pumps and chemical solutions. All operational parameters must be present within the ranges specified by the program. In the opposite case the apparatus is put into the STANDBY mode.

3.1 Apparatus preparation

3.1.1 Electric connection

The apparatus is connected to the power supply with a mains cord, and with the communication cable it is connected to a computer fitted with the communication card RS485.

3.1.2 Waste

The waste systems are connected from the cell and dispensing system, see Chapter 2.3.1 page 9 .

3.1.3 Chemicals

The bottles contain a sufficient stock of buffers, distilled water and NHD. A hose with protective atmosphere is connected to the NHD bottle.

3.2 Turning the apparatus ON

Important notice. If the apparatus has just been put into the STANDBY mode, the yellow LED must be lit before the repeated turning ON for at least 10 sec. The apparatus cannot be turned ON earlier from the computer or the key STANDBY.

3.2.1 Switch

The ready apparatus will be turned ON with the switch on the left side of the apparatus. The button of the switch and the LED STANDBY will be lit. In this state only the cooling of NHD and samples is turned ON. It is recommended that this switch should not be turned OFF at all for the time while there is some NHD in the reservoir.

3.2.2 Actual turning ON

The actual turning ON can conveniently be performed by the controlling computer. The computer is fitted with a monitor, and if you want to print results during the work of the analyser, it is also fitted with a printer connected in advance with the cable CENTRONIC to the computer. (The power supply of the printer need not be turned ON).

An advantage of the activation of the analyser by using the program `Chromulan` is the control of the pump 2 depending on the pump 1. Pump 1 only starts pumping after the heating of the column to a temperature pre-set by the program, and pump 2 must always feature a pressure smaller than the pressure of pump 1. Another precondition for the activation of the pump 2 is the reactor temperature higher than 70 ^{o}C. If the apparatus is not ready within 30 minutes (for example the pumps are not flooded), the program starts indicating an emergency condition. The operators can take over, for a certain period of time, the responsibility for the values situated out of the range limits by entering the command F9 and for the emergencies by confirming during the message PUT ASIDE - EMERGENCY Y/N by entering N. During the time of the apparatus preparation the reporting of the conditions out of range and of emergencies is limited to a minimum, and it is also suitable to monitor the running-in of the apparatus. It is recommended to be in an area where you can hear alarms for the time of identification of the first sample.

Also the apparatus which is not controlled by computer can be activated by pressing STANDBY. The values are set in the controlling units of the modules in such a way that the apparatus shall not be damaged.

3.3 Turning the apparatus OFF

The apparatus will be turned OFF by the computer after the processing of all samples. It will be followed by the flushing of the reactor by stopping the pump 2 and after a certain time it will start turning individual modules of the apparatus OFF and at the end the apparatus will pass to the STANDBY mode. In this mode also electromagnetic valves are turned OFF and the column is not endangered by NHD.

3.3.1 Turning OFF due to an error

If the computer stops communicating with the apparatus for the time of one minute, the apparatus starts preparing itself for the passage to the STANDBY mode. It will switch electromagnetic valves of the pump 2 to H_2O and starts to turn OFF individual modules of the apparatus on a sequential basis.

3.3.2 Turning OFF by operators

Termination of operation may be decided about by the operators pressing the key STANDBY and thus the apparatus will immediately be put aside.

3.4 Inserting of samples into the dispensing disk

It is carried out by removing the disk for the micro test tube. First slide the ring on the centre of the disk upwards and while pulling this ring, remove the disk from its installation.

Remove old micro test tubes and insert the new ones.

Put the disk on the carrier in such a way that it can latch as low as possible with its pivots into the five holes of the carrier. Slide the ring with the groove evenly downwards and thereby attach the disk into the carrier.

3.5 Flooding of the liquid inlets towards the pumps

During the first start-up or after the connection of hydraulic paths it is necessary to monitor the tightness of all hydraulic joints and parts. Most leakage will be indicated by liquid drops collected in the tray under the pumps. Furthermore it is necessary to monitor the connection of the cell, reactor, dispensing valve and upper closure of the column. All leakage on the column will be revealed on its lower end and on the hose from the bottom closure of the column.

3.5.1 Bleeding

Bleeding is carried out by opening the corresponding air release valve and increasing the pumping rate subsequently, exclusively through the command Purge F3. After the entering of this command the pump starts pumping much faster (8 ml/min). An advantage of the function Purge is that it controls the value of the pressure, and if the air release valve is not open it will stop the pump at a pressure of 1 MPa and in the upper line of the display it will write Error. Each Error must be reset by the command OFF F5.

3.5.2 First flooding

If the pump has not been flooded yet , it is necessary to proceed as follows:

Turn the apparatus ON, see 2.7.1 page 13

Open the air release valve of the pump 1.

By using the keys VALVES | 1 | ENTER open the valve of the buffer 1.

By using the keys PUMP 1 | F3 start the pumping of the pump 1 at a higher rate (function purge). If the pump fails to draw the buffer by itself, it is suitable to flood the pump with the help of the connection of a syringe to the waste pipe of the air release valve by using a thick-wall hose and syringe suction.

Other buffers will be drawn by the pump probably without a help of this kind. It is sufficient to switch between individual buffers 2, 3, 4, 5 and 6 at the VALVES mode.

The flooding will be terminated by the command OFF or ON. After the closing of the air release valve it is possible to check the oscillation of pressure on the display or computer and to find out the quality of the bleeding.

The same procedure will be applied to the bleeding of the second pump. PUMP 2, VALVES, NHD, H_2O.

3.5.3 Irregular pumping

If the pump pumps irregularly, it is possible to take over the keyboard control and carry out the bleeding.

At the time of preparation it is possible to check also all buffers. Open the display by using the key VALVES and then it will be possible to switch between individual buffers by using the numbers 1 to 6 + the key ENTER, or by using the keys F4 and F5 it is possible to transfer H_2O or NHD into the pump 2.

3.6 Starting of analysis

The actual starting of the analysis of the samples prepared will be carried out by means of computer. It will start with the running- in part of the analytical cycle and

then it will be followed by the analysis of the first sample. More information can be found in user manual for `Chromulan`

4. ROUTINE MAINTENANCE AND SERVICING

4.1 Regular maintenance

Perform the filling of operational solutions and check all joints for tightness in regular intervals. The preparation of buffers and ninhydrin is carried out according to the instructions which are available in the program `Chromulan` and in the independent part of the instructions focused on chemistry. Since the solutions are chemical substances of various categories, it is necessary to observe safety regulations.

4.1.1 Safety regulations for the work with Na buffers

These regulations concern the compounds of citric acid and sodium chloride, which are the ingredients commonly contained in foodstuffs and therefore it is not necessary to observe any special safety regulations.

4.1.2 Safety regulations for the work with Li buffers

These buffers are similar to Na buffers but they are more aggressive against metals. It is not necessary to observe any special safety regulations for these buffers either.

4.1.3 Safety regulations for the work with NHD

Use always rubber gloves while working on the preparation of the agent. If your skin gets into contact with this substance, wash the contaminated place with water and pour sodium pyrosulphite on it. If you find blue stains on the skin, pour sodium pyrosulphite on them as well and wash them with lukewarm water. In the case of consumption try to induce vomiting and immediately call for medical aid.

4.1.4 Bottling of operation liquids

The pouring of operation liquids into bottles shall be carried out from the apparatus area, and only properly dried bottles can be put into the apparatus. If you spill the solution onto the apparatus, wipe it as soon as possible with cellulose wadding.

4.1.5 Check for tightness

During the start-up, during the time of the first analysis and if you intend to leave the apparatus in operation for a longer time (night, weekend) it is necessary to carry out visual checks for tightness of all joints and parts through which the liquid flows. Check the tray situated in the front part of the apparatus, connection of the reactor and detector cell, dispensing valve and the column. Any leakage of the column will be revealed on its lower end.

4.1.6 Check of waste

Perform daily checks of the waste canister and pour its content to the sewerage system in time.

4.2 Parts which are subject to wear

This subsection concerns the parts which are subject to wear depending on the load and/or time.

4.2.1 Electromagnetic valve hoses

If a hose is worn due to a permanent compression, it is possible to restore it for a shorter time by sliding it gently within the spring collet of the valve. It is appropriate to replace hoses of all valves after one year of operation. Put the obliquely cut hose with its oblique side through the collet of the electrically opened valve, see 1.75, pull it out with tweezers and cut it evenly on both ends for the length required. Slide it on the corresponding metal couplings.

4.2.2 Peristaltic pump hoses

These hoses are loaded by pumping and still more by tensioning across the pump head assembly, also when the pump does not work. The spare part kit contains also complete hoses. It is possible to prepare other hoses by using the basic material and their design. The length of a silicone hose (clearance 1mm, wall thickness 0.5mm) is 80 mm. Practical lifetime is about 2 months. We recommend you to replace both hoses at the same time. Thus it is possible to ensure reliable flushing of pistons of the main pumps and thus also a good lifetime of the most important parts of the analyser.

4.2.3 detector lamp

It is a halogen lamp attached in the metal holder. It is attached by means of two screws M3 and connected with terminals to the power supply system. The voltage on the terminals is 5 V DC. The lifetime is approximately 2000 hours and generally the lamp works without any problems for its entire lifetime. After the replacement no adjustment is made. The fact that the lamp is lit can be seen when looking behind the keyboard of the apparatus.

4.3 Error message printout

If the analyser is switched OFF by the program `Chromulan` for other reasons than the processing of all samples, the information panel of the program called up by **F9**will remain in the condition for which the apparatus has been put aside. It is possible to read temperatures, pressures and to find the error message. On the sequence screen **F4**the line which was not performed flashes red. If you move cursor to this line and press **ENTER**the corresponding error message will be displayed. The numbers displayed can be conveniently recorded for the analysis of the failure and for possible agreement with the service station.

4.3.1 General information about the state of the dispenser

0 Dispenser is in the basic position
1 Sample is ready in the loop
2 Dispenser is operating
3 It is not possible to set the sensitivity of the sensors
4 Error of sensor 1 or no sample
5 Error of sensor 2 or a bubble

6 Error in the dispensing sequence
7 Error in the arm motor
8 Error in the peristaltic system motor
9 Error in some of the DC motors
10 Error in the positioning of the disk

4.3.2 Status information from the dispenser

State	Error	Description
0	-	Standby mode
Preparation of dispensing		
1	-767	close the valve, check the arm
2	-766	wait for the pulling out of the arm, then cleaning
3	-765	wait for water in both sensors, then volume flushing
4	-764	flushing with a certain volume, then air separation
5	-763	wait for air in sensors, then fixed volume of air
6	-762	drawing of a fixed volume of air
7	-761	reserve
Beginning of the actual dispensing sequence		
8	-760	waiting for the disk, then start of the arm downwards
9	-759	waiting for the arm and beginning of suction
10	-631	suction into sensor 1, then suction of a certain volume
11	-757	completion of the volume suction, then the arm upwards
12	-756	travel of the arm upwards, then suction into sensor 2
13	-630	suction into sensor 2, then time synchronisation
14	-754	suction of a sample, slightly behind sensor 2
Waiting for the command inject		
15	-639	time synchronisation, then dispenser to INJECT
16	-752	completion of the dispenser movement to INJECT
17	-751	waiting for a certain time, then dispenser to LOAD
18	-750	dispenser to LOAD, then flushing of the loop
19	-749	flushing of the loop, then end or calibration
20	-748	reserve
Calibration of sensors		
21	-747	beginning of calibration - reserve
22	-746	beginning of the flushing of the peristaltic system
23	-745	peristaltic system is flushing, then preparation of the calibration of sensors
24	-744	calibration of sensors for water, then drying
25	-743	drying
26	-742	drying by peristaltic system, then air calibration
27	-632	air calibration, then filling with water
28	-740	completion of the filling with water

4.4 Waste disposal

When the instruments operating life is over dispose it in respect to valid regulations, also it can be returned to the vendor or producer for liquidation.

Warning: Instrument contains parts (PCB's) which are rated as hazardous waste.

5. ACCESSORIES AND SPARE PARTS

5.1 Basic accessories

90000026	Ion exchanger for column LG FA (free a.a., biogenic amines)*	20ml
90000027	Ion exchanger for column LG ANB (hydrolyzát)*	20ml
90000025	Ion exchanger for precolumn LG 0804	20ml
AA 4500	Empty column with caps	2 pcs
AA 4529	Spacer tube 60 mm	4 pcs
AA 4528	Spacer tube 30 mm	2 pcs
AA 4621	Column filler cap, capillary, threaded joint and sealing	1 pc
90000028	Syringe with tubing for column filing	1 pc
AA 4611	Precolumn tube	1 pc
AA 4631	Precolumn filler cap with sealing	1 pc
AA 1781	UNF10 through bolt, PEEK	10 pcs
AA 1782	UNF10 flow-cell through bolt, PEEK	2 pcs
AA 1783	UNF10 through bolt, stainless steel	3 pcs
AA 1784	UNF10 long through bolt, stainless steel	1 pc
AA 1785	Ferrule, stainless steel	4 pcs
AA 1787	PEEK coupler	1 pc
5KAP0006	PTFE capillary O.D. 1.6mm, I.D. 0.3mm	6 m
5KAP0007	PTFE capillary O.D. 1.6mm, I.D. 0.8mm	1 m
5HAD0004	Silicon tubing O.D. 5mm	0.5 m
5HAD0002	Silicon tubing O.D. 3mm	1 m
5HAD0003	Polyamide waste tubing	1 m
AA 2520	Peristaltic pump loop	6 pc
AA 2170	Mounting device for peristaltic pump loop	1 pc
AA 2140	Sampler needle	1 pc
AA 4250	Tungsten lamp	1 pc
5SKL0032	Bottle set	10 pcs
AA 5203	Eppendorf vial 1.5 ml	200 pcs
3VOD0003	Feed cable	1 pc
	Comunication cabel	1 pc
	Fuse T 1 A	2 pcs
	Fuse T 1.6 A	2 pcs
	Fuse T 2.5 A	2 pcs
	Fuse T 3.15 A	2 pcs
	Fuse T 6.3 A	2 pcs
90000034	Spanner number 7mm	1 pc
	Spare vial 20ml	1 pc

* Depends on specified usage

6. INDEX

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7. TABLE OF CONTENTS

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7.1 List of picturtes and tables