

# Amino Acid Analyzer Model A200 User Manual

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## 1. Welcome

Congratulations on acquiring the **Knauer-aminoSys Amino Acid Analyzer**. This manual will guide you through the most important topics of handling the instrument and its software.

## 2. Guarantee Statement

Dr. Ing. Herbert KNAUER GmbH hereby guarantees the instrument shipped for a period of 12 months, beginning from the date of shipment from Berlin. The instrument supplied has been tested prior to shipment to ensure that it meets its published specifications. Operation inconsistent with this User's Manual or damage caused by unauthorized service personnel renders this guarantee null and void. Damage caused by wear-and-tear and to consumable parts such as fuses and seals is not covered by the guarantee. The general warranty conditions are not valid if the defect is due to accident, unauthorized modification, unauthorized repair attempt, incorrect operation or transport damage.

KNAUER instruments are properly packaged so that safe delivery can be assured. In any case, the instrument should be unpacked and checked for visible transport damage and complete delivery immediately after receipt. In the unlikely event of any components being either damaged or incomplete, please inform us at the address given below within three working days of delivery. You are also strongly advised to contact the delivery and/or logistics firm who delivered the instrument.

If, after carrying out the necessary control procedures, we find a fault or defect covered by the guarantee, replacement or repair will be carried out free of charge, at our discretion. Packing and transport costs are borne by the purchaser.

## Declaration of Conformity

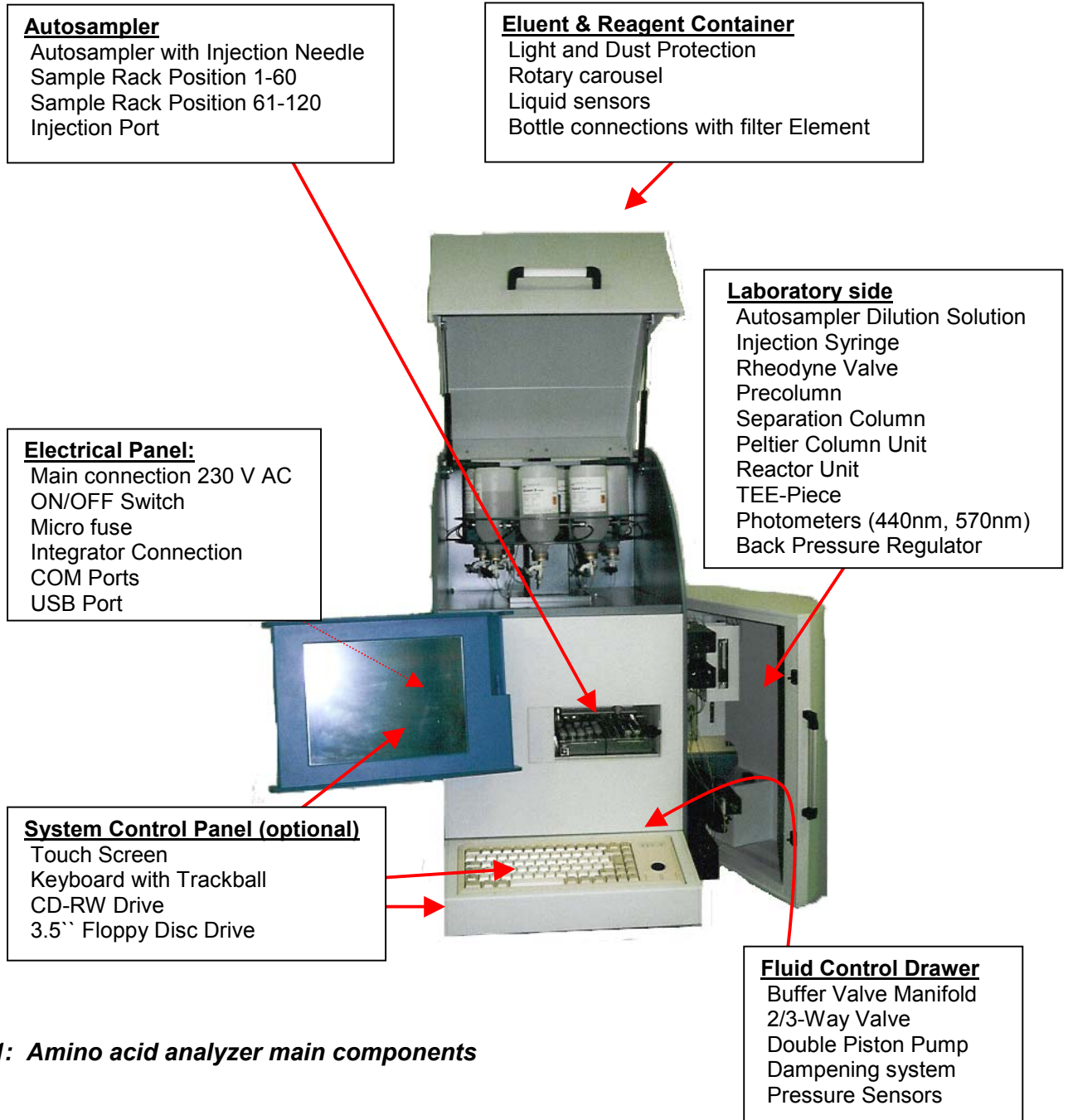
KNAUER also verifies that the instrument was tested in a typical configuration and has passed the necessary requirements (EN 98/694/EG, Document No. 398L0064) to bear the CE shield.

## Service Department and Hotline

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### 3. Graphical Overview of Components

The Amino Acid Analyzer has 6 main components :



**Fig.1: Amino acid analyzer main components**

## 4. Description of Components

### 4.01 Eluent & Reagent Container

The Eluent Rack contains a carousel with 8 bottles, each 500 mL buffer. The buffer types are:

Name	Function	Abbreviation
Eluent A	Elution of Amino Acids	A
Eluent B	Elution of Amino Acids	B
Eluent C	Elution of Amino Acids	C
Eluent D	Elution of Amino Acids	D
Eluent E	Elution of Amino Acids	E
Eluent F	Column-Regeneration	F
Reactor Wash Solution	Rinsing Solution	W
Reagent for Derivatization	Ninhydrin-Dye-Solution	R

The carousel cover protects the buffers against light and dust contamination.

Each bottle is controlled through an electro-optical system. The control software aminoControl™ warns the user if buffer volume gets low.

The filter-spikes prevent contamination of the eluents.

Exchanging buffers is very easy: Carefully remove spike from the bottle and remove the empty bottle. Insert the spike into the rubber grafting of a new bottle and install it into the rack.

Recommended is the ventilation of the pump after changing eluents or – in general – every week. This makes sure that air bubbles within the fluid system will not disturb analysis and measurement.

### 4.02 Autosampler

Two sample racks are provided for 2 x 60 sample vials (1.5 mL standard). The sample racks are cooled with Peltier elements at a constant temperature of about 10 °C.

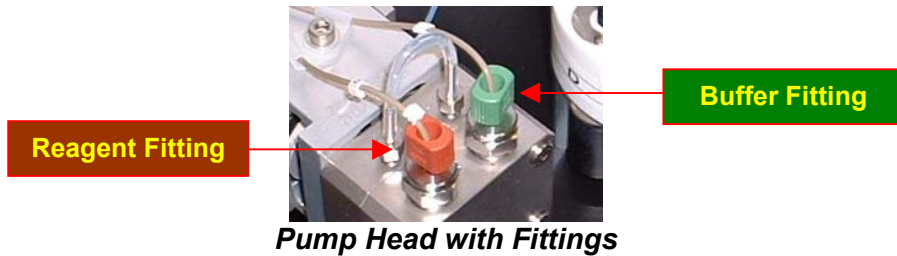
Sample extraction & injection will be done through a syringe pump in free programmable 1 µL steps in a range from 1 to 50 µL out of a defined vial position. The whole sample volume is transferred via the injection port and the Rheodyne valve onto the separation column using a motor injection valve. An automatic rinsing step ensures prevention of cross-contamination. This dedicated xyz-drive autosampler injects up to 120 samples with a variable injection volume between 1 and 50 µL. Its high reproducibility and its full system integration ensure very good performance.

### 4.03 Fluid Control Drawer

This component contains the following elements: buffer valve manifold, 2/3 way valve, double piston pump, valve block and dampening.

The tubes going down from the Eluent Rack enter the Buffer Valve Manifold which controls the flow of Eluents A to F. The flow of 'Reagent for Derivatization' and 'Reactor Wash Solution' is controlled via the 2/3-Way Valve. Both valve output tubes (P = buffer-side, and R = reagent side; always look at the little white tube makers) enter the pump head and are separately promoted to the dampening unit.

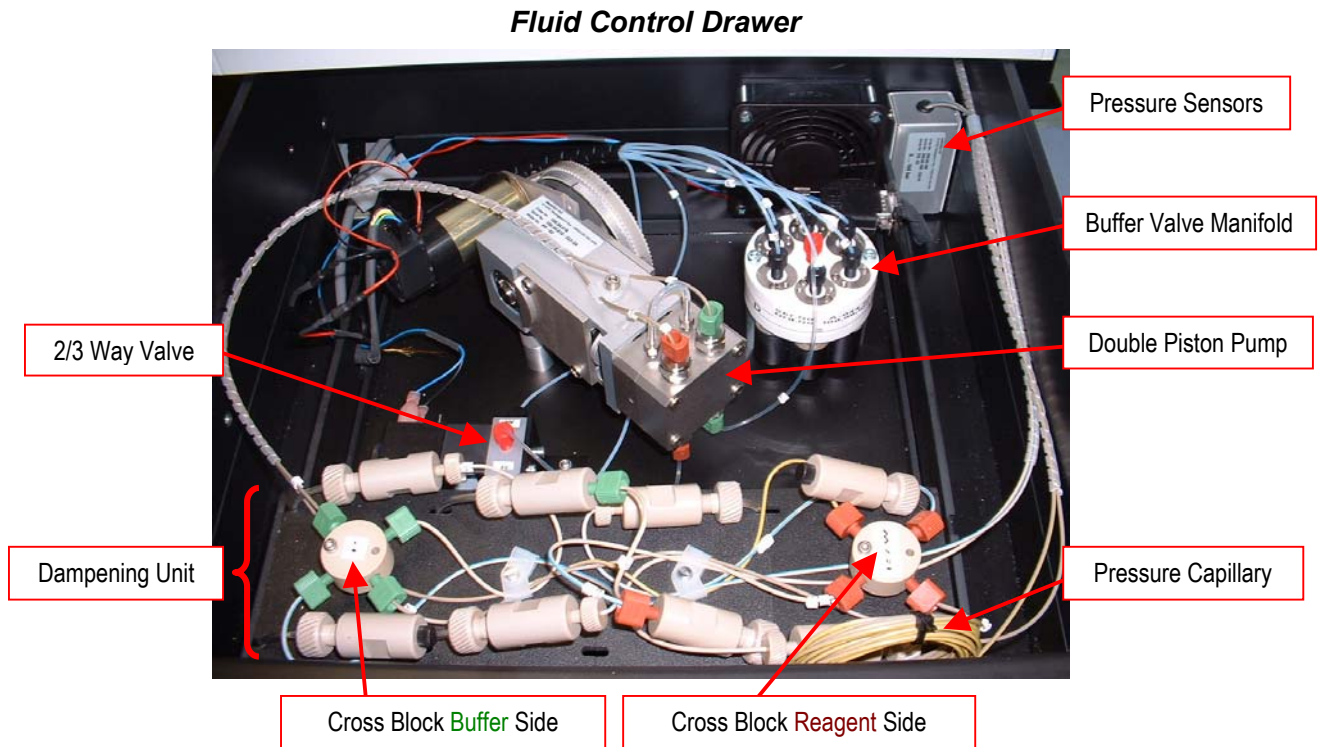
The tubes carrying the eluents have **green** fittings and the tubes carrying reagent or reactor washing solution are connected with **brown** fittings. This picture demonstrates the assignment of colors (at the pump head):



The dampening neutralizes the pulsation caused by the pump. This is important to minimize the baseline noise.

The cross block on the dampening unit facilitates dampening and measurement of pressure. From the reagent-side cross block the “pressure capillary” is directed to the outside, where it meets the TEE piece. From the buffer-sided cross block, the capillary is directed to the pre-column.

The following picture illustrates the arrangement of elements in the Fluid Control Drawer:



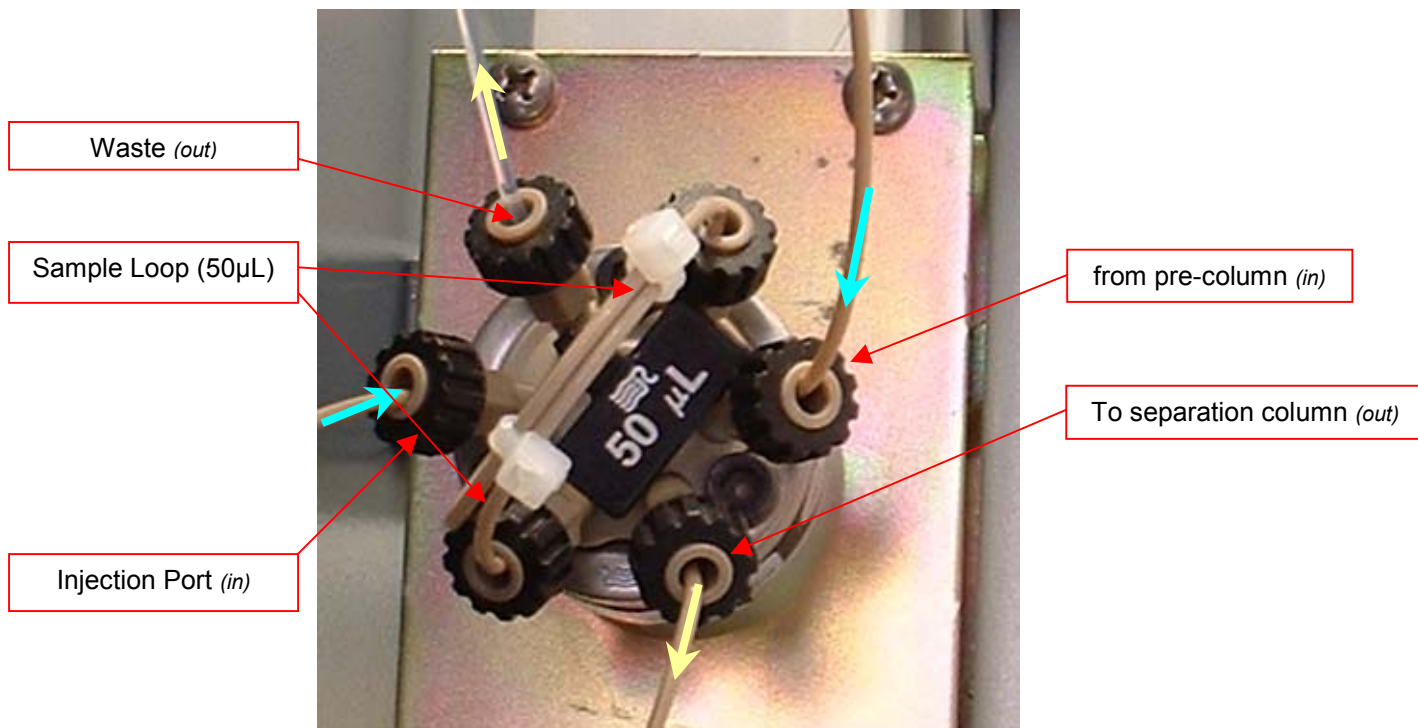
#### 4.04 Laboratory Side

##### (a) Pre-Column

The eluents coming from the dampening unit enter the pre-column. The purpose of this column is to remove crude contaminants and ammonia from the eluent buffers. The outgoing tube enters the Rheodyne valve.

##### (b) Rheodyne Valve

One tube coming from the pre-column is entering at one position of the Rheodyne valve. Furthermore, a tube coming from the autosampler injection unit is also entering the Rheodyne valve. Two tubes leave the Rheodyne valve: one tube delivers waste during loop wash, the other outgoing tube goes directly to the separation column. Finally, the Rheodyne valve has a sample loop with a defined volume of 50 $\mu$ L to store the sample prior to injection.



**The Rheodyne Valve – incoming & outgoing tubes**

Depending on the current process, the Rheodyne valve switches between several states: in case of a loop wash, the sample loop tube is filled with Autosampler Dilution Solution (ASD) and consequently this buffer is discarded, as the outgoing tube is directed to the waste bottle. In case of sample injection, the Rheodyne valve directs the sample loop content, previously filled by the autosampler injection unit, onto the separation column. In case of elution the eluents coming from the pre-column and entering the Rheodyne valve are directed to the separation column.

### **(c) Separation Column**

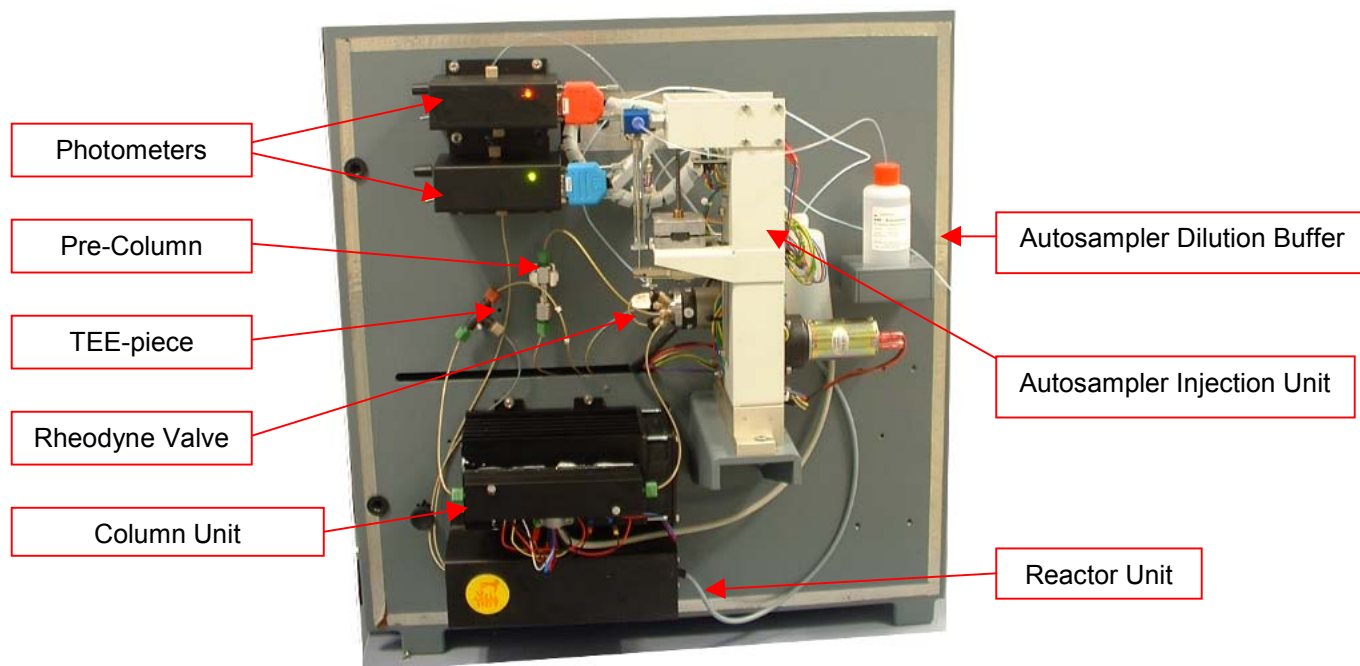
The Separation Column is enclosed in a special column oven for temperature control. This column is the “analytical heart” of the amino acid separation with a special ion exchange resin. The head of the column has a designated batch number, e.g. T37.

### **(d) TEE-piece**

After the separation process within the column has happened, the outgoing buffer (eluent) containing the current eluted amino acids, are directed to the TEE-body. This is the element where the Reagent for Derivatization and the eluted amino acids are mixed together. The outgoing tube of the mixer directs the solution into the reactor unit.

### **(e) Reactor Unit**

The reactor unit is the location where the amino acids react at a constant environment (time and temperature) with the Reagent for Derivatization. The optical density as a result of this reaction is measured by the subsequent photometers and their signals are sent to the internal/external PC for data acquisition.

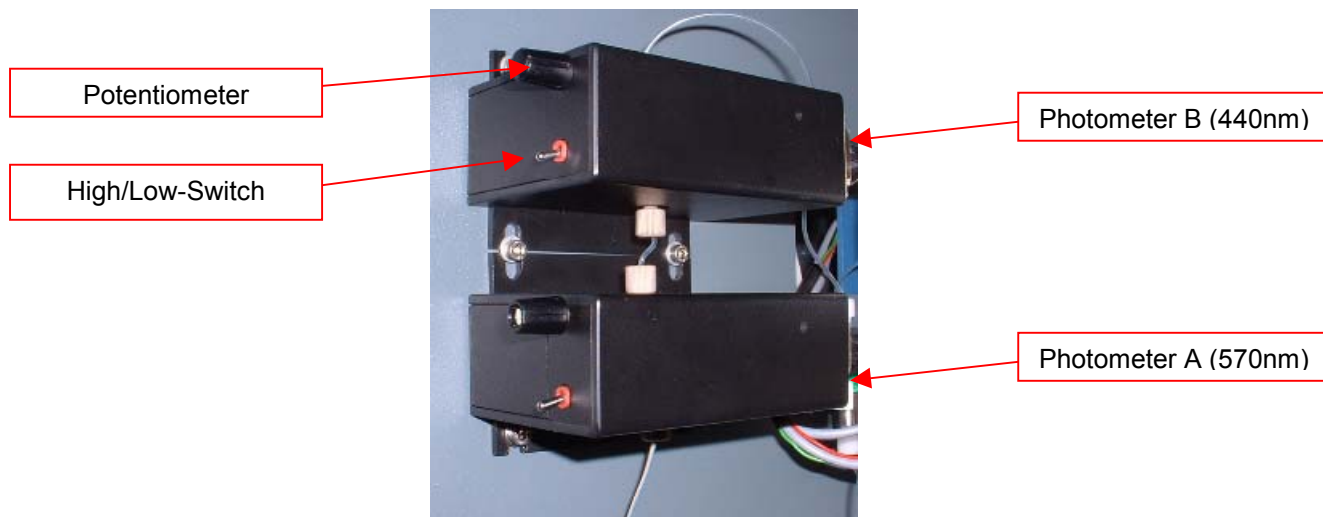


**Side panel of instrument**

**(f) Photometers**

Two photometers are installed to measure the optical density at 570 nm and at 440 nm of the flowthrough coming from the reactor unit. Each photometer has a potentiometer to adjust the height of the baseline. Further, the sensitivity of each photometer can be controlled via the High/Low-Switch.

The outgoing tube of photometer 2 passes a back pressure regulator to hold up the pressure within the system.



**The Photometers**

**5. Chemical Background**

The separation of the amino acids is done via ion exchange chromatography. The most important parameters that influence the elution of the amino acids are the normality of lithium ions, the pH and the column temperature. During the 1950's Stein and Moore developed this smart method of detection: Following chromatographic separation the amino acids react quantitatively with ninhydrin at a temperature between 100 and 130°C. The reaction product is a Schiff-base that becomes decarboxylated. Subsequent hydrolysis results in ninhydrin derivatives carrying an amino group. These derivatives react at their middle carbonyl-c-atom with a second ninhydrin

molecule which results in a blue violet dye (Ruhemann's Purple), absorption maximum at 570nm. Proline and Hydroxy-Proline undergo a slightly different reaction, where the chromophore is detectable at 440 nm. It's important to understand that identification of amino acids takes place not over its derivatives but through retention time during chromatography. The quantification is done using the measurement of Ruhemann's Purple adsorption.

## 6. Installation

After the analyzer has been placed at the desired location, the analyzer must be prepared. Follow these steps. For the control software "aminoControl™" there is a separate instruction to consult, but for the installation process not necessary.

- 1) remove all transportation locks
- 2) prepare the Reagent for Derivatization according to the delivered instructions. Write down the date of activation on the bottle.
- 3) Install all eluents and buffers into the carousel. Its very important to assign the correct bottle to the given locations. A note on the tube helps you to identify the right position, e.g. "A" for eluent A and R for "Reagent for Derivatization"
- 4) Place the autosampler racks 1-60 and 61-120 into the autosampler drawer
- 5) Remove the cap of the Autosampler Dilution bottle and place the bottle behind the Injection Unit. Install the special cap with tube connection.
- 6) Open the bottom drawer and remove both upper pump head fittings.
- 7) Switch on the instrument and log-in to Windows 2000 via "administrator" as username and "a200" as password
- 8) Start "aminoControl" by double clicking the icon, login as User with high priority
- 9) Take the supplied syringe with adapter and screw it onto the buffer side of the pump head.
- 10) In aminoControl enter the "Manual Mode/pressure and fluid control" and choose Eluent A.
- 11) Prime about 2mL of buffer with your syringe to remove air from buffer valve manifold and pump head.
- 12) Choose "Eluent B" in aminoControl and prime 2 mL with the syringe.
- 13) Go on with the other eluents until "Eluent F" has been ventilated. Now re-install the fitting. Discard the fluid within the syringe.
- 14) Now ventilate the reagent side: Open the fitting, install the syringe, choose Buffer "R" in aminoControl and prime about 2 mL.
- 15) Repeat for Buffer "W" and close with the fitting.
- 16) If all air has been removed, start the pump using aminoControl within Manual Mode at a pump flow of 0.18 mL/min for about 15 min with Eluent F and Buffer W.
- 17) The system should be now be free of air bubbles. Stop the pump.
- 18) Install Pre-Column and separation column. The column oven should be hand-screwed. Check if all fittings have pressure tightness.
- 19) Now choose buffer "W" and eluent "F" at a flow of 0.18 mL/min in Manual Mode. Start the pump.
- 20) Check the coordinates of the autosampler: Its important that the needle plunges correctly in the three positions Home/Wash-Position, Injection Port and Vial 1:
  - a) in the area "coordinates vial 1" press the button "Goto XY" and check, if the position is directly centered about the test vial. Check by manually pressing down the needle unit, if this is correct. Otherwise adjust coordinates (1 step = 0.1 mm) and re-do.
  - b) Now check the depth of needle injection by pressing the button "goto XYZ" for position 1. It's better to start with lower values and then increase values slowly (to avoid needle bending).
  - c) Continue to adjust coordinates at Home/Wash-Position
  - d) Do the same with the Injection Port: remove the gasket and make sure that the needle goes deep enough to touch the bottom of the port; on the other hand the needle must have a little bit of scope. If your are satisfied with your settings, install the gasket of the injection port.
- 21) Now press the button "Loop Wash" and watch, whether fluid is coming out of the waste tube from the Rheodyne valve. Repeat "Loop Wash" until the waste flows out.

- 22) Take a look at the pressure of the buffer side: it should be between 70 to 90 bar at 40°C column temperature. The pressure on the reagent side should have between 10 and 30 bar if “Reagent” is chosen; for “Wash Solution” you should have between 0 to 5 bar.
- 23) Now adjust the settings of the photometer:  
Go to “Manual Mode/fluid & pressure control” and choose Eluent A & Reagent at a flow rate of 0.18 mL/min. After the photometer signal (measured with your acquisition software) reaches a constant rate, correct the baseline height using the potentiometer. Preferably the baseline should be at 0 mV for good results.

## 7. Guide to aminoControl™

This is a quick step guide to amino acid analysis with the analyzer using the control software “aminoControl™”

Most of the elements used in this software explain themselves by showing “*tool tips*” which appear if you pause above an interactive element such as buttons or form fields. Although the software is self-explaining and easy to handle, the user should be familiar with basic explanatory of Windows™ Operating System.

Use the track ball on the instrument keyboard or the touch screen for data entry and manipulation.

Before starting make sure that your acquisition software, e.g. ChromGate, is correctly installed and connected with the analyzer.

### 7.01 Software Structure

aminoControl™ is designed to run with Windows 2000.

The software is structured into 4 main sections that can be entered by clicking on the buttons on the left. Every **main section** has a different number of **subsections** (tabs) containing certain **elements** (buttons, number pads etc.).

The following screenshot and table elucidate the software structure & functions:

The screenshot displays the aminoControl software interface. On the left, a vertical sidebar contains four **Main Sections**: Manual Mode, Edit Program Mode, List Program Mode, and Autosampler Run Mode. The central area is divided into **Subsections** (Sample Container, Program Table, Summary) and a main control panel. The control panel includes a temperature profile graph with stages W-F, a parameter table, and a status information section at the bottom. The status information section contains fields for username, authorization, login, actual date, program state, eluent rack, column temp, reactor temp, samples temp, pressure buffer, pressure reagent, photometer 570, photometer 440, sample number, sample ID, program name, elapsed time/since inj, pumpflow, and operating condition. A 'Cancel' button is also present.

1.	<b>Manual Mode</b>	<p>This main section contains functions to manually control and calibrate elements of the instrument. Please do <b>not</b> use these functions unless you are an expert and know what you are doing !</p> <p>The Manual Mode contains the following subsections:</p> <ul style="list-style-type: none"> <li>• <b>Pressure and Fluid Control</b></li> <li>• <b>Column Temperature Control</b></li> <li>• <b>Reactor Temperature Control</b></li> <li>• <b>Auto Sampler</b></li> <li>• <b>Service</b></li> </ul>
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2.	<b>Edit Program Mode</b>	<p>Within this area the user can create, load, delete and modify programs for analysis. The sequence of these programs with regard to the sample positions are designated in the <b>List Program Mode</b>. The following subsections are available:</p> <ul style="list-style-type: none"> <li>• <b>Program</b></li> <li>• <b>Run Time</b></li> <li>• <b>Pump Flow</b></li> <li>• <b>Buffer</b></li> <li>• <b>Column Temperature</b></li> <li>• <b>Reactor Temperature</b></li> <li>• <b>Photometer</b></li> <li>• <b>Setup</b></li> <li>• <b>Results</b></li> </ul>
3.	<b>List Program Mode</b>	<p>This main section helps in assigning programs to your vial positions and running the system. Two subsections will guide you:</p> <ul style="list-style-type: none"> <li>• <b>Sample Container</b></li> <li>• <b>Program Table</b></li> </ul>
4.	<b>Run Program</b>	<p>Here you can track the actual parameters of the running program. You'll get information and can intercede in these sections:</p> <ul style="list-style-type: none"> <li>• <b>Sample Container</b></li> <li>• <b>Program Table</b></li> <li>• <b>Summary</b></li> </ul>
5.	<b>Status Information</b>	<p>The bottom area of aminoControl™ is reserved for system parameters concerning operation mode, login, pressure, temperature, sample and program information.</p>

## 7.02 Login and Authority Levels

The login dialog box asks for a username and a password. Furthermore, the authority level must be chosen. aminoControl™ distinguishes three user types depending on the password:

- a) Low Authority User: This level is for users performing routine amino acid analysis without changing and deleting programs.
- b) High Authority User: For users who can create, modify and delete programs and control basic functions within **Manual Mode**.
- c) Service: This user has privileges to control all functions of this software and should be used only by the qualified personnel.

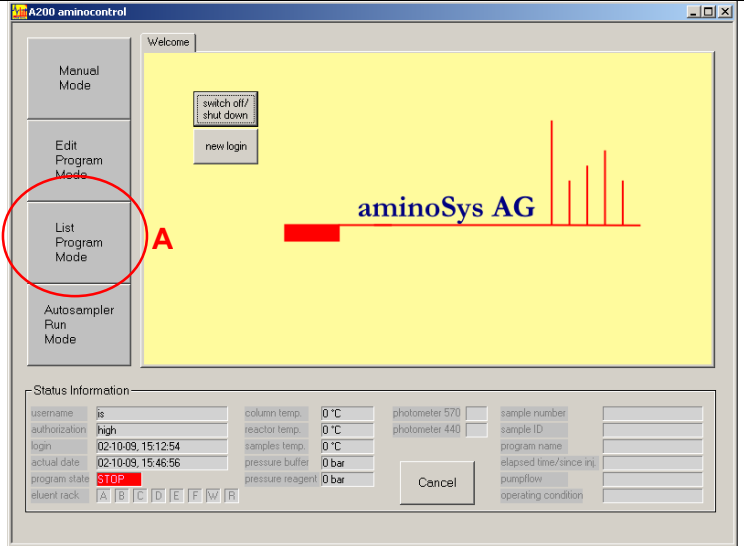
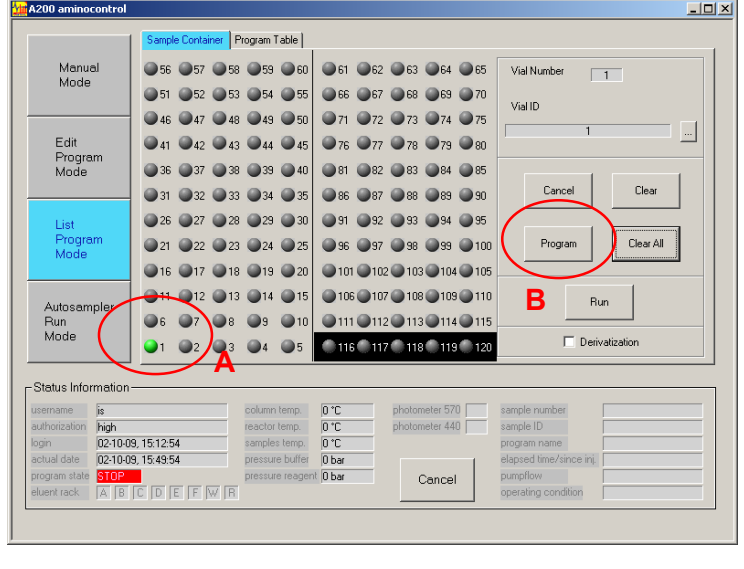
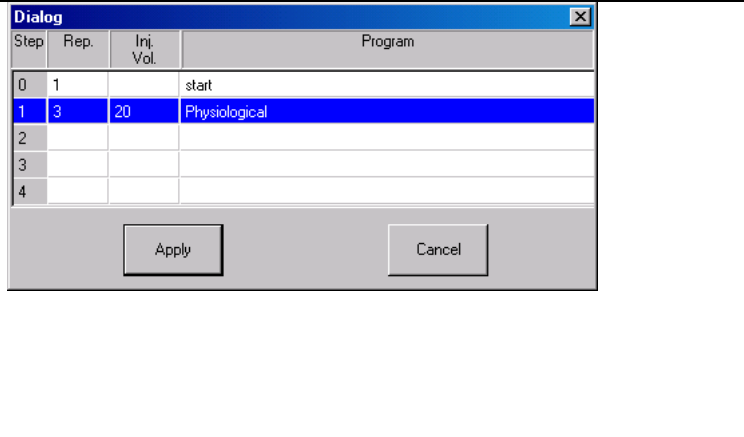
The user name entered does not have any effect on the authority level granted. The 3 preset passwords for aminoControl are shown below:

<b>Authority-Level</b>	<b>Password</b>
Low	amino1
High	amino2
Service	admin

## 7.03 Getting Started

- 1) Start the software by double-clicking onto the aminoControl icon
- 2) Assign your authority level by entering your username and the appropriate password

## 7.04 Running a predefined program

<p>1. Choose the <b>“List Program Mode”</b> on the left hand side (A). (the red circle and the letter next to it help you in identifying what is being described)</p>																									
<p>2. Place the sample-containing vial into the autosampler, e.g. in position 1. Click on position 1 in the <b>Sample Container</b> subsection and its position turns green (A). If the sample has already been assigned to a program sequence, the position is yellow. Now click on the <b>Program</b> button on the right side (B) and a window will pop up. In the upper right it is possible to assign a sample name.</p>																									
<p>3. Create a program sequence for this sample in position 1. The first program is should be a <b>“Start”</b>-program for system equilibration. This is followed by an analytical program such as <b>“Physiological”</b>. In that row the amount of repetitions (here: 3), the injection volume (here: 20µL) and the program name is assigned, e.g. to analyze a standard sample.</p> <p>Click <b>Apply</b> to continue...</p>	 <table border="1" data-bbox="751 1292 1498 1727"> <thead> <tr> <th>Step</th> <th>Rep.</th> <th>Inj. Vol.</th> <th>Program</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>1</td> <td></td> <td>start</td> </tr> <tr style="background-color: #0000FF; color: white;"> <td>1</td> <td>3</td> <td>20</td> <td>Physiological</td> </tr> <tr> <td>2</td> <td></td> <td></td> <td></td> </tr> <tr> <td>3</td> <td></td> <td></td> <td></td> </tr> <tr> <td>4</td> <td></td> <td></td> <td></td> </tr> </tbody> </table>	Step	Rep.	Inj. Vol.	Program	0	1		start	1	3	20	Physiological	2				3				4			
Step	Rep.	Inj. Vol.	Program																						
0	1		start																						
1	3	20	Physiological																						
2																									
3																									
4																									

4. Click on vial **2** and on **Program** to assign the program sequence for the next vial, e.g. the unknown sample. Again, 3 repetitions and 20 µL injection volume with program “**Physiological**” is selected within the dialog box.

In this example of a program sequence, only these two samples will be analyzed. The very last program should be always the “**Holiday**” program which rinses the system and regenerates the columns.

The screenshot shows the A200 aminocontrol software interface. The 'Program Table' section displays a grid of vial positions from 1 to 120. Vial 2 is selected. A 'Dialog' window is open, showing a table with columns for Step, Rep., Inj. Vol., and Program. The table contains the following data:

Step	Rep.	Inj. Vol.	Program
0	3	20	Physiological
1	1		Holiday
2			
3			
4			

The 'Run' button is highlighted with a red circle labeled 'B'.

4. Change to the subsection **Program Table** and press **Validate (A)** to find out if all parameters have been entered correctly (*Invalid entries disappear from the table !*).

This subsection gives an overview of the assigned program steps. Modifying, inserting or deleting can be done in this area using the buttons below or by directly changing the given values.

If you want to add more samples to be analyzed you can do it from this table here. You can also go back to the subsection **Sample Container** and choose a vial position, following step 3.

Click on **Run (B)** to start the chromatographic process.

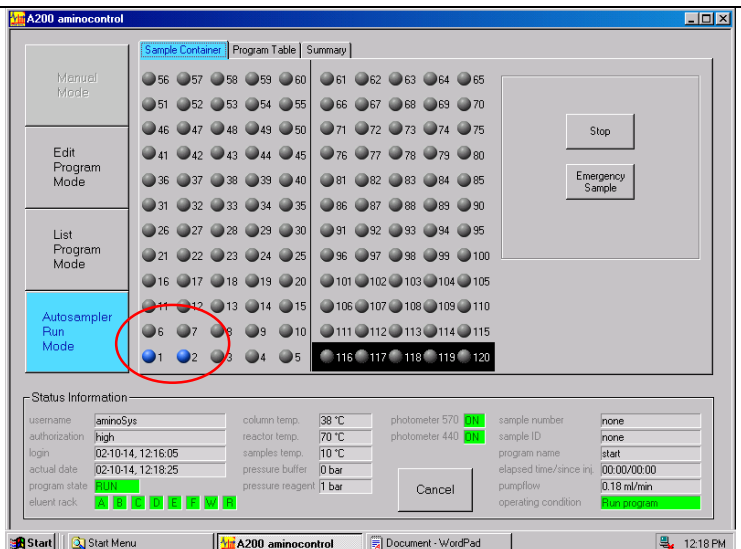
The screenshot shows the A200 aminocontrol software interface. The 'Program Table' section displays a table with columns for Step, Sample Number, Sample ID, Rep., Inj. Vol. in µl, and Program. The table contains the following data:

Step	Sample Number	Sample ID	Rep.	Inj. Vol. in µl	Program
0	1	1	1	1	start
1	1	1	3	20	Physiological
2	2	2	3	20	Physiological
3			1		Holiday
4					
5					
6					
7					
8					
9					
10					
11					
12					

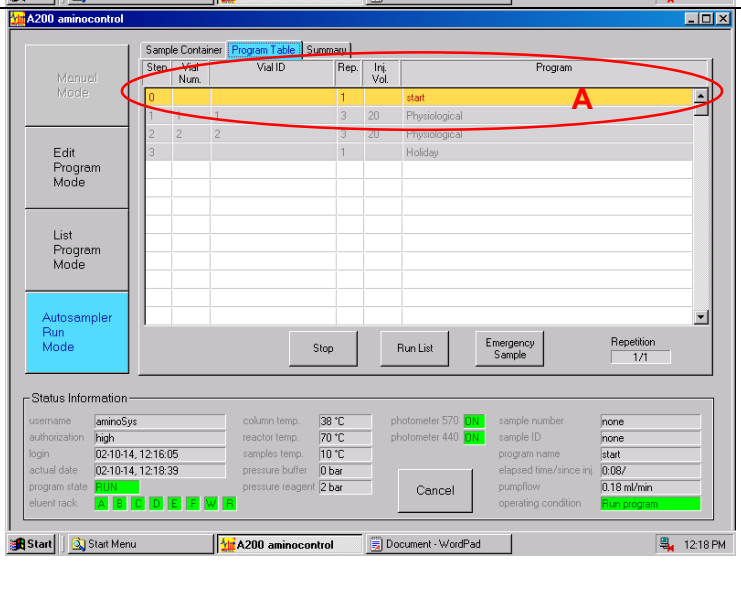
The 'Run' button is highlighted with a red circle labeled 'B' and the 'Validate' button is highlighted with a red circle labeled 'A'.

5. The aminoControl™ software jumps automatically to the main section **Autosampler Run Mode** and the subsection **Sample Container**. The assigned samples/vials will turn blue.

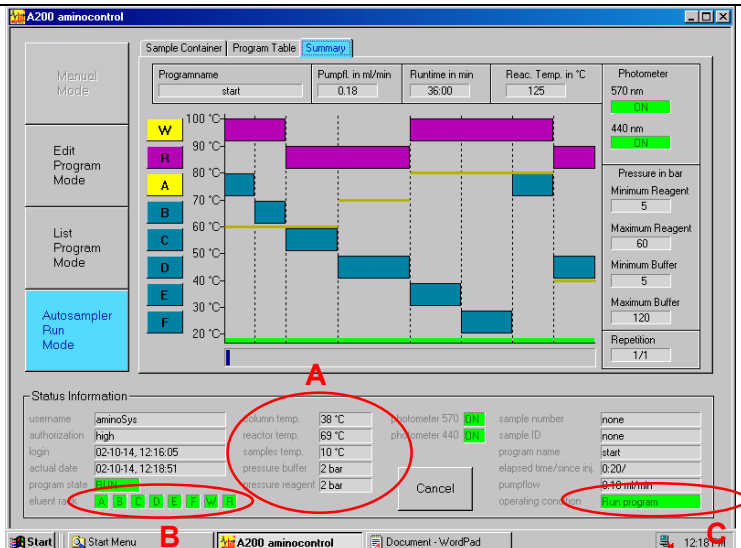
(To interrupt this run, click on the **Stop** button and follow the instructions)



6. The subsection **Program Table** shows the currently running step of the running program (yellow background, **A**) and also the subsequent steps (grey background). By clicking the **Run List** button, you can watch forthcoming sequences. If you continue programming sequences and press the **Run** button they will be attached to that run list. After the actual program sequence has finished the next sequence follows (“LIFO principle”). Via the **Emergency Sample** button you can introduce additional samples (positions 116 to 120 in the autosampler rack) in between your running sequence.



7. The subsection **Summary** gives a graphical overview of the currently running program. You can identify which buffer is currently running (yellow colored, left to the diagram) and the blue bar under the diagram shows the elapsed run time. At the bottom, temperature and pressure parameters can be tracked (**A**). Fields with a green background indicate that the system is idle, e.g. the filling status of the eluents in the eluent rack (**B**) or the operating condition (**C**).



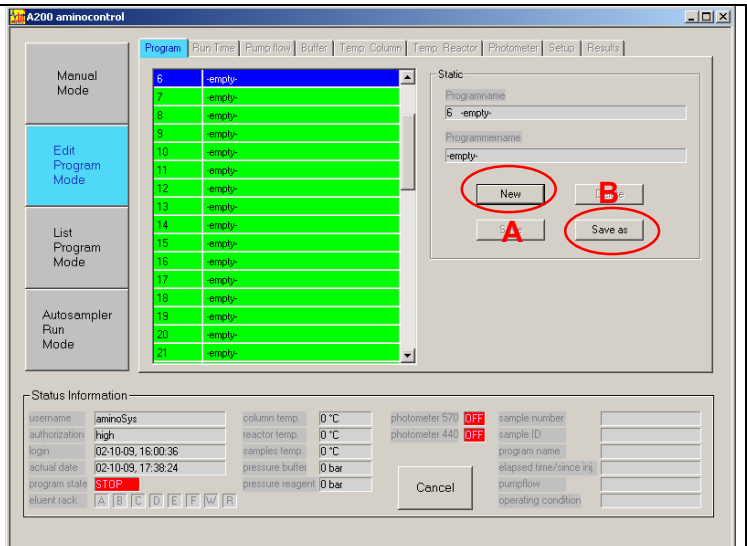
8. When this sequence is finished, the next repetition or new step will follow automatically.



## 7.05 Creating a program

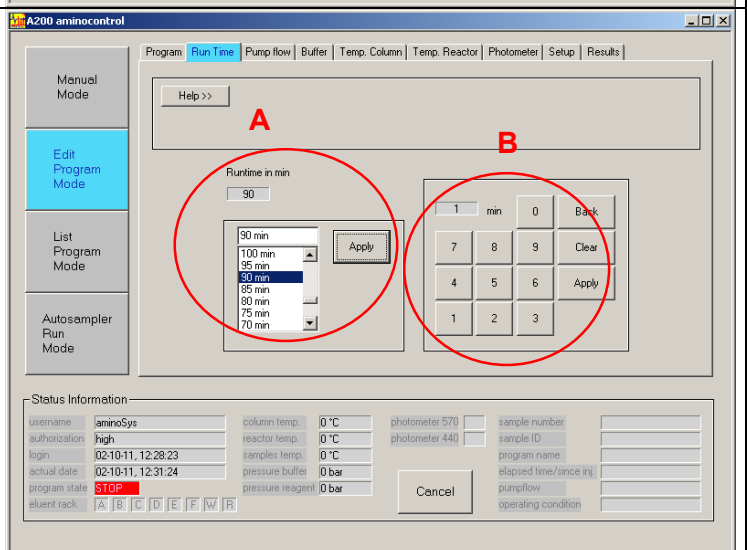
1. Change to **Edit Program Mode** and click **New** for a new program (A). Enter a program filename after clicking **Save as** (B).

A program file will be always signed with the programmers name and only this user can delete it. Clicking on a filename, e.g. "physiological test program" makes the subsections become available and guide the user to build the program parameters.

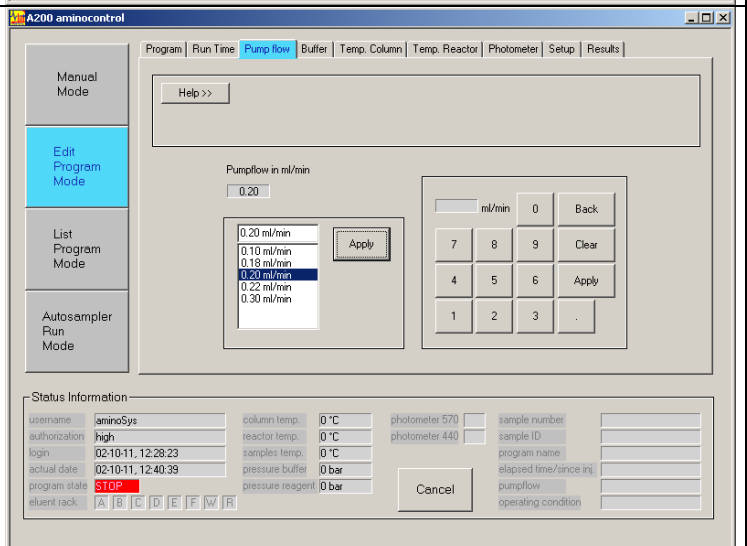


2. Change to subsection **Run Time** and assign the total running time of the program. In case you are not sure about the accurate total running time, it's better to enter a higher value (because finally, reducing run time is easier than adding run time !). Use default values (A) or enter a custom value (B). In any case, click **Apply** to save this value.

This kind of data entry (default vs. custom values) will be used in subsequent subsections.



3. Proceed with the subsection **Pump Flow** and assign a value. A value of less than 0.30 ml/min is recommended.



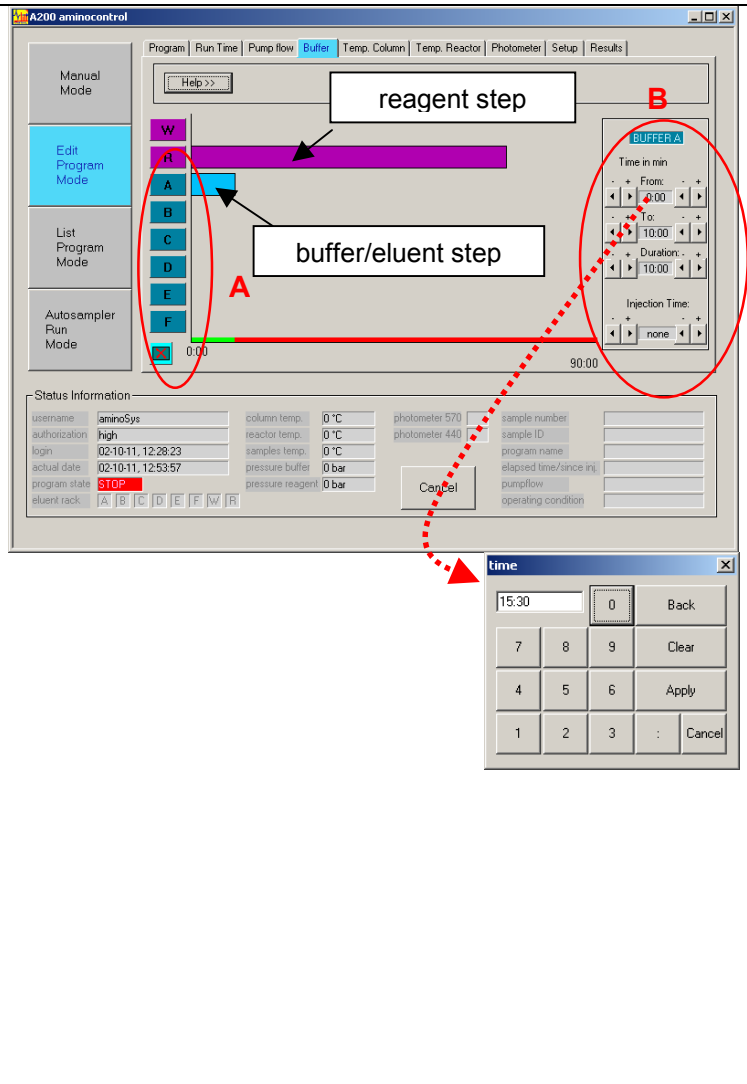
4. Enter the **Buffer** subsection. Now design the sequence of buffers and injection time.

For **every** programmed buffer step (**A** to **F**) must be an accompanying **Reagent** or **Wash Buffer** step chosen. Avoid combining **F** and **R** as this can cause reagent precipitation, followed by tube clogging.

A gap in the sequence of buffers is not acceptable and is indicated by a **red segment** while the **green segment** signals a valid composition !

To create a buffer step, click on the buttons **A** to **F** or **W & R** (**A**) and a box will appear in the diagram. To modify, select the box by clicking on it (the box becomes highlighted). Delete the selected box by clicking on **X** below button **F**.

Adjust buffer running time (start, end, duration) of the selected buffer step with **◀▶** buttons on the right-hand side of the screen (**B**). Adjust minutes (left) or seconds (right). Clicking on the time display between the buttons brings up a number pad, allowing the user to enter a custom time (e.g. 15 or 15:30 min).



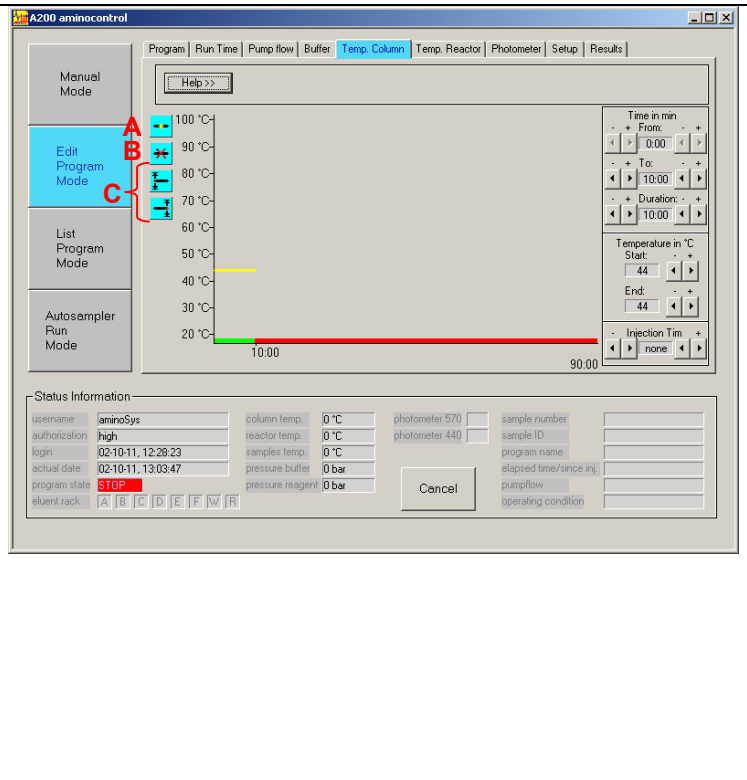
5. In the subsection **Temp. Column** the temperature-profile of the separation column has to be defined. Like for the buffers, it is important to define the temperature for the whole running time. A valid entry is indicated by the green segment at the bottom of the diagram.

By clicking on the top symbol left to the diagram (**A**) a new temperature step is created in the diagram (yellow line).

Use control tools on the right hand to assign starting, ending and duration time. Additionally, assign starting and ending temperature in °C.

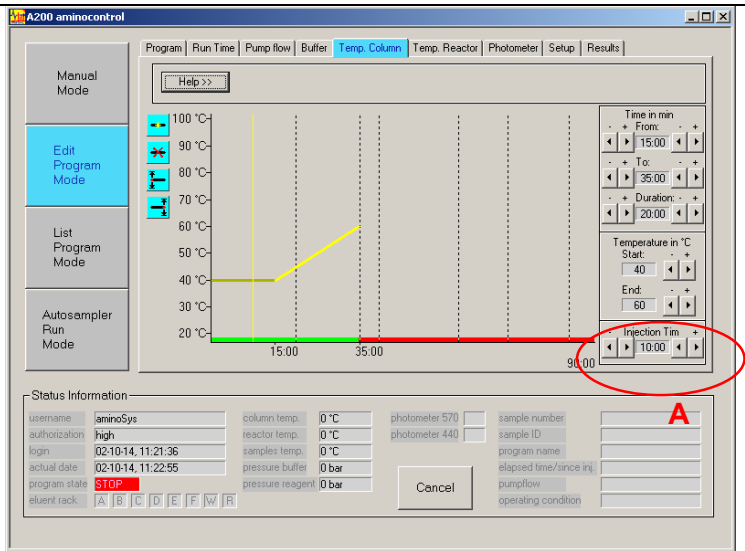
By clicking on a temperature step it becomes highlighted and can then be deleted with the 2<sup>nd</sup> button on the left (**B**).

The buttons below make the temperature snap-in to the prior or following step (**C**). This generates a temperature gradient.

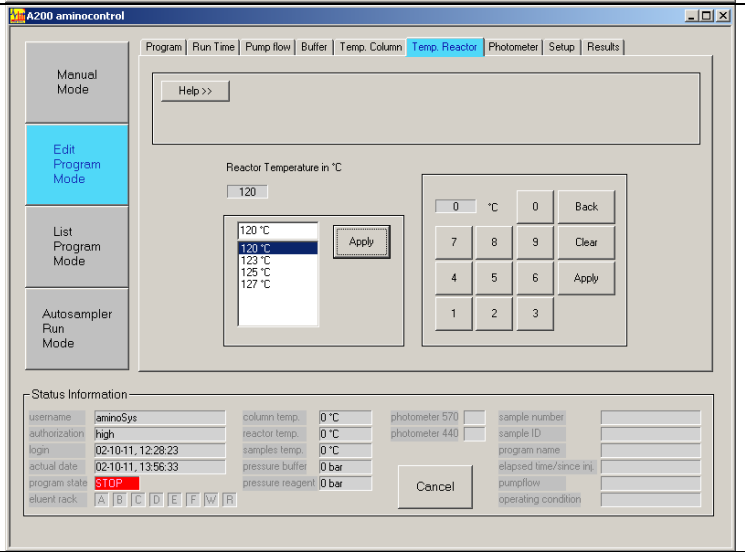


6. Define the injection time with the buttons or by double-clicking on the time digits to open a number pad.

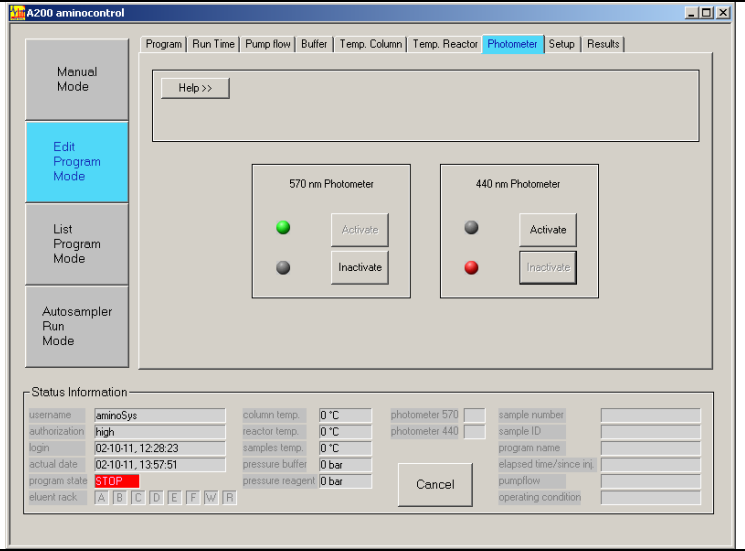
For every program, the injection should always happen after a short pre-equilibration time: Between 10 and 15 minutes is a good reference point.



7. Define the reactor temperature in the next subsection **Temp. Reactor**. Proceed as described for **Run Time**. A value of 120°C is a good starting reference point. In case you are not sure, it's better to choose a lower temperature than a higher to prevent reagent precipitation. Note that the reactor temperature setting is valid for the entire program !



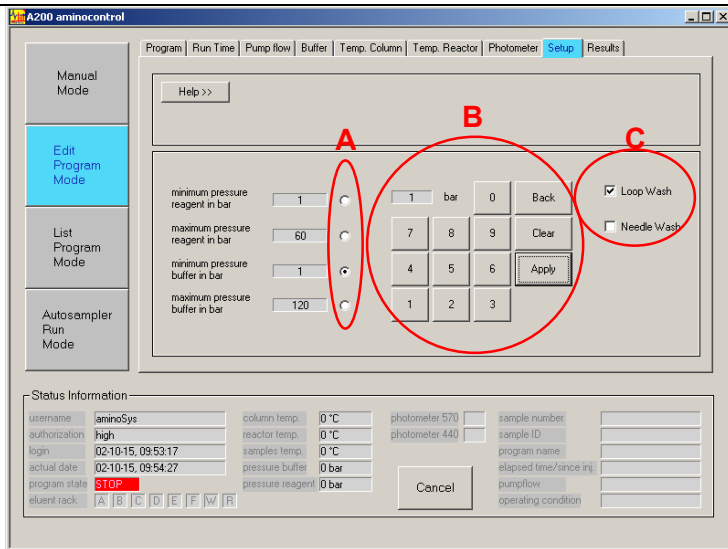
8. The subsection **Photometer** is a place holder for future developments. (At the moment both photometers - 570nm and 440 nm - are active anyway.)



9. In the subsection **Setup** the maximum and minimum pressure levels, for both eluent and reagent sides must be set. Click on the radio buttons to change assignment (**A**). Use the number pad to enter the values and press **Apply** (**B**). Use the presented values as a reference.

The recommend min/max values are 1 and 60 bar for the reagent and 1 and 120 bar for the buffer.

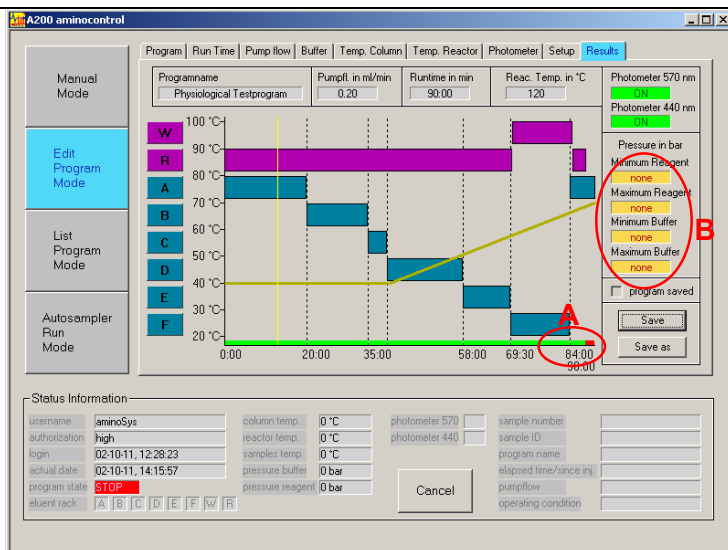
A loop and/or a needle wash can optionally be programmed introduced by activating the appropriate check boxes (**C**).



10. The last subsection **Results** gives an overview of the assigned parameters. This is the opportunity to check if all of the necessary parameters have been entered correctly. Often committed mistakes:

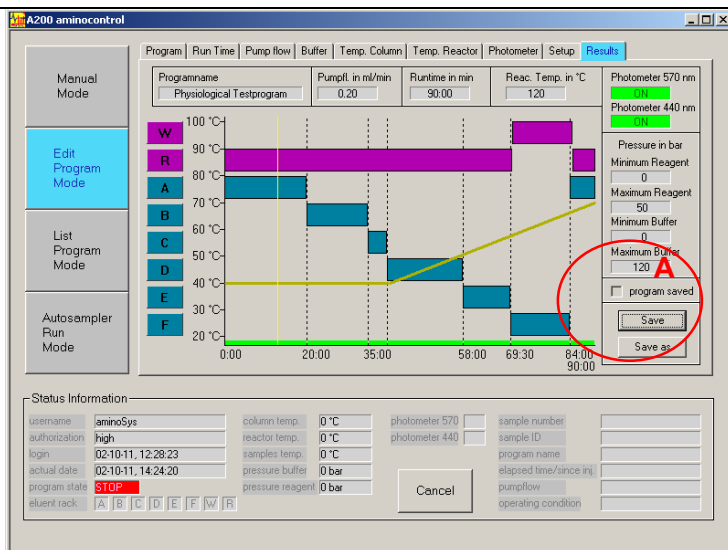
1. (**A**) The small red segment at the bottom of the diagram indicates, that something regarding buffer selection or column temperature design is wrong. The error occurred because the last reagent step is not long enough: Eluents AND reagent/wash buffer must be delivered in parallel. The user must go back to subsection **Buffer** and extend the duration time of "R".

2. (**B**) The yellow background of these fields indicates that data is missing: the minimum/maximum pressure levels have not been entered yet. The user must go back to subsection **Setup** and make the required entries.



11. After correcting and updating entries, the program sequence is valid. An invalid program doesn't appear in program table in the **List Program Mode**.

Don't forget to store your program using the **Save** button or make a copy with a new filename with **Save as**. If program parameters were changed since last saving, the checkbox near to "program saved" will be deactivated (**A**).

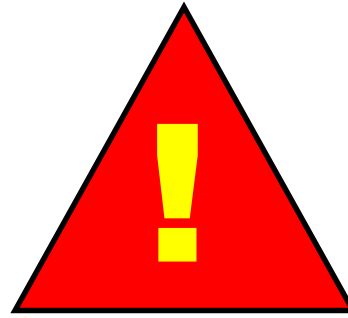


## 7.06 Manual Mode

1. The **Manual Mode** is dedicated only to qualified personnel and service technicians. Within this section you can control the functional elements manually and calibrate internal settings. The *tool tips* will give you helpful information about the element's function.

**Attention:**

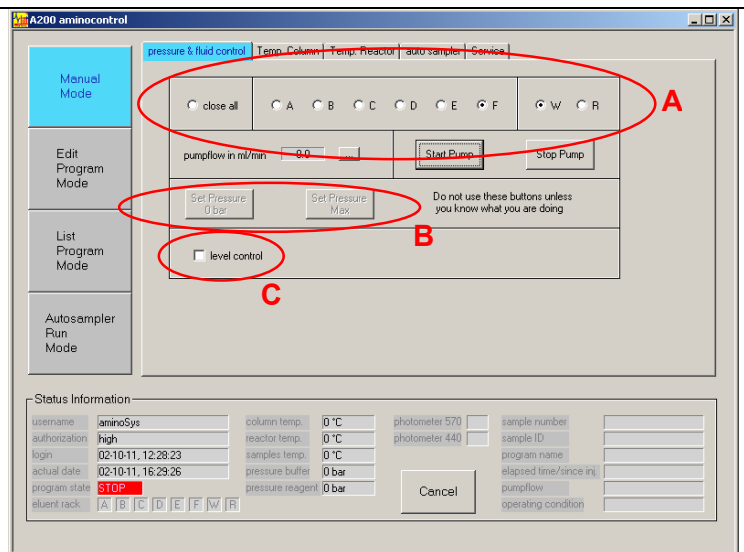
Improper handling and calibration can be harmful to the instrument resulting in loss of warranty.



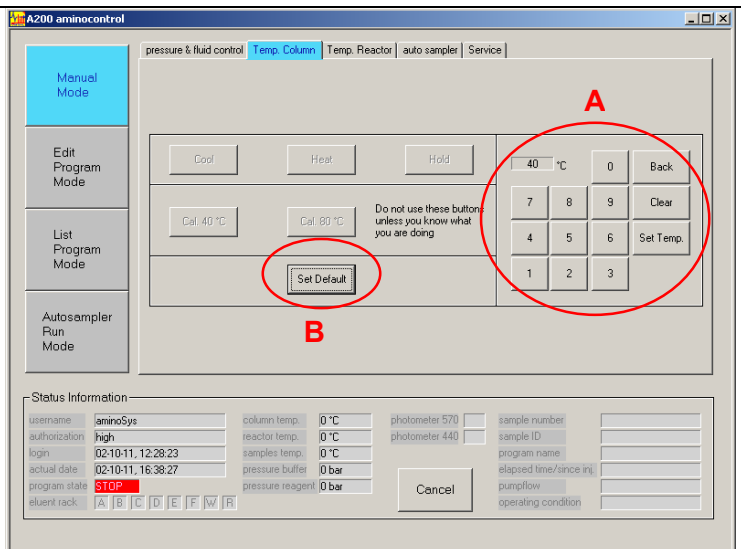
2. The first subsection **Pressure & Fluid Control** enables the user to control the pump and the buffer manifold valve. One can choose an eluent and buffer combination or close the buffer valve manifold using the radio button **close all**. The pump flow has to be set before starting the pump with **Start Pump**. Be patient as pump actions take a few seconds to process (A). Furthermore, system calibration of the pressure is possible from this subsection (B). These buttons (deactivated in picture) are very critical !

The checkbox next to level control, toggles the eluent rack filling level control on and off (C). If level control is activated the user is warned when the volume of any of the eluent & buffer bottles becomes low. This is indicated by a dialog box and an orange background of the affected buffer/eluent in the eluent rack within the **Status Information**.

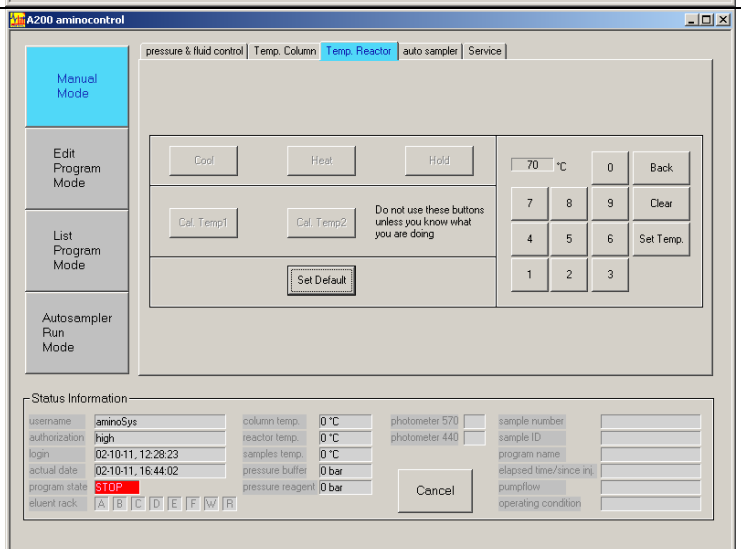
If level control is deactivated, no dialog box appears, but the buffer background turns red when the level becomes low.



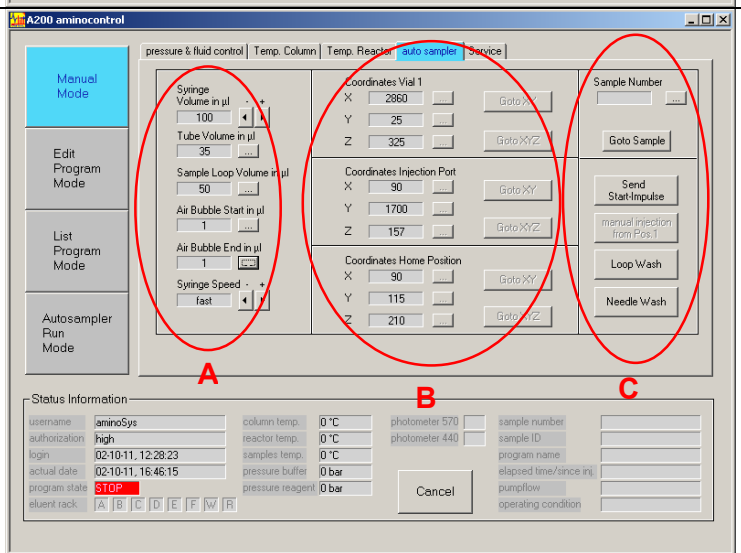
3. The subsection **Temp. Column** enables to user to manually control the column temperature. The temperature can be set manually by entering custom values (A) or by using the **Cool**, **Heat** and **Hold** buttons which are deactivated in this view. Be careful when using the **Heat** button: there's no temperature control in this subsection and inattentive handling can cause a fire hazard ! The **Cal**-buttons are for calibrating the system. The **Set Default**-button sets the column temperature to 40°C (B).

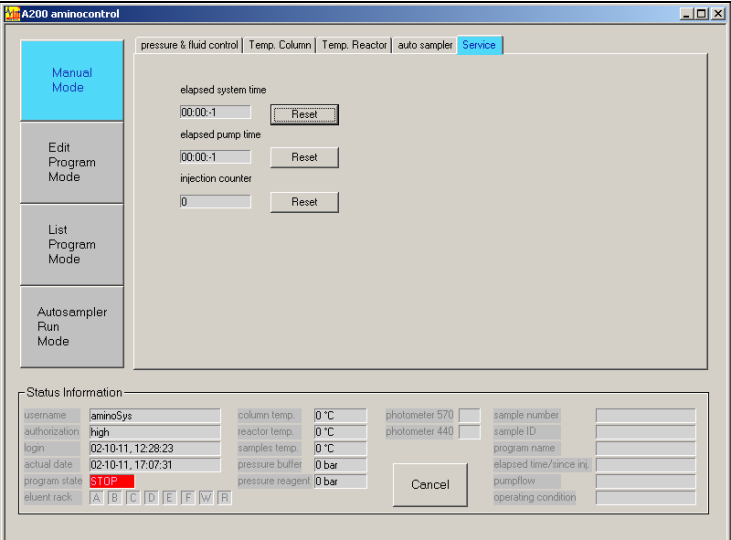


4. The subsection **Temp. Reactor** enables the user to manually control the reactor temperature. The layout is the same as for column temperature. Again, instrument calibration should only be performed by a service technician or knowledgeable personnel.



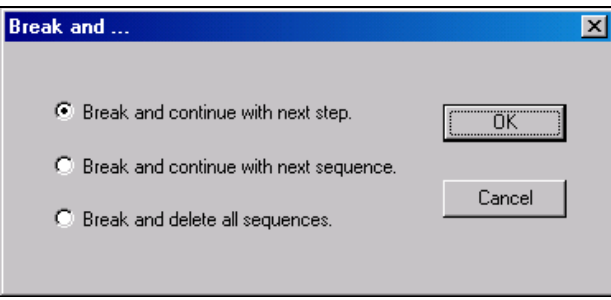
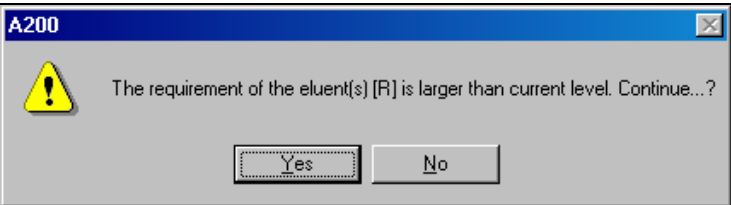
5. The subsection **auto sampler** controls the injection and coordinate parameters of the autosampler. The settings on to the left side control different parameters of the injection unit and the syringe speed (A). Take the given values as reference parameters. The middle area of this subsection contains the autosampler coordinates which can be adjusted (x, y and z-values) (B). **Goto XY** moves the autosampler to the given XY-position. **Goto XYZ** also moves to the given XY position, but additionally moves the autosampler needle down in the Z position ! The buttons on the right move the autosampler to a user-defined rack position (**Goto Sample**). Further, a **Start-Impulse** can be sent manually to the data acquisition computer, a **manual injection** from position 1 can be made as well as **loop wash** and

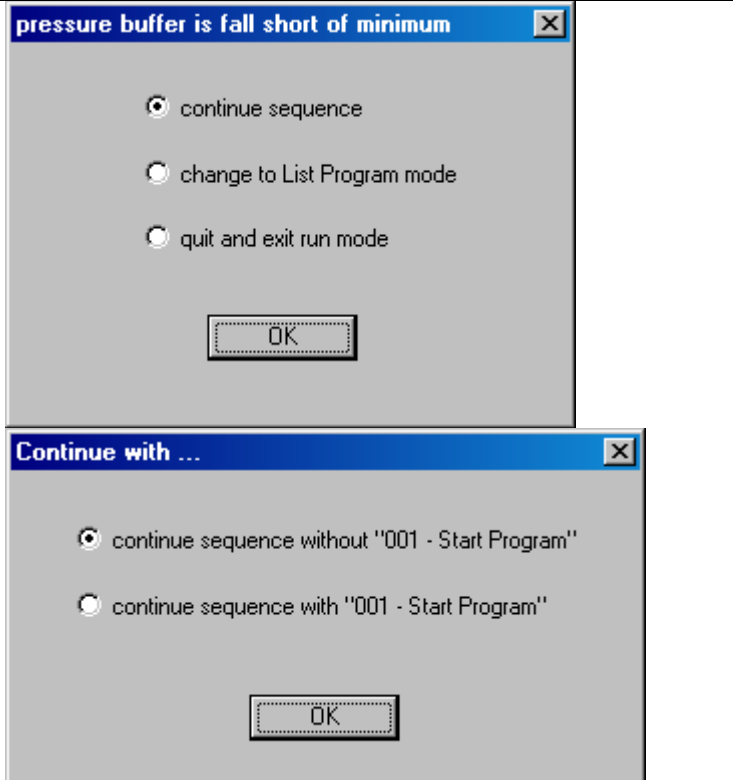


	<b>needle wash.</b> (C).
6.	<p>The <b>Service</b> subsection gives system running information.</p> 

### 7.07 Breaks and Error Messages

This software was specifically designed for the Amino Acid Analyzer and especially created to minimize critical situations. However if an error does occur (e.g. the maximum pressure is exceeded), the reactor temperature cools down automatically to 80°C and the column temperature to 40°C to avoid damage to these components. The analysis process also automatically to conserve samples & buffers. Dialog boxes will guide the user to continue or stop the analysis.

<p>1. Clicking the Stop button while a program is running will result in the “break” dialog box being displayed. Choose the desired option and click <b>OK</b>. The first option continues within the sequence, the second option leaves the current sequence and starts the next one while the last option stops the entire running process.</p>	
<p>2. In case the eluent level alert has been activated (in the Manual Mode), aminoControl™ calculates the amount of buffer required, depending on the program. If the amount is higher than the current measured level, the user is warned with this kind of dialog box. In this case the volume of Reagent R is low. If the user chooses “Yes” to continue the run, the dialog box will appear again after each program is run.</p>	

<p>3. If buffer or reagent pressure is not within the programmed range (here: buffer pressure is lower than the set minimum pressure), aminoControl™ lets the user know with this dialog box.</p> <p>If the user chooses “continue sequence”, another dialog box asks the user whether to continue with or without the Start Program.</p>	 <p>The image displays two sequential dialog boxes from the aminoControl software. The first dialog box, titled "pressure buffer is fall short of minimum", presents three options: "continue sequence" (which is selected), "change to List Program mode", and "quit and exit run mode". An "OK" button is located at the bottom. The second dialog box, titled "Continue with ...", follows the first and offers two choices: "continue sequence without '001 - Start Program'" (selected) and "continue sequence with '001 - Start Program'". It also features an "OK" button at the bottom.</p>
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## 8. Troubleshooting

Although the analyzer is a robust chromatographic system, the occurrence of problems cannot be avoided. The following topics will help the user to identify the cause of a problem (“•”) and gives suggestions to manage the situation (“→”).

The most common errors are the minimum and maximum pressure errors. The standard procedure following this event is to remove air from the pump – a procedure that should be done once a week. Furthermore it is recommend to include a flushing program at the end of a program sequence but before the “holiday” program starts. The flushing program consists of one step with Eluent F and Reactor Wash Solution at a column temperature of 70°C and a reactor temperature of 115°C at flow rate of 0.20 ml/min for 60 minutes. This prevents the reactor tubing from clogging.

It is important to document any events occurring with the instrument for effective troubleshooting. Do not hesitate to consult your distributor for advice.

### 8.01 Maximum/Minimum Pressure Errors

#### (a) “Minimum Pressure Buffer” or “Minimum Pressure Reagent”

- The *minimum pressure buffer value* of the current program is too high. If the pressure goes below this value this error message occurs.

- reduce the minimum pressure value (e.g. from 10 to 5 bar)

- The volume of hydration for the pump is too low

- exchange spikes because the filter membranes of the bottles can be impure and clogged

- remove the upper fitting of pump (buffer side) and remove air from pump with a syringe

- check ball valve of pump for proper function in flow direction and adequate pressure against the flow through – exchange if necessary

#### (b) “Maximum Pressure Buffer”

- the buffer tubing is clogged

- open fittings and check tubings of the following units in the given sequence:

- photometer, reactor, column unit, pre-column, Rheodyne valve

- if pressure drops to 0 bar after opening of fittings, the examined element was clogged

- read next how to continue with the identified element...

- photometer is clogged because of coagulated reagent

- Check date of reagent activation with co-reagent. If this is older than appr. 4 weeks use a fresh activated reagent bottle

- reduce reactor temperature and exchange reagent;

- rinse the photometer with syringe & H<sub>2</sub>O and flush the system with Reactor Wash Solution

- reactor is clogged because of coagulated reagent

- remove photometer tubings from reactor and flush reactor at a low flow rate (e.g. 0.05 mL/min) with Eluent F and Reactor Washing Solution for appr. 30 minutes at 115°C

- Carefully watch the pressure behavior

- separation column or pre-column unit is clogged

- remove the column and re-install it in opposite direction to flush the frit at the column inlet

- flush with Eluent F and Reactor Wash Solution (0.05 to 0.22 mL/min)

- if pressure normalizes, re-install column in original direction

- Rheodyne valve is clogged

- exchange tubing between valve and separation column

### **(c) "Maximum Pressure Reagent"**

- reagent tubing is clogged because of coagulated reagent  
→ open fittings and check tubing of the following units in the given sequence: photometer, reactor, mixer, pressure capillary  
→ if pressure drops to 0 bar after opening of fittings, the element was clogged
- photometer is clogged because of coagulated reagent  
→ reduce reactor temperature and exchange reagent with a fresh activated bottle; rinse the photometer with syringe & H<sub>2</sub>O  
→ regenerate the system with Reactor Wash Solution
- reactor is clogged because of coagulated reagent  
→ remove photometer tubings from reactor and flush reactor at a low flow rate (e.g. 0.05mL/min) with Eluent F and Reactor Washing Solution for appr. 30 minutes; Carefully watch the pressure behavior
- tubing is clogged at input/output of photometer  
→ remove fittings and cut about 5mm of tubing's end
- Clogging of reagent capillary  
→ cut about 5 mm of capillary's end at the 4-way-block (dampening) or from the mixer
- Clogging at photometer because of expired reagent  
→ Check date of reagent activation with co-reagent. If this is older than appr. 4 weeks use a fresh activated reagent bottle

## **8.02 Chromatographic Events**

### **(a) Small peaks**

- incorrect injection volume  
→ check injection volume of the current program (often committed mistake: 2µL instead of 20µL)
- Reagent for Derivatization has expired  
→ check date of activation with co-reagent: if > 4 weeks use a fresh activated bottle
- Air bubbles within the check valves  
→ remove air from pump
- Incorrect working auto sampler  
→ call for service

### **(b) Injection peak increases**

- waste-tubing is too long or at a low level  
→ install waste collector at the same height as the column unit

### **(c) Baseline shows strong fluctuations**

- air bubbles in photometer  
→ flush system with Eluent F and Reactor Wash Solution; if this doesn't work flush photometer with propanol and check back pressure regulator for impurities
- Clogging of bottle filter membrane  
→ if one of the bottles is deformed/shows buckling, the filter membrane can be clogged: exchange spike

#### **(d) Baseline increases**

- ammonium concentration is too high: after 2 to 3 hours running time of the column without regeneration the pre-column is saturated with  $\text{NH}^{3+}$  and releases  $\text{NH}^{3+}$  to the separation column.  
→ regenerate the columns with Eluent F and Reactor Wash Solution following every single run; if this doesn't work exchange pre-column.

#### **(e) Heavy noise in Baseline**

- tiny leakage  
→ check if fittings are installed correctly without leakage and watch for pressure fluctuations  
→ clean the back pressure regulator
- small air bubbles in the photometer  
→ rinse photometer with syringe &  $\text{H}_2\text{O}$

#### **(f) No peaks detectable**

- no sample available  
→ although trivial, check for correct vial position of your sample and if enough sample volume is available
- Injection needle is bent and doesn't extract sample and/or doesn't fit into the injection port  
→ install a new needle (for temporary use only: re-bend the needle if possible)
- Injection needle doesn't inject into the port precisely  
→ remove injection-port and check for impurities  
→ check injection gasket for correct diameter of the needle: clean gasket from impurities and slide needle through gasket
- clogging of needle  
→ remove FEP-tubing from needle and clean needle with provided spring steel wire
- no flow of buffer, reagent or wash solution  
→ check pressure and see troubleshooting of "Min Pressure Buffer/Reagent"

#### **(g) Bad peak separation, retention time of amino acids is delayed**

- pH or concentration of sample is not correct  
→ use Sample Dilution Buffer for sample preparation and dilution  
→ check pH and – if necessary - adjust to pH 2.2

#### **(h) Peaks are partially fused**

- running time of the eluents (A to E) or column temperature require optimization  
→ amino acid is diluted during change of eluent ("buffer hop"): prolong running time of affected eluent  
→ reduce column temperature to increase the retention time  
→ prolong running time and column temperature can improve separation performance

#### **(i) Unsteady baseline level**

- problems with flow of reagent  
→ check filter element of reagent bottle for impurities, exchange spike if necessary
- tiny precipitate within reagent capillary near to a fitting  
→ cut about 5 mm of capillary's end at the 4-way-valve (dampening) or from the mixer
- running times of Eluent A, F and Wash Solution are too short

→ increase running time of Eluent A and of Eluent A & F and Wash Solution in the regeneration program

- back pressure regulator is clogged
- clean with propanol in an ultrasonic cleaner

**(j) Chromatogram is “spiky”**

- air bubbles in system
- remove air bubbles from pump
- remove air bubbles from photometer
- flush the system with Eluent F and Reactor Washing Solution in the Manual Mode

### **8.03 Other Events**

**(a) Injection needle bent**

- operator has changed parameters of the autosampler during the injection process
- check autosampler setup parameters in Manual Mode

- FEP-tubing from needle is too tense
- ease the tube tension

**(b) Column or Reactor unit doesn't heat**

- call for service

**(c) “Can't read pump time from Pic” or “Can't read the injection counter from Pic”**

- shutdown aminoControl and Windows 2000 - restart your system

## 9. Appendix A: Sample Preparation

### 9.01 General Aspects

For amino acids analysis from any sample, the user must conduct a sample preparation step. In general two different approaches are distinguished:

1) Analysis of FREE amino acids in fluids of physiological or industrial origin (e.g. plasma, urine, tissue or wine, juice, cell culture medium etc.). These sample types have to be deproteinized before analysis.

2) Analysis of BOUND amino acids in proteins or peptides from industrial or laboratory origin (e.g. feedstuff or recombinant proteins). These sample types are hydrolyzed before analysis.

### 9.02 Sample preparation of free amino acids in fluids

#### (a) Introduction

Deproteinisation of physiological fluid samples prior to amino acid analysis is usually accomplished using the compound 5-sulphosalicylic acid (SSA). However, the conditions employed during the deproteinisation vary according to the different concentration of proteins in the sample.

The following precautions have to be taken:

- a) The sample must be completely free of proteins and large peptides.
- b) Where possible, maintain the molar concentration of salts in the sample below the level where they interfere with the application of sample to the analytical column.
- c) The pH of the sample should be controlled to avoid impact on chromatographic separation (optimal is pH 2.2).
- d) Perform sample preparation at low temperatures, e.g. 4°C, to prevent heat labile amino acids from degrading.
- e) If possible, all samples should be filtered using a 0.2µm membrane filter before injection into the system. Use a spin column to avoid loss for small volumes of sample.
- f) Centrifugation must always be performed at highest speed, equal or higher than 10<sup>4</sup> g.

Of the two methods described, Method A ((b)) has the advantage that no pH adjustment of the sample is necessary before injection into the system. Method B ((c)) has the disadvantage that if significant amounts of protein are present in the sample then complete deproteinisation is not achieved.

To prevent protein contamination of the analytical column, e.g. in case or large amounts of protein in the sample, use Method B. If you have only small amounts of sample and expect lower amino acid concentrations, Method B should also be applied. After using Method B the pH of the sample must be adjusted prior to injection.

Urine samples can be deproteinized using either method except in cases where pathological abnormalities such as proteinuria are suspected. In these cases and in the analysis of serum, plasma and liquor, Method B should be used.

**(b) Procedure for larger sample amounts (Method A)**

- 1) Make up a 10% SSA solution in water and add 200 $\mu$ L to a 1.5 ml-reaction tube
- 2) Cool SSA-solution and your sample to 4°C
- 3) Add 800  $\mu$ L of sample to the tube containing 10% SSA solution and immediately mix well (for smaller sample volumes make sure that you have at least a final concentration of 2% SSA)
- 4) Incubate at 4°C for at least 1 h
- 5) Centrifuge the mixture for 5 minutes in a high speed centrifuge to spin down precipitate
- 6) Carefully transfer supernatant
- 7) Filter supernatant through a 0.2  $\mu$ m filter
- 8) Continue directly with analysis or store supernatant at -20°C
- 9) Prior to analysis following storage, carefully raise the temperature from -20°C to 4°C
- 10) Mix 1 volume of supernatant with 1 volume of sample dilution buffer

**(c) Procedure for very small sample amounts (Method B)**

- 1) Place 50 mg (per 1 mL sample) of solid SSA in your reaction tube
- 2) Cool tube to 4°C
- 3) Add 1 mL of sample and immediately mix well
- 4) Incubate at 4°C for at least 1 h
- 5) Centrifuge the mixture for 15 minutes at 4°C in a high speed centrifuge to spin down the precipitate
- 6) Carefully transfer supernatant
- 7) Filter supernatant through 0.2 $\mu$ m filter
- 8) Adjust pH of supernatant to pH 2.2 using 0.3 M lithium hydroxide (approx. 10-20  $\mu$ L of hydroxide will be required for each 50  $\mu$ L of sample). If sufficient sample volume is available, control pH with pH meter or narrow range pH paper.
- 9) Continue directly with analysis or store supernatant at -20°C
- 10) Prior to analysis following storage, carefully raise the temperature from -20°C to 4°C
- 11) Mix 1 volume of supernatant with 1 volume of sample dilution buffer

**(d) Remarks**

- 5-sulphosalicylic acid (SSA) from Sigma: #S-7422
- EDTA as anticoagulant may lead to artifacts caused by the presence of contaminants which also react with the derivatization reagent
- amino acid concentrations in serum are generally higher than in plasma, due to release of amino acids from cellular constituents during the clotting process
- hemolysis increases serum amino acid concentrations
- tryptophan is destroyed by SSA. Deproteinisation with 1 part of sample with 4 parts 10% trichloroacetic acid can be performed to analyze tryptophan. An excess of trichloroacetic acid might be removed by 5 times ether extraction
- use an internal standard (e.g. Norleucine) to control sample preparation efficiency
- changes in amino acid composition during storage:
  - glutamines very decomposes quickly; it's only stable at less than -70°C
  - $\gamma$ -glutamic acid concentration increases
  - phosphoethanolamine hydrolysis into ethanolamine and phosphate

**9.03 Sample preparation of free amino acids from tissue**

The extraction of free amino acids out of tissues should be done as soon as possible after tissue removal. Otherwise the sample has to be frozen & stored using liquid nitrogen to preserve amino acids from degrading.

The procedures should be done on ice:

- 1) homogenize 10 to 20 mg of tissue with 300 $\mu$ L 0.5 N Perchloric acid.
- 2) Centrifuge the homogenate 10 min at 4000g
- 3) Add 70 $\mu$ L Potassium hydrogen carbonate to 250 $\mu$ L of supernatant to neutralize
- 4) Mix well and incubate 15 min on ice
- 5) Mix again and centrifuge for 10 min at 6000g
- 6) Mix 375  $\mu$ L sample dilution buffer with 250 $\mu$ L of the supernatant
- 7) The sample pH should be 2.20, otherwise adjust
- 8) The sample is now ready for analysis.

#### 9.04 Sample preparation for protein hydrolysate analysis

Before any protein or polypeptide can be subjected to amino acid analysis, the sample must be hydrolyzed to cleave the peptide bonds between the amino acids. Acid hydrolysis using hydrochloric acid is the most common hydrolysis method in use. However, the severe conditions of this method can partially or totally destroy some amino acids. Some amino acids are difficult to cleave and require different conditions to achieve complete hydrolysis.

The cleavage of the peptide bonds is dependent on the amino acid sequence and can the cleavage-rate can vary from protein to protein. Therefore it is often necessary to treat the samples with various hydrolysis-times (12, 18, 24, 48 and 72 h) to find the optimal one.

Hydrolysis must take place under O<sub>2</sub>-free conditions to avoid uncontrollable oxidation. Therefore, the hydrolysis container should be evacuated or filled up with nitrogen.

Anyway, the sulphur-containing amino acids methionine and cystine aren't stable during hydrolysis. For this reason, the user must oxidize the sample using formic acid to generate stable oxidized products of methionine and cystine. Because this treatment partially destroys Tyrosine and Histidine, two sample preparations must be done to measure all amino acids: with and without oxidation.

Another important factor is the ratio of sample volume to acid volume, which becomes relevant if carbohydrates are within the sample. The higher the amount of carbohydrates, the higher the volume of acid. The best relation between sample and acid must be between 1:10 and 1:1 (mg sample : mL hydrochloric acid).

The next chapters explain field-tested methods for preparation.

##### (a) Hydrolysis with hydrochloric acid

- 1) weigh 1 mg of protein in your hydrolysis tube
- 2) add 1 mL of 6 N hydrochloric acid (Tryptophan will be partially kept if 2 – 3 mg indolpropionic acid or thiolglycol acid are added)
- 3) freeze solution and evacuate tube with vacuum pump
- 4) Close tube by melting
- 5) Incubate in an oven at 110°C for 12 to 72 hours. For reproducible hydrolysis results use an oven with air agitation.
- 6) Chill tube in ice after hydrolysis
- 7) centrifuge to remove solid particles
- 8) dehydrate using a rotary evaporator at 40°C
- 9) re-dissolve in 1 mL aqua<sub>dest</sub>
- 10) treat with rotary evaporate again to remove remaining acid
- 11) for measurement of cysteine: re-dissolve in 1 mL aqua<sub>dest</sub> and adjust pH to 6.50 with ca. 3 drops of pyridine. Heat for 1 h to 105°C and dehydrate again. This procedure transforms two molecules of cysteine to cystine.
- 12) Re-dissolve residue in 1 to 2 mL of sample dilution buffer
- 13) The sample is ready for analysis

##### (b) Hydrolysis with methanosulfonic acid

The method should be used for analysis of tryptophan together with the other amino acids, but only feasible for pure proteins and peptides.

Dehydration with rotary evaporator is not done as this procedure will not remove the methanesulfonic acid.

- 1) weigh 1 mg of protein in your hydrolysis tube
- 2) add 1 mL of 4 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl)-indol
- 3) freeze solution and evacuate tube with vacuum pump
- 4) Close tube by melting
- 5) Incubate in an oven at 120°C for 20 hours. The oven should keep a very constant temperature. For reproducible hydrolysis results use an oven with air agitation.
- 6) Chill tube on ice after hydrolysis
- 7) centrifuge to remove solid particles
- 8) neutralize solution with 1 mL of 3 to 4 N sodium hydroxide solution
- 9) dilute with 1 to 2 mL aqua<sub>dest</sub>
- 10) dilute 1:1 with sample dilution buffer
- 11) the sample is ready for analysis

### **(c) Hydrolysis of feedstuff**

This method should be used for sample material like grain or feedstuff etc. that contains other components besides proteins. For reliable measurement of amino acid composition, the sample weight has to be related to the total amount of nitrogen. Lipids in the sample must be removed before analysis.

Procedure:

- 1) Weigh sample material with 32 mg nitrogen into a 1000 mL flask
- 2) Add 800 mL 6 N hydrochloric acid
- 3) Install a reflux condenser and incubate the solution at 110°C in an oil bath for 24 h. During this hydrolysis step, aerate solution with nitrogen to avoid sample oxidation
- 4) Cool down to room temperature
- 5) Filtrate through 0.22 µm membrane
- 6) Dehydrate using a rotary evaporator at 60°C (but not too dry !)
- 7) Re-dissolve the still humid residue in 20 mL aqua<sub>dest</sub> and dehydrate again
- 8) Repeat the above washing process
- 9) Re-dissolve in 50 mL sample dilution buffer
- 10) The sample is ready for analysis

### **(d) Oxidation for measurement of Cystine and Methionine**

Cystine and Methionine must be transformed to their stable oxidized forms, cysteic acid and methionine sulfone, before hydrolysis for accurate quantitative measurement. Note, that this oxidation step destroys tyrosine and histidine.

Before starting with the hydrolysis procedure, mix a fresh portion of performic acid:

- a) fill 10 mL of 30% H<sub>2</sub>O<sub>2</sub>-solution in a graduated flask
- b) add to 100 mL with formic acid
- c) cool down to +2°C
- d) incubate 45 min in an refrigerator

Then continue with the hydrolysis procedure:

- 1) weigh homogenous sample that contains 32 mg nitrogen
- 2) add 100 mL performic acid and mix well
- 3) close flask and incubate 15 hours at +2°C
- 4) add carefully (drop per drop) 12 mL of HBr and stir continuously to avoid heating (this procedure eliminates excessive performic acid)
- 5) dehydrate the solution in a rotary evaporator
- 6) wash with 20 mL aqua<sub>dest</sub> and evaporate again
- 7) Repeat the washing procedure.

8) The residue is now ready for hydrolysis.

### (e) Alkaline Hydrolysis

The alkaline hydrolysis destroys more amino acids than the acidic hydrolysis, but tryptophan is preserved for 80%. For this reason, the alkaline hydrolysis is used for tryptophan-analysis of heterogeneous sample material (which cannot be used with methanosulfonic acid hydrolysis).

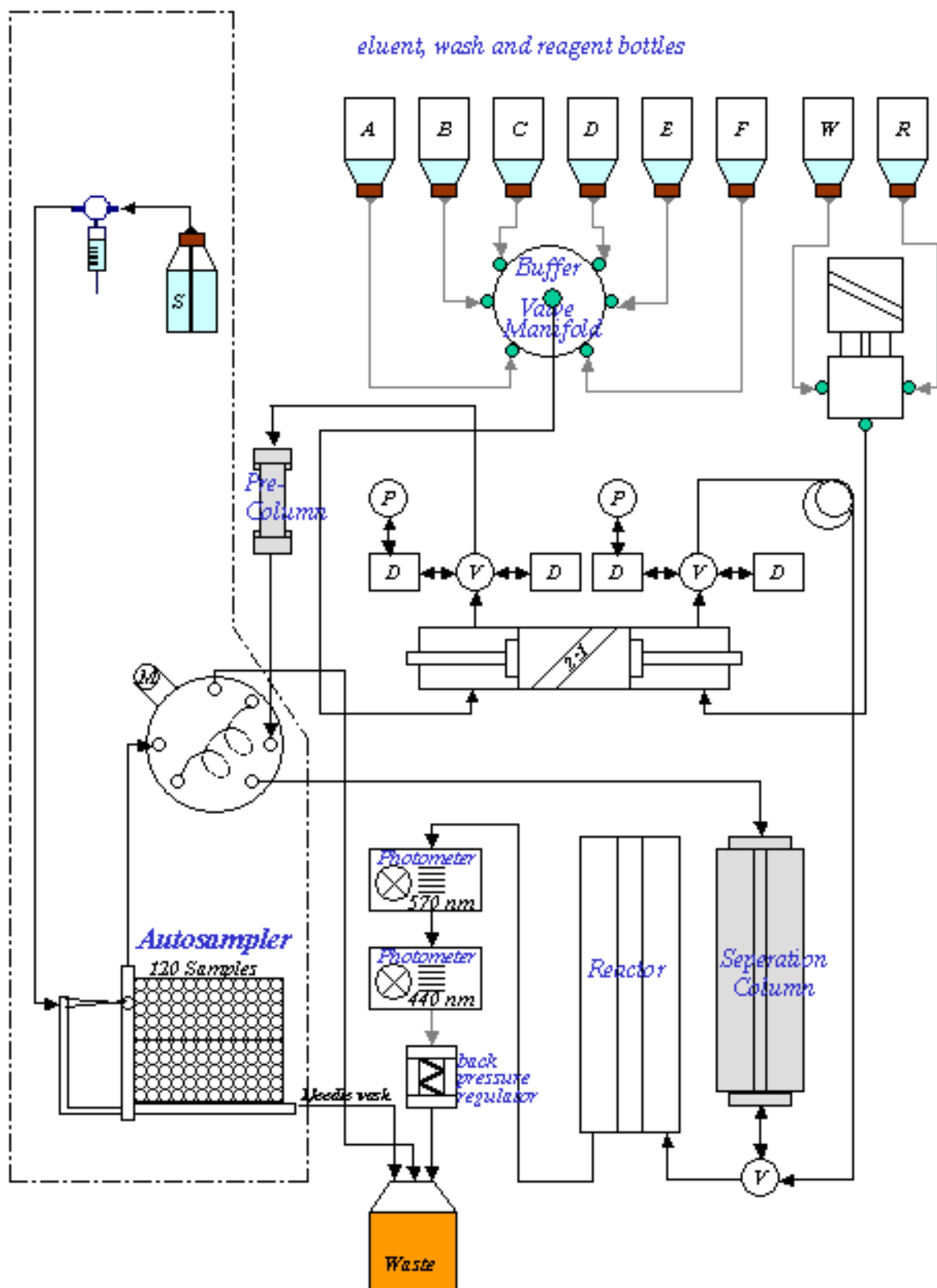
Barium hydroxide is used as base source, because  $Ba^{++}$  ions can be precipitated with  $CO_3^-$  or  $SO_4^-$  after hydrolysis. Other bases like sodium hydroxide can be used, but elimination of  $Na^+$  ions after hydrolysis is not possible which results in high salt concentrations that impair subsequent analysis.

The precipitation of Barium-ions is accompanied with loss of amino acids because of co-precipitation. Use Norleucine as internal standard to correct yields.

Procedure:

- 1) fill 200 mL of a 0.005 N HCl solution into a hydrolysis tube
- 2) add the protein solution (5 to 10 mg)
- 3) add solid Barium hydroxide ( $Ba(OH)_2 \times H_2O$ ) and aqua<sub>dest</sub> to the solution until you have a 4 N solution with a ratio of 0.2 mL/mg protein (heat to 110°C to dissolve Barium hydroxide).
- 4) Freeze solution
- 5) Evacuate the hydrolysis tube
- 6) Close tube by melting
- 7) Incubate at 110°C for 24 to 74 hours
- 8) Chill tube on ice
- 9) Add solution together with 10 mL hot aqua<sub>dest</sub> in a 100 mL centrifuge tube
- 10) Blow  $CO_2$  onto the fluid surface for 10 min while shaking
- 11) Centrifuge the precipitated salts
- 12) Rinse 3 time with 5 mL aqua<sub>dest</sub>
- 13) Reduce volume to 1 mL with a rotary evaporator at 28°C
- 14) Filtrate concentration through a 0.22  $\mu m$  membrane
- 15) dehydrate the solution in a rotary evaporator
- 16) re-dissolve in 5 mL sample dilution buffer
- 17) the sample is ready for analysis

## 10. Appendix B: Liquid Flow Scheme



## 11. Appendix C: Electrical and Chemical Precautions

The Amino Acid Analyzer was constructed and designed for safe and effective usage. Nevertheless, the user must keep in mind that working with electrical devices and handling with chemicals can causes hazards. Therefore, precautions have to be taken:

