# LabAlliance™ Gradient Control

Version 1.6.0.x

## User's Manual

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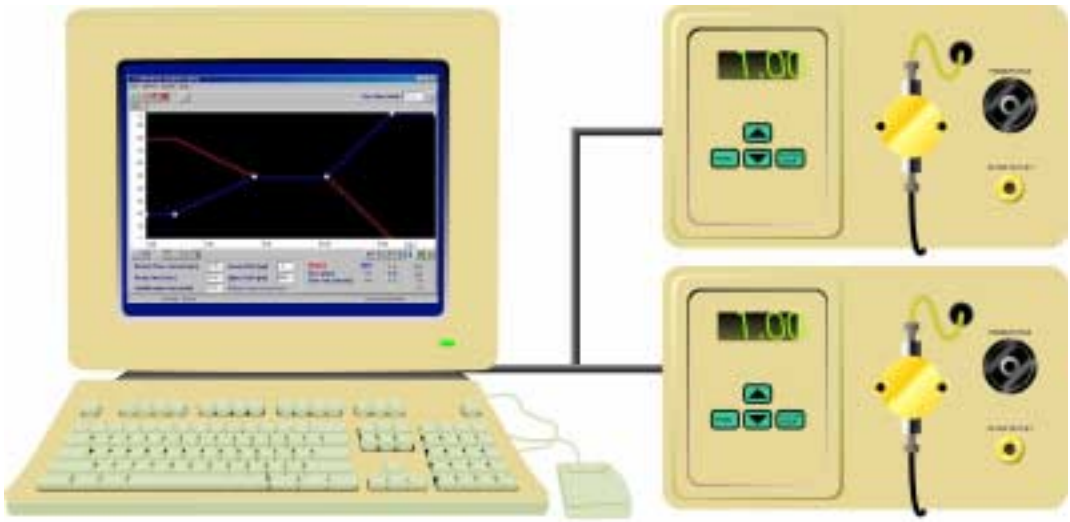
The LabAlliance Gradient Control software is a PC based HPLC pump control system designed to run under Windows. Any combination of LabAlliance/SSI HPLC pumps may be used to make a controlled HPLC gradient system.

Control of the pumps is highly intuitive. With RS232 cables that link the pumps through the com ports of a PC, a pair of HPLC pumps is easily transformed into a binary high-pressure gradient system. A special pump cable provides remote start input capability from a manual injector. You may also extend the system capability to enable control of a high-pressure quaternary gradient by merely adding two more pumps. Using MPX II System Interface up to four pumps can be controlled using one PC serial port.



*Typical Binary Gradient System Setup*

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# LIST OF FIGURES

## CONVENTIONS USED IN THIS MANUAL

| | |
|---|---|
| **File\|Save** | Program menu commands, selection by mouse click or by pressing *Alt* + underlined command character |
| *<Enter>* | Keys to press, shown in pointed brackets in italics |
| **[Save]** | Program control keys, shown in brackets in bold |
| *Solvents* | Program objects (windows, fields, functions), shown in italics |

# INTRODUCTION

*LabAlliance Gradient Control* Software is suitable for programming of binary, ternary, or quaternary gradient systems. It is also applicable to the computer control of an isocratic HPLC system. The designing of a gradient profile is as easy as drawing with a pencil. The unique **Graphic Gradient Editor™** (GGE) is implemented (Figure 1). This graphic programming tool allows you to create/edit the definition of gradient curve points (nodes) with a simple mouse click. Each node is specified by time, composition of mobile phase, and flow rate. The concentrations and the flow rates of consequent nodes are linearly interpolated. A very useful option is displaying the chromatogram in the background of the GGE area – this option is not available with only the pump control software. The usual method of directly filling in the gradient table is also available. The graphic presentation of the gradient profile is then dynamically updated.

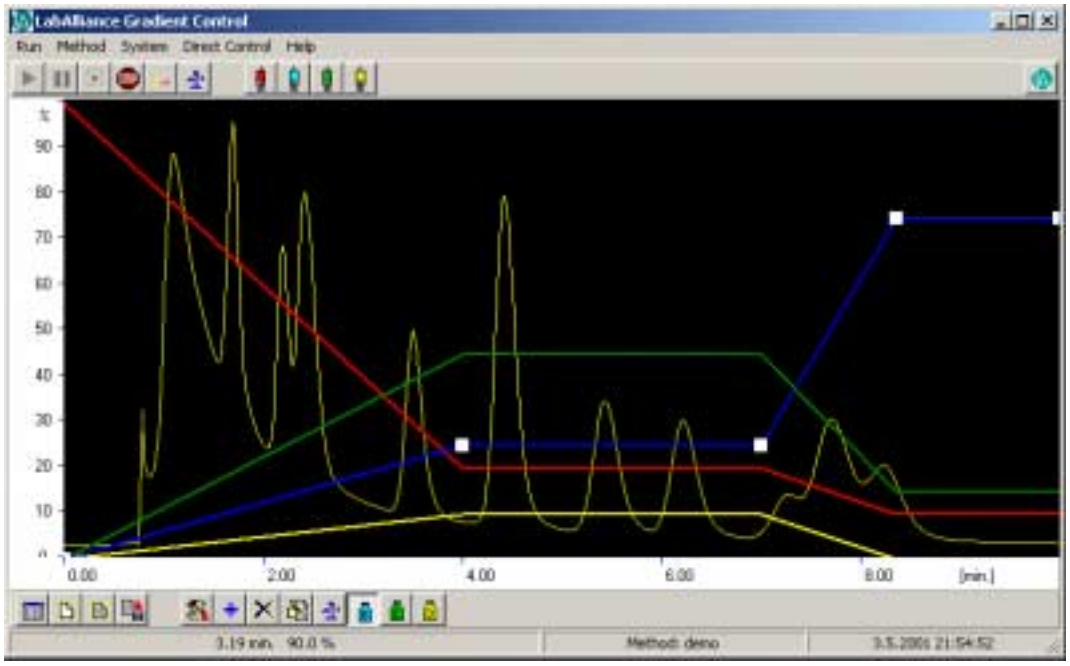



**Figure 1: LabAlliance Gradient Control main window**

# PROGRAM INSTALLATION AND CONFIGURATION

LabAlliance Gradient Control installation has the following steps (in the recommended order):

- Program installation
- Hardware Key installation
- System configuration

## *Program installation*

Insert CD-ROM into the drive and follow the instructions on the screen. If the CD-ROM Autoplay does not start or if you are installing the software from floppy discs, run the *setup.exe* file from the CD-ROM. After the installation, the software behaves as a demo version. In order to activate the full-version features, hardware key installation is required.

## *Hardware Key installation*

After installing the software all functions are active except the communication between the computer and the pumps. This version can be used for demonstration purposes.
To activate the communication (full version feature), the hardware key has to be correctly installed. Prior to installation of the hardware key, shut down the computer. Depending on the choice of hardware key made during purchase of the software, install the device to the printer (parallel) port or the USB port. If you did not specify the type of key, the default type is for the parallel port.
The second part of hardware key protection is the Sentinel hardware key driver. The Sentinel System Driver provides a communication path between Gradient Control Software and the Sentinel hardware key. The driver installation uses the Windows Installer. This allows you to install the driver for any Windows 9x/NT/2000 system just like a regular application. Use the following procedure.

1. Run the "RainbowSSD539.exe" from the Sentinel directory of the installation CD.
2. If you do not have the correct version of the Windows Installer, it will automatically be installed for you.
3. If setup detects an older version of the Sentinel System Driver, you will be prompted to confirm the upgrade. The older version is uninstalled immediately after you confirm the upgrade.
4. Select "complete" installation. This will install both the parallel and USB driver. Keep in mind that if your operating system

does not support USB (e.g. Windows NT), the installer will not install it even if you select this key option.

5. Continue with the installation, select "Install."
6. If you are running Windows 95/98/ME, you will have to reboot the computer when the installation is completed. Windows NT and 2000 do not require a reboot.

Upon restarting the computer and bringing up the gradient program, the full version features will be available without any restrictions.

## *System configuration*

*External Synchronization Setup*

LabAlliance Gradient Control software allows you to synchronize the operation with other software (such as data acquisition software) and/or injection device (manual injector, autosampler).

Go to **System|Synchronization Setup** to open synchronization setup dialog window (Figure 2). Check 'Enabled' to enable.

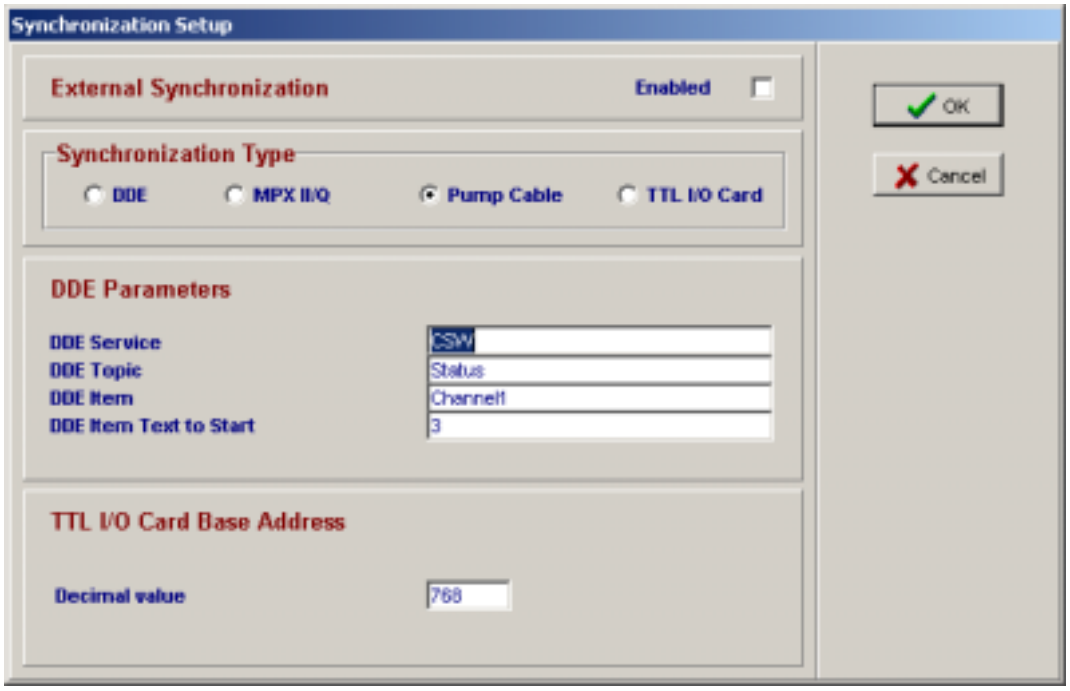



**Figure 2: Synchronization Setup**

Synchronization can be performed by different ways:

- DDE Communication

- TTL/contact closure compatible connection through MPX Pump Interface box
- Contact closure/TTL using pump cable with external start option
- TTL compatible I/O card installed into PC

DDE (Dynamic Data Exchange) is done through the internal Windows communication tool. One application assigned as the DDE server sends commands to another application assigned as the DDE client. In the present case, the client (Gradient Control software) can be triggered from another Windows software, if the latter is capable of submitting the necessary signaling data as DDE server. An example of the DDE synchronization with a channel 1 of the LabAlliance Chromatography Station for Windows (CSW) software is shown in Table 1. The *DDE* option in the *Synchronization Type* must be selected. The Gradient Control working cycle is started immediately when CSW channel 1 state turns to RUN.

**Table 1: Example of DDE parameter settings for channel 1 of CSW software**

| | |
|---|---|
| DDE Service | CSW |
| DDE Topic | Status |
| DDE Item | Channel1 |
| DDE Item Text | 3 |

Check the appropriate radio button (MPX II/Q) or Pump Cable, if the other type of synchronization is required.
Check or uncheck the Enabled to enabling/disabling of the synchronization.
By pressing **[OK]**, the new settings will be accepted and the window will close. By pressing **[Cancel]** the window will close without any changes.

### *Pump Setup*

Each pump of the gradient system is controlled via one of computer or MPX II serial communication (RS232) port. The pump parameters and communication port must be selected in the pump setup dialog (Figure 3). This can be accessed by **System|Solvent Delivery System**.

## Using MPX II System Interface

When using the MPX II Interface, connect the PC port to free com port on PC. The pumps are connected to the MPX-A,B,C, D outputs. On the MPX Panel of *Pump Setup* dialog select the communication port to which the MPX II Interface is connected.

## Max. flow, Max. pressure

Specify the pump type by the maximum flow rate and pressure from the drop down lists.

## Com port

Select the communication port to which the pump is connected. When the pump is connected to the MPX II interface, select the appropriate MPX output channel (e.g. MPX-A) for the pump. The pumps B, C, D must be enabled by check box on pump panels to be accessible from the gradient control software.

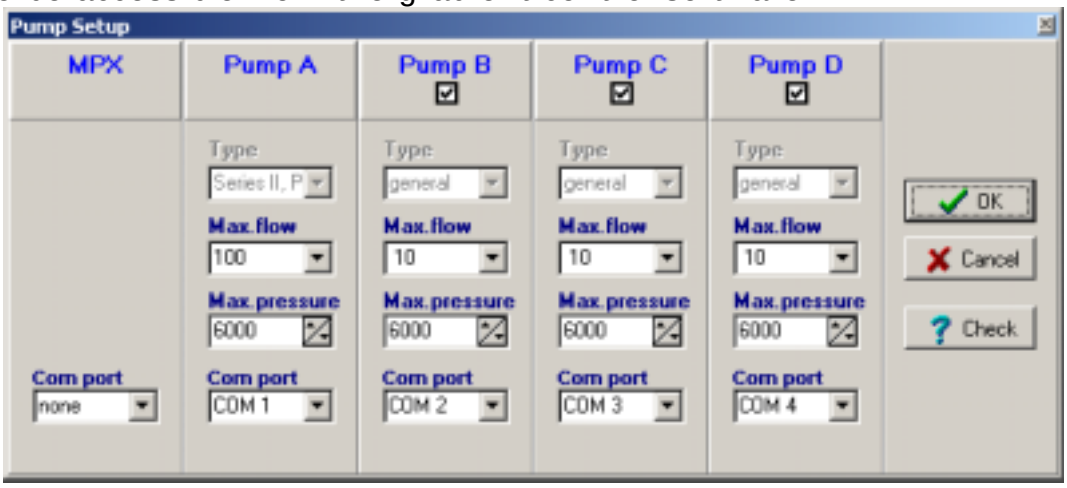



**Figure 3: Pump Setup Dialog**

By pressing [OK], the new settings will be accepted and the window will close. **In order to use the new settings, the program has to be restarted.** By pressing [Cancel] the window will close without any changes. The [Check] button performs a simple test of com port assignment. Incorrectly assigned pumps are automatically disabled.

*Directories Setup*

Directories Setup Dialog allows you to set the default directory for saving the gradient method files. The dialog box may be accessed through **System|Default Directories**.

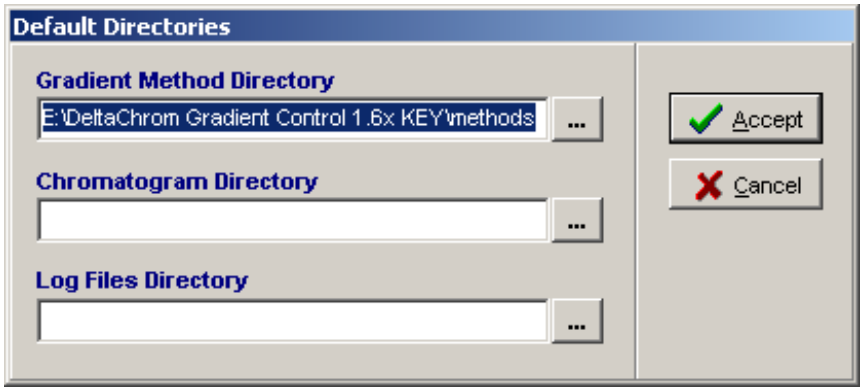



**Figure 4: Setting Directories**

# PROGRAM CONTROLS

## Graphic Gradient Editor Window

The basic tool in the Graphic Gradient Editor (see Figure 1) is the mouse. When the mouse cursor moves inside the GGE window, the actual coordinates (time and concentration) are displayed within the Status Bar. Depending on a selected editor mode, the left button mouse click performs one of the following operations: add node, delete node, edit node.
Right button click opens the Manual Setting window (Figure 5), which allows you to enter the values manually.

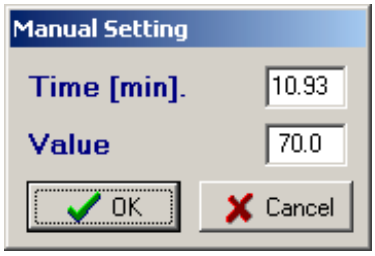



**Figure 5: Manual Setting Window**

## Program Menu

### *Run*

*Start*
Starts the working cycle of the *LabAlliance Gradient Control.* Depending on the external start option (disabled/enabled) the system starts performing the gradient (Run phase), or awaits an external start.

*Stop*
Immediately stops the gradient run, and goes to the initial solvent composition through the *Delay* phase. When the external start option is enabled, the system waits for the next injection start signal.

*Abort*
Immediately stops the gradient run, and goes to the initial composition and the *Idle status* through the *Delay* phase.

*System Exit*
Exits the *LabAlliance Gradient Control* software.

### *Method*

*New*
Clear the gradient time-table and GGE environment. Sets the default Run time, flow rate and 100% of solvent A.

*Open*
Opens the gradient method from disk.

*Save*
Saves the gradient method to the disk.

*Default Setting*
Opens the Method Default Setting Dialog.

*Init*
Sends the initial composition and flow rate to the pumps and initiates pumping.

### *System*

*Synchronization Setup*
Sets up the external synchronization option.

*Solvent Delivery System*
Sets up the pump types and communication ports used.

*Flow Calibration*
Activates the flow calibration wizard.

*Default Directories*
Opens the Default Directories Setup dialog.

### *Help*

*About*
Displays a current version of the *LabAlliance Gradient Control* software and its serial number. This information is required when contacting technical support.

## Toolbars

### *Editor Toolbar*

The Editor Toolbar (Figure 6) is located under the GGE area. It controls most of the GGE functions and Method handling functions. The toolbar buttons are accessible only in the *Idle status* of the gradient control software.

**Figure 6: The Editor toolbar**

*Show Time Table* 
Displays the gradient method table (Figure 10). A detailed description of the gradient time table is available on page 18.

*New Method* 

Clears the GGE area and the gradient table. The default solvent composition of 100% of *A* and *default flow rate* is set.

*Open Method* 
Opens the gradient method from a disk.

*Save Method* 
Saves the gradient method to the computer disk.

*Method Default Setting* 
Opens the Method Defaults dialog (see detailed description on page 15).

*Add Node* 

Add, Delete and Edit Node buttons switch the Graphic Gradient Editor mode. Add and Edit modes also require selection of one of the Flow Rate, Solvent B, Solvent C, Solvent D buttons.
The Add Node mode allows insertion of a new node into the gradient method.

*Delete Node* 
A left mouse button click within the GGE area will cause the nearest node to be deleted.

*Edit Node* 
A left mouse button click within the GGE area will cause a modification of the nearest node. The concentration of the selected solvent is updated according to the cursor position, and the concentrations of the other solvents are at the same time recalculated.

*Flow Rate* 
Selects the flow rate as editable parameter.

*Solvent B, C, D* 
Alternatively selects the solvent B, C, or D as an editable parameter for GGE operations Add or Edit.

### *Run Control Toolbar*

The Run Control Toolbar (Figure 7) is located in the upper part of the gradient control main window.

**Figure 7: Run Control Toolbar**

*Start* 
Has the same function as the menu command **Run|Start**. Starts the working cycle of *LabAlliance Gradient Control.*

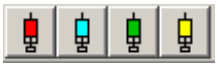
*Stop* 
Has the same function as the menu command **Run|Stop**. It immediately stops the gradient run, and returns to the initial solvent composition through the *Delay* phase. When the external start option is enabled, the system waits for the next injection (start).

*Abort* 
Has the same function as the menu command **Run|Abort**. It immediately stops the gradient run, and returns to the initial solvent composition and to the *Idle status* through the *Delay* phase.

*Panic Abort* 
Immediately stops the gradient and pumps. It will be necessary to re-init and flush the pumping system after the *Panic Abort* command has been used.

*Init Method* 
Has the same function as the menu command **Method|Init**. It sends the initial composition and flow rate to the pumps and starts pumping.

*Show Pump Status Window* 
Opens the pump status window.

*Prime buttons* 
Activates the PRIME function on each A, B, C, and D pump.
After initialization (  ), select the pump to be primed.

*About* 
Has the same function as the menu command **Help|About**.

## Pump Status Panel

The actual status of the gradient system is shown within the Status Window (Figure 8). Status parameters (Status, Time, Flow rate, Pressure and solvent proportions) are continuously updated.
The main program window of gradient control software can be minimized by  (*Hide Main Window*) and restored by  (*Show Main Window*) buttons. The *Panic Abort* (  ) button has the same function as desribed above.
The pump status panel can be closed by *Cancel* (  ) button.

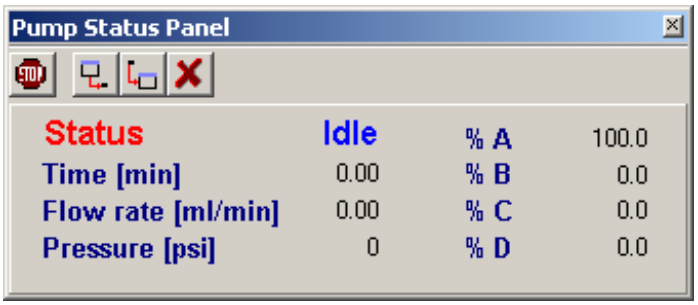



**Figure 8: Gradient Status Window**

## Method Default Setting

The Method Default Settings Window (Figure 9) maintains general parameters of the gradient method.
*Run time* specifies the total method duration.
*Default Flow rate* is used when a new gradient method is being created.
*Delay time* is the time taken for the solvent composition to go back from the final to the initial value. The final and initial values are linearly interpolated.
*Lower* and *Upper pressure limit*s are continuously compared with the current pressure. If the value exceeds the limit, the solvent delivery system is stopped.

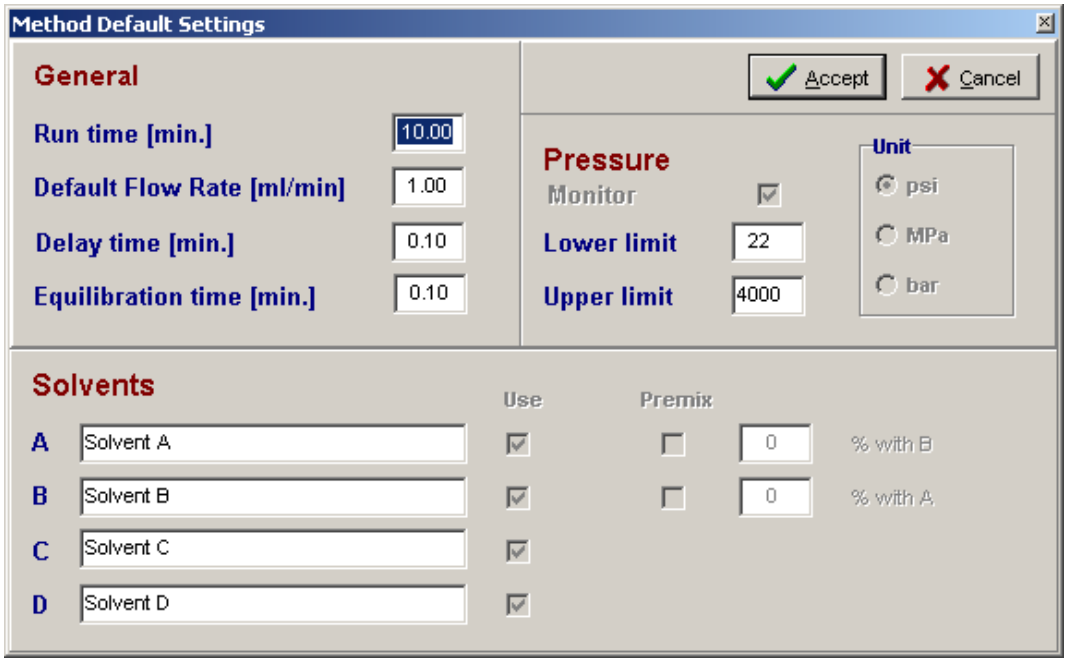



**Figure 9: Method Default Settings**

## Time Table

The Gradient *Time table* is an alternate method of programming the gradient. The gradient method created in GGE can be also modified in this *Time table*.

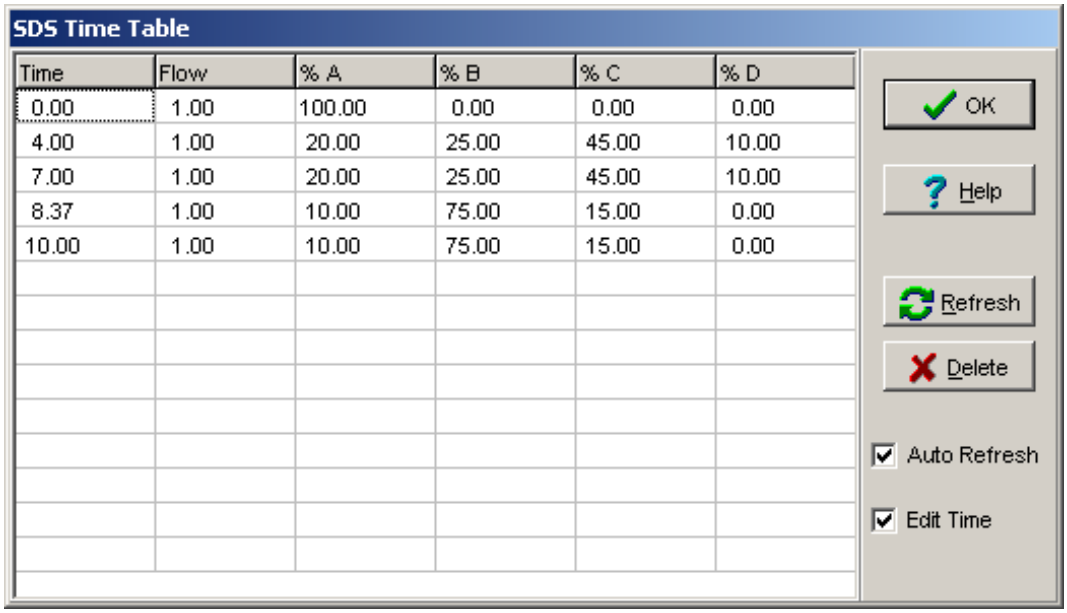



**Figure 10: Gradient time table window**

***[Ok]*** *button*
Closes the *Time table* and accepts all changes made to the gradient method. The graphic presentation in the GGE area is also updated.

***[Refresh]*** *button*
Updates the GGE main window according to *Time Table*.

***[Delete]*** *button*
Deletes the current row of the *Time table*.

***[Auto Refresh]*** *check box*
Immediately sorts the *Time table* and updates the GGE area, after the *Time table* a content has been modified.

***[Edit Time]*** *check box*

This option must be enabled in order to change the 'Time' column values.

# PRINCIPLES OF OPERATION

## Working cycle

Figure 11 displays the working sequence of the gradient system. The first run begins with the *Run phase*. The mobile phase composition and the flow rate are defined by the profiles displayed within the GGE area.
The *Run phase* is followed by the *Delay phase*. During the *Delay time* the solvent composition and flow rate will be changed from the final to the initial values.
If the *External start* option is enabled, the gradient system is then equilibrated - *Equilibration phase*. The system then waits for the next injection.

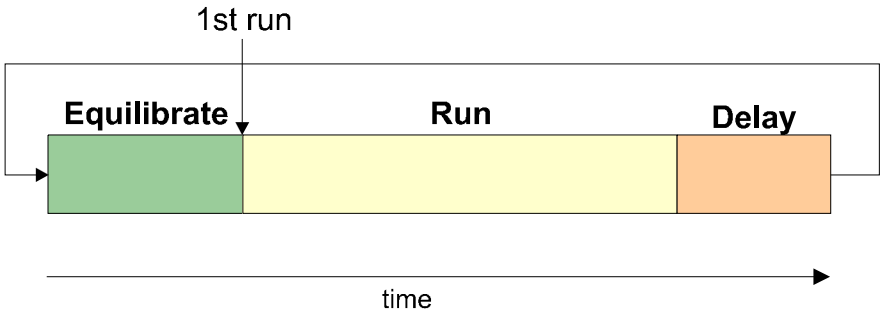



**Figure 11: Working Cycle of the Gradient Control**

## Gradient Method

Gradient Method maintains all general parameters and solvent concentration profiles. It can be saved to disk for future use. The method file header contains the parameters from the *Method Default Settings window*. Then follows the gradient *Time table*.

## Solvent Priority

Recommended order for solvent proportioning at each node of the gradient profile should be as follows:

| Step | GGE Mode | Current Solvent | B | C | D | A |
|---|---|---|---|---|---|---|
| 1. | Add Node | B | Define 0 to 100% | 0 | 0 | 100-B |
| 2. | Edit Node | C | From step 1. | Define 0 to (100-B) | 0 | 100-B-C |
| 3. | Edit Node | D | From step 1. | From step 2. | Define 0 to (100-B-C) | 100-B-C-D |

## Basic Operation

The programming of the gradient method can be performed by the *Graphic Gradient Editor* (GGE) or by the Gradient *Time table*. Within the GGE, the chromatogram can be placed in the background of the GGE area – this option is not available with only pump control.
The preferred method for setting a gradient from literature data is the *Time table*.
In order to create a new gradient method, there are several steps to be followed:

1. Fill in all the items in the *Method Default Settings window* (*Default run time, flow rate*, *Delay time*, *Equilibration time*, *Lower Limit*, *Upper Limit*).
2. Press the **[New method]** button on the Editor toolbar.
3. Using the **[Add node]**, **[Edit node]** and **[Delete node]** (or Time table) with respect to solvent priority define the gradient profile.
4. Save the method to the disk using the **[Save method]** button on the Editor toolbar.

### *Add*

Follow these steps to add a new node:

1. Click the **[Add Node]** button (on the Editor toolbar).
2. Click **[Solvent B]** button on the same toolbar.
3. Move the mouse cursor to the desired position on the GGE area. Look for an appropriate value of time and the concentration on the status bar if necessary.
4. Click the left mouse button

### *Edit*

Use the following steps to modify the existing node:

1. Click the **[Edit Node]** button on the Editor toolbar
2. Click the solvent or flow rate buttons on the Editor toolbar
3. Move the mouse cursor near to node that needs to be changed. Look for an appropriate value of the concentration on the status bar.
4. Click the left mouse button

It is not possible to edit the gradient while the system is running.

### *Delete*

Click the **[Delete]** button on the Editor toolbar to switch to the delete mode. Move the mouse cursor near to node that needs to be deleted and then click the left mouse button.

### *Save Method*

Click the **[Save method]** button on Editor toolbar to activate the save dialog. The last method saved is automatically opened when the *LabAlliance Gradient Control* program is restarted.

## Running the gradient

To start the gradient run:

1. Create or open an appropriate gradient method (verify the correct setting of the *External start enabled* option; menu **System|Synchronization Setup**).
2. Click the **[Init Method]** button on the Run control toolbar. The **Idle** status on the *Pump Status Panel* is changed to **Init**.
3. Wait until the signal of the HPLC detector stabilizes.
4. Click the **[Start]** button on the Run control toolbar. The status is now changed to **Waiting** (External start enabled) or **Run** (External start disabled)
5. Inject the sample.

## Stop the gradient

They are four ways to stop the gradient run:

1. Automatically, after the *Delay* phase when *External start* option is disabled. The final status is **Idle**. The pumps are still running.
2. By clicking the **[Stop]** button. Gradient program stops the *Run* phase and performs the *Delay* phase. The *Delay* phase is followed by the *Equilibrate* phase and the **Waiting** status (*External start* option enabled), or the **Idle** status (*External start* option disabled). The pumps are still running.
3. By clicking the **[Abort]** button. Gradient program stops the *Run* phase and performs the *Delay* phase followed by the **Idle** status. The pumps are still running.
4. By the **[Panic Abort]** button, which immediately stops all pumps.

## Direct pump control

The gradient software allows you to control all configured pumps directly from *Direct Control Panel* (Figure 12) accessible by **Direct Control** from menu.

After initialization (  ), the pumps can be controlled independent of each other. Enter the flow rate and press the [**Run]** (  ) button. To stop the pump press the **[Stop]** (  ) button on the corresponding pump panel. The **[Stop]** button on the upper (main) toolbar will stop all pumps.

The **[Exit Direct Control]** button (  ) closes the pump control panel.

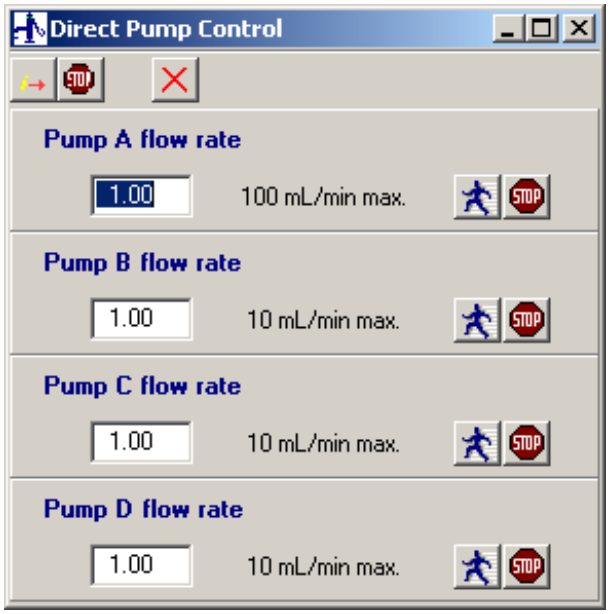



**Figure 12: Direct pump control panel**

## Flow Calibration

**System|Flow calibration** activates the *Flow calibration wizard* (Figure 13). Follow the instructions. The calibration results are stored in the computer memory. Calibration should be repeated when pump hardware was changed. The default setting of flow correction factors is 1.

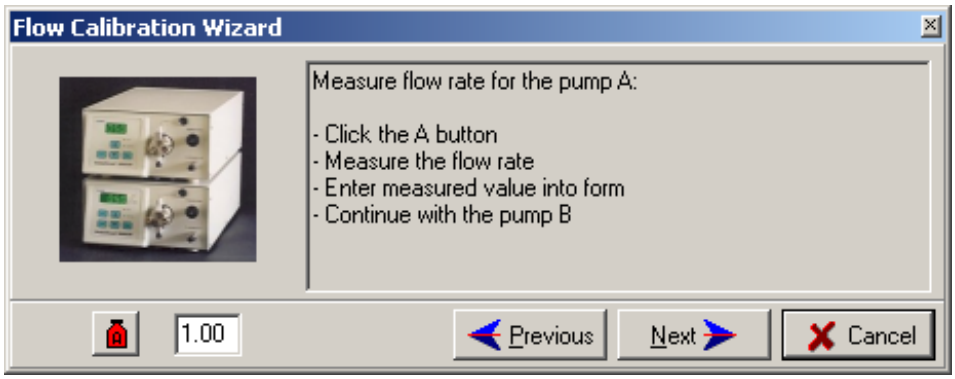



**Figure 13: Flow calibration wizard**

## CONTACTS FOR TECHNICAL SUPPORT

Should you have further questions, comments, or suggestions concerning the LabAlliance Gradient Control software, contact your distributor.

When contacting technical support, please provide the following information:

| | |
|---|---|
| User | |
| Product name | LabAlliance Gradient Control software |
| Program version | For example: 1.6.0.22 |
| Serial number | For example: 2101041 |