Panlab
An Affiliate of Harvard Bioscience, Inc.

USER MANUAL

PROTOWIN 1.1.01

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1. INTRODUCTION

PROTOWIN represents the evolution of PROTO5, the application for data acquisition from organ baths preparations from PANLAB.

PROTOWIN is a fully integrated portable package enabling researchers to carry out a thorough analysis of isolated tissue response to drugs in a large number of vessels simultaneously.

The application controls simultaneous data acquisition from the baths for periods only limited by the computer storage capacity. An analysis of the dose-response curves for each of the baths can be performed either once the data acquisition period is over, or even while data acquisition is being carried out. This analysis includes the study of the fitness of the observed values to theoretical models (linear and non-linear models) and a potency comparison between selected dose-response curves.

Data is shown on the computer screen while it is being acquired using the well-known Microsoft® Windows® graphical user interface. It can be stored on disk for later reviewing or for filing purposes. The data can also be stored in ASCII and now, in Microsoft® Excel® format making it possible to perform additional statistical analysis using standard packs.

1.1. New features

PROTOWIN includes the following new features:

- No additional communication cards are needed to connect organ bath device. Standard easy-to-plug USB 2.0 port is now used. This allows PROTOWIN to be execute even in a laptop PC.
- Fully Microsoft® Windows® compatible application.
- Graphical User Interface, mouse device and keyboard are used to interact with the application.
- Simultaneous visualization of multiple baths data acquisition.
- Up to 200 samples per second (200 Hz) can be acquired.
- Automatic backup system to avoid data lost while acquiring.
- Standalone experiment files to store multi-session data.
- Linear and sigmoid curve fitting calculations.
- Microsoft® Excel® data exportation format.
- Facilities for data illustration (bitmap exportation of graphical analysis results).

2. INSTALLATION OVERVIEW

The installation procedure is completely described for reading during the installation procedure. Therefore, they are stand-alone and can be executed separately as per your needs: acquisition (USB Key must be connected), analysis, reporting (printer must be installed), etc.

First of all, please check that your user has administrative rights on the PC or laptop in which the software or device is to be installed. Please contact your IT staff in order to clarify this issue before the installation procedure will be done.

Additionally, you will find details about how to configure some requirements which should be fulfilled to be able to install this system.

2.1. Requirements

PROTOWIN needs the following equipment:

- A fully compatible computer with at least:
  - 2,2 GHz Pentium® processor (Celeron not supported).
  - 2 Gb of RAM
  - HD 250 Gb (150 MB of free hard disk space).
  - Graphics: 1024x768 pixels and 32-bit true color.
  - 1 free USB port for the USB key.
- Connection interface:
  - 1 free USB port for the hardware device.
- Operating system supported:
  - Microsoft® Windows® 11 64bits
  - Microsoft® Windows® 10 32bits and 64bits
- Third-party software required:
  - Microsoft Excel ® (only for data exported in Excel format).
  - Microsoft Word ® (only for reports in Word format).

If external software is not available, some analysis reports cannot be generated. Please contact your IT staff in order to install the external software before analyzing sessions.

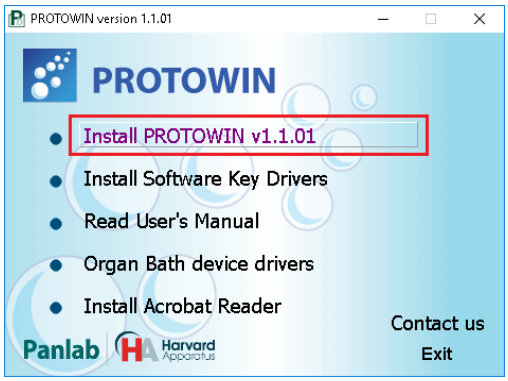
- Printer (recommended)

  At least one "virtual printer" must be correctly installed. Please refer to 2.4.1 for more details.

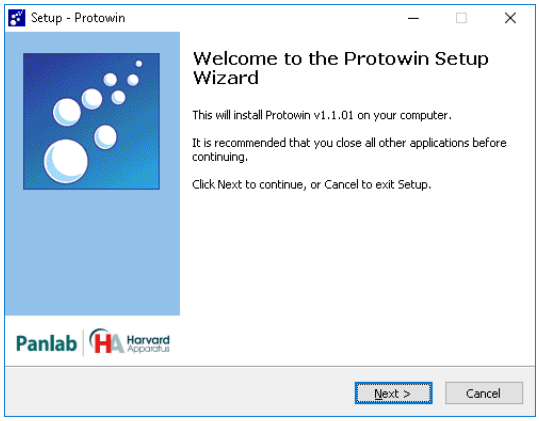
2.2. Installing Software

PROTOWIN software is delivered within a single USB flash drive. The USB flash drive contains the software installation tool, this User's Manual in PDF format and other components required to work in specific conditions.
Due to security reasons of the Windows® operating system, a user with administrative rights is required to install the software and other components. Please contact your IT staff before installing the software.
Once you get the administrative rights to install the software, please follow these steps:

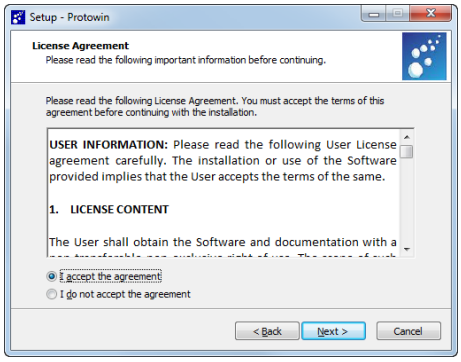
- Plug the USB flash drive in a free USB port of your computer and wait until Windows® installs it as a new removable drive.
- Access the new removable drive detected and execute the PANLAB.EXE file. A window will be shown, as below:



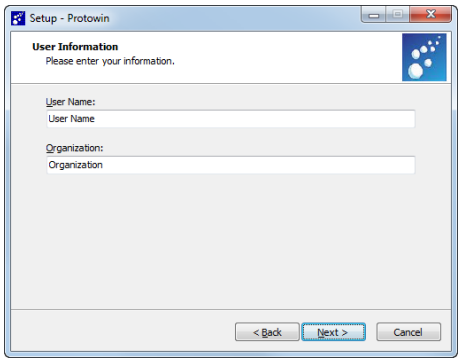
- Press the [Install PROTOWIN v1.1.01] button to start the software installation.
- An installation wizard will appear. Press the [Next] button to start the software's installation.



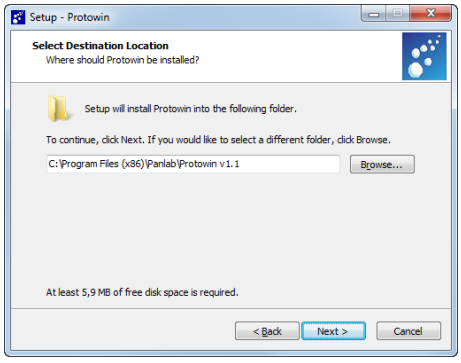
- Read the **License Agreement** and check the **"I accept the agreement"** option to continue the installation of PROTOWIN. Then press the [Next] button to start the installation.



- In the next window, introduce the name of the user and the company in the correct field. Press [Next] button to continue.



- During the installation process the software is installed in a new folder called [Panlab\PROTOWIN v1.1] created under the Programs Files folder. If desired, the installation program allows you to choose another folder to locate the software. The location of the software is independent of the data folder, which is defined by the user using the corresponding options of the program.



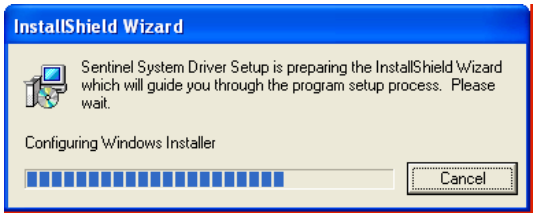
- Press the buttons [Next] and [Install] following the Install Wizard until reaching the [Finish] button.
- A new shortcut will appear on your desktop. Use it for executing the program later.

2.3. Installing software protection key

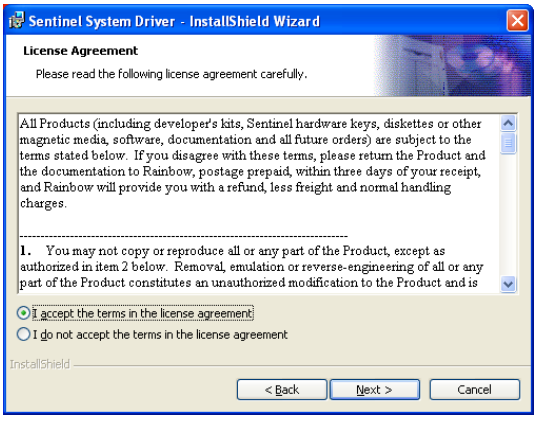
PROTOWIN software is delivered with a USB protection key that avoids fraudulent use of the application in a computer which does not have it installed.
Before installing the drivers, all applications must be closed and all USB SuperPro keys must be removed.
In order to do a correct USB key protection installation, please follow the steps below:

- Press the [Install Software Key Drivers] button to start the driver's installation.

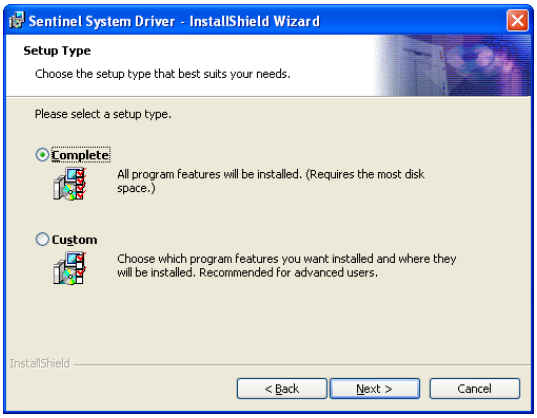
- Automatically a USB key wizard installation will be shown. When the Welcome screen appears, click [Next] to continue.



- Chose [I accept the terms in the license agreement] and click [Next] button to continue.

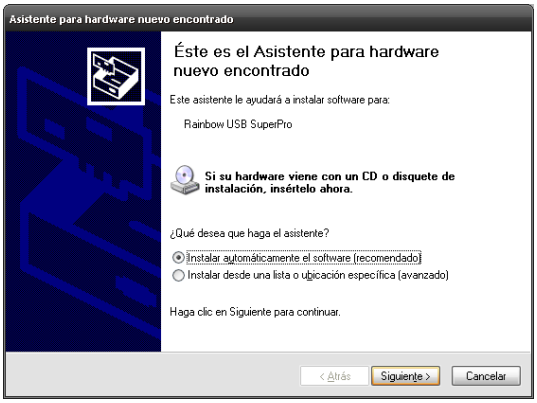


- On the incoming windows please select the [Complete] option of setup type and click [Next] and [Install] buttons to continue.

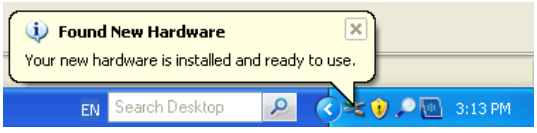


- After pressing [FINISH] button, you must reboot the system. Thus, your computer will recognize the USB security key.
- Did you reboot the computer? If you answer yes, please connect the USB key. In the lower right corner of the screen will appear the next message.

- The wizard for installing the drivers will run when your computer detected correctly the USB key. This process will need some minutes depending on your PC.
- Choose [Automatic Installation] and press the [NEXT] button.



- Wait while the wizard looks for the drivers until it asks you to press the [FINISH] button.
- Finally, a new message will appear in the lower right corner of your screen. The USB key was installed correctly.



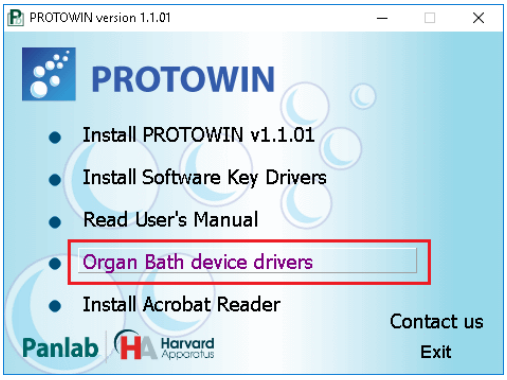
- Important remark: This step has to be repeated for each USB port of your computer.

2.4. Installing the organ bath

PROTOWIN is designed to control organ bath devices from PANLAB connected to a PC (laptop or desktop) through an USB 2.0 port.

Before plugging the organ bath unit, the device drivers included in the PROTOWIN USB Flash drive must be installed.

In the main installation window, choose Organ Bath device drivers.



Installation process will automatically start. Please note that up to 20 minutes can be needed to finish the process. When it finishes, please reboot your PC.

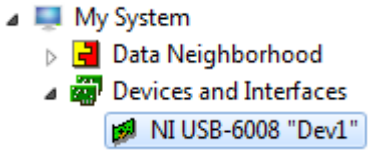
Continue the installation process according to the system you have.

2.4.1. Installing a 2 or 4 channels organ bath

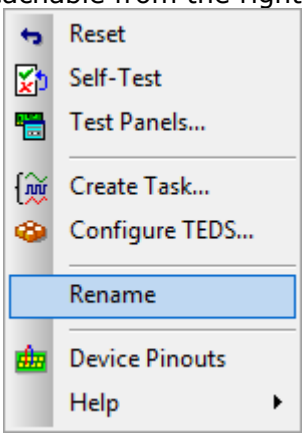
When your system is composed of a BR4740A unit, for proper installation you should follow these steps:

1) Install the organ bath driver software, as explained in the previous chapter.
2) Connect the BR4740A module in a free USB 2.0 port.
3) Check that the new device is recognized by the system and that the name assigned is "Dev1".

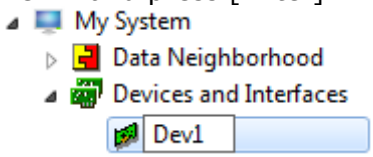
a. Open the NI-MAX application using the shortcut located on the desktop.
b. The USB NI-6008 "Dev1" item should appear in the "Devices and Interfaces" section.



c. If a different name has been assigned, modify it using the "Rename" command from the context menu, reachable from the right mouse button.



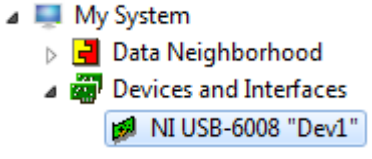
d. Type "Dev1" and press [Enter].



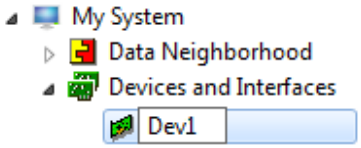
2.4.2. Installing a 6 or 8 channels organ bath

If your system is composed by two BR4740A units, please follow these steps for proper installation:

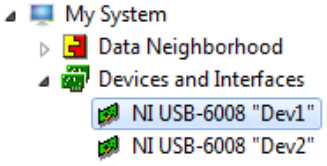
1) Install the organ bath driver software as explained in the previous chapter.
2) Connect the first BR4740A module that handles channels 1 to 4 to a free USB 2.0 port.
3) Check that the new device is recognized by the system and that the name assigned is "Dev1".
   a. Open the NI-MAX application using the shortcut located on the desktop.
   b. The USB NI-6008 "Dev1" item should appear in the "Devices and Interfaces" section.



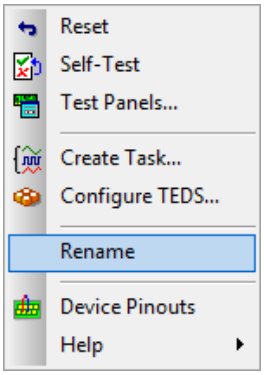
c. If a different name has been assigned, modify it using the "Rename" command from the context menu, reachable from the right mouse button.
d. Type "Dev1" and press [Enter].



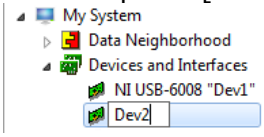
4) Connect the second BR4740A unit that handles the channels 5 to 6 or 8 to a different free USB 2.0 port.
5) Check that the new device is recognized by the system and that the name assigned is "Dev2".
   a. In the NI-MAX application, the USB NI-6008 "Dev2" item should appear in the "Devices and Interfaces" section.



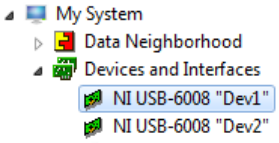
   b. If a different name has been assigned, modify it using the "Rename" command from the context menu, reachable from the right mouse button.



   c. Type "Dev2" and press [Enter].



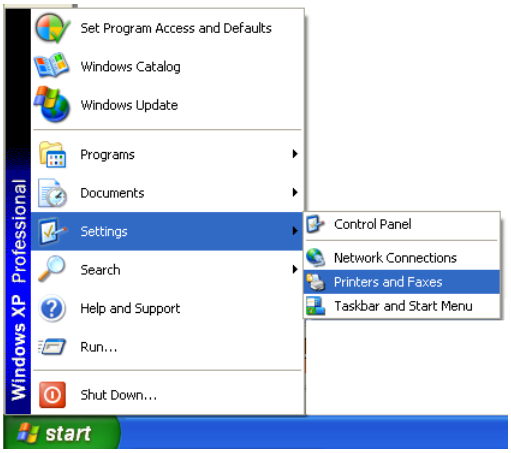
6) At the end you will have the following configuration:



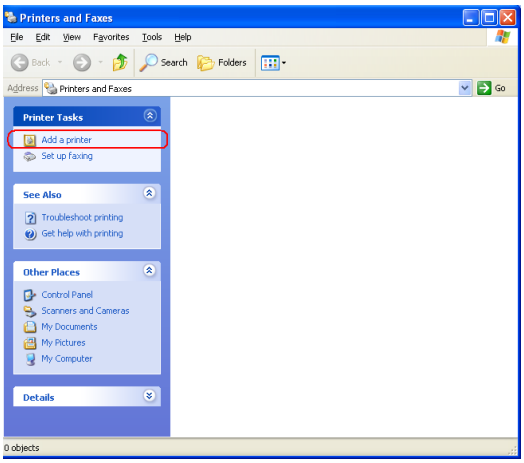
2.5. Installing a printer by default

If there is not a printer installed in your PC, one virtual printer must be installed by default. If your PC is running Windows 10, there are two virtual printers installed by default (Microsoft XPS Document Writer and Microsoft Print to PDF) and no additional action is required; but for earlier Windows versions, the next steps must be followed to fulfil the system requirements:

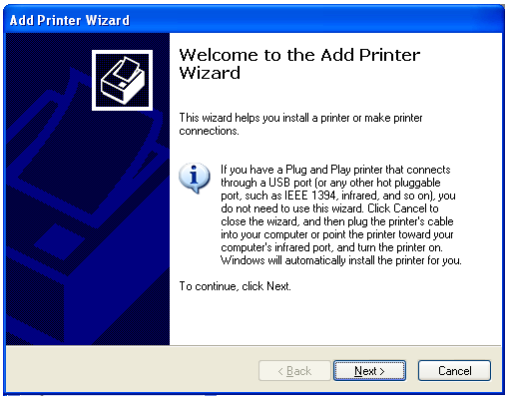
- Go to [Printers and Faxes] option of your system. The access is possible by clicking on [START – Settings].



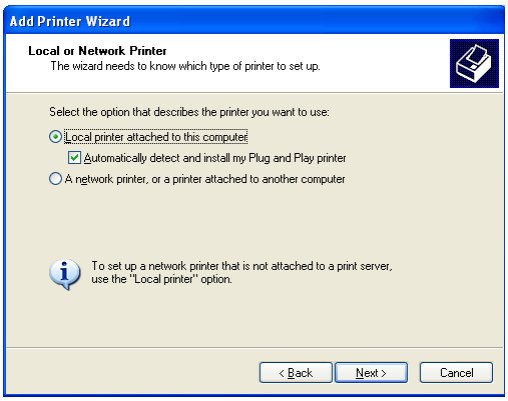
- In the [Printers and Faxes] window press on the [Add printer] button.



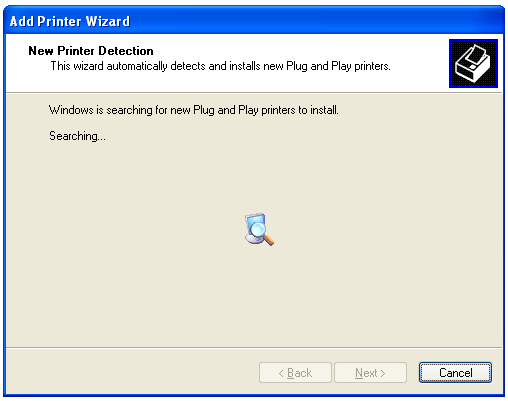
- The Welcome to the add printer wizard appears, click [Next] to continue.



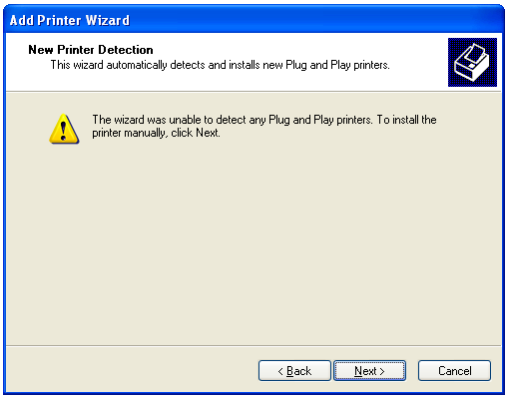
- As this procedure is for installing a virtual printer, the options must be selected as is shown in the next window. Press [Next] button to continue.



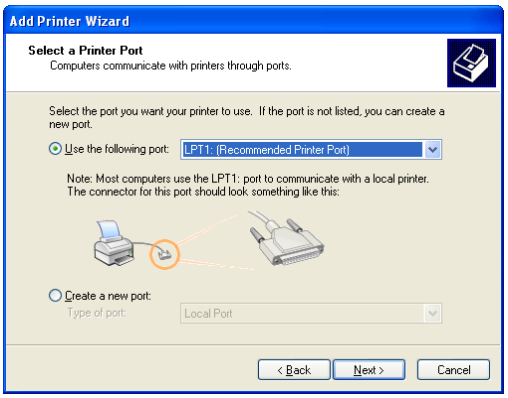
- The wizard will search the drivers for the virtual printer. This process will need some minutes depending on your PC.



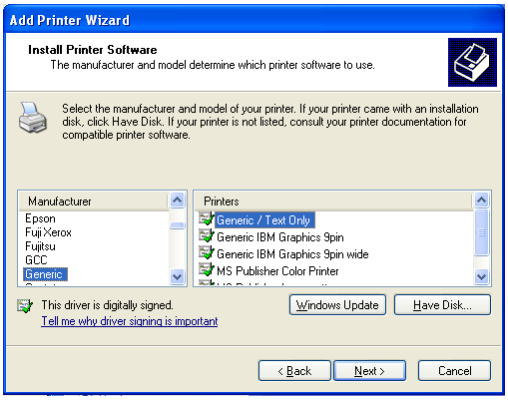
- The wizard notifies that there is not a real printer present. Press [Next] button to continue.



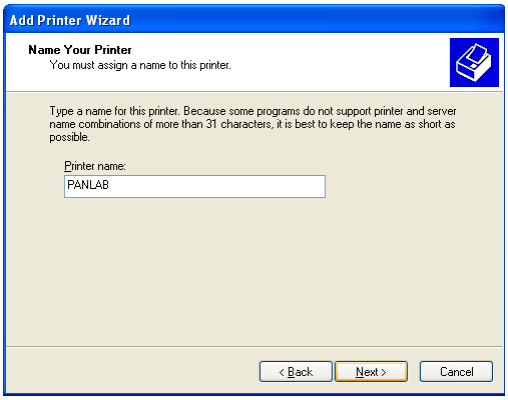
- Obviating the previous message, the options for the next window must be selected as is shown in the next picture before pressing the [Next] button.



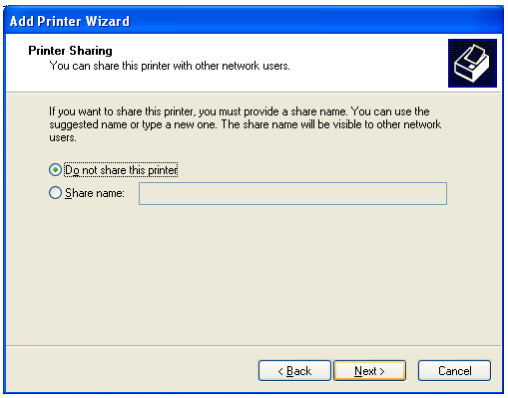
- The virtual printer will be a [Generic / Text Only] thus these options must be selected in the next window before pressing the [Next] button.



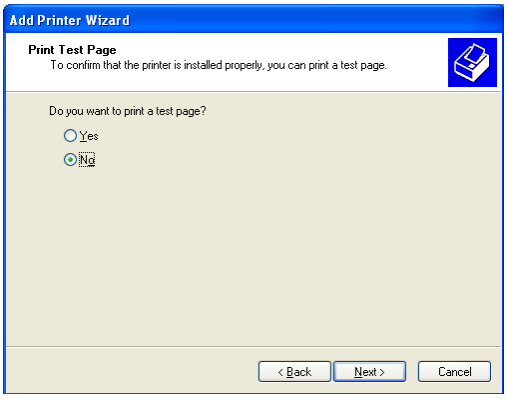
- This virtual printer can be called [PANLAB] thus it can be easy identified when a real printer will be connected to the system. The option [No] must be select before press [Next] button.



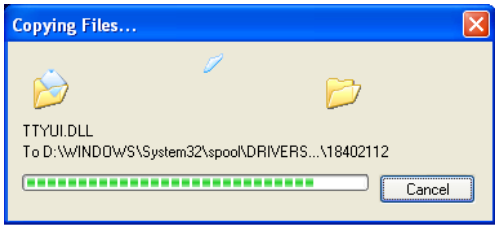
- Of course, is not necessary to share PANLAB printer thus select the [Do not share this printer] and press [Next] button to continue.



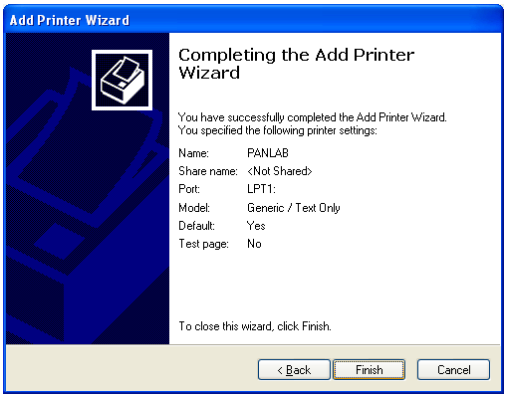
- Neither is not necessary print a test page thus selects [No] option for answering to the wizard and press [Next] button to continue.



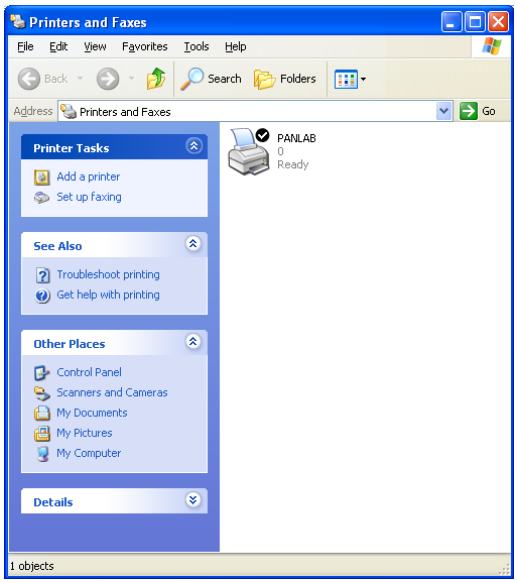
- The wizard will copy the drivers for the virtual printer. This process will need some minutes depending on your PC.



- When the virtual printer is successfully installed the wizard shows you a window as the next picture. Press [Finish] button.



- A new icon will appear into [Printers and Faxes] window called [PANLAB]. Close the window and launch the software.



3. GENERAL PROCESS

Studies of isolated tissue response to drugs using PROTOWIN can be carried out in several ways. General process could be divided into four main steps:

- **Experiment arrangement**: includes the configuration of the system, the creation of the experiment file, the arrangement of baths and vessels and the setting of all properties such as organ name, antagonists, etc.

  This task is needed only the first time the experiment is created but, of course, information can be changed during its execution.

- **Data acquisition**: its aim is to obtain data from organ bath devices. Data is organized into sessions (that represent a working day) and contain one or more **cycles**.

  Of course, several sessions can be executed during the same experiment.

- **Pre-analysis**: Defined cycles are now grouped into **curves** and initial regression calculations can be done.

  Current session ends once all curves are built and parameterized.

- **Post-analysis**: Saved experiment file can be retrieved and analyzed in the same **PROTOWIN** site or even in another computer without protection key.

  Analysis will return Child Plot, numerical data and graphical illustrations that can be exported into ASCII files and Microsoft® Excel® documents.

The rest of this User Manual will cover each of the previous steps in more detail.

4. EXPERIMENT ARRANGEMENT

4.1. Configuration

Before starting a new experiment, PROTOWIN site should be configured and calibrated in order to get right values.

[Config] menu allows set up:

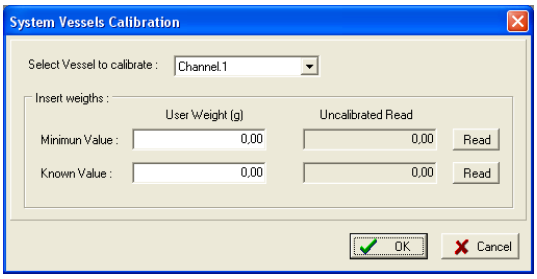
- Organization Info: name and details of your organization.
- Default folders: folders used to store experiment files, graphical illustrations (BMP) and numerical reports (Excels and ASCII files).
- Vessel calibration: explained in the next point.

4.2. Vessel Calibration

PROTOWIN must be informed as to what value represents the different organ bath readings in order to obtain reliable values for weights.

In order to calibrate vessels:

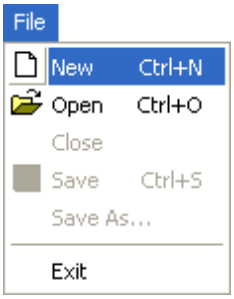
1. Select Config > Vessel Calibration option.



2. Select the desired vessel channel in the list.
3. Let force isometric transducer free of weight and click Read button associated to Minimum Value.
4. Enter known weight (any weight measures are possible) in Known Value field.
5. Put a known weight in the transducer and now, press [Read] button associated to Known Value.
6. Repeat steps from 2 to 6 for each vessel.

It is important that the highest calibration value corresponds to the maximum expected tissue tension. Otherwise, change the maximum calibration value until the desired work range is reached.

4.3. Preparing the experiment



To create a new experiment file, select [File - New] option.

Automatically an experiment is created with a bath window.

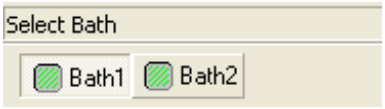
Experiment files can store multiple sessions data, only limited by the free memory of your PC, and organized with the following structure:

- Experiment
  - Baths
    - Sessions
      - ♦ Acquired data from a set of vessels.

4.3.1. The experiment window

Once a new experiment is created, the main window automatically shows the following elements:

- A toolbar, allowing:
- Arrange bath windows with  ,  and 
- Add and remove baths with  and  (see chapter 4.3.4).
- View and set bath properties with  (see chapter 4.3.6).
- Set basal line with  and  (see chapter 4.4.4).
- Create, edit and remove cycles with  and  (see chapter 4.5).
- A set of bath windows, explained in chapter 4.3.4.
- A bath selection bar, which allows User to select the current bath window among the available ones.



The bath selection bar also shows the name of the bath windows and the state of them ( means stopped and  means acquiring).

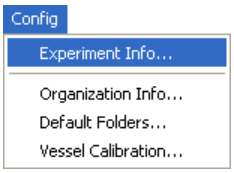
4.3.2. The signal preview window

In the course of any experiment, the input signal from the vessels can be monitored using the signal preview window provided that there is no session running.

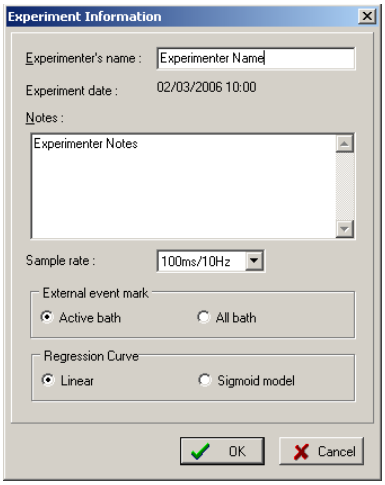
The experimenter can use this option in order to determine when to start or resume the acquisition process. Once the data acquisition starts, the preview window stops showing the signal. Later on, when the data acquisition is paused or stopped, the preview window visualizes the signal again.

The check option in the bottom of this window determines whether to show/hide automatically the preview window when the session acquisition finalizes/starts respectively.

4.3.3. Experiment information



Before starting a data acquisition session, experiment information should be entered through Config > Experiment Info menu option.



Sample rate is a very important parameter that should be selected because data precision, amount of stored data and processor effort will be directly affected by this parameter.

Sample rate represents the time interval in which a value is read from the organ bath device so, the higher it is:

- The higher the precision.
- The higher is the size of the file in the hard disk.
- The higher are the PC processor requirements.

PROTOWIN is able to acquire up to 200 samples per second (200 Hz = 5ms). However, additional processor speed and memory will be required (minimum of 2 GHz and 1Gb of RAM)

In this way, the maximum recording length of a session depends on the next factors:

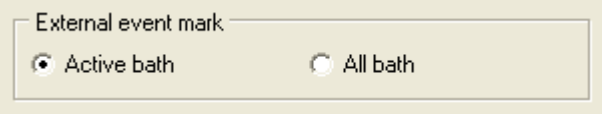
- Sample rate: when faster, less recording time.
- Number of vessels acquiring data: when more vessels, less recording time.

- Free memory in the system: when there is more memory, more recording time.

The next table illustrates the previous, based in a system with Windows XP, 512 Mbytes of memory and 300 Mbytes of free memory available:

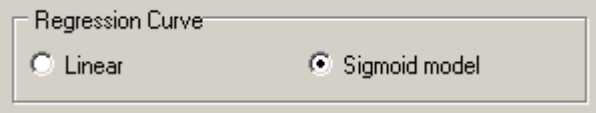
Sample Rate (Hz)	Number of Vessels			
	1	2	4	8
200	18:12	9:06	4:33	2:16
100	1.5 days	18:12	9:06	4:33
50	3.0 days	1.5 days	18:12	9:06
20	7.6 days	3.8 days	1.9 days	22:45
10	15.2 days	7.6 days	3.8 days	1.9 days
5	30.3 days	15.2 days	7.6 days	3.8 days
2	75.9 days	37.9 days	19.0 days	9.5 days
1	151.7 days	75.9 days	37.9 days	19.0 days
1/2	303.4 days	151.7 days	75.9 days	37.9 days
2/10	2.1 years	1.0 years	189.6 days	94.8 days
1/10	4.2 years	2.1 years	1.0 years	189.6 days
1/20	8.3 years	4.2 years	2.1 years	1.0 years
1/50	20.8 years	10.4 years	5.2 years	2.6 years
1/60	24.9 years	12.5 years	6.2 years	3.1 years

External events can be registered by pressing the pedal or push button connected to the amplifiers box. Events are a useful tool to, for example, visually identify the inoculations times in the graph.



Events are registered in the current active bath or in all baths depending on the option selected in the "External event mark" section. Each event is drawn in the record as a vertical line.

Moreover, regression curve model must be selected. By default, linear model is selected.



4.3.4. The bath window

Acquired data are read from an organ bath device connected to the system. The graphical representation of the organ bath device is called 'bath window'.

At least one bath window is needed to acquire data during an experiment. PROTOWIN automatically creates the first bath window when an new experiment file is generated but they can be also manually created using [+] button.

The name of the new bath window is automatically assigned with the word 'Bath' followed by a sequential number (starting from 1).

When a bath is created, a new button with its name is included in the bath selection bar located at the bottom of the main window.

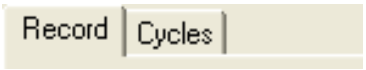
Selected bath window can be destroyed with the [-] button.

Acquired data from the bath is lost when the window is destroyed. That is why a confirmation is requested before completely destroying of the window.

Bath windows can be automatically arranged using  ,  and  buttons to facilitate viewing all acquisition channels simultaneously.

Each bath window has three main elements:

- Record area
- Cycles area



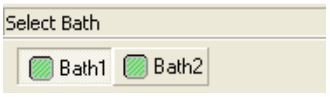
- Session control area

4.3.5. Vessels selection

An organ bath is formed by several vessels. However, not always all available vessels should be used to carry out a session.

Whether you want to choose from which vessels data acquisition will be done, please proceed to:

1. Show bath window, by pressing on the corresponding button in the bath selection bar.



2. In the bath window, click on desired vessel buttons with the right mouse button and check 'Selected' option in the dropped menu.

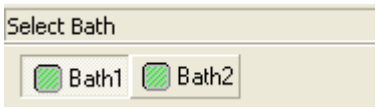
Vessel button will become as [Vessel] showing that is selected to capture data from it (red led) and also to view real-time graphic representation (pressed button).

4.3.6. Bath and vessels properties

Organ bath additional settings should be entered before starting a session.

To do that:

1. Show bath window, by pressing on the corresponding button in the bath selection bar.



2. Press 'Bath properties' button  on the toolbar.
3. Select cycle response interpretation from the list:

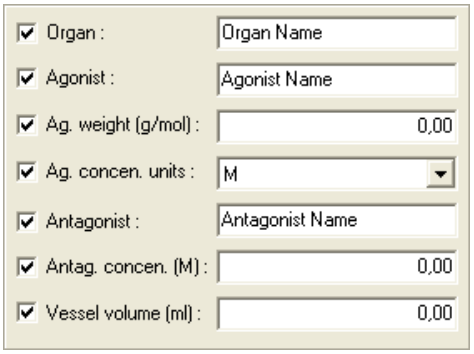
- Differential: cycle response will be measured as the difference between final and initial value.
- Integral: cycle response will be measured as the sum of all the values covered by the cycle.

4. If needed, enter a bath description.
5. Select 'Vessels' tab.
6. Select bath vessel from the list.
7. Set the following vessel properties:

- Organ: name of the organ to be experimented on in this bath.

- Agonist: name of the agonist solution to be experimented with.
- Ag. Weight: molecular weight (g/mol) of the agonist.
- Ag. Concen. Units: measurement units in which agonist concentration will be entered.
- Antagonist: name of the antagonist solution to be experimented with.
- Antag. Concen: antagonist concentration to be experimented with.
- Vessel volume: volume (ml) of the vessel.

Checked marks allow changes to be automatically applied to all vessels. If a different value is needed for another vessel, uncheck the field before changing the value.



8. If needed, set additional comments for the bath.
9. If needed, repeat steps 1 to 8 for each vessel.

4.3.7. Saving the arranged experiment

Now the experiment is arranged and prepared to start the data acquisition process. That is why this is the first moment in which experiment should be saved.

To do that:

1. Press 'Save' button  in the toolbar.
2. Given that experiment has not assigned name, PROTOWIN will ask you to set it and to choose the folder in which experiment file will be stored.

Default folder for experiment files can be set from Config -> Default Folders menu option. Please note that PTF extension is assigned to PROTOWIN experiment files.

This file extension (PTF) is associated with PROTOWIN so application can be launched with an experiment file making double click in the file icon in the Windows® Explorer®.

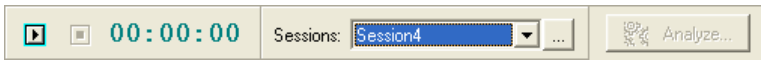
'Save' button will be then disabled showing that no more changes should be saved. 'Save' button will be enabled again in the next change you make in the experiment.

An experiment file can be stored with a different name (or in a different folder) using File > Save as menu option.Data acquisition

4.4. Starting a session

Once an experiment is correctly arranged, sessions can start.

Each bath window has its own session control toolbar (located at the bottom left side) like this:



To start a data acquisition session:

1. Press 'Start session' button  in the session control toolbar. A new session will start and its name will be shown in the list of sessions, pointing that it is the current session.

Please remember that the protection key must be correctly installed in the PC before starting a data acquisition task.

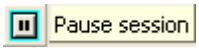
Bath selection button led will become red () and graphs of selected vessels will automatically start plotting recorded values in the record area.

Session chrono will also start, continuously showing the current session duration (hh:mm:ss).

2. Adjust balance control in the organ bath device in order to align current readings with the 0 value.
3. Now, put the organ tissue in the vessel.

As PROTOWIN can be executed in a laptop PC, please be sure to disable power management control because data loss can arise. Although PROTOWIN is designed to avoid this situation (showing a message), it is highly recommended to do that. Please refer to your laptop PC User Manual.

4.4.1. Pause started session



Pausing a data session acquisition:

Press "Pause session" in order to pause the acquisition process in the started session. This way the system interrupts the recording of the signal and the chrono indicator stops showing the time progress.

This option can be used for saving system hard disk space in lengthy experiments where it is necessary to wait too much time between two consecutive doses.

4.4.2. Resume paused session

Press "Resume session" in order to continue the acquisition process in a previously paused session. The chrono indicator shows the time progress again and the graph continues painting the signal.

Use the pause and resume options properly in order to accumulate different chunks of data in the same recorded session.

4.4.3. Record area

Record area is designed to show multiple real-time acquisition data plots and to allow the user to manage cycles.

Record area will contain as many graphics areas as vessels buttons were pressed but only 'selected' vessels will really acquire data.

A unique X axis shows the session acquisition time (hh:mm:ss). Y axis represents the measured weight in each vessel (in the units used during its calibration process).

Record area is designed to allow user the following tasks:

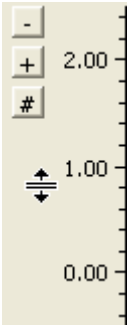
- Time zoom-in and zoom-out: using  and  buttons located at the bottom right side of the record area. The zoom list  can be also used.

Zoom is only applied in the time scale (X axis). Y axis maximum and minimum values are automatically adjusted when calibration process is done.

- Horizontal scroll: using horizontal scroll bar located just below time axis.

Automatic horizontal scroll (plot following) can be done by pressing  button located at the end of the horizontal scroll bar.

Each vessel chart has a vertical zoom tool bar on the left side of the Y axis. This tool bar appears when the mouse cursor is moved over the Y axis.



Press the buttons  "Zoom In" /  "Zoom out" in order to enlarge/ reduce the vision range in the Y axis respectively.

Press the button  "Auto Adjust" in order to adjust the vertical vision range to the height of the acquired signal.

The vertical vision range can be scrolled up and down by pressing and dragging the mouse along the vertical axis. Once you visualize the desired value release the mouse button and the graph will be repainted.

4.4.4. Setting basal level (base line)

Basal level should be set for the application to carry out right future calculations such as cycle responses.

To do that:

1. Make protocolar adjustments with the organ bath device until getting your zero-level value.
2. Click on the white area of the vessel record. A blue frame will appear rounding vessel plot.
3. Press 'Edit baseline' button  located in the main toolbar. A horizontal red line called 'baseline' will appear on the plot showing the current basal level.
4. Drag the 'baseline' up and down until the zero-level value.
5. Finish the process by pressing 'Set current baseline' button  . Following read samples will automatically be adjusted according to the new base line position.

4.5. Defining cycles

Cycle is understood as the set of data associated with an observed effect.

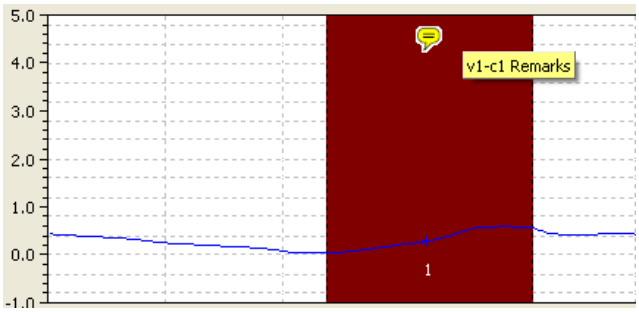
Cycle definition is a repetitive operation that should be done to make future calculations and analysis.

4.5.1. Drawing cycles in the plot

Cycles can be created both while acquiring data and while session is stopped.

To create cycles, you should switch to cycle edition mode by pressing 'Cycle editor' button  in the main toolbar.

Now, you can drag and drop into the plot to define the start and end of the cycle. When mouse button is released, a colored numbered region is shown over the plot indicating that a cycle has been created successfully.



When several cycles have been created, you can select one of them by clicking on it. Then, the region will become brown colored.

Cycle ends can be modified by dragging them horizontally with the mouse.

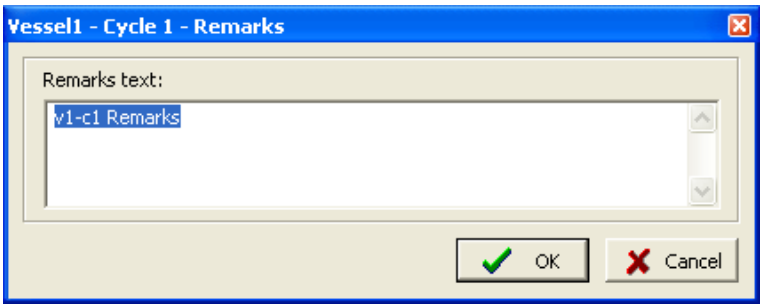
Selected cycle can be deleted by pressing 'Delete cycle' button  in the main toolbar.

4.5.2. Cycle remarks

A comment can be attached to each cycle in the chart. The system shows a balloon icon in the upper central side of the cycle.

When the mouse cursor is over the balloon icon, a hint popup label is displayed with the note comment.

In order to edit the comment of a cycle, click over the cycle remark icon. The system shows the remark edition window.

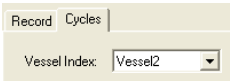


Type the remark text and press Ok for accepting and closing the remark editor window.

The balloon icon will change his color in order to identify that the cycle has a comment note.

To erase a comment note, just click the remark icon, leave the remark text box empty and then click the Ok button.

4.5.3. Defining cycles properties



Each new cycle is automatically included in the cycles table that is located in the 'Cycles' tab of the record area.

In this table, each cycle of the selected vessel is represented by a row with the following fields of information:

- Curve Index: number of the curve in which the cycle is grouped.
- Cycle Index: number of the cycle shown in the plot.
- Concentration: antagonist supplied dose. The first column shows measure in the units defined in the vessel properties. The second column always shows the measure in molars.

This value should be manually set by the experimenter.

To do that, simply double click in the cell and change the value. Then, press Enter key to finish.

- Contraction – Experience: experienced contraction calculated according to the interpretation method selected in the vessel properties (differential / integral).

This value is automatically calculated by the application taking into account the read value at the ends of the defined cycle:

- In differential mode, this value is calculated as the maximum value of the interval.
- In integral mode, this value is calculated as the integral of the signal in the cycle.

Manual changes can be done on white colored fields. In order to identify the situation, curve will be colored as 'Edited' when the rest of its properties have been correctly set.

- Contraction - % Max: estimated percentage value for each cycle response, in relation to the maximum observed response in the curve.
- This value remains undefined until a maximum response is selected in the curve (see later how to select a maximum effect).
- Contraction - % Est: this column joins estimated percentage value for each effect, according to the curve fitting between the decimal logarithm of the dose and the percentage observed effect (as calculated in the % MAX column).
- This value remains undefined until a valid dose-response curve is defined (see later how to define a dose-response curve).
- Contraction - % Ctr: means the percentage value of each observed effect in respect of the maximum observed value in the control curve is expressed.
- Cycle Interp.: allows User to select a cycle interpretation different to the method selected during vessels configuration.
- Dose Type: indicates the condition associated with each dose. Available options are:
- Null Not Graph: Null dose to be excluded in graphic procedures. This dose will be excluded from the dose-response curve calculus and will also be excluded from the graphic representation of the dose-response curve.

  This type of dose status should be used to eliminate an erroneous determination.
- Null Yes Graph: Null does to be included in graphic procedures. These type of doses will be included in the graphic representation of the dose-response curve but will be ignored in the dose-response curve calculus.

  By using this status, the lower part (e.g. doses under 16%) of the dose-response points can be included in the graphic representation but excluded from the calculus.
- No Max: Not maximum dose. These doses will be included in the dose-response curve calculus and will be represented in the dose-response curve graph.
- Max Not Calc: Maximum dose to be excluded from dose-response curve calculus. These doses are used to obtain the percentage effect of the "No Max" doses.

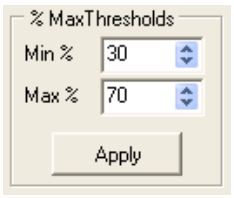
  Nevertheless, they are excluded from the dose-response curve calculus and included in the graphic procedures. By using this status, the obtainment of the percentage effect for each dose, related to the current maximum effect, is

possible. At the same time these maximum doses are excluded from the dose-response calculus (allowing the higher portion – e.g. over 84% - of the curve to be excluded from it) but including these points in the graphic representation of the curve.

- Max: Maximum dose. Only a dose in a dose-response curve can be defined as "Max". This dose is included in the dose-response calculus and in the graphic representation of the curve and also used in the percentage effect of the "No Max" doses.

  Should more than one Max dose be declared, the application will work out their average and take resulting values as Maximum effect

- Cycle Remarks: Shows the comment notes of the cycle.
- The comment notes are saved with the rest of the experiment information. The next time an experiment is loaded, the comment notes will be retrieved again.
- The cycle comment note can be edited from here. Double click over the comment icon in the cell and type the text in the remark window. After accepting the changes, the new text will appear in the corresponding cell.



Doses type of the current curve can be assigned automatically using then "% Max Threshold" box. By pressing the "Apply" button, the application does the following tasks:

1. Find the cycle MAX of the curve: If the curve has not any cycle defined as MAX, **PROTOWIN** search the cycle with a maximum effect and set it as MAX.
2. Only the cycles whose effect in % with respect to the cycle MAX (value in column "% Max") is between the two thresholds are marked as "No Max" and are used to calculate the DE50. The other cycles are marked as "Max Not Calc" or "Null Yes Graph".

   This functionality is a useful tool for linear regressions to quickly discard the cycles which effects are not between the lab standard thresholds.

Cycles table always show currently selected cycle in light blue color.

Cycles table can be exported to Microsoft® Excel® format using 'Export' button.

5. PRE-ANALYSIS

5.1. Defining dose-response curves

Defined cycles should be grouped into dose-response curves. By default, there are as many dose-response curves as vessels used in the data acquisition.

However, in each vessel, as many dose-response curves can be defined as desired.

5.1.1. Grouping cycles into curves

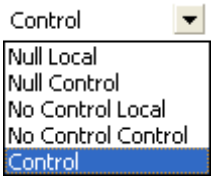
In order to do that, split operations should be applied to the current curve, obtaining a second curve with the rest of the cycles:

1. In the cycles table, click on any cell of a desired cycle.
2. Press 'Split' button , located at the right side of the table.

A new curve will be created and next cycle and the rest of the list will be automatically associated to the new curve.

To revert the operation, curve joining is possible with the 'Join' button.

5.1.2. Status of a dose-response curve



Associated with each of the defined dose-response curves, a status exists, which indicates the form in which the curve in question participates in the calculations thereafter.

The meaning of each dose-response curve status (Curve Type field in the table) is the next:

- Control: it is a reference curve, in respect of whose maximum effect, the percentage values shown in the % Ctr column are calculated.

  This definition only affects the current vessel. If more than one control curve is defined, the percentage values in the % Ctr column are calculated in respect to the average maximum effect of each of them (independently of the dose with which each of the maximum effects was obtained).

- No Control: these are the curves that should be included in the calculus but are not considered as controls. Note that these curves are, in fact, divided into "Local" or "Control" curves as described below.

- Null: in this case, the affected dose-response curve will NOT be considered in subsequent calculations and to such effect will be non-existent. Note that these curves are in fact divided into "Local" or "Control" curves, as described below.

- No Control Local / Null Local: In a Local curve (regardless of if the curve is a Null or No Control curve), the curve fitting is calculated using the % Max column as "y" values.
- No Control Control / Null Control: In a Control curve (regardless of if the curve is a Null or No Control curve), the curve fitting is calculated using % Ctr column as "y" values.

In this way, when obtaining the "% Est" column values (as estimated from the curve fitting), choosing between the control curve or the own curve maximum effect reference is possible for the Null or No Control curves. In a Control curve, the curve fitting is always obtained from the "% Max" column, as in a Local curve.

5.2. Curve regression

Regress...

Curve regression for the selected curve can be viewed by pressing [Regress…] button.

5.2.1. Curve regression models

As stated before, PROTOWIN is enabled to fit the dose-effect values both to a straight line or to a sigmoid curve.

The final selection between them depends on the "shape" of the plotted values and the nature of your experimental conditions.

The regression curve model can be selected through the Config – Experiment Info menu option.

5.2.1.1. Linear regression

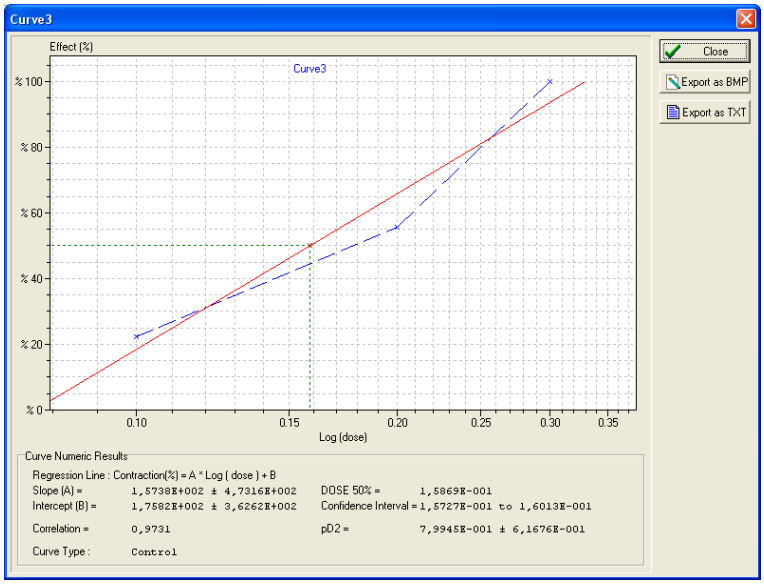
The linear regression model fits the dose-effect values (obtained from the defined cycles) to a straight line, obtaining the parameters that charity it from the following equation:

$y = A * x + B$

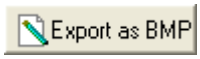
Being:

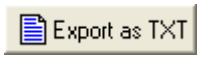
- X: Dose logarithm (base 10).
- Y: Effect %.
- A: Slope of the straight line (gradient).
- B: Intercept point with the Y-axis.

The following image shows the linear regression of an example curve:



Main area plots the dose logarithm against effect percentage, allowing to easily determine the dose-effect 50% (DE50).





Graphic can be exported in a bitmap file (BMP) for illustration purposes by clicking 'Export as BMP' button.

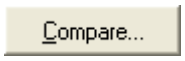
Curve numeric results can also be exported in a text file (TXT) by clicking 'Export as TXT' button.

Linear curve regressions can be generated only if all the following conditions are fulfilled:

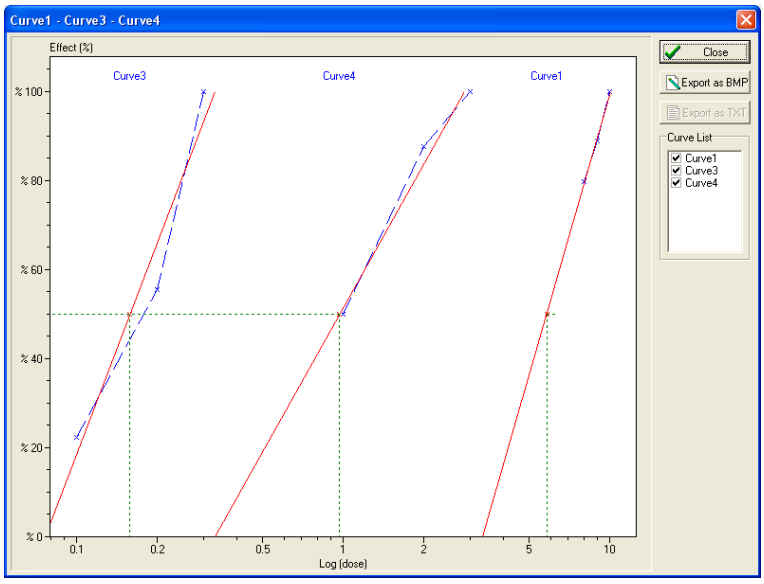
1. At least three cycles must be assigned to the curve.
2. At least one of the three cycles must be defined as 'Max'. The rest of the cycles must be defined as "No Max'.

If a curve or its cycles are not correctly defined, a message at the bottom of the cycles table will indicate the reason of the problem.

5.2.2. Curve regression comparative



When more than one curve has been correctly defined and selected to be drawn (Draw Curve column is checked), then the 'Compare' button is enabled to allow generating a curve regression comparison graphic including selected curves:



'Curve List' section allows User to choose which curves should be included in the final graphic.

Again, this final graphic can be exported as a bitmap file (BMP) for illustration purposes.

5.2.2.1. Sigmoid regression

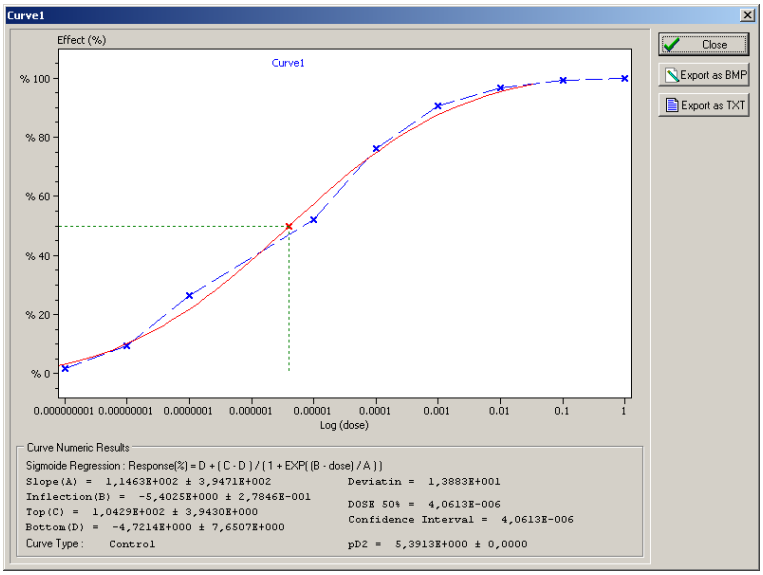
The sigmoid regression model fits the dose-effect values (obtained from the defined cycles) to a sigmoid curve (a.k.a. statistical function), obtaining the parameters that charities it from the following equation:

$$y = D + \frac{C - D}{1 + e^{-\frac{x-B}{A}}}$$

Being:

- X: Dose logarithm (base 10).
- Y: Effect %.
- A: Slope of the straight-line section of the curve.
- B: Curve inflection point.
- C: Top asymptote of the curve.
- D: Bottom asymptote of the curve.

The following image is a sample of a successful sigmoidal curve fitting in which the curve numerical results (parameters of the sigmoid function) are calculated and shown:



As a non-linear calculation, some sigmoidal curve fitting cannot be successfully solved. There are two main reasons because a sigmoidal curve fitting could not be solved:

1. There are not enough defined cycles: In general, at least six cycles must be assigned to the curve although more cycles could be required if they don't provide a clear sigmoidal curve shape.
2. The points cloud generated by the assigned cycles does not have a sigmoidal curve shape. In that case, try calculating the linear regression instead.

If a curve or its cycles are not correctly defined, a message at the bottom of the cycles table will indicate the reason for the problem.

5.3. Stopping the session

Current started session can be manually stopped by pressing  button in the session control toolbar.

All acquired data is automatically saved into the session.

5.4. Saving and loading the experiment file

5.4.1. Saving changes

When any change has been done in the current experiment, [save] button  and [File – Save] menu option is automatically enabled to allow you to store the changes in an experiment file.

If experiment file name has not been set yet, then a 'Save as' dialog is shown to allow User to choose both destination folder and file name.

Experiment files have PTF extension. Default destination folder can be configured through Config > Default Folders menu option.

If the experiment file name has been set previously, then changes are automatically stored in the file so previous file is updated.

5.4.2. Automatic backup

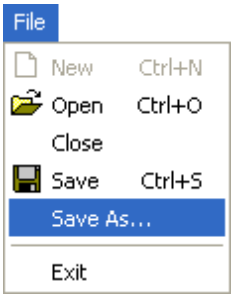
In order avoid data lost due to computer or application malfunction, PROTOWIN is designed to automatically save a copy of the experiment **every 5 minutes only while data acquisition is started**.

Backup security system will then generate a new experiment file (in the folder where the application had been installed) which name includes the start date and time of the experiment.

In case of disaster, the backup experiment file will include the last experiment status.

If this file were also corrupted, a second backup file (with BAK extension) is available but, in this case, information belonging to the last 5 minutes will be lost.

Backup files will be deleted when saving the experiment file. If experiment is closed then the application will ask for keeping or not the backup files.



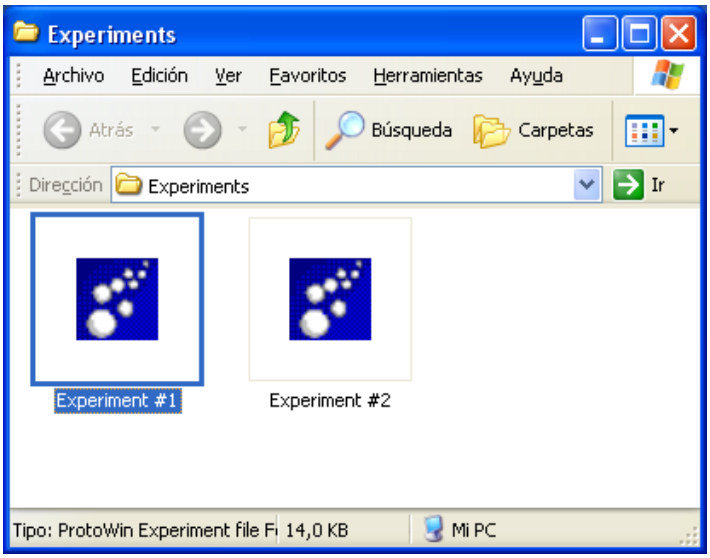
5.4.3. Saving with a different name

If desired, current experiment file can be saved with a different filename. To do that, select 'File > Save as' menu option.

5.4.4. Loading an experiment file

Saved experiment files can be retrieved (loaded) in two ways:

- If PROTOWIN application is not running
- Use Microsoft® Explorer® (through My PC icon in the Windows® desktop) to locate your experiments folder.



- double click on the experiment file.

  PROTOWIN application will run and automatically load the selected experiment file.

- While PROTOWIN application is running
- Close your current experiment file selecting File > Close menu option.

  Please note that if changes were done and they were not saved previously, then application will ask you for confirmation before closing the experiment, allowing you to save just in this moment.

- Click on  button or select File > Open menu option.
- Locate your experiments folder (if needed) and double click in the experiment file.

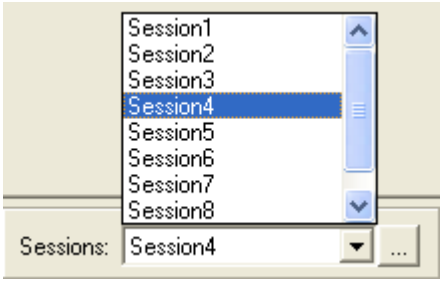
When an experiment file is loaded, its name is shown in the title bar of the window and 'Record' tab is automatically selected.

Please read the next section to know how to retrieve stored session information.

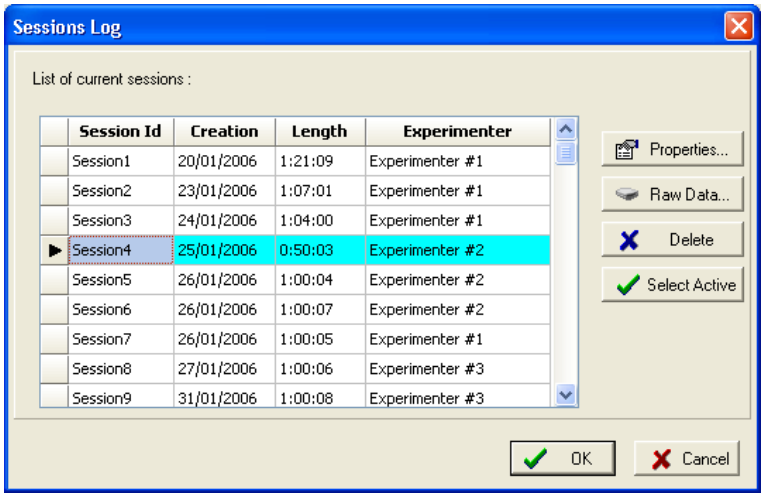
5.5. Managing sessions

All stored sessions are included in the list that is located in the session control bar.

If a session is selected in that list, then all records, cycles, curves and edited values are automatically retrieved and shown in the application.



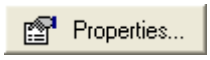
By pressing  button located at the right end of the sessions list, a Sessions Log window will appear:



Sessions Log window shows the list of all the sessions indicating:

- Session Id: the number of the session.
- Creation: the session's start date.
- Length: the session's time duration (hh:mm:ss)
- Experimenter: the Experimenter's name (set through Config -> Experiment Info menu option before starting the session). This field can be manually modified.

5.5.1. Session properties



'Properties' button allows user to review and edit the bath properties which were set when the session was started.

Please refer to section 4.3.6. Baths and vessels properties' to a more detailed explanation of the session properties.

5.5.2. Raw data

If needed, you can press "Raw Data" button to see the raw samples in more detail.

This read-only information is useful for testing purposes.

- The raw data table can be exported to a CSV file by pressing 'Export' button.

5.5.3. Selecting a concrete session

When Sessions Log panel is shown, a blue background color points the current session.

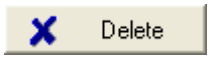
Session4	25/01/2006	0:50:03	Experimenter #2

Users can select a different session by pressing 'Select' button.

This selection method is useful when a high quantity of sessions is stored in the experiment and selecting the session of a concrete date becomes very difficult.

If a session is selected in that list, then all records, cycles, curves and edited values are automatically retrieved and shown in the application.

5.5.4. Deleting sessions



Whether you want to completely delete a session and all its acquired data, 'Delete' button should be used.

As a critical operation, sessions deleted are firstly marked with a red background color.

When the User exits the Sessions Log panel, then a confirmation question is given in order to avoid accidental data erasing. Only if question is replied in affirmative then sessions are unavoidably deleted.

If current session is deleted, then a new current session is automatically selected by the application.

6. DATA ANALYSIS

PROTOWIN is prepared to acquire data from many different experimental situations. It does not matter which experimental design is being used, the program will acquire the current tissue response and will transform it to the current experimenter tension units.

'Analyze' button will make the application to enter into Analysis module.

In this module, main Record/Cycles window will be changed by a different window with two tabs:

- PA2 Calculus: allowing the calculation of PA2.
- Schild Plot: shows the graphical representation (linear regression) of the calculated pA2.

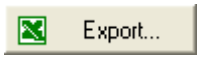
6.1. PA2 Calculus

pA2 values are determined by the Schild method. PROTOWIN calculates a linear regression for which "y" is the quotient of the DE50 values of the antagonist and those for the same agonist in the presence of different concentrations of the antagonist under study, and "x" is the value of the different antagonist concentrations (Appendix A describes the procedure used by the application for this calculation).

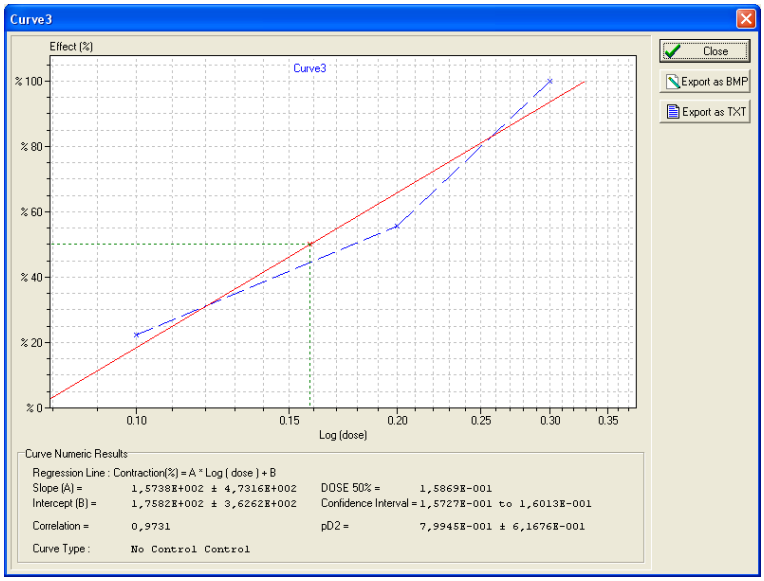
pA2 Calculus tab shows the list of all defined dose-response curves with the following additional information:

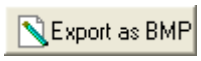
Session	Vessel	Curve	Cycles	Antagonist	DOSE	Curve	Draw
1	1	1	3	0,0000E+000	1,0000E+000	Control	☑
1	1	2	3	3,1600E-007	4,1600E+000	No Control L	☑
1	3	3	3	1,0000E-006	1,8780E+001	No Control C	☑
1	3	4	3	5,6200E-006	5,7230E+001	No Control L	☑
1	4	5	3	1,0000E-005	3,1720E+002	No Control L	☑

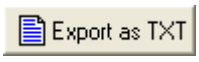
- Cycles: number of cycles associated with the curve.
- Antagonist: vessel's antagonist concentration. This value can be manually edited in this tab.
- DOSE 50%: calculated DE50. This value can be manually edited in this tab.
- Curve: Dose-response curve type. This value cannot be changed in this tab and should be modified through Cycles tab.
- Draw: check the box whether you want to include the dose-response curve in the calculations.
- The table information can be exported to a Microsoft® Excel® document pressing 'Export' button.



- Pressing ‘Regression’ button makes the application calculate and show the regression curve of the selected dose-response curve.



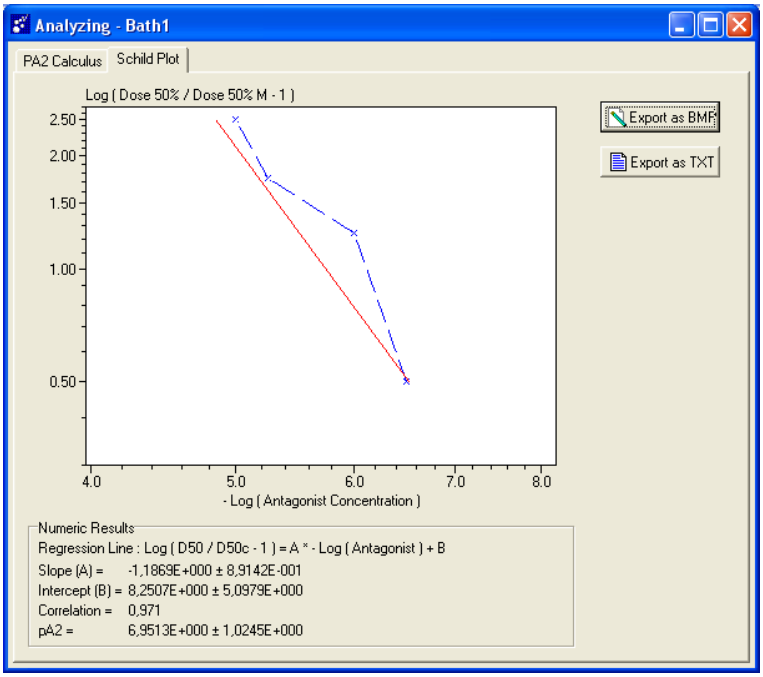




Again, regression curve plot can be exported as BMP for illustration purposes or as a text file (TXT) using ‘Export as BMP’ or ‘Export as TXT’ buttons.

6.2. Schild Plot

The Schild Plot corresponding to the selected dose-response curves (‘Draw’ column checked) is obtained and shown automatically by entering in the ‘Schild Plot’ tab.



A bitmap file for illustration purposes can be obtained by pressing ‘Export as BMP’ as long as a text format file with the numerical results can be generated using ‘Export as TXT’ button.

7. APPENDIX A – STATISTICAL PROCEDURES USED BY PROTOWIN

7.1. Linear Regression

Linear curve fitting is done through the least squares method to obtain the straight line that "best fits" the set of (x,y) data points (in which x data is expressed as decimal logarithm of dose and y is the observed effect).
The linear equation can be expressed as:

$$y = mx + b$$

In this equation *m* is the slope of the line and *b* is its intercept on y axis.
The slope is computed from the equation:

$$m = \frac{(\Sigma x_i)(\Sigma y_i) / N - \Sigma(x_i y_i)}{(\Sigma x_i)^2 / N - \Sigma(x_i)^2}$$

and the y-intercept from:

$$b = \bar{y} - m\,\bar{x}$$

where all summaries are from i = 1 to N (being N the number of points), $\bar{x}$ and $\bar{y}$ are the mean values of x and y ([summatory (x_i)/N] and [Summatory (y_i)/N] respectively).
The sum of squares (SS) about regression is given by:

$$SS = \Sigma(y_i - \hat{y}_i)^2$$

where y_i denotes the expected y_i value for the $\{x_i, y_i\}$ values according to their regression line.
The estimated standard error (SE) of the slope *m*, the y-intercept *b* and the x-intercept x' are given by the equations:

$$S.E.(m) = s\left[\frac{(\Sigma x_i)(\Sigma y_i) / N - \Sigma(x_i y_i)}{(\Sigma x_i)^2 / N - \Sigma(x_i)^2}\right]^{1/2}$$

$$S.E.(b) = s\left[(1/N) + \frac{\bar{x}^2}{\Sigma(x_i - \bar{x})^2}\right]^{1/2}$$

$$S.E.(x') = \left|\frac{s}{m}\right| \left[(1/N) + \frac{(\bar{y} / m)^2}{\Sigma(x_i - \bar{x})^2} \right]^{1/2}$$

in which the standard error of the estimate s is obtained from

$$[SS / (N - 2)]^{1/2}$$

The confidence intervals for each parameter are obtained by multiplying the respective estimated standard errors by the appropriate Student's t value for [N – 2] degrees of freedom.

The correlation coefficient r is obtained from the equation:

$$r = \frac{\Sigma x_i y_i - N \bar{x} \bar{y}}{\sqrt{(\Sigma x_i^2 - N\bar{x}^2)(\Sigma(y_i^2 - N\bar{y}^2))}}$$

(From R.J. Tallarida and R.B. Murray, 'Manual of Pharmacological calculations', Springer – Verlag, New York, 2nd ed., 1987)

7.2. Sigmoid Regression

Sigmoid regression is done through the Levenberg–Marquardt (LMA) algorithm to obtain the sigmoidal curve (a.k.a. statistical function) that "best fits" the set of (x, y) data points (in which x data is expressed as decimal logarithm of dose and y is the observed effect).

This generic algorithm provides a numerical solution to the problem of minimizing a function, generally nonlinear (sigmoid curve in our case), over a space of parameters of the function (the parameters stated in 5.2.2).

The detailed description of the algorithm can be found at K. Levenberg (1944), 'A Method for the Solution of Certain Non-Linear Problems in Least Squares ',The Quarterly of Applied Mathematics 2 and at D. Marquardt, 'An Algorithm for Least-Squares Estimation of Nonlinear Parameters', SIAM Journal on Applied Mathematics 11.

7.3. PA2 Calculation

In order to achieve quantitative information about an antagonist substance it is common to determinate the pA2 as a measure of the affinity of the competitive antagonist for its receptor.

As it is widely stated, the presence of a B concentration of a competitive antagonist produces the displacement to the right of an agonist's dose-response curve. The greater the value of B, the greater is the displacement. The KB, dissociation constant for the antagonist at concentration B may be computed for the equation:

$$(A' / A) = 1 + (B / KB)$$

assuming A and A' are the agonist dose-ratio for equal effects.

In practice, and in order to minimize the error of a single determination, several concentrations of antagonist are used.

The $(A' / A)_i$ dose ratio can be obtained for each of its corresponding B_i values, from i = 1 until N (N being the number of antagonist doses).

When the decimal log of (A' / A – 1) is plotted against the decimal log of B, a straight line with the unity slope and intercept – log (KB) is obtained. This later value is called the pA2.

PROTOWIN uses the previously described method to fit the straight line (see 8.1. Linear Regression). No checking of parallelism between the dose-response lines are made.

7.4. Unit conversions

The change between the user concentration units and the program (molar) concentration unit is performed according to the following expressions:

1 M = 1 [M]

1 mg/ml = [1 / (10^3 * MW)] [M]

1 mg/ba = [1 / (10^3 * Bv * MW)] [M]

1 mg/ml = (1 / MW) [M]

1 mg/ba = (1 / Bv * MW) [M]

Where

[M] = [Molar units]M

W = Molecular weight (g / mol)

Bv = Bath volume (ml)

8. CONTACT INFORMATION

We are available to help you with your questions and concerns. Should you hit a roadblock or need some additional training, please feel free to visit the HBIO Behavioral Support Center at https://support.behavior.hbiosci.com to find articles and helpful information in our knowledge base or submit a ticket. We are happy to help!

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