

KSV Monolayer Kit

Basic experiments for
KSV Langmuir and Langmuir-Blodgett
Instruments

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1. Introduction

The study of Langmuir and preparation of Langmuir-Blodgett films can be a tricky task for beginners and researchers not really acquainted with the techniques. There are many steps involved in order to get proper results for such measurements. However, overall there is no magic involved and already by performing a few measurements with the LB technique it will give a good overview of the capabilities of the instrument. Although the instrument gives a feeling of a really macroscopic instrument it will open a whole new world to a technique studying interactions at a molecular level.

The purpose of this manual is to introduce the user to the technique itself and give the capabilities of running the instrument for further studies. This manual will describe step-by-step how to successfully perform an isotherm, a dipping experiment, and short description of relaxation/stability/kinetics and hysteresis measurements. This manual includes a lot of the same things that are described in the LB instrument manual but in a more compact form. If more detailed information is needed about the software itself or the function of the instrument then please read the LB instrument manual.

This manual will not include any extensive discussions about the properties and behaviour of the monolayers at the air-water interface and the effect of subphase temperature or composition, as there is a vast amount of literature available for such information. More detailed information about this can be found in the literature listed at the end of this manual.

KSV Instruments welcomes any suggestions for further development or improvement of the LB products or this manual. All suggestions and enquiries should be addressed to:

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2. Content of Monolayer Kit

The monolayer kit delivered for every KSV LB system is a plastic box with small compartments containing the following components:

1. Polyethene powder-free gloves
2. Surfactant and lint-free tissues
3. One soft paintbrush for cleaning the trough, and one 50 mm glass beaker to keep the cleaning solution in
4. Aspirator that can be attached to a tap, equipped with rubber tubing and disposable polypropylene tips for cleaning the subphase surface
5. Spatula for preparing monolayer sample and subphase solutions
6. 3 pcs of 5 ml glass tubes for sample and cleaning solutions
7. Microliter syringe (50 μ l) for use for spreading monolayer material
8. 100 pcs of glass slides for LB deposition purposes, 20 mm x 20 mm
9. Tweezers for glass slides
10. Wilhelmy plate of Platinum
11. 100 pcs of Wilhelmy plates made of paper + hook
12. Calibration weight for calibration of the balance
13. Leveling tool for leveling the instrument
14. Tool set

3. Other laboratory equipments and components required

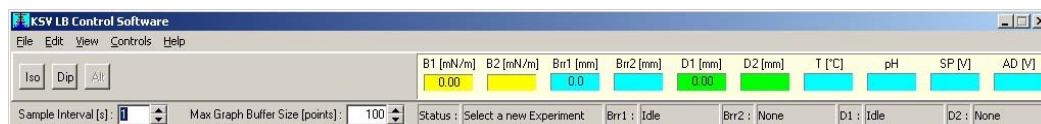
1. At least 1.5 m x 1.5 m free bench space in a sufficiently clean, draught and dust free environment
2. Running water and a sink (max. 2.5 m from the instrument) to connect the aspirator pump. Alternatively, a vacuum pump and flask could be used
3. A supply of pure de-ionized and organic free water for cleaning the trough and preparing the subphase. Recommended suppliers are Elga, Millipore, Barnstead...
4. Laboratory balance with minimum accuracy of 0.1 mg
5. Monolayer material (for example Stearic acid or DPPC)
6. Some metal salt to dissolve in the subphase (preferably CdCl_2 , MnCl_2 or TbCl_3).
7. Volatile solvents for preparing the spreading solution of the monolayer material (for example Hexane, Chloroform, or Methane)
8. Pure ethanol for cleaning purposes
9. Computer running Windows 2000/XP equipped with the KSV Win LB software for controlling the instrument

4. Checking critical definitions and parameters

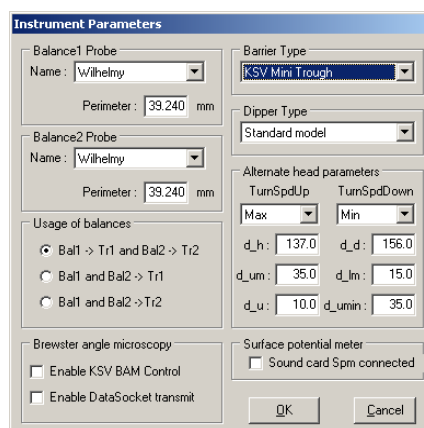
Please, install your instrument and calibrate the balance as is described in the corresponding LB instrument manual and start the **KSV Win LB software**, and further from the **Main Menu** start the **KSV LB control software** by pressing the



button. The following window will then appear:



Hereafter, make sure that the **Instrument Parameters** have been set correctly by choosing **Edit** → **Device Parameters** in the **LB Control Software** window. The Instrument Parameters screen should appear as follows:

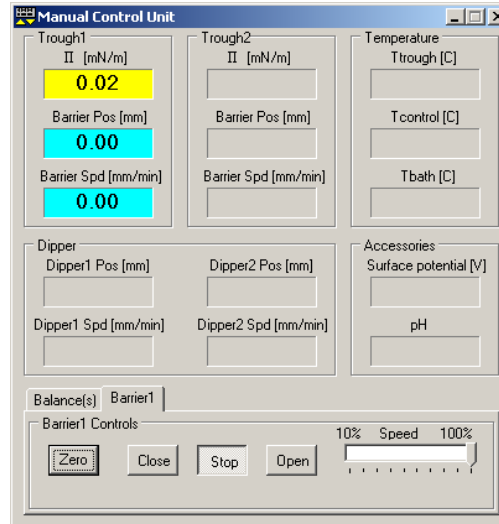


Barrier and **Dipper Type** should be defined according to what LB instrument model you have. All other default settings are OK pre-supposed you are using the standard Pt Wilhelmy plate delivered with the instrument, and you do not have an alternate deposition system. If using a custom Wilhelmy plate then the dimensions of this has to be defined into the **KSV Win LB Software** database, and then choose that newly defined Wilhelmy plate as the **Balance Probe** in the **Instrument Parameters** window above. Having the exact and correct dimensions for the Wilhelmy plate that is used is really crucial in order to be able to measure the surface pressure accurately. Furthermore, if you have an alternate deposition system then also define the correct parameters delivered with the instrument for it in this window.

Once the parameters have been set you do not have to make the changes every time unless you change your configuration, the software will remember the parameters in the future.

Then it is recommended to check that the value for the trough area has been correctly defined in the software. This is very important especially when using custom made troughs, but is also recommended for standard troughs delivered with the instrument. Start this by placing the trough and barriers to their positions and open the barriers so much that there is only a space of about 0.5-1 mm between the back of the barrier and the trough edge. This can be done

by pressing the **Open** button in the **Barrier** page of the software **Manual Control Unit**.



If the **Manual Control Unit** is not activated, then activate it by pressing the **Control Panel** on top of the **Main Menu**.

Now measure in mm the distance between the barriers and the width of the trough as accurately as possible (normally a normal ruler is sufficient to determine these to a accuracy of 0.5 mm), and then calculate the area of the trough in mm^2 . Hereafter, go to the **KSV WIN LB** software database by choosing **Edit** → **Database** in the **LB Control Software** window and further choose the **Troughs** in order to check if the dimensions of the trough are the same that you have measured. If not, change the values or define a new trough in the database. After changing values or making a new definition in the database, please remember to press the post button (✓) to verify changes.

5. Isotherm measurements

We will in this section perform 3 isotherm measurements; 2 measurements with Stearic acid (SA) and 1 measurement with Dipalmitoylphosphatidylcholine DPPC. The idea of these measurements is to first measure the SA isotherm on pure water with a pH of about 5.5 as this isotherm is so well known with clearly distinctive points that can be used as check points to confirm that the measurement is OK, and secondly demonstrate the effect on the SA monolayer caused by adding a small amount (5×10^{-3} M MnCl_2) of salt in the subphase. SA has a molecular weight of 284.5 g/mole, and due to its well known properties it can be regarded as a standard material for Langmuir films.

DPPC is used to demonstrate the appearance of a special phase called Liquid Expanded – Liquid Condensed (LE-LC) where a liquid and solid phase co-exist.

Normally it is recommended that the concentration of the monolayer material in the spreading solution is around 0.5 - 1 mg/ml. However, this is just a rule of thumb and one can use any other concentration if needed as long as the **EXACT** concentration is known. In the examples below we will use a concentration of about 1 mg/ml for SA and DPPC monolayer material in the spreading solutions. The most common spreading solvents used are Hexane and Chloroform. However, sometimes the monolayer material can not be dissolved in these and a mixture of solvents need to be used. In such a case it is important to remember that the solvent used should be as volatile and as water insoluble as possible in order to achieve good and reproducible results.

The amount of monolayer material sample solution depends first of all of the concentration of the spreading solution, but also on what type (size) of trough is used. If the concentration is around 1 mg/ml, the amount of the monolayer sample solution spread on the subphase is 20-25 μl for a KSV Minitrough, and 60-80 μl for a KSV Standard and Alternate trough. Often, the exact amount needed to be spread is found out simply by experience by performing a few measurements.

5.1. Preliminaries

Every time you are preparing a measurement with your KSV L or LB instrument there will be a series of procedures that are recommended to do in order to achieve the best outcome of your studies. These includes:

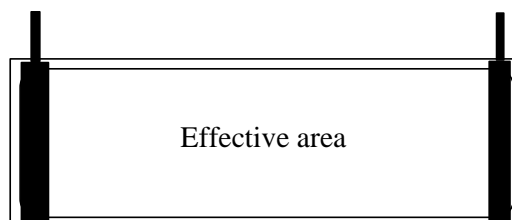
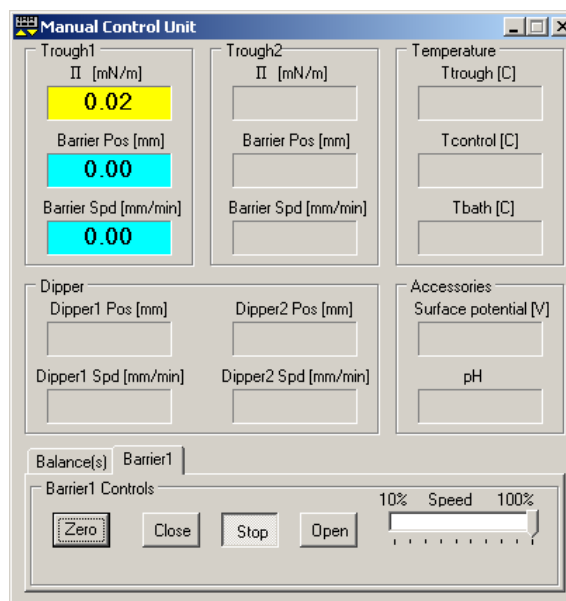
- Wash your trough and barriers thoroughly. For most cases using a soft brush to “paint” the trough and barriers with pure ethanol (or other organic solvent), and then rinsing with pure ion exchanged water has shown to be a sufficient way to clean the trough and barriers.

NOTE! The barriers do not tolerate Chloroform as a cleaning solution, but this does not restrict the use of Chloroform as a spreading solvent for the monolayer material.

During cleaning it is advisable to use rubber gloves, because touching your trough or barriers with your bare hands can later contaminate your subphase surface, which further affects the outcome of your

measurements and results. If you have not used the trough for a long time it is good to rinse it thoroughly with detergent (Decon, Helmanex, Fairy) and hot water, and after that use the cleaning procedure described above. Note that with the KSV trough, it is easy (and advisable) to move the trough to a sink where it can easily and thoroughly be cleaned.

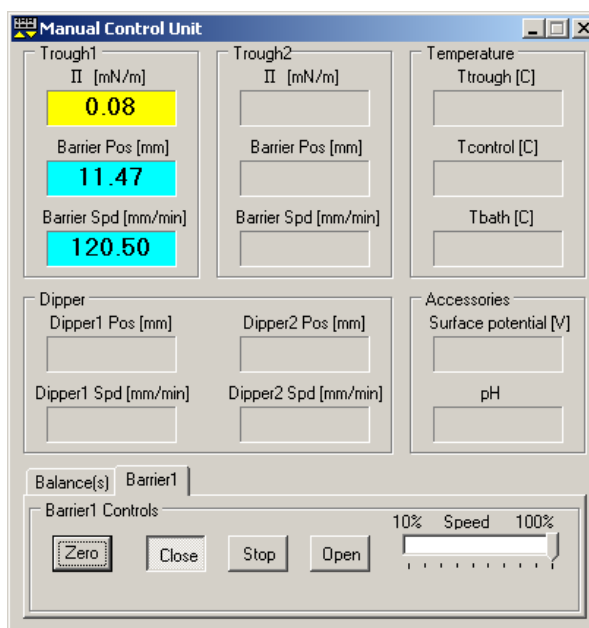
- Place the cleaned trough and barriers on their positions on the instrument and move the balance(s) to the center of the trough and the optional Dipper so that it is not disturbing the surface pressure measurement. The position of the Dipper at this point is not crucial as long as it is not in the way of the balance.
- Move the barriers with the software **Manual Control Unit** (or the optional physical Manual Control Unit if the instrument is equipped with it) so that there is not left a space more than about 0.5-1 mm between the back of the barrier and the trough edge. See picture below. At this point also move the barrier safety switches to these positions, if not already done.



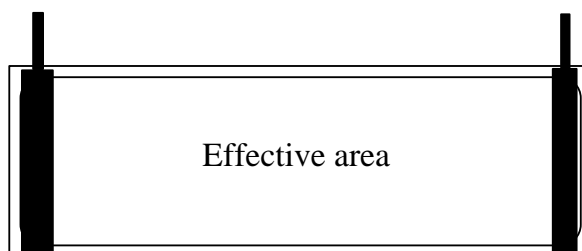
Now we are all set for making the actual isotherm measurement.

5.2. Isotherm of Stearic acid on water subphase

1. Now find some pure water (preferably ion exchanged water with a resistivity of $18 \text{ M}\Omega\text{cm}^{-1}$) and fill the trough with this so that the surface of the water subphase is a couple of mm above the edges of the trough. Pure ion exchange water should have a pH in the range 5.4-5.8 and using this water as such is sufficient. No adjustment of the pH is necessary.
2. Wait until the temperature of your subphase has stabilized to the temperature you have chosen before continuing.
3. Use the software **Manual Control Unit** (or the optional physical Manual Control Unit if the instrument is equipped with it) to close the barriers to the center and simultaneously clean the liquid surface between the barriers by using the aspirator (or a suction pipette) until the barriers are as close together as possible. Hereafter, open the barriers to the zero position and repeat the above procedure. This procedure should be repeated 2-3 times.



The last time keep on cleaning the liquid surface between the barriers until the water level equals or is just a little bit higher than the trough edge level. Then move the barriers back to zero position (see figure below).



4. Rinse the Wilhelmy plate with pure ethanol and ion-exchanged water. Hang the plate on the surface balance and lower the plate into the subphase so that about two thirds of the plate is above the surface.

Every now and then it is advisable to flame clean your probe, especially if it does not seem to be wetted completely when immersed in the subphase.

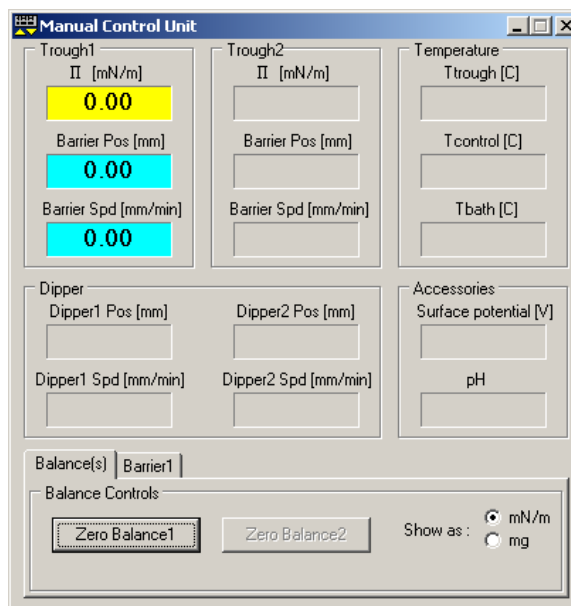
If you use your Wilhelmy plate for the first time you should clean it with solvent and flame it prior to use. Then store it in some water-soluble organic solvent (for example ethanol). To flame clean your probe, make sure you have a strong flame source comparable to a bunsen burner. Low heat flames will leave residues on your plate, which can lead to significant error. Hold the probe in the hottest part of the flame until the entire plate glows brightly for a few seconds. It is advisable to flame the Wilhelmy plate every now and then but it is not necessary to do it every time you use the plate. Rinsing with ethanol and water should be sufficient for most cases.

5. After hanging the Wilhelmy plate on the balance zero the balance and barrier positions from the software **Manual Control Unit** and check the cleanliness of the subphase by compressing the barriers together and looking on the balance value on the display. If the surface pressure value stays below 0.2-0.3 mN/m during this compression then the subphase surface can be regarded as clean and you can proceed to the next step. However, if the surface pressure value increases above 0.3 mN/m, then it is recommended to clean the surface once more as was described in point 3 above.

The barrier position should always and **ONLY** be zeroed when the barriers are at the zero level as shown in the image under point 3 above. This is very important in order to determine the molecular area as accurately as possible.

The balance should always and **ONLY** be zeroed **BEFORE** spreading any of the monolayer material to the air-water interface. This is very important in order to determine the correct surface pressure values as a function of the molecular area.

6. Clean the syringe by placing the needle in a bottle containing pure Hexane or Chloroform and fill and empty the syringe about 5-6 times. Then heavily shake the SA bottle, open the cap of the SA bottle, place the needle in the bottle and take about 20 μl (for KSV Minitrough) or about 60 μl (for KSV Standard trough) of the ~ 1 mg/ml SA solution into the syringe.
7. Just before spreading the SA solution on the subphase surface it is **very important** that you **ZERO** the balance and barrier positions from the software **Manual Control Unit**. When zeroing the barrier position, make sure that they are a zero position as shown in the image under point 3 above.



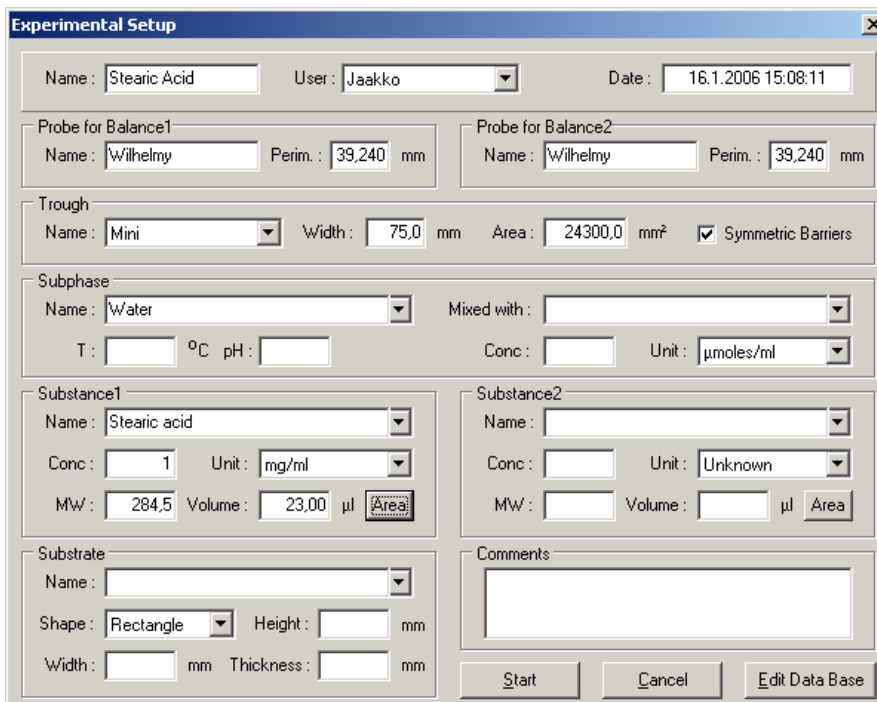
8. Spread all of the SA solution carefully on the surface by first forming a small drop of the solution on the tip of the syringe needle and carefully make the drop touch the subphase surface. Repeat this until you have spread all the SA solution in the syringe. Do **NOT** let the drop **FALL** on the surface because this can cause some loss of your surfactant to the bulk and/or uneven spreading of your substance. While spreading follow the surface pressure reading on the **Interface Unit** display and make sure that you spread the substance slowly enough so that the surface pressure value does not exceed 0.5 mN/m at any point. This ensures that the monolayer material is able to spread properly over the surface. Let the solvent evaporate for at least 10 minutes.

After spreading the monolayer material do **NOT** zero the balance value anymore.

9. During the waiting time for the evaporation of the solvent start the isotherm measurement by pressing the **ISO** button in the **KSV LB Control Software** window.



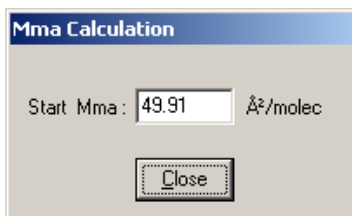
This will bring up the **Experimental Setup** window where all necessary parameters for the isotherm measurements are defined (see the instrument LB manual for more precise definitions of the fields in the Experimental Setup window).



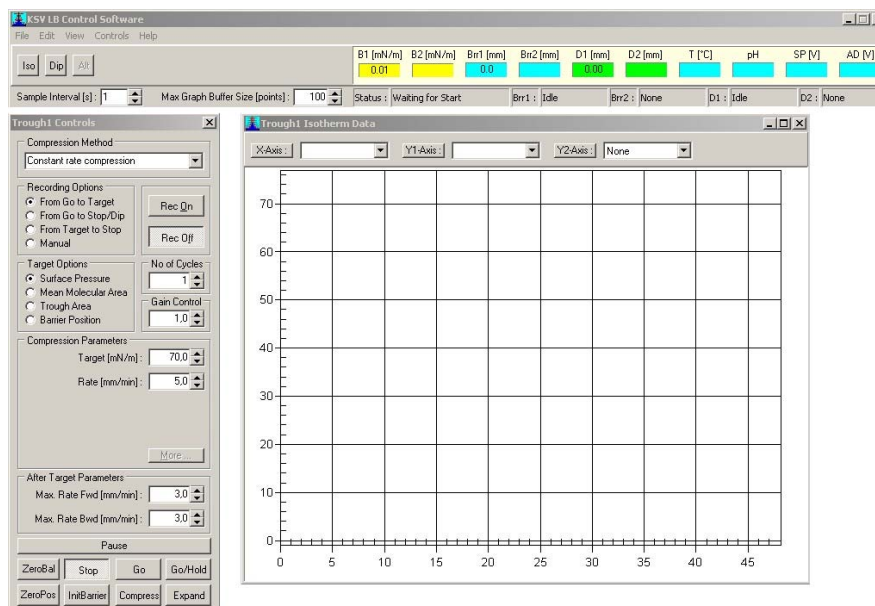
Fill in the fields as above and change the fields to fit your own information accordingly. The most important parameters in the **Exeperimental Setup** window to be defined for correct results are:

- **The exact concentration of the monolayer material in the spreading solution**
- **The exact volume of the solution spread on the subphase**
- **The molecular weight of the monolayer material.**

There is an **Area** button in the **Substance** field that can be used for checking the initial area per molecule before deciding how much of the monolayer containing solution should be spread. This feature is especially useful when using monolayer materials with known properties. For a Stearic acid monolayer this area should be at least $> 35 \text{ \AA}^2/\text{molecule}$ to make a proper isotherm measurement. In the case above the area is as shown in the window below:



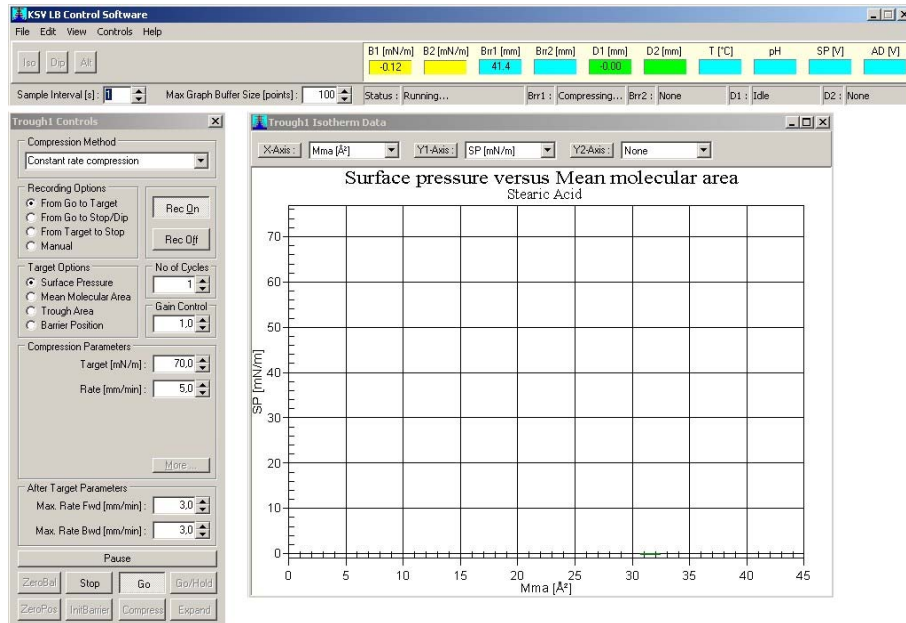
After completing the **Experimental Setup** window press the **Start** button. This will not start the actual measurement or any compression yet, but it will open the **Trough Controls** and **Trough Isotherm Data** windows as shown below.



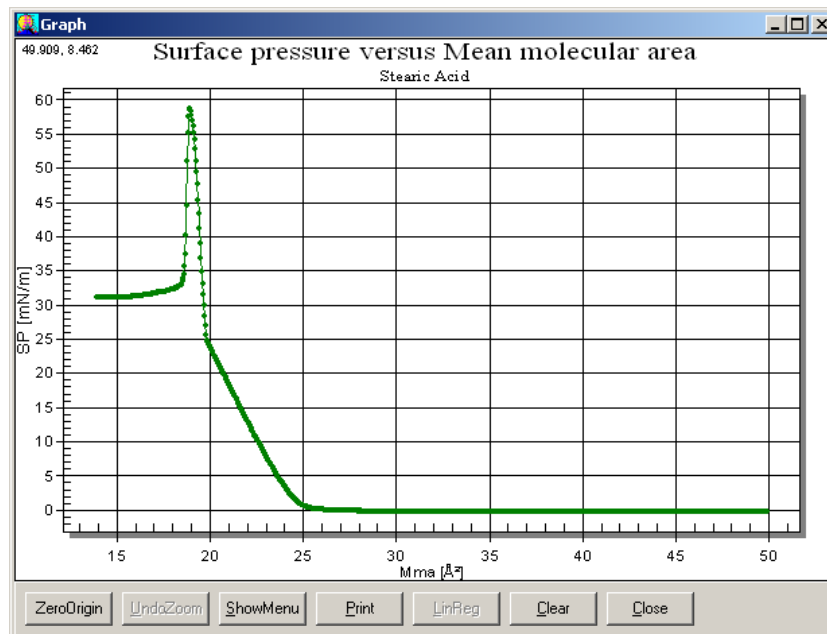
Set the parameters in the **Trough Controls** window as shown above. For a more precise description of options and fields in the **Trough Controls** window please see the LB instrument manual. The **Target** surface pressure in the **Compression Parameters** box is set so high that we make sure that the collapse of the monolayer is reached.

In practice a surface pressure higher than 72.8 mN/m can not be reached as this is the surface tension of pure water that is used as the subphase. The compression **Rate** should be sufficiently low to give the monolayer time to reorganize during the compression process. The most common rates lies between 5-20 mm/min.

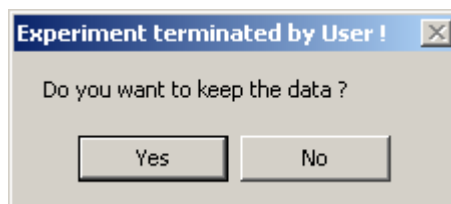
10. After at least 10 minutes has passed from the spreading of the monolayer material on the subphase surface, press the **GO** button in the **Trough Controls** window. This will inactivate all the other buttons in the **Trough Controls** window except the **STOP** and **GO** buttons, and start the compression of the barriers. The surface pressure as a function of the area will be plotted on-line in the **Trough Isotherm Data** window as the compression proceeds. The **Status:** line in the **KSV LB Control Software** window will also change from **Waiting for Start** to **Running...** The **Compression Parameters** in the **Trough Controls** window can be changed while the measurement is running and will be activated immediately after the change has been done.



- When the monolayer has reached the collapse press the **STOP** button in the **Trough Controls** window. The collapse can either be seen as a sudden decrease in the surface pressure or as a leveling out at high surface pressure.



Pressing the **STOP** button will open a small window asking if the data is to be stored.

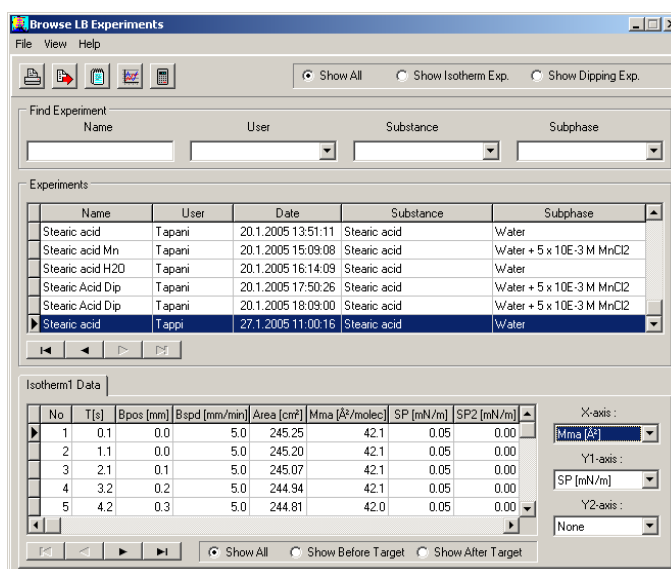


Pressing the **YES** button in this window will stop the software of controlling the barriers and the experiment is finished. Alternatively, the barriers can be let to hit the safety switch, which will stop the measurement automatically. In the latter case, please make sure before the measurements that the barriers do not hit and break anything especially the dipper, balance or Wilhemy plate.

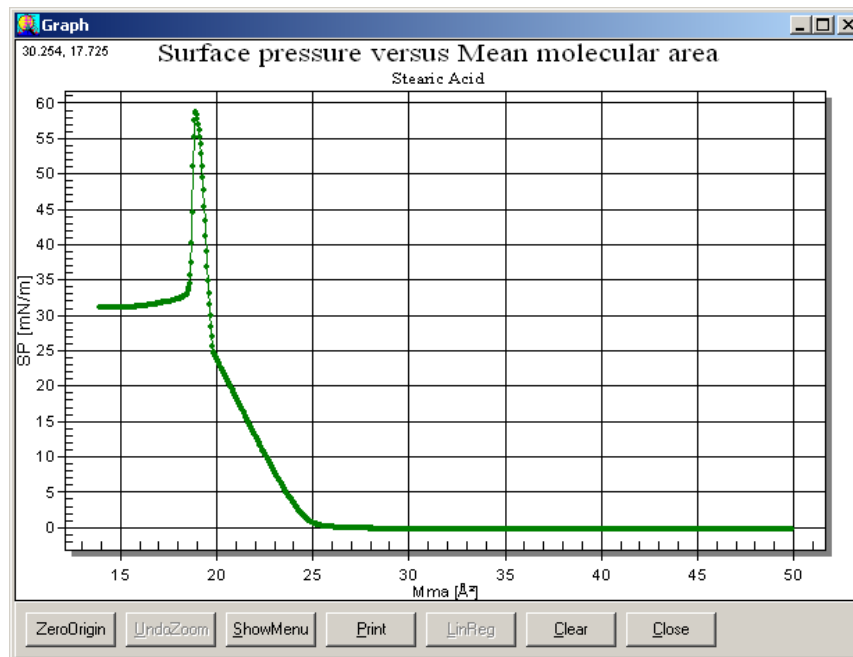
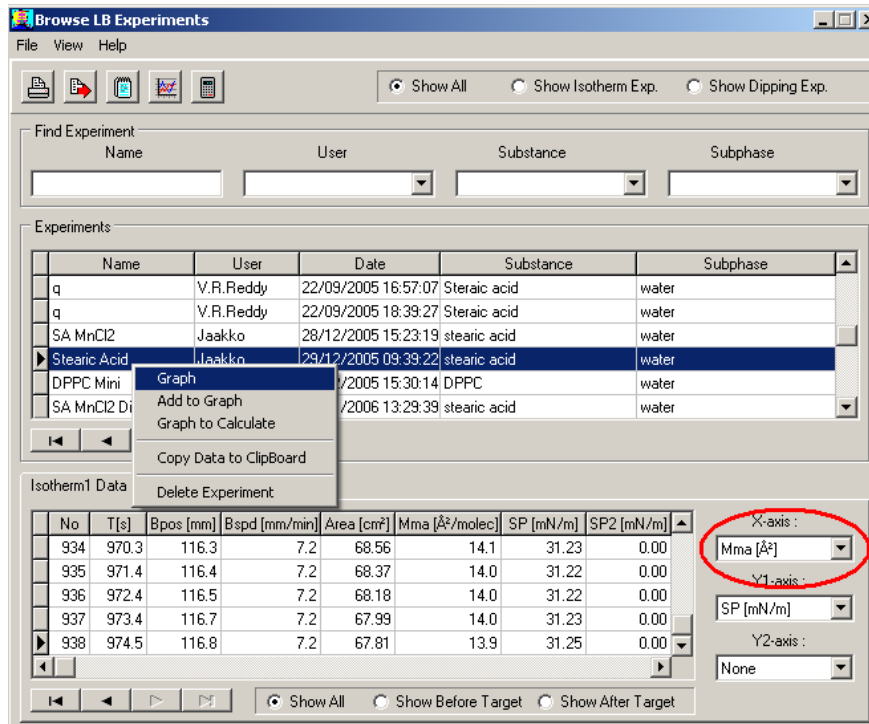
The data collected is stored in the software database and can be retrieved later for analysis through the **Browse LB Experiment** software, which can



be activated by pressing the icon in the **Main Menu**.



- The isotherm can then be re-plotted by highlighting the measurement of interest, right-click on the mouse and then choose **Graph**. It is advisable to change the **X-axis** value to **Mma [Å²]** before plotting the isotherm in order to get the molecular area.



The distinctive points that should be seen in the measured SA isotherm are; a) the surface pressure start to increase at a Mean Molecular Area (MMA, Å²) around 25 Å²/molecule, and b) There is a clear change in the slope of the surface pressure vs. area curve at a surface pressure of about 25-26 mN/m.


One can clearly see that the isotherm of SA on pure water can be divided into 3 distinctive regions i.e.

The region where the molecular area is $> 30 \text{ \AA}^2$ and the distance between the individual SA molecules is large i.e. the area available per SA molecule is sufficiently large not to induce any interaction with neighboring molecules. This region can in simple definitions be called the **gaseous** state of the monolayer.

In the intermediate region where the surface pressure rises slowly with the molecular area between $20\text{-}30 \text{ \AA}^2$ the molecules starts to feel each other and interacts leading to the formation of a **liquid** like state.

When the monolayer is still compressed to lower areas i.e. around and below 20 \AA^2 , the molecules in the monolayer is packed very closely to each other and reaches a so called **solid** state, which is seen as a very steep and fast increase in the surface pressure. By extrapolating a linear line through this **solid** state to zero surface pressure one then obtains the extrapolated mean molecular area for the SA molecule on a pure water subphase, which should be in the range of $20 \pm 1 \text{ \AA}^2/\text{molecule}$.

These definitions of phases are very rough and there are examples in the scientific literature for a more precise definition of the different phases appearing in the isotherm.

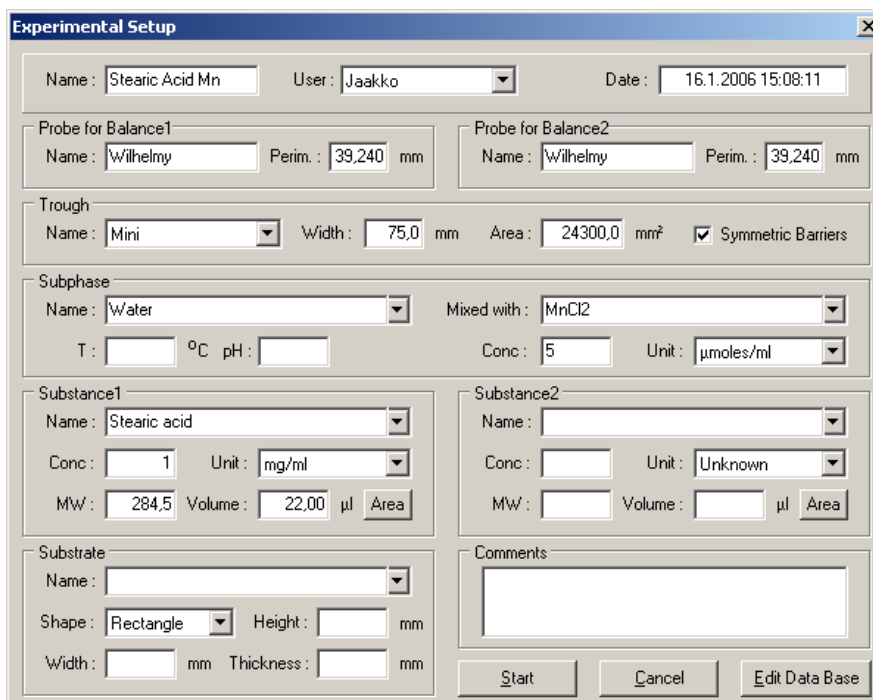
13. After the experiment is finished clean the water surface with a suction pipette (aspiration). Take off the Wilhelmy plate, rinse it with water and pure ethanol and store it in a beaker with a water-soluble solvent. Remove the barriers after removing the subphase from the trough. Clean the trough and barriers as described in section 5.1.
14. If performing a second experiment repeat the above procedure from the beginning.
15. After making more measurements, you still have the possibility to use other functions in the data reduction capabilities of the LB software by clicking the **Browse LB Experiments** icon, . Please, see the LB instrument manual for details.
16. After making all the experiments and data reduction for the day shut down the LB software and the computer. It is also advisable to clean the Wilhelmy plate, trough and barriers after the last measurement for the day and store the parts in a dust free environment.

5.3. Isotherm of Stearic acid on MnCl₂ subphase

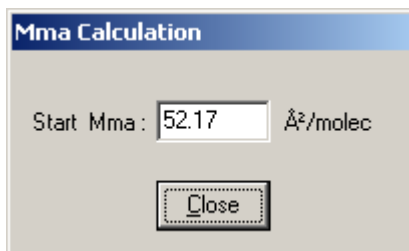
Prepare the trough for a new measurement by going through the steps described in the Preliminaries in section 5.1. Then proceed with the isotherm measurement exactly as you did with SA on pure water above with the following exceptions:

1. Instead of using pure water as a subphase, prepare a solution where you dissolve a small amount of salt for example 5×10^{-3} M (0.1979 g in 200 ml of water) of (MnCl₂ × 4 H₂O) –salt, and pour this liquid in the trough.
2. Then proceed as described in the points 2. – 9. for the SA monolayer on pure water subphase above.

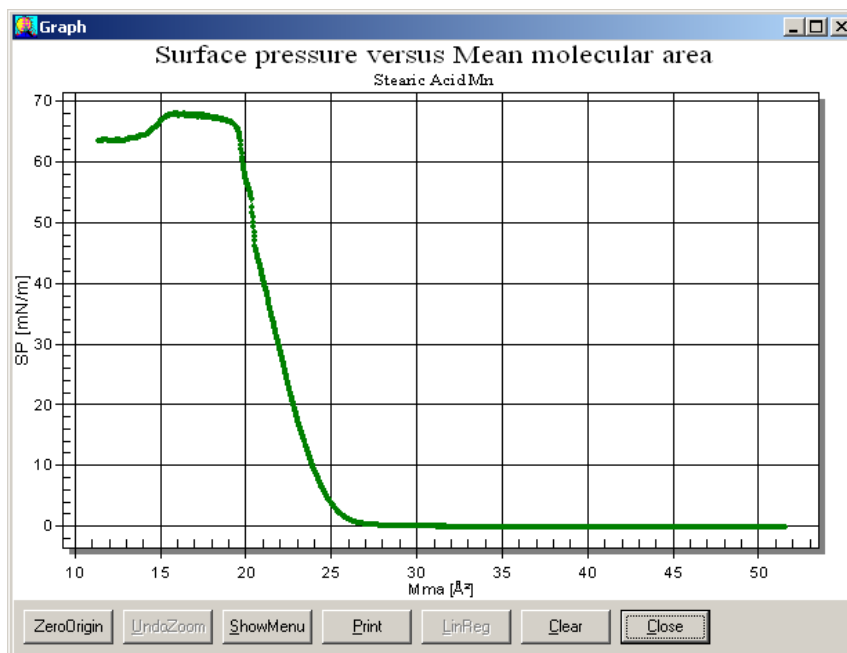
When you reach point 9. fill in the **Experimental Setup** as shown below and press the **Start** button.



The starting area in this case will then be the following:



3. Hereafter, proceed as described in the points 10. – 17. for the SA monolayer on pure water subphase above.
4. The SA isotherm on a MnCl₂ subphase should look like the following.



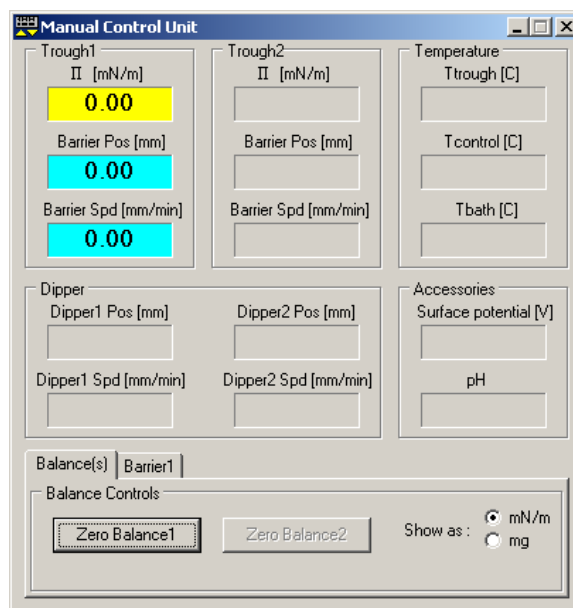
The distinctive features of the SA isotherm on a MnCl_2 subphase are; a) that the surface pressure starts to rise at around $25 \text{ \AA}^2/\text{molecule}$, and b) the surface pressure start to increase with a steeper slope almost immediately after the initial rise of the surface pressure has happened and no clear region with smaller slope is visible as was the case with the SA isotherm measured on pure water. The disappearance of the clear liquid-solid transition compared to the SA monolayer on pure water subphase is due to the interaction of the Mn^{2+} ions with the hydrophilic and dissociated carboxylic ($-\text{COOH}$) headgroup of the SA monolayer. The slightly larger extrapolated mean molecular area ($\sim 24 \text{ \AA}^2/\text{molecule}$) for the SA isotherm on a MnCl_2 -subphase compared to pure water subphase is due to the fact that the tilt angle of the hydrocarbon chains on a MnCl_2 is different than on a pure water subphase

There are a few interpretations how the ions in the subphase interact with the monolayer. One is that the ions in the subphase just screen the charges of the polar head groups of the monolayer material. The second is that the interaction of SA and Mn^{2+} ions leads to a so called “soap” formation, which means that on average each free Mn^{2+} ion in principle binds 2 SA molecules and each $\text{Mn}(\text{OH})^+$ complex binds in principle 1 SA molecule.

6. Isotherm of Dipalmitoylphosphatidylcholine (DPPC) on pure water subphase

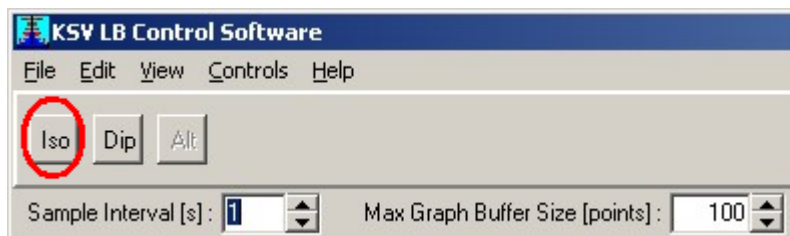
Prepare the trough for a new measurement by going through the steps described in the Preliminaries in section 5.1. Then proceed with the isotherm measurement exactly as you did with SA on pure water above with the following exceptions:

1. Clean the syringe by placing the needle in a bottle containing **pure** Hexane or Chloroform and fill and empty the syringe about 5-6 times. Then heavily shake the DPPC bottle, open the cap of the DPPC bottle, place the needle in the bottle and take 20 μl (for KSV Minitrough) or about 60 μl (for KSV Standard trough) of the DPPC solution into the syringe.
2. Just before starting to spread the DPPC solution on the subphase surface it is **very important** that you **zero** the balance and barrier positions once more from the software **Manual Control Unit**.



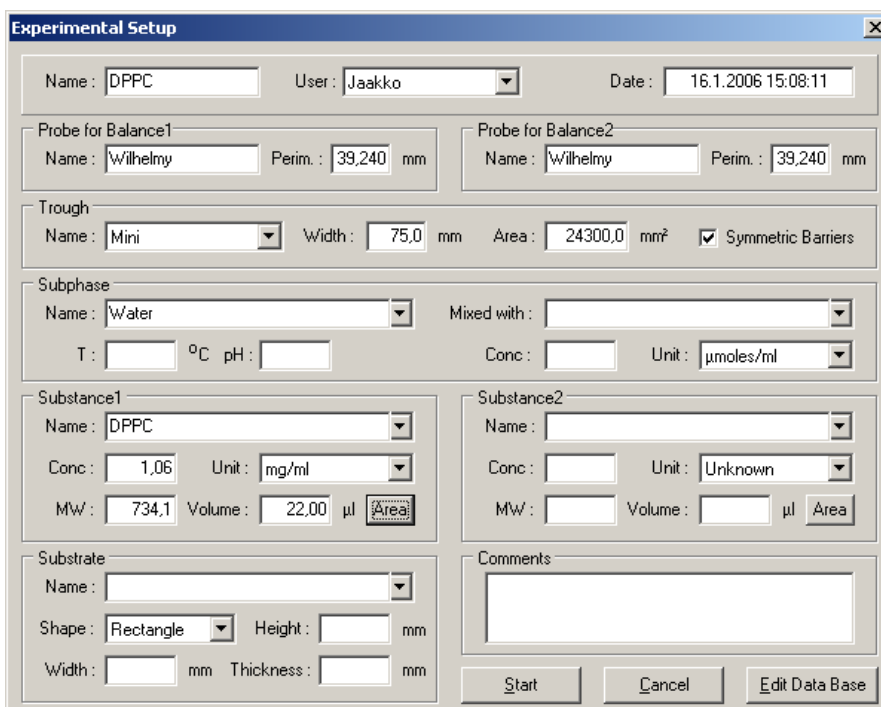
3. Spread all the DPPC solution carefully on the surface by first forming a small drop of the solution on the tip of the syringe needle and carefully make the drop touch the subphase surface. Repeat this until you have spread all the SA solution in the syringe. Do **NOT** let the drop **FALL** on the surface because this can cause some loss of your surfactant to the bulk and/or uneven spreading of your substance. While spreading follow the surface pressure reading on the **Interface Unit** display and make sure that you spread the substance slowly enough so that the surface pressure value does not exceed 0.5 mN/m at any point. This ensures that the monolayer material is able to spread properly to the surface. Let the solvent evaporate for at least 10 minutes.

- During the waiting time for the evaporation of the solvent start the isotherm measurement by pressing the **ISO** button in the **KSV LB Control Software** window.



This will bring up the **Experimental Setup** window where all necessary parameters for the isotherm measurements are defined (see the instrument LB manual for more precise definitions of the fields in the Experimental Setup window).

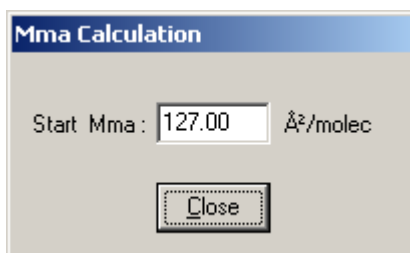
NOTE ! We will now in this case spread 22 μ l of a DPPC solution onto the pure water subphase.



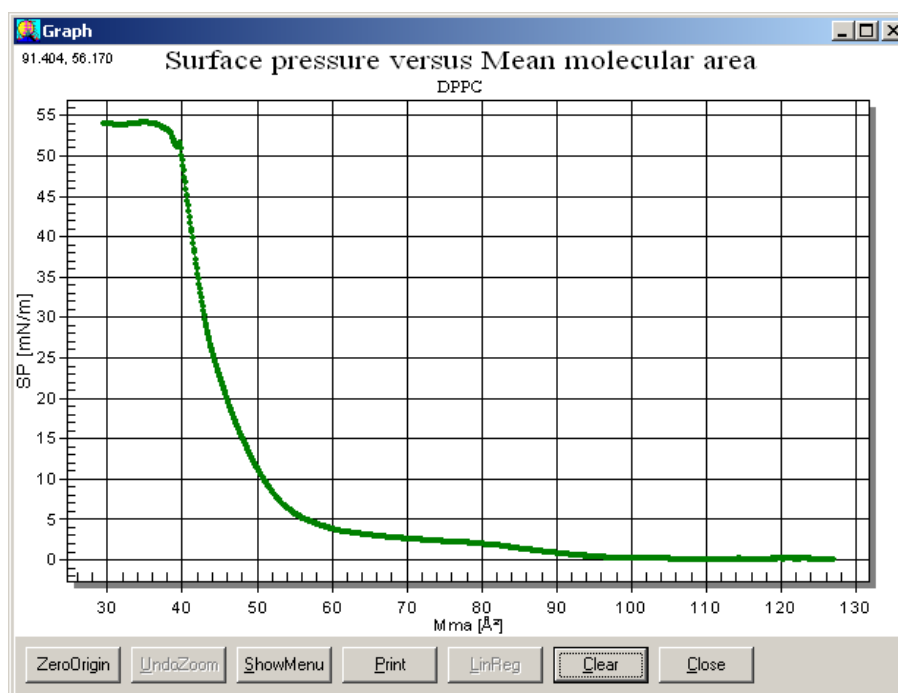
Fill in the fields as above and change the fields to fit your own information accordingly. The most important parameters in the **Experimental Setup** window to be defined for correct results are:

- **The exact concentration of the monolayer material in the spreading solution**
- **The exact volume of the solution spread on the subphase**
- **The molecular weight of the monolayer material**

There is an **Area** button in the **Substance** field that can be used for checking the initial area per molecule before deciding how much of the monolayer containing solution should be spread. This feature is especially useful when using monolayer materials with known properties. For a DPPC monolayer this area should be at least $> 110 \text{ \AA}^2/\text{molecule}$ to make a proper isotherm measurement. In the case above the area is as shown in the window below:



5. Hereafter, proceed as described in the points 10. – 17. for the SA monolayer on pure water subphase above.
6. The DPPC isotherm on a pure water subphase should look like the following



The distinctive features of the DPPC isotherm on a pure water subphase are; a) that the surface pressure starts to rise at an area of about $100 \text{ \AA}^2/\text{molecule}$ on a pure water subphase at room temperature, b) that there appears a plateau region below 10 mN/m (depending on the level of contamination of the used DPPC material). For the most pure DPPC the plateau should be at 8 mN/m and almost horizontal. This plateau is a phase

transition where the monolayer goes from a liquid expanded (LE) to a liquid condensed (LC) phase, and during this plateau these two different phases co-exist, and c) the extrapolated area from the condensed region is around $47 \pm 1 \text{ \AA}^2/\text{molecule}$.

7. Deposition (Dipping) measurement

We will in this section perform one dipping experiment by transferring 9 layers of the SA monolayer from a 5×10^{-3} M $\text{MnCl}_2 \times (4 \text{ H}_2\text{O})$ subphase onto a glass slide. The Mn^{2+} ions have been added to the subphase for stabilizing the monolayer and enabling the deposition of multiple layers. Without the salt in the subphase only one monolayer of SA would be transferred to the glass slide, and this would also probably be peeled off when trying to deposit the next layer on top of it.

7.1. Preliminaries

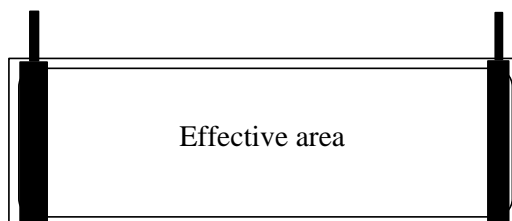
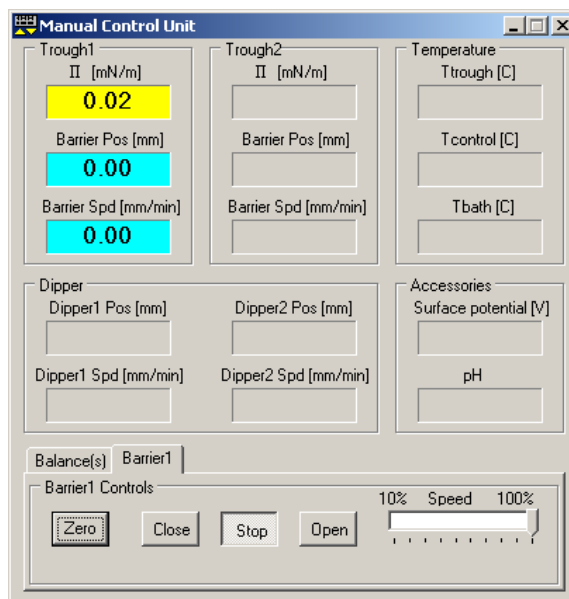
Every time you are preparing a measurement with your KSV L or LB instrument there will be a series of procedures that are recommended to do in order to achieve the best outcome of your studies. These includes:

- Wash your trough and barriers thoroughly. For most cases using a soft brush to “paint” the trough and barriers with pure ethanol (or other organic solvent), and then rinsing with pure ion exchanged water has shown to be a sufficient way to clean the trough and barriers.

NOTE! The barriers do not tolerate Chloroform as a cleaning solution, but this does not restrict the use of Chloroform as a spreading solvent for the monolayer material.

During cleaning it is advisable to use rubber gloves, because touching your trough or barriers with your bare hands can later contaminate your subphase surface, which further affects the outcome of your measurements and results. If you have not used the trough for a long time it is good to rinse it thoroughly with detergent (Decon, Helmanex, Fairy) and hot water, and after that use the cleaning procedure described above. Note that with the KSV trough, it is easy (and advisable) to move the trough to a sink where it can easily and thoroughly be cleaned.

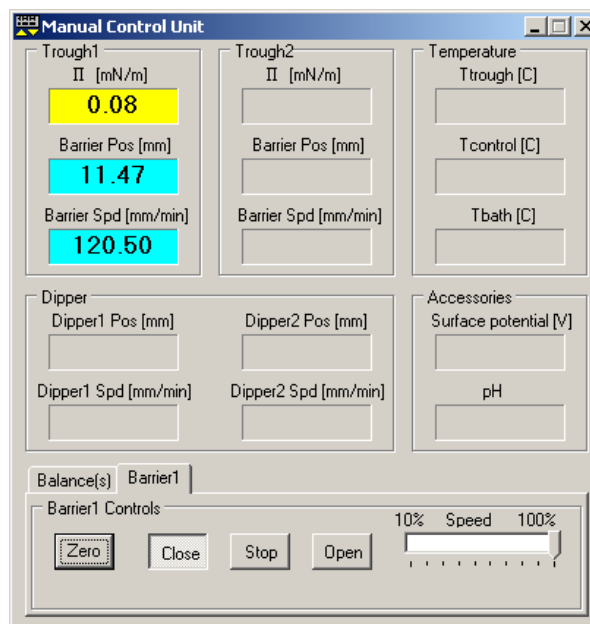
- Place the cleaned trough and barriers on their positions on the instrument and move the balance(s) to the center of the trough. Place Dipper so that it enables the dipper to immerse and withdraw the solid sample into the subphase at the point where the dipping well is located, but not disturbing (touching) the Wilhelmy plate.
- Move the barriers with the software **Manual Control Unit** (or the optional physical Manual Control Unit if the instrument is equipped with it) so that there is not left a space more than about 0.5-1 mm between the back of the barrier and the trough edge. See picture below. At this point also move the barrier safety switches to these positions.



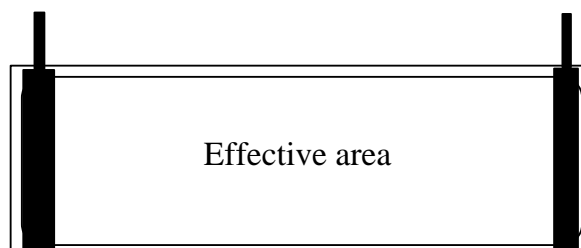
Now we are all set for making the actual deposition measurement.

7.2. Deposition of Stearic acid from a MnCl_2 subphase onto glass

1. Now freshly prepare a solution containing some salt, for example 5×10^{-3} M $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ and fill the trough with this so that the surface of the subphase is a couple of mm above the edges of the trough.
2. Wait until the temperature of your subphase has stabilized to the temperature you have chosen before continuing.
3. Use the software **Manual Control Unit** (or the optional physical Manual Control Unit if the instrument is equipped with it) to close the barriers to the center and simultaneously clean the liquid surface between the barriers by using the aspirator (or a suction pipette) until the barriers are as close together as possible. Hereafter, open the barriers to the zero position and repeat the above procedure. This procedure should be repeated 2-3 times.



The last time keep on cleaning the liquid surface between the barriers until the water level equals or is just a little bit higher than the trough edge level. Then move the barriers back to zero position (see figure below).



4. Rinse the Wilhelmy plate with pure ethanol and ion-exchanged water. Hang the plate on the surface balance and lower the plate into the subphase so that about two thirds of the plate is above the surface.

Every now and then it is advisable to flame clean your probe, especially if it does not seem to be wetted completely when immersed in the subphase.

If you use your Wilhelmy plate for the first time you should clean it with solvent and flame it prior to use. Then store it in some water-soluble organic solvent (methanol, ethanol). To flame clean your probe, make sure you have a strong flame source comparable to a bunsen burner. Low heat flames will leave residues on your plate, which can lead to significant error. Hold the probe in the hottest part of the flame until the entire plate glows brightly for a few seconds. It is advisable to flame the Wilhelmy plate every now and then but it is not necessary to do it every time you use the plate. Rinsing with ethanol and water should be sufficient for most cases.

5. After hanging the Wilhelmy plate on the balance zero the balance and barrier positions from the software **Manual Control Unit** and check the cleanliness of the subphase by compressing the barriers together and looking on the balance value on the display. If the surface pressure value stays below 0.2-0.3 mN/m during this compression then the subphase

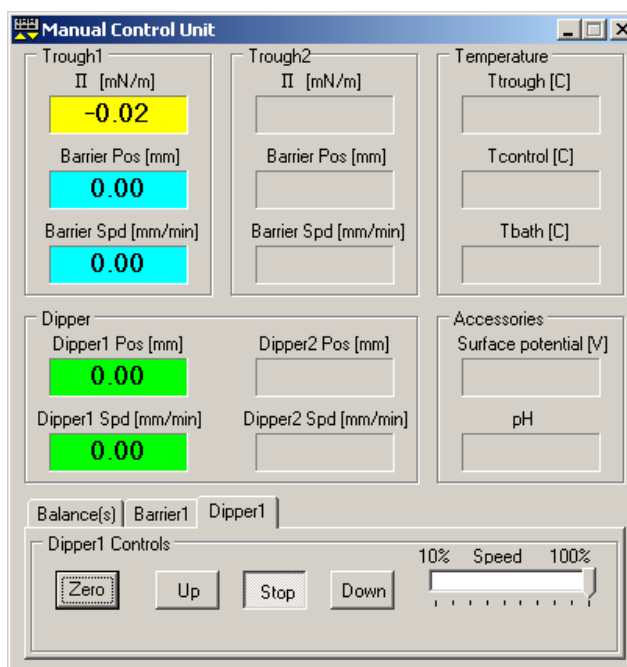
surface can be regarded as clean and you can proceed to the next step. However, if the surface pressure value increases above 0.3 mN/m, then it is recommended to clean the surface once more as was described in point 3 above.

6. Clean the glass slide by immersing it in for example in chromic acid or *piranha* solution for a few minutes. Hereafter, rinse it thoroughly with ion exchanged water and dry the glass by sucking with the aspirator. Other cleaning procedures can also be used.

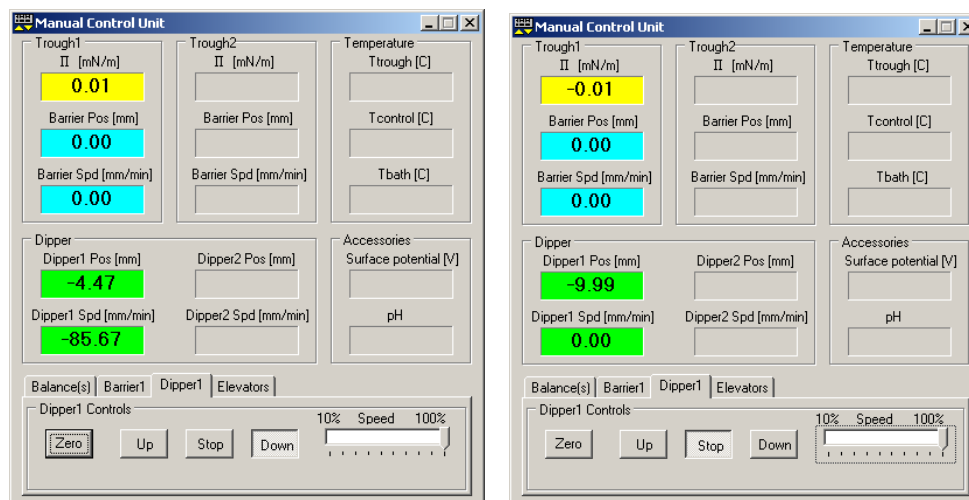
NOTE! Chromic acid and *Piranha* solutions are very toxic and corrosive, therefore utmost care and proper safety cautions should be kept in mind while using these liquids.

7. When the glass is dry place it in the dipper sample clip holder, and attach this holder to the dipper arm by using an Allen wrench. Make sure that you place the sample clip holder so that the glass slide is **parallel** with the barriers. The quality of the deposited film can be largely affected by the orientation of the solid substrate relative to the barriers. A parallel orientation has been shown to give the best quality LB layers.
8. Hereafter, lower with the help of the software **Manual Control Unit** the glass slide so that it **JUST** touches the subphase surface and stop the dipper movement.

This will be your Dipper zero point. Now at this point **IT IS VERY IMPORTANT** that you zero the dipper position from the software **Manual Control Unit**.



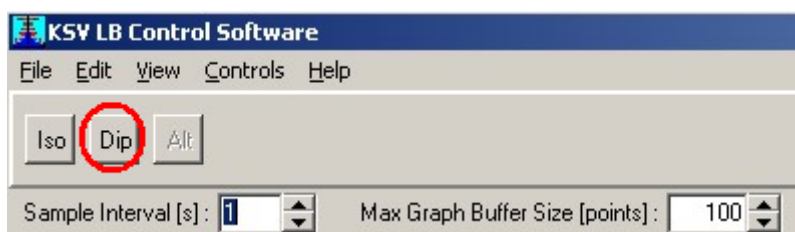
9. After the zero position of the dipper has been defined, lower the glass slide with the help of the software **Manual Control Unit** down to the depth (height) you want the slide to be coated (10 mm in this example). Hereafter, it is very important **NOT** to zero the dipper position anymore in order to be able to calculate the quality of transfer (Transfer Ratio, T.R.) during the measurement.



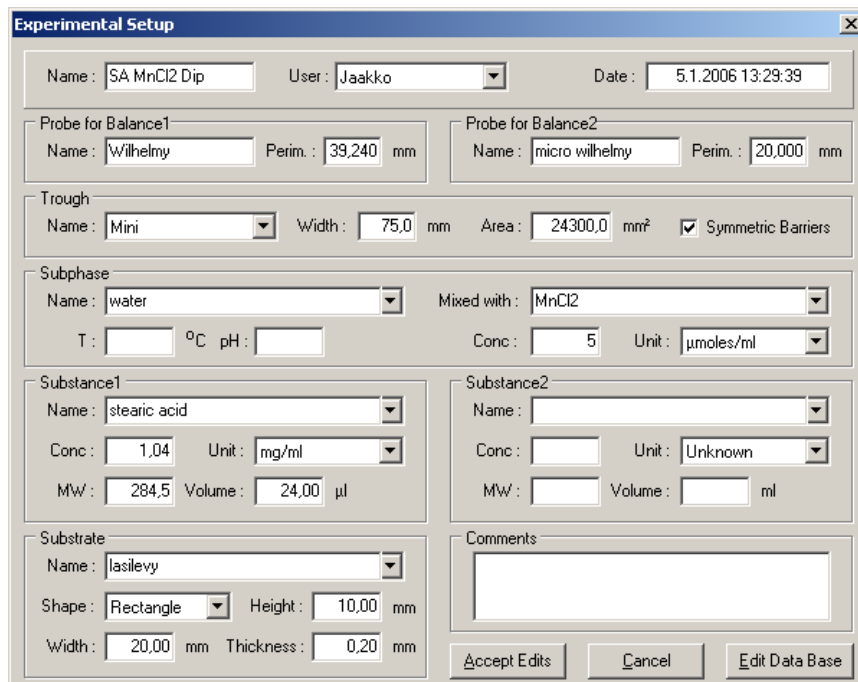
Lowering the solid substrate into the subphase

Solid substrate in its starting position for deposition

10. Clean the syringe by placing the needle in a bottle containing pure Hexane or Chloroform and fill and empty the syringe about 5-6 times. Then heavily shake the SA bottle, open the cap of the SA bottle, place the needle in the bottle and take about 25 μl (for a KSV Mini trough) or about 80 μl (for a KSV Standard trough) of the SA solution into the syringe.
11. Just before starting to spread the SA solution on the subphase surface it is **very important** that you zero the balance and barrier positions, but **NOT** the dipper position from the software **Manual Control Unit**.
12. Spread all of the SA solution carefully on the surface by first forming a small drop of the solution on the tip of the needle and carefully make the drop touch the subphase surface as was described in the isotherm measurement section. Remember, while spreading follow the surface pressure reading on the **Interface Unit** display and make sure that you spread the substance slowly enough so that the surface pressure value does not exceed 0.5 mN/m at any point. This ensures that the monolayer material is able to spread properly to the surface. Let the solvent evaporate for at least 10 minutes.
13. During the waiting time for the evaporation of the solvent start the isotherm measurement by pressing the **DIP** button in the **KSV LB Control Software** window.



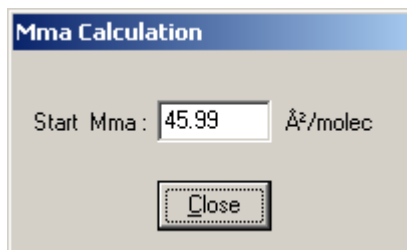
This will bring up the **Experimental Setup** window where all necessary parameters for the Dipping measurements are defined (see the instrument LB manual for more precise definitions of the fields in the Experimental Setup window).



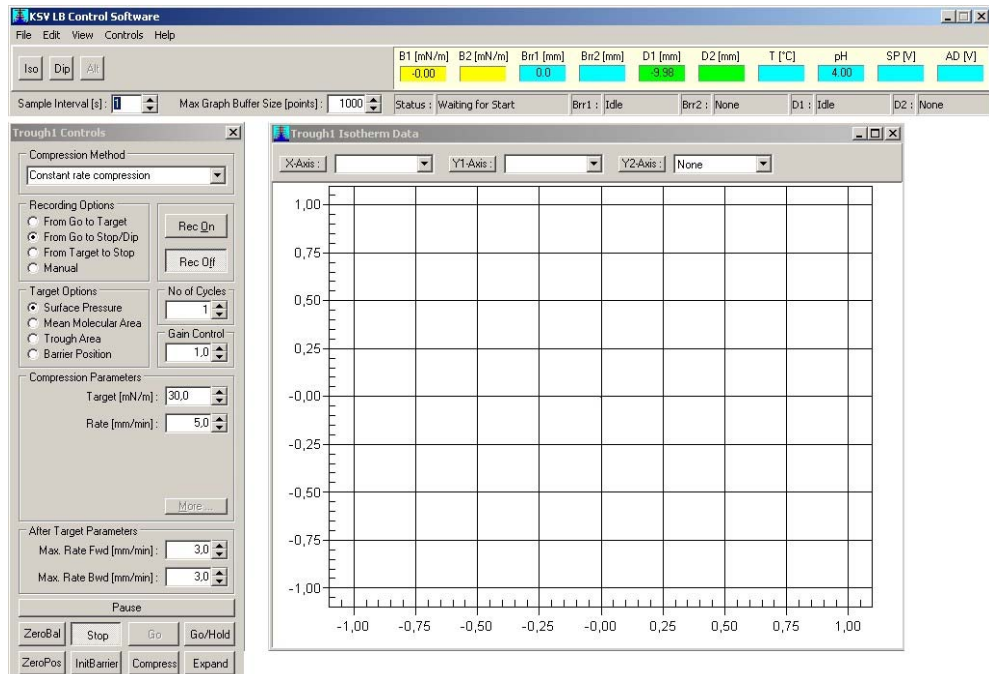
Fill in the fields as above and change the fields to fit your own information accordingly. The most important parameters in the **Exeperimental Setup** window to be defined for correct results for the dipping measurement are:

- **The shape of the glass slide (solid substrate)**
- **The exact width of the glass slide (solid substrate)**
- **The thickness of the glass slide (solid substrate)**
- **The depth (Height) at which the glass slide (solid substrate) was lowered above or is to be lowered to during the dipping measurement.**

Pressing the **Area** button in the **Substance1** box will show the starting area of compression per molecule:



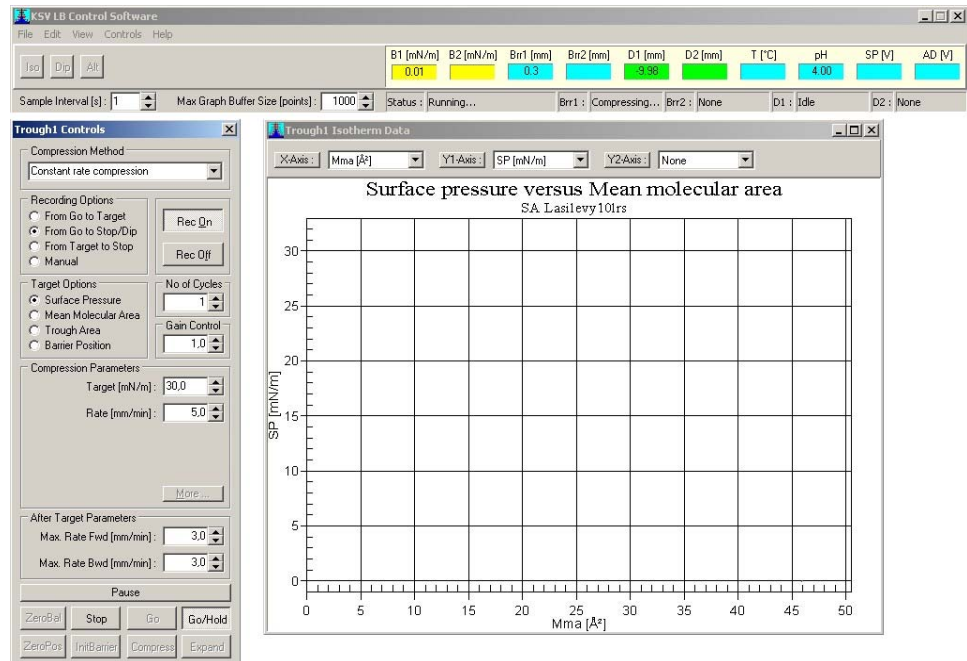
14. After completing the **Experimental Setup** window press the **Start** button. This will not start the actual measurement or any compression yet, but it will open the **Trough Controls** and **Trough Isotherm Data** windows as shown below.



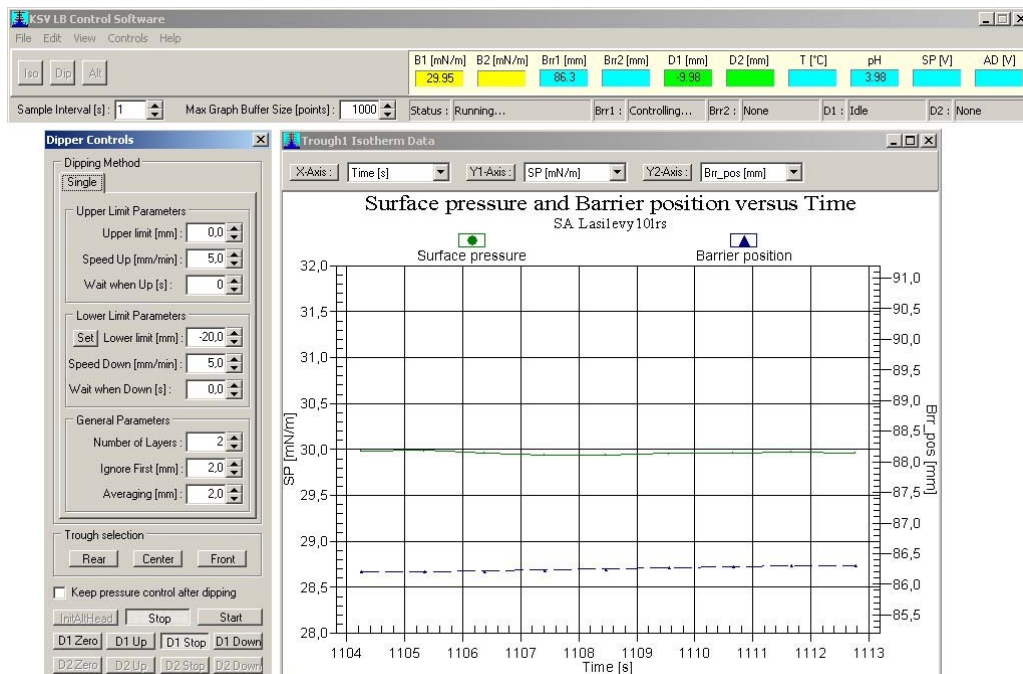
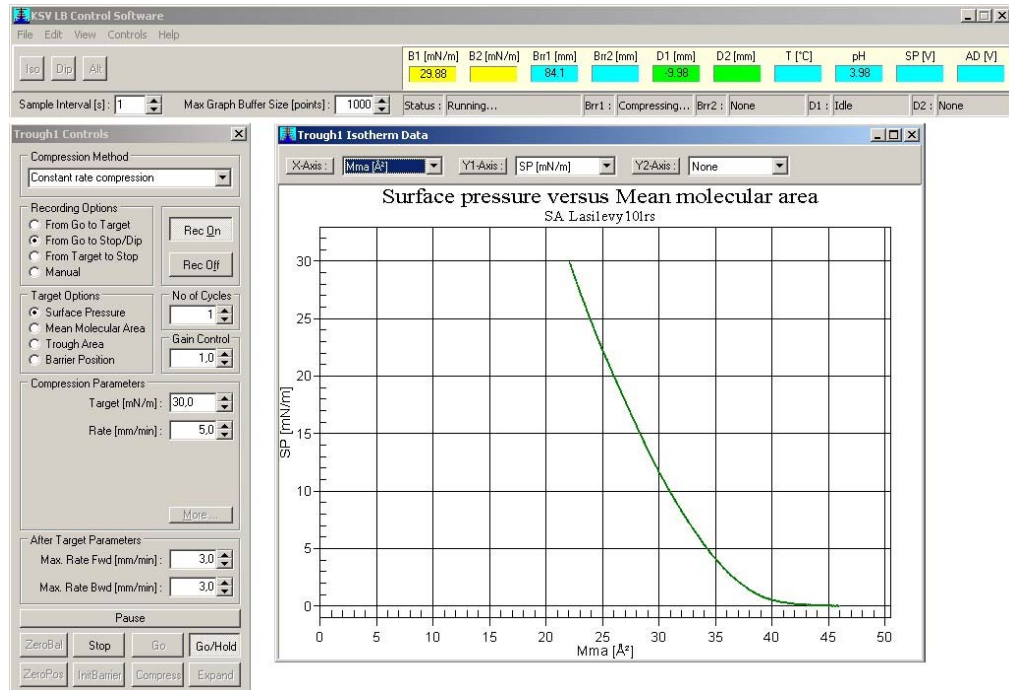
Set the parameters in the **Trough Controls** window as shown above. For a more precise description of options and fields in the **Trough Controls** window please see the LB instrument manual.

NOTE! This is the point where the surface pressure at which the monolayer is deposited is defined. In this case we have chosen 30 mN/m as the target surface pressure at which the monolayer will be transferred to the glass slide (solid substrate).

- After at least 10 minutes has passed from the spreading of the monolayer material on the subphase surface, press the **GO/HOLD** button in the **Trough Controls** window. This will inactivate all the other buttons in the **Trough Controls** window except the **STOP** button, and start the compression of the barriers. The surface pressure as a function of the area will be plotted on-line in the **Trough Isotherm Data** window as the compression proceeds. The **Status:** line in the **KSV LB Control Software** window will also change from **Waiting for Start** to **Running...** The **Compression Parameters** in the **Trough Controls** window can be changed while the measurement is running and will be activated immediately after the change has been done.



16. When the monolayer reaches the target pressure the **Trough Controls** window will disappear and the **Dipper Controls** window will automatically appear. The graph is also changed to show the surface pressure and barrier position as a function of time.



17. Now wait for the monolayer to stabilize for at least 10 – 20 minutes. During this waiting period define the parameters in the **Dipper Controls** window as shown below.



Dipper Controls

Dipping Method: Single

Upper Limit Parameters:

- Upper limit [mm]: 2,0
- Speed Up [mm/min]: 5,0
- Wait when Up [s]: 1200

Lower Limit Parameters:

- Lower limit [mm]: -10,0
- Speed Down [mm/min]: 5,0
- Wait when Down [s]: 0,0

General Parameters:

- Number of Layers: 9
- Ignore First [mm]: 2,0
- Averaging [mm]: 2,0

Trough selection: Rear, Center, Front

Keep pressure control after dipping

InitAltHead, Stop, Start

D1 Zero, D1 Up, D1 Stop, D1 Down

D2 Zero, D2 Up, D2 Stop, D2 Down

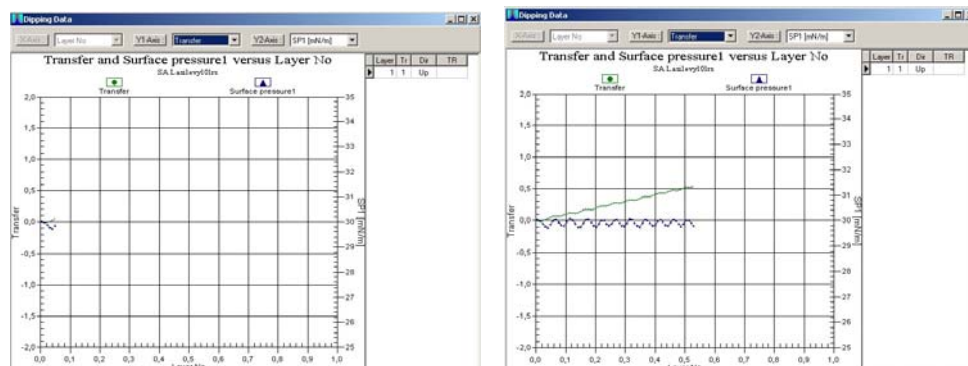
Note that defining the minimum depth (Height) can simply be done by pressing the set button beside the field reserved for this parameter. This is the case only when depositing on a hydrophilic (water loving) substrate. When using a hydrophobic substrate (water repelling) you need to decide the immersion depth in beforehand and define it here before starting the deposition.

The upper limit has been put to 2 mm in order to make sure that the glass slide (solid sample) will clearly be raised above the air-water interface during the upper waiting (drying) period. This, because if the glass slide is in contact with the interface the transferred layer it does not have any possibility to completely dry before the next layer is deposited on the slide. If complete drying is not allowed it might happen that the previously deposited layer is peeled off during the next deposition cycle.

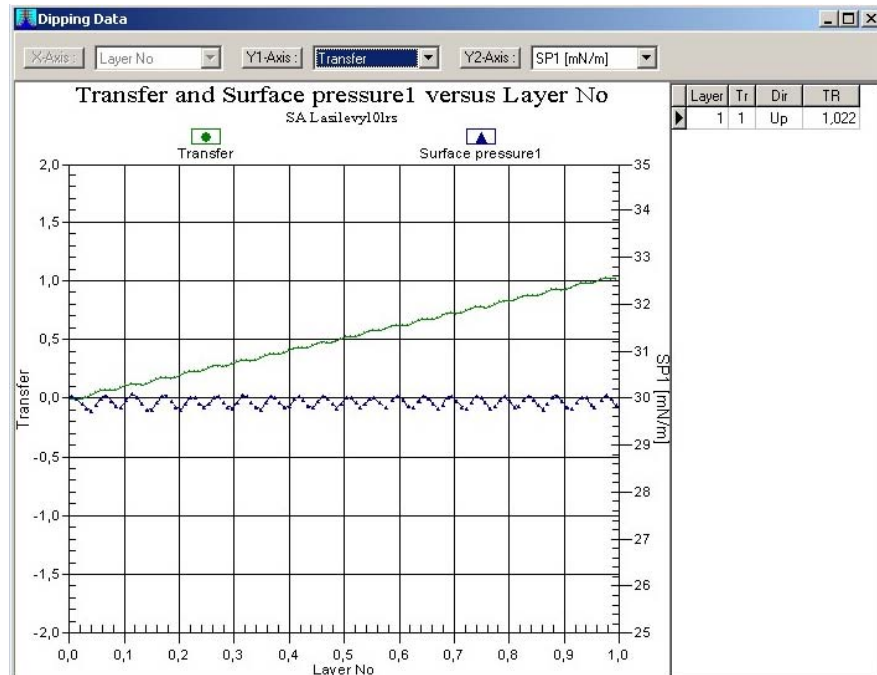
Normally the deposition speeds are kept around 5-10 mm/min to ensure enough slowly and smooth transfer of the layer.

18. After the monolayer have been stabilised for at least 10 minutes then press the **Start** button in the **Dipper Controls** window.

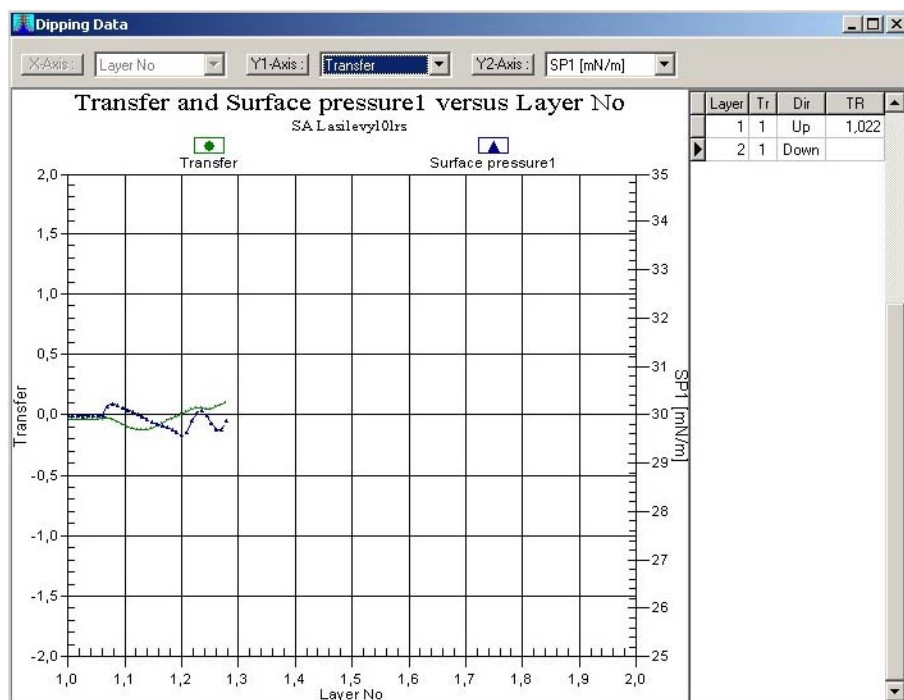
This will initiate the dipping process and the glass slide is started to be lifted out from the subphase, while simultaneously the instrument keeps the surface pressure constant. The graph also changes to show the surface pressure and the transfer ratio as a function of time as the dipping proceeds.



After the first layer has been deposited the graph show the calculated transfer ration, and the glass slide is kept in air for a sufficiently long time (normally 15-20 minutes) to enable complete drying before depositing the next layer(s).



First layer deposited.



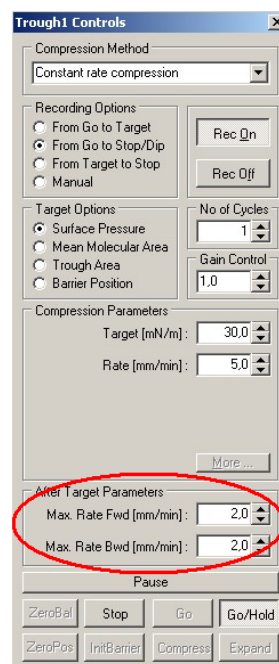
Second layer under transfer (deposition).

If the surface pressure starts to suddenly decrease this might be due to that the barriers can not keep the pressure that was defined for the deposition. In this kind of case one could try to increase the barrier speeds from the

Trough Controls window by first opening it again by choosing **Controls** → **Trough Controls**.

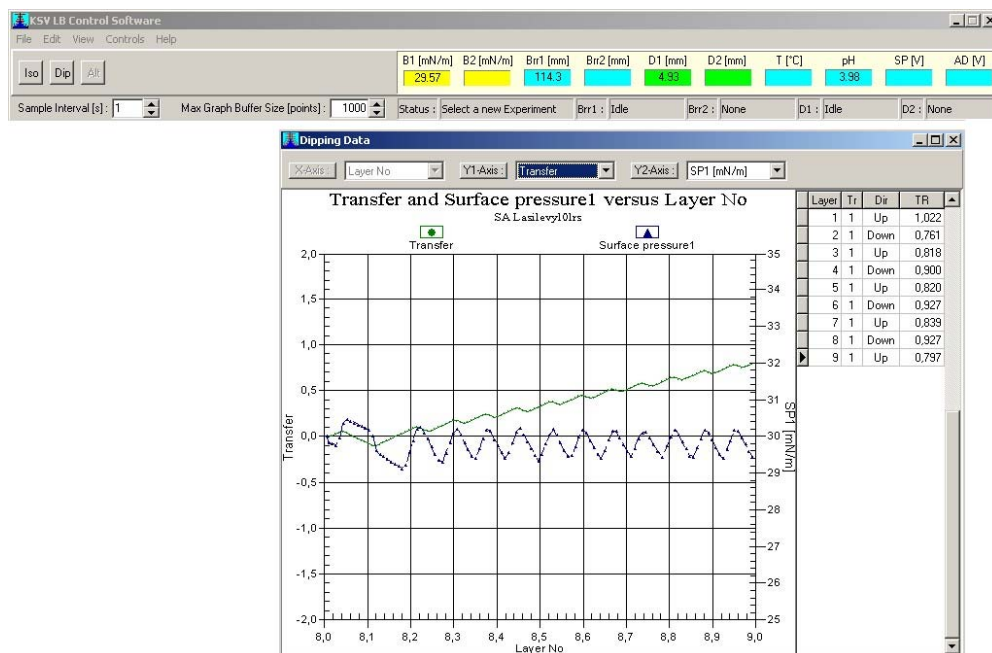


This will open the **Trough Controls** window. In order to change the barrier speed after the target pressure have been reached adjust the **Max. Rate Fwd** and **Max. Rate Bwd** in the **After Target Parameters** box.

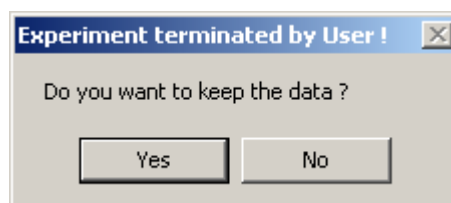


The parameters in the **Dipper Controls** window can also be changed during the measurement and will be activated for the next deposited layer if necessary.

- After all layers (9 in this example) defined in the **Dipper Controls** window have been deposited the software automatically stops and leaves the **Main Menu** and the **Dipping Data** window with the T.R. values on the right hand side visible on the desktop.




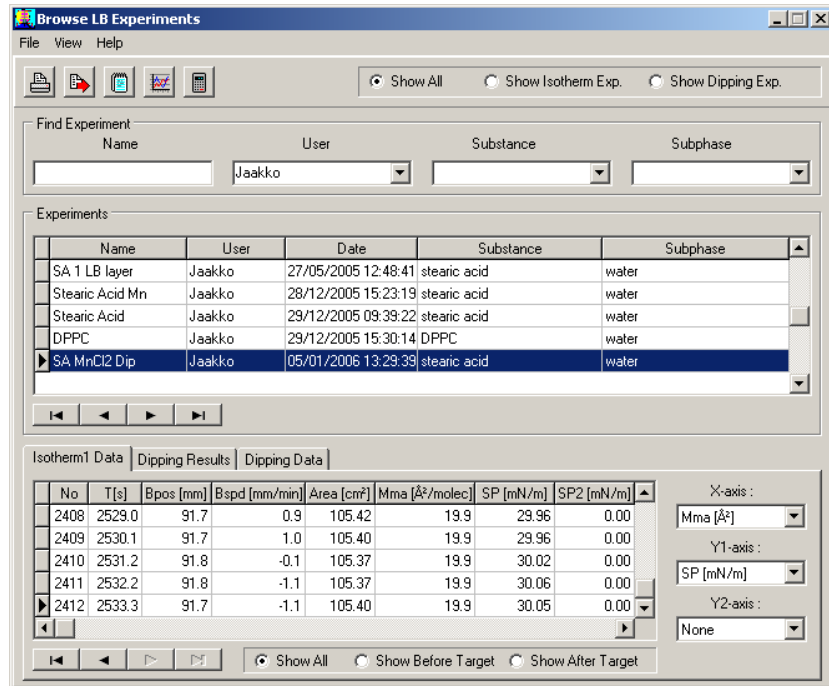
You can also stop the deposition process before all the defined layers have been deposited at any time by pressing the **STOP** button. The software will in that case ask for confirmation before interrupting the measurement.



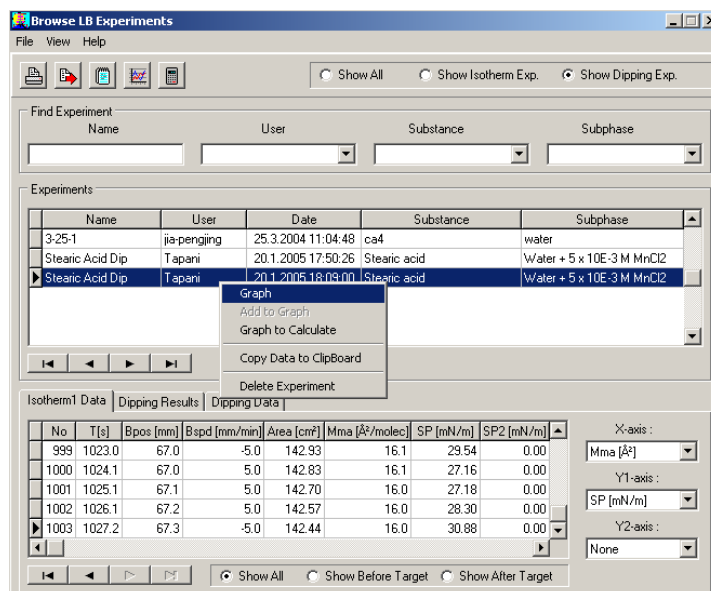
Pressing the **Yes** button will stop the instrument and store the data recorded so far.

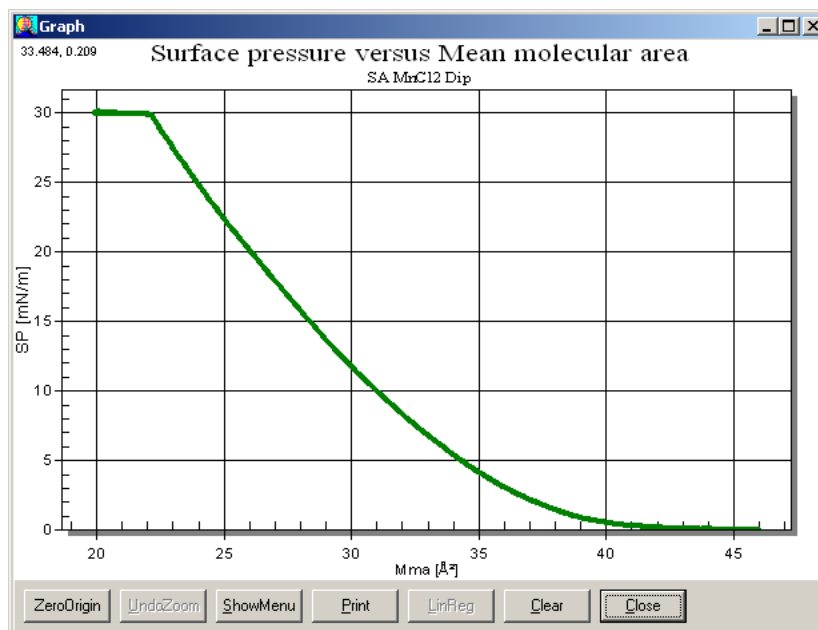
20. The data collected during the measurement is stored in the software database and can be retrieved later for analysis through the **Browse LB**

Experiment software, which can be activated by pressing the icon  in the **Main Menu**.

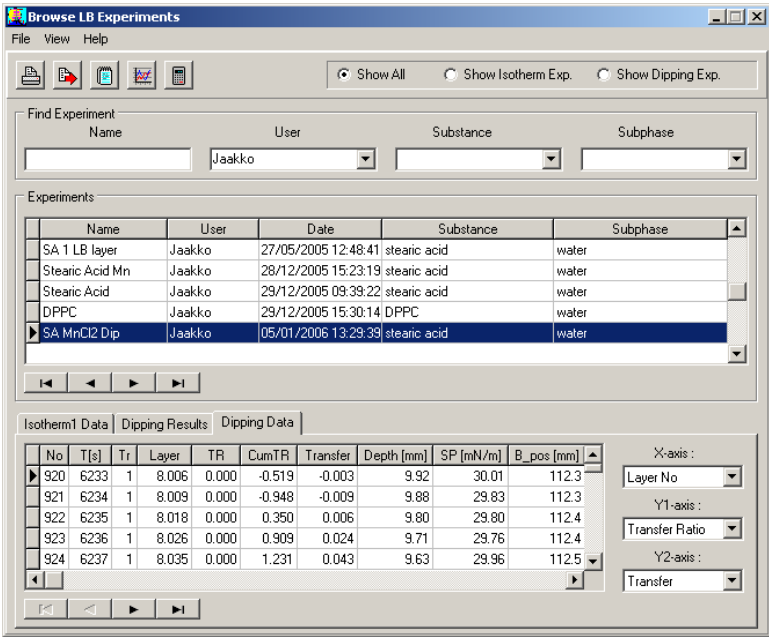


21. The isotherm part or the deposition data can then be re-plotted by highlighting the measurement of interest, activate the **Isotherm Data** page and right-click on the mouse and then choose **Graph**.





22. The dipping part of the deposition data can then be re-plotted by highlighting the measurement of interest, activate the **Dipping data** page and right-click on the mouse and then choose **Graph**. The transfer ratio window should look something similar to what is shown below. A transfer ratio value of 1 means optimum deposition, while a lower value means not so good transfer and a higher value indicates instability in film or multilayer deposition at the same time.



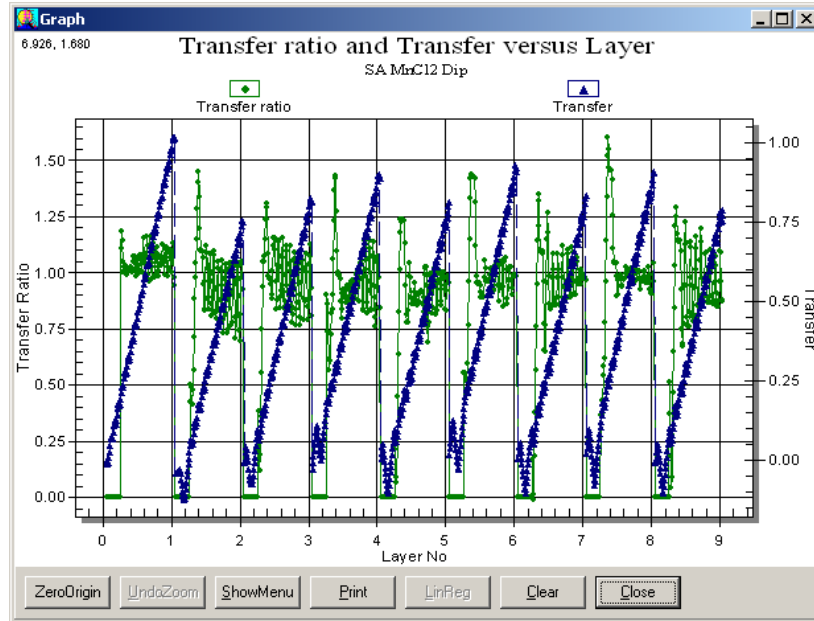
Browse LB Experiments window showing a list of experiments and a detailed view of dipping data for "SA MnCl2 Dip".

Experiments table:

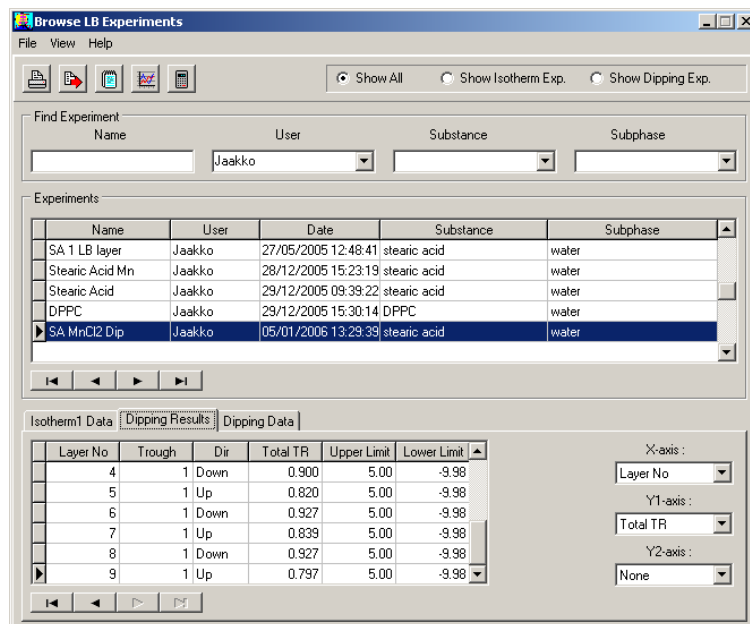
Name	User	Date	Substance	Subphase
SA 1 LB layer	Jaakko	27/05/2005 12:48:41	stearic acid	water
Stearic Acid Mn	Jaakko	28/12/2005 15:23:19	stearic acid	water
Stearic Acid	Jaakko	29/12/2005 09:39:22	stearic acid	water
DPPC	Jaakko	29/12/2005 15:30:14	DPPC	water
SA MnCl2 Dip	Jaakko	05/01/2006 13:29:39	stearic acid	water

Dipping Data table:

No	T[s]	Tr	Layer	TR	CumTR	Transfer	Depth [mm]	SP [mN/m]	B_pos [mm]
920	6233	1	8.006	0.000	-0.519	-0.003	9.92	30.01	112.3
921	6234	1	8.009	0.000	-0.948	-0.009	9.88	29.83	112.3
922	6235	1	8.018	0.000	0.350	0.006	9.80	29.80	112.4
923	6236	1	8.026	0.000	0.909	0.024	9.71	29.76	112.4
924	6237	1	8.035	0.000	1.231	0.043	9.63	29.96	112.5

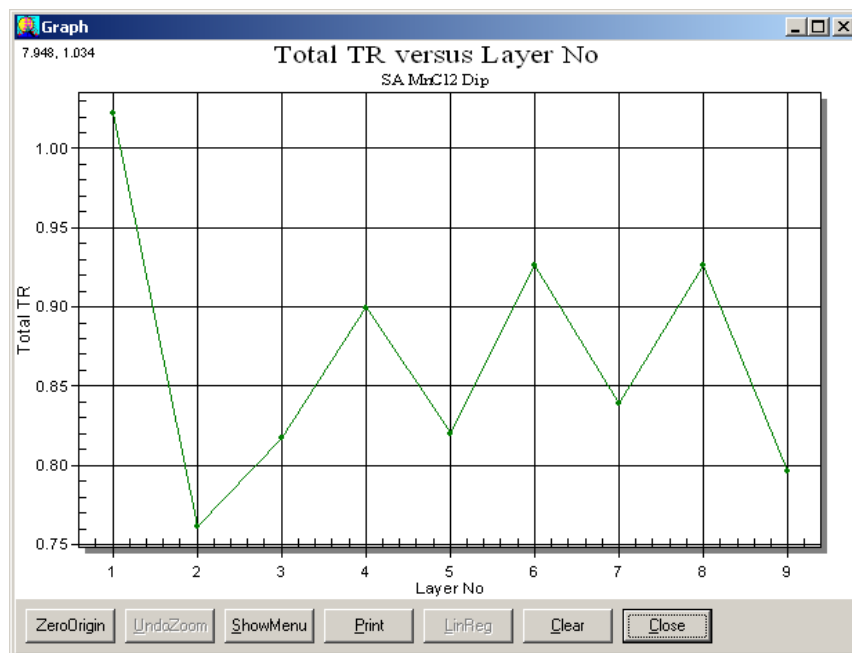




23. The Transfer Ratio (T.R.) values for each layer can be re-plotted by highlighting the measurement of interest, activate the **Dipping Results** page and right-click on the mouse and then choose **Graph**.



Browse LB Experiments window showing a list of experiments. The "SA MnC12 Dip" experiment is selected. Below the list, the "Dipping Results" tab is active, displaying a table of dipping data for Layer No. 4 to 9.

Layer No	Trough	Dir	Total TR	Upper Limit	Lower Limit
4	1	Down	0.900	5.00	-9.98
5	1	Up	0.820	5.00	-9.98
6	1	Down	0.927	5.00	-9.98
7	1	Up	0.839	5.00	-9.98
8	1	Down	0.927	5.00	-9.98
9	1	Up	0.797	5.00	-9.98



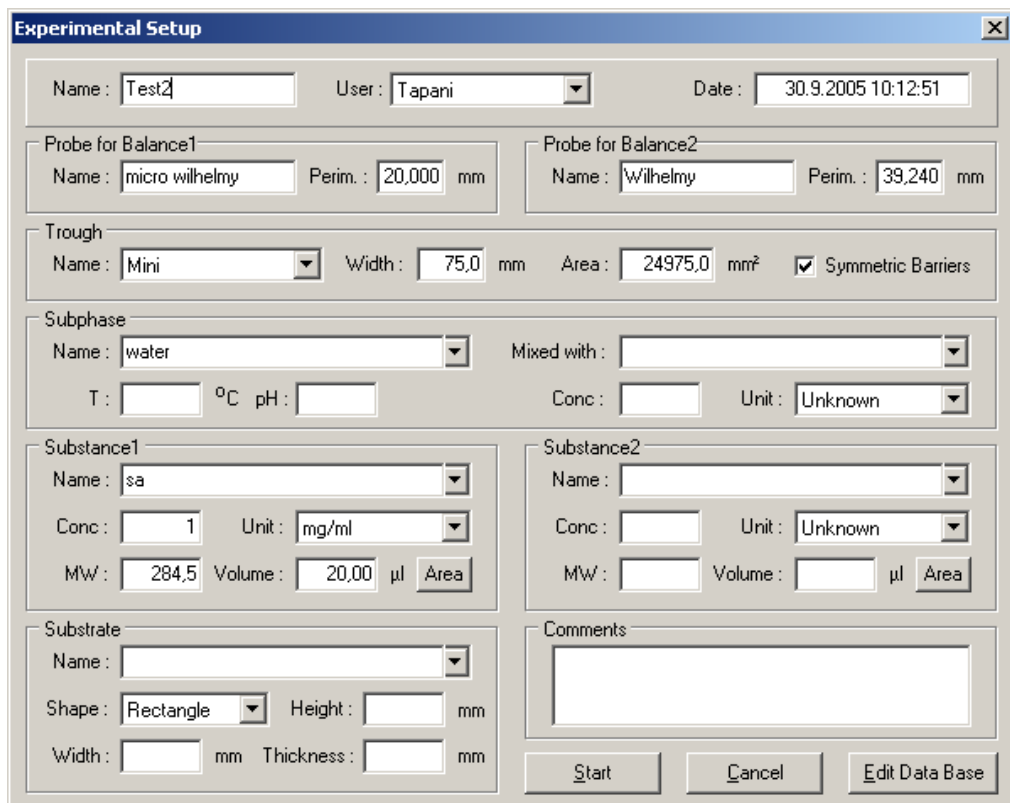
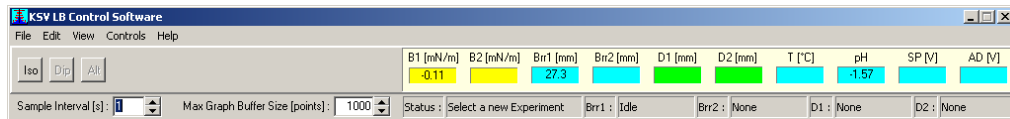
24. After the experiment is finished clean the water surface with a suction pipette (aspiration). Take off the Wilhelmy plate, rinse it with water and pure ethanol and store it in a beaker with a water-soluble organic solvent. Remove the barriers after removing the subphase from the trough. Clean the trough and barriers as described in section 5.1.
25. If performing a second experiment repeat the above procedure from the beginning.
26. After making the measurement, you still have the possibility to use other functions in the data reduction capabilities of the LB software by clicking the  **Browse LB Experiments** icon, . Please, see the LB instrument manual for details.
27. After making all the experiments and data reduction for the day shut down the LB software and the computer. It is also advisable to clean the Wilhelmy plate, trough and barriers after the last measurement for the day and store the parts in a dust free environment.

8. Other interesting basic measurements – Relaxation/Stability/Kinetics and Hysteresis

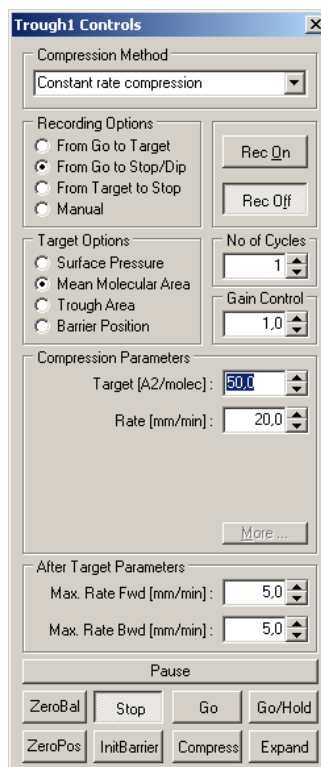
8.1. Relaxation/Stability/Kinetics measurements

The relaxation/stability/kinetics measurements in the KSV WinLB software is made in the following way:

1. Prepare the measurement as you would prepare a normal Isotherm measurement.
2. Start the Isotherm measurement (ISO button) and fill in the **Experimental setup**.

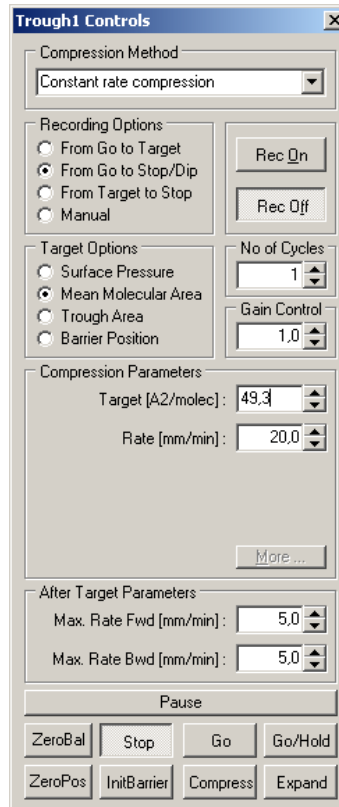


3. After filling the **Experimental setup** and pressing the **Start** button the **Trough1 Controls** window will appear.

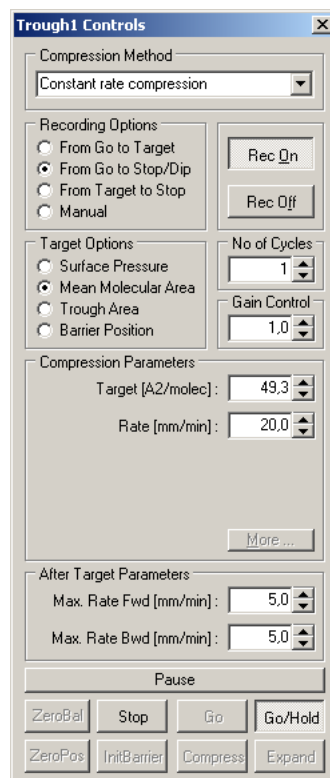


4. From the **Trough1 Controls** window choose as **Compression Method: Constant Rate Compression** and as **Recording Options: From Go to Stop/Dip**.
5. Next choose the appropriate **Target Options**. Note that the **Compression Parameters** will change according to what **Target Option** is chosen. Define the Target value in the Compression Parameters box.

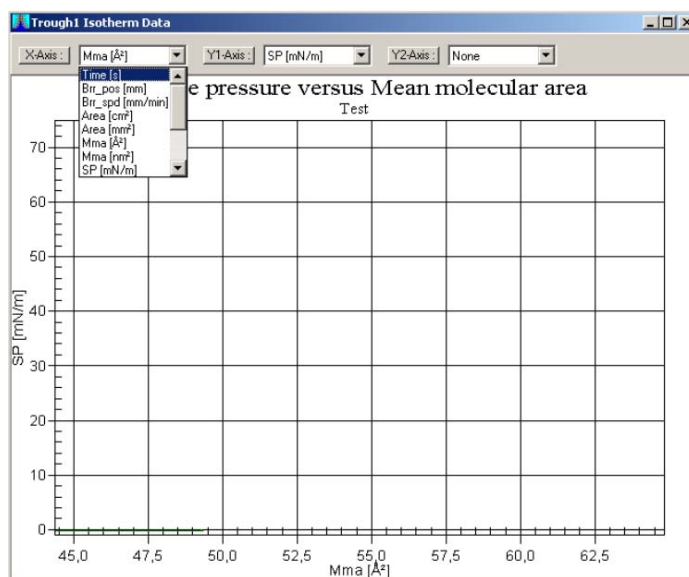
If non-integer units are required, then double-click to highlight the Target value and then just type in the required value. In this case we use 49.3 Å²/molecule.



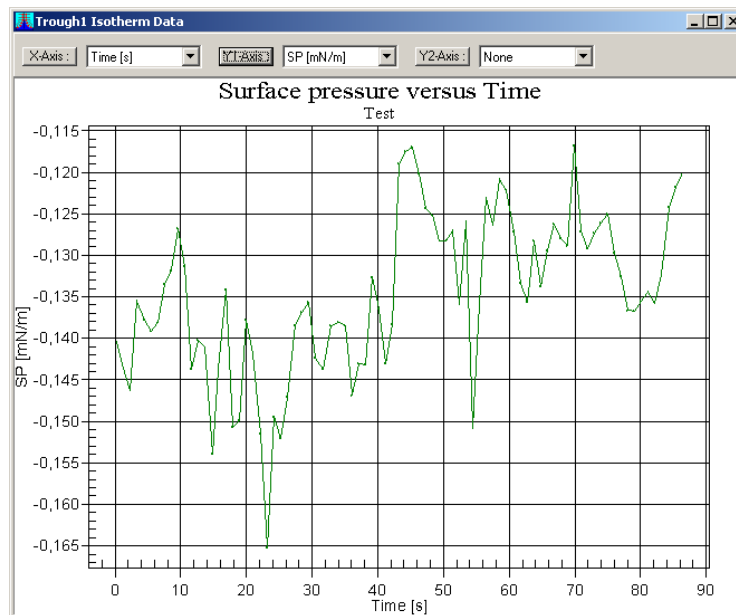
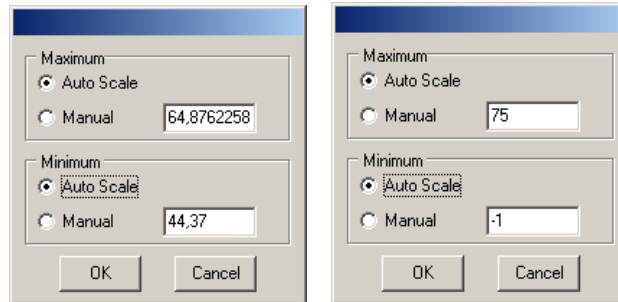
- a) If you want to keep the surface pressure constant and monitoring the trough/molecular area select as the target option: **Surface Pressure** and set the appropriate **Compression Parameters**.
 - b) If you want to keep the mean molecular area constant and monitoring the surface pressure select as the target option: **Mean Molecular Area** and set the appropriate **Compression Parameters**.
 - c) If you want to keep the trough area constant and monitoring the surface pressure select as the target option: **Trough Area** and set the appropriate **Compression Parameters**.
 - d) If you want to keep the barrier position constant and monitoring the surface pressure select as the target option: **Barrier Position** and set the appropriate **Compression Parameters**.
6. Here we will use option 5 a). After the appropriate **Target Options** have been chosen and the **Compression parameters** are defined and you are ready for starting the actual measurement, then press the **GO/HOLD** button.



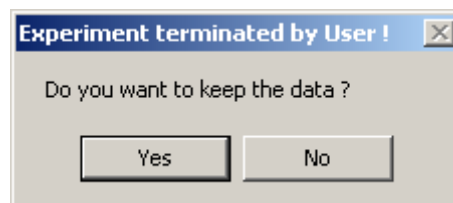
7. Now the measurement will continue until you press the **STOP** button.
8. In order to see the data recorded as a function of time after the *Target* is reached you need to change the X- and Y-axis from the drop down menus in the *Trough1 Isotherm Data* graph according to your needs.



It is also worthwhile to change the scaling to be *Auto Scale* by pressing the *X-axis* and *Y-axis* buttons beside the drop down menus.



9. After the measurement has continued the required time press the *Stop* button. This will bring you up a screen asking to save the recorded data.



10. Now the measurement can be plotted in the *Browse Experiment* section by choosing the appropriate X- and Y-axis for the graph.

Browse LB Experiments

File View Help

Show All Show Isotherm Exp. Show Dipping Exp.

Find Experiment
Name User Substance Subphase

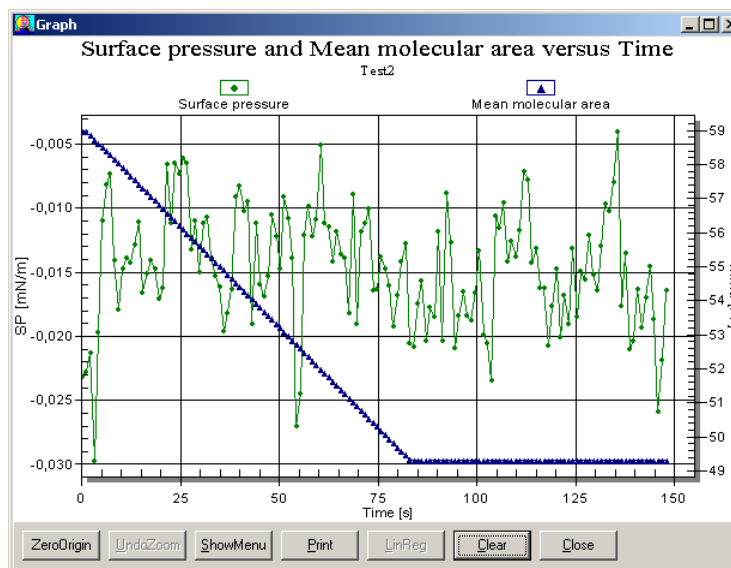
Name	User	Date	Substance	Subphase
myoglob_0_5_30	Maria	19.9.2005 13:06:43	myoglobin	water
myoglob_0_5_60	Maria	19.9.2005 14:54:36	myoglobin	water
myoglobin_30_2	Maria	20.9.2005 8:33:00	myoglobin	water
myoglobin_30_J1	Maria	20.9.2005 11:33:38	myoglobin	water
If	Tapani	30.9.2005 9:58:47	sa	
Test	Tapani	30.9.2005 10:06:00	sa	water

Experiments

Isotherm1 Data

No	T[s]	Bpos [mm]	Bspd [mm/min]	Area [cm ²]	Mma [$\text{\AA}^2/\text{molec}$]	SP [mN/m]	SP2 [mN/m]
174	178.4	27.3	0.0	208,74	49,3	-0,14	0,00
175	179.5	27.3	0.0	208,74	49,3	-0,15	0,00
176	180.5	27.3	0.0	208,74	49,3	-0,15	0,00
177	181.5	27.3	0.0	208,74	49,3	-0,14	0,00
178	182.6	27.3	0.0	208,74	49,3	-0,15	0,00

X-axis: Time [s]
Y1-axis: SP [mN/m]
Y2-axis: None

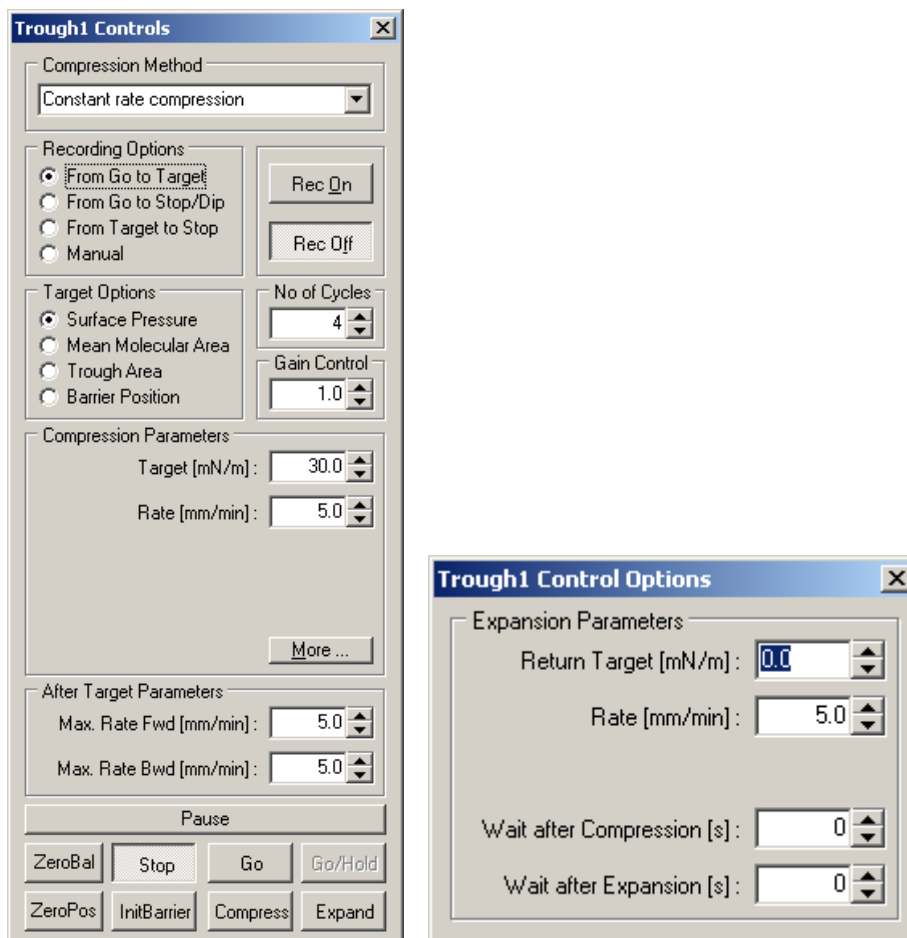


NOTE! If you do not want to record the compression part of the measurement then choose as the Recording Option: From Target to Stop in the Trough1 Controls window.

8.2. Hysteresis measurements

The Hysteresis measurements in the KSV WinLB software is made in the following way:

1. Prepare the measurement as you would prepare a normal Isotherm measurement.
2. Start the Isotherm measurement (ISO button) and fill in the **Experimental setup**.
3. After filling the **Experimental setup** and pressing the **Start** button the **Trough1 Controls** window will appear.
4. From the **Trough1 Controls** window choose as **Compression Method: Constant Rate Compression** and as **Recording Options: From Go to Target**.
5. Now define the number of compression cycles you want the instrument to perform in the **No. of cycles** field. In the example below the number is set to 4. This means that the instrument performs 2 compressions and 2 expansions i.e. 2 complete compression-expansion cycles. As soon as you increase the **No of cycles** to more than one the **More...** button in the **Compression parameters** box will be activated. By pressing the **More...** button additional parameter window needed for the expansion cycles will appear.
6. Next choose the appropriate **Target Options**. Note that the **Compression Parameters** as well as the parameters in the box activated by pressing the **More...** button will change according to what **Target Option** is chosen.



7. After the appropriate **Target Options** have been chosen and the **Compression and Expansion parameters** are defined and you are ready for starting the actual measurement, then press the **GO** button.
8. Now the measurement will do the amount of compressions+expansions defined in the **No of cycles** field. In the example above the instrument will make 2 compressions and 2 expansions.

9. Using Wilhelmy Paper Plates for KSV Langmuir and Langmuir-Blodgett film balances

In some cases, especially when performing compression-expansion hysteresis measurements with the KSV Langmuir and Langmuir-Blodgett film balances it is preferable to use Wilhelmy plates made of paper instead of the standard Wilhelmy plate of Platinum delivered with the instrument. Although the Platinum plate is practical for its reusable property and easy cleaning procedure (burn it in a Bunsen burner) there might be problems when using it for compression-expansion cycles. This problem appears in such a way that the surface pressure never reaches zero surface pressure during the expansion after a compression cycle. The reason for this is that there has been a slight deposition of the monolayer material to the platinum plate surface, and this has slightly modified the contact angle of the subphase against the platinum plate (the contact angle should be zero, i.e. complete wetting, in order to be able to measure correct values).

Despite of the problem that might appear in the compression-expansion cycle experiments, the platinum Wilhelmy plate is still highly suitable for single compression isotherm measurements and LB deposition experiments. Of course, paper plates can as well be used for these standard experiments if necessary.

However, for those interested in using paper plates this document will guide how to use them in combination with a KSV Langmuir and Langmuir-Blodgett film balance.

Shortly, the use of paper plates with a KSV film balance includes the following steps:

1. Prepare a rectangular piece of paper (filter paper) with known dimensions (width) and make a hole on top of the paper plate so that it can be hanged on the balance hook.
2. Define this new **Probe** into the **KSV LB Control Software Database**.
3. Define in the **KSV LB Control Software Instrument Parameters** section the newly defined paper plate as the default probe (Wilhelmy plate) for your coming experiments.
4. Shut down and restart the **KSV Win LB** software in order to load the newly defined paper plate as the default probe (Wilhelmy plate) for your experiments.

9.1. Preparing the paper plate

The paper plate intended to be used for L- and LB film balance measurements should be prepared and cut out of clean laboratory filter paper. It is very important to be able to determine the dimensions (width, and hence the perimeter = 2 x width) of the paper plate accurately, as the perimeter of the plate is used to calculate the measured surface pressure values by dividing the

measured force acting on the paper plate probe by the perimeter of the paper plate.


Another thing that is important is that the paper plate can be placed on the LB balance hook so that it is hanging vertically.

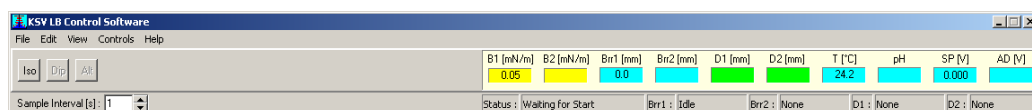
KSV provides ready cut paper plates with dimensions: width = 10.3 mm (Perimeter = 2 x width = 20.6 mm)

NOTE! The paper plates should be soaked in a separate beaker containing the subphase liquid at least for 30 minutes prior to use. This is a precaution to dissolve any contaminations and remove any solid particles from the filter paper.

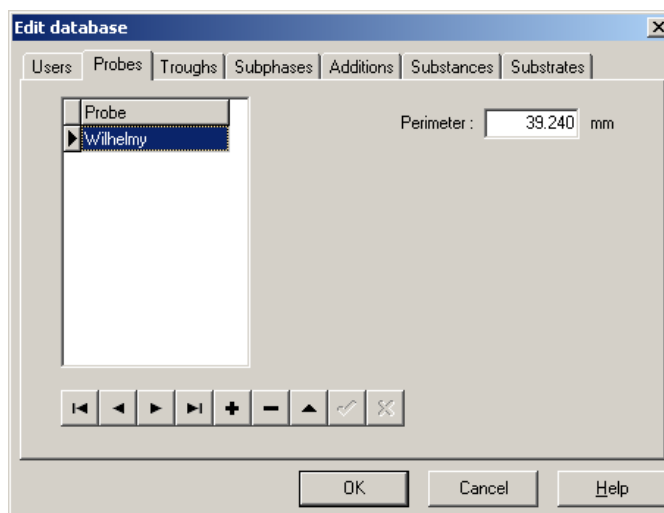
9.2. Defining the probe into the KSV Win LB software

Start the *KSV Win LB software*, and further start the *KSV LB control*

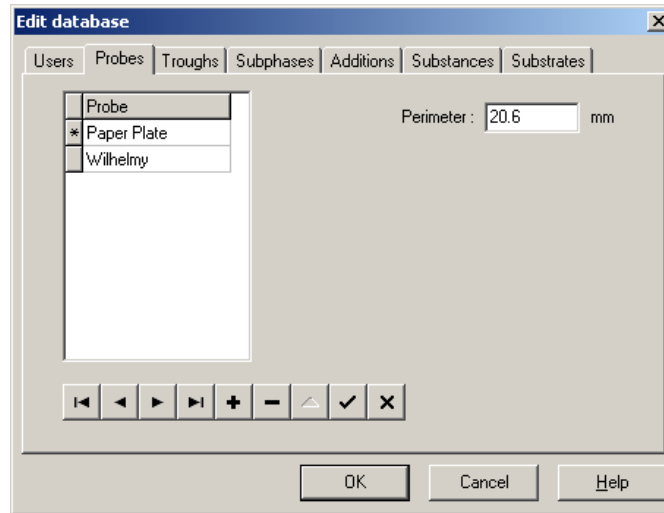
Software from the **Main Menu** window by pressing the,  icon. This will open the *KSV LB Controls Software* window:



Choose **Edit** → **Database** in the *KSV LB Control Software* window to open the **Edit Database** window.




Choose the **Probe** page and add a new probe by pressing the **+** - button, fill in the name and perimeter of the probe and then press the **√** - button.

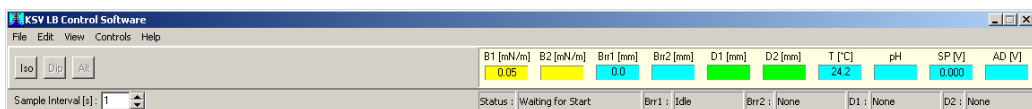


Hereafter, press the **OK** button. Now the new paper plate probe is defined into the **KSV Win LB software** database.

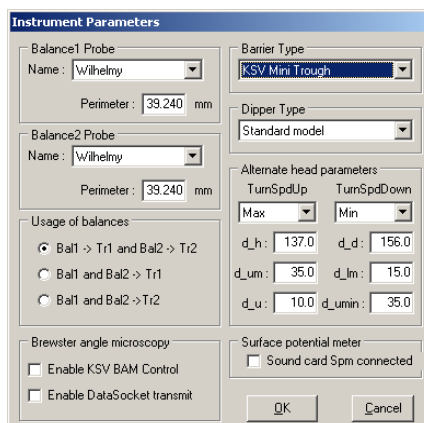
9.3. Defining the paper plate as the default probe for the experiments

The default probe used for L- and LB experiments is defined in the **Instrument Parameters** section in the **KSV Win LB software**. To define the paper plate inserted in the database as the default probe start the **KSV LB Control**

software from the **Main Menu** by pressing the, , icon. This will open the **KSV LB Controls software** window:



Choose **Edit** → **Device Parameters** in the **KSV LB Control Software** window to open the **Instrument Parameters** window.



Choose the newly defined paper plate as the probe in the drop down menu in the **Balance1 Probe** box in this window. Hereafter, press the **OK** button.

9.4. Restarting the KSV Win LB software

In order to load the paper plate as the default probe for your experiments shut down the **KSV Win LB software** completely and restart it again.

Now the paper plate has been defined as the default probe in the **KSV Win LB software** and now the L- and LB experiments can be performed as usual. In order to change the probe back to the standard platinum Wilhelmy plate, then just change the probe for the **Balance1 Probe** in the **Instrument Parameters** window back to the Wilhelmy probe, and then restart the **KSV Win LB software**.

10. Further Reading

General Books & Reviews

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