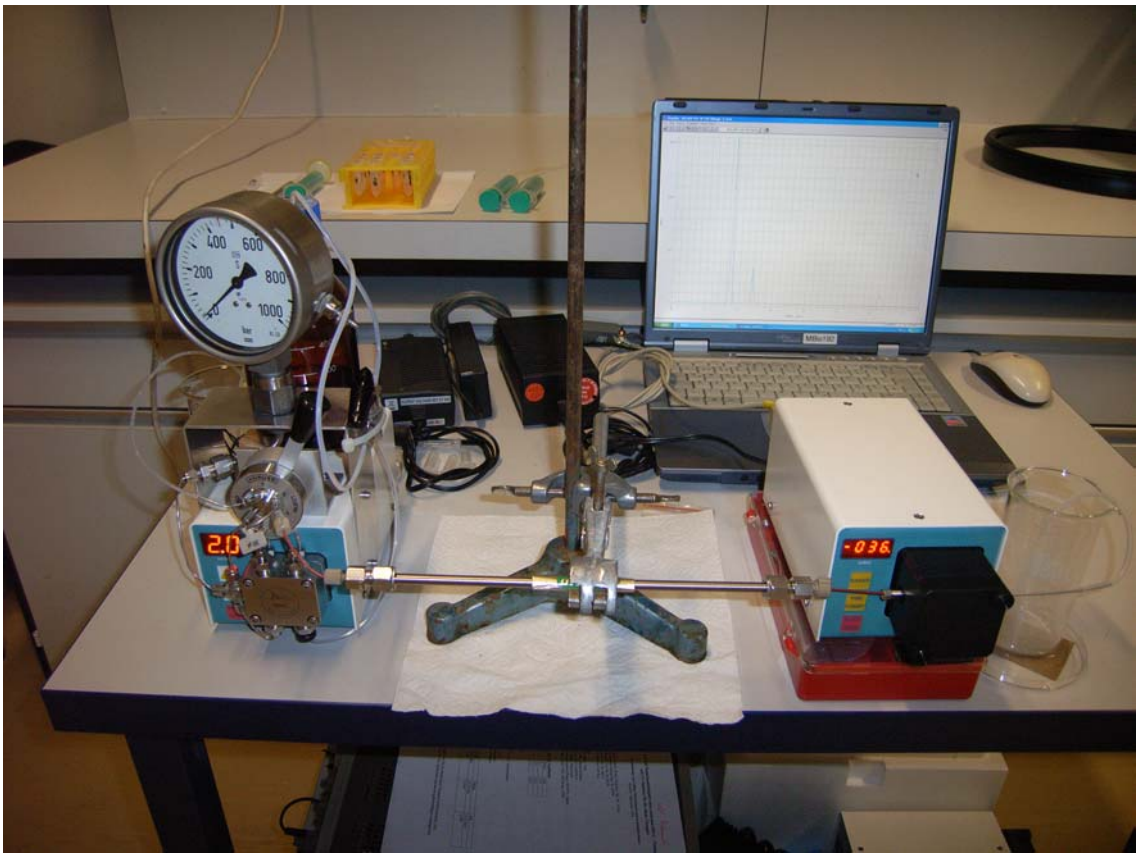
**Quick Setup Instructions for the Knauer HPLC-System**
For windows XP (using CHANCE vers. 2.0)

Compiled by P. Madl
Vers. 1.0 June 2011



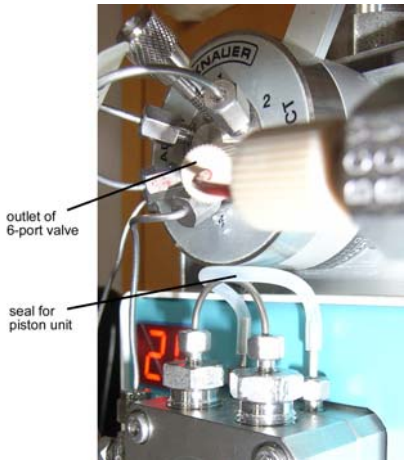
**1. Hardware Setup**:

Result

1.1. Attach RS 232 interface cable (designated as PC) to COM-port-1 of the laptop.

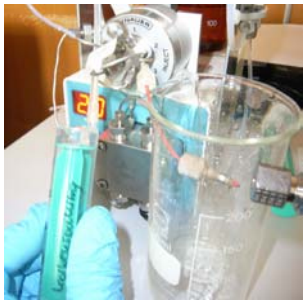
1.2. connect the other end of the RS232 cable with the peripheral device (Knauer K-200 detector).

1.3. boot both laptop and peripheral devices (Knauer pump k-200 & detector k-120 – make sure that pump is powered up but not yet working – dot-point of the flow-rate indicator shoudl not flashing).




1.4. unplug seal of piston / compression unit to attach syringe filled with cleansing solution (e.g. 70% isoprop. : 30% $H_2O$ ).

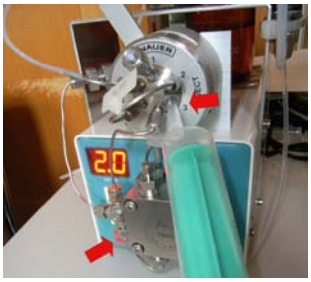
1.5. flush piston / compression unit with cleansing solution & reattach seal.



1.6. attach solvent flask (e.g. 15% acetonitrile : 0.1% formic acid) to HP-pump.

1.7. use a separate syringe with adapter and hook to outlet of 6-port valve.

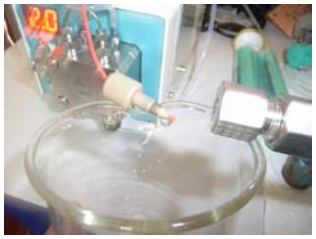
1.8. activate pump and suck entrapped air out of the pumping unit until solvent drips according to the flow-rate as indicated on the display of the HP-unit (k-200).



1.9. Repeat procedure with the lever of the 6-port valve switched into the other position.

pressure gauge should still be 0.

1.10. once flow is established, stop the pumping cycle.



1.11. attach one end of the connecting capillary to the outlet of the 6-port valve and the other one to the separation column.

1.12. hook outlet of separation column onto detector (k-120).

1.13. re-activate the pump and make sure that solvent is pumped through the column, detector all the way into the receiving waste bin at the end of the flow line.



check pressure gauge!
…. check also for leaks!

1.14. once flow is established, stop the pumping cycle and ….

…. proceed with the next section (SW-setup) ….

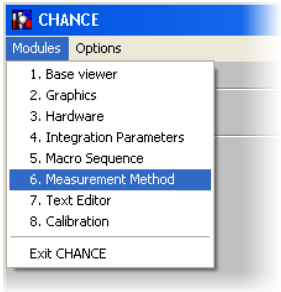
**2. Software Setup & Measurement**:

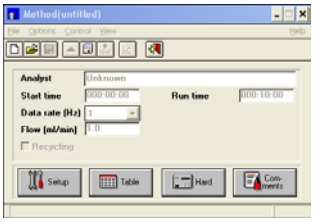
Result

2.1. boot CHANCE software by double-clicking the ECW-icon.

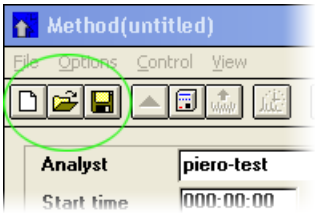

…. opens 'CHANCE's' main window

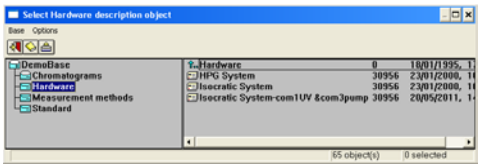

2.2. load measurement method:




2.3. select on of the file tabs:



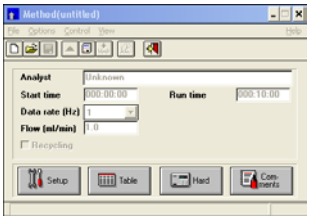



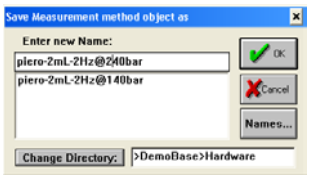

i) when working with an already existing configuration, proceed with step **2.5** (**OPEN** file)

i) if hardware config. needs to be established (**NEW** file) continue with step **2.4**.

2.4. **NEW** – creating a new measurement configuration; click the '**blank**' tap and:

i) select method (e.g. isocratic system);
i) enter the analyst's name;
i) define run time (e.g. 000:08:00)
i) select data rate (e.g. 2 Hz)
i) define flow rate (e.g. 2.0 mL/min)
i) confirm with **SAVE** by
i) enter a new 'measurement method' and specify destination **directory** (e.g. >hardware)

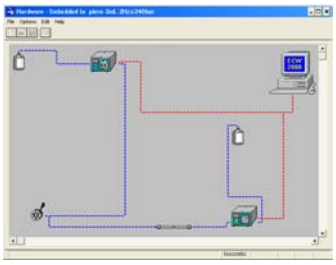


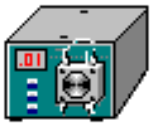
2.4.a. configure peripheral hardware:

i) click on the hardware (HARD) icon:

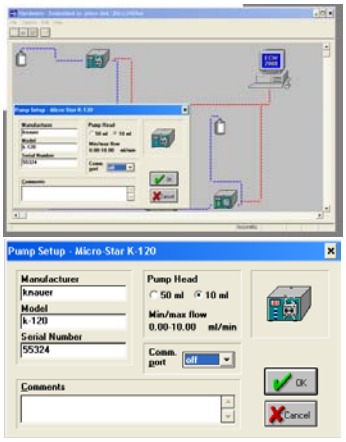

…. opens 'pump's' set-up window



2.4.b. activate the pump icon (double click pictogram):



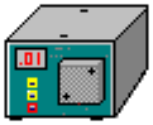
…. opens 'pump's' set-up window



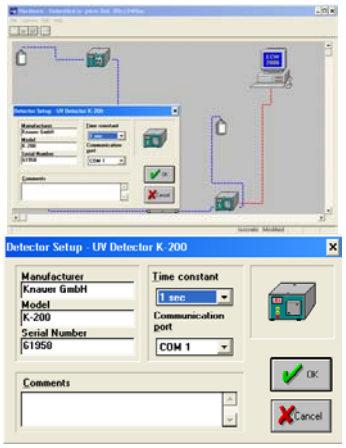

In that window, tick the appropriate tag:

i) pump head (e.g. 10 mL)
i) COM-port (off)

2.4.c. activate the pump icon (double click pictogram):



…. opens 'detector's' set-up window

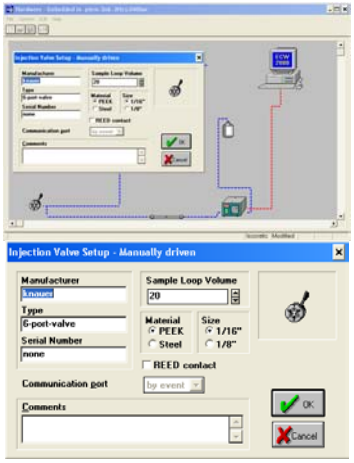



In that window, tick the appropriate tag:

i) time constant (e.g. 1 sec)
i) COM-port (e.g. COM 1)

2.4.d. activate the 6-port icon (double click pictogram):

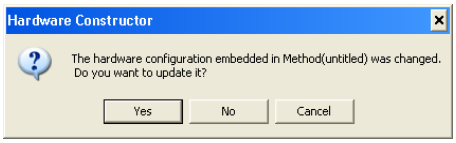
…. opens 'port's' set-up window



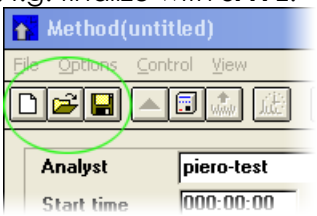

In that window, tick the appropriate tag:

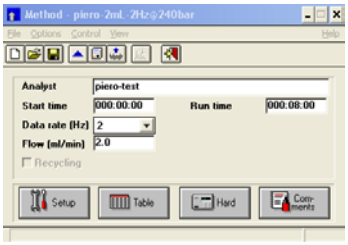
i) choose loop volume (e.g. 20 µL)
i) select material (e.g. peek)
i) select size (e.g. 1/16")

2.4.f. close hardware config window by confirming changes with YES:

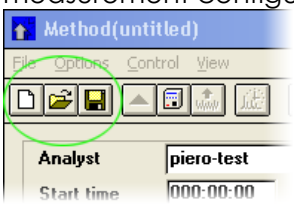


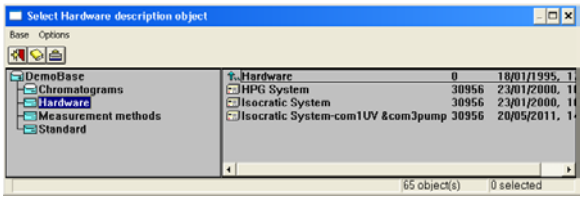

2.4.g. finalize with **SAVE**:



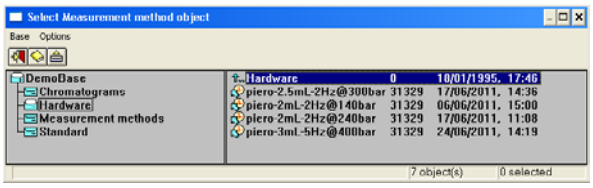

2.5. **OPEN** – loading an existing measurement configuration:




Ticking 'open' provides the window with the general features:

i) select hardware;
i) choose the appropriate config.; e.g. 2mL/min @ 240 bar;
i) check **flow rate at HP-pump** – if there is a mismatch adjust with the ↑ or ↓ arrows);
i) select data rate (e.g. 2 Hz)
i) define flow rate (e.g. 2.0 mL/min)
i) confirm with SAVE by entering a new 'measurement method'



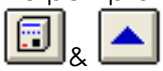


2.5.a. start chromatographic run (**blank run, w/o analyte**):

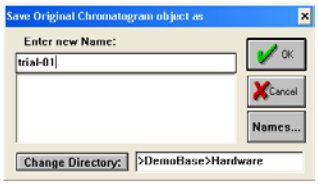
i) activate pump at HP-unit (arrow) - be sure that pump operates at same flow rate as used above; i.e. 2mL/min;

i) observe pressure gauge ….

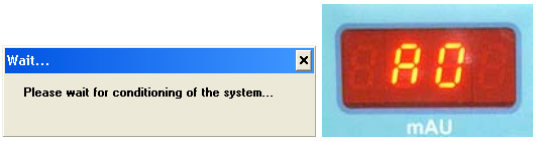
i) once operating pressure is reached, click the start icon for the pump and the measurement



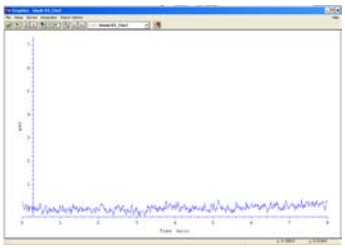
i) name scan (i.e. blank-01) and confirm




…. opens the 'hold-on' window




i) observe the detector's display for autozeroing (AO) ….

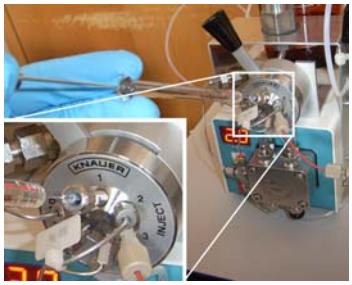


…. system opens timeline and plots the live readout values of the detector (k-120) ….

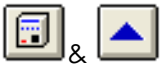
2.5.b. start chromatographic run (**w/ analyte**):

i) flush syringe thoroughly with solvent (at least 3 times - dispose runoff into waste bin);

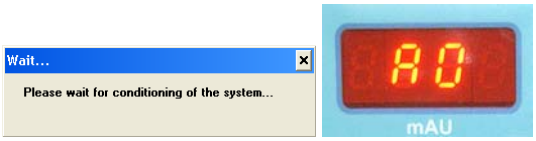
i) inject solvent into the loop (since the loop holds e.g. 20 µL, inject at least 60 µL);




i) click the start icons and ….

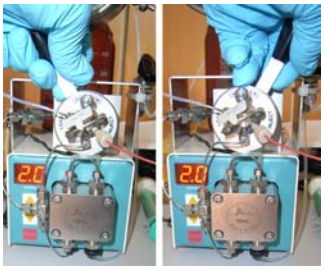


i) name scan (i.e. sample-01) and confirm




i) …. once "AO" appears, turn lever of 6-port valve to route content of 20µL standby-loop onto the chromatographic column ….

bypassed



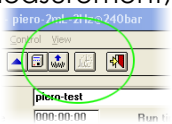
loaded

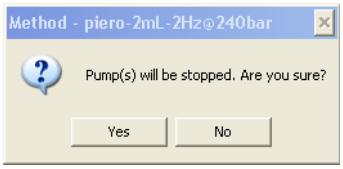
2.5.c. finalize measurement by

i) deactivating the pump,

i) pulling back the lever of the 6-port valve and

i) terminating the measurement;'



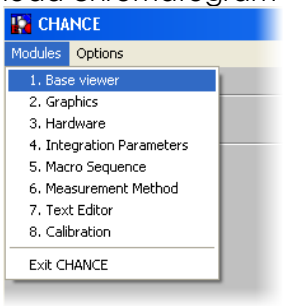



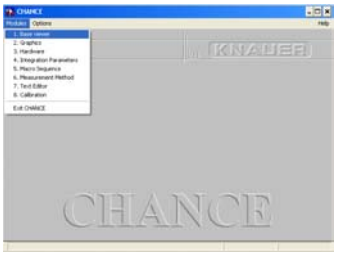
**3. Data Evaluation**:

Result

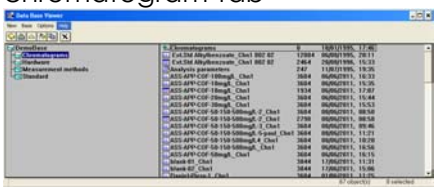
3.1. graphic data evaluation:

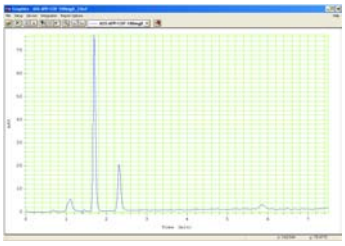
i) load chromatogram



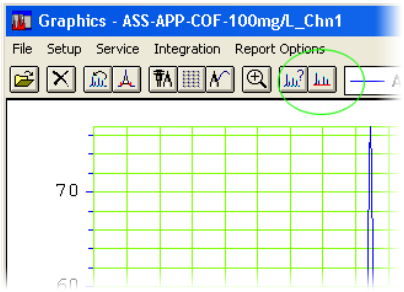



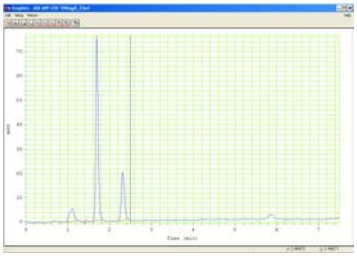

i) select appropriate file from the chromatogram-tab



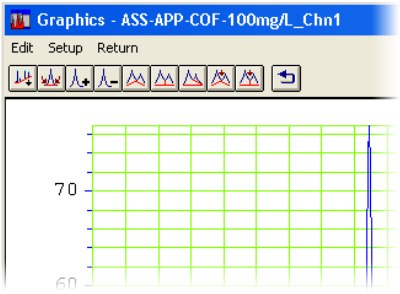


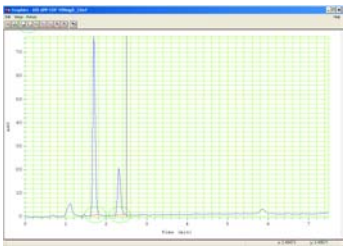
i) click the edit-tab



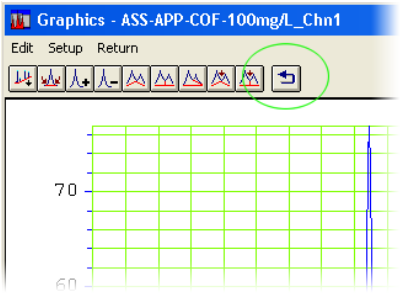



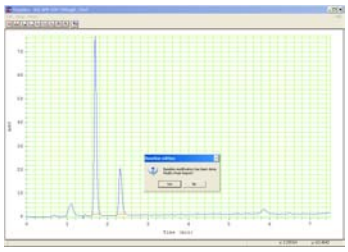
i) use the appropriate icons to mark the peaks of interest for further data evaluation



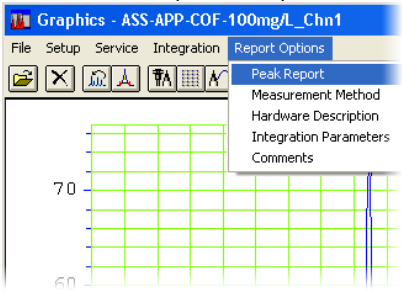
i) use right mouse-click to exit edit mode and

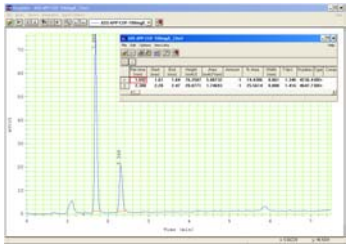


i) tab-return to switch to the previous menu.

i) activate the peak-report tab



3.2. shutting down software;

i) terminate all windows:

i) exit main frame by clicking the (CLOSE) function at the top right corner.