

AKTA ION EXCHANGE CHROMATOGRAPHY SOP

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Materials/Reagents/Equipment	Vendor
1. 0.2 um filtered Water + 0.05% sodium azide	
2. 0.2 um filtered buffers required for method to be run.	
3. ~300 mL extra buffer A to wash, prime lines	
4. 100% EtOH	
5. 20% EtOH	
6. Columns required for method to be run	
7. collection tubes 12 x 75 mm (up to 4.5 ml)	VWR #60818-383
18 mm (up to 12.5 ml)	Sarstedt tubes

Procedure

I Performing a Purification Method

A. Preparing the system for the IEX MC purification method

1. If computer is not on, start it. Log on as Administrator with ***** password. Start Unicorn software by double clicking on the Unicorn 4.11 icon on the desktop. Log in as yourself. The instrument should always be on in the cold box. Unicorn will communicate with the instrument and acknowledge when the instrument is ready to operate. There are four windows available in Unicorn. They can be accessed from tabs at the bottom of the screen.

The **Main menu** will appear on the screen. From here purification methods can be selected to run or edit and result files can be selected to view or edit.

The **Method editor** window which allows for the writing or editing of existing methods

The **Evaluation** window allows for analyzing previous runs.

The **System Control** window is where manual operations are controlled and all methods are monitored.

Click on the System Control tab. This screen has 4 windows.

Run Data is where current values are observed for any selected parameters

Curves is where selected data is graphed vs. time

Flow Scheme is where manual control of the system is initiated

Logbook is where actions are recorded

2. It is important to know how many samples of what volume will be processed for proper set up of the system.
3. Place instrument lines into the respective buffer. Make sure buffer amount is sufficient for method to be run. 500 ml of each buffer should be sufficient for 2 runs. The method

“large scale IEX” is used for one sample and “large scale IEX pump wash” is used for more than one sample. The “large scale IEX pump wash” method automatically washes the sample pump in-between samples.

inlet position	buffer	volume
A11	buffer A no salt	160 ml/sample + 100 ml
B1	buffer B buffer A + 1 M salt	70 ml/sample + 80 ml
<i>the following are for the pump wash option</i>		
S 7	100% EtOH	100 ml/sample + 100 ml
S8	buffer A no salt	65 ml/sample + 100 ml

4. Change the 20% EtOH pump wash solution. Empty the waste container.
5. Check that the pressure limit of the system and sample pumps is set to match the pressure limit of the column to be used (ie SP and Q HiTrap columns have a working pressure limit of 0.3 MPa).
6. Click on the instrument control icon. Double click on the Pump A icon in the Flow Scheme window to open the Instruction dialogue box.
7. Select Pump - PumpWashExplorer. Select A11 on, A2 off, B1 on, B2 off. Select Execute to start the macro. If air bubbles are seen in the buffer lines, they can be purged at the pump head with a syringe. Pumps are located behind the instrument door. Pump A on the left and pump B on the right. This will use ~30 ml of each buffer.
8. Manually flush the sample line to be used. Place the sample line in a container of buffer A. Select: Pump – SampleFlow and input 20 ml/min as flow rate. A 30 second flush at 30 ml/min. should be sufficient. In addition, lines S7 and S8 should be flushed if these buffers were changed for this run. Stop the sample pump by inputting 0 for SampleFlow value.
9. The column will be connected to a pair of rotary valves. Column valve V2 is the column inlet valve and column valve V3 is the column outlet valve. Each of the column valves has the valve position marked on the side of the valve body. Position 1 is used as a bypass and as such no column will be connected there. With the system pump running at 0.5 ml/min. (Pump – Flow – FlowRate = 0.5), direct the system flow to the column position desired (Flowpath – ColumnPosition – position X). Connect the inlet of the desired column to the appropriate tubing from column valve V2. The columns’ outlet will connect to the parallel port on column valve V3. After a column has been connected, increase the flow rate to the maximum used for the method (4 ml/min. for 5 ml HiTrap). Observe the column connections for leakage and tighten fittings as required. After the column has been connected, return the system flow to 0 ml/min. and the column valves to Position1Bypass.
10. Fill the fraction collector rack with tubes. Reset the fraction collector by selecting: Frac – Reset_Frac_Position – Execute.

11. Place the sample line(s) into the container of sample. Confirm that the line is free of bubbles as these may prematurely activate the air sensor and stop sample flow.
12. Ensure that the system outlet line to be used (outlet valve V4, positions F3 – F6, loop 1, loop 2) is connected to a container of sufficient capacity to hold the flow through.
14. When all preparations are complete, click the END button on the menu bar. This will stop the manual operations.

B. Selection of Method and Variables

1. There are two methods, “**large scale IEX**”, and “**large scale IEX pump wash**” programmed on the instrument for automated IEX purification. “larger scale IEX pump wash” should be used if more than one sample is to be processed. The details of these methods are viewed in the Method Editor window. Open the User file and select the method of interest. There will be three panes to view.

Gradient shows %B buffer over the course of the method

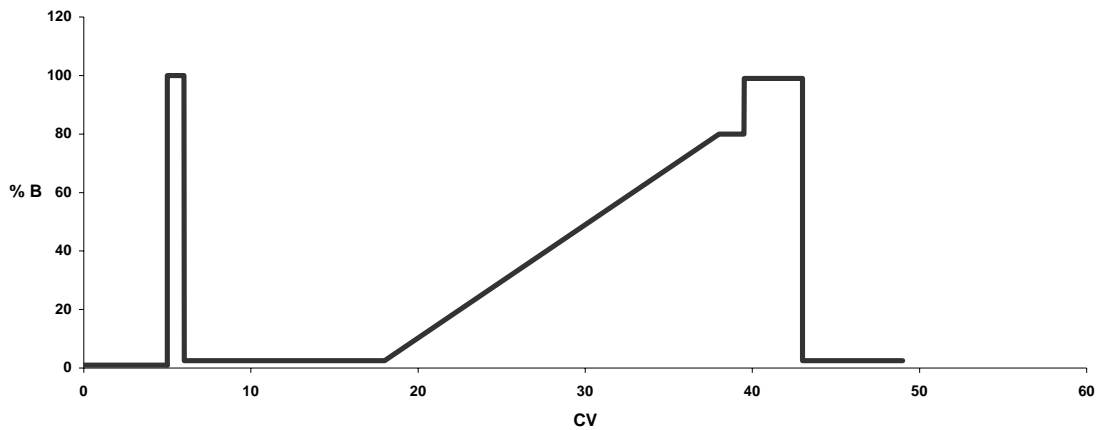
Text shows the instruction blocks for the method

Instruction box this is where changes can be made to the method.

2. The method **large scale IEX** is a convenient method. It uses automated sample injection via the sample pump as well as variables for column selection, pump rates, column equilibration, gradient elution etc. These variable parameters include:

1 CV 100% B blank wash
 7 CV column equilibration with X % B
 discrete sample loading and flow through collection
 5 CV post sample load wash with 1% B
 20 CV X – 80% B (X – 800 mM NaCl) gradient
 3 CV 100% B (1000 mM NaCl) wash
 5 CV X% B re-equilibration

Gradient for large scale IEX



Note: Please consult an experienced AKTA user if a modified method is desired.

- To start a method, go to the System Control window and click the Run button. Select the method to be run. A series of instruction windows will come up that will require the operator to acknowledge and/or input parameters. They are:

Scouting: this is the page where certain default parameters can be changed to fit the experiment to be performed. Sample volume should be 150 – 200% of actual volume because the air detector will stop sample flow prior to air entering the system. If a different column is selected other than the default, carefully check the values for all scouting parameters.

Gradient: A graphic view of the %B during the course of the run.

Method information: various information about the method to be run including duration and buffer requirements.

Result name: the location and name of the results file is defined and the method started.

C. Analysis of results

- Results of completed runs can be analyzed from the Evaluation window. Files may be selected from the Results pane of the main window or by opening the desired file directly from the Evaluation window.
- When the file is opened, a chromatogram of the run is displayed. By selecting the **Chromatogram Layout** icon from the menu bar various attributes of the chromatogram can be selected; which curves to be viewed, X and Y axis attributes etc. The desired chromatogram can then be printed for reference with fraction analysis. De-select **thick lines** and select **landscape** for the best image.

D. Shutting down the system

1. When the runs are complete, Manual control will have to be initiated again to clean the system. Any of the soiled sample and outlet lines will be rinsed with H₂O, 0.05% NaN₃. This is accomplished by placing the sample lines to be cleaned in a container of H₂O, 0.05% NaN₃. Place the outlets lines in a waste container. Check that the pressure limit of the sample pump is set to 0.3 MPa. Have the column valve set to the Bypass position and the Injection valve set to the inject position. Follow the instructions as in A.8 to activate the sample pump and sample valve. After the final sample line has been rinsed, switch the outlet valve from waste, to F3, F4 etc. until all outlet lines have been rinsed.
2. Prior to disconnecting the IEX columns for storage, rinse them with 3CV of H₂O, 0.05% NaN₃ from a sample line. **Lower the sample flow to 4 ml/min. for a 5 ml column or 1 ml/min. for a 1 ml column before directing the flow to the column position desired.** Columns will be disconnected from the system for storage. With the injection valve in the inject position, H₂O, 0.05% NaN₃ can be pumped through the columns at 0.5 ml/min. with the sample pump to maintain a liquid stream while the columns are being disconnected. The details of column connection are found in A.9 above.
3. The buffers (A11, B1, S8) should be removed from the system and replaced with H₂O, 0.05% NaN₃ for system storage. Flush the water through these buffer lines with PumpWashExplorer as in A.7 and A.8 above.
4. Click End to stop manual control of the system and end data collection.
5. Empty the waste container. Fill all the H₂O, 0.05% NaN₃ bottles if they are low in level.
6. Log off the system.