

SOP: ÄKTA pure Chromatography System Operation

Approvals

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1. Purpose

1.1. This procedure describes the operation of the ÄKTA pure Chromatography System, controlled by Unicorn 6.3 software.

2. Scope and Applicability

2.1. Applies to chromatography of proteins, etc using a column installed on the GE ÄKTA pure Chromatography System and controlled by Unicorn 6.3 software.

3. Summary of Method

- 3.1. Method writing (programming)
- 3.2. Priming the pump rinsing system
- 3.3. Priming Inlets and purging pump heads
- 3.4. Assembly and installation of a 10 ml Superloop sample chamber.
- 3.5. Calibration of the pH electrode/detector.
- 3.6. Manual control of the instrument
- 3.7. Equilibration of system and column
- 3.8. Fraction collector setup
- 3.9. Application of sample
- 3.10. Washing and elution of column
- 3.11. Regeneration of system in preparation for subsequent run
- 3.12. Procedures for cleaning and short or long term storage of the system

4. References

- 4.1. Unicorn 6.3 Users Guide (electronic)
- 4.2. ÄKTA pure 25 Users Guide (electronic)
- 4.3. Manufacturer's literature/spec sheets for media
 - 4.3.1. HiTrap SP HP 5 information booklet (GE)
 - 4.3.2. L-Lysine HyperD reference sheet (Pall)
 - 4.3.3. Capto Q information booklet (GE)
 - 4.3.4. Ni²⁺ IMAC Profinity reference sheet (BioRad)
- 4.4. Batch Record for Downstream Processing of t-PA: TFF Operation

5. Definitions

5.1. N/A

6. Precautions

- 6.1. Routine care should be exercised in handling of buffers and samples of biological materials, which may have harmful biological activity in the case of accidental ingestion, needle stick, etc.
- 6.2. User should read and be familiar with general good practice as outlined in the ÄKTA pure Cue Cards located near the instrument.

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- 6.3. Avoid damaging the threads through the use of excessive force when connecting plastic fasteners.
- 6.4. Care must be taken to avoid air in the fluid path, which could damage the pumps or give spurious and uninterpretable readout from the UV and/or conductivity detectors.
- 6.5. Gloves and protective eyewear should be worn when handling samples and reagents (buffers), however it is preferable that the user remove gloves prior to entering commands via the computer keyboard or mouse.
- 6.6. Buffers must be degassed and filtered prior to use with the AKTA pure instrument. Samples should be centrifuged at 10000xg for 5 min before injection/introduction into the fluid path.
- 6.7. Equipment calibration check: The AKTA pure system calibration is automatic; baseline for measurements of A280 and conductivity are zeroed at the beginning of a chromatography run. Further adjustment is beyond the scope of this document and should be referred to a qualified technician.

7. Responsibilities

- 7.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
- 7.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.

8. Equipment and Materials

- 8.1. AKTA pure chromatography system
- 8.2. Additional Lab Equipment: pH meter, balance
- 8.3. Lab Utensils: Beakers (250, 500ml), 500 ml graduated cylinders
- 8.4. Reagents: Filtered deionized water (MilliQ or similar), 20% ethanol
- 8.5. Lab Supplies: Filters (0.2 µm) and bottles for vacuum filtration and degassing of all chromatography buffers. Syringe (1ml). Tubes for fraction collector (3 ml – 15 ml capacity).
- 8.6. Column cleaning solution for HiTrap SP (GE) – 0.1M NaOH
- 8.7. Column cleaning solution for Lysine HyperD (Pall) – 0.1M NaOH;
- 8.8. Column cleaning solution for Canto-Q (GE) – 0.1M NaOH
- 8.9. Column cleaning solution for Ni²⁺ IMAC Profinity (Bio Rad) -

9. Procedure

- 9.1. Sample Collection and Preparation is beyond the scope of this document, however the system is compatible with a very broad range of biological samples, with the caveat that the sample must be free of debris or particulate matter. The operator will require 0.6 - 10 ml of sample per sample injection (see below). The goal (analytical vs preparative) of the chromatography will dictate the amount of sample needed and/or applied to the column. Concentration of the sample and buffer components/pH should be chosen to be compatible with the mode of separation.
- 9.2. Reagent Recommendations: Buffers should be prepared from the highest available grade reagents and water, filtered through a 0.2 µm filter and degassed thoroughly. Buffer preparation is beyond the scope of this document. Examples of buffers for specific applications where t-PA is the protein/biopharmaceutical of interest are:

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9.2.1. HiTrap SP HP Cation exchange column:

9.2.1.1. Buffer A = 0.2M sodium acetate, pH 5.0, 0.01% Tween 80

9.2.1.2. Buffer B: 0.2M sodium acetate, pH 5.0, 0.01% Tween 80, 1M NaCl

9.2.2 L-Lysine Ceramic HyperD Affinity column:

Buffer A: 20mM sodium phosphate, pH 7.5, 0.01% Tween 80

Buffer B: 20mM sodium phosphate, pH 7.5, 0.01% Tween 80, 1M NaCl

Elution Buffer B2: 20mM sodium phosphate, pH 7.5, 0.01% Tween 80, 0.2M arginine hydrochloride

9.2.3 Ni²⁺-IMAC column

Buffer A: 20mM sodium phosphate, pH 7.0, 0.5M NaCl 0.01% Tween 80

Buffer B: 20mM sodium phosphate, pH 7.0, 0.5M NaCl, 0.01% Tween 80, 0.5M imidazole

9.2.4 Protein-A Sepharose column

Buffer A: 20 mM sodium phosphate, pH 7.0

Buffer B: 0.1 M sodium citrate, pH 3.0

9.3. Method writing in Unicorn 6.3.

For the Unicorn 6.3 software to be able to control the AKTA pure instrument, it is necessary to assemble programmed steps into a method. The steps vary for a given column and protein of interest, but typically include Sample Loading, Column Washing, Elution, and Equilibration. Often more steps are required. In most situations, the method will be written and stored in the Method Navigator within Method Editor; refer to the Batch Process Record or Process SOP for the protein of interest to find the name of the specific method. In the event a new or modified method is required, here are the (minimum) steps needed.

9.3.1. In the Unicorn software, navigate to the Method Editor window.

9.3.2. Under the File menu, choose New Method.

9.3.3. In the pop-up window, choose a pre-defined method for the type of chromatography you will be doing, then click OK.

9.3.4. Each step in the program is referred to as a 'Phase' in the software. The phases for the predefined method will appear in the center pane of the Method Editor window.

9.3.5. Click the Method Settings phase to display its properties in the right hand pane. If you are using a GE Life Sciences prepacked column, find and select it in the Column Type dropdown menu. Its properties and suggested flow rate will automatically be chosen. If your column is not listed, enter the Column Volume, Pressure Limit and Flow Rate (specified by the manufacturer) in their respective boxes. Choose Inlets, usually A1 and B1; these can be specified for each step (phase) as you progress through method writing.

9.3.6. While still in the Method Settings pane, click the Start Protocol button. A pop-up window appears that allows the operator to choose items that will be presented for operator's information/approval when a run of the method is initiated. Check the boxes for Method Information (which will show the expected volume and time required, allowing one to check that the pumps will not run dry due to inadequate supply of buffer), and for Questions. Click the button labeled Define Questions. This allows the operator to specify useful information that must be entered before

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the run can start, which may include sample characteristics, buffer composition/preparation date, operator, etc. Follow the prompts to enter questions, check the Mandatory and Display in Chromatogram boxes, then Preview to confirm the appearance.

- 9.3.7. Click the button for Equilibration phase in the center pane to display its properties in the right pane. Confirm that 'Reset UV monitor' is checked. Check 'Fill the system with the selected buffer' to quickly flush inappropriate buffers between the pumps and the injection valve.
- 9.3.8. Click the Sample Application phase in the center pane to display the parameters.
 - 9.3.8.1. Uncheck the box 'Use the same flow rate as in Method Settings' – typically a capture method such as ion exchange or affinity separations achieves higher binding efficiency at lower flow rates.
 - 9.3.8.2. Select the Loop type and enter the sample volume to be injected. In the 'Empty loop with' box, enter a volume sufficient to flush all sample from the loop.
 - 9.3.8.3. Confirm that the correct inlet lines are specified; most methods use A1 and B1.
 - 9.3.8.4. If you suspect that your sample will exceed the capacity of the column, check the 'Interrupt sample application at UV' and enter a value (which will likely have to be determined empirically). A better approach is to limit the quantity of sample applied so that the operator is confident that the capacity of the column will be adequate to the task.
 - 9.3.8.5. In the 'Fractionate' section, choose the path that the eluent should follow. It is customary to collect the unbound protein in a container ('using outlet valve' directs the flow-thru to the tube labeled 'Out'; place it in a clean flask) or in fractions ('using fraction collector').
 - 9.3.8.6. Fractionation settings: choose 'Fixed outlet' using outlet valve. Choose 'fixed volume fractionation' to collect the flow-thru in constant volume fractions with the fraction collector.
- 9.3.9. Click the Column Wash phase button in the center pane.
 - 9.3.9.1. Check the box to 'Use the same flow rate as in Method Settings'.
 - 9.3.9.2. Confirm the inlets for A and B, and that the 'Fill the system....' box is unchecked.
 - 9.3.9.3. Select a value for 'Wash until', which should be at least 10 column volumes (for the small columns used in this course). Select the radio button for 'the following condition is met', choose Stable UV, set the Accepted UV fluctuation to 0.1mAU (experience may dictate a change, so revisit this setting on occasion), and set the Maximum wash volume to 20 CV.
 - 9.3.9.4. Fractionate: column wash is diverted 'in waste (do not collect)'.
- 9.3.10. Click the Elution button in the center pane and make these settings:
 - 9.3.10.1. Check the box for 'Use the same flow rate as in Method Settings'
 - 9.3.10.2. Confirm Inlet A and B specify the tubing that is placed in buffers A and B.
 - 9.3.10.3. Gradient elution is the default selection.
 - 9.3.10.3.1. Choose the 'Start at' concentration of buffer B, usually 0%. If greater than 0%, check the 'Fill the system....' box.

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9.3.10.3.2. Specify the Type of gradient (step or linear), target % B and total volume (recommend 10 CV initially) for the gradient in this segment.

9.3.10.3.3. Add more segments as needed. Commonly a final elution of 2-3 CV at high strength eluting conditions (e.g. 1M NaCl for an ion exchange separation) is included.

9.3.10.3.4. Fractionate settings should be for 'using fraction collector' and 'fixed volume fractionation'. Set the Fixed fractionation volume to a volume that represents about 1/20 of the total gradient volume.

9.3.11. Click the (second) Equilibration button to set parameters for re-equilibration of the column for subsequent runs.

9.3.11.1. Confirm that the 'Reset UV Monitor' box is unchecked.

9.3.11.2. Check the 'Use the same flow rate....' box.

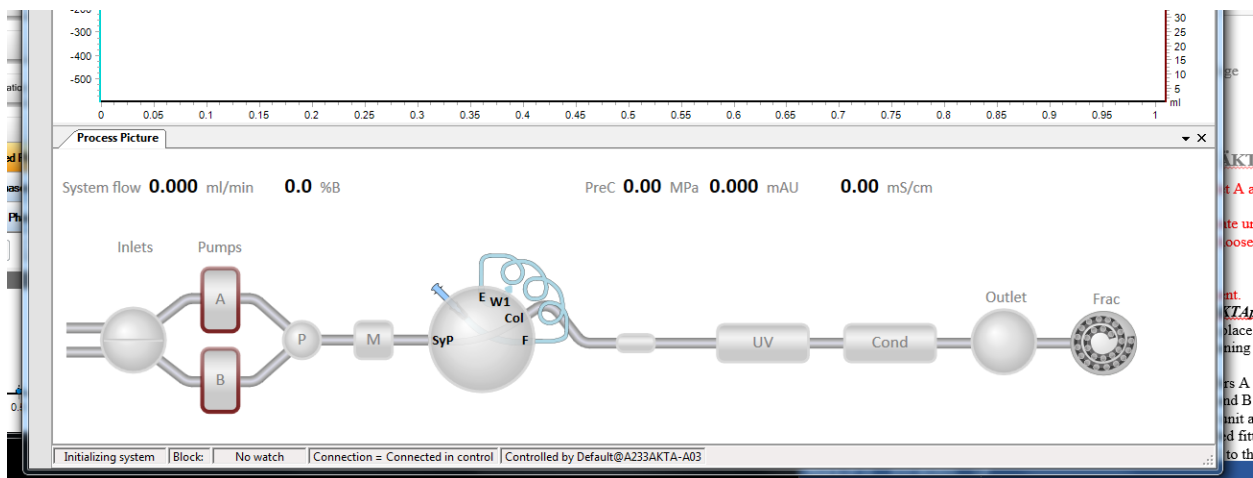
9.3.11.3. Confirm that Inlet A and B are correct and correspond to the buffers A and B.

9.3.11.4. Set the 'Equilibrate until' volume to 5 CV.

9.3.12. Under the File menu, choose Save and name the method uniquely; select the correct folder and Save.

9.4. Manual Control of the instrument.

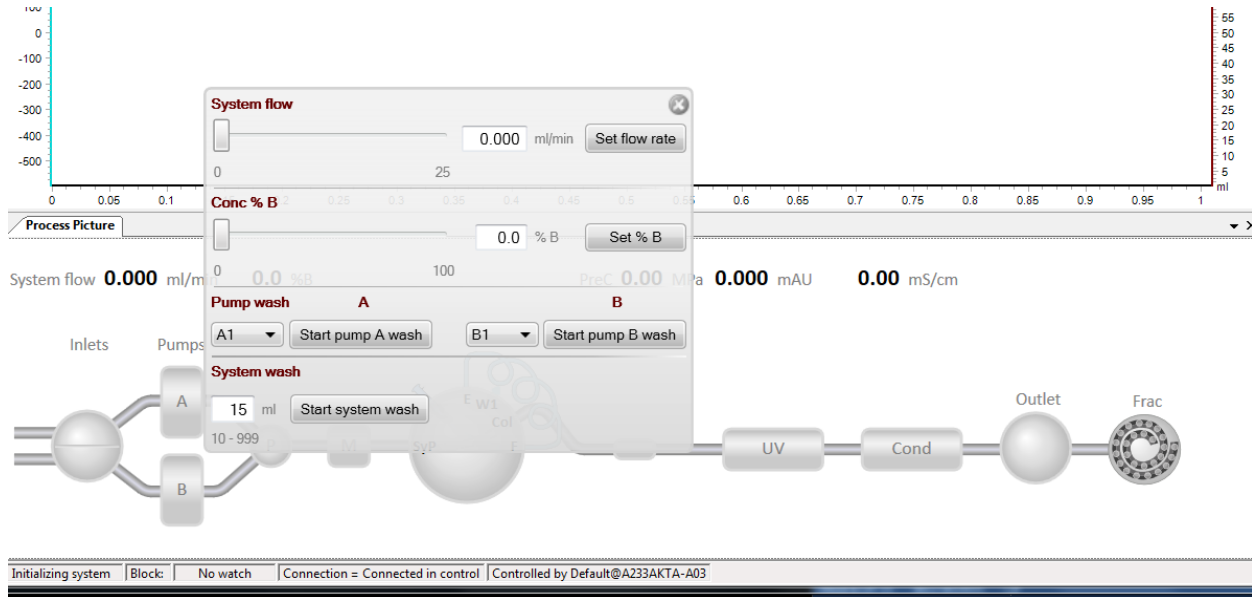
The AKTA pure instrument may be controlled 'manually' via the System Control window lower pane graphic interface (or 'Process Picture'), as pictured here.



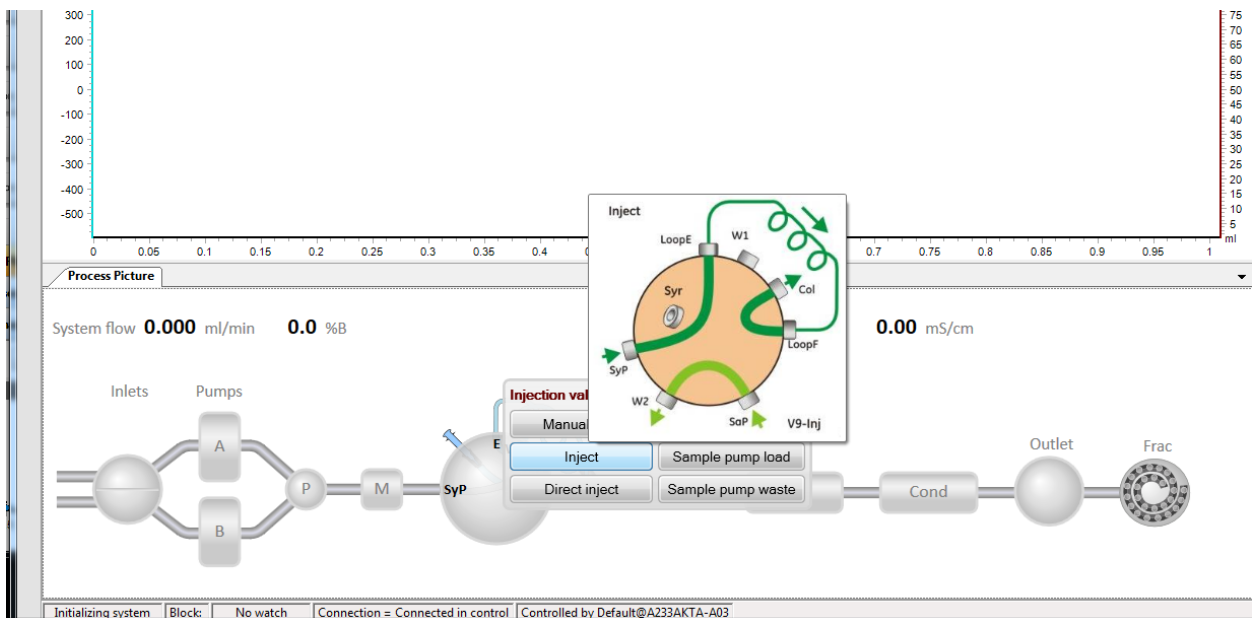
9.4.1. A control panel that allows selection of inlet pumps/lines A1, A2 B1 And B2 will appear when one clicks on the small bisected circle on the left side.

9.4.2. By clicking on either Pump A or Pump B in the depiction, you can access a dialog box which allows for quick setting of flow rate and buffer composition, as well as convenient pump wash features as shown:

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9.4.3. The large circle (approximately the center of components) in the diagram represents the sample injection valve. Upon clicking it, one will see a panel of 6 options for the position of the valve (five are visible in the diagram below). Hovering over each option causes a new diagram to appear that shows the flow path through the valve for that particular selection; shown here is the Inject position, with flow coming from the system pumps (SyP), going through LoopE valve inlet, carrying contents of the sample loop out through LoopF valve outlet and onto the column (Col).



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- 9.4.4. Clicking the UV detector icon reveals a command to 'Auto Zero UV'.
- 9.4.5. The small circle to the right is the Outlet valve. By clicking on it with the electronic mouse pointing device, the operator may select whether the eluent goes to Waste, the Outlet tubing, or the Fraction Collector.
- 9.4.6. Various parameters of the current run condition are displayed directly above the manual control interface. Pay particular attention to pre-column pressure (PreC x.xx MPa) to avoid over pressurizing/damaging the column. Most of our columns have a limit of 0.5 MPa.
- 9.5. Priming the pump rinsing system. The pump piston rinsing system protects the seal that prevents leakage between the pump chamber and the drive mechanism of the pump. Priming of the pump rinsing system is done before the run
 - 9.5.1. Remove the pump rinsing liquid tube from the holder located on the right-hand bottom corner of the system.
 - 9.5.2. Fill the pump rinsing liquid tube with 50ml of 20% ethanol
 - 9.5.3. Place the pump rinsing liquid tube back in the holder
 - 9.5.4. Insert the inlet tubing to the system pump piston rinsing system in the rinsing solution tube. (Note: Make sure that the inlet tubing reaches close to the bottom of the rinsing solution tube)
 - 9.5.5. Connect 25 to 30 ml syringe to the outlet tubing of the system pump piston rinsing system. Draw liquid slowly into the syringe.
 - 9.5.6. Disconnect the syringe and discard its contents.
 - 9.5.7. Fill the rinsing solution tube so that the tube contains 50 ml of 20% ethanol.
- 9.6. Prime inlets and purge pump heads. Before using the system pumps it is important to: Prime the inlets (fill the buffer inlets with liquid) and to Purge the system pumps (remove air from the pump heads)
 - 9.6.1. Prime the Inlets.
 - 9.6.1.1. Make sure that all inlet tubing that is to be used during the method run is placed in the correct buffer.
 - 9.6.1.2. Turn on the ÄKTA pure system.
 - 9.6.1.3. Open the unicorn 6.3 software
 - 9.6.1.4. Open the **system control** module in the unicorn 6.3 software
 - 9.6.1.5. In the **Process Picture** click on the buffer inlets
 - 9.6.1.6. Select the position of the inlet to be filled. Select the positions in reverse alphabetical order and start with the highest number. For example, if all four inlets are to be filled, fill them in the following order: B2, B1, A2, and A1. The inlet valve switches to the selected port.
 - 9.6.1.7. Connect 10 ml syringe to the purge valve of right pump head of the pump system B. Make sure that the syringe fits tightly
 - 9.6.1.8. Open the purge valve by turning it counterclockwise about three quarters of a turn. Draw liquid slowly into the syringe until liquid reaches the pump and no air bubbles are visible in the line.

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- 9.6.1.9. Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
- 9.6.1.10. Repeat step 9.6.1.6. to 9.6.1.9. for each piece of inlet tubing and their respective pump heads. For example, when priming inlet B1 select left pump head of the pump system B.
- 9.6.2. Purge System pump B
 - 9.6.2.1. Make sure that the piece of waste tubing connected to the injection valve port W1 is placed in a waste vessel
 - 9.6.2.2. In the **Process Picture** click on the **injection valve** and select **System pump waste**. The injection valve switches to waste position. This is necessary to achieve a low back pressure during purge procedure.
 - 9.6.2.3. In the **Process Picture** click on the pumps
 - 9.6.2.4. Set **Conc % B** to 100% B and click **Set % B**. only system pump B is active
 - 9.6.2.5. In the **Process Picture** click on the buffer inlets B2. The inlet valve switches to the selected port.
 - 9.6.2.6. In the **Process Picture** click on the pumps
 - 9.6.2.7. Set the **System flow** to 1.0 ml/min. Click **Set flow rate**. A system flow starts
 - 9.6.2.8. Connect a 10 ml syringe to the purge valve of the left pump head of system pump B. Make sure that the syringe fits tightly into the purge connector.
 - 9.6.2.9. Open the purge valve by turning it counterclockwise about three quarters of a turn. Draw a small volume of liquid slowly into syringe.
 - 9.6.2.10. Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
 - 9.6.2.11. Connect the syringe to the purge valve on the right pump head of System pump B, and repeat step 9.6.2.8 to 9.6.2.10. Keep the system flow running.
- 9.6.3. Validate purge of pump B
 - 9.6.3.1. In the **Process Picture** click on the **Injection valve** and select **Manual Load**.
 - 9.6.3.2. Make sure the pump flow is on.
 - 9.6.3.3. In the **Chromatogram** pane check the **PreC pressure**. If the PreC pressure does not stabilize within a few minutes there may be air left in the pump. Refer AKTA pure system handbook for a troubleshooting guide.
- 9.6.4. Purge System pump A
 - 9.6.4.1. Purge both pump heads of pump system A by repeating step 9.6.2.1. to 9.6.2.11. but replace Set **Conc % B** to 0% and click **Set % B** in step 9.6.2.4.
- 9.6.5. Validate purge of pump A
 - 9.6.5.1. Repeat step 9.6.3.1. to step 9.6.3.3.
- 9.7. Preparation and installation of Superloop 10 sample injection device. The Superloop is stored disassembled and requires assembly and filling with buffer prior to installation on the AKTA pure instrument, as described here. Refer to diagram XXX. Gloves should be worn during handling of the Superloop parts.
 - 9.7.1. Rinse/wet O-rings on the end pieces and movable seal with deionized water.
 - 9.7.2. Insert the movable seal into the graduated glass tube from the bottom (zero) end. Observe proper orientation of the seal; the end with the O-ring should be closest to

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the bottom. Using a glass rod with smooth end or a plastic pipette, push the seal into the tube until the O-ring is between the 1ml and 2 ml graduations.

- 9.7.3. Mount the glass tube on a lab stand with clamp. Working over a sink or container to catch any overflow, pipet enough buffer A into the upper portion of the tube to fill it.
- 9.7.4. Mind the liquid that will squirt from the tubing; direct it into the sink. Insert one of the inner end pieces (with tubing attached) into the glass tube, contacting the liquid meniscus to eliminate air bubble entrapment. Press the end piece completely into the glass tube.
- 9.7.5. Invert the tube in the clamp/support and wet the movable seal with a small amount of 20% EtOH (or buffer A if it contains a detergent). It may be necessary to use the pipet to eject any air bubbles that stubbornly adhere to the glass and/or movable seal. When bubbles have been eliminated, completely fill the tube with buffer A. Minding the liquid that will squirt from the tubing, insert the remaining inner end piece with tubing attached.
- 9.7.6. Rotate the bottom inner end piece so that the slotted end (inside the glass tube) aligns with the small notch inside the glass tube. This alignment is important to establish and maintain; otherwise backpressure in the pumps could increase and prevent completion of the run.
- 9.7.7. Remove the glass tube with end pieces from the clamp.
- 9.7.8. Attach the bottom outer end piece by threading it onto the glass tube.
- 9.7.9. Slide the plastic protective jacket over the glass tube and seat it firmly into the bottom outer end piece.
- 9.7.10. Attach the top outer end piece to the remaining exposed threaded end of the glass tube.
- 9.7.11. To install the assembled Superloop 10 onto the ÄKTA pure instrument, place the lab support and clamp near the instrument on the left side, then mount the Superloop in the clamp. Adjust clamp vertically and horizontally as needed to place the Superloop in close proximity to the injection valve.
- 9.7.12. Attach the tubing on the top of the Superloop to the injection valve port labeled 'loop E' using the threaded connector. Confirm that the tubing is firmly attached and will not easily pull out of the fitting.
- 9.7.13. By default, the injection valve should be in the 'Manual Load' position upon booting up the instrument. Using the manual control feature in the Unicorn software, confirm that the valve is in Manual Load position. If not, switch the valve to the correct position by clicking the injection valve on the system control diagram, then selecting 'Manual Load'.
- 9.7.14. Fill a 1 ml syringe with buffer A, attach it to the injection port and inject a small volume of buffer, so that liquid just fills the valve port labeled 'loop F'.
- 9.7.15. Attach the bottom Superloop tubing to the injection valve port labeled 'loop F' using the threaded connector. Confirm that the tubing is firmly attached and will not easily pull out of the fitting.

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- 9.7.16. Attach the top Superloop tubing to the injection valve port labeled ‘loop E’ using the threaded connector. Confirm that the tubing is firmly attached and will not easily pull out of the fitting.
- 9.7.17. The Superloop is now ready to be flushed (see section 9.6) and prepared for sample injection.
- 9.8. ***Filling the Superloop with Buffer A.*** This step is important to ensure that the sample chamber and backside of the loop are equilibrated with the starting buffer for a given chromatographic run. Most of the residual ethanol and other compounds in the injection valve flow path will be expelled in the process.
- 9.8.1. Place degassed Buffer A atop the instrument and insert inlet tubing A1 into the buffer container, ensuring that the filter rests on the bottom of the container.
- 9.8.2. Use the pump priming syringe connected to pump A1 to draw 10 ml of buffer A through the inlet A1 tubing and close the priming valve. Using the manual control panel in the System Control window, click on Pump A in the diagram and select “Pump A Wash”.
- 9.8.3. Upon completion of the wash, set the flow rate to 1 ml/min. Click on the injection valve depiction and select “Inject”.
- 9.8.4. When the Superloop movable seal arrives at the zero position, change the injection valve position to ‘Manual Load’. Allow the pump to continue at 1 ml/min.
- 9.8.5. Fill a 10 ml syringe with buffer A and inject sufficient volume to completely fill the Superloop. See section 9.10 for more information on sample injection.
- 9.8.6. Once again, change the injection valve position to ‘Inject’ using the manual control feature of the software interface. Increase the flow rate to 2 ml/min.
- 9.8.7. When the Superloop movable seal is at the zero position, stop the pump. Click the ‘Stop’ icon (a solid square) in the toolbar near the top of the System Control window.
- 9.9. Start-up and preparation of AKTA pure Instrument and computer:
Degassed buffers should be in place prior to turning on the AKTA pure instrument. Equipment start-up requires turning on the instrument and, separately, the computer connected to it.
- 9.9.1. Place the degassed buffers A and B on top of the AKTA pure instrument.
- 9.9.2. Locate Inlet tubing A1 and B1 (atop the instrument and resting in water or 20% ethanol). Each has a filter unit attached, which distinguishes them from A2 and B2; those end in a male threaded fitting and will not be used for a two-buffer procedure.
- 9.9.3. Transfer tubing Inlet A1 to the buffer A bottle.
- 9.9.4. Transfer tubing Inlet B1 to the buffer B bottle.
- 9.9.5. The On/Off switch for the instrument is located on the right side toward the rear of the housing. Switch to the ‘On’ position. Audible emanations from within the instrument cabinet indicate that the AKTA pure system is going through its brief initialization sequence.
- 9.9.6. The computer On/Off switch is located on the front of the Dell desktop computer unit, near the top of the case. Press in to turn on the computer.
- 9.9.7. Login to the computer using credentials provided by the College.

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- 9.9.8. Double click the Unicorn 6.3 icon on the desktop to open the software which controls the instrument functions. Click OK in the “Log In – Unicorn” dialog box that appears.
- 9.9.9. Open the System Control window (under Tools menu, if not opened automatically on startup).
- 9.9.10. The top pane of the window will show the current state of the instrument, and the bottom pane shows the fluid path and manual controls. If the window is blank, go to the System menu and select Connect to Systems, check the box by AKTA pure 25 and click OK.
- 9.9.11. Confirm that the correct column is attached to the system. If not, refer to Section 9.4 (Installing/Changing a Chromatography Column on the AKTA pure Chromatography System).
- 9.9.12. Under the File menu, choose Open and select the method specified in the process SOP
- 9.9.13. A dialog box appears that allows the method to be started. Click Start to initiate flushing of the pumps and equilibration of the column.
- 9.9.14. While the equilibration method is running, prepare the fraction collector for later steps by filling the carousel with clean tubes.
- 9.9.15. Allow the program to run to completion, about 15 minutes.
- 9.10. Calibration of the pH Electrode
Calibration of the pH detector is performed daily, when the instrument is in use. The calibration procedure utilizes ordinary pH standards found in the lab. Calibration is dictated by a method built into Unicorn.
 - 9.10.1. Obtain three small beakers and pH standards for pH 4.01 and pH 7.0, as well as a 10 ml syringe and a bottle of MilliQ water.
 - 9.10.2. In the Unicorn System Control window, choose ‘Calibration’ from the System menu. From the drop-down menu under ‘Monitor to calibrate’, select ‘pH’.
 - 9.10.3. Click the ‘Prepare for Calibration’ button. You will hear the valve switch to the calibrate position.
 - 9.10.4. Follow the on-screen instructions for both pH standards. Enter the pH of the first pH standard buffer in the *pH for buffer 1* field.
 - 9.10.5. Fill a syringe with approximately 10 ml of the first pH standard buffer (pH 7). Connect the syringe to the Luer connector of pH valve port **Cal** and inject the buffer. When the *Current value* is stable, click the *Calibrate* button.
 - 9.10.6. Thoroughly rinse the syringe with 3-4 changes of MilliQ water. Wash the pH flow cell by injecting water into pH valve port **Cal**.
 - 9.10.7. Enter the pH of the second pH standard buffer in the *pH for buffer 2* field. Fill a syringe with approximately 10 ml of the second pH standard buffer. Connect the syringe to the Luer connector of pH valve port **Cal** and inject the buffer. When the *Current value* is stable, click the *Calibrate* button.
 - 9.10.8. The calibration date and time are displayed in the dialog, along with values for *Calibrated electrode slope* (should be $\geq 80\%$) and *Asymmetry potential at pH 7* (should be within the interval ± 60 mV. If the conditions are met, click the *Close*

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button to switch the pH valve back to the default position and to close the **Calibration** dialog.

- 9.10.9. If values for the slope and potential are not within acceptable values, clean the pH electrode and repeat the calibration procedure. If this does not help, replace the electrode.
- 9.11. Installing/Changing a Chromatography Column on the AKTA pure Chromatography System.

It is imperative that the following operations be performed in such a way as to prevent the introduction of air bubbles into the column and fluid path, which is achieved by making liquid-to-liquid (drop-to-drop) contact prior to inserting the threaded fitting into its position.

 - 9.11.1. Have on hand a few paper lab towels and a 250 ml beaker to catch waste.
 - 9.11.2. Remove tube connector from the UV detector inlet by unscrewing the knurled fastener.
 - 9.11.3. Initiate flow manually at 0.5 ml/min collecting waste in the beaker or towel.
 - 9.11.4. Remove the plug from the column inlet and place a few drops of 20% ethanol in the inlet, filling it to insure the absence of air. Also add drops of 20% ethanol to the UV detector inlet.
 - 9.11.5. As a droplet emerges from the inlet tubing, touch it to the liquid in the column inlet and begin to thread the fitting in, leaving slight looseness of threads so that liquid escapes around the fitting and pressure buildup in the column is prevented.
 - 9.11.6. Remove the column bottom plug and screw the column directly into the UV detector inlet.
 - 9.11.7. Tighten the column inlet fitting just enough to prevent leaking.
 - 9.11.8. The column is now ready to equilibrate in buffer (step 9.3.12) prior to performing a chromatography run.
- 9.12. How to fill a sample loop.
 - 9.12.1. Fill an appropriately sized syringe with sample, taking care to remove bubbles.
 - 9.12.2. Connect the syringe to the injection port (see Fig. 4).
 - 9.12.3. Open the System Control module in the Unicorn software.
 - 9.12.4. In the Process Picture click the Injection Valve and select Manual Load to confirm that the valve is in, or switch the valve to, manual load position.
 - 9.12.5. Depress the syringe plunger to inject sample into the sample loop, and leave the syringe attached to the port (prevents sample loss due to siphoning). Regarding amount to inject, if sample preservation is a consideration, inject only enough to fill the loop. If sample is abundant and/or relatively easy to generate, inject about twice the loop volume to insure complete filling.
- 9.13. Performing a chromatography run:
 - 9.13.1. Place the fraction collector tube 1 near the outlet tubing from the instrument (refer to attachment Fig 1) so that it will touch the arrow on the white paddle of the fraction collector arm. Note: To rotate the carousel, reach around the left side of the collector to find a rubber roller pressing against the carousel (Fig 2). Pull the roller away from the carousel (Fig. 3); the carousel will rotate freely as long as the roller is held. When the first tube is in the correct position, release the roller.

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- 9.13.2. Gently raise the arm and swing it into position against tube 1.
- 9.13.3. Place all 'Waste' tubing, labeled W, W1 & W2 in 1 L Erlenmeyer flask.
- 9.13.4. Place the tube labeled Out in a 125 ml Erlenmeyer flask.
- 9.13.5. Using a 1 ml syringe, aspirate 0.6 ml of the tPA sample into the syringe, expel any bubbles and insert the loaded syringe into the injection port.
- 9.13.6. Inject the sample into the port to fill the 0.5 ml sample loop.
- 9.13.7. Open the Unicorn software and navigate to the System Control window.
- 9.13.8. Under the File menu, choose Open and select the method with file name "*HiTrapSP tPA Production*".
- 9.13.9. In the dialog box that opens, enter operator's name, sample notes.
- 9.13.10. Click Next; note the time and and volume for the run; make sure there is excess buffer A and B.
- 9.13.11. Click Next. Record the buffer composition of each buffer and the sample identity.
- 9.13.12. Click Next. Enter a filename composed of the method name, date, operator or group initials, for example "*HiTrapSP tPA 16May15 CertGroup*".
- 9.13.13. Click Start. The instrument should begin to execute the method, as evidenced by a soothing hum from the pumps and drops of liquid falling into tube 1 from the fraction collector outlet.
- 9.13.14. Observe that the fraction collector is receiving drops.
- 9.13.15. Monitor the computer screen for error messages or warnings.
- 9.13.16. Allow the method to run to completion, at which time the system will be re-equilibrated and ready for subsequent runs by repeating section 9.4.
- 9.14. Equipment shut-down and short term (less than 3 days) storage
 - 9.14.1. After completion of the final separation of the day, transfer Inlet tubing A1 and B1 to a flask of degassed Milli-Q water (250 ml or greater).
 - 9.14.2. In the Unicorn software, open the System Control window.
 - 9.14.3. Under the File menu, choose Open, then select the method 'System Short Term Storage'.
 - 9.14.4. Click Start.
 - 9.14.5. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
 - 9.14.6. Place the pH valve in the 'Calibration' position (System Control window; System\Calibrate menu). Fill a 10 ml syringe with pH electrode storage solution and inject 9 ml it into the calibration port. Leave the syringe attached.
- 9.15. Turn off the instrument or perform the long term storage routine as needed (section 9.6).
- 9.16. Cleaning of the Superloop 10 sample holder- short term.
 - 9.16.1. For short term storage of the Superloop on the AKTA instrument, inject 2 ml Milli-Q water into the sample chamber.
 - 9.16.2. Pump it out to waste by temporarily disconnecting the outlet tubing that is connected to the injection valve at port 'loop F'.

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- 9.16.3. Using manual control in the System Control window of Unicorn, set the flow rate to 2 ml/min and the injection valve position to Inject. Allow pump A to run until the Superloop chamber is empty.
- 9.16.4. Reconnect to 'loop F'.
- 9.16.5. Repeat steps 9.6.1 thru 9.6.4 three times.
- 9.16.6. Inject 10 ml water into the sample chamber of the Superloop.
- 9.17. Equipment shut-down and long term (3 days or more) storage
 - 9.17.1. After completion of the System Short Term Storage method, transfer Inlet tubing A1 and B1 to a flask of degassed 20% ethanol (250 ml or greater).
 - 9.17.2. In the Unicorn software, open the System Control window.
 - 9.17.3. Confirm that the pH valve is in the 'Bypass' or 'Restrictor' position.
 - 9.17.4. Under the File menu, choose Open, then select the method 'System Long Term Storage'.
 - 9.17.5. Click Start.
 - 9.17.6. Allow the method to run to completion, as indicated by an audible tone and onscreen window.
 - 9.17.7. Turn off the instrument.
 - 9.17.8. Remove the Superloop from the instrument and carefully disassemble it. Handwash all parts with a general-purpose lab cleaner, rinse well and allow to air dry. Store the dried components in their original box.
- 9.18. Cleaning the system
 - 9.18.1. Column Removal – have paper towels on hand to catch drips. You will also need an appropriate plug for each end of the column.
 - 9.18.1.1. Use the Manual Control in the System Control window to set the flow rate of buffer or water to 0.5 ml/min.
 - 9.18.1.2. Disconnect the column outlet from the UV detector and fit the connector with a plug, slowly so that emerging droplets displace *all* air from the plug. Leave the plug slightly loose so as to prevent backpressure buildup.
 - 9.18.1.3. Slowly disconnect the tubing from the column inlet, allowing liquid to fill the cavity where the connector attaches. Attach the tubing directly to the UV detector inlet and stop the flow of buffer/water.
 - 9.18.1.4. Completely tighten the column outlet plug but be careful not to overtighten and strip the threads.
 - 9.18.1.5. Carefully insert a plug into the column inlet threads, displacing liquid but not allowing air to enter.
 - 9.18.1.6. The column may now be stored.
 - 9.18.2. Minimal Cleaning – After every day of use, perform short-term storage (9.10).
 - 9.18.3. Thorough Cleaning – Should be performed weekly.
 - 9.18.3.1. Remove the column (9.12.1) from the system prior to thorough cleaning of the system with 0.5M NaOH.
 - 9.18.3.2. Immerse all pump inlet tubes in a container of 0.5M NaOH.
 - 9.18.3.3. **Important:** Switch the pH electrode valve to 'Bypass' or 'Restrictor' position to prevent damage to the electrode.
 - 9.18.3.4. Run the method 'System Clean'.

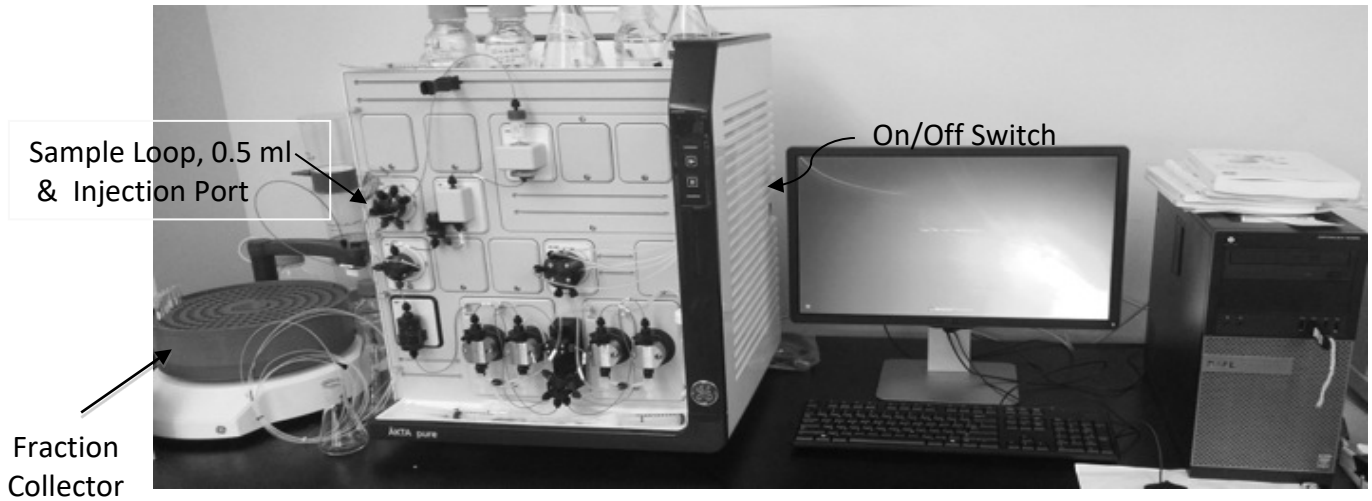
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- 9.18.3.5. Remove the pump inlet tubing from the NaOH container, rinse each carefully with a squirt bottle of MilliQ water and place in a flask of filtered and degassed MilliQ water.
- 9.18.3.6. Run the method 'System Short Term Storage'.
- 9.19. Chromatogram printout
 - 9.19.1. In the Unicorn software interface, open the Evaluation window.
 - 9.19.2. In the Result Navigator pane, click the Results tab.
 - 9.19.3. Locate the file of interest and double click its name to display your chromatogram in the right pane.
 - 9.19.4. *Optional:* Click the Customize button to open a dialog box that allows you to specify what curves display and the scale of each axis. Recommended are the UV Chrom curve, Conductivity, and Fraction Number.
 - 9.19.5. Click the Report button, check the Default report in the selection window and click Preview.
 - 9.19.6. Under File, choose to Print (or Save as PDF to use a different printer).

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10. Attachments/Figures

Fig. 1. Diagram of AKTA pure instrument, fraction collector and computer



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Fig. 2. ÄKTA pure Instrument Features

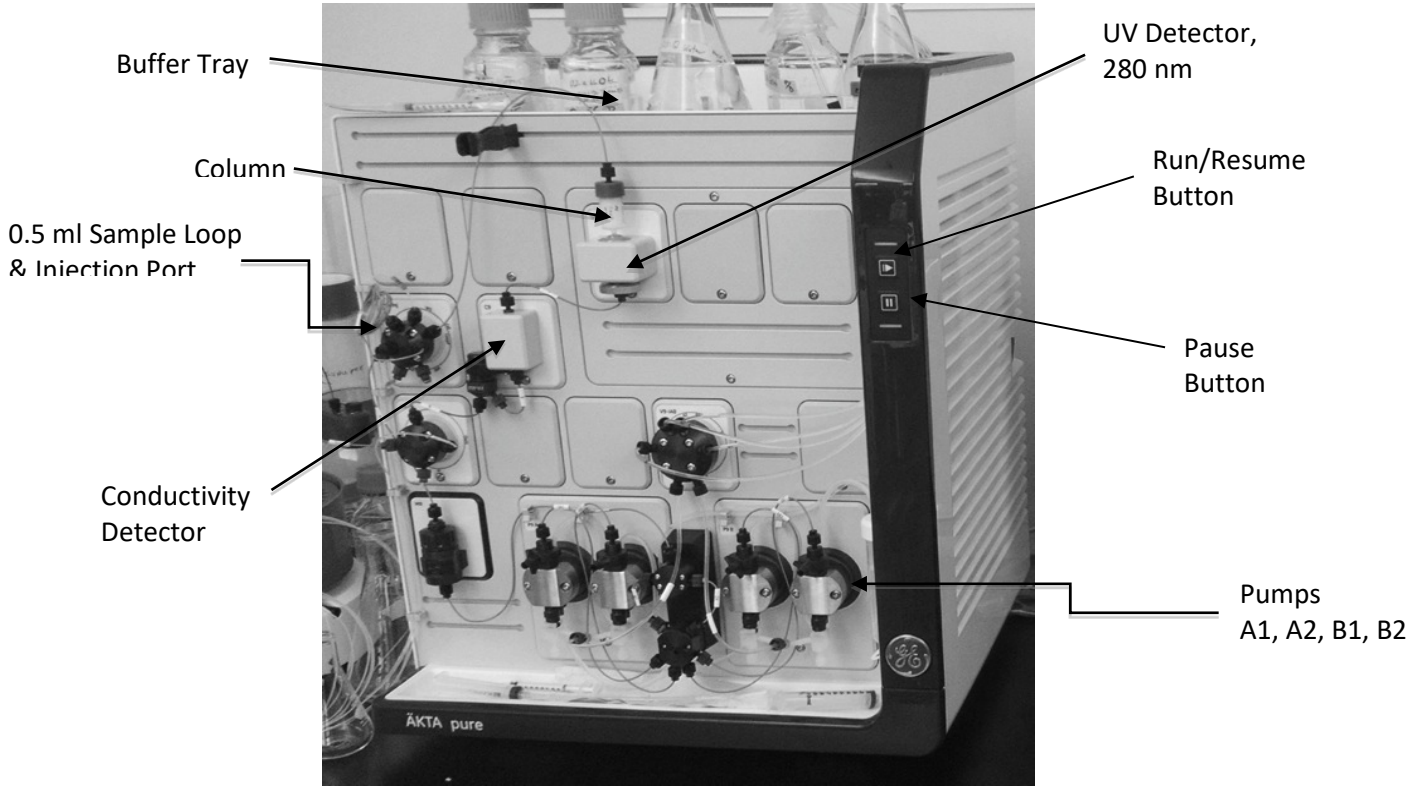
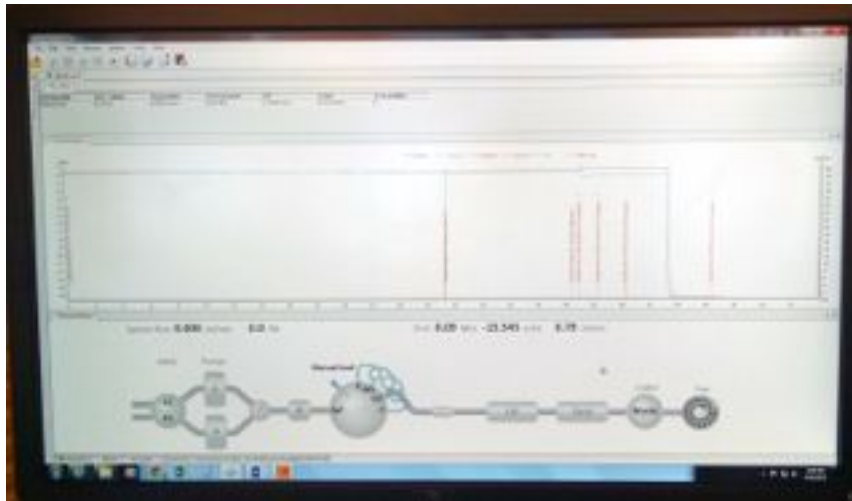


Fig 3. System Control window within the Unicorn 6.3 software.



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Fig. 4. Detail of Injection Port with Syringe in Place.

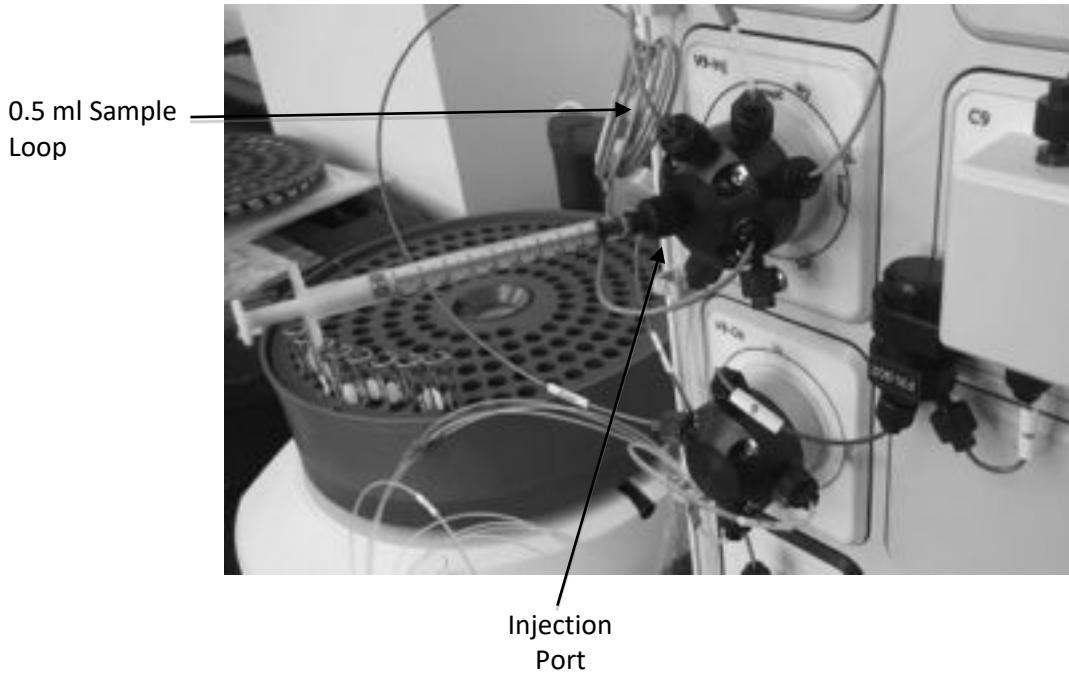
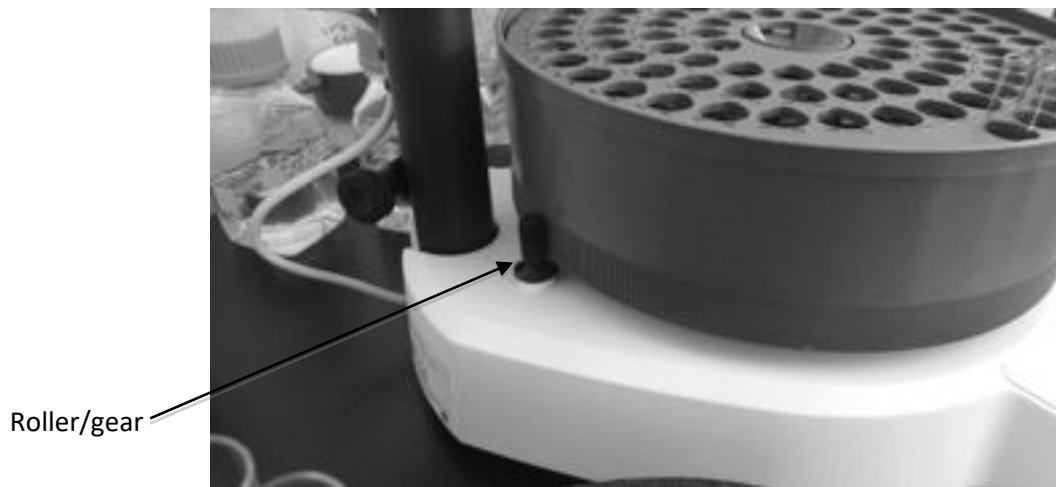


Fig. 5. Fraction collector carousel rubber advancement roller/gear.



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Fig. 6. *Release of roller to allow free rotation of the carousel.*

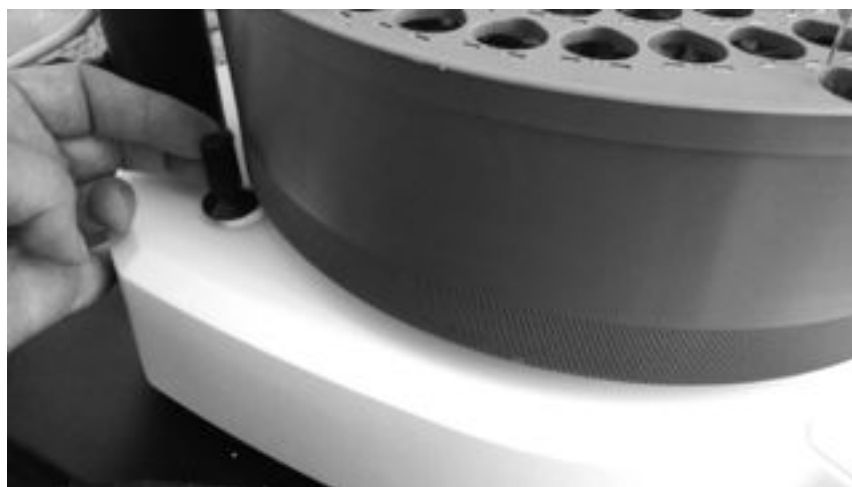


Fig. 7. *Location of tube #1 under the fraction collector drip outlet.*



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11. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	16JUL15	David Frank	Initial release
1	21APR16	David Frank	Minor changes
2	10JAN20	Hetal Doshi	Added priming of the pump rinsing system, priming of inlets and purging of pump heads