Crystallizer Operating Manual: Antisolvent Crystallization

Fall 2014 - C

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Introduction

The processing of biochemicals and pharmaceuticals is just as important to chemical engineering today as petrochemical production and oil refining are. Such processing involves operations such as crystallization, ultracentrifugation, membrane filtration, preparative chromatography and several others, all of which have in common the need to separate large from small molecules, or solid from liquid. Of these, crystallization is the most important from a tonnage standpoint; it is commonly employed in the pharmaceutical, chemical and food processing industries. Important biochemical examples include chiral separations [1]; purification of antibiotics [2]; separation of amino acids from precursors [3]; and many other pharmaceutical [4], [5]; food additive [6], [7], [8], [9]; and agrochemical [10] purifications.

The basic strategy in crystallization is to reduce the solubility of the solute of interest and can be carried out by a variety of methods including cooling, evaporation, pH swing [4], chemical modification/reaction, and non-solvent addition (frequently referred to as antisolvent crystallization). The control of crystal morphology and size distribution is critical to overall process economics, as these factors determine the costs of downstream processing operations such as drying, filtration, and solids conveying. If you are unfamiliar with crystallization, you should consult a specialized textbook or Chapter 27 of your Unit Operations textbook [11].

Our experimental crystallization apparatus enables study of key facets of *antisolvent crystallization*: (a) effects of key parameters such as supersaturation and cooling/heating rates on solids content, morphology and crystal size distribution; (b) on-line control of crystallization processes.

While an on-line video microscope is widely used in actual crystallization processes to monitor morphology and size distribution [12], such an instrument is at present beyond our budget (cost ~$80 K), so we use an offline microscope to measure from 10-1000 μ crystal sizes, a typical size range for crystallizations of biologicals and other materials of interest.

The experiment and process described in this operating manual is antisolvent crystallization, generating sodium chloride crystals from an aqueous solution by addition of the antisolvent ethanol which renders the solute less soluble in water, in a manner not dissimilar from experimental work of others in the field [13].

System Overview

The crystallization apparatus employed in antisolvent crystallization mode is composed of a subset of the entire apparatus built originally for salicylic acid crystallization. The principal components include the crystallizer vessel with agitator, heat transfer coil (A); a conductivity meter (B); an ethanol feed flask for the antisolvent supply (C); a variable speed peristaltic pump for ethanol antisolvent addition (D); and a temperature-controlled circulating bath to heat or cool the contents of the crystallizer vessel through the heat transfer coil (E). Figure 1 below can be used to identify these components.

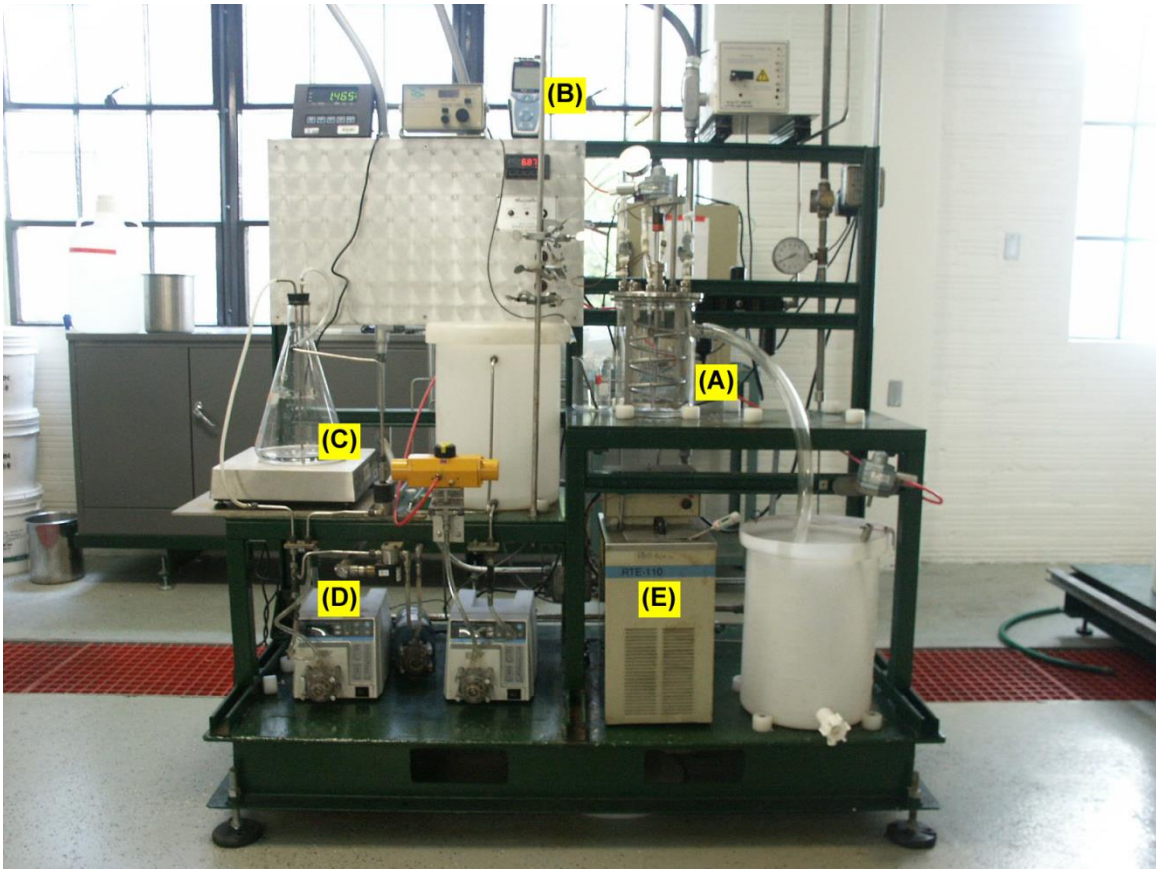


Figure 1: Photo of Crystallizer unit in antisolvent crystallization mode.

The crystallization itself takes place in a baffled ~5 L glass vessel equipped with an air-driven agitator, thermocouple, conductivity probe, sampling port and extra ports. Crystallization vessel temperature is controlled by circulation of heat transfer fluid, the temperature of which is thermostatically controlled using its own temperature controller and control thermocouple. This bath is capable of delivering 5-to-50°C glycol to the crystallizer heat transfer coil.

Filtered air is supplied to the agitator. The crystallizer normally does not require cleaning. If you are told to clean it, use soap and hot water and rinse with DI (deionized) water. This is true as well for all other glassware used in this experiment. To disassemble the crystallizer, remove the agitator unit with an Allen wrench, remove all probes and place them on Kimwipes™ (remove conductivity probe slowly and carefully – it can easily break). Rinse the conductivity probe with

DI water only and put it in either DI water or buffer solution. Leave everything else on a CLEAN (e.g., covered by Kimwipes™) surface. Then disconnect the overflow line and feed lines (quick connect fittings) and lift the internals from the glass vessel.

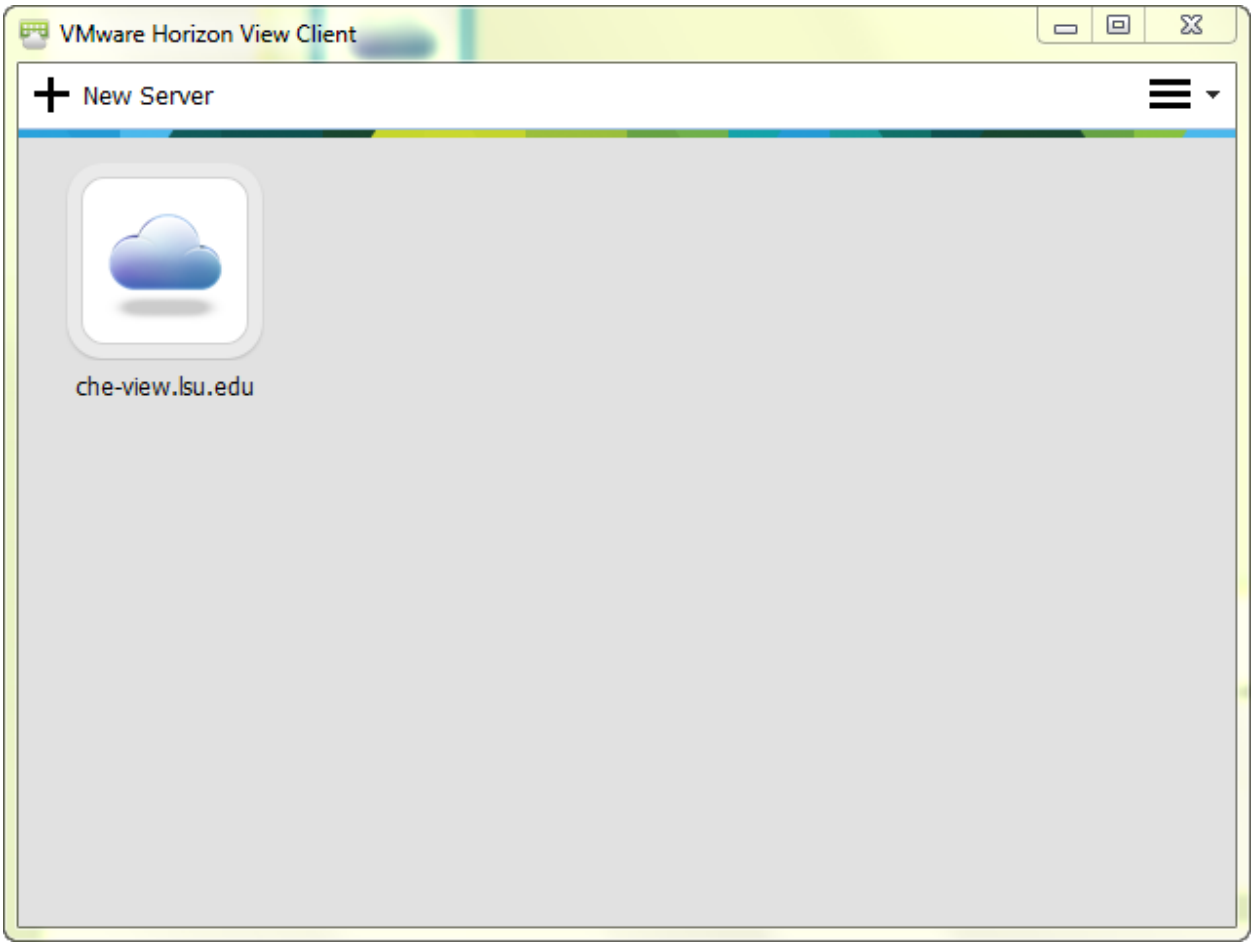
Chemicals for the experiment include: nominally 95% ethanol (the remainder is water), high-purity sodium chloride, and deionized water. All these are stocked in either the unit equipment cabinet or in the Analytical Lab.

Operating the Crystallization Experimental Equipment

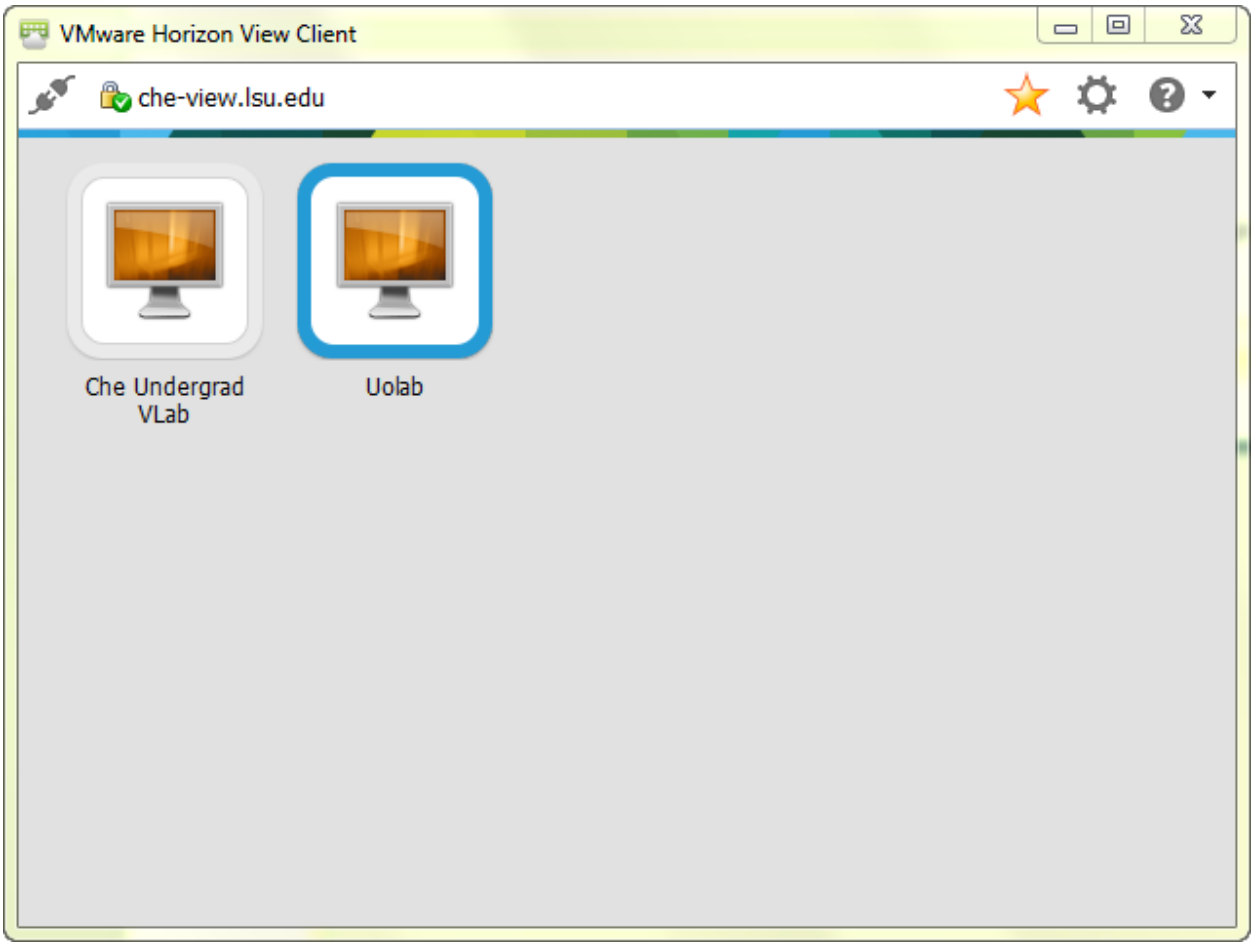
Understanding Experion PKS Control System Terminology

The purpose of the next few paragraphs is to explain how to use Honeywell's Experion to run this equipment.

Access to the Honeywell Experion DCS application is through a virtual machine using VMware Horizon View software. After logging into one of the computers in the UO Control Room, among the icons on the desktop shown is the **`VMware Horizon View Client`** icon. Double-clicking this icon should bring up the following popup with the **`che-view.lsu.edu`** icon showing. If that icon does not appear, then click **New Server**; complete the requested information to add it. The following should show:



Double-click the **che-view.lsu.edu** icon. If an additional login menu appears, login using your LSU ID and password. At this point the following view should appear:



Virtual machines are available for conventional ChE desktops (shown as **Che Undergrad VLab**) and for access to the Honeywell DCS (shown as **Uolab**). Double-click the **Uolab** icon. A splash screen for the virtual machine should appear with an **OK** button. Click **OK**. The VMware software should start up a virtual machine and show you the desktop of that machine.

Open the Honeywell Station software either by double-clicking the **Station** icon if visible or by navigating using the following pathway:

Start>All Programs>Honeywell Experion PKS>Client Software>Station

If login credentials are proper, the **Station** program will appear and – *exercise patience here* – the following default splash screen within it:

The Honeywell login process is now complete. From the **Unit** item on the Station menu, select **CRU**. This is an acronym for **CR**ystallizer **U**nit. The **CRU** P&ID schematic, shown in Figure 2, will appear.

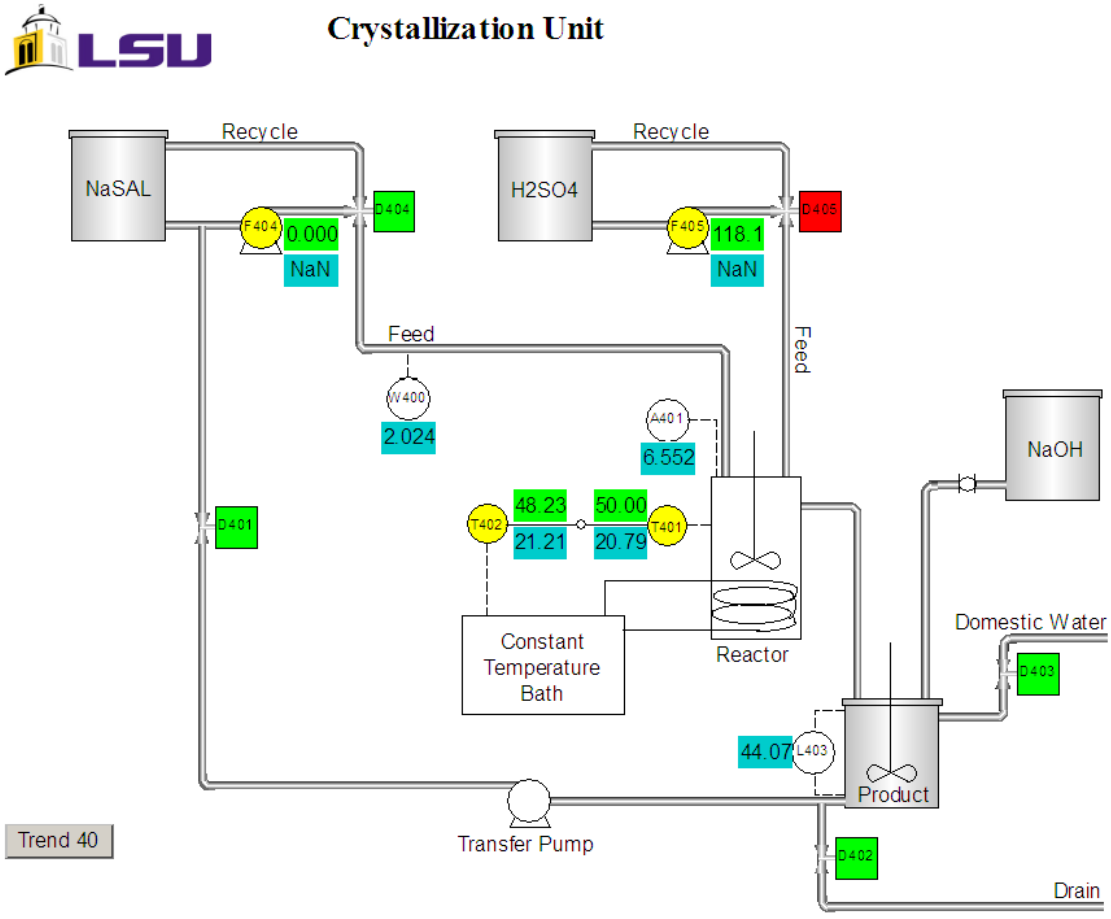


Figure 2: Crystallizer unit schematic from Honeywell Station display (The NaSAL, H2SO4, and NaOH labels are correct only for the salicylic acid mode of unit operation. In NaCl antisolvent mode, the NaSAL vessel is replaced by a large flask for ethanol addition and neither of the other two vessels is used.)

This schematic is much like a process and instrumentation diagram (i.e., P&ID), and the crystallizer is controlled from it. There is also a schematic (obtained by clicking the Trend 40 button) which shows trends of the analog values associated with the unit.

On the main schematic, each measurement transmitter and continuous controller is represented by a circle containing its tag name. The first letter of the tag name indicates the type of measurement: **A** for analyzer (pH in this case), **T** for temperature, **L** for level, **F** for flow, and **W** for weigh scale. There are also five discrete controllers; that is, solenoid valves which either start or stop the flow in a line, or switch the flow from one line to another. When you click on a circle representing any measurement or continuous controller, or a square representing a solenoid valve, a faceplate will appear in the lower right corner of the schematic. It contains the tag name, engineering units, description, and PV for transmitters. For continuous and discrete controllers, additional values and controls are available (see below).

To change an analog value from a schematic or from a faceplate, single click the value (if change is allowed, its background color will change), and then enter the new desired value. To change the mode of a continuous controller, use the combo box labeled **MD** near the bottom of its faceplate (more about controller modes below). To change the output of a device controller, use the **OP** radio buttons on the right side of its faceplate.

At the start of a run, all continuous controllers should be in manual mode (signified by yellow backgrounds in the circles), and all solenoids should be either closed or in recycle (signified by the green backgrounds in the squares). Now open the Ethanol Recycle Solenoid (D404) for practice (click on the solenoid valve on the left side of the schematic, and then click on the upper **OP** radio button on the right side of the faceplate). Notice that the solenoid valve changed from green (representing closed) to blue (representing changing positions) to red (representing open) when you open the valve. When you close the solenoid, the color sequence will be from red to blue to green. You may not see the blue state because the solenoid changes very quickly.

A transmitter's most important value is the measurement of the ***process variable*** itself, abbreviated as **PV**. This value is shown in cyan (light blue) near the circle.

Controllers have several additional values, the most important of which is the set point or **SP**. This is just like the speed setting on a cruise control - the controller will manipulate its output (the throttle position in this case) to move the **PV** to the **SP** and hold it there. The **SP** (in green) and the **PV** (in cyan) are shown near the circle representing the controller. The **OP** always has units of percent (0-100%) and the **SP** has the same engineering units as the **PV**. On the crystallizer, pH is measured in pH units, flow rates are measured in ml/min, levels are measured in percent full, and temperatures are measured in degrees C.

When the controller mode is **MANual**, the **OP** is held until the operator changes it. If you want to change the **OP**, simply click the **OP** in the faceplate, type in the new value, and press **ENTER**. The new **OP** will be held until you change it again. ***Note that you can change an*** **OP** ***only while a controller is in*** **MAN**. For practice, click the Ethanol (labeled NaSAL) flow rate controller (to call up its faceplate) and change the output to 50%, then to 100%, and back to -6% (-6% is known as "tight shutoff" in the PKS system). Notice the small bar under the control valve on the schematic – its length is proportional to the output.

When the mode is not **MAN**, the controller uses the **PV**, **SP** and tuning constants to calculate a new **OP**. When the mode is **AUTOmatic**, you may enter a new **SP** to be used for control. ***Note that changing an*** **SP** ***affects the*** **OP** ***only while a controller is in*** **AUTO**.

Notice that the circle representing a continuous controller is filled with a background color, which indicates the current mode of the controller - yellow means the mode is **MAN,** and white means the mode is **AUTO**.

Display Navigation

When you logged into Flex Station, you used an item from the menu bar to call up the main **CRU** schematic. There are several additional ways to go from one display to another. For example, you can enter the tag name of a control module in the **Command** field at the top of the screen and press **F12** to call up the detail display. Try it with the ethanol (labeled NaSAL) flow rate controller (F404). For continuous and discrete controllers, the detail display has 7 tabs, and for a measurement, only three.

Most of the toolbar buttons are used for navigation – some require a name or number to be entered, and some go directly to the display. Most of the same functions are on the function keys. For example, to return to the previous display, click  , or press **F8**. To return to the display before that, do it again.

From most displays (both system displays and custom schematics such as **CRU**), double clicking any value associated with a control module will take you to its detail display. From a detail display, click  or press **F2** to return to the main **CRU** schematic. On most custom schematics there may also be buttons to quickly get you from one display to another.

Understanding Trend Schematics

There is a button on the **CRU** schematic to call up trends. The button is labeled Trend 40 and displays **PV**s and **SP**s of the principle variables on the unit.

At the bottom of the trend is the legend with all the **tag.block.parameters** associated with the traces. The checkboxes in the **Pen** column indicate which traces are currently on the trend. Click on the chart area of the trend and a white hairline cursor appears on the chart and the values at the hairline cursor appear in the **Reference** Value column of the legend. Along the bottom of the chart area is a horizontal scroll bar which allows you to scroll the chart area back and forth. Along the left axis are the low and high ranges of the selected trace. These ranges allow you to change the range of the trace for the selected parameter. Practice by changing the range of one of the flow controllers to 20-80.

Immediately above the left side of the chart area is a combo box which allows you to select one of the traces (you may also click anywhere on the line for this trace in the legend area). When you select an active trace, it is highlighted (thicker) in the chart area. Above the right side of the chart area is the **Period** combo box which allows you to select how much data (time-wise) is displayed in the chart area. To the right of that is the **Interval** combo box which allows you to select the interval between points in the chart area. Practice changing to a different period and interval. Leave the period set to 1 day and the interval at 1 minute for now.

For practice, scroll back until some variation in some of the traces appears. Notice that the timestamps below the chart area change as you scroll. Find some local max or min in one of the traces and click or drag the hairline to it. Now change the period back to one hour and notice that the cursor is centered on (or at least near) the local max or min. If necessary, move the hairline so it is exactly on the peak or valley and notice that the values, as well as the date and time, are shown in the **Reference Value** column in the legend. Now return the trend to the current time by clicking  .

All changes you make to the trend can be saved by clicking the familiar Windows Save icon just above the right end of the chart area next to the word **(Modifed)**.

Saving Data into Excel

Successful completion of the objectives of this experiment will require analysis of a great deal of data. To collect this data, an Excel workbook containing a Visual Basic Add-In is provided. On the Desktop, look for a folder named

Excel SpreadSheets

Within that folder, open the folder named

snr

and double click on **CRURecorder.xls.** The workbook will open with a **Start** button, the experiment name, a collection frequency **Combo Box**, and a **Stop** button on the top line. Click on the **Start** button, and the workbook will start collecting the relevant data at the specified collection frequency. These data will be extremely useful in analyzing your results. While the workbook is collecting data, it may be scrolled, but you should not attempt to do anything else in this instance of Excel until after you click on the **Stop** button. If you do, the collector may stop and you may lose valuable data.

When you finish a run, click on the **Stop** button and cut or copy whatever data you need to your daily workbook in a separate instance of Excel. Let the workbook collect data while you finish reading this introduction.

CRU Tag Names and Descriptions with Engineering Units

Variables on the CRU are measured and reported by the Honeywell PKS system and are as shown in Table 1 for the antisolvent mode of operation.

Table 1: Tag names, descriptions, and engineering units for Crystallizer process variables (Tags in blue-bordered boxes are relevant to the antisolvent mode of operation. Treat other tags as Out of Service when in this mode.)

Tag Name	Description	Engineering Units
W400	EtOH Feed Weigh Scale	kg
A401	Reactor pH **(OOS)**	pH
D401	Product Recycle Solenoid **(OOS)**	Open/Closed
D402	Product Drain Solenoid **(OOS)**	Open/Closed
D403	Product Water Supply Solenoid **(OOS)**	Open/Closed
D404	EtOH Feed Solenoid	Recycle/Feed
D405	H2SO4 Feed Solenoid **(OOS)**	Recycle/Feed
F404	EtOH Flow Rate Control	ml/min
F405	H2SO4 Flow Rate Control **(OOS)**	ml/min
L403	Product Tank Level **(OOS)**	Percent
T401	Reactor Temperature	Deg C

T402	Bath Temperature Control	Deg C

(OOS) = Out of Service

Operating the Crystallizer Itself

Use the following procedures to operate the crystallizer:

1. If an operating temperature other than ambient (20 to 21°C) will be used, start up the temperature controlled glycol recirculation system.

2. Rinse the crystallizer and insert frame with deionized water to remove any particulates from the crystallizer vessel. (Doing so reduces the variation in heterogeneous nucleation caused by stray particulates and accumulated chemicals on the crystallizer hardware.)

3. Charge the ethanol feed flask with sufficient volume of 95% ethanol. (This material can be found in the flammables cabinet in the Analytical Laboratory.) For most experiments, the appropriate volume of ethanol is twice that of the water that will be used in the crystallizer vessel itself. For example, if 1 L of water will be used in the experiment, then 2 L of ethanol should be available for addition during the run. Antisolvent addition rate and water charge volume will dictate the amount of materials needed and the maximum length of the run.

   If knowing the ethanol content of the antisolvent feed with more certainty is desired, one of the Hewlett-Packard gas chromatographs used for alcohol distillation work can be used to ascertain this. A known standard in the desired range can be made from 200 proof ethanol and distilled water.

   The peristaltic pump may need to be calibrated for the feed used. A pump calibration will allow you to set your desired rate. Very low rates (perhaps as low as 2 to 4 mL/min) of antisolvent addition are possible even with this large pump – if careful calibration is pursued and periodic calibration checks are made. Use of the weigh scale will provide an independent (and perhaps less uncertain) method of knowing just how much antisolvent has been fed in those cases where a considerable amount of antisolvent will be used.

   Sometimes the tubing at the pump head will leak. In this case, the tubing must be replaced (see the Lab Coordinator regarding this maintenance item.)

   Make sure that the flask stopper has a hole in it, or else a vacuum will be drawn and uneven flow will result.

4. Fill the reactor with 34.0 grams of NaCl per 100 mL of water used. (This concentration is suitable for experiments conducted at ambient temperature – roughly 20 to 21°C.) For example, use 340 g of NaCl for 1 L of water (though actual charge quantity must be determined by the requirements of each experimental program). For volumetric accuracy volumetric flasks should be used for volume measurement. For superior accuracy the flask can be weighed before and after it is filled to obtain the actual mass of water added to the crystallizer.

5. After the deionized water and NaCl have been added to the reactor, operate the pneumatic stirrer at 10 psi or other operating pressure to establish agitator RPMs at the desired level (too fast may result in material splashing to the wall and depositing crystals; too slow may result in failure to keep all crystals suspended). A handheld tachometer is available for this purpose. This will facilitate salt dissolution.

6. If an operating temperature other than ambient (20 to 21°C) will be used, start up the temperature controlled circulating bath using the T402 controller with the set point desired. Any value from 5°C to 50°C is achievable by the bath. Insulation of the crystallizer vessel may be required.

7. Once the NaCl is dissolved, take an initial concentration sample for analysis.

8. Begin the antisolvent addition, insuring that the recycle valve is in the correct (i.e., feed) position. Verify that the feed pump is operating. Since this process mode is semi-batch crystallization, the most important crystallization behavior will occur early in the process. So, set sampling protocol in recognition of this issue, taking sample sets every 5 minutes for the first 20 to 30 minutes of the experiment. After that, samples might be taken every 10 – or even 15 – minutes. (This protocol should be reviewed with the experiment objectives in mind. It's possible that more or less frequent sampling may make sense.)

9. If program requirements necessitate the use of crystallizer overflow, equip the vessel with Tygon™ tubing from the vessel exit port to the collection tank at the bottom right of the unit. To drain the collection tank, open tank discharge valve (D402) and allow the tank to drain to the floor drain.

10. After all the desired ethanol has been added, turn off the peristaltic pump using the Honeywell system. Do not turn off the stirrer yet. Let the crystallizer sit for another 20 minutes without solvent addition. Take a final set of samples at the end of the 20 minutes.

Shutting Down and Cleaning the Crystallizer

1. To shut down the system, set the feed 3-way valve to recycle, set the collection tank discharge valve to closed, set the feed pump output to 0%, return the temperature controlled circulating bath set point to 20°C, manually turn off the circulating bath, and shut off the feed pump and agitator.

2. Empty and clean the crystallizer for the next experiment. Undo all of the connections and take the crystallizer into the Analytical Lab. Decant the liquid into a waste container supplied by the Laboratory Coordinator. Pour the crystals into a container specified for re-crystallized NaCl. Rinse the glass vessel and the stainless steel coil and baffles to remove any residual NaCl. After the reactor is sufficiently clean, it can be returned to the unit and reconnected.

Disposal of Spent Materials

Various spent or waste materials are produced from this unit: a) antisolvent from calibration of the antisolvent feed system b) various liquid samples, c) various dried samples, and d) remaining material in the crystallizer vessel after semi-batch operations. None of these materials are deemed hazardous to the environment. Use the following procedures to dispose of these materials:

1. Dispose of a), b), and d) by pouring down the drain in the Unit Operations Lab laboratory sink while extensively flushing with water from the sink faucet.

2. Dispose of material described in c) above by triple-rinsing bottles and faucet-washing of filter papers or other solids-containing lab ware. Discard washed filter paper in ordinary lab trash can.

Gravimetric Analysis for Concentration Measurements

Insight into the extent of super-saturation can be accomplished by measuring solution concentration through gravimetric analysis techniques. This involves the use of a syringe to remove samples from the reactor at predetermined time intervals. The contents of the syringe are then filtered through a syringe filter before being put in a vial. The vial's mass is measured on a mass balance before and after it is filled. After filling the vial, it is then put into an oven at 110˚C to evaporate the solvents from the vial. After the vial has been in the oven for 24 hours then it can be weighed for a third time.

With these three different vial mass measurements the combined mass of solvents and the mass of dissolved NaCl can be determined. This data can subsequently be rearranged to give a popular solubility measurement of (mass solute) / (mass solvent). Crystal yield can also be determined by taking a second sample at the same time. The exact prior procedure is carried out with one exception. This time the syringe filter is not used. After drying, this vial will contain all of the dissolved NaCl and all of the

crystallized NaCl. Since the dissolved NaCl was determined from the previous vial the crystallized NaCl per kg solvent can be easily determined.

Crystal Product Sampling, Slide Preparation, Photography and Size Determination

Product sampling

Product sample involves taking a sample with a tubing-equipped syringe and releasing the fluid onto a piece of filter paper using a Buchner funnel and vacuum. Once the liquid has passed through the filter, then the filter paper is oven dried at 50˚C for 24 hours. This procedure is used for each of the samples taken during a run.

If determining mean crystal size and/or size distribution as part of your study, extract one (1) sample vial from crystallizer contents at each experimental measurement time.

Slide preparation (See also the Microscopy Video on the uolab.com website)

(Much of the language in this and the next subsection has been only mildly edited from a description of the method used by Jarrell and Vocke [14].)

After the samples have been dried, use a spatula to scrape a small amount of crystals from the filter paper to a glass microscope slide. Make sure the slide is clean by polishing it with Kimwipes™. Do not put too many crystals onto the slide; too many crystals will make it difficult to see the individual crystals under the microscope. Use the spatula to center the crystals on the slide.

Then, put a small amount of ethanol onto the crystals. Put just enough to suspend some of the crystals. NOTE: Small crystals can be dissolved by ethanol; be very careful when dealing with the samples taken in the beginning of a run.

Gently press another glass slide down on top of the crystals; use the top slide to form a single layer of crystals on the bottom slide. Remove the top slide. Spread the crystals around by sloshing and adding a little more ethanol.

Prepare two to three slides for each sample of dried crystals as part of a replication process to estimate uncertainty.

Clean the slide stage area on the microscope with Kimwipes™. Slides can be reused, but they must be rinsed with deionized water, dried with paper towels, and again polished with Kimwipes™.

Preparing the microscope

After the samples are dried they are ready to be photographed with the microscope.

Turn on the microscope stage illumination by turning the knob on the Leitz Wild Heerbrugg polarizing light microscope power supply. Set the voltage meter to 10 volts by adjusting the knob. (Feel free to adjust this to as high as 12 volts later if necessary to get adequate illumination.)

The microscope itself has four different objectives (6x, 12x, 25x, and 50x), which can be selected by turning the large ring on which these values are stamped. The appropriate objective depends on the size of the crystals in the sample, but in many cases the 25x magnification will prove suitable. To aid in the measurement of particles, the Leitz Wild Heerbrugg is equipped with a USB microscope camera. The camera fits into the side tube on the side of the microscope with one of the supplied adapters. Since the camera is not parfocal with the microscope's eyepiece, its focus must be set independently of the eyepiece focus. This is done through the camera's included software.

Logging into the thin-client virtualized desktop for crystal microscopy work

The monitor and keyboard nearest the microscope operates as interface to a virtualized desktop running on the department server. To log in to this virtualized desktop, the display shown in Figure 1 should be present on the screen. (If you are given a choice of servers, select **`che-uolab-v29.lsu.edu`**.)

Figure 3 - Login splash screen in microscope thin-client display

Enter your username and password, making sure that the Domain field contains LSU. The Windows 7 based virtualized desktop should open.

Connecting the microscope camera to the network

The microscope camera is a USB device which must be physically and logically connected to the network before it can be accessed by the camera software. This is done through the SX Virtual Link application desktop icon. Double-click the icon to start the program. The display shown in Figure 4 should appear.

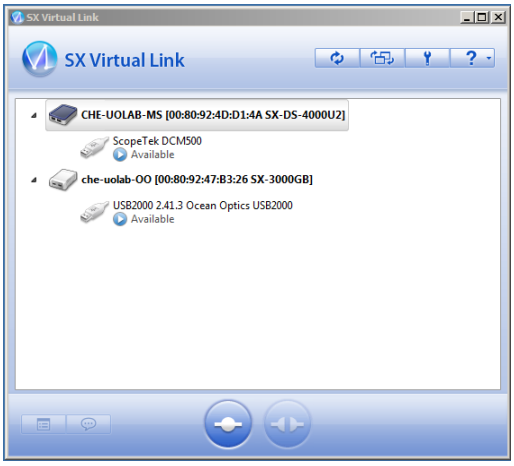


Figure 4 - SX Virtual Link interface BEFORE connecting the camera

As shown in the figure, there may be several USB devices to choose from. The microscope camera is shown as the ScopeTek DCM500. Hover over that link and double-click. A transient message indicating the connection operation is taking place will appear and disappear, leaving the SX Virtual Link interface with the appearance shown in Figure 5, indicating that the camera is successfully connected to the network.

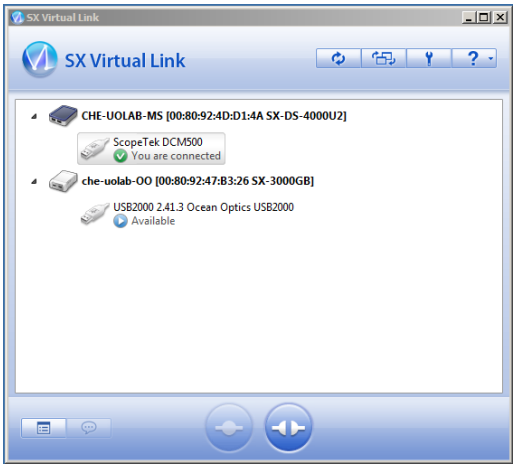


Figure 5 - SX Virtual Link interface AFTER connecting the camera

At this point, the SX Virtual Link application interface can be minimized.

Camera operation and picture taking using the MD900 AmScope camera and software

The first step to taking photographs of your crystals is to open the AmScope 3.1 software by double-clicking its icon on the virtual desktop. The Start Page opens, on which should be seen the AmScope MD900 listed under the Live Capture link. Single click that AmScope MD900 link. A Video window should open. If microscope illumination is powered on, its settings are appropriate and a slide with crystals has been placed on the microscope stage, crystals should appear in that window. However, initially, the image may look unfocused.

Focus the camera image using the large focusing knobs on the vertical mount of the microscope. Once the image is focused, check to see if the magnification of the crystals being viewed is appropriate. Hint: they must be large enough for you to be able to draw a line from one side to another – a technique you will use later in crystal sizing. If the crystals appear too large then choose a smaller objective. Likewise, if they are too small choose a larger objective. The image will need to be refocused with every objective change. You also have the ability to use software zoom (see drop down box near top of program screen) to bring the field into large view on the computer's monitor. A value of 40 to 50% usually works well.

Now the crystals are ready to be photographed, to provide an image such as the one shown in Figure 6.

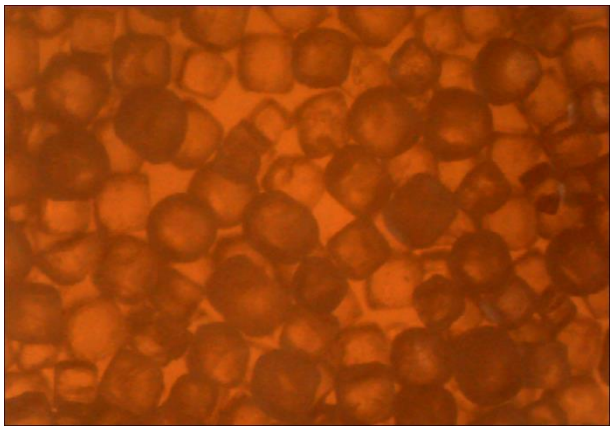


Figure 6 - Photo of reagent grade NaCl crystals taken at 12x magnification

This can be done either individually or sequentially. Under the Capture menu item, find the Capture a Frame button, to take an individual photo. Pictures can also be taken at set intervals by clicking the timer toolbar button. This will open a window that will ask you where the files are to be saved. It will also ask you what type of image file you want. Save pictures in .tiff format. Picture files can be named to keep track of sample, slide, and photo number – a great aid in retrieval of data and vital if analysis is not done immediately.

The other options dictate how often the camera will take pictures and how many it will take. Note that the timer option will automatically save the photos while the individual method requires manual saving for each picture taken.

Take three to four different field-of-view pictures from each slide by move the slide on the microscope stage, so as to maximum the availability of crystal sizing information.

Analysis of crystal size from a photo

Once the crystal photographs are taken they are ready to be analyzed. This is easily done with the software.

First, open the photo in question, if not already open. Then, open a new layer by clicking on the LAYER toolbar and choosing NEW. Click OK on the window that opens. Next, go to the OPTIONS toolbar and click the Annotations option. In the new window select line under the object heading. Switch the line color to a color other than black. Green or white might be a good choice – but try colors and see what's most visible to you on the photo on question. This helps in remembering what crystals you have already measured.

Second, look at the bottom of the window and look for a yellow ruler. Make sure the word '***pixels'*** is displayed next to it. If it is not then right click on the yellow ruler and select pixels.

Now it is time to actually measure the crystals. This can be done by clicking the line icon on the toolbar and choosing any line. Left click on one side of your crystal and move the line to the other side of the crystal. Left click a second time to finish the line. The photograph in Figure 7 is an example of the results of this process.

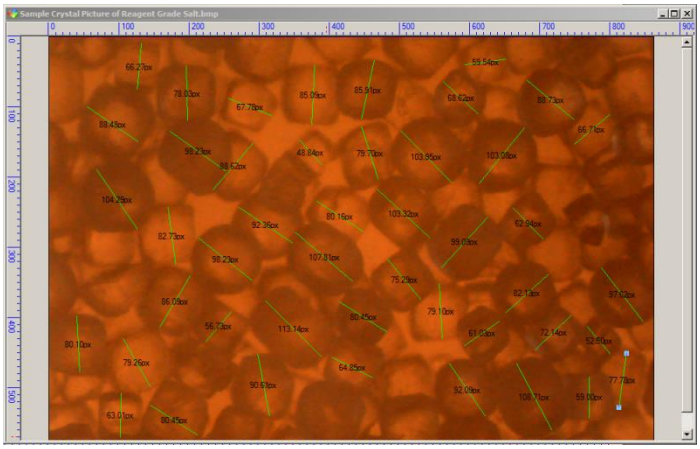


Figure 7 - Sized crystals from the previous photograph

The line details should show up on the bottom of the picture in the Annotations Manager window. Under the column called Length are the numbers of pixels that each line drawn contains. If you do not see this then click on VIEW toolbar and make sure that Annotations Manager is checked. An example of the contents of the Annotations Manager window can be seen in Figure 8.

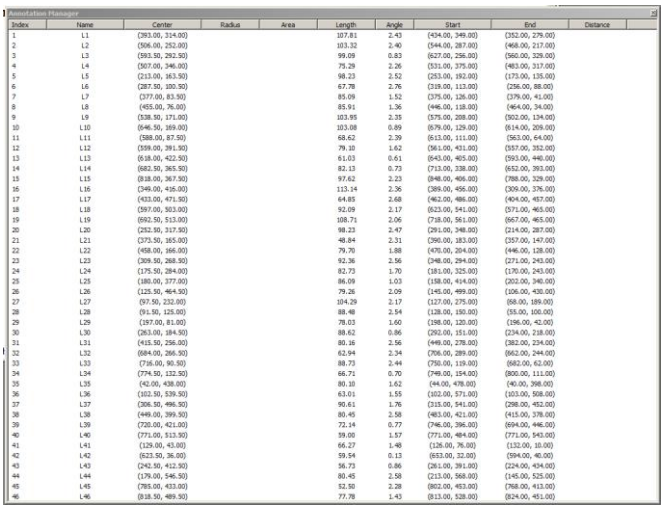


Figure 8 - Annotations Manager window containing crystal sizing information

Repeat the measuring process for all of the crystals in the frame. Selecting crystals to measure would establish a bias in sizing – not something one would want to do.

Once the crystals are measured, right click on the Annotations Manager pane at the bottom and select the Export option. The data can either be sent to Excel™ or to the clipboard via the current layer option. If the Excel™ option is selected a new Excel window is opened, containing the data from the Annotations Manager, as shown in Figure 9.

	A	B	C	D	E	F	G	H	I
1	Name	Center	Radius	Area	Length	Angle	Start	End	Distance
2	L1	(393.00, 314.00)			107.81	2.43	(434.00, 349.00)	(352.00, 279.00)	
3	L2	(506.00, 252.00)			103.32	2.4	(544.00, 287.00)	(468.00, 217.00)	
4	L3	(593.50, 292.50)			99.09	0.83	(627.00, 256.00)	(560.00, 329.00)	
5	L4	(507.00, 346.00)			75.29	2.26	(531.00, 375.00)	(483.00, 317.00)	
6	L5	(213.00, 163.50)			98.23	2.52	(253.00, 192.00)	(173.00, 135.00)	
7	L6	(287.50, 100.50)			67.78	2.76	(319.00, 113.00)	(256.00, 88.00)	
8	L7	(377.00, 83.50)			85.09	1.52	(375.00, 126.00)	(379.00, 41.00)	
9	L8	(455.00, 76.00)			85.91	1.36	(446.00, 118.00)	(464.00, 34.00)	
10	L9	(538.50, 171.00)			103.95	2.35	(575.00, 208.00)	(502.00, 134.00)	
11	L10	(646.50, 169.00)			103.08	0.89	(679.00, 129.00)	(614.00, 209.00)	
12	L11	(588.00, 87.50)			68.62	2.39	(613.00, 111.00)	(563.00, 64.00)	
13	L12	(559.00, 391.50)			79.1	1.62	(561.00, 431.00)	(557.00, 352.00)	
14	L13	(618.00, 422.50)			61.03	0.61	(643.00, 405.00)	(593.00, 440.00)	
15	L14	(682.50, 365.50)			82.13	0.73	(713.00, 338.00)	(652.00, 393.00)	
16	L15	(818.00, 367.50)			97.62	2.23	(848.00, 406.00)	(788.00, 329.00)	
17	L16	(349.00, 416.00)			113.14	2.36	(389.00, 456.00)	(309.00, 376.00)	

Figure 9 - Exported Excel file with crystal sizing information

If the clipboard option is selected then the data is copied to the windows clipboard and can be copied and saved to a program of your choosing.

Converting AmScope pixel measurement values to actual sizes

In order to convert line length in pixels to an actual length in microns, the following table is used. The particular value depends on the objective that was used when the photograph was taken.

Note the footnote carefully.

Table 2: Pixel size versus microscope objective power

Objective	**Microns per pixel[1]**
6x	**3.23**
12x	**1.62**
25x	**0.775**
50x	**0.388**

[1] Data is for a full resolution 2592 x 1944 pixel image. If you are using images of lower resolution (e.g., 864 x 600 pixels), then an additional multiplier (e.g., 3, as in the case of the aforementioned 864 x 600 pixel image) – over and above the ones indicated above – will be required to convert from pixels to microns.

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