

# ANALYTICAL CHEMISTRY 256

## ROLE - PLAYING LAB

1998-1999 Academic Year  
©Prof. John P. Walters  
St. Olaf College  
Northfield, MN 55057

Introduction to Role-Playing and Laboratory Computing  
Defining the Kinds of Role-Playing Responsibilities  
Round-Robin Certification of Laboratory Glassware  
Production Quality Control Lead Analysis  
Statistical/Chemical Evaluation of Lead Data  
Semi-Automated Weak Acid Titration  
Graphical Analysis of Weak Acid Titration

### Designing a Mock Robot Experiment

Executing the Mock Robot Experiment  
The Incredible Edible Easter Egg Grass Advertising Dilemma  
The Downsizing Dilemma  
The Broken Pill Coating Machine Assembly Line Shutdown Dilemma  
The Instrument Purchase Payment Release Dilemma  
Closure

#### ROLES:

<b>Manager:</b>	Designing the mock/real robot experiment interface
<b>Chemist:</b>	Designing and Preparing the Indicator Buffer Solutions
<b>Software:</b>	Designing the mock/real robot control language
<b>Hardware:</b>	Designing the mock/real robot command responses

#### OBJECTIVES:

The mock robot experiment is to reveal, in conjunction with operation of a real Zymark Zymate II lab robot, before a purchase is made, what problems the presence of a robot in the analytical lab may cause. These problems will relate to personnel resistance to the machine (**Staff** problems), programming problems, operating problems, and special needs for the solutions to feed the robot. The first part of the experiment is to learn and design a set of procedures that will expose the presence of problems, and give actual data on which to base a robot purchase. The second part is to execute the designed experiment, and make the purchase recommendation. The final objective is to have the Company collectively function **as** a whole mock robot, and in so doing record an isosbestic point for the indicator compound Brom-Cresol-Green. The Zymark Zymate II Laboratory Robot work gives a practical reference.

#### MANAGEMENT INTERVIEW:

The first part of the experiment is a consultation with **Staff**, discussing the plan for executing the mock robot experiment and projections on what information will be obtained. Assurance that the indicator **Chemistry** is understood will be sought.

Special attention should be directed to "contingency plans", should the expected responses not be obtained.

How well the mock robot functions have been mastered by **Manager's Staff** will be a critical discussion point.

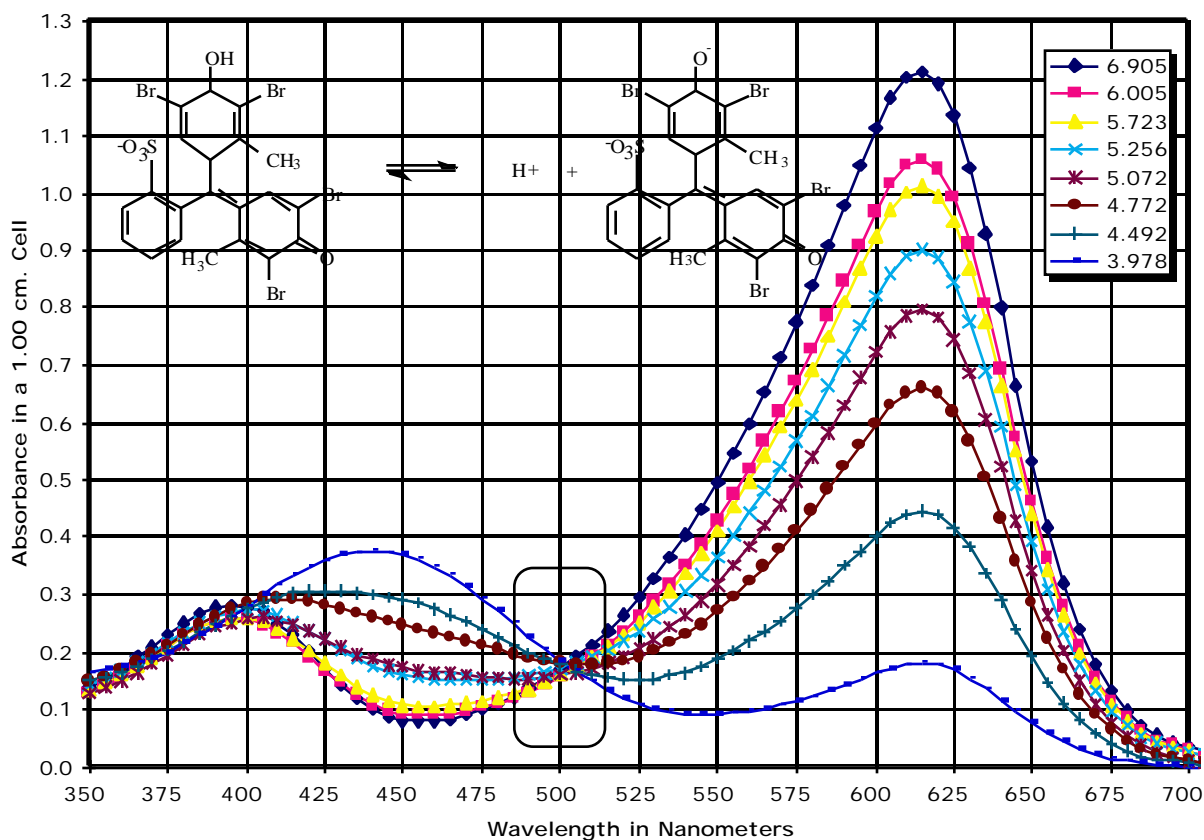
## Manager's Problem

Robots in the laboratory are here as an established fact. The installed base, while still developing, is large enough that **Upper Manager's** everywhere are beginning to look at them as possible cures to some ills in clinical and analytical laboratories. The new "combinatorial chemistry" uses robotics, if not actual robots, and appears to be a market driven force for assuring their increased presence in pharmaceutical research laboratories as well as analytical service organizations. There is a lot of "water cooler wisdom" circulating about them, even to the point of myths about "thinking" robots; yet, there is not enough actual performance based information to allow a critical evaluation of how they will work in a new context without actually purchasing one (upwards of \$90,000).

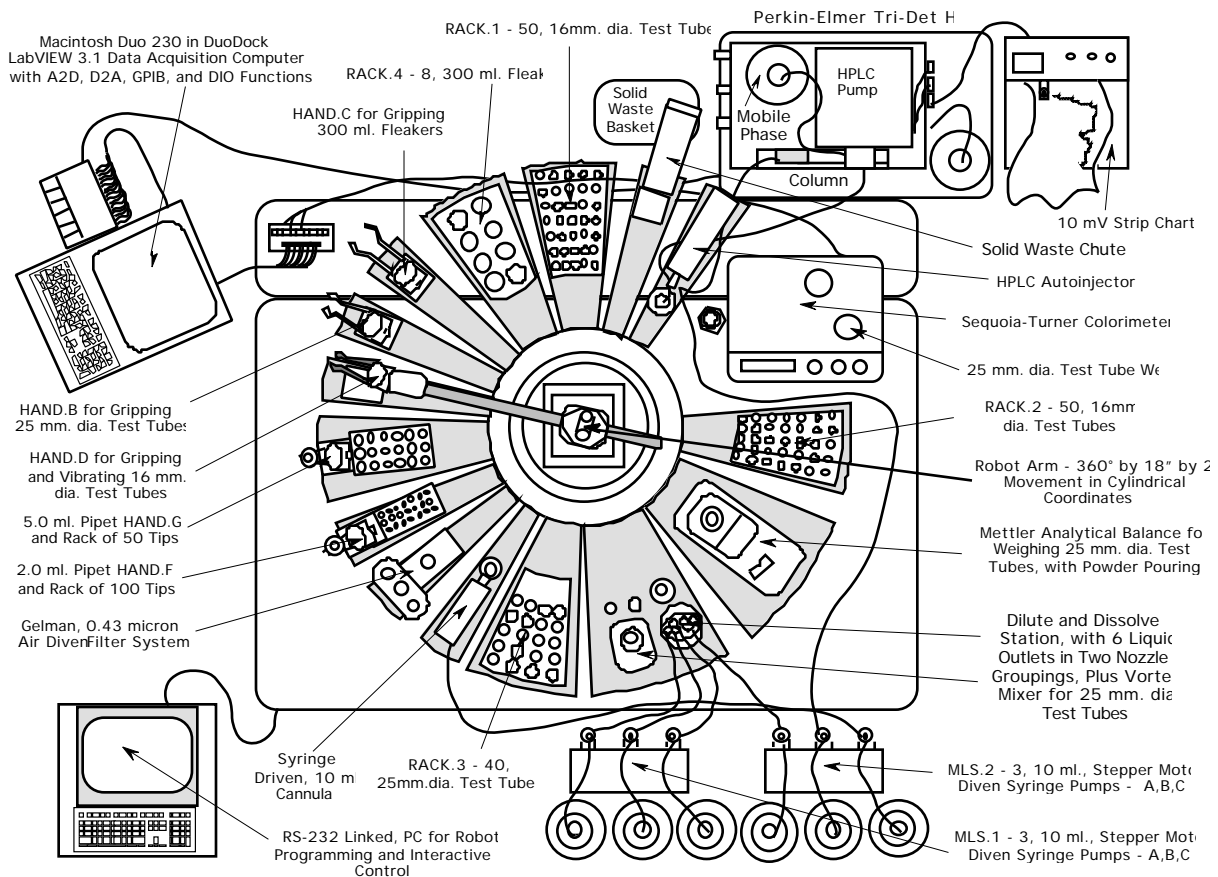
This experiment has to do with a potential purchase of a lab robot. The system in question is a Zymark machine, of the "Py" design. It is intended to fit into a "clinical" analytical lab, where a great deal of spectrophotometric assay of physiological samples is done. While the primary goal of the experiment is to assess the total impact of the robot, it is clear that a chemical task will have to be used as a pilot that will "stress" the whole system. What is needed is one that will require multiple solutions prepared to precise total compositions.

The central analytical task presented here is to record an "perfectly defined" isosbestic point using a parallel detection, charge coupled device ("CCD") spectrophotometer. An example of an isosbestic point, perhaps not so perfectly well defined, produced by adjusting the pH of a brom-cresol green indicator solution is shown in the boxed area on the diagram below.

For many years, "autoanalyzers" have been used in the clinical analytical laboratories. They work, as long as the method they are doing is rigidly defined. They are not especially flexible, by intended design. The laboratory robot, on the other hand, is "teachable". It has a "command language" that can be communicated either by direct electrical connection between the robot and controller, or by speech recognition algorithms. Some even "learn" from past experiences, because they are programmed with "expert systems" to behave as if they can learn.



A robot can execute a variety of mimics of human motor skill tasks, such as pipetting, weighing, and moving powders between stock vials and solutions, stirring, pouring, and transporting things like spectrophotometer cells between racks and instruments. It appears, at least on the surface, that a laboratory robot (such as that shown below) improves over an autoanalyzer by offering more flexibility in being adapted to complex, new tasks.



But, is this true? Is it a prudent decision to assume that a robot is teachable, and if so, that it will be taught? Ponder for the moment that if the robot is teachable, such presupposes that there will be a human somewhere in the lab who will want to teach it, and teach it to do something other than pour reagents on itself. Teaching is a subjective activity, usually done between human and human or human and animal, and done with a measure of subjective affection for the learner. Can it be expected that an employee will be willing not only to tolerate an automation that conceivably could eliminate his/her job, and also do so with enough acceptance to be willing to periodically teach it to do new tasks in response to new needs?

This is a management problem! **Manager** now has the task, given to her/him by **Upper Management**, to determine if the company **Staff** (here modeling a clinical analytical laboratory) can define enough of the problems that will come with a robot to guide in making, or not making, the purchase. This is an example of a real "quality circle" kind of decision - all are involved past the token level, and all are involved **before the fact**.

Purchasing an actual robot to make the evaluation is not financially reasonable in this example. Instead, what **Manager** is to do is, first, to operate and to observe the operation of a Zymark Zymate II laboratory robot doing equivalent tasks, and then to build a robot, using **all of the Staff roles to mimic the various functions** that a real laboratory robot would perform. This is called a "Mock Robot". It behaves as if it were mechanical, but it is composed of highly specialized roles played out by human beings.

Because a mock robot uses people to behave like instruments, its behavior and design can be based on intuition, reading, and the observing of real robots, on film and in the lab. For example, in a

mock robot, **Hardware** can mimic the motor skill part of the robot, **Software** to mimic the controller and data handling part, **Chemist** to mimic the "solution maker" part, and **Manager** to mimic the decision making part.

The mock robot, when given a demanding chemical task, using proven equipment and concepts, and when asked to gather data in a way that will allow chemical decisions to be made as the data come are acquired (rather than later), is expected to reveal the basic flaws in whatever automated system might later be purchased.

In this way, a group of people, here the Company, can use their assigned specialties to "role-play" a machine, and by so doing anticipate before the fact what adjustments in their community would have to be made if such a machine were actually included in it. This, at first, may seem artificial. But, considering that a real robot can cost well in excess of \$90,000, even the mimic can be a good management strategy.

To evaluate the exercise, **Manager** will have to make careful plans to observe all of the effects and happenings when the mock robot runs. The players will have to do strictly only their own roles, and communicate only by **established and pre-agreed** routes.

Communication using only defined words, signals, and language capabilities will be required. And, in many places during the time the experiment is run, only **Manager** may be capable of actual decision making, adaptive thought. Other players may be required to just act without thought and under obedient response to highly explicit commands from **Manager** or perhaps **Software**.

When the mock robot has completed the experiment, all of its "parts" will get together and discuss with **Manager** what the expected problems will (might?) be should a real robot be brought into the lab.

**Manager** will be expected to have some readings to share; much has been written about the assets and liabilities of a lab robot from other peoples perspectives. The **Chemistry** done by **Chemist** and **Hardware** will have been sophisticated enough that some of the most obvious advantages, compromises, and liabilities of a real robot would have appeared.

The mock robot experiment has to be done in two steps, since the first part is to view a video highlighting laboratory robotics, study the Kidd Creek chemistry (see **Appendix A** to this experiment) and observe the evolution of a control language by operating the Zymark Zymate II professional lab robot. From these experiences, it should be possible to design the mock robot. In this design, **Manager** has a critical role. It is **Manager** who determines what is to be done in the first afternoon's work to reach the chemical objective.

**Manager** also has to determine what needs to be practiced while the group can move around and teach each other. **Then, in the second part, the Staff are restrained to those tasks that have been defined in the first part.** Like the real robot, only a few of them may communicate creatively, and only **Manager** will be free to **move around the bench and talk**. If the first week is poorly managed, the second may well fail.

### *The Givens*

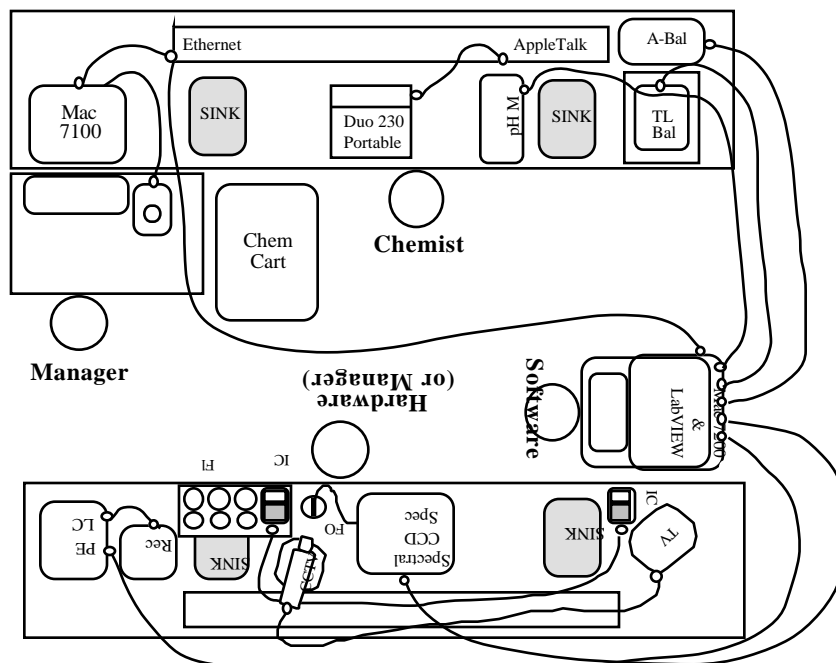
While there are a lot of choices for **Manager** to make in the first part of the experiment, there have to be some "givens" in order to make it manageable on a "two afternoon" schedule. First "givens" concern **the physical space**, then **the measuring instrument**, then **the control/acquire computer**, and lastly **the wet Chemistry**. In order then, here are the givens.

### **The Physical Space**

Some robots can move in place, and some can move between places. The mock robot is confined to the lab bench space. When the mock robot is actually operating as a robot in the second part of the experiment, **the Staff making up its functional parts are restricted to where they can move**, and to whom they can talk! These restrictions are very real, and important if the mock

experiment is to work.

To make this clear, look at the diagram below while reading here. Note that there are four possible positions for the **Staff** people who make up the mock robot. (If a three person Company is operating, **Manager** can substitute for **Hardware** at the spectrophotometer when the mock robot is actually turned on.) The bench has been arranged deliberately so that certain robot functions are performed at these regions.



For example, **Chemist** has the north side of the bench, including the electronic analytical balance (**A-Bal**), the electronic top loading balance (**TL-Bal**), the digital pH meter (**pHM**), the two sinks (**sink**), and the open wet area between the two sinks.

When the mock robot is in operation, **Chemist** is free to move along this region of the bench, **but nowhere else**. Note that this is literally a physical restriction; **Chemist** may not move away from that region of the bench for any reason without "turning off the robot".

If any other tools are needed for **Chemist**

other than what has been pre-arranged to be on that side of the bench before the robot is turned on, then it has to be called a management failure and turned off! This includes going to the stockroom!

Similarly, **Hardware** has the west end of the bench, including the **intercom (IC)** to talk to **Software** on the east end, the Spectrophotometer (**CCD**) with its dipping fiber optic (**FO**) probe, **fleaker (FI) tray**, and closed circuit television camera (**CCTV**). When the mock robot is in operation, **Hardware** is free to move just along the west end of the bench, **but nowhere else**.

The **ONLY WAY** that **Hardware** can talk to **Software** is using agreed-upon speech patterns via the intercom! Once the robot has been turned on, **Hardware** cannot say, for example, "What am I supposed to do?", unless **Manager**, **Software**, and **Hardware** have agreed beforehand that this is a speech pattern that **Hardware** will be able to use!

If **Manager** has not made sure that **Software** and **Hardware** have developed a full control language that **Hardware** will understand (using some of the suggestions to follow here), then a management failure may result when the mock robot has to be turned off because **Hardware** cannot do what **Software** wants!

However, **Software**, who basically has only the intercom (**IC**), computer (**7200**), and closed circuit TV monitor (**TV**) to use, is **firmly anchored** at the east end of the bench in front of the computer, and **cannot move from there**. **Software** thus is free to do anything except move! And, **Software** cannot use any tools other than his/her computer, TV monitor, and intercom to make **Hardware** do anything. **Software** has no physical interaction with either **Chemist** or **Hardware**.

When the mock robot is operating, only **Manager** is free to move around the bench. **Manager** is mostly located at the southwest end of the bench at the executive computer but may, when needed to do something for the other "parts" of the mock robot, get up and move around the bench.

But, not even **Manager** may leave the bench **area** until the mock robot is "off". If supplies are needed that have not been placed on the bench during the first part of the experiment, only a billable consulting request to **Upper Management** can provide them. And, almost certainly, this will be billed to compensate for a management failure. This can be costly!

### Communication Limitations

The **Mock Robot Floor Plan** describes the spatial limitations. More needs to be said about the communication limitations. **Manager** may "talk" (using human speech) to any of the mock robot "parts" (**Staff**), assuming for that a real robot would have "listening" capabilities (electronic speech recognition) from its "owner".

However, when the mock robot is operating **Chemist** may not talk to anyone other than **Manager**. And, more severely, **Software** may talk to **Hardware**, but never face to face, and **only over the electronic intercom**. And, even then, only pre-arranged speech patterns may be used. In other words, **Software** and **Hardware** must be linked by trained speech recognition.

While this may appear artificial and severe, in fact this is why such communication channels as ISDN (*Integrated Services Digital Networks*) are being developed. And, in the professional world, robots are used in place of humans, and can in actual fact only hear and respond to literal phrases that must be programmed into them.

The closed circuit TV and intercom, in combination with the RS-232 link between the control computer and the Spectral Instruments CCD spectrophotometer, constitute a "mock ISDN" link that is quite realistic in a professional sense.

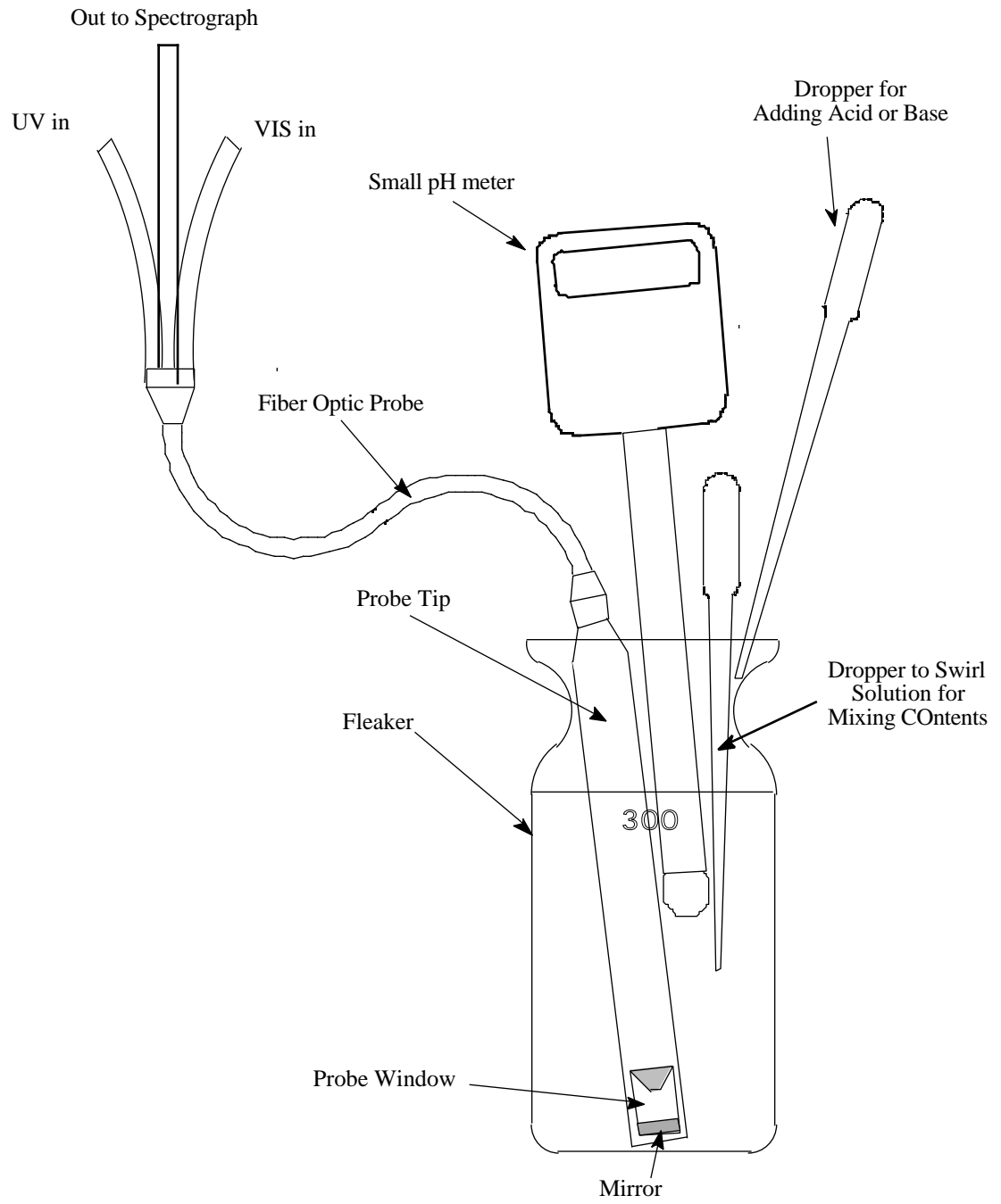
These then are the givens; the physical space conditions that apply when the mock robot is operating during the second part of the experiment. During the first part, **Manager** must make sure that all of the mock robot "parts" (the **Staff**) are prepared well enough to operate under these restrictions, and still meet the chemical objective.

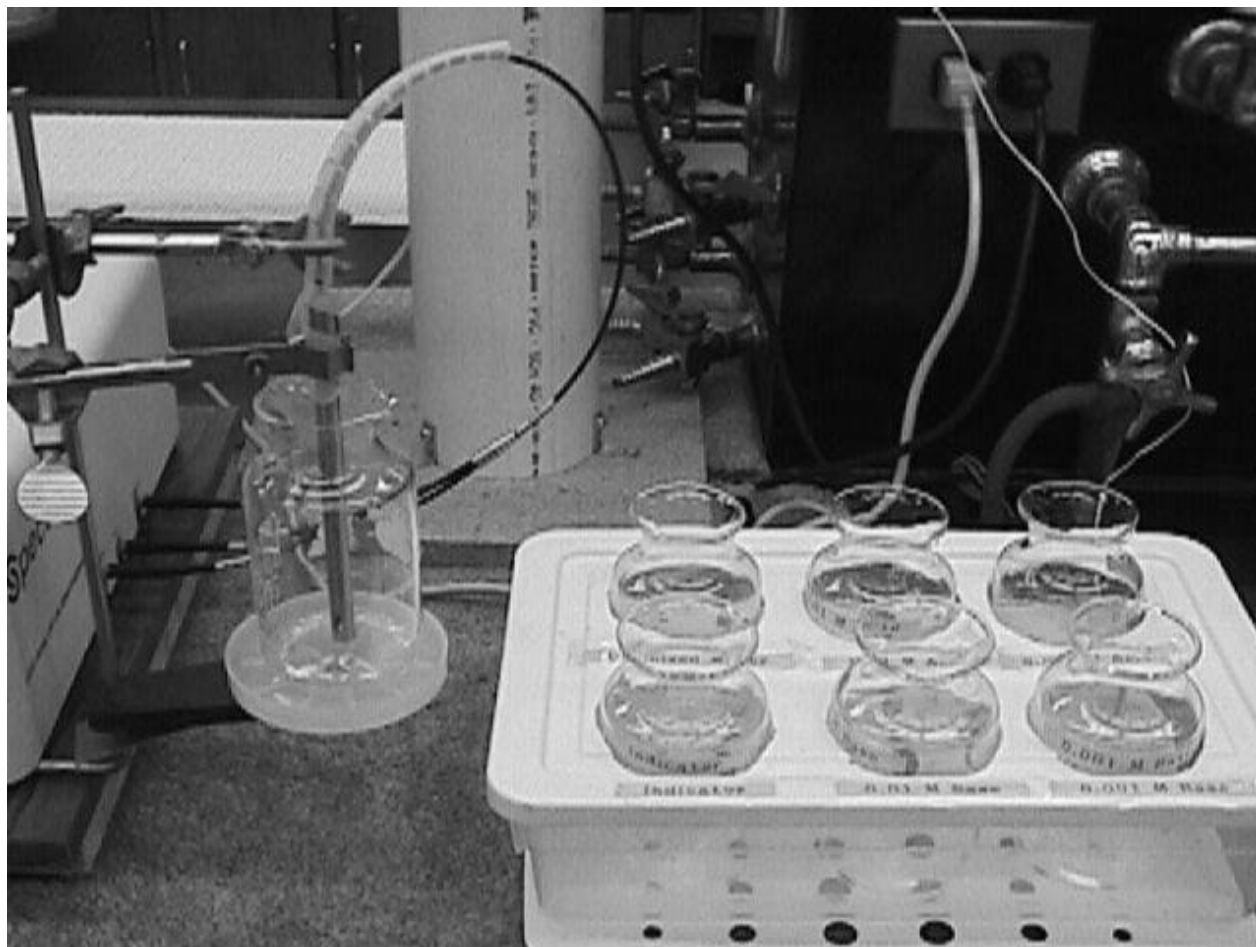
### The Measuring Instrument

There are a host of ways an automatic device (like an autoanalyzer) can make a measurement, but the laboratory robot (as a special case) instead feeds another measuring instrument. Here, the "measuring" instrument is a Spectral Instruments model SI400 Charge Coupled Device ("CCD") parallel spectrophotometer with single channel fiber optic probe. The SI400 is reasonably "smart", but has no motor skills or functions. **Hardware** must feed it.

The SI400 is "smart" in that it understands a set of commands presented to it through an RS-232 serial connection (1200 baud) from a LabVIEW front panel located on a remote Mac computer. The mock robot must operate the spectrophotometer by receiving a "blank" or "Sample" command from **Software** operating a control program on the LabVIEW VI.

The SI400, unlike other spectrophotometers, does not have a manually operated cell compartment. Instead, it has a single channel, fiber optic probe that is dipped into the solution whose spectrum is to be measured. The mock robot will thus have to have a set of "fiber optic handling" commands that it can operate in response to verbal commands from **Software**. These are considered a basic part of the robot package and are needed to load the cells into the spectrophotometer. A pictorial layout of the **cell parts tray** in front of the spectrophotometer is also shown on the following page, and the range of operations the mock robot may do for cell manipulation indicated.



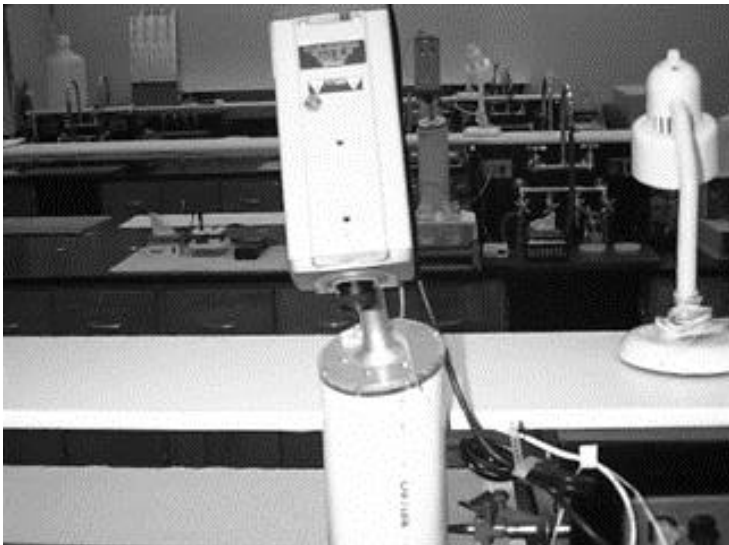


The one or the other of the two fleakers closest to the spectrophotometer fiber optic probe is inserted under the probe. When you are measuring a blank spectrum, use the fleaker marked “Deionized Water”. When you are ready to begin measuring the isosbestic point, use the fleaker marked “Indicator”. Do not move the fiber optic probe as changes to the radius of curvature can result in damage to the fiber optics. Carefully remove or insert fleakers under the probe. **Upper Management** or **Staff** will tutor on this.

You will not need to insert the probe into either of the other four fleakers marked “0.01 M Acid, 0.001 M Acid” or “0.01 M Base, 0.001 M Base”. These just hold the disposable droppers that are used to force the indicator through its transition point, while the spectrum is being measured. Stirring or mixing can be performed with the assistance of a disposable dropper.



To the right of the spectrophotometer will be the intercom that connects to **Software** (at the other end of the bench), a basket of paper towels to wipe the fiber optic probe, a box of tissues to soak the liquid out of the inside of the probe, and one or two other fleakers and droppers. **Manager** also may want to install some additional equipment here.



The tray of fleakers is televised using a CCTV camera positioned above it. The video to receive the image is located at the other end of the bench near **Software**. The idea is that **Software** will have no visual contact with what happens at the spectrophotometer, instead being able only to see the few fleakers into which **Hardware** dispenses acid or base, and the spectral results therefrom. This is in keeping with the premise that **Hardware** cannot think, and **Software** cannot act, once the mock robot is on.



**Hardware** will have to measure the pH of the indicator solution, and verbally report it to **Software**. For this, there is a dipping pH meter, electrode, and a set of tissues available next to the spectrophotometer. The pH electrode is placed in the fleaker alongside stainless steel the fiber optic probe tip.

*Remember that you must **never** add HCl to a solution containing stainless steel - it will dissolve it. Use only  $H_2SO_4$  for adjusting the pH of the indicator solution. If you use HCl with stainless steel, then the stainless will be corroded and the end of the fiber optic ruined.*

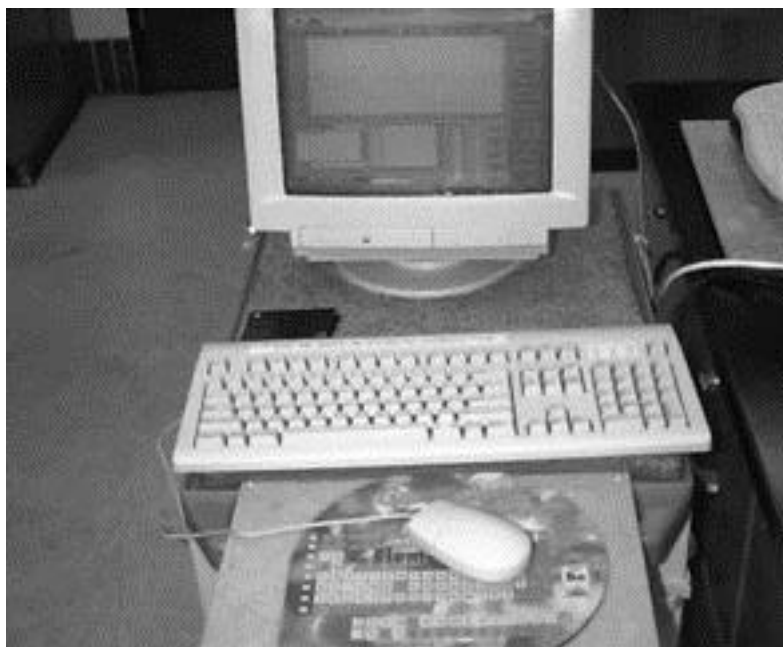


Directions from **Software** to **Hardware** can only be verified by **Software's** observation of the results. The verification can be visual, but only by looking at an image on the TV monitor nearby. Verbal commands are given through the intercom. **Hardware** can speak into the remote intercom without having to press the button on it. **Software** must first depress the button, and then speak.

Whether a command has been effective and produced the desired result can only be determined when

**Software** obtains the spectrum of the indicator solution. This takes about 20 seconds. The complete spectrum, between 200 and 900 nanometers may be displayed on the control computer, or whatever portion **Software** desires.

**Software** may use the command language developed in the first period to tell **Hardware** to add acid, or base, mix the solution using a disposable dropper, and then observe the results.



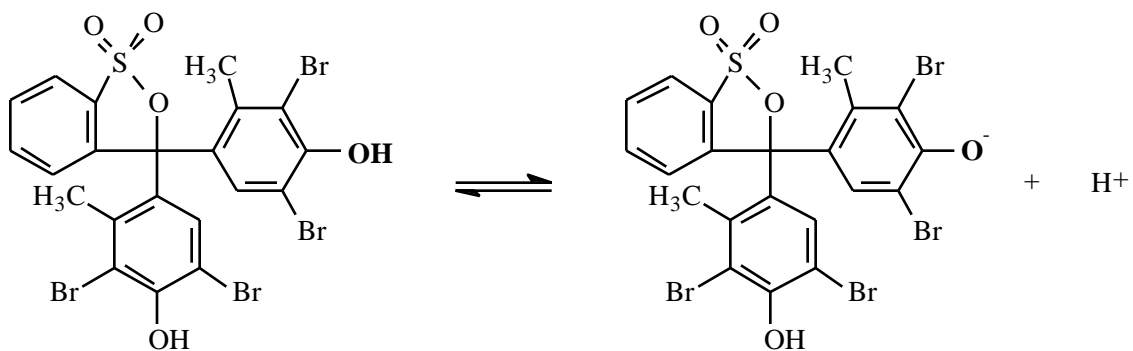
Some good results for bromcresol purple and bromcresol green are shown on the last few pages of the next lab manual section. The isosbestic points that you can achieve with this method can be almost perfect. The probe does not move between additions of acid or base. As the transition range for the indicator is traversed, you can add the more dilute acid or base so you can adjust the pH very slowly. The volumes are large enough that once the ionic strength is fixed it does not change much at all.

Bromcresol purple has a larger transition range than bromcresol green, so it is experimentally easier with it to obtain regularly separated spectra.

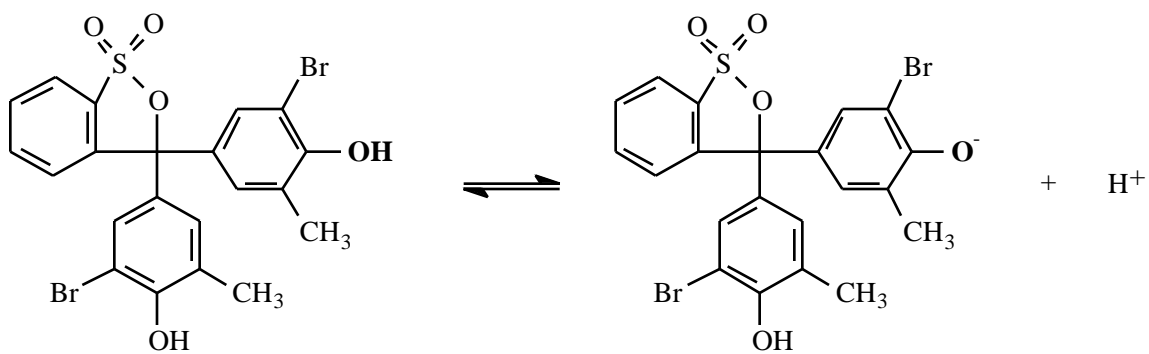
### Wet Chemistry

Prior to the testing of the mock robot **Chemist** will have to supply **Hardware** with an indicator solution of fixed ionic strength using potassium nitrate ( $\text{KNO}_3$ ). As you explored in the Weak Acid experiment, ionic strength does have an effect on many areas of chemistry, in particular, acid-base chemistry. Given the transition interval and concentration of the indicator **Manager** and **Chemist** must decide on an appropriate concentration of potassium nitrate to use that will fix the ionic strength during the experiment.

Bromcresol Green Indicator - Transition Range = 3.8 - 5.4 (pKa = 4.7)



Bromcresol Purple Indicator - Transition Range = 5.2 - 6.8 (pKa = 6.3)



**Appendix A**  
**1987 International Symposium on Laboratory Robotics Videotape Summary**

Chemistry 255/256  
 Prof. John P. Walters  
 St. Olaf College

**The Kidd Creek, Iron Ore, Robotic Method Chemistry**

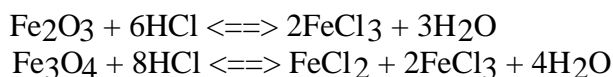
Duration: 14 minutes

Start: 00:27:25

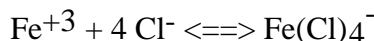
End: 00:40:50

I am really pleased to present even part of the chemistry that was being shown on the Kidd Creek video. As you probably suspected, it is not simple chemistry. In fact, not even iron ore is simple, in the sense that we have single component samples in our lab. Iron ore is a natural sample, with a complex blend of minerals and other components. The chemistry you have been observing in the video involves that complexity in two steps. The first is "extraction" of the iron as the ferrous and ferric species from the ore, followed by oxidation of any remaining metals and/or the dissolved species.

The principal iron ores are hematite ( $\text{Fe}_2\text{O}_3$ ), limonite (hydrated  $\text{Fe}_2\text{O}_3 \cdot x\text{H}_2\text{O}$ ), siderite ( $\text{FeCO}_3$ ), and magnetite ( $\text{Fe}_3\text{O}_4$ ). Iron is efficiently extracted from these ores with hot, concentrated HCl (but not with concentrated  $\text{HNO}_3$  or  $\text{H}_2\text{SO}_4$ .) The efficiency of the HCl extraction is due to the formation of soluble ferric chlorides.



There are other reactions that go on to solubilize the iron species, one example of which is the formation of chloro complexes of ferric iron with the excess  $\text{Cl}^-$ :



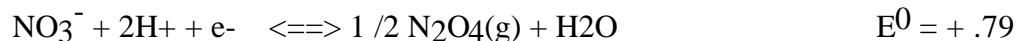
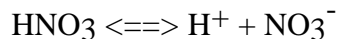
Metal	$E^0$	Metal	$E^0$
Au	+1.4	<i>Co</i>	-0.277
Pt	+1.2	<i>Cd</i>	-0.402
Ag	+0.799	<i>Fe</i>	-0.440
Hg	+0.798	<i>Cr</i>	-0.710
Cu	+0.345	<i>Zn</i>	-0.762
Bi	+0.320	<i>Mn</i>	-1.05
Sb	+0.212	<i>Al</i>	-1.67
H	0.0000	<i>Mg</i>	-2.34
<i>Pb</i>	-0.126	<i>Na</i>	-2.712
<i>Sn</i>	-0.136	<i>Ca</i>	-2.87
<i>Ni</i>	-0.250	<i>K</i>	-2.922

The ore has to be very finely ground (recall the video showed a brown powder being weighed out) otherwise it is attacked very slowly by the hydrochloric acid.

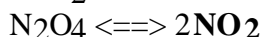
If, then, the HCl is all that is needed to dissolve the iron ore sample, why was the  $\text{HNO}_3$  added, and what was the brown gas that came off from the mixture? When a mixed acid like HCl and  $\text{HNO}_3$  is used, and it is blended in a 3:1 proportion, it is called "aqua regia". Metals below hydrogen in the

electromotive series can only be dissolved by an oxidizing acid, like  $\text{HNO}_3$  or a mix of  $\text{HNO}_3$  and HCl. Consider these metals. They are shown in italics in the following table.

When nitric acid is added to the solution, depending on its temperature and concentration, it shows a standard potential of about a volt relative to hydrogen. This means that it will oxidize all of the metals from silver down to their ions, and produce a mixture of products.



The  $\text{HNO}_2$  and the  $\text{N}_2\text{O}_4$  have equilibria that lead to  $\text{NO}_2$  :



and  $\text{NO}_2$  is the brown gas we saw coming off in the video. While I cannot say what was being oxidized in the iron ore samples that were being dissolved, it probably was metallic, and the  $\text{HNO}_3$  may even have been doing something like oxidizing  $\text{Fe}^{+2}$  to  $\text{Fe}^{+3}$ . The dissolving of the oxides however would be only due to the  $\text{HCl}$ .

The “aqua regia” mixture of  $\text{HCl}$  and  $\text{HNO}_3$  is interesting in its own right, in that the  $\text{HNO}_3$  can actually oxidize the  $\text{Cl}^-$  from dissociation of the  $\text{HCl}$  (it depends on the activities of these species in these very concentrated solutions)!



Even though this standard potential is higher than those for  $\text{NO}_3^-$  reduction, concentrations can be adjusted so that when  $\text{HCl}$  and  $\text{HNO}_3$  are first mixed together,  $\text{NO}_2$  and  $\text{Cl}_2$  come off as a noxious mixed gas. It is interesting!

### HPLC Sample Preparation of Bio-Synthetic Insulin, Eli Lilly Robotic Method Chemistry

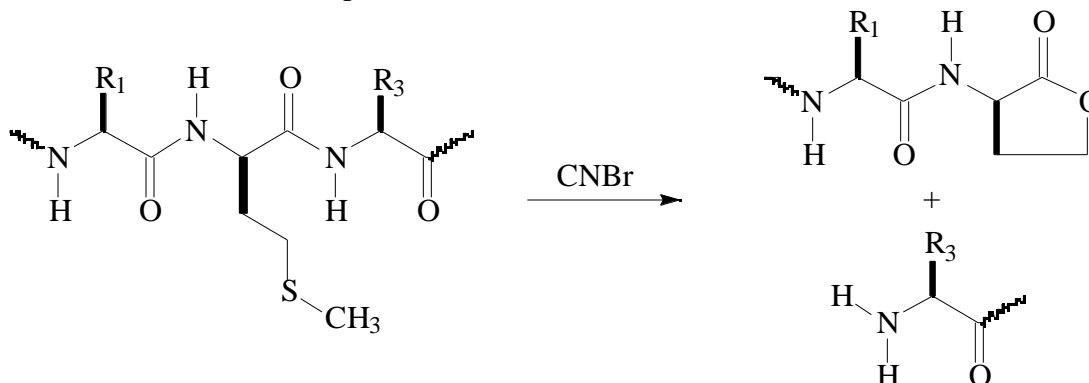
Duration: 11 minutes

Start: 00:00:30

End: 00:11:00

#### Cyanogen bromide (CNBr)

- cleaves peptides on carboxyl side of methionine residues
- immobilization reaction with proteins



**Notes and Questions to consider while viewing:**

Robot provides routine sample preparation for High Performance Liquid Chromatography

Six different reagents are added to the fermentation broth sample prior to analysis

How does robot ensure all reagents make it into the proper containers without spillage?

What are the advantages of a robot's modular construction?

What role does the technician play? Is it important to the overall process?

What is the limiting step in the entire analysis method?

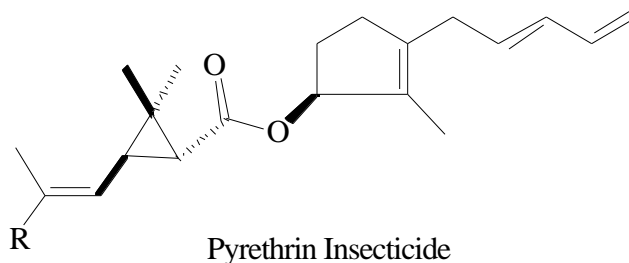
**Pesticide Residue Analysis at FMC Corporation**

Duration: 8 minutes

Start: 00:41:30

End: 00:49:40

Robot performs routine sample preparation (solid phase extraction) of insecticide residues for subsequent analysis using High Performance Liquid Chromatography.

**Notes and Questions to consider while viewing:**

What does the "prologue" program do to the robot at the beginning of a run cycle?

Does the series of solvents used in the solid phase extraction process "make sense" given the above general structure for pyrethroid type insecticides?

What is the purpose for vortexing the sample tube with the rinsing solutions?

Why does the robot have to "regrip" in order to pour substances out of containers?

Why does the method call for rinsing the sample container multiple times?