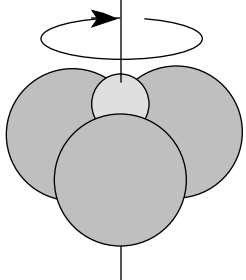


Fall, 2001 Chemistry 382/378 Role-Playing Lab in Instrumental Analysis Experiment #10 Aspirin Hydrolysis		©Prof. John P. Walters Dep't. of Chemistry St. Olaf College Northfield, MN 55057 507-646-3429 walters@stolaf.edu
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Introduction

This experiment involves the entire class (all three Companies) working interdependently to get Mike the Robot set up and running to gather data that will allow the pseudo first-order rate constant of the hydrolysis of aspirin to be determined. The experiment requires excellent lab and communication skills. It will take two full weeks to complete, including the time that Mike prepares the sample and loads it into the Spectral Instruments SI-420, CCD spectrometer to gather data 24 hours a day for 7 days.

Aspirin is a very old therapeutic substance (see Appendix C and D for a discussion of this.). Records of its use for alleviating the symptoms of rheumatism go back to Pliny the Elder and beyond. But, aspirin reacts with water and loses its effectiveness, as follows.

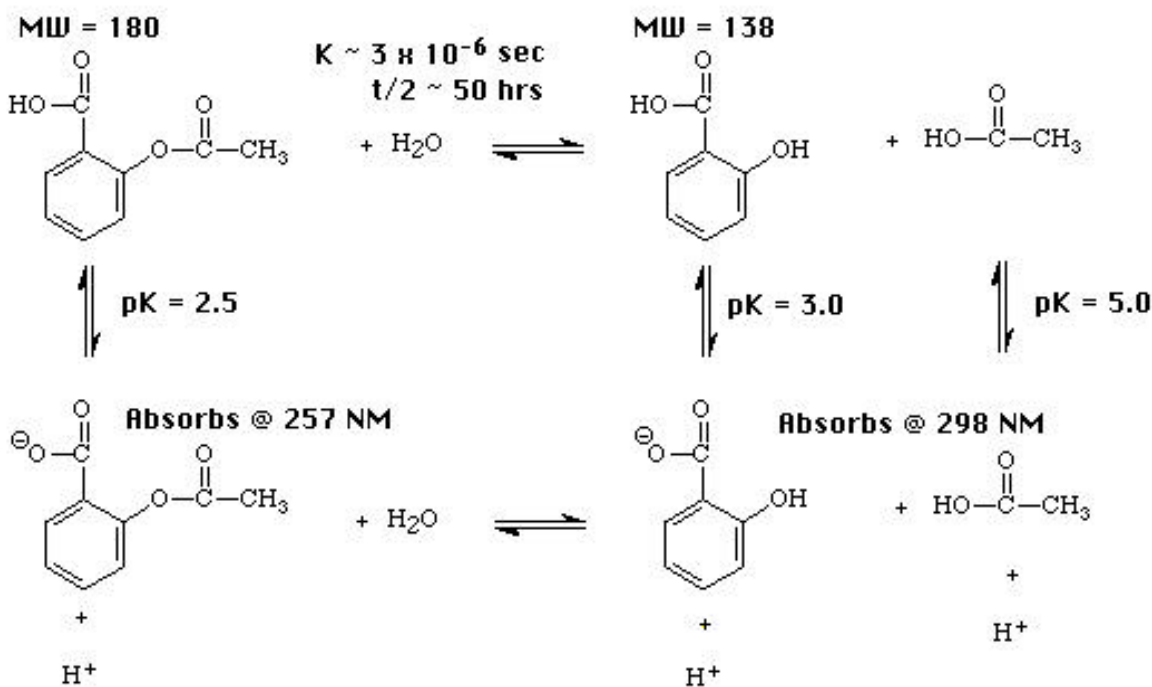


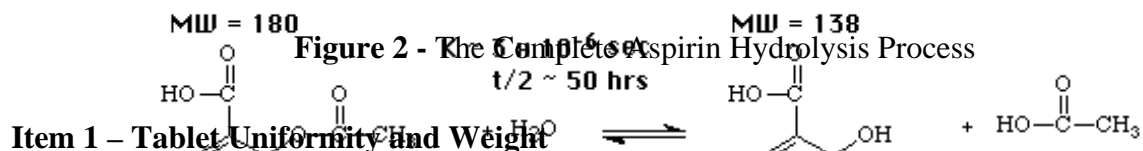
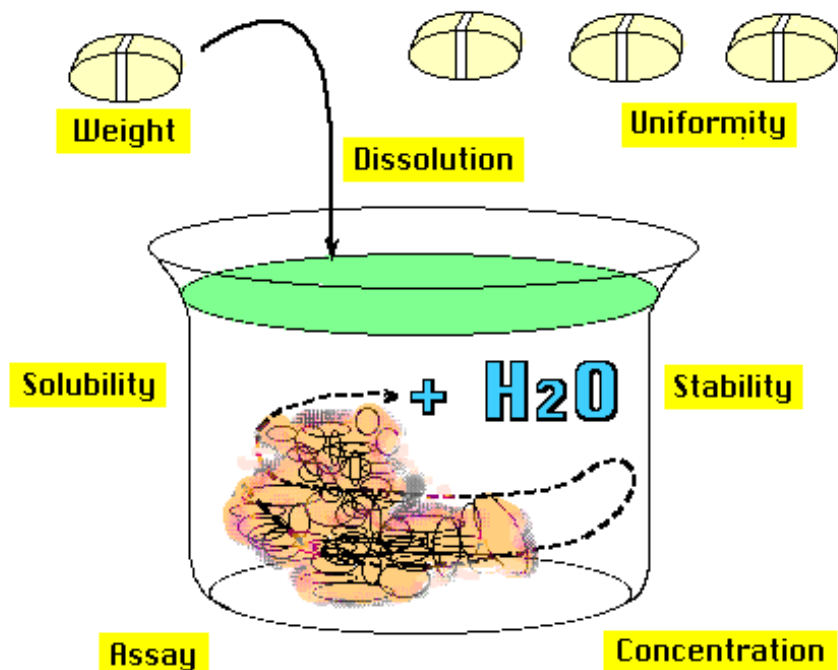
Figure 1 - The hydrolysis processes for aspirin (simplified)

As is evident, the hydrolysis of acetylsalicylic acid (aspirin) to salicylic acid is slow. It has a half-life of around 50 hours when the pH is between 5 and 9. This is fortunate. It does, however, make it a challenge to determine just what the rate is, since measurements will have to be taken over several days in order to make a rate plot. That is what we are interested in doing here.

The Basic Procedure

Determining the rate of aspirin hydrolysis involves considerably more than just monitoring the change in concentrations of hydrolysis reaction reactants or products with time. Figure 2 shows what has to be considered.

Rate Constant for the Hydrolysis of Aspirin in Buffered Solutions.



Not all aspirin are equivalent. They do not contain exactly the same amount of active ingredient. They do not all weigh the same. The first task in this experiment is to determine how uniform they are in terms of pill weight, and from that number, expressed as a percentage, search the literature to find out what the USP tolerance on their purity is.

To account for small changes in purity and weight, we will do this experiment by first preparing a saturated aspirin solution, and then filtering out the excess, undissolved aspirin and starch binder. This solution should contain a known concentration of aspirin, if the solubility of aspirin is known. Experimentally determining the solubility would be difficult. There are literature values that can be used to arrive at an estimate of the concentration of acetylsalicylic acid in solution.

Labels above the structures: Absorbs @ 257 NM, Absorbs @ 298 NM.

Item 2 – Aspirin Solubility

Note that our whole experiment will depend on the concentration of aspirin in solution, since that will determine the concentration of salicylic acid, and that will determine how much the solution absorbs. Some appropriate literature values to determine this are shown on the following page.

Issopoulos, PB “Micelle-assisted dissolution for the analysis of aspirin by second-order derivative potentiometry”, FRESNIUS JOURNAL OF ANALYTICAL CHEMISTRY, **358**, 0633 (1997).

A new potentiometric and/or visual method for the titrimetric determination of aspirin(ASPR), either in pure form or in enteric-coated tablets is proposed. The necessary enhancement of the low solubility of ASPR (3.3 mg/mL, water) is achieved using an aqueous cationic micelle medium of 0.9 mmol/L hexadecylpyridinium chloride (CPCL). The titration is performed with 0.10 mol/L NaOH aqueous solution and the exact end-point is determined from the second-order derivative graph. The pH at the equivalence point is calculated to be 7.64, while the pK alpha is measured as 3.56 (25 degrees C). The accuracy and precision of the proposed method are found to be satisfactory.

aspirin in water at 25 °C : 0.33 g/100 mL
 solubility of aspirin in water is 3.3 mg/ml at pH=3.6
 solubility of aspirin in water = 0.33 g/100 mL
 solubility of salicylic acid in water = 0.22 g/100 mL

These data all were abstracted from various citations on the web. It is likely that they all trace to the number given in the Merck Index, especially since they are generally all the same number. We have seen that they are too low. This is likely due to the pH. We find that the solubility increases as the pH increases. Since we have typically buffered between 5 and 7, we usually find our solubility low by about a factor of 1.5 to 2. It is interesting that the solubility of salicylic acid, the substance we actually monitor, is lower than acetylsalicylic acid. This would imply that we cannot work in a saturated solution for long, since hydrolysis to any significant degree would cause precipitation. Fortunately, the hydrolysis is slow enough that we can filter and dilute the acetylsalicylic acid before enough salicylic acid forms to precipitate. Over a longer time (2 weeks or so) it will.



If pH then $[\text{H}^+]$

If pH then solubility

We will need to select both dilution factors and analytical wavelengths where the molar absorptivity of salicylic acid is not so large that the solution will become “black” when about five half-lives of hydrolysis have occurred. This is a particularly important experimental design consideration. Reference spectra for some buffer solutions (see next section) and for both aspirin and salicylic acid are shown in Figure 1 on the next page.

Item 3 – Choosing an Analytical Wavelength from Reference Spectrum

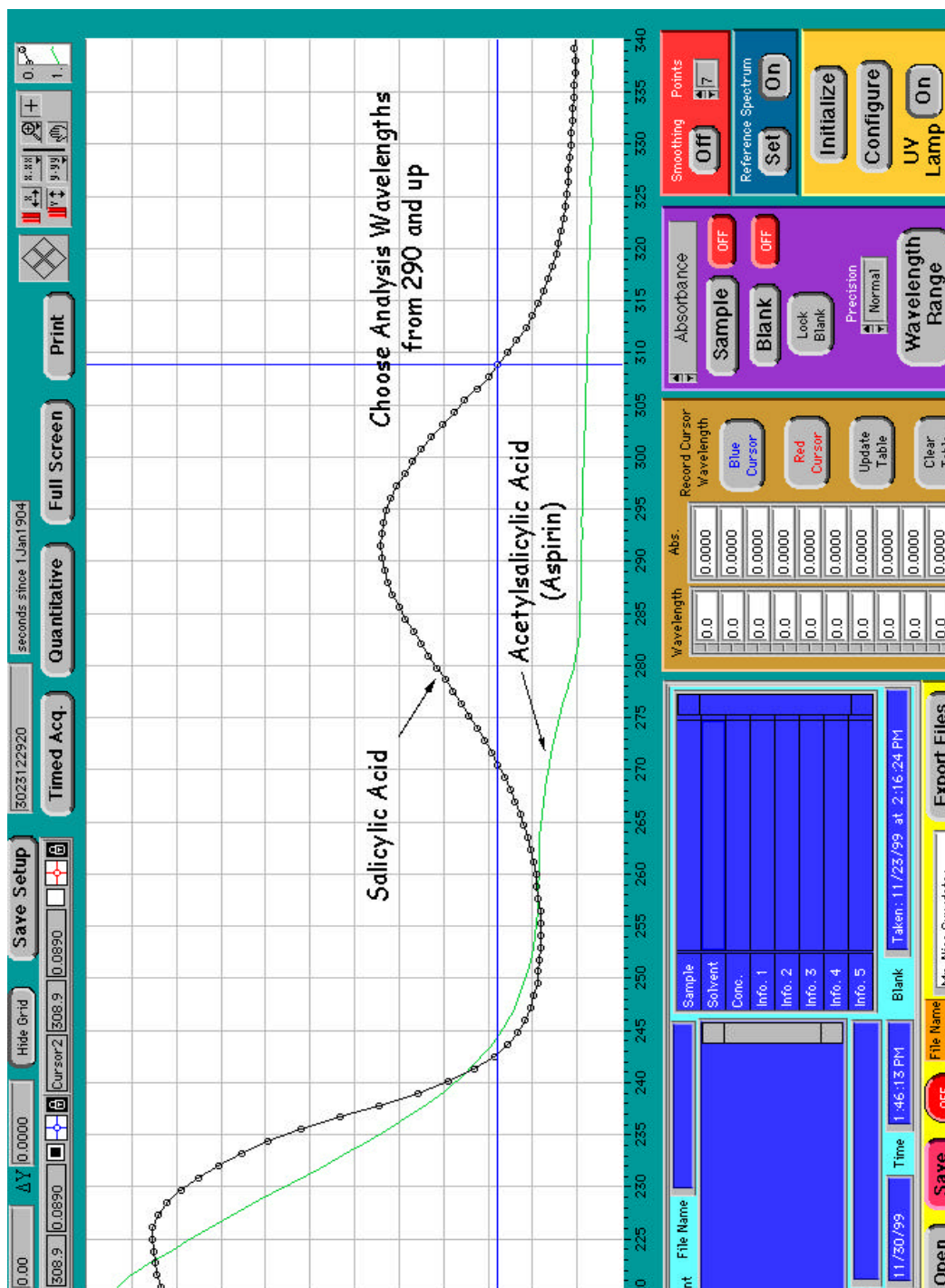
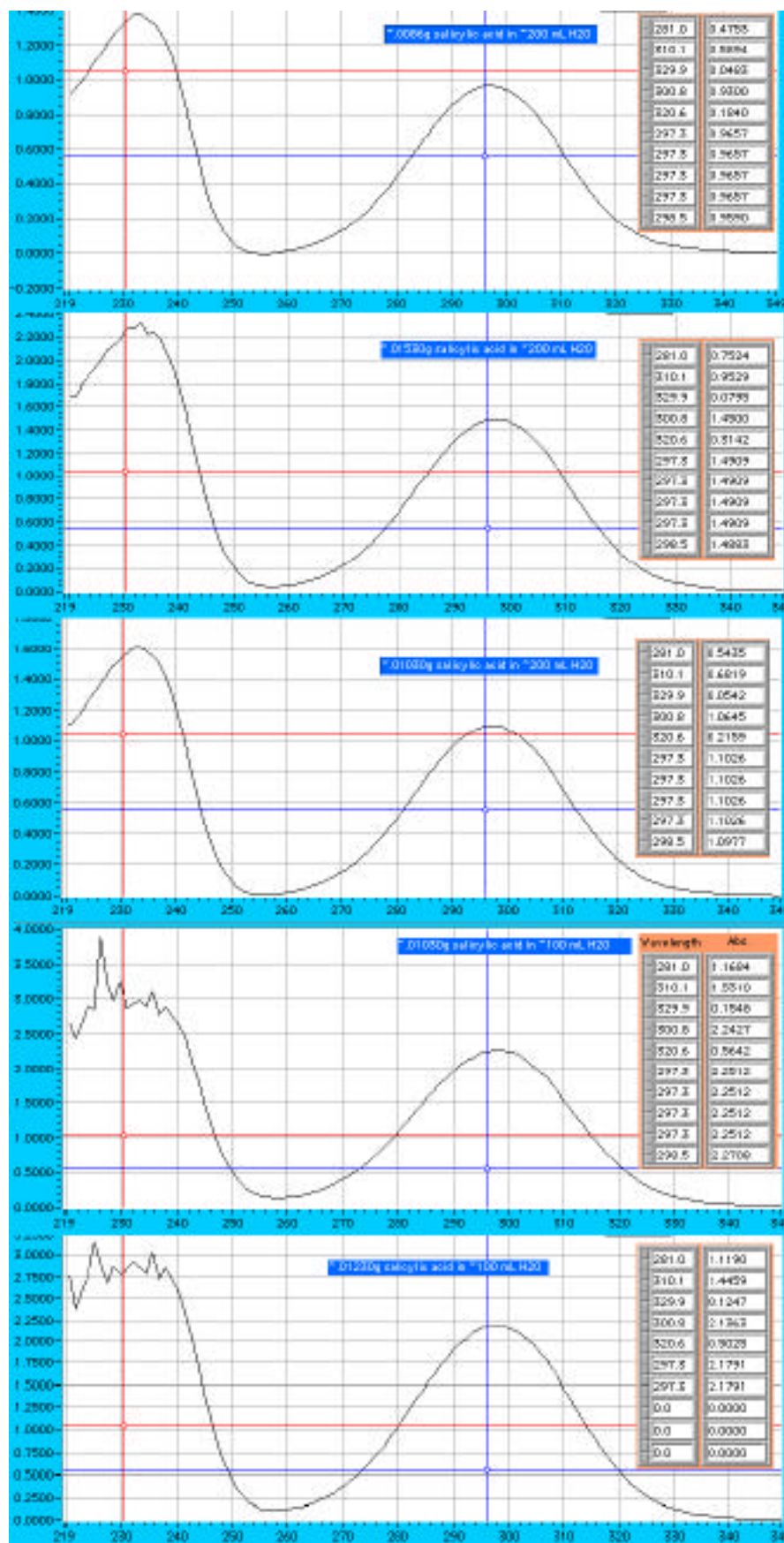


Figure 3 - The Range of Analytical Wavelengths for SA in the Presence of ASA.

When analytical wavelengths are chosen, they must not be interfered with by the absorption of the buffer solution, or other possible solution components. For simplicity, there should be little or no overlap of the aspirin and salicylic acid spectra at the analytical wavelengths chosen. They must also account for the molar absorptivity of Salicylic acid, and its concentration, so that the solution doesn't become too dark to measure reliably over time. These are all considered on the following pages. The examples are only illustrative – you will have to choose your own's.

Item 4 – Determining the Molar Absorptivity of Salicylic Acid:



Knowing the solubility of aspirin in solution is only the first part of knowing what the maximum absorbance will be when hydrolysis is complete. Two additional pieces of information are needed. The first is the cell path length. Here that is 1 cm. The second is the molar absorptivity of salicylic acid at any wavelength, accompanied by a pure salicylic acid spectrum that shows how absorbance changes with wavelength.

To determine the molar absorptivity requires that a working curve be made and its slope determined. To do this, the students of the 1999 class prepared a set of standards of salicylic acid. Measured weights of high purity salicylic acid were dissolved in known volumes of pH 6 buffer solution. Full spectra of each solution were taken, and are shown here as Figure 3. One wavelength (298.5 nm) was selected, and the working curve shown in Figure 4 on the next page prepared.

Figure 4 - Salicylic acid spectra taken to prepare working curve in Figure 4.

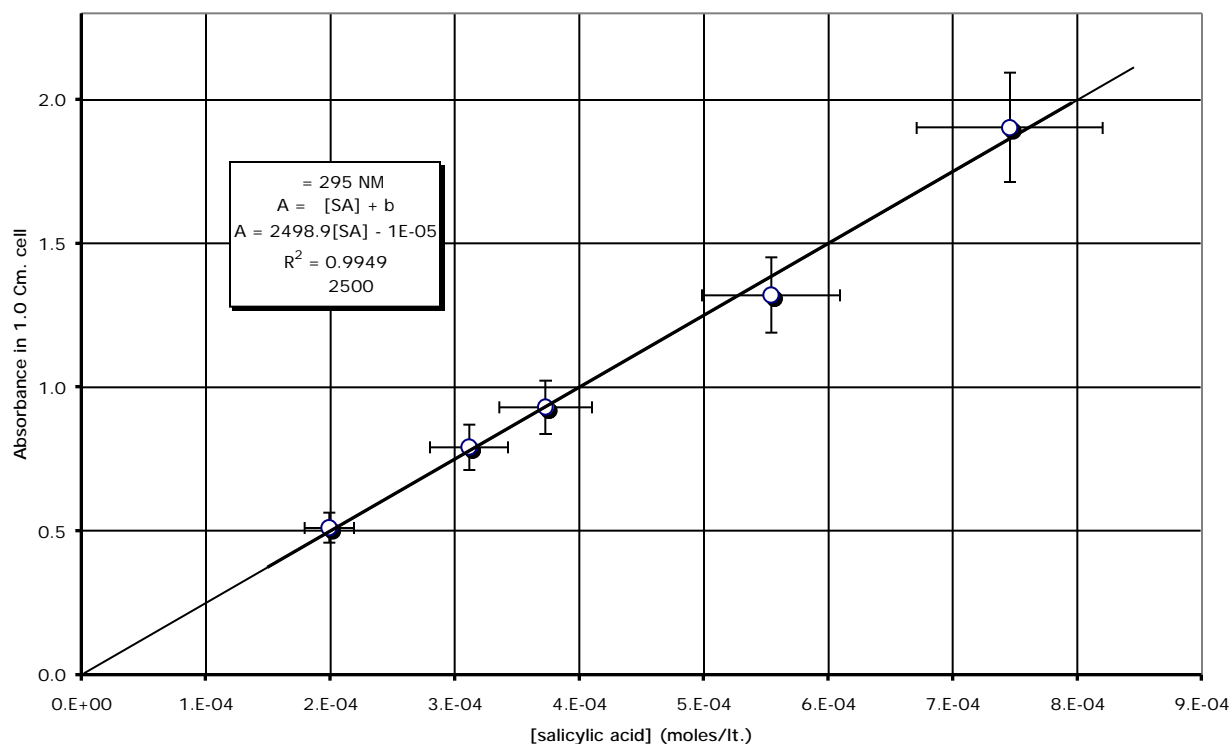


Figure 5 - Working curve for salicylic acid prepared by the class of 1999.

Unfortunately, these data do not agree with those of the class of 2000, as shown in the table below.

[salicylic acid] (M)		*cell path length is 1.0 cm*		
1.215E-02				
Dilution Factor	Dilution Concentration (M)	λ (nm)	Absorbance	Molar Absorptivity (cm ⁻¹ M ⁻¹)
1.5 mL:18.5 mL	9.11E-04	295.5	2.9688	3258.53
0.5 mL:19.5 mL	3.04E-04	295.5	0.9947	3275.32
	Average Molar Absorptivity			
	3266.92			

There is no way to reconcile these data. Either set of data could have a determinate error. The pH of the buffer solutions could have been different. It is unlikely that an actual measurement error occurred. Experience seems to indicate that the class of 1999 is too low, while that of 2000 is too high. The class of 2001 will either have to determine new data or choose analytical wavelengths over a range that would bracket the two molar absorptivities shown above. It is a management decision.

Item 5 – What pH to Make the Hydrolysis Measurements at

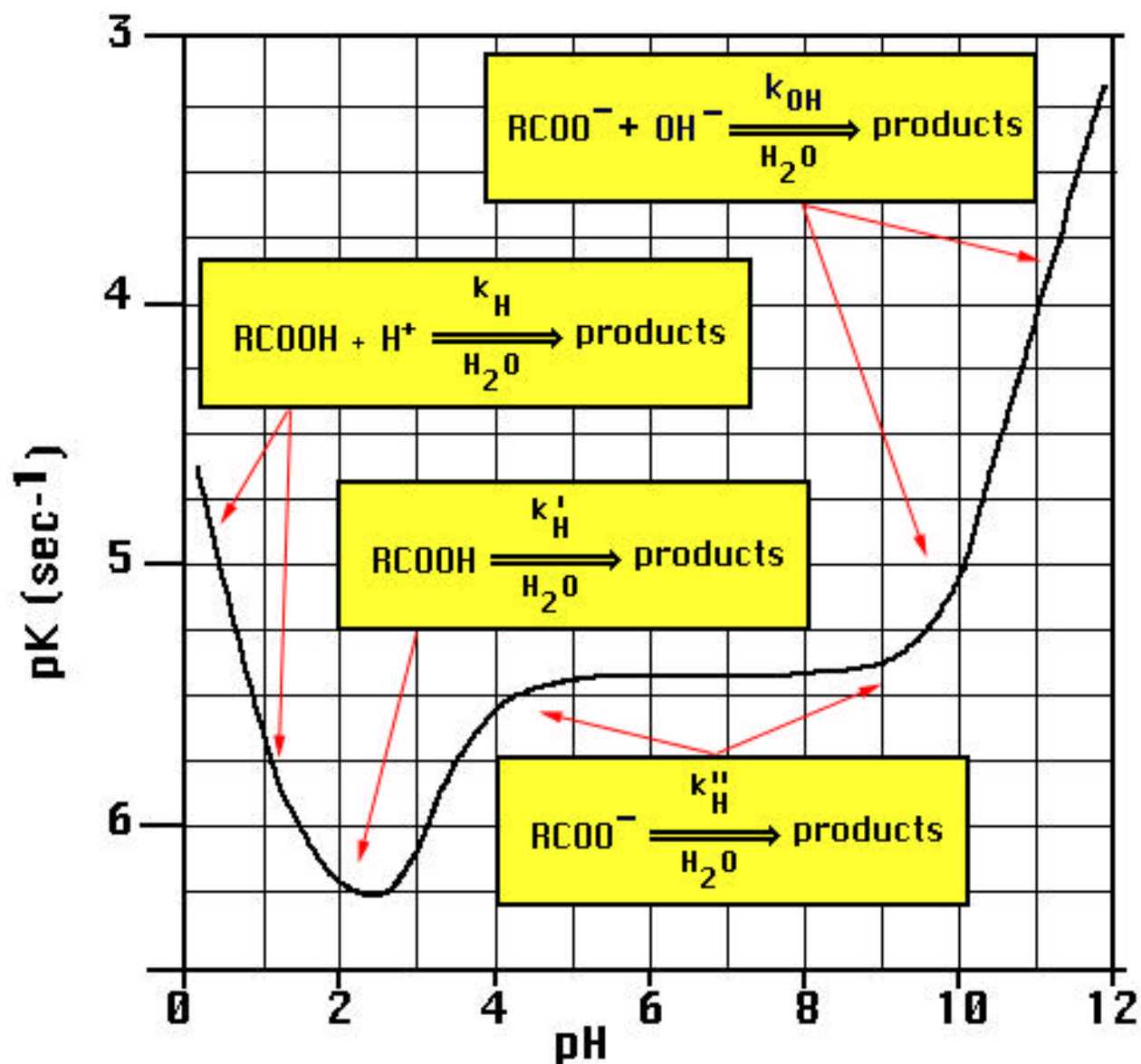


Figure 6 - The Dependence of the Pseudo First-Order Hydrolysis Rate Constant on pH

Aspirin hydrolysis is not as simple a mechanism on the molecular level as it first seems. There are four known mechanisms, each having its own rate constant. The mechanisms are controlled by the solution pH. If the pH is low, a rapid, acid-catalyzed hydrolysis occurs, as shown in Figure 2 above. If the pH is near the pK values of acetylsalicylic acid or salicylic acid, then the rate of hydrolysis slows significantly. When the pH is high enough that most of the acetylsalicylic acid and salicylic acid are predominantly ampholytic mixtures, having the hydrolysis occur from the acetylsalicylic acid anion stabilizes the rate. It is easy to see why so many classes have selected a pH near 6 in the past. However, the solution can be made highly acidic if shorter measurement times are desired. It should not be made too basic, as the buffer may etch the absorption flow cell!

Item 6 –Selecting a Suitable Chemical Buffer

Simply choosing a pH based on rate mechanism is not adequate. The buffer may itself absorb significantly in the UV spectral region where the salicylic acid concentration is to be measured. Consider the spectra shown below. Clearly, the KHP and commercial phosphate buffer are unsatisfactory choices for certain analytical wavelengths you might have selected because of their spectral overlap with the salicylic acid maximum absorbance regions..

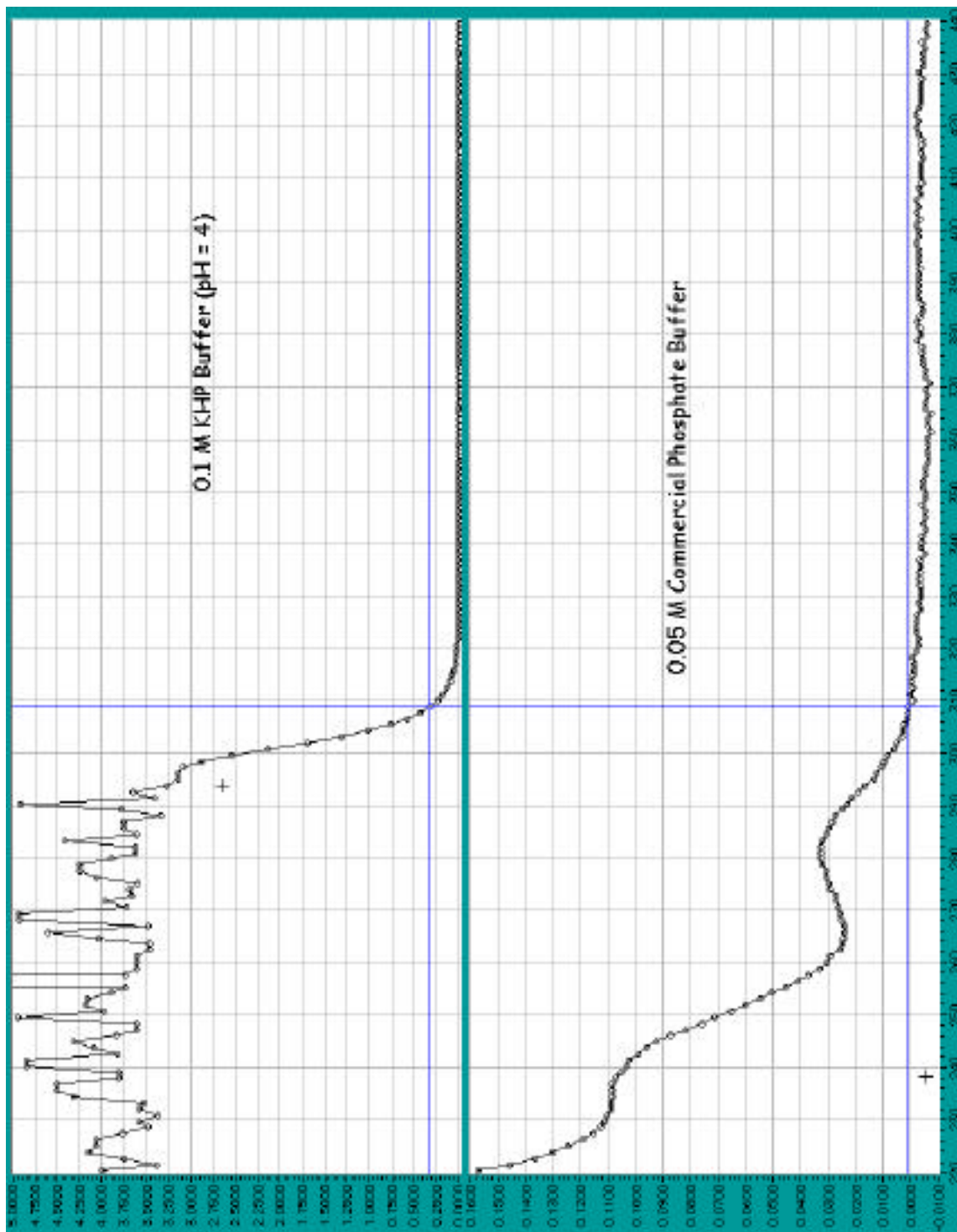


Figure 7 - Two unsuitable (for some analytical wavelengths) buffers

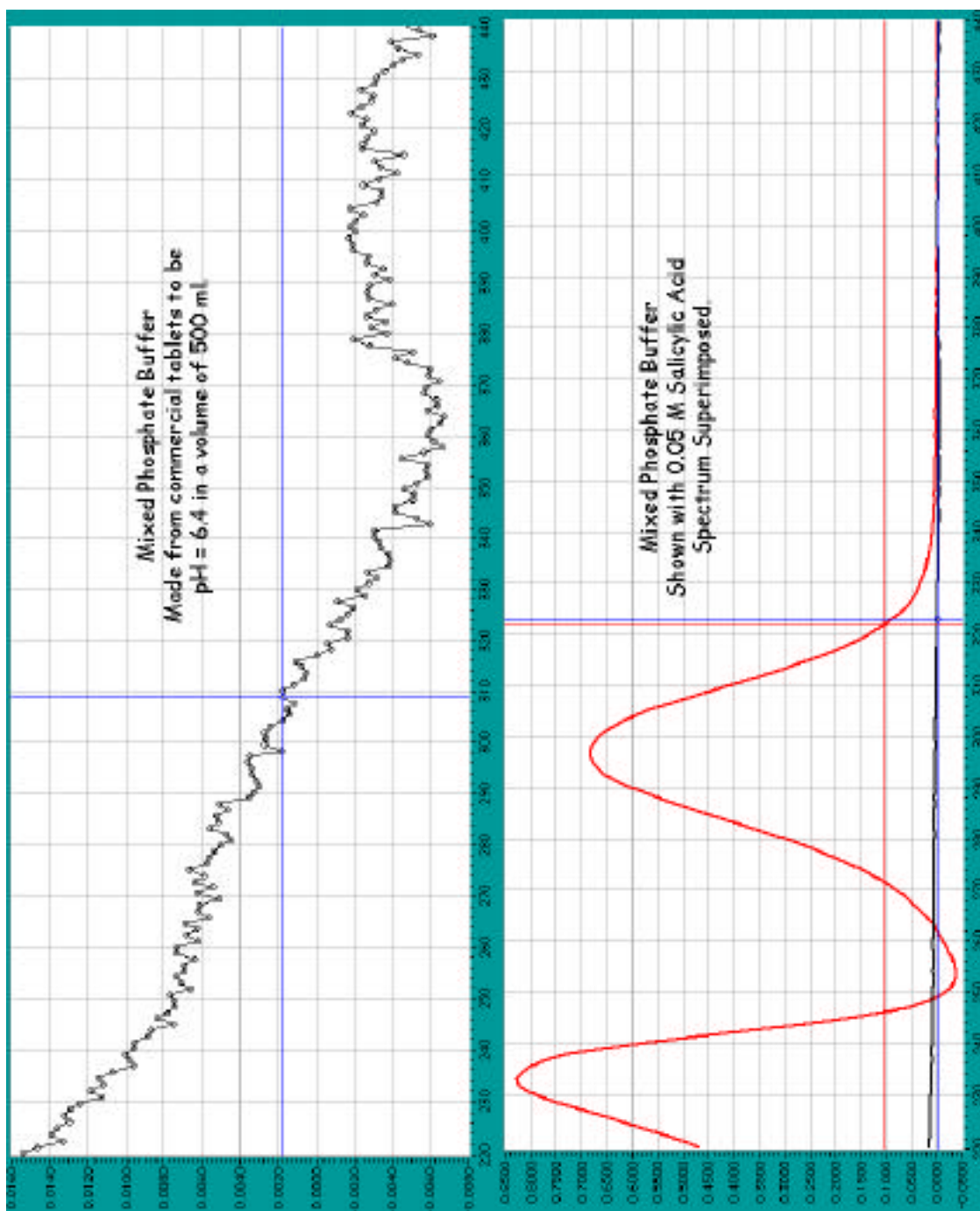


Figure 8 - A suitable (for most analytical wavelengths) buffer

For the mixed phosphate buffer, very little absorbance overlap occurs at wavelengths of 300 nanometers and higher. These are some analytical wavelengths you might have selected for measuring the hydrolysis rate by the appearance of salicylic acid. The class of 1996 discovered this information. The buffer is available in tablet form. It is simple to prepare solutions of it.

Item 7 – Determining How long Measurements have to be Taken

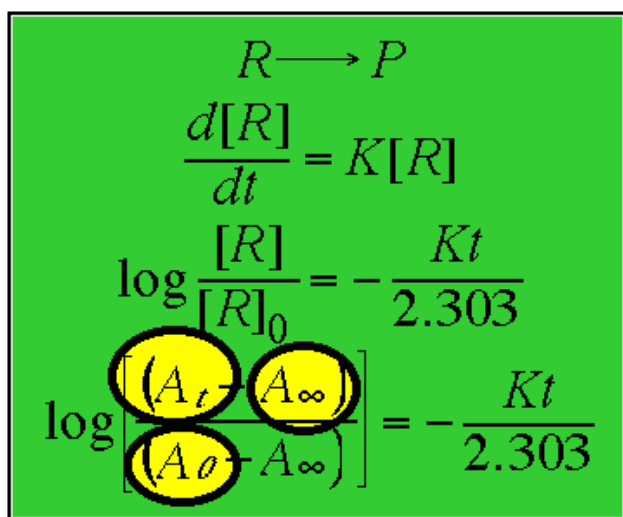


Figure 9 - The Effects of A_∞
effect and learned that:

We can use previous class results to decide on how many days the SI-420 instrument has to be programmed to take data. But, special attention needs to be directed to deciding at what time A_∞ has to be measured.

This is deceptive. Note that A_∞ appears in both the numerator and denominator of what is called the “reduced absorbance parameter” shown in Figure 9. (The rate equations that produce this final equation are derived in Appendix B.) This signals that its value can introduce significant non-linearity in the rate plot at both early and late times. The 1999 class studied this

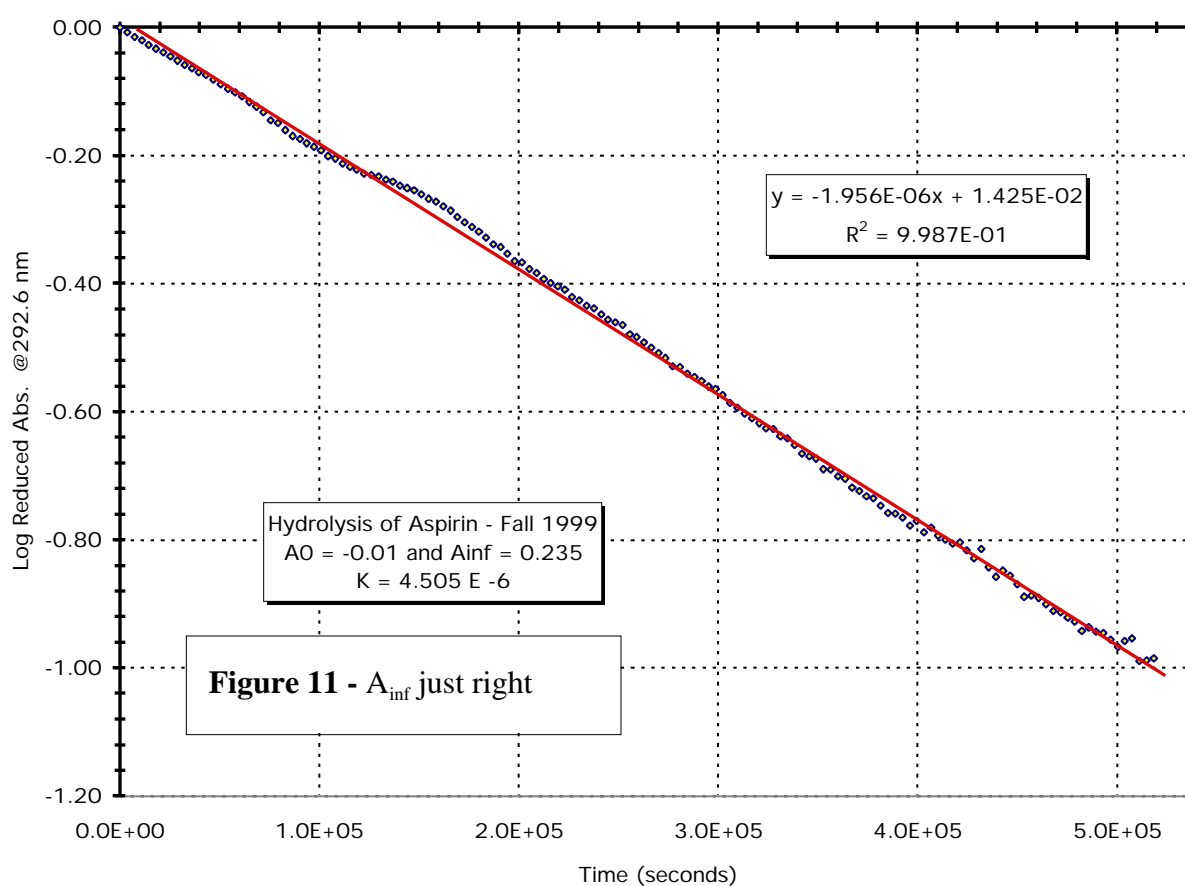
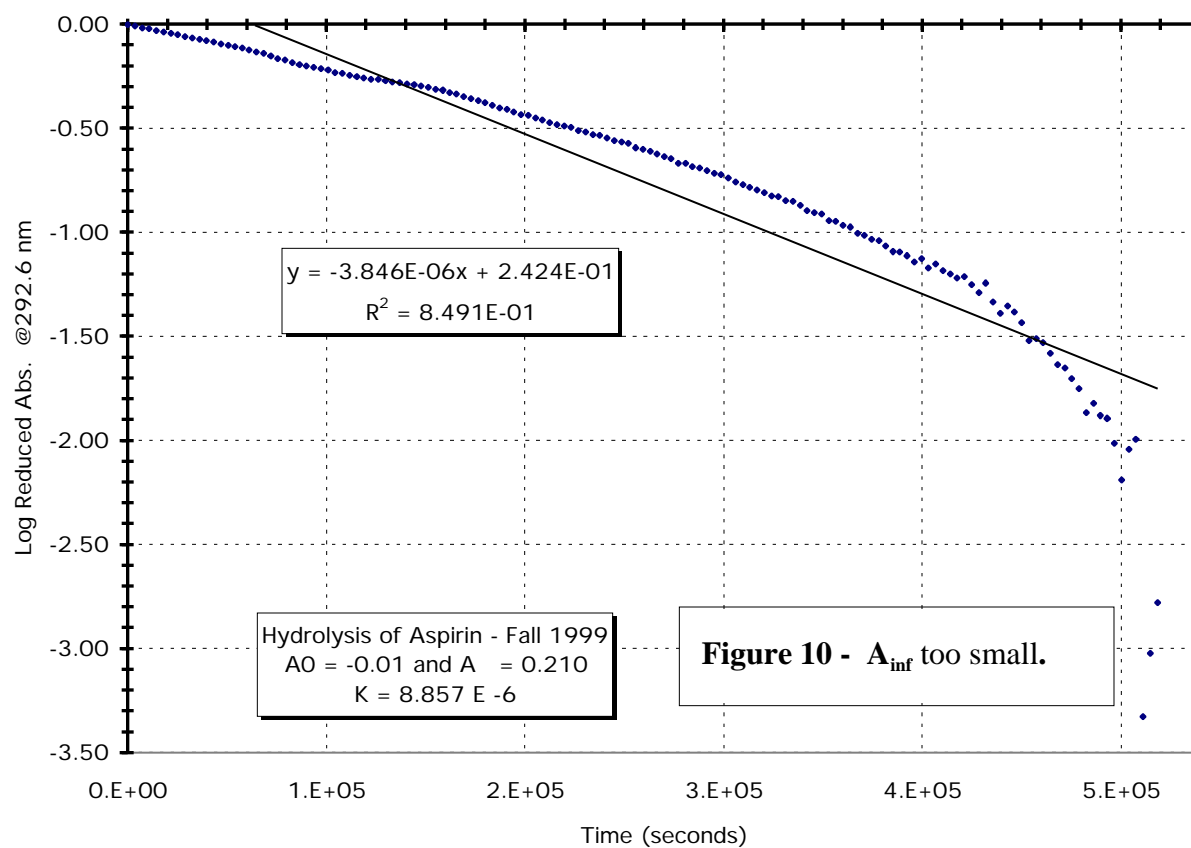
A	K
0.210	8.857E-6
0.235	4.505E-6
0.260	3.358E-6
0.285	2.718E-6

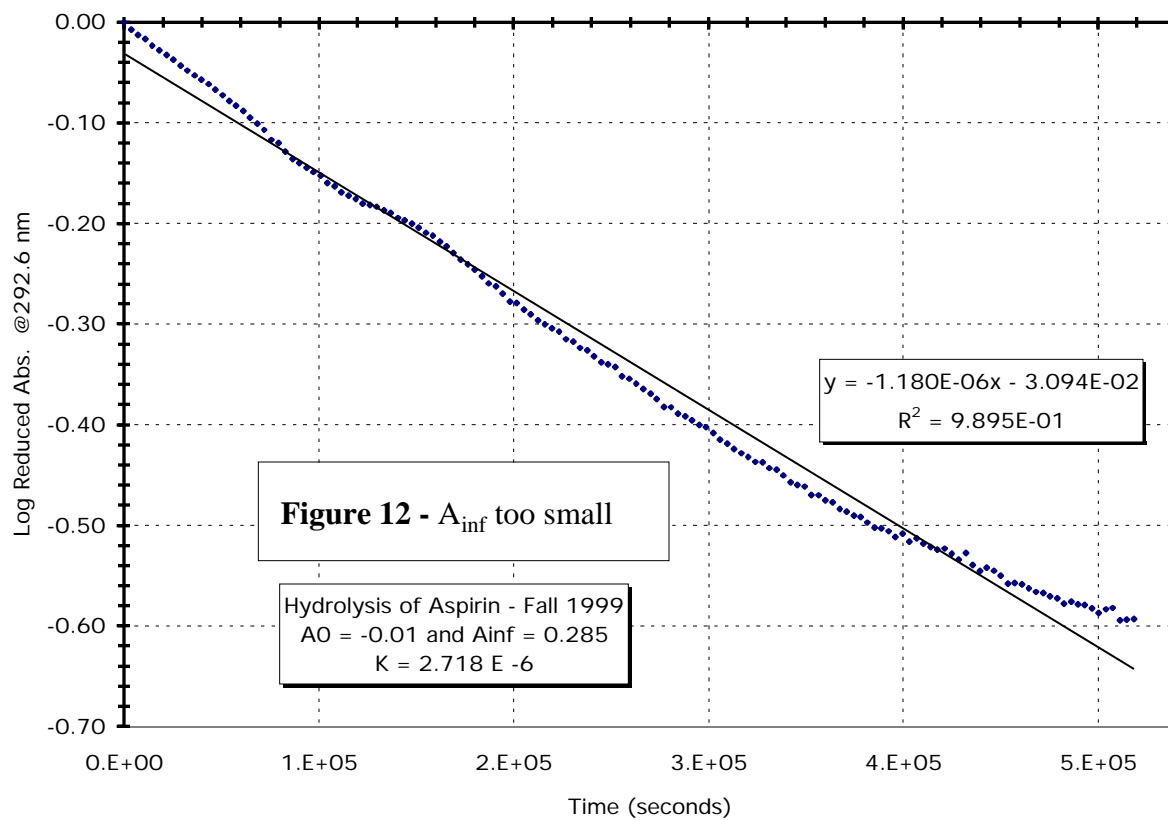
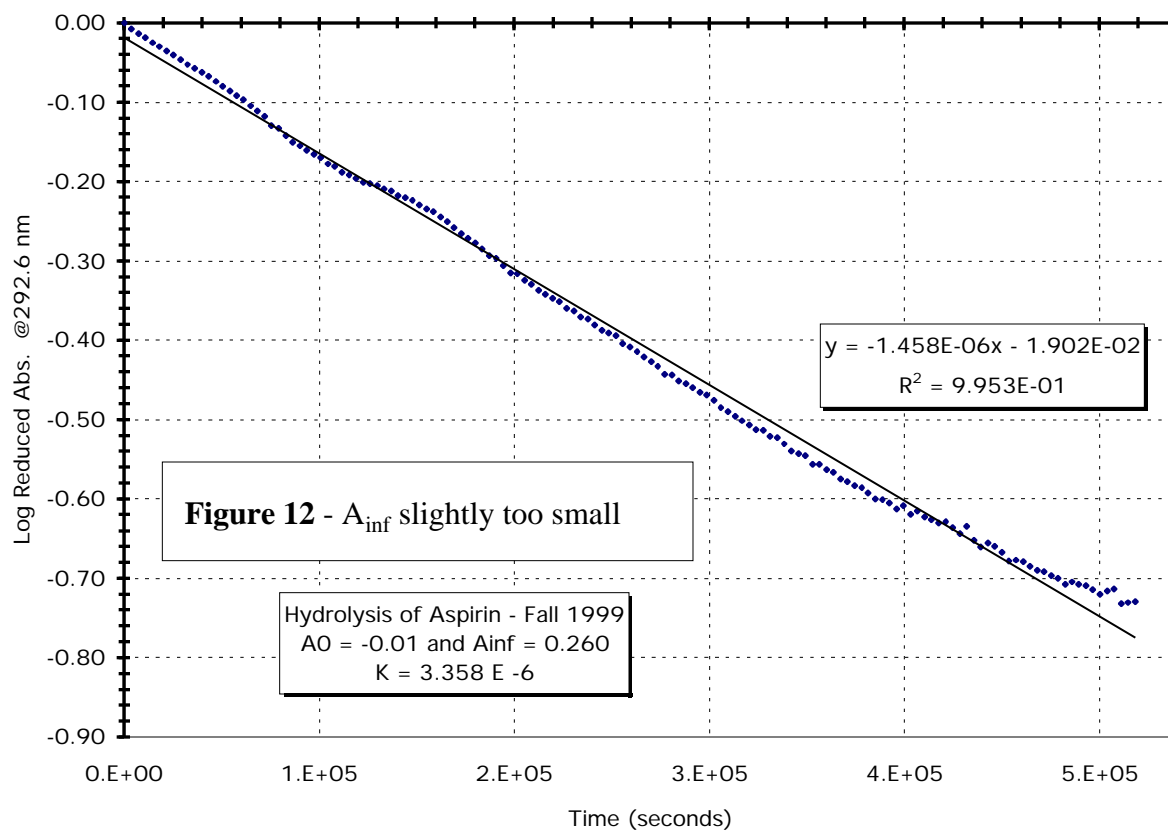
A_0	K
-0.035	4.505E-6
-0.010	4.505E-6
0.015	4.505E-6
0.040	4.505E-6

“The value of A_0 does not seem to affect the rate constant greatly (or within our range, at all). However, the A_∞ value greatly affects the rate constant due to the curvature it causes in the reduced parameter rate plot.”

The way in which A_∞ influences the entire rate plot is best seen graphically. This is shown in the next four figures, Figure 10, Figure 11, Figure 12, and Figure 13. Only the value in Figure 11 is correct. It alone produces a straight-line reduced absorbance parameter rate plot.

One question is if it is legitimate to simply stipulate an A_∞ that linearizes the rate plot? Strictly speaking, the answer is no. To get an actual A_∞ would require at least 7 or 8 half lives of elapsed time. Leaving the solution in the absorption cell for this length of time could lead to precipitation of salicylic acid due to its solubility. You will have to decide how to deal with this apparent dilemma.





Item 8 – Setting up the Robot/SI-420 Combination for Time Measurements

The way the measurements will be made involves the Spectral Instruments model 420 CCD spectrometer and a flow cell fed by the cannula sector on the robot. Note first the SI-420 instrument shown in Figure 14.

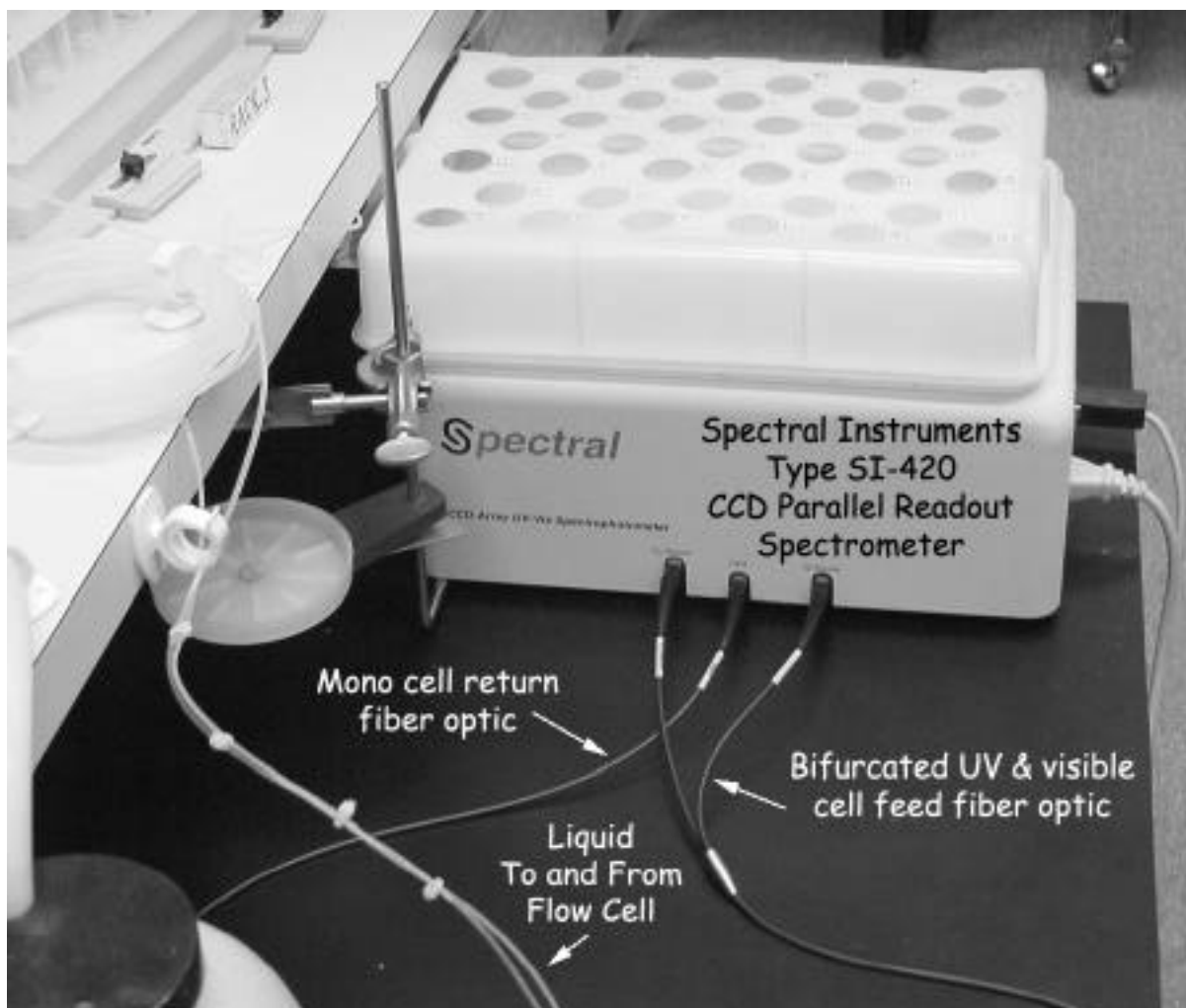


Figure 14 - The Spectral Instruments model 420 (SI-420) CCD parallel readout spectrometer.

This spectrometer is a single beam instrument that depends on the stability of its light sources to achieve stable absorbance readings. It has an external cell holder. Light is fed to the cell holder in both the UV and visible spectral regions from two independent sources via a bifurcated fiber optic. It is returned from the cell to the internal Ebert “over and under” type spectrometer and CCD detector by a single fiber optic. These are labeled in Figure 14.

The key to this measurement system is the flow cell and the SI-420 program for taking timed readings shown in Figure 15. The light path in the flow cell is low enough that when air bubbles form in the cell over the long time required measuring the salicylic acid formed, they go to the top out of the light path. Thus, neither the concentration in the cell nor the path length changes over illumination and measurement times as long as six days.

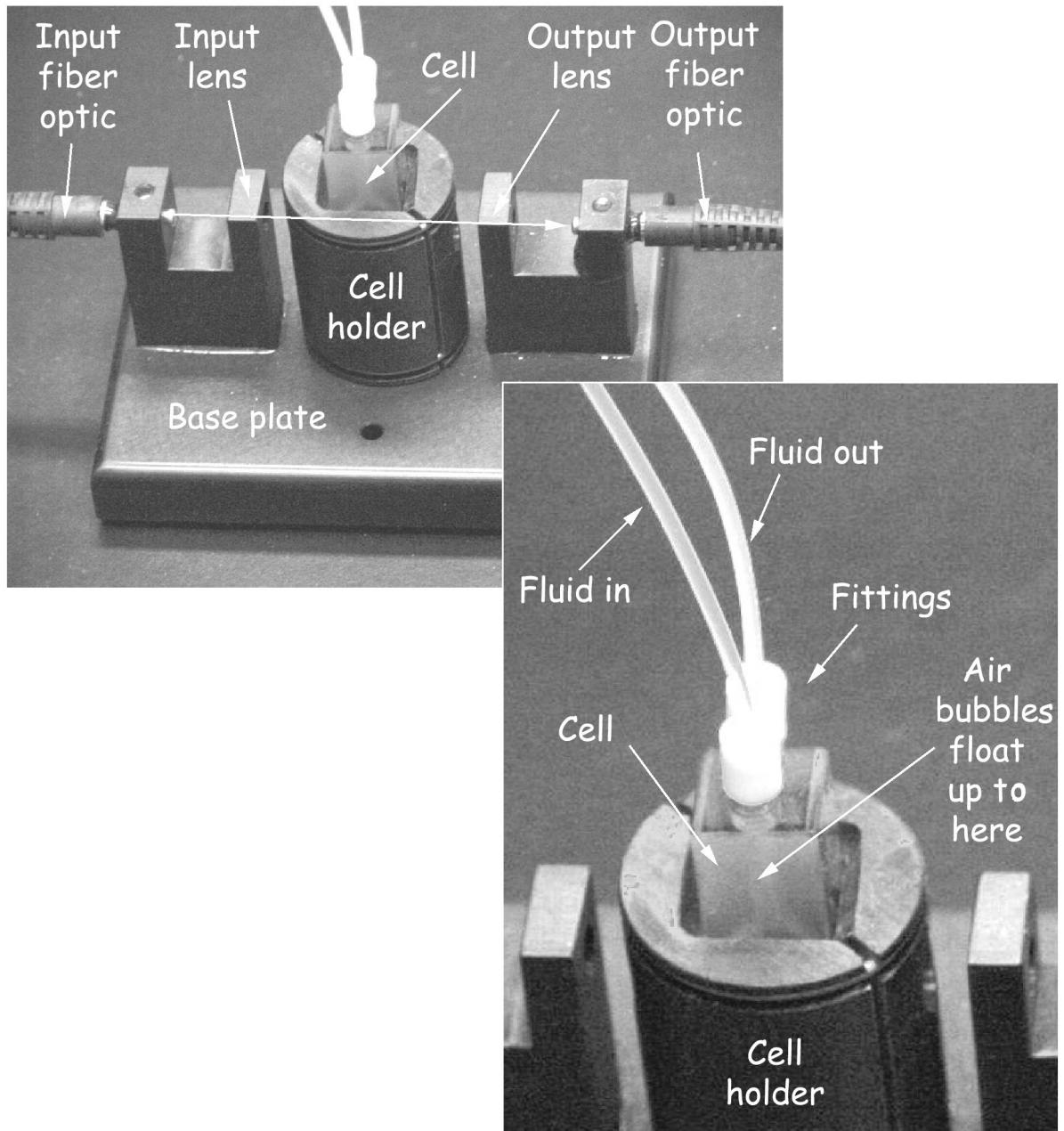
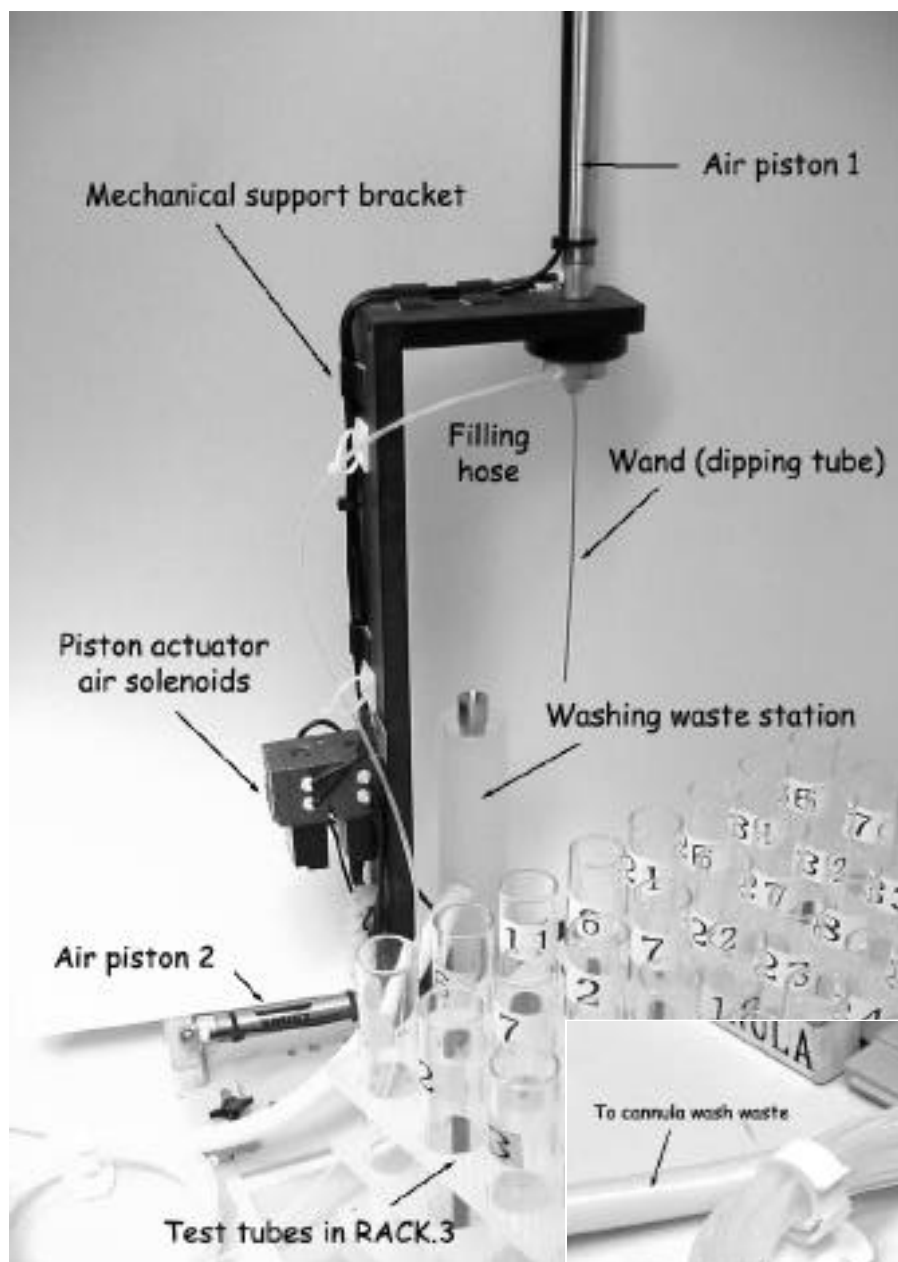


Figure 15 - Cell and holder showing light (top) and fluid (right) paths.

Item 9 – Filling the Flow Cell

The hoses at the top of the flow cell transport liquid through the cell by the aspiration action of the cannula. The robot controls the cannula. The pictures on the next pages show the cannula in operation.



wand down into the waste station for washing out the flow cell, the storage coil, and the wand. This important task is the only way to rinse out the flow cell between samples and prevent residual sample-to-sample contamination.

The flow cell and cannula wand have to be fed from a 25 mm diameter test tube containing the exact solution that is to be measured, as follows.

The cannula is a hollow, stainless steel wand, or dipping probe, that is connected to the flow cell through a large coil of tubing. A syringe in the MLS.2 master lab station draws liquid up through the wand from a test tube held under it. If the syringe solenoid is reversed, the syringe action forces liquid out of the wand. These actions move the liquid back and forth through a storage coil. For this experiment, the coil was cut and the flow cell inlet and outlet tubes shown in Figure 15 spliced into it.

The air pistons shown move the washing waste station back and forth under the wand, and the

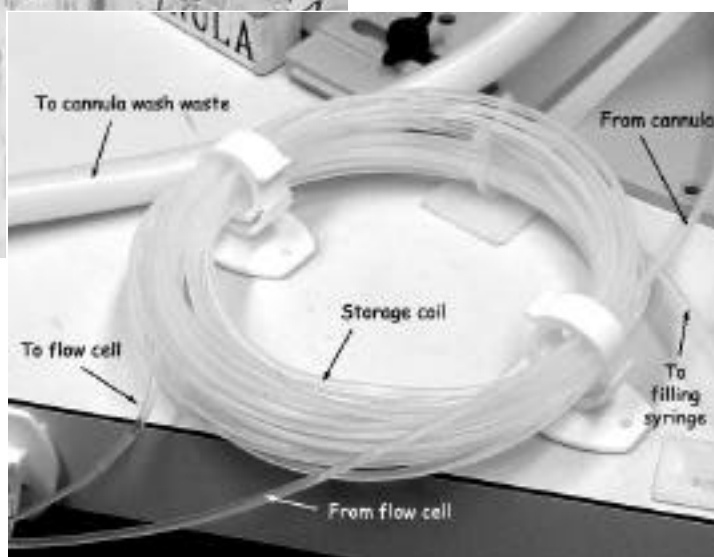


Figure 16 - The general layout of the cannula and the storage coil

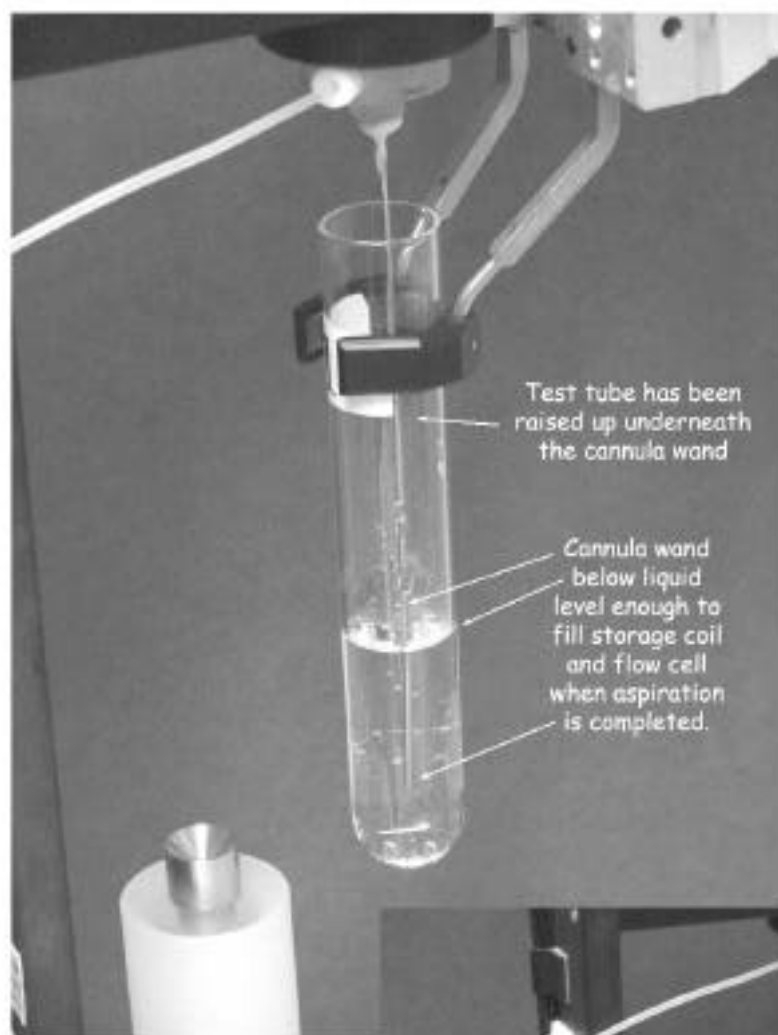
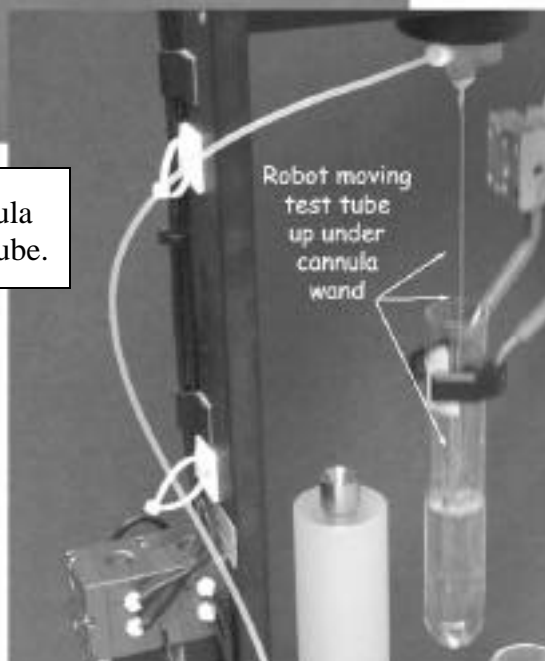


Figure 17 - How the cannula wand is filled from a test tube.

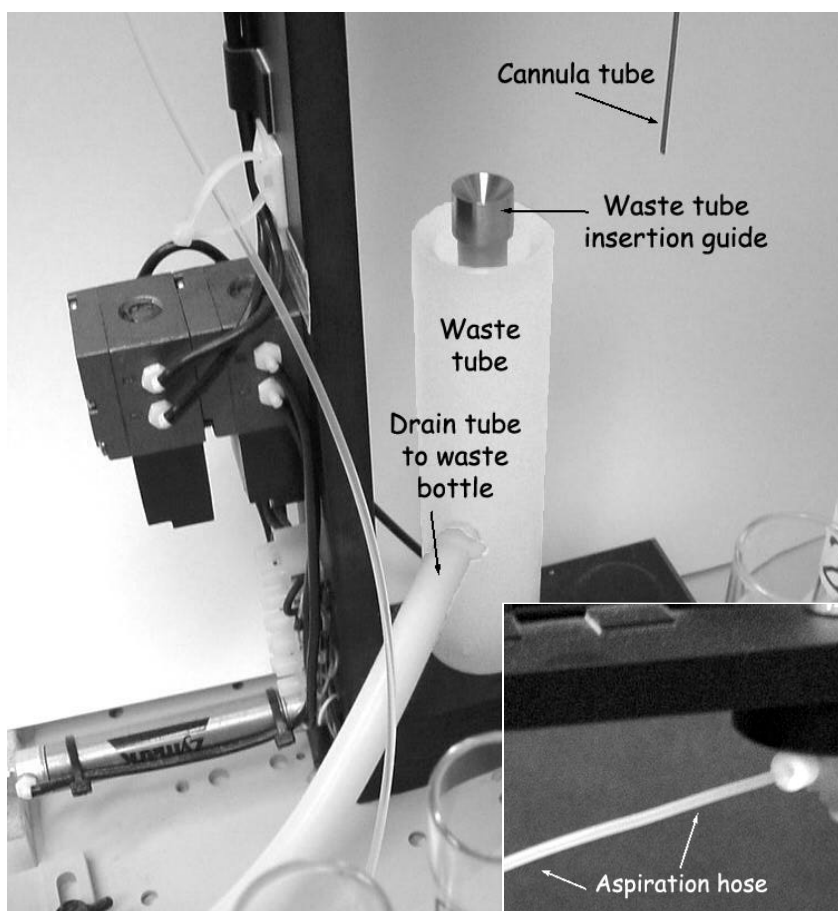


The robot moves the test tube you have selected underneath the cannula wand. Then, knowing the amount of liquid in the tube, and its diameter, it raises the tube just enough to allow the storage coil and flow cell to be filled by one aspiration cycle.

If desired, this cycle can be reversed and the material aspirated into the flow cell can be flushed back into the test tube. This is called “dispensing” it. Unfortunately, a little bit of water almost always accompanies the dispensing, slightly diluting the sample over what it originally was. For one cycle, this is not serious. But, for a dozen or so, it will produce noticeable effects.

The cure is to wash out the cannula wand between aspiration

and dispensing cycles, or between samples, or both. This generates waste and uses up rinse solution and fresh sample, but it also ensures uncontaminated samples. The way this wash cycle is accomplished is shown in Figure 18 on the next page:



The cannula is designed for easy washing. The wand (or cannula tube) needs to be filled with fresh water. Then, that water has to be back flushed through the tube after it has been pushed down inside of a small waste tube. That waste then is forced down a larger drain tube into a collection container. These processes are shown in these three figures, top,

middle, and bottom. The figure at the right shows the cannula wand just as it is about to be inserted into the waste tube, where it will be washed and flushed into the container below.

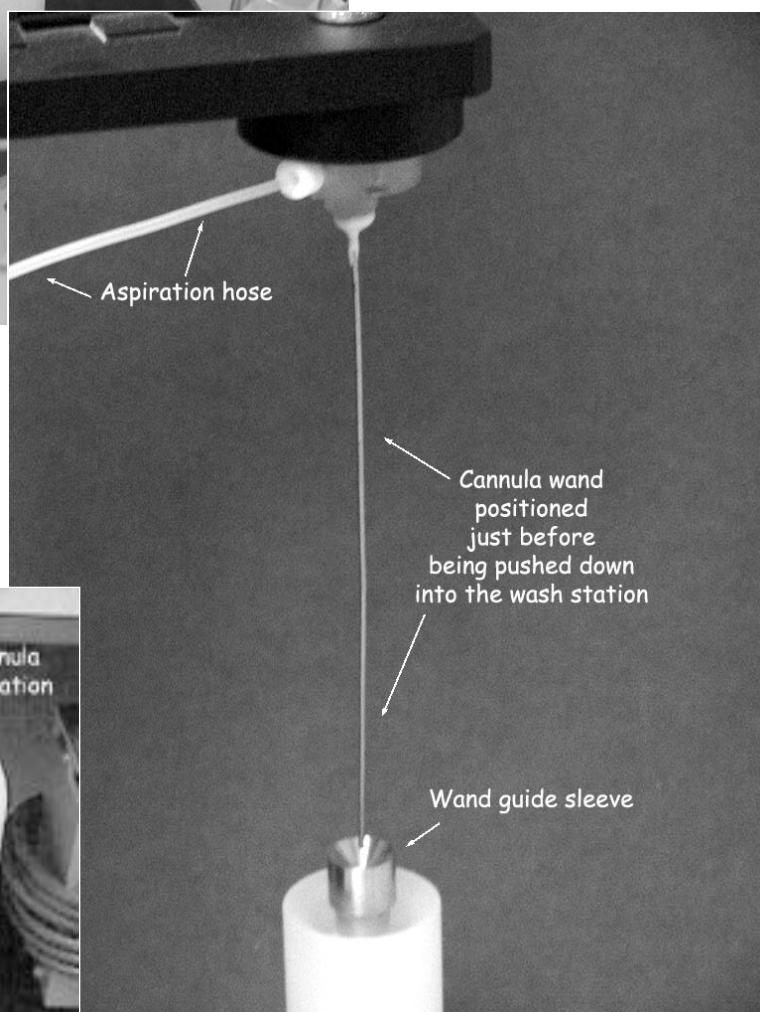


Figure 18 - Washing the wand

Item 10 – Filtering the Saturated Aspirin Solution

It is important to realize that the aspirin, when dissolved in buffer solution, will produce a saturated solution. Excess aspirin and starch binder will cloud the saturated solution. The solution will have to be thoroughly mixed and then filtered before progressive dilutions are made.

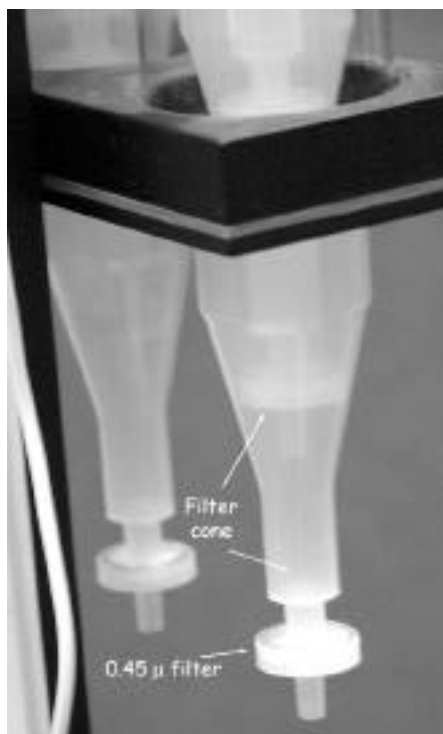


Figure 19 - Filter and cone

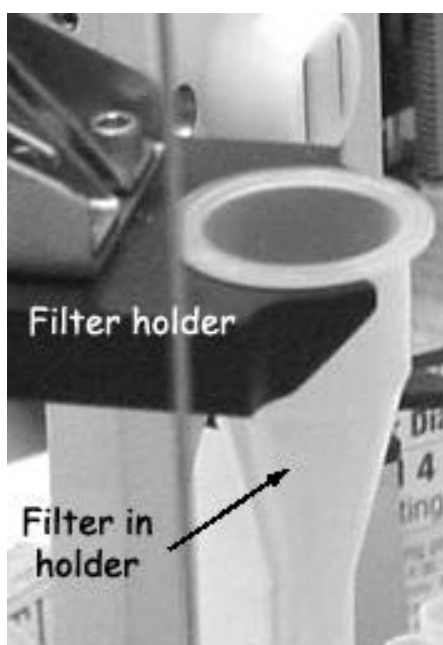


Figure 20 - Filter in holder

The filters themselves are 0.45-micron “Gelman” devices. They attach to the bottom of a plastic filter cone by friction. The combined assembly is stored in a three-column filter holder (Figure 19).

Robot hand B has the right finger sizes to grab a filter cone from the filter holder and transport it to the filter holder (Figure 20). The filter holder has an air-actuated lid that is attached to an accordion hinge. When pressurized, this hinge snaps down onto the top of the filter cone (Figure 21, 25) and high-pressure air is applied to the contents of the cone. This forces any liquid inside the cone through the Gelman filter itself.

Liquid has to be transferred into the filter cone in a separate series of steps. These are in the next figures.

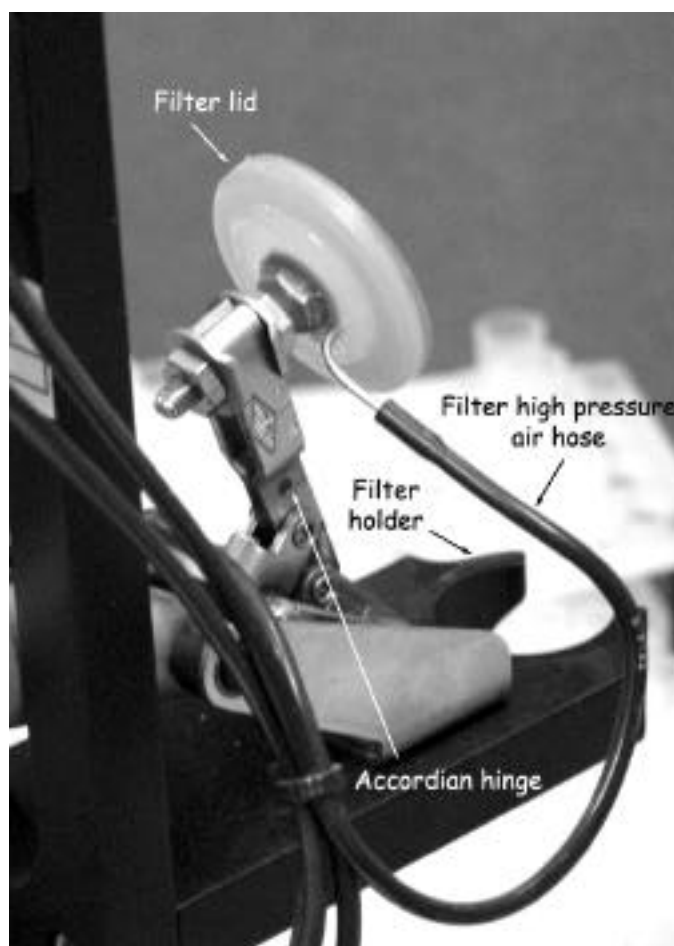


Figure 21 - Filter apparatus, showing holder and lid

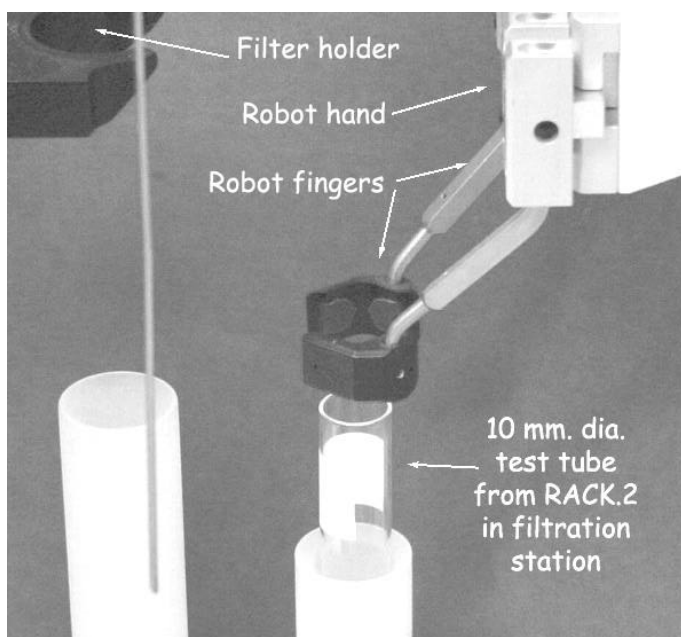


Figure 22 – Filtrate-receiving test tube put in filtration station

A tube has to be selected from RACK.2 to hold the filtrate. After this is done, the robot picks up the tube and places it in the filtration station, as shown in Figure 22.

A pipet hand then is selected, and a tip attached (typically the 5 ml tip). This is then moved over the tube in RACK.3 that contains the undissolved aspirin and starch binder suspension to be filtered (Figure 23). An aliquot is drawn up, and tip is moved over the filtration station cone, where it is dispensed (Figure 24). More than one aliquot may be transferred to the filter cone. Some judgment is needed, since the filtration rate will slow as more liquid is processed.

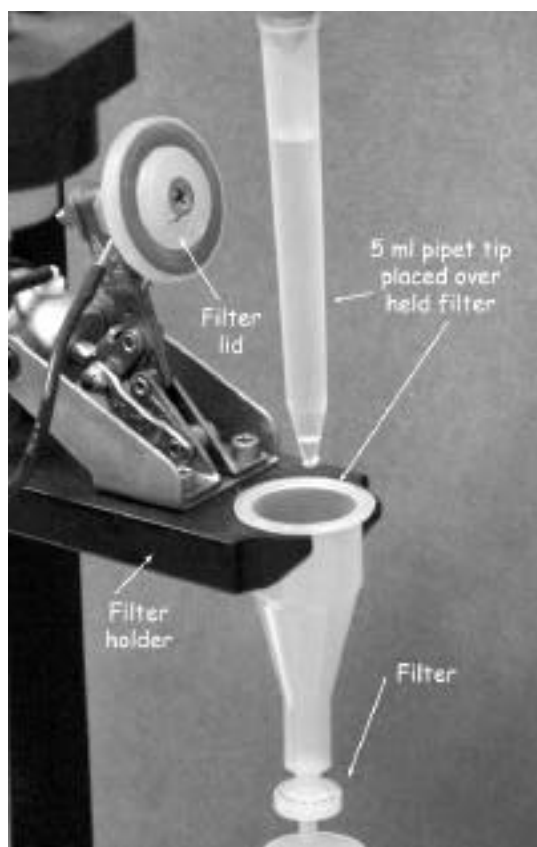


Figure 24 - Dispensing liquid into filter cone for filtration

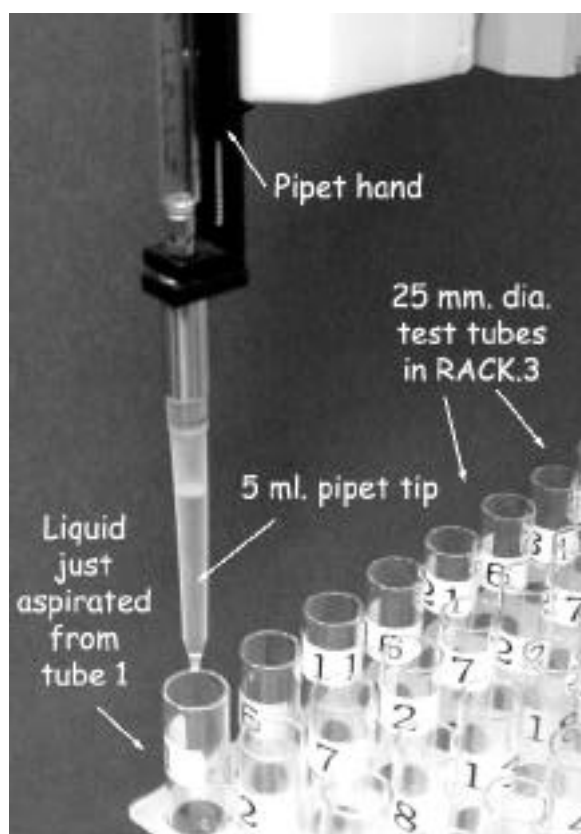


Figure 23 - Pipet hand aspirating liquid to

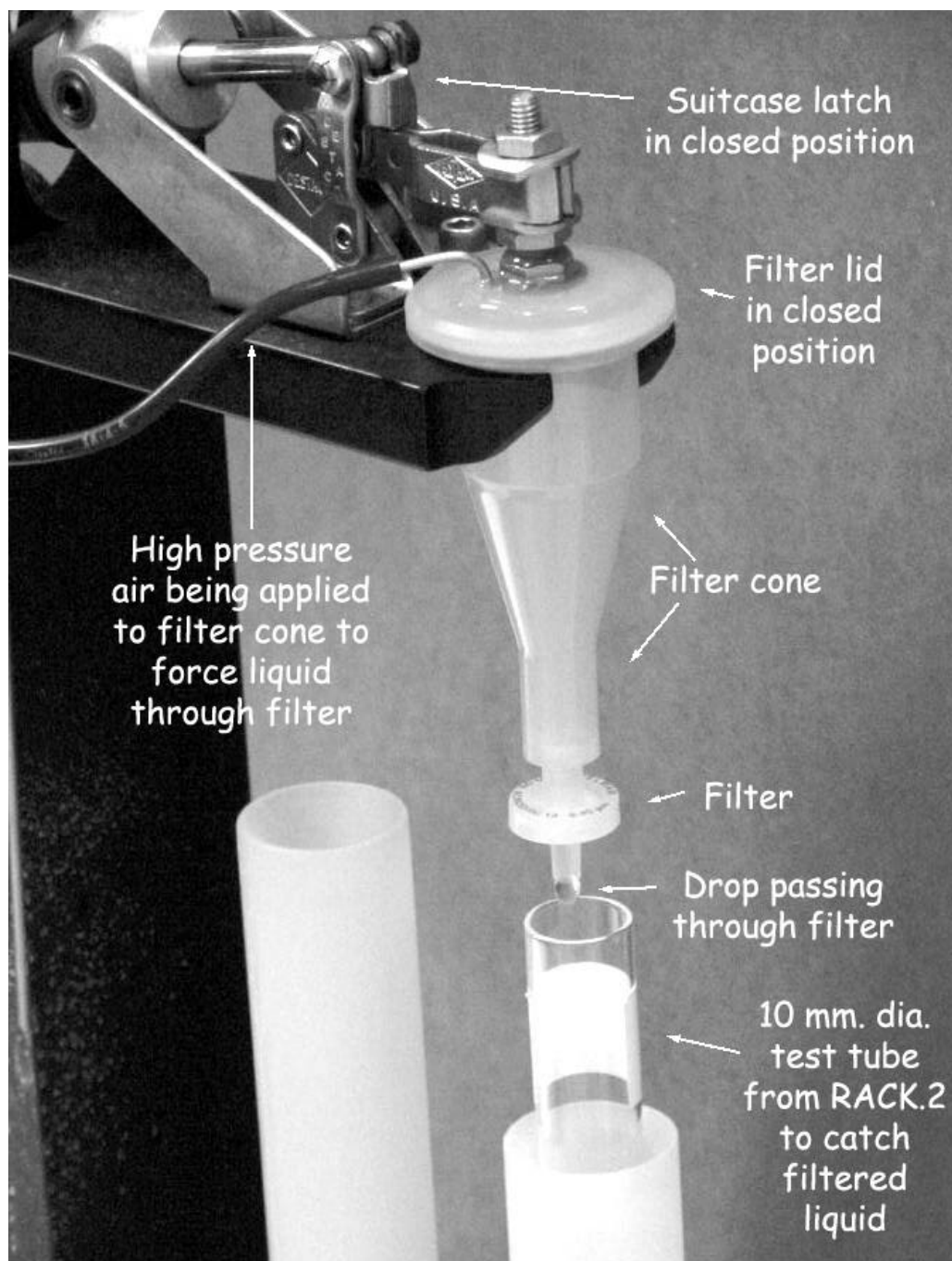


Figure 25 - Filtration in action

the filtration station. The air pressure is held for an adjustable time, typically 60-90 seconds. Then the filter is discarded and the tube put back in RACK.2

As first filtered, the aspirin solution is saturated and must be diluted. This amounts to withdrawing an aliquot from the tube in RACK.2, transferring it to a tube in RACK.3, adding buffer solution from the MLS, vortex mixing, and returning the tube to RACK.3. If serial dilutions are needed, the entire process is repeated with a new pipet tip. The amounts for the aliquot and diluent have to be calculated beforehand..

The complete filtration action is shown here in Figure 25. The filter lid has clamped down on top of the filter cone due to the action of the air-actuated accordion hinge moving the suitcase latch into a closed position. .

High-pressure air is applied to the sealed assembly, and the liquid is forced through the filter, drop wise.

After passing through the filter, the filtrate is collected in the 10-mm. dia. test tube taken previously from RACK.2 and placed in

Item 11 – Setting up the Computer/SI-420 Measurement Parameters

The actual taking of data is done after the robot has finished preparing the sample. Robot sample preparation takes about 30 minutes, depending on the kind of program that you have written and any complications that result from it. After it is complete, there should be one, known, 25 mm.

Diameter test tube in RACK.3 containing the diluted sample plus buffer that constitutes the sample to monitor. This then would be aspirated into the flow cell using the cannula, and the measurements started.

Presumably the SI-420 CCD parallel spectrometer would have been set up to take readings at select wavelengths as a function of time. For example, the class of '99 selected 5 wavelengths to monitor. Their raw data, fitted with cubic polynomials, are shown below. They based their wavelength choices on Figure 3.

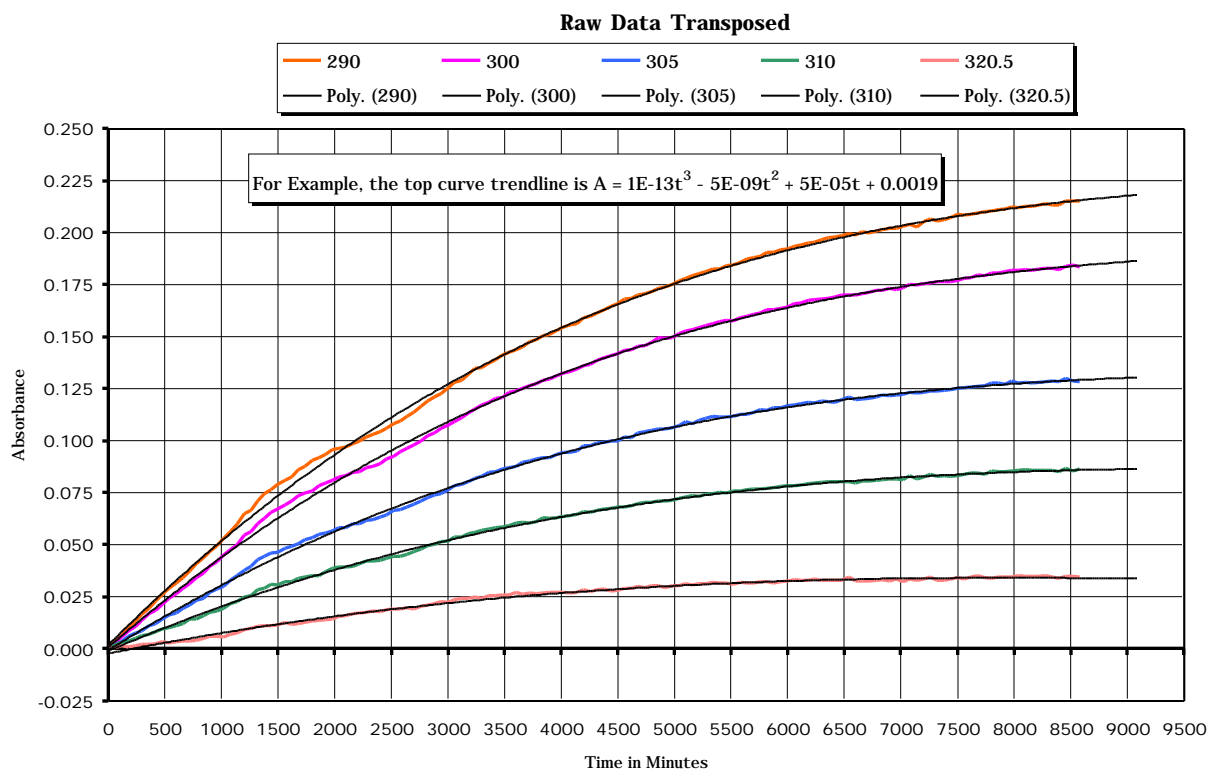


Figure 26 - Raw data from the class of '99, transposed from rows to columns before plotting.

Some trials can be made before the final hydrolysis run is started to determine if enough of the right wavelengths have been chosen. There is a weekend between the Thursday section's programming and the start of the Thanksgiving break run during which a test can be made of the system.

Full Spectrum:
Set the Time Interval, Number of Spectra (Acquisitions), and File Information.
Any wavelengths set will be saved with the spectrum and vaues will be displayed in the main front panel.

Clear

Wavelengths

0.00
0.00
0.00
0.00
0.00
0.00
0.00
0.00
0.00
0.00

Ok

Time Interval Sec/Min **Number of Acquisitions**

5 Sec. 0

File Name is "Series.###"

Data Series: **Start at #:**

0

Set Path %

Figure 27 – Internal LabVIEW® Table to set up measurement times on SI-420

The wavelengths and times for measurements are set into the above table, The time interval is how long between measurements, either in seconds or minutes. The number of acquisitions is as expected. It must be set large enough to allow enough points to be acquired for a reliable rate plot. The data series is just a descriptive number, such as 001. Measurements can be made at 10 wavelengths, which are entered in the right hand table. These are the wavelengths chosen from Figure 3.

SI 400 Timed Acquisition.vi

Cursor1 0 -0.0005 Cursor2 40 -0.0000

ΔX 40.00 ΔY 0.0005

Close **Hide Grid**

Absorbance

Start

Mode

Discrete Wavelength

Setup

Print

Wavelengths

1	0.00
2	0.00
3	0.00
4	0.00
5	0.00
6	0.00
7	0.00
8	0.00
9	0.00
10	0.00

Time Remaining

0

Time Until Next Aq.

0

Samples Taken

0

Comment **File Name** blank2

Sample

Solvent 20 cc isopropanol/H water

Conc.

Info. 1

Info. 2

Info. 3

Info. 4

Info. 5

Operator

Date 11/15/99 Time 9:57:40 AM Blank Taken: 11/15/99 at 9:57:32 AM

Open File **Save to File** **Save as Text** **File Name Filter**

Figure 28 - The LabVIEW® panel for acquiring data at selected times

several short time practice runs to learn parameter-setting effects.

After the acquisition time interval, number of acquisitions, and wavelengths have been entered, and the run is ready to be started, the above panel will appear. This is where all parameters have to be entered. When the SI-420 is set up, the Company doing this part of the work should do

Item 12 – What are the lab section responsibilities?

Each lab section has different responsibilities for making the whole project work. These will be discussed in detail during the staff meeting prior to the start of the work. In general, they fall along the following lines:

1. Tuesday – week 1
 - a. Decide on the pH
 - b. Prepare enough buffer at this pH
 - c. Verify all molar absorptivity measurements and calculations, or do new
 - d. Specify analytical wavelengths
 - e. Manually test and verify all dilution steps on robot
2. Wednesday – week 1
 - a. Decide on measurement times
 - b. Load all parameters into SI-420
 - c. Load all solutions and supplies into robot
 - d. Manually test and verify all filtration steps on robot
 - e. Take reference spectra on SI-420 of all solution
3. Thursday – week 1
 - a. Prepare complete robot program (see example in Appendix A)
 - b. Test and verify robot program
 - c. Test and verify SI-420 parameters
 - d. Test and verify molar absorptivities and dilution steps
 - e. Test and verify analytical wavelengths
4. Tuesday – week 2
 - a. All sections gather
 - b. Verify robot deck
 - c. Verify robot initialization
 - d. Verify robot program
 - e. Verify SI-420 program and parameters

When all three Managers approve these responsibilities, the Managers start the robot for the Thanksgiving break run. Tuesday's Manager actually starts the robot. Wednesday's Manager starts the SI-420 when the robot finishes. Thursday's Manager signs up one person from each Company to process the data when the break is over.

The raw data will be in an Excel ready file that can be transferred from the SI-420 LabVIEW program to Excel by the people that Thursday's Manager selects. The data will look something like what is shown on the next page in Figure 27. It would be wise to have tested out transfer and processing of some sample data before returning from the break.

0	0.12112	0.1114	0.10446	0.08729	0.06593	0.04471
5400	0.1817	0.17855	0.16803	0.14061	0.10253	0.06602
10800	0.23971	0.24212	0.22868	0.19027	0.13577	0.08523
16200	0.29645	0.30315	0.28736	0.23901	0.16894	0.10389
21600	0.35292	0.36557	0.34438	0.28551	0.20085	0.12114
27000	0.40597	0.42355	0.40231	0.33315	0.23198	0.13937
32400	0.45822	0.48135	0.45578	0.37668	0.26218	0.15645
37800	0.50783	0.53452	0.50715	0.41933	0.29119	0.17308
43200	0.55385	0.58622	0.55595	0.46017	0.32048	0.19068
48600	0.59831	0.63545	0.60342	0.49953	0.34778	0.20855
54000	0.64399	0.6862	0.6508	0.54043	0.37657	0.22639
59400	0.69161	0.73549	0.69885	0.58207	0.40503	0.24334
64800	0.73717	0.78853	0.74856	0.6228	0.43399	0.26121
70200	0.78452	0.83877	0.79888	0.66306	0.46227	0.27779
75600	0.83043	0.88969	0.84621	0.70334	0.48998	0.29428
81000	0.87702	0.94006	0.89435	0.74355	0.51756	0.31126
86400	0.92447	0.98958	0.94316	0.78354	0.54461	0.3267
91800	0.96819	1.03842	0.98768	0.82221	0.57208	0.34227
97200	1.01121	1.08849	1.03469	0.8611	0.59835	0.35803
102600	1.05437	1.13165	1.08005	0.89822	0.62318	0.37342
108000	1.09601	1.18015	1.12516	0.93397	0.64853	0.38729
113400	1.13588	1.22597	1.16699	0.96918	0.67319	0.40133
118800	1.1774	1.27065	1.2078	1.00232	0.69683	0.41441
124200	1.21706	1.3109	1.2494	1.03833	0.71789	0.42901
129600	1.25539	1.35305	1.28724	1.06799	0.74115	0.44102
135000	1.29117	1.39443	1.32527	1.09978	0.76147	0.45426
140400	1.32771	1.43682	1.36598	1.1315	0.78386	0.46645
145800	1.36717	1.47662	1.40672	1.16443	0.80528	0.47907
151200	1.40074	1.51559	1.4483	1.19757	0.82839	0.49101
156600	1.43901	1.55206	1.48303	1.22925	0.84862	0.50354
162000	1.47794	1.597	1.52314	1.26043	0.87027	0.51517
167400	1.51595	1.63212	1.56061	1.29037	0.89258	0.52759
172800	1.55559	1.67798	1.59716	1.32478	0.91268	0.53944
178200	1.59377	1.71908	1.63406	1.35177	0.93443	0.55234
183600	1.62372	1.75617	1.6721	1.38449	0.9553	0.56372
189000	1.65853	1.79037	1.70638	1.41292	0.97398	0.57576
194400	1.69095	1.83093	1.7486	1.43968	0.99101	0.58631

Figure 27 - Raw data in spreadsheet form

The leftmost column contains the times, either in hours or seconds as the SI-420 has been programmed. Each subsequent column contains the absorbancies at some different wavelength. The column headings will not be in the raw data, and will have to be added. The data are shown here in spreadsheet format. To get them here, the SI-420 will have to be set so it saves absorbancies for all selected wavelengths at each time a measurement is made. When the data are transferred to spreadsheet, they should be done so by copy and “paste special”, with the “transpose” button selected to convert a row to a column.

Appendix A:
An Example EasyLab Program for Preparing and Diluting Aspirin Sample
 (Class of 2000)

Prologue3

“How many samples this time?”

1

rack.3.index=1

get.from.rack.3

put.into.balance

obtain.weight

printer.on

*** You need to get this to work

print weight.value

*** Our data value was 31.3451

rack.2.index=1

get.from.rack.2

pour.contents

put.into.rack.2

obtain.weight

print weight.value

*** Our data value was 31.7181

printer.off

get.from.balance

mls.1.volume.c=20

dilute2

put.into.vortex

vortex.time=30

vortex.timed.run

get.from.vortex

put.into.rack.3

Timer (1)=5*60

*** You need to remember to program these steps

Wait for timer

We waited for over 5 minutes before filtering.

put.filter.in.filtration.station

rack.2.index=6

get.from.rack.2

put.tube.in.filtration.station

filter.exercise

number.of.5ml.prewets=0

get.5ml.tip

rack.3.index=1

move.over.rack.3

pipette.volume=4

aspirate.5ml.tip

move.over.filtration.station

dispense.5ml.tip

filter.sample

move.over.rack.3

pipette.volume=4

aspirate.5ml.tip

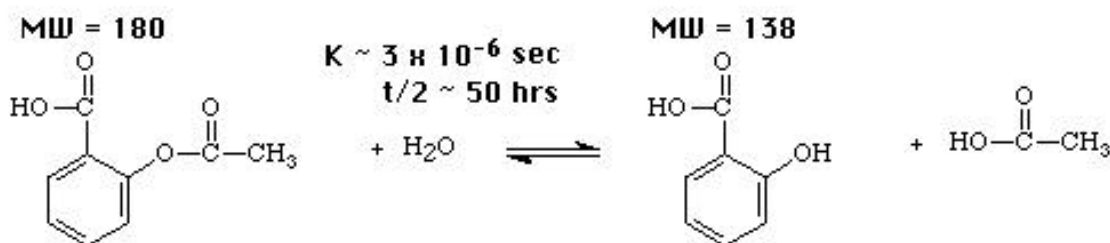
```

move.over.filtration.station
dispense.5ml.tip
dispose.to.waste.      ***    Do this step here to avoid droplets on pipette
tip from falling onto the table.
filter.sample
get.filter.from.filtration.station
dispose.to.waste
get.tube.from.filtration.station
put.into.rack.2
number.of.2ml.prewets=0
get.2ml.tip
move.over.rack.2
pipette.volume=0.5
rack.3.index=2
move.over.rack.3
dispense.tip
dispose.to.waste
get.from.rack.3
mls.1.volume.c=19.5
dilute2
put.into.vortex
vortex.time=30
vortex.timed.run
get.from.vortex
can.wash
can.blank
can.sample
can.aspirate
can.sample
***    Don't forget to pre-set the wavelength and time values in the SI-420
***    Once the SI-420 is done taking spectra (as in 7.5 days, or 648,000 seconds later), we'll
        need to dispense the sample back into the tube
can.dispense
put.into.rack.3
park.hand

```


Appendix B: The Rate Equations

In developing the following material, please refer to Figure 1 below. Both the salicylic acid and the acetylsalicylic acid absorb strongly in the UV spectral region, and the slow reaction can be followed that way. It is spectrally most convenient to follow the appearance of the reaction product, salicylic acid.



To understand how kinetics works, let's begin by assuming that the concentration of water in the hydrolysis reaction does not change, and that the solution is buffered about the pK_a of acetic acid so that any acetic acid liberated during the hydrolysis will not change the pH. These are the conditions for a "pseudo first order reaction", which we denote as:



$[\text{R}]_0$ is the equilibrium concentration of R at $t = 0$, and $[\text{R}]$ is the equilibrium concentration of R at "infinity". We can state the mass balance immediately:

$$[\text{R}_0] = [\text{R}] + [\text{P}]$$

where $[\text{P}]$ is the equilibrium concentration of the product at any t .

Since the reaction is pseudo first order, the differential rate law is simply:

$$\frac{d[\text{R}]}{dt} = -K[\text{R}]$$

Integrating this then:

$$\int_{[\text{R}_0]}^{[\text{R}]} \frac{d[\text{R}]}{[\text{R}]} = -K \int_0^t dt$$

Which gives the familiar "ln" function:

$$\ln \frac{[R]}{[R_0]} = -K$$

or in terms of \log_{10} :

$$\log_{10} \frac{[R]}{[R_0]} = - \frac{K}{2.303}$$

This expression is now ready to use with the appropriate initial values. They are as follows:

$$\text{If } t = 0 \text{ then } [R] = [R]_0$$

$$\text{If } t = 0 \text{ then } [P] = 0$$

$$\text{If } t = 0 \text{ then } A = A_0 = \epsilon_R b [R]_0$$

Next, consider what happens at infinite time:

$$\text{If } t = \infty \text{ then } [P] = [R]_0$$

$$\text{If } t = \infty \text{ then } A = \epsilon_P b [R]_0$$

These basic relationships can be used (with a little algebra) to derive a general relationship relating the rate constant to just experimentally measurable absorbencies (the ϵ 's and b 's need not even be known!). Let's begin with the mass balances, using the limiting conditions just described:

$$\text{If } [R]_0 = [R] + [P]$$

$$\text{Then } [P] = [R]_0 - [R]$$

$$\text{And then } A_t = \epsilon_R b [R] + \epsilon_P b [R]_0 - \epsilon_P b [R]$$

$$\text{But recall } A = \epsilon_P b [R]_0$$

$$\text{So that } A_t - A = \epsilon_R b [R] - \epsilon_P b [R]$$

$$\text{and thus } A_t - A = (\epsilon_R b - \epsilon_P b) [R]$$

$$\text{and finally } [R] = \frac{A_t - A}{\epsilon_R b - \epsilon_P b}$$

We now can evaluate the ϵ 's in terms of the limiting absorbencies, A and A_0 as follows:

$$p^b = \frac{A}{[R]_0}$$

$$r^b = \frac{A}{[R]_0}$$

$$r^b - p^b = \frac{[A]_0 - [A]}{[R]}$$

We can now write the ratio:

$$\frac{[R]}{[R]_0} = \frac{(A_t - A)}{(A_0 - A)}$$

and it follows then that:

$$\log_{10} \frac{[R]}{[R]_0} = \log_{10} \frac{(A_t - A)}{(A_0 - A)}$$

or, in terms of what we will measure in the lab:

$$\log_{10} \frac{(A_t - A)}{(A_0 - A)} = - \frac{Kt}{2.303}$$

In the lab, we will be taking a series of measurements of A_0 , A_t , and A . If the aspirin is hydrolyzing as expected, in a pseudo first order manner, then a plot of the left hand side of the above equation, which we will call the log of the reduced absorbance A_R , against time as the independent variable, should produce a straight line, with a slope of $-(K/2.303)$. If there is a problem, the line will not be linear. Since we are working from a methods development perspective, we can choose to make problems deliberately if we wish, such as dissolving the aspirin in water rather than buffer, or making the wrong buffer, or setting dilutions incorrectly, or missing the A_0 or A value, to see the effect on the results. This kind of deliberate "bugging" is a standard approach for developing an analytical method, done to apply known stresses to the method to see how it recovers.

Appendix C: Some Critical Dates in the History of Aspirin

200 B.C.

Greek physician Hippocrates prescribes the bark and leaves of the willow tree (rich in a substance called salicin) to relieve pain and fever.

100 A.D.

Willow leaves are mentioned in the writings of Greek surgeon Dioscorides.

200 A.D.

Romans Pliny the Elder and Galen, a doctor, describe Willow leaves in writings.

1832

A German chemist experiments with salicin and creates salicylic acid (SA).

1897

Felix Hoffmann (pictured at left), a chemist at Bayer in Germany, chemically synthesizes a stable form of ASA powder that relieves his father's rheumatism. The compound later becomes the active ingredient in aspirin named--"a" from acetyl, "spir" from the spirea plant (which yields salicin) and "in," a common suffix for medications.

1899

Bayer distributes aspirin powder to physicians to give to their patients. Aspirin is soon the number one drug worldwide.

1900

Bayer introduces the first aspirin in water-soluble tablets--the first medication to be sold in this form. This new product form cuts costs in half

1915

Aspirin becomes available without a prescription.

1948

Dr. Lawrence Craven, a California general practitioner, notices that the 400 men he prescribed aspirin to hadn't suffered any heart attacks. He regularly recommends to all patients and colleagues that "an aspirin a day" could dramatically reduce risk of heart attack.

1980

The FDA approves the use of aspirin to reduce the risk of stroke after TIA in men (transient "ischemic attack" or "mini-stroke" -- the occurrence of stroke warning signs).

1982

British pharmacologist John R. Vane is awarded the Nobel Prize for Medicine by discovering aspirin's basic mechanism of action. He noted that the action of acetylsalicylic acid is due to inhibition of prostaglandin synthesis.

Appendix D: The Pharmacology of Aspirin - A miracle drug

A marvelous discussion of how aspirin has developed over the years, and why it works, can be found at the web site:

<http://www.chemsoc.org/gateway/chembyte/cib/jourdier.htm>

Since I do not have permission to reproduce this article as [part of this experiment, it will be up to you to read it on the web, or dump a printed copy for your own person use to include here. It is very worthwhile to read.



Figure 29 - Class of 2000 students, Niels Sveum (top left), Andy Lantz,(top right), and Dave Erickson (bottom right) watching Mike the robot do all their work.

Appendix E:**MSDS for Aspirin**-----
1 - PRODUCT IDENTIFICATION

PRODUCT NAME: ASPIRIN
 FORMULA: 2-CH3COOC6H4COOH
 FORMULA WT: 180.16
 CAS NO.: 50-78-2
 NIOSH/RTECS NO.: V00700000
 COMMON SYNONYMS: ACETYLSALICYLIC ACID
 PRODUCT CODES: 0033
 EFFECTIVE: 06/30/86
 REVISION #02

PRECAUTIONARY LABELLING
BAKER SAF-T-DATA(TM) SYSTEM

HEALTH	- 1 SLIGHT
FLAMMABILITY	- 1 SLIGHT
REACTIVITY	- 0 NONE
CONTACT	- 1 SLIGHT

HAZARD RATINGS ARE 0 TO 4 (0 = NO HAZARD; 4 = EXTREME HAZARD).

LABORATORY PROTECTIVE EQUIPMENT
 SAFETY GLASSES; LAB COAT

PRECAUTIONARY LABEL STATEMENTS

CAUTION
 MAY CAUSE IRRITATION DURING USE
 AVOID CONTACT WITH EYES, SKIN, CLOTHING. WASH THOROUGHLY AFTER
 HANDLING. WHEN NOT IN USE KEEP IN TIGHTLY CLOSED CONTAINER.

SAF-T-DATA(TM) STORAGE COLOR CODE: ORANGE (GENERAL STORAGE)

2 - HAZARDOUS COMPONENTS

COMPONENT	%	CAS NO.
ACETYLSALICYLIC ACID	90-100	50-78-2

3 - PHYSICAL DATA

BOILING POINT:	140 C (284 F)	VAPOR PRESSURE(MM HG):	N/A
MELTING POINT:	133 C (271 F)	VAPOR DENSITY(AIR=1):	N/A
SPECIFIC GRAVITY:	1.40 (H2O=1)	EVAPORATION RATE:	N/A
		(BUTYL ACETATE=1)	

SOLUBILITY(H₂O): SLIGHT (0.1 TO 1 %) % VOLATILES BY VOLUME: 0

APPEARANCE & ODOR: WHITE CRYSTALLINE POWDER WITH A SLIGHT CHARACTERISTIC ODOR.

4 - FIRE AND EXPLOSION HAZARD DATA

FLASH POINT (CLOSED CUP: N/A

FLAMMABLE LIMITS: UPPER - N/A % LOWER - N/A %

FIRE EXTINGUISHING MEDIA

USE WATER SPRAY, CARBON DIOXIDE, DRY CHEMICAL OR ORDINARY FOAM.

SPECIAL FIRE-FIGHTING PROCEDURES

FIREFIGHTERS SHOULD WEAR PROPER PROTECTIVE EQUIPMENT AND SELF-CONTAINED BREATHING APPARATUS WITH FULL FACEPIECE OPERATED IN POSITIVE PRESSURE MODE.

TOXIC GASES PRODUCED

CARBON MONOXIDE, CARBON DIOXIDE

5 - HEALTH HAZARD DATA

THRESHOLD LIMIT VALUE (TLV/TWA): 5 MG/M³ (PPM)

TOXICITY:	LD50 (ORAL-RAT)(MG/KG)	- 1000
	LD50 (ORAL-MOUSE)(MG/KG)	- 815
	LD50 (IPR-RAT)(MG/KG)	- 390

CARCINOGENICITY: NTP: NO IARC: NO Z LIST: NO OSHA REG: NO

EFFECTS OF OVEREXPOSURE

DUST MAY IRRITATE EYES.

TARGET ORGANS

NONE IDENTIFIED

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY EXPOSURE

NONE IDENTIFIED

ROUTES OF ENTRY

NONE INDICATED

EMERGENCY AND FIRST AID PROCEDURES

INGESTION: IF SWALLOWED AND THE PERSON IS CONSCIOUS, IMMEDIATELY GIVE LARGE AMOUNTS OF WATER. GET MEDICAL ATTENTION.

INHALATION: IF A PERSON BREATHES IN LARGE AMOUNTS, MOVE THE EXPOSED PERSON TO FRESH AIR. GET MEDICAL ATTENTION.

EYE CONTACT: IMMEDIATELY FLUSH WITH PLENTY OF WATER FOR AT LEAST 15 MINUTES. GET MEDICAL ATTENTION.

SKIN CONTACT: IMMEDIATELY WASH WITH PLENTY OF SOAP AND WATER FOR AT LEAST 15 MINUTES.

6 - REACTIVITY DATA

STABILITY: STABLE HAZARDOUS POLYMERIZATION: WILL NOT OCCUR

CONDITIONS TO AVOID: MOISTURE

DECOMPOSITION PRODUCTS: CARBON MONOXIDE, CARBON DIOXIDE

7 - SPILL AND DISPOSAL PROCEDURES

STEPS TO BE TAKEN IN THE EVENT OF A SPILL OR DISCHARGE

WEAR SUITABLE PROTECTIVE CLOTHING. CAREFULLY SWEEP UP AND REMOVE.

DISPOSAL PROCEDURE

DISPOSE IN ACCORDANCE WITH ALL APPLICABLE FEDERAL, STATE, AND LOCAL ENVIRONMENTAL REGULATIONS.

8 - PROTECTIVE EQUIPMENT

VENTILATION: USE ADEQUATE GENERAL OR LOCAL EXHAUST VENTILATION TO KEEP FUME OR DUST LEVELS AS LOW AS POSSIBLE.

RESPIRATORY PROTECTION: RESPIRATORY PROTECTION REQUIRED IF AIRBORNE CONCENTRATION EXCEEDS TLV. AT CONCENTRATIONS ABOVE 5 MG/M³, A DUST/MIST RESPIRATOR IS RECOMMENDED.

EYE/SKIN PROTECTION: SAFETY GLASSES WITH SIDESHIELDS, PROPER GLOVES ARE RECOMMENDED.

9 - STORAGE AND HANDLING PRECAUTIONS

SAF-T-DATA(TM) STORAGE COLOR CODE: ORANGE (GENERAL STORAGE)

SPECIAL PRECAUTIONS

KEEP CONTAINER TIGHTLY CLOSED. SUITABLE FOR ANY GENERAL CHEMICAL STORAGE AREA.