Stopped-flow Kinetics

Introduction

A number of conventional kinetic techniques measure reactions on a time scale of minutes or hours. The most straightforward way to measure the rate of chemical reactions is to mix two reagent solutions and observe the subsequent change. This operation is best carried out in the observation cell of a spectrophotometer if a change in the UV-visible spectrum is produced. There are two factors that limit the minimum half-life of reactions that can be studied in this way: the speed of mixing and the speed of observation. The fastest way to fill the observation cell is by the stopped-flow technique.

The figure on page IX-7 shows a schematic diagram of a stopped-flow apparatus that fits easily into a spectrophotometer. To perform a run, two drive syringes are filled with reagent from reservoirs and the contents are briskly expelled into the flow circuit using the drive plate. A small volume (250 μL) of each reagent is displaced through the mixer to fill the observation cell. The flow is stopped when the syringe's plunger hits the mechanical stop. The reaction initiated by mixing proceeds in the observation cell and the change in absorbance is monitored by the spectrophotometer. The minimum observable half-life is determined by the spectrophotometer electronics. The freshly mixed solution is only about 20 ms old, but not all commercial spectrophotometers can follow absorbance change in the millisecond time range. Half-lives of down to about 0.5 s can be measured using stopped-flow in Cary 219 spectrophotometer (using the chart recorder). For shorter half-lives we monitor the signal change using an older Beckman spectrophotometer which has been modified for a faster time response. A Macintosh computer is used to collect and analyze the data, giving a system response time of about 0.05 seconds. Try to verify this number in your experiments.

The reaction that you will be studying is that between ferric and thiocyanate ions in perchloric acid solution[1]:

$$Fe^{3+}_{(aq)} + SCN^{-}_{(aq)} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} FeSCN^{2+}_{(aq)} \quad \text{(IX.1)}$$

where k_f and k_r are the rate constants for the forward and reverse reactions. The kinetic analysis is given in Shoemaker *et al.*[2]. The instructor will demonstrate the use of the stopped-flow apparatus. Since the apparatus is fragile and expensive, exercise great care in carrying out the operations. The base and support brackets are made of Acetal, which is attacked by most acids and bases, including perchloric acid. Any spills should be cleaned up immediately.

The rate measurements should be carried out at several temperatures in the range 5 to 30°C, starting at the lowest temperature. For each temperature, the rate constant k_f is calculated from the average value of three tracings of rise of absorbance with time. Also determine the energy of activation for the reaction.

Procedure

Two solutions are provided:

1. 0.020M $Fe(NO_3)_3$, 0.14M $NaClO_4$, 0.20M $HClO_4$ (color coded red)
2. 0.0020M NaSCN, 0.14M $NaClO_4$, 0.20M $HClO_4$ (color coded green)

Please be careful not to contaminate or dilute these solutions. Waste solutions are handled with glassware that is color coded pink.

Data Collection:

0. Turn on the constant-temperature circulator bath and set the temperature to 5°C, if it is not already on. On the RTE-100, hold down "display" while adjusting course and fine knobs to the desired temperature. It may take 10-15 min for the temperature to begin dropping.

1. Read page IX-8 describing the operation on the SFA-11. Practice operation of the apparatus with distilled, deionized water and be sure to remove all air bubbles from the viewing region of the cuvette. Remove the waste solution periodically with a Pasteur pipet and dispose of the waste in the appropriately labeled flask. This is important when using the perchloric acid-based reagents because the apparatus has a slow leak, and perchloric acid reacts with the base material.

2. Close the shutter on the Beckman spectrophotometer and turn the instrument on. Adjust the wavelength to 460nm. *What color is 460nm?*

3. With the shutter on the spectrophotometer closed, adjust the dark current for a 0% transmittance reading.

4. Insert the stopped-flow apparatus cuvette into the cuvette holder of the spectrophotometer, secure it with black masking tape, and cover the sample-holder portion of the spectrophotometer with black cloth. Adjust the slits on the spectrophotometer to read 100% transmittance.

5. Close the shutter and check the 0% transmittance (infinite absorbance) reading. Open the shutter and check the 100% transmittance (zero absorbance) reading.

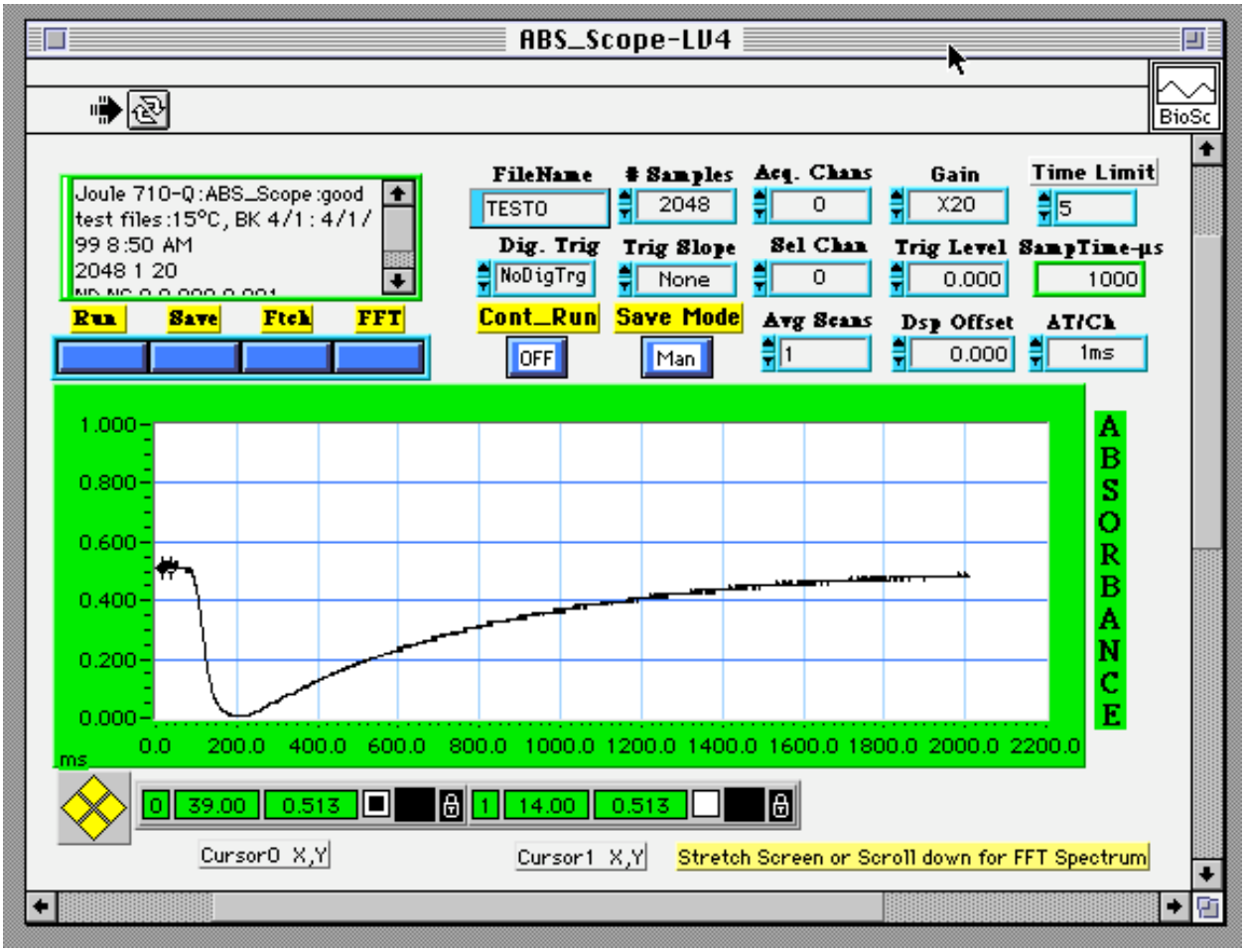
6. Load the Fe^{3+} and SCN^- sample solutions into the appropriate syringes. Inject samples until the waste solution is a dark orange. Watch the meter for a change in the absorbance. When all of the water has been flushed, the absorbance should be about 0.5 or 0.4. When the (transparent) reagents are injected, the absorbance should drop to a low value (<0.2), then return to the maximum value. Your objective is to measure the "decay-time" for this process.

7. When you are satisfied with the measurements that you see on the meter, turn the meter off and plug in the coaxial cable that is attached to the Macintosh to the meter box.

8. To collect data on the Macintosh, you will use a data collection routine "ABS_scope_5.1" written by Bryce Babcock in the LabVIEW™ programming environment. An upgrade is scheduled for the 2003-2004 academic year, so your details may vary. Open the program and examine the options available. The default settings are probably:

    2ms = Time Base (ΔT/Ch)
    2048 = # Samples
    Cont(inuous run) = off

    The only parameters that you will need to change are the number of samples and the time base. At higher temperatures, the decay rate will be faster, and you will want to decrease the time base. Resist the temptation to open menus, click on buttons, and do all of the things that you normally do with a new Macintosh program. You run the risk of altering the data collection routine. In particular, do not Open..., or Save from the File menu, as this will open or save a new data collection program (not a data file). If you have changed several parameters, but want to get back to the original settings, under OPERATE choose "Reinitialize All to Default."

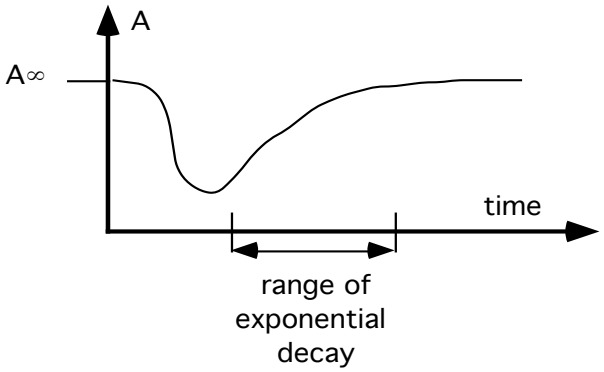


9. To start a run, click on the blue "RUN" button. Immediately after clicking, push in the plunger of the stopped-flow apparatus to start the reaction. This synchronization will take some practice.

10. Wait a minute or so for the program to process the data. The run button will blink yellow briefly . Eventually, you will see a plot of your data appear on the screen.

11. If you like the data, click on the blue "SAVE" button. The save dialog is rather strange. (It looks like an open-file procedure). The first time create a folder for your data by selecting "new," entering a folder name, and clicking on "folder." Then save the file by clicking "new," entering the file name, and clicking on "file."

    It will save you a great deal of time during data analysis if you record in your lab notebook the time over which the decay is exponential.



    The first 100 or so data points are worthless, because the reaction has not yet started. The final 100 or so data points are also worthless because the reaction is essentially complete. The return to A_∞ is still exponential at long times, but the resolution of the data collection is not high enough to pick up the small differences between A_∞ and A_t. The "art" of data analysis will come later when you must decide exactly where to truncate the data for your fit. A range where the absorbance lies between 10 and 90 % of its maximum range is reasonable. You should also note the approximate value of A_∞, the infinite time absorbance.

12. Collect two or three data scans at each of four or five temperatures in the range 5 to 30˚C. (The data below room temperature will probably be more reliable.) For temperatures above 5°C, the reaction will be faster and you can use 1 ms for the time base (ΔT/Ch) with 2048 samples. You may want to make even further adjustments, such as reducing the number of samples, as you continue to raise the temperature.

13. When you are finished, quit the LabVIEW™ routine by clicking on Quit in the File menu. Please also unplug the coaxial cable from the meter-box, turn off the spectrophotometer, turn off the circulator bath, and rinse the stopped-flow apparatus thoroughly with water.

Data Processing

1. The data files that you have saved are in text-file format so that a word processor, a spreadsheet or a plotting program may read it. You may choose any analysis program, such as Excel, Kaleidagraph, DeltaGraph, or Igor. The first line lists the file name including path where it was saved plus the date and time. The second line lists the number of samples collected (2048 in most cases), the number of channels collected, and the gain. The third line lists 4 triggering parameters followed by a very important number: The "sample time interval." This tells you how many milliseconds between each absorbance reading. Following these data collection parameters are the actual absorbance reading, all in one column.
2. Open the analysis program and open the data file of interest. (NOTE: if you are using Kaleidagraph and some data columns disappear, 1. set-up importing "text" but then say cancel just before starting, 2. then import normally.)
3. You will need to generate a time column for your data. You can use the series function in Excel to step up from 0 in increments of the "sample time interval."
4. Scroll to the very end of the file and record the maximum absorbance reading from column one. Column two contains the time in milliseconds. If the run did not go to completion, it may be safer to use the initial (pre-injection) value for A_∞.
5. Delete or mask the leading and ending series of data points, so that you have a set of points where exponential decay is observed. (Masking in Kaleidagraph tells it to ignore these certain points.) The range where data points are exponential is somewhat arbitrary, and is best chosen by examining a plot. Hopefully, you recorded the proper range when you collected the data.
6. Generate a new data column of the natural log of your final absorbance minus the absorbance at any given time, $\ln (A_\infty - A_t)$.
7. Prepare a graph of $\ln(A_\infty - A_t)$ versus time, and find the slope of this line using a linear-least-squares approach. The slope of this line is equal to $-([Fe^{3+}] + \frac{1}{K_{eq}})k_f$, where k_f is the rate constant for the reaction $Fe^{3+} + SCN^- \rightarrow FeSCN^{2+}$. Use the equilibrium constant, $K_{eq} = (146 \pm 5) M^{-1}$ at 25°C. Note that you will have to adjust the equilibrium constant for the temperature that you used.
8. Alternatively, instead of step 6 and 7 you can directly plot A_t vs. t, and use an exponential fitting function to the data. The fit routine will adjust the initial and final value of the absorption automatically. Instead of the slope of the linear fit use the exponential decay rate. There are advantages to either approach.
9. The Arrhenius equation, $k_f = A \exp(-E_a / RT)$ may be used to calculate the activation energy, E_a, for the reaction. Prepare a plot of $\ln(k_f)$ versus (1/T).

========== Kaleidagraph ==========

"File" "Import" "text" {default setting of tab-delineated is good}
You can rename the columns (such as "Time" and "Absorbance") by double clicking on the current column names, "A", "B", ...(Kaleidagraph may make the first data point the column headers--you can easily afford to lose the first data point, so just write over it with your column names)

To do the data manipulation, you need to add more columns in the data section, under "Data" choose the appropriate command.
Then, "Windows" "Formula Entry" "c3=c2-c1" or "c4=c2^2."

Next, **make a graph**
From the menu at the top, choose "Gallery," "Linear," and then "Scatter" or "Line." Pick the appropriate X and Y axes.
If you want to find the best-fit line, choose "Curve Fit" and "Linear."
If Kaleidagraph does not automatically display the equation, choose "Plot" and "Display Equation."
Be sure to look at the line it gives you to make sure the line is reasonable.

Once you have your graph, you can make all kinds of changes--double click on the title or axes text and play with the font, etc. Also, you might find more points you want to delete.

If you make several plots, the previous ones are hidden. To see them, choose "Windows" and "Show Plot."

========== Excel 4.0 ==========

Open one of your data files.
If you don't know exactly which range of data you want to keep and what to throw out, you can make a quick graph and throw out at least those points that are clearly not going to be good (all of the initial stuff and much of the end)
In cell c1, type "=-a1" {return}.
Now copy this to the rest of the column. Either use cut and paste, or highlight the appropriate cells and use apple-D to "fill down."

Making a graph
Highlight the numbers you've entered.
Select button second from right at top toolbar. (The one that's a combination bar graph, line, and scatter plot)
Go to a free area of your worksheet and highlight a region where you want to place your graph.
Answer the questions (try x-y scatter first, format 1 or 2)
Play with options.
Once you've made a graph of the data in the form where it should be linear, you might identify more data points at either end to delete.

Then, to get the **slope** of the numbers.
Go to a blank cell. From the top menu items select "Formula," "Paste Function," "Statistical," "Slope" "OK"

Where it asks for "Known-Y's" highlight the y-data with the mouse. Then with the mouse go back to the formula, highlight the words "Known-x's" and then go to the spreadsheet and highlight your x-data. Press enter
Find the **intercept** similarly.

Lastly, to get this **line onto our graph** you calculate the y-points from the slope and intercept and your x-data, put these into a new column, and graph your y data and the calculated y-line against the x-points.

References

1. J. F. Below, Jr., R. E. Connick, and C. P. Coppel, *J. Am. Chem. Soc.* **80**, 2961 (1958).
2. D.P. Shoemaker, C.W. Garland, J.I. Steinfeld, and J.W. Nibler, *Experiments in Physical Chemistry*, 4th ed. (McGraw-Hill Book Co., New York, 1981). The third or fifth edition of this text is acceptable. Copies are available on the CHEM 302 reserve shelf or from the chemistry office.

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From the screen click the Start button first and second the arrow. To save the data the red light on the top of the menu should be on or running.

After saving the data press the red button to stop the run. Remember that the saving is always as a new file.

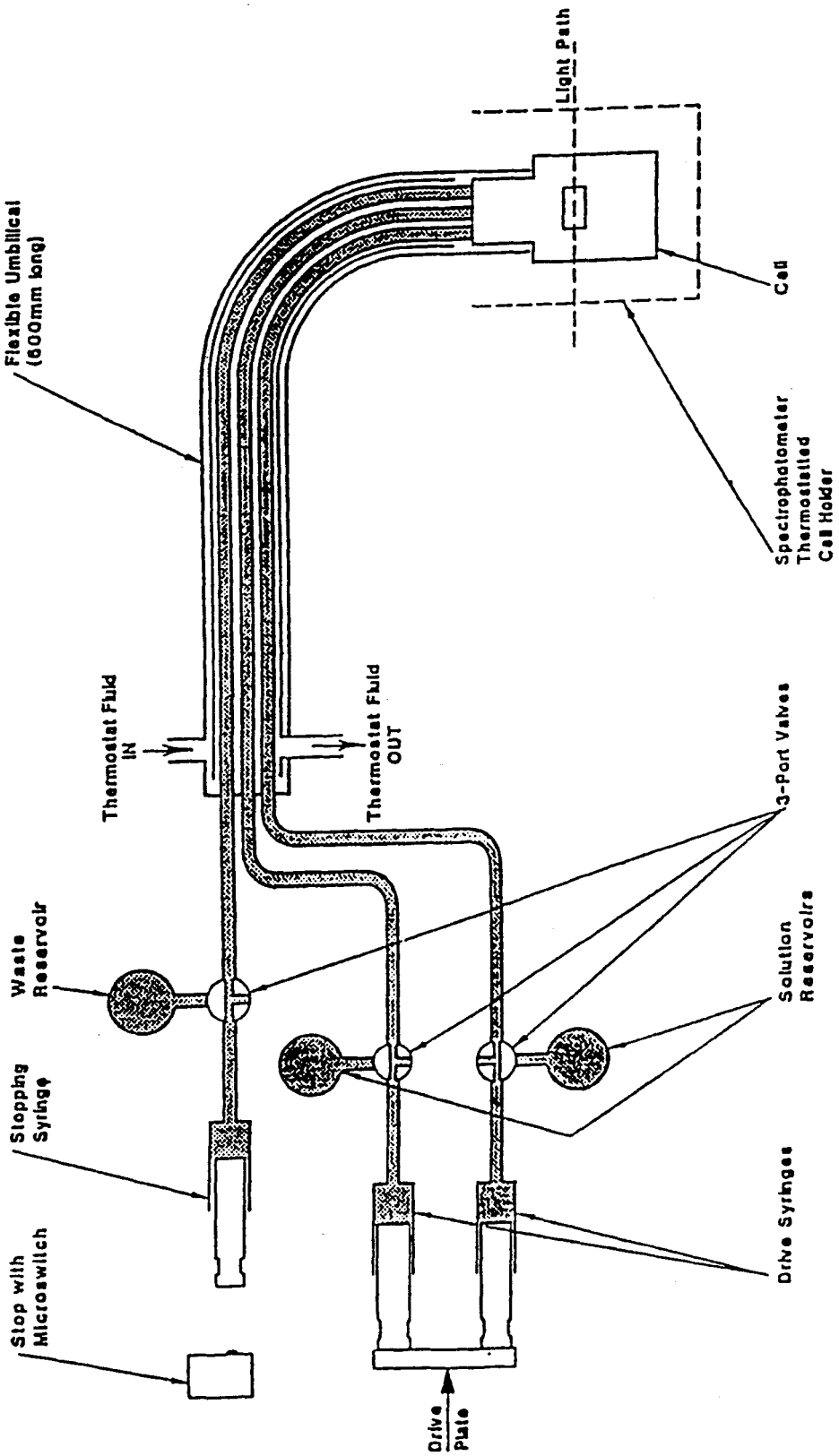
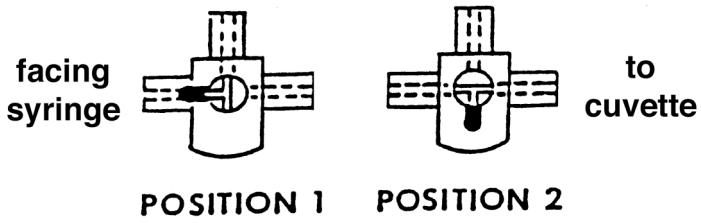


Fig: 1: Schematic of Stopped Flow Apparatus

Operation of the SFA-11

There are two positions for the three-way taps. In position 1 the operating lever points horizontally towards the syringe. In position 2 the lever points vertically downwards.



1. Half - fill the open syringes, with their reagent solutions.
2. Turn all taps to 1. Push and pull rapidly each drive syringe piston so as to fill the drive syringes and expel air bubbles. Pull the drive syringe pistons out as far as possible. Push the stopping syringe piston in as far as possible.
3. Turn all taps to 2. Push firmly on the drive plate. The best way to do this is to squeeze the drive plate and the stopping block firmly together, using the thumbs. and first fingers of both hands, one on each side.
4. Turn all taps to 1. Push the stopping syringe in as far as possible, and pull the drive syringe pistons out as far as possible.
5. Repeat 3 and 4 a few t times with the taps set at 1, push and pull the waste syringe piston to and fro, so as to expel all the air bubbles.
6. To prepare the unit for a run, now that all air bubbles have been expelled, turn all taps to 1, fill the drive syringes and empty the waste syringe. Refill the reservoir syringes as required to prevent further air being drawn in.
7. To perform a run, turn the taps to 2, start the data-capture system, and squeeze the drive plate and stopping block firmly together. The spectrophotometer signal may vary slightly with how hard this squeeze is. For reproducibility adopt the same procedure for each run. . If the run is complete in less that a minute or so, keep squeezing until the reaction is complete. If the reaction is slower, release the pressure as soon as the flow stops.

SPECTROPHOTOMETER REPONSE TIME.

A useful check on the spectrophotometer and data-capture system response time may be obtained by observing the initial part of the run. At the start of the push the old, reacted, solution is swept out of the sample cell in about 20 milliseconds, and there is a rapid absorbance change as the new, unreacted, solution comes in. The observed time for this process will be the response time for the spectrophotometer and data-capture system, which is normally much greater than 20 milliseconds.