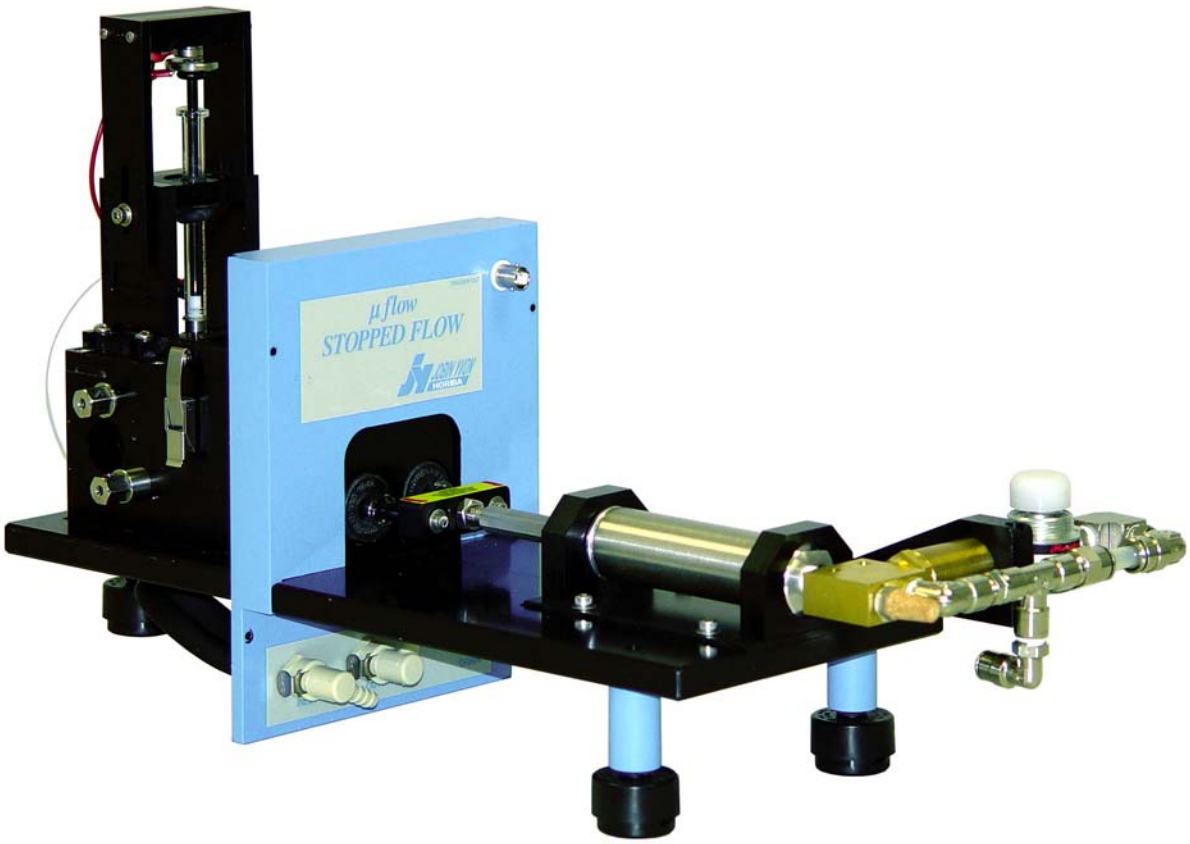
F-3009 MicroFlow



Operation Manual

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March 2002

Part Number 81068

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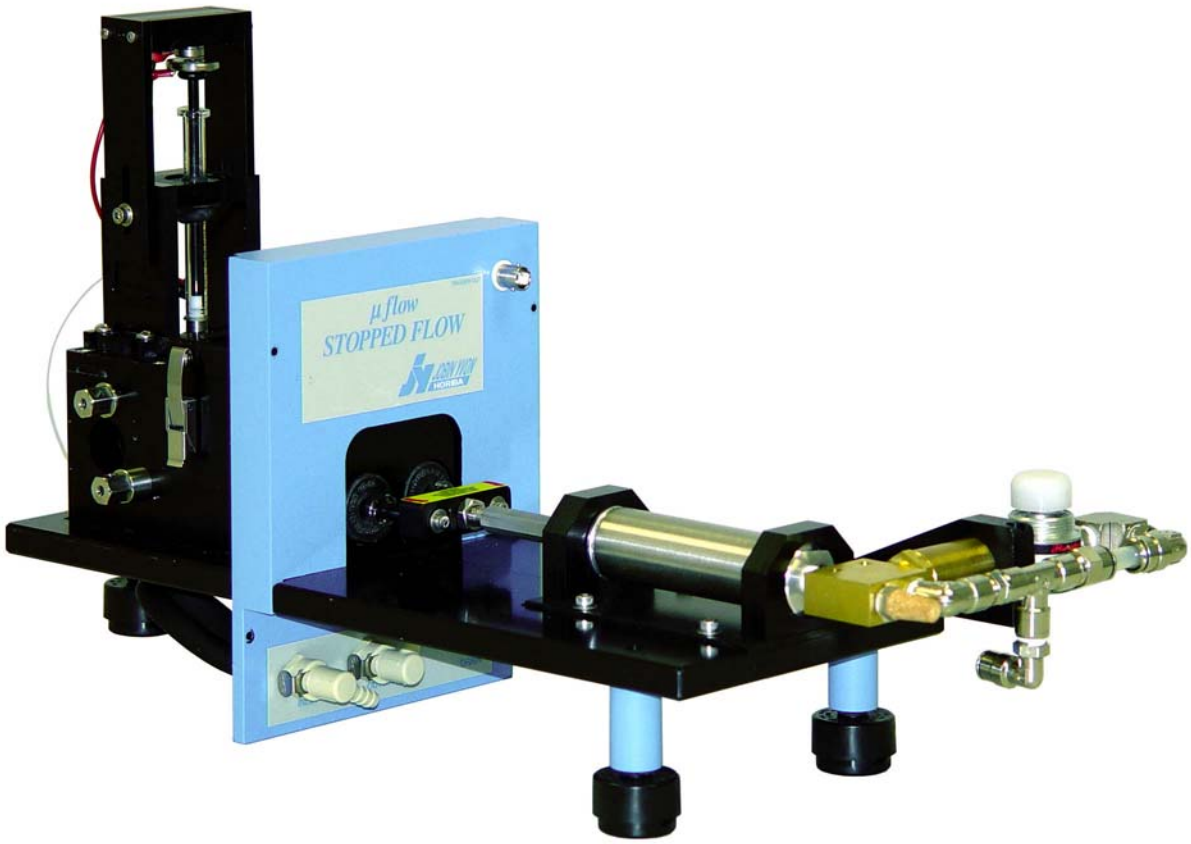
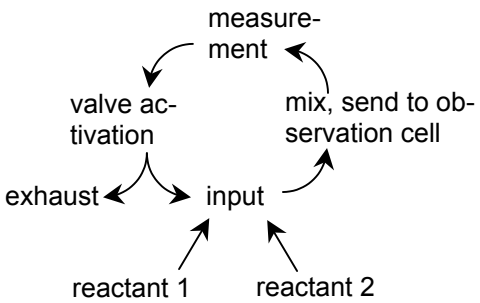
0: Introduction



About the MicroFlow

The MicroFlow is used with the Fluorolog®-3, FluoroMax®-3, and Fluorolog®-Tau-3 spectrofluorometers and the DataMax time-based scan-type to study moderately fast reactions from the point of mixing onwards. The reaction kinetics of two reactants may be examined under different mixing ratios, by varying temperatures, and repetitively. A schematic of the MicroFlow's operation cycle is shown here:

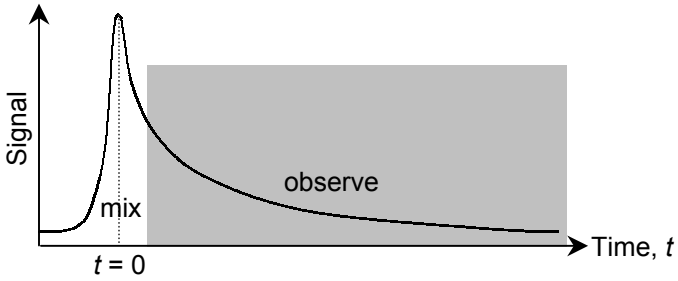


The MicroFlow permits observation of the reaction rate of two reactants vigorously forced through a mixing chamber and into an observation cell. The two solutions are contained in drive syringes driven simultaneously by an air-operated plunger or manu-

ally. Reagent volumes are varied by changing the drive syringes. The syringes are borosilicate glass with a controlled inner diameter, and they contain precision-machined-Teflon® plunger-tips. This glass-and-Teflon® construction provides a nearly inert flow-path for almost any reaction. The syringes rely on an interference fit for a seal between the plunger-tip and inside of the glass syringe. This fit allows interchanging of common parts for easy replacement.

The thermostatting chamber-cover contains a thermometer well to monitor temperatures during a reaction. We recommend a thermometer with a range of –5 to 50°C with 0.2°C divisions. The well encloses ~ 60 mm of the thermometer, so that the chamber temperature can be observed from 10–45°C.

After leaving the observation cell, the mixed reactants advance the piston of a stop-syringe , which is brought to a halt against an adjustable barrier, the "stop block" (see photograph at right). Upon contacting the stop block, an electrical circuit is completed, triggering the start of data-acquisition at time $t = 0$, as shown in the graph below.



Stop block with electrical contact.

After observation of the reaction, the MicroFlow empties the solution in the stop-syringe. Two 5-mL fill-syringes are reservoirs for loading more solution into the drive syringes. Exhaust- and fill-valve levers fill the drive syringes and empty the stop-syringe.

The valves, mixing chamber, and observation cell are made of chemically inert Kel-F®, Teflon®, glass, and quartz. A push-button air valve controls the air- (or nitrogen-) operated plunger to advance the drive pistons. The air piston operates at a maximum line pressure of 90 psi (0.62 MPa).

This manual explains how to operate and maintain the MicroFlow. The manual also describes measurements and tests essential to obtain accurate data. For a complete discussion of DataMax, see the *DataMax Operation Manual* (data-acquisition information) and the *Grams/32® User's Guide* (post-processing instructions for data manipulation).

Note: *Keep this and the other reference manuals near the system.*

Symbols used in this manual

Certain symbols are used throughout the text for special conditions when operating the instruments:

Warning:

A hazardous condition exists, or danger exists that could damage the equipment. Jobin Yvon Inc. is not responsible for damage arising out of improper use of the equipment.

Note:

General information is given concerning operation of the equipment.

1: Theory of Operation

Path-length

The total path-length of the observation cell for absorbance measurement is 10 mm. When a fluorescence measurement is made at a right angle to the flow, the path length is ~ 2 mm.

Dead time

The *dead time*, T_d, is the time required for the reacting solution to flow from the mixing point to the observation point. The dead time, in s, is calculated by

$$T_d = \frac{V}{U}$$

where V is the volume of the reacting solution in mL, and U is the flow velocity in mL s^{-1}. At a particular flow velocity, the dead time is governed by the cell used. For absorbance measurements using the 10-mm path-length, V between mixing and observation is 0.04 mL. For fluorescence measurements using the 2-mm path length, $V = 0.02$ mL. At a flow velocity of 20 mL s^{-1}, the dead times are 2.0 ms and 1.0 ms, respectively.

The first observation can be made only after T_d. If the half-life of the reaction is equal to T_d, then 50% of the reaction is completed by the time the solution reaches the point of observation. In general, observation of the remaining 50% of the reaction is sufficient for kinetic analysis.

Efficiency of mixing

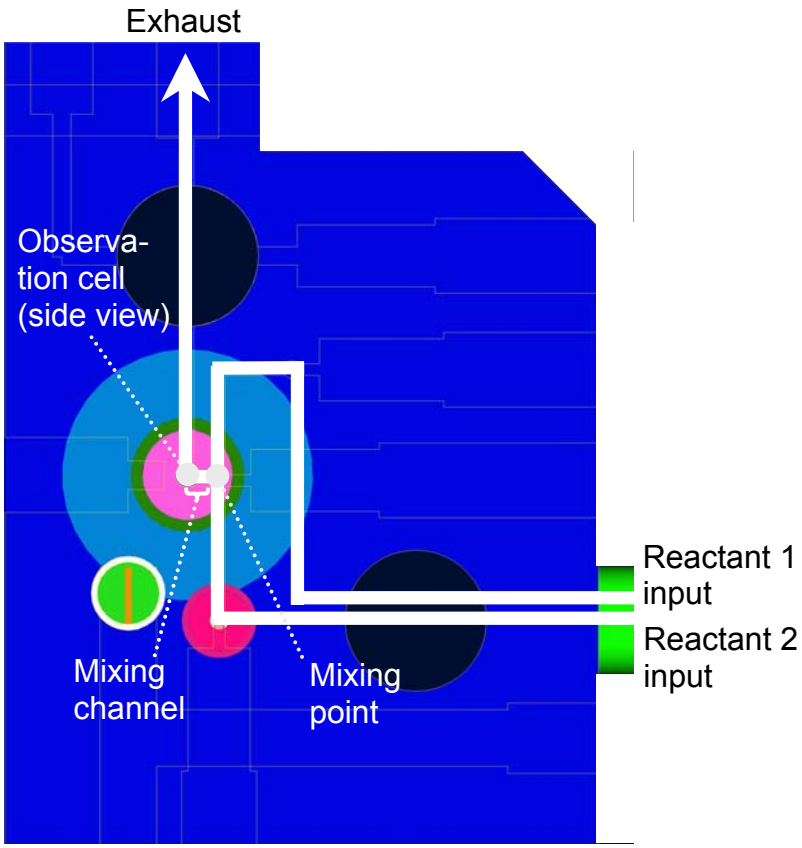
The efficiency of mixing is found by observing the rate of formation of blue peroxychromic acid, CrO_5, and calculating the third-order rate constant, k, for this reaction. The rate expression is

$$\frac{d[CrO_5]}{dt} = k[H^+][H_2O_2][HCrO_4^-]$$

The reaction is run under pseudo-first-order conditions; conditions may be varied to give half-lives from 0.001 to 0.1 s. The measured pseudo-first-order rate constant then is used to calculate the third-order rate constant.

Transport time

The transport time is the interval during which the reactants flow. The volume of solution delivered must be sufficient to fill the volume in the tubing between mixing point and observation point. If this is true, the mixed reactants from a previous experiment are flushed through the observation area and replaced with fresh solution.



Internal flow path of the mixing block, shown from the side. Reactant 1 input is offset slightly above Reactant 2 input for clarity; it ought to be directly behind Reactant 2 input.

Above is a schematic of the side of the mixing block. The white arrow shows the flow direction. The volume of the observation area is ~ 32 µL (i.e., 2 mm dia. × 10 mm long), and the volume of the mixing channel is ~ 2 µL. Therefore, at least 34 µL of freshly mixed reactants are required per experiment. Empirically, for best reproducibility, at least twice the volume of total reactant (~ 80 µL) is required to flush the system. At a flow rate of 20 mL s^{-1}, the transport time is 4.0 ms.

2: Requirements & Installation

Surface requirements

- A sturdy table- or bench-top.
- Surface must hold 6.8 kg (15 lbs.) beyond the current instrument's weight.
- Surface should accommodate 6" × 18" (15 cm × 43 cm) extra from side of the existing instrument's sample compartment.
- Overhead clearance should be at least 15" (38 cm).

Environmental requirements

- Temperature 59–86°F (15–30°C)
- Maximum temperature fluctuation ± 2°C
- Ambient relative humidity < 75%
- Low dust levels
- No special ventilation
- Available regulated pressure source (maximum 90 psi, 0.62 MPa for 2.5-mL syringes supplied)

Warning: *Excessive humidity can damage the optics.*

Unpacking and installation

Introduction

The MicroFlow is delivered in a single packing carton. All cables, software, and manuals ordered with the system are included with the delivery.

Examine the shipping boxes carefully. Any evidence of damage should be noted on the delivery receipt and signed by representatives of the receiving and carrier companies. Once a location has been chosen, unpack and assemble the equipment as described below. To avoid excessive moving and handling, unpack the equipment as close as possible to the selected location.

Warning: *The MicroFlow is delicate. Mishandling may seriously damage its components.*

Note: *Many public carriers will not recognize a claim for concealed damage if it is reported later than 15 days after delivery. In case of a claim, inspection by an agent of the carrier is required. For this reason, the original packing material should be retained as evidence of alleged mishandling or abuse. While Jobin Yvon Inc. assumes no responsibility for damage occurring during transit, the company will make every effort to aid and advise.*

MicroFlow carton contents

Quantity	Item	Part number
1	MicroFlow	F-3009
1	Sample-chamber lid assembly	352420
1	Removable valve-handle (wrench)	992010
1	Nylon tubing, 144" (366 cm) long	535056
2	2.5-mL plastic syringes	
1	Trigger box	TRIG-15/25
2	Hose clamp	60352
2	#4-40 Phillips screws, ½" long (for FluoroMax®); or 5/8" long (for Fluorolog®)	61555 or 61552
2	#4-40 Phillips screws, 1¼" long (for FluoroMax®); or 1½" long (for Fluorolog ®)	61553 or 600105
1	Cable (BNC male–double banana plug), 48" (122 cm) long	980044
1	*MicroFlow Operation Manual*	81068

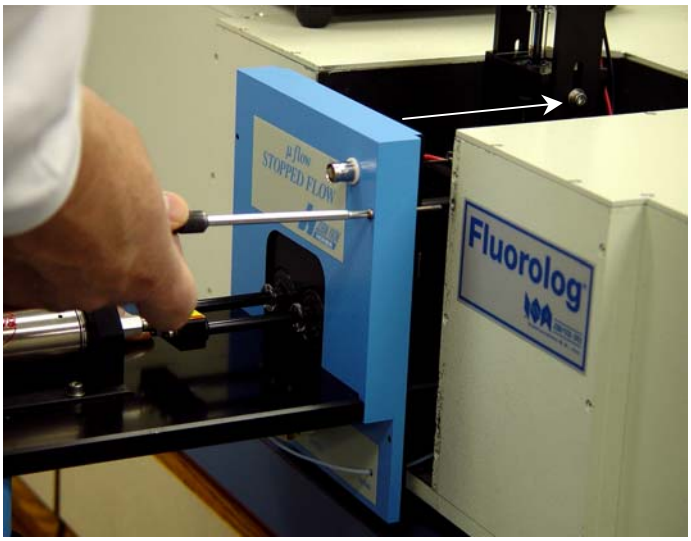
Note: *For a MicroFlow to be mounted in a FluoroMax®, 2 pairs of screws, ½" and 1¼" long, are included. For a MicroFlow to be mounted in a Fluorolog®, 2 pairs of screws, 5/8" and 1½" long, are included.*

MicroFlow unpacking and installation

1. Carefully open the shipping carton.
2. Remove the foam-injected top piece and any other shipping restraints in the carton.
3. Carefully lift the MicroFlow from the carton, and rest it on the laboratory bench.
4. Place the accessory in its permanent location.
5. Inspect for previously hidden damage.

   Notify the carrier and Jobin Yvon Inc. if any is found.
6. Check the packing list to verify that all components are present.
7. Close spectrofluorometer's emission shutter.
8. Remove the existing sample holder from the spectrofluorometer's sample compartment.
9. Slide the MicroFlow into the sample compartment.

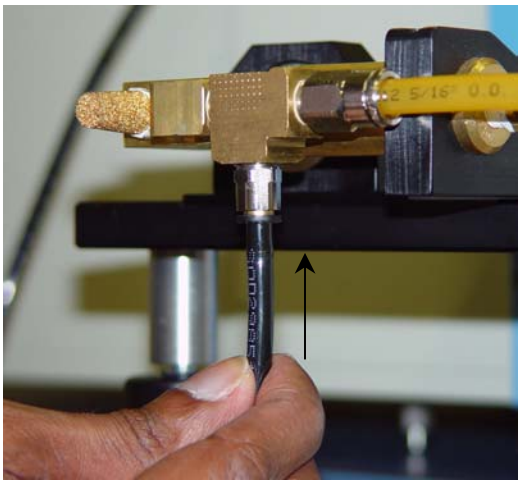
   Be sure that the electrical connections in the back of the MicroFlow mate properly to the instrument.



10 Secure the front panel of the MicroFlow to the instrument with the four #4-40 stainless-steel screws.

11 Connect the air or N_2 supply to the air-supply nozzle on the MicroFlow, using 5/16" (8-mm) I.D. hose.

Pull out the black ring, while inserting the hose.



12 Turn on the air or N_2 supply.

See the chart on the next page for maximum safe operating pressure.

Warning: *Never exceed a line pressure of 90 psi (0.62 MPa). Use a 1% regulator and gauge to control line pressure accurately. Always wear protective goggles when working with pneumatic systems.*

13 Place the free end of the Teflon® drain tube into an appropriate waste-solution receptacle (e.g., 250-mL Ehrlenmeyer flask).

Warning: *Always follow local laws concerning disposal of hazardous wastes.*

Maximum safe operating pressure according to drive syringes used

Drive-syringe volume ratio	Syringe volumes (µL)	Maximum safe operating pressure (psi)	(MPa)
1:1	500, 500	19	0.13
	1000, 1000	47	0.32
	2500, 2500	90	0.62
2:1	250, 500	13	0.090
	500, 1000	30	0.21
4:1	250, 1000	27	0.19
5:1	100, 500	13	0.090
	500, 2500	60	0.41
10:1	50, 500	10	0.069
	100, 1000	23	0.16
	250, 2500	60	0.41
20:1	25, 500	11	0.076
	50, 1000	22	0.15
25:1	100, 2500	60	0.41
40:1	25, 1000	21	0.14
50:1	50, 2500	55	0.38
100:1	25, 2500	56	0.39

3: Preparation for Use

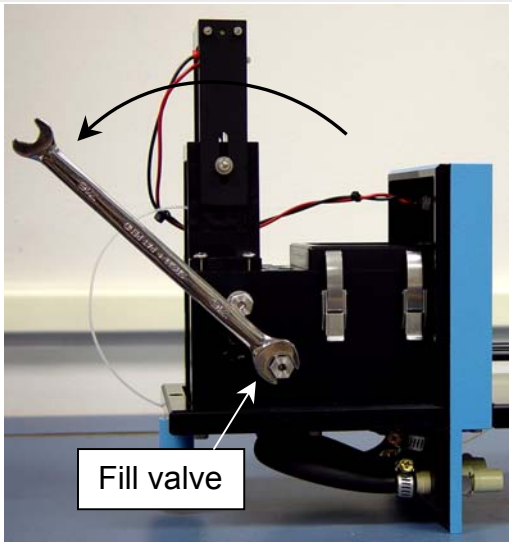
1 Fill the plastic fill syringes with distilled or de-ionized water.

Note: *For first-time cleaning, use 100 mL of 50:50 water-ethanol mixture (instead of pure water) to clean out contaminants.*

2 Rotate valve handle to fill position.

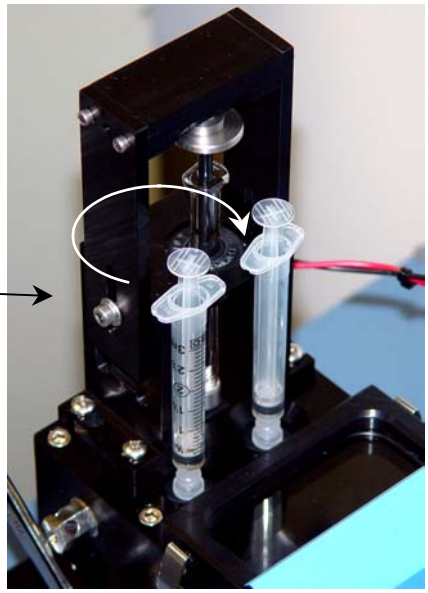
Note: *Opening or closing a valve requires four steps:*

- *Move the handle.*
- *Remove the handle.*
- *Replace the handle.*
- *Continue moving the handle another ~1/4 turn until the valve "grips" properly.*



3 Insert the tips of the fill syringes into the top of the mixing block (while facing the accessory).

4 Rotate the syringes clockwise until they feel tight.



If this is the first time the MicroFlow is cleaned, wait 5 min before proceeding further. This helps any deposits in the system dissolve and get flushed from the system.

5 Flush the reactor.

a Be sure that the fill-valve lever is open (vertical) as shown in step 2.

Warning: *Do not overtighten syringes!*

b Remove the two screws that join the drive syringes to the drive block.

c Manually depress one of the fill syringes, while guiding the corresponding drive syringe toward you.

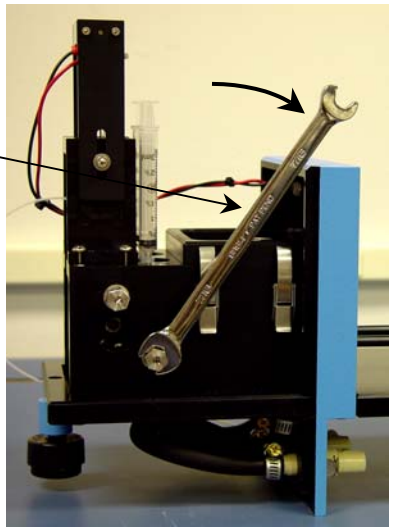
Note: *This procedure may be performed on one or both sets of corresponding syringes simultaneously.*

d Reverse step c, that is, manually push the drive syringe inward while guiding the fill syringe upward.

e Repeat steps b through d three to five times, to ensure complete cleaning.

f Depress both drive syringes to empty them completely.

g Rotate the fill valve forward to closed position.



h Remove the fill-valve lever and the fill syringes.

i Empty the fill syringes.

j Refill them with de-ionized water.

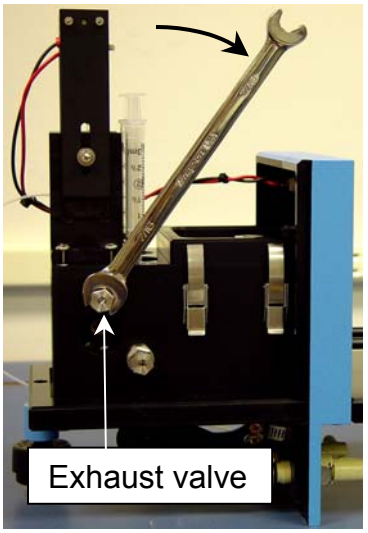
k Install the fill syringes.

Note: *Detaching each syringe from the drive block, to fill each drive syringe individually, may be more convenient.*

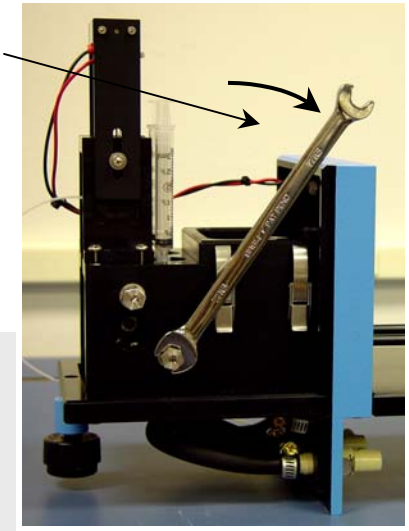
l Repeat the flushing process.

m Flush several times with de-ionized water.

6 Place exhaust-valve lever in the Run position (forward).



7 Close the fill valve by moving the fill-valve lever to the Run position (forward).

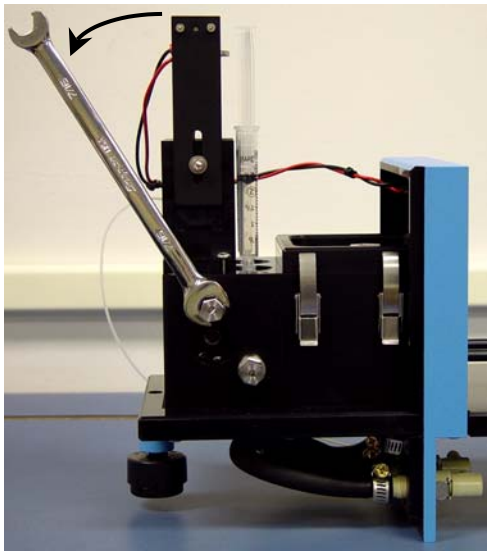


8 Remove the fill-valve lever from the valve.

Note: *Keep the exhaust valve in the Run position before removing the fill-valve lever.*

9 Expel spent reactants from the stop syringe.

a Open the exhaust valve by moving the exhaust-valve lever to the exhaust position (back-ward).

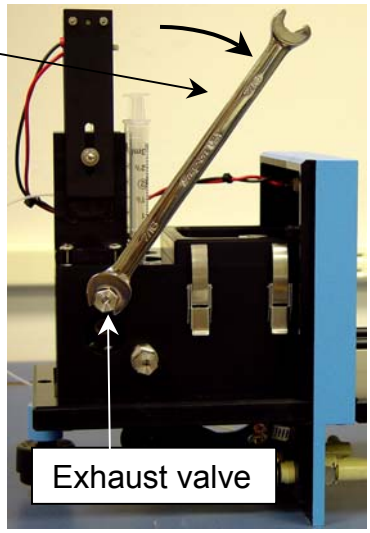


b Manually push down the piston of the stop syringe.

This removes spent reactants through the Teflon® drain tube into the waste receptacle.

10 Close the exhaust-valve lever (forward).

11 Leave the Microflow filled with water.



Note: *When the Microflow is not in use, store it filled with distilled water. This prevents drying of reagents in the internal passages.*

12 Check for bubbles in the system by manually pushing in on the drive block until the pistons are completely depressed.

Warning: *Do not press the pneumatic valve button while adjusting the pistons. Failure to attach the drive syringes* ***before*** *pneumatic activation of the drive block can damage severely or shatter the drive syringes.*

Warning: *Make sure that both drive syringes are secured to the drive block. The syringes are attached to the drive block using the screws provided; check that they are tight.*

4: Data Acquisition

Warning: *Do not use the MicroFlow when the* **Real Time Display** *is open. Spurious peaks in the data may result, or the signal may drop to zero (with no detectable noise).*

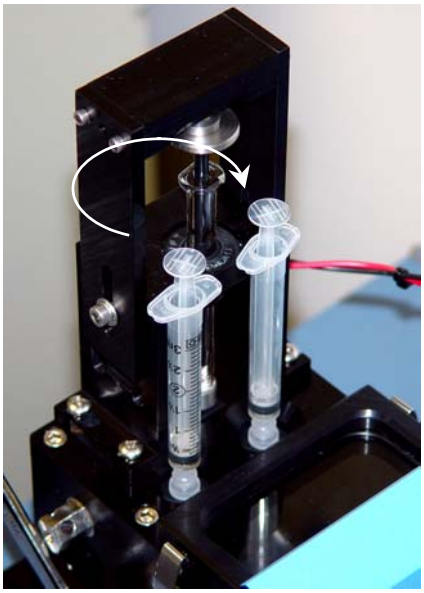
1 Using the solvent or buffer for the system under study, clean out the MicroFlow according to Chapter 3, steps 1–10.

2 Fill the plastic fill syringes with re-agents.

3 Insert them into the top of the mixing block.

The graduated markings should face away from you.

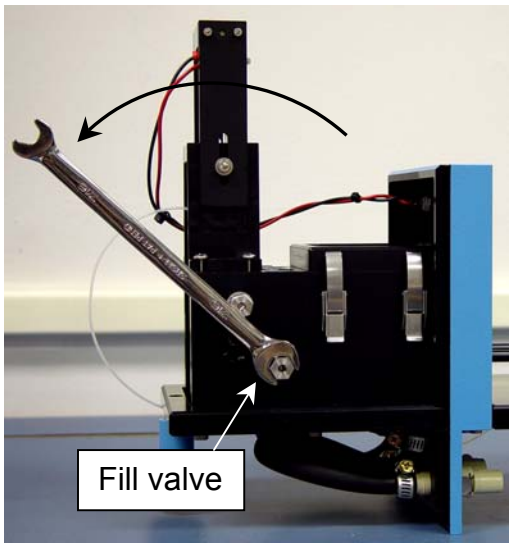
4 Turn them clockwise until tight.



Warning: *Do not overtighten syringes!*

5 Place the fill-valve lever onto the fill valve.

6 Open the fill valve by moving the fill-valve lever backward to the fill position.

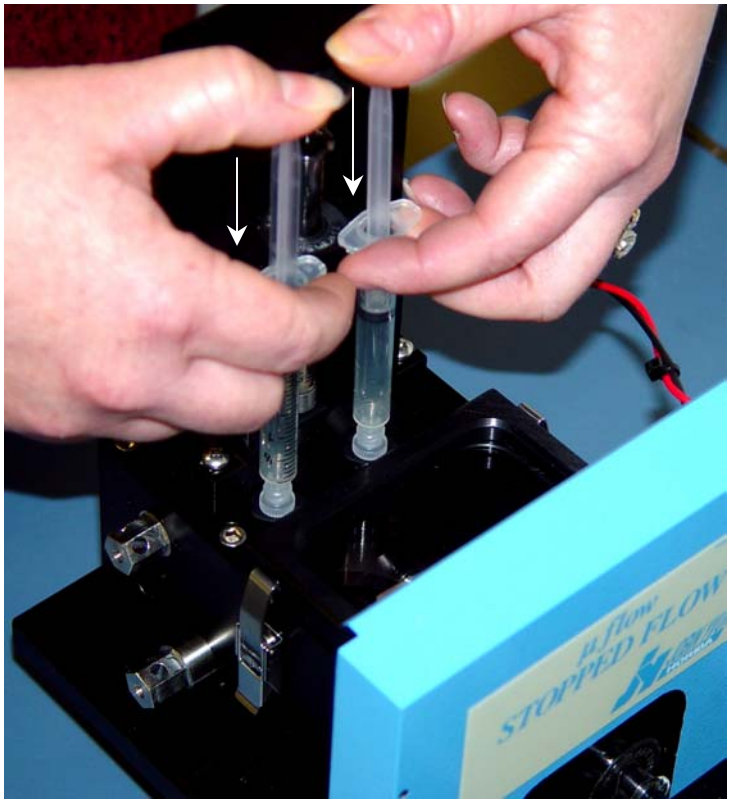


Note: *Opening and closing a valve requires four steps:*

- *Move the handle.*
- *Remove the handle.*
- *Replace the handle.*
- *Continue moving the handle another ~1/4 turn until the valve "grips" properly.*

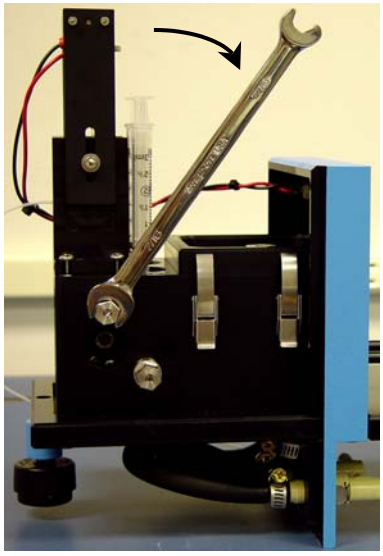
7 Manually depress both fill syringes.

As the syringes are depressed, the reactants are transferred to the drive syringes housed in the thermostatted chamber. The filling process continues until the drive block is fully retracted against its internal stop.



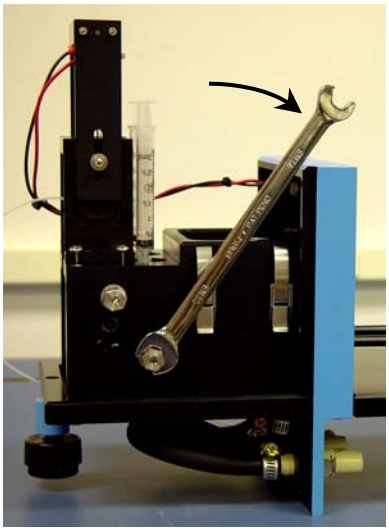
Note: *Detaching each syringe from the drive block, to fill each drive syringe individually, may be more convenient.*

8 Place exhaust-valve lever in the Run position (forward).



9 Close the fill valve by moving the fill-valve lever to the Run position (forward).

10 Remove the fill-valve lever from the valve.

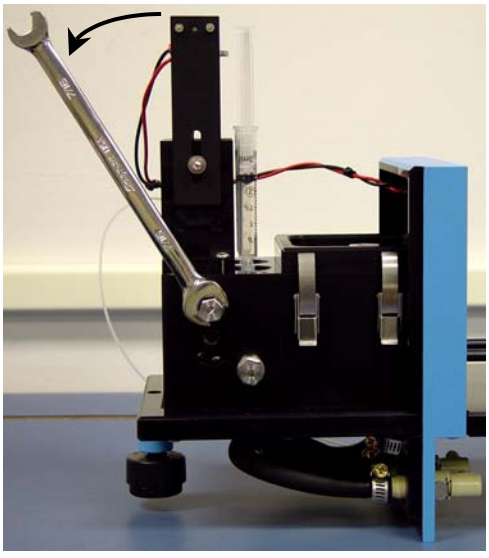


Note: *Keep the exhaust-valve lever in the Run position before removing the fill-valve lever.*

11 Replace the cover plate of the reactor and the sample-chamber cover.

12 Expel spent reactants from the stop syringe.

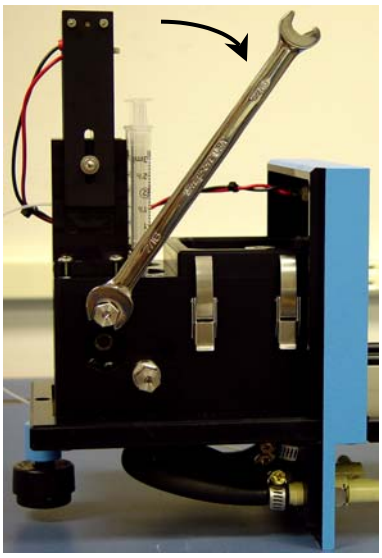
a Open the exhaust valve by moving the exhaust-valve lever to the exhaust position (backward).



b Manually push down the piston of the stop syringe.

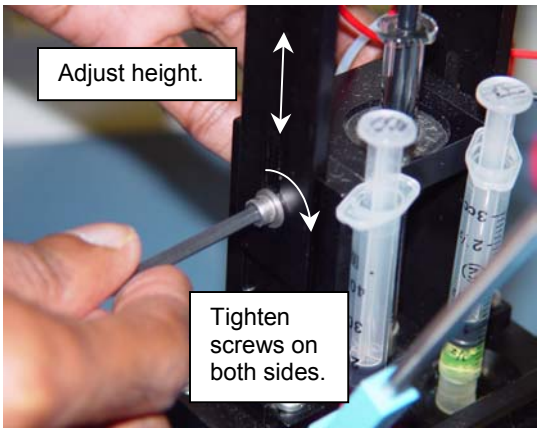
This removes spent reactants through the Teflon® drain tube into the waste receptacle.

13 Close the exhaust-valve lever (forward).



14 Adjust the stop-block adjustment for the total mixing volume.

Raise or lower the stop block to offer the desired volume. The screws must be tight, or the stop block moves when stop occurs. Use the 9/64" Allen key provided.

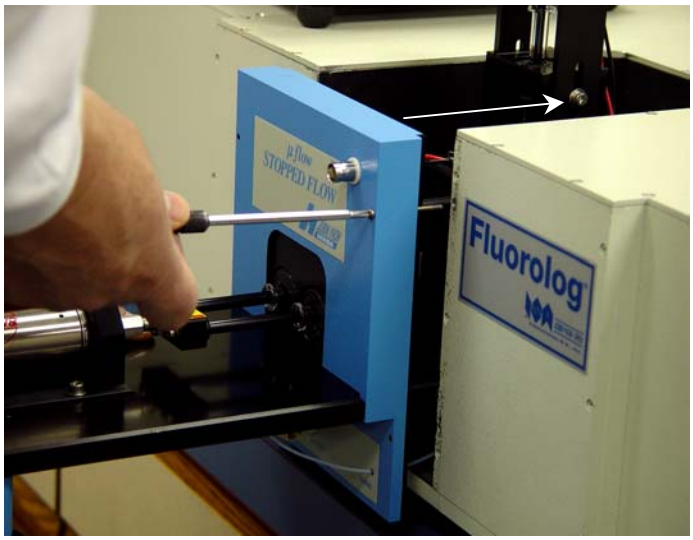


Note: *The height of the stop block is proportional to total mixing volume.*

Note: *The stop block must be secured, the fill-lever valve must be removed, and the exhaust valve must be in the correct position.*

15 Slide the MicroFlow into the sample compartment.

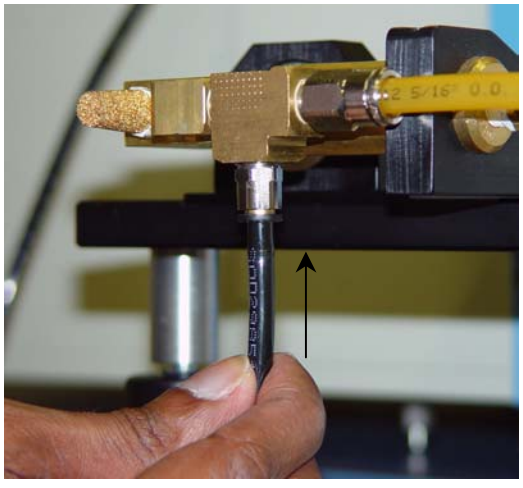
Be sure that the electrical connections in the back of the MicroFlow mate properly to the instrument.



16 Secure the front panel of the MicroFlow to the instrument with the four #4-40 stainless-steel screws.

17 Place the free end of the Teflon® drain tube into an appropriate waste-solution receptacle (e.g., 250-mL Ehrlenmeyer flask).

Warning: *Always follow local laws concerning disposal of hazardous wastes.*

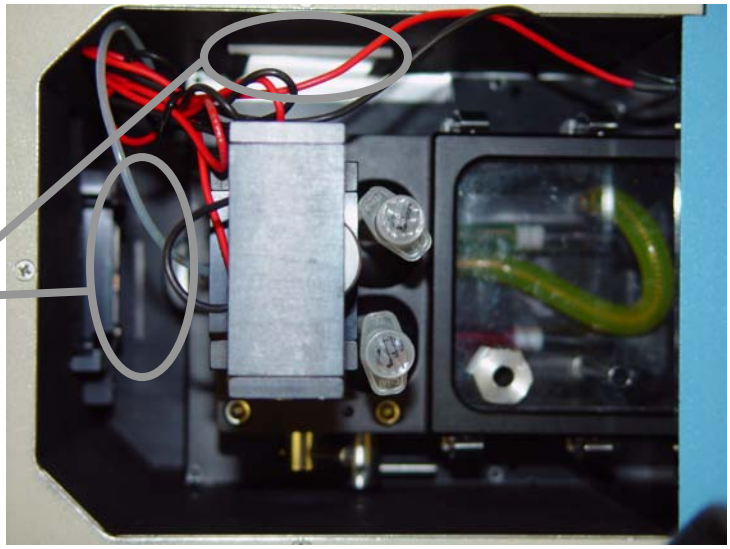


18 Connect the air or N_2 supply to the air-supply nozzle on the MicroFlow, using 5/16" (8-mm) I.D. hose.

Pull out the black ring, while inserting the hose.

19 Be sure the exhaust valve is forward.

20 Check that the stop-block wires are out of the optical path.



21 Place the stop-block cover over the MicroFlow.

22 Turn on the air or N_2 supply.

See the chart in Chapter 2 for maximum safe operating pressure.

Warning: *Never exceed a line pressure of 90 psi (0.62 MPa). Use a 1% regulator and gauge to control line pressure accurately. Always wear protective goggles when working with pneumatic systems.*

23 Set up DataMax for a time-based scan.

a Press **ENTER** when the setup is complete.

b When the reactor and instrument are ready, DataMax tells you when to trigger.

Warning: *Make sure that both drive syringes are secured to the drive block. Failure to attach the drive syringes **before** pneumatic activation of the drive block can damage severely or shatter the drive syringes.*

24 Push the pneumatic button to activate the drive block.

This forces the reactants in the drive syringes through a mixing chamber at high velocity, into the observation cell, and finally into the stop syringe. The piston of the stop syringe moves upward until its motion is arrested by the adjustable slide stop. When the stop piston contacts the slide stop, an electrical circuit triggers data acquisition.

Warning: *Never exceed a line pressure of 90 psi (0.62 MPa). Use a 1% regulator and gauge to control line pressure accurately. Always wear protective goggles when working around pneumatic systems.*

5: Maintenance

After use

1 Flush the MicroFlow thoroughly with distilled water.

See Chapter 3 for detailed directions on cleaning.

Storage

2 Leave the MicroFlow filled with distilled water.

This prevents drying of reagents in the internal passages.

Warning: *If reagents are left in the module for an extended time, they may tend to thicken or dry out. This impedes proper functioning of the MicroFlow, or may cause the MicroFlow to fail.*

6: Troubleshooting

For difficulties with the MicroFlow, consult the table below to see if the question is answered here. Otherwise, reach Spex® Fluorescence Customer Service at Jobin Yvon Inc. by phone, fax, or e-mail. Before contacting us, please follow the instructions below:

1 Note the problem and record any error messages.

2 See if the problem is listed below.

If so, try the suggested solutions. Be sure to note carefully the steps taken to remedy the problem and the result. Refer to the appropriate section of this manual (and the instrument and software manuals, if necessary).

Problem	Cause	Possible Remedy
Flow is restricted	Dried reagents are in the accessory	Flush module with distilled water. If this does not help, call Spex® Fluorescence Service.
No flow	Dried reagents are in the accessory	Flush module with distilled water. If this does not help, call Spex® Fluorescence Service.
	Air pressure is insufficient to drive the pistons	Check gas pressure. Change gas cylinders, if required.

Warning: *Never exceed a line pressure of 90 psi (0.62 MPa). Use a 1% regulator and gauge to control line pressure accurately. Always wear protective goggles when working with pneumatic systems.*

3 If the problem persists, or is not listed,

Call Spex® Fluorescence Service by phone at (732) 494-8660, or fax at (732) 549-5125. Outside the United States, call the local distributor. You may also reach us at our web page www.isainc.com/Fluor

When you contact the Spex® Fluorescence Service Department, have the purchase date, serial number, system configuration, and software version available. Be prepared to describe the malfunction and the attempts, if any, to correct it. Note any error messages observed and have any relevant spectra (sample, polarization ratio, xenon-lamp scan, water Raman scan) ready for us to assist you.

7: Glossary

Dead time, T_d

The time required for the reacting mixture to flow from the point of mixing to the observation cell. The dead time may be found from

$$T_{\mathrm{d}} = \frac{V}{U}$$

where V is the volume of the reacting solution, and U is the flow velocity. *See also* **Observation cell**.

Efficiency of mixing, η

The mean change in potential energy during a turbulent event. Mathematically,

$$\eta = \frac{\text{rise in background potential energy}}{\text{total energy lost}}$$

$$= \frac{\Delta E_b}{\Delta E_b - \Delta E_T}$$

First-order reaction

A reaction in which the rate is directly proportional to reactant A's concentration, i.e.,

$$\text{rate} = k[\mathrm{A}]$$

where k is the rate constant. The integrated form of this rate law with respect to time is then

$$\ln([\mathrm{A}]_0) - \ln([\mathrm{A}]) = kt$$

where $[\mathrm{A}]_0$ is the starting concentration of A, and t is the time since the start of the reaction. *See also* **Rate expression, Rate constant**.

Flow velocity

The velocity of the liquid flowing through the MicroFlow, measured in mL s^{-1}.

Half-life

The time for a species to diminish to half its original concentration.

Kinetics

The study of the rates and processes of chemical reactions. Kinetics studies often prove useful in determining the microscopic details of reactions, such as the sequence of bond formation, properties of intermediates, etc.

Mixing ratio

The ratio of the two drive syringes' volumes, e.g., 1:1, 2:1, 4:1, etc.

Observation cell

The volume in the MicroFlow in which the optical observation is made.

Order of reaction The exponent in the rate expression. *See also* **Rate expression**.

Path length The distance that light must travel through the sample in the observation cell. *See also* **Observation cell**.

Pneumatic valve A valve activated by pressurized gas.

Pseudo-order reaction A reaction run under conditions where certain species' concentrations change little, so that their concentrations are essentially constant over the course of the reaction. These conditions are used to isolate the kinetic effects on a reaction while other species' concentrations change in a predictable manner.

Rate constant, *k* A numerical proportionality constant inserted into the rate law, which is fixed under temperature and pressure. The rate constant usually is highly dependent upon temperature. *See also* **Rate Law**.

Rate expression (rate law) Describes how the rate of reaction depends on the concentration of all species present. The rate law usually is written in terms of the reactants. Rate laws may be differential equations with respect to time, or integrated rate laws including time. With careful choice of the *y*-axis, a straight line often may be found. *See also* **Rate of Reaction**.

Rate of reaction The change in concentration of reactants or products in a chemical reaction with time. A rate with respect to an individual species participating in a reaction may be defined, for a generalized reaction, in which $aA + bB \rightarrow$ products,

$$\text{rate} = k\,[A]^m\,[B]^n$$

where m is the order of the reaction with respect to reactant A; n is the order of the reaction with respect to reactant B; and the overall order of the reaction is $(m + n)$. *See also* **Order of reaction, Rate constant**.

Second-order reaction A reaction in which the rate is proportional to reactant A's concentration squared, i.e.,

$$\text{rate} = k[A]^2$$

where k is the rate constant. The integrated form of this rate law with respect to time is then

$$\frac{1}{[A]} - \frac{1}{[A]_0} = kt$$

where $[A]_0$ is the starting concentration of A, and t is the time since the start of the reaction. *See also* **Rate expression, Rate constant**.

Transport time The time from mixing to observation, during which the reactant mixture flows to the observation cell. *See also* **Observation cell**.

Zeroth-order reaction

A reaction in which the rate is independent of reactant A's concentration:

$$\text{rate} = k[\text{A}]^0$$

that is,

$$\text{rate} = k$$

where k is the rate constant. The integrated form of this rate law with respect to time is then

$$[\text{A}] = [\text{A}]_0 - kt$$

where $[\text{A}]_0$ is the starting concentration of A, and t is the time since the start of the reaction. *See also* **Rate expression, Rate constant**.

8: Technical Specifications

Dimensions	6" × 18" × 13" (width × length × height) 15 cm × 46 cm × 33 cm (width × length × height)
Weight	13 lbs. (5.9 kg)
Fill-syringe volume	5 mL
Maximum pressure for pneumatic control	90 psi (0.62 MPa)
Pneumatic air (gas) inlet	For 5/16" (8-mm) I.D. hose
Mixing channel volume	~ 2 µL
Observation chamber	
Path length	10 mm (absorption) 2 mm (right-angle fluorescence)
Volume	~ 32 µL
Thermometer well	60 mm long

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