Introduction to Sipper Systems

A sipper system allows you to automate many of the tasks associated with handling individual cuvettes. A flow cell with a relatively small volume[1] is placed in the beam and sample solution is pumped into and out of it by a peristaltic pump controlled by the instrument software.

Automated sample handling with a sipper pump can bring considerable efficiency to a laboratory, but thoughtful experiment design is required to get the best results in terms of both data quality and operational efficiency. This introduction describes the sipper's capabilities and programming options. It also provides advice and recommendations for programming or selecting sipper action sequences to meet particular laboratory needs or scenarios.

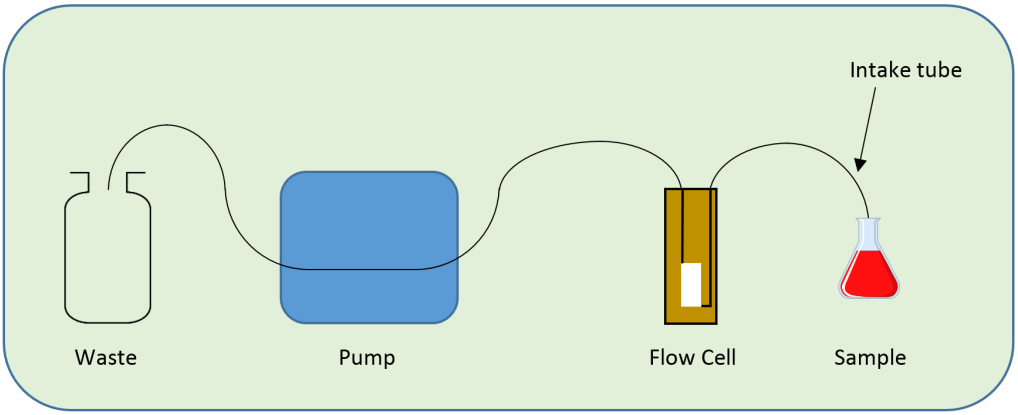
Advantages of using a sipper:

- No need to handle or wash cuvettes
- No risk of breaking expensive cuvettes
- No risk of contaminating optical faces of cuvettes
- No risk of error introduction due to differences in transmittance between cuvettes
- Lower level of user training required compared to using cuvettes

[1] 160 µL is the standard volume supplied and recommended – this size cell has a window that allows the entire light beam to pass without clipping.

- Samples can be handled while wearing bulky protective gloves
- Volume of sample required is no larger than standard measurements
- Sample can be returned after measurement
- High throughput possible when sample is discarded

Block diagram of a sipper system



NOTICE Flow cells are connected so that the sample enters at the bottom of the chamber and leaves from the top. This arrangement is necessary to allow air to exit the chamber. If the cell were to be connected in the opposite direction an air pocket would be trapped in the top of the cell. Air bubbles or air pockets in the chamber cause incorrect and inconsistent absorbance readings to be recorded.

Tips for Successful Sipper Use

1. The recommended amount of liquid required to rinse a flow cell system that is filled with sample or solvent is six times the volume of the system. This is the flow cell chamber plus the volume of the tubing between the flow cell and the sample (a.k.a. the “intake tube”). For a 160 µL cell with a typical amount of tubing that is approximately 1.1 mL.
2. The flow cell should be connected to the tubing such that the inlet connects to the bottom of the chamber and the outlet connects to the top of the chamber. This has several important effects:
    a. Any air bubbles drawn into the cell during sipping will rise to the top of the chamber and be forced out of the outlet. This is a good thing. Bubbles cause inconsistently high ABS readings.
    b. When air is deliberately drawn into the system it will not be trapped in the top of the chamber as an air pocket.
    c. You cannot empty the chamber by pumping air in the forward direction. Pumping air forwards will empty approximately 30% of the chamber and then simply blow bubbles through the remaining liquid.

d. You can empty the chamber by pumping air in the reverse direction. Air enters from the top of the chamber and forces liquid out the bottom. The walls of the chamber will remain wet; however, so the a small amount of sample or rinse will "carry-over" even when you "blow out" the chamber, and you must run a little extra of the next sample through and out of the chamber to compensate for that.

3. If you have a small amount of sample, keep the length of tubing between the cell and the intake as short as possible.

   Drawing air in pushes sample from the intake tube into the chamber. This can reduce the total amount of sample required to make a measurement. Be careful to draw only enough air to push the sample to within approximately 5 cm of the top of the cell. Do not draw air into the chamber as that will corrupt your measurement.

4. If you program an air gap before and after the sample you can recover the sample effectively.

5. To rinse the cell completely and leave it empty:

   a. Calculate the volume of the chamber plus the volume of the intake tubing. This is "the volume" in the following steps, and can be rounded to 0.200 mL in a typical 160 µL cell setup.

   b. Pump rinse water through the cell (6 times the volume)

      This removes the previous sample and leaves only clean water (or other solvent) in the chamber.

   c. Pump at least 3 times the volume of air – this pushes most of the rinse well out of the way, leaving only clean water filling approximately 70% of the chamber.

   d. Return 2 times the volume back out of the intake tube.

      This blows the residual water out of the chamber, leaving it empty.

6. The rinse solution is not entirely harmless. Rinse solution left in the chamber dilutes the first portion of the next sample. If you rinse and pump only in the forward direction, you need to sip enough sample to carry that solvent out.

7. Allow a few seconds of settle time between pumping and measuring. The liquid in the cell chamber must be still during measurement. Failure to allow the sample to settle can lead to inconsistent measurements.

8. Over time, the portion of the intake tubing that dips into the sample may become contaminated with sample material in spite of the use of cleaning steps. There are two recommended strategies for dealing with this issue.

      i. Leave the intake tubing significantly longer than the minimum required to cover the distance from sample to flow cell. Use an air gap to compensate for the extra volume. As the tip of the tubing becomes contaminated, trim off the contaminated portion. When the tubing becomes too short, replace it with new flow-cell tubing.

ii. Place a connector in the intake tubing line so that you can replace the portion of the tubing that dips into the sample periodically. This means that you save money by not needing to replace the flow-cell connectors, but you do allow your sample to contact the in-line connector material, introducing potential for contamination, carry-over and air leaks.

Example Flow Cell Sequences[2]

The needs of each laboratory differ in terms of:

- How much sample is available
- Whether the sample must be recovered or can be sent to waste
- Desired or acceptable complexity of the workflow

Precious sample and/or small quantity of sample

Sip enough sample to fill the flow cell chamber plus a little to wash out residual rinse solution and to avoid drawing air bubbles into the cell chamber.

Sip | Draw air | Settle | Measure | Return | Rinse | Draw Air | Return

Sequence explained

1. Sip enough sample to fill the chamber and carry and remaining small amount of rinse solution moisture out of the flow cell.
2. First draw air carries the sample into the cell, leaving an air gap in the intake tubing.
3. Settle and measure to get the data.
4. Return the sample back out of the intake tube to the user. This volume includes the air in the intake tube and enough additional volume of air to fully evacuate the system.
5. Rinse – draw 6 times the volume of the system through the cell.
6. Draw enough air through the system to push the rinse into the waste container completely.

> **Note** If the sipper programming does not allow you to specify the length of each air gap individually you can combine a sip or rinse step with a draw air step by programming a larger volume for the sip or rinse and removing the sample or rinse solution part way through the pumping time. Air will be pumped for the remainder of the time.

[2] Sipper control software may specify "prompt" steps where the software prompts the user to present the next sample, a waste container, or a rinse solution before it performs the next step. In this section we do not call out those prompts as part of the sequence.

7. Return air back out through the intake tube.

   This will push residual rinse solution out of the chamber and out of the intake tube. Place a waste container under the intake tube to catch the drops.

Following this sequence:

- The sample has been returned to the user. It is very slightly diluted with rinse solvent because there will always be a trace of solvent in the system after the rinse step.
- The rinse solvent is mostly in the waste container at the "end" of the circuit, with a few drops in a second waste container from step 7.
- The system is clean, empty and ready to repeat the cycle with the next sample.

Note Smaller volume flow cells are available but the smaller window size cuts off some of the spectrophotometer beam, reducing photometric performance. If the absorbance values measured are below 1.0, however, this will not make a noticeable impact on data quality.

The minimum recommended chamber volume is 50 µL.

High throughput pumping sequence no. 1

If you have lots of each sample and it can go to waste you can use the next sample as the rinse.

Sip | Settle | Measure

Sequence explained

1. Sip 7 times the volume of the system.

   As you sip each sample it flushes the previous one out of the flow cell and away to the waste container. The chamber is left filled with the next sample.

2. Settle and measure the sample.

   This sequence provides high efficiency of measurement with minimum user interaction and no requirement for a separate rinse solution. It does require a larger volume of each sample (approximately 1.4 mL with a standard 160 µL cell).

High throughput pumping sequence no. 2

If you have less of each sample and it can go to waste you can blow out the previous sample in a return step so that you need less of the next sample to act as a rinse.

Sip | Settle | Measure | Return

Sequence explained

1. Sip approx. 3 times the volume of the system.

   As you sip each sample it flushes the small remaining quantity of the previous one out of the flow cell towards the pump. The chamber is filled with the next sample.

2. Settle and measure the sample.

3. Return (2 times the volume sipped).

   The sample is pushed back out of the intake tube and additional air drawn from the back end of the circuit pushes all sample out of the flow-cell chamber leaving only a trace of the sample solution behind.

   This sequence provides high efficiency of measurement with low user interaction and no requirement for a separate rinse solution. It requires less volume of sample (approximately 0.7 mL with a standard 160 µL cell) than sequence no. 1. With some experimentation to determine the impact of sample carry-over on the results, it may be possible to "tune" the volume required to an even lower amount. Note that this sequence does not send anything to the waste container beyond the pump. Liquid is drawn in and pumped out through the intake tube. The "tail end" of the sipper pump tubing must be open to the air to allow excess air to be drawn in for the return step.

Single direction with rinse, everything goes to waste

A common traditional pumping sequence.

Sip | Draw air | Settle | Measure | Rinse | Draw air

Sequence explained

1. Sip enough sample to wash residual rinse solution (approximately 70% of the volume of the chamber) out of the cell and leave it filled with pure sample solution.

2. Draw air to pull the sample into the chamber – this step reduces the total quantity of sample required and makes a significant difference if the tubing run from the flow cell to the sample is long.

3. Settle and measure the sample.

4. Rinse the tubing and cell with solvent to remove all traces of the previous sample.

5. Draw air to clear rinse solvent from the intake tubing and push as much rinse solvent as possible out of the cell chamber.

This sequence has three main advantages:

- The rinse between samples eliminates the possibility of sample carry-over.
- Material flow is all in one direction. Everything goes to the waste container.
- Very simple and intuitive work-flow for technicians – measure the sample, rinse the system, repeat.

Troubleshooting Sipper Systems

Problem	Possible Cause	Solution
Inconsistent absorbance readings	Air bubbles in the flow cell chamber. To check, remove the flow-cell from the cell holder after the sample measurement (when it should be full of liquid) and inspect the chamber with a magnifying glass and a bright light (for example, mobile phone flashlight) shining through from the back.	1. Check that an air gap following the sample sip step is not drawing air into the chamber. 2. Check technique used during sample presentation for sipping. Is the tube being kept below the surface of the sample? 3. Check that any in-line tubing connectors do not leak. You can usually see air bubbles appearing inside the PTFE tubing on the pump side of a leaking connector. 4. Check the flow-cell connectors for leaks. Replace the connectors and tubing if necessary. 5. Check the flow cell for cracks.
Incorrect volume pumped	Sipper calibration is incorrect	1. Repeat the sipper calibration process in the control software. 2. Verify that the same size and material pump tubing is being used now as when the calibration was performed.
	Pump tubing is worn or damaged	Remove the pump tubing from the pump-head and inspect it. If the tubing is: • flattened out and sticking to itself • beginning to flake or crumble • turning cloudy in the case of silicone tubing Replace the tubing.
	Connector between flow-cell tubing and pump-head tubing is leaking – causes lower than expected volumes to be pumped	Check that the connector and the connections to the tubing are tight. If necessary, trim the tubing and replace the cone on the flow cell tubing and/or the zip tie on the pump-head tubing.

Problem	Possible Cause	Solution
Sipper pump does not turn when it should	Pump is not properly connected or powered	Check the power and data connections to the pump. If the pump has a power/ready light, verify that it is lit.
	Sipper accessory is not recognized or selected in software.	Check that the sipper accessory is selected and recognized in the experimental method in software.
	If a sipper is disconnected and reconnected while the software is running, the software may have trouble establishing communication.	Save your current work. Power off both the sipper and the spectrophotometer. Close the software if running the system from a remote computer. Power the sipper ON first, then restart the instrument and control software.

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Thermo Fisher Scientific Inc.

PN 269-331400_A