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Best Practices for Optimizing UV-Visible Autosampler Accessory Performance

Michael W. Allen, Ph.D., Thermo Fisher Scientific, Madison, WI, USA

Key Words

- Autosampler
- Flow Cell
- Sipper
- Spectrophoto-meter
- UV-Visible

Introduction

Autosampler accessories, for use with UV-Visible spectrophotometers, greatly increase the sample throughput of a laboratory by automating the analysis of solutions. Once method parameters are established, autosamplers can continuously analyze samples without the need for operator intervention. Getting the best results from an autosampler system requires optimization of key parameters affecting the quality of the analysis.

This technical note describes the parameters affecting an analysis performed with an autosampler accessory. A complete description of each parameter is given along with typical values for a CETAC® ASX-260 or ASX-520 autosampler interfaced to a Thermo Scientific spectrophotometer running VISION*lite*™ auto software. Use these parameters as a starting point for optimizing your laboratory's methods.

Factors to Optimize Performance

Sipper Configuration

A sipper accessory is used to pump fluid through the flow cell. The sipper accessory is controlled by an electrical connection to the spectrophotometer. For best performance, the sipper should be plumbed in a manner so that the sample is pulled into the inlet of the flow cell. Pulling the solution through the flow cell helps reduce the possibility that air bubbles are introduced into the flow cell by the peristaltic pump on the sipper.

The sample should flow in the following order: CETAC autosampler sampling probe → flow cell inlet → flow cell outlet → sipper inlet → sipper outlet → waste.

Flow Cells

The choice of flow cells is important for optimizing performance of the spectrophotometer equipped with an autosampler. For best results, a sloping roof flow cell should be selected. These cells allow any bubbles that may form in the system to travel along the upper roof of the flow cell that gradually slopes upward from inlet to outlet. This sloping roof keeps the bubbles out of the light path of the cell, preventing them from interfering with the measurement. This configuration also promotes the elimination of the bubbles from the flow cell.

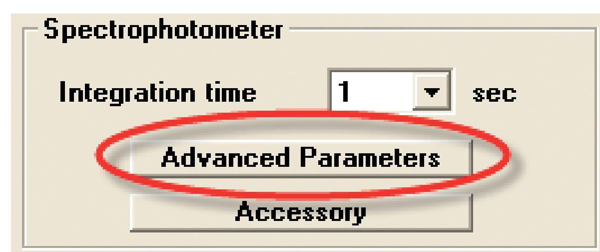
Spectrophotometer Parameters

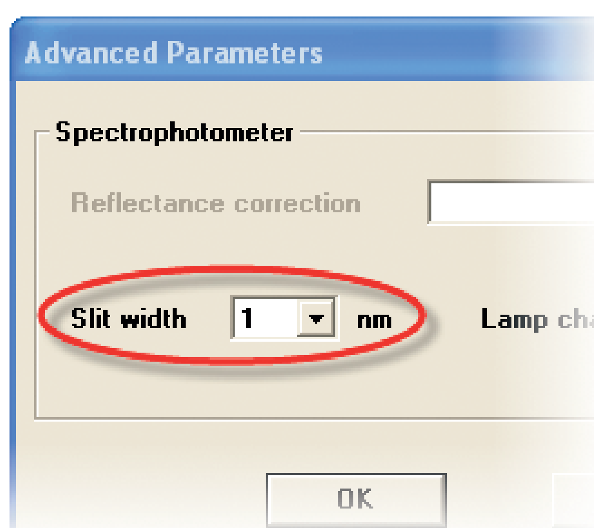
Spectral Bandwidth

The spectral bandwidth (SBW) directly influences the resolution of the spectrophotometer. The largest SBW capable of resolving a peak should be used for the measurement. **As a rule of thumb, the SBW should be at least one-tenth (1/10) of the width of the peak at half-height.** The width of the peak at half-height is sometimes referred to as the full width at half-maximum or FWHM. For example, if a peak is 20 nm wide at half-maximum, the minimum SBW that should be used is 2 nm.

A higher SBW sends more energy to the sample, helping to increase the signal-to-noise ratio. Some flow cells have apertures that are smaller than the sample beam, therefore it important to use the highest SBW possible to obtain accurate quantitative results.

To adjust the SBW choose *Advanced Parameters* in the Thermo Scientific VISION*lite* auto software and then adjust the Slit width selection box on the dialog.

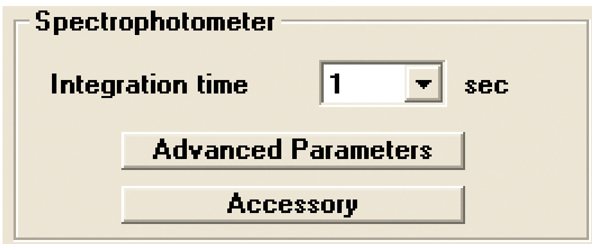




Integration Time

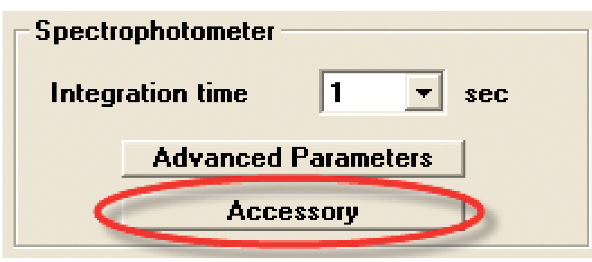
Integration time defines the amount of time over which the sample is measured. This parameter should be optimized to give the best results in the shortest amount of time. For example, there is no benefit to using a 10-second integration time, if the quality of the measurement is not improved over the results obtained with a 5-second integration time.

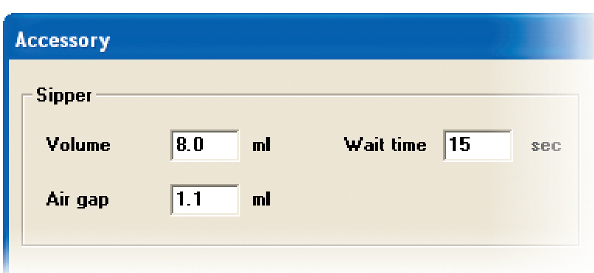
The integration time is controlled by the list box on the left-hand side of the screen. **The recommended integration time for measurements in a flow cell is 3 seconds.**



Sipper Parameters

Sipper parameters are controlled from the Accessory box on the main screen. The sample volume, wait time, and air gap are all controlled from the sipper section of the Accessory dialog box.





Sample Volume

The Sipper Volume control allows you to determine the volume of liquid pulled from each standard or sample. **The Sipper Volume should be a minimum of 10 times the volume of the flow cell.** This theoretically results in a sample carry-over of less than 1.0%. However, it is often necessary to use more than 10 times the nominal volume of the flow cell depending on the efficiency of the rinsing procedure.

Keep in mind that the solution used to wash the autosampler probe and tubing remains in the flow cell after the washing step. Therefore, it is necessary to run sufficient sample through the flow cell to eliminate carryover from the washing step.

Wait Time

Wait time allows you to set the time that elapses after the sample volume and air gap are collected before the measurement is made. This time allows the contents of the flow cell to settle and for any bubbles that may be in the system to rise to the top of the flow cell out of the light beam. **The minimum recommended wait time for a 10 mm flow cell is 15 seconds.**

Air Gap

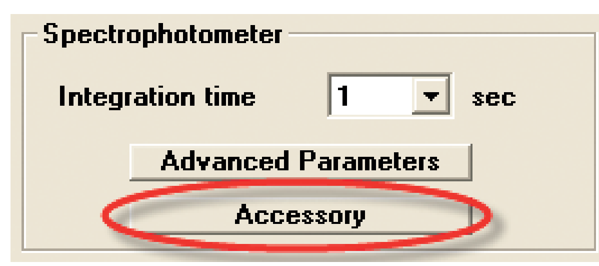
The air gap is the volume of air that is pulled into the autosampler tubing after the sample volume has been extracted. The autosampler probe will move out of the container of solution above the last standard or sample and pull air into the autosampler tubing.

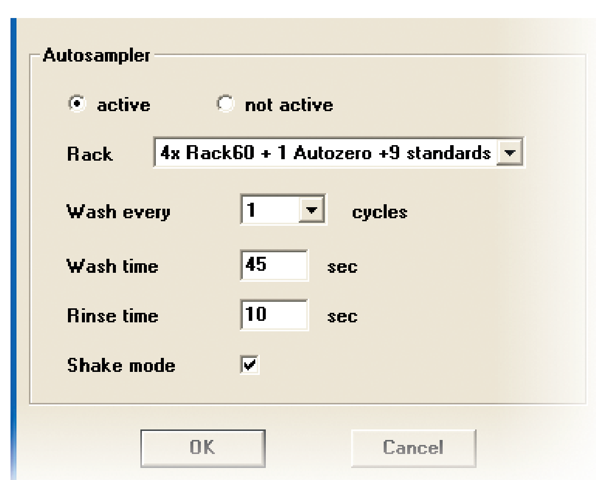
The air gap should be large enough to separate the liquids in the autosampler and flow cell tubing. The air gap should never extend into the inlet of the flow cell and introduce air into the flow cell before a measurement is made.

Depending on the length and the diameter of tubing used in the system, the air gap may vary significantly. **Using standard autosampler probe tubing and flow cell tubing (as supplied) the air gap should be between 1.0 and 2.0 ml.**

Autosampler Parameters

Autosampler parameters are controlled from the Accessory box on the main screen. The *Wash every, Wash time, Rinse time*, and *Probe shake* mode are all controlled from the Autosampler section of the Accessory dialog box.





Wash Every

This setting allows you to set how often the sample probe of the autosampler is washed. During the washing process, the autosampler will move the probe to the rinse/wash station and will pull solution from the rinse/wash station through the autosampler probe. Keep in mind that this solution passes into the flow cell tubing and eventually into the flow cell.

A continuous supply of washing liquid should always be available to the rinse/wash station of the autosampler. If aqueous solutions are analyzed, deionized water is usually sufficient for washing the probe. However, if your samples are prepared in another matrix, such as an organic solvent, we recommend using that solvent for washing and rinsing the autosampler probe.

The probe washing step can be eliminated by setting this value to zero. **For best performance, we recommend that the probe be washed between every cycle.**

Wash Time

This setting determines the amount of time the sipper pump will run to pull liquid from the rinse/wash station through the autosampler probe.

Depending on the length and the diameter of tubing used in the system, the wash time may vary significantly. **Using standard autosampler probe tubing and flow cell tubing (as supplied) the wash time should be 45 seconds for best results.**

Rinse Time

The rinse time parameter determines how long the sample probe of the autosampler is rinsed. During the rinsing process, the autosampler will move the sample probe up and down in the rinse/wash station as fluid is pumped through the rinse/wash station. The rinse/wash liquid supplied to the autosampler will be used for this process. As mentioned previously, the solution or solvent used in the rinse/wash station should closely match the sample matrix for the best results.

The rinse time can be set to zero seconds to bypass the rinsing step. **For best results, the recommended rinsing time is a minimum of 10 seconds.**

Shake

The shake feature causes the autosampler probe to vibrate rapidly as it is withdrawn from a standard or sample. Shaking dislodges any drops of liquid that may be clinging to the autosampler probe. **For best results, we recommend always using the Shake feature.**

Typical Configuration

The following method parameters should be used with 10 mm pathlength flow cells with standard autosampler/sipper tubing:

Parameter	Value
Flow Cell Nominal Volume	0.440 ml
Spectral Bandwidth	2.0 nm
Signal Averaging Time	5 sec
Sample Volume	8 ml
Air Gap	1.1 ml
Wash Time	45 sec
Rinse Time	10 sec
Wait Time	15 sec
Shake Mode	On

Conclusion

Proper configuration of an autosampler system is key to accurate and reproducible results. In this technical note, we have explained the function and optimization of each of the parameters associated with autosampler, sipper, and spectrophotometer performance. Following the suggestions in this document will help you get the most value and performance from your spectrophotometer and autosampler system. Use this document as a starting point for developing your own methods, optimizing parameters for your individual standards and samples.

Product Information

Thermo Scientific UV-Visible Spectrophotometers

Evolution 100, 300, 600
Helios Alpha, Beta, Gamma, Delta
BioMate 5
AquaMate
UV1

Autosamplers

222-239700	CETAC ASX-520 4-Rack Autosampler
222-239800	CETAC ASX-260 2-Rack Autosampler

Software

869-127200	VISION*lite* auto

Sipper Accessory (Evolution 300/600)

10010401	Smart Sipper

Sipper Accessories (Evolution 100, UV1/3xx/5xx, Helios Alpha/ Beta/ Gamma/ Delta, BioMate 5, AquaMate)

9423UV64250E	SuperSipper
9423UV64270E	MiniSipper

Accessories/Consumables

268-815800	Sample Probe for 222-239700 and 222-239800 Autosamplers
268-815900	Sample Rack, 90-position, for 8 mL vials, pkg of 1
268-816000	Sample Rack, 60-position, for 14 mL vials, pkg of 1
268-816100	Sample Rack, 40-position, for 20 mL vials, pkg of 1
268-816200	Sample Rack, 24-position, for 30 mL vials, pkg of 1
268-816300	Sample Rack, 21-position, for 50 mL vials, pkg of 1
268-816400	Sample Vials, 8 mL vials, package of 1000 polypropylene, 13 mm x 100 mm
268-816500	Sample Vials, 14 mL vials, package of 1000 polypropylene, 16 mm x 100 mm
268-816600	Sample Vials, 50mL vials, package of 500 polypropylene, 30 mm x 115 mm
268-816700	Sample Vials, 30 mL vials, package of 500 polypropylene, 25 mm x 95 mm
268-816800	Sample Vials, 20 mL vials, package of 500 polypropylene, 21.5 mm x 100 mm

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+61 2 8844 9500
Austria
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