Autosampler For Spectrophotometric Analysis

GX-271 Liquid Handler as an autosampler for a Shimadzu UV1800

TECHNICAL NOTE TN222

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INTRODUCTION

A general liquid handling application is to use a liquid handler as an autosampler for a spectrophotometer. The traditional manual method for filling a flow-through cuvette is to use a peristaltic pump (a sipper) to draw the sample through the cuvette. This can easily be replicated using a Gilson GX liquid handler.

Example applications are:

- The determination of chlorophyll in surface water
- The Molybdenum Blue method for determination of phosphate in Coca Cola
- Protein Matter and Lipolysis Index in milk analysis

Applications and customers require different levels of traceability of the samples, as well as reporting. This technical note will explain different hardware configurations, as well as software options, to use Gilson liquid handlers with TRILUTION® LH Software as autosamplers for spectrophotometric analysis.

SIPPER CONFIGURATION

The sipper configuration uses a MINIPULS® 3 Peristaltic Pump or a VERITY® syringe pump to draw the sample through the flow cuvette. Figure 1 shows the flow path. The syringe pump can be replaced by a MINPULS 3. In that case the outlet of the peristaltic pump goes to the waste.

When using a peristaltic pump, no rinsing is possible. Instead, samples can be aspirated in succession without the need of resetting the pump resulting in faster processing times. Using a syringe pump, the sample can be easily returned to the source vial or tube and the system can be rinsed from the reservoir. Flow-through cuvettes have a directional flow for filling to reduce the chance of air bubbles being trapped in the system. Make sure that the cuvette is installed with the flow direction towards the pump so that the sample is aspirated through to fill the cuvette.

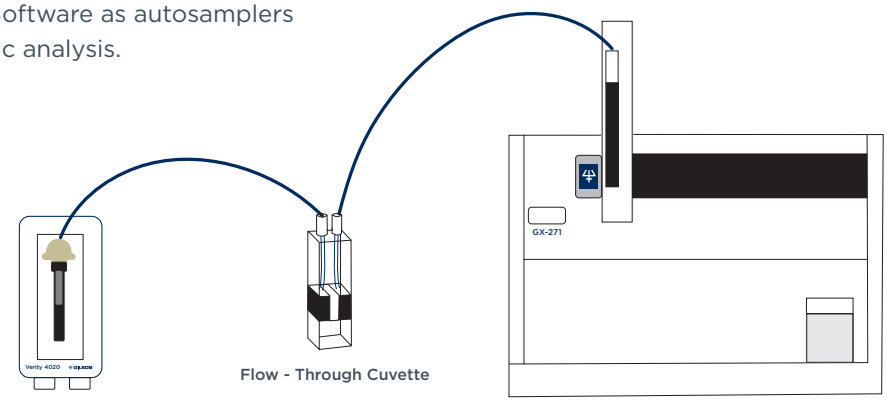

Figure 1
Sipper configuration

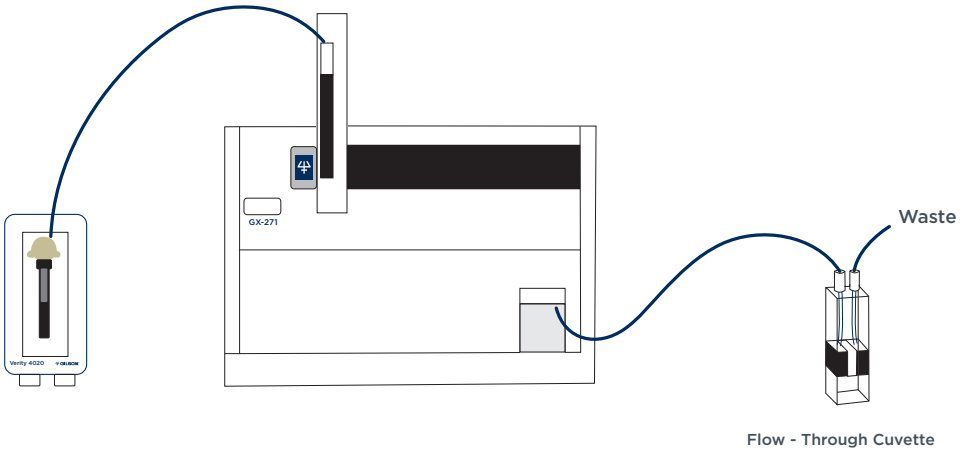

Figure 2
Transfer port configuration

TRANSFER PORT CONFIGURATION

As an alternative to the sipper configuration, a transfer port can be used to push samples into the flow-through cuvette as depicted in Figure 2.

This configuration frees up the liquid handler to perform more pipetting. For example, derivatization of the samples can be performed. When the probe is not inserted in the transfer port there is an open connection to the flow-through cuvette. This might cause air bubbles being trapped in the liquid flow to the flow-through cuvette. To avoid this effect a 2-way solenoid shut off valve can be inserted in the waste tubing of the flow-through cuvette.

METHOD

The connection tubing and cuvette need to be rinsed with the sample to ensure that the concentration of the sample in the cuvette is the same as the concentration of the sample in the sample tube, making it possible to take a measurement. A larger rinse volume leads to a more accurate measurement and reduces carryover. The rinse volume can be determined by manually drawing a test sample through the cuvette using a syringe. Once the measurement readout stabilizes, the aspirated volume can be read from the syringe.

The flow rate has a large role to play in the rinsing process. A high flow rate ensures a lot of circulation in the cuvette resulting in a lower volume needed to rinse the system. Flow rates up to 75mL/min were tested. In the end, a flow rate of 50mL/min was used.

Using the transfer port configuration an extra volume needs to be aspirated to compensate for the laminar flow in the transfer tubing. This extra volume is discarded after the measurement. Not setting the extra volume may result in stable and reproducible measurement values; however, these are not representative of the actual sample. The size of the extra volume depends on the sample volume, flow rate and transfer tubing material and diameter. To determine the extra volume, measurements of a test sample can be compared. First fill the cuvette manually as the procedure above and note the read-out value of the spectrophotometer. Then use the autosampler to fill the cuvette. The read-out value should be the same. If this is not the case, then increase the extra volume until the measurement provides the same read-out value for both the manual and automated filling method.

CONTACT CLOSURE COMMUNICATION

The most basic form of communication between the autosampler and the spectrophotometer is contact closure. In the case of a Shimadzu spectrophotometer, a Gilson connection kit is available from Shimadzu.

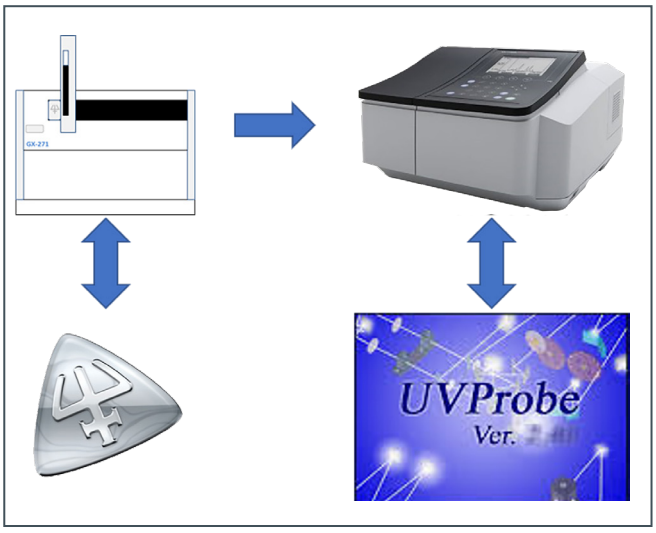

Figure 3
Communication through contact cable

This enables communication as schematically represented in Figure 3. For other devices check with the supplier what the options are for communication with the data acquisition software. Once the sample is inserted in the flow cell, TRILUTION LH sends a 0.1 minute pulse through the liquid handler to the spectrophotometer to indicate the sample is ready for measurement. The Shimadzu/Gilson connect cable uses the sipper port on the spectrophotometer to read the input. The Shimadzu software UVProbe is configured to read the sipper input and take a reading upon contact closure.

This solution lets TRILUTION LH determine the order in which the samples are measured but there is no communication to the software of the spectrophotometer to link this information to the measurements. The sample table is entered in the software of the spectrophotometer. To improve sample traceability the “log variable” function of TRILUTION LH may be used. This function can create a log file during the run to prove which sample was offered when and in what order to the spectrophotometer.

A custom task was created to inject the sample into the transfer port. There is an option included to use a solenoid valve to close the waste line after the cuvette. A 24V output or a solenoid output (for the GX-271) can be selected to control this valve. The extra volume and air gap can either be pushed into the transfer port or can be discarded into the inside rinse. Finally, a rinse option can be used to rinse the probe and the transfer port.

In the task there is an additional option to create a log file for the injections. There is a message field to enable a clear log message. Inside the task, the well number is added to this string. In the log file (that can be found in: Documents\TRILUTION LH 4.0\Export\Variable Logs folder) for each injection, a line is added that contains a time stamp, the message and the well number.

SHIMADZU UV1800 THROUGH EXCEL

For a more integrated solution the Shimadzu UV1800 can be read through an Excel macro creating a complete report of sample information,

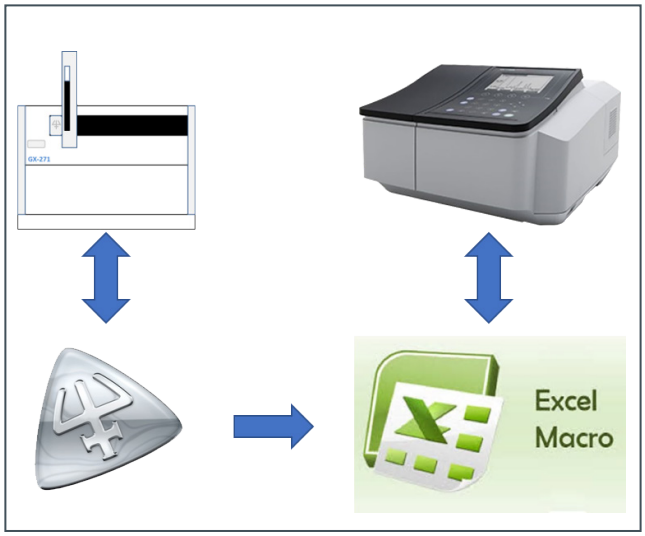

Figure 4
Communication through Excel

measurement data and calculations in an Excel spreadsheet as shown in Figure 4. UVProbe is not utilized for this solution, only the device drivers are required to enable communication to the spectrophotometer. This Excel macro can be controlled through GEARS making TRILUTION LH the controlling software. The sample table is entered in TRILUTION LH. The sample name is reported through GEARS into the Excel report improving sample traceability. The current version of the software allows two measurements per sample to be reported using a fixed wavelength.

The communication between GEARS and the Excel macro uses RS232 communication. To enable this, a USB-USB cable was created to loop two USB-RS232 converters to communicate with each other.

Two custom tasks are available for TRILUTION LH (Autozero and Measure). The measure task requires a well number and sample name. The well number is also used as an index number to determine the location in the excel spreadsheet. For the sample name, the default “Sample Description” column of the application table of TRILUTION LH cannot be used. This information cannot be accessed from the method. Instead a separate variable, like “Sample Name” must be created for the user to enter the sample name.

CONCLUSION

The GX-271 Liquid Handler and TRILUTION LH can be used as an autosampler for filling a flow-through cuvette in a spectrophotometer with the following benefits:

- Increased reproducibility due to automated filling of the cuvette
- Additional liquid handling can be included
- Derivatization reaction times can be standardized in automation
- Depending on the spectrophotometer an integrated solution is feasible using GEARS
- Improve traceability between sample and measurement results

EXAMPLE CONFIGURATIONS

SIPPER CONFIGURATION	
2614101	GX-271 LH, ETHERNET, WITH Z DRIVE
F155001	MP3 DRIVE UNIT 0.01 TO 48 RP, 110/220V
F117604	PUMP HEAD, R1 SINGLE CHANNEL SS
F117949	TUBING, PVC, 3.18MM ID, BLK/WHT, 12/PKG
	SPECTROPHOTOMETER
	FLOW CUVETTE
	CONNECTION CABLE FOR CONTACT CLOSURE COMMUNICATION
	TUBING TO CONNECT CUVETTE TO PROBE AND MINIPULS TUBING

TRANSFER PORT CONFIGURATION	
2614101	GX-271 LH, ETHERNET, WITH Z DRIVE
SPL-1785A-HDW	INJ PORT HOLDER,GX-27X 125MM
2749767	TRANSFER PORT
31130001	4020 SINGLE SYRINGE PUMP
25025345	SYRINGE, 10ML, 215/235
	SPECTROPHOTOMETER
	FLOW CUVETTE
	CONNECTION CABLE FOR CONTACT CLOSURE COMMUNICATION
	TUBING TO CONNECT CUVETTE TO TRANSFER PORT AND WASTE