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AZURA

Flow cell cartridge
Supplement

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Note: Please read the corresponding technical documentation for handling and safety reasons.

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1. Product information

This supplement provides useful information for the KNAUER LightGuide flow cell cartridges and KNAUER PressureProof flow cell cartridges. These flow cells are compatible with the following AZURA® detectors: DAD 6.1L, DAD 2.1L, MWD 2.1L. As all wetted parts are biocompatible the flow cells are suitable for LC and Bio and FPLC applications (except article no. AMC-19XA and AMD59XA).

1.1 KNAUER LightGuide flow cell cartridges

The KNAUER LightGuide flow cell cartridges combine maximum light throughput (due to total internal reflection) with minimal peak dispersion (due to the small cell volume) to guarantee an optimized signal to noise (S/N) ratio.

The LightGuide technology stands for:

- Total reflection technology for excellent light transmission
- Low cell volume enabling detection of fast eluting peaks

The High Sensitivity KNAUER LightGuide flow cell is ideal for trace analysis. The Standard KNAUER LightGuide flow cell is ideal for high throughput and high resolution.

1.2 KNAUER PressureProof flow cell cartridges

The KNAUER PressureProof flow cell cartridges are optimized for conventional and high flow HPLC and FPLC applications. These flow cells feature:

- Increased pressure stability (up to 300 bar)
- Extended flow rate range (up to 20, 100 and 200 ml/min)

2. Installation

With the delivery you receive an accessories kit that includes a waste tubing kit, capillary (LightGuide only) and fittings. Use the waste tubing kit to connect the flow cell to the waste container and the other components to connect the flow cell to the

system. Before connecting to the flow cell, flush your column with mobile phase to keep the flow cell clean.

Process

1. Unpack the flow cell cartridge.
2. Remove the hoods from the light inlet and outlet ① (see Fig. 1).
3. Remove the hole plugs from the inlet and outlet cartridge ② .
4. Insert the flow cell into the detector. For further details, consult the detector's user manual.
5. Connect the capillaries. For further details, consult the waste tubing kit's supplement.

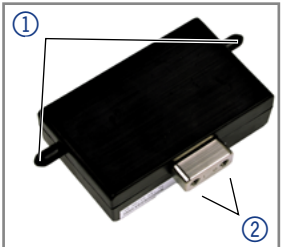

Fig. 1 Flow cell

Keep the hoods and hole plugs for later use, e. g. storage. The hoods and hole plugs protect the flow cell against contamination and solarization.

Note: The accessory kit for the LightGuide flow cell cartridges includes capillaries optimized for fast analytical (U)HPLC (0.13 mm ID). When working with higher flow rates, this capillary should be exchanged for one with a larger inner diameter.

3. Troubleshooting

Problem	Solution
Contamination	Clean the light inlet or outlet with a lens cloth moistened with alcohol or a cotton swab.
Leak caused by overpressure	Reduce the flow/pressure. If the leak continues, the flow cell must be replaced (no repair possible).

Problem	Solution
Increased baseline noise/ reduced sensitivity	Clean the flow cell

Consult the detector's user manual for additional information on troubleshooting.

4. Cleaning

Note: In the case of using acetonitrile or mixtures containing acetonitrile as mobile phase in combination with the LightGuide flow cells, it is necessary to clean the flow cell in regular intervals for maintaining the performance of the cell. For this purpose flush the flow cell every 24 hours for at least 1 hour with pure methanol at 1 ml/min. Bypass your installed column for this flush step.

Do not let buffers stay for a long time in the flow cell. We highly recommend flushing the flow cell cartridge after running an application with buffers. The following solvents are recommended for cleaning:

- Water (when using buffers)
- Ethanol or methanol
- Isopropanol

Never touch the light inlet and outlet as this could lead to contamination which reduces the performance of the flow cell (intensity, wavelength accuracy). Should this occur however, clean with a lens cloth moistened with alcohol or a cotton swab.

To diagnose this issue, we recommend generating an intensity spectrum (via your chromatography software under Diagnostics). Dirty fiber optic ends result in little or no UV light (see Fig. 2).

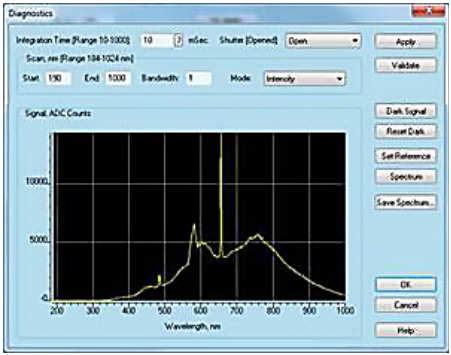

Fig. 2 UV spectrum with contaminated fiber optics ends

4.1 Advanced flow cell cleaning type 1

This section describes a cleaning procedure for aggressive cleaning of LightGuide flow cells.

Preparation of chemicals

All chemical reagents should be of at least ACS-Grade, preferably HPLC-Grade. This procedure involves the use of caustic and flammable reagents. Consult the manufacturer's MSDS for necessary safety precautions.

Prerequisite

The following 3 cleaning solutions:

Cleaning solution 1: 0.5M Potassium Hydroxide in 100 % Ethanol (briefly, 7.013g KOH in 250 ml EtOH). After thorough mixing, the solution should be filtered through a 20 µm pore size filter.

Cleaning solution 2: 100 % Methanol

Cleaning solution 3: Ultrapure water, Type I per ASTM D1193-99 or equivalent.

Note: Grade 1 ultrapure water per ISO 3696 differs significantly from the above classification.

Cleaning procedure

The preferred method of cleaning involves the use of a spectrophotometer in "monitor mode" throughout the entire cleaning process. This allows the technician to observe the extent of performance improvement as a function of time.

The simplest cleaning method involves using a peristaltic pump to flow each cleaning solution through the sample cell in numerical order. It is recommended the pump is configured to "pull" through the cell to avoid possible contamination from degraded peristaltic pump tubing. Each solution is cycled for approximately 3-4 minutes, with a bolus of air introduced between each solution. This procedure is repeated until there is no noticeable improvement in sample cell performance. The flow direction can be reversed between cycles to ensure thorough cleaning.

To lessen the time required for the above cleaning method, large bubbles of air can be introduced into the sample cell alternately with the cleaning solutions. This method uses a laminar flow profile and radial diffusion to effectively "scrub" the inside of the sample cell.

Note: It is imperative that Solution #2 immediately follows Solution #1 to remove residue remaining on the optical components. Failure to do so will result in poor flow cell performance.

Another possible cleaning method involves the use of two syringes of appropriate volume connected to the liquid ports of the sample cell. In numerical order, each cleaning solution is introduced into the sample cell and flushed back and forth between the syringes 10-12 times.

This procedure is repeated until there is no noticeable improvement in sample cell performance.

Final rinsing procedure

Once the technician identifies the point where subsequent cleaning cycles no longer improve the performance of the flow cell, the unit should be flushed with ultrapure water for a period of at least 15 minutes to ensure all cleaning solutions have been completely removed and there are no persistent residues that might affect flow cell performance or stability.

4.2 Advanced flow cell cleaning type 2

Note: This cleaning type is recommended only for LightGuide flow cells.

KNAUER recommends to carry out this cleaning procedure at regular intervals (every two weeks) when using acetonitrile-containing eluents in order to extend the life of the flow cells.

Materials

- Potassium hydroxide (pellets)
- Hydrogen peroxide solution (30 %)
- Water (MilliQ)
- A syringe with Luer Lock adapter for UNF 10/32 threading (Volume: at least 5 ml)
- Waste tube
- Hole plug

Production of the cleaning solution

1. Carefully dissolve 5.7 g of potassium hydroxide while stirring in 10 ml of water under heat generation.

2. Slowly add the solution with 6 ml of 30 % hydrogen peroxide solution while stirring (heat and gas evolution).
3. Finally mix the resulting solution with 10 ml of water and use directly for cleaning.

Note: Always prepare the cleaning solution freshly before cleaning to ensure an optimum performance.

Cleaning procedure

1. For cleaning, the flow cell may need to be rinsed with water beforehand. The flow cell must not contain any residues of organic solvents!
2. A syringe with Luer Lock adapter is filled with at least 5 ml of the cleaning solution and connected to the inlet port of the flow cell.
3. The outlet port of the flow cell is connected to a waste tube and connected to a waste container.
4. Slowly and carefully flush the flow cell with the cleaning solution.
5. Remove the waste tube from the flow cell and close the port with a hole plug.
6. Remove the syringe from the inlet port and close it with a hole plug as well.
7. Leave the cleaning solution in the flow cell for at least 2 hours. For a more extensive cleaning, it is recommended to let the cleaning solution work overnight for at least 12 hours.
8. After the exposure time, remove the hole plugs, fill a Luer Lock syringe with at least 5 ml of water and connect it to the inlet port. Install the waste tube again at the outlet port.

9. Slowly and carefully flush the flow cell with water. Then install the flow cell in the system and flush with water for 15 minutes at a flow of 1 ml/min.
10. Check the light intensity via the diagnostic function. The intensity at 220 nm should be at least 3500 ADC counts. If necessary repeat the cleaning steps 2 to 9 and check the light intensity at 220 nm again .

5. Storage

Note: Never store the flow cell in pure water to prevent microbial contamination.

1. Flush the flow cell cartridge.
2. Disconnect the capillaries.
3. Close the inlet and outlet with the hole plugs.
4. Release the flow cell from the detector.
5. To secure the light inlets and outlets, replace the hoods.

6. Technical data

6.1 Standard LightGuide

Path length	10 mm
Capillary connection	1/16"
Flow cell volume	2 µl illuminated volume (0.8 µl dispersion volume)
Maximum flow rate	5 ml/min
Maximum pressure	50 bar
Wetted materials	Quartz (SUPRASIL)/Teflon®/ stainless steel/PEEK/Titanium

6.2 High Sensitivity LightGuide

Path length	50 mm
Capillary connection	1/16″
Flow cell volume	6 µl illuminated volume (2 µl dispersion volume)
Maximum flow rate	5 ml/min
Maximum pressure	50 bar
Wetted materials	PEEK/quartz (SUPRASIL)/Teflon®/ stainless steel/Titanium

6.3 Analytical PressureProof

Path length	10 mm
Capillary connection	1/16″
Flow cell volume	10 µl
Maximum flow rate	20 ml/min
Maximum pressure	300 bar
Wetted materials	Titanium/quartz (SUPRASIL)/ PEEK

6.4 Semi-Preparative PressureProof

Path length	3 mm
Capillary connection	1/16″
Flow cell volume	2 µl
Maximum flow rate	100 ml/min
Maximum pressure	300 bar
Wetted materials	Titanium/quartz (SUPRASIL)/ PEEK

Path length	10 mm
Capillary connection	1/16″
Flow cell volume	10 µl
Maximum flow rate	200 ml/min
Maximum pressure	300 bar
Wetted materials	Titanium/quartz (SUPRASIL)/ PEEK

7. Repeat orders

Name	Order no.
Standard LightGuide flow cell cartridge, 10 mm	AMC19XA
High Sensitivity LightGuide flow cell cartridge, 50 mm	AMD59XA
Analytical PressureProof flow cell cartridge, 10 mm	AMC38
Semi-Preparative PressureProof flow cell cartridge, 3 mm	AMB18
Semi-Preparative PressureProof flow cell cartridge, 10 mm	AMC37
Accessory kit LightGuide	FMC19XA
Accessory kit PressureProof	FMC38
Waste tubing kit LightGuide	A9842
Waste tubing kit PressureProof	A9843

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