Eliminating Baseline Problems

Chromatograms *should* reflect the separation of analyte peaks as accurately as possible. Baseline anomalies not only affect data presentation, but can also lead to problems with identification and quantitation of analytes.

Baseline problems include noise, wandering, drift, ghost peaks, and negative peaks. These symptoms can have both mechanical and chemical origins. This article highlights some tips that you can apply in your lab to reduce or remove these anomalies. The tips are organized by root cause:

- UV detector – flow cell and lamp
- Pump, mobile phase, and mixer
- Column and column temperature

The article also describes how the latest HPLC technology eliminates sources of baseline artifacts, or makes it easier to diagnose and fix them.

UV detector – flow cell and lamp

A mechanical problem with the flow cell is a common cause of baseline anomalies. To check the flow cell, remove it from the instrument and examine the window surfaces for dust, cracks, or leaks. Shine a flashlight on the windows to aid in the observation. Rinse the outside of the flow cell with water, followed by methanol, to remove any salt, dust, or hazy deposit that may be present. Use lint-free lens paper to wipe the cell dry, or use a compressed air canister to dry the cell. If the windows are cracked or leaking, you can rebuild most UV flow cells with kits from the manufacturers.

To clean the inside of the cell, reinstall it, then start the pump at a low flow rate and look through each window while shining a light from the opposite direction, to see if there is debris or an air bubble trapped inside. Flush the flow cell with water, followed by methanol, to remove these interferences. The new-

generation flow cells in the Agilent 1290 Infinity LC System incorporate optofluidic waveguides, which are simpler to maintain (**Figure 1**).

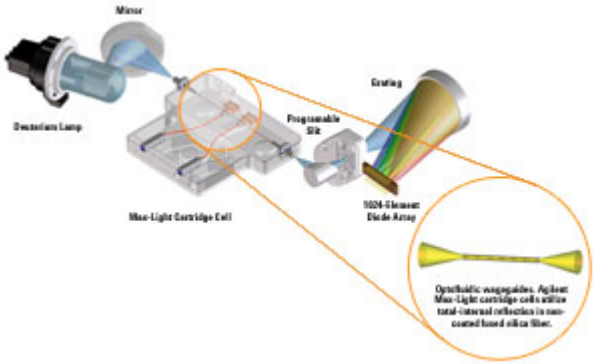

Figure 1. The fused cells in optofluidic waveguides do not have removable windows, so the only maintenance you need to do is flush the cell and clean the optical interfaces. (Click here to see this image larger.)

It is important to degas both aqueous and organic mobile phases for gradient operation. Inadequate degassing of the mobile phase can cause air bubbles to form in the flow cell, since the high pressure that keeps bubbles entrained at the front of the column is released before the flow cell.

A weak lamp can be a source of short-term noise and wandering baselines. After you correct any problems with the flow cell, reinstall it and perform a lamp intensity test by following the manufacturer's recommended procedure. Assess a drifting baseline with and without flow to see if the rest of the system is contributing to the apparent flow cell or detector problem. Replace the lamp(s) if necessary.

With the Agilent 1200 Series Diode Array Detector SL and 1290 Infinity Diode Array Detector, the lamps have RFID tags which track the age, burn time, and number of ignitions. Such data are useful not only to know when to replace the UV lamp, but also for troubleshooting any degradation in data quality.

Pump, mobile phase, and mixer

Baseline wander is also caused by pump pressure pulsations. To check for this problem, overlay the pressure trace and the baseline of a blank injection to see if there is any correlation between the two. Pressure pulsations are typically due to leaking pump seals, worn or scratched pistons, and/or check-valve problems, but may also be caused by incorrect compressibility settings, poorly degassed solvent, or bubbles trapped within the pump. The latest pump technologies used in the Agilent 1290 Infinity Binary Pump reduce these pressure pulsations.

Contaminated mobile phase can also cause baseline problems, so use freshly prepared mobile phases. If you use buffer salts or additives, use only HPLC-grade chemicals, and replace the mobile phases frequently. Discard any buffer

solutions that are held at room temperature for more than two days, especially if they are near-neutral pH, which favors microbial growth.

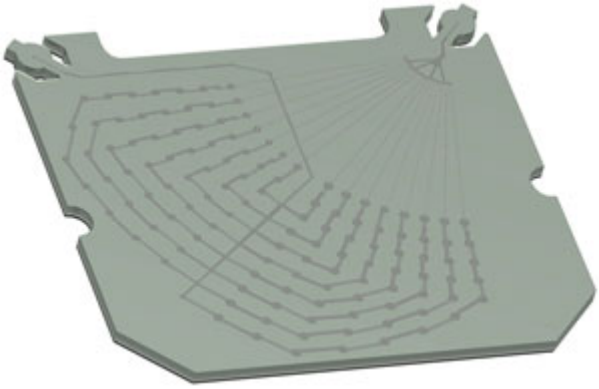

Figure 2. Microfluidic mixers, such as this Jet Weaver mixer in the 1290 Infinity LC, provide better mixing without adding delay-volume to gradient analyses.

Byproducts from bacterial or algal growth can produce ghost peaks or baseline problems. Filter the aqueous mobile phase as described in our earlier article. Look for evidence of bacterial and algal growth by the coloration of the inline filter. If such contamination occurs, rinse the reservoirs and the HPLC flow path (excluding the column) with water, followed by isopropyl alcohol, and then water again. Replace the inline filters with new ones.

Inadequate mixing during gradient generation or isocratic blending can cause periodic fluctuations of the baseline. Insufficient mixing manifests itself most often when you add modifiers only to the aqueous mobile phase in a gradient run, or when there is a significant imbalance in the UV absorbance of the mobile phase components. Add enough of the modifier to the second mobile phase to balance out the absorptivity difference. Be aware that the same amount of modifier in an aqueous solution may not give the same absorptivity in an organic solution. A good example is trifluoroacetic acid (TFA), where the UV spectrum of TFA is different in water than in acetonitrile. Use TFA in acetonitrile at about 85% of the amount used in the water.

Column and column temperature

Column-related problems can also cause baseline fluctuations. Impurities from samples or mobile phases can build up in the column and eventually leach out, causing ghost peaks, negative peaks, a wandering baseline, or drift. These symptoms can occur in addition to poor peak shape. Follow the column manufacturer's recommendation to clean the column, or replace it if you have used it for a long time.

Figure 3. To avoid contaminating the flow cell while cleaning the column, disconnect the column from the detector and divert the effluent flow directly to a waste container.

On rare occasions, bleeding or leaking silica from the column can cause baseline symptoms. As you wash the column, collect the effluent in a small, clean beaker for observation.

Another possible cause of baseline noise or wandering is refractive index change due to the temperature difference between the column and the flow cell. If the column temperature is more than 20 °C above ambient, consider using a secondary heat exchanger to reduce the temperature of the mobile phase that exits the column to as close to the flow cell temperature as possible. Note that with the new Agilent 1290 Infinity LC, temperature changes between the column and flow cell have minimal impact on the baseline, since the new detector design dramatically reduces refractive index effects.

If you follow good LC practices, you can avoid many of the problems that lead to unstable baselines. Taking proper precautions will ensure higher overall performance and save significant time. For additional timesaving HPLC tips, visit the Agilent podcast Web site or the Agilent support page for frequently asked LC questions

www.chem.agilent.com

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