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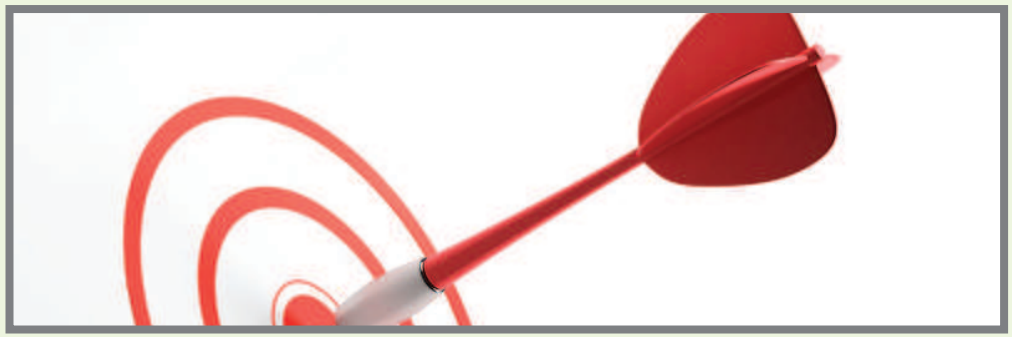
Tech Tip

Capillary Conditioning —

The Foundation of Precision in CE



In previous issues we saw the importance of a controlled, constant electro-osmotic flow (EOF) for proper precision. In this issue we will therefore look into the different strategies for capillary conditioning, as a good conditioning procedure is crucial to a stable EOF.

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Capillary Conditioning — The Foundation of Precision in CE

In previous issues we saw the importance of a controlled, constant electro-osmotic flow (EOF) for proper precision. In this issue we will therefore look into the different strategies for capillary conditioning, as a good conditioning procedure is crucial to a stable EOF.

A Fused Silica Capillary is Not an Inert Open Tube

Fused silica is the most commonly used material for capillaries in capillary electrophoresis (CE). We discussed previously that although we think of fused silica as a rather inert tube to perform our separation in, in fact the use of silica results in an electro-osmotic flow. The electro-osmotic flow is a "chemical" flow, not a constant flow controlled by a pump as in LC. The silanol groups from the capillary wall deprotonate depending on the pH of the background electrolyte BGE. Cations from the BGE then form a so-called double layer at the wall and when the voltage is applied, create a flow to the cathode, the negative electrode. It is thus easy to understand that anything happening to the wall will cause fluctuations in the EOF and improper conditioning results in high variability in the migration times of analytes. On top of this, some analytes show quite strong solute — wall interactions that can cause extensive band broadening. This is all the more reason to look into the conditioning of the capillary wall.

Treatment of a New Capillary

Although most labs have empirically found procedures for capillary conditioning and these procedures can vary distinctively between each other, there is general consensus that new bare fused silica capillaries should be treated with sodium hydroxide. Usual procedures work with 0.1–1 M NaOH with rinses/flushes for 20 min–1 h. This is then followed by a flush with water and the method's background electrolyte (BGE).

Already in the early 1990s the group of Khaledi published an extensive study on the conditioning of a capillary (1). The group of Wätzig also did some intensive studies and looked at the inner surface of CE capillaries with X-ray photoelectron spectroscopy (e.g., 2). Both showed the importance of an NaOH wash of a new capillary. The sodium hydroxide treatment gives a controlled surface hydroxylation and might also remove other debris left from the production of the capillary. Wätzig et al. furthermore showed the presence of organic material on the capillary wall of a new capillary. This was mostly removed with an NaOH flush. Sometimes a longer wash than described above might be needed. If the intended pH range of the method is in the interval where the EOF varies strongly with the pH, i.e. pH 4–7, a longer rinse improves precision (2). Do not wash the capillary with an organic solvent directly after the NaOH wash as this can cause anomalous behaviour of retention time and current (1).

For coated capillaries, check with the vendor's instruction, as some coatings are unstable in NaOH. For non-aqueous CE (NACE), avoid flushing with aqueous solvents.

Why Method Preconditioning?

With method preconditioning I mean here the preconditioning that

we perform between injections in a sequence and that is often programmed with the run method in the software. We set out to discuss preconditioning for EOF stability purpose, and thus good reproducibility, but there are more reasons for preconditioning steps that we might perform. These can be:

- Precision through reproducible and repeatable EOF and therefore mobilities and migration times
- Control over the direction of the EOF or suppression of EOF
- Refresh the separation medium, prevention of buffer depletion
- Reproducible reduction of wall interactions
- Prevention of carry-over from highly-concentrated sample components
- Flush out late-migrating components that are of no interest for the analysis.

The last one especially is often forgotten. Since the capillary is just an open tube, we can flush out anything that would migrate after our components of interest have migrated out.

Preconditioning is Part of Method Development

There are many different kinds of preconditioning steps performed. In the sidebar is an overview of steps to be considered. It is important to realize though that each application requires its own preconditioning procedure and that the investigation of appropriate steps should be part of method development. The best way to approach that is by starting simple. Always put in a BGE rinse before injecting the sample. Depending on its composition, pH and the samples you are working with, it might be sufficient to do just that. If so, do not spoil the wall equilibrium by adding unnecessary steps. If not, the preconditioning procedure can be increased step by step, depending on the issues met.

Not all steps described in the sidebar are rinsing steps, as we have found in practice that simple steps such as dipping the capillary in water or BGE, or applying (reversed) voltage can also greatly improve method performance. The latter also reduces carry-over.

Pre-sequence Conditioning

At the start of a new sequence, the (dedicated) capillary is installed in the instrument and a pre-sequence conditioning is performed. Usually, it suffices to perform a shortened version of the new capillary treatment (e.g., a 10 min wash with NaOH and conditioning with BGE). The application of the voltage might shorten the conditioning time needed. If a capillary is stored overnight in BGE, only a short BGE conditioning often suffices.

As we discussed in the previous issue of *CE Solutions*, static adsorbed coatings such as SMIL are becoming more and more popular for active EOF control and sample adsorption prevention. These coatings have shown to be very stable and effective. If properly applied, these coatings are stable for many runs, and it is not needed to have the coating polymer present in the BGE. Therefore, these kind of coatings are usually applied by rinsing the capillary with subsequent solutions of the coating components at the start of

Table 1

Preconditioning step	Remarks
BGE	• Obligatory, simplest possible preconditioning step, often sufficient • Cleans out sample components • Refreshes the BGE to avoid buffer depletion effects • Some BGE components interact with the surface, which in its turn can affect the equilibration time needed
Applied voltage	• Stabilizes the EOF • Apply a voltage with polarity opposite to the run voltage to reduce carry-over
Water	• To bracket solvents that are not compatible
NaOH	• If harsher treatment of the capillary wall is needed (highly concentrated samples (analyte and/or matrix), sample components with strong wall interactions) • Not compatible with some capillary coatings
Strong acids, such as H_3PO_4 or HCl	• Harsher treatment than BGE or water, but without deprotonation of the silanol groups of the capillary wall • Zeta-potential decreased (EOF slower) using the same pH in the BGE if acidic rinse is applied instead of an NaOH wash. This effect is less when applying EOF-modifying BGE components
Organic solvents	• Other cleaning properties than aqueous solutions • Do not use directly after NaOH rinse • Some solvents, such as acetonitrile, can swell the polyimide outer coating • Some solvents are not compatible with coated capillaries
Detergents, such as SDS	• Might be beneficial for some matrices, such as biological samples (3) • Some detergents cause permanent changes of the capillary wall
Wait step	• Time to equilibrate • In some software the way to programme a dip
Dynamic coating solutions	• Do not always need to be added to the BGE, sometimes it suffices to flush in between runs or before the start of a sequence
Dip capillary end	• Cleaning the injection end of the capillary by dipping into water after preconditioning and before sample injection can improve injection precision

Figure 1

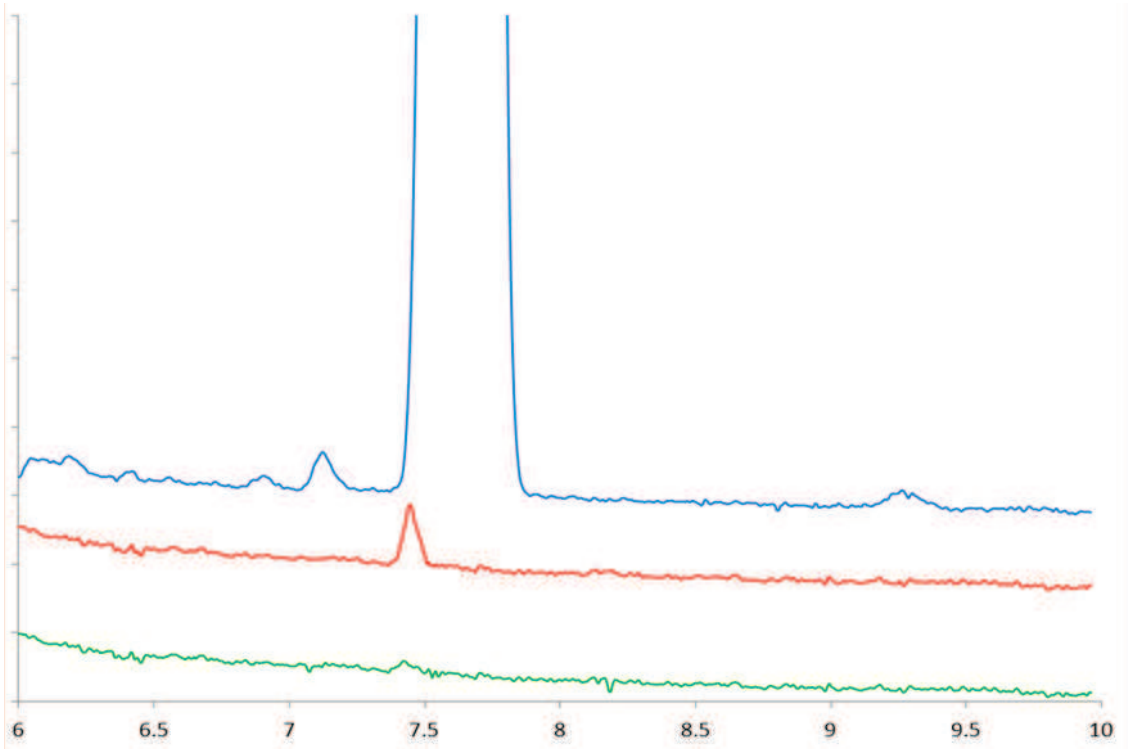

Figure 1: This example illustrates a simple and effective step to get rid of carry-over. The red trace shows the injection of a blank after the injection of a standard. The peak observed is carry-over from the main component of the previous standard injection and varies between 0.2 – 0.3 % of the nominal standard concentration. In the blue trace, the injection of a sample, the impurity peak before the main component is about 0.17 % (corrected peak area) of the main component. The green trace is also the injection of a blank after injection of a standard, only now an extra step was added to the pre-run conditioning: a voltage reversed to the run voltage (-20 kV instead of 20 kV) was applied for 1 minute as the final step in the preconditioning.

the day as part of the pre-sequence conditioning. Some of them are even stable for several days and then just a BGE pre-sequence rinse might suffice.

Storage of the Capillary

For most equipment and software combinations, you can create a separate method that is programmed at the end of a sequence and that automatically performs the shut-down procedure you want it to. For short-term storage, for example, between sequences or overnight, it is often best to clean the capillary and then fill it with running buffer, making sure that the capillary ends are immersed in the BGE. Always leave a capillary filled with (salt) solutions with the ends immersed to prevent evaporation of the solvent and crystallization of the salts, as salt crystals will block the capillary.

For long-term storage, the bare fused silica capillary needs to be cleaned properly with NaOH, for example. The final washing step is an extensive water wash before the capillary is blown dry. The latter can be achieved by flushing with air via empty vials. For coated capillaries, again, follow the vendor's instructions.

The Tiny Details

Use filtered (pore size ≤ 0.45 µm) and degassed solutions. Always programme the capillary conditioning such that rinsing liquids are not flushed in the BGE vials, especially not in the vials used during the high voltage step in the run. This is to prevent composition and pH changes in the BGE vials, and furthermore to maintain constant liquid levels. If the liquid levels are not level between inlet and outlet during the voltage run, there will be a hydrodynamic flow on top of the electro-osmotic flow in the capillary. The profile of this hydrodynamic flow is parabolic, and results in additional band broadening, which in its turn could destroy resolution. On top of that, the migration times of the analytes will not be constant during the sequence. With wrongly programmed conditioning in long sequences, the outlet vial level will gradually increase compared to the inlet vial, resulting in gradually increasing migration times.

For the same reason, conditioning with BGE should not be performed from the same vials as the run inlet and outlet vials.

The best is to use dedicated waste vials at the outlet for the conditioning procedure. The waste vial should not

be empty but contain some liquid to prevent a drop hanging down from the capillary. Especially with viscous liquids such as gels this is important. For example, empty waste vials for CE-SDS applications resulted in blocked capillaries, something easily prevented by putting some water in the waste vial.

If the sequence is very long or if the preconditioning procedure is extensive, check that the waste vial does not overflow midway through the sequence. When a voltage step is programmed, the inlet and outlet vial should contain BGE.

Be Explicit

Describe the conditioning and storage procedures clear-cut in your method. Since preconditioning is dependent on the aim of the method, the BGE and the samples, it should be an integral part of method development. Often sub-optimal preconditioning is a significant aspect of method problems. A well-developed capillary conditioning procedure is crucial to a stable EOF and the foundation of a precise and reproducible CE method.

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Cari Sänger has more than 20 years of experience in pharmaceutical and chemical analysis. Her aim is to stimulate people to keep growing and learning, striving to get the best out of themselves. Cari is an independent, reliable, scientific people-manager and a globally recognized expert on separation science, especially within the capillary electrophoretic techniques. Cari's focus is primarily on implementation, knowledge transfer and good working practices.

Ask the Doctor

Cari Sänger is available to answer your specific method development and troubleshooting CE questions. Submitted Q & As will also form the basis of future CE Solutions.

NOTE! *"Help! I need a method to separate ____" Unfortunately, this is a question that Cari can't help you with. However, here are a few hints: (1) do a literature search using 'Pub Med' or one of the free search engines; (2) a good source of methods are Electrophoresis Journal of Chromatography A and B issues; (3) consult the applications literature of various manufacturers (4) visit Chrom Forum at www.chromforum.org*

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