CE Solutions

separation | science
new horizons in chromatography and detection techniques

Cari Sänger is best known as one of the world's foremost CE troubleshooting authorities. Separation Science and Cari Sänger have collaborated to offer this digital learning platform providing valuable advice on everyday issues, problems and challenges faced by CE practitioners. Importantly, you will also have the opportunity to interact with Cari through our online questions submission system.

Tech Tip

The CE Capillary

The small diameter of the capillary in capillary electrophoresis makes for a very good heat dissipation compared with conventional electrophoresis. As a consequence, much higher voltages can be applied before heat dissipation becomes an issue. The high voltage, typically up to 30 kV, means that the separation becomes very fast and efficient and gives high plate numbers. In this issue of *CE Solutions* we will focus on the capillary as used for CE. We will look at the typical choices of length and diameter for method development. We will also look at coated capillaries and discuss both permanent coatings and adsorbed coatings.

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What is a capillary?

A capillary is a thin tube and for CE we typically use fused silica capillaries. Mostly used inner diameters are 50 µm and 75 µm. Common capillary lengths for a CE separation are 30 – 60 cm. For CE-MS, you normally need a longer capillary of around 1 m in order to be able to make the connection of the CE to the MS. On the outside the capillary is covered with a protective coating, usually polyimide. On the inside, you can also use coatings, so it is important to distinguish between them. When we talk about coated capillaries we are not talking about this outer coating, but about the an additional coating that is added on the inside of the capillary to enhance separation.

Detection window

Fused silica meets most of the requirements one can have for a capillary. It is a rather inert material, inexpensive and easy to handle. As fused silica is transparent for UV light, it is possible to use UV-VIS detection by looking straight through the capillary. Of course you then have to remove the protecting coating on the outside of the capillary. The easiest way to remove the polyimide coating is to burn off the coating with a ordinary cigarette lighter or, in a more sophisticated manner, with a specially designed window burner.

Capillary ends

The capillary can be cut to the desired length with a ceramic cutter or with a special capillary cutting

Figure 1

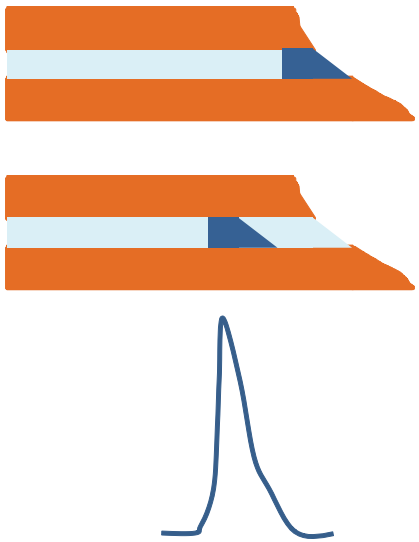


Figure 1: Injection artefacts caused by a badly cut capillary. If the capillary cut is not straight, this will lead to injection artefacts. In this case, the sample is not injected as a neat zone. Consequently, a tailed peak will result.

device containing a diamond. It is important that the cut is straight to avoid extra band broadening. To get a straight cut, make a small cut or flaw in the capillary with the ceramic cutter without applying too much pressure. Do not bend the capillary while cutting. After making the flaw, a little bending is sufficient for cleaving the capillary. So, it is actually quite similar as cutting a window-pane. Check with a magnifying glass or under the microscope. Even the outlet of the capillary should be straight. If not, or if broken, this might affect your separation (e.g., Figure 1). There are examples where a broken outlet resulted in sloping baselines or tailing peaks. Do not saw with the cutter. Sawing gives a rough cut and debris might end up in the capillary, disturbing your analysis.

It is usually advantageous to remove the polyimide coating a few millimetres from the inlet and outlet of the capillary. This will reduce carry-over and give better precision. This is important especially when using solvents that make the polyimide swell, such as acetonitrile (Figure 2). Removing the polyimide from the inlet and outlet is not advisable for some coated capillaries, as it might damage the inner coating.

Capillary length

In selecting the appropriate capillary length for your application, there are a few things to consider. Fundamentally, the resolution in a CZE separation is independently of the capillary length. Short capillaries give fast separations and high field strengths can be applied. For that reason you can find several examples in literature where people use the so called 'short-end injection'. This is done as follows. The common CE equipment is restricted to minimum capillary lengths of around 30 cm total length. In effect that means 20–24 cm effective separation length to the detector and 8–11 cm from the detector to the outlet. In order to use a shorter capillary, you can inject on the outlet end and reverse the applied voltage. The separation is then from outlet to detector, so over the short-end.

A longer capillary could give additional resolution, at the cost of a longer analysis time, if you use CE modes that make use of chromatographic interactions, such as MEKC or chiral separations. An additional advantage to a longer capillary is that you can inject more, a longer plug, before the injection plug length starts to contribute to the band broadening.

Capillary diameter

The smaller the capillary diameter, the more efficient the heat dissipation, so the higher the voltages that can be applied for fast and efficient separations. However, because detection is usually UV detection performed on-capillary, the capillary diameter is also the detection path length. Lambert-Beer's law teaches us that for sensitive detection, you'd

Figure 2

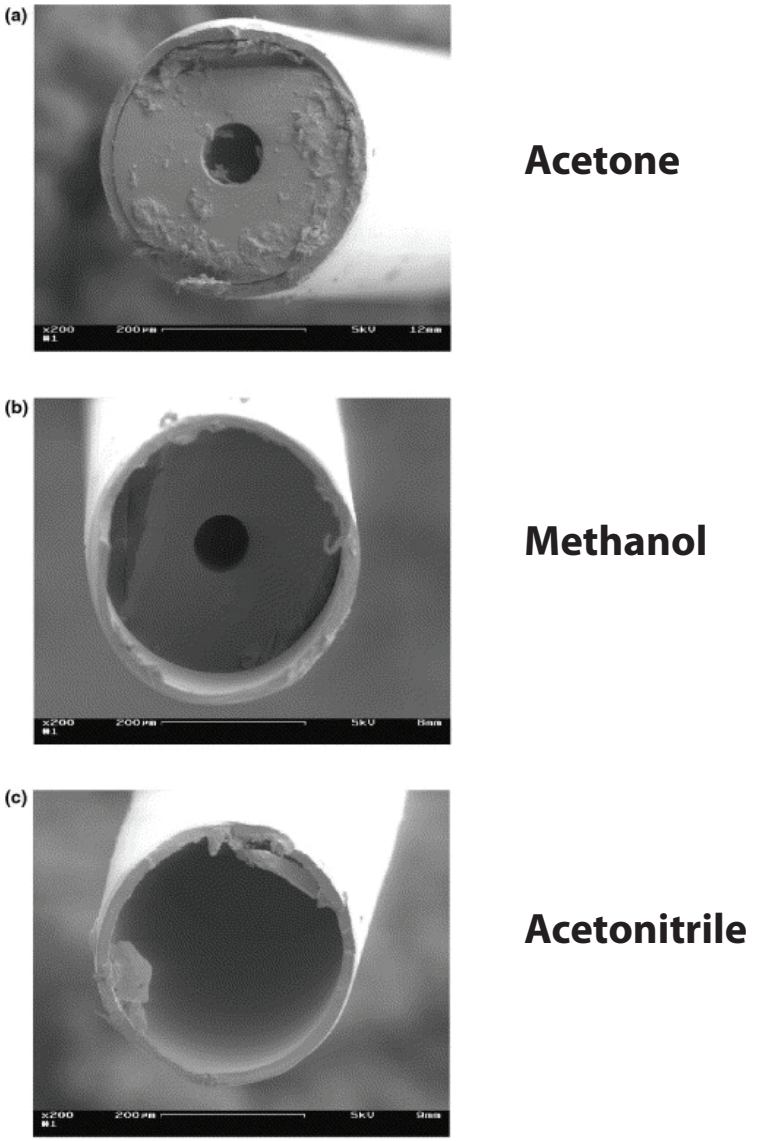


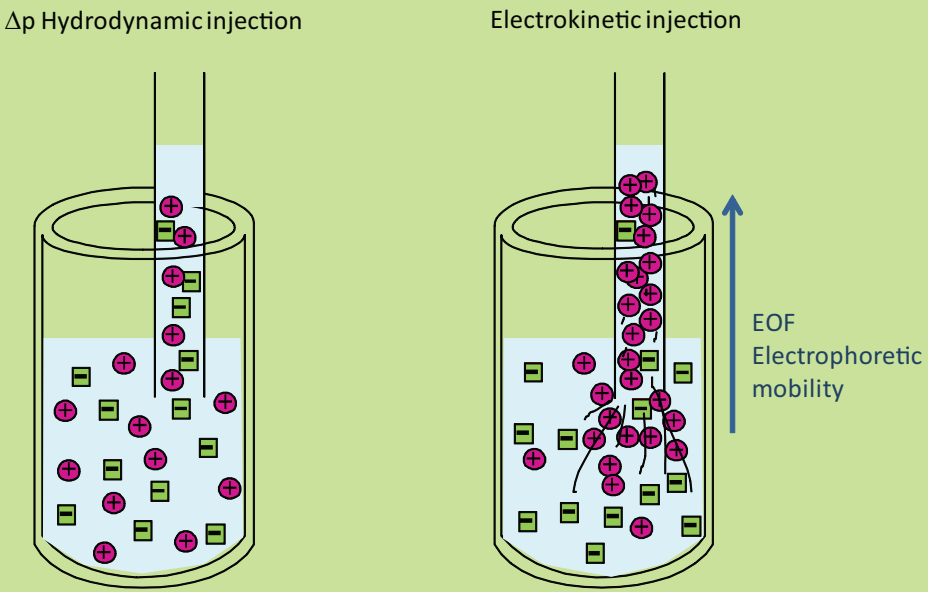
Figure 2: Some solvents cause swelling of the polyimide coating. These impressive and demonstrative pictures come from a publication of F Baeuml and T Welsch, *J. Chromatogr. A* 961 (2002) 35–44. It shows what happens if the fused silica capillary end with the polyimide coating on the outside is immerged in solvent. In methanol the polyimide swells a little. In acetonitrile it swells substantially. So much so in the latter the proper end of the capillary is no longer visible, as the polyimide sticks out like a too long sleeve.

Injection

In capillary electrophoresis, you can either inject hydrodynamically, or in other words with pressure difference, or electrokinetically, which is through applying an electric field.

The volume injected with hydrodynamical injection depends on the capillary diameter, the length, the applied injection time and pressure difference and the viscosity of the liquid. With Poisseuille's law you can estimate the injected volume V:

$$V = \frac{\Delta p \, d^4 \, \pi \, t}{128 \, \eta \, L}$$



Δp is the pressure difference, d the capillary diameter, t the applied injection time, η the viscosity and L the total length of the capillary. As the injected volume depends on the pressure difference over the capillary, it is important for repeatability that the vial at the outlet of the capillary during injection has a constant level. That is, this vial should not be the waste vial, which is probably the vial in the system with the most variable liquid level.

The electrokinetically injected volume depends on the strength and direction of the applied voltage and the injection time, but also on the total capillary length, the capillary diameter and electro-osmotic mobility. On top of that, the electrophoretic mobility of the analyte plays a role. If an analyte has a high mobility towards the outlet electrode, this analyte will be injected in a larger amount than an analyte with a lower mobility. From an analyte with a mobility towards the inlet electrode, a little will only be injected if the electro-osmotic flow is towards the outlet electrode. So electrokinetic injection is a selective injection mode. This means, that the composition of the sample vial will change during injection. So multiple injections from the same vial is not recommended.

$$Q = \frac{(\mu_e + \mu_{eo}) \, V \pi r^2 \, C \, t}{L}$$

The amount Q of an analyte injected in relation to the electrophoretic and electro-osmotic mobilities μ_e and μ_{eo}, the applied Voltage V, capillary radius r, analyte concentration C, injection time t and total capillary length L.

want longer detection path lengths, not shorter. So depending on the detection properties of your analytes and the required sensitivity of the application, you have to select the optimal diameter. You can check if the heat dissipation for the selected BGE is still sufficient or not by making an Ohm's plot, as explained in the previous issue of *CE Solutions*.

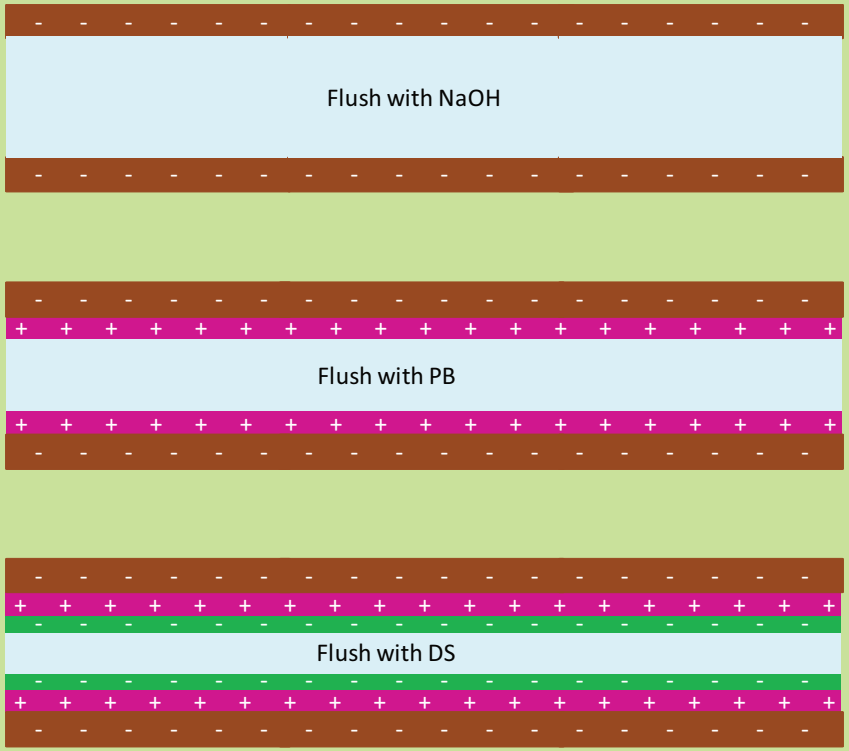
Injected volume

Both the capillary length and the diameter have a big influence on how much you inject into the capillary, see sidebar. The most common way of injection is hydrodynamically by replacing the inlet vial for the sample vial and applying a pressure difference (over-pressure on the inlet or under-pressure on the outlet). Poiseuille's law teaches us that volume displacement through a tube by a pressure difference for a certain time is inverse proportional to the length of the tube and is proportional to the fourth power of of the diameter, d^4. So if the capillary is halve the (total) length, twice as much gets injected. If the diameter reduces from 75 µm to 50 µm, the injected volume is 5 times less if you don't change the injection pressure and time.

Coated capillaries

Sometimes it is better to use coated capillaries instead of bare fused silica capillaries. For instance, if no or a reversed electro-osmotic flow EOF is wanted. Or if components from

SMIL coatings



Double and triple layer coatings are also called SMIL coatings. SMIL stands for Successive Multiple Ionic polymer Layers. They are a form of static adsorbed coatings. Commonly applied polymers are polybrene PB, dextran sulphate DS and poly (vinyl sulfonic acid) PVS. 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. This gives more flexibility to optimise the BGE independent of the coating.

The procedure for coating is simple and can be performed at the start of the day or sequence. First, the capillary is flushed/rinsed with sodium hydroxide to wash the wall and get a proper negative charge. The a solution of PB in water is flushed through for some minutes, followed by a water flush and the a solution of DS or PVS in water and a last water flush. If a triple layer is required, add another flush of PB and water. Finally fill the capillary with the BGE and the system is ready for use.

the sample stick to the capillary wall and make poor repeatability and the separation irreproducible. We divide the capillary coatings into two groups, permanent coatings and adsorbed coatings. Permanent coatings are covalently bonded, whereas adsorbed coatings can be flushed off.

Examples for permanent coatings are poly (acryl amide) PAA, poly (vinyl alcohol) PVA, poly (ethylene glycol) PEG and poly (ethylene oxide) PEO. Some of them eliminate the EOF. These coated capillaries can be purchased from different vendors. With the capillary comes a document showing a batch test for the effectiveness of the EOF elimination. It will also tell you under what conditions the coating is stable, what kind of solutions are incompatible with the coating and how to pretreat, clean and store the capillary.
Adsorbed coatings can be divided again into two groups, static adsorbed and dynamic coatings. Examples of the static adsorbed coatings are the polymer coatings such as polybrene PB, dextran sulphate DS and PVS, poly (vinyl sulfonic acid). These coatings have shown to be very stable and effective as double and triple layer coatings, or also called SMIL coatings (see sidebar). If properly applied, these coatings are stable for many runs, and it is not needed to have the coating polymer present in the BGE, giving more flexibility to optimise the BGE independent of the coating.
The other group of adsorbed coatings are the so called dynamic coatings. These have to be present in the BGE for an effective, repetitive and reproducible effect. Dynamic coatings can be ionic surfactants such as CTAB, monoamines, such as triethanolamine, diamines as putrescine, polyamines as spermine etc. To demonstrate how well-used these coatings are, two examples from the pharmacopoeias. Triethanolamine is being used in the pharmacopoeial method for the enantiomeric purity of S-ropivacaine and Putrescine is being used in the method for the separation of EPO isoforms.

Capillary history: one application per capillary

The electro-osmotic flow is a "chemical" flow, as we saw in the *first issue*. 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. You can easily imagine that when you are developing a method and testing different BGE compositions, that these can leave traces at the capillary wall. Even so certain sample components. Therefor it is good practice to test the optimised conditions after method development on a new capillary and to stick to one application per capillary.

The capillary, an inert tube to keep the separation together?
We set out thinking that the capillary is just a mechanical device for performing very efficient electrophoresis in. But we have seen that in fact the capillary is much more than that. Good CE practice taught us that the capillary ends need to be well-cut, with the polyimide removed. For method development purposes, the capillary length and diameter are parameters of importance, both having an impact on the detectability and injected volume of the sample. The importance of the quality of the EOF shows from all the attention people have paid over the years to capillary coatings. Selecting the proper coating and developing an efficient coating procedure is a major part of method development.

So far, we have not discussed capillary conditioning. As this is a chapter in itself, we will take that up in a future issue of *CE Solutions*.

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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