CE Solutions

SeparationScience
PREMIER LEARNING FOR ANALYTICAL CHEMISTS

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

Detection in Capillary Electrophoresis

Previously we went through the fundamentals, the separation medium BGE, the capillary and injection in capillary electrophoresis. In this issue of *CE Solutions* we will reach the end of the system, the detector. We will discuss the most popular detection principles in use in industry and CE-related good working practice.
Click here to read more..

Ask the Doctor

Through 'CE Solutions' you will be able to ask questions directly. So if you have problems with low signal, detection, precision or any other CE issues then *click here* to contact Cari.

Detection in Capillary Electrophoresis

Previously we went through the fundamentals, the separation medium BGE, the capillary and injection in capillary electrophoresis. In this issue of *CE Solutions* we will reach the end of the system, the detector. We will discuss the most popular detection principles in use in industry and CE-related good working practice.

UV Detection

The usual detection techniques for liquid separation are also available for CE and UV detection is by far the most common. Modern equipment comes with build-in UV/VIS single wavelength or diode array detectors, specially designed to focus the beam of light into the capillary. Absorption is a function of the path length (Lambert-Beer's law). As we detect on-column in CE, the path length is directly related to the capillary diameter. Compared to other liquid separation techniques, this means that the path length is rather short, which limits detection sensitivity. The loss in sensitivity from the path length is partly compensated for by the high efficiency in CE and by the possibility to use low UV wavelengths. High efficiency means reduced band broadening and a higher concentration in the sample bands. With the aqueous background electrolytes (BGEs) that we usually employ in CE, we can go down to 190–200 nm for UV detection. Such low wavelengths usually mean a significant gain in sensitivity and wider applicability. Thirdly, we learned in the past years a lot about injection stacking techniques. With stacking, low sample concentrations end up in higher detection concentrations (CE Solutions, Issue 5).

Indirect UV Detection

For compounds that do not exhibit UV absorption, indirect UV detection can be used. Indirect UV detection is universal and non-selective. In the BGE, an absorbing co-ion is dissolved, also called the UV-probe. The non-absorbing analyte band displaces the absorbing UV-probe. So at the position of the non-absorbing analyte band there is less absorption and the signal will show a negative peak. The amount of displacement depends on the charge of the UV-probe and the analyte ions and on their mobilities. A measure of the amount of displacement is called transfer ratio. The highest transfer ratios are obtained when the mobility of the

UV-probe matches the mobility of the analyte. Moreover, the mobilities of the analytes compared to the UV-probe will determine the shape of the peaks. If the mobilities are similar, symmetric peaks are obtained. If the mobilities differ from the mobility of the UV-probe, electromigration dispersion occurs and we observe the typical triangular peaks.

Consequently, the UV-probe should match the analytes at hand.
The advantages of indirect UV detection are its universal and non-selective applicability and the possibility to determine absorbing and non-absorbing analytes simultaneously. The disadvantages are that analytes can show up as positive and negative peaks and that you cannot use the diode array detector for additional identity confirmation. For identification of the analytes it is therefore important that the migration times are highly reproducible. To increase reproducibility, the use of an internal standard and comparing relative migration times or relative mobilities is recommended.

There are several commercially available kits for the indirect UV detection of small cations and anions, organic acids and forensic anions, for example. Typical applications are drug counter ions, fermentation broths, carbohydrates, metal ions and explosive residues etc.

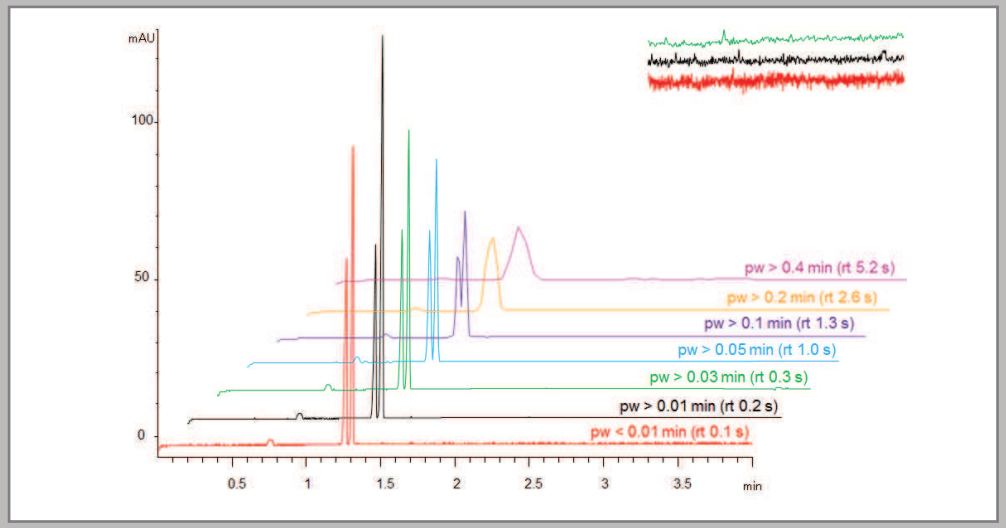
Fluorescence Detection

A fluorescence detector is almost as straight forward to use as a UV-detector. The fluorescence detector comes as a module that can be exchanged with the UV or DAD module within the instrument or you can add a module to your instrument. For a good detection signal, a strong light source is needed for excitation. For many years this has been a laser (laser induced fluorescence LIF), but now also LEDs are appearing on the market.

The advantages of LIF detection are its sensitivity and its selectivity. Its selectivity is not only an advantage, but can also be a disadvantage. Either the analytes of interest need to show native fluorescence, or labelling is required. Many labelling reagents have been developed over the years and are commercially available. Usually, labelling requires derivatization during the sample preparation.

Contactless Conductivity Detection

Contactless Conductivity Detection (CCD) is rapidly gaining foothold within industry. The CCD detector is now commercially available with a simple mechanical construction and is relatively cheap. Two cylindrical electrodes, the actuator and pick-up electrode, are placed around the capillary, without the need to remove the polyimide coating. The capillary does not need to be fused silica, but can be of any non-conducting material, such as PEEK®, Teflon® etc. Also rather small internal diameters, such as 10 µm, can be used. The detector measures the difference in conductivity between the BGE and the analyte bands. For optimal sensitivity, the difference in conductivity between BGE and analyte should be as high as possible. So for CCD, zwitterionic buffers with low conductivity, such as a MES-Histidine buffer, are frequently used. Alternatively you can apply indirect conductivity detection if the conductivity of the analytes is low.



Detector Sampling Rate

This figure illustrates the effect of the sample rate setting for the detector. The detector sampling rate (in Hz), or response time (rt), determines how often a data point is recorded to construct the electropherogram. This data point is actually the average of the time interval set as sampling rate. The sampling rate does not affect the separation itself, only the way it is recorded. If the sampling rate is set too fast, the noise is higher (and the raw data file bigger), see enlargement. If the sampling rate is too slow, it looks like losing resolution. This will also affect the quantitative results! In the picture above, as an extreme you see that the separated bands look like one peak. Another drawback of a too slow sampling rate is that there are too few data points per peak which impedes integration.

The proper setting for the sampling rate is connected to the peak width (pw). In some software the sampling rate is therefore set by adjusting the peak width. Select the narrowest peak of interest for this. The black trace shows the appropriate settings for this separation.

Sample: p-hydroxyacetophenone and vitamin C; BGE: 20 mM borate buffer pH 9.3; Capillary: 50 µm x 33.0 (24.5) cm fused silica; V = 25 kV; T = 25 °C; injection 50 mbar x 5 s; DAD 280 nm with 10 nm bandwidth. The UV lamp during recording was at the end of its lifetime and only just passed the lamp intensity test.

Figure 1

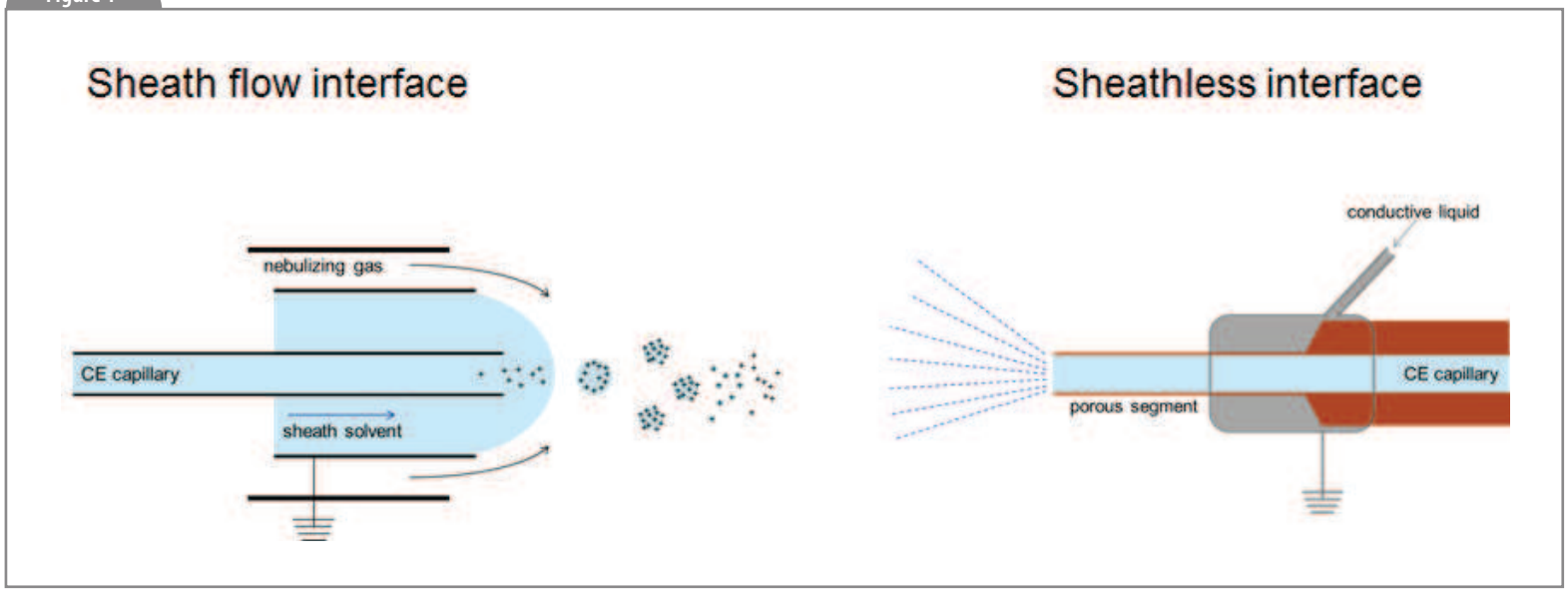


Figure 1: Schematic representation of the sheath flow and sheathless interface for coupling capillary electrophoresis to mass spectrometry.

Table 1

Diameter (µm)	Injection pressure		Injection time (s)	Injected volume (nl)	Injected plug length (% of total length)
	(mbar)	(psi)			
25	50	0.7	80	12	7.2
50	50	0.7	5	12	1.8
75	10	0.15	5	12	0.8
25	50	0.7	20	2.9	1.8
50	50	0.7	5	12	1.8
75	22	0.3	5	26	1.8
25	50	0.7	5	0.7	0.4
50	50	0.7	5	12	1.8
75	50	0.7	5	59	4.0

Table 1: Changing the capillary diameter has a huge influence on the amount injected. In this table you see different settings for maintaining the same injected volume or injection plug length. For convenience, pressures are given both in mbar and psi.

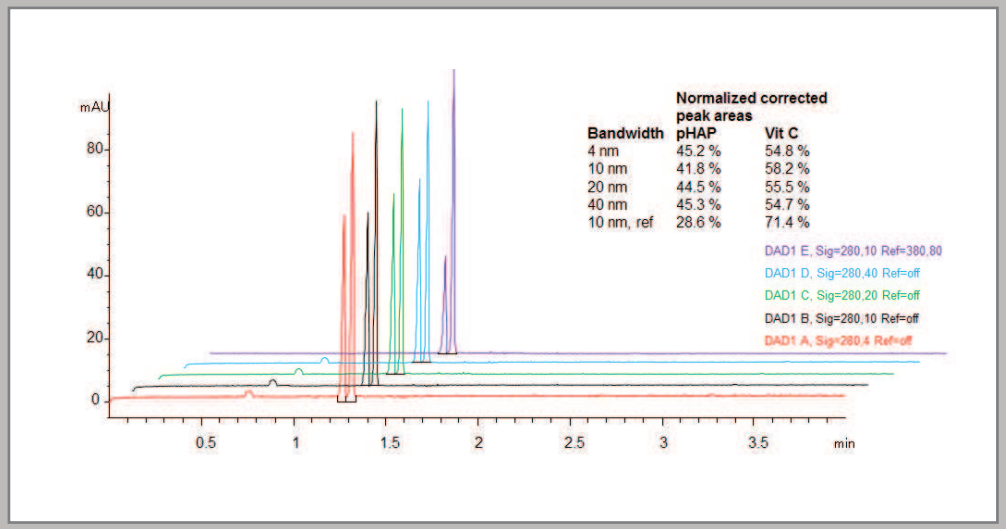
Contactless conductivity detectors can easily be used in parallel to other detectors such as UV/DAD, LIF or MS.

CE-MS

There are many applications for which CE-MS is an interesting combination. The most common ionization mode is electrospray ionization. Commercially available is a sheath flow interface.

The function of the sheath liquid is both to ground the capillary end and to deliver a microliter flow for robust spraying. A typical sheath liquid is made of 50 % methanol / 50 % water and contains some formic or acetic acid. A typical flow-rate is 4 µL/min. Around the needle with the sheath liquid flows the nebulizing gas that is needed for a stable spray. Advantages of the sheath flow interface are its relative robustness and independence of the electro-osmotic flow. A disadvantage is that solutes are diluted on leaving the capillary, at the cost of sensitivity.

Another type of interface that is being tested for commercial launch is the sheathless interface. The capillary outlet is etched in such a way that only a very thin layer of silica remains. This thin layer of silica is porous and conducts current. The capillary end is grounded through a conductive liquid around the porous part of the capillary. The grounding liquid does not reach the outlet. This means that the flow out of the capillary is an undiluted nanoflow, resulting in increased sensitivity, which is a major advantage. The disadvantage is that



UV Detection Wavelength and Band Width

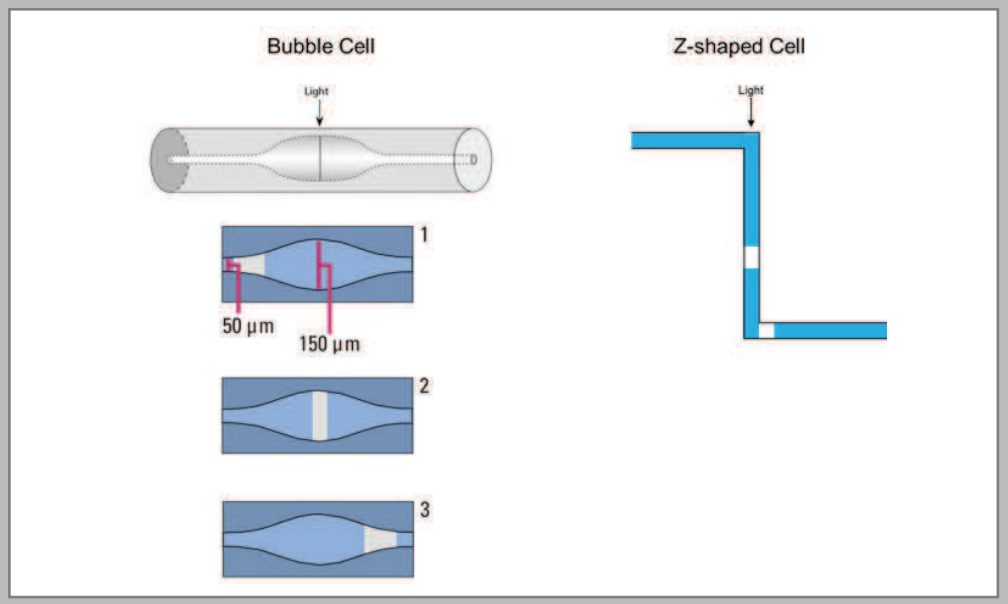
If you use a diode array detector for single wavelength detection, you can select the detection wavelength and bandwidth for the UV signal. There is no standard optimum setting for the bandwidth, as this figure shows. During the same run, the 280 nm signal was recorded with different settings for the bandwidth. Depending on the settings, even different quantitative results were obtained! In this case, especially using a reference wavelength gave wrong results. The percentages in the table are corrected peak area percentages.

Sample: p-hydroxyacetophenone and vitamin C; BGE: 20 mM borate buffer pH 9.3; Capillary: 50 µm x 33.0 (24.5) cm fused silica; V = 25 kV; T = 25 °C; injection 50 mbar x 5 s.

you need a net flow towards the capillary outlet. There is not much information yet on the robustness of this interface.

Good CE Detection Practice

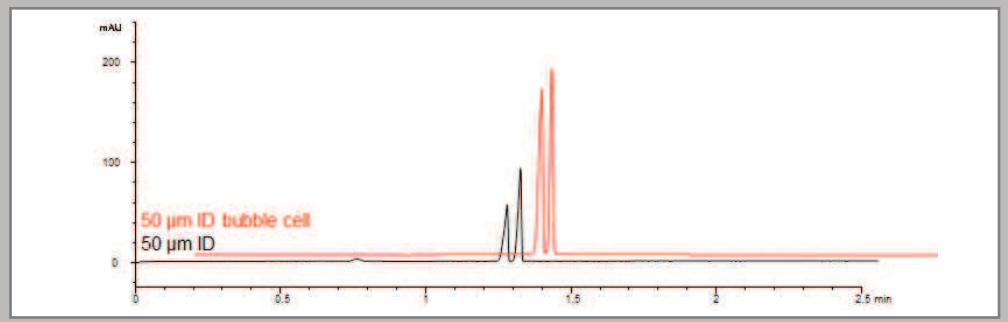
Most of the above mentioned detection techniques provide on-column detection. The advantage of this is that there is no additional band broadening after separation from transport to the detector. But a consequence from on-column detection is that the analytes move with different velocities through the detector. The early migrating analytes move fast and their bands result in narrow peaks. The late migrating analytes move slow and their bands result in broader peaks, as they move for a longer time through the detection window. If we would use peak areas for quantification, this would result in a bias towards slower migrating peaks. To correct for the difference in velocity through the detection window, we correct the peak area either for the velocity (e.g.,



Increasing Detection Path Length

One way of increasing detection path length is by increasing the capillary diameter, but this is often accompanied with loss of efficiency due to excessive Joule heating. An alternative way to increase the detection path length is to widen the capillary only at the detection window. Examples are the bubble cell or the z-shaped cell. Bubble cells (picture courtesy of Agilent Technologies) are available with an increased path length with factor 3 or 5. In a z-cell, detection is performed through a part of the capillary in a construction that is z-shaped. The advantage is that the detection path length is no longer dictated by the capillary diameter. The drawback is that the resolution between the analyte bands should be wider than the path length of the z-cell. If the bands would be simultaneously inside the detector cell, the bands are separated but show as reduced resolution or even as one peak.

Electropherogram: Sample p-hydroxyacetophenone and vitamin C; BGE 20 mM borate buffer pH 9.3; Capillary 50 µm x 33.0 (24.5) cm fused silica with or without bubble cell; V = 25 kV; T = 25 °C; injection 50 mbar x 5 s; UV detection 280 nm with 10 nm bandwidth.



32Karat software) or for the migration time (e.g., ChemStation software). Do not use corrected peak areas in capillary isoelectric focussing (cIEF). In cIEF the analytes are separated in the pH-gradient throughout the capillary and only upon mobilization after the separation they migrate through the detector window.

For any photometric kind of detection, it is key that the light is focused on the core of the capillary and that there is not too much stray light. Use the appropriate interface/aperture. If you have a BeckmanCoulter instrument with the black plastic aperture, please inspect the aperture regularly. With time, the UV light will degrade the plastic of the aperture and the detection slit becomes larger. Replace the aperture at least every year.

Furthermore, the detector settings should match the fast and efficient bands in the CE method at hand (see sidebars). For example, if the sampling rate is too slow, the raw data does not contain sufficient measurements in a peak in order to recognize it as such or to integrate properly. If the sampling rate is too high, noise levels will be unnecessarily high as well.

For sensitive detection, you would like to choose a larger inner diameter, but then the risk of issues with excessive Joule heating is higher. If you test different capillary diameters for optimal sensitivity, please adjust the injection settings. See the example of Table 1 for a 33 cm long capillary.

If you want a smaller internal diameter for better heat dissipation, but also want to inject more, take a longer capillary. With a longer capillary, you can inject a larger volume without exceeding the optimal plug length (but at the cost of longer run times). The optimal plug length without stacking mechanisms is 1-2 % of the capillary length. With simple stacking such as field amplified stacking (see CE Solutions, Issue 5), as a rule of thumb up to 5 % of the capillary length is feasible. Other ways to increase sensitivity are increasing the detection path length, (e.g., by using a bubble cell or a z shaped detection cell.)

It is Just a Detail, but…

With the detector we have reached the end of the CE system. Although at first hand detection looks like just recording the separation, there is more to it. As seen before with the other parts of the CE-system, the right or wrong choices make or break the separation and sensitivity. Attention to CE details seems tricky at first because in our enthusiasm it is easy to forget that any new technique has its own good working practice. (Unconsciously) copying good working practices from one technique to another is not applicable. We have discussed many of the CE good working practice aspects throughout the issues of CE Solutions so far and will pay more attention to them in the upcoming troubleshooting examples.

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 trouble-shooting 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*

Click here to submit your question