High-sensitivity detection cell for the Agilent Capillary Electrophoresis system

Technical Note

Introduction

Detection sensitivity is often cited as the most significant limitation of capillary electrophoresis (CE). This is a result of both small sample volumes (1–50 nL) and the short detection path length associated with on-capillary detection. Whereas on-capillary detection is simple, the UV-visible detection path length is limited to the internal diameter of the capillary. In practice the path length is further reduced because the capillary has a circular cross section, and the actual path length is approximately 80 % of the internal diameter.[1]

To improve sensitivity of CE analyses, Agilent Technologies developed extended path length capillaries, known as bubble cell capillaries. These capillaries increased the internal diameter of the capillary at the point of detection by about a factor of three and thereby increased sensitivity by the same amount.

Agilent also developed the high-sensitivity detection cell which effectively decouples the detection cell from the rest of the capillary. The high-sensitivity detection cell for the Agilent CE system represents a major advance in detection technology for capillary electrophoresis, increasing sensitivity by an order of magnitude.

The high-sensitivity detection cell is available as a kit (Agilent part number G1600-68723) which includes the cell, capillaries, preassembled ferrules, finger-tight fittings and cassette.

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The high-sensitivity detection cell
The high-sensitivity detection cell is a decoupled detection cell for direct UV-visible absorbance detection with the Agilent CE system. The increased path length of the high-sensitivity detection cell provides an order of magnitude increase in sensitivity and an increase in linear range while at the same time enhancing spectral analysis.

The high-sensitivity detection cell is constructed using microfabrication techniques and is designed so that it is fully decoupled from the separation capillary. The low volume of the high-sensitivity detection cell has negligible impact on peak shape, making it ideal for applications which demand sensitivity (trace analysis), increased linear range (chiral excess, impurity analysis) and high resolution. The reusable cell body, with replaceable capillaries, makes the high-sensitivity flow cell extremely flexible and reduces the cost of achieving high sensitivity UV-visible absorbance detection in CE.

The high-sensitivity detection cell has a unique design – constructed from silica parts which are fused together to form the detection cell. The schematic in figure 1 illustrates the increased path length relative to the capillary dimensions. The high-sensitivity detection cell is 100 µm square with a path length of 1.2 mm and a volume of 12 nL. The light path through the cell is made entirely of black fused silica to minimize stray light and to define the aperture for diode-array detection. In addition the reflective interior functions as a light pipe, ensuring almost 100 % transmission of the light which enters the detection cell. The detection cell is flanked by flat clear windows. All these properties combine to provide not only an order of magnitude increase in sensitivity, but also a significant improvement in the linear range and unsurpassed spectral fidelity with the diode array detector.

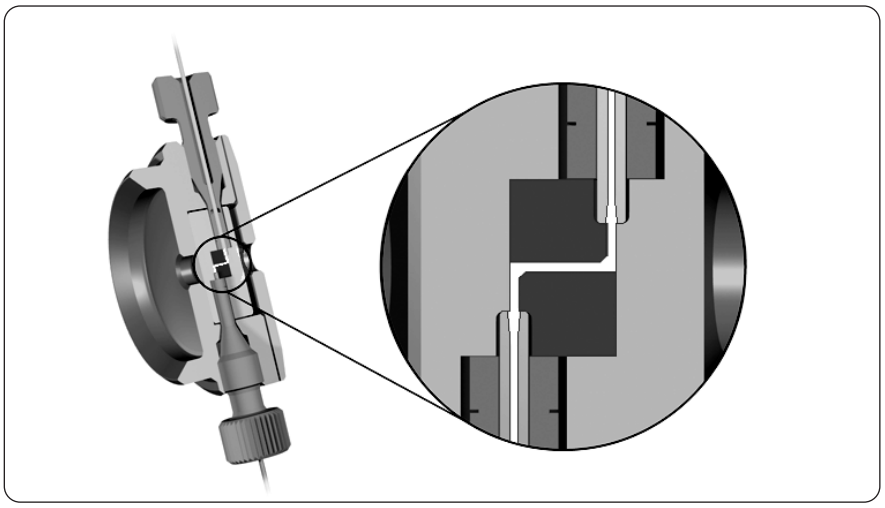

Figure 1
Schematic of the new high-sensitivity detection cell for the Agilent Capillary Electrophoresis system.

The high-sensitivity detection cell can be fitted in any version of Agilent CE systems. As shown in figure 1 the cell is incorporated in an optical interface which is nearly identical to that used for standard and bubble-cell capillaries. This makes capillary and cassette installation the same as for other capillaries. Figure 1 also illustrates the decoupled design which allows capillary replacement.

The capillaries are attached to the cell by preassembled ferrules and finger-tight fittings. Replacement of capillaries can be performed within 2–3 minutes.

Preservation of peak shape is fundamental to the design of the cell and capillary coupling. Because poor capillary coupling and alignment would result in peak broadening and distortion, the capillary couplings of the high-sensitivity detection cell have a unique geometry. The detection cell is designed for use with Agilent capillaries of 75 µm internal diameter which are flared to 100 µm at the point of connection to the flow cell. This is illustrated in figure 2 which also

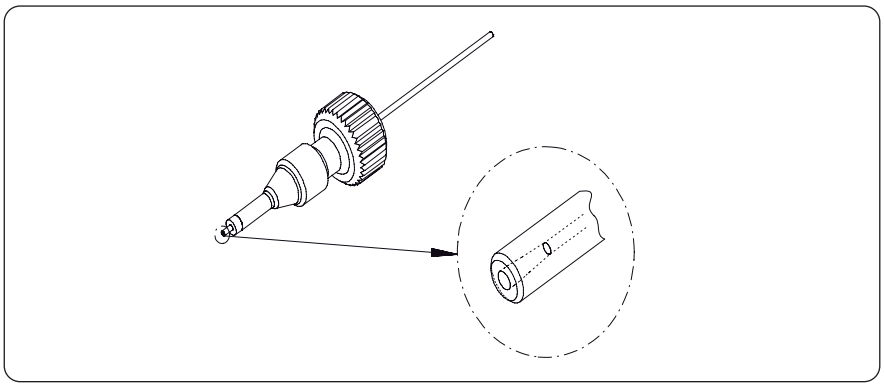

Figure 2
Schematic of capillary connection.

shows how the outer edge of the capillary is beveled to complete the no-dead-volume connection to the flow cell.

Performance

The high-sensitivity detection cell can be used for both CE and CEC applications and is especially useful where increased sensitivity or linear dynamic range is required. The application areas demanding these attributes include chiral analysis in the pharmaceutical industry, analysis of dilute biological samples, analysis of small polar compounds of environmental interest and in trace analysis of components in biochemical analysis.

Naphthalene sulfonic acids are of interest in environmental analysis where they occur at very low concentrations. The previous lack of sensitivity associated with CE made such an analysis extremely problematic. However with the increase in sensitivity available using the high-sensitivity detection cell, these analytes can be quantified easily. Figure 3 shows the analysis of naphthalene sulfonic acids on a 75 µm id standard capillary. The signals obtained are minimal although the separation exhibits a reasonable resolution. Figure 3 also shows a comparison with the high-sensitivity detection cell coupled to a 75 µm capillary of the same length and using the same injection parameters. The signal-to-noise ratio (S/N) obtained with the high-sensitivity detection cell is about 10 times greater than with the 75 µm id standard capillary. With respect to resolution, a slight reduction may be found when the interpeak volume is smaller than the cell volume (about 12 nL). These losses are often minimal, as shown here, and the separation can usually be optimized for use with the high-sensitivity detection cell.

The sensitivity limit of CE is often exceeded when analyzing dilute biological samples with conventional capillaries. A possible solution may be to load more sample, however, this is complicated further when complex, multicomponent biochemical samples, such as tryptic digests of proteins, must be analyzed. The use of large volume injections to overcome sensitivity limitations can cause losses in resolution and eliminate any benefits obtained from using a high resolution technique like CE.

Figure 4 compares the analysis of a tryptic digest of myoglobin using a standard capillary and using the high-

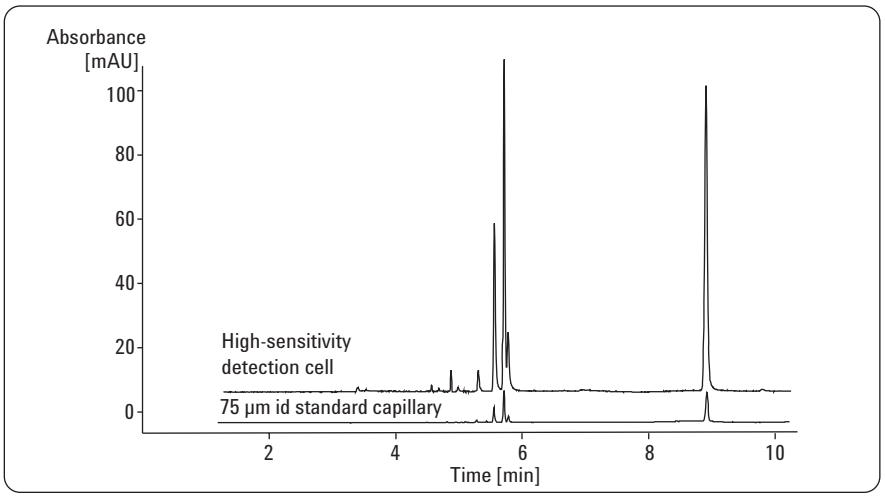

Figure 3
Comparison of the high-sensitivity detection cell and a standard capillary for the CZE analysis of naphthalene sulfonic acids.

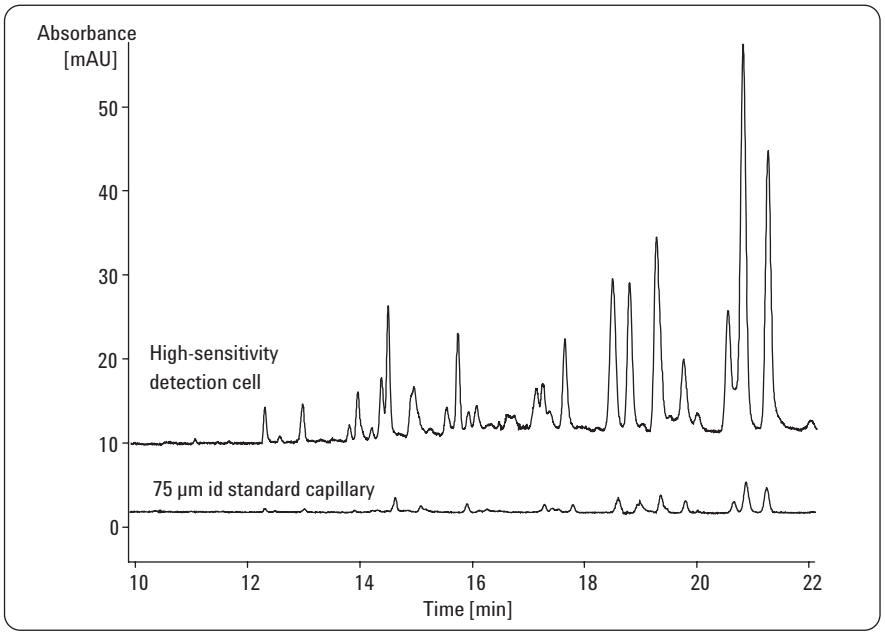

Figure 4
Analysis of dilute sample of 5 µM myoglobin tryptic digest using the high-sensitivity detection cell.

sensitivity detection cell. The analysis was performed at low pH with a 72 cm effective-length capillary and detection was performed at 200 nm where the peptide bond has maximum absorbtion. With a sample concentration of about 5 pmol/µL the 75 µm id standard capillary was unsuitable for analysis of such a dilute sample (despite the low detection wavelength). Using the high-sensitivity detection cell under similar conditions clearly improved the sensitivity of the analysis. The three peaks that migrated at about 24 minutes illustrate how well resolution was preserved even when transferring a method directly from a standard capillary to the high-sensitivity detection cell.

Analysis of chiral drug compounds is easily performed by CE because the chiral selector (for example, cyclodextrin) is dissolved in the buffer rather than bound to a stationary phase as in high-performance liquid chromatography (HPLC). This enables more rapid method development, improved efficiency and resolution, and reduced cost of both analysis and method development. CE is frequently the method of choice for chiral analyses. Regulatory requirements demand that pharmaceutical manufacturers demonstrate the chiral purity of any chiral drug substance and the acceptable criteria for presence of excess enantiomer is about 0.1–0.5 %. This requirement therefore demands that the analysis not only has adequate sensitivity but also that the linear range is such that the minor component may be quantitatively reported as (area/area) percent of the main component. To date, the success of CE for this purpose has been limited.

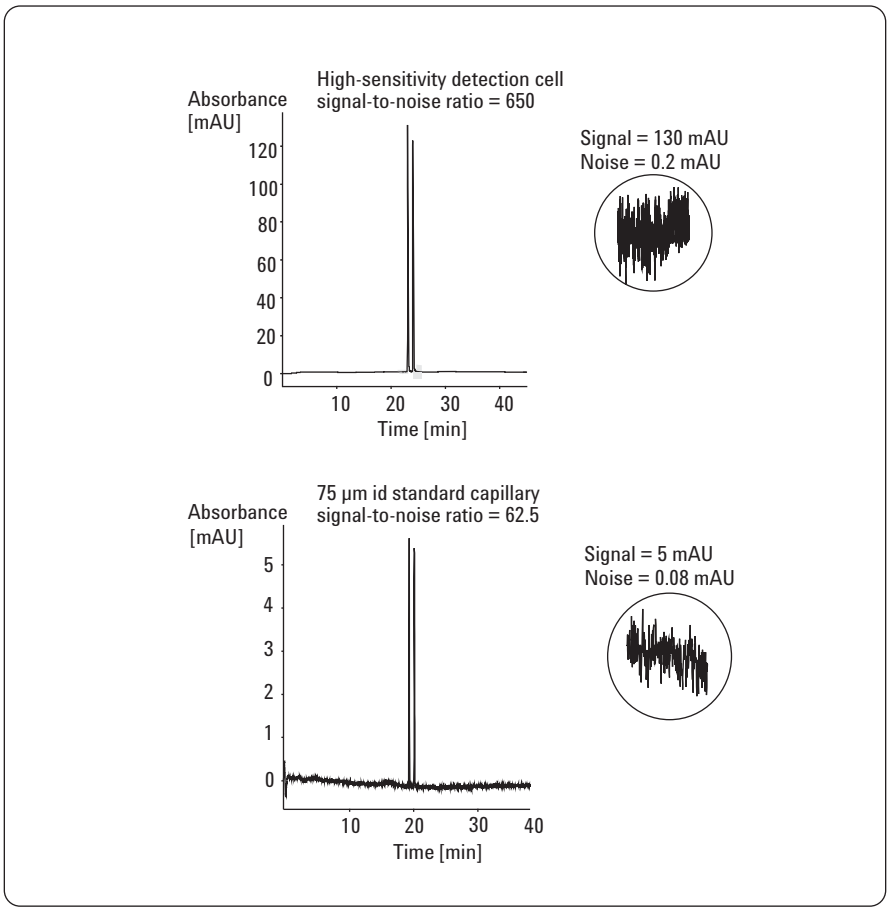

Figure 5
Increased sensitivity with the high-sensitivity detection cell.

Figure 5 shows the chiral separation of epinephrine on a 75 µm id standard capillary, using 20 mM dimethyl-ß-cyclodextrin in a 50 mM tris-phosphate buffer at pH 2.4. The S/N ratio obtained for a 75 mbar·s injection (about 20 nL) of a 100 µM solution of (+/-) epinephrine was 62.5. The S/N ratio using the high-sensitivity detection cell increased to 650, giving a true increase in sensitivity of more than tenfold.

Conventionally the linear range of CE is limited to 300–500 mAU because of stray light associated with the small size, curvature and transparent walls of the capillary. With the high-sensitivity detection cell the construction of the cell body from black fused silica eliminates stray light and the signal is linear beyond 1300 mAU with less than 1 % deviation and to more than 2000 mAU with less than 3 % deviation from

linearity (figure 6). This is of the same order of magnitude as found in HPLC.

This combination of extended linear range and increased sensitivity makes the high-sensitivity detection cell extremely well suited for the quantitative analysis of chiral excess. Figure 7 shows the analysis of S-form of a basic drug in the presence of trace amounts of the R-form. The R enantiomer is present at a levels of about 0.05 % area/area with S/N ratio of > 3.

Another advantage of the rectangular cell design is excellent compatibility with diode-array detection, allowing the spectral analysis of components. Spectra may even be obtained from peaks with very low S/N ratio as shown in figure 7 where the excess enantiomer can be clearly identified by its absorption spectra. The high-sensitivity detection cell allows full use of the functionality of the diode-array detector, including the use of spectral libraries for analyte identification and peak purity determinations even at such low trace concentrations.

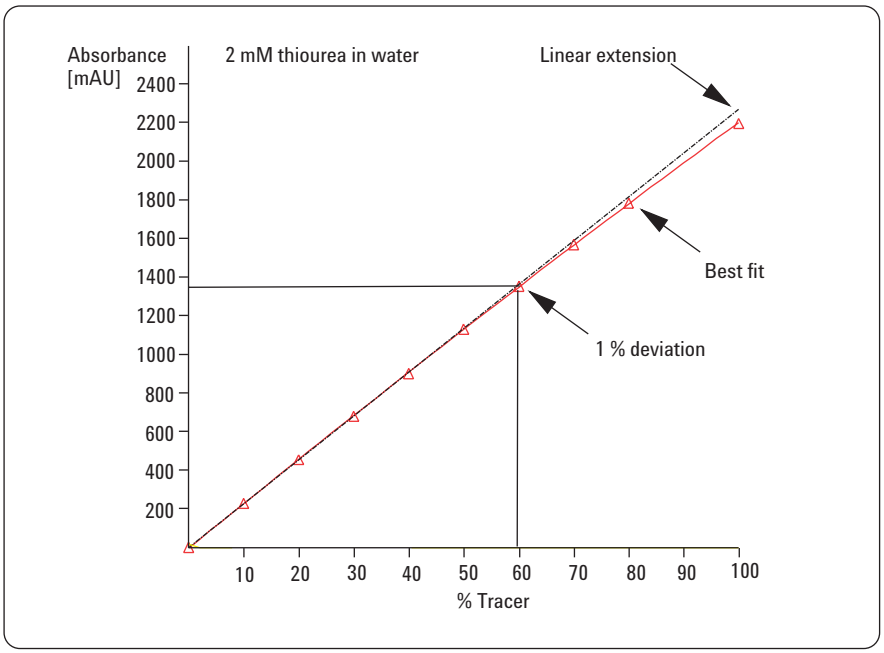

Figure 6
Extended linear range with the high-sensitivity detection cell.

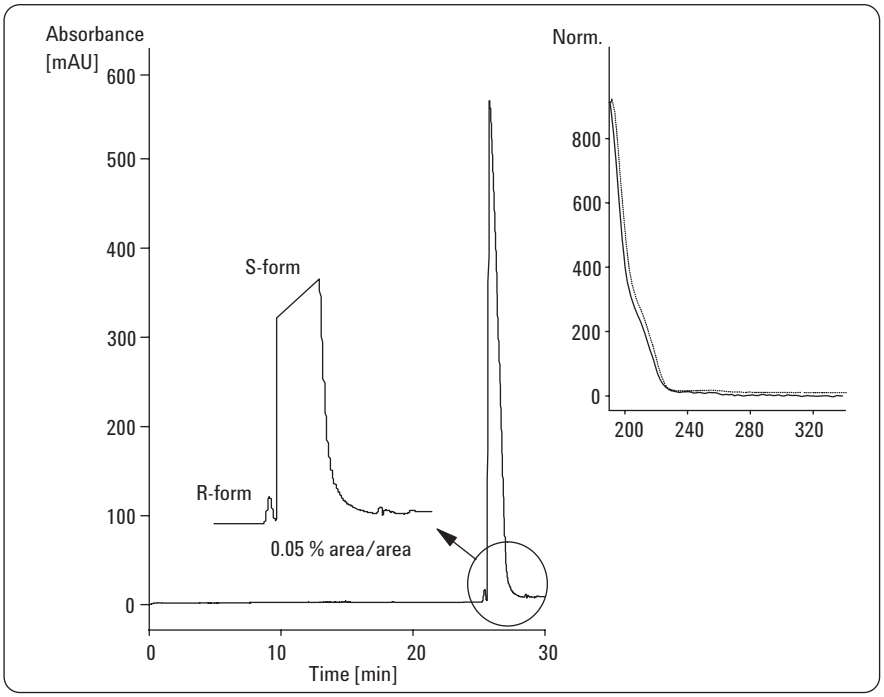

Figure 7
Chiral excess determination with the high-sensitivity detection cell.

Table 1 compares the high-sensitivity detection cell with a standard capillary and with another available option for improvements in CE sensitivity, the Z-cell.[2] The Z-cell is integral to the capillary and is created by bending the capillary longitudinally for 3 mm and using this section for detection. Whereas the Z-cell is used extensively in HPLC, its use in CE has been somewhat limited.

Capillary type	Standard 75 µm id capillary	High-sensitivity detection cell	Z-cell
Cell volume	3 nL	12 nL	12 nL
Expected detection pathlength	0.075 mm	1.2 mm	3 mm
Effective detection pathlength	0.06 mm	1.2 mm	1.07 mm
Signal increase	-	20-fold	14.3-fold
Noise increase	-	2-fold	1.4-fold
S/N increase	-	10-fold	10-fold
Linearity	< 500 mAU at 1 %	1400 mAU at 1 % deviation from linearity	~ 600 mAU
Resolution	-	3 mm between zones to maintain resolution	3 mm between zones to maintain resolution
Spectral fidelity	-	Improved over standard capillary	Incompatible with diode-array detector

Table 1
Comparison of high-sensitivity detection cell, standard capillary and Z-cell.

Both the high-sensitivity detection cell and the Z-cell have an increased cell volume of 12 nl, therefore in both cases peaks must travel about 3 mm apart in order to preserve resolution. The expected path length of a standard capillary is the internal diameter of 0.075 mm, however, because of the capillary curvature the effective path length is reduced to about 0.06 mm. The expected path length of the Z-cell is 3 mm, but for the same reasons this produces an effective path length of only 1.07 mm.[2] The rectangular design of the high-sensitivity detection cell means that its expected and effective path length is the same, that is, 1.2 mm. This means that it will provide a full 20-fold increase in signal compared to a standard 75 µm id capillary. However, the noise is also increased and therefore the real increase in S/N ratio is about tenfold. The Z-cell provides a similar increase in S/N ratio of about 10, however, because of light leakage its linear range is limited to about 600 mAU. The high-sensitivity detection cell is linear to more than 2000 mAU, allowing detection and quantitative analysis over four orders of magnitude, and its rectangular shape improves its spectral qualities over that of a standard capillary. Further, the Z-cell is integral to the separation capillary while the decoupled high-sensitivity detection cell can be reused many times with only low-cost replacement of capillaries.

Conclusion

The high-sensitivity detection cell is a significant advance in the efforts to increase the sensitivity and utility of CE and is a major response to the concerns of users in all analytical areas.

- The high-sensitivity detection cell provides a true increase in sensitivity of an order of magnitude as indicated by increases in S/N, allowing the use of CE in trace analysis.
- The improved linear range beyond 2000 mAU rivals that of HPLC and allows determination of low levels of impurities and chiral excess.
- The decoupled capillary design allows capillaries to be replaced independently of the cell body thus reducing costs.
- Micromachined flared and beveled capillaries maintain peak shape by ensuring proper alignment and coupling to the cell body.
- The materials and design of the high-sensitivity detection cell enhance spectral analysis by diode-array detection.
- The high-sensitivity detection cell is designed to be used in any version of the Agilent CE system.

References

1.
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2.
Moring, S.E., Reel, R.T., van Soest, R.E., "Optical improvements of a Z-shaped cell for high sensitivity UV absorbance detection in capillary electrophoresis", *Anal. Chem., 65, 3454–3459*, **1993.**

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