Agilent Capillary Electrophoresis System — Technical Description

Product Note

The Agilent CE system is a versatile, fully automated system for high performance capillary electrophoresis (CE) separations, comprising an Agilent CE instrument controlled through an Agilent CE ChemStation.

The Agilent CE system is supported by a range of specialized capillaries, buffers, method development and total solutions kits, educational and applications literature and a worldwide network of CE specialists.

The design objective was to develop a complete easy-to-use system that provides high sensitivity diode-array detection, improved quantitative analysis, full automation, the capability of performing all modes of capillary electroseparation to the highest standards available (including CEC*), and at the same time bearing in mind the future requirements of the user. This has been achieved by innovative detector design, high precision control of operational parameters, versatile automated operational features and provision of a fully operational core instrument which is equipped to perform all CE separation modes and is designed to facilitate low-cost upgrade for CE-MS interfacing.
The instrument is controlled through the Agilent CE ChemStation using a self-explanatory, intuitive graphical user interface. The ChemStation control offers many advanced operational features to ensure versatility in all capillary electrophoretic separations. Every operation, from method development and routine operation to demanding research, has been designed to be straightforward and easily mastered. With this vision Agilent has created a precision engineered instrument allowing the analyst fully featured operation from routine analysis to cutting-edge research.

This product note presents the technical details of the full CE offering from Agilent with descriptions and examples of relevant features and benefits.

* for abbreviations see back page

The Agilent Capillary Electrophoresis Instrument

The Agilent CE instrument (figure 2) contains all functional hardware for performing CE separations. The instrument houses a high-voltage power supply, carousel for autosampling and fraction collection, injection system, on-capillary high-sensitivity diode array detector, offline buffer replenishment station, capillary cartridge and is capable of accepting an external gas pressure for performing CEC and CGE. The instrument includes features that allow economic conversion for CE-MS interfacing. Each of these is described in detail in the following sections.

Figure 2
The Agilent Capillary Electrophoresis instrument

Sample Injection

Two injection modes are provided:
- pressure injection, and
- electrokinetic injection.

Although pressure injection is used most frequently, electrokinetic injection may be used when the capillary contains a matrix which does not allow pressure injection (for example with fixed gel CGE), and for selectively injecting only one type of ion in non-EOF analyses. Injection using either mode may be performed from the long end of the capillary or from the post-detector (short) end of the capillary with equal precision.

Pressure Injection

Sample introduction into the capillary is achieved by applying pressure to the injection vial after insertion of the capillary. The quantity injected is dependent on the pressure and the length of time the pressure is applied.

The Agilent CE system uses a unique mechanism which constantly controls the pressure and corrects for the rise-time effects of valves and for any leakage during injection. Figure 3 shows the profile of pressure against time during pressure injection. When applied, the pressure to the sample vial is increased gradually to its maximum (A1). This is then reduced to a value about half of the maximum (A2) and a correction time (t2) is inserted. The process is repeated (A3, A4) in order to apply precisely the injection parameters. In this way the total area under the graph is calculated as the product of the pressure and time settings to provide accurate injection.
The pressure system valves only operate at atmospheric pressure avoiding any detrimental effects from valve-induced pressure fluctuations. This results in accurate and highly reproducible injections (figure 4) as well as exceptional linearity over a broad pressure range, even when injection times are very short (figure 5).
When using pressure injection both the time and the applied pressure can be selected (time range: 0 to 1000 seconds, pressure range: -50 to 50 mbar). Accurate and reproducible injection is possible from volumes as low as a few µl. For CEC separations the capillary is packed with stationary phase and therefore presents a high back-pressure. In this case the external pressure source (up to 12 bar) can be used to inject sample.

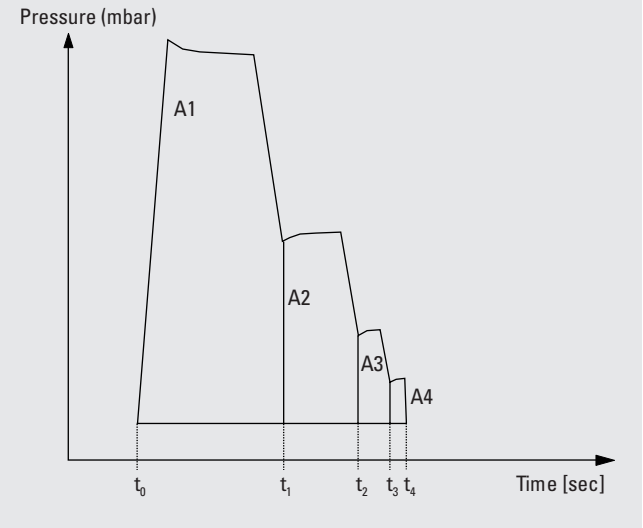


Figure 3
Self-correcting pressure injection profile

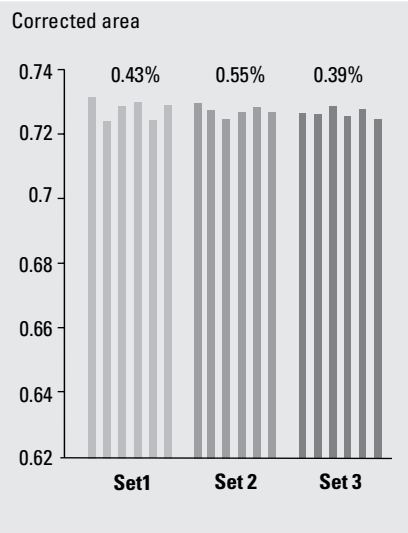


Figure 4
Reproducibility of corrected peak area for 3 sets (N=6) of injections of *p*-hydroxyacetophenone separated by CZE

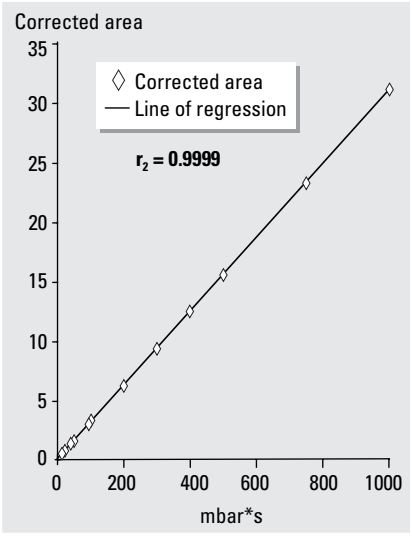
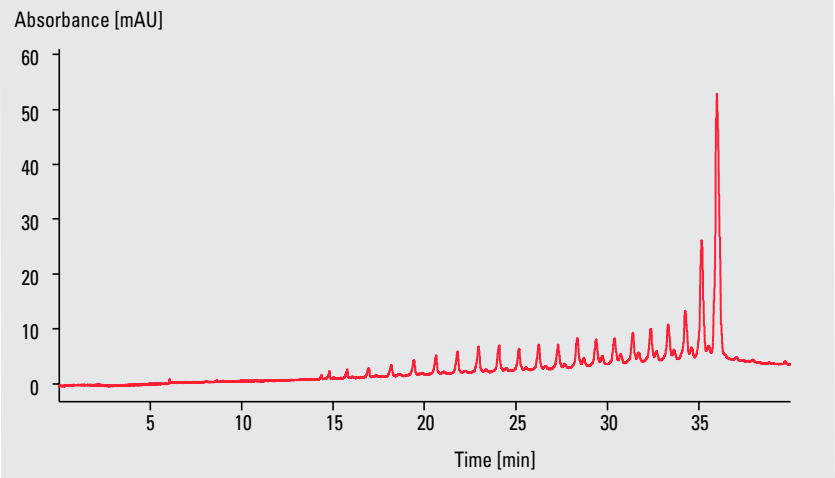


Figure 5
Linearity of peak corrected area for MECC separation of paracetamol with pressure injected amount (mbar*s)



Capillary	PVA-coated leff 24.5 cm id100 µm
Buffer	Agilent oligonucleotide buffer
Inject	7s @ -10kV
Voltage	-25 kV
Temperature	30 °C
Detection	260 / 8 nm DNA filter

Figure 6
Oligonucleotide separation using the Agilent Oligonucleotide Analysis Kit (part number 5063-6530)

Electrokinetic Injection

Electrokinetic injection is performed by placing the sample vial at the capillary inlet and applying an electric field. When the anode end of the capillary is inserted into the sample vial the injected aliquot will not truly represent the sample make-up. Positively charged ions will migrate into the capillary due to their electrophoretic mobility whereas neutral species will enter the capillary through the pumping action of the EOF. Anionic species will only enter the capillary if the EOF is greater than their own negative mobility. Inserting the cathode end into the capillary or reversing the power supply polarity results in the opposite process. The quantity loaded is dependent on the electroosmotic mobility, the ions electrophoretic mobility and the amount and duration of the applied voltage. If the EOF is suppressed only one ion species will be introduced into the capillary, depending on the polarity of the electric field. In CGE with fixed crosslinked gels applying sufficient pressure for sample injection may deform the gel or extrude it from the opposite end. Figure 6 shows the separation of synthetic oligonucleotides injected using electrokinetic injection.

Injection Program

The Agilent CE Chemstation allows the flexibility of defining a number of functions during the injection part of a method. Available parameters for definition are:

- inlet and outlet vials, injection pressure (-50 to 50 mbar for 0 to 1000 s),
- injection voltage (-30 to 30 kV for 0 to1000 s), and
- *wait* time before or after injection.

This allows a variety of injection techniques, for example:

- injection of a buffer plug after the sample plug to minimize sample loss through thermal expansion,
- injections from multiple vials readily allowing the use of transient ITP or stacking to improve sensitivity,
- injection from multiple vials for online spiking for peak identification, and
- washing the capillary end by dipping into water and waiting thus reducing the risk of sample carryover.

Short-end Injection

Full control over sample-vial access allows the use of the post-detector capillary length as the effective separation length. Using this functionality, the sample is placed at the outlet end of the capillary and a negative pressure is applied for a set time. By reversing the polarity of the electric field, the short, 8.5-cm length of capillary may be used for separation with the same degree of precision as for normal injection (figure 7). This may be used for:

- quick purity check,
- rapid screen of analytical parameters, and
- very short analysis time.

For more information see Agilent publication number 5963-3403E.

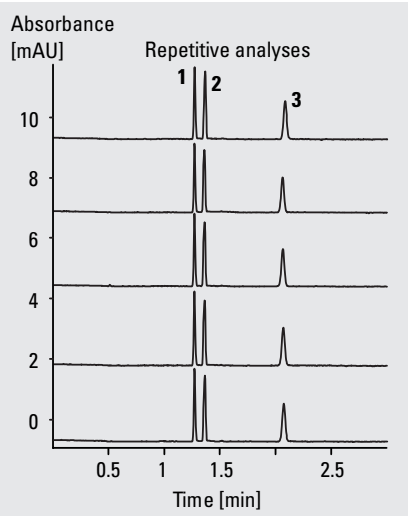


Figure 7
Short-end injection for fast analysis of paracetamol (1), caffeine (2) and 4-hydroxyacetophenone (3) by MECC

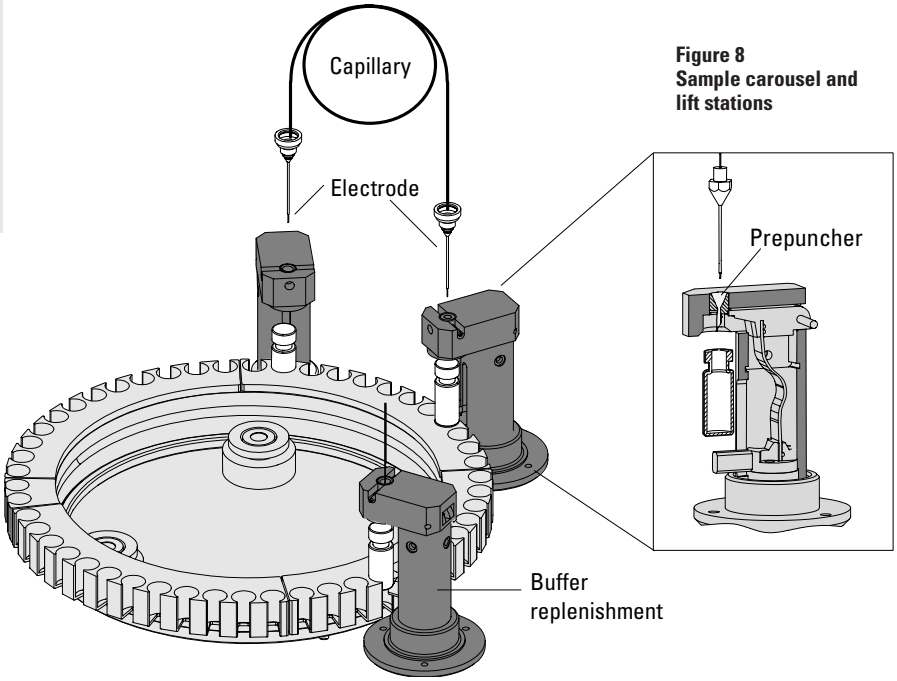


Figure 8
Sample carousel and lift stations

Sample Carousel

The Agilent CE system is equipped with a 48-position carousel (figure 8). The carousel can be thermostated simply by connecting an external water bath to the instrument (10 to 40 °C). Two vial lift stations are situated around the carousel to place vials at the capillary inlet and outlet. Further, a third vial lift station can automatically replenish, fill or empty any vial offline before or during the analysis.

The carousel design allows random access to any vial at all times. The carousel can be accessed during analyses because the vials are lifted above the carousel when electrophoresis is performed. The anode and cathode vials can be emptied and refilled with buffer offline so that no valuable sample locations in the carousel are occupied by additional buffer vials, thus maximizing the sample vial capacity.

Sample or buffer evaporation is avoided by the combination of carousel thermostating and the use of fully-sealed vials. The vial caps are pierced in the vial stations immediately before insertion of the capillary and electrodes or the replenishment needle.

Sample vials can be exchanged at any time during an analysis or during a sequence so that the actual sample capacity can be increased. For routine analysis only two vial locations are needed for the running buffer. For method development more than 20 different buffers can be tested in a sequence. In addition, a separate waste vial can be selected so that all flushings from preconditioning can be collected separately to prevent fouling of the running buffer. Random access to the vials enables a sequence of multiple analyses to be made from a single sample with different buffer systems which is mandatory for automated method development.

Random access to all vials also allows the carousel to operate as both buffer and sample tray and to function as a fraction collector (see page 10).

Buffer Replenishment

The Agilent CE system is equipped with an automated buffer replenishment station which can access any vial before or during an analysis, empty it, and refill it with fresh buffer from a large volume reservoir (about 500 ml). Replenishment can be programmed to refill vials to a preset level to automatically correct unequal vial levels. For more information see Agilent publication number 5963-3296E.

Increased Reproducibility

During electrophoresis, electrolysis of the buffer solutions results in changes in buffer composition and pH. This can result in unstable electroosmotic flow and can also cause changes in selectivity. This is of particular importance when the buffer is chosen for properties other than its buffering capacity (for example in indirect detection techniques) or when the chosen buffer is not a true buffer but is acting merely as an electrolyte. In addition when running a series of samples at high pH (> 7) the pumping action of the EOF can reduce the buffer volume of the inlet vial — this can result in laminar flow in the capillary due to unequal vial levels. On average buffer replenishment should be performed every three to five analyses.

High Sample Throughput

The buffer reservoir for replenishment has a capacity of 500 ml which ensures sufficient capacity for > 300 analyses when replenishing both inlet and outlet vial after each run (figure 9).

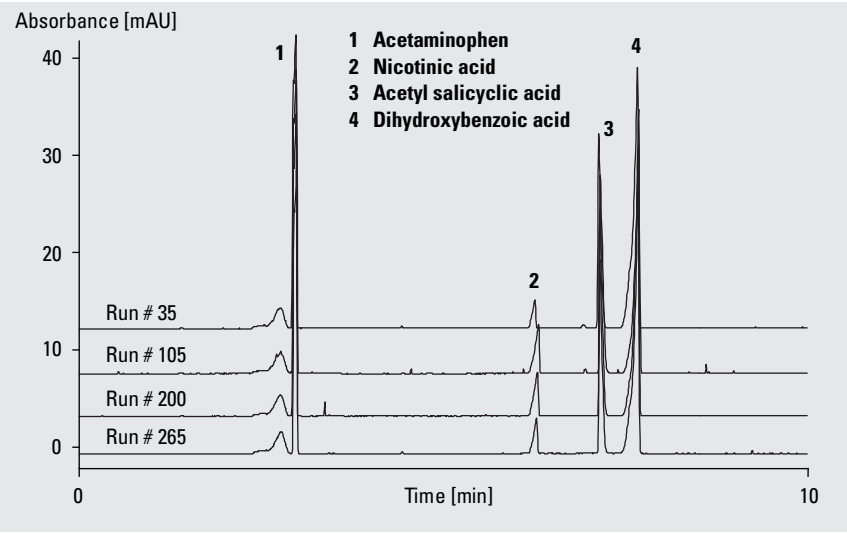


Figure 9
Extended operation using buffer replenisment

Capillary id	50 µm
Length	56 cm
Buffer	89 mM Tris-Borate, pH 8.2
Injection	100 mbars
Voltage	25 kV
Current	13 µA

Capillary Cassette and Alignment Interface

The Agilent CE system is equipped with a cassette that is easily opened without using tools and allows quick exchange of capillaries (figure 10). This allows rapid exchange of capillaries when changing methods. Further, the ability to rapidly remove and install a new capillary ensures minimum downtime, with the entire process taking less than 1 minute.

The cassette contains two feet that make leveling of the capillary ends easy and straightforward. The precision engineered capillary alignment interface ensures autoalignment of the capillary detection window when inserting the cassette into the detector opening. Because capillary alignment can affect sensitivity it is important that the capillary is properly aligned in the incident light path. The dimensions of the slit are of major importance in reducing stray light and therefore increasing sensitivity and detector linear dynamic range (for more information see Agilent publication number 5963-1891E). Alignment interfaces are available for all standard capillary dimensions and in addition special interfaces are available for use with the Agilent Extended Light Path capillaries (see table 1).

The alignment interfaces are color-coded similarily to Agilent capillaries to ensure correct selection. Any capillary from a third-party vendor may be used with the system, provided that it has a minimum total length of 33 cm with a minimum post-detection window length of 8.5 cm and a standard outer diameter of 365 µm. The design of the alignment interface and capillary cassette also provides the necessary protection when using packed capillaries for capillary electrochromatography.

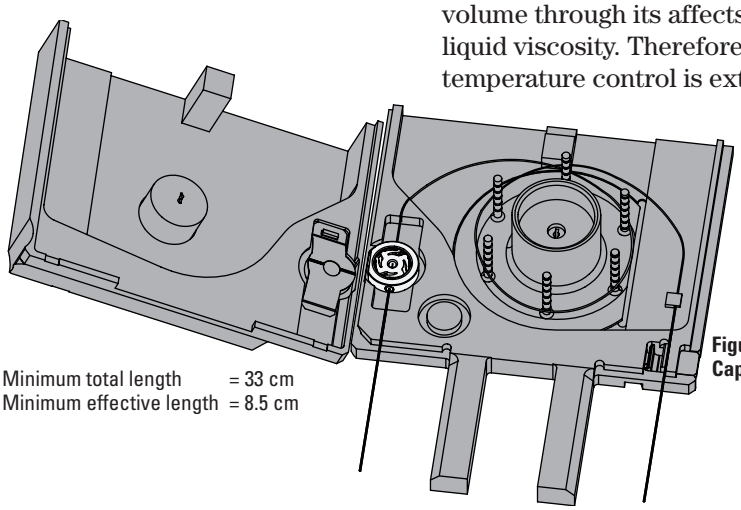


Figure 10
Capillary cassette

Capillary Thermostatting

The Agilent CE system provides stable and efficient capillary thermostating through the use of high-velocity recycling air flow (10 m/s) which is thermostatically regulated by a Peltier element. Capillary temperature is one of the many operational parameters which can be optimized in capillary electrophoresis. Temperature affects an analyte's electrophoretic mobility in CZE separations, partition coefficients in MECC and CEC and even the injected sample volume through its affects on liquid viscosity. Therefore, stable temperature control is extremely important to ensure precision, reproducibility and selectivity. By actively monitoring and correcting for variations in ambient temperature, the system is independent of external temperature fluctuations. The capillary temperature can be regulated between 10 degrees below ambient (minimum 4 °C) up to 60 °C. This efficient high-velocity air flow thermostating is capable of ensuring stable temperatures and effective capillary cooling even when operating with high concentration, high power buffers (figure 11), for more information see Agilent publication number 5963-3975E. The actual temperature of the capillary compartment is recorded throughout the analysis and can be stored with analysis data. The ability to achieve rapid temperature equilibrium allows the use of temperature gradients.

Capillary id	75 µm
Length	80.5 cm
Buffer	105 mM phosphate, pH 2.5
Injection	150 mbars
Voltage	30 kV
Temperature	35 °C
Current	130 µA
Power	4.8 W/m

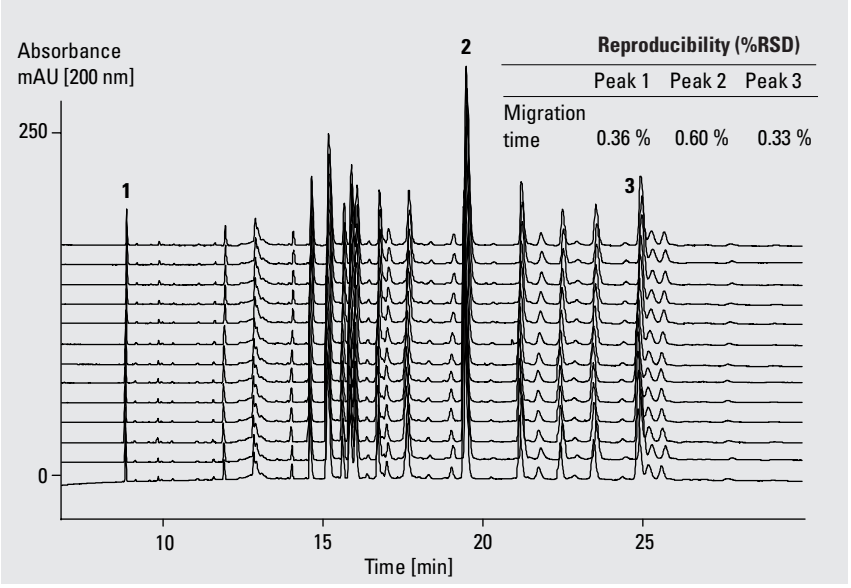


Figure 11
Reproducibility of peptide map of human hormone through efficient thermostating under high power conditions

High-Voltage Power Supply

The Agilent CE system has a power supply capable of applying voltages over the range from -30 kV to +30 kV. The high-voltage power supply is the heart of any CE instrument and as such it must supply a stable voltage during the course of an analytical run. This is achieved by constantly monitoring the applied voltage throughout the analysis and actively correcting for voltage fluctuations. The actual voltage applied during the run can be recorded and viewed during analysis and stored with other analytical data. The power supply provides for rapid rise and decay times of around 1 second, which is of particular importance where fraction collection of peaks is performed (see page 10). The system has safety features built in which limit maximum current and power levels of 300 µA and 6 W respectively. The polarity of the applied voltage is fully controlled by software providing a true positive or negative polarity with the outlet electrode held at ground potential. The software control enables the construction of voltage, current or power gradients, and the application of voltage ramps which is of great importance when analysing heat-sensitive analytes in low conductivity solutions.

High Sensitivity Diode Array Detector

The Agilent CE system is equipped with a high-sensitivity diode array detector (table 3). In CE the capillary itself acts as the detector flow cell. Unlike many detectors in CE instrumentation, which have been adapted from LC detectors, the optical design of the Agilent CE detector has been specifically developed for UV-visible absorbance detection in capillary-shaped flow cells. In conjunction with the capillary alignment interfaces the optical design of the detector is optimized for both standard capillaries and for Agilent Extended Light Path capillaries. Wavelength calibration is performed automatically on replacing the capillary cassette and can be performed manually at any time. The response time of the detector is sufficient to detect and acquire spectra of high efficiency peaks with peak widths less than 0.02 min.

High Sensitivity and Linear Dynamic Range

The design of the capillary alignment interfaces reduces the amount of stray light reaching the detector allowing a linear dynamic range up to 500 mAU with a 75-µm id capillary, with less than 1 % deviation from linearity. This typically covers a concentration range of three orders of magnitude. The linear dynamic range of the detector allows detection of a wide range of analyte concentrations without resorting to sample dilution. This provides great utility in the analysis of trace impurities in the presence of a major component and also for determination of chiral excess. With the use of the Agilent Extended Light Path capillaries the linear dynamic range is extended significantly beyond 500 mAU (figure 12).

Spectral Acquisition for Peak Identification and Peak Purity Analysis

The Agilent CE ChemStation built-in diode array detector allows a range of tasks. Simple spectra, isoabsorbance plots, 3-D plots, and other evaluation can take place online while analysis is in progress. For more information see Agilent publication number 5963-6977E.

The Agilent CE Chemstation, which controls the system, enables full spectral information to be retrieved as well as single wavelength and multiple wavelength detection.

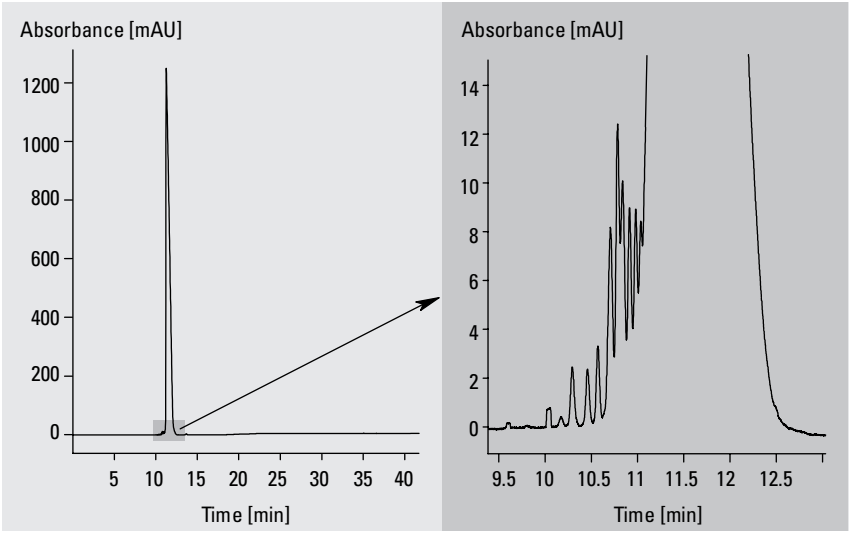


Figure 12
Analysis of trace impurities in polylysine

Spectral library searches and peak purity algorithms can greatly facilitate method development in CE. The peak purity algorithm is especially useful for differentiating peak shapes distorted by mobility differences between analyte and run buffer from those that represent actual impurities (figure 13).

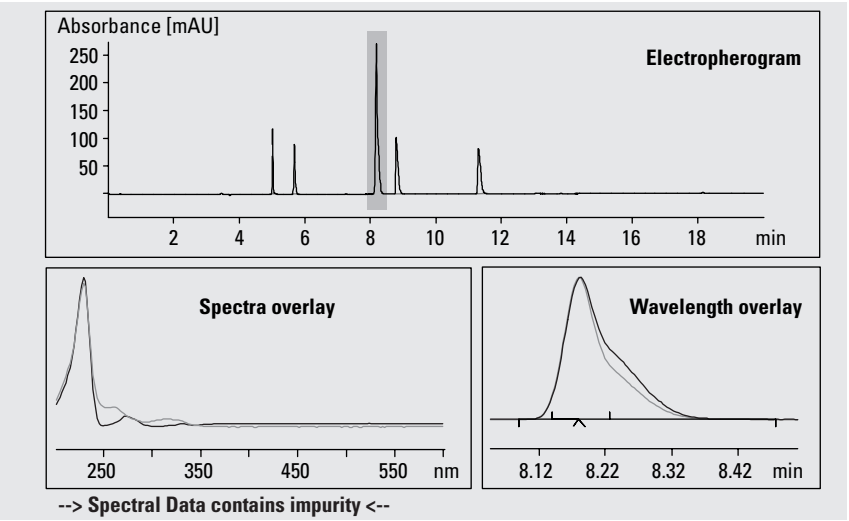


Figure 13
Peak-purity analysis

Agilent Extended Light Path Capillaries

To further enhance sensitivity, Agilent Extended Light Path capillaries (*bubble cell*) can be used with the Agilent CE system. These capillaries contain an expanded diameter section (bubble) at the point of detection (figure 14). The bubble manufacturing process is computer controlled to provide tolerances of less than 3 %. Agilent provides capillaries with a range of normal and extended light paths (table 1). This design results in corresponding increases in sensitivity over conventional straight capillaries of the same id, for more information see Agilent publication number 5963-1889E. In the region of the bubble the electrical resistance is reduced and thus the field decreases. Concomitant to this is a proportional decrease in flow velocity due to the expanded volume of the bubble. When the zone front enters the bubble its velocity decreases and the zone contracts or stacks. The sample zone expands radially (across the capillary) and contracts longitudinally (along the capillary), keeping the total volume constant. This results in an unchanged zone concentration but an increase in optical path length corresponding to the extension factor (figure 16).

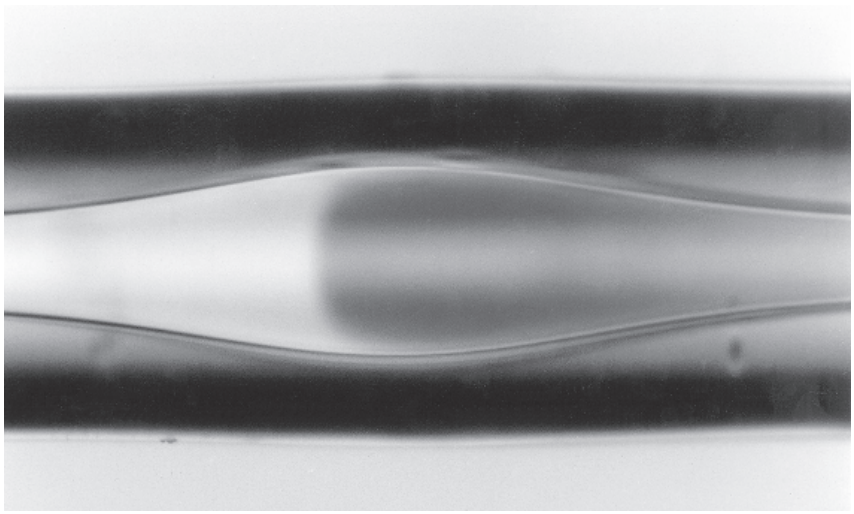


Figure 14
Bubble cell with dye front pushed through to highlight the extended path

Table 1
Capillary internal diameters, bubble factors and alignment interface dimensions

Capillary id (µm)	Bubble factor	Optical pathlength (µm)	Alignment slit dimensions (µm)	Color code
25	5	125	120 x 80	Black
50	1	50	40 x 620	Green
	3	150	145 x 145	Red
75	1	75	55 x 620	Blue
	2.7	202	200 x 150	Yellow
100	1	100	55 x 620	Blue

25 µm id capillaries with extended light paths of 125 µm can be used for applications which use high power buffers e.g. high salt or SDS concentrations (see figure 15). The dimensions of the alignment interfaces used with the bubble cells are designed specifically to maintain light throughput by being extended radially, and to maintain resolution by being reduced axially. This maintains sensitivity without sacrificing resolution. A detailed description is given in Agilent publication number 5963-1889E.

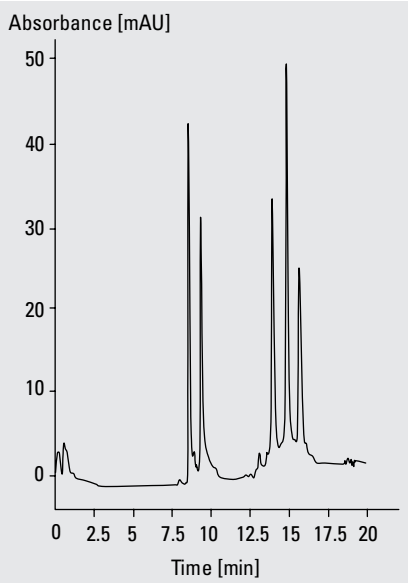


Figure 15
Separation of protein standards in a 25 µm id capillary with bubble using 150 mM phosphate, ph 7.0 with 200 mM ammonium sulfate

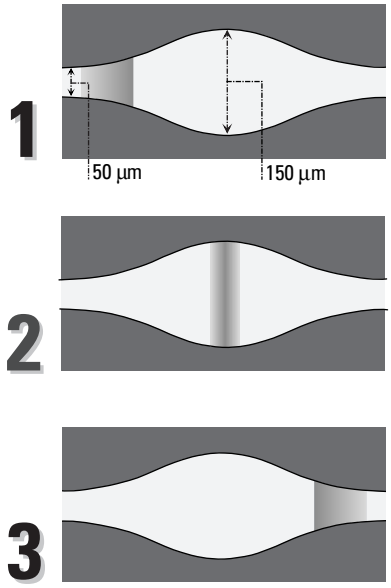


Figure 16
Mechanism of zone concentration using Agilent Extended Light Path capillaries

Capillary Gel Electrophoresis Operation

High Pressure for Viscous Replaceable Gels

The Agilent CE system can accept an external gas supply up to 15 bar which is internally regulated from 2 to 12 bar. Provision of higher pressure is of benefit in the CGE analysis of proteins and oligonucleotides where highly viscous replaceable gels are used. When using a viscous polymer matrix, necessary for high resolution SDS-PAGE, dsDNA and oligonucleotide separations, capillaries of smaller internal diameter (≤ 50 µm) require relatively high pressure (about 8 bar) in order to replace the capillary volume within a reasonable inter-analysis time frame. With the high-pressure option available as a core composite of the Agilent CE system, such separations in the bioscience field are made more accessible. High pressure is also invaluable for flushing 25 µm id capillaries.

Spectral Filter

Linear polyacrylamide (LPA) is used in a variety of capillary gel electrophoresis applications and is also used in some coated capillaries. However, LPA is susceptible to degradation when exposed to high intensity light sources at low UV wavelengths. In order to facilitate the use of LPA and LPA-coated capillaries without resorting to changing the detector, Agilent has developed spectral filters designed specifically for use in nucleic acid analysis. For more information, see Agilent publication 5963-9807E. This filter allows higher light trans-mission levels than are available with conventional interference filters which give light transmission of about 15 % found in fixed wavelength detectors. For analysis of nucleic acids the spectral filter provides high light

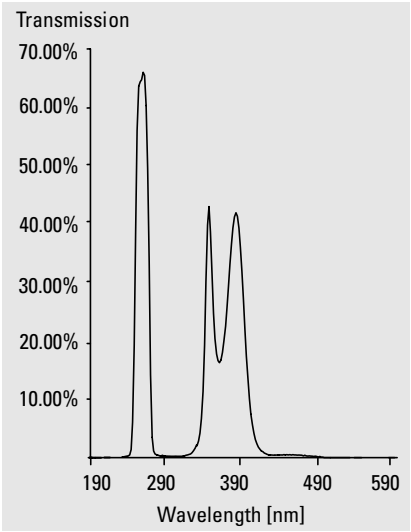


Figure 17
Spectral filter for oligonucleotide analysis

transmission at the detection wavelength range of 254 – 260 nm (bandwidth 4 nm) with lower transmission levels at 360 nm which is necessary for cassette recognition (figure 17). The light throughput at 260 nm is about 68 % transmission. This is much larger than is found with fixed wavelength detectors, which use intereference filters. In this case the light throughput is around 15 % resulting in significantly higher baseline noise. The spectral filter is also recommended for use with the Agilent ssDNA kit whre it significatntly extends its lifetime (figure 18). For more information see Agilent publication number 5963-9870E.

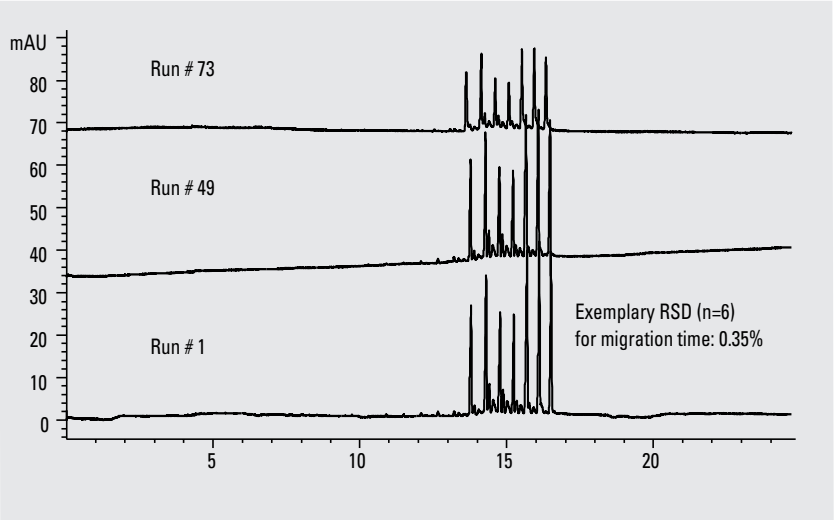


Figure 18
Long term stability testing of CGE for oligonucleotide analysis using spectral filter

Fraction Collection

Although in capillary electrophoresis only very small quantities of analytes are injected, fraction collection can be desirable for further analysis using a different CE mode, protein sequencing, mass spectrometry or other offline analytical techniques. The Agilent CE system offers fully automated, software-controlled fraction collection in three different modes. Fractions may be collected by pressure elution, by electrokinetic elution or from CIEF analyses. The fraction collection mode is selected from the Instrument menu of the graphical user interface, for more information see Agilent publication numbers 5963-1251E and 5963-3506E .

In each case the DAD signal is used as the peak sensor, allowng time-independent fraction collection. In all three modes up to four peaks may be collected individually by defining their migration time, or multiple peaks over a set time range can be collected. The peak detector may also be used to specify collection of those peaks which have an absorbance above a user-defined threshold. Because the sample carousel also acts as the fraction collection vial holder, the location of the vial for fraction collection can be specified. Where multiple fractions are being collected the Agilent CE instrument will collect the first fraction in the specified vial and subsequent fractions in correspondingly incremented vials. For fraction collection it is essential that the power supply decays in < 1 s.

Fraction Collection using Pressure Elution

Pressure elution of fractions from the capillary can be used in most CE modes. The user must input information on the time taken for migration of the buffer from the inlet to the detection window using the elution pressure of 50 mbar (figure 18). This low pressure ensures minimum distortion of other analyte zones still within the capillary and allows collection of multiple fractions from a single run. The software calculates when the peak will reach the end of the capillary and places the collection vial at the outlet end at the appropriate time. Fractions may be collected in as little as 5 µl of solvent. Figure 18 shows the reinjection of a peptide fraction collected by pressure elution from separation of a protein tryptic digest.

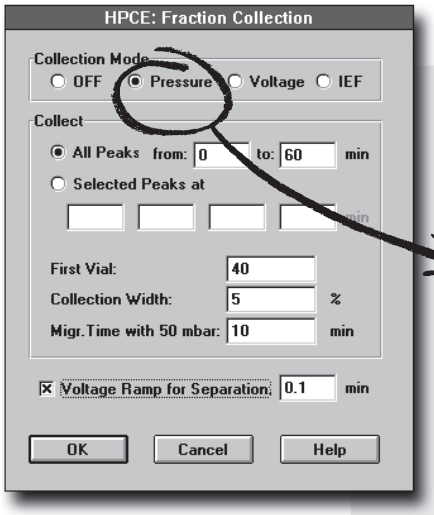


Figure 19
Window for entering pressure elution fraction collection parameters

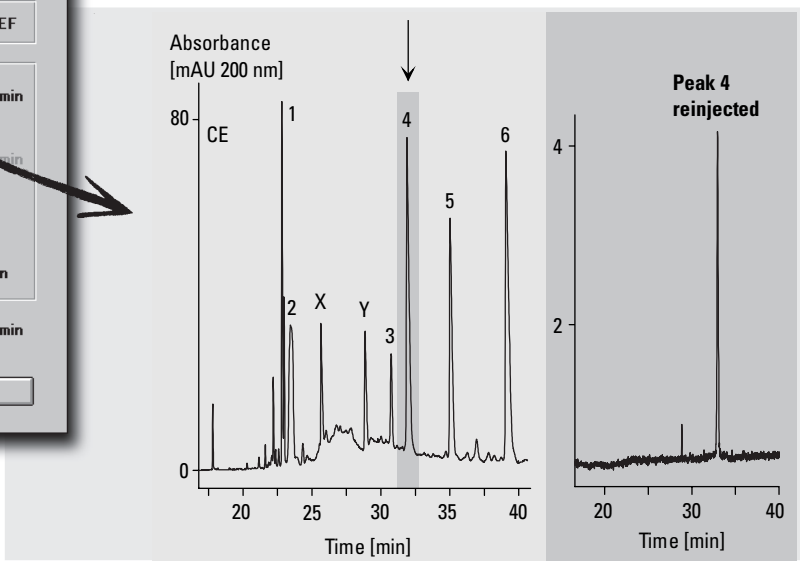


Figure 20
Reinjection of pressure collected fraction from peptide map single run using CZE

Fraction Collection using Electrokinetic Elution

In this case the user is prompted for the capillary dimensions and time of the peaks of interest (figure 21). Electrokinetic elution of fractions is particularly appropriate when the capillary contains a pressure resistant matrix, for example, fixed gels, viscous polymers or packed solid phase particles (figure 22).

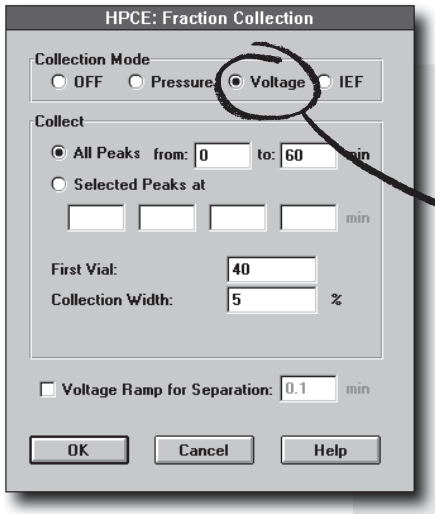


Figure 21
Window for entering electrokinetic elution fraction collection parameters

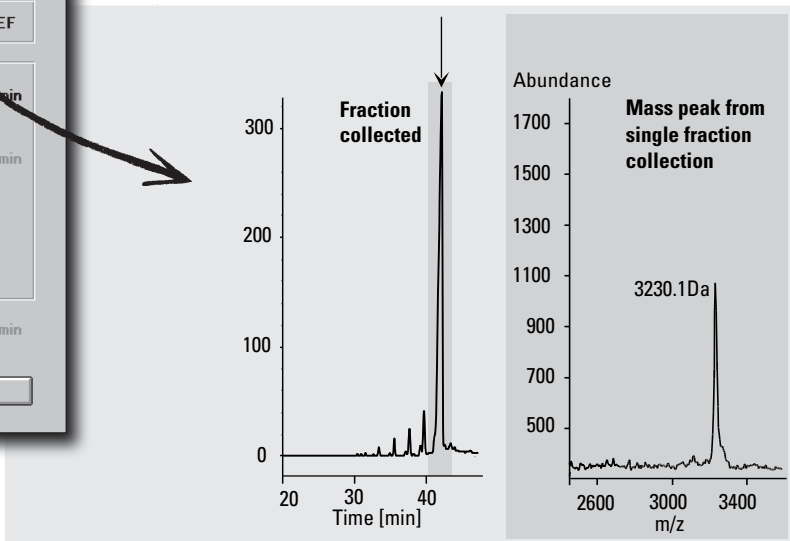
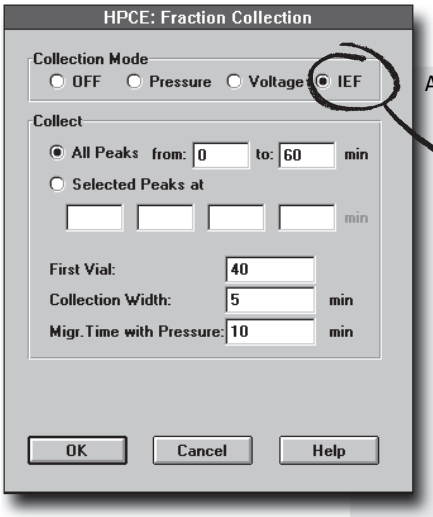


Figure 22
MALDI-TOF analysis of single fraction collected from CGE oligonucleotide separation

Fraction Collection from CIEF

In CIEF pressure and voltage are applied simultaneously to mobilize the separated components while maintaining the integrity of the sharply focused zones. Using the fraction collection option for CIEF the user provides information on the capillary dimensions and migration time using pressure (figure 23).

Figure 23
Window for inputting CIEF fraction collection parameters



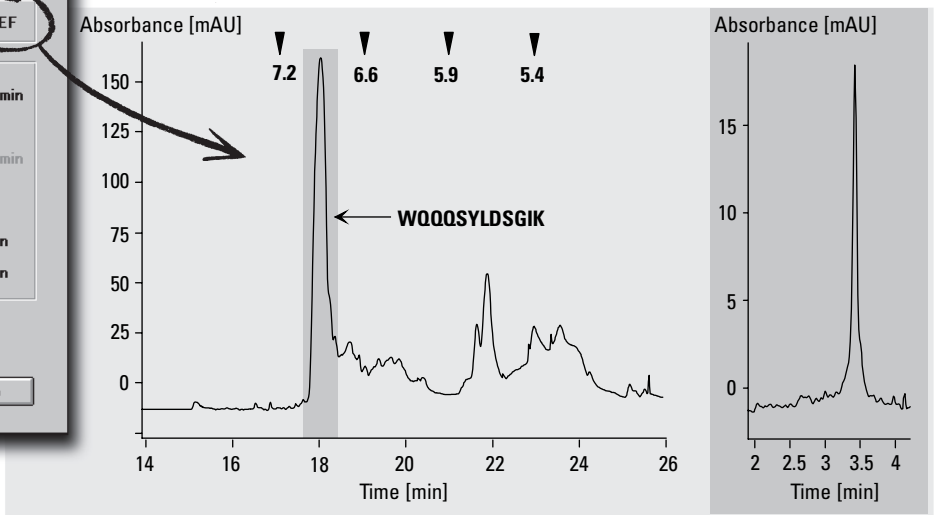
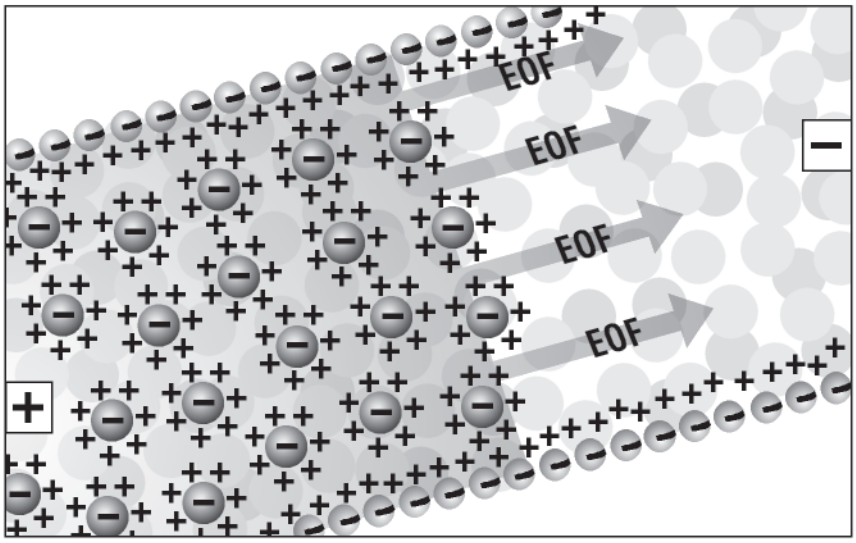


Figure 24
Refocussing of proteins isolated from micropreparative by CIEF

Capillary Electro-chromatography (CEC)

CEC is a true fusion of CE and LC and is, at present, an emerging technique in the first exciting stages of investigation. The technique may be used to separate neutral molecules, or mixtures of charged and/or neutral species. Similarly to LC, separations are achieved through partition between a mobile phase and a stationary phase. The motive force which propels neutral molecules through the capillary bed is the electroosmotic flow (EOF). The benefits of this are that the EOF has a uniform flow-velocity profile unlike LC which provides a more variable flow profile with an increased contribution to band broadening from eddy diffusion. The more uniform velocity flow profile greatly reduces this major contribution to band broadening.

Figure 25
Graphical representation of EOF generation in CEC



Since the flow is generated within the capillary, longer columns containing very small particles (< 3 µm) may be used which are prohibited by pressure constraints in LC. For a comprehensive description of the technique see Agilent publication number 5964-5930E. Typically, in CEC separations, microbubble formation can occur as a consequence of applying high voltage across a packed capillary bed. This phenomenon can be avoided by ubiquitous application of pressure to both inlet and outlet vials which eliminates bubble formation and separation breakdown, while introducing no laminar flow because of the equally applied pressure.

The Agilent CE system is designed to internally regulate an external pressurized gas supply (≤ 15 bar) to provide single or dual-vial pressurization at 2 to 12 bar. Regulated pressure can be applied to either or both of the buffer reservoirs. The external gas pressure source should be oil-free air or nitrogen.

The CEC operational mode is selected from the graphical user interface which changes to indicate that an external high pressure source is connected and to indicate the external pressure supplied (figure 26). The Agilent CE system controls both the rate of application of pressure and the pressure applied. All CEC functionality is included in the Agilent CE instrument hardware and software control is provided as standard in the Agilent CE ChemStation. Although an external gas supply is needed for high-pressure and CEC operation (see figure 27), the Agilent CE instrument is fully functional for all other CE modes without this external gas pressure.

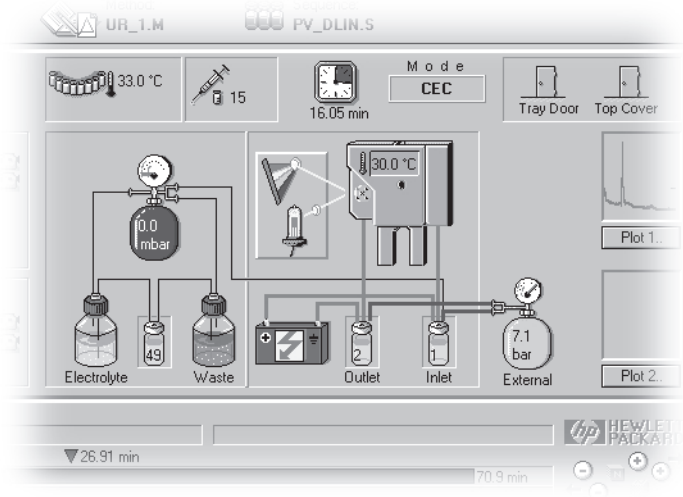
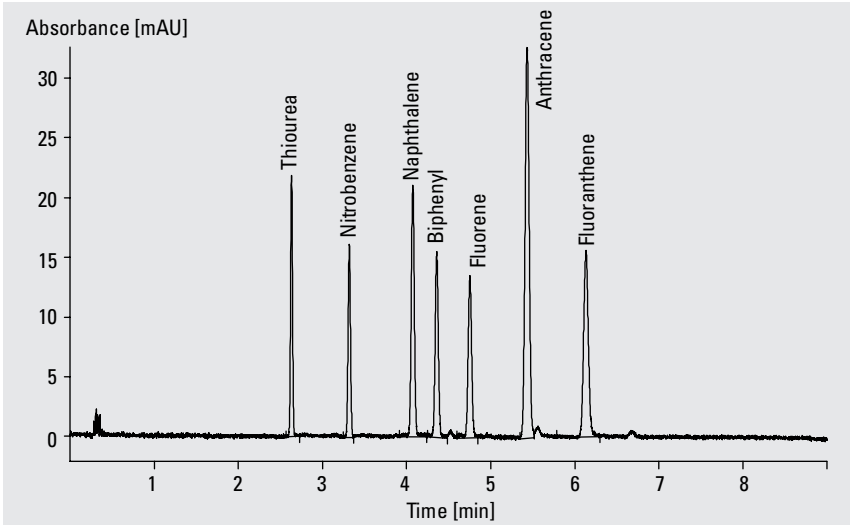


Figure 26
Part of the graphical user interface for CEC operation window



Column	Spherisorb ODS1, 3 µm 250 x 0.1 mm
Mobile phase	80/20 ACNTris-HCl 50 mM, pH 8
Voltage	25 kV
Injection	5 k, 3 s
Pressure	10 bar both sides
Temperature	20 °C
Plate number	50,000 – 60,000
Symmetry	0.95 – 0.98

Figure 27
CEC separation of polyaromatic hydrocarbons

Capillary Electrophoresis/ Mass Spectrometry

The interfacing of capillary electrophoresis with mass spectrometric analysis is widely viewed as an optimum conjunction of separation power with a universal detection principle which also provides highly selective identification data. The Agilent CE instrument is fully fitted as standard with the features necessary for conversion to CE-MS operation. This provides a versatile and fully automated front end for CE-MS separations. Designing the Agilent CE instrument such that it has built-in features which enable its conversion to CE-MS capability greatly reduces the cost of opting for CE-MS analysis.

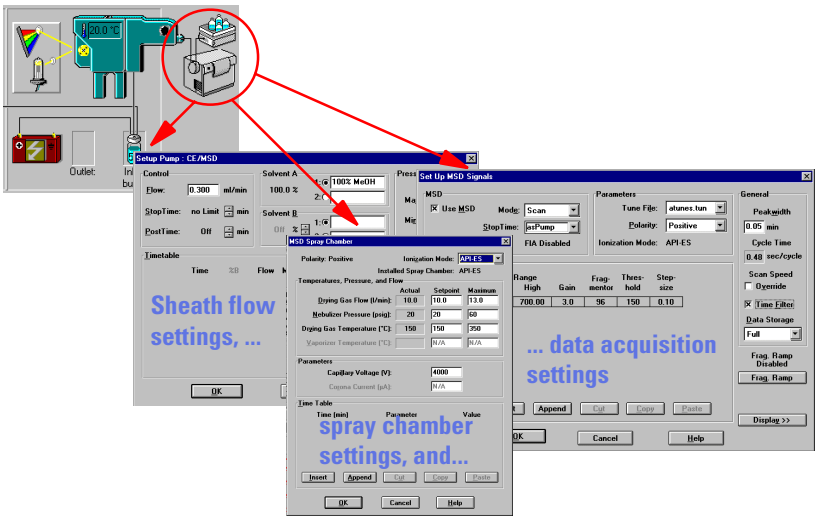


Figure 28
Single point instrument control for CE-MS

The Agilent CE-MS adapter kit (part number G1603A) includes all parts to allow interfacing of the Agilent CE to most ESI-MS instruments and includes:

- capillary cassette designed to allow thermostating of the capillary throughout its position within the instrument (figure 29),
- ground cable,
- capillary safety sleeve,
- warning labels, and
- plastic UV capillary interface to allow tandem UV/MS detection (figure 30).

The capillary can be passed through the DAD for method development or directly into the MS for routine operation. The CE-MS probe for non-Agilent MS should be purchased from the MS vendor. The Agilent CE sprayer kit (part number G1607A) is designed to interface perfectly with the Agilent 1100 Series MSD and the Esquire-LC Ion Trap LC-MS $^{(n)}$. The Agilent CE ChemStation supplied as standard already contains the necessary software for CE-MS operation. CE-MS option may be selected from the GUI toolbar. When using CE-MS software (part number G2201AA) the Agilent 1100 Series MSD and the Agilent 1100 Series isocratic pump used to supply the sheath liquid can be controlled directly from the GUI as shown in figure 28 (For more detailed information refer to publication number 5968-1328E).

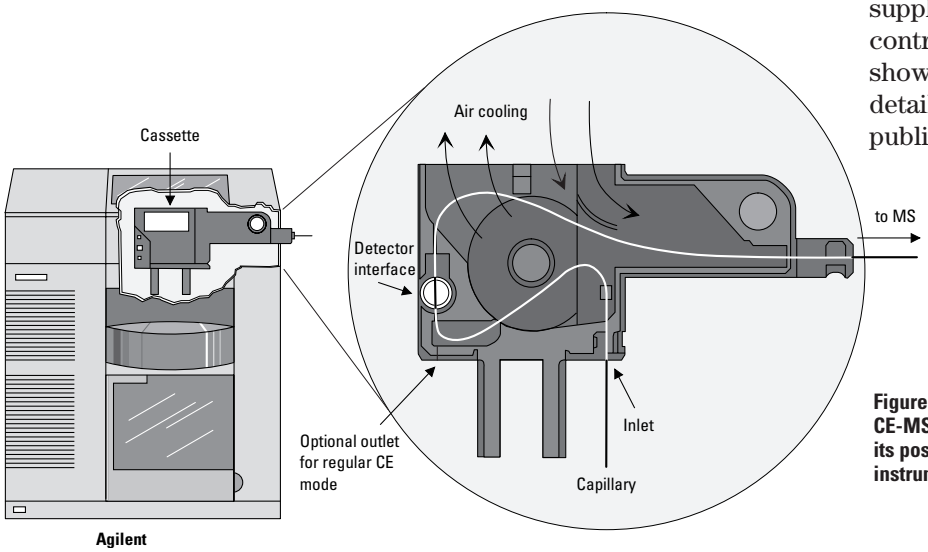


Figure 29
CE-MS cassette design and its position within the instrument

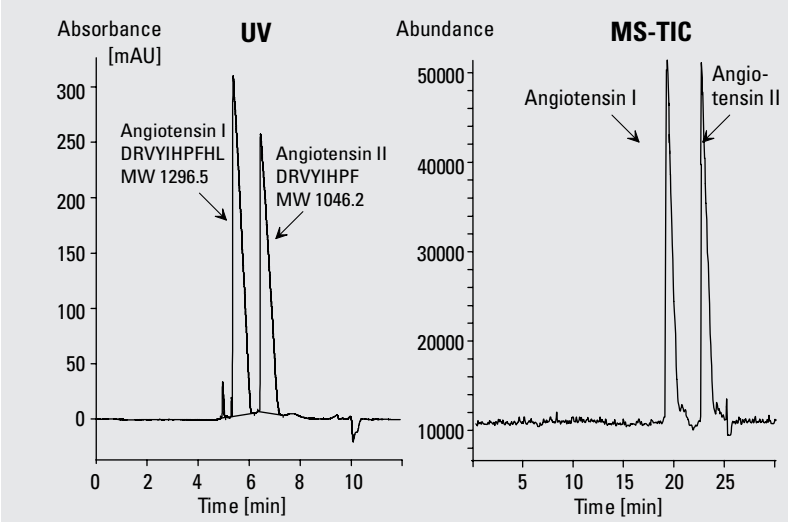


Figure 30
CE-MS of peptide mixture

Agilent CE ChemStation

The Agilent CE ChemStation comprises instrument control and data handling software, personal computer and printer. The software runs within Microsoft® Windows and MS-DOS® operating environments. Data analysis procedures range from standard integration protocols and reporting to advanced spectral library searches and peak-purity analysis. The Agilent CE ChemStation is part of a standardized platform for running CE, GC and LC instruments from Agilent Technologies.

Instrumental Control

The Agilent CE system is operated from the Agilent CE Chemstation using a graphical user interface (figure 31). A schematic diagram of the Agilent CE instrument guides the user through the programmable parameters. This interface also allows direct access to all method parameters, including control of the vial tray, capillary flushing, and so on. The user is informed about the actual status of the analysis by real time indicators.

CE Mode Selection

The CE operational mode may be selected from the GUI. This allows operation in all normal CE modes and additionally includes CEC, CGE and CE-MS. The GUI changes to indicate the operational mode selected.

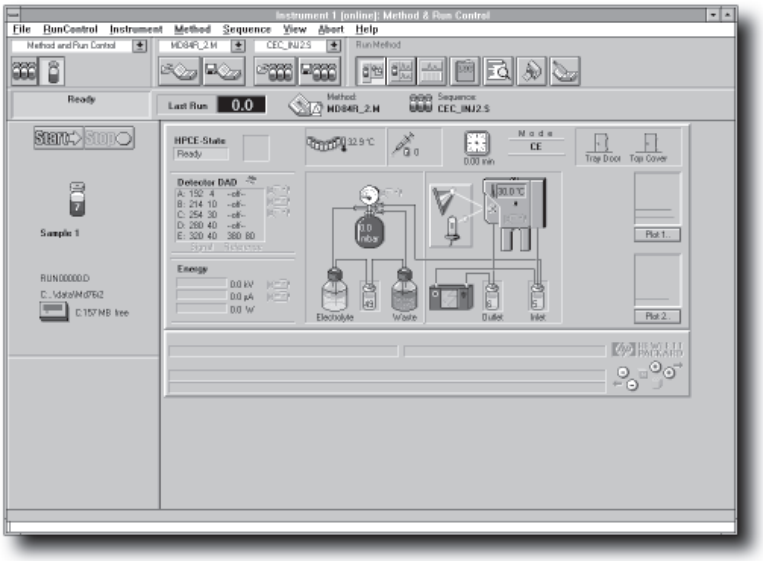


Figure 31
Graphical user interface for CE

Method and sequence programming

Method programming takes place from a single screen using easy to recognize icons (figure 32). The intuitive design of this interface reduces the time necessary for familiarization and facilitates rapid training. Table 2 lists all the method parameters that can be programmed. Individual methods and sample series can be combined in a sequence to fully automate any series of analysis methods and samples.

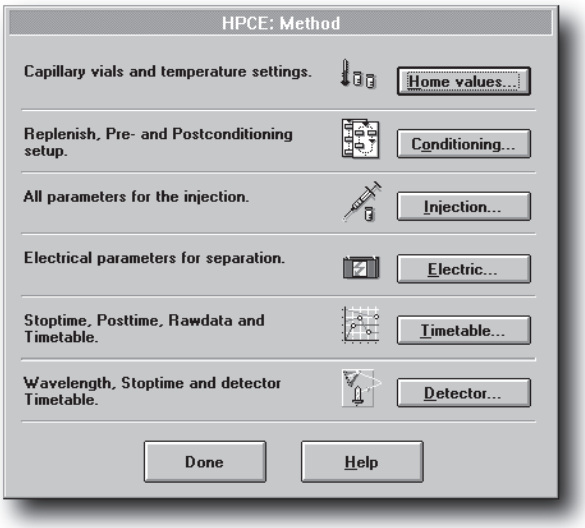


Figure 32
Method set-up window

Sequences for Method Development and High Sample Throughput

Sequences may be constructed to contain any number of methods and to analyze numerous samples. This is of major benefit in method development where numerous methods with varying operational parameters may be used to optimize a separation. Additionally different buffer vials may be programmed within methods. This allows automated method development which may be performed unattended.

Full access is available to the sample tray during operation allowing samples to be removed and replaced during a sequence thereby increasing the sample throughput available. Additionally sequences can be updated or modified while the sequence is running.

Data Analysis and Reports

The ChemStation provides for autointegration, integration and manual integration.

- Autointegration can be used to set up initial integration parameters based on characteristic signals.
- Integration integrates the electropherogram according to the values in the integration events table. This may be used to adjust integration results by changing integration events and reintegrating the signal.
- Manual integration may be performed.

The user has a choice of 15 different report styles or may choose to customize the report to suit their own requirements (table 3). There are a number of CE specific parameters which are included in the report and CE specific functions for calibration.

CE Specific data reports and analysis:

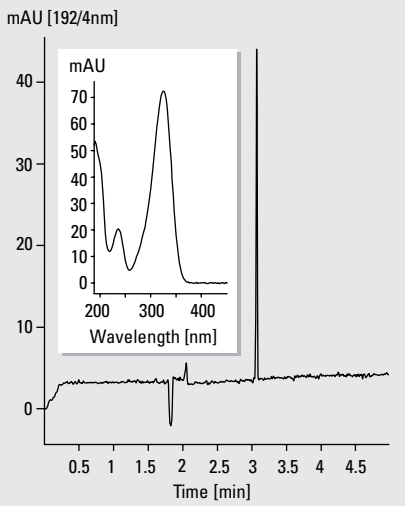
- corrected area (peak area / time),
- apparent mobility and effective mobility, (see publication number 5968-2232E)
- MW determination from SDS-protein or DNA analyses,
- pI determination for CIEF analyses.

Quantitative reporting of peak areas as corrected peak area (area/time) is required for accurate reporting of peaks separated by CE, and is mandatory for chiral analyses. Data analysis options also include the ability to perform sequence summary reports of calibrated compounds. This is a major benefit in performing precision calculations for system suitability.

Function	Parameters/ Settings	Values
Power supply	Polarity	Positive or Negative
	Voltage	0 – 30 kV
	Current	0 – 300 µA
	Power	0 – 6 W
Capillary conditioning	Inlet /outlet vial	Vial number 1– 48
	Wait	0 – 1000 min
	Voltage	0 – 30 kV
	Current	0 – 300 µA
	Power	0 – 6 W
	Flushing	1 bar
	Pressure	0 – 50 mbar (programmable)
	High pressure	2 – 12 bar (includes HV)
		Both: Inlet/outlet 2 – 12 bar with/out simultaneous voltage
Buffer replenishment	Inlet/outlet	Vial number 1– 48
	Time	0 – 1000 min
	Buffer level	0 – 1.8 cm
Injection	Pressure	0 – 50 mbar
		0 –1000 s
	Electrokinetic	0 – 30 kV
		0 – 300 µA
		0 – 6 W
		0 – 1000 s
	Program	Vial number 1 – 48
		Voltage (0 – 30 kV)
		Current (0 – 300 µA)
		Power 0 – 6 W
		Pressure 0 –50 mbar
		Flushing 1 bar
		High pressure 2 –12 bar
		Wait 0 –1000 min
Detector	Wavelength	190 – 600 nm
	Reference wavelength	190 – 600 nm
	Bandwidth	2 – 400 nm
	Peak width	0.01 – 1 min
	Response time	0.1 – 20 s
	Spectral data rate	0.16 – 5 spectra/s
	Signals	5 simultaneously
	Spectra	All spectra
		All in peak
		Peak baseline and apex
		Threshold
Capillary cassette	Temperature	10 degrees below ambient to 60°C (not below 4°C)
		Full capillary details entered in a table.
Sample carousel	Temperature	10 – 40 °C
	Vials	1– 48
Timetable	Voltage	-30 – 30 kV
	Power	0 – 6 W
	Current	0 – 300 µA
	Inlet vial	1 – 48
	Outlet vial	1 – 48
	Capillary temperature	10 degrees below ambient to 60 °C
	Lower current alarm limit	
	Polarity	Positive or negative
	Pressure	0 – 50 bar
	High pressure	2 – 12 bars with/out simultaneous voltage
Fraction collection	Electrokinetic	
	Pressure	
	CIEF	

Table 2
Programmable instrumental parameters

Figure 33
Typical electropherogram for OQ/PV test sample (*p*-hydroxyacetophenone) illustrating short run time and spectra



Report Style	Features
Short	Quantitative text results of all integrated signals
Electropherogram + short	Electropherogram plus quantitative text results
Electropherogram + detail	Electropherogram, quantitative text and calibration curves
Header + short	File header, quantitative text results.
GLP + short	Header, sample information, instrument conditions, logbook, electropherogram, quantitative results
GLP + detail	Header, sample information, instrument conditions, logbook, electropherogram, quantitative results and calibration curves
Short + spectrum	Instrument conditions, electropherogram, quantitative results and peak purity plots
Detail + spectrum	Header, instrument conditions, electropherogram, quantitative results and peak purity plots
Full	Header, sample information, instrument conditions, electropherogram, quantitative results and peak purity plots
Library search	Produces a calibrated report including library search results, peak numbers, ,migration times, librarysearch match factors, amounts, compound names
Performance	Migration time, peak are, peak height, signal description, true half-height peak-width, symmetry, efficiency and resolution
Performance + library search	Combines the performance and library search styles
Performance + noise	Combines the performance report style with noise calculations
Performance + extended	All parameters from the peak performance calculation and individual plots of each peak. Additionally - peak start and end, skew, excess, peak width, USP tailing factor, tie interval between data points, number of data points, statistical moments, plates, plates per metre, selectivity and resolution; all method relevant information (instrument, capillaries, sample and acquisition parameters and electropherogram)
HPCE mobility	Quantitative text results plus apparent mobility

Table 3
Report styles available from Agilent CE ChemStation

Provision for Regulatory Compliance

Operation Qualification / Performance Verfication (OQ/PV)

In the use of capillary electrophoresis in regulatory compliant environments there is an increasing demand for operational qualification/performance verification (OQ/PV). Using experience in regulatory issues and in capillary electrophoresis, Agilent Technologies has developed a kit which provides instructions, methods and materials for performing OQ/PV for the Agilent CE system. The procedure tests the following instrumental parameters:

- temperature accuracy and stability,
- voltage accuracy and stability,
- detector noise and drift,
- detector wavelength accuracy internally (holmium oxide filter),
- detector wavelength accuracy externally (using test analyte),
- detector linearity,
- injector reproducibility,
- injector linearity, and
- replenishment system functionality.

The tests has been developed to identify and test functions of an Agilent CE system which should be validated for regulatory compliance.

Installation Qualification (IQ)

Agilent can also supply the appropriate test procedures and certification necessary for installation qualification as an aid to the validation of methods and operation within regulatory compliant environments.

Capillary Electrophoresis Kits and Accessories

Agilent Technologies offers a range of capillaries, buffers, kits for method development and total solutions for CE analysis (table 4). Full details and ordering information may be found in the Agilent CE supplies catalog. Additionally numerous applications and educational literature are available in the form of application notes and booklets, CE and GLP primers, and scientific publications (figure 35).

Capillaries	• Bare fused silica capillaries of varying dimensions with precision cut ends for accurate and precise quantitation • Agilent Extended Light Path capillaries for high sensitivity applications • PVA coated capillaries for protein analysis and suppressed EOF analyses • CEP coated capillaries for suppressed EOF analysis and replaceable gel CGE • ODS packed capillaries for CEC
Buffers	• CZE buffers covering a wide pH range • MECC buffers for CE of neutral molecules • Gel buffers for DNA analysis
Kits	• Chiral method development kit • dsDNA analysis kit • ssDNA analysis kit • Inorganic anion analysis kits • Organic acids analysis kit • OQ/PV and IQ kits for regulatory compliance • Cation solutions kit • Forensic anion solutions kit • Plating bath analysis kit

Table 4
Kits and accessories for CE available from Agilent Technologies

Agilent Technologies Application Support

Agilent Technpologies application support includes educational and applications materials:

- primers and CD-ROMs covering introduction, theory, appli cations and GLP requirements,
- a wide range of application notes in bioscience, food and pharmaceutical analysis, and
- an international network of CE specialists.

Many of the scientific articles and application notes may be accessed through the Agilent home page on the worldwide web at: http://www.agilent.com/chem

List of abbreviations

CE	Capillary electrophoresis
CZE	Capillary zone electrophoresis
MECC	Micellar capillary electrochromatography
CGE	Capillary gel electrophoresis
CIEF	Capillary isoelectric focussing
CE-MS	Capillary electrophoresis/mass spectrometry
OQ/PV	Operational qualification/ performance verification
IQ	Installation qualification
CEC	Capillary electrochromatography
LC	Liquid chromatography
GUI	Graphical user interface
SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
MW	Molecular weight
LPA	Linear polyacrylamide
dsDNA	Double-stranded deoxyribonucleic acid

Publication Number 5965-6512E

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Innovating the HP Way