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Evaluating CE-MS as an Orthogonal Method for Pharmaceutical Impurity Profiling

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Introduction

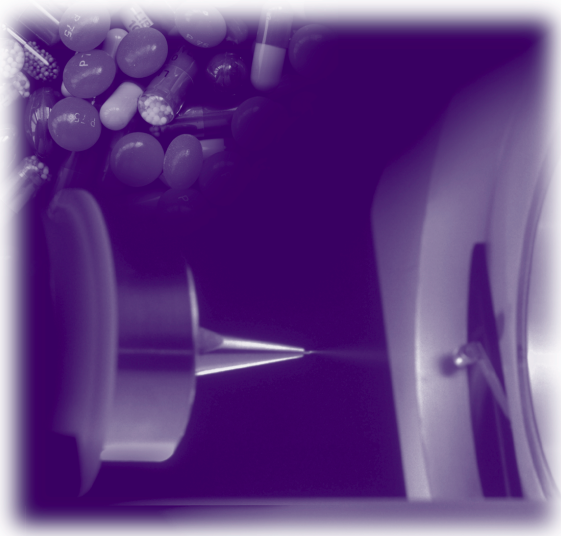
Reversed-phase HPLC methods are applied extensively during quality control of pharmaceutical products. Since capillary electrophoresis (CE) is based on a different separation principle and consequently results in a unique selectivity compared to RP-HPLC, it can be used advantageously as an orthogonal method. A CE instrument equipped with a photodiode array (PDA) UV detector and coupled to a mass spectrometer (MS) provides even more selective information that can be helpful in identification and structural elucidation purposes.[1]

In this paper, the implementation of the capillary electrophoresis–mass spectrometry technique in impurity profiling of pharmaceutical products is reported. Critical parameters, like sensitivity, repeatability, and the reproducibility of the approach, were investigated. The quality of the MS/MS spectra obtained at a low concentration level was also evaluated.

Experimental

Instrumentation

A P/ACE™ MDQ–ThermoElectron LCQ® Advantage CE-MS/MS system (Beckman Coulter, Fullerton, CA) equipped with electrospray ion source



and quadrupole ion trap mass analyzer was employed. Instrument control and data acquisition were performed with Xcalibur® Ver. 1.3 software.

Capillary Electrophoresis Method

A 375 µm OD x 75 µm ID uncoated fused-silica capillary (Composite Metal Services, Ilkey, UK) of 80 cm total length was employed. Background electrolyte composition was the following: 100 mM ammonium acetate-acetonitrile-methanol (50-25-25). Galantamine hydrobromide (0.5 mg) spiked with the related compounds was dissolved in 5 mL water and was hydrodynamically injected as the sample solution at 36 mbar for 10 seconds. The sample injected ("selectivity batch") contained the active compound and impurities at 0.2 % level which usually occur in real samples. A voltage of 30 kV was applied during the analysis. The capillary was rinsed before each run with the background electrolyte for 5 minutes. The samples and the capillary were thermostatted at 25°C.

A typical electropherogram obtained using off-line UV detection (λ=214 nm) can be seen in Figure 1. Formulas of the components are illustrated in Figure 2.

Mass Spectrometry Conditions

The source was operated in positive ion mode. The optimized parameters of the MS detector included a spray voltage of +4.5 kV, capillary temperature at 220°C. Nitrogen sheath gas was set to 5 arbitrary units and no auxiliary gas was used.

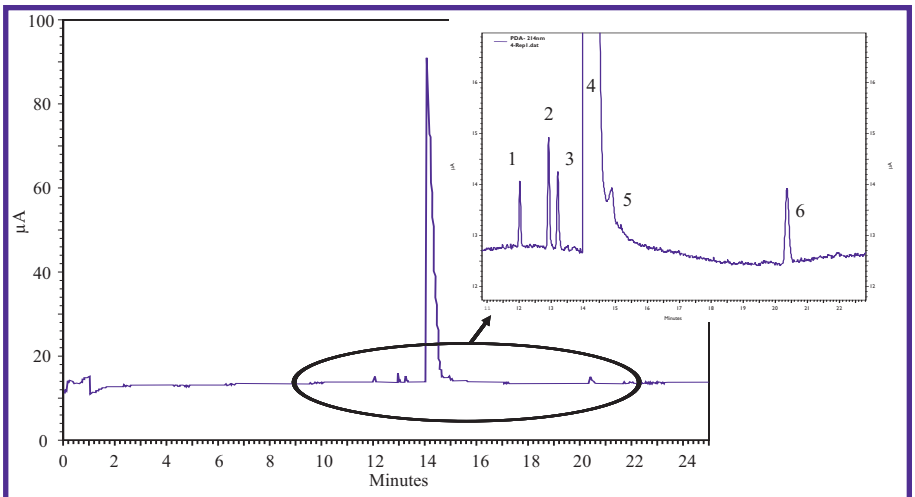


Figure 1. *Separation of galantamine and related compounds. Conditions: see in Experimental.*

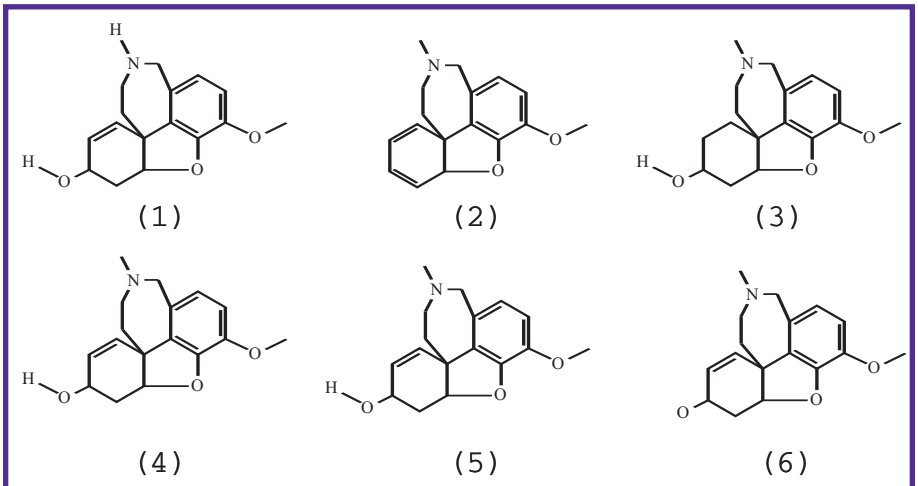


Figure 2. *Formulas of galantamine and related compound. (1): norgalantamine, (2): R116937, (3): dihydrogalantamine, (4): galantamine, (5): epi-galantamine, (6): narwedine.*

The sheath liquid was methanol/isopropanol (50:50) + 1% formic acid, a flow rate of 5 mL/min was performed. The ion transmission efficiency from the ion source to the ion trap was optimized by automatic tuning. MS spectra were acquired in a mass range of *m/z* 50–650 in full-scan mode. During MS/MS experiments, protonated molecular ions were isolated in the ion trap (isolation width: 1 *m/z*) as precursor ions and product ion full scan spectra were recorded after collision induced decomposition using helium as the collision gas. Three microscans were carried out with a maximum injection time of 200 msec.

Results and Discussion

Sensitivity

Sensitivity is one of the most important parameters to be investigated. The concentration of 0.5 mg/mL was regarded as 100% and this solution was diluted 10, 100, 1000, and 10,000 times. CE-MS electropherograms were acquired in the order of increasing concentration and are shown in Figure 3. Total Ion Current (TIC) electropherograms can be seen Figure 3a, Selected Ion Mode (SIM) electropherograms in Figure 3b.

The 0.1 % level is hardly detected in the TIC electropherogram, although this limit can be improved by employing mathematical algorithms like CODA.[2] However, 0.01% can be easily detected with SIM, but, of course, this only applies when the molecular mass of the impurity is known.

In our experience, the sensitivity of the applied CE-MS system is suitable for detecting impurities at lower than 0.1% level when appropriate data filtering is used. For example, with the Xcalibur software, it is possible to plot the masses of ions that appeared as a function of time. In this way, the present ions can be revealed.

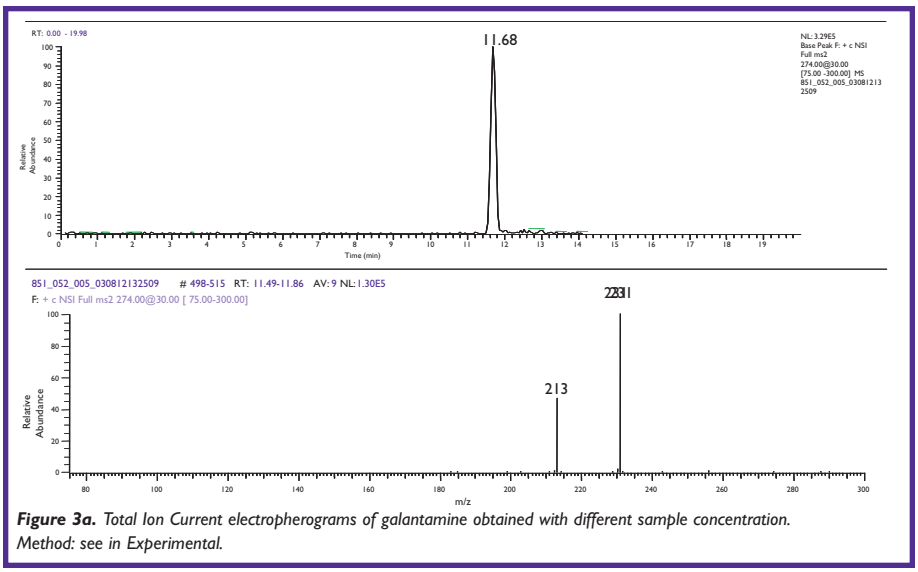


Figure 3a. *Total Ion Current electropherograms of galantamine obtained with different sample concentration. Method: see in Experimental.*

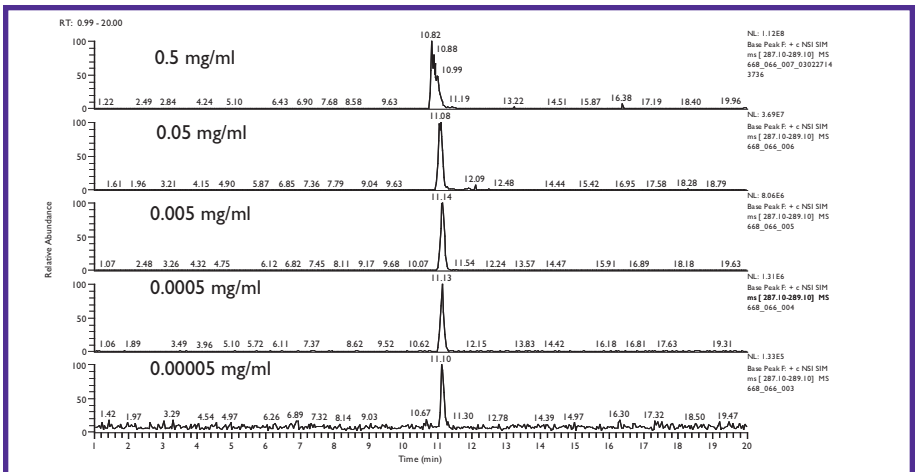


Figure 3b. *Selected Ion Mode electropherograms of galantamine obtained with different sample concentration. Method: see in Experimental.*

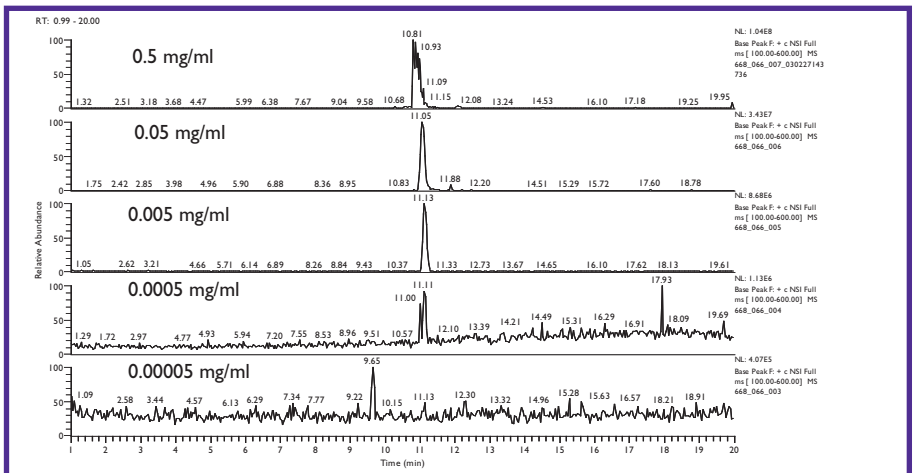


Figure 4. *Reproducibility of separation of galantamine and related compounds. Method: see in Experimental.*

Repeatability and Reproducibility

Using the method described above, we achieved excellent intra-day repeatability with a migration time RSD of galantamine at less than 0.5%. The inter-day repeatability was also suitable with the migration time RSD of galantamine at 2.7%. The reproducibility of the approach is also very important, especially for a multinational company. A new technique can only be considered for implementation if the same results can be obtained across different laboratories. To test this reproducibility across sites, the CE-MS separation of galantamine and related compounds was performed at both our site (Beerse, Belgium) and at the Beckman-Coulter site (Fullerton, California, USA). The electropherograms obtained are shown in Figure 4. The same separation and comparable detection limit were observed at both sites.

MS/MS Opportunities

The MS/MS capability of the ion trap detector is useful for both identification and structure elucidation of unknown compounds. Figure 5 illustrates an excellent signal-to-noise ratio obtained in the electropherogram of norgalantamine acquired in Selected Ion Mode (*m/z* 274) for a compound present at 0.2% level in the sample. The MS/MS spectra of norgalantamine were also acquired in the same run, of which the average can be seen in the bottom of Figure 5. This spectrum consists of ten consecutive scans, and the quality of the spectrum is excellent.

Conclusion

CE-MS/MS is a very powerful and promising technique. Since the separation principle is different than in HPLC, a unique selectivity is obtained that provides novel opportu-

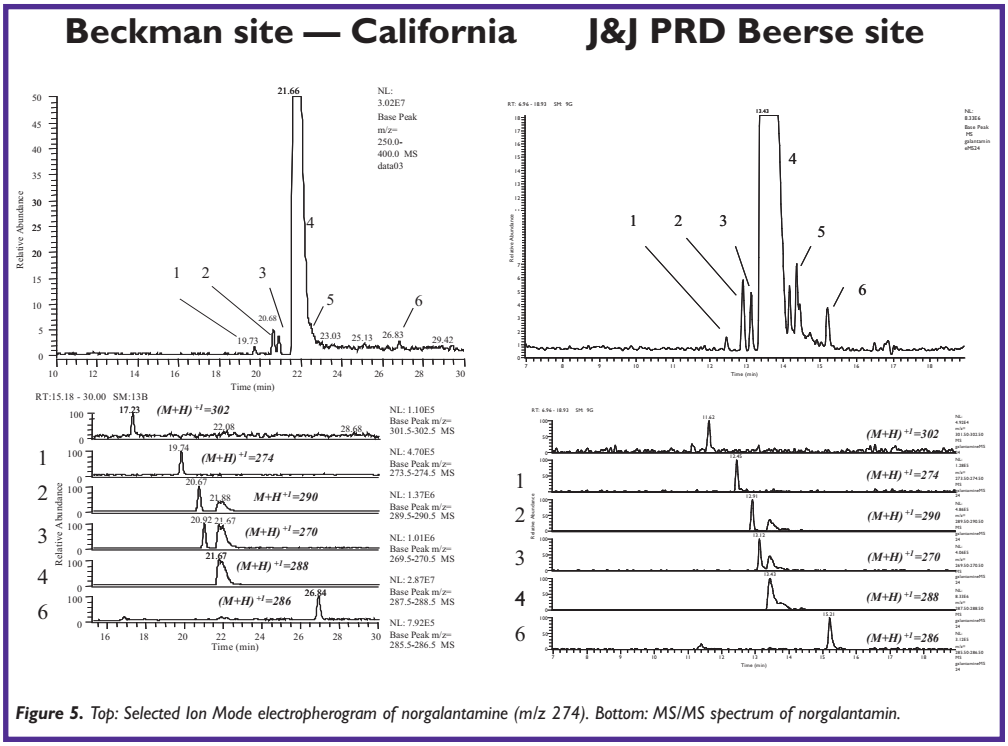


Figure 5. *Top: Selected Ion Mode electropherogram of norgalantamine (m/z 274). Bottom: MS/MS spectrum of norgalantamin.*

nities to develop orthogonal methods. By employing UV-PDA and MS detection together, the selectivity of the method is further improved. Moreover, MS detection results in an increased sensitivity. The CE-MS/MS approach was successfully applied in our laboratory as an orthogonal chromatographic methodology during impurity profiling of drugs. According to our experience, the applied system generally meets our expectation—i.e., the sensitivity, repeatability, and reproducibility are excellent and allow the detection of impurities at less than 0.1% in pharmaceutical samples. These promising results are encouraging us to continue the application of CE-MS as an orthogonal technique besides HPLC for impurity profiling.

References

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CE—It's Working Everywhere You Are: HPCE 2004

The 17th International Symposium on Microscale Separations and Capillary Electrophoresis (HPCE) was held in Salzburg, Austria, February 8–12, 2004. More than 500 scientists from Europe, North America and Asia participated.

Scientific Programs

The technical program focused on all aspects of separation methodologies and solutions to analytical problems implementing microscale techniques. The program included oral and poster sessions with emphasis on: Theory and Methodologies, Detection Schemes including Labeling Strategies, LC/CE-MS, NMR, IR Hyphenation, Chip & Nano Technology, Novel Stationary & Column Design and Affinity & Screening Strategies.

The social program was also of high quality. Salzburg is the birthplace of Wolfgang Amadeus Mozart, so it was fitting that conference attendees were treated to a Chamber Concert of Mozart's music at the Salzburg Residenz, a place where Mozart himself had performed.

Exhibition

The conference exhibition included representation from 22 different companies. The Beckman Coulter exhibition featured a 20-foot booth with the central theme of "CE, It's working everywhere you are." Featured products were our ProtemeLab™ PA 800 Protein Characterization System, P/ACE™ MDQ System, and the CEQ™ 8800 Genetic Analysis System.

Beckman Coulter sponsored two luncheon workshops, both of which were fully attended. Our workshop "Applying CE Technology" covered implementation tips to ensure trouble-free operation and our second workshop was a P/ACE™ Users Discussion which was an open forum where attendees were able to discuss the way they were using capillary electrophoresis (CE) in their laboratories.

HPCE '04 created a venue which brought the community together and provided a superb forum for us to exchange technology and ideas.

Integrating P/ACE™ MDQ and ProteomeLab™ PA 800 to Applied Biosystems/MDS Sciex Mass Spectrometers

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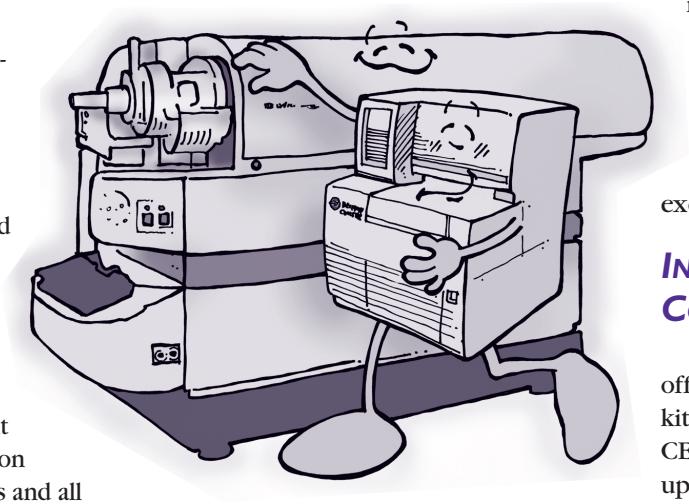
Upgrade Kits Are Now Available

A new line of CE-MS kits for the conversion of Applied Biosystems/MDS Sciex ion sources for capillary electrophoresis (CE) operation is now available. Ion sources modified with these kits implement special electrospray emitter tips and a sheath liquid junction to produce a highly sensitive and exceptionally stable signal from the outlet of Beckman Coulter CE systems. Each kit includes a detailed instruction booklet, a set of emitter tips and all necessary parts required to make the modification of the ion source. Each kit also includes an instrument license for the use of the sheath liquid junction.

Virtually all of the Applied Biosystems/MDS Sciex mass spectrometers can now be easily coupled to Beckman Coulter capillary electrophoresis systems using one of our new CE-MS upgrade kits. Capillary electrophoresis instruments have been coupled to different mass spectrometers and ionization instruments since the late 1980s since mass spectrometry (MS) provides diverse ways to qualitatively and quantitatively detect and decode CE data. Structure elucidation, accurate mass determination, and quantitation add another dimension to analysis through multiple reaction monitoring (MRM). The selectivity and specificity of MS also allows unambiguous detection and identification of analytes. The high separation power of CE forms an ideal combination with the superior detection that mass spectrometry provides. These new upgrade kits for CE-MS were purposely designed to make the fusion of CE with MS as worry-free as possible.



Mode of Operation

To overcome the limitations of prior unions, scientists at Applied Biosystems/MDS Sciex have developed a special electrospray emitter with optimized tip shape that generates an intense and highly stable signal from the CE outflow (see Figure 1). The coupling with the mass spectrometer is achieved via a sheath liquid junction that adds a make-up flow to the nL/min electroosmotic flow created in the CE capillary. The sheath liquid flow is a key element for creating ideal ion spray conditions in the ion source by closing the electrical circuit between the CE and the electrospray capillary. The sprayer assemblies are modified to allow a large-size CE capillary to fit inside precisely up to the tip of the special electrospray emitter to minimize dead volume. A syringe supplies the sheath liquid through the void space between the capillary and the electrode. Methanol is often used as a sheath liquid because of its excellent electrospray characteristics.

Instrument Configurations

Applied Biosystems/MDS Sciex is offering two different CE-MS upgrade kits to their regular ion sources: The CE-MS IonSpray®/MicroIonSpray® upgrade kit and the CE-MS Turbo V® upgrade kit. These upgrade kits allow customers to interface CE devices to Applied Biosystems/MDS Sciex mass spectrometers. The IonSpray/MicroIonSpray upgrade kit can be used to upgrade a standard IonSpray® source or a NanoSpray® source housing with a MicroIonSpray head mounted in it. The open architecture of the NanoSpray source housing provides easy access to the inner components of the interface and optimal control over the spray position. The Turbo V upgrade kit can be used with the highly sensitive API 4000® triple quadrupole and 4000 Q TRAP® systems. With this configuration, a modified probe is used to ensure proper connections to a CE capillary (Figure 1A). This configuration offers the ease of use and robustness associated with orthogonal spraying.

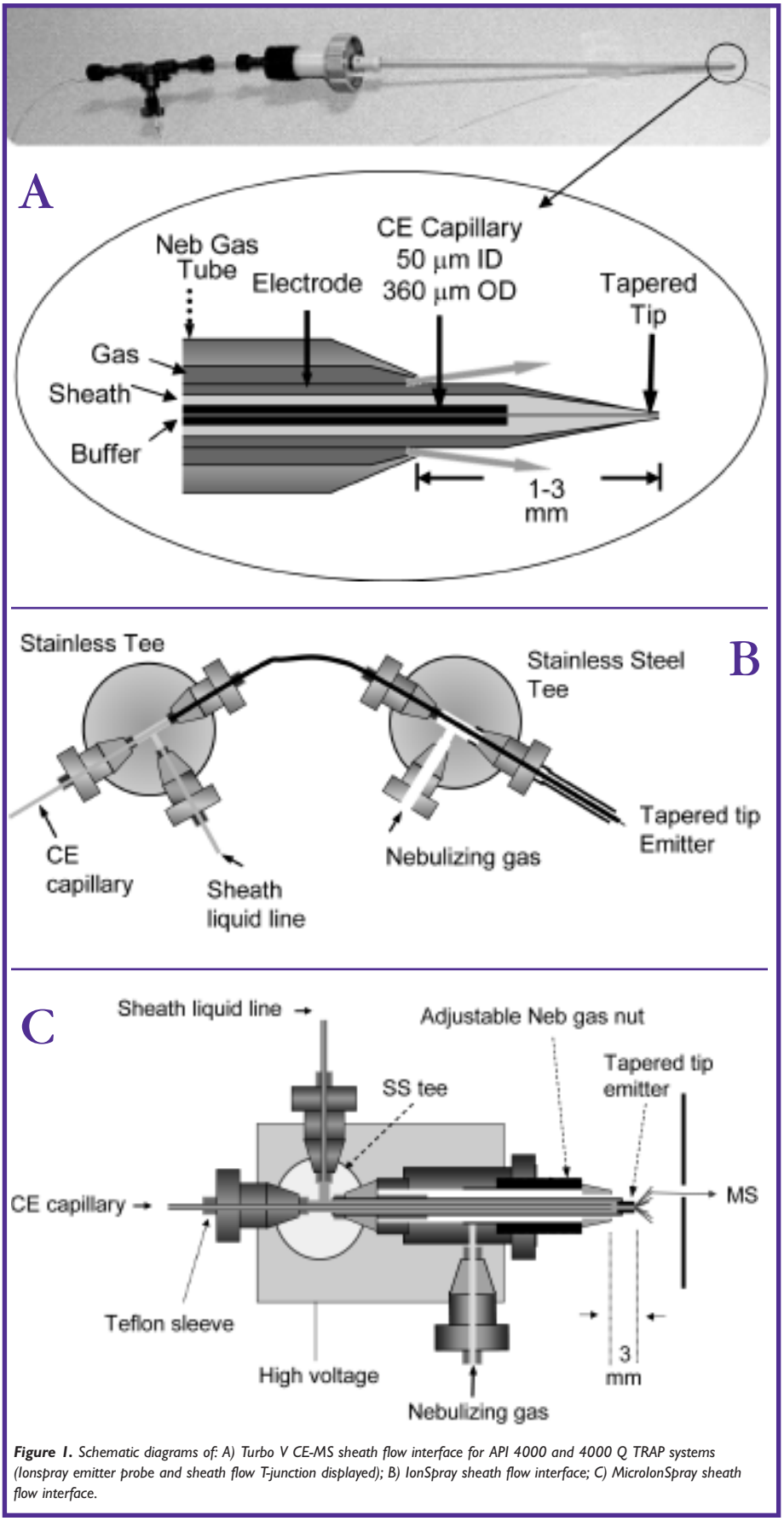


Figure 1. *Schematic diagrams of: A) Turbo V CE-MS sheath flow interface for API 4000 and 4000 Q TRAP systems (Ionspray emitter probe and sheath flow T-junction displayed); B) IonSpray sheath flow interface; C) MicrolonSpray sheath flow interface.*

Separation of Angiotensins

In Figure 2, we illustrate the separation of a standard mixture of angiotensins injected electrokinetically as obtained on a QSTAR® system (QqTOF) coupled with an automated CE system (P/ACE™ MDQ, Beckman Coulter). A modified IonSpray source (as illustrated in Figure 1B) was used to conduct CE-MS analysis of the angiotensins in positive electrospray ionization (ESI) mode. Angiotensin I and angiotensin II (10^{-4} M) were prepared in the CE running buffer (50 mM NH_4Ac, pH 3). The IonSpray interface was set to +5.2 kV and +30 kV was applied at the inlet of the CE capillary. Signal stability of 0.8% RSD in electrokinetic introduction and 2.4% RSD in hydrodynamic introduction were observed. Excellent reproducibility in migration time and peak height, of less than 2% and 5%, respectively, were also observed for injected samples.

Profiling and Quantitation of Antibodies

CE-SDS combined with UV and laser induced fluorescence (LIF) detection is routinely used in purity assays for monitoring the manufacture process of therapeutic proteins. CE-MS promises to be a valuable addition to this process adding another dimension of separation power. In Figure 3, we illustrate the analysis of therapeutic antibodies using capillary zone electrophoresis coupled to a Turbo V source for API 4000 triple quadrupole system. In this example, 95 nL of a 1 µM solution is injected hydrodynamically (4 psi, 20 sec) into a PVA coated capillary (50 µm I.D. x 100 cm x 365 µm O.D.). The sheath liquid is 50/50/0.1% MeOH/H_2O/formic acid. In this example, two comigrating antibodies can be easily discriminated and quantitated using ion chromatograms extracted from the spectral data set.

Conclusion

A new set of upgrade kits for Applied Biosystems/MDS Sciex mass spectrometers allows the coupling of standard CE capillaries to standard ion sources. A sheath liquid junction and specially shaped electrospray emitters provide high sensitivity and exceptional signal stability. Virtually all of the Applied Biosystems/MDS Sciex mass spectrometers can now be routinely converted to CE-MS operation. This hyphenated technique allows analysts to combine the high separation power of capillary electrophoresis with the highly specific and selective detection by mass spectrometry.

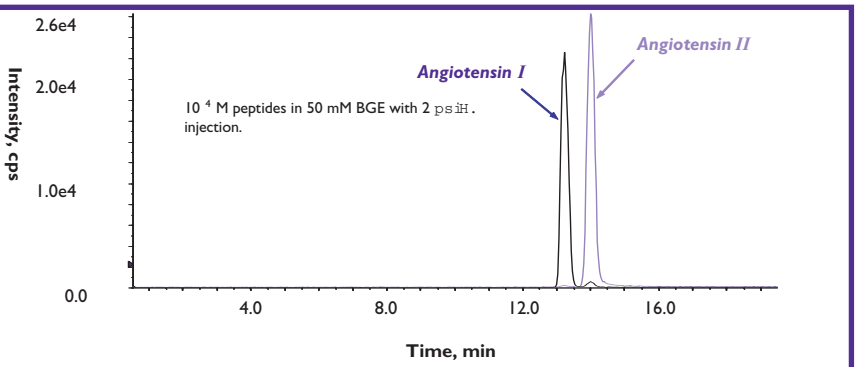


Figure2. *Separation of angiotensins by capillary electrophoresis using single ion monitoring (SIM) on a QSTAR system.*

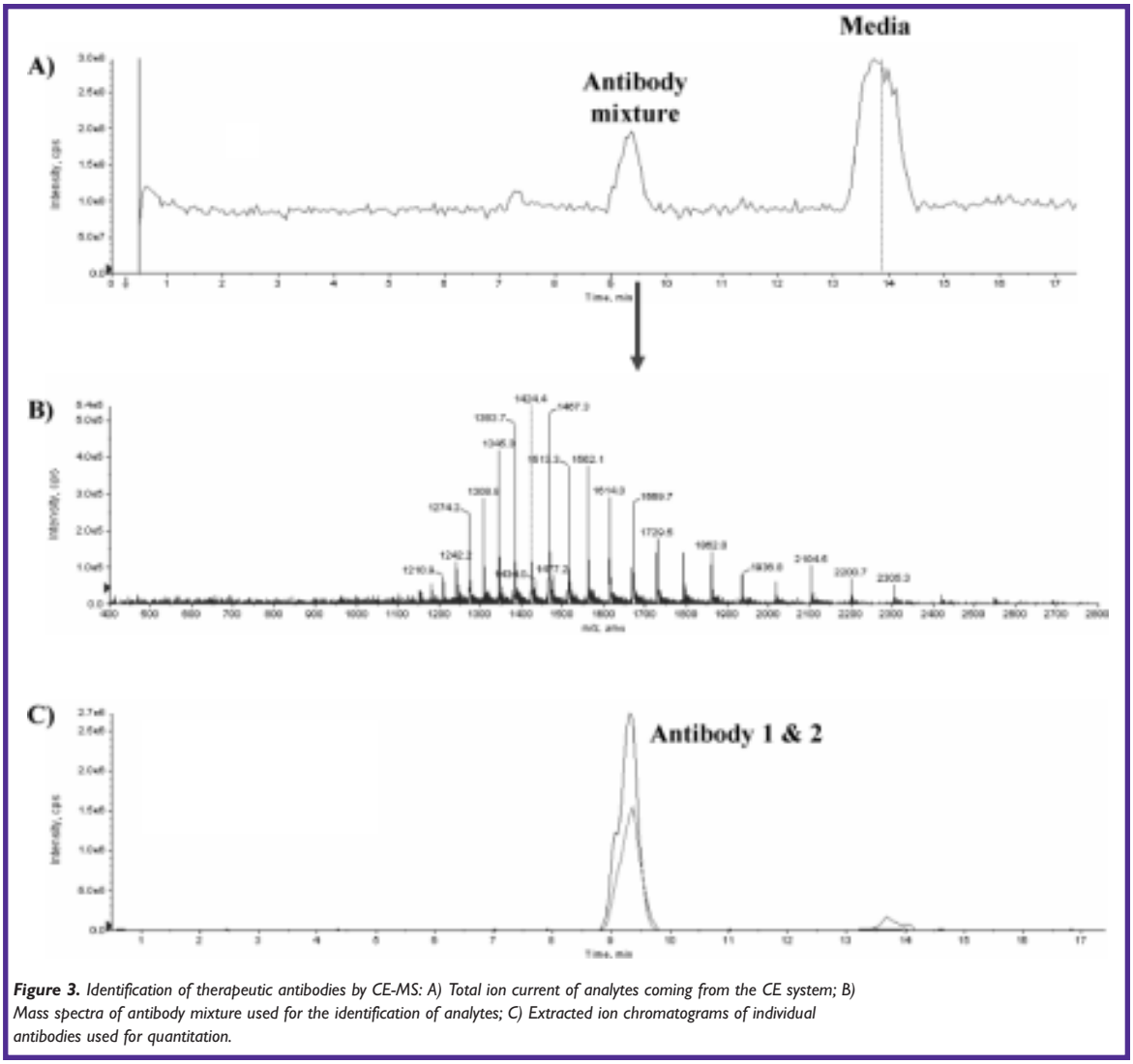


Figure 3. *Identification of therapeutic antibodies by CE-MS: A) Total ion current of analytes coming from the CE system; B) Mass spectra of antibody mixture used for the identification of analytes; C) Extracted ion chromatograms of individual antibodies used for quantitation.*

Capillary Electrophoresis as a Powerful Characterization Tool in Nanotechnology

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Introduction

Nanotechnology is an emerging multidisciplinary area of research that is spearheading new discoveries, applications, and solutions in such diverse areas as biotechnology, materials science and engineering, and the pharmaceutical industry. It is fundamentally driven by the unique properties of materials in the nanometer dimension which differ markedly from those of individual molecules, atoms, and bulk materials. This paper demonstrates for the first time the power of capillary electrophoresis in the characterization of the fundamental properties of nanodimensional systems. We demonstrate that, using CE,

(1) charged and uncharged nanoparticles can be separated,

(2) their zeta potentials and net charges can be calculated,

(3) association between negatively charged nanoparticles and positively charged metal ions and metal complexes can be discerned,

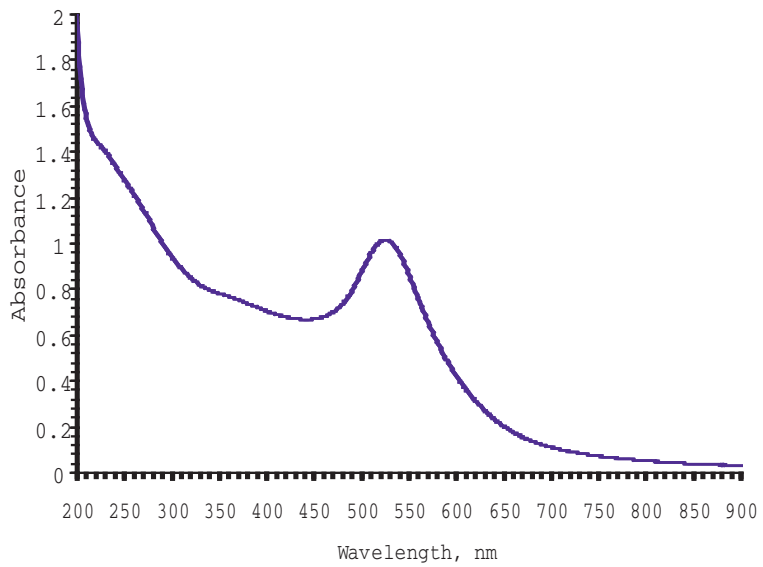
(4) association constants for such equilibria can be obtained, and

(5) predictions regarding dependence of fundamental properties on size can be made.

Experimental Approach

A Beckman Coulter P/ACE™ MDQ instrument with UV-visible detection and capillaries of 75 µm inner diameter was employed in these studies. Gold nanoparticles of 15 nm diameter derivatized with monolayers of tiopronin ($CH_3CH(SH)C(O)NHCH_2COOH$) (Figure 1) were examined at pH = 10.6 under the following conditions: 1) varying concentrations of $NaNO_3$ (0–25 mM) at 10 kV; 2) different dielectric constants by the addition of dioxane at 20 kV and 10 mM $NaNO_3$ (Figure 2); and 3) in the presence of different concentrations of the complex $Cu(en)_2^{2+}$ (Figure 3).

All CE experiments were conducted at 25°C in silica capillaries of 75 µm inner diameter and length of 50 cm to the UV-Vis detector and total length of 57 cm. New capillaries were conditioned by washing with 1 M NaOH at 20 psi for 30 minutes followed by H_2O wash for 5 minutes. The studies reported here were conducted with a run buffer of 1 mM NaOH (pH = 10.3) at either 10 kV (Figure 2) or 20 kV (Figure 3). The capillaries were washed with 1 M NaOH for 2 minutes at 20 psi, followed by H_2O and run buffer rinse for 2 minutes each at 20 psi. Nanoparticle samples were injected for 20 seconds into the run buffer. The nanoparticles were detected either at 254 nm or 553 nm.



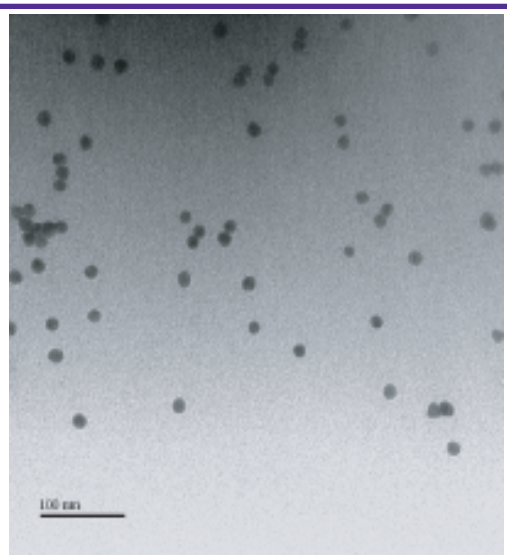


Figure 1. *UV-Vis spectrum of 15 nm Au-Tiopronin nanoparticles and their TEM.*

Results and Discussion

The significant results from these studies that have been submitted as a full paper to the Journal of the American Chemical Society are:

- The high molar absorptivities of the thiolated nanoparticles (~10^9 $M^{-1}cm^{-1}$) in the 520-550 nm enable the investigation of their fundamental properties by CE at the subnanomolar concentrations (Figure 1).
- CE can efficiently separate nanoparticles of different charges and sizes (Figures 2 and 3).
- The shape of the capillary electropherogram is a function of the monodispersity of the nanoparticles. Highly symmetrical gaussian-shaped electropherograms are obtained with monodiosperse particles and the peak shape is severely distorted with polydispersity.
- The charged nanoparticles, unlike charged ions and molecules, are characterized by a zeta potential. This zeta potential is markedly different from that of the capillary wall at low ionic strengths but approaches the wall zeta potential at high ionic strengths.
- The zeta potential is sensitive to the structure of the thiol ligand on the gold surface as evident from the dependence of these on the ionic strength of the run buffer for the different thiols.
- The zeta potentials and hence the electrophoretic mobilities of the charged nanoparticles are dependent on the dielectric constant of the medium. This dependence is sensitive to the structure of the ligand.
- Association between the negatively-charged nanoparticles and positively-charged ions and complexes occurs in H_2O and H_2O-doxane mixtures and this association constant can be calculated from the electropherograms.

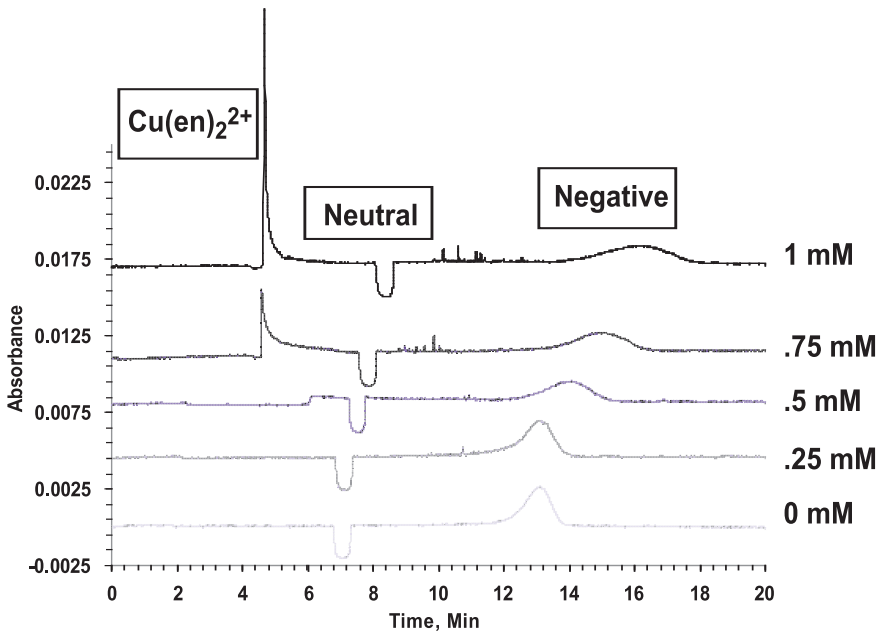


Figure 2. CE of neutral and negatively charged 15 nm Au-tiopronin nanoparticles in the presence of $Cu(en)_2^{2+}$.

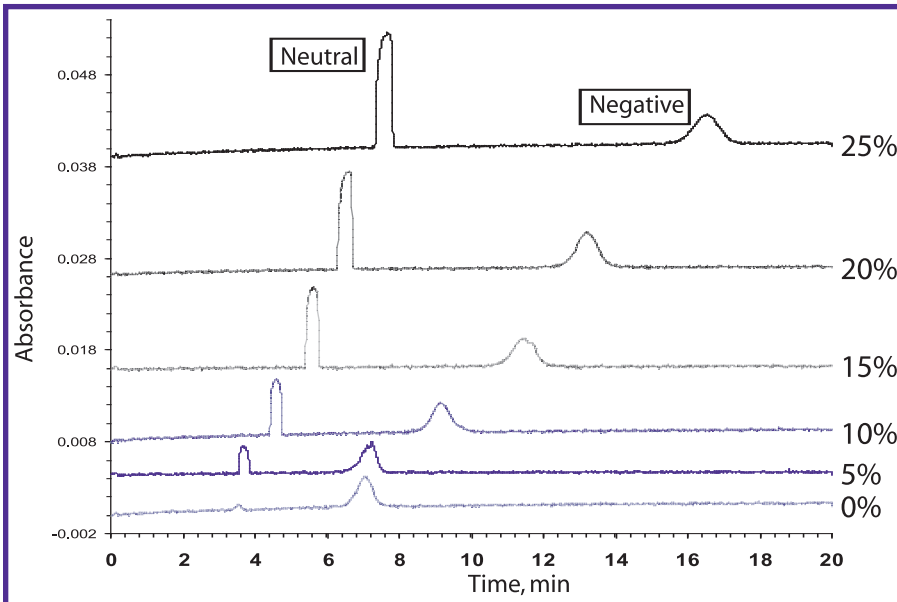


Figure 3. CE of 15 nm Au-tiopronin nanoparticles in the presence of $Cu(en)_2^{2+}$.

In summary, capillary electrophoresis is a very useful and powerful tool for the fundamental characterization of charged and neutral nanoparticles.

Tips for Measuring the Capillary and Marking the Correct Window Position

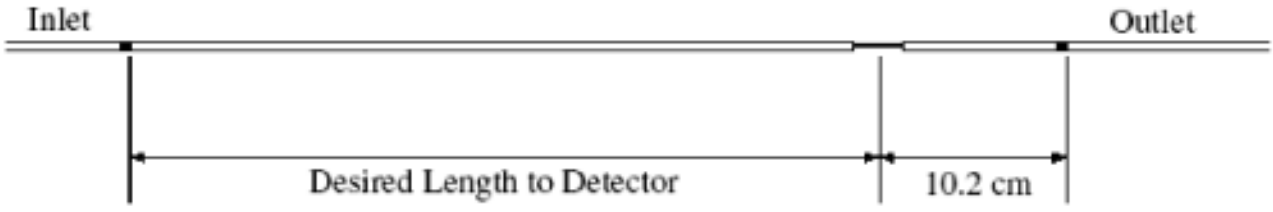
Analyte mobility can be a valuable parameter in assigning peak identification, in that regardless of variation in electroosmotic flow, the mobility of an analyte remains constant at a defined buffer pH. Your 32 Karat® software that controls the P/ACE™ MDQ and ProteomeLab™ PA 800 systems allows you to identify peaks based on their calculated mobility, however, if the capillary lengths are not accurately measured, the assignment of peak identification between different capillaries will be compromised. To ensure accurate capillary measurement we recommend that you use the following simple procedure:

1. Referring to Figure 1 measure 10.2 cm from the middle of the capillary window to the outlet end. Using a felt tip marker, mark the position.
2. Measure the desired length from the middle of the capillary window to the inlet end. Using a felt tip marker, make a mark on that position.
3. Do not remove the excess capillary at this time. Install the capillary into the cartridge, and cut-off the excess capillary only after the capillary is locked in place. Rather than use the template—simply cut the capillary at the marks you placed with the felt tipped pen.

To ensure consistent measurements, place a masking tape template on the edge of a bench and mark the measurements on the tape, then simply line the capillary up with the marks when preparing a new capillary. This will avoid having to pull out the ruler every time you install a new capillary.

NOTE: Do not touch the capillary window as it is fragile and finger oils can reduce detection sensitivity.

Once the capillary is installed, make sure the new measurement is appropriately noted in the software—identifying the total capillary length and the distance to the detection cell.



Reminder:
Carefully inspect the ends of the capillary to ensure a clean and smooth cut. A rough or jagged edge may introduce artifacts into your results.

Confusing CE Terminology – A Historical Perspective

Have you ever wondered why the polarity of the power supply is referred to as "normal" and "reverse?" Or why the left side of the capillary is referred to as "inlet" while the right side is referred to as "outlet?"

The answer to both of these questions may relate to historical convention. The following is a brief explanation.

Normal and Reverse Polarity

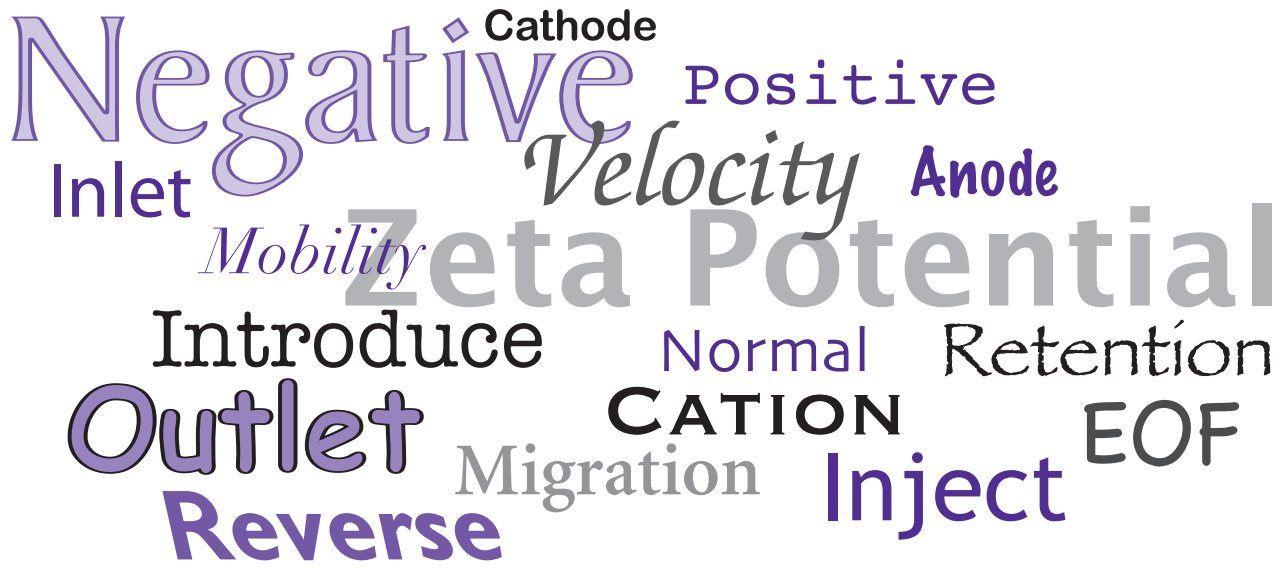
When the first automated CE system, the P/ACE™ 2000, was commercialized in 1989, bare fused-silica separations were the primary methods being used. In this mode of operation, the strong electroosmotic flow generated from the capillary surface forced most separations to be done using an anode to cathode configuration (where the sample is introduced at the anode and detected close to the cathode). As this was the primary means of operation, this mode of electrophoresis established the convention of "normal" polarity for the power supply. For the rare occasion where the EOF became reversed, one would use the reverse polarity to ensure that the analyte was swept by the detector. By today's standards, this designation really no longer applies since we now use the reverse-polarity designation much more frequently than the normal polarity. However, the original terminology is still in use. Normal polarity references anode (+) at the left electrode while cathode (-) is at the right electrode (when facing the system). Reverse polarity references cathode (–) at the left electrode while anode (+) is at the right.

Quick Memory Tip: Cations (+) migrate to the Cathode (-), while Anions (-) migrate to the Anode (+)

Inlet and Outlet

Once again, when the P/ACE 2000 was first commercialized, sample introduction was fixed on the left side of the capillary cartridge, allowing the longest path from sample introduction to the ultimate detection at the capillary window. As the sample was introduced on the left, this became known as the capillary inlet. Following the detection window was the short side of the capillary, where the analyte ultimately exited the capillary, and this became known as the capillary outlet. Today, however, samples may be introduced on either the long side or short side of the capillary—rendering the terminology of inlet (sample goes in) and outlet (sample goes out) interchangeable. However, the historical convention of inlet and outlet have been retained on the modern P/ACE MDQ platform such that, when facing the system, the left side is referred to as the system inlet and the right side is referred to as the system outlet, even though you may frequently introduce the sample into the outlet and terminate the separation at the inlet.

So much for historical convention, eh?



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CE in the Biotechnology and Pharmaceutical Industries

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August 13 – 16, 2004
Sponsored by the California Separation Science Society (CaSSS)

The goal of this Symposium is to provide a forum for the discussion of recent developments in CE analysis of protein, nucleotide, and small-molecule pharmaceuticals. The presentations and workshops will be devoted completely to practical concerns to strengthen the use of CE within the biotechnology and pharmaceutical industries. Applications will highlight uses of CE in various areas of product development including high-throughput screening, formulation studies, process development, product characterization, validated lot release, and stability testing. The Symposium will feature presentations from leading experts within the industries. The workshops will allow for open discussions of specific techniques and/or applications including carbohydrate analysis, MEKC, CZE, cIEF, SDS-protein, CE/MS, oligonucleotide, and chiral separations as well as validation of CE assays for product release.

The Symposium will begin on Friday, August 13, with a full day optional practical course from expert lecturers. Following the optional course, the Symposium will continue with three full days of seminars, practical workshops and technical seminars on different CE applications.

Who Should Attend?

This Symposium is designed for all individuals (industrial, regulatory or academic) who use, or would like practical information about, the state-of-the-art use of capillary electrophoresis in the various areas of biotechnology and pharmaceutical development, including discovery, formulation studies, process scale-up, characterization, product testing, lot release, and stability. The upcoming conference is conveniently located in San Juan, Puerto Rico, to facilitate worldwide attendance.

Abstract Submission Deadline

The Organizing Committee invites scientists from industry, government regulatory agencies and academia to submit abstracts on their current work by June 15, 2004 to be considered for an oral seminar or workshop presentation. July 12, 2004 is the deadline for poster abstract submissions.

For More Information

Contact the California Separation Society website at www.casss.org

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