BECKMAN COULTER

PACE Setter

Volume 5, Issue 1 • April 2001

The worldwide newsletter for capillary electrophoresis

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PACE Setter

Volume 5, Issue 1 • April 2001

The worldwide newsletter for capillary electrophoresis

Applying Enantioselective CE in the Pharmaceutical Industry

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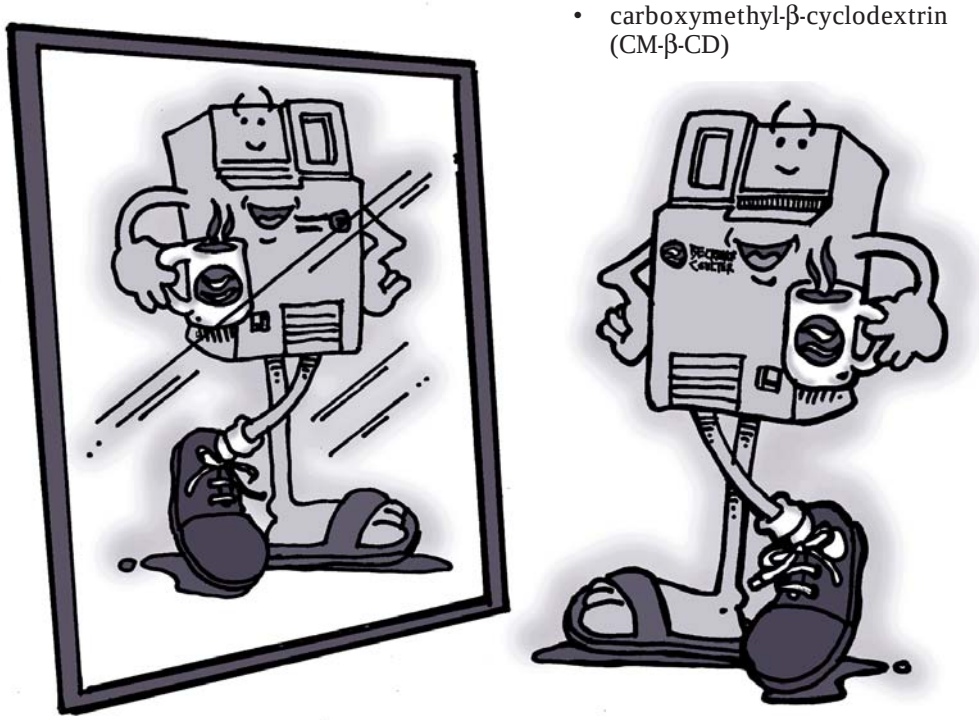
Why Use CE?

Enantioselective CE has, over the last 10 to 15 years proved to be an efficient tool for chiral separation[1]. Generally CE offers high efficiency, short time of analysis, and minimal solvent/reagent consumption in comparison with other separation techniques. Although most chiral selectors can be used, it is primarily cyclodextrins and cyclodextrin derivatives that are best suited for CE. Other important selectors like macrocyclic antibiotics and proteins can be used but, due to their UV-absorbance, are easier to employ in LC methods where they are immobilized on the column. It is, however, our impression that most analytical scale chiral separations can be accomplished using CE with a limited number of cyclodextrin derivatives.

Method Development

In our strategy for method development, we screen eight chiral selectors using two types of buffers: 50 mM sodium dihydrogenphosphate, pH 2.5, and 20 mM sodium tetraborate, pH 9.3. The choice of buffer pH depends on the type of analyte and chiral selector. Bases are analyzed at low pH. Acids are analyzed at high pH or, alternatively, at low pH using highly sulfated cyclodextrins. Neutrals are analyzed at low/high pH using highly sulfated cyclodextrins or, alternatively, at high pH using carboxymethyl β-cyclodextrin. The selectors that we are screening are the following (usually at 20 kV normal polarity, bare fused-silica, 20 cm effective length, 50 mm i.d., 20°C, and a selector concentration of 20 mM):

- α-, β- and γ-cyclodextrin (α-CD, β-CD, and γ-CD, 15 mM)
- 2,3,6-trimethyl-β-cyclodextrin (TM-β-CD)
- 2,6-dimethyl-β-cyclodextrin (DM-β-CD)
- (2-hydroxy)propyl-β-cyclodextrin (HP-β-CD)
- highly sulfated α-, β-, and γ-cyclodextrin (HS-α-CD, HS-β-CD, and HS-γ-CD, reversed polarity, *i.e.*, anode at detector, and 15 kV)
- carboxymethyl-β-cyclodextrin (CM-β-CD)



In our screening procedure, type of selector is first evaluated. In order to speed up the method development, arrays of 2 mL vials containing different chiral selectors and buffer

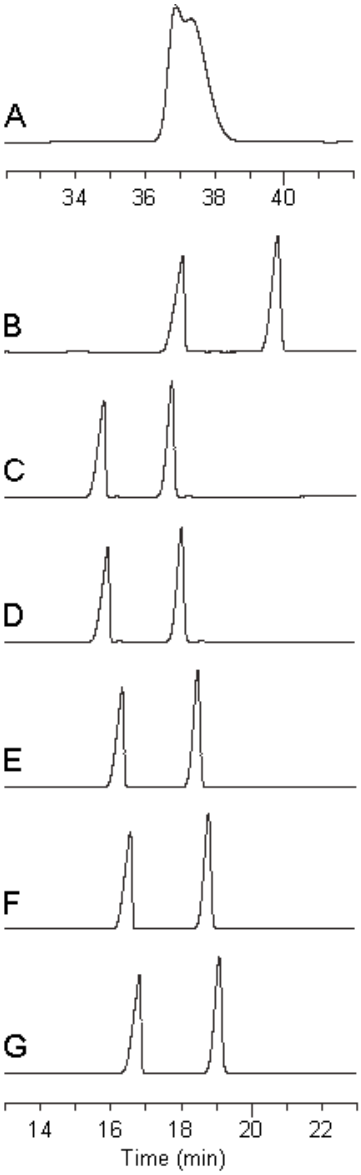


Figure 1. *(A-B) An example of selectivity and retention differences between sulfated γ-CD batches from two different manufacturers. According to the manufacturers, the degree of substitution should be approximately 12-13 sulfate groups per CD. (B-D) A comparison of three different batches of "raw" HS-γ-CD from one manufacturer. (E-G) Three batches of the HS-γ-CD commercially available from Beckman Coulter, Inc. These lots are formulated by blending the raw, synthetic material to meet a tight specification.*

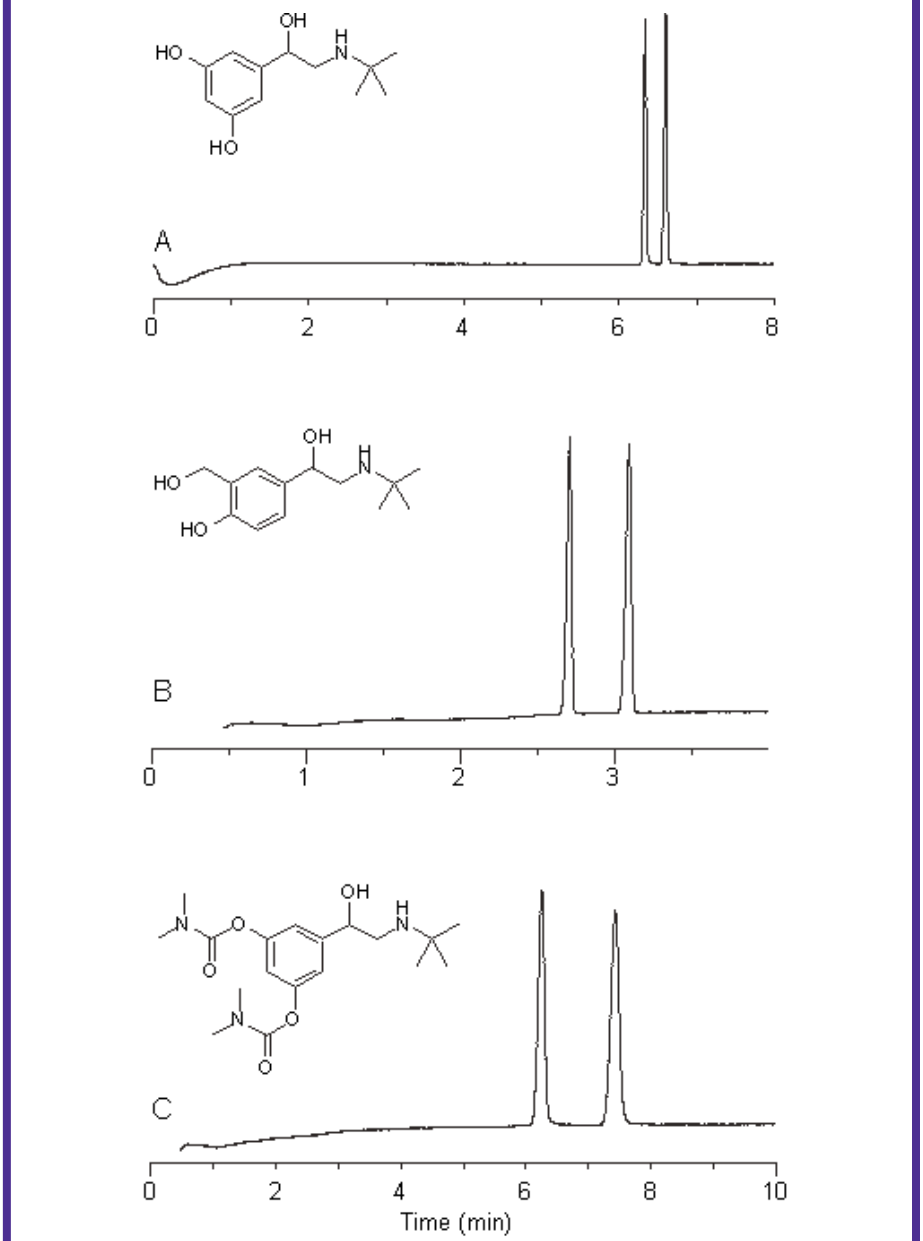


Figure 2. *The enantiomers of (A) terbutaline, (B) salbutamol (short end injection mode), and (C) bambuterol separated with the same system based on HS-β-CD.*

combinations can be kept in the freezer. For those selectors that display changes in peak shape, the influence of CD concentration is evaluated at high (50 mM) and low levels (2 mM). Finally, for the most promising selector(s) the CD concentration is fine tuned together with an optimization of temperature, capillary length, and field strength. If necessary, the addition of a modifier (*e.g.*, methanol) is evaluated.

For basic drugs, peak shape improvements may be obtained by altering the composition of the pH 2.5 buffer. A buffer based on triethanolamine and phosphoric acid should result in mobility matching and minimized wall interactions for bases[2,3]. For the analysis of neutral compounds, systems combining a negatively charged CD (HS-β-CD or CM-β-CD) to create electrophoretic mobility and a neutral CD to create chiral selectivity are an additional possibility[4]. For primary amines, we also consider the use of a crown ether, 18-crown-6-tetracarboxylic acid, as chiral selector[1,5].

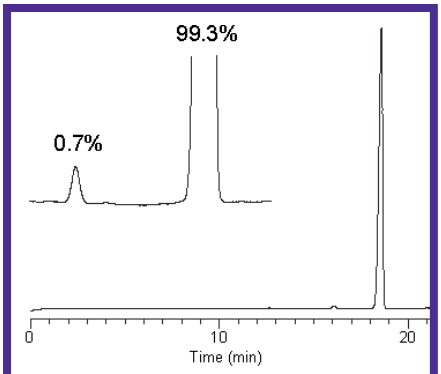


Figure 3. *Another example, a proprietary amine for which HS-γ-CD gave a better selectivity than 17 other selectors including macrocyclic antibiotics, proteins, and derivatized cellulose.*

If more than one selector gives an acceptable result, batch-to-batch reproducibility, number of manufacturers, and cost should be taken into account. Batch-to-batch variations are a potential problem for derivatized CDs. Thus it is advisable to always investigate the ruggedness of a new method by evaluating different batches and manufacturers of the chiral selector. Different manufacturers may have products with different degrees and/or positions of substitution. Consequently, there can be very large selectivity and retention differences as illustrated in Figure 1A-B for sulfated γ-CD from two different manufacturers. It should, however, be stressed that derivatized CDs can also be produced in a reproducible way as shown in the same figure (Figure 1B-G) for our evaluation of six batches of HS-γ-CD from Beckman Coulter, Inc. More data on the characterization of HS-CDs and the comparison with other types of sulfated CDs can be found in references 6 and 7.

In order to meet the requirement from drug regulatory authorities to be able to determine related impurities at 0.05% level, both good resolution and a thorough optimization of the signal-to-noise ratio is needed. Parameters to optimize are injected amount and volume, sample focusing, capillary dimensions, as well as detector wavelength, bandwidth, sampling frequency, and filter settings. It should also be noted that a sufficient signal-to-noise ratio often is difficult to obtain for chiral separations based on techniques like GC and LC due to poorly separated and/or tailing peaks.

EXAMPLES

As we are working with pharmaceutical compounds, most of our analytes are bases. For these types of compounds, it is our experience that HS-CDs and HS-β-CD in particular provide the broadest selectivity and the fastest separations of the CDs listed above. Figure 2 illustrates one example where the enantiomers of three amino alcohols, terbutaline, salbutamol and bambuterol, are separated with the same CE system based on HS-β-CD. The enantiomers of terbutaline and bambuterol have previously been separated using other CDs[8]. To our knowledge, however, only HS-β-CD can separate all of them under the same conditions. Another example is shown in Figure 3 for a proprietary amine with an enantiomeric purity of 99.3%. For this amine, HS-γ-CD gave a better selectivity than 17 other chiral selectors including the CDs listed above, proteins, macrocyclic antibiotics, and derivatized cellulose. Similar results have also been obtained for other types of amines.

CONCLUSIONS

It is our impression that enantioselective CE is an important tool for pharmaceutical analysis—perhaps the most important tool for analytical-scale chiral separations. Among the chiral selectors that are available for CE today, HS-CDs seem to provide not only the most general selectivity but also the fastest separations. The separation of enantiomers for more than 140 pharmaceutical compounds have been reported[6-7,9-12]. An additional advantage is that bases, acids, and neutrals can be analyzed using the same systems.

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In-Process Analysis of Plasmid Copy Number for Fermentation Control

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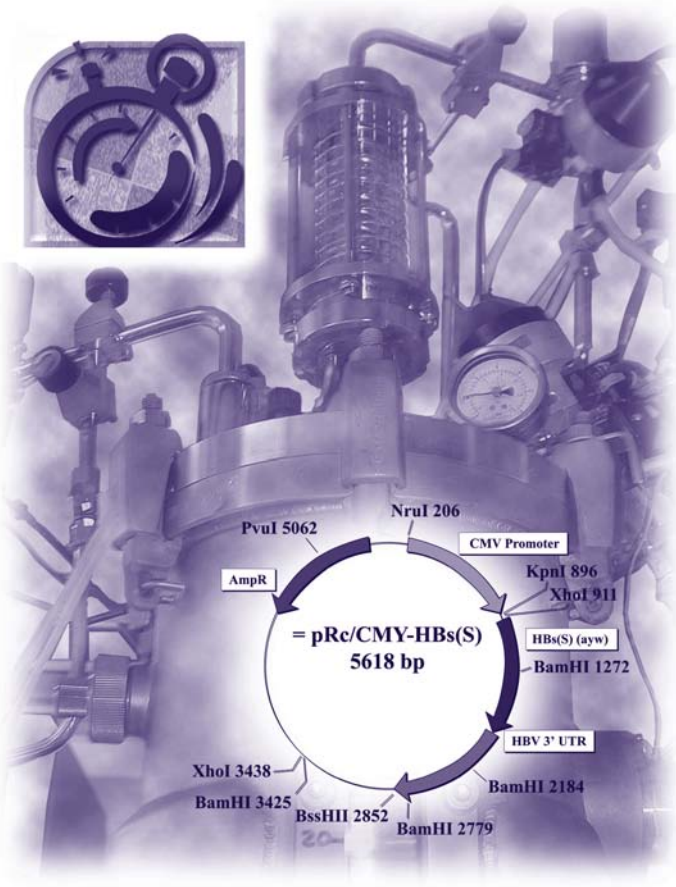
Introduction

In biotechnology, the plasmid copy number, defined as the number of plasmids per cell, has a high impact on the productivity of recombinant microorganisms. A high plasmid copy number (*i.e.*, a high gene dosage) usually leads to high expression of plasmid-encoded genes and, therefore, overproduction of recombinant proteins. Since plasmid DNA became a pharmaceutical product itself as a vector for therapeutic genes in gene therapy or DNA vaccination trials[(1)], the microbial production of plasmid DNA in *Escherichia coli* is of great interest. In these cultivation processes, the plasmid copy number is an important control parameter for product concentration and productivity of the process. The quantitation of plasmid copy number during cultivation needs a quick DNA isolation in combination with a fast and reliable analysis method offering in-process controls in less than 30 minutes. In the literature, however, numerous direct or indirect methods for determination of plasmid copy number are described, like measuring the activity of the plasmid-encoded product[(2)] or dot blotting[(3)], as well as quantitation by UV absorbance[(4)], HPLC[(5)], or gel electrophoresis[(6)] after isolating plasmid DNA from the cells. All these methods have the disadvantage of being too time-consuming to be useful for plasmid copy number determination during a bacterial cultivation. For this application, the procedures for both the DNA isolation and its quantitative analysis has to be accelerated compared to common procedures. The quick isolation method for plasmid DNA is of primary importance because the fast analytical method should not be slowed down by time consuming isolation. On the other hand, the completeness of plasmid isolation is of interest for getting reliable results as well.

A novel method, based on the fast quantitation of plasmid DNA with capillary gel electrophoresis after a modified rapid DNA isolation, allows determination of plasmid copy number for in-process control in fewer than 30 minutes.



Experimental

Analyses were performed using a P/ACE™ 2050 CE instrument from Beckman Coulter equipped with a LIF detector (488/520 nm). Coated capillaries (DB-17; J&W Scientific, Folsom, CA, USA) with a length of 30 cm to the detector window, 100 µm I.D., and a coating thickness of 0.1 µm were used for separation. The capillary was flushed with run buffer consisting of 89 mM Tris, 89 mM boric acid, 2 mM EDTA (pH 8.4), and 0.1% (w/w) hydroxypropylmethylcellulose (HPMC, Sigma, Deisenhofen, Germany). Just prior to analysis, the intercalating dye YOYO (Molecular Probes, Eugene, OR, USA) was added to the run buffer (1 µL YOYO in 15 mL run buffer). After DNA pre-staining with YOYO, the samples were injected hydrodynamically for two seconds. Electrophoresis was carried out at 300 V/cm and 30°C. Plasmid DNA concentration was determined by the corrected peak area (peak area migration time ratio) using a calibration curve

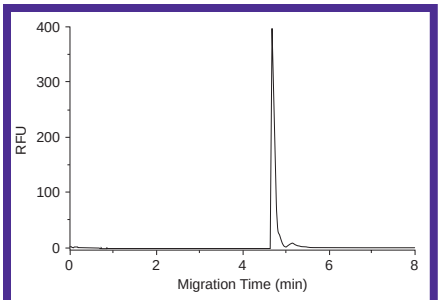


Figure 1. *Electropherogram of the reference plasmid standard for quantitation.*

of a reference plasmid standard (PlasmidFactory, Bielefeld, Germany) with concentrations from 3 to 2000 pg μL^{-1}.

For quantitation, small amounts of plasmid DNA were quickly isolated from bacteria culture in 20 minutes using commercially available kits based on silica technology, like NucleoSpin from Macherey-Nagel (Düren, Germany) or QIAprep Spin from QIAGEN (Hilden, Germany). However, this kit technology is not sufficient for purification of plasmid DNA to be used in pre-clinical or clinical applications but in quantitation it is helpful for separation of plasmid DNA from genomic DNA, RNA, and proteins.

RESULTS

The quantitation of plasmid DNA with capillary gel electrophoresis can be performed in fewer than six minutes (Figure 1). In contrast to our report in the *P/ACE Setter* of October 2000 (Volume 4, Issue 2—"Assessing the Homogeneity of Plasmid DNA: An Important Step toward Gene Therapy"; see also www.CGEservice.com), the separation of different plasmid structures resulting in longer analysis time is not necessary for the determination of plasmid copy number because, in this case, the quantitation of the total plasmid amount is required. The electropherogram shows that the plasmid purity reached with the quick isolation method is sufficient. Only one peak is obtained, representing the plasmid DNA. Other nucleic acid contaminants, like RNA or chromosomal DNA, do not disturb the measurements. Approximately 20 minutes for plasmid sample preparation plus 10 minutes for CGE analysis results in a total of a 30-minute method for determining the plasmid copy number. The completeness of plasmid isolation could be demonstrated by comparing with other plasmid purification methods[7].

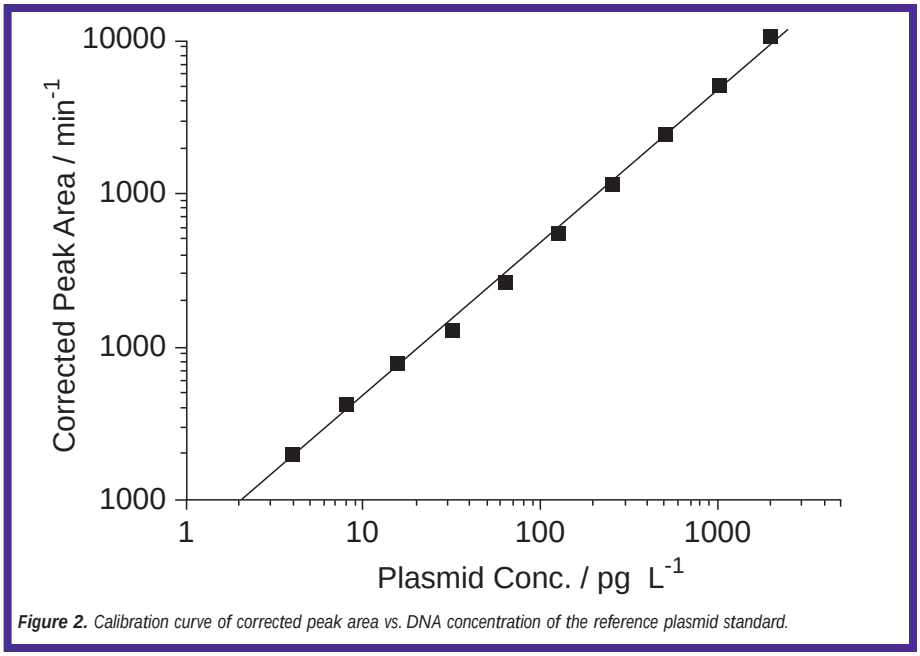


Figure 2. *Calibration curve of corrected peak area vs. DNA concentration of the reference plasmid standard.*

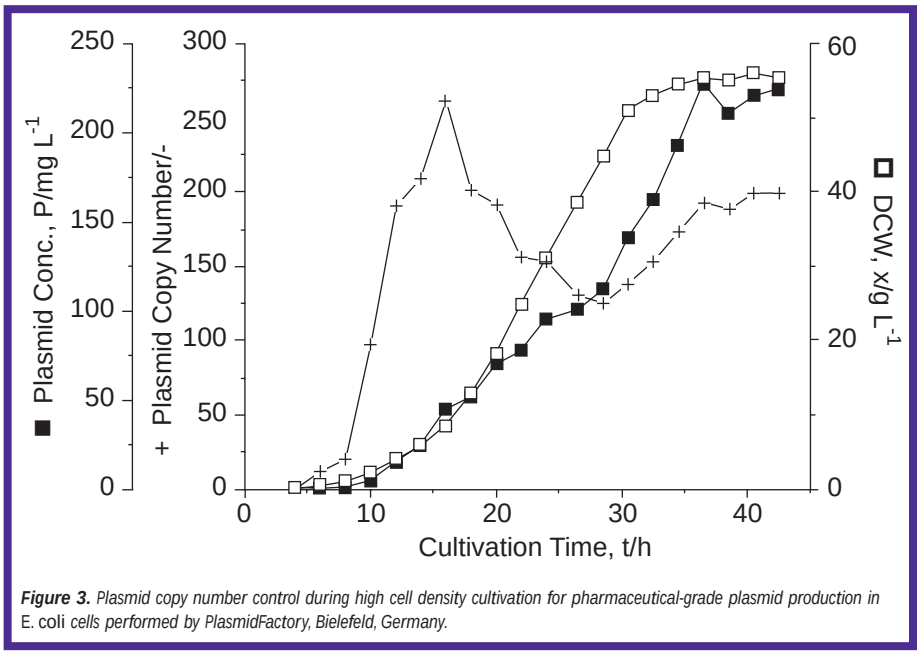


Figure 3. *Plasmid copy number control during high cell density cultivation for pharmaceutical-grade plasmid production in* E. coli *cells performed by PlasmidFactory, Bielefeld, Germany.*

Figure 2 shows the calibration curve of corrected peak area (peak area migration time ratio) versus DNA concentration of a highly purified pBR322 plasmid standard. The linear range extended over three orders of magnitude from 3 to 2000 pg μL^{-1}, respectively. Fortunately, the use of the calibration curve is independent from the size of the quantified plasmids.

The in-process control of plasmid copy number during a high-cell-density fed-batch cultivation for pharmaceutical, industrial-scale manufacturing of plasmid DNA performed by PlasmidFactory is demonstrated in

Figure 3. The fed-batch cultivation process enables high biomass yields and, thus, high product yields by controlling the nutrient level in the cultivation medium. At the beginning of the cultivation, the formation of biomass and production of the 7.6 kbp plasmid pUK21CMVβ in the *E. coli* cells does not follow synchronously. The plasmid copy number, which is calculated from plasmid concentration, plasmid size, and number of cells, varies from 10 to 250 during cultivation. Maximum plasmid copy numbers are reached at the end of the batch mode (at 15 h cultivation time) and at the beginning of the stationery phase, indicating the optimum point of time for harvesting the cells to get a maximum product yield.

Conclusions

Capillary gel electrophoresis offers fast quantitation of quickly isolated plasmid DNA resulting in an in-time method for the determination of plasmid copy number, which is an important parameter for in-process control of bacterial cultivations. The rapid determination of plasmid copy number in fewer than 30 minutes makes it possible to control process productivity and plasmid stability and to indicate the point of time for cell harvest.

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How to Bee a Protein Chemist

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Beckman Coulter, Inc.

Introduction

Complex natural protein mixtures provide a difficult analytical challenge. Capillary electrophoresis–tandem mass spectrometry (CE-MS/MS) provides a powerful tool with which to attack this problem and offers new capabilities to the scientist engaged in protein research. The identification of the protein constituents in a mixture can be performed rapidly without time-consuming protein purification steps.

In this approach, components are identified by CE-MS/MS analysis of peptides produced by partial enzymatic digestion of the entire mixture. The high resolving power of the CE system separates the peptides in as few as 20 minutes. Automated ion selection and fragmentation coupled with computer analysis of the fragment ion pattern provides user-friendly identification of components in the mixture. The speed and low reagent consumption of this method facilitates the testing of multiple buffer chemistries. Data from several different systems is presented here; each provides a different perspective on this complex mixture.

Animal venoms provide interesting model systems for studying the effectiveness of this approach. These venoms consist of multiple proteins with a variety of biological activities mixed with inactive proteins and non-protein components. The technique described here is applicable to other natural protein mixtures as well as to protein mixtures created by biotechnology. This report will describe studies with the venom of the honeybee, Apis mellifera.

The honeybee has been valued for centuries as a source of honey and beeswax. Bee products have long been used in folk medicine. In recent years, the venom of these insects has been studied as a potential source of therapeutics for the treatment of a variety of conditions, including rheumatoid arthritis, multiple sclerosis, and local inflammation. Whether or not bee venom is effective against disease, the separation scientist finds bee venom to be an easily obtained mixture of bioactive substances that serves well as a model for the analysis of other protein mixtures.

The mixture of components in bee venom, shown in Table 1, provide some challenges to the analyst. Melittin composes 65-80% of a typical bee venom preparation. The remaining components are relatively minor. Melittin and some of the other components have significant hydrophobic properties and tend to form multimers. Many of the components of bee venom have very high pI values. The larger proteins are compactly folded and not very accessible to digestive enzymes.

Table 1: Major Components of Bee Venom

Component	MW
Apamin	2089
Melittin	2848
Secapin	2867
Mast Cell Degranulating Protein	3354
Phospholipase A2	15239
Hyaluronidase	40837

Serotonin, dopamine, nor-epinephrine, and histamine are also present.

EXPERIMENTAL

Dried bee venom (Sigma, St. Louis, MO) was dissolved in water and clarified by centrifugation. Preliminary analysis of the crude venom was performed on a P/ACE™ MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA) with PDA detection or coupled to an LCQ DUO MS/MS system (Thermo-Finnigan, San Jose, CA). See the figures for details of the separations. To generate fragments, crude venom was dissolved in ammonium carbonate buffer at pH 8.3 and digested with 1% w/w trypsin without prior reduction. After

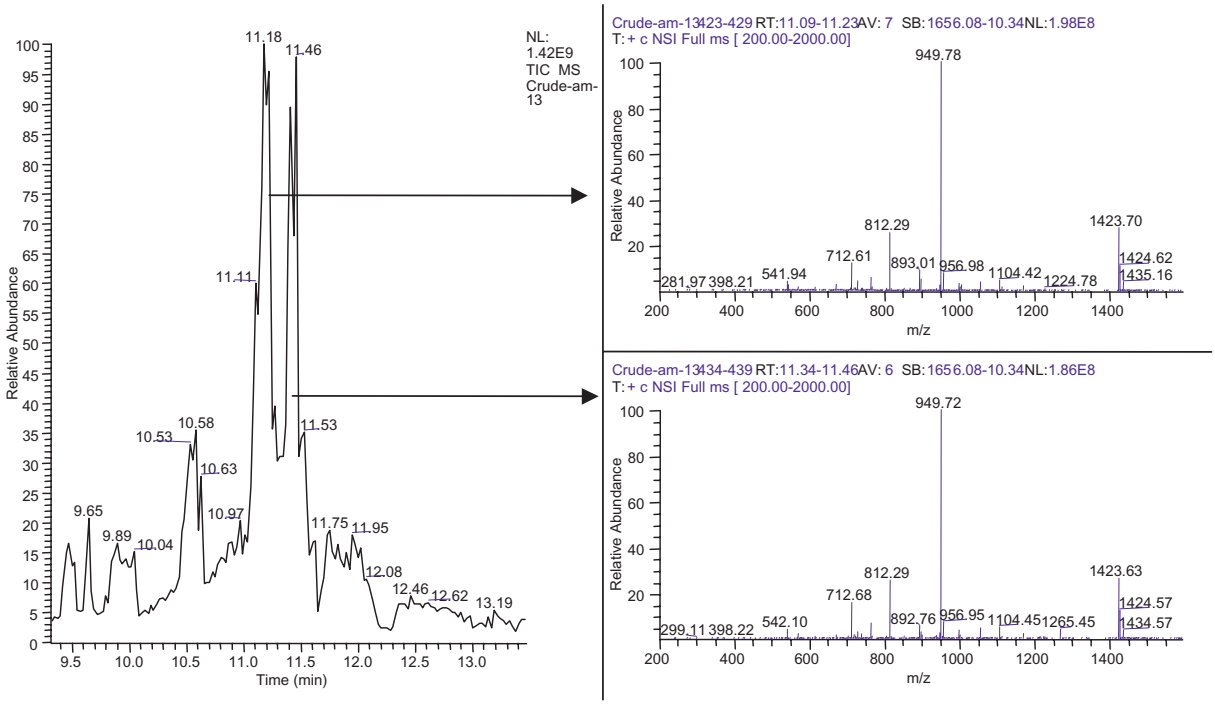

Figure 1. *CE-MS of undigested bee venom; Amine coated capillary 50 µm x 80cm Ld/Lt 85:15; 1N HOAc:Acetonitrile; 30 kV, cathode at outlet; total ion current; ion at m/z of 949.78 is triply charged.*

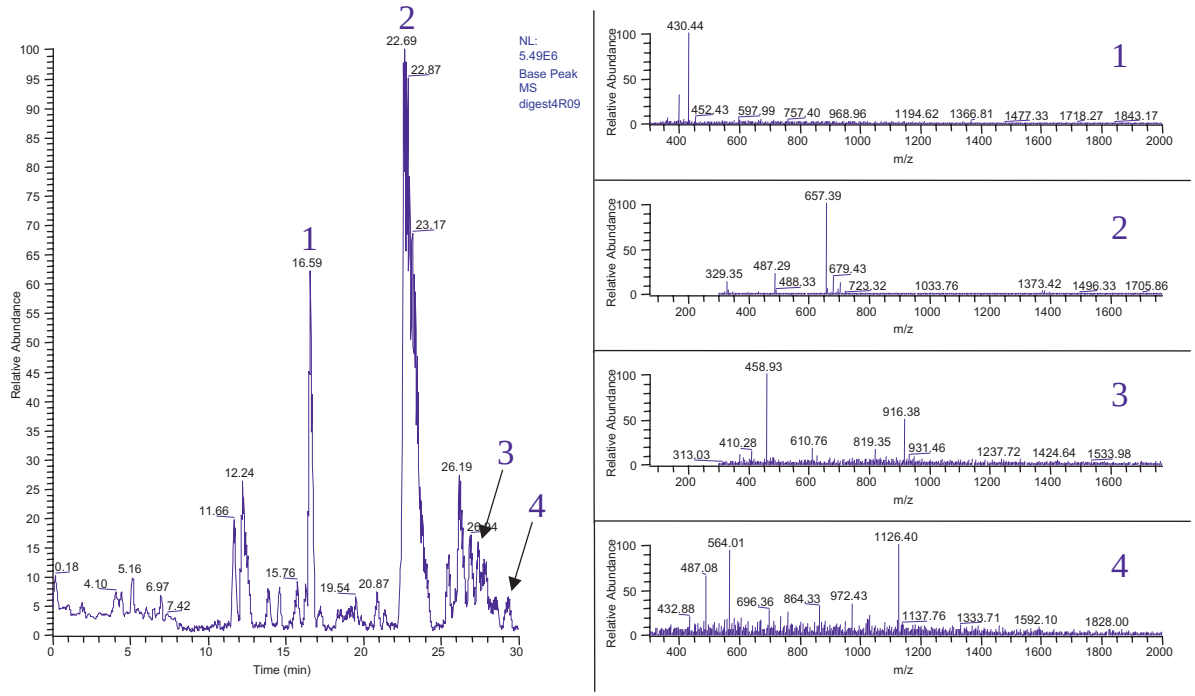

Figure 2. *Analysis of tryptic digest of bee venom. Labeled components are peptides from Phospholipase A2 (1 and 3), Melittin (2), and Secapin (4). Automated CE-MS/MS; PEO capillary 50 µm x 80 cm Ld=Lt; 1N HOAc; 30 kV, cathode at outlet; components identified by TurboSequest.*

digestion, the mixture was reacted with excess Tris (carboxyethyl) phosphine to reduce disulfide bonds, then adjusted to pH <2 with acetic acid to stop the reactions. Peptide identification was preformed with the TurboSequest software from ThermoFinnigan. CE separation conditions are shown in the figures.

Results and Discussion

Using this technique, we were able to verify the presence of the protein components listed in Table 1. Melittin is by far the dominant component and could be identified without digestion. It is known to form dimers, tetramers, and higher-order aggregates. Figure 1 indicates that it may be possible to separate these multimers by CE. In the MS, they dissociate into the monomer.

Using CE-MS/MS on the tryptic digest of bee venom results in a fairly complex profile. By using an amine-coated capillary, the complete analysis of the sample is possible in 20 minutes. The Xcalibur software allows the MS to be programmed in "data dependent triple-play" mode. In this mode, individual ion species in a peak are automatically isolated and fragmented, so that even unresolved components can be isolated. The triple-play data can be read by the TurboSequest program (ThermoFinnigan, Inc.) and the peptide fragmentation pattern matched to known proteins in a sequence database. Figure 2 shows data from such an experiment.

In addition to the known components, a peak was found in the crude venom with an m/z of 1003. This was determined to be a triply charged species. The mass of the parent molecule can be calculated to be about 3010 which does not correspond to any known venom component. Work is under way to identify this protein.

Conclusions

In this study, most of the known components of crude bee venom were identified with relatively simple techniques and without sample purification. A previously unreported protein of mass 3010 was found in this sample. CE-MS/MS combined with enzymatic fragmentation provides a simple and rapid method for the identification of proteins in complex mixtures. The technique described here does not require the purification of individual proteins prior to analysis. CE is a "protein friendly" separation technique, particularly with samples that tend to aggregate or bind to surfaces. The speed and low buffer requirements of CE simplify method development and optimization. The P/ACE™ MDQ–LCQ DUO combination provides a turnkey system approach to the analysis of complex protein mixtures.

Training Course

Quality Control Of Drugs by Capillary Electrophoresis: A Practical Approach

May 14-17, 2001
Faculty of Pharmacy
University of Montpellier

Objective

This training course aims to show through lectures, laboratory sessions, and computer simulations how it is possible to optimise the criteria required for validating an assay method for an active drug and its degradation products. Particular attention is paid to the quantitative aspects of the technique.

Public Concerned

Technical executives and technical staff of Development and Quality Control laboratories from pharmaceutical, chemical, agro-chemical, and cosmetic industries; laboratories involved in the quantitative aspects of the CE; those wishing to acquire training in specialised analytical techniques for quality control.

Location and Date

Faculté de Pharmacie, Montpellier, France, from 2pm Monday, 14th May, 2001, through 5pm Thursday, 17th May, 2001.

Programme

The programme includes:

- Principles (CZE, MECC); instrumentation; background review of applications (impurities, assay, identification, chiral separations and small ions)
- Optimisation of selectivity, repeatability, and sensitivity
- Quantitative procedures
- Method validation, method transfer
- Chiral separations
- Analysis of small ions
- Non-aqueous CE
- Microemulsion CE
- Electrochromatography

Teaching will be given as lectures, laboratory sessions, and computer simulations by Dr. K. D. Altria (GLAXOWELLCOME; Ware, UK).

To register, or for more information on this course, please contact:

Prof. H. Fabre or Dr. M. D. Blanchin
Laboratoire de Chimie Analytique - Faculté de Pharmacie - F-34060 MONTPELLIER CEDEX 2 FRANCE

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ICES—THE INTERNATIONAL COUNCIL OF ELECTROPHORESIS SOCIETIES INVITES YOU TO ATTEND THE MEETING

ICES-2001: An Electrophoretic Odyssey

GLAXOWELLCOME CONGRESS CENTER
10-14 JUNE 2001
UNDER THE AUSPICES OF THE UNIVERSITY OF VERONA (ITALY)

Dear Friends and Colleagues,

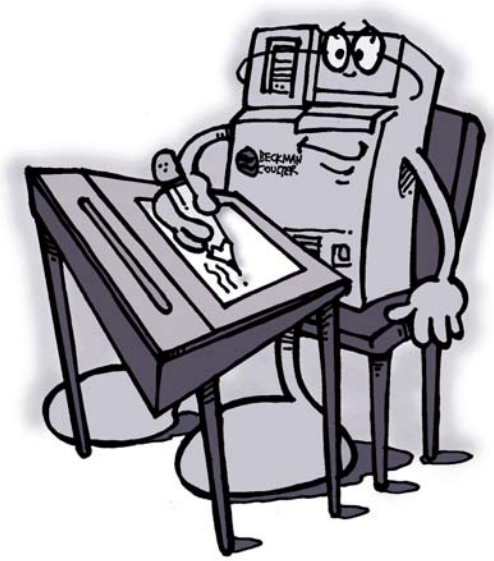
In fact Dear "Electrophoreticists" (let me use this neologism so that we can have our niche in Separation Science, just like those working in chromatography, who call themselves "Chromatographers"), on behalf of ICES (the International Council of Electrophoresis Societies), I have the pleasure of inviting you to our 10th International Congress, to be held in Verona during June 10-14, 2001. Our Congress, as you are aware, takes place every other year, the last three having been held in Paris (1995), Seattle (1999) and Tokyo (1999). It was in Tokyo that ICES selected the University of Verona as the site for the Congress of the third Millennium, a selection which, although being a big honour for our University, filled me with "Fear of Flying", i.e. of being unable to carry out such a task of great responsibility. We are doing our best for assembling a great meeting, but certainly the main contribution will come from all of you, the future participants, the Electrophoreticists who want again to meet each other, to renew old acquaintances and to make new ones, and to positively contribute to the success of this meeting by presenting your newest data in the field.

A great contribution to the present ICES meeting has already come from GlaxoWellcome who generously donated their congress facilities, a unique setting where the ICES-2001 will take place (you can have a glimpse at these facilities by browsing into our web site, although the pictures there do not render justice to the elegance and beauty of the place). Companies dealing with electrophoretic products are also generously contributing, but, again, the ultimate contribution will be your participation, which we hope will be in great numbers.

Thus, I hope you will not miss this unique opportunity of being "electrophoresed" into the third Millennium by coming to a meeting offering the most advanced topics in the field and some of the top speakers available today in the scientific arena. But let me also finish with a note of caution: if you decide to attend this meeting, be an early planner! Due to severe limitations in accommodations in town, we are strictly forced to limit attendance to 400 participants, which represents the maximum capacity of our Congress Center as well as the maximum number of rooms we could reserve in town. I hope what happened recently at the third Siena meeting on "Proteome Analysis" (a classic in the field by now) will not happen to you. Although attendance there had also been limited to 400, in the end, approximately 800 applications poured in and 400 scientists had to be left out (believe me, it was a fierce battle for the latecomers to try to win a seat in the parterre!). In addition, quite a number of the latecomers, due to hotel room limitations, had to find accommodations as far as 30 Km out of town. I would not want this to happen to you. Remember that the participants at our meeting will be a tiny minority among the visitors storming the town to enjoy this magnificent setting and to stroll along the banks of the Adige river (yes, June is a magnificent month, with the glorious light of the summer and the long, long days due to the daylight saving time schedule). Plan your trip well in advance and reserve your hotel very early in the game. I would not want you to have to spend your nights under the bridges of the Adige river (romantic, yes, but hardly pleasant).

With my best wishes,

Prof. Pier Giorgio Righetti



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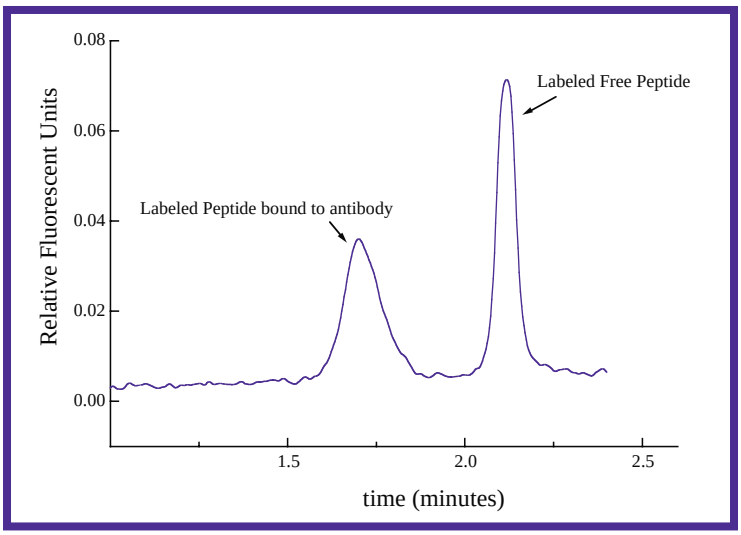
Dr. Schmerr Wins 2001 "Putting CE to Work" Award

Searching for the "Holy Grail"

The "Putting CE to Work" Award celebrates innovation in CE analysis, with the recipient being chosen by scientific peers on the merits of applying CE technology to solve key analytical challenges—essentially Putting CE to Work. This year, entries were submitted from around the world with the selection and presentation made during the 2001 P/ACE Users celebration, held in conjunction with HPCE Symposium in Boston, MA. The winning entry was submitted by Dr. Mary-Jo Schmerr from the National Animal Disease Center, Ames, IA, and was titled, "Searching for the Holy Grail." We extend our congratulations to Dr. Schmerr and invite you to consider planning your entry for next year's award.



Dr. Mary-Jo Schmerr
National Animal Disease Center

Transmissible spongiform encephalopathies are a group of fatal neurodegenerative diseases in animals and humans. The most famous member of this family is bovine spongiform encephalopathy or "mad cow disease." The oldest known member of these diseases is sheep scrapie. The causative agent of these diseases is an altered form of a normal host glycoprotein (prion protein). Most of the diagnostic tests are post-mortem tests. Tissue biopsies performed in live animals are risky, expensive, and painful. Since blood is readily available and easily obtained, a blood assay is the "Holy Grail" for testing for TSEs.

The electropherogram shown represents a competition immunoassay for the presence of the abnormal prion protein in a blood sample from a scrapie-infected sheep. We used 0.2 M Tricine, pH 8.0, containing the additives 0.1% N-octyl glucoside and 0.1% BSA as our buffer. The additives prevented the sample from binding to the capillary walls. Uncoated glass capillaries, 20 µm I.D. x 20 cm to the detector, were used for the analysis. The voltage was 26 kV with a current of ~12 µA. Samples were stored at 7°C until they were run. The running temperature of the capillary was 20°C. The analysis was done on a P/ACE™ MDQ using LIF for detection.

A peptide from prion protein, amino acid positions 218-232 was labeled during synthesis through a gamma butyric acid bridge with fluorescein. Theoretically, each peptide was labeled with the dye. Rabbit antibodies to this peptide were purified over a column that had the corresponding peptide linked to the matrix. A single peak was obtained when the peptide was run alone. The affinity purified antibody was titrated with the peptide so that the ratio of the peaks of the bound peptide to the free peptide would be ~1. The presence of the prion protein would displace the fluorescein–peptide from the antibody resulting in a lower signal for the immunocomplex peak and an increase in the free peptide peak. After testing several normal animals, the "cutoff value" was determined as 70% of the antibody control. The ratio of the peaks on the electropherogram is 0.47 and, thus, a positive sample. This sheep was clinically normal when the test was performed but died of clinical scrapie approximately 14 months later. We have determined that the amount of abnormal prion protein in 10 mL of blood ranges from 2-25 pg. This combination of the extraction protocol for the abnormal prion protein and the sensitivity of CE-LIF system makes a test using blood possible for the TSEs. Thus, the "Holy Grail" is within reach.

HYPHENATIONS

HARRY WHATLEY

Optimizing Sample Introduction Conditions in CE-MS/MS

Sample introduction into the separation capillary for CE-MS/MS is performed by positive pressure displacement or by electrokinesis. In most CE-MS systems, sample is introduced while the MS system is in a "standby" mode, with the start of MS data acquisition being triggered by the start of the CE separation step.

It is the "standby" status of the MS system that concerns us here. In many systems, the spray voltage, sheath gas, and sheath liquid are left on during the sample introduction step, unfortunately resulting in poor reproducibility. The impact of this effect may range from unexpectedly increased or decreased quantities loaded to the complete loss of sample from the capillary.

Effect of Sheath Gas

Consider the sheath gas. Depending on the capillary position in the spray needle and the velocity of the sheath gas, a venturi effect can develop. The moving gas stream creates an area of low pressure at the capillary tip. Because pressure injection depends on a pressure difference between the two ends of the capillary, a diminished pressure at the outlet end will cause the sample volume introduced to be greater than expected. In extreme cases, the shape of the sample plug may be distorted by laminar flow, or air may be drawn into the capillary as the inlet end is moved from vial to vial.

Effect of Spray Voltage

Spray voltage is somewhat more complex. This is the voltage applied between the sprayer tip and the heated capillary in the MS. Because the CE and the MS are at a common ground potential, the spray voltage and the separation voltage can influence each other. The capillary voltage is approximately the algebraic sum of the applied capillary voltage and the spray voltage. This can be verified by noting the change in the CE capillary current as the spray voltage is changed. In separation mode, the applied capillary voltage is usually much greater than the spray voltage. During sample introduction, however, the presence of voltage on the sprayer tip can result in a significant current flow down the CE capillary. Depending on the nature of the sample, this current can either reduce or enhance the amount of sample loaded. Under the right conditions, the sample may even back-migrate out the end of the capillary during the few seconds when no pressure or voltage is being applied to the CE capillary.

Our Recommendation

Turn off the spray voltage and the sheath gas during the sample introduction step. Fortunately, the LCQ series mass spectrometers can be programmed to turn off the offending elements until the CE separation has begun. This is accomplished by using multiple tune files in the acquisition method. First, the MS system is tuned as usual to maximize sensitivity to the ions of interest. After saving the tune file, it is modified. Spray voltage and/or sheath gas are set to zero. The file is saved with a new name. The Xcalibur software allows the user to program multiple segments for MS acquisition, and each segment can have a different tune file. The spray conditions during the "standby" phase will be those defined in the tune file of Segment 1. By creating a short Segment 1 with our "zero volts-zero gas" tune file, those factors will not be present during sample introduction. A length of 15 to 30 seconds is sufficient for this segment, which only needs to be longer than the time programmed for the ramp-up of the separation voltage (usually ten seconds). No ions will be produced in this segment, but no sample is expected to travel the length of the capillary in so short a time.

Effect of Sheath Liquid

Sheath liquid flow is not likely to create a problem with sample introduction. And since the sheath liquid provides the electrical connection for the outlet end of the CE capillary, we advise you to keep the sheath liquid flowing at a low rate (2-5 µL/min) during sample introduction.

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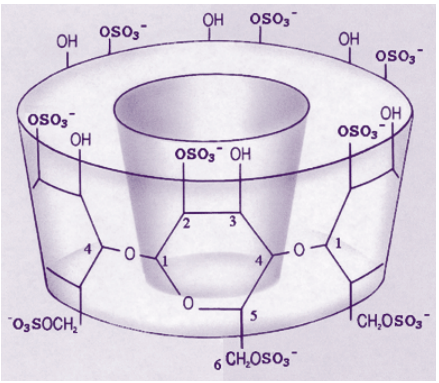

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713351	**HS γ Cyclodextrin (100 mL) 20% w/v**
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