BECKMAN COULTER

P/ACE Setter

The worldwide newsletter for capillary electrophoresis

Volume 4, Issue 1 • June 2000

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The worldwide newsletter for capillary electrophoresis

Advancing Atherogenesis Research: High-Resolution Analysis of Oxidized Low-Density Lipoproteins

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Polyunsaturated fatty acids are known to alter the composition of lipoproteins, rendering them more susceptible to oxidation. Recent data[1] suggest that subfractions of lipoprotein classes including modified oxidized low-density lipoproteins (OxLDL) are responsible for an increased lipid accumulation in the vascular wall, contributing to plaque formation – a pathological process known as atherogenesis. The various steps in oxidative modification of LDL can be monitored using different methodologies with varying degrees of complexity (conjugated dienes; lipid peroxides; thiobarbituric acid-reactive substances, or TBARS; loss of polyunsaturated fatty acids; relative electrophoretic mobility on agarose gel; or apo B-100 fluorescence). However, most of these methods show little sensitivity and/or specificity.

In this study, we propose capillary isotachophoresis (CITP) as a sensitive, automated method to detect and measure oxidized LDL. To underline the inherent advantages of this approach, we compare CITP with classical lipoprotein electrophoresis using agarose gels.

Instrumentation

Capillary electrophoresis experimentation was carried out using a P/ACE™ 5500 System from Beckman Coulter (Fullerton, USA). Bare, fused-silica capillary (internal diameter 180 µm) was purchased from Alltech (Deerfield, USA), a Model L-60 ultracentrifuge with a Ti 50.2 rotor

from Beckman Coulter (Munich, Germany), and an electrophoresis chamber from Helena Laboratories (Beaumont, Texas) were all used in our experimentation.

Methods

LDL Isolation and Oxidation

LDL was isolated from pooled human serum of healthy volunteers in a density range between 1.019 and 1.063 g/ml by a sequential ultracentrifugation procedure. After isolation, LDL was aliquoted into nine samples. Oxidation of LDL was performed at 37°C in a shaking water bath by dialyzing 8 aliquots against oxygenated PBS containing 5 µm $CuSO_4$ as a catalyst. Oxidation was stopped after 2, 4, 6, 8, 10, 12, 15, and 24 hours.

One aliquot was not oxidized and used as control. Native LDL and the oxidized LDL samples were dialyzed (3 x 12 hours) against 100-fold volume of degassed PBS at 4°C to remove $CuSO_4$.

Capillary Isotachophoresis

Native and oxidized LDL fractions (100 µl of each) were incubated with 50 µl of a Sudan Black B solution (1% - solution Sudan Black B in ethylene glycol [w/v]) for one hour at 5°C. Prestained samples (100 µl) were mixed with 30 µl spacer solution (TAPSO, BICINE, CHES, ASP [each in the concentration 10 mg/ml, Ser [12,5 mg/ml], Ala-Gly, Val-Gly, EPPS, Gly-His, Gln, AMPSO [25 mg/ml], and 400 µl cellulose containing leading electrolyte for a better separation of the lipoprotein subfractions.

A fused-silica capillary (180 µm i.d., length: 37 cm, C1-coated) was placed in the capillary electrophoresis system by connecting the inlet to the cathode and the outlet to the anode. The capillary was filled

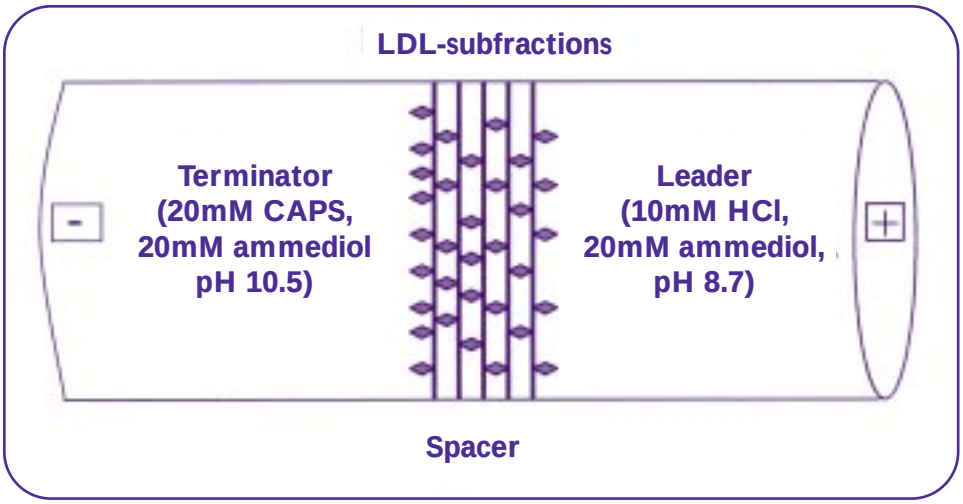

Figure 1. Mechanism of capillary isotachophoresis.

with the cellulose containing leading electrolyte (10 mM HCl, 20 mM ammediol, 0.25% cellulose, w/v, Figure 1). After a 30-second pressure injection (0.5 psi) of the prestained samples, the lipoproteins were separated using the terminating electrolyte (20 mM CAPS with 20 mM ammediol, pH 10.5). The applied voltage was 18 kV.

Electrophoresis on Agarose Gel

Agarose gel electrophoresis was performed as described by the manufacturer's instructions (25 minutes, 90 volts). The separated LDL bands were visualized by cholesterol-specific staining.

Results

Capillary Isotachophoresis

Native LDL was separated into six subfractions (Figure 2). Oxidation of isolated LDL resulted in (i) changes in the lipid contents of the separated six LDL subfractions (oxidation grades 2-24 hours) with increased leading LDL peaks and decreased terminating LDL peaks, and (ii) an increased migration time of LDL subfractions (oxidation grades 4-24 hours, Figures 3 and 4).

Electrophoresis on Agarose gel

The increase in the negative charge of OxLDL was visible on the agarose gel after an oxidation time of four hours. This increase in electrophoretic mobility continued to an oxidation time of 24 hours (Figure 5).

Discussion

With agarose gel electrophoresis, the presence of oxidized LDL is identified by an increased migration time of LDL fraction. Unfortunately, the separation of LDL into subfractions is not possible using this method. Schmitz, *et al.*, developed a capillary isotachophoresis (CITP) procedure for the effective separation of lipoproteins into subfractions, employing Sudan Black B as a marker for lipids.[2-4] We extended this work by combining the separation of lipoprotein subfractions by capillary isotachophoresis with specific enzymatic derivatization of cholesterol and triglycerides.[5] Here we show that the oxidized LDL subfractions can be detected and measured by capillary isotachophoresis.

We demonstrate the CITP method to be highly sensitive, as changes in peak area of the separated LDL subfractions were detected after only two hours of oxidation. The leading LDL peaks increased, while the terminating LDL peaks decreased in paral-

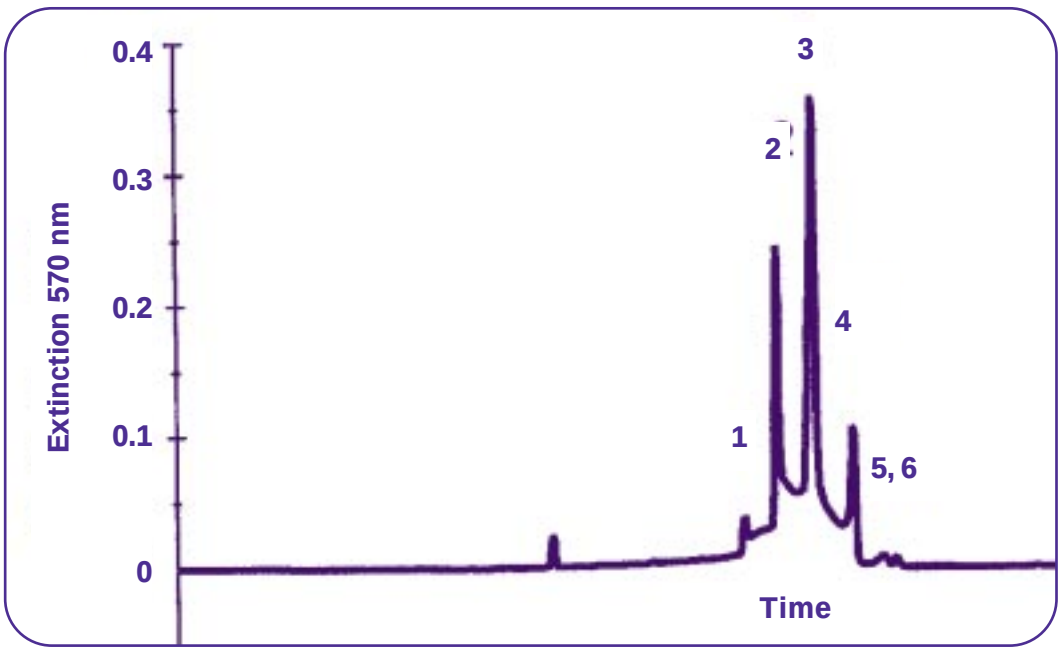

Figure 2: Capillary isotachophoretic pattern of isolated native LDL with the density 1.063 ≤ d ≤ 1.21 g/ml.

lel throughout the duration of oxidation. The LDL samples, oxidized for 4-24 hours, also exhibited an increased migration time of the fractions. We suggest that the changes in lipid content of the separated LDL subfractions are due to small modifications of apolipoprotein lysine, hystidine, and proline residues. We propose the CITP technique as a sensitive tool to investigate the changes of LDL occuring *in vivo*.

In summary, our results show that CITP is a useful tool to detect the oxidation grade of LDL regarding the lipid pattern and the electrophoretic mobility of the separated subfractions.

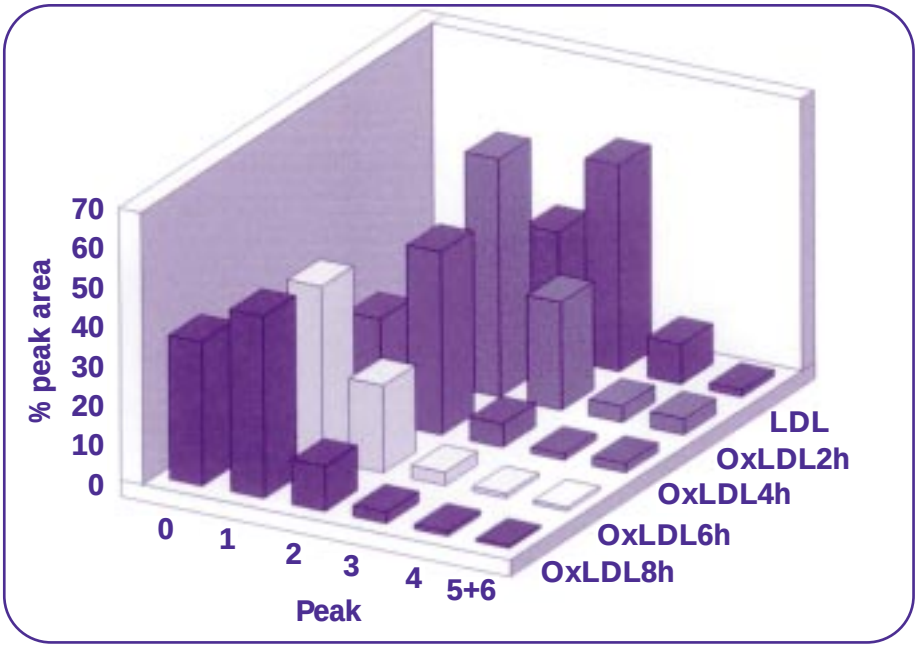

Figure 3: Capillary isotachophoretic changes of percentage peak area of isolated LDL during the oxidation process, x-axis: LDL-subfractions 1,2,3,4,5+6,0=all peaks migrating in front of LDL subfraction number 1, y-axis: percentage area of the peaks, z-axis: oxidation grade of LDL: native LDL, OxLDL 2 h, OxLDL 4 h, OxLDL 6 h, OxLDL 8 h.

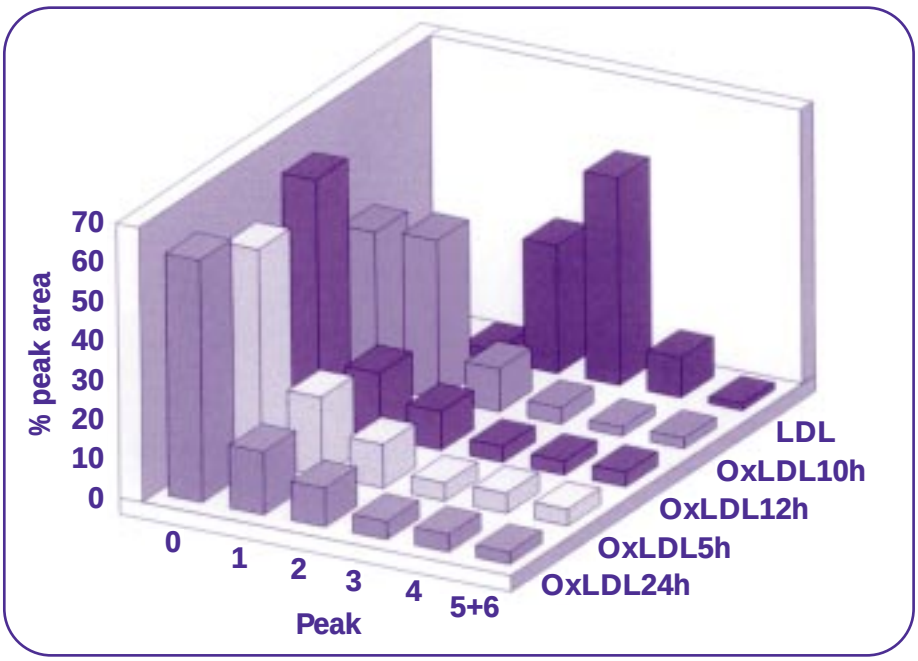

Figure 4: Capillary isotachophoretic changes of percentage peak area of isolated LDL during the oxidation process, x-axis: LDL-subfractions 1,2,3,4,5+6, 0=all peaks migrating in front of LDL subfraction number 1, y-axis: percentage area of the peaks, z-axis: oxidation grade of LDL: native LDL, OxLDL 10 h, OxLDL 12 h, OxLDL 15 h, OxLDL 24 h.

REFERENCES

1. Witztum, J. L., Steinberg, D., J Clin *Invest* 1991, *88*, 1785-92
2. Schmitz, G., Möllers, C., *Electrophoresis* 1994, *15*, 31-39
3. Schmitz, G., Borgmann, U., Assmann, G., *J. Chromatogr.* 1985, *320*, 253-262
4. Nowicka, G., Schmitz, G., Klin. *Wochenschr.* 1990, *68*, 119-120
5. Zorn, U., Wolf, Ch.-F., Wennauer, R., Bachem, M. G., Grünert, A., *Electrophoresis* 1999, *20*, 1619-1626

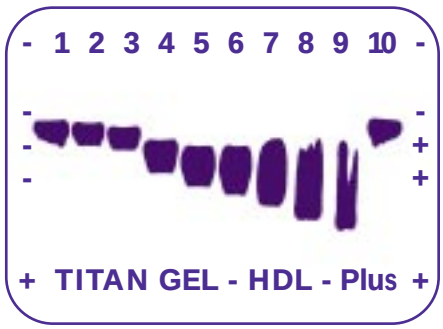

Figure 5: Agarose gel electrophoresis of isolated native LDL (lane 1 and 10) and LDL oxidized for 2 (lane 2), 4 (lane 3), 6 (lane 4), 8 (lane 5), 10 (lane 6), 12 (lane 7), 15 (lane 8), and 24 hours (lane 9).

Using Binding Isotherms to Accelerate CE Methods Development: Part II – Complex Systems

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In part I of this article[5] we defined binding isotherms and described how they are used in our lab to accelerate CE methods development. The examples that we gave focused on a single additive system. However, these same properties can be applied to more complex systems where multiple additives are used.

The use of individual capacity factors allows for the quantitative prediction of the analyte migration behavior when there is more than one additive in a CE system. When an analyte interacts with two additives with 1:1 stoichiometry, the equilibrium can be described as:

$$2A + C + D \Leftrightarrow AC + AD \qquad (1)$$

where A is the analyte, C and D are the additives, and AC, AD are the complexes. The analyte species present in this equilibrium are the free analyte (A) and each of the analyte-additive complexes (i.e., AC, AD). The capacity factors for species A is 1 while the AC complex is K_{AC} [C] and the k' for species AD is K_{AD}[D].

The net analyte mobility in this case is described by:

$$v\mu_{ep}^{A} = \frac{k'_A \mu_{ep,A}}{k'_A + k'_{AC} + k'_{AD}} + \frac{k'_{AC} \mu_{ep,AC}}{k'_A + k'_{AC} + k'_{AD}} + \frac{k'_{AD} \mu_{ep,AD}}{k'_A + k'_{AC} + k'_{AD}} \qquad (2)$$

The capacity factor for a particular species is independent of any other equilibrium in the system. Therefore, the capacity factors in Eq. 2 can be measured individually using one additive at a time if there is no interaction between different additives. Methods for detecting interactions between additives and the effect of such interactions on the binding isotherms are discussed in another paper[1].

In multiple additive CE, the net analyte migration behavior can be described by multivariate binding isotherms. An example of such isotherms for a two-additive system is shown in Figure 1. This figure shows the simulated effect of two additives, β-cyclodextrin (β-CD) and HP-β-CD, on the mobility of three analytes, phenol, p-nitrophenolate, and benzoate in 160 mM borate buffer at pH 9.1. The net electrophoretic mobility values are calculated from Eq. 2. The x-z plane of Fig. 1 corresponds to the corrected electrophoretic mobility values, $v\mu_{ep}^{A}$, of the analytes at different β-CD concentrations while HP-β-CD is absent, and the y-z plane shows the $v\mu_{ep}^{A}$ values at different HP-β-CD concentrations while there is no β-CD. The surfaces in Fig. 1 are the $v\mu_{ep}^{A}$ values calculated for various combinations of the concentrations of the two additives.

Rotating Fig. 1 so that the x-z plane is facing the reader, as shown in Fig. 2(a), the range of the $v\mu_{ep}^{A}$ values that can be achieved at each β-CD concentration by varying the concentration of HP-β-CD for each analyte is demonstrated by each projected surface. Similarly by rotating Fig. 1 so that the y-z plane is facing the reader, the ranges of values that can be achieved at each HP-β-CD concentration, by varying the β-CD concentration, are shown by the projected $v\mu_{ep}^{A}$ surfaces in Fig. 2(b). The projected surfaces show the obtainable analyte mobilities at any concentration of one additive by changing the concentration of the other.

It was noted in Fig. 2(b) that at a certain HP-β-CD concentration, there is a twist in the mobility surface where the net electrophoretic mobility of p-nitrophenolate stays the same regardless of the amount of β-CD added to the system. The concentration at the inversion point (line) is called the dengsu additive concentration (DAC) for an analyte and a certain pair of additives ("Dengsu" means "same speed" in Chinese). The conditions for a valid

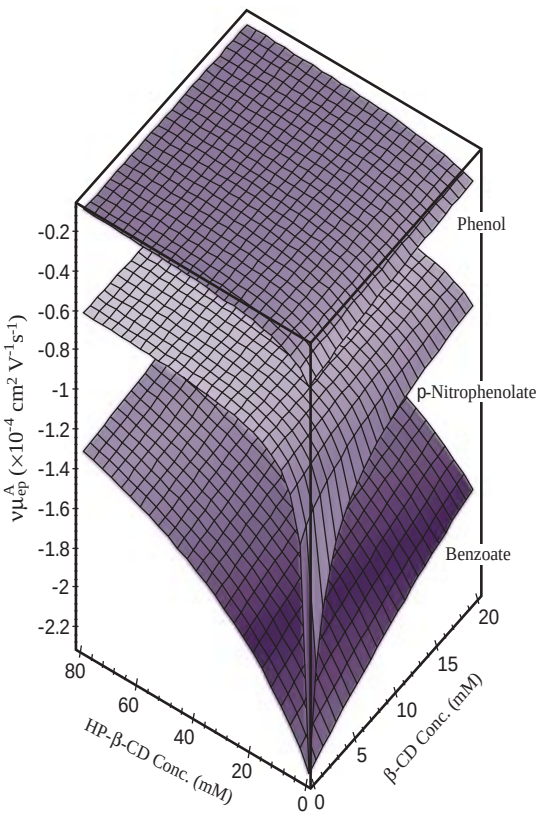

Figure 1. Effects of two additives on the electrophoretic mobilities of the analytes. The effect of b-CD and HP-b-CD concentrations on the net electrophoretic mobilities of the analytes.

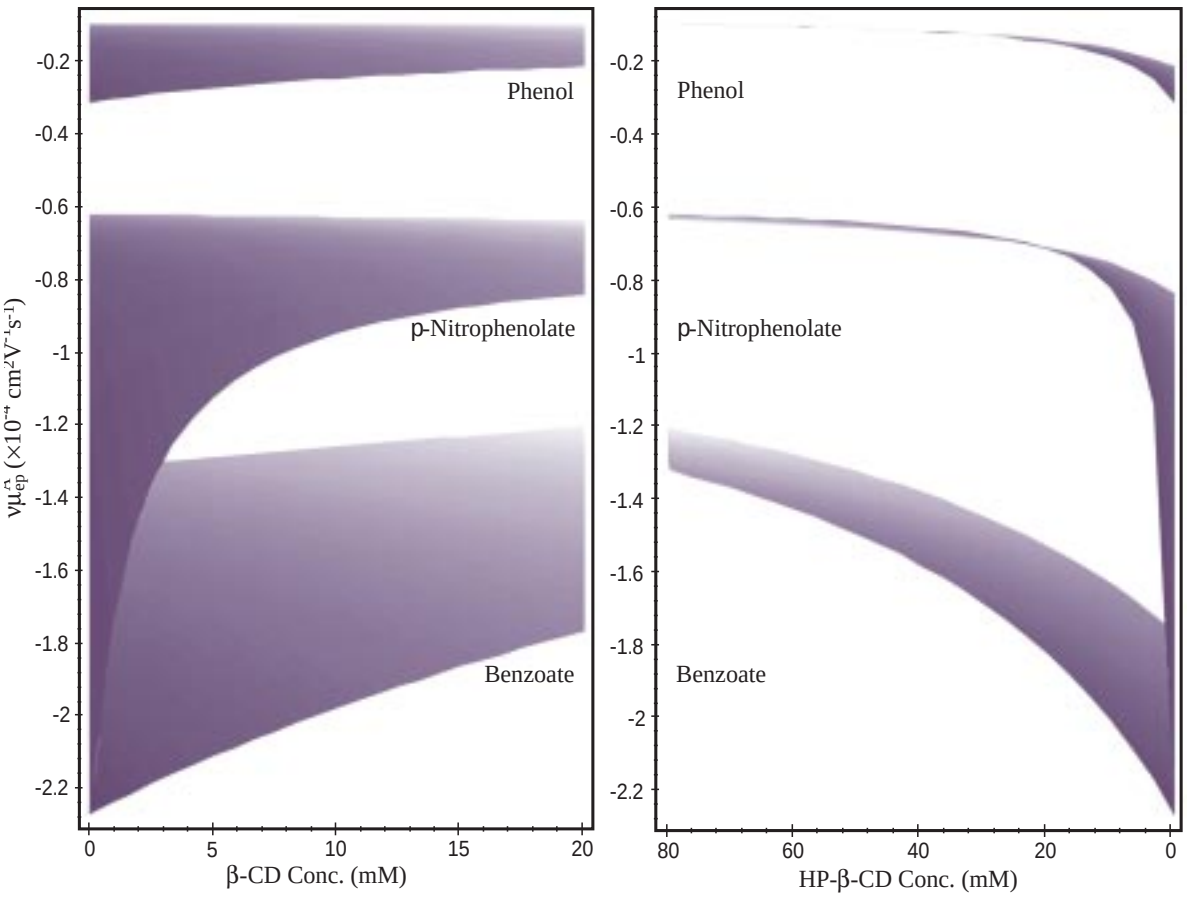

Figure 2. (a) and (b): Projected vμAep planes of the analytes when Figure 1(a) is rotated so that x-z or y-z plane is facing the reader. Reprinted with modification from Peng et al., Electrophoresis, 1997, 18, 706-716, with kind permission from Wiley-VCH Verlag Gmbh.

DAC to exist, and the properties of the dengsu point are discussed in detail in previously published papers[1,2]. Because this inflection point is a general phenomenon in multiple equilibria systems, applications of this unique property are not limited to separation science. Another interesting phenomenon for multiple additive CE is that the variability of the net analyte mobility depends upon which additive concentration is kept constant and which is varied. In Figures 2(a) and 2(b), it is evident that the analyte mobility can be varied more effectively by fixing the β-CD concentration and varying the HP-β-CD concentration.

Concluding Remarks

Binding isotherms can be used to visualize how additives affect the mobility of an analyte, and how individual capacity factors can be used to derive equations that describe analyte mobility in CE systems with any number of equilibria. The importance of describing such complicated interactions and the ability to predict the effects of these interactions on analyte mobilities are valuable to study the binding of biological molecules. Equilibria involving biomolecules are often complex. Several molecules can compete for a single binding site, or the binding of several molecules may be required to induce the biological activity. More details on understanding these complex situations can be found in other papers published by our group[1,3,4].

References

1. Bowser, M.T.; Kranack, A. R.; Chen, D. D.Y. *Anal. Chem.* 1998, *70*, 1076-1084.
2. Peng, X.; Bowser, M.T.; Britz-McKibbin, P.; Bebault, G. M.; Morris, J.; Chen, D. D.Y. *Electrophoresis* 1997, *18*, 706-716.
3. Kranack, A. R.; Bowser, M.T.; Britz-McKibbon, P.; Chen, D. D.Y. *Electrophoresis* 1998, *19*, 388-396.
4. Bowser, M.T.; Chen, D. D.Y. *Anal. Chem.* 1998, *70*, 3261-3270.
5. Chen, D.Y., *P/ACE Setter*, Beckman Coulter, Inc, Vol. 3, Issue 3, 1999.

Pharmacokinetics Profiling of New Drug Candidates: A Key process in Drug Discovery

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Nortran Pharmaceuticals, Inc.

One of the challenges of Drug Discovery is developing analytical methods for the pharmacokinetics (PK) profiling of new drug candidates. Important to this process is the development of rapid, generic methods that allow the screening of large numbers of compounds isolated from complex sample matrices. Capillary electrophoresis (CE) is being used routinely in our Lab to quantify drugs in blood plasma, the brain and the heart. The high efficiency and the lack of interference from the matrix makes CE a fast and easy analytical tool for PK screening.

Sample preparation includes the precipitation of proteins with trichloroacetic acid, basification with sodium hydroxide, followed by the liquid-liquid extraction with ether. CE conditions are basically the same (adjustment of the voltage may be necessary) for all compounds of Nortran's antiarrhythmic amine series. The following method is typically used:

INSTRUMENT: P/ACE™ MDQ–Beckman Coulter, Inc.
BACKGROUND ELECTROLYTE:
100 mM phosphate buffer, pH 2.5
SEPARATION VOLTAGE: 25 KV
CARTRIDGE TEMPERATURE: 20° C
CAPILLARY: 60(50) cm, 75 µm ID, uncoated
INJECTION: 1 psi, 10 seconds

We find the choice of internal standards to be quite straightforward, as most basic drugs can be easily resolved using this method. In our experience, only two different internal standards have been required for the quantitation of more than 40 of our drug candidates. The biological matrix poses few problems, the major one being a faster deterioration of the capillary surface compared to non-biological samples. No interference peaks were observed in the analysis of rat, dog, monkey and human plasma, rat brain and rat heart tissue. Figures 1 and 2 show representative electropherograms of rat plasma and rat brain extracts.

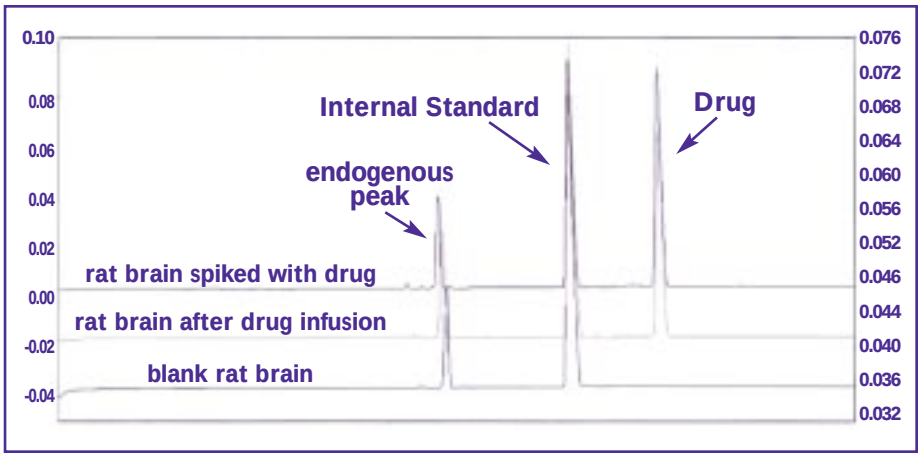


Figure 1. Electropherograms of extracted rat plasma samples (shown offset). CE conditions: see text.

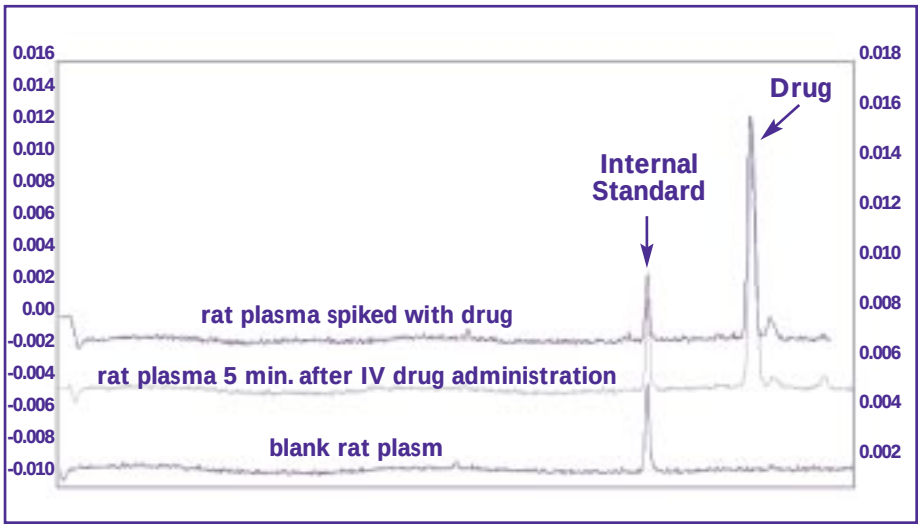
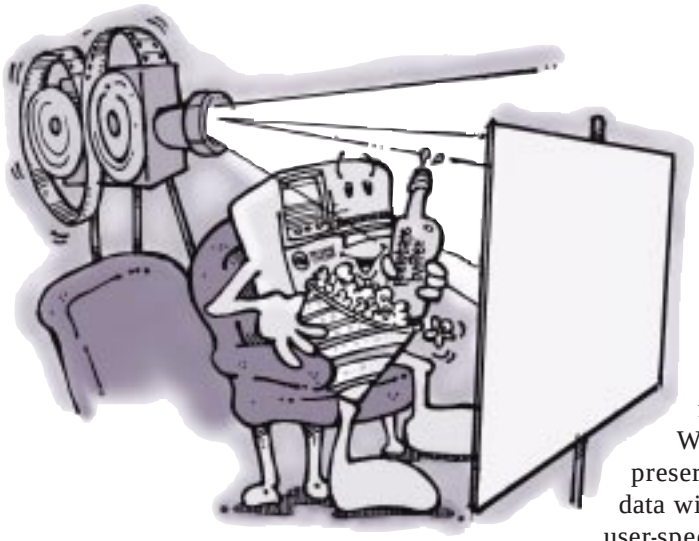


Figure 2. Electropherograms of extracted rat brain tissue (shown offset). CE conditions: see text.

We currently use this method for the analysis of drugs for PK profiling, bioavailability determinations, plasma protein binding, drug levels at activity (plasma, heart), and drug levels at convulsion (plasma, brain). Our CE instruments practically run 24 hours per day, seven days a week – generating a significant amount of data for our organization. The Limits of Quantitation (LOQ) we have obtained are as low as 100 ng/ml, which seems to be satisfactory for our purposes. The major advantages of this technique have been fast and transferable method development (one fits all), good accuracy and precision, rapid analysis times, low cost and less waste.

New Product Preview



Now previewing at a trade show near you are two new CE-based products from Beckman Coulter. These previews offer you a look at what will be available to you this summer. Both products are planned to be available for purchase July 14, 2000, with product shipments scheduled for August—pricing, part numbers and greater detail will be available at that time. Please contact our CE home page at **www.beckmancoulter.com/cenews** to keep up to date on these exciting new products.

32 Karat™ Software for P/ACE™ MDQ Capillary Electrophoresis (CE) Systems has been previewed at both HPCE 2000 and the Pittsburgh Conference. Designed to address the demands of the biotechnology and pharmaceutical industry, this new platform will help you comply with FDA regulations such as 21 CFR Part 11, concerning electronic data records and signatures. 32 Karat for CE will also provide powerful new capabilities for analyte identification.

This new software is an extension of the 32-Karat platform that Beckman Coulter first introduced for HPLC early last year. The 32 Karat platform, developed entirely in 32-bit code, fully utilizes the functionality of WindowsNT*, and preserves the integrity of data with features such as user-specific log-in, independent audit trails and results sign-off.

In addition to extending the 32-Karat platform to CE, this software adds new analysis dimensions that significantly increase the accuracy of compound identification. New peak identification algorithms allow the P/ACE MDQ to combine an analyte's mobility with spectral information for accurate identification against a large database of drugs. These capabilities will extend the applicability of CE into routine drug screening applications. Mobility-based analysis will improve the robustness of CE assays, making it easier to put CE to work in a regulated environment. This software will be available this summer – including a special program to assist you to migrate from your current software platform.

The P/ACE MDQ - Finnigan LCQ*DUO CE-MS/MS System, a result of a collaboration with ThermoQuest Corporation, was also previewed at both HPCE 2000 and Pittsburgh Conference. Developed for both the analysis of basic (amine containing) pharmaceuticals and the characterization of proteins, this new application system combines the separation advantages of capillary electrophoresis (CE) with versatile mass spectrometry detection. Xcalibur* software from ThermoQuest (Operating on the Windows* NT platform) will integrate and control both the CE and MS systems. This system will utilize electrospray ionization and ion trap MS/MS technology. Both Beckman Coulter and ThermoQuest will work together to provide installation, customer training and service.

Beckman Coulter has led the CE field since the technique was commercialized, and ThermoQuest offers the best ion trap mass spectrometry technology. We believe this collaboration brings a valuable combination of technologies to you. Once again, this product will be available for sale July 14, 2000.

*A special note — the Editors at Pittcon gave the P/ACE MDQ-Finnigan LCQ*DUO CE-MS/MS system the Silver Award for the Best New Product shown. A very good start to a very exciting new product.*

HPCE 2000 – Saarbrücken, Germany

The 13th International Symposium on High Performance Capillary Electrophoresis and Related Microscale Techniques, held in February in Saarbrücken, Germany, was a mix of new invention and applied technology—providing literally something for everyone. Although the setting did not boast a tropical climate, this charming city on the border of Germany and France was host to a very successful meeting on capillary electrophoresis technology.

The program unofficially began with five workshops: Microfabricated Fluidic Devices, Capillary Electrochromatography (CEC), Troubleshooting in CE, CE in Biotechnology, and Proteomics. The meeting was opened in high ceremonial fashion by Professor Heinz Engelhardt of Saarland University on Monday, Feb. 20, and was closed on Thursday, Feb. 24, with an invitation to HPCE 2001, which will be held in Boston, Mass. (USA), Jan. 13-18.

As the acknowledged leader in capillary electrophoresis, Beckman Coulter takes a strong interest in this meeting, with primarily three main objectives in mind: 1) to support the scientific exchange of CE information, 2) to present our latest enhancements to the P/ACE™ product line, 3) to thank our customers for their support of CE technology.

Exchange of Scientific Information

The most important goal of the HPCE meeting is the exchange of scientific information. To promote this exchange, Beckman Coulter once again invested in being a major supporter of this conference. This included providing the largest display at the exhibition with active instrument and software demonstrations, and participation in the scientific program with the following presentations. If you wish to review the presentations, copies may be downloaded from the internet at **www.beckmancoulter.com/cenews**.

Industrial Seminars

1) **Applying CE Technology—Part I: Successful Implementation**
Jeff Chapman, Strategic Marketing Manager, CE Technology—Beckman Coulter, Inc., Fullerton, CA
2) **Applying CE Technology—Part II: Validating and Transferring CE methods**
Joel Aliphon, CE Product Marketing Manager, North America Operations—Beckman Coulter, Inc., Fullerton, CA

Session Lectures

1) Applying Capillary Electrophoresis in Routine Clinical Analysis—Cynthia R. Blessum, Beckman Coulter, Inc., Brea, CA
2) Particle-Loaded Monolithic Sol-Gel Columns for Capillary Electrochromatography. Characterization of the Supporting Gel and Its Influence Upon Physical and Electrochromatographic Properties—Michael P. Henry, Beckman Coulter, Inc., Fullerton, CA

Poster Session

1) On the Interaction of Enantiomers of Alpha-Amino Acids and Derivatives with Highly Sulfated Cyclodextrins—Albert Fu-Tai Chen, Beckman Coulter, Inc., Fullerton, CA
2) Particle-Loaded Monolithic Sol-Gel columns for Capillary Electrochromatography. Characterization of the Monolith and its Physical and Electrochromatographic Properties, C. K. Ratnayake, C. S. Oh, M. P. Henry, Beckman Coulter, Inc., Fullerton, CA

New Product Previews

For the 11th year in a row, Beckman Coulter was proud to showcase new CE-based products at HPCE—an example of our continued commitment toward CE technology. This year, two new major products were previewed.

1) P/ACE MDQ–Finnigan LCQ*DUO CE-MS/MS System—an integrated solution for the characterization of proteins and the analysis of pharmaceuticals. This new system optimizes the interface of capillary electrophoresis with an ion-trap mass spectrometer capable of MS/MS experimentation. This new product is a result of a collaboration between Beckman Coulter and ThermoQuest Corporation.
2) 32 Karat™ Software for P/ACE MDQ—a new fully 32-bit software operating on the Windows* NT platform, designed to help users comply with FDA regulations such as 21 CFR part 11, concerning electronic data records and signatures. 32 Karat software for CE will also provide powerful new capabilities for analyte identification.

P/ACE Users Celebration

Beckman Coulter hosted the annual P/ACE users appreciation event. More than 200 P/ACE users attending HPCE were whisked away by motorcoach across the German border into Forbach, France, to enjoy an evening of jazz, casino games, gourmet cuisine, and to act as reviewers in the "Putting CE to Work" contest. As an update to the CEparation of the Year award, this year's contest focused on separations that were judged for merit in applying CE technology—in other words. "Putting CE to Work." The winning separation was selected from more than 21 entries and was presented to Dr. Philippe Schmitt-Kopplin, GSF—National Research Center for Environment and Health—Attaching/Fresing, Germany. The title of the winning entry was "Putting CE to Work in a Chromatographer's World." Although only one award was given, all posters were winners, clearly identifying ways in which CE technology is being effectively applied.

Congratulations to all who participated, as all the entries were of exceptionally high quality. Next year's "Putting CE to Work" award will be held in Boston.

We encourage all of you who wish to submit an entry to plan ahead, as the Putting CE to Work Award offers much distinction and notoriety—and, well, there is usually a good prize, too!

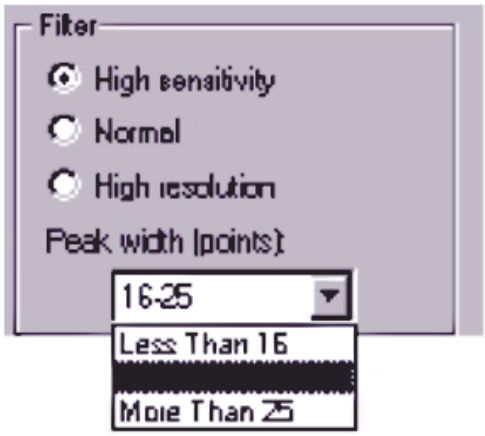
Approximately 200 attended the two workshops (opposite page) held by Beckman Coulter. Collage (above) shows Beckman Coulter's exhibit graphics; a view of the historic Johanneskirche in Saarbrücken; casino games at the users meeting at the Burghoff Congress Center (Forbach Castle) in France; and the presentation of the "Putting CE to Work Award" to Dr. Philippe Schmitt-Kopplin, GSF.

Tips on Optimizing Signal Conditioning

By its very nature, digital data is inherently filtered, as every sampling process applied to a detector signal imparts a band-limiting character to the data. In the design of instrumentation, goals of reducing baseline noise and maximizing signal resolution are achieved by various forms of signal processing. The degree and type of electronic filtering will define key attributes of your electropherogram, including peak shape, resolution and baseline noise. As with most things, choosing the type of processing to apply to the signals becomes a compromise between these attributes. For this reason, there are often differences in the choices provided by various manufacturers.

Some systems fix the electronic filters so they are invisible to the user. The trade-off is usually a "one setting must fit all" approach, which by no means optimizes your signal. A common approach to signal conditioning involves the use of filters that are specified by a rise time (the P/ACE™ 5000 system uses these). However, choosing the correct rise time is not always an intuitive task, as your use of this parameter is usually built up from your experience with that given instrumentation. For this reason, rise time is usually not used to its fullest potential and may, in some circumstances, result in an unflattering compromise that you did not intend.

With the P/ACE MDQ, we chose to develop a more intuitive approach to allow you to define the best signal processing for your data; this choice was in response to feedback from CE users like you. The optimization process here is an easy one:



The first step is to choose the detector sampling (data) rate. The system choices are 0.5, 1, 2, 4, 8, 16 and 32 Hz (datapoints per second). In most cases a data rate producing 16 to 25 points at baseline (4 sigma) is a good choice. As an example, 4 Hz would be a good data rate for a peak that is 5 seconds wide (20 data-points). However, electropherograms sometimes have peaks with widths varying more than the 16- to 25-point range. In these cases the data rate should be set so the "average" peak has 16 to 25 points. To handle narrower or wider peaks, the MDQ provides a second setting -- peak width in number of data points (that can be set differently for different time ranges in the electropherogram.)

Once you have a chosen a data rate, your next step is to define, generally, how wide your peaks will be. Usually you would select the 16–25 data points setting. However, within the limits accommodated by the available data rate and peak width parameter ranges, doubling (or halving) both the data rate and peak width will produce equivalent results. *Note: It is important to choose a peak width setting that matches the number of data points in your peak, as this will define the operating range for your signal conditioning. Choosing the wrong setting or ignoring these settings by simply choosing defaults may result in less optimal detection performance.*

Once you have set up these parameters you can choose to optimize your data to favor resolution or sensitivity. Conversely you can choose the "Normal" setting, which is a balance between the two parameters. With these three filters, you can effectively optimize the signal conditioning to match the type of analysis that you are trying to achieve.

1

Select Data Rate

2

Identify Number Data Points defining Peak

3

Choose Filter type:

- ***High Sensitivity***
- ***High Resolution***
- ***Normal***

CE Application Information Available from Beckman Coulter

Protein Analysis

A-1861A	cIEF of Human Monoclonal Antibodies
A-1736	Micropreparative Method for Microsequencing of Peptides
A-1740	Analysis of Proteins by Sample Stacking
A-1745B	Resolution of Glycoforms, Ribonuclease B
A-1771A	Hemoglobin Analysis by cIEF
A-1777A	Characterization of Proteases by CE-LIF
A-1778A	Digoxin Immunoassay using CE with LIF Detection
A-1791A	Quantitation of Bioactive Peptides in Serum
DS-749	Analysis of Recombinant Human Growth Hormone

DNA Application Information

A-1726	High Sens.Analysis of Nucleotides (MECC approach)
A-1748A	CE of dsDNA Fragments by UV & LIF
DS-816	Purity Assessment of Synthetic Oligo's
R-8034	Multi-target PCR analysis using CE with LIF detection
A-1788A	Competitive RNA-PCR by CE and LIF Detection for Quantitation of Cellular mRNA

Pharmaceutical Application Information

A-1747A	Salbutamol Related Impurities
DS-766	Separation of Antibiotics and Corticosteroids by MECC
DS-821	Analysis of Flavonoids by MECC
DS-802	Determination of Drug-Related Impurities
DS-804	Analysis of Water Soluble Vitamins
A-1859A	CZE Replaces GC/NPD for Screening Basic Drugs (Forensic Toxicology)

Chiral Analysis

A-1728	Chiral Separation of CE/Pharmaceutical Compounds with Cyclodextrin Buffers Using P/ACE Capillary Electrophoresis
DS-836	Chiral Separations of Basic Drugs with Modified Cyclodextrin-Containing Buffers by P/ACE Capillary Electrophoresis
CD-8238A	Reflections on Chirality CD-ROM

Carbohydrate Analysis

R-8144	CGE of Reducing Oligosaccharides, APTS labeled
R-8163A	NATURE Reprint: High-resolution carbohydrate profiling by capillary gel electrophoresis

Application Primers

266924	Micellar Electrokinetic Chromatography
360643	Introduction to Capillary Electrophoresis
727484	CE in Biotechnology: Protein and Peptide Analysis
538703	Quantitative Application of CE in Pharmaceutical Analysis
607397	Analysis of Nucleic Acids
725036	High Performance Capillary Electrophoresis of Carbohydrates

Miscellaneous

T-1775A	Practical Online CE-Mass Spectrometry
T-1823A	Comparison of Air & Liquid Cooling in CE
T-1842A	Mobility Determinations in CE
T-1839A	Method Conversions from 2000/5500 to MDQ
T-1860A	Precision of Capillary Electrophoresis (MDQ)
D-8239A	CE Expert (general purpose calculator for CE)
AR-8571A	Putting Capillary Electrophoresis to Work (LC•GC Reprint)
AP-8508A	Capillary Electrochromatography

P/ACE Setter – Back Issues

NL-8412A	Volume I, Issue I, Summer 1997
NL-8413A	Volume I, Issue II, Fall 1997
NL-8415A	Volume II, Issue I,Winter 1998
NL-8416A	Volume II, Issue II, Spring 1998
NL-8417A	Volume II, Issue III, Summer 1998
BA985182	Volume II, Issue IV, Fall 1998
BA983134	Volume III, Issue I,April 1999
NL-8320A	Volume III, Issue II, June 1999
NL-8408A	Volume III, Issue III, September1999
NL-8516A	Volume III, Issue IV, December 1999

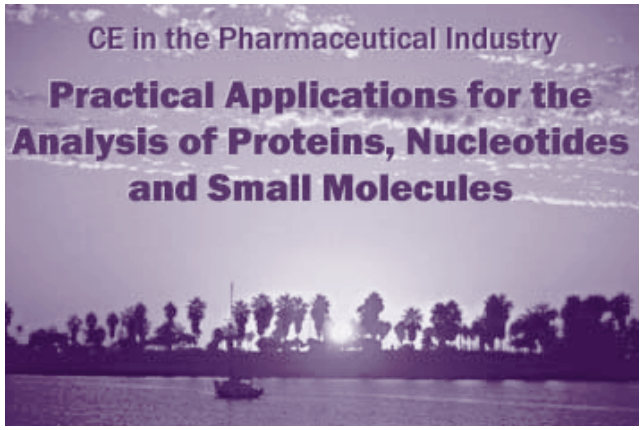
Legend:

xxxxxxx: Available at www.beckmancoulter.com/cenews (go to CE Home Page, Publications)

A/DS/T = Technical Application Information Bulletins AR = Article Reprints DS = Data Sheets AP = Application Primer

MEETING NOTICE

The goal of this symposium is to provide a forum for the discussion of recent developments in CE analysis of small molecules, nucleotides, and proteins. The presentations and workshops will be dedicated to practical concerns to strengthen the use of CE within the pharmaceutical industry. Applications will highlight uses of CE in various areas of product development, including formulation studies, process development, product characterization, validated lot release, and stability testing.



This meeting is being held Aug. 28-29 in San Diego, Calif. (USA), and is sponsored by the California Separation Science Society (CaSSS). For information on the program and call for abstracts, contact the CaSSS website at *www.casss.org*.

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