BECKMAN COULTER.

PACE Setter

Volume 8, Issue 2 • September 2004

The worldwide newsletter for capillary electrophoresis

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Industrial-Scale Carbohydrate Profiling Using High-Speed CE with Automated Batch Sampling from a 96-Well Plate

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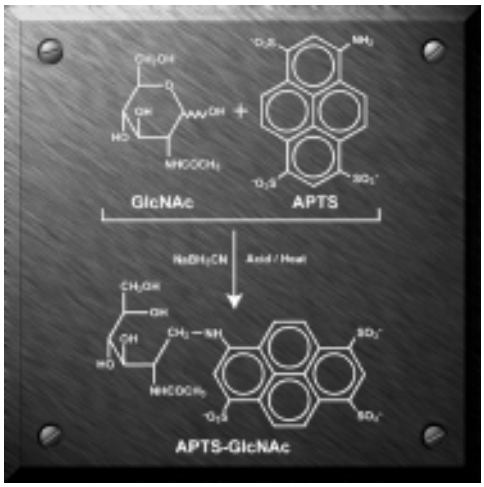
Introduction

Analysis of complex carbohydrates continues to be of great interest to the biotechnology and biopharmaceutical industries as these molecules are widely distributed in nature and play important roles in various metabolic, signaling, energy conversion, storage, and structural processes. The post-translational modification of proteins may further play an important role in the biology of disease and disease progression and ultimately be the source of important biomarkers for diagnosis and targeting therapeutic disease intervention.

Glycoconjugates, in the form of glycoproteins and glycolipids, are some of the most structurally complex molecules with diverse intra- and intercellular functionalities. Challenges in carbohydrate analysis arise from the structural diversity of monosaccharides, their sequence and linkage modes in oligo- and polymer chains, as well as the lack of chemically functional groups for electric-field-mediated separation and chromophores for detection.[1]

Capillary electrophoresis with laser-induced fluorescence (LIF) detection has been widely applied to oligosaccharide profiling and complex carbohydrate characterization, generating high-resolution and high-sensitivity analysis that is superior to many other analytical methods.[2]



Using suitable fluorescent labeling procedures, charged derivatives of various carbohydrates can be formed, enabling attomole level detection[3] and high-resolution analysis, solving such difficult separation problems as resolving positional isomers. APTS (8-aminopyrene-1,3,6-trisulfonate) is one of the most popular of such derivatization agents, providing very bright fluorescence with good spectral characteristics.[4]

In this newsletter, we describe our implementation of a high-speed carbohydrate profiling method that allows unattended batch sample processing from 96-well plates after a single-step derivatization procedure. The technology described here should bring high-performance carbohydrate screening and characterization within reach of a life sciences lab without the need for additional costly equipment and help expedite the pace of large-scale systems biology discovery from a single-capillary CE platform.

Materials And Methods

Chemicals

Lithium acetate buffer containing polyethylene oxide EOF suppression additive, APTS (8-aminopyrene-1,3,6-trisulfonic acid trisodium salt), and the maltooligosaccharide ladder standard were all obtained from Beckman Coulter (Fullerton, CA) in the form of the Carbohydrate Labeling and Analysis Kit (PN 477600). Cello-oligosaccharides, glucose, xylose, arabinose, galactose, glucuronic acid, and sodium cyanoborohydrate $NaBH_3CN$ (1 M in tetrahydrofuran) were from Sigma-Aldrich (St. Louis, MO).

Carbohydrate Derivatization

Aliquots of oligosaccharide standard solutions (0.5–10 µL), containing approximately 5–50 nmol reducing sugars, were dried in a centrifugal vacuum evaporator and labeled through reductive amination by the addition of 2 µL 0.2 M APTS in 15% acetic acid and 2 µL 1M $NaBH_3CN$ in tetrahydrofuran (as described in the Carbohydrate Labeling and Analysis Application Guide). The labeling reaction was incubated for one hour at 75ºC, followed by the addition of 100 µL CE-grade water to stop the reaction. Prior to CE analysis, the APTS-labeled samples were diluted further 50–100 fold in water, resulting in high nanomolar concentration range of the derivatized carbohydrates. The labeled oligosaccharide samples were then analyzed by the ProteomeLab™ PA 800.

Capillary Electrophoresis

All capillary electrophoresis experiments were performed on a P/ACE MDQ capillary electrophoresis system (Beckman Coulter) with the cathode on the sample introduction side and anode on the outlet side, since the negatively charged APTS-labeled oligosaccharides migrate toward the anode under the applied electric field. For the CE analysis, bare, fused-silica capillaries of 50 µm ID were used (Polymicro Technologies, Phoenix, AZ). For high-speed oligosaccharide profiling, sample introduction was carried out from the outlet (right side) sample tray, and although the total column length was 30 cm, the effective separation length was only 10 cm. For the monosaccharide analysis, samples were introduced from the left sample tray with a total capillary length of 60 cm and an effective separation length of 50 cm. In either case, the 25 mM lithium acetate buffer (pH 4.75) containing polyethlylene oxide polymer provided sufficient dynamic coating to suppress electroosmotic flow at the separation pH of 4.75. Samples were injected by reverse vacuum for 5–10 seconds at 0.5 psi and separated at 500 V/cm electric field strength. Separations were monitored on column by LIF detection (3 mW Ar-ion laser, excitation and emission wavelengths 488 and 520 nm, respectively). The capillary was thermostatted at 20ºC using recirculating liquid coolant (FluorInert®). The data was acquired and processed with the 32 Karat™ workstation (Beckman Coulter). The capillaries were first preconditioned by rinsing with 1 M NaOH for 10 minutes followed by water for 5 minutes, 1 M HCl for 10 minutes, water for 5 minutes, and running buffer for 5 minutes. The washing procedure between runs was 1 M HCl for 0.5 minutes, water for 0.5 minutes, and running buffer for 1 minute.

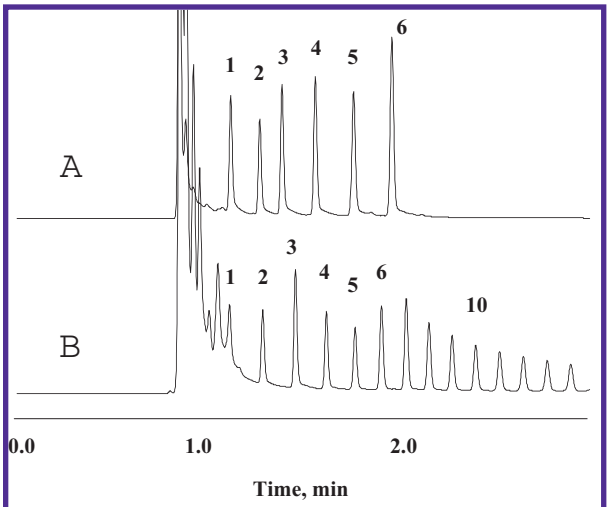


Figure 1. *High-resolution oligosaccharide profiling by capillary electrophoresis. Traces: (A) cello-oligosaccharide mixture; (B) Malto-oligosaccharide ladder. Numbers above the peaks correspond to the degree of polymerization (DP) of the oligosaccharides. Conditions: bare, fused-silica capillary (50 µm ID); effective separation length: 10 cm; total capillary length 30 cm. Electric field strength: 500 V/cm; running buffer: 25 mM lithium acetate with PEO modifier (pH 4.75); sample introduction: 5 sec, vacuum 0.5. Typical current generated was 17–18 µA.*

Results and Discussion

Figure 1 illustrates an example of high-speed, high-resolution oligosaccharide separation using a bare, fused-silica capillary with a 10-cm effective separation length. Traces A and B depict the electropherograms of the cello-oligosaccharide mixture (degree of polymerization, DP 1-6) and maltooligosaccharide ladder (DP 1-14), respectively. Note that there are well-observed differences in the migration times of these oligosaccharides with the same degree of polymerization but different linkages, e.g., maltotriose vs. cellotriose (Peak 3), maltotetraose vs. cellotetraose (Peak 4), etc. It is especially interesting that while the maltotriose and maltotetraose migrate more slowly than the cellotriose and cellotetraose, the maltohexaose migrates faster than the cellohexaose. We consider these migration time variations to be due to hydrodynamic radius differences of the molecules, having either α 1,4 (malto-oligomers) or β 1,4 (cello-oligomers) linkages, providing a basis for their capillary electrophoretic separation. High efficiency of differentiation between structural isomers of carbohydrates as well as high speed and simplicity of the described CE method represent advantages over HPLC approaches.

Using high field strengths and a relatively short capillary (10 cm), we readily accomplished large-scale batch sequence of 96 samples processed unattended overnight. Figure 2 presents an overlay of 96 traces of the cello-oligosaccharide standards obtained with this high-speed method. In each trace, the first, large, fast-migrating peak is the excess un-reacted APTS. The 96-well sample plate was processed in 8 hours, corresponding to approximately 4.5 minutes per sample—this includes separation buffer replacement, sample introduction,

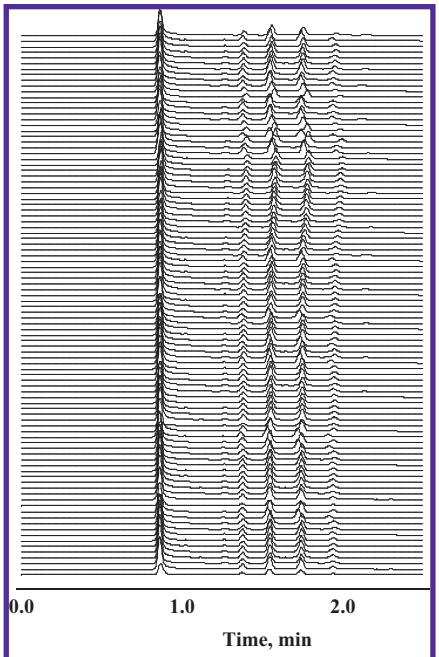


Figure 2. *Ninety-six electropherograms obtained by automated batch sample processing from a 96-well microtiter plate. Conditions as described in Figure 1.*

separation, and all necessary robotic movements. It is important to note that there appears to be no pronounced drift or shift in migration time of the peaks through the entire sequence run (migration time RSD < 2.5%).

Monosaccharide analysis by CE has been previously demonstrated using borate-based buffer systems at high pH (>9), taking advantage of the resolution enhancement due to the complexation between the polyhydroxy compounds (sugars) and borate.[5] Good separation performance was also demonstrated using capillary zone electrophoresis[6] as well as micellar electrokinetic chromatography.[7] However, in most cases, an internal standard is required to account for possible migration time shifts due to shifts in electroosmotic flow. In the course of our work, we have found that for the monosaccharides of our interest, comparable monosaccharide separation performance can be obtained using the CE conditions described above for oligosaccharide profiling. Moreover, the suppressed EOF typically rendered more reproducible performance and significantly shorter capillary regeneration time. Figure 3 illustrates a well-resolved separation of a five monosaccharide mixture in fewer than 5 minutes, employing the same lithium acetate separation buffer containing a polyethylene oxide additive that was applied above for oligosaccharide analysis. Due to its additional charge, glucuronic acid (1) migrates the fastest, preceding xylose (2), arabinose (3), glucose (4), and galactose (5).

Precise quantification is also an important consideration for carbohydrate analysis in industrial settings. Errors are usually associated with sample preparation, as well as electrophoresis-related factors such as injection variation and laser intensity fluctuations. Calibration by appropriate external standards can eliminate some of these factors, but the introduction of internal standards more accurately compensates for any run-to-run variability (data not shown). By adding a known amount of appropriate standard to the samples, precise concentrations of the separated sugars can be calculated based on their peak areas (velocity corrected) relative to the internal standard. In this case, the internal standard should not co-migrate with any of the peaks of interest. Quantitative calculations can be readily accomplished in an automated fashion using the PA 800 32 Karat™ data system.

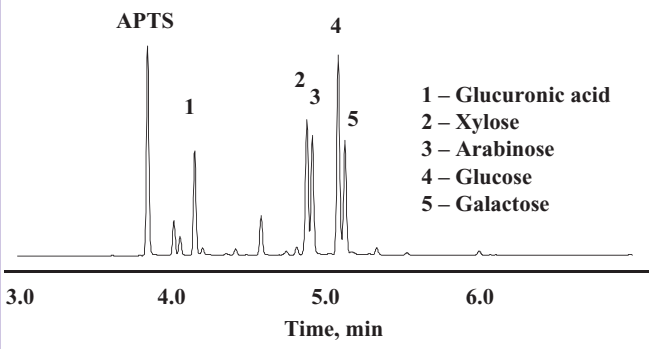


Figure 3. *Monosaccharide analysis by capillary electrophoresis under very low EOF condition. Peaks: 1—glucuronic acid, 2—xylose, 3—arabinose, 4—glucose, and 5—galactose); Conditions: same as in Figure 1, except a bare fused-silica capillary with 50 µm ID, 50 cm effective separation length, 60 cm total length was used. Electric field strength: 500 V/cm. Once again, the PEO polymer within the separation buffer served to effectively reduce EOF.*

Summary

In this newsletter, we have introduced a high-speed CE method for large-scale carbohydrate analysis useful for both oligosaccharide profiling and monosaccharide analysis. The approach proved to be efficient for industrial-scale carbohydrate characterization in our laboratory. This technique could also be readily adapted to a multicapillary array system to further increase parallel processing.

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Rapid Separations of Sugar Phosphates and Anomers

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Phosphate sugars are key components of the glycolytic energy metabolism. They have been analyzed by anion exchange chromatography, HPLC, and enzymatic methods. Capillary zone electrophoresis (CZE) provides a rapid way to separate and quantitate these sugars in complex mixtures. Many of these phosphate sugars have similar mobilities, making resolution difficult. Torralba[1] and Shen[2] used CZE in bare silica capillaries, applying reverse polarity, with an EOF modifier to achieve rapid separations. Under reverse polarity the phosphate sugar anions migrate to the detector at the anode, and the EOF creates a bulk flow in the same direction to shorten the separation time. Ciringh[3] and Shaeper[4] used bare silica capillaries with normal polarity where the natural EOF drags the anions toward the detector at the cathode. Ciringh's studies demonstrated that for many of the sugars the optimum buffer pH was 5.8. The power of EOTrol® HR in these separations was demonstrated by separating glucose-1-phosphate (G-1-P) and glucose-6-phosphate (G-6-P), and then by showing that it also facilitated the rapid separation of the α and β anomers of glucose-1-phosphate. Separating the α and β anomers is difficult because they are almost identical structurally. Analysis of mixtures is normally conducted by enzymatic methods.

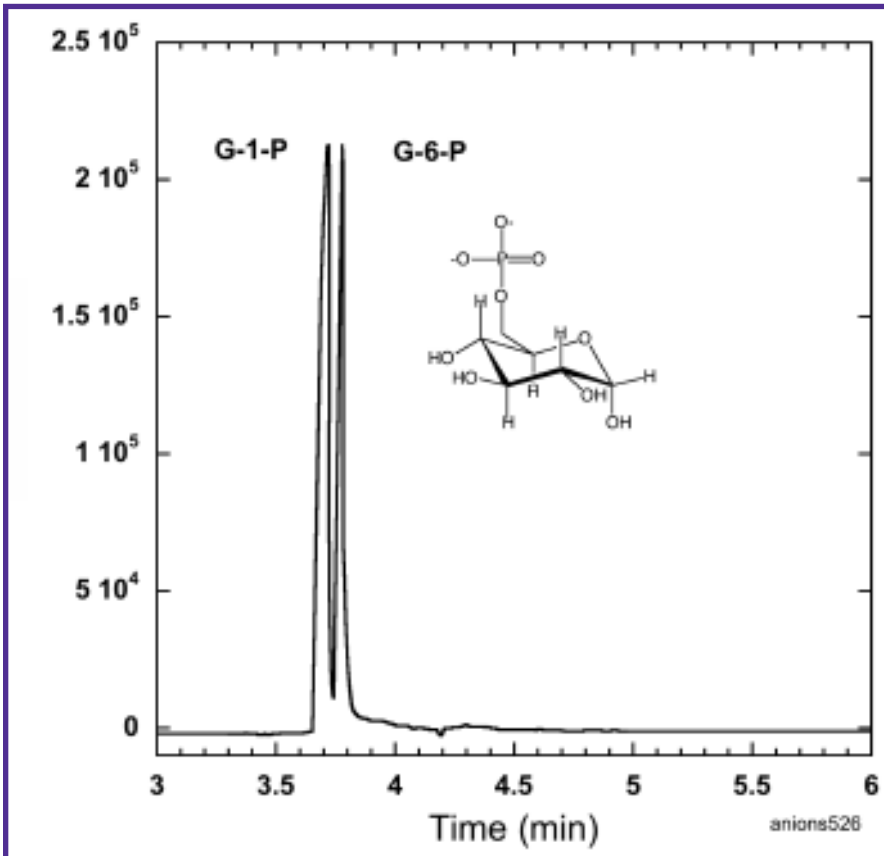

Figure 1. Separation of sugar phosphates in a bare silica capillary. EOTrol HR in the separation buffer provides a dynamic coating. Separation buffer: 6 mM Tris-Sorbate, pH=5.8, 0.15% (w/v) EOTrol HR. Sample Injection: 5 cm height difference between sample vial and outlet vial for 8 seconds. Sample was 1 mg/mL of each sugar phosphate. Separation conditions: Total length, L = 57 cm, analysis length, l = 43 cm, 75 µm i.d., 20 kV, reverse polarity. Indirect detection at 256 nm.

Experimental

Analysis was conducted in a benchtop system with the detection window at the anode end. The system included a Glassman high voltage power supply and a Hyperquan UV absorbance detector operating at 256 nm and set at 0.05 absorbance units full scale per volt (0.05 AUFS). Data was collected with an Alltech AllChrom data system. The sorbate anion serves as the probe for indirect detection of the sugar phosphates. New capillaries were conditioned for use by rinsing with 0.1 M NaOH for 30 minutes, followed by a 5-minute water wash, a 20-minute rinse with 0.1 M HCl, and then a 5-minute water wash. Capillaries were rinsed with 3 capillary volumes of the separation buffer before each measurement.

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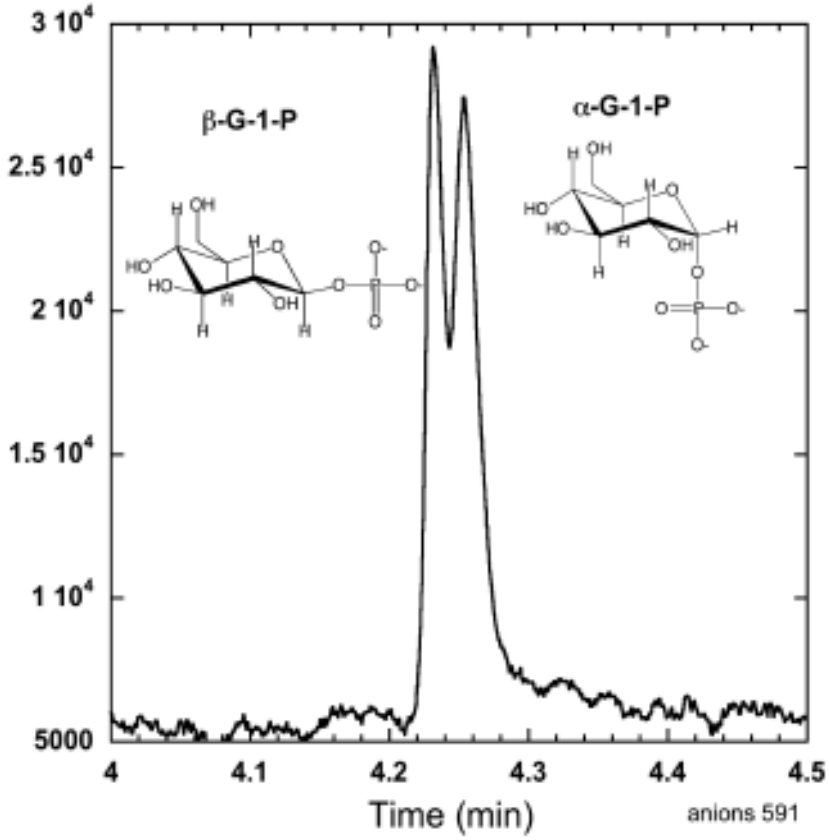

Figure. 2 Separation of anomers of glucose-1-phosphate in a bare silica capillary. EOTrol HR in the separation buffer provides a dynamic coating. Method has not been optimized to give baseline resolution. Separation buffer: 2.5 mM Tris-Sorbate, pH=5.8, 0.15% (w/v) EOTrol HR. Sample Injection: Electrokinetic injection (5 sec at 5 kV) of solution with 60 µg/mL of each anomer. Separation conditions: Total length, L = 57 cm, analysis length, l = 43 cm, 100 µm i.d., 20 kV, reverse polarity. Indirect detection at 256 nm.

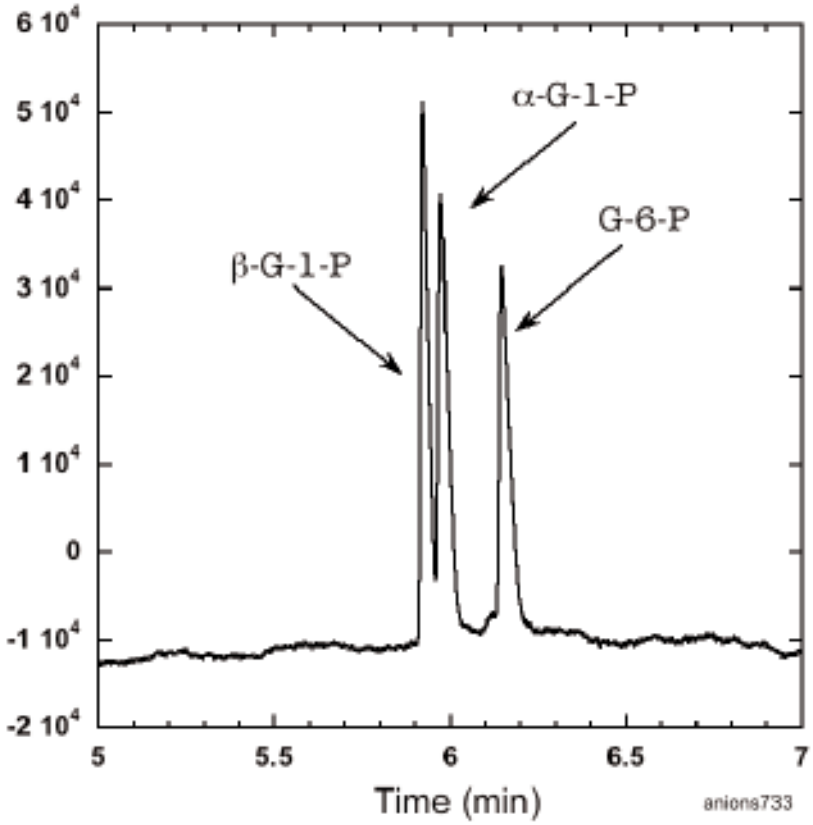

Figure 3. α and β glucose-1-phosphate (G-1-P) and glucose-6-phosphate (G-6-P) are separated in a bare silica capillary. EOTrol HR in the separation buffer provides a dynamic coating. Separation buffer: 6 mM Tris-Sorbate, pH=5.8, 0.15% (w/v) EOTrol HR. Sample Injection: Electrokinetic injection (5 sec at 5 kV) of sample containing 1 mg/mL each of β-G-1-P, α-G-1-P, and G-6-P. Separation conditions: total length, L = 68 cm, analysis length, l = 53 cm, 75 µm i.d., 25 kV, reverse polarity. Indirect detection at 256 nm.

Identifying Organoarsenic Compounds with CE-MS/MS

Emma Edwards, Russell Pickford, and Jane Thomas-Oates, University of York, UK

Introduction

Although capillary electrophoresis (CE) has previously been interfaced with single-stage mass spectrometry (MS) for the detection of organoarsenic compounds, false positives due to matrix interference can complicate data and lead to mistaken identities, especially when analysing samples in complex biological matrices. The organoarsenic species are of low molecular weight, and, therefore, mass spectral responses are in the mass-to-charge range where there is considerable background signal due to interfering analytes and chemical noise. Use of tandem mass spectrometry (MS/MS) has the potential to avoid observing much of this interference while improving the selectivity and limit of detection of analysis, and to produce characteristic, structurally diagnostic fragment ions from the analytes. This newsletter article demonstrates the application of CE-MS/MS to the characterisation of organoarsenic compounds. The P/ACE™ MDQ system is coupled to the Thermo Electron LCQ Deca XP Plus ion trap mass spectrometer via a sheath-flow interface and used to separate and analyse a mixture of six charged and neutral organoarsenic compounds.

Experimental

- CE instrument: P/ACE™ MDQ with CE-MS interface kit
- Ion trap mass spectrometer: LCQ Deca XP Plus (Thermo Electron)
- Capillary: 75 µm ID x 80 cm
- Capillary temperature: 25°C
- Background electrolyte: 10 mM formic acid
- Applied voltage: 20 kV
- Sheath liquid: 1:1 (v/v) methanol:water 1% formic acid at 1 mL min^{-1}
- Electrospray capillary voltage: 5800 V

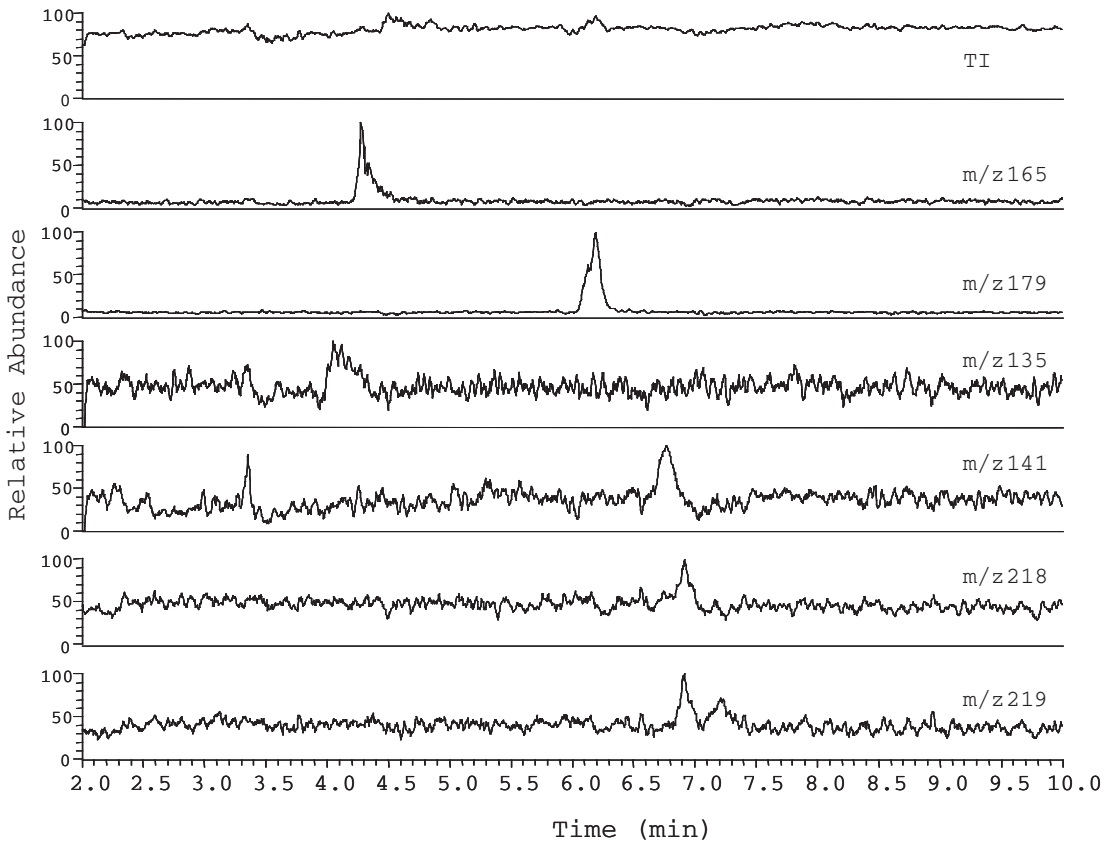

Figure 1.

- Tandem MS conditions: helium gas, collision energy setting 30%, activation q value 0.25, activation time 30 ms
- Sample: mixture of 6 arsenicals, each at a concentration of 1 ppm (arsenobetaine, dimethylarsinic acid, 4-hydroxyphenylarsonic acid, 4-nitro phenylarsonic acid, monomethylarsonic acid and the tetramethylarsonium ion)

Results and Discussion

A solution containing a mixture of the 6 organoarsenic compounds (each at 1 ppm) was analysed using the combined separating power of CE and detection using an ion trap mass spectrometer. Peaks can be seen in the extracted ion electropherograms for all of the arsenicals analysed (Figure 1), although, as expected, the cationic species had a much better MS response than the neutral species. There was little or no separation between the phenyl-containing species and monomethylarsonic acid, but the selectivity of detection allows their differentiation.

Product ion spectra were obtained for all species during CE separations. Data obtained for a separation of arsenocholine (*m/z* 165) and arsenobetaine (*m/z* 179) are shown as an example in Figure 2. The CE-MS/MS total ion electropherogram was acquired by selecting the relevant precursor ion and recording its collision induced fragments as follows: precursor ion *m/z* 165 over the time from 0 to 4.0 min, and precursor ion *m/z* 179 from 4.0 to 6.8 min. This resulted in an intense, narrow peak corresponding to arsenobetaine and a weak peak for the arsenocholine. The product ion data produced contain structurally diagnostic fragment ions for each of the two analytes which allow their identification.

This is the first demonstration of the combined use of capillary electrophoretic separations with tandem mass spectrometry for the analysis of organoarsenic compounds. Application of this technique could be of particular value in aiding peak assignment in complex samples such as food matrices. With development of a suitable method for clean up for samples from complex matrices, CE-MS/MS will have an obvious place in the toolkit for analysis of arsenic-containing compounds.

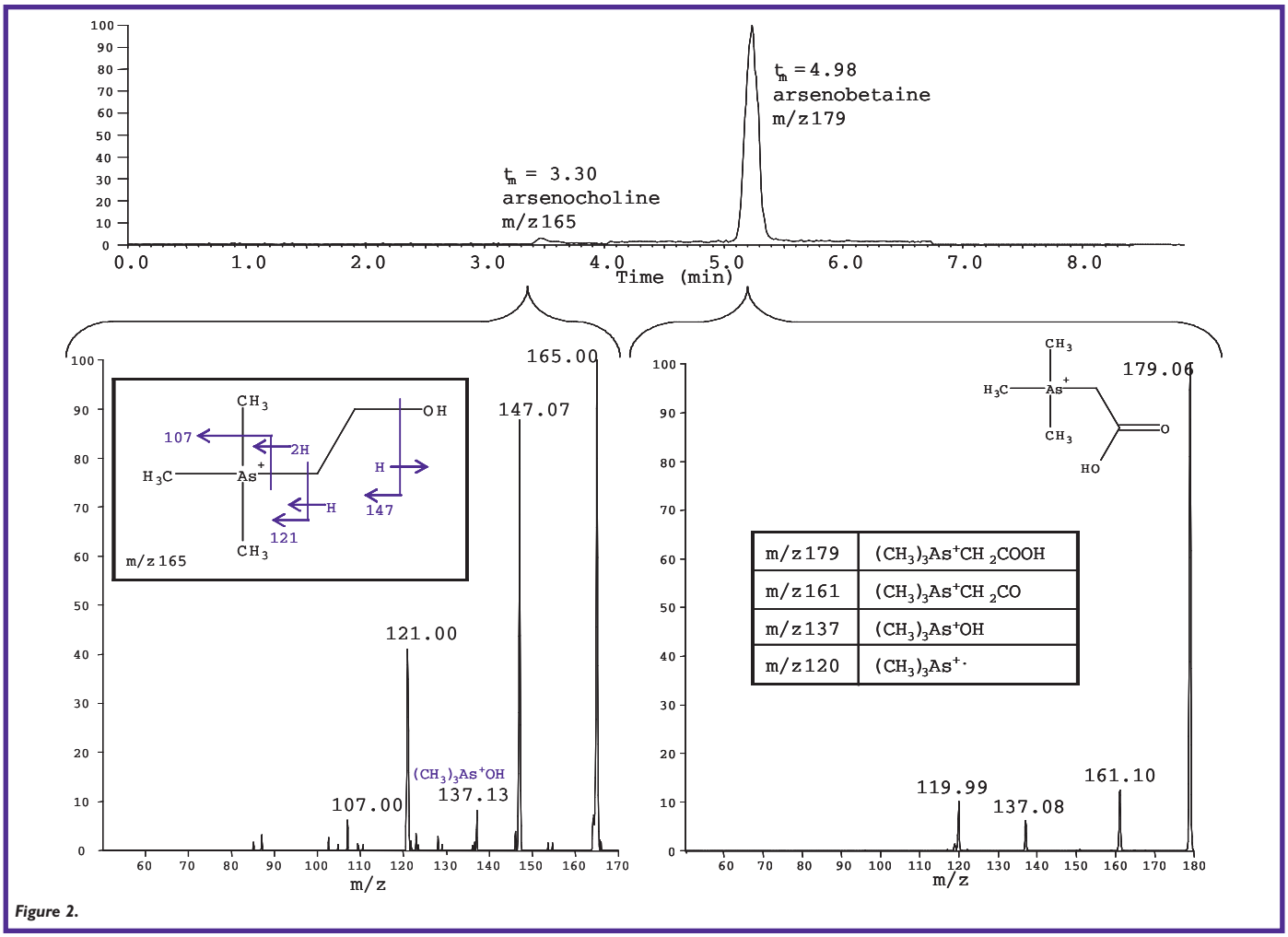

Figure 2.

Capillary Isoelectric Focusing with the ProteomeLab™ PA 800 for the Characterization of a Recombinant Monoclonal Antibody

V. F. Knudson,
H. Huang, and
M. H. Simonian
Beckman Coulter, Inc.

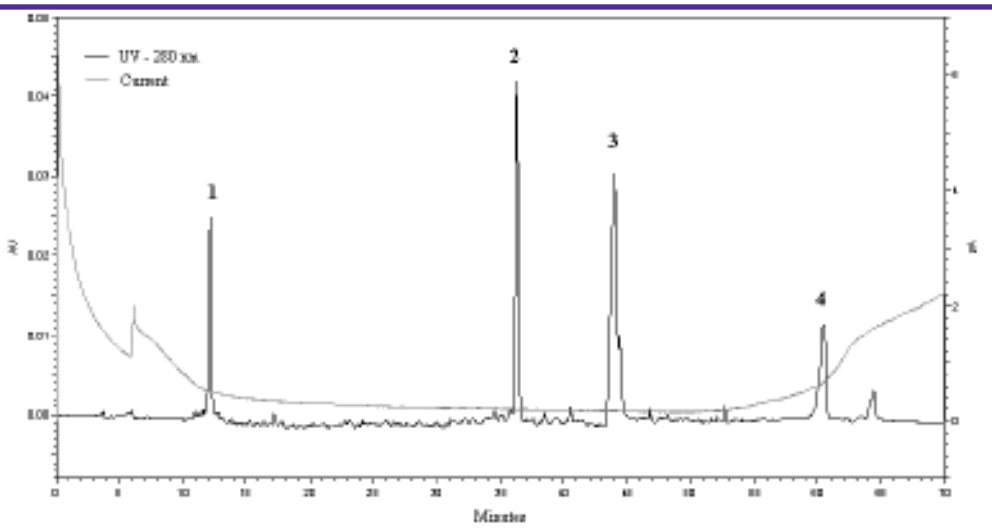

Figure 1. Electropherogram of standard markers detected using wide-range ampholyte (pI 3.0–10.0). Peak 1: ribonuclease A (pI 9.45); 2: carbonic anhydrase II (pI 5.9); 3: β-lactoglobulin A (pI 5.1); and 4: CCK flanking peptide (pI 2.75). The red line illustrates the typical current profile of CE separation.

Introduction

The ProteomeLab™ PA 800 Protein Characterization System provides analytical solutions for determining a protein's isoelectric point and molecular weight, generating a peptide map, and evaluating oligosaccharide profiles.

Capillary Isoelectric Focusing (cIEF) is a powerful technique for separating complex mixtures of proteins or peptides and identifying their charged isoforms. There is an increasing interest in characterizing and monitoring the charge heterogeneity of recombinant monoclonal antibodies in many laboratories and biopharmaceutical companies.[1-3] The cIEF technique is used in combination with mass spectrometry (MS) to determine the identity and relative quantity of isoforms. The coupling of these two techniques has allowed remarkable advances to be made in the separation of proteins and peptides.[4-6]

For the cIEF technique, protein samples are introduced into a capillary with a mixture of ampholyte buffers which are a polymerized mixture of monomers that contain weakly acidic and weakly basic groups. By using an acidic solution on the anode side of the capillary (anolyte) and a basic solution on the cathode side (catholyte) and applying an electric field, the ampholyte molecules orient themselves with respect to their pKa values, generating a pH gradient within the capillary.
Proteins migrate through the ampholyte mixture and focus at a pH where they become electrically neutral (isoelectric point or pI). After focusing, the zones must be mobilized to the detection window by applying pressure together with focusing potential which prevents zone dispersion. The capillary used for cIEF is usually coated (neutral) to eliminate electroosmosis and minimize protein adsorption.

Successful separation by cIEF requires carrier ampholytes that generate minimum baseline noise and support calibration with pI markers. The pI markers are selected for their linear relationship between their detection time and pI value.[7-8]

This bulletin describes the application of the cIEF technique with the ProteomeLab PA 800 for the rapid, qualitative analysis of complex peptides and proteins in the pI range of 3 to 10. As an example, a recombinant monoclonal antibody, IgG1k, was characterized for pI. Two different pH range ampholytes were used: one for separation and pI prediction of proteins with pI values over a wide range from 3.0 to 10.0 and another for high-resolution separation of acidic proteins with pI values from 5.0 to 7.0.

Experimental

Instrumentation and Reagents

CE separations were performed on a ProteomeLab PA 800 System with UV detection and 32 Karat™ software (version 7.0) using the methods and reagents described in the system application manual.

The neutral coated capillary, 50 µm ID x 30.2 cm (20 cm effective length to detector), was used for separation to eliminate electroosmotic flow.

The ProteomeLab™ Capillary Isoelectric Focusing Kit was used. This kit contains a neutral coated capillary, cIEF separation gel, ampholyte buffers, and protein standard markers. Monoclonal mouse IgG1k was purchased from Sigma-Aldrich (St. Louis, MO) and Bio-Lyte® 5/7 Ampholyte was purchased from Bio-Rad (Richmond, CA).

Preparation of Working Solutions and Samples

The anode electrolyte solution (anolyte) was prepared as a mixture of filtered cIEF separation gel (polymeric solution) and phosphoric acid (91 mM final concentration). The cathode electrolyte solution (catholyte) was 20 mM sodium hydroxide. The acidic rinse buffer was 10 mM phosphoric acid H_3PO_4.

To make the sample/ampholyte solution, 10 µL of concentrated IgG1k sample (5 mg/mL) was added to the selected ampholytes prepared as described below.

Two different pH range ampholytes were used for analysis: **Wide-range ampholyte** (pI 3.0–10.0) was prepared as a mixture of 4 µL of cIEF ampholyte (pI 3–10) and 200 µL of cIEF gel. For calibration of pI to migration time, a total of four markers were added to the ampholyte mixture: 2 µL each of carbonic anhydrase II (pI 5.9) and CCK flanking peptide (pI 2.75), and 4 µL each of ribonuclease A (pI 9.45) and β-lactoglobulin A (pI 5.1). For separation and pI prediction of the IgG1k sample, two standard markers were added to the sample/ampholyte mixture: 2 µL of carbonic anhydrase II (pI 5.9) and 5 µL of ribonuclease A (pI 9.45). **Narrow-range ampholyte** (pI 5.0–7.0) for the high-resolution separation of acidic proteins was prepared as a mixture of 3 µL of cIEF ampholyte (pI 3–10), 6 µL of Bio-Lyte 5/7 ampholyte, and 200 µL of cIEF gel.

CE Separation Method

The monoclonal antibody mouse IgG1k was detected by UV absorbance at 280 nm. During separation, the capillary temperature was stable at 20°C.

For every separation run, prior to introduction of the sample/ampholyte, the capillary was rinsed with acid (10 mM H_3PO_4) and water to clean the capillary surface and then filled with sample/ampholyte mixture at 30 psi for 1.50 minutes. The focusing step was performed for 6.00 minutes at a constant voltage of 15 kV with normal polarity for a 30.2 cm neutral coated capillary. The mobilization step was performed for 64 minutes at a constant voltage of 21 kV with normal polarity and 0.5 psi pressure applied at the anode side of capillary. The current was stable at 1 µA.

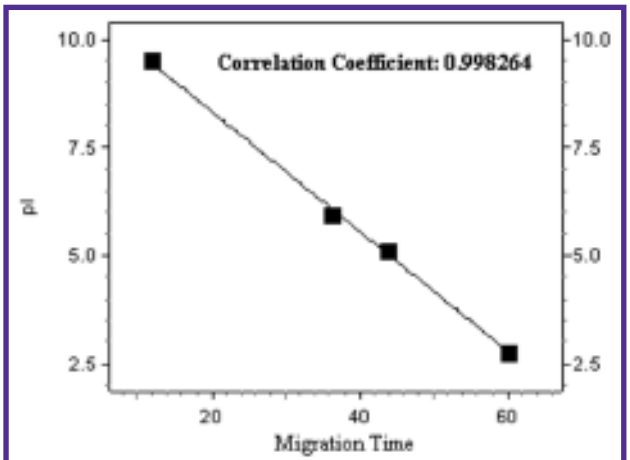

Figure 2. Linearity of the pI values of standard markers versus their migration times obtained from the electropherogram in Figure 1.

Results and Discussion

Calibration of the pH Gradient

For the pI value calculation of the proteins of interest, a wide pH range ampholyte for linearity of assay detection was developed. The correlation between the pI values of standard pI markers and their migration time was determined. Figure 1 shows the electropherogram of the protein pI standards with the wide-range ampholyte carrier (pI 3.0–10.0). The relationship of pI to migration time has good linearity with a correlation coefficient equal to 0.998 (Figure 2).

Separation and pI Prediction of IgG Isoforms

To identify the isoforms of immunoglobulin IgG1k and predict their pI values, the cIEF separation was performed with wide-range carrier ampholyte (pI 3.0-10.0) in the presence of two suitable markers closely related to the analyzed sample in terms of pI values: carbonic anhydrase II, pI 5.9, and ribonuclease A, pI 9.45 (Figure 3). Since the separation time of the pI 5.9 marker was established at 35 minutes, the time of the sample mobilization step was reduced to 39 minutes.

High-Resolution Separation of IgG

After the pI values of the IgG isoforms were predicted, the ampholyte solution was modified for detection in a narrow pH range that significantly increased the resolution of the peaks of interest. Figure 4 shows a profile of the IgG1k separation using the narrow-range ampholyte (pI 5.0-7.0) carrier for high-resolution separation of acidic proteins. The predicted pI values are the same as those with the wide-range ampholytes.

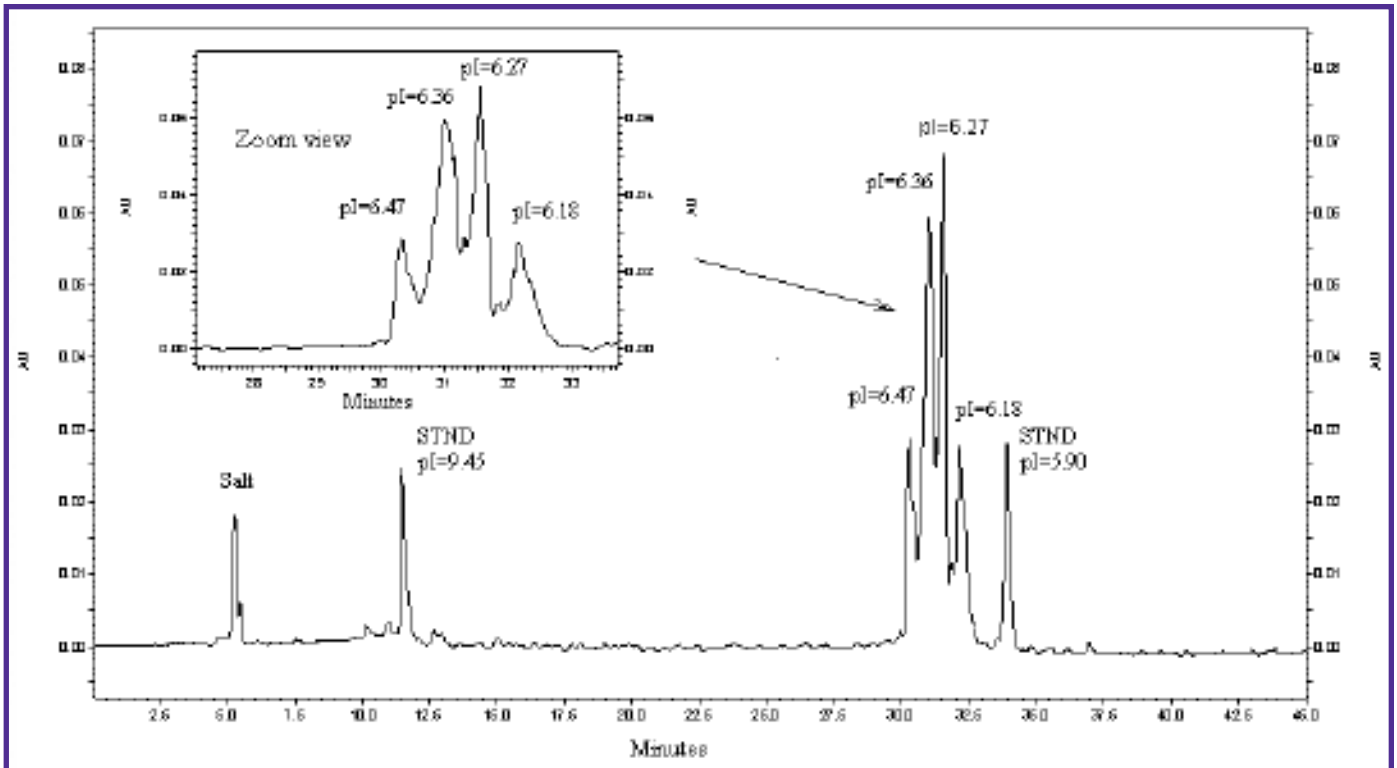

Figure 3. cIEF of monoclonal mouse antibody IgG1k along with two standard protein markers (carbonic anhydrase II, pI 5.9, and ribonuclease A, pI 9.45) using the wide-range ampholyte (pI 3.0–10.0). The pI values for the IgG1k isoforms are automatically calculated by the qualitative analysis feature and annotated on the peaks by the software.

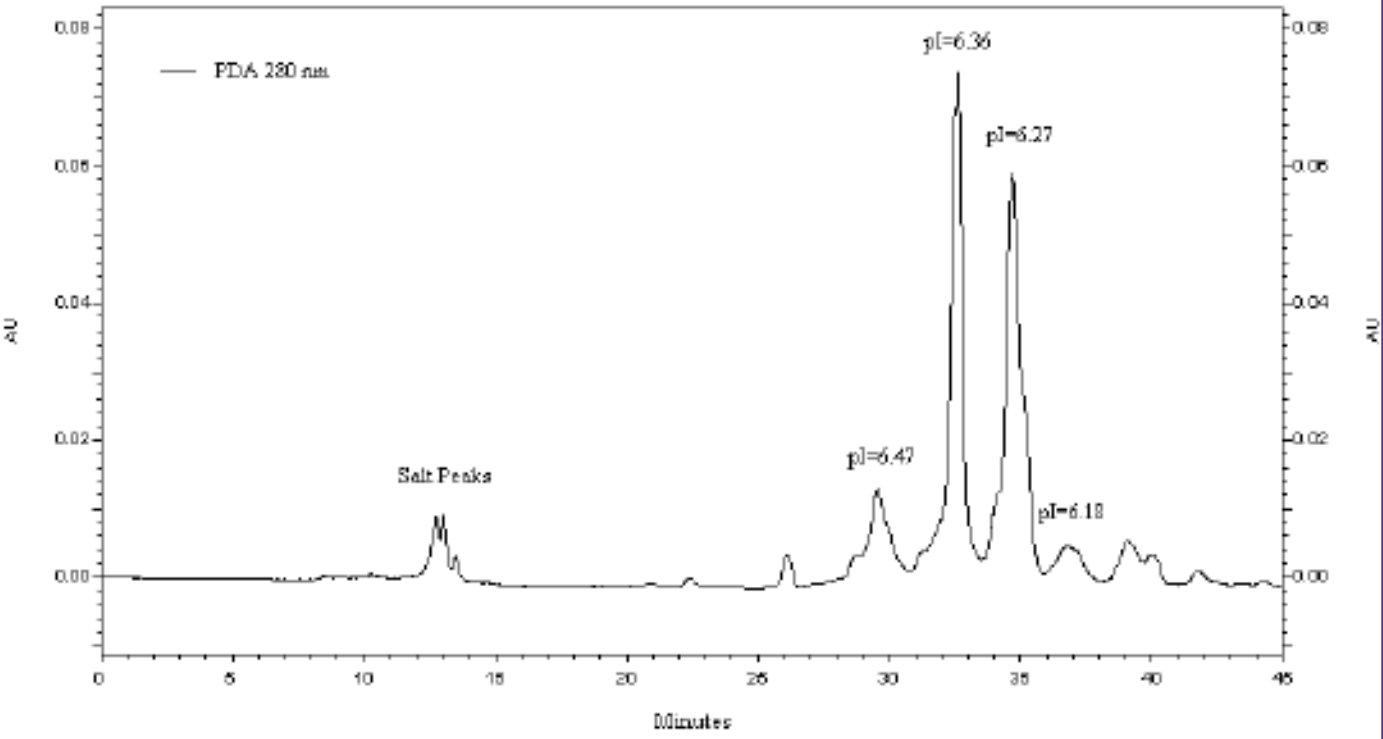

Figure 4. cIEF profile of monoclonal mouse antibody IgG1k using the narrow-range ampholyte (pI 5.0–7.0) for high-resolution separation of acidic proteins.

Conclusion

We have demonstrated the application of cIEF with the ProteomeLab™ PA 800 for separating proteins based on pI and then determining the pI value of a protein. Furthermore, this technique was able to resolve charged isoforms present in a heterogenous protein. Ampholytes with a wide pH range were shown to have good reproducibility with the selected pI markers which enabled a linear pH calibration profile. The high-resolution separation that results from cIEF could precede analysis by MS.

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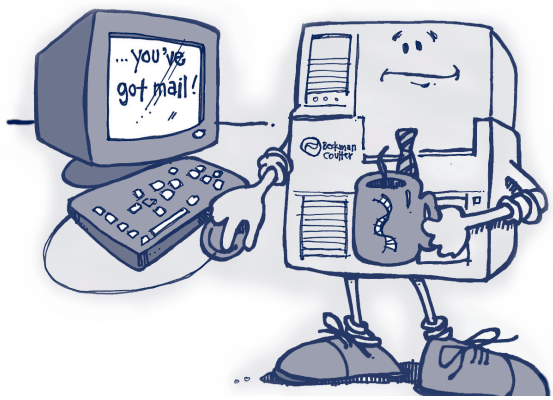

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The 15th Annual Frederick Conference on CE/Proteomics

The focus of this meeting is the scientific exchange and dissemination of the technical aspects of Capillary Electrophoresis (CE) and proteomics. We invite discussion of creative and innovative new approaches and techniques. We encourage participation at this conference and recommend expedient registration and abstract submittal.

The format will include oral and poster presentations by invited speakers and individual conference participants. Invited speakers will provide expert overviews of the basic aspects and applications of CE, capillary electochromatography (CEC), micellar electrokinetic capillary chromatography (MECC), resolution multidimensional separations, CE/MS/MS and nano-HPLC/MS/MS separation and identification of peptides and proteins. Also, the application of CE to the separation of small ions as well as large biomolecules with emphasis on pharmaceutical, biochemical, and biomedical applications. Plenary lectures will be given by distinguished invited speakers.

The conference will convene at 9:00 a.m. on Monday, October 18, 2004 on the NCI at Frederick campus and is scheduled to adjourn Tuesday, October 19. Morning and afternoon sessions will include lectures and individual presentations. Exhibitions of the most recent CE instrumentation and equipment will be on site Monday and Tuesday.

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