BECKMAN COULTER

PACE Setter

The worldwide newsletter for capillary electrophoresis

Volume 4, Issue 2 • October 2000

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The worldwide newsletter for capillary electrophoresis

Assessing the Homogeneity of Plasmid DNA: An Important Step toward Gene Therapy

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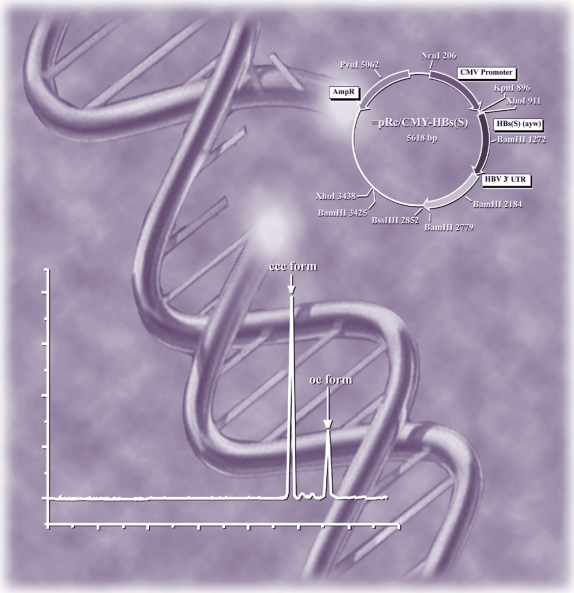
Introduction

Plasmids are extra-chromosomal, double-stranded DNA molecules that may exist in several forms differing in topology and size. However, as plasmid DNA is being used as vectors for therapeutic genes, the development of good analytical processes to assess both purity and heterogeneity is of great importance.

With pharmaceutical-grade plasmid DNA, an adequate homogeneity of the final product is achieved when more than 90% of the molecules exist as the supercoiled covalently closed circular (ccc) form.[1,2] This is the most compact form where the circular and covalently closed DNA helix is interwoven in itself, like a twisted rubber band. If one of the DNA strands is broken, the circular molecule relaxes under loss of coiling. This relaxed structure is called the open circular (oc) or nicked form. These topological plasmid structures may exist as different sizes, such as monomers and dimers, creating additional heterogeneity. Finally, linear plasmid structures are generated when both strands are cleaved at the same position.

Agarose gel electrophoresis (AGE) has been the primary method used to assess the homogeneity of plasmid DNA, but this approach has some major disadvantages. The AGE method is manual, only semi-quantitative, and the assignment of bands to plasmid structures is difficult since the electrophoretic mobility of plasmids of different shapes changes with the electrophoresis operating conditions.[3,4]



A more powerful routine technology for the quantification of plasmid forms is capillary gel electrophoresis (CGE).[5] This automated approach offers high resolution, high sensitivity, and high reproducibility to this analysis. In this paper, we highlight a method allowing the separation and quantitation of all plasmid DNA forms (including oligomeric structures) typically present in a bacterial plasmid preparation.

Experimental

Capillary Electrophoresis

Analyses were performed using a P/ACE™ 2050 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 (pH 8.4) consisting of 89 mM Tris, 89 mM boric acid, 2 mM EDTA, 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 pre-staining with YOYO at a DNA-base pair-to-dye molar ratio of 5:1, the plasmid

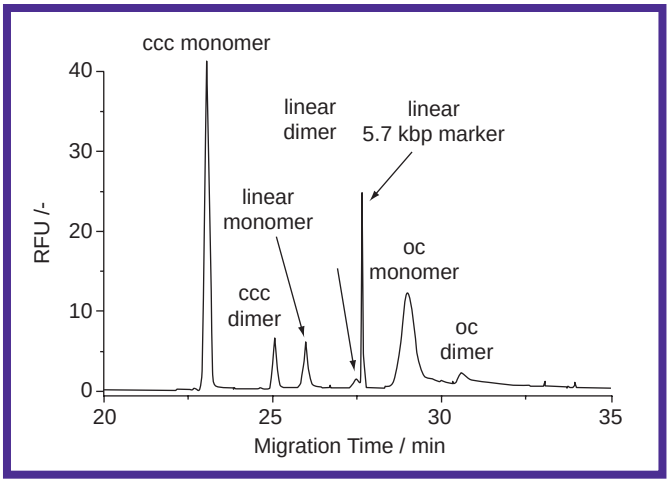


Figure 1. Separation of pUC19 (2.7 kbp) plasmid structures.

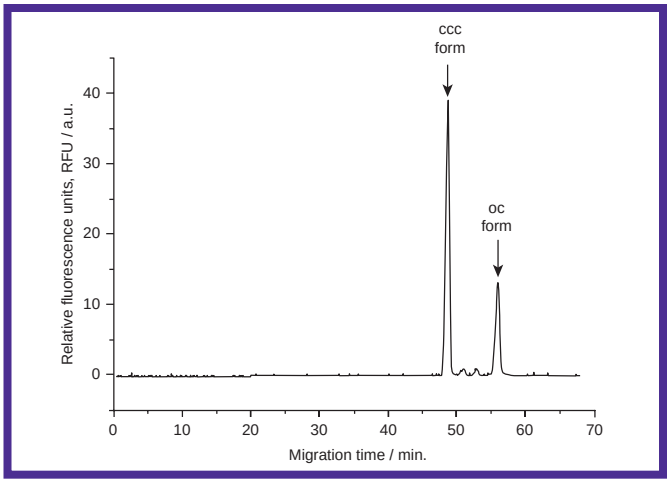


Figure 4. Electropherogram of typical plasmid prep.

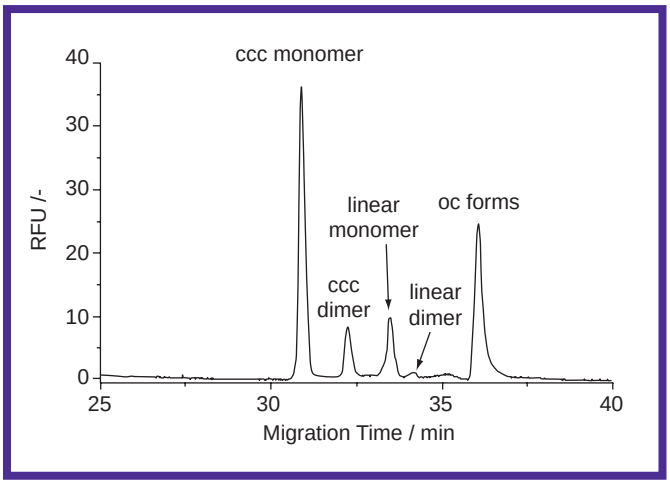


Figure 2. Separation of pCMV-S2S (5.7 kbp) plasmid structures.

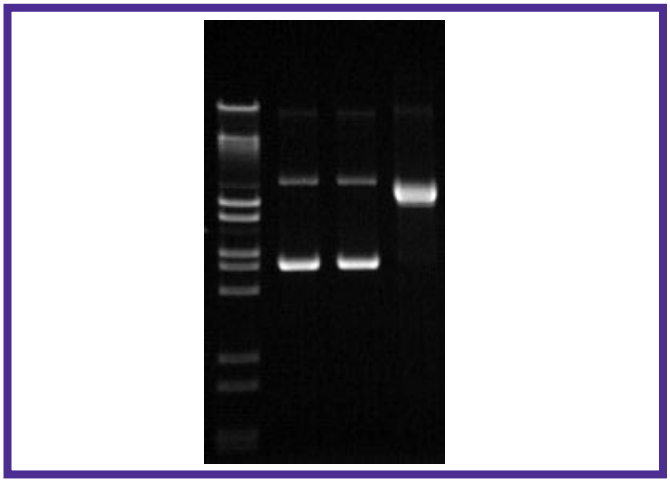


Figure 5. Agarose gel electrophoresis: two untreated plasmid samples and one sample of the plasmid transferred into the oc-form.

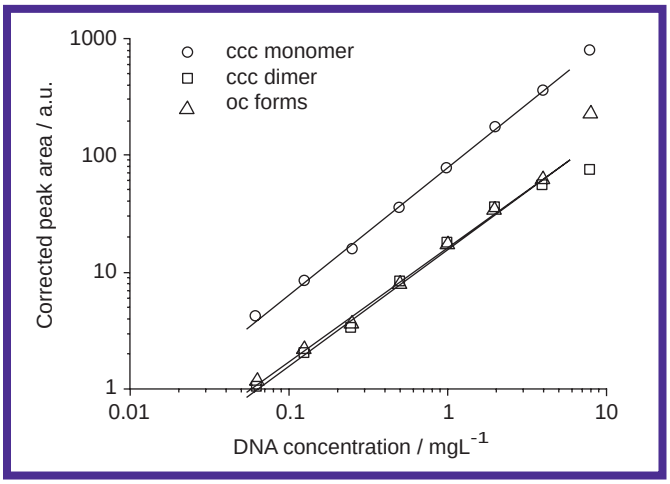


Figure 3. Linear correlation of corrected peak-area vs. DNA concentration of each plasmid structure of pCMV-S2S.

samples were introduced hydrodynamically. Electrophoresis was carried out at 100 V/cm with the capillary thermostatted to 30°C.

Plasmid Preparation

pUC19 (2.7 kbp) and pCMV-S2S (5.7 kbp) plasmid DNA were isolated from overnight cultures of *Escherichia coli* using QIAGEN* Plasmid Maxi Kit (QIAGEN, Hilden, Germany). Linear plasmid DNA was prepared by digesting purified DNA with restriction endonuclease *Eco*RI (Roche Diagnostics, Mannheim, Germany). UV-treated plasmid DNA was irradiated with UV light resulting in single-strand breakage and relaxation of ccc to open circular structures.

Results

An electropherogram of a mixture of untreated, linearized, and UV-irradiated pUC19 plasmid DNA is presented in Figure 1. All monomeric and dimeric plasmid structures can be separated with baseline resolution. The order of migration is governed by the topology of plasmid structures. Supercoiled ccc molecules (monomers and dimers) have the most compact structure with the highest electrophoretic mobility—appearing earlier than linearized (monomers and dimers) forms that are followed by the open circular forms. This order of migration is further supported by the 5.7 kbp internal linear standard migrating more slowly than the linear pUC19 dimer of 5.4 kbp size.

When larger plasmids are analyzed, the same order of migration holds true. Figure 2 shows an electropherogram of a mixture of untreated, linearized, and UV-irradiated pCMV-S2S plasmid samples (5.7 kbp). The most compact ccc monomer and dimer structures migrate faster than linear and open circular structures. However, the resolution of oc monomers and dimers does appear to decrease with increasing plasmid size.

To accurately quantify the different forms of plasmid DNA, a linear correlation between the corrected peak area and the concentration of each structure should be demonstrated (as shown in Figure 3). In this example, an excellent linear correlation was achieved for the analysis of pCMV-S2S over a wide concentration range (0.06 to 4.0 mg/L). The supercoiling ratio of this plasmid sample was determined to be 86 ± 1.5% independent of the concentration applied. Figure 4 illustrates an electropherogram of a typical plasmid preparation, in comparison to the agarose gel image shown in Figure 5.

Conclusions

We have demonstrated the feasibility of using CGE as a quality control tool to assess the homogeneity of plasmid DNA for the purposes of gene therapy or genetic vaccination. This technique performs in a fast, automated, and highly reliable manner. The same order of migration, independent of plasmid size, is attained - simplifying the plasmid identification process.

This analytical method can be used as a both a purity and heterogeneity assay during the cultivation of plasmid-bearing cells, during the purification of plasmid DNA, and during the formulation of plasmid-based therapeutics. This assay provides a basis for reliable stability studies and allows the establishment of quality assurance standards for plasmid DNA structural homogeneity.

References

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Using CEofix* to Develop Automated Routine Hemoglobin Assays

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INTRODUCTION

Electroosmotic flow (EOF) refers to the bulk flow of electrolyte that is generated at the wall of a fused-silica capillary while in the presence of an electric field. Although the characteristic plug-flow of EOF allows much higher efficiencies than does pressure-driven flow, two important elements require attention when developing routine assays utilizing EOF:

1. The intensity of EOF is a function of pH since the amount of ionized silanol is pH dependent. At acid pH, EOF is very low and, at basic pH, EOF is high. At intermediate pH, EOF is poorly reproducible using the same capillary and badly reproducible from capillary to capillary.
2. Ion pairing and hydrophobic interactions between the capillary wall and the analyte may result in peak broadening with a resultant loss in resolution. With large proteins like fibrinogen, a complete loss of the analyte can result as the fraction becomes adsorbed to the capillary wall.

Control of EOF and of wall interaction are, consequently, key factors to get optimum results from CZE.

PRINCIPLE

We describe here a method allowing the above elements to be controlled using a dynamic coating of the capillary. The principle of this coating is as follows: a buffer containing a polycation is introduced first. The polycation sticks strongly to the capillary wall due to a large number of charge interactions. The running buffer is then introduced, containing a polyanion that sticks to the first layer of polycation to form a double layer. This double layer is immobilized on the capillary wall.

The final result is that the last layer (polyanion containing sulfate groups rather insensitive to pH variation) confers a large number of negative charges to the capillary wall, creating a stable and elevated EOF in the presence of an electric field.

This coating is dynamic. It is removed after each run by a simple 1 min rinse with NaOH (0.2 N).

We have used this coating to effectively implement two important hemoglobin assays:[1,2,3,4]

1) HbA1c % measurement and Hb variants at pH 4.6
2) HbA2 % measurement and Hb variants at pH 8.7

Since the coating is dynamic, the switch from the HbA1c analysis at pH 4.6 to HbA2 analysis at pH 8.7 is immediate and doesn't require any re-equilibration of the capillary.

CEOFIX A1c KIT

This kit allows quantification of the HbA1c fraction, the carbamylated Hb, the Hb A1A, A1B, A1D, F, S, C, O-arab, and others that are well separated. CV on HbA1c % determination is typically <3% (Figure 1).

CEOFIX A2 KIT

This kit allows quantification of the HbA2 fraction and of the Hb F, S, C, D, G, E, O-arab, and others that are also well separated (Figure 2). Even in the case of homozygous SS, measurement of relative percent of Hb F and Hb A2 is possible (Figure 3).

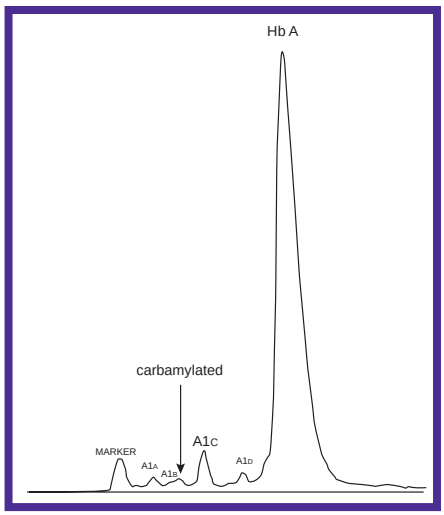

Figure 1. *Experimental Conditions: The instrument used is a P/ACE™ 5000 from Beckman Coulter and CEofix A1 Kit. Capillary (25 µm, 27 cm total length) is first rinsed for 0.5 min with the "initiator," which is maleic acid (32 g/L) and arginine, to pH 4.6 supplemented with a polycation. It is then rinsed for 1 min with the running buffer, maleic acid (32 g/L) and arginine, to pH 4.6 supplemented with polyanion. The sample is introduced by voltage for 5 sec at 8 kV (20 µL whole blood mixed with 100 µL hemolyzing buffer). Electrophoresis is performed at 14.2 kV, 26°C, for 4 min with detection using a UV/Vis detector at 415 nm. The capillary is then rinsed for 1 min with NaOH (0.2N).*

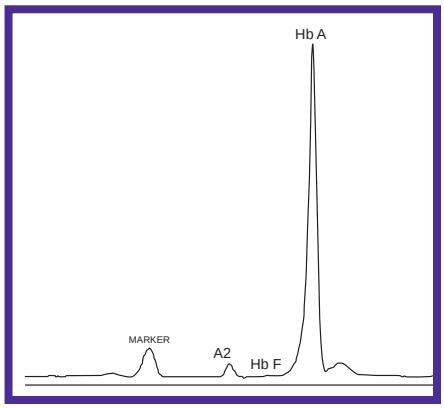

Figure 2. *Experimental Conditions: The instrument used is a P/ACE 5000 from Beckman Coulter and CEofix A2 Kit. Capillary (25 µm, 27 cm total length) is first rinsed for 0.5 min with the "initiator," taurine (115 g/L) and arginine, to pH 8.7 supplemented with polycation. It is then rinsed for 1 min with the running buffer, taurine (115 g/L) and arginine, to pH 8.7 supplemented with polyanion. The sample is introduced by pressure for 3 sec (20 µL whole blood mixed with 100 µL hemolyzing buffer). Electrophoresis is performed at 10.5 kV, 26°C, for 6 min and detection is performed at 415 nm. The capillary is then rinsed for 1 min with NaOH (0.2 N).*

Orthogonal Information

As the migration patterns of the various Hb variants are different, the use of these two Hb kits can generate greater confidence in characterizing the pattern. Electrophoresis of blood hemolyzates from three members of a family are shown in Figure 4. From top to bottom, we show the father and the mother, both heterozygous A/S, and then the child, homozygous S/S.

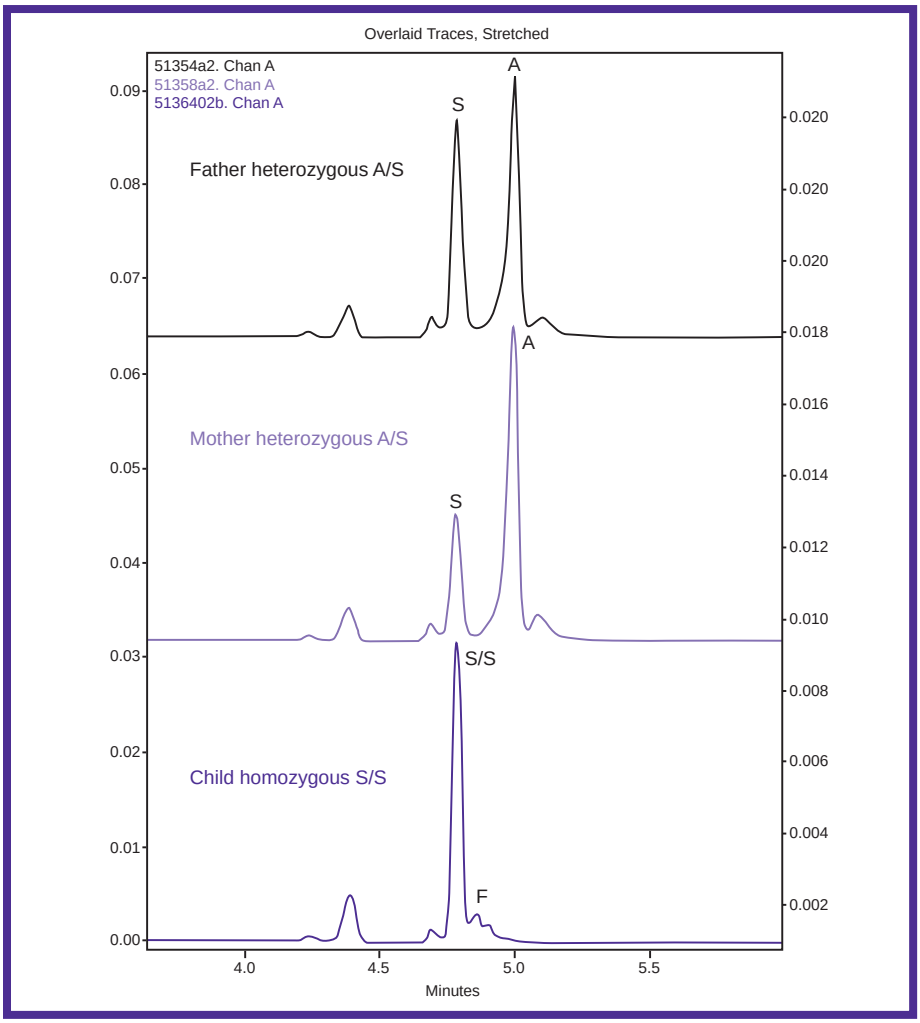

Figure 3. *The separation of three samples from the same family using the CEofix A2 kit. Experimental conditions as described above. This example shows the heterozygous HbA/S character of the parents and the homozygous HbS/S of the child.*

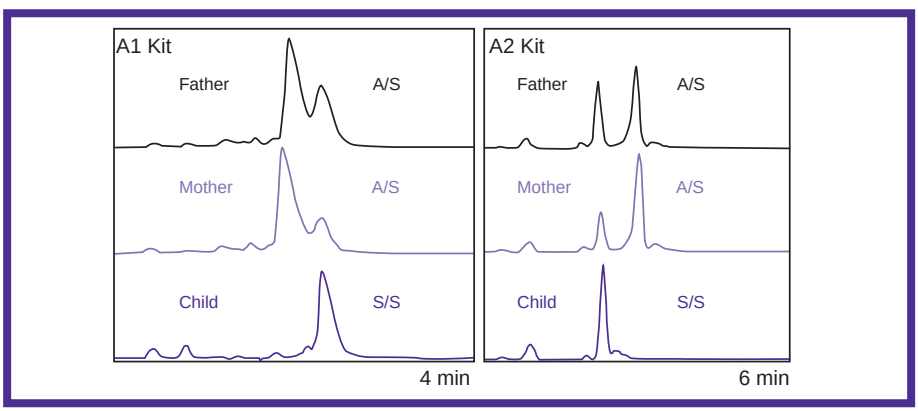

Figure 4. *Electrophoresis of blood hemolyzates from three members of a family are shown.*

Conclusion

The double coating technique represents valuable progress for routine protein separations by CZE. It not only regulates the EOF and suppresses the re-equilibration steps between buffers but also it avoids protein adsorption on the capillary wall. We have successfully implemented this approach in the development of robust assays for the analysis of hemoglobins.

Editor's Comment

The P/ACE 5000 has been developed as a research instrument and is not listed as a medical device with the FDA.

The chemistries described above are a product of Analis, s.a, of Belgium. For more information on these chemistries – you may contact the Analis website at www.analis.com.

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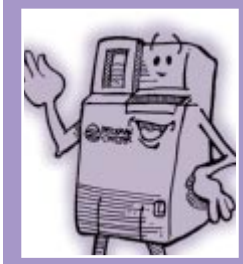
This Is the Dawning of the "Age of Mobility"

DR. Q'S REVIEW



Whether used to describe high-speed transportation or a means to performance success, the term *mobility* may elicit different responses depending upon the context of its use. Although lateral mobility has become synonymous with career development in the corporate culture of America, it has earned a much more favorable connotation in the nomenclature of analytical technology. It is providing a rebirth in the way in which we view capillary electrophoresis (CE)—a change in mindset that we like to call "CE- thinking."

The traditional approach to peak identification has been through the comparison of migration times between sample peaks and standard peaks analyzed under the same conditions. This approach has provided excellent results within CE. However, when there is change in the operational conditions from run to run, either as selected by the operator or resulting from a change in sample matrix or capillary surface, the direct comparison of migration times is prevented.[1]

The most common source of migration time shift is from changing electroosmotic flow. Even under low pH conditions, slight changes in EOF will shift an analyte's migration time. These changes have further impact when you consider making comparisons between different instruments—which functionally may be applying different field strengths, different capillary temperatures, and even different capillary lengths at the point of detection.

To respond to this challenge, we propose taking a different view of electropherogram analysis: decoupling peak shift from analyte separation. This can be accomplished through the evaluation of an analyte's mobility. As the separation of an analyte within an electric field is a result of differences in that analyte's mobility, using this parameter to interpret the separation and identify the peaks makes a lot of sense. In this manner, a change in mobility can be a diagnostic indicating a change in the analyte, providing important information to the analytical chemist.

Mobility is, in fact, the velocity of an analyte per unit field strength, typically measured in units of cm^2 $volts^{-1}$ $seconds^{-1}$. This parameter represents a physical characteristic of a molecule that considers its charge, size, shape, and hydrophobicity within a given buffer system and can be reproduced with high precision.

P/ACE™ MDQ software includes the algorithms necessary to calculate an analyte's effective mobility—a measurement of the apparent mobility minus the contribution by electroosmotic flow (EOF).

$$\mu = \mu_{app} - \mu_{eof}$$

The apparent mobility of an analyte is the sum of both the analyte's true mobility and a mobility contribution from the electroosmotic flow.

$$\mu_{app} = \frac{v}{E} = \frac{L_d L_t}{Vt} \text{ (apparent mobility)}$$

L_d = capillary length to detector
L_t = capillary total length
V = average voltage up to migration time of the peak
t = migration time of the peak
v = velocity

Important to this calculation is the use of actual voltage history rather than programmed voltage. The P/ACE MDQ records the voltage history at every data-point such that an average voltage at any migration time may be calculated. This allows the voltage ramp and any voltage variation that occurs during the run to be accounted for, improving both the accuracy and precision of mobility determination.

$$v = \frac{\sum_{i=1}^{n} v_i}{n}$$

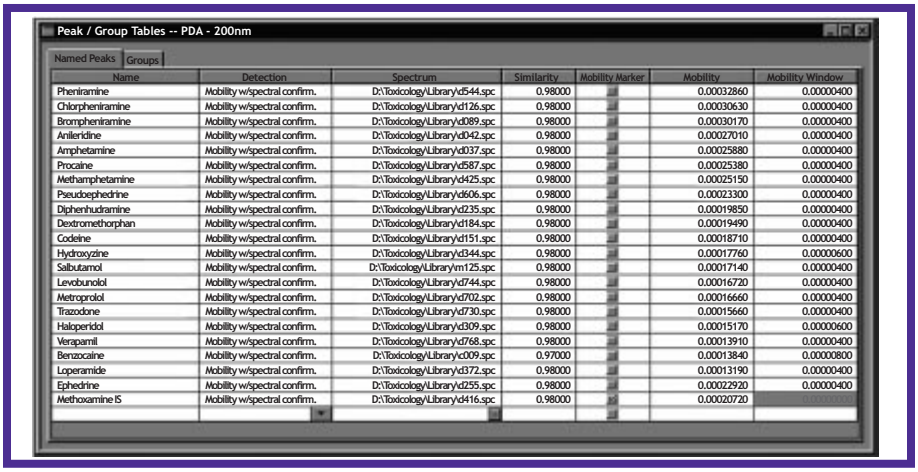
n = data point number at peak migration time
V_i = voltage at data point i

To calculate the effective mobility of an unknown, P/ACE MDQ soft -ware requires you to first identify a mobility marker. Although one's first impression is to use a neutral marker (mobility of 0.000000 cm^2 $volts^{-1}$ $seconds^{-1}$), transformation of the mobility equation allows the use of any marker with a known mobility. In fact, we recommend choosing a mobility marker that is chemically similar to your analyte as this will not only consider changes in EOF but will also compensate for extraneous analyte interactions which may occur with either the sample matrix or capillary surface. You can select your marker from a large compendium of compounds with known mobility values[2] or you can determine the value yourself using established methods.[3]

$$\mu = L_d L_t \left(\frac{1}{Vt} - \frac{1}{V_{ref} t_{ref}} \right) + \mu_{ref}$$

V = average applied voltage up to migration time of the peak of interest
L_d = capillary length to detector
L_t = capillary total length
t_{ref} = migration time of reference peak in the current run
μ_{ref} = defined mobility for the reference peak
V_{ref} = average applied voltage up to migration time of reference peak
t = migration time of the peak of interest

The results are impressive. With care paid to the preparation of your buffer, coefficients of variation (CV) in mobility of less than *0.3%* can be expected from run to run, day to day, capillary to capillary and instrument to instrument.[4]

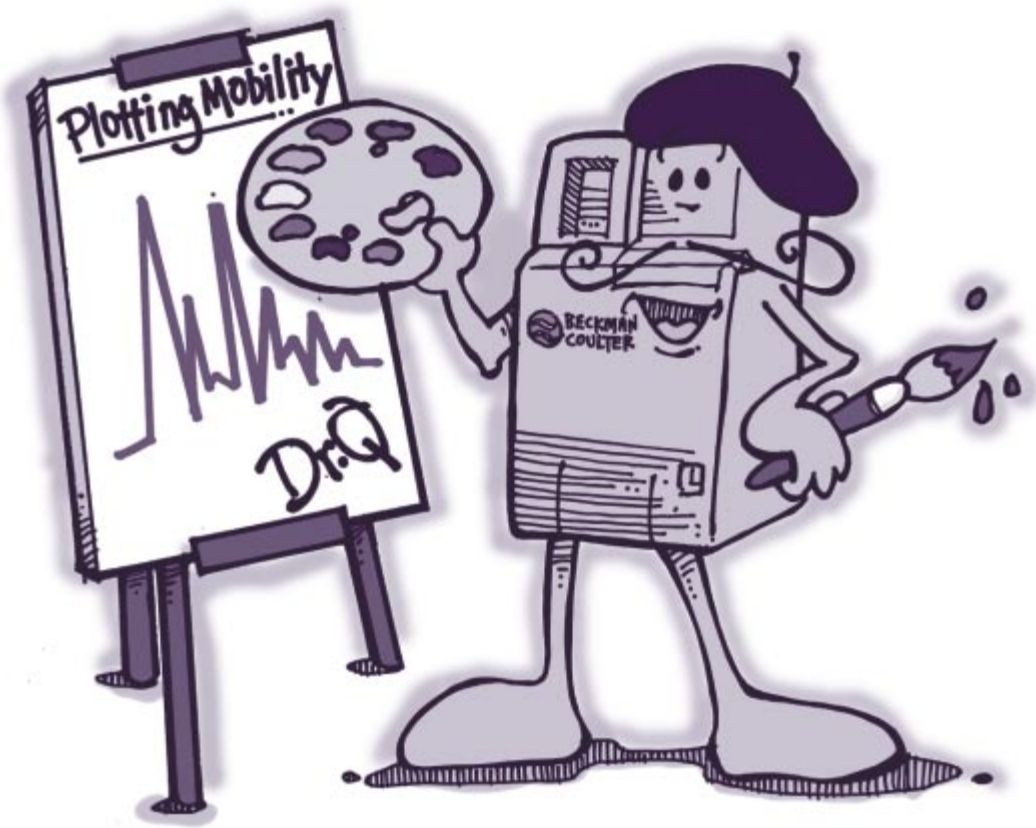


Name	Detection	Spectrum	Similarity	Mobility Marker	Mobility	Mobility Window
Pheniramine	Mobility w/spectral confirm.	D:\Toxicology\Library\d544.spc	0.98000		0.00032860	0.00000400
Chlorpheniramine	Mobility w/spectral confirm.	D:\Toxicology\Library\d126.spc	0.98000		0.00030630	0.00000400
Brompheniramine	Mobility w/spectral confirm.	D:\Toxicology\Library\d089.spc	0.98000		0.00030170	0.00000400
Anileridine	Mobility w/spectral confirm.	D:\Toxicology\Library\d042.spc	0.98000		0.00027010	0.00000400
Amphetamine	Mobility w/spectral confirm.	D:\Toxicology\Library\d037.spc	0.98000		0.00025880	0.00000400
Procaine	Mobility w/spectral confirm.	D:\Toxicology\Library\d587.spc	0.98000		0.00025380	0.00000400
Methamphetamine	Mobility w/spectral confirm.	D:\Toxicology\Library\d425.spc	0.98000		0.00025150	0.00000400
Pseudoephedrine	Mobility w/spectral confirm.	D:\Toxicology\Library\d606.spc	0.98000		0.00023300	0.00000400
Diphenhudramine	Mobility w/spectral confirm.	D:\Toxicology\Library\d235.spc	0.98000		0.00019850	0.00000400
Dextromethorphan	Mobility w/spectral confirm.	D:\Toxicology\Library\d184.spc	0.98000		0.00019490	0.00000400
Codeine	Mobility w/spectral confirm.	D:\Toxicology\Library\d151.spc	0.98000		0.00018710	0.00000400
Hydroxyzine	Mobility w/spectral confirm.	D:\Toxicology\Library\d344.spc	0.98000		0.00017760	0.00000600
Salbutamol	Mobility w/spectral confirm.	D:\Toxicology\Library\m125.spc	0.98000		0.00017140	0.00000400
Levobunolol	Mobility w/spectral confirm.	D:\Toxicology\Library\d744.spc	0.98000		0.00016720	0.00000400
Metroprolol	Mobility w/spectral confirm.	D:\Toxicology\Library\d702.spc	0.98000		0.00016660	0.00000400
Trazodone	Mobility w/spectral confirm.	D:\Toxicology\Library\d730.spc	0.98000		0.00015660	0.00000400
Haloperidol	Mobility w/spectral confirm.	D:\Toxicology\Library\d309.spc	0.98000		0.00015170	0.00000600
Verapamil	Mobility w/spectral confirm.	D:\Toxicology\Library\d768.spc	0.98000		0.00013910	0.00000400
Benzocaine	Mobility w/spectral confirm.	D:\Toxicology\Library\c009.spc	0.97000		0.00013840	0.00000800
Loperamide	Mobility w/spectral confirm.	D:\Toxicology\Library\d372.spc	0.98000		0.00013190	0.00000400
Ephedrine	Mobility w/spectral confirm.	D:\Toxicology\Library\d255.spc	0.98000		0.00022920	0.00000400
Methoxamine IS	Mobility w/spectral confirm.	D:\Toxicology\Library\d416.spc	0.98000		0.00020720	

Figure 1. *Advanced peak identification—2D peak ID strategies combine mobility with spectral signature confirmation providing greater confidence in the identification of an analyte.*

Such precision makes mobility an ideal parameter for both interpreting electropherograms and identifying peaks. To harness this power further, Beckman Coulter has included several new mobility features within 32 Karat™—a new control & analysis software for the P/ACE MDQ series.

With this software, you have the option of identifying peaks by either migration time or mobility. As an analyte's physical mobility is highly reproducible, one can rapidly establish universal peak tables to be used across a variety of methods, assays, and laboratories. Furthermore, mobility detection may be linked with spectral confirmation, providing you with an even higher degree of confidence in analyte detection. Such two-dimensional peak identification strategies are already being applied in drug screening regimens (see Figure 1).



Mobility plots have also been added to the list of user-selectable traces. By simply identifying a mobility marker and selecting the mobility trace option, 32 Karat software will automatically generate an electropherogram with the x-axis plotted as mobility. By utilizing this feature, you can remove variability associated with capillary length, applied voltage, temperature, sample matrix, and electroosmotic flow. This will allow you to more easily interpret the electropherogram and rapidly identify "real change" that may be occurring with an analyte.

Figures 2 and 3 highlight the impact of plotting electropherograms with either migration time or mobility on the x-axis. In this example, our benzoic acid test mix was run under several different applied voltages. This dramatic operational change had minimal effect on the mobility plot, while

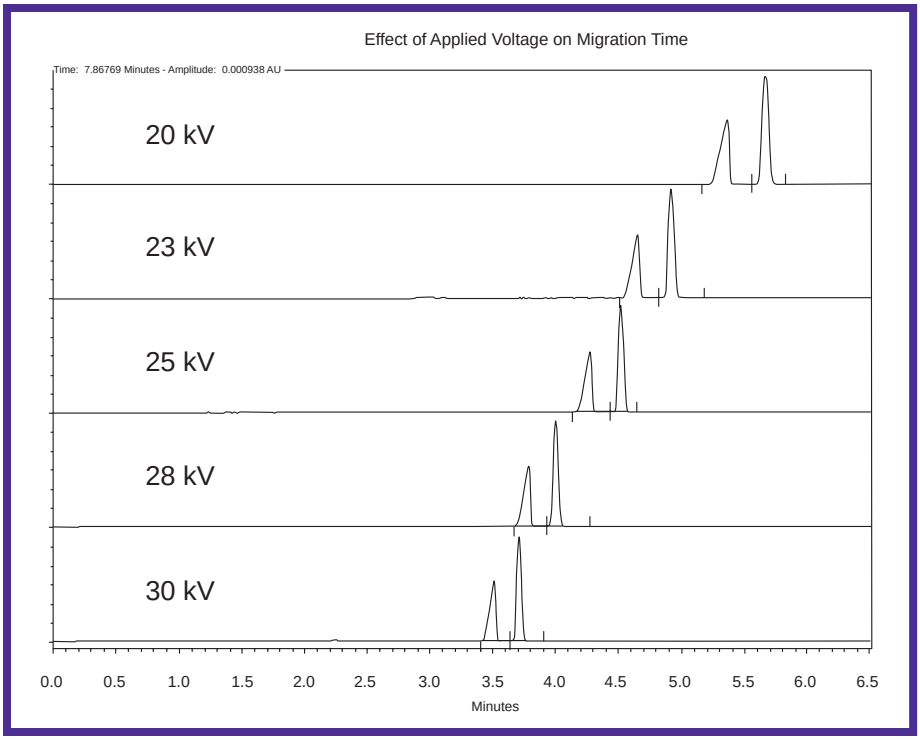


Figure 2. *p-hydroxy-benzoic acid and p-hydroxyphenylacetic acid resolved in a 60 cm capillary (50 cm to detector), 100 mM borate buffer, pH 8.3, capillary temperature thermostatted at 25°C. Plots differ by the voltage applied (30, 28, 25, 23 and 20 kV). X-axis plotted as migration time.*

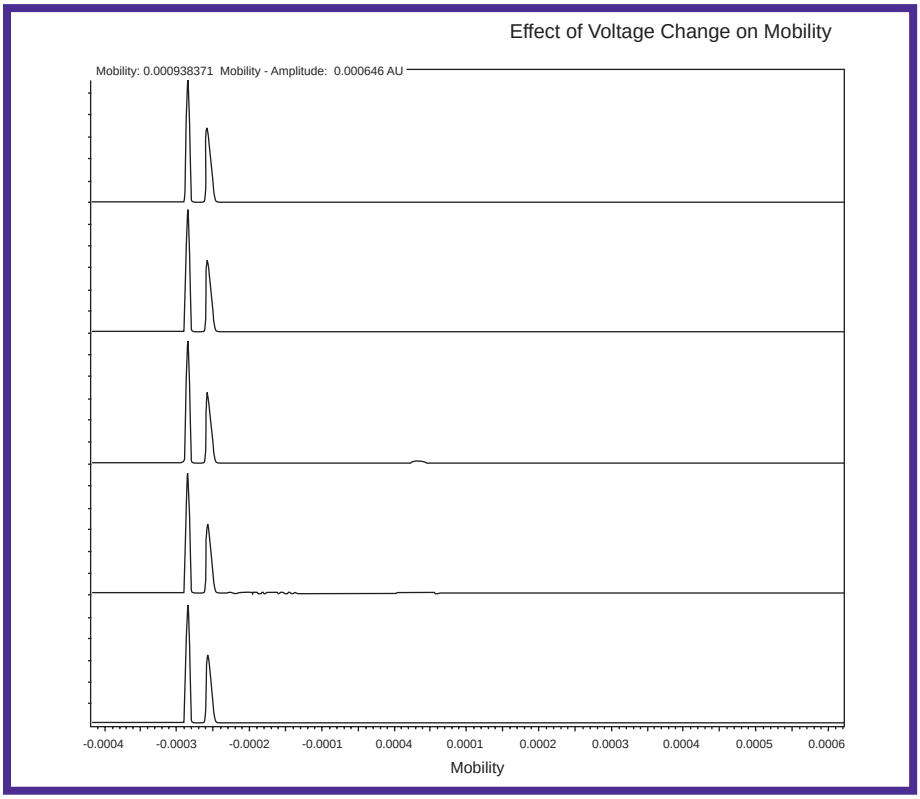


Figure 3. *p-hydroxy-benzoic acid and p-hydroxyphenylacetic acid resolved in a 60 cm capillary (50 cm to detector), 100 mM borate buffer, pH 8.3, capillary temperature thermostatted at 25°C. Plots differ by the voltage applied (30, 28, 25, 23 and 20 kV). X-axis plotted as mobility.*

the migration time plot resulted in an expected shift.

Another good application of the mobility plot is for the measurement of receptor–ligand interactions where the analysis is concentrated around the shift of an analyte's mobility. By plotting mobility, you can assess these changes more easily as shifts due to EOF and general operational change will not be reflected in the electropherogram.

SUMMARY

Capillary electrophoresis has made significant advances over the last decade, moving from a research technology to one of applied routine use for complex polar analytes. Many who looked at the technique in its early years and concluded that it was not for them could well find it worthwhile to check it out today. As with this new age of mobility—we have only just begun.

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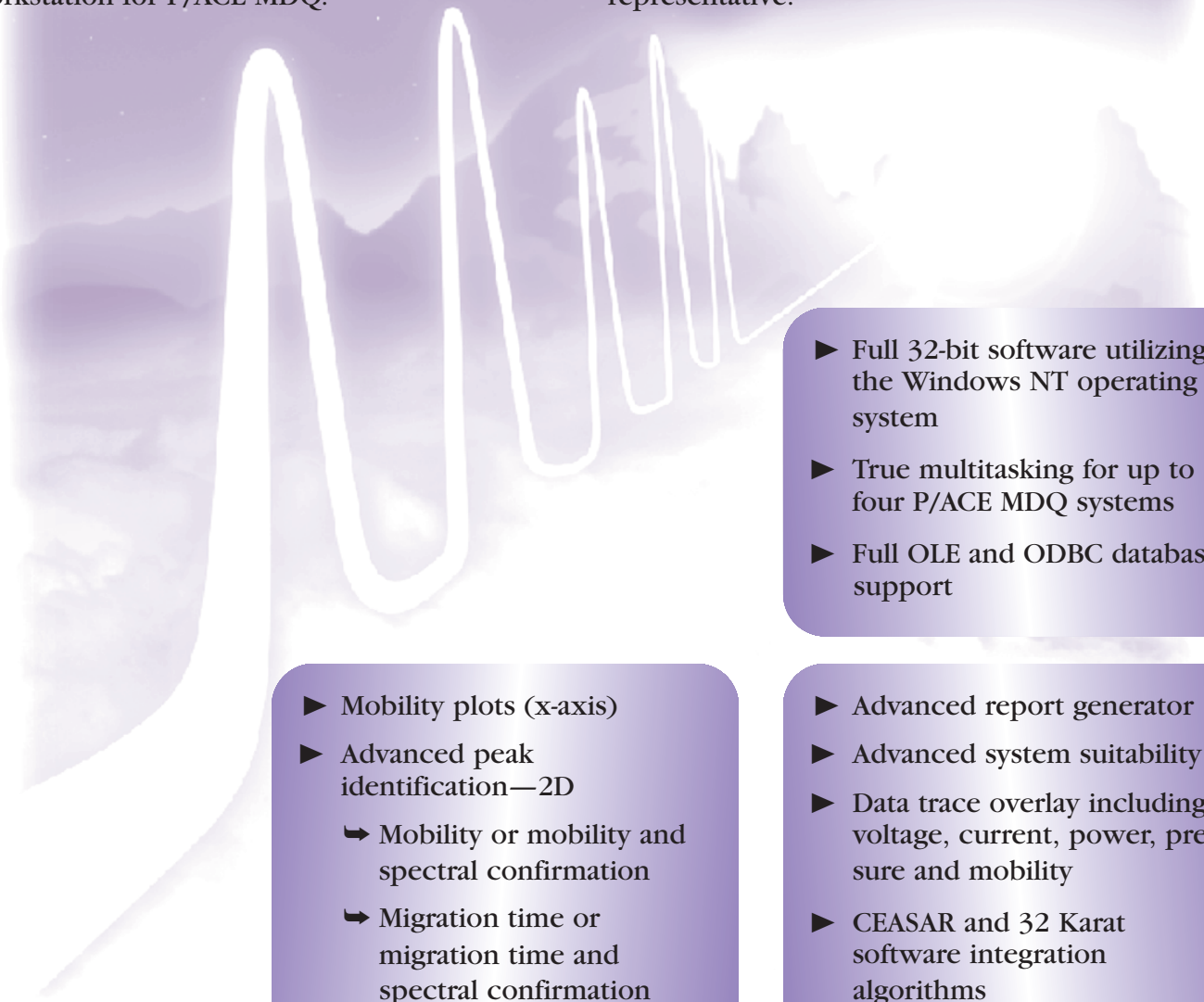
3. Williams, B., Vigh, G. Fast, accurate mobility determination method for capillary electrophoresis. *Anal. Chem. 68,* 1174-1180 (1996)
4. Hudson, J., Malcolm, M., Golin, M. Advancements in forensic toxicology, CZE replaces GC/NPD as the screen of choice for basic drugs. *P/ACE Setter, Vol. 2,* Issue 4 (1998)

Special Offer: 32 Karat Workstation

If you currently own P/ACE™ MDQ software, you are entitled to a special offer to assist you in migrating to our new 32 Karat™ Workstation for P/ACE MDQ.

To learn more about this special offer or to receive a demonstration of this new software, please contact your local Beckman Coulter representative.



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- Software operation wizards

Analysis of Nucleoside Reverse Transcriptase Inhibitors by CE-MS

X. Cahours, Ph. Morin, L. Agrofoglio, M. Dreux
Institut de Chimie Organique et Analytique, Orleans, France

The analysis of nucleoside reverse transcriptase inhibitors has been achieved by on-line capillary electrophoresis–mass spectrometry (CE-MS) in positive-ion mode. The separation of four of four antiretroviral drugs used in HIV therapy (AZT, ddA, D4T, 3TC) has been achieved using the following two procedures:

1. an acid system for the cationic nucleosides separation (ddA, dA, A, C, 3TC)
2. a basic system for the anionic nucleosides separation (AZT, T, D4T, U, and G)

MS-MS detection has been also investigated to reach a low-detection limit for nucleoside antiretroviral drugs (between 5 $\mu g/L^{-1}$ for ddA to 20 $\mu g/L^{-1}$ for D4T).

Experimental Conditions

Acid System

CE conditions:

Capillary dimensions: 70 cm x 50 µm I.D. (150 µm O.D.); electrolyte: formic acid/ ammonia (pH 2.5; I = 10 mM); applied voltage: +25 kV; injection: 20 s (50 mbar); nucleoside concentration: 10 mg/l^{-1}.

MS conditions

Make-up: MeOH/H_2O (95/5) + 0.5% formic acid; 5 $\mu L/min^{-1}$; ionspray voltage: +5 kV; nebulizer gas: air.

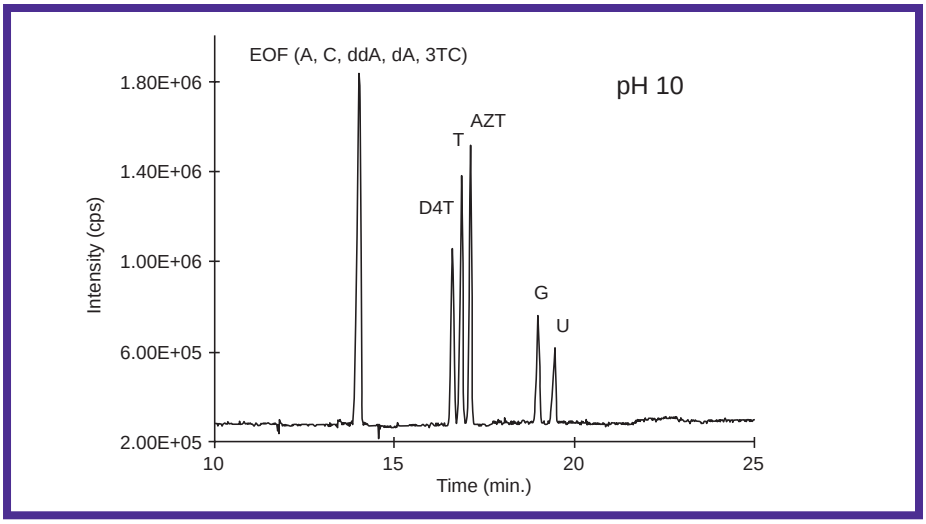

Figure 1. *Illustrates the analysis of the anionic nucleosides using a basic buffer system.*

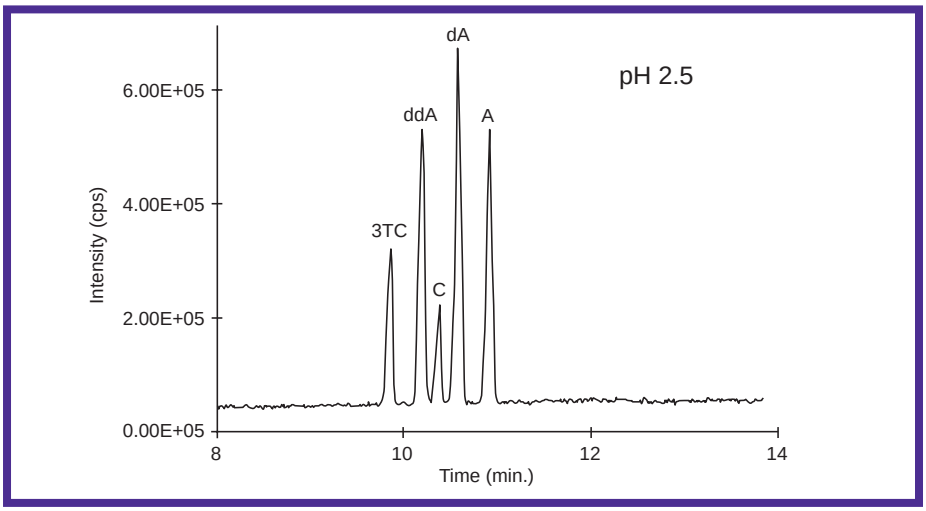

Figure 2. *Illustrates the analysis of the cationic nucleosides using an acid buffer system.*

Basic System

CE conditions

Capillary dimensions: 95 cm x 50 µm I.D. (150 µm O.D.); electrolyte: formic acid/ ammonia (pH 10; I = 50 mM); applied voltage: +25 kV; injection: 20 s (50 mbar); nucleoside concentration: 10 mg/L^{-1}.

MS conditions

Make-up: MeOH/H_2O (95/5) + 0.5% formic acid; 5 $\mu L/min^{-1}$; ionspray voltage: +5 kV, nebulizer gas: air.

CE separation has been performed on a P/ACE™ MDQ apparatus (Beckman Coulter, Fullerton, CA, U.S.A.)

MS detection was carried out on an API 300 triple quadrupole mass spectrometer apparatus (Perkin-Elmer Sciex, Toronto, Canada).

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NEW PRODUCT ANNOUNCEMENT

The CEQ™ 2000XL — Genetic Analysis System

Beckman Coulter, Inc., has just announced the next-generation CEQ™ 2000 DNA analysis system for fully automated DNA sequencing and fragment analysis. The new CEQ 2000XL has been advanced to allow increased read lengths while shortening run times. The readlength/cycle time achieved by the CEQ 2000XL leads the industry and allows an operator to optimize the choice of long fast reads or short fast reads by simply changing a method—there is no need to change gels or capillary lengths.

In DNA sequencing and fragment analysis, a laboratory's productivity is measured by its ability to effectively handle analytical challenges rapidly and accurately. The CEQ 2000XL is a system that adapts to a broad spectrum of requirements, tailored to your specific needs. By focusing on the key elements of automation, performance, and the quality of results, Beckman Coulter has delivered a highly responsive instrument that helps you achieve meaningful results in a timely, cost-effective manner.

To find out more about this new product, contact your local Beckman Coulter representative or visit us online at www.beckmancoutler.com/ceq2000

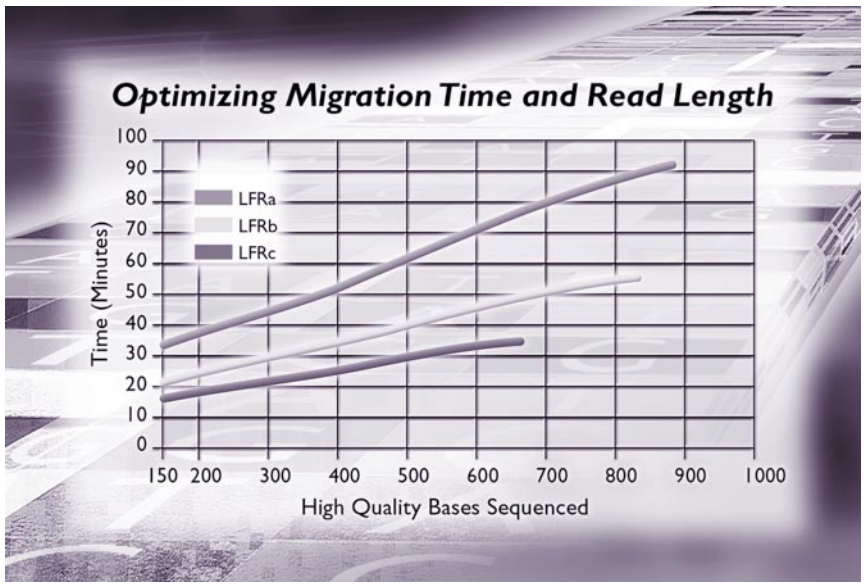

Figure 1. *Optimum sequencing performance for all sample types may be achieved without changing capillary length or gel.*

Volume 4, Issue 2 • October 2000

Ready Your Submission for Putting CE to Work

Capillary electrophoresis is an exciting in-solution technique that continues to challenge all of our imagination. With the goal of recognizing creative thinking and to promote the application of CE, Beckman Coulter once again invites you to submit an example of how you have used CE to solve an analytical challenge in your laboratory—in effect—how you have put CE to work.

Conditions of entry are as follows:

- The separation must have been performed on a P/ACE™ Series instrument.
- Only one entry per scientist.
- Your entry should fit on no more than two pieces of 8.5″ x 11″ or A4 paper. One sheet should illustrate at least one electropherogram. The second sheet should describe the separation and its significance to your work.
- Entries must be received no later than Friday, December 29, 2000. Attendance not required to be considered for the award.

Please include your name, address, e-mail, phone, organization, and title of your entry on the cover sheet, NOT the actual poster.

All entries will be judged by attendees of the 2001 P/ACE User Celebration, to be held January 17 at the New England Aquarium in Boston. Watch for your invitation!

Send your entry to:

Putting CE to Work Award
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Find back issues of *P/ACE Setter* and more information on capillary electrophoresis at:
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