BECKMAN COULTER

P/ACE Setter

The worldwide newsletter for capillary electrophoresis

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Using Binding Isotherms to Accelerate CE Methods Development

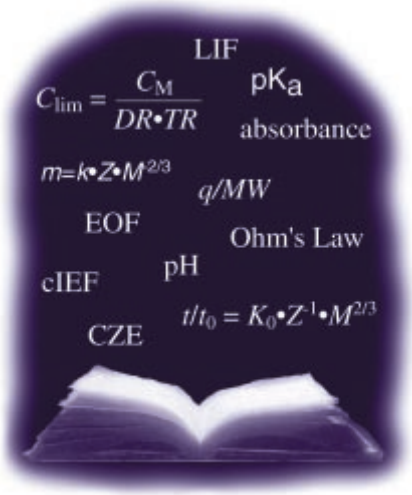
David D.Y. Chen
Department of Chemistry, University of British Columbia, Vancouver, BC, Canada

Methods development can be a very time-consuming process, often relying on a combination of personal experience and trial and error to determine the best conditions for a given separation. This approach can often lead to sub-optimum conditions, as the concentration of additive, pH or even choice of background electrolyte (BGE) may not always be directly intuitive.

In our laboratory, binding isotherms have proven to be a very useful methods development tool, as they provide us with the necessary information to select the optimum BGE/additive system for our analysis. This approach can be applied to separations based on protein binding, protein–sugar interactions, antigen–antibody complexes, glycopeptide interactions, cyclodextrin complexes, as well as chiral separations and non-aqueous separations.

What Is a Binding Isotherm?

A binding isotherm is a mathematical expression that describes changes in the net electrophoretic mobility of an analyte as a function of the additive concentration. This is represented in the form of a binding isotherm plot. When an analyte migrates in the BGE, the charge to size ratio determines its electrophoretic mobility. When an additive capable of interacting with the analytes is introduced, the net mobility of the analytes will be



determined by the mobilities of both the free and complexed analyte along with the capacity factor of each species. An analyte can take many different physicochemical forms that together, through various types of equilibria, constitute its total amount in a system. Each of these forms is defined as a species of this analyte.

The capacity factor, k', is defined as[1]:

$$k'_i = \frac{\text{the amount of analyte in species i}}{\text{the amount of free analyte}} \qquad (1)$$

Therefore, the capacity factor for the free analyte is equal to 1. The capacity factor for the complex formed in a 1:1 interaction between the analyte and an additive is the product of the equilibrium constant and the additive concentration (e.g., $K_{AC}[C]$). Figure 1 shows the binding isotherms of a hypothetical pair of analytes. There are three constants that determine the shape of a binding isotherm: the free analyte mobility, the mobility of the analyte–additive complex, and the equilibrium constant. The two mobilities determine the mobility of the analyte at zero and infinite additive concentrations (assuming solubility is not a problem). The equilibrium constant determines the shape of the binding isotherm. Higher K gives a sharper curvature. Each curve can be expressed mathematically by:

$$\mu_{ep}^{A} = \frac{k'_A}{k'_A + k'_{AC}}\mu_{ep,A} + \frac{k'_{AC}}{k'_A + k'_{AC}}\mu_{ep,AC} = \frac{1}{1 + k'_{AC}}\mu_{ep,A} + \frac{k'_{AC}}{1 + k'_{AC}}\mu_{ep,AC} \qquad (2)$$

where A is the analyte, C is the additive, AC is the complex, μ_{ep}^{A}, $\mu_{ep,A}$, and $\mu_{ep,AC}$ are the net, free, and AC complex mobilities of the analyte, respectively, and k'_A and k'_{AC} are the capacity factors of the free analyte and the AC complex (1 and $K_{AC}[C]$), respectively.

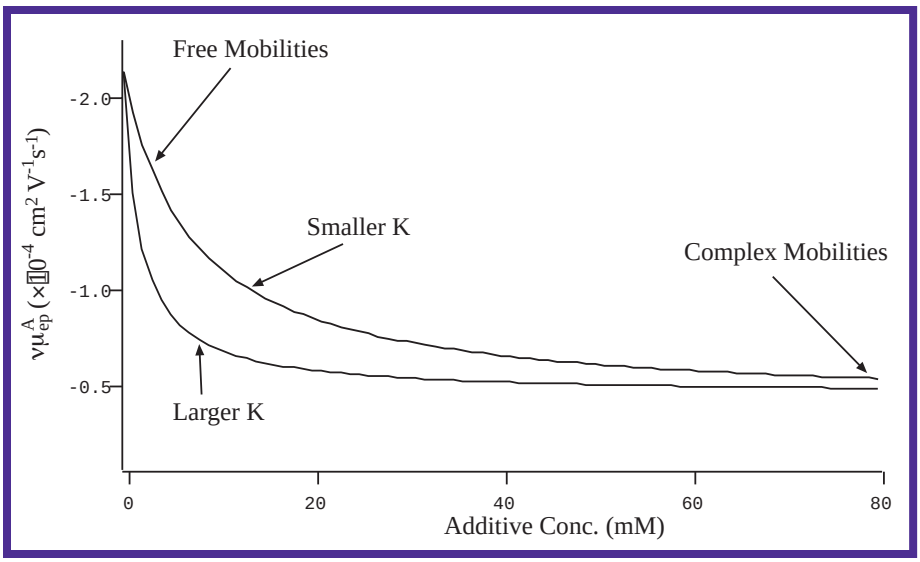


Figure 1. Binding isotherms of a pair of hypothetical analytes.

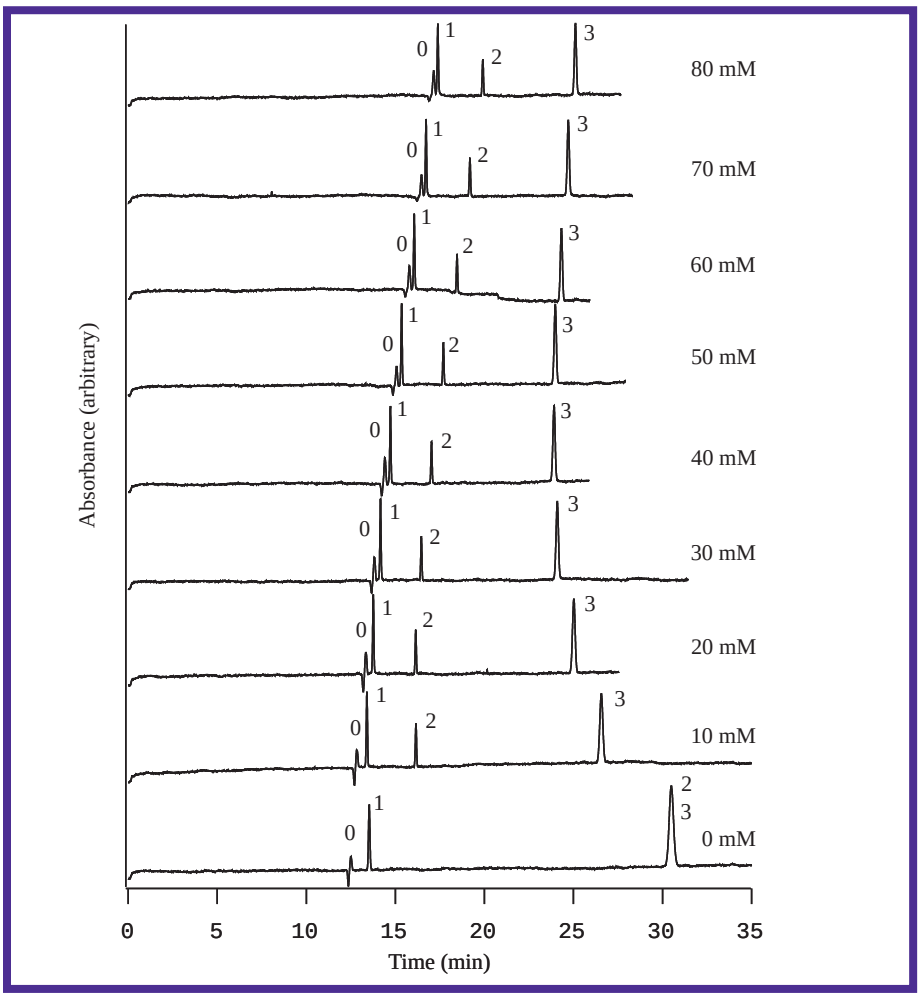


Figure 2. Electropherograms demonstrating the effects of the viscosity and the additive concentration on the migration times of the analytes for HP-β-CD concentrations ranging from 0 to 80 mM. The peaks are: 0. Methanol (EOF marker), 1. Phenol, 2. p-Nitrophenolate, and 3. Benzoate. Reprinted with modification from Peng et al., Electrophoresis, *1997, 18, 706-716, with kind permission from Wiley-VCH Verlag Gmbh.*

What Is the Correction Factor?

When Eq. 2 is used to describe the mobility of the analyte over a range of additive concentrations, the individual mobilities of the analyte species must remain constant. Unfortunately, these mobilities are often influenced by the changes in physical properties of the BGE when an additive is introduced into the system.[2] Figure 2 shows an example of the effects of such changes. It is obvious that the EOF marker (Peak 0) migrates more slowly at higher additive concentrations even though everything else has been kept constant. The additive in this case is hydroxypropyl-β-cyclodextrin (HP-β-CD).

These physical changes can be accounted for by introducing a correction factor (ν) into Eq. 2:

$$\nu\mu_{ep}^{A} = \frac{1}{1 + k'_{AC}}\mu_{ep,A} + \frac{k'_{AC}}{1 + k'_{AC}}\mu_{ep,AC} \qquad (3)$$

In this case, $\nu = \eta/\eta^0$, where η is the viscosity of the buffer with additive, and η^0 is the viscosity of the buffer when the additive concentration is zero.[3] The correction factor normalizes the observed mobilities to those that would occur if the additive did not affect the buffer viscosity. Depending on the additive and the experimental conditions, viscosity or other correction factors may or may not be needed. Care must be taken to ensure that any changes in the electrophoretic mobility caused by changing the additive concentration are only due to the association of the analyte and the additive, and not to other factors. Experimental results can be misinterpreted if the physical changes of the system are not considered.

How to Obtain Binding Isotherms

For simple 1:1 interactions, the following procedure should be used:

First, an appropriate BGE system should be chosen in which the analytes are stable and, preferably, charged. Some analytes may be unstable in acidic or basic environments, and some may bear different charges when the pH is varied (*e.g.,* zwitterionic compounds). If the analyte, *e.g.,* epinephrine, is easily oxidized by the oxygen dissolved in the BGE, an antioxidant such as sodium metabisulfite should be added as well. The free analyte mobilities are obtained from the migration times using this BGE. We should keep in mind that proton is one of the most potent additives. Therefore, it is important to keep its concentration (pH) constant in order to have reproducible results. In addition, some analytes can be separated by simply adjusting the pH of the BGE.

Second, a series of six to eight BGE with various additive concentrations are made, and the net mobilities of the analytes are measured in these solutions. The optimal additive concentration range is related to the strength of the interaction, but the rule of thumb is that the concentrations should include a few points at the higher end, which is often determined by the solubility of the additive. The rest of the points should be evenly spaced to cover as wide a range as possible. Detailed experimental procedures and information on avoiding excessive errors have been published.[2-7] A nonlinear regression based on Eq. 3 gives the binding isotherm.

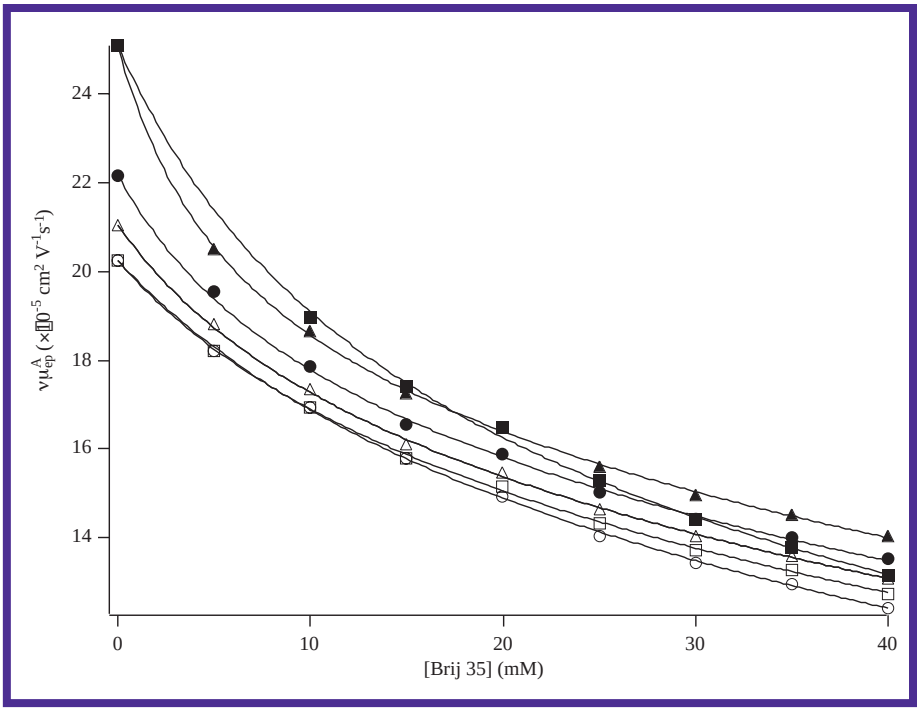


Figure 3. Binding isotherms of 6 porphyrin acids when Brij 35 is used as the additive. The symbols representing the porphyrin acids are: ■ mesoporphyrin, ▲ coproporphyrin, ● pentacarboxylporphyrin, Δ hexacarboxylporphyrin, □ heptacarboxylporphyrin, and ○ uroporphyrin.

How to Use Binding Isotherms

To achieve the best resolution, one should simply choose the additive concentration where the difference of the two mobilities is large enough. Even in cases where both the free mobilities and the AC complex mobilities are similar, the analytes can still be separated if the equilibrium constants are different. This can be explained by the pair of binding isotherms shown in Figure 1. Because the equilibrium constant determines the curvature of the binding isotherm, larger K values lead to a faster change and reach the complex mobility at a lower additive concentration. An optimum resolution can be found when the ratio of the two mobilities is the highest. Therefore, adding more additives to the system doesn't always lead to better resolution, and the optimum separation conditions can be easily missed without the use of binding isotherms.

The example in Figure 2 shows how the mobility of an analyte is affected by the additive concentration. Peak 1 (phenol) is weakly acidic under the experimental conditions, and migrates close to the EOF marker (Peak 0) when no HP-β-CD is added. Even though the migration time of Peak 1 does not change much with the increasing concentration of this additive, it does not imply that there is no interaction. The fact that Peaks 0 and 1 become closer at higher additive concentrations tells us that an interaction is present. However, because the difference between the mobilities of the free analyte and the AC complex is very small, this additive can do little to alter the mobility of the analyte. Peaks 2 and 3, *p*-nitrophenol and benzoic acid, comigrate without the additive, meaning that their free mobilities are the same. However, as the concentration of additive changes, the behavior of the two analytes is quite different. With 10 mM additive,

Peak 2 migrates significantly faster, but adding more additive does not cause further significant changes in its mobility. This is because Peak 2 has a larger binding constant (K), thus the net mobility approaches the complex mobility more quickly. The curve with the higher K value in Figure 1 demonstrates the mobility change in this situation. Peak 3 has a smaller K value, and the migration time changes more slowly, as demonstrated by the curve with a smaller K value in Figure 1. The binding isotherms tell us that only a small amount of additive is needed in this case.

Binding isotherms are especially important when the sample contains many analytes. Figure 3 shows an example in which six porphyrin acids are to be separated. Even though eight conditions were tested, the optimum condition (*i.e.,* 23 mM BRIJ 35*) would still have been missed if binding isotherms were not plotted.[4] It should be noted that the acids are also separated when the BRIJ 35 concentration is 35 mM, and one would have been satisfied using this condition. However, using 23 mM BRIJ 35 not only saves the amount of additive, but also saves time, as the migration time is shorter.

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 immediate benefit is a more rational approach to designing a separation system. The importance of describing such complicated interactions and the ability to predict the effects of these interactions on analyte mobilities also can be applied 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.[5,8,9]

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You can contact Dr. Chen by e-mail at: chen@chem.ubc.ca

Learn how binding isotherms can be used in more complex systems in the next issue of *P/ACE Setter.*

P/ACE Series CE Goes Hollywood

Chances are they won't win an Oscar, but several Beckman Coulter instruments have been finding a lot of work in the motion picture business. The P/ACE™ 5510 System braved the perils of the deep in the summer action-thriller "Deep Blue Sea." The Warner Bros.* film, which premiered in the United States in July, tells the story of genetically

enhanced sharks that terrorize workers at a marine facility 60 miles off the coast of Baja California. The P/ACE was employed to characterize a protein crucial for nerve regeneration, but of course the shark had other ideas! See if you can spot the P/ACE 5510 — it makes a rather dramatic appearance! Not to be outdone, the P/ACE MDQ is being used in the production of "Molly," which stars Elisabeth Shue as a challenged young woman whose genius is unleashed after receiving a miraculous medical treatment. The MGM film is scheduled for release in the United States in early September 1999.

Making CE Methods Robust

In past issues of *P/ACE Setter,* I have discussed the effects of buffers, sample matrix and capillary coating on our CE separations. Following these tips, you now have an idea of the right buffer pH, type of capillary, and wavelength to use. You have accomplished this by attempting to separate your analytes with a variety of buffers, perhaps running with a diode array detector, and monitoring the EOF with a neutral marker.

Our discussion now will focus on optimizing conditions to reach that compromise between speed and resolution, making sure our method is both robust and reproducible. Luckily, CE analysis and re-equilibration times are typically quite short.

Dedicate Your Capillary

Until now, you have probably been using one or two capillaries to scan your samples over an array of buffers. You have chosen the buffer and the pH at which you wish to work. However, the capillary you will now use should only see that specific buffer — in other words, dedicate the capillary. The first time you use the capillary — or any capillary for that matter — you should have a capillary wash method (this was discussed in an earlier article). When you wash your capillary between runs, remember to keep it simple. If you are working with a phosphate buffer at a low pH, there is probably no reason to wash with NaOH — use 1 N phosphoric acid instead. I prefer to use a water rinse in between my acid/base wash and buffer rinse. I also rinse with buffer for at least 10 to 20 capillary volumes. How do you calculate that volume? Visit www.beckmancoulter.com and download the "CE Expert." This calculates the physical parameters of your CE experimentation and allows you to transfer methods between platforms with ease. I use a start-up method for my first run of the day, which stabilizes my system. I run five minutes with my acid or base, five minutes with water, and five minutes with buffer. I then apply a low voltage (e.g., 5-10 kV) for 15 minutes. This gives my lamp 30 minutes to warm up and preps my capillary for the day of analysis ahead.

Inject into Sample Buffer

When introducing my sample into the capillary, I always inject into my sample buffer. This improves the precision of my injection from run to run. Also, if using a P/ACE™ MDQ system, make sure to purchase the new cartridge seals, Part Number 144873. These seals hold the capillary tighter and prevent capillary slippage, increasing sample injection precision as well.

Choose Large-Volume Buffer Tray

If you will be running the same method, you should also consider Part Number 144824, the large-volume buffer reservoir, which allows you to hold 60 mL of buffer — less need to change buffer, less ion depletion, and more runs per day (your software will need to be version 1.5 or greater). Also, if you have been using a diode array detector for methods development, you might consider switching to a UV detector for routine use, if for no other reason than to simply decrease your file size. The P/ACE MDQ was designed for transferring methods from any platform to quality assurance or "routine" analysis. The large buffer reservoir simplifies the repetitive running of a single method (see Figure). The UV detector also simplifies the method using a single-wavelength detector. The idea is to simplify and automate the methods as easily and accurately as possible.

Notes from the Lab

Mark T. Flocco

Use Internal Standard

If you will be quantifying your data, the utilization of internal standards is smart science. Also remember to use the proper CE variables, mobility and corrected area. This will keep your RSDs low and reproducibility high. One last tip: Work on cutting the edges of your capillary neatly and squarely. Use a magnifying glass or dissecting scope to check your cuts. Practice on discarded pieces of capillary. Developing a skill on capillary cutting will go a long way in increasing precision from capillary lot to lot.

Mark Flocco is a Field Marketing Specialist with Beckman Coulter.

Beckman Coulter Celebrates 10th Anniversary of the P/ACE Series

Capillary electrophoresis (CE) was developed in the late 1970s showing much promise for the analysis of highly polar samples. As the future potential of this technology was clear, Beckman Instruments secured several important CE-related patents and, in the mid-1980s, began development on the P/ACE™ (Project/Automated Capillary Electrophoresis).

1989: On July 24, the first P/ACE 2000 system was shipped. This fully automated CE offered instrument control of all operations, pressure and electrokinetic injection, an autosampler, fractionation capability, automated methods development, and precise capillary temperature control (liquid).

1990: Enhancements were made to the cartridge design, providing more effective temperature control, improved detection sensitivity and user assembly.

1991: Beckman introduced the first LC-CE system, linking liquid chromatography and capillary electrophoresis under the Gold™ software control and analysis workstation. This system recognized the need for specialized data handling as capillary electrophoresis utilizes on-column detection. It featured advanced data analysis such as velocity normalized peak area and mobility calculations. In the same year Beckman introduced the first eCAP™ chemistry kit; the ssDNA 100 chemistry, developed to provide a turnkey approach to oligonucleotide purity analysis.

1992: The next major milestone was the launch of the world's first laser-induced fluorescence (LIF) detector, boasting sensitivity enhancements up to 1,000 times that of UV. The LIF system design was modular, adapting to all existing P/ACE systems. These modules employed a unique plug-and-play design, which is automatically sensed and configured by the Gold workstation. This same year Beckman introduced the eCAP SDS 200 kit for the separation of proteins by molecular weight (MW) and a software upgrade to allow the automatic calculation and assignment of MW. As CE-mass spectrometry began to show promise, a CE-MS adapter to bridge the two techniques was developed. The final crowning achievement for 1992 was in addressing sample stability. Beckman developed a sample cooling ring that controlled the temperature of the samples independently from the buffers. Not only could this tray be used for cooling, but also for heating — a key requirement to stabilize and/or accelerate enzymatic reactions.

1993: The P/ACE 2200 System was launched, boasting many new enhancements, including simultaneous voltage and pressure. This capability soon became invaluable for the interface of CE-to-mass spectrometry, as it allowed rapid movement of analytes into the MS. A new high-sensitivity cartridge was also developed. Two new chemistry kits for the analysis of nucleic acids were introduced; the eCAP dsDNA 1000 and the LIFluor™ dsDNA 1000. The latter included a novel intercalating dye (enhanCE), to provide fluorescence of unlabeled DNA. This was also the introduction date for the P/ACE 5000 Series, a new CE platform that added diode-array detection to the growing stable of P/ACE detectors. The P/ACE 5000 Series boasted better detection sensitivity and improved temperature control, allowing the user to — once again — push the "current limits" of methods development.

1994: With the hardware well-developed, the next major investment was in chemistry. Three new chemistries focused on minimizing surface interactions were introduced. These were: 1) The eCAP Amine capillary methods development kit for small molecules; ideal for basic drug analysis. 2) The eCAP Amine methods development kit for proteins which provided a good surface for the analysis of basic proteins. 3) The eCAP Neutral Capillary Methods development kit, ideal for for a wide range of acidic and basic proteins.

1995: Beckman introduced a 635 nm diode laser which provided excitation of the "very stable" cyanine dyes. The first in a series of "chiral analysis" kits also was introduced. These kits included buffers, cyclodextrins and methods for the rational development and optimization of a chiral separation. This was followed by the introduction of the eCAP ds-DNA 20,000 kit, allowing the separation of large-fragment DNA — and eCAP IEF 3-10 kit, providing isoelectric point screening of proteins.

A "continuum philosophy" using technology developed within the bioresearch organization to design analyzers for its clinical diagnostics business was adopted. The Paragon CZE® was born from this develop-

ment. This instrument, a multi-capillary serum protein analysis system, is intended for routine-use clinical diagnostic laboratories.

1996: The eCAP™ *N*-linked oligosaccharide profiling kit was introduced, bringing improved sensitivity, resolution and automation to complex carbohydrate analysis. This release also coincided with the development of a LIF-only P/ACE™ 5000 System. The eCAP ssDNA 100-R kit was introduced, featuring a replaceable gel to streamline the method of assessing oligonucleotide purity. Later in the year, P/ACE Station software was introduced — a powerful new Windows* 95-based, CE-specific software platform.

1997: In March, Beckman introduced another new CE platform — the P/ACE MDQ — an advanced capillary electrophoresis system especially designed for the biotechnology/pharmaceutical industry. This platform can be configured to meet the demands of research, methods development and quality control. The system utilizes a high degree of automation with features such as the 36-pair buffer array and includes a 96-well plate format for compatibility with other automated interfaces. The software includes a sophisticated graphical user interface, to simplify programming, and uses the CAESAR integration algorithm to improve CE analysis.

1998: Beckman Coulter introduced two new options for the P/ACE MDQ: 1) An external detector adapter, which allows the capillary to be interfaced to off-column detectors, extending the powerful P/ACE temperature control right to the point of interface. 2) A large-volume buffer tray. This was designed to replace the buffer array once methods are developed and transferred to a routine-use environment. The tray allows repetitive analysis from hundreds of samples without the need to exchange buffer. A key contribution in 1998 was the introduction of the highly sulfated cyclodextrins, a family of reagents (α, β and γ) demonstrating unprecedented resolution for the separation of enantiomers .These reagents are manufactured with very tight specifications to ensure consistent results from lot to lot. A CD-ROM tutorial describing the fundamentals behind methods design, along with a website (www.beckmancoulter.com/chiral38) highlighting pharmaceuticals already analyzed by this methodology, was introduced. This same year, Beckman Coulter launched the CEQ™ 2000, a multicapillary array DNA analysis system, providing the world's first truly automated DNA sequencer — yet another example of applying CE technology toward the development of dedicated analyzers.

1999: CE development continues: Beckman Coulter introduced the P/ACE MDQ dual-wavelength, laser-induced fluorescence detection module. This detector allows dual-wavelength input for simultaneous excitation of multiple analytes, along with dual-emission detection. Although available as an option to existing P/ACE MDQs, this detector has been integrated into four new application-based systems: The P/ACE MDQ DNA System, P/ACE MDQ Molecular Characterization System, P/ACE MDQ Glycoprotein Analysis System and P/ACE MDQ Carbohydrate System. The latter includes a new high-purity dye, intended for labeling monosaccharides (APTS-M). A CD-ROM tutorial on macromolecule analysis was also developed and can be obtained online at www.beckmancoulter.com/glycoprotein.

2000 and beyond: So there you have it — a decade of CE development accelerating toward the next millenium. With the P/ACE Series instrumentation, Beckman Coulter has established the largest productive base of CE instruments and users in the world. Beckman Coulter continues to lead the field of capillary electrophoresis, strongly committed to further CE research and development. Our direction is guided by you, to apply this core technology to systems providing key analytical solutions.

New!
APTS-M, Monosaccharide Labeling Reagent, 20 mg

APTS-M or 8-aminopyrene-1,3,6-trisulfonic acid, trisodium salt, is synthesized at Beckman Coulter as a highly purified reagent ideal for labeling the reducing terminus of monosaccharides. Its (-)3 charge provides high mobility for very rapid analysis by capillary zone electrophoresis.

Q: How does APTS-M differ from Part No. 501309 — CE-Grade APTS?

A: CE-Grade APTS was designed for the analysis of oligosaccharides. Low molecular weight reagent peaks may comigrate with labeled monosaccharides. APTS-M has been selectively purified to remove these low molecular weight species. APTS-M is stored lyophilized with citric acid, in a ready-for-labeling state.

APTS Formula:	$C_{16}H_8NO_9Na_3S_3$
APTS Formula Weight:	523.39
Product Name:	APTS-M
Description:	Purified APTS, lyophilized with citric acid
Part Number:	**725898**
Container:	Amber, glass vial (screw cap)
Vial Contents:	20 mg APTS; 80 mg Citric Acid
Storage Condition:	2 – 8°C (light sensitive, keep in dark)
Shelf Life:	3 years from date of manufacture
Excitation Wavelength*:	488 nm
Emission Wavelength*:	520 nm

*of the reaction product (after labeling)

DNA Fragment Analysis on the CEQ 2000

CEQING SOLUTIONS

SUSAN STONE

In 1998, Beckman Coulter introduced the CEQ™ 2000 DNA Analysis System, providing DNA investigators with cost-effective automation of DNA sequencing. In 1999, the CEQ application menu has been extended to include molecular size measurements of DNA fragments. This Fragment Analysis feature allows standard DNA genotyping assays to be conducted in a fully automated fashion.

Fragment Analysis on the CEQ 2000, like DNA sequencing, will utilize a 96-well thermocycler plate format for the samples. This format provides seamless continuity between sample preparation with liquid handlers and thermocyclers and fragment analysis on the CEQ 2000, thus reducing the chances of sample mix-up or cross-contamination. The separation vehicle is an array of eight capillaries.

The benefits:

- Automatic sample loading
- No gel plates to clean
- No gels to pour
- No gel pictures to take
- High-sensitivity fluorescent detection replaces radioactivity

As with sequencing, capillaries for fragment analysis will be automatically rinsed and filled with denaturing gel prior to each separation. Eight samples will be run simultaneously in approximately 45 minutes. The operator will be able to multiplex each sample using Beckman Coulter's family of WellRED fluorescent dyes. These fluorescent dyes will be the identifier tags for the specific DNA fragments. One of the dyes is used for the internal sizing standard, while the other three dyes are available for the assay markers.

The CEQ 2000, Windows NT-based software will provide automatic molecular sizing of unknowns. The new genotyping software enhancements will allow for automatic locus and allele tagging and allele list generation. Analyzed data will also be exportable to common genotyping packages.

If you would like to learn more about these Fragment Analysis capabilities, please contact your local Beckman Coulter representative.

Susan Stone is Strategic Market Manager for Genetic Analysis with Beckman Coulter.

A Step Toward Understanding Interactions with Chiral Selectors

All α-amino acids except that of glycine contain a chiral α carbon center. Variation in the side chain of α-amino acids or their derivatives is ideal to develop a model for the systematic analysis of chiral selector interactions. As α-amino acids derivatized with 5-dimethyl-amino-1-naphthalene-sulfonyl moiety (DNS-AA) are readily available, they have become one of the most actively studied species for this purpose.[1-10]

Dr. Chen's Corner

Fu-Tai A. Chen

In this report we present a comprehensive study on the interactions of DNS-AA with a class of highly sulfated cyclodextrins (HS-CDs) produced at Beckman Coulter.[11-13] The CE-based chiral separations were performed using a generalized procedure containing 5% (w/v) HS-CD in phosphate buffer, pH 2.5 with a 50 μm I.D. × 31 cm (10 cm effective length) fused silica capillary. At pH 2.5, all DNS-AAs will be positively charged, migrating toward the cathode, while HS-CDs — being negatively charged — migrating toward the anode.

Table 1 summarizes the separation results of the 13 DNS-AAs, which we ran in HS-α, β, and γ-CD. All migration times reported were normalized with a pyrenetetrasulfonic acid (PTS) marker added to each sample. PTS provides a good marker for this purpose, as it is highly negatively charged and has no interaction with the HS-CDs.

Observations

All the DNS-AAs carry a net mobility toward the anode, indicative of the complexation between the two species and the negatively charged HS-CDs. Each DNS-AA migrates faster in HS-γCD than that in the HS-α and β-CD, characteristic of a stronger affinity of DNS-AA to the HS-γCD. Chiral analyses of all DNS-AAs tested were better resolved in HS-γCD than in the HS-α and β-CD. As the DNS moiety is the common denominator of all species studied here, its interaction with each HS-CD is expected to be similar, if not identical. The DNS-AAs differ from each other in the structure of the side chain that produces significant differences in the migration time.

The size of the hydrophobic cavity and the stereochemistry of the functional moieties around the binding site in HS-CDs play a central role in providing the complexation and chiral differentiation. The overall analysis of DNS-AAs in HS-CDs showed that HS-γCD provides rapid enantiomeric separation of all species studied, mostly within 3 minutes. The resolution for all DNS-AAs in HS-γCD were well above 2.0 except that of the DNS-valine (R=0.5), while that in the HS-α and β–CD produced poor or no resolution — with a substantially longer migration time.

Interpretations

A comparison of the electropherograms of dansyl aliphatic and aromatic amino acids in HS-γCD give us a clue to the potential interactions that may be occurring (Figure 1). All R-isomers of DNS-AAs migrate faster than their corresponding S-isomers, indicative of higher affinity of R-isomer to HS-γCD. The R-isomer of DNS-phenylalanine migrates at 1.35 minutes followed by DNS-norleucine (1.91 min), DNS-leucine (2.06 min), DNS-norvaline (2.13 min), DNS-α-aminobutyric acid (2.26 min) and

Table 1. Chiral Analysis of DNS-AAs in HS-CDs

	HS-αCD		HS-βCD		HS-γCD	
	Resolution	MT(min)	Resolution	MT(min)	Resolution	MT(min)
DNS-α-aminobutyric acid	0.0	*7.64*	1.0	6.12	3.7	2.26
DNS-Aspartic acid	0.0	*8.00*	0.0	*7.27*	11.2	*2.58*
DNS-glutamic acid	0.0	*6.30*	0.5	*7.40*	3.2	*2.99*
DNS-leucine	3.3	*5.46*	0.4	*5.21*	3.8	*2.06*
DNS-methionine	1.3	*7.59*	0.0	*7.26*	10.0	*2.30*
DNS-norleucine	0.9	*5.04*	0.0	*5.92*	6.5	*1.91*
DNS-norvaline	0.0	*6.40*	0.0	*7.00*	2.1	*2.13*
DNS-phenylalanine	2.7	*4.87*	0.0	*4.35*	4.8	*1.35*
DNS-threonine	0.2	*8.88*	0.7	*7.43*	2.0	*4.09*
DNS-valine	0.0	*8.06*	0.2	*6.94*	0.5	*2.51*
DNS-homoproline	ND		ND		2.9	*2.12*
DNS-tryptophan	0.8	3.5	2.0	3.15	3.3	1.69
DNS-serine	0.7	7.7	0.4	6.58	1.0	3.80

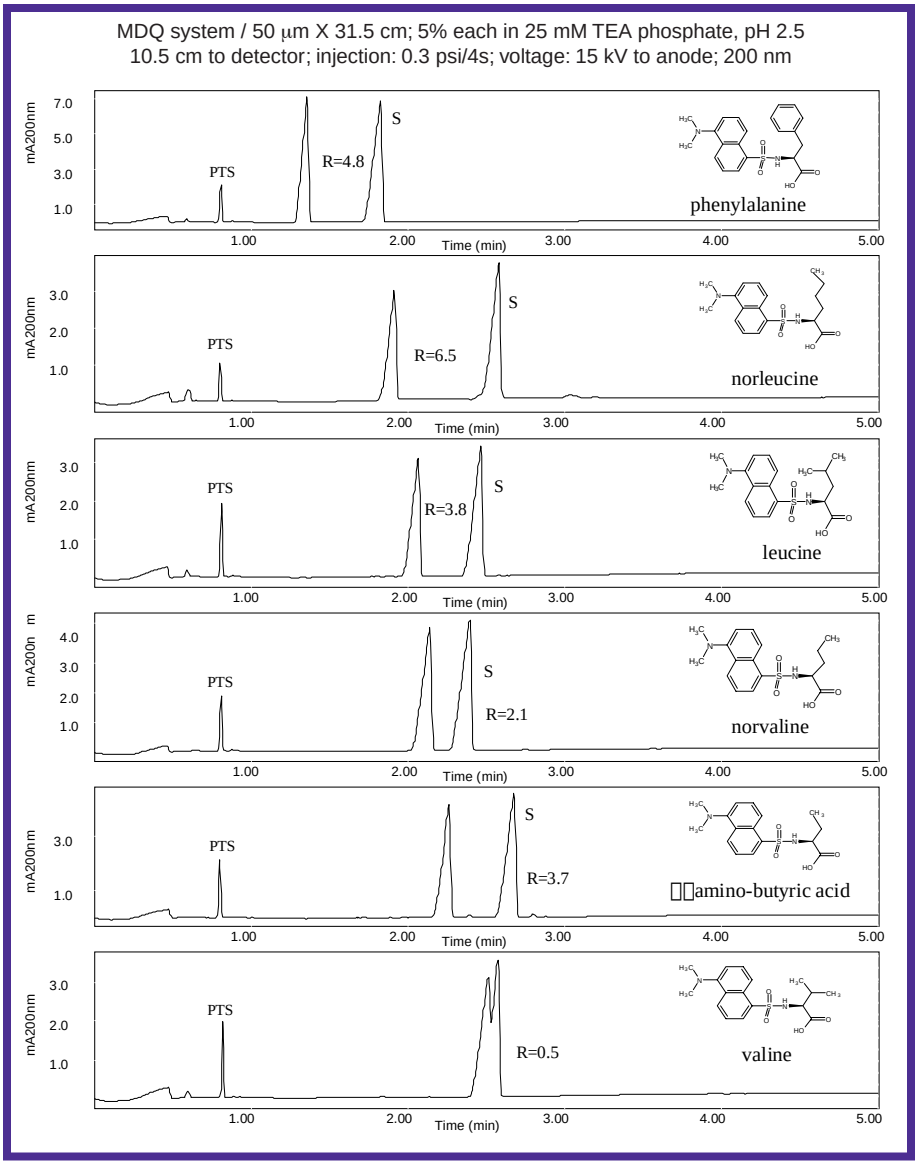

Figure 1. Chiral analyses of DNS- aliphatic and aromatic AA enantiomers by CE in HS-γCD.

DNS-valine (2.51 min). The dansyl group has a positively charged tertiary amino group, which interacts strongly with the sulfate ester at either 6- or 2-position of the glucopyranosyl ring of HS-γCD. The α-carboxylic acid group of the DNS-amino acids is essentially non-ionized at pH 2.5 and could provide hydrogen bonding site with the 3-hydroxyl group of the HS-γCD. The remaining components of aromatic and aliphatic residues are the variable portions of the DNS-AAs and are pivotal for interacting with the wider side of the hydrophobic pocket in HS-γCD. The benzyl moiety in DNS-phenylalanine has the highest affinity to the hydrophobic binding cavity of the HS-γCD, followed by n-butyl (DNS-norleucine), 2-methylpropyl (DNS-leucine), propyl (DNS-norvaline), ethyl (DNS-butyric acid) and isopropyl (DNS-valine). The benzyl group extends to the interior of the hydrophobic pocket of HS-γCD, presumably to yield the most stable complex. Meanwhile, the isopropyl moiety in DNS-valine produces the least stable complex among the DNS-AA species (Figure 1), presumably due to the less favorable interaction of the geminal dimethyl moiety near the rim of the cyclodextrin cavity that is relatively hydrophilic.

The R-isomers of DNS-aspartic and glutamic acids migrate at 2.58 and 2.99 minutes with resolution of 11.2 and 3.2, respectively. The side chain carboxylic acid is non-ionized at buffer pH 2.5 and is one carbon atom apart between each species, yet produce such a profound difference on the chiral resolution. Similarly, DNS-norleucine, leucine and methionine contain four atoms on the side chain, counting from the α-carbon, that migrate at 1.91, 2.06 and 2.3 minutes, but with very different resolution of 6.5, 3.8 and 10.0, respectively, in HS-γCD, demonstrating pronounced resolution differences with only subtle changes in the side chains of DNS-AAs.

Proposed Mechanism

The dansyl group is the common denominator in all DNS-AAs with the positively charged tertiary amino group in close proximity to the sulfate ester at the 2-position of the glucopyranosyl ring of HS-γCD. The α-carboxylic acid group of the DNS-AA is essentially non-ionized at pH 2.5, which could provide hydrogen bonding site with the 3-hydroxyl group of glucopyranosyl ring around the rim of HS-γCD. This leaves the remainder of the side chain to roam into the cavity of HS-γCD. The fact that all DNS-AA racemates in Table 1 were resolved with relatively short migration times in HS-γCD supports the hypothesis that the larger cavity in HS-γCD not only accommodates the side chain, but also interacts with a portion of the naphthylsulfonamide moiety of the DNS — AAs. A proposed model for this interaction is illustrated in Figure 2. Such an arrangement would be consistent with subtle differences in DNS-AA side chains having such a dramatic impact on chiral resolution.

Dr. Fu-Tai A. Chen is Manager of Probe Technology, Instrument Systems, Beckman Coulter.

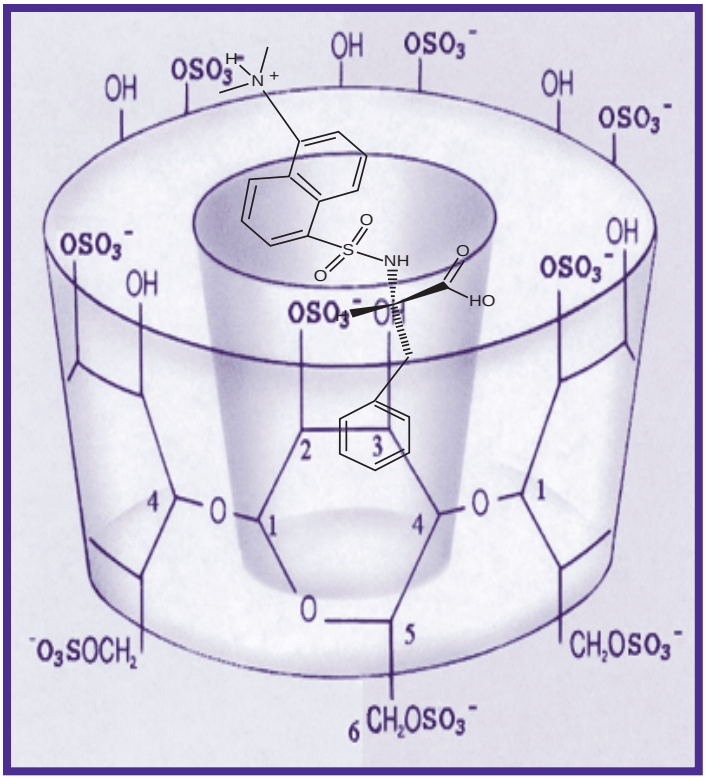

Figure 2. Proposed Mechanism of HS-γCD interaction with DNS-Phenylalanine.

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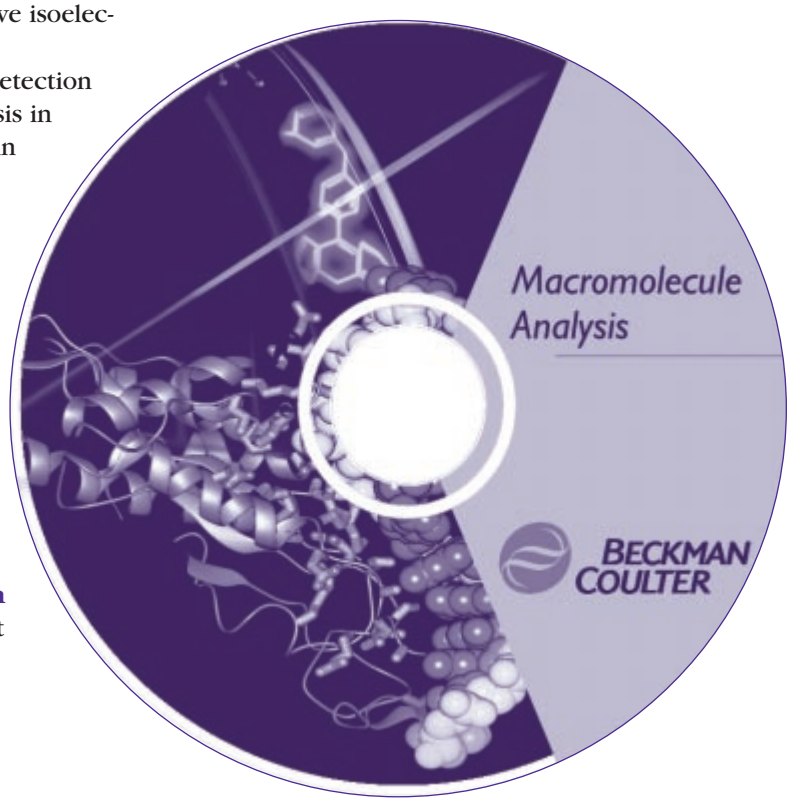

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