Products

♦ Brain Microdialysis Probes for Transgenic Mice

New brain microdialysis probes with intracerebral cannulae have been developed by Bioanalytical Systems, Inc. (BAS) for use in transgenic mice, rats and other rodents. The MBR line of probes expands the offerings of microdialysis probes available from BAS for studies in brain, dermis, bile, blood vessels and other tissues.

These lightweight probes are secured in the guide by a low-insertion force elastomeric fit which accommodates the fragile skull of a mouse. Their small size makes them ideal for implanting multiple probes in a single animal. Guide cannulae can be implanted as close as 3.2 mm on center. Probes implanted without a guide can be placed within 2.4 mm of one another. Multiple MBR probes can be implanted in a rat, and depending upon the targets, two MBR probes may even be implanted in a single mouse.

MBR probes are available with 1 or 2 mm membrane lengths. The membrane offers a 38,000 MWCO (molecular weight cutoff) and is suitable for a broad range of neurotransmitters and drugs. A 24-karat gold coating on both the probe and guides provides an inert surface compatible with tissues and biological fluids.

♦ Bioanalytical Systems, Inc. has just introduced a unique system, CHADS for Vials™, for labeling lab samples legibly, quickly and easily.

Chads are thin, flexible, pre-numbered tags, printed on waterproof and solvent-resistant material. They use no adhesive and are available in a variety of colors.

Chads adhere to the vials using friction. The hole in the Chad is slightly smaller than the diameter of the vial, so when the vial is inserted into the hole, the Chad is stretched tightly around the vial. When the vial is lifted, the Chad clings to its neck, whether the vial is capped, stopped, open or closed. The Chads come in sheets, so you can label one vial at a time, or label an entire rack of 192 vials (enough for two 96-well plates) in one motion. Load them into a fraction collector, a centrifuge, autosampler or freezer; when the vials are thawed, the Chads will be just as legible as the day they were applied. You will have no more tedious hand labeling or smudged, illegible labels with the Chads system.

For more information or to request a free sample and a copy of the company's 3-minute demonstration CD, go to ***www.culex.net***.

♦ Automated Serial Blood Sampler for Research Animals

Bioanalytical Systems, Inc. has introduced the Culex®Automated Blood Sampler (ABS). The system provides for robotic sampling of blood (10-250 µL per sample) from cannulated rats for research in pharmacokinetics, drug metabolism and drug safety assessment.

Four freely-moving animals are housed on a wheeled cart with refrigerated microfraction collectors, as well as collectors for urine and feces. Sterilized tubing sets maintain aseptic transfer of blood and replacement saline. A notebook computer enables the user to program independent collection protocols for each animal. Time and volume of blood draws are designated, along with the option to dilute the sample with specified volumes of heparinized saline. Food and water are provided ad lib and simultaneous drug infusions, microdialysis or implanted biosensors are feasible. Samples are collected in vials compatible with 96-well plates for centrifugation and sample preparation. Both serum and plasma can be managed. The blood sampling process does not stress the animals. For example, sleep is not disturbed. Animals can be maintained on the system for five days or more with excellent catheter patency. ***(www.culex.net)***

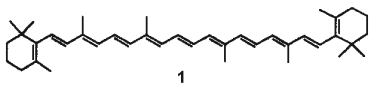
In the EC Literature

Complied by:
Adrian W. Bott, Ph.D.
Bioanalytical Systems, Inc.
West Lafayette, IN

Email:
awb@bioanalytical.com

♦ Electrochemical Properties of Natural Carotenoids

D. Liu, Y. Gao and L.D. Kispert, J. Electroanal. Chem. 488 (2000) 140-150.



1

The electron transfer properties of carotenoids (e.g. **1**) are of considerable interest due to their involvement in photosynthetic processes. The electrochemical behavior of 12 carotenoids was investigated in this study using cyclic voltammetry and (Osteryoung) square wave voltammetry (on a BAS 100B/W). Simulation software (DigiSim®) was used to extract thermodynamic and kinetic parameters from the cyclic voltammograms.

It was found that some carotenoids showed a single (two-electron) oxidation process, whereas others showed two sequential one-electron oxidations. (This difference in behavior appeared to be related to the presence of a carbonyl group.) It was also observed that the cationic carotenoids were involved in a number of homogeneous chemical reactions, including deprotonation and disproportionation reactions. The stability of the cations also appeared to be related to the presence of a carbonyl group. Cations containing this group were typically less stable than those that did not.

♦ Parameters Important in Tuning the Response of Monolayer Enzyme Electrodes Fabricated Using Self-Assembled Monolayers of Alkanethiols

J.J. Gooding, P. Erokhin and D.B. Hibbert, Biosens. Bioelectron. 15 (2000) 229-239.

The optimization of biosensors based on the immobilization of enzymes on an electrode surface continues to be an area of intensive study. In this article, the parameters affecting the performance of an enzyme electrode based on the immobilization of glucose oxidase on a gold surface using self-assembled monolayers of alkanethiols were assessed using a theoretical model in addition to experimental observations made using a BAS 100B.

Both theory and experiment showed that these enzyme electrodes had good sensitivity and a broad dynamic response, which was attributed to the short distance between the electrode surface and the immobilized enzymes. The response was shown to be dependent upon the rate of enzyme turnover, and hence was sensitive to the enzyme loading and the concentration of the mediator in the sample solution. (In contrast, variation in the rate of mass transport had little effect.) However, it was noted by the authors that further characterization of these electrodes was required (e.g. their effectiveness in biological samples and their long-term stability).

♦ A Micro Flow Injection Electrochemical Biosensor for Organophosphorus Pesticides

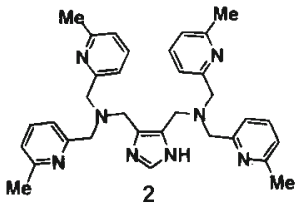
T. Neufeld, I. Eshkenzai, E. Cohen and J. Rishpon, Biosens. Bioelectron. 15 (2000) 323-329.

Organophosphorus pesticides inhibit esterase enzymes, and hence there have been a number of methods proposed for the quantitative determination of these pesticides based on their inhibition of, for example, acetylcholine esterase (AChE). In this study, the inhibition of AChE by dimethyl 2,2-dichlorovinyl phosphate was studied by flow injection analysis using screen-printed electrodes covered by an enzymatic membrane. (The amperometric measurements were conducted using a BAS potentiostat.) The enzymatic activity was monitored by measuring the current due to the oxidation of ferrocyanide at the electrode surface (which is generated by the reduction of ferricyanide by thiocholine, a product of the enzyme reaction).

Since the inhibition of the enzyme was not fully reversible, any sensor based on the above protocol would have to be disposable. However, it was observed that such a disposable sensor would have a number of advantages including good sensitivity and speed, small size and low cost. It would also allow measurements of small sample volumes and could readily be used on site with little training required.

♦ Synthesis and Characterization of Imidazolate-Bridged Dinuclear Complexes as Active Site Models of Cu, Zn-SOD

H. Ohtsu, Y. Shimazaki, A. Odani, O. Yamauchi, W. Mori, S. Itoh and S. Fukuzumi, J. Am. Chem. Soc. 122 (2000) 5733-5741.



2

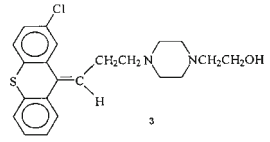
Copper-zinc superoxide dismutase (SOD) catalyzes a two-step dismutation of superoxide to oxygen and hydrogen peroxide through the redox activity of the copper ion and is important in preventing oxidative damage. As is the case with many metalloproteins, the structure-activity relationships of SOD have been elucidated using coordination complexes as models of the active site.

In this study, novel dinucleating ligands containing an imidazolate bridging group (e.g. **2**) were used to synthesize dinuclear copper-zinc coordination complexes as models for SOD. The analogous copper-copper complexes and mononuclear copper complex were also synthesized for comparative purposes. These complexes were characterized by X-ray crytallography, magnetic susceptibility, ESR and cyclic voltammetry (using a BAS 100B). The molecular structures of all the complexes showed coordination of a solvent molecule which could be substituted to allow coordination of superoxide. The cyclic voltammograms of the dinuclear copper complexes showed two redox processes, consistent with significant metal-metal interaction.

The potential of the first reduction was about 200 mV positive of the redox potentials of the mononuclear complexes, as was the redox potential of the copper ion in the copper-zinc complex. The superoxide dimutase activities of the dinuclear complexes were significantly greater than those of the mononuclear complexes, and it was proposed that this increased activity was due in part to the modulation of the copper redox potential by the second metal ion.

♦ Voltammetric Investigation of Oxidation of Zuclopenthixol and Application to Its Determination in Dosage Forms and in Drug Dissolution Studies

Z. Senturk, S.A. Ozkan, Y. Ozkan and H.Y. Aboul-Enein, J. Pharm. Biomed. Anal. 22 (2000) 315-323.



3

Zuclopenthixol (**3**) is a thioxanthine neuroleptic that is used for schizophrenia and other psychoses. In this study, the redox properties of **3** were studied and then used for pharmaceutical analyses.

It was shown by cyclic voltammetry using a BAS 100B/W that **3** undergoes 3 pH-dependent irreversible oxidations, the first of which was assigned to a 2-electron oxidation to the sulfoxide. The linear sweep and differential pulse voltammograms were also recorded. Since the peak currents were larger in the differential pulse voltammograms, this technique was used for quantitative determinations. The conditions for quantitative measurements were optimized with respect to the pH and the potential pulse parameters. Under the optimized conditions, the calibration curve was linear in the range 8 x 10^{-7} - 2 x 10^{-4} M, with a detection limit of 2.2 x 10^{-7} M and a standard deviation of 1.5% for the peak current.

This method was used for determination of **3** in tablets and oral drops as well as tablet dissolution studies. The results from this method were comparable with those from the standard spectrophotometric method, with the voltammetric method having the advantages of speed and simplicity.

DigiSim is a registered trademark of Bioanalytical Systems, Inc.

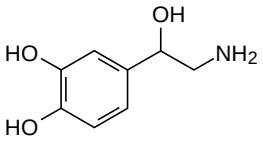
In the LC Literature

Complied by:
Bruce P. Solomon, Ph.D.
Bioanalytical Systems, Inc.
West Lafayette, IN

Email:
bp@bioanalytical.com

♦ Chronic Morphine Treatment and Withdrawal Increase Extracellular Levels of Norepinephrine in the Rat Bed Nucleus of the Stria Terminalis

J.A. Fuentealba, M.I. Forray and K. Gysling, J. Neurochem. 75 (2000) 741-748.



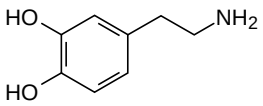
norepinephrine

The role of the ventral bed of the stria terminalis in morphine addiction was studied by determining levels of norepinpehrine (NE) and glutamate (Glu) following chronic morphine administration. NE was determined from *in vivo* microdialysis samples by electrochemical detection using a BAS LC-4C amperometric detector with a glassy carbon electrode set to +650 mV vs. Ag/AgCl. NE was separated on a BAS UniJet microbore column. Glu was determined by fluorescence detection of *o*-phthalaldehyde-derivatized samples separated on a C_{18} column.

Morphine treatment significantly increased NE concentrations. Inducement of withdrawal symptoms by administration of naloxone caused a further increase. Glu concentrations were not significantly influenced by any treatment.

♦ The Potent, Selective mGlu2/3 Receptor Agonist LY379268 Increased Extracellular Levels of Dopamine, 3,5-Dihydroxyphenylacetic Acid, Homovanillic Acid & 5-Hydroxyindole-3-Acetic Acid in the Medial Prefrontal Cortex of the Freely-Moving Rat

J. Cartmell, K.W. Perry, C.R. Salhoff, J.A. Monn and D.D. Schoepp, J. Neurochem. 75 (2000) 1147-1154.



dopamine

LY-379268 is an experimental drug with potential antipsychotic action. This study looked at the effects of its administration on catecholamines and their metabolites in freely-moving rats. Extracellular levels of these analytes were sampled from the prefrontal cortex using BAS *in vivo* microdialysis equipment (5-mm guide cannula, 4 mm probe, liquid swivel, syringe pump and fraction collector). Detection was with a BAS dual amperometric detector fitted with a glassy carbon electrode. DOPAC, HVA and 5-HIAA were detected by oxidation at an upstream electrode set at +700 mV (vs. Ag/AgCl), while dopamine was detected by subsequent reduction at a downstream electrode set at +100 mV.

Systemic LY-379268 increased extracellular levels of dopamine, DOPAC, HVA and 5-HIAA about two and a half times.

In the MD Literature

Complied by:
James F. Gitzen, Ph.D.
Bioanalytical Systems, Inc.
West Lafayette, IN

Email:
jgitzen@bioanalytical.com

♦ The Role of Afferents to the Locus Coeruleus in the Handling Stress-Induced Increase in the Release of Noradrenaline in the Medial Prefrontal Cortex: a Dual-Probe Microdialysis Study in the Rat Brain

H. Kawahara, Y. Kawahara, B.H.C. Westerink, European J. of Pharmacology, 387, 279-286, 2000.

Microdialysis probes were implanted in the locus coeruleus (LC) and the ipsilateral medial prefrontal cortex (PFC) in order to identify the neurotransmitter system(s) involved in the handling stress-induced increase in noradrenaline (NA). Following surgical recovery, one of various receptor (a2, $GABA^A$, $GABA^B$, cholinergic, CRF, NMDA and non-NMDA glutamate)-specific antagonists was retro-dialyzed into the LC of each animal while NA was sampled at the PFC. During drug exposure the animals were handled for ten minutes to induce the NA stress response.

The authors found that a2, NMDA glutamate and CRF receptors altered the handling stress-induced NA response, while blocking glutamatergic, GABA and cholinergic receptors had no significant effect.

[With their small 3.2 mm footprint, BAS' new miniature MBR brain microdialysis probes and guides, developed for doing microdialysis in mice or for the implantation of multiple brain microdialysis probes, would be ideal probes and guides for such multiple probe studies. See the description of the new MBR probe on page 76 and 93 of this issue of *Current Separations*. J.G.]

♦ Microdialysis Sampling of the Isothiazolone, PD-161374, and its Thiol and Disulfide Metabolites.

M. Ye, D.T. Rossi, C.E. Lunte, J. of Pharmaceutical and Biomedical Analysis, 2000, 24, 273-280.

Traditionally, drug metabolism studies are conducted by withdrawing blood, cleaning up the sample so it can be analyzed, followed by the actual sample analysis. In the case of some drugs however, the sample clean-up steps can alter the distribution of the drug's metabolites. PD-161374, an isothiazolone, is just such a drug whose metabolite distribution is skewed by the sample clean-up. In this case, the distribution of the isothiazolone, thiol and disulfide forms of the compound are severely disturbed by the protein precipitation stage of the sample clean-up. This causes important information about the relative distribution of these compounds to be lost, and possibly invalid conclusions to be drawn.

Vascular microdialysis samples do not require such sample clean-up prior to analysis. This is because the large molecular weight compounds in blood that normally interfere with analysis will not cross the dialysis membrane. Thus vascular microdialysis samples are clean samples that can typically be analyzed directly without sample clean-up. Ye and colleagues used this to their advantage to enable them to determine reliably the proportions of isothiazolone, thiol and disulfide forms of PD-161374 both *in vitro* and *in vivo*. During the *in vitro* microdialysis work they also collected blood aliquots which underwent conventional clean-up and analysis. Their paper vividly demonstrates how dramatically the sample clean-up process can alter the results for such compounds.

♦ Potassium Permanganate Can Mark the Site of Microdialysis in Brain Sections.

M-H Sun, L. Hildebrandt, A.K. Curran, R. Darnall, G. Chen, J.J. Filano, J. of Histotechnology, 2000, 23:2, 151-154.

Confidently identifying the site of a microdialysis probe membrane can sometimes be a challenge in sections of brain and other tissues. This is especially true if histological processing produces any tears or cracks in the tissue, which may be indistinguishable from a probe track. Shrinkage of the tissues can also complicate probe track location and identification.

To eliminate these potential problems, the authors infused a 1% filtered solution of potassium permanganate through the probe or guide cannula. They note that potassium permanganate reacts with brain tissue to produce insoluble tar-like manganese dioxide reaction products that leave a contrasting visual mark at the infusion site. And unlike the use of some other histological dyes, the resulting indicator does not tend to washout during staining with cresyl violet, hematoxylin, or eosin. The mark also facilitates tissue sectioning since the probe target site is more evident.

MD Q&A

James F. Gitzen, Ph.D.
Bioanalytical Systems, Inc.
West Lafayette, IN

Email:
jgitzen@bioanalytical.com

♦ What is the molecular weight cut-off of your microdialysis probes? Do you have probes with large cut-offs for sampling small proteins?

Molecular Weight Cut-Offs (MWCO) of what are fundamentally kidney dialysis-type membranes are rather a nebulous concept when applied to microdialysis. The manufacturers of the membranes give a MWCO value, but they usually don't define how those numbers were derived. Since the manufacturers typically don't reveal how they determined a particular MWCO, most people, myself included, cannot know for certain how they arrive at a given value.

One might envision their test procedure to be somewhat similar to doing protein purification by dialysis, much as you might have done in an undergraduate biochemistry lab. In such a scenario, one can imagine the manufacturers filling some membrane fibers with markers of various molecular weights. They would then seal the membrane ends and put the filled membranes in a beaker containing several liters of buffer, and then let the buffer and the membranes stir for x hours while dialysis occurs, until everything comes to equilibrium. Naturally, even in this situation, as the MWCO of the analytes increases toward the membrane's cut-off, the amount of those higher MW analytes crossing the membrane declines compared to lower MW compounds, since the larger the analyte, the harder it is to get it though a membrane pore of any given size.

If this is indeed how it is done, that would certainly explain why the MWCOs reported for a given membrane are usually considerably higher than the MWCOs actually seen when doing microdialysis. With microdialysis, since the lumen of the membrane is being continually perfused, the kinetics of microdialysis dictate that you typically would never reach equilibrium with the surrounding solution. As a result, since the larger analytes would not have an opportunity to reach equilibrium, larger analytes are much less likely to cross the membrane, at least not in anything approaching detectable levels. This is probably why, even though a membrane manufacturer may rate a particular membrane at some MWCO, often the effective MWCO one can actually expect to see under the kinetics of microdialysis is perhaps 20-30% of the rated MWCO. For example, the membranes used in BAS probes are rated at 30-38K Daltons (Da). However, in practice, when used for microdialysis we would not expect to see recovery of analytes higher than perhaps 6-7K Da.

We have had a report that insulin will cross the membrane, but in its monomeric form insulin is ~6K Da. The same pattern is true of most kidney dialyzer membranes. For example, with microdialysis probe membranes which are rated at 20K Da, effectively speaking, under microdialysis conditions the largest molecular weight compounds which typically cross (though in low quantities) are in the 5-6K Da range.

In addition to these basic kinetics issues which, as I envision it are due at least in part to the static versus actively perfused use of the membrane, effective MWCO numbers are further complicated by differing perfusion flow rates, different lengths of membranes on different probe types, globular versus linear analytes and different membrane manufacturers apparently using different approaches to rate their membranes.

For sampling larger compounds by microdialysis, the solution is not simply to make probes with bigger membrane pores so larger analytes can be dialyzed through them. This is because when larger pores are used, the pore size quickly becomes large enough that water can cross the membrane; so rather than doing MD, one starts to pump very finely filtered water from the probe into the tissue. This is decidedly undesirable.

Technical Note

Meloney Cregor and
Bruce P. Solomon, Ph.D.
Bioanalytical Systems, Inc.
West Lafayette, IN

Email:
mcregor@bioanalytical.com
bp@bioanalytical.com

♦ The Effect of Blood-Drawing Method on Plasma Catecholamine Concentrations in Rats

The BAS Culex® Automated Blood Sampler (Culex ABS) is designed for automated blood collection without the extensive handling and restraints normally associated with manual blood sampling. To see just how much of an effect this humane sampling technique would have, we compared catecholamine levels for the same rats bled via Culex and via a traditional method.

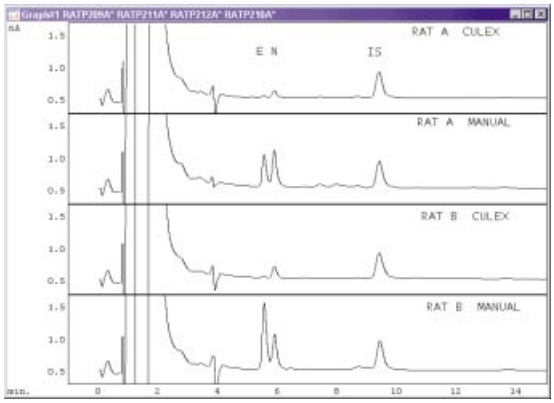
It is not surprising that analytes considered to be stress hormones would show wide fluctuations in titer, depending upon an animal's stress level at the time of sample collection. For example, awake rats had up to three times higher norepinephrine levels, and up to five times higher epinephrine levels, compared to anesthetized rats (1). So how is one to obtain basal measurements of these compounds? One common method is to use anesthetized animals (2,3). But the use of an anesthetic creates artificial conditions that may affect the results. A better method would be to connect the animal to a sampling device, then allow it to resume its normal activities in a stress-free environment before actually taking samples. This is the function of the Culex ABS.

Blood Drawing

Two male Sprague Dawley rats were implanted with BAS Jugular Catheters (P/N CX-2010) in the right external jugular vein. Both animals were placed in BeeKeeper bowls to recover, and connected to the Culex ABS to maintain patency.

F1

Chromatograms of extracted plasma from rat blood drawn by Culex ABS and manually. E, epinephrine; N, norepinephrine; IS, internal standard. LCEC conditions essentially outlined in the BAS Plasma Catecholamine Kit manual.



T1

Plasma catecholamine concentrations for two rats, from blood drawn by Culex ABS and by a traditional manual method.

	RAT A pg/mL PLASMA		RAT B pg/mL PLASMA	
	Epinephrine	Norepinephrine	Epinephrine	Norepinephrine
Culex	65.9	251.9	57.5	483.6
Manual	1302.2	1365.6	3003.8	1200.1

At 24 hours post catheter implant, blood was collected from rat A by Culex ABS, while rat B blood was manually drawn. A 48-hour blood collection was the reverse of that done at 24 hours: rat B by Culex ABS and rat A manually.

Culex ABS

Five blood samples consisting of 200 µL blood and 50 µL of heparinized saline (10 U/mL) were collected and pooled. Plasma was separated by centrifugation, then stored at - 80 °C until analysis.

Manual

The rat was removed from Culex maintenance and placed in a rodent restrainer. Using the catheter, 1 mL of whole blood was manually drawn into a 3 mL syringe pre-filled with 250 µL of heparinized saline (10 U/mL). The animal was then returned to the Culex system for catheter maintenance. The sample was centrifuged and the plasma stored at - 80 °C until analysis.

Catecholamine Determination

Epinephrine and norepinephrine were determined using the BAS Plasma Catecholamines Kit (MF-9016) as follows:

250 µL plasma was added to a 12x68 mm concial glass centrifuge tube along with 20 µL internal standard (alpha-ethyl norepinephrine, 400 pg) in eluting solution, 13 mg AAO and 400 µL reagent A. The tube was vortexed every other second for 7.5 minutes, centrifuged for one minute and the supernatant discarded.

The AAO was washed twice with reagent B (750 µL each), centrifuged for one minute after each wash and the supernatant discarded.

Eluting solution (130 µL) was added to the tube, mixed, allowed to stand for two minutes, mixed again and centrifuged for one minute. A 100 µL aliquot of the supernatant was removed to an autosampler vial and 75 µL injected.

An external standard solution was made by combining 20 µL of a solution of epinephrine and norepinephrine (100 pg) and alpha-ethyl norepinephrine (400 pg) with 110 µL eluting solution.

Results

T1 lists the plasma catecholamine concentrations for the two rats. **F1** presents the respective chromatograms. There were substantial differences between the two collection methods: epinephrine levels were 20-50 times higher for the traditional drawing method, while norepinephrine levels were two to five times higher. These results suggest that a bias is introduced when basal catecholamine concentrations are determined by traditional blood-drawing techniques.

References

1. F.M. Siri and C.D. Kauer, Life Sci. 37 (1985) 1923-1931.
2. P. Prados, S. Higashidate and K. Imai, Biomed. Chromatogr. 8 (1994) 1-8.
3. B.B. Fredholm, L.O. Farmebo and B. Hamberger, Acta Physiol. Scand. 105 (1979) 481-495.

♦ BCEIA 2001: The Ninth International Beijing Conference and Exhibition on Instrumental Analysis

BCEIA 2001 will be held October 17-20, 2001 at the Beijing Friendship Hotel (Conference) and the Beijing Exhibition Center (Exhibition) in Beijing, the capital of China.

The International Beijing Conference and Exhibition on Instrumental Analysis (BCEIA), sponsored by the Ministry of Science and Technology, has been a biennial international event in China since 1985. Its aim is to promote academic exchanges among scientists of all countries and trade cooperation between Chinese and foreign partners.

BCEIA has been the largest and the most influential international conference and exhibition on instrumental analysis ever held in China, enjoying an international status matching that of the Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy (Pittcon) in the United States.

Conference

The BCEIA 2001 Conference will feature a plenary session with specially invited lectures by internationally prominent scientists on applications of multiple instrumental analysis to current focal problems of the environmental, material and life sciences. In addition, separate sessions for oral and poster presentations in the fields of Electron Microscopy, Mass Spectrometry, Spectroscopy, Chromatography, Magnetic Resonance and Electroanalytical Chemistry will be covered.

Conference Proceedings

Abstracts of contributed papers of either oral or poster presentation prepared in two camera-ready pages following the format to be specified in detail in the Second Circular will be published in separate volumes for each field as the Proceedings of the Conference.

Conference Language

The official language of the conference will be English.

Exhibition

The Exhibition will be a large-scale (floor area about 10,000 m^2) show of newly developed equipment and technology in all the relevant fields of instrumental analysis from various countries. Associated technical seminars will be held simultaneously.

Correspondence

BCEIA 2001 General Services Office
Room 585, 54 Sanlihe Road
P.O. Box 2143, Beijing 100045
P.R.China
Tel: +86 10 6851 1814
+86 10 6851 1133 Ext.1585
Fax: +86 10 6851 1814
Email: conf@bceia.org
office@bceia.org
expo@bceia.org
Web: www.bceia.org

♦ Peter Kissinger: Scientist, entrepreneur, and "still basically twelve years old."

By Lillian Price
Special to The Lafayette Leader

He's a spiffy dresser, highly articulate, a cross between Tom Oliphant, the Boston Globe reporter on PBS' Lehrer Report, and actor John Malkovich. Behind the black-and-white checked jacket, and the natty bowtie (hand-tied, of course), lies a nonstop talker (by his own admission) with a droll, off-the-wall sense of humor.

Tall, thin and bespectacled, he looks like the college professor he is. Or was. Peter Kissinger, the chairman and CEO of Bioanalytical Systems, Inc., taught for many years in the Department of Chemistry at Purdue while running his own company. Now he devotes most of his time to the business.

But it's obvious that teaching is second-nature to him. When he wants to explain to a visitor what his company does, he's up at the blackboard in an instant drawing diagrams and arrows, waxing poetic about science, the wonders of pharmaceuticals, the pleasures of art and literature, and a life spent passionately engaged in all of the above.

He is a man of broad interests. A major one is writing. Besides publishing numerous professional papers and editing several journals, he has summarized his research career in a 12-page booklet he calls "Fishing for Molecules in Biological Soup"; he has written a handbook for supervisors entitled, "Homo Sapiens Operating Manual, the BAS Way," a treatise which might have been written by satirist Dave Barry (whose name, in fact, appears in the index); and writes for a company newsletter called "Bylines" in which he asks deep philosophical questions like, "Are we basically good (trustworthy) or basically bad (untrustworthy)?", and dispenses opinions on such aesthetic issues as the proper socks for office wear.

He is an obsessive e-mailer. He predicted that during the several hours he spent in this interview, there would be more than 50 e-mail letters waiting for him on his computer. He has written an essay about his wife Candice called "The Queen Bee" in which, among other things, he talks about the 350,000 bees she keeps in her apiary in the yard of their house.

In fact, Dr. Kissinger often compares the keeping of bees to his own business - and to life in general: "Like beekeeping, we're developing instruments (honeycombs), and seeing people use them."

Or: "Religion may help some people, but it is certainly not required for a caring community. I study bees. They are selfless. They

cooperate for the good of the hive. Some nurture the kids, some search for food, some keep things tidy, and some guard the door to keep predators away. They have no soul. We should do better than they do. Often we don't act like it."

He continues: "When Candice and I work with bees we wake at five a.m. to get to the Farmers' Market at six. When we sell the honey, the idea of someone buying it, using it, and coming back for more is very satisfying to us."

Which is why Dr. Kissinger started Bioanalytical Systems, Inc. It was half- hobby and half-business when he brought it to Purdue in the mid-seventies. Today it employs about 250 people and has two subsidiaries in England.

Bioanalytical Systems, Inc., is a pharmaceutical research and development company. It develops tools of measurement for scientific research; for example, it has helped develop new ways to monitor chemicals like neurotransmitters in the brain which are critical to certain central nervous system diseases like Alzheimer's, Parkinson's, schizophrenia and stroke.

Understanding the biochemistry behind these diseases, says Dr. Kissinger, may lead to the development of drugs to do battle with them. Recently, BAS worked with Eli Lilly to help develop a drug called Zyprexa for schizophrenia.

One important aspect of his work is determining interactions among different drugs. AIDS therapy, for example, involves taking cocktails of drugs, the effects of which must be monitored in the blood. "We're very dependent on hundreds of thousands of chemical measurements," says Dr. Kissinger. "That's where my passion is."

Dr. Kissinger feels strongly that the high prices of prescription drugs are justified in light of the years of time, effort and funds devoted to their development. Finding the one molecule out of thousands that will work for a particular disease, testing it in the laboratory and conducting clinical trials, he states, can cost up to $500 million.

His enthusiasm and love for his work is apparent, but Dr. Kissinger is just as enthusiastic about other aspects of his life. He loves canoeing (he met his wife on a canoeing trip), flying airplanes (he's retired from that), swimming in lakes and tramping through woods.

"I'm still basically 12 years old," he says. "It's a nice age. Scientists must stay young to maintain their curiosity."

And he has strong opinions on many subjects. For example:

Television: "An abomination. If America falls it will be because of sitcoms, and our acceptance of politicians who make stuff up."

Vietnam: "Shocking to my academic friends. I did not oppose the war in Vietnam, only the way we went about it - which was shameful."

Trust: "It strikes me that we have become too suspicious. This has bankrupted many of our souls. My feeling is there are too many rules, too much checking of the checkers who are checking the work."

His is a sharp wit, often directed against himself. In his handbook for supervisors, for example, he discusses the uselessness of giving unsolicited advice: "I always tell my sons the old adage, 'reading is for the mind as exercise is for the body.' The more I say this the less they read and the more they sleep."

In that same book he points out that while some people thrive in a small business, others just don't fit. "I acknowledge that some people cannot be helped without medication or at least a good massage. Some will do better with a dog or a cat as a friend than with me spewing words at them. Some people need jail time."

He urges his supervisors to treat their subordinates as individuals. "We are all totally unique (so far)," he writes. "One of our Vice Presidents recently referred to me as 'odd,' and I think that is an astute observation. He, too, is odd, and so are the other 5,999,999,998 of you Homo Sapiens."

Dr. Kissinger doesn't mind being called odd; in fact, one suspects he takes some pride in the characterization. More than once in this interview he referred to "my odd personality." By his own admission, he wears bowties because "I get attention."

Being unconventional, going against common expectations, is a state he admires. In his supervisor handbook, for example, he touts the benefits of his small company, where "individual personalities can shine rather than be suppressed to some common standard" adding, "Can you imagine me at General Motors? The people at Ford would love to see me there."

But more than giving his employees an environment in which they can feel free to be different, Dr. Kissinger sees his company as advancing the health of society as it seeks to improve the quality of life. "You can see the benefits," he says excitedly. "It's science, chemistry, computers, math. But, at the end of

the day, does Grandma feel better or not? That's what motivates me."

Beginnings

Peter Thomas Kissinger was born on December 19, 1944 on Staten Island, New York. Living close to New York City with its diverse population and cultural offerings gave him a deep appreciation of other cultures, and stimulated an already budding interest in science.

But it wasn't just the vibrant city. Though his father, who passed away last year, was an accountant by profession, he was interested in science. As a youngster he and his friends had fiddled with radio, and he encouraged his son to experiment as well.

Young Peter built a few radio transmitters as a high school student, and became an amateur radio operator in Morse code. He built a polarograph, borrowing parts from two uncles, a high school teacher, and Beckman Instruments. It won a prize at a science fair and sits today among other old instruments in a museum case in the company building, thanks to the love of a proud mother.

Patricia Kissinger, now 81, lives in East Aurora, New York and is a homemaker. When her son was young, she and her husband were very much involved in their community of New Dorp. Dr. Kissinger describes his parents as "very determined," the kind of people who "have a plan and stick with it!" He recalls their advice to him: "Be nice to people who are down on their luck."

Asked what his own counsel to others would be, he replies with two bits of Kissingerian wisdom: "Worry more about what you do than what others think you do," and "Dislike no one for more than five minutes. Hate is a waste of time and energy."

His father was not his only role model for scientific endeavors. His uncle, Roscoe Ammon, had a factory in New Hampshire where he made aircraft instruments, some for the early space flights. Mr. Ammon knew Alan Shepard, a fact that fascinated his young nephew who viewed great scientists and inventors as heroes.

Peter also admired military people, especially naval officers (as an award for winning a science fair, he was taken on a cruiser); and writers, especially Hemingway and Twain. He saw himself alternately as a pilot flying aircraft off carriers, as a journalist, and as a scientist.

It's no surprise that today one of Dr. Kissinger's heroes is Winston Churchill, because "he had great balance, was interested in science and technology, was a military officer, a member of several different parties, and supported his family as a journalist."

Real life, however, sometimes gets in the way of dreams. Peter Kissinger's first job was cutting grass in a cemetery. "I cut the grave of Cornelius Vanderbilt and his many relatives," Dr. Kissinger recalls. "Working around the dead made me realize I'd better get to work and study harder in high school. At $1.25 an hour, this didn't seem like a career for me in 1960."

While his favorite subject in high school was science, he also enjoyed English. He is grateful to his English teacher, Miss Ernst, to his Latin teacher, Miss Price, and to other fine instructors he had in college and graduate school. Only many years later, he says, did he realize how much they did for him.

The making of a scientist

With his broad interests in science and the arts, and somewhat shy socially, Peter chose the small campus of Union College in Schenectady, New York, which, Dr. Kissinger notes, has a good balance in science, engineering and the liberal arts.

While he was at Union, he worked for General Electric, and the experience introduced him to the excitement of industrial research. He graduated in 1966 with a bachelor's degree in chemistry, and proceeded to the University of North Carolina in Chapel Hill to work with Charles Reilley, a noted analytical chemist.

Four years later, he received a PhD in analytical chemistry. He then spent two years as a research associate at the University of Kansas, and three years as an assistant professor at Michigan State University, before joining the Purdue faculty in 1975. A year before coming to West Lafayette, he founded Bioanalytical Systems.

Dr. Kissinger loves teaching and the debating of ideas. He is awed by the idea of being able to influence so many young people in their careers. "Teaching at a university," he states, "is a route to everlasting life. You teach, and others will pass the knowledge on to the next generation. It's reincarnation!"

In the 20-some years since the founding of his company, Dr. Kissinger has seen it grow and flourish. He retains his post at Purdue and also does a good bit of traveling, speaking at universities, pharmaceutical research and development centers, and other scientific venues.

Though Dr. Kissinger considers teaching his greatest success, he also derives intense satisfaction as a scientist working with teams that are contributing to the better health of millions of people. Often, as he was describing his life and his work, he would exclaim, "It's so much fun!"

The chemist and CEO speaks

What skills do you believe are essential to a young scientist?

The curiosity of a five or six year-old.

Liberal Arts skills. The best scientists are those who appreciate the arts. Often we scientists and engineers are too narrowly engaged in our specialty; we don't break through to the big stuff, don't innovate. I look at science as a liberal art itself.

A sense of humor. Lots of science doesn't work. Many trails are dead ends. It's the thrill of a discov-

ery that allows you take the disappointments.

How was it growing up in the sixties?

Very exciting. Many of us were intrigued by science and technology - plastics, antibiotics, rockets. We were utilizing scientific discoveries made during the war. Homes were filled with refrigerators, Teflon-covered cookware, TV sets, stereos, Pampers. We weren't afraid of genetically-modified organisms. We were excited by them.

As a chemist, how do you respond to the public fear of chemicals?

I picked up a bottle of shampoo recently that said "not tested on animals." It has been tested on animals; they mean this particular combination was not. And that combination will be tested on one animal - you!

Everything in our world is chemical. We have the idea that chemicals are not natural. Every flower is filled with chemicals, every bird. The press does a tremendous disservice in not educating the public about this.

Community activities

Dr. Kissinger has been involved with numerous local organizations, among them The Greater Lafayette Community Foundation, the Greater Lafayette Museum of Art, the Chamber of Commerce, and Greater Lafayette Progress.

He is a member of the newly-formed "Vision 2020," a group of people from different segments of the population brought together to present their ideas about the future of Lafayette.

Awards

In 1998 Dr. Kissinger received the "World of Difference Award" from the Indiana Health Industry Forum for his contributions to medical research.

Just a few weeks ago, he was elected a Fellow of the American Association for the Advancement of Science. He is also a Fellow of the American Association of Pharmaceutical Scientists.

On behalf of his company, Dr. Kissinger has established the Reilley Award to recognize excellence in scientific research. The prize is presented every year at a conference of the Society for Electroanalytical Chemistry, an organization he founded 15 years ago.

Family

The Kissingers have two sons: William (Bill), 19, who attends Valparaiso University, and Samuel, 15, a student at Harrison High School.

Personal Preferences

Favorite vacation

A family trip to England and Wales, "climbing over old castles and enjoying the mince pies and sherry in 500 year-old pubs."

Hobbies

Collecting wooden toys and models of WWII military Jeeps. (Mrs. Kissinger gave her husband a real 1943 Jeep for their 20th wedding anniversary.)

Favorite comics

Calvin and Hobbes, The Far Side, Dilbert.

Pets

"I've had four cats: Sniffy as a kid; Epinephrine and Norepinephrine before I got married, and then Dopamine. This year's Nobel prize in medicine was awarded for dopamine research."

Favorite reading

History and biography, especially books by or about Winston Churchill. Authors Mark Twain, Ernest Hemingway. (He is halfway through the Harry Potter books. So is his mother.)

Earliest national event remembered

The launching of Sputnik in 1957, "especially running out in the backyard hoping to get a glimpse of it above the oak trees."

Most famous people met

Photographer Ansel Adams, General Anthony McAulliff (Battle of the Bulge) and Richard Nixon.

BASI NEWS

Pete T. Kissinger, Ph.D.
Bioanalytical Systems, Inc.
West Lafayette, IN

Email:
pete@bioanalytical.com

♦ Culex® passes milestone with repeat orders from pharmas

Our new robotic product for preclinical pharmacokinetics has passed an important milestone. Pharmaceutical companies who purchased a unit(s) last summer and fall have placed orders for additional units during the second quarter. Why is this important? The pharmaceutical industry is highly regulated and R/D ventures are very expensive. We've all heard the quote of $500-600 million to get a new drug through clinical trials and onto the market. With the combination of rigorous regulation and high cost (the two are related) the industry cannot afford to adopt new technology in a cavalier fashion.

Culex presents a major shift in the way things are done at the interface between discovery research and preclinical studies. This is the point where potential drug substances first see a laboratory animal after the in vitro screening process has weeded out many possibilities. While we all would like to see the way to bypassing expensive animal work and going directly to humans, this will not happen in the foreseeable future. The numbers of candidate chemical entities is accelerating, and it is important to confirm key issues such as oral absorption, blood/brain barrier penetration and rates of metabolism and elimination in complete mammalian species, not just in cell culture studies.

I recognize that many of our shareholders are not trained in pharmaceutical research. If anyone would like to learn more, I'd be happy to explain these things in layman's terms. Just ask by mail or to *pete@bioanalytical.com*. (Pharmacokinetics [PK] describes and explains the concentration of a drug in blood as a function of time after dose. Ultimately the PK properties of a drug determine the amount in a dose and the frequency of dose. Both combine to assure the patient receives enough drug to provide appropriate therapy, but not enough to compromise safety.)

For any new technology, pharmas must validate its use by comparing the advantages and disadvantages versus conventional technology. This is a complex process, everyone is very busy, and the industry is necessarily conservative. The easiest thing is to do nothing and continue with methodology that has been in place for twenty years. Therefore, we were delighted that a number of companies saw the Culex as sufficiently innovative to take a chance on it and invest time validating it in parallel with conventional techniques. Naturally this added to their workload and expense.

The benefits are clear: Culex improves data quality. In fact, it demonstrates deficiencies in conventional methods. The old way can place animals under untoward stress, leading to distortions in pharmacokinetic results. Culex reduces the number of animals required to get high quality information. It provides opportunity to combine several objectives in one experiment, for example, systemic pharmacokinetics and pharmacodynamics as well as animal behavior indications. It reduces substantially the labor expended per animal and replaces manual record keeping with computer records.

A robot such as the Culex requires care and feeding. Pharmas typically want to use "Culex-ready" animals that have been carefully prepared with Culex catheters from BAS. The four leading suppliers of rodents to pharma companies—Harlan, Charles River, Hilltop and Taconic—are now able to provide such animals. Thus pharmas can have subjects that match up with Culex delivered to their R/D centers. Once again, this saves a great deal of expensive labor and provides a just-in-time advantage for R/D throughput. As these suppliers have risen on the learning curve, BAS has improved the manufacturing and packaging of our other Culex "razor blade" components. These include animal containment systems, catheters, Culex tubing sets and software, which have all advanced in response to customer suggestions and the ingenuity of our in-house team.

♦ Culex launched in Europe

Right on schedule, as announced last Spring, Culex was launched in Europe on January 1, 2001. The ramp-up will take time, largely due to the differences in regulatory affairs for animal research in Europe, especially in the UK. We are currently working with the appropriate agencies. We understand that one animal supplier, Charles River, has just begun the process of supplying catheterized rodents in Europe. Traditionally, pharmas were required to do the needed surgery in their own facilities.

As mentioned earlier, change is slow in pharma research. It will likely take several years for this new approach to be adapted. We are actively talking about Culex to leading pharmas in Europe. To be honest, most are global companies who have already been using Culex in the USA. Our hope is that their American colleagues will share their enthusiasm and therefore facilitate installations in Europe. On the other hand, caution is needed due to cultural and regulatory differences. We expect to have positive results in Europe in the fourth quarter of the current fiscal year.

♦ Japan remains a special challenge

Our launch plan included Japan being on board by January 1, 2002. Maintaining our policy of realistic disclosure to shareholders, I have to say that we are behind schedule. There are language issues with software, and special challenges with the Japanese distribution system,

providing local service and (again) meeting regulatory requirements. These challenges are very much on my front burner, along with the current economic problems in Japan. In general, Japanese pharmas are much smaller than the global companies we have been working with. This, too, must be kept in mind as we go forward.

♦ New Preferred Provider Relationships

BAS and Pharmacia management teams recently signed a Master Agreement awarding BAS preferred vendor status. BAS will provide preclinical research and bioanalytical services assisting Pharmacia in new drug development. This agreement recognizes our excellent multi-year relationship and an exceptional level of effort by our Evansville and West Lafayette contract research groups. The agreement places BAS among a handful of select vendors chosen for their quality, regulatory performance and flexible, personable, timely service. Pharmacia sees this Master Agreement as a two-way relationship and is committed to advising and assisting key vendors toward improved operations. The preferred vendor recognition process has been in place since 1995, intended to improve value for both Pharmacia and vendor shareholders. This agreement follows recognition of BAS as a preferred vendor by Eli Lilly in 1999, and by one other major drug developer who is among the top five, worldwide. We expect to disclose more about this third agreement in the near future.

♦ New Vetronics Windows Software Launched

As everyone including Bill Gates knows, software is not something easily developed on a schedule. We now can assure shareholders that software for our Vetronics (veterinary electronics) products has been totally revamped and began shipping in the quarter just ended. Likewise, we signed an agreement with a major chain of veterinary hospitals which is installing our BAS Vetronics units in all their units around the USA.

♦ Final Word

I'm frequently asked how the Greenspan effect or layoffs in the auto and technology sectors have impacted BAS. My answer: not at all. Healthcare research spending continues at a torrid pace. The budget of the National Institutes of Health (NIH) is again seeing major increases and pharmas/biotechs are spending to replace approved drugs with even safer and more effective therapies. If a general recession occurs, it would clearly impact us over time, but not in the short term. As we enter our third quarter, I feel like a Bull in the midst of many sharp-toothed Bears. Drug development remains an excellent place to be in the current climate. (We do, of course, like the concept of lower interest rates! Go, Allan, go!)