

For Immediate Release  
April 30, 1991

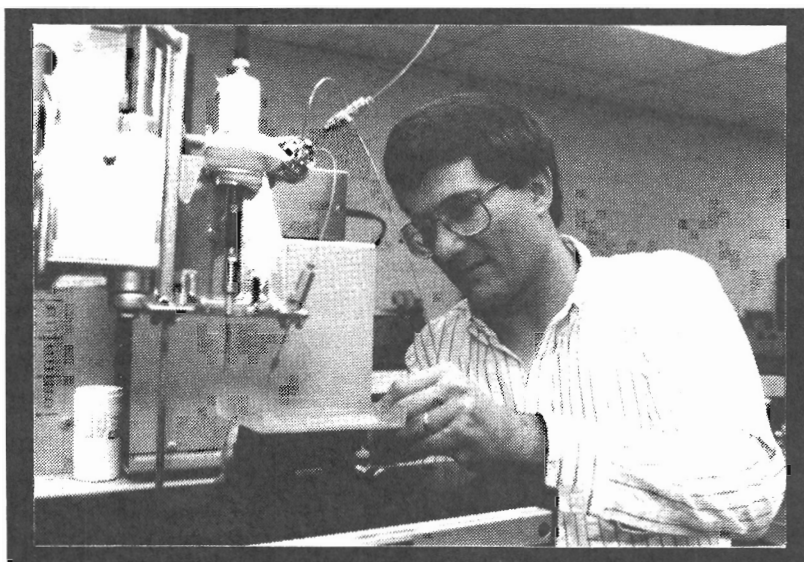
Georgia Institute of Technology  
Research Communications Office  
Atlanta, Georgia 30332-0800  
404-894-3444

## IDENTIFYING COMPLEX BIOMOLECULES: NEW INTERFACE SYSTEM COMBINES GEL ELECTROPHORESIS & MASS SPECTROMETRY

An new interface system which combines two widely used analytical techniques -- gel electrophoresis and mass spectrometry -- will give scientists a faster and more accurate means of identifying complex biological molecules such as peptides and proteins.

The experimental interface, developed by chemists at the Georgia Institute of Technology, would provide a better means of identifying biological molecules separated by gel electrophoresis.

"It is a very balanced match of a sensitive and specific spectroscopic analytical method with a very good separation method," said Dr. Kenneth L. Busch,



*Dr. Kenneth Busch prepares interface device to capture material which has been separated by gel electrophoresis for analysis by mass spectrometer. (Color slide/B&W prints available).*

associate professor of chemistry at Georgia Tech. "Complex mixtures are separated into individual components with the gel electrophoresis, and then you have a very specific means of detection to identify each separated component."

Busch described the technique in a paper before the Division of Analytical Chemistry of the American Chemical Society meeting

April 17.

Gel electrophoresis uses differences in molecular size and electrical charge to separate complex biomolecules. First, the samples under study are placed into a gel composed of cross-linked polymers, and then an electrical potential gradient is established across the gel.

- OVER -

### FOR MORE INFORMATION:

ASSISTANCE: John Toon,  
(404) 894-3444

RESEARCHER: Dr.  
Kenneth Busch, (404) 894-  
4030

WRITER: John Toon

The electrical charge causes the molecules to migrate through the gel. The size of the molecules and differences in their electrical charges govern the movement, causing identical molecules to cluster together. The molecules can then be stained with dyes or identified by radioactive tracers, producing a distinctive pattern of separated bands that help identify the chemicals present.

Although heavily used in both biological research and medical testing, electrophoresis is hard-pressed to provide the detailed specific and quantitative information that is essential for

*"It is a very balanced match of a sensitive and specific spectroscopic analytical method with a very good separation method." Dr. Kenneth Busch*

positive identification of a molecule, Busch noted.

Mass spectrometry, however, can provide such information. By breaking molecules into smaller components and measuring the masses of those components, mass spectrometers produce a "fingerprint" spectrum that can be used to positively identify a molecule. But before chemical samples can be analyzed with the mass spectrometer, mixtures must be separated and individual samples must be prepared.

Until recently, separating and preparing large biomolecules has been difficult. Small molecules like those of drugs can be separated by liquid or gas chromatography, but that separation technique doesn't work well for large biological molecules.

To solve the problem, the Georgia Tech chemists first use conventional gel electrophoresis to separate the mixture being analyzed into its components. After focusing on the band containing the compound of interest, they introduce a solvent which begins to break down the gel structure. A small ultrasonic probe then liquefies the gel within a small band and removes a few microliters of solution.

The solution is then filtered to remove the gel, buffers, surfactants, solvents and other extraneous materials from the sample under study. The properly-prepared sample can then be sent into the mass spectrometer for analysis.

Removing a sample from an individual band in the electrophoretic gel requires less than three minutes, allowing scientists to process many samples in a relatively short period of time, Busch noted. Because there are no moving parts in the homogenization probe, it can be quickly cleaned and readied for the next sample.

Though the procedure works well at the experimental level, Busch and Graduate Student Stephen Brown plan to refine it for more routine operation and to study alternative and more rapid methods for removing samples from the gel.

For instance, further miniaturization of the probe would allow removal of samples from electrophoretic bands that are not well separated. Currently, said Busch, the device can remove a section of gel that measures approximately one millimeter by 2-3 millimeters.

But not all bands are that well resolved. Busch hopes to refine the device to provide spatial accuracies into the tenths of millimeters, near the limits of what current gel electrophoresis technology can resolve.

The Tech interface was developed for the continuous flow fast-atom bombardment (FAB) mass spectrometer, which is just one type of instrument in use. Busch would like to adapt the interface for other types of ionization techniques, including the new electrospray equipment.

Though useful for many complex molecules of less than 15,000 daltons, the electrophoresis-mass spectrometry combination based on FAB cannot now be used to analyze larger biological molecules. The use of new electrospray ionization technology would permit such analysis, and Busch said the interface device is compatible with that advanced technology -- as well as with other ionization techniques under development.

The research group is also studying laser desorption ionization mass spectrometry, which uses laser energy to sample a section of the section of gel for study. Lasers can be more tightly focused on the area of interest, Busch noted, but the high temperatures generated at the surface tend to damage the sample.

Two-dimensional gels pose another challenge because they contain many more sample spots, and may provide less material for analysis. Expected improvements in the sensitivity of the device should improve its ability to obtain information from small samples, Busch said.

###