Modular Automated Processing System (MAPS) for Bioanalysis

Sample processing is undisputedly the limiting step in analysis and characterization of biological samples. Tools to automate customized sample preparation are being created to flexibly and conveniently address this need. Addressing this step is important since faster, more selective methods to characterize microliter volumes of biological matrices is a current focus in bioanalytical research. Although sensitive separation and detection methods such as capillary electrophoresis and mass spectrometry enable identification of proteins, they have strict sample preparation constraints. These methods require minimal amounts of salts, surfactants, contaminants and, preferably, significant enrichment of the target analytes. Particularly challenging is the fact that each biological system requires a custom-designed sample-processing protocol, composed of laborious and complex multistep operations that are sample-size limited in bench-top analysis.

In the field of bioweapons detection, around-the-clock analysis of possibly toxic samples requires hands-off processing. Sandia scientists and engineers generated a modular automated processing system (MAPS) which enables construction of continuous computer-controlled protocols customized for any biological-related applications. It is essentially a toolbox that contains a set of processing units and the supporting hardware infrastructure to process microliter-volume samples in a sterile, biocompatible environment. Operations routinely used in bioanalytical laboratories, such as concentration, fractionation, lysing, filtering, sieving, desalting, contaminant removal, digestion and buffer exchange are miniaturized and adapted to function in an on-line format. Custom-built interconnect hardware including Captite^{TM} fittings, manifolds, and flow-switching valves designed by Ron Renzi (8125) provide nanoliter dead-volume connections to effectively counteract sample broadening due to diffusion. Low-pressure pumps controlled by Bruce Mosier's (8125) software enable precise handling of microliter volumes. Each multistep protocol can be adapted to use our tools, then built and optimized on a breadboarded platform into a robust computer-controlled device.

At the heart of this system are the biocompatible cartridges designed by Gabriela Chirica (8324) and Ron Renzi, which enable construction and assembling of packed beds of 1-µl to 500-µl volumes. Figure 1 shows the simple design of the refillable cartridges in which polyesther membranes confine the packed material, introducing essentially no extra volume. A kit of inserts provides interchangeable internal volumes and diameter/length ratios to accommodate the amount of sample and packing rigidity/strength requirements specific to each application.

The most attractive features of the cartridges are the interchangeable geometry and the fact that they can be packed with any type of material. For example, anion exchange and hydrophobic interaction media packed in 2-µl volumes enabled solid phase extraction (SPE) of proteins with a 200-fold increase in protein concentration. Compared to commercially available alternatives for protein concentration, our design is much more resistant to clogging (due to the use of 10-µm mesh size polyester membranes rather than 0.5- to 2-µm metal frits) and is one-tenth as expensive. The larger opening of the retaining membranes allows unrestricted passage of particulates such as cells. Direct on-

line SPE concentration of viruses and spores was demonstrated with a dynamic range of three orders of magnitude. Currently no commercial counterparts exist that concentrate intact organisms at the microliter level.

Cartridges wrapped with resistive wires provide excellent reproducibility and temperature control for lysing viral samples at 90° C. As part of a Laboratory-Directed Research and Development-funded collaboration between Mike Kent (8340) and Gabriela Chirica, the temperature-controlled cartridges were used for testing thermosensitive poly (N-isopropylacrylamide) hydrogels synthesized at Sandia/New Mexico for bacteria sequestration.

MAPS processing components often translate traditional batch-mode processes such as centrifugation or lysis to continuous-flow operation. Chemicals such as dyes, salts or detergents that are added to affect adsorption and/or solubility often interfere with subsequent analysis, necessitating their removal. Centrifuge-operated vials packed with sieving media are commonly used to isolate interferents. However, for a portable, automated unit the centrifuge-based method had to be replaced. Gabriela Chirica and Jaime Lachman used the cartridges to achieve complete isolation of interferents from a 10-µl sample in 70 seconds with excellent reproducibility. This work has been accepted for publication in *Analytical Chemistry*. The MicroChemBioLab proteomic-based biological detector team led by Brent Haroldsen (8125) and Victoria Vandernoot (8321) received a Sandia Employee Recognition Award in 2006.

As development of processing modules and supporting hardware continues, the MAPS system is reaching the critical mass that enables tackling more difficult problems, including analysis of complex matrices such as those necessary for food analysis or cancer sample preparation. Licensing agreements between Sandia and commercial vendors have the potential to bring this versatile toolbox to every biology research laboratory.

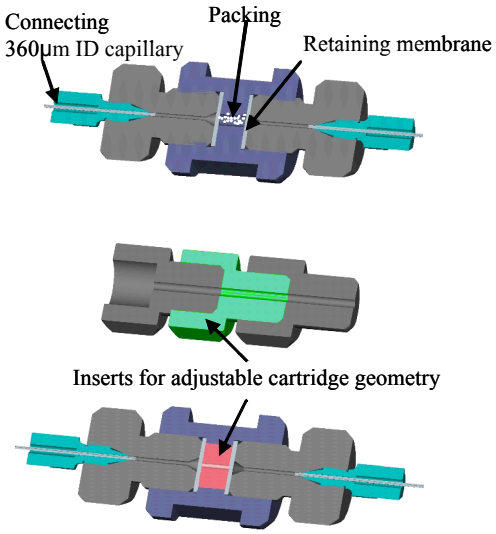

Figure 1. Sandia-designed cartridges offer interchangeable internal volumes (1 – 500 µl), an essential feature for flexibility in protocol optimization.

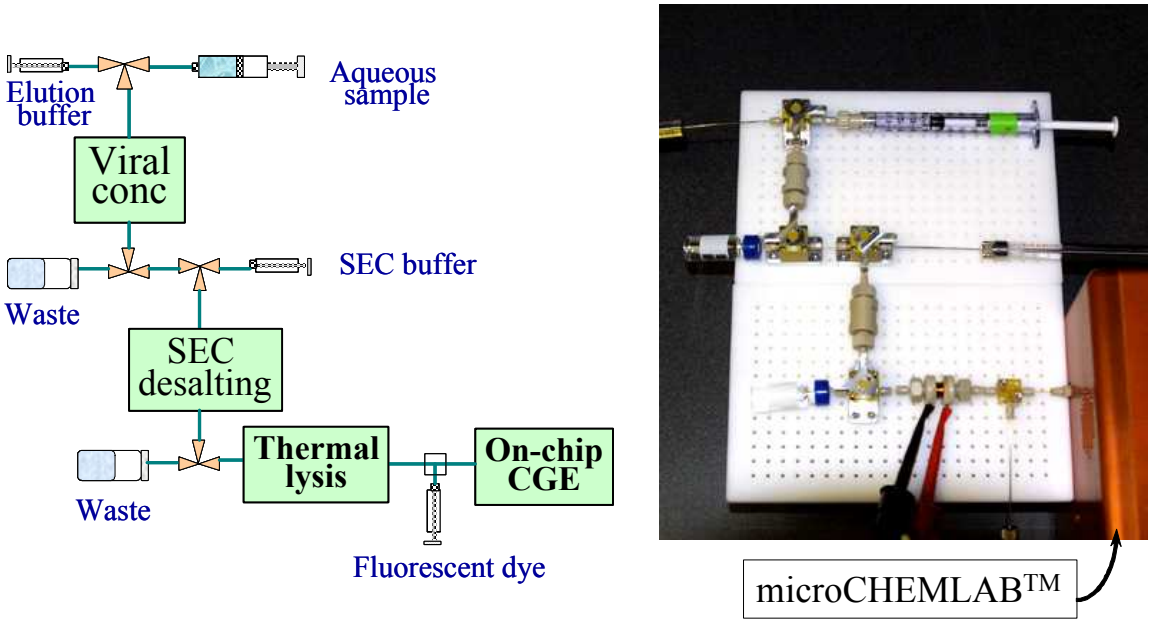

Figure 2. Prototype MAPS for concentration, desalting, lysis and labeling of viral samples prior to on-chip analysis on the microChemlab™ device, as part of a Department of Homeland Security-funded project (Jaime Lachman, Todd Lane (8321), Gabriela Chirica and Mark Allendorf (8324)).

Gabriela Chirica obtained her Ph.D. in analytical chemistry and biochemistry from Oregon State University in 2001, after which she joined Sandia as a limited-term employee in Microfluidics Dept. 8324. She is interested in developing microscale systems for selective separation of biological species from molecules to cells to identify and characterize effectors.