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MODULAR DESIGN FOR AUTOMATED PROCESSING OF COMPLEX BIOLOGICAL SAMPLES

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ABSTRACT

Early warning and protection of military and population against potential biological attacks require small footprint instrumentation that performs all operations, including sample preparation, at the push of a button. Recent research has focused on improving separation and detection, yet to attain exquisite sensitivity with "real-life" samples extensive processing is needed. Sample preparation is not suitable for field analysis in its current format: multistep labor intensive processes, bench-top instrumentation operating discontinuously.

We introduced a modular automated sample processing system (MAPS) which addresses the need for reliable, automated high-throughput processing readily prototyped/tailored for sample-specific applications. MAPS consists of a set of microfluidic modules connected with custom-designed fittings, manifolds, and miniature flow-switching valves assembled on a breadboarded platform. Using MAPS, we demonstrated approximately 100-fold concentration of proteins, spores and viruses. The intact viruses were desalted on-line and analyzed using µChemLab™, the Sandia-developed portable chip-based platform.

INTRODUCTION

The need for early detection of potential biological attacks as well as point-of-care analysis is driving the development of analytical instrumentation towards miniaturized, highly sensitive devices that can be readily deployed for on-site characterization. Sandia National Laboratories has developed a series of portable detection systems which can rapidly detect and analyze chemical warfare agents, toxic industrial chemicals, explosives (µChemLab™ Chemical Detection gas-phase system [1]) and biotoxins, viral and bacterial protein signatures (µChemLab™ Bio-Detection liquid phase system [2]). Both systems demonstrated reliable operation during field testing.

These platforms, focused on enabling portable analysis and detection, can now be taken a step further and applied for more complex samples. To maintain the sensitivity characteristic of µChemLab™ Bio-Detection, such samples require processing for removal of contaminants/interferents and enrichment of target analytes. Existing protocols and devices for sample preparation are mostly batch mode processes which use benchtop instrumentation, manual sample handling. This makes field analysis essentially impossible and reduces significantly the number of samples that can be processed. Furthermore, during analysis of samples containing bio-weapons manual pipetting and operating with open vials can pose a safety risk to the operator.

At Sandia we developed a modular automated processing system (MAPS) which enables construction of continuous computer-controlled protocols customized for any bio-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 closed, sterile, biocompatible environment.

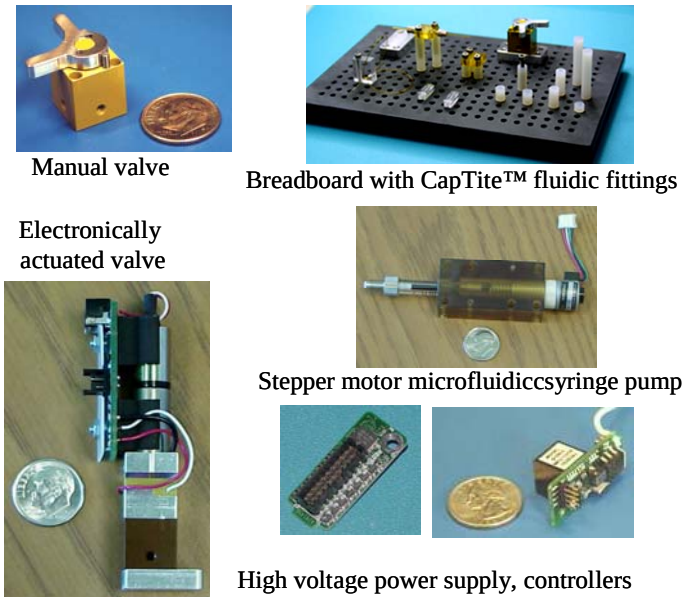

Figure 1. Hardware elements of the modular automated processing system (MAPS) used for building microfluidic system for integrated sample preparation.

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™ fittings, manifolds, and flow-switching valves provide nanoliter dead volume connections while low pressure pumps enable precise handling of microliter volumes.

In a previous study, we built an on-line module that replaced the centrifugation-based protocol commonly used for buffer exchange and interferent removal [3]. The same function was reproducibly executed in an enclosed system which was coupled directly to the detection device. A fully automated train which processes aerosol collected samples containing spores was later on field tested at Edgewood [4].

In this paper we investigated the feasibility of viral concentration from aerosol collected samples and water to enable early detection in the case of a bio-warfare attack. The main challenge is to concentrate the viruses in a very small volume of sample which is compatible with subsequent analysis on Sandia's chip-based µChemLab™ Bio-Detection system. Selective capture and concentration of viruses was achieved with adsorption-elution interactions, or solid phase extraction (SPE), to benefit from high surface areas and higher grafting densities. Gentle elution buffers used in SPE improve the overall recovery rate without interfering with culturing methods; this allows subsequent culturing to test viral viability.

The microfluidic–based analysis poses a volume/device scaling challenge: the starting sample can be 5- 20ml while the volume needed for on-chip analysis is 2µl. At this scale the best approach is to use microliter-volume concentration cartridges. The basic design of commercially available cartridges incorporates frits to retain the adsorptive material. The frits have small pore diameters which often times mechanically trapped bacterial cells and spores. During testing of commercially available concentration devices, we observed rapid clogging due and limited reusability.

The above mentioned obstacles were surmounted by designing a new SPE cartridge which uses highly permeable nylon mesh filters (55 µm thick with 35 µm openings), with essentially zero dead-volumes, to retain the sorbent material in microliter-volume packed beds (Figure 2). As long as the particle diameters are larger than the mesh openings of the membranes, the adsorptive particles are retained inside the cartridge while water flows unrestricted through the packed bed. The Sandia cartridges can be packed using vacuum with any type of packing material and can be easily refilled.

The unique feature of this cartridge design is the fact that it allows unrestricted flow through of microorganisms enabling rapid access to the adsorptive packing. We tested these cartridges (5-500µl volume) packed with materials of various surface chemistries for retention and release of proteins, spores and viruses.

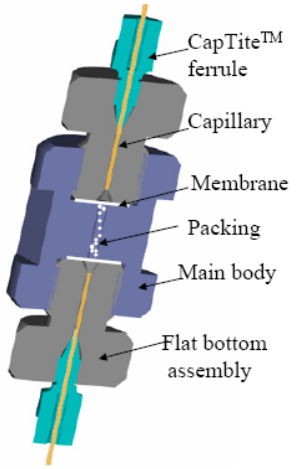

Figure 2. Cross-sectional representation of the Sandia microliter volume cartridge used for protein, viral and bacterial concentration.

EXPERIMENTS and RESULTS

Optimization of the operating conditions for viral concentration has been carried out using the setup illustrated in Figure 3.

A sample containing bacteriophages T2 was loaded on a 15 µl cartridge packed with Toyo Pearl DEAE material (TosoH Biosciences); the absorbance of the eluted liquid was analyzed using a UV detector. The resulting absorbance profile was initially flat, indicating no residual T2 in the liquid eluted off the cartridge. The maximum amount of microorganisms retained is dictated by the cartridge size, flow rate and type of adsorbent material. Our preliminary studies with bacteria spores and viruses indicated that the capture mechanism based on anionic interactions works for both viruses and bacteria. Anion exchange retention of bacteriophages T2 occurred at pH 8 conditions and low ionic strength; these operating conditions are compatible to the on-chip separation. Notably, the sample capacity of ion-exchange packings is extremely high: the 15µl cartridge was loaded with 10^{12} T2 particles before the increase in signal was observed. This indicated that for the present application (analysis of large volumes of water) saturation of the adsorption sites is not likely to occur.

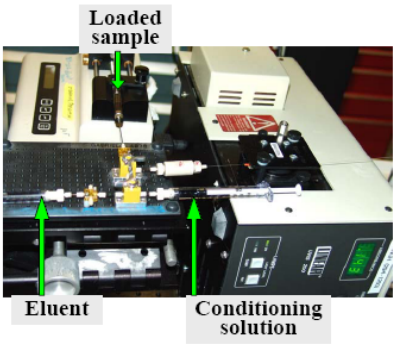

Figure 3. Experimental setup for monitoring the T2 breakthrough after sample retention on our SPE cartridges.

A 3 ml solution of 10^8 viruses/ml was loaded for 9 min at 30 µl/min flow rate on a 15 µl cartridge packed with anion exchange material ToyoPearl DEAE 650S. As we switched from the loading sample to the elution solvent (500 mM NaCl), the viruses were released from the packed bed in a concentrated plug and the corresponding UV absorbance was recorded.

Figure 4 illustrates the virus peaks obtained in 4 consecutive experiments. Overall, a 100 fold concentration ratio was recorded. The reproducibility in peak areas observed over 40 runs indicate that there is essentially no carry-over between sample–to-sample, the Spectrum filters are permeable to viruses and, the enclosed system, although operating with large concentrations of salt, is very robust.

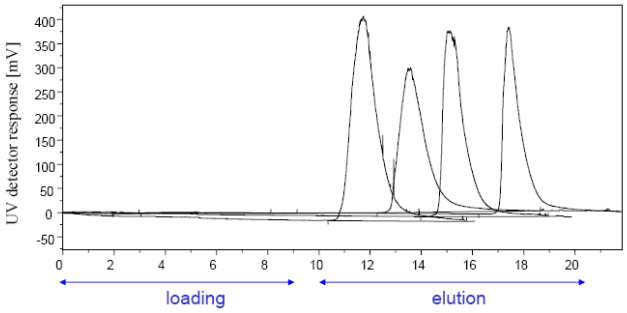

Figure 3. Loading of diluted viral samples, followed by release of a concentrated plug of T2 from an anion-exchange packing material.

500mM NaCl is needed to elute the viruses off the cartridge. Since salt interferes with electrophoretic separation, desalting prior to on-chip analysis is needed. Once again, handling small volume is a challenge for current sample processing methods and devices. Sandia cartridges packed with different materials can be readily coupled in MAPS to build a continuous microfluidic system. We therefore packed cartridges with various size exclusion resins, of which ToyoPearl HW 65 was the best suited for this application. Due to their larger size, the bacteriophages were excluded from the pores while salt and other low molecular weight analytes entered the pores, travelled a longer path and were thereby separated. The desalted, concentrated viral sample was now compatible to on-chip separation.

CONCLUSIONS

We designed devices that enable continuous processing of liquid samples containing bioparticulates, such as viruses and bacteria. Our modular system enables integration of numerous continuous steps for automated, fully enclosed processing of potentially biohazardous samples in a fieldable device. 100 fold concentration ratio was recorded for enrichment of water samples containing bacteriophages T2. The microliter-volume sample of concentrated viruses was subsequently desalted to enable on-chip analysis using μChemLab^{TM}.

ACKNOWLEDGEMENTS

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