Integrated sample preparation and analysis of biological agents using the hand portable capillary electrophoresis instrument, μChemlab.

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Abstract

Rapid identification of viral and bacterial species is dependent of the ability to manipulate the biological agents into a form where they can be analyzed directly. Many of these species of interest, such as bacterial spores, are inherently hearty and very difficult to lyse or solubilize. Standard protocols for spore inactivation include chemical treatment, sonication, pressure and thermal lyses. While these protocols are effective for the inactivation of these agents they are less well suited for sample preparation for analysis using capillary electrophoresis techniques. In order to overcome this difficulty we designed a simple capillary device to perform thermal lyses of vegetative bacterial cells and Bacterial spores. Using an ethylene glycol buffer to super heat bacterial spores we were able to perform rapid flow through lyses and solubilzation these agents. This device was then coupled to sample preparation station for on-line fluorescent dye labeling and buffer exchange for direct analysis using a miniaturized capillary electrophoresis instrument. Using this integrated device were we enabled to perform sample lyses, labeling and protein fingerprint analysis of vegetative bacterial cells, bacterial spores or viruses in less than 10 minutes. The described device is simple, inexpensive and easily integratable with various microfluidic devices.

Results and discussion

Field portable analysis of unknown biological agents both critically dependent on appropriate versatile instrumentation, but also the ability to prepare samples for analysis and perform. We have recently developed a portable mCCE analysis platform, which is able to perform such field ready analysis (figure 1). While this system has the capability of performing such analyses, preparing samples in such an environment remains a significant challenge.

The focus of this study was to devise a scheme which would enable to rapid solubilization of a wide variety of agents that could be easily integrated into a simple device for rapid for preparation and analysis of proteins for microchannel gel electrophoresis. In order to accomplish this we first developed an ultra high temperature solubilization protocol for bacterial spores using ethylene glycol as a carrier solvent. Second we adapted this protocol to simple flow capillary lysing apparatus (figure 2) which had the capability of performing flow-through high temperature lyses of organisms as hearty a bacillus subtilis spores. Third this lyser was then integrated with a sample preparation station with for fluorescent dye labeling and sample buffer mixing with the lysate of the bacterial and viral agents.

Using this scheme we demonstrated complete solubilization of Bacillus anthrasis, Cereus, and Subtilis spores. The lysates of the cells could be easily labeled with the fluorescent dye fluorescamine, mixed with sample buffer directly injected into a miniaturized capillary electrophoresis instrument. Using this configuration we were able to generate specific protein fingerprints of these bacterial spores (figure 3). We further found this protocol was adaptable for the flow through preparation and analysis of virus containing samples. The scheme and the developed device represent the ability to rapidly and universally prepare samples of viruses, bacteria and bacterial spores for protein fingerprint analysis using a miniaturized CE platform.

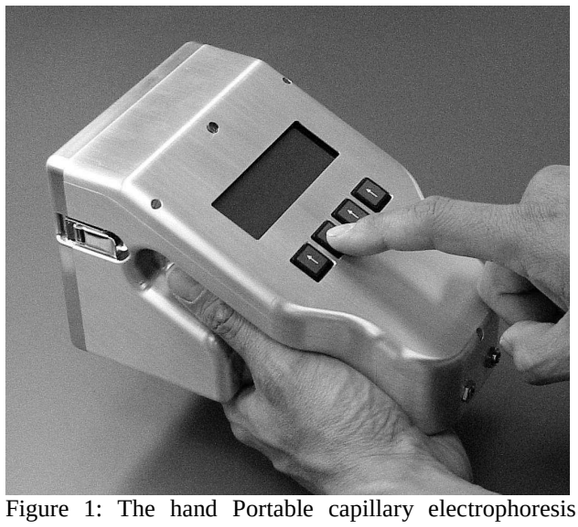

Figure 1: The hand Portable capillary electrophoresis device, μChemlab.

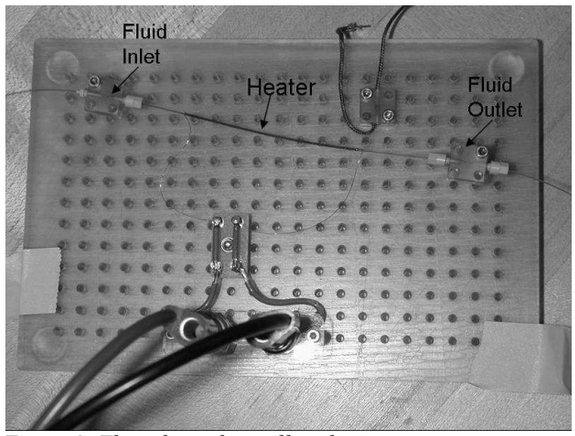

Figure 2: Flow through capillary lysing apparatus.

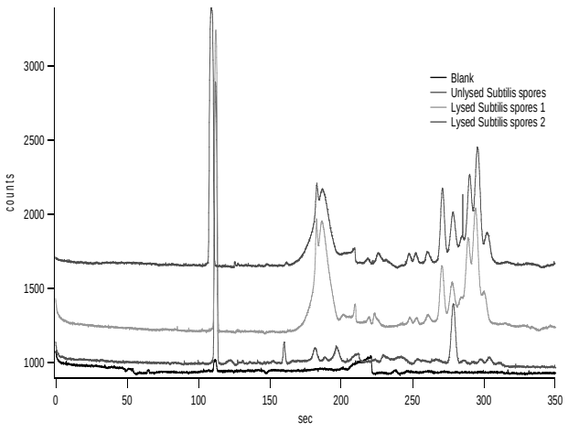

Figure 3: Protein signatures generated from B. Subtilis using the flow through capillary lysing apparatus integrated with μChemlab.