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**GRADIENT ELUTION MOVING BOUNDARY
ELECTROPHORESIS FOR USE WITH
COMPLEX SAMPLES AND DETECTION OF
TOXINS**

STATEMENT OF GOVERNMENT RIGHTS

This invention was made with government support under the National Institute of Standards and Technology (NIST)/ Applied Research Associates Cooperative Research and Development Agreement (CRADA) No. CN-10-0001. The United States government may have certain rights in this invention.

TECHNICAL FIELD

The present invention relates generally to methods of detecting toxins using electrophoretic separations, and relates particularly to methods of detecting toxins using enzymatic assays coupled with gradient elution moving boundary electrophoresis. The present invention also relates to methods of performing electrophoretic separations of complex samples, and relates particularly to methods of performing electrophoretic separations of complex samples using gradient elution moving boundary electrophoresis. Additionally, the present invention also relates to a device for varying with respect to time the bulk flow of a fluid, and relates particularly to a device for varying with respect to time the variable bulk flow of a fluid in a separation channel of an electrophoretic device.

BACKGROUND

Chemical warfare involves the use of toxins as weapons to kill, injure, or incapacitate. Toxins are poisonous substances produced by living cells, organisms, and/or artificial processes. Some examples of toxins include nerve agents and pesticides. Many nerve agents and pesticides are organophosphates, which function by irreversibly inhibiting acetylcholinesterase.

Acetylcholinesterase is a serine esterase that is anchored to the surface of the post-synaptic membrane. Acetylcholinesterase hydrolytically degrades acetylcholine to yield acetate and choline. Inhibition of acetylcholinesterase results in an increase in the concentration of acetylcholine, a neurotransmitter responsible for the influx of Ca^{2+} ions and an action potential that triggers muscle contraction. Thus, when acetylcholinesterase is inhibited, acetylcholine triggers repeated influxes of Ca^{2+} ions and repeated muscle contractions, resulting in paralysis and eventual death by suffocation. Thus, the detection of toxins is of wide import. Moreover, the ability to detect toxins in the field is necessary.

Many detection mechanisms are known in the art. Several examples of detection mechanisms include ion mobility spectrometry, differential mobility spectrometry, Raman spectroscopy, and capillary electrophoresis. These detection mechanisms are limited, however, in that they require the use of devices which, while technically portable, are heavy and bulky. Moreover, capillary electrophoresis is limited with regard to toxins as toxins must typically be detected at lower concentrations than is permitted by conductivity detection. As a result, additional embodiments for the detection of toxins are needed.

Moreover, the ability to detect toxins in complex samples, such as soil, is desirable. Typically, the separation and purification of complex samples using traditional separation techniques is tedious. In the field of capillary electrophoresis,

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complex samples containing particulates must be filtered before they may be analyzed to prevent particulates from entering the separation capillary. When particulates enter the separation capillary, the accuracy of detection is compromised due to a variety of factors, including: a noisy detector signal, interruption of electrophoretic current, clogging of the separation capillary, and fouling of the inner surface of the separation capillary resulting in irreproducible measurements. Thus, additional embodiments for detecting toxins in complex samples are needed.

Recently, gradient elution moving boundary electrophoresis ("GEMBE") has been developed. GEMBE is an electrophoretic separation technique which combines electrophoresis and pressure-based separation techniques. More particularly, GEMBE involves applying an electric potential to a sample and controlling the variable bulk flow of the sample, to obtain sequential separation of sample components. GEMBE is advantageous over other electrophoretic separation techniques in that it does not require sample injection, does not employ moving parts, provides electrophoretic separations over short separation lengths, and provides high data quality.

We have discovered that GEMBE may be employed to indirectly detect the presence of toxins in a sample by reacting the sample with an enzyme in a reaction medium containing a substrate for the enzyme. Additionally, we have discovered that GEMBE may be employed to perform electrophoretic separations of complex samples with little or no sample preparation. Finally, we have developed a novel, field portable device for varying with respect to time the bulk flow of a fluid, which may be employed in GEMBE.

SUMMARY

In one embodiment, a method of indirectly detecting the presence of toxins in a sample using electrophoretic separations is provided, the method comprising reacting the sample with a signaling enzyme in a reaction medium containing a substrate for the signaling enzyme, wherein the signaling enzyme converts the substrate to a product, introducing a run buffer into a separation channel having an inlet end, selectively introducing at least one of the substrate and the product of the reaction medium into the inlet end of the separation channel, electrophoretically separating the substrate and the product by applying an electric potential (i.e. field strength) across the separation channel and varying with respect to time the bulk flow of the run buffer in the separation channel, wherein the substrate and the product are sequentially detected and quantified, and determining the rate of conversion of the substrate to the product, wherein a change in the rate of conversion is indicative of the presence of toxins.

In another embodiment, a method of performing electrophoretic separations of complex samples having charged particulates and oppositely charged analytes is provided, the method comprising introducing a run buffer into a separation channel having an inlet end, selectively introducing the oppositely charged analytes in the complex sample into the inlet end of the separation channel, and electrophoretically separating the charged particulates and the oppositely charged analytes by applying an electric potential across the separation channel and varying with respect to time the bulk flow of the run buffer in a direction substantially aligned with the electric potential, wherein the oppositely charged analytes are sequentially detected and quantified.

In yet another embodiment, a device for varying with respect to time the flow of a fluid in a separation channel of an electrophoretic device having a buffer reservoir in fluid con-