

METHOD AND DEVICE FOR ISOELECTRIC FOCUSING WITHOUT CARRIER AMPHOLYTES

This is a continuation-in-part application of application Ser. No. 08/026.635 filed Mar. 5, 1993, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a device and method for isoelectric focusing of ampholytes contained in a buffer. The focusing facilitates fractionation of ampholytic components.

2. Description of the Prior Art

Isoelectric focusing is an electrophoretic method that has been used previously to separate ampholyte analytes, such as proteins, based on differences in their isoelectric points. Analytes are placed in an electrostatic field produced in a medium such as agarose gel with a well-defined pH gradient. Analytes are initially protonated and deprotonated depending on the pH of the buffer in which they are located and they migrate in the electrostatic field towards their respective isoelectric points where the net charge of the analytes is zero and therefore their mobility is nil. Ampholyte analytes can be concentrated and focused in narrow zones frequently giving resolution between analyte bands better than 0.01 pH units. Isoelectric focusing using capillaries has advantages over the gel format because of superior speed and because the capillary can have an inside diameter as small as 5 μm which allows analysis of very small samples. When a capillary is used, a pH gradient is created using carrier ampholytes, which are polyaminopolycarboxylic acids. These carrier ampholytes are expensive and introduce complexity in purifying the proteins. In addition, they interfere with ultraviolet detection. It is also known to create a pH gradient, which results from a temperature gradient, by using a system of two circulating baths at different temperatures attached to each end of the separation vessel. Unfortunately, the temperatures are not stable due to Joulean heating and this procedure is very inconvenient. In all of the prior art methods that do not use carrier ampholytes, the pH gradient is created separately from an electric current that is used for the actual separation or fractionation of the ampholytes.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a device and method of isoelectric focusing and fractionation where an electric current is used to create a temperature gradient along a separation vessel and that same electric current is used to create an electric field gradient for isoelectric focusing and fractionation. It is a further object of the present invention to provide a device and method for isoelectric focusing and fractionation where a temperature gradient is created due to the physical characteristics of a separation vessel using a power source that generates a constant current.

A device used for isoelectric focusing of ampholytes contained in a buffer has an elongated separation vessel with two ends. The vessel contains the buffer and the vessel has an elongated tapered portion so that a temperature gradient can be created within contents of the vessel using a power source that generates a constant current. The power source has one terminal connected at one end of the vessel and another terminal connected at the other end of the vessel. The power source is connected to provide means with said tapered portion to create a temperature gradient longitudi-

nally along the buffer in said vessel between said ends and to simultaneously focus said ampholytes, with a detection system to monitor progress of said focusing.

A method of isoelectric focusing of ampholytes contained in a buffer uses an elongated separation vessel with two ends. The vessel has physical characteristics such that a temperature gradient can be created longitudinally within contents of the vessel using a constant current from a power source. The power source and an imaging detection system are arranged to monitor progress of said focusing. The method comprises the steps of filling the vessel with a buffer containing ampholytes, connecting the power source to create a temperature gradient longitudinally along said buffer in said vessel and monitoring the progress of said focusing using said detection system.

A method of fractionating ampholytic components of biological material contained in a buffer uses an elongated separation vessel with two ends. The vessel has physical characteristics such that a temperature gradient can be created within contents of the vessel using a constant current generated by a power source having one terminal connected at one end of said vessel and the other terminal connected at the other end. There is a reservoir for the terminals at each end of the vessel. One of the reservoirs is a cathodic reservoir and the other reservoir is an anodic reservoir. There are several separate anodic reservoirs. The method comprises the steps of activating the current until all components of the buffer having a pI, which is low enough, pass through the vessel into a first anodic reservoir, replacing the first anodic reservoir with a second anodic reservoir and activating the system with a slightly lower current than that which was used with the first reservoir, thereby causing part of the ampholytes located by focusing at one end of the vessel and having a low enough pI to be charged negatively and to migrate into the second anodic container, replacing the second anodic container with a third anodic container and repeating the process with an even lower current to cause another part of the ampholytes located by focusing at one end of the vessel with a low enough pI to migrate into the third anodic container, continuing to repeat the process with successive anodic containers and successive reductions in current until sufficient fractions of ampholytic components are obtained.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings:

FIG. 1 is a schematic perspective view of part of an isoelectric focusing system;

FIG. 2 is a schematic side view of an isoelectric focusing system;

FIG. 3a is a schematic side view of a cone-shaped capillary with ampholytes separated into sample zones;

FIG. 3b is a graph of a temperature gradient along the capillary of FIG. 3a;

FIG. 3c is a graph of the pH gradient along a length of the capillary;

FIG. 4 is a side view of a cone-shaped capillary with a reservoir at each end;

FIG. 5 is a sectional side view of a capillary having a constant inside diameter and a conductor of varying thickness;

FIG. 6 is a schematic perspective view of a continuous flow separation vessel having a V-shaped cross-section;

FIG. 7 shows the magnitude of successive absorption signals along the length of a capillary with a coated interior wall; and