

decreased because of the increase in the band's width and increase of the capillary diameter at the new position.

From FIG. 7, it can be seen that the isoelectric focusing technique of the present invention can be used not only for analytical and preparative separations but also for preconcentration and purification of biological samples prior to analysis. From FIG. 8, it can be seen that the isoelectric focusing system can be used for trapping and concentrating target ampholytes (for example, proteins, peptides, amino acids or any other substances having an isoelectric point) in the capillary followed by mobilization of analytes towards the detection or collection point by increasing the electrical potential.

#### EXAMPLE #1

Cone-shaped capillaries of 4 cm in length were used as the separation concentration channel and were drawn from 5 mm inside diameter glass tubes. The i. d. of one end of the capillary was 0.2 mm and another end was 1 mm. The capillary was mounted on a cartridge and its two ends were connected to buffer reservoirs as shown in FIG. 1. In some experiments, the inner wall of the capillary was coated with non-cross-linked poly (acrylamide) to eliminate electroosmosis by the reported way. Cross-linked poly (acrylamide) could also be used. The separation was driven by a high-voltage DC power supply (Spellman, Plainview, N.Y.), and the separation voltage was about 1 kV. The anode was inserted into the buffer reservoir at the narrower end of the capillary, and the another end of the capillary was connected to ground.

The protein sample used in the experiment was human hemoglobin (Sigma, St. Louis, Mo.) which contained two major isoforms; methemoglobin (75%) and oxyhemoglobin (25%). All chemicals were reagent grade, and solutions were prepared using deionized water. The buffer as 0.05 M TRIS buffer at pH 7.3. This buffer has a large temperature co-efficient of pH (dpH/dT is  $-0.028K^{-1}$  at  $250^{\circ}C.$ ) (10). Protein solutions were prepared in the TRIS buffer. The solutions were filtered using 0.2- $\mu$ m pore size cellulose acetate filters (Sartorius, Gottingen, Germany).

A UV-vis absorption imaging detector was employed for the monitoring of the protein zones focused inside the capillary. As shown in FIG. 2, the light source of the detector was a halogen lamp. The sensor was a 1024 pixel CCD (Type S3903-1024Q, Hammamatsu, Hammamatsu City, Japan). A bandpass coloured filter (400 nm–600 nm) was used to cut near IR and ultraviolet radiations of the lamp. The light beam was first collimated as shown in FIG. 2, and then focused into the capillary by three cylindrical lenses. The image of the capillary was projected into the CCD sensor as shown in FIG. 2.

Two sample introduction methods were used in the experiment. In the first method, the coated capillary was filled with the buffer, and plugs of 1% agarose gel (prepared in the buffer) were placed in both reservoirs to avoid hydrodynamic flow in the system, and then the voltage was applied. After 10 minutes, a few drops of 0.1 mg/mL sample solution was added to the top of the reservoir at the cathodic end of the capillary. In the second method, the reservoirs and the uncoated capillary were filled with the protein solution and the voltage was applied. In all experiments, the current passing through the capillary was kept at about 0.8 mA by adjusting the applied voltage to about 1 kV. All experiments were done in triplicate to ensure reproducibility.

In using the isoelectric focusing system of the present invention, the reservoir 6 at the narrow end of the capillary

(anodic end) can be kept at a predetermined temperature to produce a low enough pH of the buffer to positively charge target proteins present in the reservoir (i.e. the sample that was added to the anodic reservoir). These proteins will then migrate through the capillary toward the cathode and will be trapped in the capillary at their isoelectric points or will be collected at the reservoir 4 connected to the wide end of the capillary. The system can be used to purify the biological material by fractionating it with respect to the isoelectric point. The cathodic end reservoir 4 will contain proteins which have a lower pI than the buffer in the reservoir 4 which is kept at room temperature. The anodic reservoir will contain proteins at a pI that is higher than the pH of the buffer in that reservoir while the capillary will contain proteins which have intermediate pI. The anodic reservoir 6 will be heated up in the process and that will speed up the concentration and focusing process when the sample is introduced to both the cathodic and anodic reservoirs.

Numerous variations, within the scope of the attached claims, will be readily apparent to those skilled in the art.

What I claim as my invention is:

1. A device used for isoelectric focusing of ampholytes contained in a buffer, said device comprising an elongated separation vessel with two ends, said vessel containing said buffer, said vessel having an elongated tapered portion so that a temperature gradient is created within contents of the vessel using a power source that generates a constant current, said power source having one terminal connected at one end of said vessel and another terminal connected at another end of said vessel, said power source connected to provide means with said tapered portion to create a temperature gradient longitudinally along said buffer in said vessel between said ends and to simultaneously focus said ampholytes with a detection system to monitor progress of said focusing.

2. A device as claimed in claim 1 wherein the separation vessel has side walls that extend between the two ends, said walls smoothly tapering substantially from one end to the other to form the tapered portion.

3. A device as claimed in claim 2 wherein the walls have a linear taper.

4. A device as claimed in any one of claims 1 or 2 wherein the separation vessel is a tapered capillary.

5. A device as claimed in claim 1 wherein the separation vessel is a cone-shaped capillary.

6. A device as claimed in claim 5 wherein the power source is a DC power source, a positive electrode from said power source being connected into the buffer at a narrow end of said capillary and a negative electrode from said DC source being connected into the buffer at a wide end of said capillary.

7. A device as claimed in claim 6 wherein there is a reservoir at each end of the capillary, the reservoir containing said buffer and the electrodes being connected into said reservoir.

8. A device as claimed in claim 1 wherein the separation vessel contains an elongated channel of constant inside dimensions and has a conductive surface on an outside wall thereof, the conductive surface varying in thickness between said ends to form the tapered portion, a positive electrode being connected to said outside wall at a narrow end and a negative electrode being connected to said wall at a wide end.

9. A device as claimed in claim 8 wherein the power source is a DC power source and the narrow end has a higher temperature than the wide end.

10. A device as claimed in claim 8 wherein the conductive surface completely surrounds the outside wall of the elongated channel.