On-Column Conductivity Detector for Capillary Zone Electrophoresis

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A new on-column conductivity detector for use with capillary zone electrophoresis is described. The system, constructed with inexpensive, readily available components, can analyze nanoliter samples. Peak area is linear with concentration over 3 orders of magnitude, and a small detection volume on the order of 30 pl allows the detection of about 10^3 ions. Potential applications include routine, direct analyses on samples of biological interest.

Capillary zone electrophoresis (CZE), first introduced by Mikkers et al. (I) and by Jorgenson and Lukacs (2), holds exciting promise for separating charged species in solution, particularly aqueous media. This technique is characterized by excellent mass sensitivity, low sample consumption, and high resolution. Because of the combined action of electroosmotic flow with electrophoretic separation, all species normally travel in one direction, allowing detection of positively charged, neutral, and negatively charged species at one single location along the capillary tubing. However, the size of the capillary dimensions, dictated by the need to remove efficiently Joule heating, places stringent requirements on the detection system. Indeed, this last feature has been identified by Jorgenson and Lukacs (3) as "the greatest obstacle to further development and utilization of capillaries." This paper concerns the development of an on-column conductivity detector for capillary zone electrophoresis which can extend the application of this powerful separation technique.

Conductivity is well-recognized as a universal detector in liquid chromatography (4) and the first experiments by Mikkers et al. (I) employed a potential drop between two electrodes separated along the flow direction. More recently, Boček and co-workers (5) have described the use of a conductivity cell into which the capillary is connected. They reported a level of detection of 2 x 10^{-5} M. The present work differs in that the conduction measurements are made on-column by placing the electrodes on opposite sides of the capillary wall.

EXPERIMENTAL SECTION

The on-line conductivity cell was constructed by fixing platinum wires through diametrically opposite holes in 50- or 75-μm-i.d. fused-silica capillary tubing (Polymicro Technology, Inc., Phoenix, AZ, and SGE, Austin, TX). These 40-μm-i.d. holes were made with a computer-controlled CO₂ laser. Under a microscope, two 25-μm-o.d. Pt wires (California Fine Wire Co., Grover City, CA) were placed in the holes exactly opposite to each other in order to minimize the potential difference between these electrodes when a high electrical field strength is applied. Polyethylene glycol (PEG) (MW 1000, J. T. Baker Chemical Co.), heated to liquid, is applied to the area surrounding the electrodes so that they are held temporarily in place. Once the PEG has solidified, it is carefully removed from the outside surface of the capillary. An epoxy (Miller Stephenson 907) is then used to seal permanently the electrodes in the capillary. Wires (no. 30 wire wrap, Digital,
Samples containing ions were dissolved in a buffer solution consisting of 20 mM morpholinoethanesulfonic acid (MES) adjusted by histidine to pH 6.1. All chemicals were obtained from Sigma (St. Louis, MO) and used without further purification. The serum samples were acquired from the Stanford University Medical Center and diluted, as needed, with buffer solution. The diluted serum samples were deproteinized with a filter membrane (Toya Soda, Japan) in a centrifuge.

RESULTS AND DISCUSSION

Figure 3 depicts the electrophoretic separation and detection of Rb⁺, K⁺, Na⁺, and Li⁺. The concentration of each ion is 2 x 10⁻⁶ M. The signal-to-noise ratio, at this concentration, exceeds 100. Based on a signal-to-noise ratio of 2, the detection limit is found to be about 10⁻⁷ M for Li⁺. We have been able to estimate the effective detection volume to be about 30 nL based on the determination of the cell constant (cross-sectional area of the electrodes divided by the distance between them) made by measuring the conductance of a known solution of KCl and using the literature value of the specific conductance for this solution. This volume estimate assumes that the electrodes are separated by 50 μm (the inside diameter of the capillary tube). The value obtained agrees within a factor of 1.5 with the geometrical volume based on the cross-sectional area of the electrodes and the distance between them. This implies that the actual amount detectable is 10⁻³⁶ mol, which corresponds to about 10⁹ ions. The retention time of each ion is approximately proportional to the reciprocal of its mobility. All of the peaks shown are “positive”, i.e., when each of these ions passes the detection electrodes, their mobilities are greater than the background conductivity of the buffer solution and show up as positive deviations above the base line. Thus, the areas of the peaks represent the mobility differences between the ions in the detection zone and the counterion (histidine) of the electrolyte. This suggests that, in other cases, “negative” peaks may also be observed.

It was determined that peak area is linearly related to ion concentration. A correlation-regression analysis was done on 18 concentration levels of Li⁺ extending over 3 orders of magnitude from 0.0025 to 2.0 mM. Three consecutive runs were made at each concentration level. The peak areas of Li⁺ were found to be linear over the entire range examined with a correlation coefficient of 0.993. Similar results were obtained for Na⁺.

An electropherogram showing the separation of tetramethylammonium, triethylamine, arginine, and histidine is shown in Figure 4. Since the K⁺, used as the counterion in the buffer, has a greater mobility than the sample components, the peaks are “negative”, i.e., they project below the base line. This data illustrates that this method can be used to separate
The conductivity change is an electrical signal, it is easily coupled with the electrical characteristics. We are able to control the deviation of the conductance with respect to the background electrolyte. Since the conductivity change is an electrical signal, it is easily coupled to a data acquisition system. The apparatus is economical in terms of both cost and energy. The cost for all of the materials necessary for the construction of the conductivity cell and its associated circuitry is about $200, and the power required is only a few watts, which could be furnished by a battery system. Hence, the entire system is readily made portable. Indeed, its small size suggests that it can be readily adapted to in vivo sampling.

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Registry No. Rb, 7440-17-7; K, 7440-09-7; Na, 7440-23-5; Li, 7439-93-2; (CH3)4N, 51-92-3; (CH3CH2)3N, 121-44-8; Arg, 74-79-3; His, 71-00-1.

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LITERATURE CITED


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