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Registry No. Benzene, 71-43-2; toluene, 108-88-3; ethylbenzene, 100-41-4; propylbenzene, 103-65-1; butylbenzene, 104-51-8; pentylbenzene, 538-68-1; hexylbenzene, 1077-16-3; acetophenone, 98-86-2; propiophenone, 93-55-0; butyrophenone, 495-40-9; valerophenone, 1009-14-9; hexanophenone, 942-92-7.

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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 10^6 ions. Potential applications include routine, direct analyses on samples of biological interest.

Capillary zone electrophoresis (CZE), first introduced by Mikkers et al. (*1*) 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. (*1*) 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 2×10^{-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 CO_2 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. Poly(ethylene 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,

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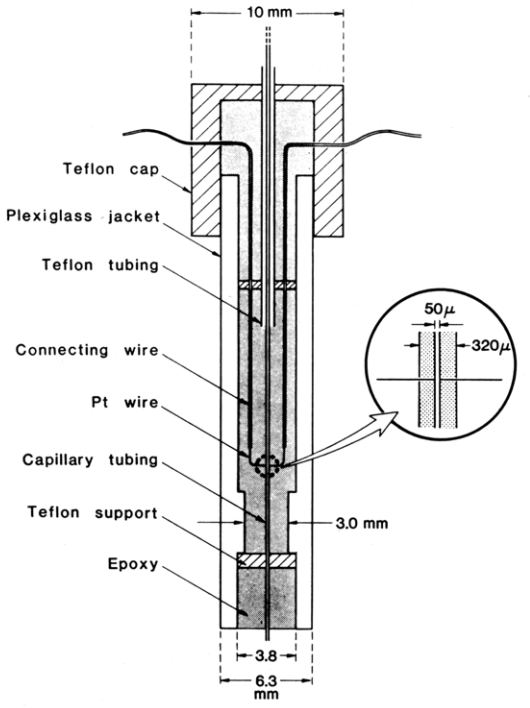

Figure 1. Diagram of the conductivity cell.

Inc.) are soldered to the platinum electrodes and the entire conductivity cell is sealed in a Plexiglas jacket. Figure 1 shows the completed structure of the conductivity cell.

The underlying principles of the ac conductivity meter are based on the work of Everaerts et al. (*6*). Figure 2 shows our circuit diagram, which was modified in several ways from the conductivity meter circuit given by Everaerts et al. (*6*), including replacement of the μ741 chip by two kinds of high-impedance input integrated circuits, LF351 and LF355. A transformer was used as a galvanic insulator between the sensing electrodes, which have a high dc potential to ground, and the electronic circuit. The oscillation frequency was set to 3.5 kHz. A low-pass filter was placed after the circuit to minimize electronic noise. The output of the conductivity meter was amplified (gain: 10 or 20) before being transmitted to a data acquisition board (DT2801, Data Translation, Inc., Marlborough, MA) in an IBM XT microcomputer.

The CZE system used was similar to that reported by Gozel et al. (*7*) except for the replacement of the laser-induced fluorescence detector with the conductivity detector. Samples were introduced either by electromigration for 5 s at 5 kV or by gravity flow for 30 s with one end of the capillary elevated 10 cm higher than the other. The estimated volume injected was about 10 nL for electromigration and about 5 nL for gravity injection. The capillary was washed with buffer after each run.

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 2×10^{-5} 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 pL 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^{-18} mol, which corresponds to about 10^6 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 conductivities 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

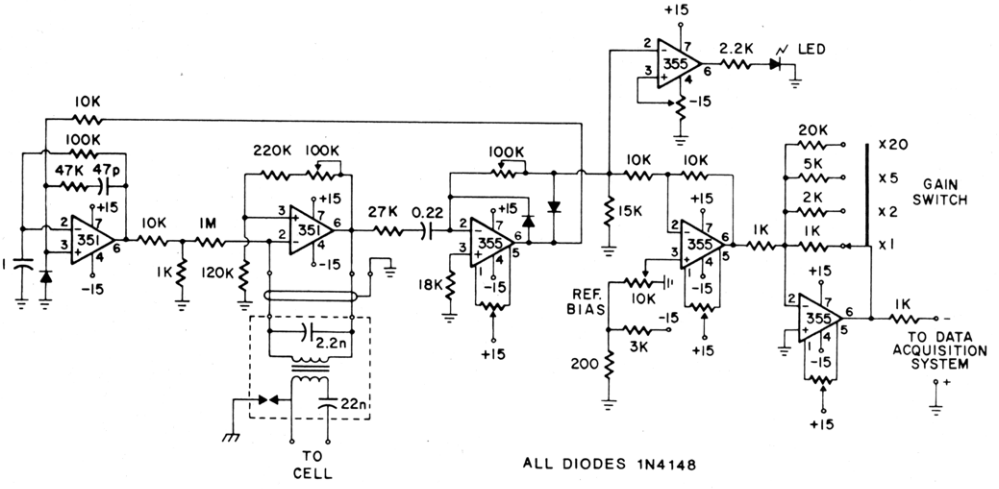

Figure 2. Conductivity detector circuit diagram.

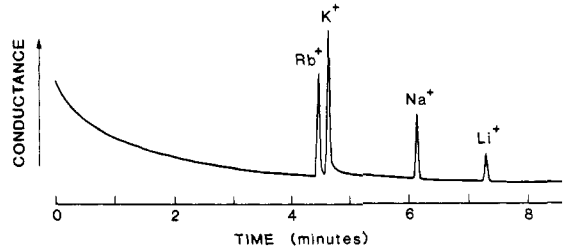

Figure 3. Electropherogram of a mixture of four cations, Rb^+, K^+, Na^+, and Li^+, at a concentration of 2×10^{-5} M: capillary inside diameter, 75 μm, length, 60 cm; buffer, 20 mM MES/His, pH 6; electromigration injection for 5 s at 5 kV; applied voltage, 15 kV.

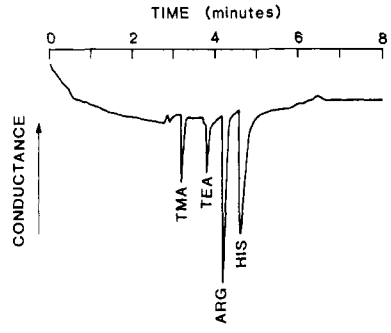

Figure 4. Electropherogram of a mixture of 8×10^{-5} M tetramethylammonium and triethylamine plus 4×10^{-5} M arginine, and histidine: capillary inside diameter, 50 μm, length 51 cm; buffer, 5 mM potassium acetate (KCH_3COO), pH 5.4; gravity injection from 10 cm for 30 s; applied voltage, 15 kV.

some organic cations. With a change in buffer conditions, organic anions can also be detected.

A normal human serum sample was subjected to CZE and the results are shown in Figure 5a. The first peak is K^+, the very broad second peak is Na^+. The peak clipping occurs due to saturation of the electronics because the Na^+ concentration in serum is so high (about 140 mM before dilution). As a result, Ca^{2+} and Mg^{2+}, which have mobilities close to that of Na^+, are obscured by the large Na^+ peak. Figure 5b is an electropherogram of a serum sample from a patient on lithium therapy. It demonstrates that the third (Li^+) peak is completely resolved from the Na^+ peak. This suggests that CZE may be useful in clinically monitoring patients taking lithium therapy.

This new conductivity detector has several advantageous characteristics. We are able to control the deviation of the distance along the capillary between the electrodes to less than 10 μm. This means that the potential difference between the two electrodes can be minimized to less than 0.3 V in a 300 V/cm electric field. This feature eliminates most of the electrochemical reactions occurring at the electrodes. Another advantage of this structural form is that excellent resolution is made possible by the very small cross-sectional area of the electrodes. There is essentially no dead volume in the detector and the detection volume is very tiny. This system can be used to detect and quantify any species causing a conductivity change with respect to the background electrolyte. Since the conductivity change is an electrical signal, it is easily coupled

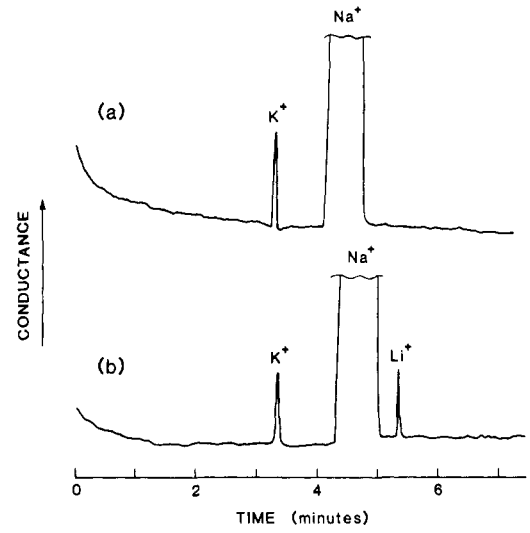

Figure 5. Electropherograms of human serum: (a) normal subject, (b) patient on lithium therapy, dilution, 1:19 with 20 mM MES/His buffer, pH 6.1; capillary inside diameter, 75 μm, length, 70 cm; gravity injection from 10 cm for 30 s; applied voltage, 25 kV. The Na^+ peaks are off scale.

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. We feel that the sensitivity of this detector is quite remarkable for a nonoptical system.

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Registry No. Rb, 7440-17-7; K, 7440-09-7; Na, 7440-23-5; Li, 7439-93-2; $(CH_3)_4N$, 51-92-3; $(CH_3CH_2)_3N$, 121-44-8; Arg, 74-79-3; His, 71-00-1.

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