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## Note

### **Quantitation of Li<sup>+</sup> in serum by capillary zone electrophoresis with an on-column conductivity detector**

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Since treatment with lithium for acute mania has grown in acceptance, the need for close monitoring of this therapy has become essential. The variation of lithium concentration in serum with time has made it necessary to establish a standard of sampling 12 h after a dose is administered. There appears to be some individual variation in tolerance to the drug, but it has been reported [1] that 1.5 mmol/l lithium in serum is the lower limit of risk for intoxication. Indeed, death may result from a lithium concentration in serum over 3.5 mmol/l. The need for a fast, convenient method for monitoring the lithium concentration in serum has been met generally by flame emission photometry [2-4] and atomic absorption spectroscopy [5-8]. In general, flame emission photometry is more sensitive for lithium determinations than atomic absorption spectroscopy, but is also the less precise of the two methods. In addition, there is some spectral interference from other ions, in particular Na<sup>+</sup> and K<sup>+</sup>, in these procedures [5]. The use of an ion-selective electrode based on ETH 1810 for the assay of lithium in serum has been reported [9], but appears to need better Li<sup>+</sup>/K<sup>+</sup> selectivity.

Electrophoresis is a well established technique for the separation of charged species. In zone electrophoresis, a narrow zone of the sample mixture is injected into the migration channel and a potential gradient is applied. Individual sample components migrate at different rates determined by their electrophoretic mobilities and separate into discrete zones provided that their mobilities are sufficiently different. Associated with the passage of current in the migration channel is the generation of Joule heating. This heat is uniformly produced across the length and cross-section of the migration channel but may only be removed at the walls of the migration channel. As a consequence, a radial temperature gradient is set up which in turn causes a radial density gradient. This has the un-

wanted effect of causing uneven migration rates of the charged species and convective zone broadening.

By far, the most common way to prevent convection is to carry out the electrophoretic separation in a supporting medium, such as in a gel or along a strip of paper [10]. However, the presence of the support introduces other zone broadening mechanisms, such as eddy migration and slow adsorption/desorption kinetics, which prevents this method from achieving high separation powers. An alternative approach is to decrease the cross-section of the migration channel, thereby increasing the surface area-to-volume ratio and hence the cooling rate. This is the idea behind capillary zone electrophoresis (CZE), pioneered by Mikkers et al. [11] and by Jorgenson and Lukacs [12]. By using an open tubular capillary column of several hundred microns or less inside diameter, separation efficiencies approaching and in some cases exceeding one million theoretical plates have been reported for selected analytes [13]. Moreover the formation of a diffuse double layer at the capillary walls causes the free liquid to have an excess of one charge. Application of a potential gradient across the capillary then causes the entire liquid to move in one direction, called electroosmotic flow [14]. The electroosmotic flow-rate typically exceeds the electrophoretic mobilities of the different species present in the sample mixture causing all species to pass as narrow zones through a detector located at a fixed distance downstream along the migration channel. In electrophoresis, as in chromatography, the sensitivity depends on the quality of the detection system. Particularly advantageous is the use of an on-column detector as this does not appreciably disturb the flow or add to the dead volume. The earliest use of an on-column detector in capillary zone electrophoresis involved UV absorption [12, 13, 15]. Recently we have developed an on-column conductivity detector for use with CZE [16]. We describe here its use in quantitating lithium ion concentrations in human serum samples.

## EXPERIMENTAL

A schematic diagram of the entire CZE-conductivity cell system is shown in Fig. 1 and has been fully described elsewhere [16]. Briefly, the capillary is filled with buffer solution using a syringe. Then, about 5 nl of sample are injected (described below) into the capillary end farthest from the detector. The detector end of the capillary is made the cathode and voltage is applied. The detector measures a constant value for the conductivity of the buffer until some sample component migrates between the detector electrodes and changes the conductivity. The background conductivity and such changes are transmitted to a data acquisition system.

Aqueous lithium solutions of fourteen concentration levels (0.0025–1.4 mM) were made to establish linearity. Three consecutive runs were made at each concentration level. Lithium carbonate standards were prepared at concentrations of 0.1, 0.5, 1.0, 1.5, and 2.0 mM and used for calibration. Lithium carbonate was chosen because it is non-hygroscopic. A stock standard solution was prepared by dissolving 369.45 mg lithium carbonate in 250 ml of water plus 100 ml of 0.1 M hydrochloric acid in a 1-l volumetric flask, mixing and diluting to the mark with

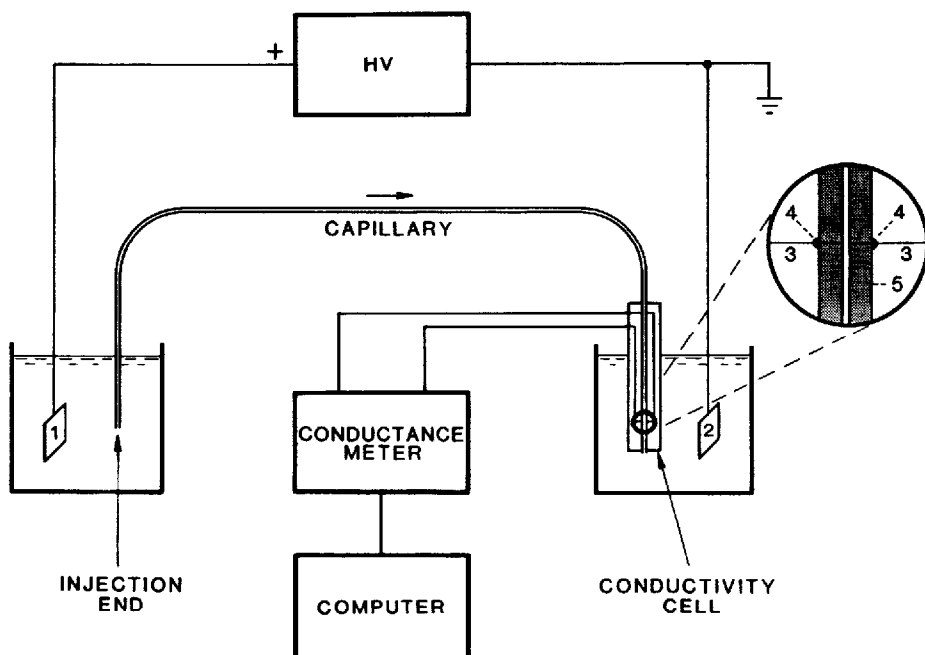


Fig. 1. Schematic diagram of the CZE-conductivity cell system. (1) Anode electrode. (2) Cathode electrode. (3) On-column conductivity cell platinum electrodes, 25  $\mu\text{m}$  O.D. (4) Epoxy resin sealant. (5) Capillary tube.

deionized water. Appropriate dilutions were made with the stock standard to provide the final concentrations desired.

Human serum and plasma samples were obtained from the Clinical Laboratories of Stanford University Medical Center and from the Palo Alto Veterans Administration Hospital. The samples were diluted 19:1 or 49:1 with 10 or 20 mM morpholinoethanesulfonic acid (MES) adjusted by histidine to pH 6.1. The diluted serum samples were deproteinized with a filter membrane (Toya Soda, Japan) in a centrifuge.

CZE was conducted in a 70 cm  $\times$  75  $\mu\text{m}$  I.D. fused-silica capillary tube (Polymicro Technology, Phoenix, AZ, U.S.A.). Samples were introduced into the capillary by gravity flow for 30 s with one end of the capillary elevated 10 cm higher than the other. Throughout this study, sample injection was made by gravity flow because we found [17] that representative sampling occurs with gravity flow but does not always occur with electrokinetic flow injection. All runs were made using 25 kV.

## RESULTS AND DISCUSSION

A normal human serum sample was subjected to CZE and the results are shown in Fig. 2a. The first peak is  $\text{K}^+$ , the very broad second peak is  $\text{Na}^+$ . The peak clipping occurs due to saturation of the electronics because the  $\text{Na}^+$  concentration in serum is so high (about 140 mM before dilution). As a result,  $\text{Ca}^{2+}$  and

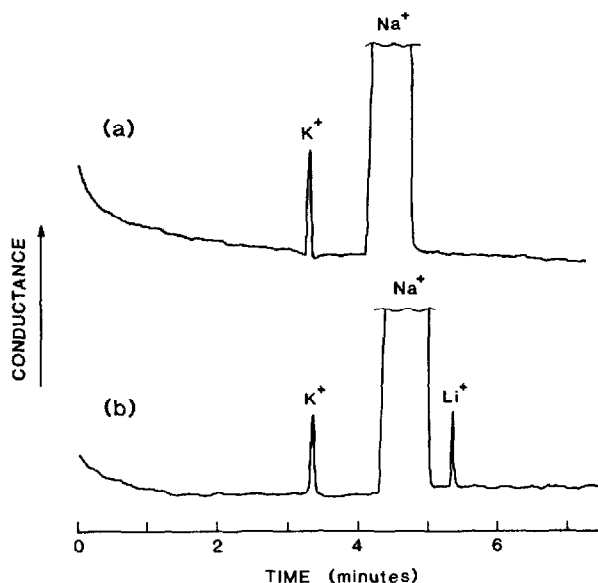


Fig. 2. Electropherograms of human serum. (a) Normal subject; (b) patient on lithium therapy. Dilution is 1:19 with 20 mM MES-His buffer, pH 6.1. Capillary I.D., 75  $\mu$ m, length, 70 cm; gravity injection from 10 cm for 30 s; applied voltage, 25 kV. The  $\text{Na}^+$  peak is off scale.

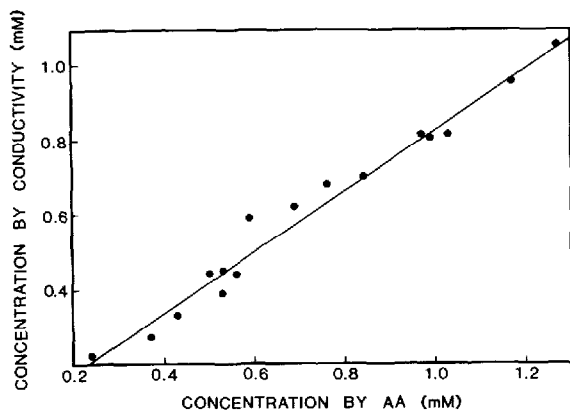


Fig. 3. Plot of the  $\text{Li}^+$  concentrations determined by atomic absorption (AA) (Palo Alto Veterans Administration Hospital) versus concentrations obtained by CZE-conductivity (this laboratory).  $y = 0.0087 + 0.825x$ ;  $r = 0.988$ ;  $n = 16$ .

$\text{Mg}^{2+}$ , which have mobilities close to that of  $\text{Na}^+$ , are obscured by the large  $\text{Na}^+$  peak. Other metal ions, such as  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ , are not seen because after dilution they are present in amounts below the detection level. Fig. 2b is an electropherogram of a serum sample from a patient on lithium therapy. It demonstrates that the third ( $\text{Li}^+$ ) peak (at a concentration of about 1.0 mM) is completely resolved from the  $\text{Na}^+$  peak.

A correlation-regression analysis was done on the data obtained from the fourteen concentration levels of  $\text{Li}^+$ . The peak area is linearly related to  $\text{Li}^+$  concen-

TABLE I  
PRECISION OF Li<sup>+</sup> PEAK AREAS

Run No.	Sample 1	Sample 2	Sample 3
1	5043	10422	2003
2	5219	9940	2043
3	5099	10437	1936
4	4807	10417	1904
5	5172	9782	1930
6	5033	10157	1922
7	4774	9997	1893
8	5015	10255	2105
9	5165	9925	2076
10	4913	9872	2045
11	5097	9716	2061
12	4938	9829	1840
13	4996	10371	2140
14	5295	9648	2025
15	5215	9896	1990
16	5171	10361	2021
17	5239	10085	2058
18	4961	10528	1903
19	5115	10447	2199
20	5058	9954	2133
Mean	5066.25	10101.95	2011.35
Coefficient of variation (%)	2.78	2.79	4.76

tration over three orders of magnitude. The correlation coefficient ( $r$ ) is 0.997. Results from the calibration runs show that the Li<sup>+</sup> peak areas are linear with concentration with  $r=0.998$ . Normal serum samples were spiked with varying concentrations of Li<sup>+</sup>. The recovery of Li<sup>+</sup> at 0.5 mM was 98.3% and at 4 mM was 92.7%.

Three consecutive runs on each of sixteen serum samples from patients on lithium therapy were made in a double-blind test environment and then compared with the atomic absorption results obtained by the Clinical Laboratory at the Palo Alto Veterans Administration Hospital. Fig. 3 is the plot of those data with a calculated correlation coefficient of 0.988.

Three of the sixteen samples with Li<sup>+</sup> concentrations markedly different from each other were selected to examine the precision of the method. Table I shows the raw data and the results of twenty consecutive determinations of each sample. All of the sample injections were done manually and probably are the major source of variability. We believe that if injections are automated, the coefficients of variation would be substantially reduced.

The instrumentation itself is very simple and low cost. Sample volumes can be less than 20  $\mu$ l with a detection limit calculated to be about  $10^{-7}$  M for Li<sup>+</sup>. Since Li<sup>+</sup> is well separated from K<sup>+</sup> and Na<sup>+</sup>, there is no interference from these two ions in the CZE analysis procedure. Although the total run time is about 4 min (including sample injection), which is longer than atomic absorption, CZE with conductivity detection appears to be a viable alternative to both atomic absorp-

tion spectroscopy and flame emission photometry for the quantitation of Li<sup>+</sup> concentration in human serum.

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