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Determination of Chloride Concentration Using Capillary Zone Electrophoresis: An Instrumental Analysis Chemistry Laboratory Experiment

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Capillary zone electrophoresis (CZE) has received a great deal of attention as a tool for the separation and detection of low-molecular-mass inorganic ions. CZE is a fast growing analytical technique that uses a low-volume sample accompanied by a high detection efficiency. Since this is a fairly new analytical technique that shows tremendous promise, it should be introduced to students at the undergraduate level. We have developed a CZE experiment that is well suited for an instrumental analysis chemistry laboratory and can be modified for other species or substances.

Experimental Procedure

Instrumentation

Electrophoresis separates charged and neutral species in aqueous solution by using an electric field (*1*). This technique can be applied to single atomic ions, proteins, and nucleic acids (*2–4*). Some examples of electrophoresis techniques are gel electrophoresis, isoelectric focusing, capillary isotachophoresis, micellar electrophoresis, and capillary zone electrophoresis.

The applicability and reproducibility of capillary zone electrophoresis is due to one important factor: electroosmotic flow (*5*). On the inner wall of the capillary, there is a formation of an electric double layer at the solid–liquid interface caused by the preferential adsorption of ions. Silanol groups on the inner wall of the capillary give up an acidic hydrogen and become negatively charged when in contact with the medium. There is an attraction between the cations or partial positive charges in the medium and the negatively charged silanol groups on the capillary wall forming the electric double layer that gives rise to electroosmosis. This flow is generated at the wall of the capillary and causes the elution of cations, anions, and neutral species at one end of the capillary (*6–8*). Since all species elute at the same end of the capillary, a single on-column detector is needed (*9*). Samples in the size range of 0.1 to 20 nL are introduced at the positive end of the capillary and migrate down the capillary under the influence of an electric field toward the detector at the negative end of the capillary. The rate of migration depends on the ion size as well as the sign and magnitude of the charge of the ion (*1*). The resulting plot of analyte concentration versus time is called an electropherogram. All species (cations, anions, neutrals) are carried by electroosmotic flow toward the detector despite the fact that anions are attracted toward the positive end and neutral species are not attracted toward either end of the capillary. Electroosmotic flow has no affect on separation, but does affect all substances in the same manner. The faster the electroosmotic flow, the faster all analytes will sweep through the capillary leaving little time for zones to diffuse.

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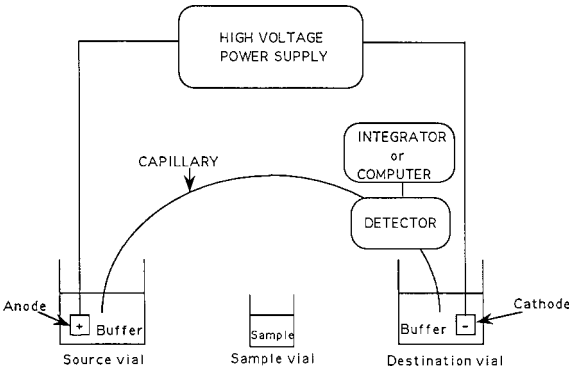

Figure 1. Schematic of capillary electrophoresis system.

Capillary zone electrophoresis uses small-diameter capillaries, enabling capillary electrophoresis to provide very fast, high-efficiency separations. Capillary electrophoresis typically uses fused silica capillaries that are 30–100 cm long, 50 or 75 μm i.d., and 375 μm o.d., with voltages of up to 30 kV (*1*). Small-diameter capillaries dissipate heat very well; therefore, high voltages, 30 kV, can be used to allow shorter separation times and higher efficiencies. Efficiencies in excess of 400,000 theoretical plates can be obtained using capillaries only 80–100 cm long. These efficiencies are excellent, especially when compared with high-performance liquid chromatography in which highs of 20,000 theoretical plates are obtained (*9*).

The main components of a typical capillary electrophoresis system as shown in Figure 1 are sample vial, source and destination vials, capillary, detector, high-voltage power supply, and data acquisition device. The technique uses an electrolyte, aqueous buffer solution, to fill the source vial, capillary, and destination vial. Samples are loaded into sample vials that typically have volumes of a few milliliters. Only a few nanoliters are actually injected into the capillary. The sample is introduced from the sample vial by the capillary inlet. There are four common techniques for sample injection into the capillary: (i) hydrodynamic injection (performed by pressure or siphoning), (ii) gravity injection (performed by raising the sample vial, causing the sample to siphon into the capillary), (iii) pressure injection (performed by pressurizing the sample vial or applying a vacuum to the destination vial), (iv) electrokinetic injection (performed by applying an electric field to the sample vial, causing the sample components to migrate into the capillary). Most commercial instruments are equipped with autosamplers and automatically inject using any one of the techniques mentioned. After the sample is introduced into the capillary at the sample vial, the capillary inlet moves to the source vial. An electric field is applied

between the source and destination vials, causing the solute to migrate through the capillary. The solute is detected by the detector; its output is sent to an integrator; and the output is displayed as an electropherogram (plot of detector responses versus time). There are a variety of detectors, but the most commonly used are UV–vis absorbance detectors. Detection limits range from 1 ppm to 1 ppb. There was a period of time when the lack of a universal detection scheme for nonchromophoric analytes inhibited the early development of separations for inorganic anions. Much progress has been made and several reports have been published recently describing the application of capillary zone electrophoresis for the analysis of organic and inorganic anions with indirect UV–vis absorbance detection (*10, 11*). Indirect UV–vis absorbance detection was developed for the detection of "transparent" ions that were separated by ion exchange chromatography (*12*). With the aid of this detector, capillary electrophoresis can be used for analysis of compounds that do not absorb enough light to be detected by UV–vis. A light-absorbing compound, chromophoric ion, is used as the run buffer, which creates a large background absorbance. Chromophoric ions in the buffer are displaced by nonabsorbing solutes in the sample. A decrease in absorbance occurs when a nonabsorbing solute passes through the detector. The resulting effect will be a dip in the baseline which gives a negative peak. To avoid the negative peak, the output polarity of the detector is reversed so that a positive peak is seen at the integrator or computer. The fact that all solutes can be detected by indirect UV–vis makes it a universal detector. A variety of chromophoric ions are available: chromate (*13*), pyromellitic acid (*14*), and phthalate (*15*) for anion determination; quinine (*16*), and malachite green (*17*) for cation determination. One criterion for selecting the chromophoric ion is that the mobility of the chromophoric ion should match the mobilities of the solutes.

Electropherograms are different from chromatograms because of the use of the UV absorbance detector (a concentration-dependent detector). For equal solute concentrations and detector responses, chromatography peak heights and widths are different. In capillary electrophoresis, the solutes move through the detector in zones of approximately the same length and same concentration; therefore, peak heights remain constant as retention gets longer. Peak areas increase with time for equal concentrations and detector responses.

Equipment

- Dionex Capillary Electrophoresis System I (CES I) with a Dionex Advanced Computer Interface using a Zenith Z386/25 computer as a chromatography workstation. The software used on the computer was Dionex AI 450 Chromatography Automatic software version 3.32.
- Fused silica capillary: 50 μm (i.d.) × 375 μm (o.d.) × 50 cm total length.
- Data analysis program capable of performing least squares analysis, e.g. *Journal of Chemical Education*: Notebook—A Column Calculator and Plotter for IBM-PC.

Reagents

- IonPhor anion PMA (pyromellitic acid) electrolyte buffer obtained from Dionex
- Deionized water
- 100.0 ppm Cl^- stock solution (typical solution preparation: 0.1653 g dried primary standard grade NaCl [assay 99.70%] diluted to 1.00 L with deionized water)
- Individual unknown samples of soluble chloride (Thorn Smith Corporation)

Procedure

A series of standards ranging from 2.00 to 10.00 ppm Cl^- are analyzed by CZE. The results are used to prepare a linear calibration curve of peak area versus concentration (ppm Cl^-), which is used to determine the percent chloride of an unknown sample.

1. Dry the unknown at 110 °C for at least one hour.
2. *Standard*. Label five 50.00-mL volumetric flasks S1, S2, S3, S4, S5. Transfer by buret or transfer pipet exactly 1.00, 2.00, 3.00, 4.00, 5.00 mL of the 100.0 ppm Cl^- stock solution into S1–S5, respectively. Dilute to the mark with deionized water and mix well. Calculate the concentration of each standard solution in terms of ppm Cl^-. NOTE: The concentration of S1–S5 should be 2.00, 4.00, 6.00, 8.00, 10.00 ppm Cl^- but the concentration depends on the exact concentration of the Cl^- stock solution.
3. *Bulk unknown solution*. Weigh out (to the nearest 0.1 mg) 0.1 g of oven-dried unknown. Quantitatively transfer to a 100.00-mL volumetric flask. Dilute to the mark with deionized water and mix well.
4. *Diluted unknown solution*. Label three 50.00-mL volumetric flasks U1, U2, U3. Transfer by micropipet exactly 0.250, 0.500, 0.750 mL of the bulk unknown solution into U1–U3, respectively. Dilute to the mark with deionized water and mix well.
5. *Data acquisition*. Fill one vial each with S1–S5, U1–U3, and one with pure deionized water (nine vials total) and obtain data. The deionized water sample is used as a background check. Use the parameters for CZE analysis as specified on Dionex IonPhor anion PMA electrolyte buffer product sheet (Document No. 034864-08: separation voltage, 30 kV; current, 9–13 μA; detector, indirect UV at 250 nm (wavelength absorbance of buffer); injection volume, gravimetric, 100 mm for 30 seconds, run time: 3.0 min, data collection rate: 10 Hz), and obtain electropherograms for all samples.
6. *Data analysis*. Using the data obtained for pure water and five chloride standard solutions (S1–S5) generate a linear calibration curve of peak area versus concentration (ppm Cl^-). Data analysis program: *Journal of Chemical Education*: Notebook—A Column Calculator and Plotter for IBM-PC is well suited for this task. The best fit to the data should be determined by a least squares regression analysis.
7. *Concentration of bulk unknown solution*. Determine the concentration of Cl^- in the diluted unknown solutions (U1–U3) by use of the linear calibration curve obtained in step 6. Calculate the concentration of Cl^- in the bulk unknown solution from each of the diluted unknown solutions (U1–U3).
8. *Percent chloride*. Calculate % chloride in the bulk unknown solutions for all three determinations (U1–U3).

$$\frac{\text{g chloride}}{\text{g sample}} \times 100\% \qquad (1)$$

NOTE: 1 ppm = 1 mg/L for aqueous solutions.

Student Results

Figure 2 is an example of the linear calibration curve obtained by a chemistry major with good laboratory skills. The slope of the line is 5590 and *y*-intercept is −883. The correlation coefficient of this least squares analysis is .9994 indicating a very good fit. The student was supplied with an unknown sample containing 57.22% chloride. The student made a bulk unknown solution using 0.1011 g of the dried unknown and diluting to 100.00 mL. The student made the three diluted unknown solutions and obtained data from the CES. Areas for U1–U3 from the CES are shown in Table 1.

Example Calculation for U1

1. Concentration of diluted unknown solution U1 by using the equation of a line:

   area = (slope)(concentration) + *y*-intercept (2)

   15310 = (5590) (concn U1) − 883

   concn U1 = 2.89_7 ppm

2. Concentration of bulk unknown solution from U1:

   (Bulk unknown concn)(0.250 mL) = (2.89_7 ppm)(50.00 mL)

   Bulk unknown concn = $579._4$ ppm

3. Calculate g Cl^- in bulk 100.00 mL sample (diluted 0.1011 g of unknown sample to 100.00 mL):

$$\left(557._4\ \text{ppm}\right) \times \frac{1\ \text{mg/L}}{1\ \text{ppm}} \times \frac{1\ \text{g}}{1000\ \text{mg}} \times (100.00\ \text{mL}) \times \frac{1\ \text{L}}{1000\ \text{mL}} = 0.0579_4\ \text{g Cl}^-$$

4. Calculate percent chloride in the unknown sample using eq 1 (57.3_1% chloride).

The results of the calculations for all three determinations, U1–U3, are shown in Table 1. As can be seen, the student's results for this experiment are very good. The average of the different determinations is in excellent agreement with the value reported by Thorn Smith (57.22%).

Conclusion

This is a short experiment that teaches many concepts. It teaches students serial dilution techniques and how to check the accuracy of the dilutions and calculate their concentration after dilution. Students tend to have problems with serial dilutions and there is a great need for more practice in pipetting techniques. Students tend to lose track of dilutions when performing an experiment. Students tend to find the concentration of the diluted sample, not realizing that they still need to calculate the concentration of the bulk sample. This experiment contains a working example of this situation and also shows the student how to use linear calibration curves. Overall, this is a good experiment that shows students how to use and interpret the results of the new technique of capillary electrophoresis. Excellent results can be obtained as long as the student has good lab techniques. Once the technique and basic concept of the procedure have been mastered, more complex and interesting experiments can be performed by modifying the procedure for other species or substances (e.g., measuring the concentration of various species in commercial products or water samples, or caffeine in beverages [*18*]).

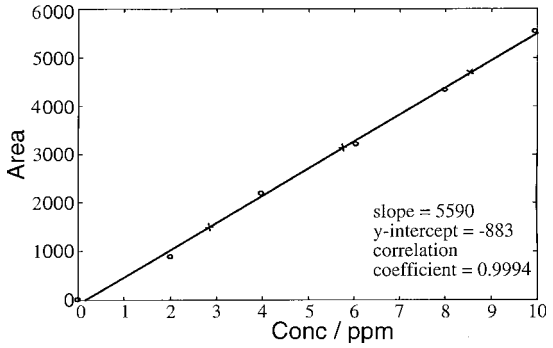

Figure 2. Calibration curve for Cl^-: standards 0, 2, 4, 6, 8, 10 ppm (∘); unknowns U1, U2, U3 (+).

Table 1. Capillary Electrophoresis Results and Calculated Data for U1–U3

Sample	Area	Concn diluted/ ppm	Concn bulk/ ppm	Cl^-/g	% Cl^-
U1	15310	2.90	579	0.0579	57.3
U2	31546	5.80	580	0.0580	57.4
U3	47582	8.67	578	0.0578	57.2
				average (SD)	57.3 (0.1)

Acknowledgment

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Literature Cited

1. Baker, D. R. *Capillary Electrophoresis*; Wiley: New York, 1995.
2. Kuhr, W. G. *Anal. Chem.*. **1990**, *62*, 403R.
3. Lauer, H. H.; Ooms, J. B. *Anal. Chim. Acta* **1991**, *250*, 45.
4. Gross, L.; Yeung, E. S. *J. Chromatogr.* **1989**, *480*, 169.
5. Hayes, M. A.; Ewing, A. G. *Anal. Chem.* **1992**, *64,* 512–516.
6. Whitehead, R.; Rice, C. C. *J. Phys. Chem.* **1965**, *69*, 233–240.
7. Stevens, T. S.; Cortes. H. J. *Anal. Chem.* **1983**, *55*, 1365–1370.
8. Altria, K. D.; Simpson, C. F. *Chromatographia* **1987**, *24*, 527–532.
9. Jorgenson, J. W.; Lukacs, K. D. *Anal. Chem.* **1981**, *53*, 1298.
10. Kenney, B. *J. Chromatogr.* **1991**, *546*, 423.
11. Harrold, M. P.; Wojtusik, M. J.; Riviello, J.; Henson, P. *J. Chromatogr.* **1993**, *640*, 463.
12. Small, H.; Miller, T. E. *Anal. Chem.* **1982**, *54,* 462–469.
13. Jandik, P.; Jones, W. R. *J. Chromatogr.* **1991**, *546*, 431–443.
14. Harrold, M. P.; Wajtusik, M. J.; Riviello, J.; Hensen, P. *J. Chromatogr.* **1993**, *640*, 463–471.
15. Henshall, A.; Harrold, M. P.; Tso, J. M. *J. Chromatogr.* **1992**, *608*, 413–419.
16. Zhang, R.; Cooper, C. L.; Ma, Y. *Anal. Chem.* **1993**, *65*, 704–706.
17. Xue, Y.; Yeung, E. S. *Anal. Chem.* **1993**, *65*, 2923–2927.
18. Conte, E. D.; Barry, E. F.; Rubinstein, H. *J. Chem. Educ.* **1996**, *73*, 1169–1170.